

USER GUIDE

applied  
biosystems®  
by *life* technologies™

# LifeScope™ Genomic Analysis Software 2.5

## Graphical User Interface

DATA ANALYSIS METHODS AND INTERPRETATION

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# About This Guide

## Purpose

This guide is designed to help you quickly perform next-generation sequencing analyses using LifeScope™ Software. These analyses support fragment, paired-end (PE) and long mate pair (LMP) library types of analyses. Specifically, the following workflows are supported:

- Whole genome resequencing
- Targeted resequencing
- Low frequency variant
- Whole transcriptome resequencing
- Small RNA sequencing
- ChIP-Seq mapping
- MethylMiner™ mapping

## Prerequisites

It is assumed that you have working knowledge of the:

- Linux® operating system
- Internet Protocol (IP) address of the LifeScope™ cluster.
- Linux environment and know how to:
  - Navigate to directories.
  - Edit and save files in a text editor.
  - Run Linux shell scripts.
  - Run basic Linux commands such as `chmod`, `ps`, `pwd`, `cd`, `echo`, `grep`, and other commands.



# PART I

## Overview



## 1

# Introduction to LifeScope™ Genomic Analysis Software

## LifeScope™ Overview

LifeScope™ Genomic Analysis Software is a modular data analysis bioinformatics tool for performing off-instrument secondary and tertiary analyses on sequence data generated by Life Technologies instruments. The resulting industry-standard files from LifeScope™ can be used with third-party visualization and analysis software tools.

After years of development on analysis tools for SOLiD® System data, in response to customer feedback, Applied Biosystems LifeScope™ Genomic Analysis Software enables fast translation of next-generation data for biologically meaningful results. LifeScope™ matches the accuracy of the next generation 5500 Series SOLiD™ Sequencers with Exact Call Chemistry (ECC) and streamlines your data analysis.

## Features

LifeScope™ Software is part of the LifeScope™ Genomic Analysis Solution. This informatics solution is comprised of genomics software combined with a specified hardware platform. LifeScope™ Software features:

- Seamless integration with the 5500 Series Genetic Analysis Systems
- Performance-tuned algorithms for the 5500 Series and ECC module
- Push-button workflows, intuitive user interface, and secure project management
- Optimized mapping and smaller file formats
- Annotated variant reports, numerous charts, and select visualization tools for simple data interpretation
- Graphically driven configuration of multistep analysis workflows
- Ability to save and reuse workflows
- Ability to resume a workflow without repeating completed analyses
- Secure project-based data management specific to your data analysis
- Projects can be stored and data reanalyzed

## Data analyses

Complementing LifeScope™ Software features are the following types of data analyses:

- Whole genome sequencing
- Targeted resequencing
- Low frequency variant
- Whole exome sequencing
- Whole transcriptome RNA sequencing
- Small RNA sequencing
- MethylMiner™ mapping
- CHiP-Seq mapping

- Detection of wide range of genomic and RNA variation, including:
  - SNP detection
  - Large and small indel detection
  - Copy number variation detection
  - Inversion detection
  - Fusion transcript detection
  - Exon counting
  - Splice finding

## LifeScope™ components

LifeScope™ Software components include:

- The core software server, which is the main software architecture that maintains the interaction of the graphical user interface (GUI) and compute engine. The server works with high performance cluster schedulers to maximize the computation demands of the finely tuned data analysis algorithms.
- The research GUI, which enables scientists to perform mapping and predefined workflows from a desktop computer. The GUI offers auto-generated charts and plots for each analysis type module run within a workflow.
- The command-line user interface, which gives bioinformaticians the power to customize workflows and manipulate every parameter available in each analysis module using specific LifeScope™ Software command-line syntax.
- The Admin GUI, which is an administration tool for managing user accounts and licensing permissions.

## Workflows

This section describes a standard workflows, a project workflow, and several analysis workflows.

### Standard workflow

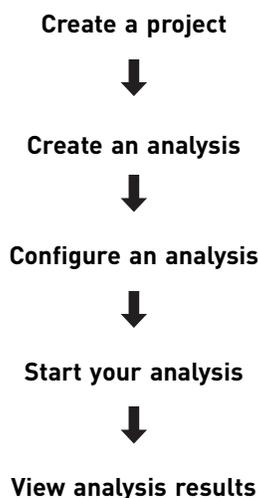
A standard workflow is a built-in series of commonly used analyses, which correspond to a common biological application. Using one of these workflows (data analyses) enables you to run an analysis with a minimum of required setup.

Standard workflows are provided for each of the data analyses listed on .

In LifeScope™, running a standard workflow requires the following steps:

1. Create a project and analysis.
2. Identify your input data (read-set from your 5500 Series Sequencer instrument).
3. Identify the reference genome.
4. Choose the data analysis (see the list [on page 23](#)) to be executed on your data.
5. Start and monitor your analysis.
6. View the results of your analysis.

## Project workflow



In *LifeScope™ Genomic Analysis Software Command Shell User Guide* (Part no. 4471875), these steps are covered in the following chapters:

- *Chapter 5, Understand The LifeScope™ Software Shell* – Introduces projects, analyses, and other concepts required for working with the LifeScope™ command shell.
- *Chapter 6, Run a Command Shell Analysis* – Describes the shell commands, including commands to create a project, create an analysis, configure an analysis, start an analysis run, and view analysis results.
- *Chapter 7, Run a Standard Workflow Analysis* – Describes LifeScope™ Software standard workflows, which provide built-in sequences of experiments corresponding to common biological applications, such as resequencing, targeted resequencing, low frequency variant detection, small RNA, whole transcriptome, ChIP-Seq, and MethylMiner™ mapping.

## Analysis workflows

In LifeScope™ Software, a workflow is a series of analysis steps, and each analysis step is dependent only on the steps that precede it. This section describes the flow of secondary and tertiary analyses. Primary analysis is done on the instrument.

### Secondary analysis workflows

Secondary analysis always starts with mapping. The input files required by the mapping tool are XSQ (eXtensible SeQuence) files.

The results of mapping and pairing in secondary analysis are used as input for tertiary analysis tools. You can only run the inversion and large indel modules on the results from mapping paired data. The diBayes, CNV, and small indel modules can be executed on the output of mapping either fragment or paired data. The whole transcriptome analysis workflow is different from the resequencing workflows because it uses fragment data to perform its own mapping.

LifeScope™ Software secondary analyses include:

- Mapping
- Mapping statistics
- Reporting
- Whole Genome Resequencing

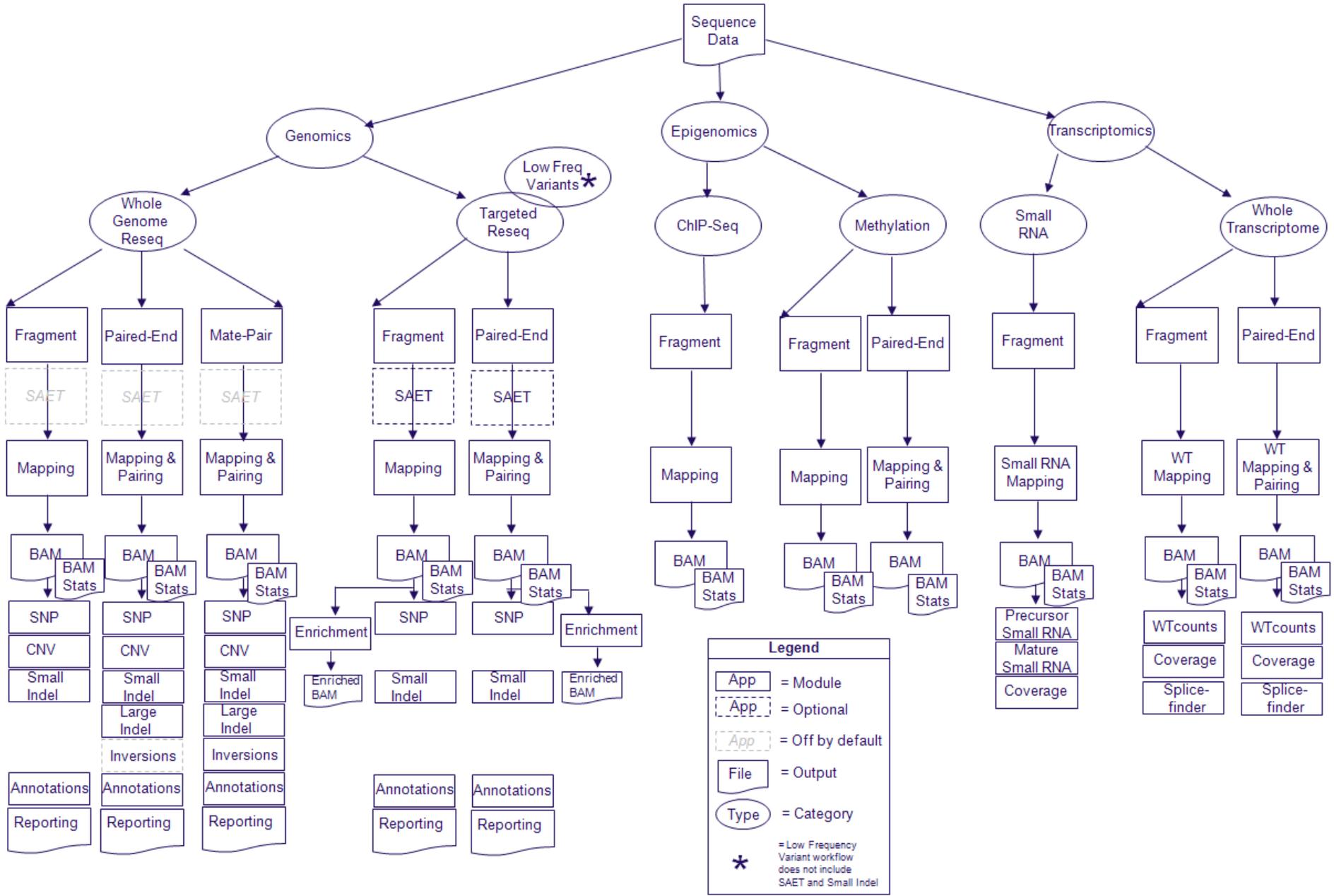
- Targeted Resequencing
- Low Frequency Variant detection
- Whole Transcriptome
- Small RNA
- ChIP-Seq
- MethylMiner™

### Tertiary analysis modules

The following table describes the data analysis modules used in workflows.

Analysis	Used in workflows...
Annotations	Whole Genome Resequencing, Targeted Resequencing, Low Frequency Variant
Human CNV	Whole Genome Resequencing, Targeted Resequencing
Inversions	Whole Genome Resequencing, Targeted Resequencing
Large indels	Whole Genome Resequencing, Targeted Resequencing
Small indels	Whole Genome Resequencing, Targeted Resequencing
SNPs	Whole Genome Resequencing, Targeted Resequencing, Low Frequency Variant
Small RNA count	Small RNA
Small RNA coverage	Small RNA
WT count	Whole Transcriptome
WT coverage	Whole Transcriptome
WT splice finder	Whole Transcriptome

The illustration [on page 27](#) shows the data analysis modules used in predetermined workflows.



The analysis workflows and the modules they execute are described in the following table.

<b>Workflow</b>	<b>Library type</b>	<b>LifeScope™ modules involved</b>
ChIP-Seq	Fragment	Secondary: <ul style="list-style-type: none"> <li>• SAET</li> <li>• Fragment mapping</li> <li>• Mapping statistics-</li> </ul>
Genomic resequencing	Fragment	Secondary: <ul style="list-style-type: none"> <li>• SAET</li> <li>• Mapping</li> <li>• Mapping statistics</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• SNP Finding</li> <li>• CNV</li> <li>• Small indels</li> <li>• Annotations</li> </ul>
	Mate-pair	Secondary: <ul style="list-style-type: none"> <li>• SAET</li> <li>• Paired mapping</li> <li>• Mapping statistics</li> <li>• Mark duplicates</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• SNP Finding</li> <li>• CNV</li> <li>• Inversions</li> <li>• Large indels</li> <li>• Small indels</li> <li>• Annotations</li> </ul>
	Paired-end	Secondary: <ul style="list-style-type: none"> <li>• SAET</li> <li>• Paired mapping</li> <li>• Mapping statistics</li> <li>• Mark duplicates</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• SNP Finding</li> <li>• Inversions</li> <li>• Large indels</li> <li>• CNV</li> <li>• Small indels</li> <li>• Annotations</li> </ul>

Workflow	Library type	LifeScope™ modules involved
MethylMiner™	Fragment	Secondary: <ul style="list-style-type: none"> <li>• SAET</li> <li>• Paired mapping</li> <li>• Mapping statistics</li> </ul>
	Paired-end	Secondary: <ul style="list-style-type: none"> <li>• SAET</li> <li>• Fragment mapping</li> <li>• Mapping statistics</li> <li>• Mark duplicates</li> </ul>
Small RNA	Small RNA	Secondary: <ul style="list-style-type: none"> <li>• Small RNA mapping</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• Small RNA count</li> <li>• Small RNA coverage</li> </ul>
Targeted resequencing	Fragment	Secondary: <ul style="list-style-type: none"> <li>• SAET</li> <li>• Fragment mapping</li> <li>• Mapping statistics</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• Enrichment</li> <li>• SNP Finding</li> <li>• Small indels</li> <li>• Annotations</li> </ul>
	Paired-end	Secondary: <ul style="list-style-type: none"> <li>• SAET</li> <li>• Paired mapping</li> <li>• Mapping statistics</li> <li>• Mark duplicates</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• Enrichment</li> <li>• SNP Finding</li> <li>• Small indels</li> <li>• Annotations</li> </ul>

<b>Workflow</b>	<b>Library type</b>	<b>LifeScope™ modules involved</b>
Low frequency variant	Fragment	Secondary: <ul style="list-style-type: none"> <li>• Fragment mapping</li> <li>• Mapping statistics</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• Enrichment</li> <li>• SNP Finding</li> <li>• Annotations</li> </ul>
	Paired-end	Secondary: <ul style="list-style-type: none"> <li>• Paired mapping</li> <li>• Mapping statistics</li> <li>• Mark duplicates</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• Enrichment</li> <li>• SNP Finding</li> <li>• Annotations</li> </ul>
Whole transcriptome	Fragment	Secondary: <ul style="list-style-type: none"> <li>• WT splice junction extractor</li> <li>• WT fragment mapping</li> <li>• Mapping statistics</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• WT counts</li> <li>• WT coverage</li> <li>• Splice finder</li> </ul>
	Paired-end	Secondary: <ul style="list-style-type: none"> <li>• WT exon sequence extractor</li> <li>• WT splice junction extractor</li> <li>• WT paired-end mapping</li> <li>• Mapping statistics</li> </ul>
		Tertiary: <ul style="list-style-type: none"> <li>• Splice finder</li> <li>• WT counts</li> <li>• WT coverage</li> </ul>

## Primary and secondary file types

The following table lists the files used in secondary and tertiary analysis.

<b>Analysis type</b>	<b>File type</b>	<b>File name extension</b>	<b>File content</b>	<b>Used in . . .</b>
Primary	Raw reads file	*.xsq	Extensible sequence file	Secondary analysis

Analysis type	File type	File name extension	File content	Used in . . .
Secondary	BAM file	*.bam	Binary Alignment sequence Map (BAM), a generic file format used to store large numbers of nucleotide sequence alignments	Tertiary analysis

## Input and output file formats

All analysis modules use specific input files and produce specific output files. The input and output file requirements vary, depending on the type of analysis that you want to perform. The following table provides the names of the input and output file types.

Analysis module	Input file types	Output file types
Mapping and pairing	*.xsq, *.csfasta, *.fasta, *.qual	*.bam
SNP Finding	*.bam	*.gff.3
CNV - singleSample		
Large indel -singleSample		
Large indel -pairedSample	—	—
Inversion	*.bam	*.gff.3, *.txt
Position error		position error
Whole transcriptome mapping	*.csfasta, *.fasta, filter reference fasta, WT *.gtf reference	*.bam

Key

\* = one or more

\*.gff.3 = public, viewer-oriented \*.gff v3



# PART II

## Installation



## 2

# LifeScope™ Genomic Analysis Software Installation

This chapter covers:

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## Overview

This chapter is intended for use by the LifeScope™ administrator. The chapter describes the LifeScope™ Genomic Analysis Software installation procedure, and system software and hardware requirements.

## Prerequisites

Some procedures in this chapter require that you:

- Know the Linux® operating system of the cluster on which LifeScope™ is to be installed
- Know the Internet Protocol (IP) address of the cluster
- Have a login (ID) on the cluster
  - Portions of the install require root access or your system administrator's assistance
- Know how to:
  - Navigate to directories in a Linux environment
  - Edit and save files in a text editor

- Run Linux shell scripts
- Run basic Linux commands such as `chmod`, `ps`, `pwd`, `cd`, `echo`, `grep`, and other commands

## Workstation requirements

Workstations are shipped with CentOS 5.7 and are ready to run LifeScope™ Software. Customer upgrade of the CentOS software is not supported.

---

**WARNING!** LifeScope™ does not operate properly on workstations running operating systems other than CentOS 5.7. CentOS 6.0 is not supported.

---

## Cluster hardware requirements

To successfully install and run the LifeScope™ Software application, we recommend that your cluster meets the following hardware requirements:

CPU Speed	2 GHz Minimum
Cores	8 Minimum
Memory	24 GB Minimum per compute node
Disk Space	500 GB of local or shared storage per node
	200 MB for LifeScope™ installation
	225 GB for reference files installation‡
	2 GB for examples installation
	25 GB for performance verification data

‡ You can use any type of shared storage to meet this requirement.

**Note:** Distributed parallel file systems such as Lustre, GlusterFS, GPFS require additional tuning to be compatible with LifeScope™ Software. For example, we have encountered instances of job failures caused by subpar small file performance.

## System requirements

LifeScope™ Genomic Analysis Software requires a Linux® cluster with a TORQUE, Sun Grid Engine (SGE), or Load Sharing Facility (LSF) resource manager, with a scheduler that can support scheduling policies and dynamic job priorities.

## Server requirements

Before you install LifeScope™ Genomic Analysis Software, be sure that your cluster meets the following requirements:

- LifeScope™ Genomic Analysis Software supports only the following distributions, and only on 64-bit platforms:
  - Red Hat Enterprise Linux 4.7 through 5.7
  - CentOS 4.7 through 5.7

**Note:** LifeScope™ Software does not support CentOS 6.0 or RHEL 6.0.

- PBS/TORQUE v2.3+, SGE v6.2+, or Platform LSF 7 Update 6.

**Note:** LifeScope™ Software requires a job submission queue on the cluster. The LifeScope™ Software installer does not create this queue on the cluster.

**Note:** If you use SGE, you must create a symmetric multiprocessing (SMP) parallel environment.

**Note:** If you use LSF, it is highly recommended that LifeScope™ Software jobs are non-preemptable by LSF.

Before you install LifeScope™ Software on the head node, pre-install compatible versions of these software packages on *all* compute nodes:

- Python 2.3 (or later version)
- Perl v.5.8.5 (or later version)

**Note:** Inversion module works with Perl versions up through 5.8.8. The more recent versions of Perl have a backwards compatibility issue which causes the inversion module to fail.

### GCC Compiler

Ensure that the GNU Compiler Collection (GCC) compiler v3.4.6 or v4.1.2 installed and configured. While LifeScope™ Software does not use the compiler itself, having the compiler installed and configured ensures that the necessary dynamically linked libraries are available.

### Check firewall restrictions

Ensure that there is no firewall restriction on the host running the LifeScope™ server. The LifeScope™ accesses the LifeScope™ license server on TCP/IP port 27000, by default. The LifeScope™ UI clients and command shell clients access the LifeScope™ server on TCP/IP port 9998 by default, although this port can be changed during the installation. Consult your system administrator to check the firewall access for LifeScope™ users.

### Check DNS hostname

The DNS fully-qualified domain name and the local Linux hostname must match. Also, the forward and reverse lookup on the DNS against the hostname and its public-facing IP address must be consistent. The LifeScope™ licensing server may not work correctly if there are DNS discrepancies. Consult the system administrator to check this requirement.

### Client machines

Java v1.6 (or higher) is required on client machines.

For Mac users who do have Java v1.6, follow these steps to update:

1. Use the Mac Software Update (**apple ▶ Software Update...**) to install.
2. Once you have installed, go **Applications ▶ Utilities ▶ Java Preferences**.
3. Select only Java SE 6.0.

### Browser requirements

LifeScope™ Software supports these browsers:

- Internet Explorer® versions 6 and 7
- Mozilla® Firefox 3.0.1

## LifeScope™ Software Administration

This section uses the acronyms in this list to describe installation roles and locations:

- **SA** – The Linux system administrator for the cluster. The administrator's root access is required for user management and copying the data drive.
- **LSA** – The LifeScope™ Software administrator. The installation instructions recommend creating a new user account for the LSA. The LSA must run the installation script.
- **LSU** – LifeScope™ Software users. These users do not have admin permissions.
- **LSBF** – The LifeScope™ Software Binaries Folder. The LSBF is the installation directory.
- **LSDF** – The LifeScope™ Software Data Folder. The LSDF is a location for LifeScope™ Software repositories, resources, and other files.

To install or run LifeScope™, one person must be designated as the master LifeScope™ user. The master user also becomes the default LifeScope™ Administrator (LSA). The LSA and regular LifeScope™ Users (LSUs) must share a common UNIX® user group.

We recommend that the LSA not share the same user account as the UNIX System Administrator (SA), because of a requirement that all LSUs be members of the LSA's primary UNIX user group. The SA's primary UNIX user group is usually `root`. Non-administrator users typically are not allowed to be members of the `root` user group.

We recommend creating a new Linux user account, `lifescape`, for the LSA. The primary UNIX user group for the `lifescape` user should be the same group as most LifeScope™ Users (for example, the group `users`). The Linux account for the LSA should have this shared group as its primary group when the LSA starts the LifeScope™ server.

After installation, LifeScope™ files and folders are owned by the LSA. All application jobs and processes are submitted by the LSA.

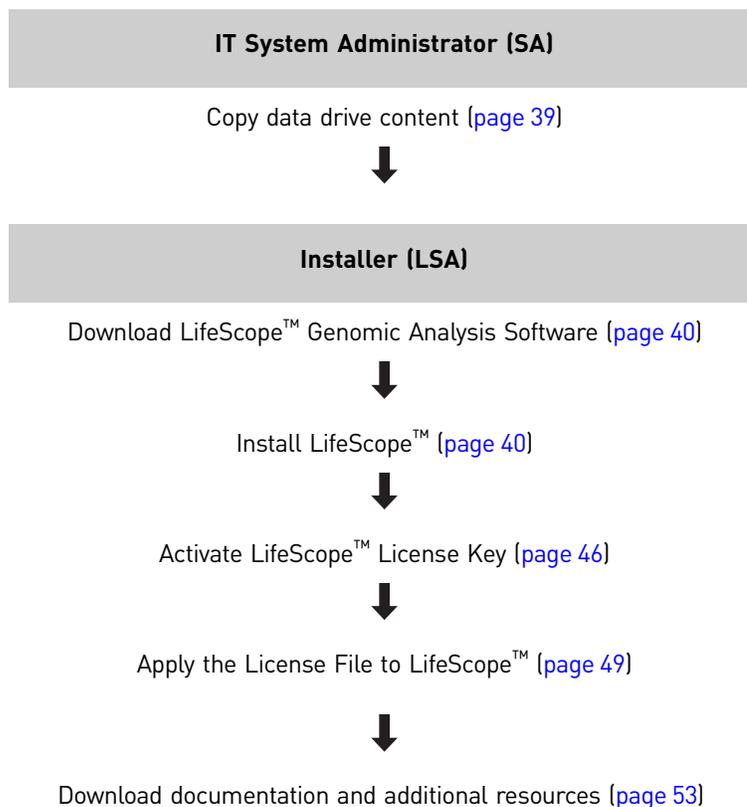
If you are not the LSA or if an LSA does not exist, please contact your System Administrator to have the master user and user group created. You must be logged in as the LSA to install LifeScope™ Software.

Identify two shared folders for holding the software and data in a suitably common location, accessible to all LifeScope™ users. Both locations should be accessible (mounted) from the compute nodes.

- Identify the LifeScope™ Binaries Folder (LSBF) to install LifeScope™ Software. This folder should be writable to LSA and readable to LSUs. Typically you may choose `/opt/lifescape`. If the LSBF does not exist, create it with appropriate permissions.
- Identify the location of the LifeScope™ Data Folder (LSDF) to hold all LifeScope™ resources, repositories, pre-installation tar files, installer, examples, test data, and other files. This location should have read and write permissions for the LSA and all LSUs. This location also should be sufficiently large to hold the initial data and the results of future analysis. Typically you may choose `/share/lifescape`. If the LSDF does not exist, create it with appropriate permissions.

Make sure that you have the appropriate read and write permissions for the LSBF and LSDF folders, before proceeding with the installation.

## Installation workflow overview



## Copy data drive content

(System Administrator) Copy the data drive content to the LifeScope™ Data Folder (LSDF) on the LifeScope™ server, and change owner : group to those of the LifeScope™ Administrator (LSA).

The data drive's file system is ext3, which typically is supported only on Linux.

The drive content includes the following:

- Files for use with hg18 and hg19 genomes:
  - Hash tables for the main mapping schemes
  - Mappability files for use with the CNV analysis module
- LifeScope™ reference repository files:
  - Hg18 and hg19 reference files and filter reference files
  - Genomic annotation files (dbSNP)
  - Annotation files from the miRNA database
- Data files for installation verification

The reference files downloaded in this step become the reference repository for your LifeScope™ Software. The location of the reference files (the `referenceData` directory) is required during installation.

---

**IMPORTANT!** If you add your own reference files to the reference repository, you must follow the instructions in the Reference Repository appendix in *LifeScope™ Genomic Analysis Software Command Shell User Guide* (Part no. 4471875).

---

## Download LifeScope™

1. (LSA) Visit the LifeScope™ project page:  
<http://solidsoftwaretools.com/gf/project/lifescop>
2. On the project page, you must:
  - a. Read and accept the LifeScope™ End User License Agreement.
  - b. Create an email with the following information:
    - Your acceptance of the LifeScope™ End User License Agreement.
    - Your LifeScope™ license key.
    - Your solidsoftwaretools account name.Send the email to:  
[lifescop@lifetech.com](mailto:lifescop@lifetech.com)  
Instructions to download LifeScope™ are emailed to you.
3. Go to the download page, as directed by the emailed instructions.
4. Download LifeScope™ .tar.gz file (for example, *LifeScope-2.0r8380-2011040333223344.tar.gz*) to the LifeScope™ Data Folder (LSDF).
5. Download the associated installer (*install.sh*) to the LifeScope™ Data Folder (LSDF).

## Install LifeScope™ Software

This procedure provides typical, general instructions for installing LifeScope™ Software. The actual procedures for your site might vary, depending on the configuration of the LifeScope™ Software cluster and the installation options you select.

---

**WARNING!** LifeScope™ Software must *NOT* be installed as root.

---

### Hardware configuration

This section explains the three types of installation based on hardware configuration. You must select your configuration when prompted by the installer in “[Select the type of installation:](#)”, [step 6 on page 42](#).

- **Standalone Workstation** – LifeScope™ Software is installed completely on the local workstation. There is no access to a cluster. Resource management software is still required on the workstation.
- **Cluster** – LifeScope™ Software is installed on a cluster with a head node, a set of compute nodes, shared storage, node local storage, and resource management software, as described in “[Cluster hardware requirements](#)” on [page 36](#).

- **Workstation with Remote Submission** – The local workstation, instead of the cluster head node, is used for running the LifeScope™ Software server. The requirements for the workstation are:
  - A regular Linux 64-bit workstation with the following:
    - 250 GB harddrive
    - 4 GB RAM
    - Dual core
  - The operating system requirements are the same as those listed for the cluster in [“Server requirements” on page 36](#).

LifeScope™ Software jobs are submitted from the workstation onto the cluster. This requires that you *first* setup the workstation as described in [“Set up a workstation with remote submission” on page 44](#).

You must be logged in as the LifeScope™ Software administrator (LSA) on the local workstation, and not on the cluster. The installer (`install.sh`) also must be run on the local workstation and not on the remote cluster. You must provide the IP address of the cluster head node, and the LSA username and password, when asked by the installer. This information is required for the workstation to automatically login to the cluster for submitting jobs.

By default the password appears as plain text in the file `<LSBF>/etc/analysis/system.properties` file. If the LSA is setup for password-less login as described in [“Configure the workstation” on page 45](#), then you can give a blank password when asked by the installer, and prevent the password from appearing in the properties file.

## Authentication realm

This section explains the authentication realms supported by LifeScope™ Software. You must supply your realm type when prompted by the installer in [“Select an authentication realm to be used with LifeScope™ Software:”, step 3 on page 44](#).

- **LifeScope** – This authentication realm refers to the LifeScope™ Software application user database. If this realm is chosen, user passwords are maintained in the software database. Passwords are stored as one-way MD5 digests for security reasons. Users must be manually created in this realm. There is no relationship between the application users and Linux® host system users. User account management can be done by the LSA using the LifeScope™ Admin module (included in this installation).
- **LDAP** – This realm refers to an LDAP-compliant authentication server (OpenLDAP, Active Directory, etc.). If this realm is chosen, user passwords are *not* stored in LifeScope™. User credentials are authenticated against the configured LDAP server. Users need not be manually created in LifeScope™ with this realm (though they may be). A valid LDAP server address and LDAP Bind DN must be provided in the configuration. User account management can be done by the LSA using the LifeScope™ Admin module (included in this installation).
- **Host** – This realm refers to the Linux host machine on which the LifeScope™ server is running. If this realm is chosen, user passwords are *not* stored in LifeScope™ Software. User credentials are authenticated against the local machine using SSH (therefore uses whatever authentication scheme SSH uses, for example, NIS, `/etc/passwd`). There is a one-to-one relationship between the application users and Linux host system users. Users need not be manually created in the LifeScope™ Software Admin Portal with this realm.

**Note:** If you change the authentication realm after installation, you must stop and restart the LifeScope™ Software server.

**Note:** Changing a realm does not remove users from the previous realm. However, users might not be able to login in the new realm, unless they both exist in the new realm and are enabled in the new realm.

## Installation instructions

1. (LSA) Go to the downloaded folder LSDF:

```
cd /path/to/<LSDF>
```

2. Run this UNIX® command:

```
chmod +x install.sh
```

to make the downloaded `install.sh` file executable.

3. Run the installer:

```
./install.sh
```

The welcome banner appears:

```
*****
This is the LifeScope™ Genomic Analysis Software installation and
licensing program
*****
```

4. Installation options include:

- 1) Install the LifeScope™ Genomic Analysis Software.
- 2) Upgrade your current installation of LifeScope™ Genomic Analysis Software.

The following options require successful installation of LifeScope:

- ```
-----
```
- 3) Re-configure the LifeScope™ Genomic Analysis Software.
  - 4) Request a LifeScope™ Software license.
  - 5) Register a LifeScope™ Software license.
  - 6) Exit this application.

Please select the operation you would like to perform (Q/q to quit) [1]:

To install LifeScope™ Software, type **1**.

5. At the prompt, type **y** to begin installation.

6. Select the type of installation:

- 1) Standalone Workstation Server (default)
- 2) Cluster
- 3) Workstation w/Remote Submission

Review **“Hardware configuration” on page 40** to determine your installation type.

7. Enter the location where LifeScope™ Software is to be installed. The default directory is `/opt/lifescopes`.

Accept the default or change the directory. Provide the location you have chosen for the LifeScope™ Software Binaries Folder (LSBF):

`/path/to/<LSBF>`

If the directory you enter already exists, you are prompted “Do you still want to use this location?”

8. Enter the location for reference files. Provide the destination location of the data drive contents (from the “Copy data drive content” step on page 39):

`/path/to/<LSDF>/referenceData`

**Note:** LifeScope™ Software reference files are required for full functionality of LifeScope™ Software. The default directory is `/data/results/referenceData`.

9. Enter the location for reads. This entry sets the location of the reads repository. You may choose

`/path/to/<LSDF>/reads`

The default directory is `/data/results/reads`.

10. Enter the location for projects. This entry sets the location of the projects repository. You may typically choose

`/path/to/<LSDF>/projects`

The default directory is `/data/results/projects`.

11. Enter the location for Binary Alignment Map (BAM) file repository. You may choose

`/path/to/<LSDF>/bams`

The default directory is `/data/results/bams`.

12. Enter the location for the scratch location for LifeScope™ Software. The default directory is

`/scratch`.

**Note:** The scratch location is used as a temporary work space for submitted jobs. For best performance, cluster and remote server installations require a scratch location on the local file system of each *compute node* of the system, not on the file system of the *head node*.

13. Enter the IP address or Fully Qualified Domain Name (FQDN) for the LifeScope™ Software server, for example: `192.168.1.27`.

The address of the system where LifeScope™ Software is installed is required to successfully access and run the software. The system address is the IP address or Fully Qualified Domain Name (FQDN) of this system or head node.

**Note:** Do not use the name `localhost` or IP address `127.0.0.1`. The address should be the public-facing name or IP address of the LifeScope™ server. The LSUs access the software using this name or IP address. Typically, the address is the domain name system (DNS) name of the server.

Test the connection to the IP address or FQDN to verify a connection to the system.

Enter the Web URL port address to be used to access the LifeScope™ Server. Default is 9998.

## Configure the license server

1. Enter the IP address or Fully Qualified Domain Name (FQDN) for the LifeScope™ License Server.

The installer tests the connection to the given IP address or FQDN to verify a connection to the system.

2. Enter the port address for the license server to be used with LifeScope™ Software.
3. Select an authentication realm to be used with LifeScope™ Software:
  - 1) LifeScope™ Software (default)
  - 2) Lightweight Directory Access Protocol (LDAP)
  - 3) Host

Review “[Authentication realm](#)” on page 41 to determine your realm type.

**Note:** If you change the authentication realm after installation, you must stop and restart the LifeScope™ Software server.

**Note:** Changing a realm does not remove users from the previous realm. However, users might not be able to login in the new realm, unless they both exist in the new realm and are enabled in the new realm.

4. Select the cluster resource manager to be used by LifeScope™ Software:
  - 1) PBS/Torque (default)
  - 2) SGE
  - 3) LSF
5. Enter the cluster-resource-manager-job-submission-queue-name to be used by LifeScope™ Software, for example, *lifescope*.
6. Enter the number of nodes available on the cluster. Default is 4.
7. Enter the number of cores for each compute node. Default is 8.
8. Enter the memory size (in GB) for each compute node. Default is 22.

---

**IMPORTANT!** Allow 2 GB for the compute nodes' OS. For example, for compute nodes with 24 GB, enter 22; for compute nodes with 48 GB, enter 46.

---

### Continue installing LifeScope™ Software

1. At the prompt “Do you want to change any of these entries?” type **n** to accept the entries.  
The selected LifeScope™ configurations are saved.
2. At the prompt, “Do you want to continue installing the LifeScope package?” type **y** to continue the installation.

## Set up a workstation with remote submission

This section describes prerequisites for the install type *Workstation w/Remote Submission*. Skip this section if your installation type is either of these:

- 1) Standalone Workstation Server (default)
- 2) Cluster

In the *Workstation w/Remote Submission* scenario, LifeScope™ is installed a workstation but submits analysis jobs to a remote cluster. The LifeScope™ server and licensing server run on the local workstation. The analysis jobs are submitted to the remote cluster using SSH. LifeScope™ services do *not* run on the cluster head node.

The workstation and cluster nodes share common file system locations through NFS mounting or other means. The installation location `<LSBF>/lifescope` and the LifeScope™ data folder `<LSDF>` must be on the common shared file system. Both the `<LSBF>/lifescope` and the `<LSDF>` folders must first be created on the cluster. (See [“LifeScope™ Software Administration” on page 37](#) for a description of `<LSBF>` and `<LSDF>`.)

The cluster head node must be accessible (via SSH) from the workstation while the analysis is executing.

As with the normal cluster installation, the LifeScope™ administrator account must exist on the cluster, as described in [“LifeScope™ Software Administration” on page 37](#).

## Configure the workstation

These instructions are only for Workstation w/Remote Submission, option 3) in [“Select the type of installation:”, step 6 on page 42](#). The IT system administrator performs these configuration steps. The commands given in this section are not applicable to all cluster and workstation combinations. The given commands are expected to serve as general guidelines to the system administrator to give an example of the procedures required. The actual commands to be used may vary depending on the system configuration.

1. Note down the UID and GID (user and group ids) of the LifeScope™ administrator (LSA) on the cluster.
2. Create a user and group on the workstation with the same UID and GID as that of the LSA on the cluster. If the LSA user account already exists on the workstation, the IT system administrator must modify the UID and GID of that user account on the workstation. For example:

```
usermod -u <UID> <LSA>
```

3. Set up password-less login for the LSA user account from the workstation to the cluster, using `ssh-keygen`, `.ssh/authorized_keys` file on the destination, etc.
4. On the workstation, mount the location of the `<LSBF>` and `<LSDF>` directories from the cluster, using the same path. The path used on the workstation must be the same as the path used on the cluster.

Example commands are:

```
mkdir -p /path/to/<LSBF>
mkdir -p /path/to/<LSDF>
mount -t nfs cluster_ip:/path/to/<LSBF> /path/to/<LSBF>
mount -t nfs cluster_ip:/path/to/<LSDF> /path/to/<LSDF>
```

These commands may require root permissions. These are example commands. The actual commands may vary according to the system administrator's choice.

You are now ready to proceed with the installation procedures described in these sections:

- [“Hardware configuration” on page 40](#)
- [“Authentication realm” on page 41](#)

- “Installation instructions” on page 42

## Activate the LifeScope™ Software License Key

(LSA) These instructions describe how to apply the License Key. The process includes (each of these steps is described below):

- Obtaining the MAC address of the server that has LifeScope™
- Activating your License Key and obtain a license file specific to your server
- Applying the license file to activate your copy of LifeScope™

When applied to LifeScope™, the core license file grants up to five users access to the software for one year.

### Prerequisites

Before activating the LifeScope™ License Key, please ensure that:

- You have downloaded and installed LifeScope™ on computer hardware that meets the LifeScope™ minimum hardware requirements (please see [www.lifetechnologies.com/LifeScope](http://www.lifetechnologies.com/LifeScope) for more details).
- You have performed the DNS hostname check described in “Check DNS hostname” on page 37.
- You are familiar with using command-line Linux®, and you have the necessary permissions to log on to the server that has LifeScope™.
- You have a LifeScope™ License Key.

### Internet connectivity

Direct internet connectivity for the host computer is highly recommended for the license activation process.

### Check the computer date

Check that the computer date matches the current date and time. Each license has a start date. If the computer date is earlier than the license start date, access to the software is disabled until the license start date. If you need to reset the computer date, only reset it *forward*. **Do not** reset the computer date backward; doing so prevents access to LifeScope™. Contact your system administrator (SA) if you need to change the date on the system.

---

**IMPORTANT!** Resetting your computer date backwards disables your license key and prevents the operation of LifeScope™.

---

### Obtain the MAC address

---

**IMPORTANT!** Run the Linux commands used in this procedure on the computer hardware that has LifeScope™ installed on it. If you want to run the LifeScope™ license server on a different machine, then you must obtain the MAC address for that machine.

---

---

**IMPORTANT!** The MAC address used for licensing LifeScope™ should be associated with a permanent Ethernet card on the host computer system. If there is a possibility that `eth0` is not a permanent Ethernet card, consult your system administrator to determine which Ethernet card should be used instead. If the Ethernet card associated with the LifeScope™ license is replaced on the host system, a new license file must be generated for your account.

---

To obtain the MAC address of the server that has LifeScope™ installed on it:

1. Run the Linux command

```
/sbin/ifconfig
```

Output similar to the one shown below appears:

```
[cora@foshtv02 ~]$ /sbin/ifconfig
eth0  Link encap:Ethernet  HWaddr 00:1C:23:BB:E1:F3
      inet addr:10.1.1.1  Bcast:10.1.1.255  Mask:255.255.255.0
      inet6 addr: fe80::21c:23ff:febb:ef3/64 Scope:Link
      UP BROADCAST RUNNING MULTICAST  MTU:1500  Metric:1
      RX packets:259842678 errors:0 dropped:0 overruns:0 frame:0
      TX packets:296616310 errors:0 dropped:0 overruns:0 carrier:0
      collisions:0 txqueuelen:1000
      RX bytes:214032251167 (199.3 GiB)  TX bytes:33928429 (315.9 GiB)
      Interrupt:169 Memory:f4000000-f4012800

eth1  Link encap:Ethernet  HWaddr 00:1C:23:BB:E1:F1
      inet addr:167.116.6.72  Bcast:167.116.6.255  Mask:255.255.255.0
      inet6 addr: fe80::21c:23ff:febb:ef1/64 Scope:Link
      UP BROADCAST RUNNING MULTICAST  MTU:1500  Metric:1
      RX packets:25669852 errors:0 dropped:0 overruns:0 frame:0
      TX packets:82980132 errors:0 dropped:0 overruns:0 carrier:0
      collisions:0 txqueuelen:1000
      RX bytes:10648346642 (9.9 GiB)  TX bytes:117855364 (109.7 GiB)
      Interrupt:169 Memory:f8000000-f8012800

lo    Link encap:Local Loopback
      inet addr:127.0.0.1  Mask:255.0.0.0
      inet6 addr: ::1/128 Scope:Host
      UP LOOPBACK RUNNING  MTU:16436  Metric:1
      RX packets:11049203 errors:0 dropped:0 overruns:0 frame:0
      TX packets:11049203 errors:0 dropped:0 overruns:0 carrier:0
      collisions:0 txqueuelen:0
      RX bytes:6069766283 (5.6 GiB)  TX bytes:6069766283 (5.6 GiB)
```

2. In the paragraph labeled `eth0`, the first line should contain text that starts with `HWaddr`. The MAC address follows this text, and should look similar to `00:30:48:9F:72:76`

## Obtain the MAC Address with the LifeScope™ Installer

**Note:** In this method, the MAC address is referred to as the “Computer ID.”

If you have difficulty finding the MAC address, the LifeScope™ Installer can find the the address for you. To use the LifeScope™ Installer:

1. From the software download directory, run

```
install.sh
```

The LifeScope™ Installer information shown below appears:

```
*****
This is the LifeScope Genomic Analysis Software installation and licensing program
*****

1) Install the LifeScope Genomic Analysis Software.

The following options require successful installation of LifeScope:
-----
2) Re-configure the LifeScope Software.
3) Request a LifeScope Software license.
4) Register a LifeScope Software license.

5) Exit this application.

Please select the operation you would like to perform (Q/q to quit) [1]: 3

To request a LifeScope product license for operating the application:
Enter the following URL into a Web browser. This will present a form to
request a LifeScope product application license.
*****
^   https://licensing.appliedbiosystems.com/activation/lifescopy   ^
*****

Enter the Computer ID listed below on the form. If this utility is being run on a
system other than the system running LifeScope, this Computer ID is invalid.
Stop and re-run this utility on the system where LifeScope is installed.
*****
*   ComputerID: 00:1C:23:BB:E1:F3   *
*****

The License Key you must enter on the form is the license key
you were given with the LifeScope system.

When the form is completed and submitted, a LifeScope product license will be sent
to the email address specified on the form. If multiple people are to receive this key,
their email addresses should be entered in the "CC'ed" field of the form.

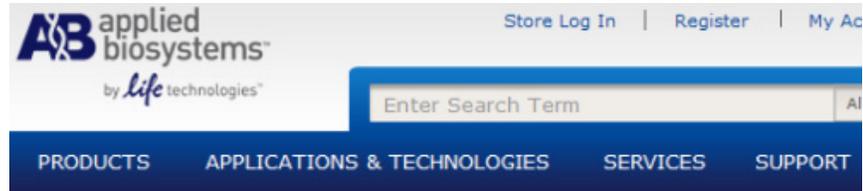
Once the LifeScope product license key is received, execute "Step 2" of this utility
to register the license with the License Server.
```

2. Choose option 3 (“Request a LifeScope product license.”) to display the information about product licensing, including the MAC address of the first Ethernet card.

## Activate License Key and obtain license file

To activate the license and obtain the license file associated with your MAC address:

1. Visit the LifeScope™ License activation page, shown below, at <https://licensing.appliedbiosystems.com/activation/lifescopy>



### LifeScope License Activation

Complete the fields below. Fields with an \* are required.

|                |                        |
|----------------|------------------------|
| *Computer ID   | <input type="text"/>   |
| *License Key   | <input type="text"/> ⓘ |
| *Email Address | <input type="text"/>   |
| Cc             | <input type="text"/>   |
| *Country       | Choose One ▾           |
| *First Name    | <input type="text"/>   |
| Middle Name    | <input type="text"/>   |
| *Last Name     | <input type="text"/>   |

2. Enter the MAC address in the **Computer ID** field.
3. Enter the License Key included in the LifeScope™ 2 Genomic Analysis Software license instructions.
4. Enter the email address where your license file (.lic) should be sent.
5. Fill in the remaining information, then click **Submit** to activate your license.

**Note:** Your license begins the moment you activate your License Key.

The License File "Lifescopy.lic" is sent to you as an attachment in an email from "RightNow AB Technical Support <[RightNow.ABTechnicalSupport@lifetech.com](mailto:RightNow.ABTechnicalSupport@lifetech.com)>".

## Apply the License File to LifeScope™

(LSA) To apply the license to your copy of LifeScope™:

1. Download the license file (.lic) to the system that has LifeScope™ has installed on it.

If you are using another computer for email, you can use tools such as ftp or winscp to transfer the .lic file to your LifeScope™ system. For more information on transferring files, contact your system administrator.

2. Move the .lic file to  
<lifescop-installed-dir>/server/licenses/  
Your LifeScope™ is now licensed.

---

**IMPORTANT!** Keep a backup copy of your license file in a safe place.

---

Later in [“Start the licensing server” on page 51](#), you start the license server and confirm that the license has been correctly applied.

You have completed the LifeScope™ licensing process.

After the license has been activated, see [Appendix D, “Administration” on page 365](#) for instructions to make LifeScope™ Software available to users. These steps include the following:

- Setting a named license per user.
- Enabling and disabling LifeScope™ Software users.

LifeScope™ Software installation is completed. However, you are recommended to do at least one installation verification step. These steps are optional but confirm that the software is functioning properly and that the LifeScope™ Data Folder (LSDF) is properly configured. The optional installation verification steps are:

- The demo analyses
- The performance verification tests

See [Chapter 3, “Verify the LifeScope™ Genomic Analysis Software Installation” on page 55](#) for instructions on optionally running example analysis workflows and optionally performing verification tests.

## Set the LifeScope™ environment

Follow the instructions in [“Set the LifeScope™ Software environment” on page 66](#) to set your PATH variable.

## Start LifeScope™ servers and check servers' status

This section describes starting the LifeScope™ servers and checking their status. Commands described in this section are run in the Linux shell (not in the LifeScope™ Software command shell).

The LifeScope™ licensing server must be started first, before the LifeScope™ web server. Only the LifeScope™ administrator (LSA) may start the licensing server.

### Start the licensing server

To start the licensing server, follow these steps:

1. Make sure LifeScope™ binaries are in the PATH environment variable. If the PATH variable is not set properly, set PATH using this Linux® command:

```
export PATH=<lifescopy-installed-dir>/bin:$PATH
```

2. Start the license server, using this Linux command:

```
lscope-lmgrd.sh start
```

If the license server is already running, a second instance is not started.

### Check the licensing server

1. Use the following command to check if the LifeScope™ licensing server is running:

```
ps -ef | grep lmgrd
```

If the LifeScope™ licensing server is not running, the Linux command prompt returns (with no output). If the LifeScope™ licensing server is running, this command displays two processes, one for the license manager daemon (lmgrd) and one for the vendor daemon (lifetech). Example output when the licensing server is running is:

```
lifescopy 20447      1  0 Mar04 00:00:00 ./lmgrd -c /opt/lifescopy/lifescopy/server/licenses/LifeScope.lic
lifescopy 20448 20447  0 Mar04 00:00:00 lifetech -T foshtdv12.na.ab.applera.net 11.9 4 -c /opt/lifescopy/lifescopy/server/licenses/LifeScope.lic -lmgrd_port 6979 --lmgrd_start 4d59fb1f
```

2. Verify the license status and the available user licenses, shown below, using the Linux command:

```
lscope-lmgrd.sh status
```

```
[panak1@fospanak2 licenses]$ cd /share/apps/lifescopeserver/licenses/
[panak1@fospanak2 licenses]$ ls -l
total 8
-rwxrw-r-- 1 panak1 panak1 1144 May  5 17:50 LifeScope.lic
[panak1@fospanak2 licenses]$ echo $PATH
/share/apps/lifescopeserver/bin:/usr/lib64/qt-3.3/bin:/usr/kerberos/bin:/usr/local/bin:/usr/bin:
/bin:/usr/X11R6/bin:/home/panak1/bin
[panak1@fospanak2 licenses]$ lscope-lmgrd.sh start
Started License Server

[panak1@fospanak2 licenses]$ lscope-lmgrd.sh status
lmutil - Copyright (c) 1989-2010 Flexera Software, Inc. All Rights Reserved.
Flexible License Manager status on Thu 5/5/2011 17:53

License server status: 27001@fospanak2.ads.invitrogen.net
  License file(s) on fospanak2.ads.invitrogen.net: /share/apps/LifeScope-2.0.r0-86630_2011
0412120300/server/licenses//LifeScope.lic

fospanak2.ads.invitrogen.net: license server UP (MASTER) v11.9

Vendor daemon status (on fospanak2.ads.invitrogen.net):

  lifetech: UP v11.9
Feature usage info:

Users of LTC.BIOSCP.USERS:  (Total of 5 licenses issued;  Total of 0 licenses in use)
Users of LTC.BIOSCP.LAUNCH: (Total of 1 license issued;  Total of 0 licenses in use)
Users of LTC.BIOSCP.CONCURRENT: (Total of 1 license issued;  Total of 0 licenses in use)
```

3. Check that you have activated the same number of licenses that you ordered by cross-verifying the number with the result of the status command.

You must start the license server every time the system undergoes a reboot.

## Start the LifeScope™ server

Only the LifeScope™ administrator (LSA) must start the LifeScope™ server. The command to start the LifeScope™ server is:

```
lscope-server.sh start
```

The server by default uses port 9998, but the default port number might be changed during installation. To run on a different port, use the following version:

```
lscope-server.sh start -p port
```

If you start the server on a port other than the default 9998, you must check that the firewall is also enabled for the new port number. See [“Check firewall restrictions” on page 37](#).

If the port is busy, the server start fails.

If the server is already running, a second instance is not started. The server issues the following message:

```
Web server already running (pid=nnnnn) on port nnnn
```

You must start the LifeScope™ server every time the system undergoes a reboot.

**Check the LifeScope™ server status**

The following command checks if the LifeScope™ server is running:

```
ps -ef | grep WebServer
```

If the LifeScope™ server is not running, the Linux command prompt returns (with no output). Example output when the web server is running is:

```
lifescope 23521      1  0 13:26 pts/1    00:00:05 java -server -Xmx3072m -XX:PermSize=512m -XX:MaxPermSize=512m -XX:+UseParallelGC com.lifetechnologies.bioscope.webservice.WebServer -b /opt/lifescope/lifescope
```

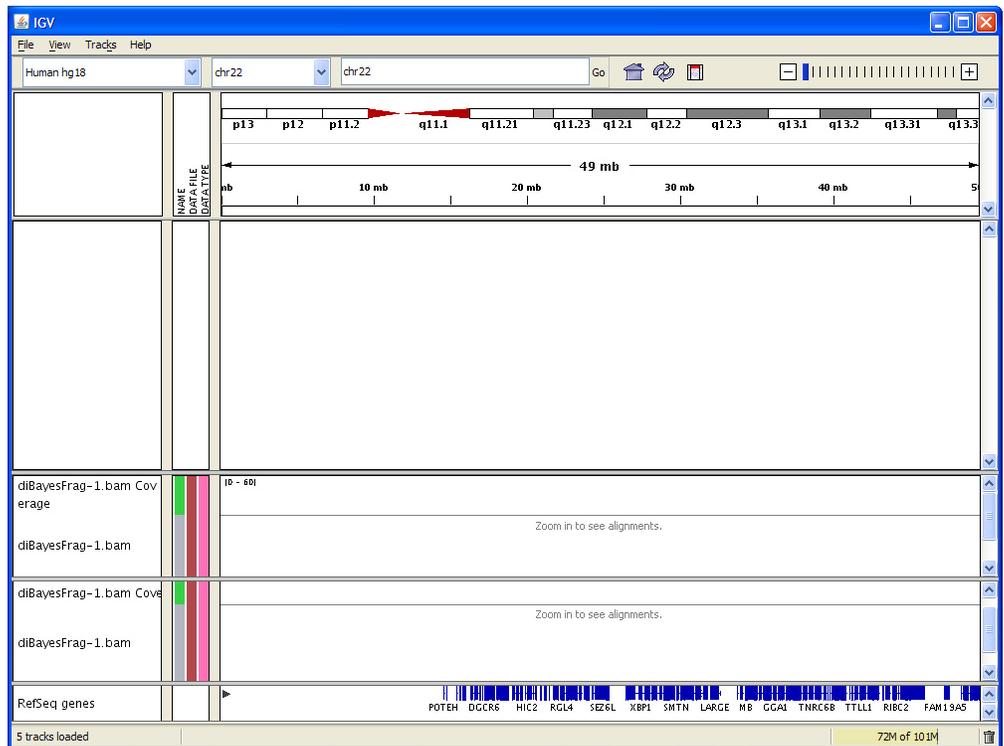
**Download documentation and additional resources**

To ensure you have the latest version of LifeScope™, please visit [http://solidsoftwaretools.com/gf/project/lscope\\_release](http://solidsoftwaretools.com/gf/project/lscope_release) and look for the Download option. You also find user documentation and the latest information and technical resources. The full resources package includes pre-installation scripts, smaller data files, the installation wizard, and performance verification scripts. Additional links and online support resources are also available at this site.

**Integrative Genomics Viewer (IGV)**

You can optionally install the Integrative Genomics Viewer (IGV), shown on page 53, available from the Broad Institute. The IGV is a visualization tool for interactive exploration of large, integrated datasets. It directly reads BAM files, which enables you to easily view and inspect alignments against the genome.

For more information about the IGV, go to [www.broadinstitute.org/igv/](http://www.broadinstitute.org/igv/).





## 3

# Verify the LifeScope™ Genomic Analysis Software Installation

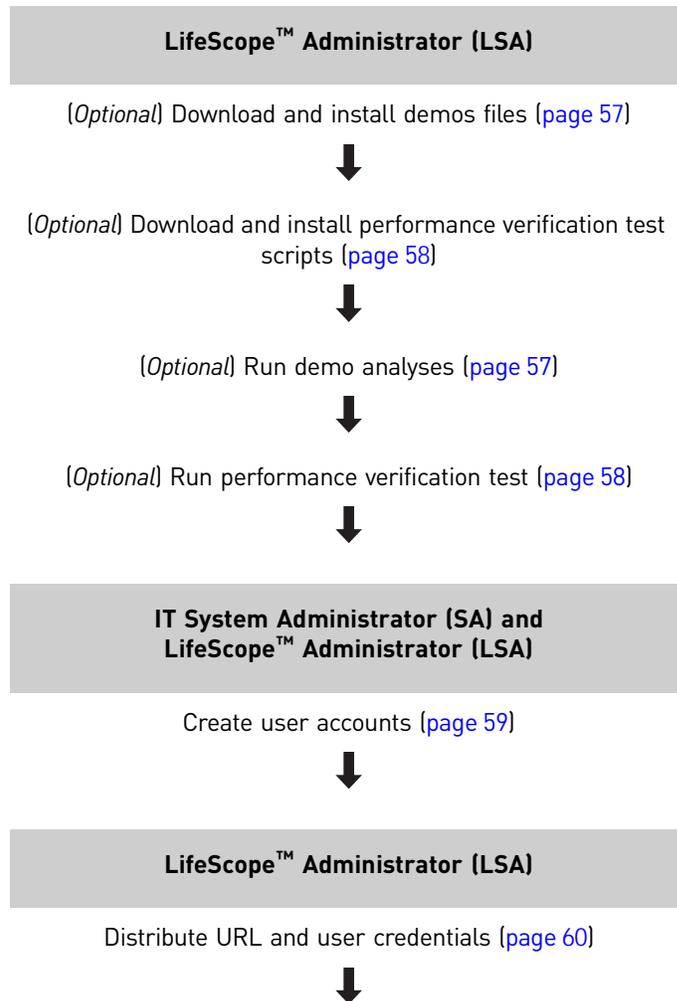
This chapter covers:

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## Overview

This chapter describes procedures and tests that the LifeScope™ administrators should perform after the software has been installed. LifeScope™ users may skip this chapter.

## Workflow



## Verify the installation

(LSA) Two mechanisms are provided to verify the installation:

- **Demos** – A set of individual analysis modules with small data sets. The demos take approximately 30 minutes or less to complete.
- **Performance verification** – A realistic computation scenario that takes approximately 24 hours to complete.

---

**IMPORTANT!** You are strongly recommended to run one or both of the verification procedures. The procedures are listed as optional because you are not required to run both of them. Run at least one of the optional verification procedures.

---

You may run the demo analyses and performance verification scripts as the LifeScope™ administrator or as a LifeScope™ user. See “Create user accounts” on page 59 create a LifeScope™ user account.

## (Optional) Download and install demos files

(LSA) This step is required in order to run the optional sample analyses, “(Optional) Run demo analyses” on page 57.

Example scripts and data for demo LifeScope™ analyses are available from the following site:

[http://solidsoftwaretools.com/gf/project/lscope\\_release](http://solidsoftwaretools.com/gf/project/lscope_release)

Included in this download are the required PLN and INI files for running individual LifeScope™ analysis modules, and scripts to run the analyses in the LifeScope™ command shell.

Follow these steps to install the demo files:

1. Download the `.tar.gz` file to the LifeScope™ Data Folder (LSDF).
2. Go to the LSDF directory and untar the `.tar.gz` file:  

```
cd <LSDF>  
tar -xzf exampleWorkflows.tar.gz
```

**Note:** The demo analyses are not recommended as the first experience for users new to LifeScope™. The standard workflows are recommended for new users to learn LifeScope™ command-line analyses. For information on standard workflows, see the workflow chapters in Part IV, “Workflows”, or refer to the workflows chapter in *LifeScope™ Genomic Analysis Software Command Shell User Guide* (Part no. 4471875).

## (Optional) Run demo analyses

(LSA) Run demo analyses, which takes approximately 30 minutes.

These instructions require the following (in addition to the software installation):

- The default reference repository, which includes hg18 and hg19 assemblies. The reference repository is created during the “Copy data drive content” on page 39 and “Install LifeScope™ Software” on page 40 procedures.
- The demos files and sample data installed during the “(Optional) Download and install demos files” on page 57.

Follow these steps to execute the demos in one run:

1. Follow the instructions in “Set the LifeScope™ Software environment” on page 66 to set the LifeScope™ environment.
2. Go to the demos directory:  

```
cd <LSDF>/examples/demos
```
3. Execute this command in the Linux shell:  

```
./runall.sh -u username
```

where *username* must be for the LifeScope™ administrator or for a valid LifeScope™ user account. Provide the password when prompted.

This script runs each demo one at a time.

## Check results

The results files are generated in the projects repository, at a location which is set during installation. Its default location is at `<LSDF>/projects`. Within the projects repository directory, the demos results are generated at the following directories:

```
username/examples/analysis_name/outputs/sample_name
```

Replace *username* with the user name used to run `runAll.sh`. The default is `lifescope`, the LSA account. The string `examples` is the project name used by all demos. *analysis\_name* is the name of the analysis module used by a demo, and is for example, `cnv`, `dibayes`, `enrichment`, etc. Each *analysis\_name* folder has an `outputs` subfolder. The final folder, *sample\_name*, is determined by the sample in the input data.

Each of the demo analyses creates a log file named `summary.log` in the `lifescope/examples/analysis_name` directory. Check for a success message in the last line of each log file.

The logs and output files are overwritten on subsequent runs executed by the same user.

## (Optional) Download and install performance verification scripts

(LSA) This step is required in order to run the optional performance verification tests, “(Optional) Run the performance verification test” on page 58.

The performance verification test data file are in the LSDF directory, from the “Copy data drive content” step on page 39.

The performance verification test scripts are available from the following site:

[http://solidsoftwaretools.com/gf/project/lscope\\_release/performanceVerificationTestScripts.tar.gz](http://solidsoftwaretools.com/gf/project/lscope_release/performanceVerificationTestScripts.tar.gz)

Follow these steps to install the performance verification test scripts:

1. Download the file `performanceVerificationTestScripts.tar.gz` to the LifeScope™ Data Folder (LSDF).
2. Go to the LSDF directory and untar the `.tar.gz` file:
 

```
cd <LSDF>
tar -xzf performanceVerificationTestScripts.tar.gz
```

## (Optional) Run the performance verification test

(LSA) Run a performance verification test, which takes approximately 24 hours.

The performance verification data files are installed during the “Copy data drive content” step on page 39. The performance verification test scripts are installed during the “(Optional) Download and install performance verification scripts” step on page 58.

Follow these steps to execute the performance verification test:

1. Follow the instructions in “Set the LifeScope™ Software environment” on page 66 to set the LifeScope™ environment.
2. Confirm that the performance verification test data is installed at this directory:  
`<LSDF>/performanceVerificationData`
3. Go to the performance verification test scripts directory:  
`cd <LSDF>/performanceVerificationTestScripts`
4. Execute this command in the Linux shell:  
`./runAll.sh -u username`  
where *username* must be for the LifeScope™ administrator or for a valid LifeScope™ user account. Provide the password when prompted.

## Monitor progress

Monitor the progress of your run by checking the output log files and also through job status commands such as `qstat`.

## Check results

The results files are generated in the projects repository, at a location which is set during installation. Its default location is at `<LSDF>/projects`. Within the projects repository directory, the performance verification results are generated at the following results directories:

`username/performanceVerification/analysis_name/outputs`

Replace *username* with the user name used to run `runAll.sh`. The default is `lifescopes`, the LSA account. The string `performanceVerification` is the project name used by the performance verification test. The analysis names used by `runAll.sh` are `genomicResequencing.20x`, `genomicResequencing.2x`, and `wholeTranscriptomePE`. The `outputs` subfolder name is fixed.

The performance verification tests creates a log file in the *analysis\_name* directory. Check for a success message in the last line of each log file.

The results files are overwritten on subsequent runs executed by the same user.

## Create user accounts

(LSA or SA) Create user accounts.

For instructions in this section that require using the LifeScope™ Admin Portal, see [Appendix D, “Administration” on page 365](#).

The method for user account creation depends on the authentication realm for your installation:

- **LifeScope realm** – (LSA) The LifeScope™ administrator creates LifeScope™ user accounts using LifeScope™. The LifeScope™ Admin Portal is recommended for user administration.
- **LDAP realm** – (SA and LSA) The Linux system administrator creates user accounts on the LDAP server. The LifeScope™ administrator must use the LifeScope™ Admin Portal to enable each LifeScope™ user.

- **Host realm** – (SA) The Linux system administrator creates Linux user accounts on the local operating system.

### Enable named users

(LSA) This step is not required if your installation uses the concurrent licensing scheme. (With concurrent licensing, all users in the authentication realm are automatically enabled for LifeScope™.)

If your installation uses a named licensing scheme, the LSA must use the Admin Portal to identify the users for the named license.

## Distribute the URL and user credentials

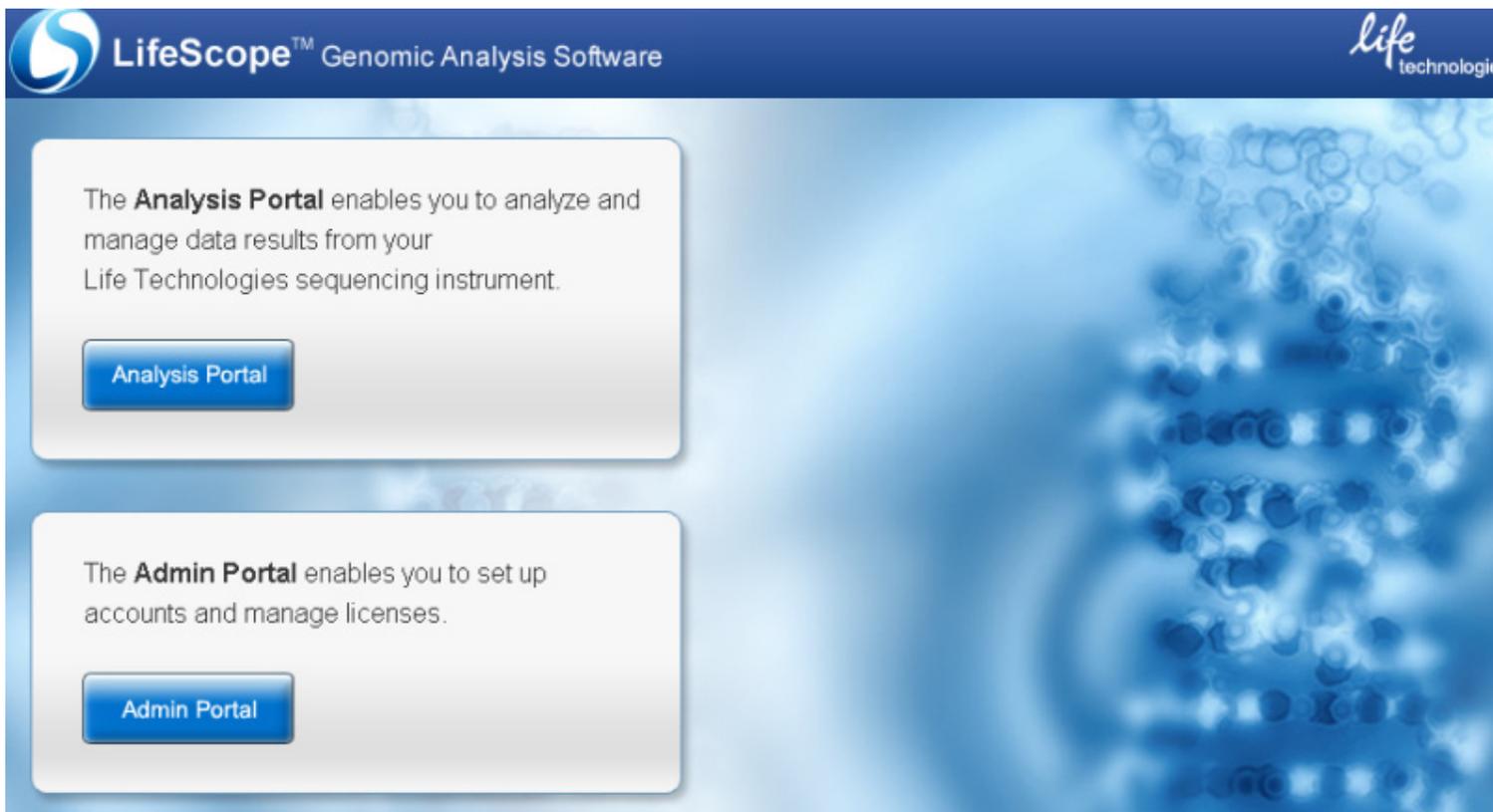
(LSA) The LifeScope™ URL follows this pattern:

`http://<fqdn of host>:<port number>/LifeScope.html`

For example, for an installation on host `orange.intranet.company.com`, using the default port 9998 for the LifeScope™ server, the URL is:

`http://orange.intranet.company.com:9998/LifeScope.html`

With the LifeScope™ servers running, verify the LifeScope™ URL on a supported browser. The following page appears:



Notify LifeScope™ users (LSUs) of the LifeScope™ URL.

If new user accounts are created for LifeScope™ users, notify them of the username and password credentials required to access the LifeScope™.

## Maintenance

### Stop the LifeScope™ server

The following cautions apply to stopping the LifeScope™ server:

- The LifeScope™ server should only be stopped for required maintenance such as license upgrades.
- Only the LSA account can stop the LifeScope™ server.
- In order to stop both the LifeScope™ server and the licensing server, you must stop the LifeScope™ server first.
- All running analyses are terminated when you stop the LifeScope™ server.

Follow these steps to stop the LifeScope™ server:

1. If you have not already done so, set the LifeScope™ environment. See [“Set the LifeScope™ Software environment” on page 66](#).
2. Check for LifeScope™ jobs running on the cluster with a command such as `qstat`. Notify LifeScope™ users that their jobs are affected.
3. Execute this Linux command:  

```
lscope-server.sh stop
```

### Stop the licensing server

The following cautions apply to stopping the licensing server:

- The LifeScope™ licensing server must not be stopped except for required maintenance such as license upgrades.
- Only the LSA account can stop the licensing server.
- You must stop the LifeScope™ server first before stopping the licensing server.
- Running LifeScope™ analyses are affected if the licensing server is stopped.

---

**IMPORTANT!** If the LifeScope™ licensing server stops accidentally while the LifeScope™ server is running, the licensing server must be restarted as soon as possible. The licensing checks fail if the licensing server is not operating, and running analysis jobs may stop.

---

Follow these steps to stop the LifeScope™ licensing server:

1. If you have not already done so, set the LifeScope™ environment. See [“Set the LifeScope™ Software environment” on page 66](#).
2. Execute this Linux command:  

```
lscope-lmgrd.sh stop
```
3. Verify that the LifeScope™ licensing server has stopped, with this command:  

```
lscope-lmgrd.sh status
```

### Upgrade

Upgrading to a new version of LifeScope™ does not require you to uninstall your existing version of LifeScope™.

Follow the installation instructions for the new version of LifeScope™. This procedure installs the new version in the `<LSBF>` alongside your existing version, and modifies the soft link `<LSBF>/lifescopy` to point to the new version. The older version of LifeScope™ is not deleted during installation.

After the upgrade, ensure that your license files are moved or copied from the older installation to the newer one.

Once the new installation has been verified, you may remove the older installation at your discretion.

## Uninstall

This section describes the steps for the LifeScope™ administrator (LSA) to uninstall LifeScope™. If you use an authentication realm that requires the IT system administrator (SA) to create user accounts, the SA deletes those user accounts as part of the uninstall process.

Follow these steps to uninstall LifeScope™:

1. (LSA or SA) Backup your LifeScope™ license file or files, from the directory `<LSBF>/lifescopeserver/licenses`.
2. (LSA or SA) Backup any required files, from the `<LSDF>` directories (these directories hold the LifeScope™ repositories):
  - Reads files (XSQ and BAM)
  - Reference files
  - Output files
  - Data
  - Projects and analyses

Notify LifeScope™ users to backup any files they might need from the `<LSDF>` directories.

3. (LSA or SA) Delete the LifeScope™ installation directory, with this Linux command:

```
/bin/rm -rf <LSBF>/lifescopeserver
```

4. (Optional) (LSA or SA) Delete the LifeScope™ data folder, with this Linux command:

```
/bin/rm -rf <LSDF>
```

5. (Optional) (SA) If you use host or LDAP authentication, delete the user accounts you created for LifeScope™ users.

(Optional) (LSA or SA) If you modified any bash profile scripts in `/etc` or in the local home directory to change the `PATH` variable, remove the LifeScope™ Software location entries in the `PATH` variable.

# PART III

## Getting Started



## 4

## Before You Begin

This chapter covers:

|                                                 |    |
|-------------------------------------------------|----|
| ■ Overview .....                                | 65 |
| ■ Verify the browser version .....              | 66 |
| ■ Set the LifeScope™ Software environment ..... | 66 |
| ■ Check LifeScope™ Software servers .....       | 67 |

## Overview

This chapter is for LifeScope™ Genomic Analysis Software users. Some procedures described in this section require that you:

- Know the IP address of the LifeScope™ cluster
- Have a login ID on the LifeScope™ cluster
- Know how to:
  - Navigate to directories in a Linux environment
  - Edit and save files in a text editor
  - Run Linux shell scripts
  - Run Linux commands such as `ls`, `pwd`, `cd`, `echo`, `grep`, and other common utilities

This chapter describes the following procedures:

- Verify your browser version
- Set your shell environment to use LifeScope™
 

**Note:** In some installations, the LifeScope™ system administrator sets your environment for you.
- Check whether the LifeScope™ servers are running

## Verify the browser version

LifeScope™ Genomic Analysis Software supports:

- Internet Explorer® versions 6 and 7
- Mozilla® Firefox 3.0.1

## Set the LifeScope™ Software environment

To set your environment to run LifeScope™ Software, add the LifeScope™ `bin` directory to your `PATH` and export the new `PATH` variable. Enter this command every time you open a Linux shell window to run LifeScope™ Software:

```
export PATH=<installdir>/bin:$PATH
```

where `installdir` is the LifeScope™ Software installation directory.

The `installdir` is the `<LSBF>/lifescopesoftware` directory defined in the administrator instructions (“[LifeScope™ Software Administration](#)” on page 37).

If BioScope™ Software is not used on the same machine, you can make your `PATH` setting permanent by modifying your local `.bashrc` file or the global `/etc/bashrc` files. Contact your system administrator for more information.

### BioScope™ Software users’ `PATH` variable

If you install LifeScope™ on a machine that also has BioScope™ Software installed and the BioScope™ Software is actively being used, you must follow these instructions:

1. LifeScope™ users must *not* use their shell profile script to update the `PATH` variable with the LifeScope™ `bin` directory. If the LifeScope™ `bin` directory is added to the `PATH`, BioScope™ Software cannot run correctly.
2. Run BioScope™ Software and LifeScope™ in different Linux® shell windows.
3. Set and export your `PATH` variable *every* time you open a Linux shell window to run LifeScope™ server or client software.

### BioScope™ profile scripts

Some customers with early versions of BioScope™ Software use a profile script to set the `BIOSCOPE_ROOT` environment variable for all users. If you have the file `/etc/profile.d/bioscope_profile.sh` on your system, you must delete this file, or remove it from the `/etc/profile.d` directory, for LifeScope™ Software to run properly.

### Clusters running both versions

If you currently run BioScope™ Software on your cluster, and need to support both BioScope™ and LifeScope™ users on the same cluster, follow these steps:

- Remove the file `bioscope_profile.sh` from the `/etc/profile.d` directory (the script cannot be in any `/etc/init` directory). Having this script in a system `init` directory prevents LifeScope™ Software from running properly. (Save the script in your own private location in order to configure the environment for BioScope™ Software users, as described below.)
- Users who run LifeScope™ Software must not have the environment variable `BIOSCOPE_ROOT` set and must not have the BioScope™ Software `/bin` directory in their `PATH`.

- Users who still run BioScope™ Software should configure their environment by copying the content of the file `/etc/profile.d/bioscope_profile.sh` into their own shell setup file (such as `.bash_profile`).

## Check LifeScope™ Software servers

This section describes checking the status of LifeScope™ servers. Commands described in this section are run in the Linux shell (not in the LifeScope™ Software command shell).

The LifeScope™ licensing server must be started first, before the LifeScope™ web server. Only the LifeScope™ administrator (LSA) may start the servers.

### Check the licensing server

Use the following command to check if the LifeScope™ licensing server is running:

```
ps -ef | grep lmgrd
```

If the LifeScope™ licensing server is not running, the Linux command prompt returns (with no output). If the LifeScope™ licensing server is running, this command displays two processes, one for the license manager daemon (`lmgrd`) and one for the vendor daemon (`lifetech`). Example output when the licensing server is running is:

```
lifescope 20447      1  0 Mar04 00:00:00 ./lmgrd -c /opt/lifescope/  
lifescope/server/licenses/LifeScope.lic  
lifescope 20448 20447  0 Mar04 00:00:00 lifetech -T  
foshtdv12.na.ab.applera.net 11.9 4 -c /opt/lifescope/lifescope/  
server/licenses/LifeScope.lic -lmgrd_port 6979 --lmgrd_start  
4d59fb1f
```

### Check the LifeScope™ server status

The following command checks if the LifeScope™ server is running:

```
ps -ef | grep WebServer
```

If the LifeScope™ server is not running, the Linux command prompt returns (with no output). Example output when the web server is running is:

```
lifescope 23521      1  0 13:26 pts/1    00:00:05 java -server  
-Xmx3072m -XX:PermSize=512m -XX:MaxPermSize=512m -  
XX:+UseParallelGC com.lifetechnologies.bioscope.webservice.  
WebServer -b /opt/lifescope/lifescope
```



## 5

## Understand LifeScope™

This chapter covers:

|                          |    |
|--------------------------|----|
| ■ Overview .....         | 69 |
| ■ Terminology .....      | 70 |
| ■ Common scenarios ..... | 72 |

## Overview

This chapter describes the concepts and terminology required when working with LifeScope™ Genomic Analysis Software. These terms are explained in more detail in this chapter.

- **Project** – A collection of analyses based on scientific experiments or computational methodologies.
- **Module** – A module is a single step in an analysis workflow. For example, mapping, SAET, and CNV are modules.
- **Analysis** – A set of modules within an analysis workflow. An analysis can contain one or more modules.
- **Analysis workflow** – A pre-defined set of modules in a workflow based on common applications areas, such as targeted resequencing or small RNA analysis.
- **Read** – Sequencing data from a single bead with a single primer set.
- **Read-set** – Sequencing data associated with an indexed (barcoded) sample from one XSQ file.
- **Read-set repository** – An indexed database of eXtensible SeQuence (XSQ) files based on individual read-sets.
- **Group** – A collection of data that can be analyzed together. Grouping allows for aggregated data (for instance, data from different lanes or different runs) to be analyzed as one set of data.

## Terminology

This section defines terminology and concepts used to describe LifeScope™

### Projects and analyses

Projects are containers for your analysis runs. You may use projects as you wish, to organize your analyses as is convenient and meaningful for you.

You create your projects at the `/projects` level. A project itself does not have a configuration or definition. A project is a way of organizing your LifeScope™ analysis runs.

Within each project, you create one or more analyses. Through the configuration of an analysis, you define the executable run.

The configuration of analysis includes these areas:

- The reference genome.
- The reads files.
- The type of analysis to be performed (which LifeScope™ modules perform the analysis). The analysis type can be any of the following:
  - A pre-defined workflow, which is a built-in sequence of commonly-used analyses, corresponding to a common biological application.
  - An analysis sequence you define, by either creating your own or customizing one of the LifeScope™ pre-defined workflows.

An example of a pre-defined workflow is the targeted resequencing fragment workflow. This workflow includes the following LifeScope™ modules:

- SAET
- Enrichment
- Mapping
- Mapping statistics (BAMStats)
- SNPs
- Small indels
- Annotations

Your projects are private and cannot be seen by other users or shared with other users.

### Naming restrictions

Project and analysis names must conform to Linux filename conventions:

- Contain only alphanumeric and underscore characters.
- Do not contain spaces.
- Must be unique (among siblings).
- Do not begin with an underscore character (this is a LifeScope™ requirement, not a Linux rule).

In sample group names and reference file names, hyphens are not permitted. This restriction also applies to the path names for reference files.

## Reads Data

The data from each lane of a 5500 Series Genetic Analysis Systems instrument results in a single XSQ output file (read). Different models of the 5500 support from 6 to 12 lanes, so typically multiple XSQ files are generated per sequencing run. You can direct your LifeScope™ runs to analyze data from specific barcodes in multiple lanes, or group the output of multiple lanes or multiple sequencing runs to be processed as a single specimen.

The type of data used in LifeScope™ Software is called a read-set, which is a group of reads belonging to one index (barcode) from one XSQ file. The smallest set of data you can specify in the shell is one index from one XSQ file.

You can optionally use groups to combine more than one index into an analysis. Reads that have been combined into a group are processed as one specimen, even if the read-sets come from different input XSQ files. Each group generates a combined set of output files.

In LifeScope™ Software, input data is added at the project level.

## Define input data

Carefully plan your analysis input. The way you define your reads input affects the behavior of your analysis. Index IDs and group names give you flexibility in handling your input data. For example:

- Combine specific read-sets from one XSQ file or multiple XSQ files into one group to be analyzed together. All read-sets within a group must be of the same library type.
- Assign read-sets to different groups, to be analyzed separately within one LifeScope™ Software run. All read-sets within a run must be of the same library type.

## Repositories

LifeScope™ Software repositories organize and persist your projects, your input data, and your reference data files.

The reads repository is a storage place in LifeScope™ Software for instrument data intended to be input data for LifeScope™ Software analyses. Data imported into the reads repository are in unmapped XSQ-format files.

Importing reads and references file into LifeScope™ Software repositories is optional, but preferred.

Files imported into the read and reference repositories are visible to all LifeScope™ Software users. However, each user has their own projects repository. The contents of your projects repository are private and cannot be seen by other users.

**Note:** In the current release, one repository cannot be split across multiple partitions or file systems (the various repositories can each be on a different partition or file system).

### The reference repository

The reference repository is initially populated during the installation process with reference files for hg18 and hg19.

## Common scenarios

This section describes how LifeScope™ handles common biologic analysis scenarios.

### Basic

A basic scenario is to run a bioinformatics analysis, such as targeted resequencing, on a sequenced data. The steps to do this in LifeScope™ are:

- Create a project
- In that project, create an analysis.
- In that analysis:
  - Add the sequenced data file.
  - Specify the reference genome for this analysis.
  - Specify the type of analysis to be performed on the data.
  - Run the analysis.

### A sample sequenced on multiple lanes

In this scenario, one sample is sequenced in multiple lanes on the sequencing instrument. The output data from the sequencing instrument is contained in multiple XSQ files.

The steps to do this in LifeScope™ shell are:

- Create a project
- In that project, create an analysis.
- In that analysis:
  - Add the XSQ data files. When you add the XSQ files, specify the same group name for each file.
  - Specify the reference genome for this analysis.
  - Specify the type of analysis or analyses to be performed on the data.
  - Run the analysis.

### Data from multiple samples

This scenario performs, in one run, the same analysis on the sequenced data from multiple specimens. The data can be in one or multiple XSQ files.

LifeScope™ keeps the data from each specimen separate, and each specimen is analyzed separately. The steps to do this in LifeScope™ shell are:

- Create a project
- In that project, create an analysis.
- In that analysis:
  - Add the sequenced data file.
  - Specify different grouping for the data.
  - Specify the reference genome for this analysis.
  - Specify the type of analysis (such as mapping, SNPs, and CNV) to be performed on the data.
  - Run the analysis.

## 6

# LifeScope™ Genomic Analysis Software Graphical User Interface

This appendix describes the components of the Applied Biosystems LifeScope™ Genomic Analysis Software graphical user interface (GUI).

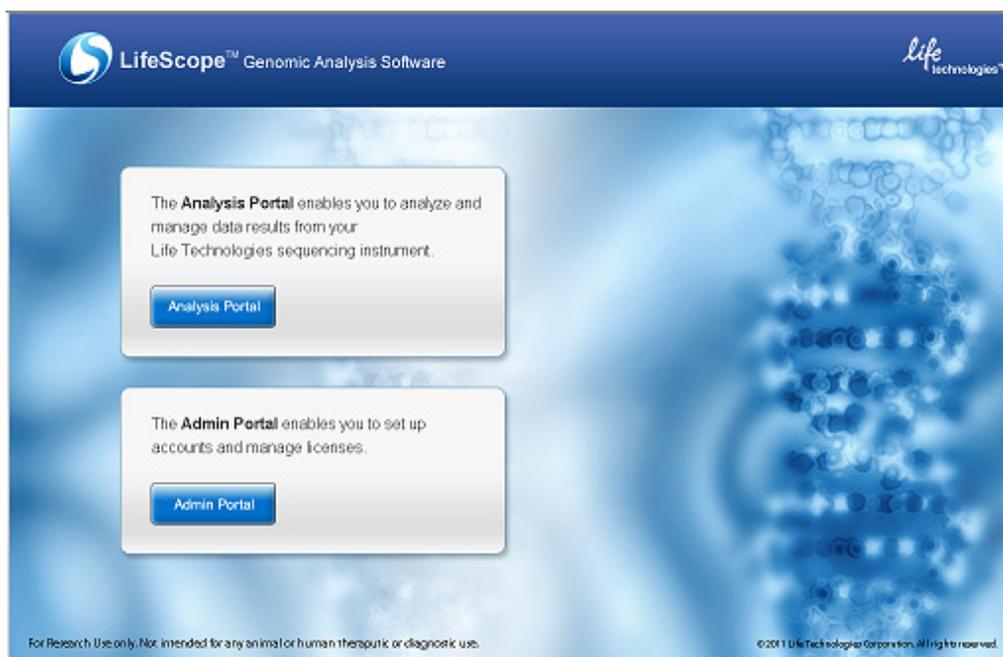
## Log in, log out

**Note:** For a description of the graphical user interface, see [Chapter 6, “LifeScope™ Genomic Analysis Software Graphical User Interface”](#) on page 73.

1. To start LifeScope™ Software, go to:

`http://<IP address>:<port number>/LifeScope.html`

where *IP address* is the address of the system or head node and *port number* is the number of the port used by the server.



- To run LifeScope™ Genomic Analysis Software, click **Analysis Portal**,
  - (Administrator) To run the LifeScope™ Software Admin tool, click **Admin Portal**.
2. In the Welcome window, type your username and password, then click **Login**.  
If you have forgotten your password, click **Forgot Password**, then follow the prompts. If you need assistance, click **Login Help**.

Welcome to  
LifeScope

Genomic Analysis  
Made Easy

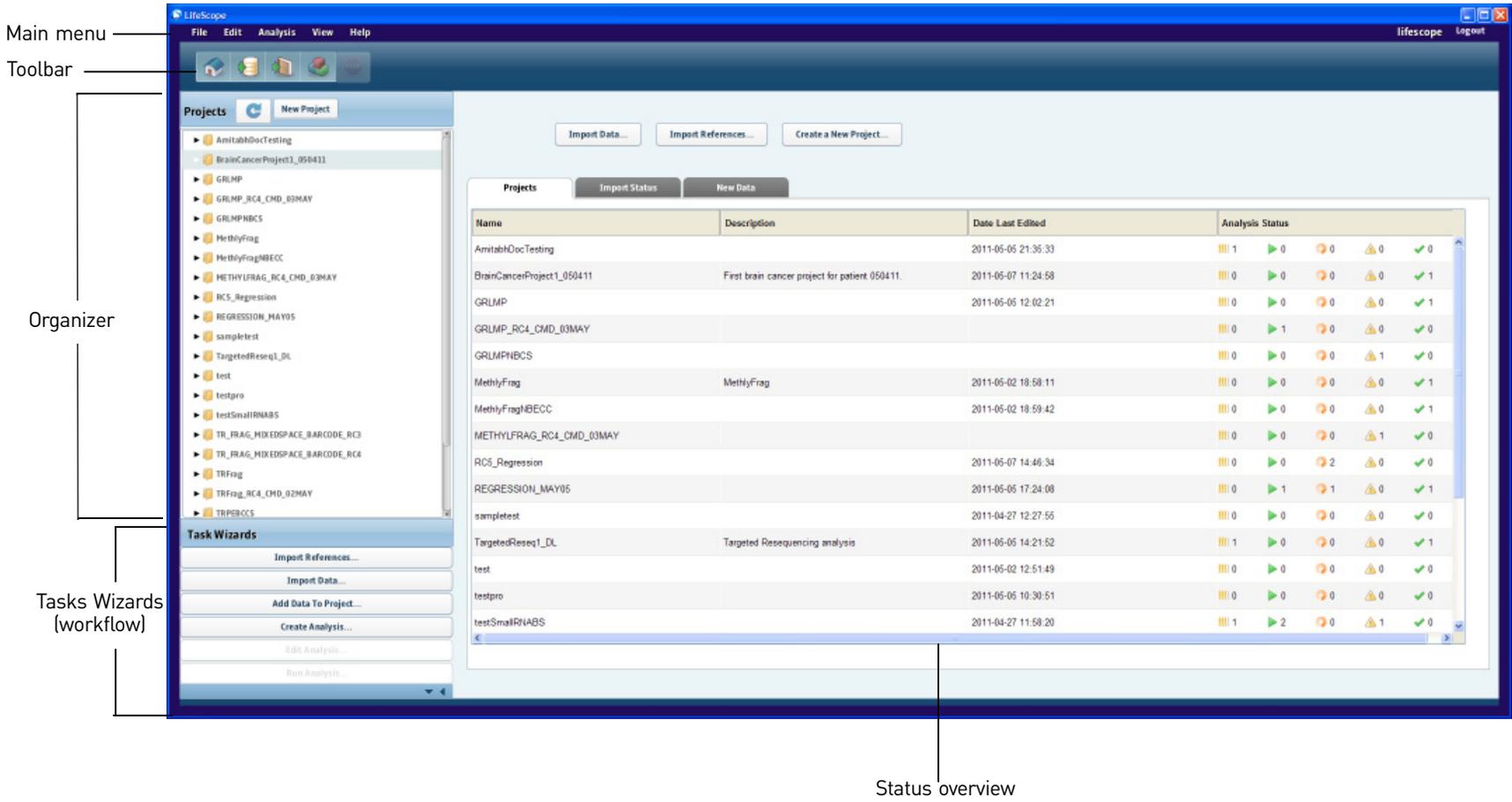
Login

\* Username:

\* Password:

[Login Help](#) [Forgot My Password](#) [Login](#)

After you log in, the home view, shown [on page 75](#), appears. The view shows a toolbar, a left panel that has a Projects and Task Wizards section, and a right panel that shows the status overview of a project and analysis results.



The following table describes the areas, components, and purpose of components in the LifeScope™ Software GUI.

| Area                                       | Components                                                                          | Purpose                                                                                                                           |
|--------------------------------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Main menu with action buttons              | File                                                                                | Create a new project, delete a project, add data, import data, import references, and exit LifeScope™ Software.                   |
|                                            | Edit                                                                                | Copy, paste, and delete text.                                                                                                     |
|                                            | Analysis                                                                            | Create, edit, delete, reuse, run, and stop an analysis.                                                                           |
|                                            | View                                                                                | View a project overview, project status, parameters, reads, and BAM data.                                                         |
|                                            | Help                                                                                | Open the user guide or tutorial; view information about LifeScope™ Software; and access Life Technologies Corporation on the Web. |
| Toolbar with navigation and action buttons |    | Return to the LifeScope™ Software home view.                                                                                      |
|                                            |    | Import data into the read repository.                                                                                             |
|                                            |    | Import references into the read repository.                                                                                       |
|                                            |   | Create an analysis. Faded (inactive) until a project is selected in the organizer.                                                |
|                                            |  | Stop running an analysis. Faded (inactive until a project is running).                                                            |

| Area                                                                                | Components                                                                                               | Purpose                                                                                                                 |
|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| Projects<br>(left panel)                                                            |                         | Refresh the list of projects to see project status.                                                                     |
|                                                                                     | <b>New Project</b>                                                                                       | Create a project.                                                                                                       |
|                                                                                     | Organizer                                                                                                |                                                                                                                         |
|                                                                                     |                         | Show contents. Click to show the contents of a folder, analysis, or module.                                             |
|                                                                                     |                         | Hide contents. Click to hide the contents of a folder, analysis, or module.                                             |
|                                                                                     |                         | Project name. Click once to show data and analyses, and the project overview and status.                                |
|                                                                                     |                         | Data. Click once to show the Read-sets and BAM Data in the status overview pane.                                        |
|                                                                                     |                         | Analysis, created and ready to review, edit, and run. Click once to show the analysis overview, status, and parameters. |
|                                                                                     |                        | Analysis running.                                                                                                       |
|                                                                                     |                       | Analysis completed.                                                                                                     |
|                                                                                     |  <b>bac_analysis2</b> | Analysis failed.                                                                                                        |
|                                                                                     |                       | Analysis module. Click to show the analysis results and the analysis overview, status, and parameters.                  |
|                                                                                     |                       | Analysis module completed.                                                                                              |
|                                                                                     |  <b>diBayes</b>       | Red text indicates that analysis module failed.                                                                         |
|  | Results. Click to show analysis results in the status overview (described in this table).                |                                                                                                                         |

| Area                                  | Components                                                                          | Purpose                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------------------------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Tasks Wizards buttons<br>(left panel) | Import References (into an analysis)                                                | Utilities for performing tasks, the order of the buttons guides you in following the workflow. Clicking a button shows steps, which are highlighted to show progress through a workflow.<br><br>Add Data To Project is faded (inactive) until a project is selected.<br><br>Create Analysis is faded until a project is created.<br><br>Edit Analysis is faded until an analysis is selected.<br><br>Run Analysis is faded until an analysis is selected. |
|                                       | Import Data (into an analysis)                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|                                       | Add Data To Project                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|                                       | Create Analysis                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|                                       | Edit Analysis                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|                                       | Run Analysis                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Status overview<br>(right panel)      | Tabs, data entry fields.                                                            | Import data and references; create a new project.<br><br>Show analysis status information, task results, and project details. Tabs include data entry fields, buttons, and interactive tables.<br><br>Placing your mouse cursor over an icon displays brief descriptions of the icons.                                                                                                                                                                    |
|                                       |    | Analysis ready to run.                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                       |    | Analysis not ready to run/or stopped                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                                       |   | Analysis running.                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                       |  | Analysis finished successfully                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                       |  | Analysis failed.                                                                                                                                                                                                                                                                                                                                                                                                                                          |

### Button labels and table titles

Button labels and table titles reflect the data type. For example, when you group XSQ data, the data is listed in a table titled “Available Data in Project.” When you group BAM data, the table is titled “BAM Data in Project.”

### Pop-up windows

Small pop-up windows can be hidden by other, larger windows that are open. If you are unable to perform an action in a pop-up window, move, minimize, or close windows that you do not need open.

## 7

# Create and Manage Projects

This chapter covers:

|                                |    |
|--------------------------------|----|
| ■ Overview .....               | 79 |
| ■ Workflow .....               | 79 |
| ■ Create a project .....       | 80 |
| ■ (Optional) Import data ..... | 81 |
| ■ Add data to a project .....  | 82 |
| ■ View project data .....      | 86 |
| ■ Delete a project .....       | 87 |

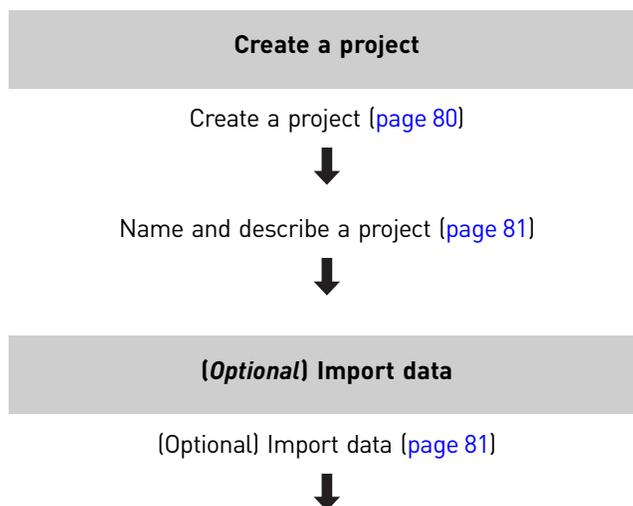
## Overview

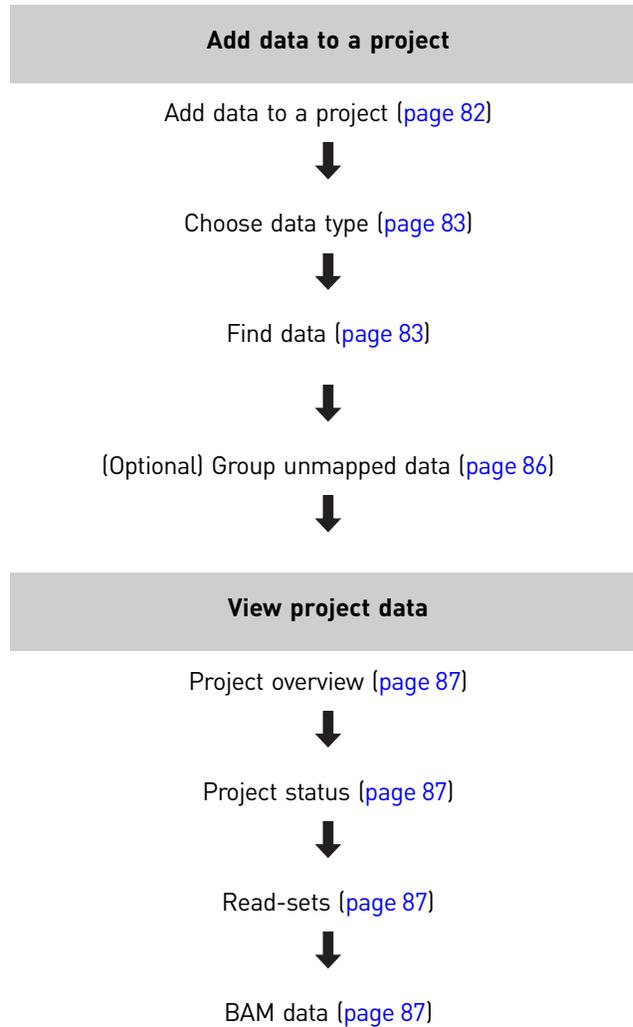
The first step in using LifeScope™ Genomic Analysis Software to perform analysis of next generation sequencing data is to create a project. Creating a project involves importing data and adding data.

(You later create an analysis within your project, and configure and run the analysis. See [Chapter 8, “Perform an Analysis” on page 89.](#))

## Workflow

This following table illustrates a standard workflow for creating a project.





## Create a project

### Prepare to create a project

#### Determine your input and reference files

Before you create a project, you must know:

- The read and references files required by your analyses.
- The regions of interest file, for targeted resequencing, low frequency variant, and enrichment analyses.
- Any non-default module parameter values.

### Log in to LifeScope™

1. If you are not already logged in, navigate to LifeScope™ Software at `http://<IP address>:<port number>/LifeScope.html` where *IP address* is the address of the system or head node and *port number* is the number of the port used by the server.

2. In the Login screen, enter your username and password, then either click **Login** or press **Enter** to open the LifeScope™ home view (shown on page 75).

## Create a project

1. In the home view, there are three ways to create a project:
  - Click **File** in the top menu, then select **New Project**, or
  - Select the **New Project** button in the Projects panel (see page 75), or
  - In the home view, click **Create a New Project** button in the status overview panel.

To exit the Enter Project Name view, click any option in the top menu, any project in the Projects organizer, or any button in the Task Wizards section.

## Name and describe a project

In the Overview tab (see page 75), provide the following details about a project:

- Name of the project, for example, "Cancer\_Study\_3."  
A project name can contain only letters, numbers, and the "\_" symbol. Other special characters are not allowed.
- A description of the project, for example, "Clinical trial: pancreatic cancer. Transcript analysis experiment. Time-course samples covering treatment period."

After you have named and described your project, click **Create New Project**.

Your project appears in the Projects list. If necessary, scroll down the list of projects to see your newly created project. Now you are ready to import references and data that you can use in your project.

## (Optional) Import data

Import data into the reads repository. The reads repository is a storage place in LifeScope™ for instrument data intended to be input data for LifeScope™ analyses. The XSQ, CSFASTA/QUAL data can be imported into the read repository. Data files that are not already in the XSQ file format to be converted to that format.

Files imported into the read and reference repositories are visible to all LifeScope™ users. However, each user has his or her own projects repository. The contents of a user's projects repository are private and cannot be seen by other users.

**Note:** Multiple files cannot have the same name. Make sure that the names of the files that you import are distinct.

**Note:** To import a 1.3 BAM file, see Appendix E, "Convert 1.3 BAM Files" on page 373. 1.3 BAM files must be converted to work with LifeScope™ 2.x. The conversion utility opens automatically in the GUI when you correctly import a 1.3 BAM file.

Import data from a desired location, such as your instrument computer or storage system, into the read-set repository. The type of data is called a read-set, which is a group of reads belonging to one barcode from one XSQ file.

1. Import data by using one of the following methods:
  - In the home view (see page 75), click the **Import Data** button (see page 76).
  - In the main menu (see page 75), click **File**, then **Import Data**.
  - In the toolbar (see page 75), click the **Import Data** button .

- In the Task Wizards panel (see [page 75](#)), click the **Import Data** button. The Import Data window appears.
2. In the Choose Data Type view of the Import Data window, select the type of data to be imported:
    - Raw unmapped (XSQ, CSFASTA/QUAL), or
    - Already-Mapped (BAM)
 then click **Next**.
  3. In the Specify Data Files view, click the  button next to the Data File 1 data entry field to open the repository file browser.
  4. In the pop-up window, navigate to the data file you want to import, select the file, then click **OK**.
 

**Note:** You can copy the data, move the data to a location on the LifeScope™ Software server or make a symbolic link to the original data. These options are available in the upper-right corner of the Import Data · Specify Data Files view.

To add another data file, click that button.  
To refrain from importing data, click **Cancel**.  
To import the data and close the window, click **Finish**.

**Note:** After you import a file, it will not appear in the reads repository until the file has been completely converted.
  5. After the data has been converted, click **Add Data**.

## Add data to a project

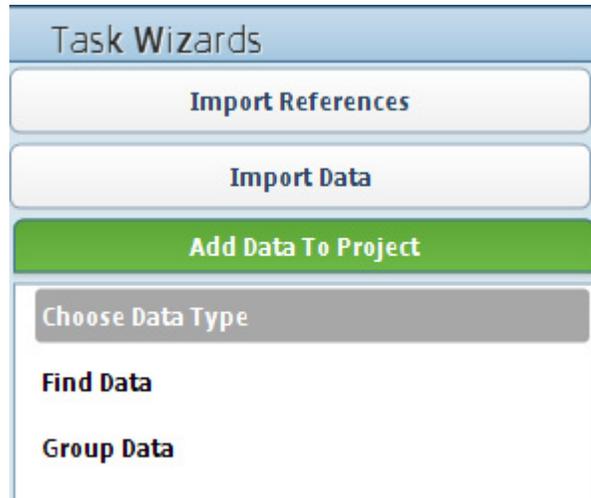
Add data from the read repository to a project by choosing a data type and finding data. You can also optionally group data.

**Note:** Multiple files cannot have the same name. Make sure that the names of the files that you add are distinct.

**Note:** If the LifeScope™ Software administrator has changed the path to the read repository since your last login, restart LifeScope™ Software to update the repository to the new path.

### Open the Add Data to Project tab

1. Open your project in the LifeScope™ Software GUI.
2. Click the Add Data to Project tab in the left Task Wizards pane.



### Choose data type

In the Choose Data Type view of the Add Data to Project window, select the type of data to add to your project:

- Raw unmapped (XSQ) data, or
- Already-Mapped (BAM) data

then click **Next** to find data. If you do not want to add data, click **Cancel** to close the Add Data to Project window.

### Find data

If you chose raw, unmapped (XSQ) data, there are two tabs with read repository filters you can use to find data:

|          |                                                                                     |
|----------|-------------------------------------------------------------------------------------|
| Basic    | Use this filter to search for data and view basic details about found data.         |
| Advanced | Use this filter to refine searches and view more specific details about found data. |

Within each tab are data entry fields, buttons, and a table of reads (sequencing data). Use the arrow buttons or page number button at the bottom of the table to navigate the table.

### Find raw, unmapped data with the Basic filter

Find data by specifying details about a read.

1. Specify any or all of the following read details by:
  - Typing the XSQ ID.
  - Typing the barcode.
  - Typing the run start time and end time.
  - Selecting the library type.

To clear the search results, click **Clear Filters**.

2. Click **Filter** to see search results in the read repository table.
3. Click the checkboxes in the left column to select data, then click **Next** to group data.

Read Repository Filter

Basic **Advanced** Clear Filters

XSQ ID:  Barcode:

Run Start Time From:  To:  Library Type:  Filter

| <input type="checkbox"/> | XSQ ID                  | Barcode        | Application    | Data Space  | Lane # | Run Start Time   | Run End Time     | Library Type | Read Len |
|--------------------------|-------------------------|----------------|----------------|-------------|--------|------------------|------------------|--------------|----------|
| <input type="checkbox"/> | diBayesFrag.xsq         | DefaultLibrary | Unknown        | Color Space | 255    | 2011-02-24 13:47 | 2011-02-24 13:47 | Fragment     |          |
| <input type="checkbox"/> | case2001_MatePair.xsq   | DefaultLibrary | Unknown        | Color Space | 255    | 2011-01-11 20:10 | 2011-01-11 20:10 | MatePair     |          |
| <input type="checkbox"/> | diBayesPE.xsq           | DefaultLibrary | Resequencing   | Color Space | 255    | 2011-02-01 10:30 | 2011-02-01 10:30 | PairedEnd    |          |
| <input type="checkbox"/> | helen_Test1111.xsq      | DefaultLibrary | Unknown        | Color Space | 255    | 2011-02-09 17:00 | 2011-02-09 17:00 | Fragment     |          |
| <input type="checkbox"/> | helen_Test2222.xsq      | DefaultLibrary | Unknown        | Color Space | 255    | 2011-02-09 17:00 | 2011-02-09 17:00 | Fragment     |          |
| <input type="checkbox"/> | test_S1_F31301452711... | DefaultLibrary | Unknown        | Color Space | 255    | 2011-01-11 20:10 | 2011-01-11 20:10 | Fragment     |          |
| <input type="checkbox"/> | helen_Test22.xsq        | DefaultLibrary | Unknown        | Color Space | 255    | 2011-02-09 17:00 | 2011-02-09 17:00 | Fragment     |          |
| <input type="checkbox"/> | test_S1_F31301463425... | DefaultLibrary | Unknown        | Color Space | 255    | 2011-01-11 20:10 | 2011-01-11 20:10 | MatePair     |          |
| <input type="checkbox"/> | solid0054_20110102_P... | DefaultLibrary | Targeted Reseq | Color Space | 255    | 2011-02-12 09:34 | 2011-02-12 09:34 | Fragment     |          |
| <input type="checkbox"/> | WT_ch17_PE.xsq          | DefaultLibrary | Unknown        | Color Space | 255    | 2011-01-11 20:10 | 2011-01-11 20:10 | PairedEnd    |          |
| <input type="checkbox"/> | chr6reads.xsq           | DefaultLibrary | NONE           | Color Space | 0      | 2011-01-13 15:34 | 2011-01-13 15:34 | MatePair     | 50,0     |
| <input type="checkbox"/> | helen_Test111.xsq       | DefaultLibrary | Unknown        | Color Space | 255    | 2011-02-09 17:00 | 2011-02-09 17:00 | Fragment     |          |
| <input type="checkbox"/> | helen_Test222.xsq       | DefaultLibrary | Unknown        | Color Space | 255    | 2011-02-09 17:00 | 2011-02-09 17:00 | Fragment     |          |

< 1 of 2 >

### Find raw, unmapped data with the Advanced filters

The Advanced tab includes an AND filter and an OR filter. To narrow your search for a specific read:

1. Click the Edit AND Filter button.  
Click the Edit OR Filter button.

In both filters, you can refine the display of found information by specifying the table column, relation, and value.

- To add a specification in the Edit AND Filter, click the **&** button.
- To add a specification in the Edit OR Filter, click the **%** button.
- To remove a specification in both filters, click the **X** button.

Click **Filter** to see search results in the read repository table.

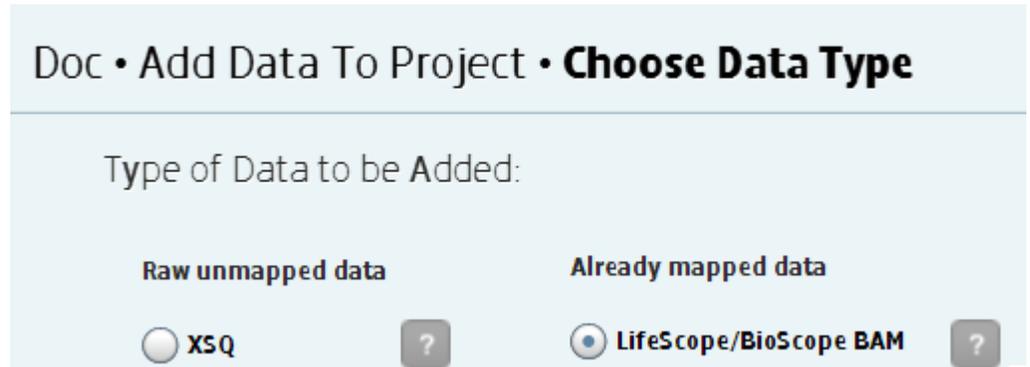
2. Select the found data that you want to add, then, click **Next** to optionally group data. If you do not want to add data, click **Cancel** to exit the Add Data window.

### Find BAM data

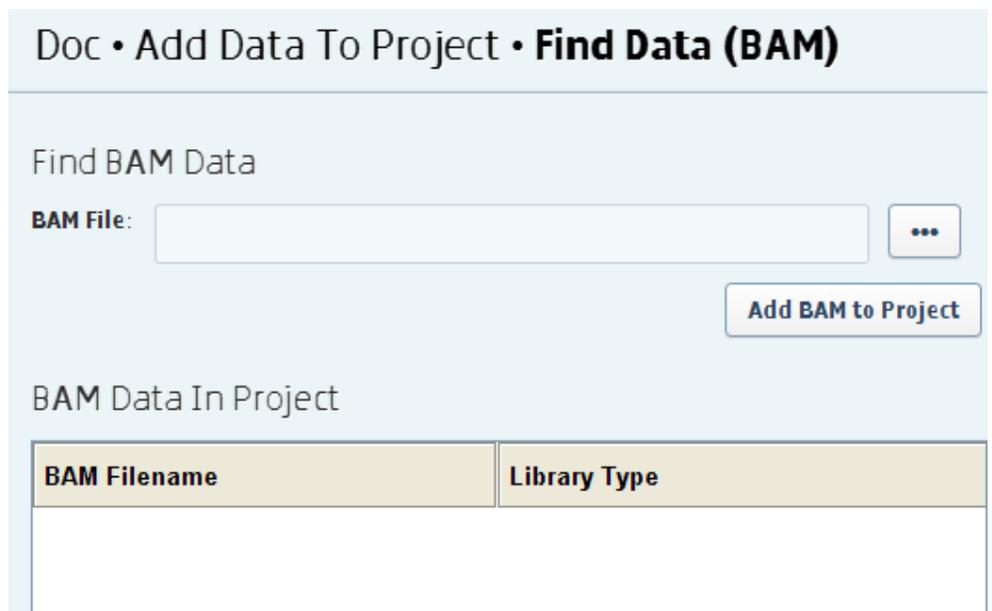
If you chose Already-Mapped (BAM) data, do the following procedure to find data:

1. Click the Add Data to Project tab in the left Task Wizards pane.

2. In the Add Data to Project • Choose Data Type screen, in the “Already mapped data” section, click the **LifeScope/BioScope BAM** button to select it.



3. Click **Next >>**. The Find Data (BAM) screen appears.



4. Choose *one* of the following methods, depending on whether or not your BAM file is already imported in to the LifeScope™ Software reads repository:
  - a. If your BAM file *is not* in the reads repository, click the ellipsis  button *above* the **Add BAM to Project** button and browse to your BAM file. Select the BAM file and click **OK**.  
The full path name of the BAM file is displayed in the Bam File field.  
Click the **Add BAM to Project** button. The BAM file now appears in the BAM Data in Project section.
  - b. If your BAM file *is* in the reads repository, it appears in the BAM Data In Project section, and you click on the BAM file name to highlight it.  
**Note:** An example of BAM file that is imported into the reads repository is not shown in the Find Data (BAM) screenshot above.
5. Click **Finish**.

## (Optional) Group unmapped data

You can optionally group unmapped (XSQ) read-sets data to add to a project. In the Add Data · Group Data view, the available data in the project is shown in the Read-Sets in Project table.

**Note:** The Add Group to Project button is faded (inactive) until you select a read-set. The Add Read-Set to Selected Group button is faded until you select a group.

To group data:

1. Select the checkbox of the data to create a group of read-sets, then click the **Add Group to Project** button.
2. In the Group Read-sets window, type a group name, then click **OK**.  
A project name can contain only letters, numbers, and the “\_” symbol. Other special characters are not allowed.  
A table with the newly created group appears in the Groups in Project section. Below the table are Edit and Delete buttons:
  - Use the Edit button to change the name of a group. You can also delete a read-set from a group, unless the group has only one read-set. A group must contain at least one read-set.
  - Use the Delete button to delete a group.

To select a group for a project, click its checkbox in the Groups in Project table.

To save the group, click **Finish**. To exit the Add Data to Project window without saving the group, click **Cancel**. To proceed, click **Add Analysis** and follow the procedure “Choose data” on page 93.

## Group BAM data

In LifeScope™, an imported .bam file is treated as a group of data. You cannot create a group of multiple, imported .bam files for tertiary analysis.

To group multiple .bam files and use the group for tertiary analysis:

1. Create a group of multiple .xsq files and run an analysis.  
After secondary analysis (mapping), the group of .xsq files is created as a .bam file.
2. Use the created .bam file for tertiary analysis.

## View project data

You can view a project overview, status, parameters, read-sets, and BAM data. To view project data:

1. Select a project in the Projects organizer (shown ).

There are two ways to view project details. Select a project in the Projects organizer (shown on page 75), then:

- Click **View** in the top menu, then select the details that you want to view, or
- Click the Overview and Status tabs.

To view the Read-Sets and BAM Data tabs, click  on the subfolder under the project name in the Projects organizer.

## Project overview

In a project overview, you can view and edit the name of a project, its description, and the persons to be alerted about the project: To show the project overview, either:

- Click **View** in the main menu, then **Project Overview**, or
- Select a project in the Projects list to display the Overview tab in the status overview pane.

To save changes that you make in the Overview tab, click **Apply Changes**. To cancel your changes, click **Revert to Last Saved Settings**.

## Project status

To show the status of a project, select a project in the Projects list, then either:

- Click **View** in the main menu, then **Project Status**, or
- Click the **Status** tab to switch from the Overview tab in the status overview pane.

## Read-sets

To show the read-sets in a project, select a project in the Projects list, then either:

- Click **View** in the main menu, then **Project Read-Sets**, or
- Select a project in the Projects list, click **Data**, then (if necessary) the **Read-Sets** tab in the status overview pane.

In the Data, Read-Sets view, click the **Create Analysis of Data** button below the Read-Sets tab to create an analysis from the listed read-sets.

## BAM data

To show the BAM data in a project, select a project in the Projects list, then either:

- Click **View** in the main menu, then **Project BAM Data**, or
- Select a project in the Projects list, click **Data**, then (if necessary) the **BAM Data** tab in the status overview pane.

In the Data, BAM Data view, you can create an analysis from the listed BAM data by clicking the **Create Analysis of Data** button below the BAM Data tab.

## Delete a project

To delete a project, click **File** in the top menu, then select **Delete Project**.



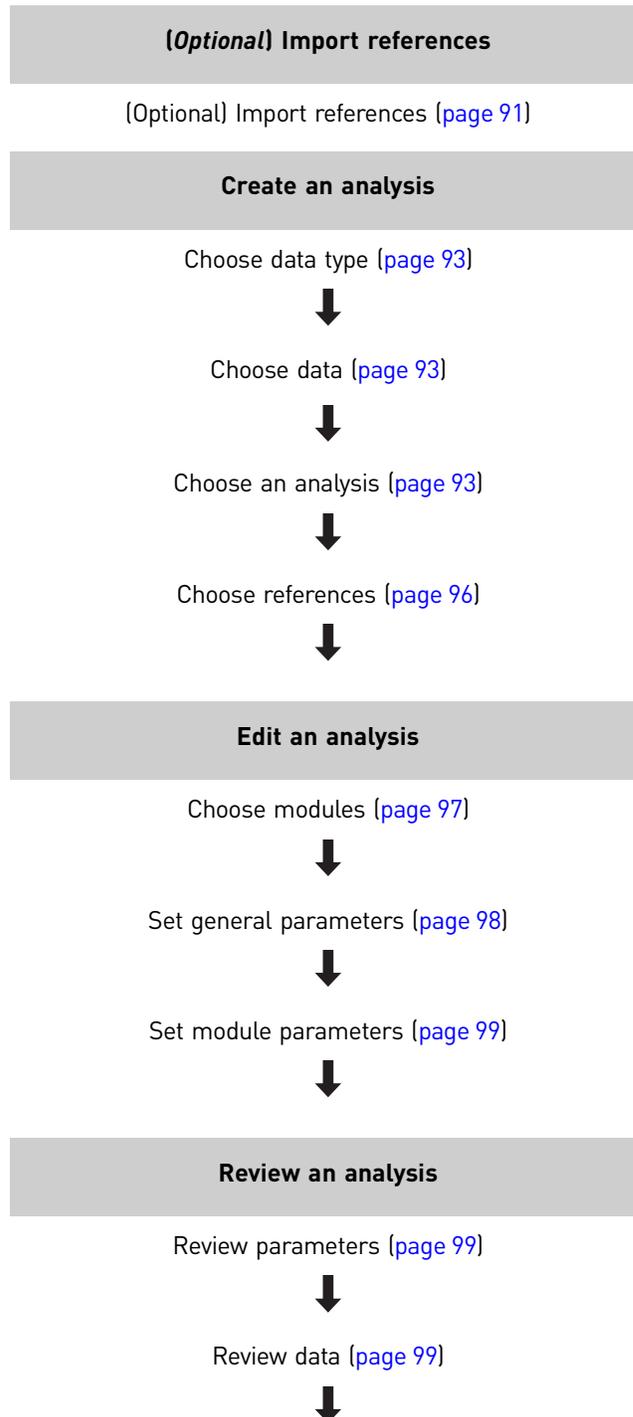
## 8

# Perform an Analysis

This chapter covers:

|                                       |     |
|---------------------------------------|-----|
| ■ Workflow .....                      | 90  |
| ■ (Optional) Import references .....  | 91  |
| ■ Create an analysis .....            | 92  |
| ■ Reuse an analysis .....             | 96  |
| ■ Edit an analysis .....              | 97  |
| ■ Review your analysis .....          | 99  |
| ■ Run and monitor your analysis ..... | 99  |
| ■ View analysis status .....          | 100 |
| ■ Delete an analysis .....            | 100 |
| ■ Resume, re-run, and re-use .....    | 101 |

## Workflow



**Run an analysis**Start your analysis now ([page 99](#))Stop an analysis ([page 100](#))

## (Optional) Import references

Import genomic repository reference files and folders from another directory into the reads repository. The reads repository is a storage place in LifeScope™ for instrument data intended to be input data for LifeScope™ analyses. Data imported into the reads repository are in unmappped eXtensible SeQuence (XSQ)-format files.

For more information about repositories, see “[Repositories](#)” on [page 71](#).

### Import references

1. Access the Import References window by using one of the following methods:
  - In the home view (see [page 75](#)), click the **Import References** button (see [page 76](#)).
  - In the main menu (see [page 75](#)), click **File**, then **Import References**.
  - In the toolbar (see [page 75](#)), click the **Import References** button.
  - In the Task Wizards panel (see [page 75](#)), click the **Import References** button.

The Import References window appears.

2. Import a reference file and/or repository folder.

Click the  buttons to open file browsers and navigate to a specific reference file and/or repository folder, for example:

```
/data/analysis/referenceData/lifetech/hg19/human_filter_reference.fasta
```

```
/data/analysis/referenceData
```

Click **OK**.

To add more reference files, click **Another Reference File**.

3. To import the references, click **Close**, or to close the Import References window without importing references, click **Cancel**.

The location of reference repository is set by the LifeScope™ Software system administrator. If you are unable to locate reference files, please contact the system administrator.

### Import your own references

You can also import your own references. Before importing your own references, validate them to prevent analysis errors or failure. Follow the procedure “[Validate the reference file](#)” on [page 30](#).

We recommend that you keep your original reference file and write a validated version of the reference file.

For more information about the reference repository, refer to the References appendix in the *LifeScope™ Genomic Analysis Software Command Shell User Guide* (Part no. 4471875).

### Import regions of interest files

You can also import your regions of interest files into the references repository. Follow these steps to import a regions of interest file:

1. Ensure that the file is a properly-formatted BED file.

**Note:** The tertiary modules using the regions of interest file fails if the file is not in BED format.

2. Follow the instructions in [“Import references” on page 91](#) to browse to your regions of interest file, using the reference file  button.

3. For the repository folder, use a location in the references repository under either the `internal` or `external` folders.

**Note:** Do not specify a location under the reference repository `lifetech` folder.

### View import status

To view the import status of files, click the Import Status tab.

Files that were successfully imported are indicated as COMPLETED in the File Transfer Status column.

Files that were not imported are indicated as FAILED! in the Conversion Status column. Failed imports cannot be removed in the LifeScope™ graphical user interface, but can be removed in the LifeScope™ command shell. For instructions on how to remove failed imports, refer to the *LifeScope™ Genomic Analysis Software Command Shell User Guide* (Part no. 4471875).

## Create an analysis

Creating an analysis involves choosing a data type, data, an analysis type, and references. Analysis types are predefined workflows. You can create a new analysis or reuse an existing analysis to create an analysis. For instructions on reusing an analysis, see [“Reuse an analysis” on page 96](#).

The following table lists the types of analysis, analysis modules, and references to chapters in this user guide.

| Analysis Type         | Includes                                                                                                    | Refer to . . .                                                                      |
|-----------------------|-------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Genomic Resequencing  | SAET, mapping, pairing, base translation, SNP finding, small indel, CNV, large indel, inversion, annotation | <a href="#">“Perform Genomic Resequencing Analysis” on page 163</a>                 |
| Whole Transcriptome   | Mapping, pairing, counts, coverage, splice finder exons, SASR gene fusions                                  | <a href="#">“Perform Whole Transcriptome Analysis” on page 203</a>                  |
| Targeted Resequencing | SAET, mapping, pairing, base translation, enrichment, SNP finding, small indel, annotation                  | <a href="#">“Perform Targeted Resequencing and Enrichment Analyses” on page 111</a> |

| Analysis Type         | Includes                                                                | Refer to . . .                                                      |
|-----------------------|-------------------------------------------------------------------------|---------------------------------------------------------------------|
| Low Frequency Variant | Mapping, pairing, base translation, enrichment, SNP finding, annotation | "Perform Targeted Resequencing and Enrichment Analyses" on page 111 |
| Small RNA             | Mapping, counts                                                         | "Perform Small RNA Analysis" on page 183                            |
| ChIP-Seq              | Mapping                                                                 | "Perform ChIP-Seq Mapping" on page 251                              |
| MethylMiner™          | Mapping                                                                 | "Perform MethylMiner™ Mapping" on page 241                          |

### Start analysis creation

There are two ways to create an analysis:

- Select a project from the Projects list, click **Analysis** in the toolbar (see [page 75](#)), then click **Create**.
- In the Task Wizards section, click **Create Analysis**.

The Create Analysis · Choose Data Type view appears in the status overview pane (see [page 75](#)).

### Choose data type

Choose unmapped (XSQ) or mapped (BAM) data, then click **Next** to choose data.

A table appears in the status overview pane. If you chose XSQ data, you will see an Available Data in Project table. If you chose BAM data, you will see a BAM Data in Project table.

#### BAM data

When you choose BAM data:

1. In the Add Data To Project window, click the  button, next to the BAM File data entry field, to open the file browser.
2. In the Browse to BAM File window, navigate to /data/results, select the BAM file that you want to add to your project, then click **OK**.
3. Click **Add BAM to Project**.

The BAM file appears in the BAM Data in Project area.

To delete a data file, click the **X** button. However, if there is only one imported data file, you cannot delete it.

If you decide to not add BAM data and instead add XSQ data to your project, click **Back**.

To refrain from adding the BAM data, click **Cancel**.

To add the BAM data and close the window, click **Finish**.

To proceed, click **Add Analysis**.

### Choose data

In the Available Data in Project table, click the checkboxes of the data for analysis, then click **Next** to choose an analysis.

### Choose an analysis

**Note:** If you added BAM data to your project, do not select the ChIP and Methyl Miner analysis modules.

In the Create Analysis · Choose Analysis view:

1. Select either **Create New Analysis** or **Reuse Old Analysis**.
2. Define the analysis with a name and description, and select an analysis type. The following table describes the analysis types.

| <b>Analysis Type</b>  | <b>Description</b>                                                                                                                                                                                                                                                              |
|-----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Targeted Resequencing | Analysis method designed for the analysis of target-enriched sequencing data. Targeted Resequencing supports SAET, mapping, SNPs, and small indels analyses. See <a href="#">Chapter 10, "Perform Targeted Resequencing and Enrichment Analyses"</a> on page 111.               |
| Low Frequency Variant | Analysis method designed for the analysis of target-enriched sequencing data. Low Frequency Variant workflows involves mapping and SNPs analyses. See <a href="#">Chapter 10, "Perform Targeted Resequencing and Enrichment Analyses"</a> on page 111.                          |
| Genomic Resequencing  | A workflow for identifying individual variants and structural variations in a genomic sample.                                                                                                                                                                                   |
| ChIP-Seq              | A combined assay and sequencing technique for identifying and characterizing elements in protein-DNA interactions. See <a href="#">Chapter 17, "Perform ChIP-Seq Mapping"</a> on page 251,                                                                                      |
| Whole Transcriptome   | Analysis method in LifeScope™ Software that aligns to a reference genome. Using the mapping results, WTA counts the number of tags aligned with exons. See <a href="#">Chapter 14, "Perform Whole Transcriptome Analysis"</a> on page 203.                                      |
| Methyl Miner          | A system for the enrichment of methylated sequences from genomic DNA, that, with the use of SOLiD® System sequencing, allows for focused evaluation of methylation patterns in genome-wide studies. See <a href="#">Chapter 16, "Perform MethylMiner™ Mapping"</a> on page 241. |

To see a list of modules for an analysis type, place your cursor over a  button. The following table describes the analysis modules.

| <b>Analysis Module</b> | <b>Description</b>                                                                                                                                                                                                                                                                                                                                                                       | <b>Used in Analysis Type</b>                   |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| Coverage               | This module calculates read coverage per position.                                                                                                                                                                                                                                                                                                                                       | Small RNA<br>Whole Transcriptome               |
| Enrichment             | This module creates statistics that provide a means to assess enrichment platform performance by: <ul style="list-style-type: none"> <li>• Looking at variations in coverage, both across all targets and on a per-target basis.</li> <li>• Addressing the uniformity and completeness of coverage within the target region.</li> <li>• Calculating the degree of enrichment.</li> </ul> | Targeted Resequencing<br>Low Frequency Variant |

| Analysis Module         | Description                                                                                                                                                                  | Used in Analysis Type                                                                                                    |
|-------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| Exon count              | This module counts and annotates the number of exons that are expressed in a gene region.                                                                                    | Whole Transcriptome                                                                                                      |
| Exon junction           | This module discovers splice junctions (introns from pre-messenger-RNAs to generate mature messenger-RNAs) and annotates the type and evidence for each discovered junction. |                                                                                                                          |
| Gene count              | This module counts the number of reads that align within genomic features.                                                                                                   |                                                                                                                          |
| Gene fusion             | This module uses mapped reads to find gene fusions.                                                                                                                          |                                                                                                                          |
| Mapping: fragment       | This module generates BAM files from gapped alignments and ungapped alignments.                                                                                              | ChIP-Seq<br>Genomic Resequencing<br>MethylMiner<br>Targeted Resequencing<br>Low Frequency Variant<br>Whole Transcriptome |
| Mapping: paired-end     | This module matches reads from the F3 and F5-P2 files of a paired-end mapping run.                                                                                           | Genomic Resequencing<br>MethylMiner<br>Targeted Resequencing<br>Low Frequency Variant<br>Whole Transcriptome             |
| Mapping: mate-pair      | Mate-pair mapping is similar to fragment mapping. Match files (which contain alignment information) are provided to pairing stage.                                           | Genomic Resequencing                                                                                                     |
| Mapping: fragment smRNA | This module maps fragmented small RNA (also known as microRNA or miRNA) reads.                                                                                               | Small RNA                                                                                                                |
| Mature small RNA        | This module generates tag counts for mature miRNA sequences.                                                                                                                 |                                                                                                                          |
| Precursor small RNA     | This module generates tag counts for precursor miRNA sequences.                                                                                                              |                                                                                                                          |
| SAET                    | This module increases mappability of reads to a reference genome.                                                                                                            | (Optional) Genomic Resequencing<br>Targeted Resequencing                                                                 |
| Small Indels            | This module detects gap alignments.                                                                                                                                          | Genomic Resequencing<br>Targeted Resequencing                                                                            |
| SNP Finding             | This module uses the diBayes algorithm to find Single Nucleotide Polymorphisms (SNPs).                                                                                       | Genomic Resequencing<br>Small RNA<br>Targeted Resequencing<br>Low Frequency Variant                                      |

3. Click **Next** to choose references.

The Create Analysis · Choose References view appears.

**Choose references** **Note:** The procedure for choosing references is the same for XSQ and BAM data types.

1. Click the Select Reference button to browse for reference.
2. In the pop-up window, select a folder in the Genomic Ref. Folders pane, select a reference file, then click **OK**.  
The referenced file appears in the Reference column.
3. To exit Choose References without saving your references, click **Cancel**. To save your changes and exit Choose References, click **Finish**. Otherwise, click **Edit** and follow the procedure “[Edit an analysis](#)” on page 97.

## Reuse an analysis

There are two methods you can reuse an analysis to create an analysis:

- **Method 1:** Select a completed analysis , click **Analysis** in the top menu, and then click **Reuse**, or
- **Method 2:** Select a project, then click **Create Analysis** in the Task Wizards section (shown [page 75](#)).

### Method 1

1. In the Create Analysis · Choose Analysis view, enter a new analysis name, then click **Next** to choose references, described [on page 96](#).
2. Click **Edit**, then follow the procedure “[Edit an analysis](#)” on page 97.

### Method 2

1. In the Create Analysis · Choose Data Type view, follow the procedure “[Choose data type](#)” on page 93.
2. Follow the procedure “[Choose data](#)” on page 93.
3. In the Create Analysis · Choose Analysis view, click **Reuse Old Analysis**, then click the  button to select an old analysis.
4. In the Select Old Analysis window, select an analysis, then click **OK**.
5. In the Choose Analysis view, click **Next** and follow the procedure “[Choose references](#)” on page 96.
6. Click **Edit**, then follow the procedure “[Edit an analysis](#)” on page 97.

## Reuse BAM data

You cannot reuse an analysis to run only BAM data.

To run an analysis with only BAM data:

1. Create an analysis, as described [on page 92](#).
2. Choose BAM data, as described [on page 93](#).
3. Edit the analysis, as described in the following section.

## Edit an analysis

**Note:** To edit Advanced, SAET, and BamStats parameters, refer to the *LifeScope™ Genomic Analysis Software Command Shell User Guide* (Part no. 4471875).

Editing an analysis involves choosing modules, setting general parameters, and setting module parameters.

**Note:** To see a brief description of the settings, place your cursor over a  button.

### Choose modules

In the Choose Modules view, you can specify pre-processing of raw reads, the method for mapping data during secondary analysis, and the modules for tertiary analysis.

#### Secondary Analysis

During secondary analysis, information from reads in XSQ-formatted input files and a FASTA-format reference file are combined to generate alignment information in an intermediate file format known as a Binary Alignment sequence Map (BAM) file.

The following table shows the type of analysis that is done during genomic resequencing and whole transcriptome analyses.

| Analysis             | Mapping                                                                                                                                                                                                             |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genomic resequencing | Fragment mapping                                                                                                                                                                                                    |
| Whole transcriptome  | <ul style="list-style-type: none"> <li>• Whole Transcriptome Exon Sequencing Extractor</li> <li>• Whole Transcriptome Splice Junction Extractor</li> <li>• Whole Transcriptome Splice Junction Extractor</li> </ul> |

#### Map data

Accept the default setting or click **Customize** to not include the BAMStats mapping module.

If raw reads need to be pre-processed with the SOLiD® Accuracy Enhancer Tool (SAET), click the **Pre-Process** checkbox.

#### Tertiary Analysis

**Note:** The procedure for choosing modules is the same for XSQ and BAM data types.

In the Tertiary Analysis Modules section, select tertiary analysis modules for targeted resequencing:

- Click a module name, then click the > button to insert it into the Include list. To include all available modules, click **All>>**.
- To generate annotated output for a module, click its Annotate checkbox.

| Modules     | Description                                                                                                                                                                                                                                                                                                                                       | Workflow                                                              |
|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|
| CNV         | Detects copy number variations in a data sample that is mapped to the reference sequence.                                                                                                                                                                                                                                                         | <a href="#">Perform Human Copy Number Variation Analysis</a>          |
| SNP Finding | Takes the color-space reads, the quality values, the reference sequence, and error information on each SOLiD® System slide as its input, and calls Single Nucleotide Polymorphisms (SNPs). For more information, refer to <a href="#">Chapter 20, “Perform SNP Finding Analysis” on page 289</a>                                                  | <a href="#">Perform SNP Finding Analysis</a>                          |
| Enrichment  | Enrichment statistics provide a means to assess enrichment platform performance by looking at variations in coverage, both across all targets and on a per-target basis.                                                                                                                                                                          | <a href="#">Perform Targeted Resequencing and Enrichment Analyses</a> |
| Inversion   | Calls inversions based on library size.                                                                                                                                                                                                                                                                                                           | <a href="#">Perform Inversion Analysis</a>                            |
| Large indel | Identifies deviations in clone insert size.                                                                                                                                                                                                                                                                                                       | <a href="#">“Perform Large Indel Analysis”</a>                        |
| Small Indel | Takes in a BAM file or a set of BAM files from Fragment, Paired-End, and Long Mate Pair libraries using the LifeScope™ Software alignment produced by LifeScope™ Software.                                                                                                                                                                        | <a href="#">Perform Targeted Resequencing and Enrichment Analyses</a> |
| Small RNA   | Used to analyze high throughput small RNA sequencing raw data.<br>The small RNA mapping module maps small RNA reads using the mapping program mapReads.<br>The small RNA whole transcriptome coverage module calculates read coverage per position.<br>The small RNA counts module generates tag counts for precursor and mature miRNA sequences. | <a href="#">“Perform Small RNA Analysis”</a>                          |
| SNP Finding | Used to call Single Nucleotide Polymorphisms (SNPs) from mapped and processed SOLiD® System color-space reads.                                                                                                                                                                                                                                    | <a href="#">“Perform SNP Finding Analysis”</a>                        |

After you have chosen modules and customized mapping, click **Next** to set the general parameters.

## Set general parameters

**Note:** To revert parameters to their default settings, click **Reset to Defaults**.

Set the parameters for files locations by clicking on the ... button and navigating to the locations.

After you set general parameters, you can optionally set module parameters. To skip setting module parameters and proceed to reviewing your analysis, click **Review**. Otherwise, click **Next** to set module parameters.

## Choose analysis space

Mapping and select tertiary modules support the option to have your analysis done in base space or color space (if the space is present in your input data). The analysis space for your runs is controlled by these factors and parameters:

- **Input data** – Whether base space and color space are present in your input data.
- **Secondary processing** – Your setting for the `mapping.in.base` parameter. This parameter controls the analysis space for secondary processing. The run fails if the input data does not have the space specified by the `mapping.in.base` parameter.

- **Tertiary processing** – Your setting for the `analysis.space` parameter.

Use the global workflow parameter `analysis.space` to enter your preference for base space or color space tertiary analysis. Three tertiary modules – SNPs, small indel, and splice finder – use your preference, in conjunction with information about whether color space is present in the BAM file and whether mapping is done in base space or color space, to determine their analysis space.

The recommended usage is to perform mapping and tertiary processing in the same space.

### Choose analysis platform

Always set `analysis.platform` to “SOLID”. Other values are reserved for future use.

### Set module parameters

Review the parameters for each module. If necessary, change the parameters.

To reject your edits, click **Cancel**. To save your edits and close the Edit Analysis window, click **Finish**. Otherwise, click **Review** to proceed.

## Review your analysis

Review the parameters and data in your analysis.

### Review parameters

Review the parameters for modules before starting an analysis run. You can show and hide the parameters for each module by clicking on the ► next to each module.

To change a parameter, click **Edit**.

### Review data

In the Data tab, review the read-only data in the Targeted Resequencing Analyses to be Run table.

## Run and monitor your analysis

You can immediately run your analysis or run it later.

### Start your analysis now

After you review your analysis and if you are satisfied with the parameters and data, click **Start Analysis**. The Run Analysis window closes.

### Start your analysis later

1. Click your project in the Projects organizer (shown on page 75) to show the analysis module, select the analysis , then either:
  - Click **Run Analysis** in the Task Wizards section, or
  - Click **Analysis** in the top menu, then **Run**.

In the Run Analysis window, you can review the parameters for analysis modules and data that will be analyzed. When you are ready, click **Start Analysis**. The Run Analysis window closes.

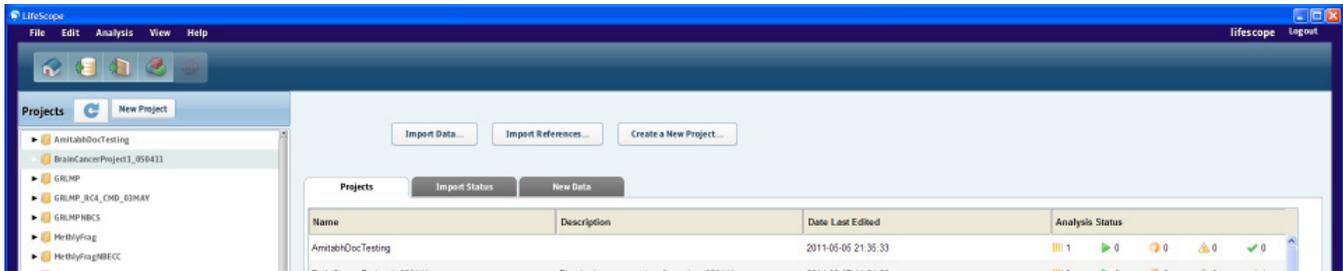
After the analysis starts, the home view (shown on page 75) appears. The progress of the secondary and tertiary analyses is shown in the Status tab.

**Stop an analysis** To stop the run of an analysis, click **Analysis** in the toolbar, then click **Stop**.

## View analysis status

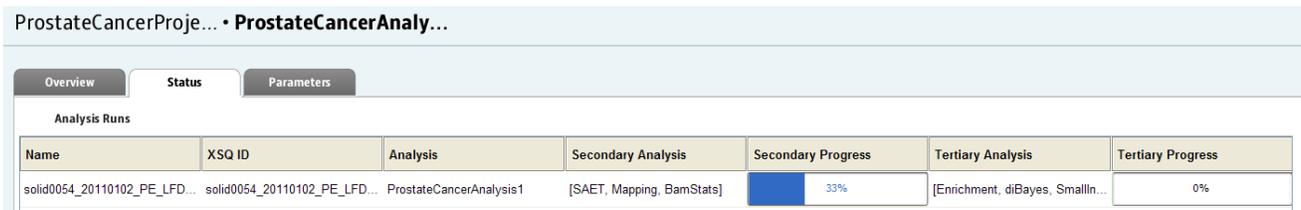
There are two ways to check the status of an analysis run:

- In the status overview Projects tab (shown on page 75). For a description of status icons, see the table on page 77.

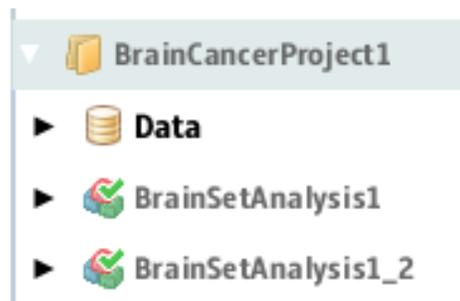


- In the Status tab for a specific analysis.
  1. In the Projects list, click the project to check the status of the analysis run.
  2. Click the **Status** tab in the status overview.
 

The Progress columns show the percentage of completion for the secondary and tertiary analyses.



When analysis is complete, a green check mark appears on the analysis name in the Projects organizer (shown on page 75).



## Delete an analysis

To delete an analysis, select the analysis in the Projects list, click **Analysis**, then **Delete**.

## Resume, re-run, and re-use

This section explains analysis reuse concepts and supported functionality for the GUI interface.

- **Resume** – A stopped job is restarted, without repeating modules that have successfully completed.

**Note:** With the `resume` command, every module that is in progress at the time the job is stopped (or fails) is processed from the beginning of that module.

The GUI does not support resuming a failed job.

- **Re-use** – An existing analysis is used as the basis for a new analysis (with a new analysis name). You can optionally change the configuration of the new analysis.
- **Re-run** – An analysis is repeated (with the same configuration). All processing is redone.

| Purpose | Support in the GUI                                                                                                                                              | Comments                                                                |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Resume  | Use the <b>Analysis &gt; Resume...</b> command.                                                                                                                 | Only stopped jobs are supported.                                        |
| Re-use  | Use the <b>Analysis &gt; Reuse...</b> command.<br><br><b>Note:</b> <b>Reuse...</b> is available only for analyses with status of Running, Completed, or Failed. | You may optionally change the configuration of the new analysis.        |
| Re-run  | Supported indirectly: use the <b>Analysis &gt; Reuse...</b> command, and run the same analysis content under the new analysis name.                             | You may optionally change the analysis configuration before restarting. |

**Note:** If all secondary processing completed successfully and the resulting BAM files are correct, use the BAM input option (not the XSQ input option) when re-using or re-running a job. If you use the XSQ option, then all secondary processing is repeated. With the BAM option, only tertiary processing is done.



## 9

## View Analysis Results

This chapter covers:

- View analysis results ..... 103
- View Results window ..... 103
- Failed analysis..... 106

## View analysis results

You can view analysis results immediately after mapping has been completed or view results at a later time. The duration for an analysis run depends on the complexity of the analysis.

**Note:** If multiple View Results windows are open, actions such as file downloads and file exports will be slowed. To improve performance, close windows that you do not need open.

### View results now

If an analysis run is brief, click **View Results** in the Secondary Progress and Tertiary Progress columns in the Status tab.

### View results later

To view analysis results at a later time:

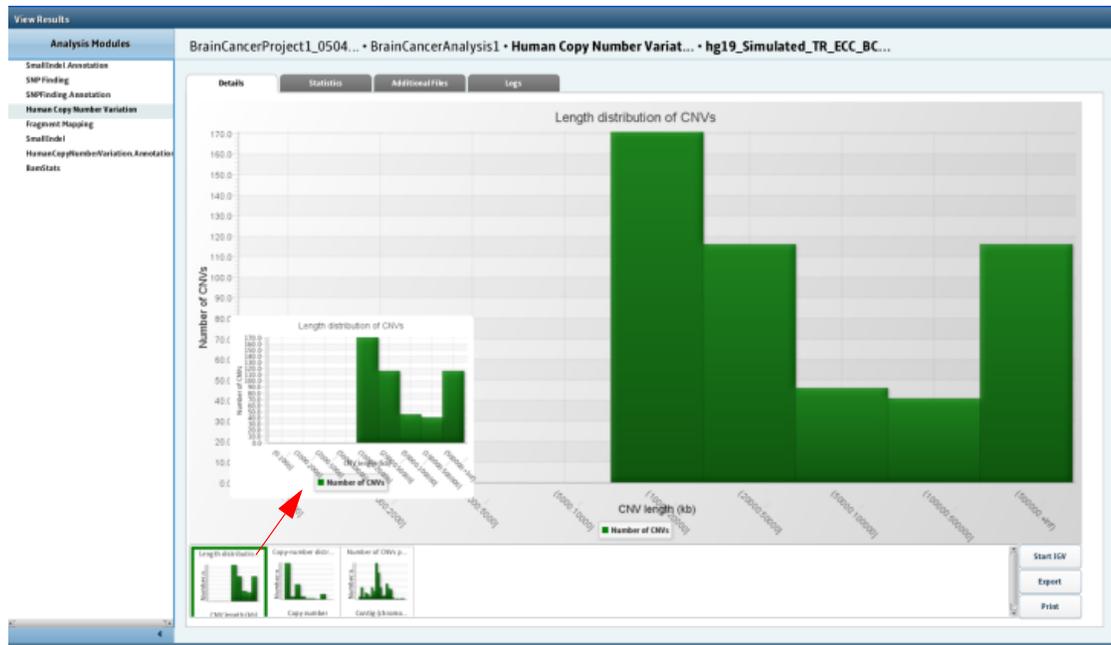
1. Select your project in the Projects organizer (shown [on page 75](#)).
2. Select the completed analysis , then either:
  - Click the Results icon  in the Projects organizer, or
  - Click the **Status** tab in the status overview (shown [on page 75](#)).
3. Click the **View Results** button in a Progress column to open the View Results window (shown [on page 104](#)).

| BrainCancerProject1... • BrainCancerAnalysis1 • Results |                               |                      |                              |                    |                                |                   |
|---------------------------------------------------------|-------------------------------|----------------------|------------------------------|--------------------|--------------------------------|-------------------|
| Data-Sets with Results in Analysis                      |                               |                      |                              |                    |                                |                   |
| Name                                                    | X5Q ID                        | Analysis             | Secondary Analysis           | Secondary Progress | Tertiary Analysis              | Tertiary Progress |
| hg19_Simulated_TR_ECC_BC1...                            | hg19_Simulated_TR_ECC_BC16... | BrainCancerAnalysis1 | [Fragment Mapping, BamStats] | View Results       | [SNP Finding, Human Copy Nu... | View Results      |

## View Results window

The left pane of the View Results window shows successful analysis modules. Annotated analysis modules that fail will not appear in the left pane of the window.

The right pane of the View Results window shows the analysis results, which include details, statistics, additional files, and logs.



## Details

The Details tab consists of a canvas that shows analysis results in the form of bar charts and pie charts, as shown above. Thumbnail views of charts are below the canvas. Buttons include Start IGV, Export, and Print.

Placing your cursor over a thumbnail view opens a magnified preview. Clicking on a thumbnail view of a chart displays it on the canvas.

You can change the colors of bars or wedges in a chart by right-clicking on those areas and selecting a color in the Change Color pop-up window.

### Start IGV

You can view BAM files and GFF3 files, described, with the Integrative Genomics Viewer (IGV) browser. In addition to a BAM file, you need a reference genome. For more information about the IGV, refer to [“Integrative Genomics View \(IGV\)” on page 350](#). For instructions on using the IGV, go to [www.broadinstitute.org/igv/](http://www.broadinstitute.org/igv/).

### Export

You can export charts as a comma-separated values (.csv), .jpeg, .gif, or .png file.

To export details:

1. Click **Export**.
2. In the Export Results window, choose to export only the displayed, magnified view of data or all data in the Details tab.
3. Select an export format.
4. Click **Browser** to navigate to a destination for the exported data.
5. Click **OK** to export the data.

## Print

Click this button to print analysis details.

## Statistics

The Statistics tab shows a LifeScope™ Software report of statistics that you can view, export, and print. You cannot edit the statistics.

## Additional Files

The Additional Files tab shows a list of result files that you can download and view with the IGV browser.

**Note:** A large file can take several minutes to download.

### Download additional files

To download a file:

1. Select the file in the Additional Files table, then click **Download**.
2. In the Confirm window, click **OK**.

After a file has been downloaded, the message “Download Done” appears.

To view a file, click **Add to IGV**.

## Logs

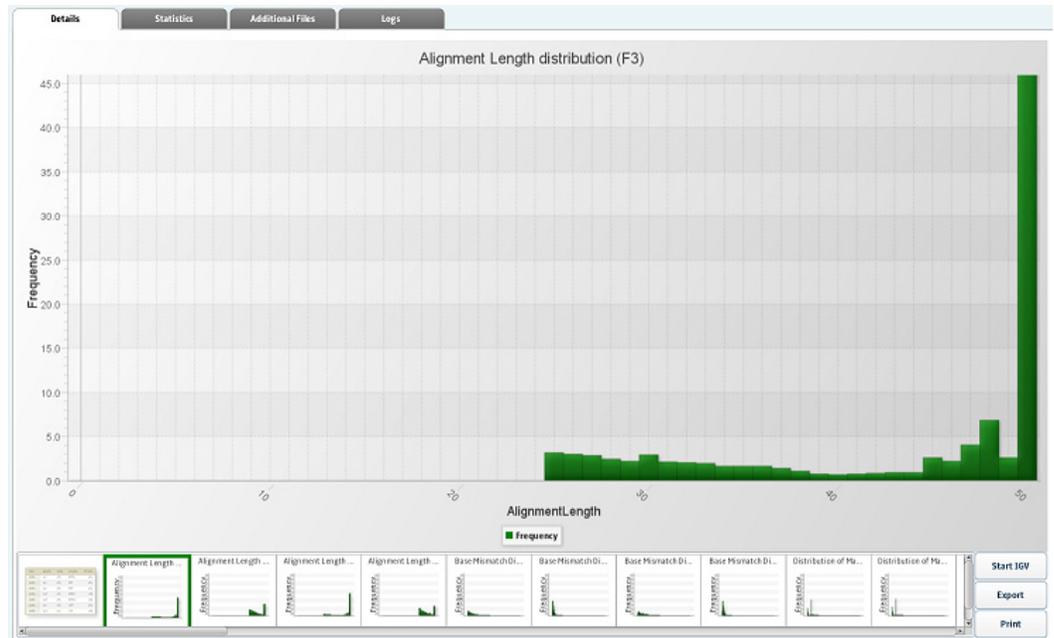
The Logs tab shows a list of log files that you can download. To download a log file, select the file in the Log Files table, then click **Download**. In the

**Note:** A large file can take several minutes to download. After a file has been downloaded, the message Download Done appears.

## Examples of view results

This section shows examples of results for BAMStats.

### Details



## Statistics

There are not statistics for BAMStats.

## Additional files

Additional files for small indel analysis output include comma-separated values (.csv), text (.txt), and wiggle (.wig) files.

The following table is an example of additional files for BAMStats results.

| Directory                                                                        | Files                                                                                     | File Type |
|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|-----------|
| bamstats/WTPE                                                                    | WTPE_statstuple.txt                                                                       | .txt      |
| bamstats/WTPE/<br>K_201001207_PE_BC_MAGNUM1_WT_FC1_BC.s<br>owmi-1-Idx_BC1-1/Misc | K_201001207_PE_BC_MAGNUM1_WT_FC1_BC.sowmi<br>-1-Idx_BC1-1.bam_chr19.POS.wig               | .wig      |
|                                                                                  | K_201001207_PE_BC_MAGNUM1_WT_FC1_BC.sowmi<br>-1-Idx_BC1-1.Mismatches.by.ReadPair.Type.csv | .ungap... |
|                                                                                  | BrainCancerAnalysis1.gff3                                                                 | .csv      |

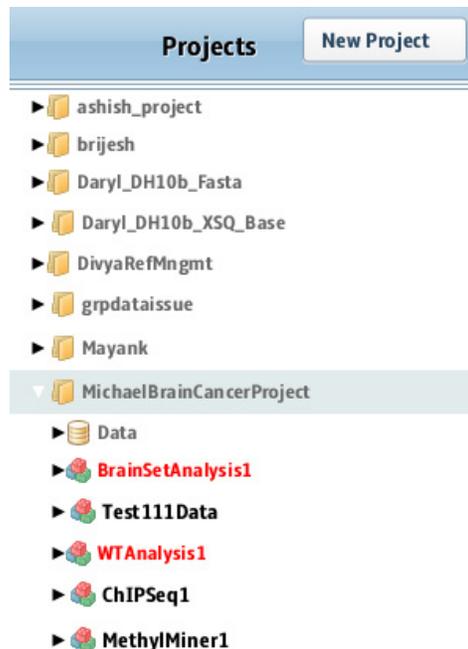
## Logs

The following table is an example of logs for BAMStats results.

| Directory | Files                                              | File Type |
|-----------|----------------------------------------------------|-----------|
| bamstats  | secondary-hg19-bamstats.20110429133358916.log      | .log      |
|           | secondary-hg19-bamstats.main.20110429133401974.log |           |

## Failed analysis

In the Projects organizer, a failed analysis is indicated by red text, as shown:



In the status overview for all projects, a failed analysis is indicated by the fault icon  in the Analysis Status column, as shown:



In the Status overview of a specific project, a failed analysis is indicated in red, as shown:

| Analysis Runs                          |                             |          |          |
|----------------------------------------|-----------------------------|----------|----------|
| Name                                   | XSQ ID                      | Analysis | Progress |
| test_S1_F31298403116375_DefaultLibrary | test_S1_F31298403116375.xsq | a2       | Fail !!  |

### View failure details

To find out the reason an analysis failed:

1. Click **Fail** in the Progress column to view the results of the analysis run.
2. In the View Results window, click a row in the Log Files table, then click **Download**.
3. In the Select Directory browser, navigate to a destination for the log file, then click **OK**.
4. Navigate to the log file and open it to see the details of the run.



# PART IV

## Workflows



## 10

# Perform Targeted Resequencing and Enrichment Analyses

This chapter covers:

- Introduction to Targeted Resequencing (TRS) analysis ..... 111
- Targeted resequencing library types ..... 112
- Targeted resequencing input files ..... 113
- Enrichment statistics input files ..... 113
- Targeted resequencing analysis modules ..... 115
- Targeted resequencing parameters ..... 116
- Perform Targeted Resequencing analysis ..... 126
- View analysis results ..... 130
- Targeted resequencing output files ..... 131

## Introduction to Targeted Resequencing (TRS) analysis

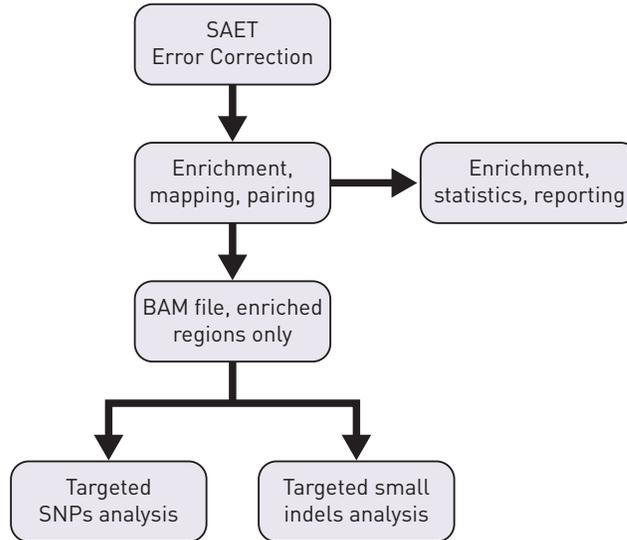
Targeted resequencing is a set of analyses designed for target-enriched sequencing data. Given a set of coordinates representing a region of interest in a genome, a library may be enriched for reads within those regions prior to sequencing.

The SAET module is enabled by default for enriched runs, as the smaller target size makes spectral error correction viable, even if it may not be practical for unenriched sequencing runs on a large reference sequence.

Targeted resequencing mapping produces a filtered BAM file for variant calling. The variant calling modules SNP Finding and Small Indel are run on the filtered data sets instead of the entire read population, and limit SNP and indel calls to the target region.

The input list of regions of interest (ROI) is provided by the user. Supported ROI input files include third-party files of baits, probes, or tiles. The input ROI files can be in BED format or in a text file as a list of “chr:start-end” values. The Targeted Resequencing mapping module sorts the input ROI file by chromosome and start position, and makes the resulting file available as output.

The following illustration shows the components of Targeted Resequencing analyses.



## Targeted resequencing library types

The Targeted Resequencing analyses support data from these library types:

- Fragment
- Paired-End
- Mate-Pair  
 Mate-pair libraries are accepted, but pairing information is not considered for mate-pair libraries.
- Libraries generated with up to 96 barcodes  
 Individual sequencing and enrichment reports are provided for each barcoded library.

Targeted Resequencing output files are compatible with third-party genome browsers, including the IGV browser.

The Targeted Resequencing Map analysis type provides analysis of sequencing runs that have been run through targeted enrichment.

### Enrichment statistics

Targeted resequencing support optional enrichment statistics, which provide a means to assess enrichment platform performance by:

- Looking at variations in coverage, both across all targets and on a per-target basis.
- Addressing the uniformity and completeness of coverage within the target region.
- Calculating the degree of enrichment.

## Enrichment statistics scenarios

The enrichment statistics module supports these scenarios:

- Genome class:
  - Human
  - Non-human multi-chromosome
  - Single chromosome
- Library type:
  - 2X50
  - 2X25
  - fragment
- Experiment type:
  - Single sample, single run
  - Multiple runs, multiple samples

## Targeted resequencing input files

| Input file                          | File extensions | Analysis modules              |
|-------------------------------------|-----------------|-------------------------------|
| eXtensible SeQUENCE (XSQ)           | .xsq            | Small Indel, SNP Finding, CNV |
| <i>(Optional)</i> Color space FASTA | .csfasta        | Mapping                       |
| <i>(Optional)</i> FAST-All (FASTA)  | .fa, .fasta     | Mapping                       |
| <i>(Optional)</i> Quality Value     | qual, *qv       | Mapping, Pairing              |
| Binary Alignment Map (BAM)          | .bam            | Small Indel, SNP Finding, CNV |

## Enrichment statistics input files

This section describes the input files required for by the enrichment statistics module.

The following table lists the two input files for the enrichment statistics module.

| File           | File format          | Associated parameter   | Description                                                                                                                                                           |
|----------------|----------------------|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| target regions | BED, GFF v3, or text | enrichment.target.file | A list of target regions, either as regions of interest (ROI) or as target lists provided by vendors of enrichment platforms (such as baits, probes, or tiles files). |
| aligned reads  | BAM                  | enrichment.input.file  | Alignments generated by the sequencing run after being matched to the entire reference genome.                                                                        |

### The input target regions file

The target regions file may be either in BED format or a list of chr:start-end elements. An example of partial content for a target region file is shown below.

```
chr13 31787000 31788297
chr13 31788404 31788957
chr13 31789616 31789695
chr13 31790390 31790601
chr13 31790852 31791495
chr13 31791644 31791981
```

The following information applies to the input target region file:

- Target region coordinates must be specified using the same reference used in mapping.
- Target regions should be sorted by chromosome/position. If not sorted, they will be sorted as the first step in the enrichment statistics module.
- After the first 3 required fields per line, any additional fields are ignored.
- The file can optionally be a list of target regions in the “chr:start-end” format.

An example of this format is: chr1:1234-4321

The first field, “chrom”, must have identifiers that match the sequence names used in the SAM file. For example, for the hg18 reference:

- Use “chr1”, not “1”.
- Use “chrX”, not “23” or “chr23”.

**Note:** A standard BED file uses zero-based indexing and a half-open interval (meaning the specified range is up to, but not including, the last position).

### The input aligned reads file

This file is a BAM-formatted file of aligned reads generated by the sequencing run after being matched to the entire reference genome.

The following information applies to the input aligned reads file:

- The BAM file must have a header listing sequence identifiers.  
The header is mandatory in the SAM specification for the binary BAM files.
- These sequence identifiers must be consistent with those used in the target list.
- The BAM file must be sorted by coordinate. The SO annotation in the header is not examined. All alignments within a chromosome must be contiguous. For sequential records, start coordinates within a chromosome must be equal or increasing.
- The BAM file may be from a fragment, paired-end, or mate-pair library.  
All reads are treated independently; no special handling is performed based on library type.
- Reads from paired libraries will tend to have improved mapping scores based on unique paired hits, and will be more likely to pass uniqueness filtering than unpaired reads.

## Targeted resequencing analysis modules

Targeted resequencing provides a mechanism to restrict resequencing analysis to specific regions of interest within the genome being studied. Targeted resequencing analysis includes the following modules:

- Mapping
- Enrichment
- SNP Finding
- Small indels

### Mapping

The map data resequencing workflow uses eXtensible SeQuence (.xsq) files to create a .bam file.

The Targeted Resequencing mapping module uses eXtensible SeQuence (.xsq) files to create a \*.bam file. Targeted Resequencing mapping also requires a regions of interest (ROI) file as input. The ROI file can be in BED, text, or tiles format.

### Enrichment

Enrichment statistics provide a means to assess enrichment platform performance by looking at variations in coverage, both across all targets and on a per-target basis.

### SNP Finding

The SNP Finding package is the module used to call Single Nucleotide Polymorphisms (SNPs) from mapped and processed SOLiD<sup>®</sup> System reads. The module takes the reads, the quality values, the reference sequence, and error information on each SOLiD<sup>®</sup> System slide as its input, and calls SNPs.

The module creates three results files:

- A list of SNPs.
- *(Optional)* A consensus \*.fasta file with the same number of bases as the reference sequence.
- *(Optional)* A list of all covered positions.
- *(Optional)* A collection of annotated files.

For information about using the module to run a Targeted Resequencing experiment, see [Chapter 10, “Perform Targeted Resequencing and Enrichment Analyses”](#) on page 111.

### Small Indel

When an indel occurs in a sequence, and that sequence is measured using color-space, the color-space sequence has a gap the same size as the indel. The color-space sequence also leaves a signature that can indicate whether there is a measurement error within the gap. The Small Indel tool targets the processing of indel evidences found in the pairing step during secondary offline data analysis.

For information about using the module to run a Targeted Resequencing experiment, see [Chapter 10, “Perform Targeted Resequencing and Enrichment Analyses”](#) on page 111.

## Targeted resequencing parameters

Parameters for Targeted Resequencing analysis include:

- General parameters
- **Secondary analysis:** Fragment Mapping (including optional BAMStats and SOLiD® Accuracy Enhancement Tool [SAET])
- **Tertiary analysis:** Small Indel, Enrichment, and SNP Finding.

### General

General parameters include:

| Parameter              | Default value | Description                                                                                                                                                                                               |
|------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| analysis.assembly.name | —             | The name of the genome assembly used in current analysis. Examples: hg18, hg19.                                                                                                                           |
| annotation.dbsnp.file  | —             | The path to the file used to annotate SNPs and small InDels. LifeTech-provided files are dbSNP_b130.tab(hg18) and 00-All.vcf(hg19).                                                                       |
| analysis.regions.file  | —             | The path to the file containing genomic regions used in targeted resequencing selection, such as a .bed format file containing exome targets.                                                             |
| annotation.gtf.file    | —             | The file containing gene and exon annotations corresponding to the genome assembly used in the analysis.                                                                                                  |
| analysis.space         | Base          | Your preference of whether analysis should be done in base space or in color space. This setting should be the same for all modules in your run. See <a href="#">“Choose analysis space” on page 98</a> . |
| analysis.platform      | SOLID         | Always specify “SOLID”. Other values are reserved for future use.                                                                                                                                         |

Use the  button to open the File Chooser and search for input files.

### Fragment Mapping

There are four categories of fragment mapping parameters: Main, Advanced, SAET, and BAMStats.

#### Main

| Parameter          | Default value | Description                                                                                                                                                                                                                                                                                   |
|--------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Add color sequence | True          | Add color sequence to BAM records: Values: <ul style="list-style-type: none"> <li>• True: Add color.</li> <li>• False: Do not add color.</li> </ul>                                                                                                                                           |
| Map in base space  | False         | Allow mapping in base space if input data has base space available. If only color space is available, then mapping will fail when base space mapping is turned on. Values: <ul style="list-style-type: none"> <li>• True: Map in base space.</li> <li>• False: Map in color space.</li> </ul> |

**Advanced**

| Parameter                        | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|----------------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| BAM soft clip                    | False         | Modify an unaligned portion of a read that needs to be presented in the BAM record. Values: <ul style="list-style-type: none"> <li>• True: Soft clip.</li> <li>• False: Do not soft clip.</li> </ul>                                                                                                                                                                                                                                                  |
| Base quality filter threshold    | 0             | Replace resulting base-calls with a quality value less than the specified value.                                                                                                                                                                                                                                                                                                                                                                      |
| Create unmapped BAM files        | False         | Create BAM files containing unmapped reads. Values: <ul style="list-style-type: none"> <li>True: Create.</li> <li>False: Do not create.</li> </ul>                                                                                                                                                                                                                                                                                                    |
| Mapping QV/threshold             | 0             | Provide control the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer.<br>Allowed values: Integers 0–1,000.                                                                                                                                                                                                                     |
| Primary output filter type       | Primary_only  |                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Reference weight                 | 15            | Used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. Adds weight (in terms of Phred score) to the signals that are compatible with reference. Color combinations that result in a variant are considered compatible with reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation.<br>Allowed values: Integers 0–100. |
| Second map gapped algorithm type | GLOBAL        | Do indel finding, and control the behavior of indel finding.<br>Allowed values: <ul style="list-style-type: none"> <li>• NONE: Turn off indel finding.</li> <li>• GLOBAL: Mapping reports global alignment up to one indel.</li> <li>• LOCAL: Mapping reports local alignment up to one indel.</li> </ul>                                                                                                                                             |

**SAET**

The SAET tab is accessible only if you select SAET pre-processing when you choose modules.

| Parameter       | Default value | Description                                                                                                                                                                                      |
|-----------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genome length   | 1000000       | Expected length of sequenced (or enriched) DNA region. For example, 4,600,000 for the E.Coli 4.6 MB genome or 30,000,000 for the entire Human Transcriptome.<br>Allowed values: Integers >= 200. |
| On target ratio | 0.5           | The expected ratio of reads that come from the targeted region.<br>Allowed values: Floats 0.0–1.0.                                                                                               |

| Parameter                              | Default value | Description                                                                                                                                                                                                                                                                                                                                               |
|----------------------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Update quality values                  | True          | Update quality value of modified calls.<br>Allowed values:<br>True: Update the QV of modified calls.<br>False: Do not update the qv for modified calls.                                                                                                                                                                                                   |
| Trusted quality value                  | 25            | Correction is applied to calls with a quality value below the value of this parameter.<br>Allowed values: Integers $\geq 1$ .                                                                                                                                                                                                                             |
| Support votes                          | 2             | The minimum number of k-mer votes required make a correction.<br>Allowed values: Integers $\geq 1$ .                                                                                                                                                                                                                                                      |
| Trusted frequency                      | 0             | The lowest multiplicity of the seed to be included in the spectrum. (If set to 0, then the value is computed internally.)<br>Allowed values: Integers $\geq 0$ .                                                                                                                                                                                          |
| Maximum corrections per read           | 0             | Maximum number of allowed corrections per read. (If set to 0, then the value is set to $\lceil \text{readLength}/8 \rceil$ ). Reduce if over-corrections are observed, or increase if under-corrections are observed.<br>Allowed values: Integers 0–9.                                                                                                    |
| Number of recursive runs               | 1             | The error correction step is repeated the provided number of times. Reduce if over-corrections are observed, or increase if undercorrections are observed.<br>Allowed values: 1, 2, or 3.                                                                                                                                                                 |
| Position of error inflation point      | 0             | Position in the read at which the error rate inflates, for instance, 35-40 for 50bp long reads. (If set to 0, then the value is equal to $0.8 * \text{readLength}$ ).<br>Allowed values: Integers.                                                                                                                                                        |
| Disable random sampling for large data | False         | Disables random sampling in spectrum building. If set to 0, then for large datasets (coverage > 300x), a subset of reads is used in spectrum building.<br>Allowed values: <ul style="list-style-type: none"> <li>• True: Disables random sampling in spectrum building.</li> <li>• False: Do not disable random sampling in spectrum building.</li> </ul> |
| K-mer size                             | 0             | Size of k-mer (>5) used in spectrum construction and error correction. (If set to 0, then the value is computed internally.)<br>Allowed values:<br>Integers 0–28.                                                                                                                                                                                         |

### BAMStats

| Parameter                    | Default value                                         | Description                                                                                                          |
|------------------------------|-------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| Input directory for BAMStats | <code>\${analysis.output.dir}/fragment.mapping</code> | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample. |

|                                                                          |                     |                                                                                                                                                                                                                                                                                                   |
|--------------------------------------------------------------------------|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Output directory                                                         | \$(task.output.dir) | The path to the output directory where BAMStats will write its chart (.cht) files.                                                                                                                                                                                                                |
| Maximum coverage                                                         | 10,000              | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br><br>Allowed values: Integers >= 0.                                                                                 |
| Maximum insert size                                                      | 100,000             | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br><br>Allowed values: Integers 0–100,000.                                             |
| Insert bin size                                                          | 100                 | Bin size for insert range distribution.<br><br>Allowed values: Integers 1–100,000                                                                                                                                                                                                                 |
| Whether to combine data from both the strands for coverage in WIG format | 0                   | Combine or do not combine data from both strands for coverage in WIG format.<br><br>Allowed values: 0–1.                                                                                                                                                                                          |
| Primary alignments only for coverage in WIG file format                  | 1                   | Use only primary alignments for coverage in WIG file format.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• 0: Do not restrict coverage in WIG file format to only primary alignments.</li> <li>• 1: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Bin size for coverage in WIG file format                                 | 100                 | The bin size for coverage in WIG file format.<br><br>Allowed values: Integers 1–100,000.                                                                                                                                                                                                          |

## Small indel

Categories for the Small Indel analysis module include Advanced and (if you selected Annotation for Small Indel output) Annotation. There are no Main parameters.

### Advanced

There are five categories of Advanced parameters: General Options, Pileup, Mapping Quality Filtering, Heuristic Filtering, and Indel Size Filtering.

#### General Options

| Parameters   | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                        |
|--------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Detail level | 0             | For BAM file inputs, the level of detail in output: <ul style="list-style-type: none"> <li>• <b>0:</b> Keeps only position information about the anchor read and no information for the ungapped alignment.</li> <li>• <b>1-8:</b> Keeps only some of the alignment's anchor alignment but none of the ungapped alignment.</li> <li>• <b>9:</b> Is most detailed, but also the slowest.</li> </ul> |

| Parameters                      | Default value | Description                                                                                                                                                                                                                                                                                                                                                                             |
|---------------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Zygosity profile name           | max-mapping   | Zygosity profile name. <ul style="list-style-type: none"> <li><b>classic</b>: Profile for classically (full read) mapped reads.</li> <li><b>max-mapping</b>: Profile for seed and extend ungapped alignments.</li> <li><b>max-mapping-v2</b>: Reserved for future use.</li> <li><b>gap-align-only</b>, and <b>no-calls</b>: Force all zygosity calls to be homozygous calls.</li> </ul> |
| Genomic region                  | -             | Names a specific genomic region to be selected from the BAM file. Only full chromosomes are guaranteed not to alter results. Specifying partial chromosomes is allowed but may result in the loss of indels near the edges of that region.                                                                                                                                              |
| Display base QVs                | False         | Display base QV scores in the GFF file. Allowed values: <p><b>True</b>: Display the FASTQ base QV scores for all of the reads used for each indel in the GFF file. FASTQ strings contain semi-colons, so adding these strings may produce a GFF file that is not compatible with certain applications.</p> <p><b>False</b>: Do not display QV scores in the output file.</p>            |
| Number of alignments per pileup | 1000          | For pileups with more than this number of reads, set the expected number of alignments per pileup. Values much higher than 1000 may result in a significant increase in computational time. <p>Allowed values: Integers <math>\geq 0</math>.</p>                                                                                                                                        |
| Random seed                     | 94404         | The random seed value used to determine which pseudo random set of reads to use when there are greater than 1000 reads in a pileup. The random number generator used is the Mersenne Twister MT19937 algorithm. <p>Allowed values: Integers <math>\geq 0</math>.</p>                                                                                                                    |

### Pileup

| Parameters   | Default value | Description                                                                                                                                                                                                                                                                                                                  |
|--------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Min num evid | 2             | Minimum number of evidences required for an indel call. This parameter does not have an upper limit, but a value higher than the average coverage level in most cases causes a significant reduction in sensitivity.                                                                                                         |
| Max num evid | -1            | Maximum number of evidences. Use -1 for no maximum. <p>Setting this value to some multiple of the average coverage could remove indels found in abnormally high coverage areas.</p>                                                                                                                                          |
| ConsGroup    | 1             | Indel grouping method. Allowed values: <p><b>1</b>: Conservative grouping of indels with 5bp max between consecutive evidences.</p> <p><b>2</b>: Lax grouping. Groups indels that are at maximum the higher of 15 or 7 times the indel size.</p> <p><b>9</b>: No grouping. Makes every indel evidence a separate pileup.</p> |

### Mapping Quality Filtering

| Parameters                 | Default value | Description                                                                                                                                                                                                                                                                                                                                      |
|----------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Max reported alignments    | -1            | Only uses those alignments where the NH field (the number of reported alignments; from the BAM record) is this value or lower. A value of -1 is to have no upper limit. The range where this is effective depends on the input BAM file's range of values of the NH tag.                                                                         |
| Min mapping quality        | 8             | Keeps only reads that have this or higher pairing qualities. For paired tags, mapping quality is for the pair (pairing quality), and for fragment, it is the single tag's map quality.<br><br>Reads that are lower than this value are filtered out.<br><br>Allowed values: Integers 0-100.                                                      |
| Min best mapping quality   | 10            | For a particular indel called with a set of reads, at least one pairing quality in this set must be higher than this value. Allows for supporting evidences to have a lower mapping quality threshold than the best read.                                                                                                                        |
| Min anchor mapping quality | -1            | Minimum mapping quality for a non-indel (anchor) tag. Effective only for paired reads, for the number of anchors queried as defined by <code>small.indel.detail.level</code> .                                                                                                                                                                   |
| Ungapped BAM flag filter   | ProperPair    | For ungapped alignments, specifies the BAM flag properties that a read must have to be included. Allowed values: A comma-separated string of one or more of these values: <ul style="list-style-type: none"> <li>• ProperPair</li> <li>• UniqueHit</li> <li>• NoOptDup</li> <li>• Primary</li> <li>• None</li> </ul> None turns off all filters. |
| Gapped BAM flag filter     | Primary       | For gapped alignments, specifies the BAM flag properties that a read must have to be included. Allowed values: A comma-separated string of one or more of these values: <ul style="list-style-type: none"> <li>• ProperPair</li> <li>• UniqueHit</li> <li>• NoOptDup</li> <li>• Primary</li> <li>• None</li> </ul> None turns off all filters.   |
| Edge length deletions      | 0             | Gap alignments that do not have this minimum length on either side of the indel will not be considered.<br>Allowed values: Integers $\geq 0$ .                                                                                                                                                                                                   |
| Edge length insertions     | 0             | Gap alignments that do not have this minimum length on either side of the indel will not be considered.<br>Allowed values: Integers $\geq 0$                                                                                                                                                                                                     |

## Heuristic Filtering

| Parameters                      | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|---------------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Perform filtering               | True          | Whether or not to perform filtering in each pileup. Allowed values: <ul style="list-style-type: none"> <li>• <b>True</b>: Perform filtering on pileups.</li> <li>• <b>False</b>: Do not perform filtering on pileups.</li> </ul> Parameters that change the makeup of pileups, such as <a href="#">Min num evid</a> are still active.                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Indel size distribution allowed | can-cluster   | Indel sizes in a pileup are allowed to have certain indel size distributions. <p>Allowed values:</p> <ul style="list-style-type: none"> <li>• <b>similar-size</b>: 75% of the reads of a pileup must have exactly the same size.</li> <li>• <b>similar-size-any-large-deletions</b>: Any pileups with at least 2 large deletion alignments, the other pileups must have similar sizes.</li> <li>• <b>can-cluster</b>: Allowed if at least one cluster of any indel size is found.</li> <li>• <b>can-cluster-any-large-deletions</b>: Any pileups with at least 2 large deletion alignments; other pileups must be able to cluster (will have indels with two more reads with larger deletions, even if they don't form good clusters).</li> <li>• <b>any</b>: Can have any size distribution.</li> </ul> |
| Remove singletons               | True          | Remove the singletons that occur when different alignment methods are combined based on identical bead ids and read sequence. <p>Allowed values:</p> <ul style="list-style-type: none"> <li>• <b>True</b>: Remove singletons.</li> <li>• <b>False</b>: Do not remove the singletons.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Alignment compatibility filter  | 1             | Alignment compatibility level. Checks color space compatibility around the gap. Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: No alignment compatibility filtering.</li> <li>• <b>1</b>: The small indel module determines whether the data contains base-space or color-space sequence.</li> <li>• <b>2</b>: Force the use of base-space sequence, if present.</li> <li>• <b>3</b>: Force the use of color-space sequence, if present.</li> </ul>                                                                                                                                                                                                                                                                                                                                  |
| Max coverage ratio              | 12            | Maximum allowed value for the ratio of number of reference alignments allowed by the <code>ungapped.bam.flag.filter</code> over the number of non-redundant indel variant reads selected with the <code>gapped.bam.flag.filter</code> . <p>Use -1 for no limit (no coverage ratio filtering).</p> <p>Allowed values: Integers.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Max nonreds 4Fit                | 2             | Maximum number of non-redundant reads where read position filtering is applied. <p>Allowed values: Integers.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

| Parameters       | Default value | Description                                                                                                                                                                                                     |
|------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Min from end pos | 9.1           | Minimum average number of base pairs from the end of the read required of the pileup, when there are at most a certain number of reads defined by <a href="#">Max nonreds 4Fit</a> .<br>Allowed values: Floats. |

### Indel Size Filtering

| Parameters          | Default value | Description                                                     |
|---------------------|---------------|-----------------------------------------------------------------|
| Min insertions size | 0             | Minimum insertion size to include.<br>Allowed values: Integers. |
| Min deletions size  | 0             | Minimum deletion size to include.<br>Allowed values: Integers.  |
| Max insertions size | 1000000000    | Maximum insertion size to include.<br>Allowed values: Integers. |
| Max deletions size  | 1000000000    | Maximum deletion size to include.<br>Allowed values: Integers.  |

### (Optional) Annotation

You can optionally annotate the mapped output of Small Indel analysis. For descriptions of the Annotation parameters, see “Annotation” on page 126.

## Enrichment

There are two categories of enrichment parameters: Main and Advanced.

### Main

| Parameter name   | Default value                          | Description                                                                                                                                                             |
|------------------|----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Report directory | <code>\${task.output.dir}</code>       | Directory where all reports will be created.                                                                                                                            |
| Output directory | <code>\${task.output.dir}</code>       | Directory where output BAMs will be created.                                                                                                                            |
| Target file      | <code>\${analysis.regions.file}</code> | File specifying target regions or regions of interest to be analyzed. It may be BED, GFF (v.3), or a list of chr:start-end elements. Multiple target files are allowed. |

## Advanced

There are two categories of Advanced parameters: general and Reports.

| Parameter name                 | Default value | Description                                                                                                                                                                                                                                                                                      |
|--------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Extend bases                   | 0             | The number of bases on either side of the target region in which alignments may be captured. The target sequence is extended by this number of bp on either side. Used by both target capture and target coverage statistics.<br>Allowed values: Integers $\geq 0$ .                             |
| Minimum mapping score          | 8             | Minimum mapping quality value (MAPQ) allowed for aligned reads. Reads below this threshold are not used.<br>Allowed values: Integers $\geq 0-80$ .                                                                                                                                               |
| Minimum target overlap         | 0.0001        | The fraction of an alignment that must be overlapped by a target in order to be classified as on target. "0.50" is interpreted as 50%, and implies that the midpoint of the read must fall within target region.<br>Allowed values: $0.0001 < X \leq 1.0$                                        |
| Minimum target overlap reverse | 0.0001        |                                                                                                                                                                                                                                                                                                  |
| <b>Reports</b>                 |               |                                                                                                                                                                                                                                                                                                  |
| Summary report                 | True          | Create or do not create the summary statistics file.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Create the summary statistics file.</li> <li>• <b>False:</b> Do not create the summary statistics file.</li> </ul>                                                 |
| Target coverage stats          |               | Output or do not output per-target coverage statistics (min, max, mean) in tabular format.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Create per-target coverage statistics.</li> <li>• <b>False:</b> Do not create per-target coverage statistics.</li> </ul>     |
| Coverage frequency             | False         | Create or do not create the per-target coverage frequency histogram.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Create the per-target coverage frequency histogram.</li> <li>• <b>False:</b> Do not create the per-target coverage frequency histogram.</li> </ul> |
| Coverage bedgraph              |               | Create a BEDGRAPH format coverage file for on-target reads.<br>Allowed values: true, false, 1, 0 <ul style="list-style-type: none"> <li>• <b>True:</b> Create a BEDGRAPH format coverage file for on-target reads.</li> <li>• <b>False:</b> Do not create a this file.</li> </ul>                |
| Genome coverage frequency      |               | Create a per-chromosome coverage frequency histogram.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Output a per-chromosome coverage frequency histogram.</li> <li>• <b>False:</b> Do not create this histogram.</li> </ul>                                           |

## SNP Finding

There are two categories of SNP Finding parameters: Main and Advanced.

### Main

| Parameter name                     | Default value | Description                         |
|------------------------------------|---------------|-------------------------------------|
| Call stringency                    | medium        | Call stringency.                    |
| Skip high coverage positions (Het) | 1             | Skip high coverage positions (Het). |
| Minimum mapping quality value      | 8             | Minimum mapping quality value.      |

### Advanced

There are six categories of Advanced parameters: general, Read filter, General position filter, Heterozygous position filter, Homozygous position filter, and Output file processing.

| Parameter name                                                       | Default value | Description                                                           |
|----------------------------------------------------------------------|---------------|-----------------------------------------------------------------------|
| Detect adjacent SNPs                                                 | 0             | Detect adjacent SNPs.                                                 |
| Polymorphism rate                                                    | 0.001         | Polymorphism rate.                                                    |
| <b>Read filters</b>                                                  |               |                                                                       |
| Include reads with unmapped mate                                     | 0             | Include reads with unmapped mate.                                     |
| Exclude reads with indels                                            | True          | Exclude reads with indels.                                            |
| Require only uniquely mapped reads                                   | 0             | Require only uniquely mapped reads.                                   |
| Ignore reads with a higher mismatch count to alignment length ratio  | 1.0           | Ignore reads with a higher mismatch count to alignment length ratio.  |
| Ignore reads with a lower alignment length to read length ratio      | 1.0           | Ignore reads with a lower alignment length to read length ratio.      |
| <b>General position filters</b>                                      |               |                                                                       |
| Require alleles to be present in both strands                        | False         | Require alleles to be present in both strands.                        |
| Minimum base quality value for a position                            | 14            | Minimum base quality value for a position.                            |
| Minimum base quality value of the non-reference allele of a position | 14            | Minimum base quality value of the non-reference allele of a position. |
| <b>Heterozygous position filters</b>                                 |               |                                                                       |
| Minimum allele ratio (Het)                                           | 0.15          | Minimum allele ratio (Het).                                           |
| Minimum coverage (Het)                                               | 2             | Minimum coverage (Het).                                               |
| Minimum unique start position (Het)                                  | 2             | Minimum unique start position (Het).                                  |
| Minimum non-reference color QV (Het)                                 | 7             | Minimum non-reference color QV (Het).                                 |
| Minimum non-reference base QV (Het)                                  | 14            | Minimum non-reference base QV (Het).                                  |
| Minimum ratio of valid reads (Het)                                   | 0.65          | Minimum ratio of valid reads (Het).                                   |
| Minimum valid tricolor counts (Het)                                  | 2             | Minimum valid tricolor counts (Het).                                  |
| <b>Homozygous position filters</b>                                   |               |                                                                       |
| Minimum coverage (Hom)                                               | 1             | Minimum coverage (Hom).                                               |
| Minimum count of the non-reference allele (Hom)                      | 2             | Minimum count of the non-reference allele (Hom).                      |

| Parameter name                                                             | Default value | Description                                                                                                                                                                                                |
|----------------------------------------------------------------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Minimum average non-reference base QV (hom)                                | 14            | Minimum average non-reference base QV (Hom).                                                                                                                                                               |
| Minimum average non-reference color QV (hom)                               | 7             | Minimum average non-reference color QV (Hom).                                                                                                                                                              |
| Minimum unique start position of the non-reference allele (Hom)            | 2             | Minimum unique start position of the non-reference allele (Hom).                                                                                                                                           |
| <b>Low frequency variant filters</b>                                       |               |                                                                                                                                                                                                            |
| Minimum average color qv of the less common allele                         | 0             | Minimum average color qv of the less common allele.                                                                                                                                                        |
| Minimum unique start position of the less common allele                    | 0             | Minimum unique start position of the less common allele.                                                                                                                                                   |
| The less common allele on both strands                                     | 0             | The less common allele on both strands.                                                                                                                                                                    |
| Maximum difference of color qvs of the most common and less common alleles | 0             | Maximum difference of color qvs of the most common and less common alleles.                                                                                                                                |
| <b>Output file processing</b>                                              |               |                                                                                                                                                                                                            |
| Output fasta file                                                          | True          | Output or do not output the FASTA file. <ul style="list-style-type: none"> <li>• <b>True:</b> Output the FASTA file.</li> <li>• <b>False:</b> Do not output the FASTA file.</li> </ul>                     |
| Output consensus file                                                      | True          | Output or do not output the consensus file. <ul style="list-style-type: none"> <li>• <b>True:</b> Output the consensus file.</li> <li>• <b>False:</b> Do not output the consensus file.</li> </ul>         |
| Compress the consensus file                                                | False         | Compress or do not compress the consensus file. <ul style="list-style-type: none"> <li>• <b>True:</b> Compress the consensus file.</li> <li>• <b>False:</b> Do not compress the consensus file.</li> </ul> |

**(Optional)  
Annotation**

You can optionally annotate the mapped output of Targeted Resequencing analysis. For descriptions of the Annotation parameters, see the following section [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on page 317.

## Perform Targeted Resequencing analysis

**(Optional) Import  
data**

You can optionally import data to be analyzed. For instructions on how to import data, see “Import Data” on page 81.

**Log in to  
LifeScope™**

1. Navigate to LifeScope™ Software at `http://<IP address>:<port number>/LifeScope.html` where *IP address* is the address of the system or head node and *port number* is the number of the port used by the server.
2. In the Login screen, enter your username and password, then either click **Login** or press **Enter** to open the LifeScope™ home view (shown on page 75).

## Create a project

1. In the home view, click **Create a New Project**.
2. If you create a project:
  - a. Type a name and description in the Enter Project Name view.  
**Note:** The name cannot have spaces or special characters.
  - b. Click **Create New Project**.
  - c. In the Projects lists, select the new project.
3. In the Task Wizards section (shown on page 75), click **Add Data to Project** to choose a data type.

## Add data to the project

**Note:** Add data from the read repository to a project by choosing a data type and finding to the new path.

1. In the Add Data to Project window, select **Raw unmapped (XSQ) data**, then click **Next** to find data.
2. In the Read Repository Filter table, select the reads you want to map and analyze. If you want to group data, click **Next**. If you do not want to group data, skip [step 3](#) and proceed to [step 4](#).
3. (*Optional*) In the Read-sets in Project table, click the checkbox of the data files you want to group, then click **Add Group to Project**. The files appear in the Groups in Project table.  
To rename a group, click the checkbox of the group in the Groups in Project table, then click **Edit**. In the Edit Group window, enter a new name.  
To remove a data file from a group, click the checkbox of the data file, then click **Delete**.
4. Click **Add Analysis** to proceed, or
  - Click **Cancel** to refrain from adding data and close the Add Data to Project window, or
  - Click **Finish** to add the data and close the Add Data to Project window.

## Create an analysis

1. In the Choose Data view of the Create Analysis window, select data files from the Available Data in Project table, then click **Next** to choose an analysis.
2. In the Choose Analysis view, enter a name for the analysis. You can optionally describe your project.  
If you are re-using an Old Analysis, select **Reuse Old Analysis** and select the name of the analysis you want to use.  
**Note:** The name cannot have spaces or special characters.
3. Select **Targeted Resequencing**, then click **Next**.

4. In the Data To Be Analyzed table, click **Select the reference for the reads** to open the repository file browser.

5. In the Browse for Reference File window, navigate to the location of the reference genome of your sample. Open the folder with your .fasta file, for example:  
     data ▶ referenceData ▶ lifetech ▶ hg18 ▶ reference ▶ human\_hg18.fasta  
 Select the file, then click **OK**.  
 The file name appears in the Reference column.  
**Note:** To change the reference file, click **Select the reference for the reads**, then select another reference file.
6. After you have created the analysis:
  - Click **Edit** to proceed, or
  - Click **Cancel** to refrain from choosing references and close the Create Analysis window, or
  - Click **Finish** to complete analysis creation and close the window.

## Edit the analysis

1. In the Edit Analysis window, accept the default settings for mapping and pre-processing data in the Secondary Analysis section.  
 If you want to exclude BAMStats, click **Customize**. In the Customize Mapping window, uncheck BamStats, then click **OK**.  
 If you do not want to pre-process data, uncheck the box.
2. In the Tertiary Analysis section, accept the included Small Indel SNP Finding, CNV, and Small Indel modules, and Annotation settings and click **Next** to set module parameters. To include all modules, click the **All>>** button.  
 To exclude a module, select it in the Include column, then click the < button. To exclude all modules, click the **All<<** button.  
 To skip setting module parameters and review the analysis, click **Review**.

## Set module parameters

This section describes the procedures for setting general parameters and parameters for the mapping, SNP Finding, and Small Indel modules. For descriptions of module parameters, see [“Targeted resequencing parameters” on page 116](#).

You can restore the default settings of parameters by clicking the **Reset to Defaults** button.

To view descriptions of parameters, place your mouse cursor over a  button.

## Set general parameters

1. Enter an analysis assembly name.
2. Set the annotation.dbsnp, analysis.regions.file, and annotation.gtf.file parameters.  
 Click the  buttons to open the File Chooser, navigate to reference files, and choose files.  
**Note:** Verify that the file path to the annotation.gtf file is correct. An incorrect file path will result in a failed analysis.
3. Set analysis.space to your preference of whether analysis should be done in base space or in color space. This setting should be the same for all modules in your run. For more information, see [“Choose analysis space” on page 98](#).

4. Always leave the default analysis.platform setting, which is “SOLID”.
5. If you want to accept the default parameters for all modules, click **Review**. If you want to edit module parameters, click **Next**.

#### Set Mapping parameters

1. Either Fragment Mapping or Paired Mapping parameters are displayed, depending on the input data for your analysis. Accept the default settings or click the tabs to edit the settings.
2. Click **Next** to edit Mapping parameters.

#### Set Small Indel parameters

Categories of Small Indel parameters include Main, Advanced, and (if you selected Annotation for Small Indel output) Annotation. Accept the default settings or edit the settings.

#### Set Enrichment parameters

1. There are two categories of enrichment parameters: Main and Advanced. Accept the default settings or edit the settings.
2. Click **Next** to edit Enrichment parameters.

#### Set SNP Finding parameters

1. Categories of SNP Finding parameters include Main, Advanced, and (if you selected Annotation for SNP Finding output) Annotation. Accept the default settings or edit the settings.
2. Click **Next** to edit Small Indel parameters.

If you are ready to start the analysis, click **Review** to proceed.

If you want to erase your edits, click **Cancel** to close the Edit Analysis window.

If you want to start the analysis later, click **Finish** to save your edits and close the Edit Analysis window.

### Review and run the analysis

**Note:** For a description of Targeted Resequencing parameters, see [“Targeted resequencing parameters” on page 116](#).

The Review Analysis view in the Run Analysis window includes two tabs: Parameters and Data. In the Parameters tab, click the  , next to parameter categories to show or hide the parameters.

1. Review the parameters. To edit the parameters, click **Edit**.
2. To review the data that will be analyzed, click the **Data** tab.
3. If you are ready to run the analysis, click **Start Analysis**.

The Run Analysis window closes.

## Checking Analysis Status

- In the Projects list, click the project to check the status of the analysis run. The Progress columns show the percentage of completion for the secondary and tertiary analyses.

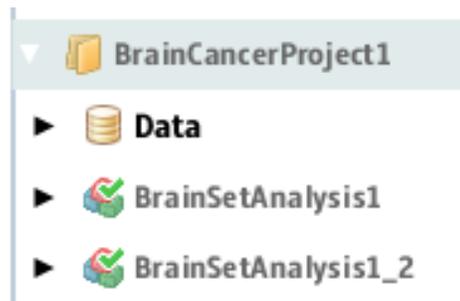
ProstateCancerProje... • ProstateCancerAnaly...

Overview Status Parameters

Analysis Runs

| Name                         | XSQ ID                       | Analysis                | Secondary Analysis        | Secondary Progress                                                                     | Tertiary Analysis                | Tertiary Progress |
|------------------------------|------------------------------|-------------------------|---------------------------|----------------------------------------------------------------------------------------|----------------------------------|-------------------|
| solid0054_20110102_PE_LFD... | solid0054_20110102_PE_LFD... | ProstateCancerAnalysis1 | [SAET, Mapping, BamStats] |  33% | [Enrichment, diBayes, SmallIn... | 0%                |

When analysis is complete, a green check mark appears on the analysis name in the Projects organizer (shown on page 75).



## View analysis results

To view analysis results:

- In the Projects organizer, click the project to show its contents.
- To view analysis results, click either the:
  - Results icon  for an overview of the analysis, then Click **View Results** in the **Secondary Progress** and **Tertiary Progress** columns to open the View Results window.
  - Completed analysis icon  for results of a specific analysis. Click the Status tab, then **View Results** to open the window.
- In the View Results window, click each analysis module for analysis details.
- Click each analysis module to see statistics, additional files, and logs.

The following table shows the results that are available for each analysis module.

| Analysis Module           | Details | Statistics | Additional Files | Logs |
|---------------------------|---------|------------|------------------|------|
| SmallIndel.Annotation     | No      | No         | Yes              | Yes  |
| SNP.Finding.Annotation    | No      | No         | Yes              | Yes  |
| SNP.Finding.targeted.frag | Yes     | Yes        | Yes              | Yes  |
| Enrichment                | No      | Yes        | Yes              | Yes  |
| SAET                      | No      | No         | No               | Yes  |
| BAMStats                  | Yes     | No         | Yes              | Yes  |

| Analysis Module | Details | Statistics | Additional Files | Logs |
|-----------------|---------|------------|------------------|------|
| Mapping         | No      | No         | Yes              | Yes  |
| SmallIndel      | Yes     | Yes        | Yes              | Yes  |

### View results in a genome browser

Output files generated by Targeted Resequencing runs are compatible with third-party browser such as the Integrative Genomics Viewer (IGV) available from the Broad Institute and the UCSC Genome Browser. See [“Pairing information in a BAM file” on page 348](#) for information on using LifeScope™ Software output files with genome browsers.

For more information on viewing analysis results, see [Chapter 9, “View Analysis Results” on page 103](#).

## Targeted resequencing output files

### SmallIndel.Annotation

#### Details

There are no details for the SmallIndel.Annotation analysis module.

#### Statistics

There are no statistics for the SmallIndel.Annotation analysis module.

#### Additional files

The following table is an example of additional files for the SmallIndel.Annotation analysis module.

| Directory                                                                                            | Files      | File Type |
|------------------------------------------------------------------------------------------------------|------------|-----------|
| SmallIndel.Annotation/<br>solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary | README.txt | .txt      |

#### Logs

The following table is an example of logs for the SmallIndel.Annotation analysis module.

| Directory             | Files                                                                                                                           | File Type |
|-----------------------|---------------------------------------------------------------------------------------------------------------------------------|-----------|
| SmallIndel.Annotation | tertiary-solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary-SmallIndel.Annotation.20110421220403452.log | .log      |

### SNP.Finding.Annotation

#### Details

There are no details for the SNP.Finding.Annotation analysis module.

## Statistics

There are no statistics for the SNP.Finding.Annotation analysis module.

## Additional files

The following table is an example of additional files for the SNP.Finding.Annotation analysis module.

| Directory                                                                                         | Files      | File Type |
|---------------------------------------------------------------------------------------------------|------------|-----------|
| SNP.Finding.Annotation/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary | README.txt | .txt      |

## Logs

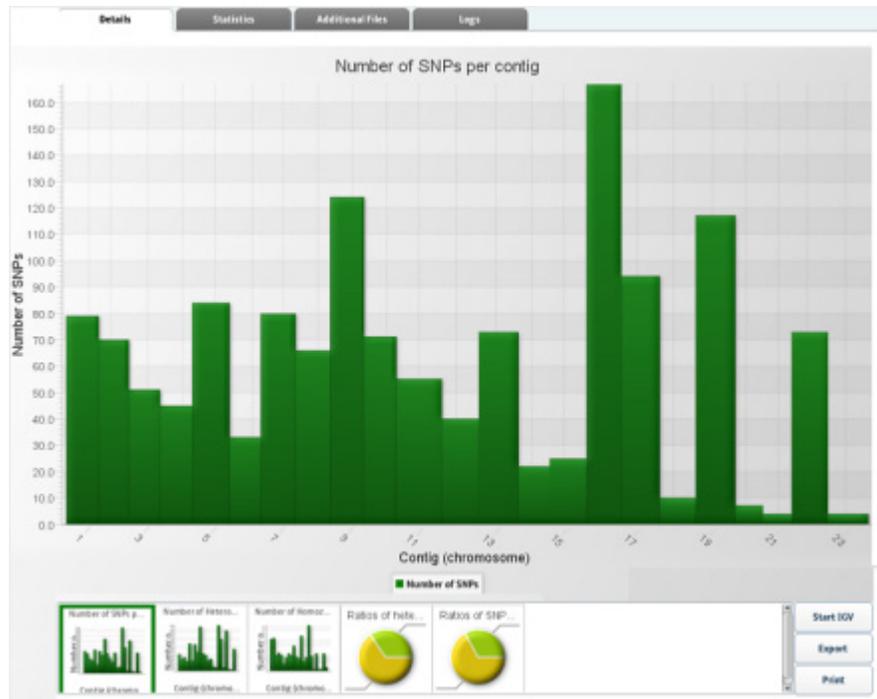
The following table is an example of SmallIndel.Annotation logs.

| Directory              | Files                                                                                                                            | File Type |
|------------------------|----------------------------------------------------------------------------------------------------------------------------------|-----------|
| SNP.Finding.Annotation | tertiary-solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary-SNP.Finding.Annotation.20110421220103477.log | .log      |

## SNP.Finding.targeted.frag

### Details

The following illustration is an example of details for the SNP.Finding.targeted.frag analysis module.



## Statistics

The following illustration is an example of statistics for the SNP.Finding.targeted.frag analysis module.

```

*****
LIFESCOPE REPORT
*****

Input File:      /panasas/lifescopetesttemp1/results/projects/corona/Da
frag/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_Default
Date:   Apr 21, 2011 10:02:31 PM
Annotation file, dbSNP: /panasas/lifescopetesttemp1/results/projects/corona/Da
Annotation file, Genes and Exons, GTF:/panasas/lifescopetesttemp1/results/projects/corona/Da

*****
Statistics Overview
*****

-- Basic Statistics -----
Number of variants                               1394
Number of heterozygous variants                  913
Number of homozygous variants                   481

-- Variant-Specific Statistics (SNP) -----
Number of transition SNPs                       935
Number of transition heterozygous SNPs          603
Number of transition homozygous SNPs           332
Number of transversion SNPs                   459
Number of transversion heterozygous SNPs       310
Number of transversion homozygous SNPs        149
Transition:Transversion ratio                   2.037 : 1.000

-- dbSNP Annotation Statistics -----
Number of          SNPs in dbSNP                1210
Number of heterozygous SNPs in dbSNP           731
Number of homozygous SNPs in dbSNP            479
dbSNP concordance                               86.80%
dbSNP heterozygous concordance                 80.07%
dbSNP homozygous concordance                   99.58%

-- Gene and Exon Statistics -----
Number (percent) of variants overlapping exon   453 ( 32.50%)
Number (percent) of heterozygous variants overlapping exon 288 ( 31.54%)

```

### Additional files

The following table is an example of additional files for the SNP.Finding.targeted.frag analysis module.

| Directory                                                                                                     | Files                               | File Type |
|---------------------------------------------------------------------------------------------------------------|-------------------------------------|-----------|
| SNP.Finding.targeted.frag/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary/contig2  | Analysis2_SNP.gff3                  | .gff3     |
| SNP.Finding.targeted.frag/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary/contig19 | Analysis2_Consensus_Calls.txt       | .txt      |
| SNP.Finding.targeted.frag/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary/contig12 | Analysis2_Consensus_Basespace.fasta | .fasta    |

### Logs

The following table is an example of logs for the SmallIndel.Annotation analysis module.

| Directory                 | Files                                                                                                                                               | File Type |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| SNP.Finding.targeted.frag | tertiary-solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary-SNP.Finding.targeted.frag.3.persistHeader.20110421214007357.log | .log      |

## Enrichment

### Details

There are no details for the enrichment analysis module.

### Statistics

The following illustration is an example of statistics for the enrichment analysis module.

```

Run      Reads On      Percent On      Reads Off      Percent Off      Enrichment Fold
/results/Analysis2.ontarget.bam      71681629      89.2239%      8657401 10.7761%  1347.43

Reads In Targets:      71681629      89.2239%
Reads Off Targets: 8657401      10.7761%
Ratio of Percent on/off Target: 8.27981
Total Target BP: 2039803
Total Genome Size: 3080436051
Ratio of target to genome: 0.00066218
Enrichment fold relative to target size: 1347.43

# Targets Not Covered  Target Bases Not Covered  Percent of Target Bases Not Covered
Percent of Target Covered >= 1X  Percent of Target Covered >= 5X  Percent of Target Covered >= 10X
Percent of Target Covered >= 20X  Average Depth of Target Coverage
1      13067  0.64%  99.36%  98.94%  98.62%  98.22%  2451.59

Number of target regions with no coverage: 1
Percent of target bp not covered: 0.64% (13067 bp)
Percent of target bp covered at >= 1X: 99.36%
Percent of target bp covered at >= 5X: 98.94%
Percent of target bp covered at >= 10X: 98.62%
Percent of target bp covered at >= 20X: 98.22%
Maximum Depth of Coverage within targets: 51306
Average Depth of Coverage within targets: 2451.59

```

### Additional files

The following table is an example of additional files for the enrichment analysis module.

| Directory                                                                              | Files                                                                                            | File Type |
|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-----------|
| enrichment/solid0054_20110102_PE_ILFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary | Analysis2.ontarget.bam.bai                                                                       | .bai      |
|                                                                                        | Analysis2_RainDance4K_targetHG18_withChr.validated.bed                                           | .bed      |
|                                                                                        | solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary_target_statistics.txt | .txt      |
|                                                                                        | Analysis2.ontarget.bam                                                                           | .bam      |

### Logs

The following table is an example of logs for the enrichment analysis module.

| Directory  | Files                                                                                                                     | File Type |
|------------|---------------------------------------------------------------------------------------------------------------------------|-----------|
| enrichment | tertiary-solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary-enrichment.main.20110421212207477.log | .log      |

## SAET

### Details

There are no details for the SAET analysis module.

### Statistics

There are no statistics for the SAET analysis module.

### Additional files

There are no additional files for the SAET analysis module.

### Logs

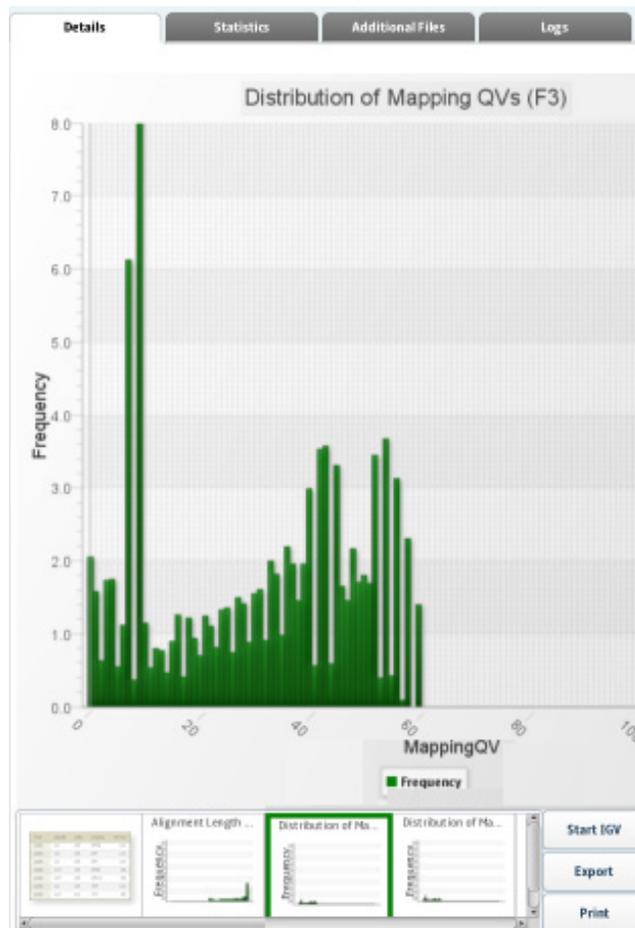
The following table is an example of logs for the SAET analysis module.

| Directory    | Files                                                        | File Type |
|--------------|--------------------------------------------------------------|-----------|
| Mapping.SAET | secondary-human_hg18-Mapping.SAET.main.20110421154741315.log | .log      |

## BAMStats

### Details

The following illustration is an example of BAMStats details.



### Statistics

There are no statistics for the BAMStats analysis module.

### Additional files

The following table is an example of BAMStats additional files.

| Directory                                                                                                                                                                      | Files                                                                                            | File Type |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-----------|
| Mapping.BamStats/<br>solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.color<br>Remake_DefaultLibrary/<br>solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.color<br>Remake-1/Misc | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.bam_chrX.NEG.wig           | .wig      |
|                                                                                                                                                                                | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.BaseQV.by.Position.csv     | .csv      |
|                                                                                                                                                                                | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.Unique.Start.Positions.txt | .sql      |
|                                                                                                                                                                                | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.bam_chrX.NEG.wig           | .wig      |

### Logs

The following table is an example of logs for the BAMStats analysis module.

| Directory        | Files                                                                      | File Type |
|------------------|----------------------------------------------------------------------------|-----------|
| Mapping.BAMStats | secondary-human_hg18-Mapping.BamStats.10.run.temp.10.20110421211327339.log | .log      |

## Mapping

### Details

There are no details for the Mapping analysis module.

### Statistics

There are no statistics for the Mapping analysis module.

### Additional files

The following table is an example of Mapping additional files.

| Directory                                                                                       | Files                                                                         | File Type |
|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-----------|
| fragment.mapping/solid0054_20110102_PE_LFD_RD_SetA_1_<br>HuRef100_F3.colorRemake_DefaultLibrary | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.bam     | .bam      |
|                                                                                                 | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.bam.bai | .bai      |

### Logs

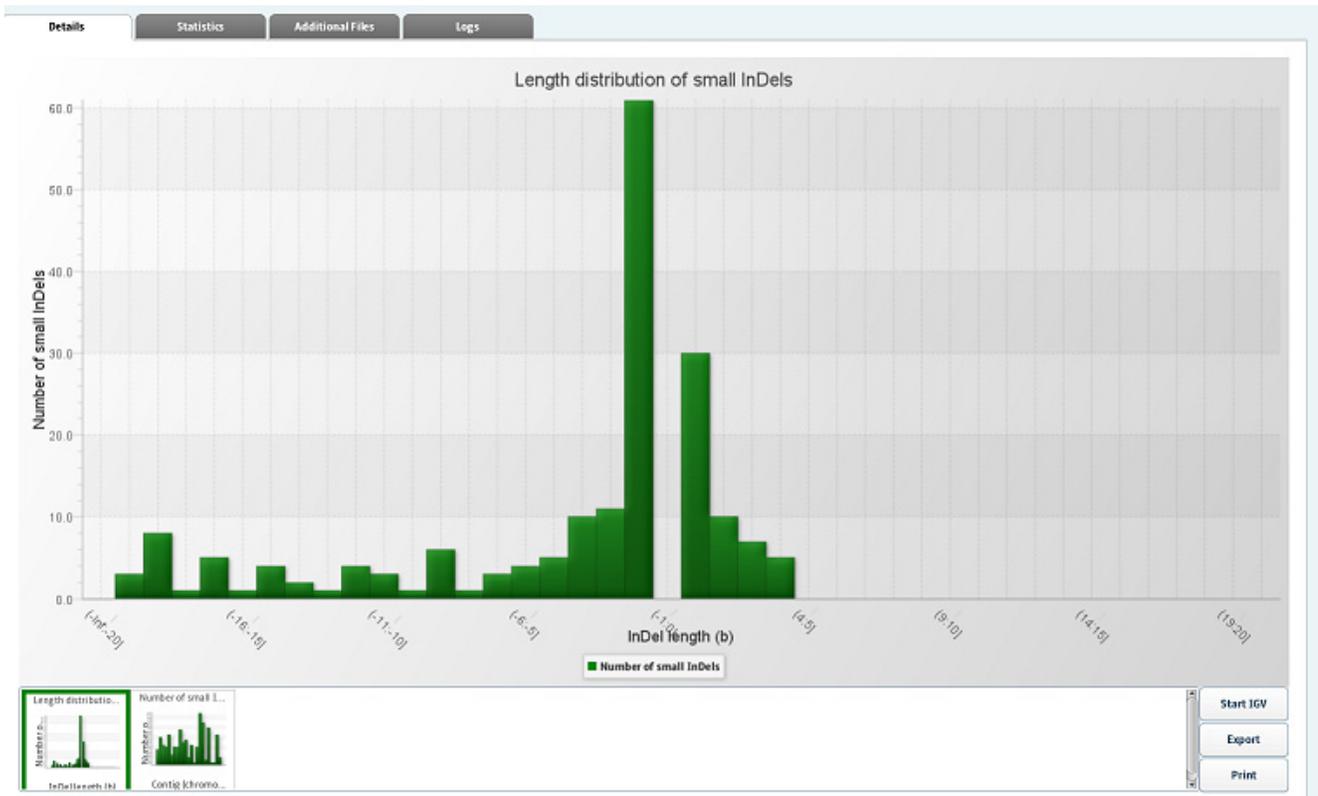
The following table is an example of logs for the Mapping analysis module.

| Directory        | Files                                                             | File Type |
|------------------|-------------------------------------------------------------------|-----------|
| fragment.mapping | secondary-human_hg18-fragment.mapping.3.run.20110421182159033.log | .log      |

Small Indel

Details

The following illustration is an example of Small Indel details.



## Statistics

The following illustration is an example of Small Indel statistics.

```

*****
LIFESCOPE REPORT
*****

Input File:      /panasas/lifescopetesttempl/results/projects/co
indel/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake
Date:   Apr 21, 2011 10:05:52 PM
Annotation file, dbSNP: /panasas/lifescopetesttempl/analysis/referenceDa
Annotation file, Genes and Exons, GTF:/panasas/lifescopetesttempl/analys
*****
Statistics Overview
*****

-- Basic Statistics -----
Number of variants                186
Number of heterozygous variants   127
Number of homozygous variants     59

-- Variant-Specific Statistics (InDel) -----
Distribution of InDel length

Length(bases)      Number
(-Inf:-20]         0
(-20:-19]          3
(-19:-18]          8
(-18:-17]          1
(-17:-16]          5
(-16:-15]          1
(-15:-14]          4
(-14:-13]          2
(-13:-12]          1
(-12:-11]          4
(-11:-10]          3
(-10:-9]           1
(-9:-8]            6
(-8:-7]            1
(-7:-6]            3
(-6:-5]            4
(-5:-4]            5
(-4:-3]            10
(-3:-2]            11
(-2:-1]            61
    
```

### Additional files

The following table is an example of Small Indel additional files.

| Directory                                                                                      | Files                        | File Type |
|------------------------------------------------------------------------------------------------|------------------------------|-----------|
| small.indel/<br>solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.color<br>Remake_DefaultLibrary | dbSnpDeployed_genes.bed      | .bed      |
|                                                                                                | dbSnpDeployed_genes.tab      | .tab      |
|                                                                                                | Analysis2.sql                | .sql      |
|                                                                                                | dbSnpDeployed_annotated.gff3 | .gff3     |
|                                                                                                | Analysis2.gff3               |           |
|                                                                                                | Analysis2.txt                | .txt      |
|                                                                                                | Analysis2.pas.sum            | .sum      |
|                                                                                                | Analysis2.align              | .align    |
|                                                                                                | Analysis2.ungapped           | .ungapped |
|                                                                                                | Analysis2.pas                | .pas      |

### Logs

The following table is an example of Small Indel logs.

| Directory   | Files                                                                                                                 | File Type |
|-------------|-----------------------------------------------------------------------------------------------------------------------|-----------|
| small.indel | tertiary-solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary-small.indel.20110421213917621.log | .log      |
|             | small.indel-solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary.20110421213922698.log          |           |

| Analysis Module           | Additional Files             | File Type                                                                   |
|---------------------------|------------------------------|-----------------------------------------------------------------------------|
| Small Indel Annotation    | dbSnpDeployed_genes.bed      | README.txt, .log                                                            |
| SNP.Finding Annotation    | dbSnpDeployed_genes.tab      | README.txt, .log                                                            |
| SNP.Finding Targeted Frag | Analysis2.sql                | Charts, .stats, .gff3, .txt, fasta, .log                                    |
| Enrichment                | dbSnpDeployed_annotated.gff3 | .stats, .bai, .bam, .bed, .txt, .log                                        |
| SAET                      | Analysis2.gff3               | .log                                                                        |
| BAMStats                  | Analysis2.txt                | Charts, .csv, .txt, .wig, .log                                              |
| Mapping                   | Analysis2.pas.sum            | .bai, .bam, .log                                                            |
| Small Indel               | Analysis2.align              | Charts, .stats, .align, .bed, .gff3, .pas, .sql, .sum, .tab, ungapped, .log |
|                           | Analysis2.ungapped           |                                                                             |
|                           | Analysis2.pas                |                                                                             |

## 11

# Perform Low Frequency Variant Analyses

This chapter covers:

|                                                                  |     |
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| ■ Introduction to Low Frequency Variant (LFV) analysis . . . . . | 141 |
| ■ Low Frequency Variant library types . . . . .                  | 142 |
| ■ Low Frequency Variant input files . . . . .                    | 143 |
| ■ Low Frequency Variant analysis modules . . . . .               | 143 |
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| ■ Perform Low Frequency Variant analysis . . . . .               | 150 |
| ■ View analysis results . . . . .                                | 154 |
| ■ Low Frequency Variant output files . . . . .                   | 155 |

## Introduction to Low Frequency Variant (LFV) analysis

The Low Frequency Variant workflows use the SNP Finding algorithm (and highly optimized parameters) to reveal SNPs present in a targeted resequencing experiment. The detection range can range from 1%-5% based on experimental conditions, acceptable sensitivity and specificity, and sequencing coverage. This workflow is ideal for detecting low frequency variants in cancer-related research projects.

We performed low frequency variant validation with fragment libraries with read length of 50, and we observed high sensitivity and specificity, with both array enrichment and PCR-based enrichment. The experimental design we recommend for low frequency variant detection is fragment sequencing with a read length of 50.

The low frequency variant workflows use unique defaults for SNPs filters and also uses these new SNPs filters:

- The color QV of the less common allele
- The color QV difference between the most common allele and the less common allele
- The unique start positions of the less common allele
- The requirement that the less common allele be present on both strands

The workflow for analysis of target-enriched data is nearly identical to a standard resequencing workflow. The low frequency variant application consists of many of the same modules as standard resequencing analysis. However, several modules have specific of target-enriched data.

The low frequency variant workflow uses the enrichment module to produce a filtered BAM file for variant calling. The variant calling module diBayes is run on the filtered data sets instead of on the entire read population. This technique limits SNP calls to the target region. SNPs module parameters are optimized for low frequency variant detection.

The input list of regions of interest (ROI) is provided by the user. Supported ROI input files include third-party files of baits, probes, or tiles. The input ROI files can be in BED format or in a text file as a list of “chr:start-end” values. The mapping module sorts the input ROI file by chromosome and start position, and makes the resulting file available as output.

Low frequency variant output files are compatible with third-party genome browsers, including the IGV browser. Low frequency variant workflows optionally produce enrichment statistics, which provide a means to assess enrichment platform performance.

### Low frequency variant SNPs parameters

The low frequency variant workflows use these SNPs parameters (which are not used in other SNPs applications):

- `dibayes.het.lca.both.strands`
- `dibayes.het.min.lca.start.pos`
- `dibayes.het.min.lca.color.qv`
- `dibayes.het.max.diff.color.qv`

### Enrichment

Low Frequency Variant analyses use the enrichment module to restrict analysis to your specific regions of interest. For information on the enrichment module, see the following sections in [Chapter 10, Perform Targeted Resequencing and Enrichment Analyses](#):

- [“Enrichment statistics” on page 112](#)
- [“Enrichment statistics input files” on page 113](#)
- [“Enrichment” on page 115](#)
- [“Set Enrichment parameters” on page 129](#)

## Low Frequency Variant library types

The Low Frequency Variant analyses support data from these library types:

- Fragment
- Paired-End
- Libraries generated with up to 96 barcodes

Individual sequencing and enrichment reports are provided for each barcoded library.

### Enrichment statistics

Low Frequency Variant analyses support optional enrichment statistics, which provide a means to assess enrichment platform performance by:

- Looking at variations in coverage, both across all targets and on a per-target basis.
- Addressing the uniformity and completeness of coverage within the target region.

- Calculating the degree of enrichment.

See [Chapter 10, “Perform Targeted Resequencing and Enrichment Analyses”](#) on page 111 for more information on enrichment.

## Low Frequency Variant input files

| Input file                   | File extensions | Analysis modules                          |
|------------------------------|-----------------|-------------------------------------------|
| eXtensible SeQUENCE (XSQ)    | .xsq            | Mapping, Pairing                          |
| (Optional) Color space FASTA | .csfasta        | Mapping, Pairing                          |
| (Optional) Quality Value     | qual, qv        | Mapping, Pairing, SNP Finding, Enrichment |
| (Optional) FAST-All (FASTA)  | .fa, .fasta     | Mapping, Pairing                          |
| Regions of interest file     | .bed            | Enrichment                                |
| Binary Alignment Map (BAM)   | .bam            | SNP Finding                               |

## Low Frequency Variant analysis modules

Low Frequency Variant analysis includes the following modules:

- Mapping
- (Optional) Pairing
- Enrichment
- SNP Finding

### Mapping

The map data resequencing workflow uses eXtensible SeQUENCE (.xsq) files to create a .bam file.

The Low Frequency Variant mapping module uses eXtensible SeQUENCE (.xsq) files to create a \*.bam file.

### Enrichment

Low Frequency Variant enrichment also requires a regions of interest (ROI) file as input. The ROI file can be in BED, text, or tiles format.

Enrichment statistics provide a means to assess enrichment platform performance by looking at variations in coverage, both across all targets and on a per-target basis.

### SNP Finding

The SNP Finding package is the module used to call Single Nucleotide Polymorphisms (SNPs) from mapped and processed 5500 or SOLiD® System reads. The module takes the reads, the quality values, the reference sequence, and error information on each SOLiD® System slide as its input, and calls SNPs.

The module creates these results files:

- A list of SNPs.
- A quartile file with coverage as well as color and base quality value distribution.

- (Optional) A consensus \*.fasta file with the same number of bases as the reference sequence.
- (Optional) A consensus calls file with a list of all covered positions.
- (Optional) A collection of annotated files.

## Low Frequency Variant parameters

Parameters for Low Frequency Variant analysis include:

- **General parameters**
- **Secondary analysis:** Fragment Mapping (including optional BAMStats)
- **Tertiary analysis:** Enrichment and SNP Finding.

### General

General parameters include:

| Parameter              | Default value | Description                                                                                                                                   |
|------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| analysis.assembly.name | —             | The name of the genome assembly used in current analysis. Examples: hg18, hg19.                                                               |
| annotation.dbsnp.file  | —             | The path to the file used to annotate SNPs and small InDels. LifeTech-provided files are dbSNP_b130.tab(hg18) and 00-All.vcf(hg19).           |
| analysis.regions.file  | —             | The path to the file containing genomic regions used in targeted resequencing selection, such as a .bed format file containing exome targets. |
| annotation.gtf.file    | —             | The file containing gene and exon annotations corresponding to the genome assembly used in the analysis.                                      |
| analysis.space         | Color         | Low Frequency Variant analyses only support color space.                                                                                      |
| analysis.platform      | SOLID         | Always specify "SOLID". Other values are reserved for future use.                                                                             |

Use the  button to open the File Chooser and search for input files.

### Fragment Mapping

The fragment mapping parameters are displayed when your analysis contains fragment data. The parameters are listed in the following table.

| Parameter                 | Default value | Description                                                                                                                                                                                                                       |
|---------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mapping QV threshold      | 0             | Provide control the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer.<br>Allowed values: Integers 0–1,000. |
| Create unmapped BAM files | False         | Create BAM files containing unmapped reads. Values:<br>True: Create.<br>False: Do not create.                                                                                                                                     |

| Parameter                        | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|----------------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reference weight                 | 8             | Used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. Adds weight (in terms of Phred score) to the signals that are compatible with reference. Color combinations that result in a variant are considered compatible with reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation.<br><br>Allowed values: Integers 0–100. |
| Second map gapped algorithm type | GLOBAL        | Do indel finding, and control the behavior of indel finding.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• NONE: Turn off indel finding.</li> <li>• GLOBAL: Mapping reports global alignment up to one indel.</li> <li>• LOCAL: Mapping reports local alignment up to one indel.</li> </ul>                                                                                                                                             |
| Base quality filter threshold    | 0             | Replace resulting base-calls with a quality value less than the specified value.                                                                                                                                                                                                                                                                                                                                                                          |
| Map in base space                | False         | Always set to False. Low Frequency Variant workflows do not support mapping in base space. Values: <ul style="list-style-type: none"> <li>• True: Map in base space. Not supported for LFV workflows.</li> <li>• False: Map in color space.</li> </ul>                                                                                                                                                                                                    |
| Add color sequence               | True          | Add color sequence to BAM records: Values: <ul style="list-style-type: none"> <li>• True: Add color.</li> <li>• False: Do not add color.</li> </ul>                                                                                                                                                                                                                                                                                                       |
| BAM soft clip                    | False         | Modify an unaligned portion of a read that needs to be presented in the BAM record. Values: <ul style="list-style-type: none"> <li>• True: Soft clip.</li> <li>• False: Do not soft clip.</li> </ul>                                                                                                                                                                                                                                                      |

## BAMStats

| Parameter                                                                | Default value               | Description                                                                                                                                                                                                                                            |
|--------------------------------------------------------------------------|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Whether to combine data from both the strands for coverage in WIG format | 1                           | Combine or do not combine data from both strands for coverage in WIG format.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: Do not generate wig files.</li> <li>• <b>1</b>: Generate the combined coverage file.</li> </ul> |
| Input directory for BAMStats                                             | \${analysis.output.dir}/bam | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample.                                                                                                                                   |
| Bin size for coverage in WIG file format                                 | 100                         | The bin size for coverage in WIG file format.<br><br>Allowed values: Integers 1-100,000.                                                                                                                                                               |

|                                                         |                                  |                                                                                                                                                                                                                                                                                               |
|---------------------------------------------------------|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Maximum insert size                                     | 100,000                          | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br>Allowed values: Integers 0–100,000.                                             |
| Insert bin size                                         | 100                              | Bin size for insert range distribution.<br>Allowed values: Integers 1–100,000                                                                                                                                                                                                                 |
| Output directory                                        | <code>\${task.output.dir}</code> | The path to the output directory where BAMStats will write its chart (.cht) files.                                                                                                                                                                                                            |
| Primary alignments only for coverage in WIG file format | 1                                | Use only primary alignments for coverage in WIG file format.<br>Allowed values: <ul style="list-style-type: none"> <li>• 0: Do not restrict coverage in WIG file format to only primary alignments.</li> <li>• 1: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Maximum coverage                                        | 500,000                          | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br>Allowed values: Integers 0–500,000.                                                                            |

## Enrichment

| Parameter name            | Default value                          | Description                                                                                                                                                                                                                                                                                      |
|---------------------------|----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genome coverage frequency | False                                  | Create a per-chromosome coverage frequency histogram. Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Output a per-chromosome coverage frequency histogram.</li> <li>• <b>False:</b> Do not create this histogram.</li> </ul>                                              |
| Target file               | <code>\${analysis.regions.file}</code> | File specifying target regions or regions of interest to be analyzed. It may be BED, GFF (v.3), or a list of chr:start-end elements. Multiple target files are allowed.                                                                                                                          |
| Coverage frequency        | False                                  | Create or do not create the per-target coverage frequency histogram.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Create the per-target coverage frequency histogram.</li> <li>• <b>False:</b> Do not create the per-target coverage frequency histogram.</li> </ul> |
| Output directory          | <code>\${task.output.dir}</code>       | Directory where output BAMs are created.                                                                                                                                                                                                                                                         |

| Parameter name                 | Default value                    | Description                                                                                                                                                                                                                                                                                     |
|--------------------------------|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Coverage bedgraph              | False                            | Create a BEDGRAPH format coverage file for on-target reads.<br>Allowed values: true, false, 1, 0<br><ul style="list-style-type: none"> <li>• <b>True:</b> Create a BEDGRAPH format coverage file for on-target reads.</li> <li>• <b>False:</b> Do not create a this file.</li> </ul>            |
| Report directory               | <code>\${task.output.dir}</code> | Directory where all reports are created.                                                                                                                                                                                                                                                        |
| Minimum mapping score          | 8                                | Minimum mapping quality value (MAPQ) allowed for aligned reads. Reads below this threshold are not used.<br>Allowed values: Integers $\geq 0-80$ .                                                                                                                                              |
| Minimum target overlap         | 0.0001                           | The fraction of an alignment that must be overlapped by a target in order to be classified as on target. "0.50" is interpreted as 50%, and implies that the midpoint of the read must fall within target region.<br>Allowed values: $0.0001 < X \leq 1.0$                                       |
| Extend bases                   | 0                                | The number of bases on either side of the target region in which alignments may be captured. The target sequence is extended by this number of bp on either side. Used by both target capture and target coverage statistics.<br>Allowed values: Integers $\geq 0$ .                            |
| Summary report                 | True                             | Create or do not create the summary statistics file.<br>Allowed values:<br><ul style="list-style-type: none"> <li>• <b>True:</b> Create the summary statistics file.</li> <li>• <b>False:</b> Do not create the summary statistics file.</li> </ul>                                             |
| Target coverage stats          | True                             | Output or do not output per-target coverage statistics (min, max, mean) in tabular format.<br>Allowed values:<br><ul style="list-style-type: none"> <li>• <b>True:</b> Create per-target coverage statistics.</li> <li>• <b>False:</b> Do not create per-target coverage statistics.</li> </ul> |
| Minimum target overlap reverse | 0.0001                           | The fraction of a Reverse Read (F5) alignment which must be overlapped by the target in order to be classified as on-target. To require 100% alignment, use 1.0 (not recommended for LFV workflows).<br>Allowed values: $0.0001 < X \leq 1.0$                                                   |

## SNP Finding

SNP Finding parameters are optimized for Low Frequency Variant analysis. New parameters specific to LFV workflows are used, and some parameters shared with other analyses have different default values for LFV workflows.

The next sections describes parameter optimizations for Low Frequency Variant analysis.

**Note:** In the discussion below, GUI users can ignore the parameter names such as `dibayes.het.min.allele.ratio` and instead use the names displayed in the UI, such as “Minimum allele ratio (Het)”.

### Set the minimum allele ratio

Use the parameter “Minimum allele ratio (Het)” (`dibayes.het.min.allele.ratio`) to set the allele ratio to a desired allele frequency.

In most experiments, setting the minimum allele ratio to be one-fifth of the lowest desired variant frequency ensures high sensitivity. For example, if you want to detect >90% of variants down to 10% frequency, set the minimum allele ratio to 2%. (This setting, of course, also generates variants with lower frequency, for example about half of variants at frequency in the sample of 2% are detected.) If you want to detect all variants down to 1% frequency, set the minimum allele ratio to 0.2%. If your coverage is not extremely high, or to increase specificity with some reduction in sensitivity, the minimum allele ratio may be set to one-third or one-half of the desired variant frequency. For example, if you are looking for variants at 1% frequency, you can set the minimum allele ratio to 0.5% or 0.33%.

### Adjust sensitivity and specificity

#### Increase sensitivity

To *increase the sensitivity* (detect more low frequency variants), or if the coverage is not extremely high, change the following parameters:

- *Reduce* the minimum number of unique start points of the less common allele (for example, to 3 or 4), with “Minimum unique start position of the less common allele” (`dibayes.het.min.lca.start.pos`).
- *Reduce* the threshold for the difference of color QVs of the most common and less common alleles (for example, to 4), with “Maximum difference of color qvs of the most common and less common alleles” (`dibayes.het.max.diff.color.qv`).

Other parameters that can be changed to *increase sensitivity* include:

- *Remove* the requirement to observe the less common allele on both strands, with “The less common allele on both strands” (`dibayes.het.lca.both.strands`).
- *Reduce* the minimum average color QV of the less common allele (for example, to 20), with “Minimum average color qv of the less common allele” (`dibayes.het.min.lca.color.qv`).
- *Reduce* the minimum map QV (for example, to 8), with “Minimum mapping quality value” (`dibayes.reads.min.mapping.qv`).

#### Increase specificity

To *increase the specificity* (call fewer false positives), parameters that can be changed include:

- *Increase* the minimum allele ratio, with “Minimum allele ratio (Het)” (`dibayes.het.min.allele.ratio`).
- *Increase* the minimum number of unique start points of the less common allele, with “Minimum unique start position of the less common allele” (`dibayes.het.min.lca.start.pos`).

- *Include* the requirement to observe the less common allele on both strands, with “The less common allele on both strands” (`dibayes.het.lca.both.strands`).
- *Increase* the minimum average color QV of the less common allele, with “Minimum average color qv of the less common allele” (`dibayes.het.min.lca.color.qv`).

### SNPs parameter table

There are several categories of SNP Finding parameters: general, Low Frequency Variant filters, Read filters, General position filters, Heterozygous position filters, Homozygous position filters, and Output file processing. The following table lists SNP Finding parameters and their default values in Low Frequency Variant workflows.

| Parameter name                                                             | Default value                 | Comments                                                                                      |
|----------------------------------------------------------------------------|-------------------------------|-----------------------------------------------------------------------------------------------|
| <b>General parameters</b>                                                  |                               |                                                                                               |
| Call stringency                                                            | low_frequency                 | The default for Targeted Resequencing is medium. Always use low_frequency with LFV workflows. |
| Skip high coverage positions (Het)                                         | 0                             | —                                                                                             |
| Minimum mapping quality value                                              | 16                            | The default for Targeted Resequencing is 8.                                                   |
| Detect adjacent SNPs                                                       | 0                             | —                                                                                             |
| Polymorphism rate                                                          | 0.001                         | —                                                                                             |
| <b>Low frequency variant filters</b>                                       |                               |                                                                                               |
| Minimum average color qv of the less common allele                         | 30                            | The default for Targeted Resequencing is 0.                                                   |
| Minimum unique start position of the less common allele                    | 7                             | The default for Targeted Resequencing is 0.                                                   |
| The less common allele on both strands                                     | 1                             | The default for Targeted Resequencing is 0.                                                   |
| Maximum difference of color qvs of the most common and less common alleles | 10                            | The default for Targeted Resequencing is 0.                                                   |
| <b>Read filters</b>                                                        |                               |                                                                                               |
| Include reads with unmapped mate                                           | True (Paired)<br>False (Frag) | —                                                                                             |
| Exclude reads with indels                                                  | True                          | —                                                                                             |
| Require only uniquely mapped reads                                         | 0                             | —                                                                                             |
| Ignore reads with a higher mismatch count to alignment length ratio        | 1.0                           | —                                                                                             |
| Ignore reads with a lower alignment length to read length ratio            | 0.9                           | The default for Targeted Resequencing is 1.0.                                                 |
| <b>General position filters</b>                                            |                               |                                                                                               |
| Require alleles to be present in both strands                              | False                         | —                                                                                             |
| Minimum base quality value for a position                                  | 28                            | —                                                                                             |
| Minimum base quality value of the non-reference allele of a position       | 28                            | —                                                                                             |
| <b>Heterozygous position filters</b>                                       |                               |                                                                                               |
| Minimum allele ratio (Het)                                                 | 0.01                          | The default for Targeted Resequencing is 0.15.                                                |

| Parameter name                                                  | Default value | Comments                                                                                                                                                                                                   |
|-----------------------------------------------------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Minimum coverage (Het)                                          | 2             | —                                                                                                                                                                                                          |
| Minimum unique start position (Het)                             | 2             | —                                                                                                                                                                                                          |
| Minimum non-reference color QV (Het)                            | 7             | —                                                                                                                                                                                                          |
| Minimum non-reference base QV (Het)                             | 28            | —                                                                                                                                                                                                          |
| Minimum ratio of valid reads (Het)                              | 0.65          | —                                                                                                                                                                                                          |
| Minimum valid tricolor counts (Het)                             | 2             | —                                                                                                                                                                                                          |
| <b><i>Homozygous position filters</i></b>                       |               |                                                                                                                                                                                                            |
| Minimum coverage (Hom)                                          | 1             | —                                                                                                                                                                                                          |
| Minimum count of the non-reference allele (Hom)                 | 2             | —                                                                                                                                                                                                          |
| Minimum average non-reference base QV (hom)                     | 28            | —                                                                                                                                                                                                          |
| Minimum average non-reference color QV (hom)                    | 7             | —                                                                                                                                                                                                          |
| Minimum unique start position of the non-reference allele (Hom) | 2             | —                                                                                                                                                                                                          |
| <b><i>Output file processing</i></b>                            |               |                                                                                                                                                                                                            |
| Output fasta file                                               | True          | Output or do not output the FASTA file. <ul style="list-style-type: none"> <li>• <b>True:</b> Output the FASTA file.</li> <li>• <b>False:</b> Do not output the FASTA file.</li> </ul>                     |
| Output consensus file                                           | True          | Output or do not output the consensus file. <ul style="list-style-type: none"> <li>• <b>True:</b> Output the consensus file.</li> <li>• <b>False:</b> Do not output the consensus file.</li> </ul>         |
| Compress the consensus file                                     | False         | Compress or do not compress the consensus file. <ul style="list-style-type: none"> <li>• <b>True:</b> Compress the consensus file.</li> <li>• <b>False:</b> Do not compress the consensus file.</li> </ul> |

**(Optional)  
Annotation**

You can optionally annotate the mapped output of the SNP finding analysis. For descriptions of the Annotation parameters, see the following section [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on page 317.

## Perform Low Frequency Variant analysis

**(Optional) Import  
data**

You can optionally import data to be analyzed. For instructions on how to import data, see “Import Data” on page 81.

**Log in to  
LifeScope™**

1. Navigate to LifeScope™ Software at `http://<IP address>:<port number>/LifeScope.html` where *IP address* is the address of the system or head node and *port number* is the number of the port used by the server.

2. In the Login screen, enter your username and password, then either click **Login** or press **Enter** to open the LifeScope™ home view (shown on page 75).

### Create a project

1. In the home view, click **Create a New Project**.
2. If you create a project:
  - a. Type a name and description in the Enter Project Name view.  
**Note:** The name cannot have spaces or special characters.
  - b. Click **Create New Project**.
  - c. In the Projects lists, select the new project.
3. In the Task Wizards section (shown on page 75), click **Add Data to Project** to choose a data type.

### Add data to the project

**Note:** Add data from the read repository to a project by choosing a data type and finding to the new path.

1. In the Add Data to Project window, select **Raw unmapped (XSQ) data**, then click **Next** to find data.
2. In the Read Repository Filter table, select the reads you want to map and analyze. If you want to group data, click **Next**. If you do not want to group data, skip [step 3](#) and proceed to [step 4](#).
3. (Optional) In the Read-sets in Project table, click the checkbox of the data files you want to group, then click **Add Group to Project**. The files appear in the Groups in Project table.  
 To rename a group, click the checkbox of the group in the Groups in Project table, then click **Edit**. In the Edit Group window, enter a new name.  
 To remove a data file from a group, click the checkbox of the data file, then click **Delete**.
4. Click **Add Analysis** to proceed, or
  - Click **Cancel** to refrain from adding data and close the Add Data to Project window, or
  - Click **Finish** to add the data and close the Add Data to Project window.

### Create an analysis

1. In the Choose Data view of the Create Analysis window, select data files from the Available Data in Project table, then click **Next** to choose an analysis.
2. In the Choose Analysis view, enter a name for the analysis. You can optionally describe your project.  
 If you are re-using an Old Analysis, select **Reuse Old Analysis** and select the name of the analysis you want to use.  
**Note:** The name cannot have spaces or special characters.
3. Select **Low Frequency Variant Detection**, then click **Next**.



**Note:** You do not make an Analysis Type selection during **Reuse Old Analysis**.

4. In the Data To Be Analyzed table, click **Select the reference for the reads** to open the repository file browser.
5. In the Browse for Reference File window, navigate to the location of the reference genome of your sample. Open the folder with your .fasta file, for example:
  - data ▶ referenceData ▶ lifetech ▶ hg18 ▶ reference ▶ human\_hg18.fasta
 Select the file, then click **OK**.  
 The file name appears in the Reference column.
 

**Note:** To change the reference file, click **Select the reference for the reads**, then select another reference file.
6. After you have created the analysis:
  - Click **Edit** to proceed, or
  - Click **Cancel** to refrain from choosing references and close the Create Analysis window, or
  - Click **Finish** to complete analysis creation and close the window.

## Edit the analysis

1. In the Edit Analysis window, accept the default settings for mapping and pre-processing data in the Secondary Analysis section.  
 If you want to exclude BAMStats, click **Customize**. In the Customize Mapping window, uncheck BamStats, then click **OK**.  
 If you do not want to pre-process data, uncheck the box.
2. In the Tertiary Analysis section, accept the included SNP Finding and Annotation settings and click **Next** to set module parameters. To include all modules, click the **All>>** button.  
 To exclude a module, select it in the Include column, then click the < button. To exclude all modules, click the **All<<** button.  

**Note:** The Enrichment module is mandatory and cannot be removed.

 To skip setting module parameters and review the analysis, click **Review**.

## Set module parameters

This section describes the procedures for setting general parameters and parameters for the mapping and SNP Finding modules. For descriptions of module parameters, see [“Low Frequency Variant parameters” on page 144](#).

You can restore the default settings of parameters by clicking the **Reset to Defaults** button.

To view descriptions of parameters, place your mouse cursor over a  button.

### Set general parameters

1. Enter an analysis assembly name.
2. Set the `annotation.dbsnp`, `analysis.regions.file`, and `annotation.gtf.file` parameters. Click the  buttons to open the File Chooser, navigate to reference files, and choose files.  
**Note:** Verify that the file paths are correct. An incorrect file path results in a failed analysis.
3. The `analysis.space` parameter is set to color space. Low Frequency Variant Detection is available only in color space.
4. Always leave the default `analysis.platform` setting, which is "SOLID".
5. If you want to accept the default parameters for all modules, click **Review**. If you want to edit module parameters, click **Next**.

### Set Fragment Mapping parameters

1. There are several categories of mapping parameters: Main, Advanced, and BAMStats. Accept the default settings or click the tabs to edit the settings.
2. Click **Next** to edit Enrichment parameters.

### Set Enrichment parameters

1. There are two categories of enrichment parameters: Main and Advanced. Accept the default settings or edit the settings.
2. Click **Next** to edit Enrichment parameters.

### Set SNP Finding parameters

1. Categories of SNP Finding parameters include Main, Advanced, and (if you selected Annotation for SNP Finding output) Annotation. Accept the default settings or edit the settings.
2. Click **Next**.

If you are ready to start the analysis, click **Review** to proceed.

If you want to erase your edits, click **Cancel** to close the Edit Analysis window.

If you want to start the analysis later, click **Finish** to save your edits and close the Edit Analysis window.

## Review and run the analysis

**Note:** For a description of workflow parameters, see [“Low Frequency Variant parameters” on page 144](#).

The Review Analysis view in the Run Analysis window includes two tabs: Parameters and Data. In the Parameters tab, click the  , next to parameter categories to show or hide the parameters.

1. Review the parameters. To edit the parameters, click **Edit**.
2. To review the data that will be analyzed, click the **Data** tab.

3. If you are ready to run the analysis, click **Start Analysis**.  
The Run Analysis window closes.

## Checking Analysis Status

1. In the Projects list, click the project to check the status of the analysis run.  
The Progress columns show the percentage of completion for the secondary and tertiary analyses.

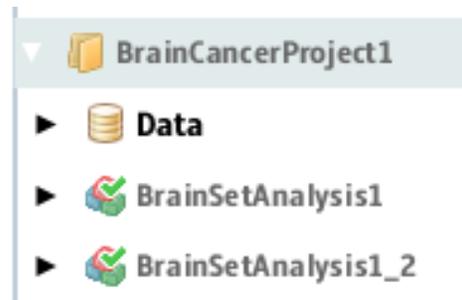
ProstateCancerProje... • ProstateCancerAnaly...

Overview Status Parameters

Analysis Runs

| Name                         | XSQ ID                       | Analysis                | Secondary Analysis        | Secondary Progress                                                                     | Tertiary Analysis                 | Tertiary Progress |
|------------------------------|------------------------------|-------------------------|---------------------------|----------------------------------------------------------------------------------------|-----------------------------------|-------------------|
| solid0054_20110102_PE_LFD... | solid0054_20110102_PE_LFD... | ProstateCancerAnalysis1 | [SAET, Mapping, BamStats] |  33% | [Enrichment, diBayes, Smallin...] | 0%                |

When analysis is complete, a green check mark appears on the analysis name in the Projects organizer.



## View analysis results

To view analysis results:

1. In the Projects organizer, click the project to show its contents.
2. To view analysis results, click either the:
  - Results icon  for an overview of the analysis, then Click **View Results** in the **Secondary Progress** and **Tertiary Progress** columns to open the View Results window.
  - Completed analysis icon  for results of a specific analysis. Click the Status tab, then **View Results** to open the window.
3. In the View Results window, click each analysis module for analysis details.
4. Click each analysis module to see statistics, additional files, and logs.

The following table shows the results that are available for each analysis module.

| Analysis Module           | Details | Statistics | Additional Files | Logs |
|---------------------------|---------|------------|------------------|------|
| SNP.Finding.Annotation    | No      | No         | Yes              | Yes  |
| SNP.Finding.targeted.frag | Yes     | Yes        | Yes              | Yes  |
| Enrichment                | No      | Yes        | Yes              | Yes  |
| BAMStats                  | Yes     | No         | Yes              | Yes  |

| Analysis Module | Details | Statistics | Additional Files | Logs |
|-----------------|---------|------------|------------------|------|
| Mapping         | No      | No         | Yes              | Yes  |

**View results in a genome browser**

Output files generated by Low Frequency Variant Detection runs are compatible with third-party browser such as the Integrative Genomics Viewer (IGV) available from the Broad Institute and the UCSC Genome Browser. See [“Pairing information in a BAM file” on page 348](#) for information on using LifeScope™ Software output files with genome browsers.

For more information on viewing analysis results, see [Chapter 9, “View Analysis Results” on page 103](#).

## Low Frequency Variant output files

**SNP.Finding.Annotation**

**Details**

There are no details for the SNP.Finding.Annotation analysis module.

**Statistics**

There are no statistics for the SNP.Finding.Annotation analysis module.

**Additional files**

The following table is an example of additional files for the SNP.Finding.Annotation analysis module.

| Directory                                                                                         | Files      | File Type |
|---------------------------------------------------------------------------------------------------|------------|-----------|
| SNP.Finding.Annotation/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary | README.txt | .txt      |

**Logs**

The following table is an example of SNP.Finding.Annotation logs.

| Directory              | Files                                                                                                                            | File Type |
|------------------------|----------------------------------------------------------------------------------------------------------------------------------|-----------|
| SNP.Finding.Annotation | tertiary-solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary-SNP.Finding.Annotation.20110421220103477.log | .log      |

**SNP.Finding.targeted.frag**

**Details**

The following illustration is an example of details for the SNP.Finding.targeted.frag analysis module.



### Statistics

The following illustration is an example of statistics for the `SNP.Finding.targeted.frag` analysis module.

```

*****
LIFESCOPE REPORT
*****

Input File:      /panasas/lifescopetesttemp1/results/projects/corona/Da
frag/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_Default
Date:   Apr 21, 2011 10:02:31 PM
Annotation file, dbSNP: /panasas/lifescopetesttemp1/results/projects/corona/Da
Annotation file, Genes and Exons, GTF:/panasas/lifescopetesttemp1/results/projects/corona/Da

*****
Statistics Overview
*****

-- Basic Statistics -----
Number of variants                1394
Number of heterozygous variants   913
Number of homozygous variants     481

-- Variant-Specific Statistics (SNP) -----
Number of transition SNPs         935
Number of transition heterozygous SNPs 603
Number of transition homozygous SNPs 332
Number of transversion SNPs      459
Number of transversion heterozygous SNPs 310
Number of transversion homozygous SNPs 149
Transition:Transversion ratio     2.037 : 1.000

-- dbSNP Annotation Statistics -----
Number of          SNPs in dbSNP      1210
Number of heterozygous SNPs in dbSNP  731
Number of homozygous SNPs in dbSNP    479
dbSNP concordance                    86.80%
dbSNP heterozygous concordance        80.07%
dbSNP homozygous concordance          99.58%

-- Gene and Exon Statistics -----
Number (percent) of variants overlapping exon      453 ( 32.50%)
Number (percent) of heterozygous variants overlapping exon 288 ( 31.54%)
    
```

### Additional files

The following table is an example of additional files for the SNP.Finding.targeted.frag analysis module.

| Directory                                                                                                     | Files                               | File Type |
|---------------------------------------------------------------------------------------------------------------|-------------------------------------|-----------|
| SNP.Finding.targeted.frag/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary/contig2  | Analysis2_SNP.gff3                  | .gff3     |
| SNP.Finding.targeted.frag/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary/contig19 | Analysis2_Consensus_Calls.txt       | .txt      |
| SNP.Finding.targeted.frag/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary/contig12 | Analysis2_Consensus_Basespace.fasta | .fasta    |

### Logs

The following table is an example of logs for the SNP.Finding.Annotation analysis module.

| Directory                 | Files                                                                                                                                               | File Type |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| SNP.Finding.targeted.frag | tertiary-solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary-SNP.Finding.targeted.frag.3.persistHeader.20110421214007357.log | .log      |

## Enrichment

### Details

There are no details for the enrichment analysis module.

### Statistics

The following illustration is an example of statistics for the enrichment analysis module.

```

Run      Reads On      Percent On      Reads Off      Percent Off      Enrichment Fold
/results/Analysis2.ontarget.bam      71681629      89.2239%      8657401 10.7761%      1347.43

Reads In Targets:      71681629      89.2239%
Reads Off Targets: 8657401      10.7761%
Ratio of Percent on/off Target: 8.27981
Total Target BP: 2039803
Total Genome Size: 3080436051
Ratio of target to genome: 0.00066218
Enrichment fold relative to target size: 1347.43

# Targets Not Covered      Target Bases Not Covered      Percent of Target Bases Not Covered
Percent of Target Covered >= 1X      Percent of Target Covered >= 5X      Percent of Target Covered >= 10X
Percent of Target Covered >= 20X      Average Depth of Target Coverage
1      13067      0.64%      99.36%      98.94%      98.62%      98.22%      2451.59

Number of target regions with no coverage: 1
Percent of target bp not covered: 0.64% (13067 bp)
Percent of target bp covered at >= 1X: 99.36%
Percent of target bp covered at >= 5X: 98.94%
Percent of target bp covered at >= 10X: 98.62%
Percent of target bp covered at >= 20X: 98.22%
Maximum Depth of Coverage within targets: 51306
Average Depth of Coverage within targets: 2451.59

```

### Additional files

The following table is an example of additional files for the enrichment analysis module.

| Directory                                                                             | Files                                                                                            | File Type |
|---------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-----------|
| enrichment/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary | Analysis2.ontarget.bam.bai                                                                       | .bai      |
|                                                                                       | Analysis2_RainDance4K_targetHG18_withChr.validated.bed                                           | .bed      |
|                                                                                       | solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary_target_statistics.txt | .txt      |
|                                                                                       | Analysis2.ontarget.bam                                                                           | .bam      |

### Logs

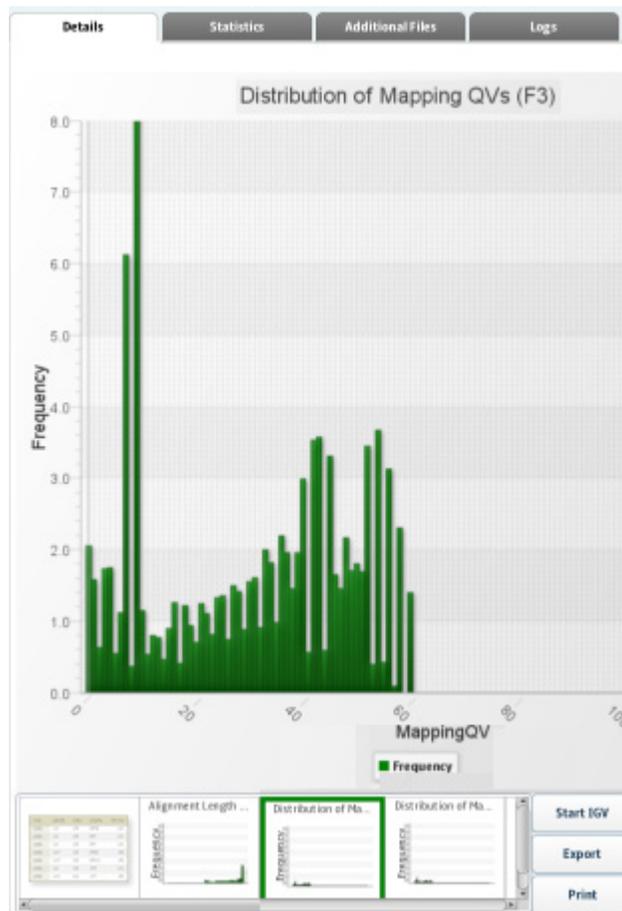
The following table is an example of logs for the enrichment analysis module.

| Directory  | Files                                                                                                                     | File Type |
|------------|---------------------------------------------------------------------------------------------------------------------------|-----------|
| enrichment | tertiary-solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary-enrichment.main.20110421212207477.log | .log      |

## BAMStats

### Details

The following illustration is an example of BAMStats details.



### Statistics

There are no statistics for the BAMStats analysis module.

### Additional files

The following table is an example of BAMStats additional files.

| Directory                                                                                                                                                                      | Files                                                                                            | File Type |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-----------|
| Mapping.BamStats/<br>solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.color<br>Remake_DefaultLibrary/<br>solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.color<br>Remake-1/Misc | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.bam_chrX.NEG.wig           | .wig      |
|                                                                                                                                                                                | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.BaseQV.by.Position.csv     | .csv      |
|                                                                                                                                                                                | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.Unique.Start.Positions.txt | .sql      |
|                                                                                                                                                                                | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.bam_chrX.NEG.wig           | .wig      |

### Logs

The following table is an example of logs for the BAMStats analysis module.

| Directory        | Files                                                                      | File Type |
|------------------|----------------------------------------------------------------------------|-----------|
| Mapping.BAMStats | secondary-human_hg18-Mapping.BamStats.10.run.temp.10.20110421211327339.log | .log      |

## Mapping

### Details

There are no details for the Mapping analysis module.

### Statistics

There are no statistics for the Mapping analysis module.

### Additional files

The following table is an example of Mapping additional files.

| Directory                                                                                       | Files                                                                         | File Type |
|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-----------|
| fragment.mapping/solid0054_20110102_PE_LFD_RD_SetA_1_<br>HuRef100_F3.colorRemake_DefaultLibrary | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.bam     | .bam      |
|                                                                                                 | solid0054_20110102_PE_LFD_RD_Set<br>A_1_HuRef100_F3.colorRemake-<br>1.bam.bai | .bai      |

### Logs

The following table is an example of logs for the Mapping analysis module.

| Directory        | Files                                                             | File Type |
|------------------|-------------------------------------------------------------------|-----------|
| fragment.mapping | secondary-human_hg18-fragment.mapping.3.run.20110421182159033.log | .log      |



## 12

# Perform Genomic Resequencing Analysis

This chapter covers:

|                                                       |     |
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## Introduction to genomic resequencing analysis

Genomic resequencing is a workflow for identifying individual variants and structural variations in a genomic sample.

Genomic resequencing output files are compatible with third-party genome browsers, including the Integrative Genome Viewer described [on page 53](#)).

## Genomic resequencing analysis library types

Genomic resequencing analysis supports data from the following library types of data:

- Fragment
- Paired-end
- Mate-pair

## Genomic resequencing analysis input files

| Input File                          | File Extension | Analysis Module               |
|-------------------------------------|----------------|-------------------------------|
| Binary Alignment Map (BAM)          | *.bam          | Small Indel, SNP Finding, CNV |
| <i>(Optional)</i> Color space FASTA | *.csfasta      | Mapping                       |
| eXtensible SeQuence (XSQ)           | *.xsq          | Small Indel, SNP Finding, CNV |
| FAST-All (FASTA)                    | *.fa, *.fasta  | Mapping                       |
| <i>(Optional)</i> Quality Value     | *qual, *qv     | Mapping, pairing              |

## Genomic resequencing analysis modules

Genomic resequencing analysis includes the following modules:

| Secondary Analysis Module | Used for Library Type |
|---------------------------|-----------------------|
| Mapping                   | Fragment              |
| Mapping and pairing       | Paired-end            |
|                           | Mate-pair             |

| Tertiary Analysis Module | Used for Library Type      |
|--------------------------|----------------------------|
| Small Indel              | Fragment, mapping, pairing |
| SNP Finding              |                            |
| CNV                      |                            |

### Mapping

The map data resequencing workflow uses eXtensible SeQuence (.xsq) files to create a .bam file.

### Small Indel

When an indel occurs in a sequence, and that sequence is measured using color-space, the color-space sequence has a gap the same size as the indel. The color-space sequence also leaves a signature that can indicate whether there is a measurement error within the gap. The Small Indel tool targets the processing of indel evidences found in the pairing step during secondary offline data analysis.

You can optionally annotate input files. Annotation adds new attributes to the General Feature Format (GFF) entries in an input file, information from publicly available sources about the variants in an input file, features intersecting the variants in an input file, or any biological function potentially changed by the variants.

For information about using the Small Indel module to run a genomic resequencing analysis, see [Chapter 23, “Perform Small Indel Analysis” on page 307](#).

### SNP Finding

The SNP Finding module is used to call Single Nucleotide Polymorphisms (SNPs) from mapped and processed SOLiD<sup>®</sup> System color-space reads. The module takes the color-space reads, the quality values, the reference sequence, and error information on each SOLiD<sup>®</sup> System slide as its input, and calls SNPs.

The module creates three results files:

- A list of SNPs.
- (Optional) A consensus \*.fasta file with the same number of bases as the reference sequence.
- (Optional) A list of all covered positions.
- (Optional) A collection of annotated files.

For information about using the module to run a Targeted Resequencing experiment, see [Chapter 20, “Perform SNP Finding Analysis” on page 289](#).

## CNV

The Copy Number Variation (CNV) analysis module detects CNV in SOLiD® System data that originates from a single human sample. Slide(s) from this sample must be mapped to the hg18 reference to facilitate correct normalization.

For information about using the module, see [Chapter 18, “Perform Human Copy Number Variation Analysis”](#) on page 263.

## Genomic resequencing analysis parameters

Parameters for genomic resequencing analysis include:

- General parameters
- **Secondary analysis:** Mapping (including optional BAMStats and SOLiD® Accuracy Enhancement Tool [SAET])
- **Tertiary analysis:** Small Indel, SNP Finding, CNV

### General

General parameters include:

| Parameter                | Default value | Description                                                                                                                                                                                                   |
|--------------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| analysis.assembly.name   | unknown       | The name of the genome assembly used in current analysis. Examples are hg18 and hg19.                                                                                                                         |
| annotation.dbsnp.file    | n/a           | The path to the file used to annotate SNPs and small InDels. LifeTech-provided files are dbSNP_b130.tab(hg18) and 00-All.vcf(hg19).                                                                           |
| analysis.mappability.dir | n/a           | The path to the directory containing binary mappability files used in CNV module.                                                                                                                             |
| analysis.regions.file    | n/a           | The path to the file containing genomic regions, such as a .bed format file containing exome targets. Required with enrichment analyses, including targeted resequencing and low frequency variant workflows. |
| annotation.gtf.file      | n/a           | The path to the file containing gene and exon annotations corresponding to the genome assembly used in the analysis.                                                                                          |
| analysis.space           | Base          | Your preference of whether analysis should be done in base space or in color space. This setting should be the same for all modules in your run. See <a href="#">“Choose analysis space”</a> on page 98.      |
| analysis.platform        | SOLID         | Always specify “SOLID”. Other values are reserved for future use.                                                                                                                                             |

Use the  button to open the File Chooser and search for input files.

**Mapping**

Categories of parameters for mapping include Main, Advanced, (if you selected SAET pre-processing) SAET, and BAMStats.

**Main**

| Parameter          | Default value | Description                                                                                                                                                                                                                                                                                   |
|--------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Add color sequence | True          | Add color sequence to BAM records: Values: <ul style="list-style-type: none"> <li>• True: Add color.</li> <li>• False: Do not add color.</li> </ul>                                                                                                                                           |
| Map in base space  | False         | Allow mapping in base space if input data has base space available. If only color space is available, then mapping will fail when base space mapping is turned on. Values: <ul style="list-style-type: none"> <li>• True: Map in base space.</li> <li>• False: Map in color space.</li> </ul> |

**Advanced**

| Parameter                     | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|-------------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| BAM soft clip                 | False         | Modify an unaligned portion of a read that needs to be presented in the BAM record. Values: <ul style="list-style-type: none"> <li>• True: Soft clip.</li> <li>• False: Do not soft clip.</li> </ul>                                                                                                                                                                                                                                                  |
| Base quality filter threshold | 10            | Replace resulting base-calls with a quality value less than the specified value.<br>Allowed values: Integers 0–1,000                                                                                                                                                                                                                                                                                                                                  |
| Create unmapped BAM files     | False         | Create BAM files containing unmapped reads. Values:<br>True: Create.<br>False: Do not create.                                                                                                                                                                                                                                                                                                                                                         |
| Mapping QV/threshold          | 0             | Provide control the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer.<br>Allowed values: Integers 0–1,000.                                                                                                                                                                                                                     |
| Primary output filter type    | Primary_only  |                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Reference weight              | 8             | Used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. Adds weight (in terms of Phred score) to the signals that are compatible with reference. Color combinations that result in a variant are considered compatible with reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation.<br>Allowed values: Integers 0–100. |

| Parameter                        | Default value | Description                                                                                                                                                                                                                                                          |
|----------------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Second map gapped algorithm type | GLOBAL        | Do indel finding, and control the behavior of indel finding.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• GLOBAL: Mapping reports global alignment up to one indel.</li> <li>• LOCAL: Mapping reports local alignment up to one indel.</li> </ul> |

### SAET

The SAET tab is accessible only if you select SAET pre-processing when you choose modules.

| Parameter                    | Default value | Description                                                                                                                                                                                                                                                |
|------------------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genome length                | 2,800,000,000 | Expected length of sequenced (or enriched) DNA region. For example, 4,600,000 for the E.Coli 4.6 MB genome or 30,000,000 for the entire Human Transcriptome.<br><br>Allowed values: Integers $\geq 200$ .                                                  |
| On target ratio              | 0.5           | The expected ratio of reads that come from the targeted region.<br><br>Allowed values: Floats 0.0–1.0.                                                                                                                                                     |
| Update quality values        | True          | Update quality value of modified calls.<br><br>Allowed values:<br>True: Update the QV of modified calls.<br>False: Do not update the qv for modified calls.                                                                                                |
| Trusted quality value        | 25            | Correction is applied to calls with a quality value below the value of this parameter.<br><br>Allowed values: Integers $\geq 1$ .                                                                                                                          |
| Support votes                | 2             | The minimum number of k-mer votes required make a correction.<br><br>Allowed values: Integers $\geq 1$ .                                                                                                                                                   |
| Trusted frequency            | 0             | The lowest multiplicity of the seed to be included in the spectrum. (If set to 0, then the value is computed internally.)<br><br>Allowed values: Integers $\geq 0$ .                                                                                       |
| Maximum corrections per read | 0             | Maximum number of allowed corrections per read. (If set to 0, then the value is set to $\lceil \text{readLength}/8 \rceil$ ). Reduce if over-corrections are observed, or increase if under-corrections are observed.<br><br>Allowed values: Integers 0–9. |

| Parameter                              | Default value | Description                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Number of recursive runs               | 1             | The error correction step is repeated the provided number of times. Reduce if over-corrections are observed, or increase if undercorrections are observed.<br><br>Allowed values: 1, 2, or 3.                                                                                                                                                                |
| Position of error inflation point      | 0             | Position in the read at which the error rate inflates, for instance, 35-40 for 50bp long reads. (If set to 0, then the value is equal to $0.8 * \text{readLength}$ ).<br><br>Allowed values: Integers.                                                                                                                                                       |
| Disable random sampling for large data | False         | Disables random sampling in spectrum building. If set to 0, then for large datasets (coverage > 300x), a subset of reads is used in spectrum building. Allowed values: <ul style="list-style-type: none"> <li>• True: Disables random sampling in spectrum building.</li> <li>• False: Do not disable random sampling in spectrum building. true?</li> </ul> |
| K-mer size                             | 0             | Size of k-mer (>5) used in spectrum construction and error correction. (If set to 0, then the value is computed internally.)<br><br>Allowed values:<br>Integers 0-28.                                                                                                                                                                                        |

### BAMStats

| Parameter                    | Default value                            | Description                                                                                                                                                                                                                                            |
|------------------------------|------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Input directory for BAMStats | <code>\${analysis.output.dir}/bam</code> | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample.                                                                                                                                   |
| Output directory             | <code>\${task.output.dir}</code>         | The path to the output directory where BAMStats will write its chart (.cht) files.                                                                                                                                                                     |
| Maximum Coverage             | 10000                                    | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br><br>Allowed values: Integers 1 – 10,000.                                |
| Maximum insert size          | 100000                                   | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br><br>Allowed values: Integers 1 – 100000. |
| Insert bin size              | 100                                      | Bin size for insert range distribution.<br><br>Allowed values: Integers 1 – 100000                                                                                                                                                                     |

|                                                                          |     |                                                                                                                                                                                                                                                                                                                |
|--------------------------------------------------------------------------|-----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Whether to combine data from both the strands for coverage in WIG format | 1   | Combine or do not combine data from both strands for coverage in WIG format.<br>Allowed values:<br><ul style="list-style-type: none"> <li>• <b>0</b>: Do not generate wig files.</li> <li>• <b>1</b>: Generate the combined coverage file.</li> </ul>                                                          |
| Primary alignments only for coverage in WIG file format                  | 1   | Use only primary alignments for coverage in WIG file format.<br>Allowed values:<br><ul style="list-style-type: none"> <li>• <b>0</b>: Do not restrict coverage in WIG file format to only primary alignments.</li> <li>• <b>1</b>: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Bin size for coverage in WIG file format                                 | 100 | The bin size for coverage in WIG file format.<br>Allowed values: Integers > 1-100000.                                                                                                                                                                                                                          |

## Small Indel

There are two categories of parameters for the Small Indel analysis module: Advanced and Annotation. There are no Main parameters.

### Advanced

There are five categories of Advanced parameters: General Options, Pileup, Mapping Quality Filtering, Heuristic Filtering, Indel Size Filtering.

#### General Options

| Parameters          | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                           |
|---------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Detail level        | 3             | For BAM file inputs, the level of detail in output:<br><ul style="list-style-type: none"> <li>• <b>0</b>: Keeps only position information about the anchor read and no information for the ungapped alignment.</li> <li>• <b>1-8</b>: Keeps only some of the alignment's anchor alignment but none of the ungapped alignment.</li> <li>• <b>9</b>: Is most detailed, but also the slowest.</li> </ul> Allowed values: Integers 0 - 10 |
| Zygoty profile name | max-mapping   | Zygoty profile name.<br><ul style="list-style-type: none"> <li>• <b>classic</b> Run classic mapping.</li> <li>• <b>max-mapping</b>: Run max mapping.</li> <li>• <b>max-mapping-v2</b>: Run max mapping version 2.</li> <li>• <b>gap-align-only</b>:</li> <li>• <b>no-calls</b>:</li> </ul>                                                                                                                                            |
| Genomic region      | blank         | Names a specific genomic region to be selected from the BAM file. Only full chromosomes are guaranteed not to alter results. Specifying partial chromosomes is allowed but may result in the loss of indels near the edges of that region.                                                                                                                                                                                            |

| Parameters                      | Default value | Description                                                                                                                                                                                                                                                                                                                                                          |
|---------------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Display base QVs                | False         | Display base QV scores in the GFF file. Allowed values:<br><b>True:</b> Display the FASTQ base QV scores for all of the reads used for each indel in the GFF file. FASTQ strings contain semi-colons, so adding these strings may produce a GFF file that is not compatible with certain applications.<br><b>False:</b> Do not display QV scores in the output file. |
| Number of alignments per pileup | 1000          | For pileups with more than this number of reads, set the expected number of alignments per pileup. Allowed values: Integers $\geq 0$ .                                                                                                                                                                                                                               |
| Random seed                     | 94404         | The random seed value used to determine which pseudo random set of reads to use when there are greater than 1000 reads in a pileup. The random number generator used is the Mersenne Twister MT19937 algorithm.<br>Allowed values: Integers $\geq 0$ .                                                                                                               |

#### Pileup

| Parameters   | Default value | Description                                                                                                                                                                                                                                                                                                      |
|--------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Min num evid | 2             | Minimum number of evidences required for an indel call.                                                                                                                                                                                                                                                          |
| Max num evid | -1            | Maximum number of evidences, use -1 for no maximum.                                                                                                                                                                                                                                                              |
| ConsGroup    | 1             | Indel grouping method. Allowed values:<br><b>0:</b> Conservative grouping of indels with 5bp max between consecutive evidences.<br><b>1:</b> Lax grouping. Groups indels that are at maximum the higher of 15 or 7 times the indel size.<br><b>9:</b> No grouping. Makes every indel evidence a separate pileup. |

#### Mapping Quality Filtering

| Parameters              | Default value | Description                                                                                                                    |
|-------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------|
| Max reported alignments | -1            | Only uses those alignments where the NH field (the number of reported alignments; from the BAM header) is this value or lower. |

| Parameters                 | Default value         | Description                                                                                                                                                                                                                                                                                                                                      |
|----------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Min mapping quality        | 8                     | Keeps only reads that have this or higher pairing qualities. For paired tags, mapping quality is for the pair (pairing quality), and for fragment, it is the single tag's map quality.<br><br>Reads that are lower than this value are filtered out.                                                                                             |
| Min best mapping quality   | 10                    | For a particular indel called with a set of reads, at least one pairing quality in this set must be higher than this value. Allows for supporting evidences to have a lower mapping quality threshold than the best read.                                                                                                                        |
| Min anchor mapping quality | -1                    | Minimum mapping quality for a non-indel (anchor) tag. Effective only for paired reads, for the number of anchors queried as defined by <code>small.indel.detail.level</code> .                                                                                                                                                                   |
| Ungapped BAM flag filter   | ProperPair<br>Primary | For ungapped alignments, specifies the BAM flag properties that a read must have to be included. Allowed values: A comma-separated string of one or more of these values: <ul style="list-style-type: none"> <li>• ProperPair</li> <li>• UniqueHit</li> <li>• NoOptDup</li> <li>• Primary</li> <li>• None</li> </ul> None turns off all filters. |
| Gapped BAM flag filter     | Primary               | For gapped alignments, specifies the BAM flag properties that a read must have to be included. Allowed values: A comma-separated string of one or more of these values: <ul style="list-style-type: none"> <li>• ProperPair</li> <li>• UniqueHit</li> <li>• NoOptDup</li> <li>• Primary</li> <li>• None</li> </ul> None turns off all filters.   |
| Edge length datelines      | 0                     | Gap alignments that do not have this minimum length on either side of the indel will not be considered.<br>Allowed values: Integers $\geq 0$ .                                                                                                                                                                                                   |
| Edge length insertions     | 0                     | Gap alignments that do not have this minimum length on either side of the indel will not be considered.<br>Allowed values: Integers $\geq 0$                                                                                                                                                                                                     |

## Heuristic Filtering

| Parameters                      | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Perform filtering               | True          | Whether or not to perform filtering in each pileup. Allowed values: <ul style="list-style-type: none"> <li>• <b>True</b>: Perform filtering on pileups.</li> <li>• <b>False</b>: Do not perform filtering on pileups.</li> </ul> Parameters that change the makeup of pileups, such as <a href="#">Min num evid</a> are still active.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Indel size distribution allowed | can-cluster   | Indel sizes in a pileup are allowed to have certain indel size distributions. Allowed values: <ul style="list-style-type: none"> <li>• <b>similar-size</b>: 75% of the reads of a pileup must have exactly the same size.</li> <li>• <b>similar-size-any-large-deletions</b>: Any pileups with at least 2 large deletion alignments, the other pileups must have similar sizes.</li> <li>• <b>can-cluster</b>: Allowed if at least one cluster of any indel size is found.</li> <li>• <b>can-cluster-any-large-deletions</b>: Any pileups with at least 2 large deletion alignments; other pileups must be able to cluster (will have indels with two more reads with larger deletions, even if they don't form good clusters).</li> </ul> <b>any</b> : Can have any size distribution (same as small .indel.require.called.indel.size=false) |
| Remove singletons               | True          | Remove the singletons that occur when different alignment methods are combined based on identical bead ids and read sequence. Allowed values: <ul style="list-style-type: none"> <li>• <b>True</b>: Remove singletons.</li> <li>• <b>False</b>: Do not remove the singletons.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Alignment compatibility filter  | 1             | Alignment compatibility level. Checks color space compatibility around the gap. Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: No alignment compatibility filtering.</li> <li>• <b>1</b>: The small indel module determines whether the data contains base-space or color-space sequence.</li> <li>• <b>2</b>: Force the use of base-space sequence, if present.</li> <li>• <b>3</b>: Force the use of color-space sequence, if present.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                       |
| Max coverage ratio              | 12            | Maximum clipped coverage/# non-redundant support ratio. Use -1 for no limit (no coverage ratio filtering). Allowed values: Integers.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

| Parameters       | Default value | Description                                                                                                                                                                                                     |
|------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Max nonreds 4Fit | 2             | Maximum number of non-redundant reads where read position filtering is applied.<br>Allowed values: Integers.                                                                                                    |
| Min from end pos | 9.1           | Minimum average number of base pairs from the end of the read required of the pileup, when there are at most a certain number of reads defined by <a href="#">Max nonreds 4Fit</a> .<br>Allowed values: Floats. |

Indel Size Filtering

| Parameters          | Default value | Description                                                     |
|---------------------|---------------|-----------------------------------------------------------------|
| Min insertions size | 0             | Minimum insertion size to include.<br>Allowed values: Integers. |
| Min deletions size  | 0             | Minimum deletion size to include.<br>Allowed values: Integers.  |
| Max insertions size | 1000000000    | Maximum insertion size to include.<br>Allowed values: Integers. |
| Max deletions size  | 1000000000    | Maximum deletion size to include.<br>Allowed values: Integers.  |

**(Optional) Annotation**

You can optionally annotate the mapped output of Small Indel analysis. For descriptions of the Annotation parameters, see “Annotation” [on page 178](#).

**SNP Finding**

There are two categories of SNP Finding parameters: Main and Advanced.

**Main**

| Parameter name                     | Default value | Description                                                      |
|------------------------------------|---------------|------------------------------------------------------------------|
| Call stringency                    | medium        | Call stringency. Highest, high, medium, low, lowest              |
| Skip high coverage positions (Het) | True          | Skip high coverage positions (Het)                               |
| Minimum mapping quality value      | 8             | Minimum mapping quality value.<br>Allowed values: Integers 0-100 |

**Advanced**

There are six categories of Advanced parameters: general, Read filter, General position filter, Heterozygous position filter, Homozygous position filter, and Output file processing.

| Parameter name                                                       | Default value | Description                                                                                             |
|----------------------------------------------------------------------|---------------|---------------------------------------------------------------------------------------------------------|
| Detect adjacent SNPs                                                 | False         | Detect adjacent SNPs.                                                                                   |
| Polymorphism rate                                                    | 0.001         | Polymorphism rate.<br>Allowed values: 0.0000 - 1.0000                                                   |
| <b>Read filter</b>                                                   |               |                                                                                                         |
| Include reads with unmapped mate                                     | False         | Include reads with unmapped mate.                                                                       |
| Exclude reads with indels                                            | True          | Exclude reads with indels.                                                                              |
| Require only uniquely mapped reads                                   | False         | Require only uniquely mapped reads                                                                      |
| Ignore reads with a higher mismatch count to alignment length ratio  | 1             | Ignore reads with a higher mismatch count to alignment length ratio.<br>Allowed values: 0.0000 - 1.0000 |
| Ignore reads with a lower alignment length to read length ratio      | 0             | Ignore reads with a lower alignment length to read length ratio.<br>Allowed values: 0.0000 - 1.0000     |
| <b>General position filter</b>                                       |               |                                                                                                         |
| Require alleles to be present in both strands                        | False         | Require alleles to be present in both strands                                                           |
| Minimum base quality value for a position                            | 20            | Minimum base quality value for a position.<br>Allowed values: Integers 0-40                             |
| Minimum base quality value of the non-reference allele of a position | 20            | Minimum base quality value of the non-reference allele of a position.<br>Allowed values: Integers 0-40  |
| <b>Heterozygous position filter</b>                                  |               |                                                                                                         |
| Minimum allele ratio (Het)                                           | 0.15          | Minimum allele ratio (Het).<br>Allowed values: 0.0000 - 0.5000                                          |

| Parameter name                                                  | Default value | Description                                                                                                                                                                                                |
|-----------------------------------------------------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Minimum coverage (Het)                                          | 2             | Minimum coverage (Het).<br>Allowed values: Integers $\geq 1$                                                                                                                                               |
| Minimum unique start position (Het)                             | 2             | Minimum unique start position (Het).<br>Allowed values: Integers $\geq 1$                                                                                                                                  |
| Minimum non-reference color QV (Het)                            | 7             | Minimum non-reference color QV (Het).<br>Allowed values: Integers 0-62                                                                                                                                     |
| Minimum non-reference base QV (Het)                             | 20            | Minimum non-reference base QV (Het).<br>Allowed values: Integers 0-41                                                                                                                                      |
| Minimum ratio of valid reads (Het)                              | 0.65          | Minimum ratio of valid reads (Het).<br>Allowed values: 0.0000 - 1.0000                                                                                                                                     |
| Minimum valid tricolor counts (Het)                             | 2             | Minimum valid tricolor counts (Het).<br>Allowed values: Integers $\geq 1$                                                                                                                                  |
| <b>Homozygous position filter</b>                               |               |                                                                                                                                                                                                            |
| Minimum coverage (Hom)                                          | 1             | Minimum coverage (Hom).<br>Allowed values: Integers $\geq 1$                                                                                                                                               |
| Minimum count of the non-reference allele (Hom)                 | 2             | Minimum count of the non-reference allele (Hom).<br>Allowed values: Integers $\geq 1$                                                                                                                      |
| Minimum average non-reference base QV (hom)                     | 20            | Minimum average non-reference base QV (Hom).<br>Allowed values: Integers 0-62                                                                                                                              |
| Minimum average non-reference color QV (hom)                    | 7             | Minimum average non-reference color QV (Hom).<br>Allowed values: Integers 0-41                                                                                                                             |
| Minimum unique start position of the non-reference allele (Hom) | 2             | Minimum unique start position of the non-reference allele (Hom).<br>Allowed values: Integers $\geq 1$                                                                                                      |
| <b>Output file processing</b>                                   |               |                                                                                                                                                                                                            |
| Output fasta file                                               | True          | Output or do not output the FASTA file. <ul style="list-style-type: none"> <li>• <b>True:</b> Output the FASTA file.</li> <li>• <b>False:</b> Do not output the FASTA file.</li> </ul>                     |
| Output consensus file                                           | True          | Output or do not output the consensus file. <ul style="list-style-type: none"> <li>• <b>True:</b> Output the consensus file.</li> <li>• <b>False:</b> Do not output the consensus file.</li> </ul>         |
| Compress the consensus file                                     | False         | Compress or do not compress the consensus file. <ul style="list-style-type: none"> <li>• <b>True:</b> Compress the consensus file.</li> <li>• <b>False:</b> Do not compress the consensus file.</li> </ul> |

**(Optional) Annotation**

You can optionally annotate the mapped output of SNP Finding analysis. For descriptions of the SNP Finding analysis parameters, see “Annotation” [on page 178](#).

## Human CNV

Categories of Human CNV parameters include Advanced and (if you selected Annotation for CNV output) Annotation. There are no Main parameters.

### Advanced

There are two categories of Advanced parameters: general and `cnv.stringency.setting`.

| Parameter            | Default Value | Description                                                                                                                                                                                                                                                   |
|----------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Window size          | 5000          | Size of the window block to be considered as a region.<br>Allowed values: Integers $\geq 100$ .                                                                                                                                                               |
| Trim distance        | 1000          | Distance in kilo bases to be trimmed from the extreme ends of the chromosome arms. Allowed values: Integers 0-100000                                                                                                                                          |
| Min quality          | 0             | Minimum quality value of the alignments. Allowed values: Integers 0-99                                                                                                                                                                                        |
| Ploidy               | 2             | General ploidy of the genome. Allowed values: Integers $\geq 1$                                                                                                                                                                                               |
| Ploidy exception     | None          | List of all the contigs whose ploidy is different to the general ploidy of the genome. Entries in the list are in the format {contig id: ploidy of the contig}, and are separated by commas. Use the string "None" to indicate no entries.                    |
| Local normalization  | False         | Whether or not genome-wide normalization or local normalization should be performed. <ul style="list-style-type: none"> <li>• <b>True:</b> Perform chromosome-arm local normalization.</li> <li>• <b>False:</b> Perform genome-wide normalization.</li> </ul> |
| Write coverage       | False         | Create coverage output files <ul style="list-style-type: none"> <li>• <b>True:</b> Create coverage output files, in WIG format.</li> <li>• <b>False:</b> Do not create.</li> </ul>                                                                            |
| Coverage window size | 1000          | Size of the window block to be considered as a region for writing coverage output. The mean coverage of all bases in each of these windows is output.<br>Allowed values: Integers: 1 – 100000                                                                 |

### `cnv.stringency.setting` parameters

| Parameter                  | Default Value | Description                                                                                                                                                                                                                                                                                                                                                                                                                              |
|----------------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Deletions min mappability  | 50            | Minimum mappability percentage for regions to be shown as copy number deletions. Allowed values: 0.000-<100.0000<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this parameter to 25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 0.</li> </ul>       |
| Insertions min mappability | 10            | Minimum mappability percentage for the regions to be shown as copy number insertions. Allowed values: 0.000-<100.0000.<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this parameter to 25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 0.</li> </ul> |

|                       |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|-----------------------|-------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Deletion min windows  | 2                                         | <p>Minimum number of windows for the regions to be shown as copy number deletions. Allowed values: Integers <math>\geq 1</math></p> <p>If this parameter is not specified, and</p> <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this parameter to 4.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 1.</li> </ul>                                        |
| Insertion min windows | 2                                         | <p>Minimum number of windows for the regions to be shown as copy number insertions. Allowed values: Integers <math>\geq 1</math></p> <p>If this parameter is not specified, and</p> <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this parameter to 4.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 1.</li> </ul>                                       |
| Deletion max p-value  | 1.0                                       | <p>Maximum p-value for regions to be shown as copy number deletions. Allowed values: <math>&gt;0.000-1.0000</math>.</p> <p>If this parameter is not specified, and</p> <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this to 0.25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 1.0.</li> </ul>                                                         |
| Insertion max p-value | 1.0                                       | <p>Maximum p-value for regions to be shown as copy number insertions. Allowed values: <math>&gt;0.000-1.0000</math>.</p> <p>If this parameter is not specified, and</p> <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this to 0.25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 1.0.</li> </ul>                                                        |
| Stringency setting    | Medium                                    | High, medium, low                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Deletion max ratio    | 0.5                                       | <p>Maximum ratio between the coverage of the region and the expected coverage, for a region to be called as CNV deletion.</p> <p>Allowed values: <math>&gt;0.000-&lt;1.0000</math>.</p> <p>If this parameter is not specified, and</p> <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then this parameter is set to 0.25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then this parameter is set to 0.7.</li> </ul> |
| Insert min ratio      | 1.25                                      | <p>Minimum ratio between the coverage of the region and the expected coverage, for a region to be called as CNVs insertion.</p> <p>Allowed values: Floats <math>\geq 1.0</math>.</p> <p>If this parameter is not specified, and</p> <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then this parameter is set to 1.75.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then this parameter is set to 1.25.</li> </ul>   |
| Gender                | Male                                      | Set the ploidy of all chromosomes for Human.                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Mappability directory | <code>\${analysis.mappability.dir}</code> | Path to the directory of mappability files.                                                                                                                                                                                                                                                                                                                                                                                                                                                     |

**(Optional)  
Annotation**

You can optionally annotate the mapped output of the analysis. For descriptions of Annotation parameters, see [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on page 317.

**Perform genomic resequencing analysis****(Optional) Import  
data**

You can optionally import data to be analyzed. For instructions on how to import data, see “Import Data” on page 81.

**Log in to  
LifeScope™**

1. Navigate to LifeScope™ Software at `http://<IP address>:<port number>/LifeScope.html` where *IP address* is the address of the system or head node and *port number* is the number of the port used by the server.
2. In the Login screen, enter your username and password, then either click **Login** or press **Enter** to open the LifeScope™ home view (shown on page 75).

**Create or select a  
project**

1. In the home view (shown on page 75), either click **Create a New Project** (described on page 80) or select a project in the Projects organizer (shown on page 75).
2. If you create a project:
  - a. Type a name and description in the Enter Project Name view.  
**Note:** The name cannot have spaces or special characters.
  - b. Click **Create New Project**.
  - c. In the Projects lists, select the new project.
3. In the Task Wizards section (shown on page 75), click **Add Data to Project** to choose a data type.

**Add data to a  
project**

Add data from the read repository to a project by choosing a data type and finding data. You can also optionally group data.

**Note:** Multiple files cannot have the same name. Make sure that the names of the files that you add are distinct.

**Note:** If the LifeScope™ Software administrator has changed the path to the read repository since your last login, restart LifeScope™ Software to update the repository to the new path.

1. In the Add Data to Project window, select **Raw unmapped (XSQ) data**, then click **Next** to find data.
2. In the Read Repository Filter table, select the read-sets you want to map and analyze.  
If you want to group data, click **Next**. If you do not want to group data, skip [step 3](#) and proceed to [step 4](#).

3. (Optional) In the Read-sets in Project table, click the checkbox of the data files you want to group, then click **Add Group to Project**. The files appear in the Groups in Project table.

To rename a group, click the checkbox of the group in the Groups in Project table, then click **Edit**. In the Edit Group window, enter a new name.

To remove a data file from a group, click the checkbox of the data file, then click **Delete**.

4. Click **Add Analysis** to proceed, or
  - Click **Cancel** to refrain from adding data and close the Add Data to Project window, or
  - Click **Finish** to add the data and close the Add Data to Project window.

## Create an analysis

1. In the Choose Data view of the Create Analysis window, select data files from the Available Data in Project table, then click **Next** to choose an analysis.

2. In the Choose Analysis view, enter a name for the analysis. You can optionally describe your project.

If you are re-using an Old Analysis, select **Reuse Old Analysis** and select the name of the analysis you want to use.

**Note:** The name cannot have spaces or special characters.

3. Select **Genomic Resequencing**, then click **Next** to choose references.

4. In the Data To Be Analyzed table, click **Select the reference for the reads** to open the repository file browser.

5. In the Browse for Reference File window, navigate to the location of the reference genome of your sample. Open the folder with your .fasta file, for example:

data ▶ referenceData ▶ lifetech ▶ hg18 ▶ reference ▶ human\_hg18.fasta

Select the file, then click **OK**.

The file name appears in the Reference column.

**Note:** To change the reference file, click on a button in the Reference column, then select another reference file.

6. After you have created the analysis:
  - Click **Edit** to proceed, or
  - Click **Cancel** to refrain from choosing references and close the Create Analysis window, or
  - Click **Finish** to complete analysis creation and close the window.

## Edit the analysis

1. In the Edit Analysis window, accept the default settings for mapping and pre-processing data in the Secondary Analysis section.

If you want to exclude BAMStats, click **Customize**. In the Customize Mapping window, uncheck BAMStats, then click **OK**.

If you do not want to pre-process data, uncheck the box.

- In the Tertiary Analysis section, accept the included Small Indel SNP Finding, Human CNV, and Small Indel modules, and Annotation settings and click **Next** to set module parameters. To include all modules, click the **All>>** button.  
 To exclude a module, select it in the Include column, then click the < button. To exclude all modules, click the **All<<** button.  
 To skip setting module parameters and review the analysis, click **Review**.

### Set module parameters

This section describes the procedures for setting general parameters and parameters for the mapping, Small Indel, SNP Finding, and CNV modules. For descriptions of module parameters, see [“Genomic resequencing analysis parameters” on page 165](#).

You can restore the default settings of parameters by clicking the **Reset to Defaults** button.

To view descriptions of parameters, place your mouse cursor over a  button.

### Set general parameters

- Enter an analysis assembly name.
- If your analyses include the enrichment module, set the `analysis.regions.file` parameter.  
 Click the  buttons to open the File Chooser, navigate to reference files, and choose files.
- Set the `annotation.dbsnp` and `annotation.gtf.file` parameters.  
 Click the  buttons to open the File Chooser, navigate to reference files, and choose files.  
 Verify that the file path to the `annotation.gtf` file is correct. An incorrect file path will result in a failed analysis.
- Set `analysis.space` to your preference of whether analysis should be done in base space or in color space. This setting should be the same for all modules in your run. For more information, see [“Choose analysis space” on page 98](#).
- Always leave the default `analysis.platform` setting, which is “SOLID”.
- If you want to accept the default parameters for all modules, click **Review**. If you want to edit module parameters, click **Next**.

### Set mapping parameters

- There are three categories of mapping parameters: Main, Advanced, and BAMStats. Accept the default settings or click the Main, Advanced, and BAMStats tabs to edit the settings.
- Click **Next** to edit Small Indel parameters.

### Set Human CNV parameters

- There are two categories of SNP Finding parameters: Advanced, and Annotation. There are no Main parameters. Accept the default settings or edit the settings.
- After you have edited module parameters:

- Click **Review** to proceed, or
- Click **Cancel** to erase your edits and close the Edit Analysis window, or
- Click **Finish** to save your edits and close the Edit Analysis window.

#### Set SNP Finding parameters

1. There are three categories of SNP Finding parameters: Main, Advanced, and Annotation. Accept the default settings or edit the settings.
2. Click **Next** to edit CNV parameters.

#### Set Small Indel parameters

1. There are three categories of Small Indel parameters: Main, Advanced, and Annotation. Accept the default settings or edit the settings.
2. Click **Next** to edit SNP Finding parameters.

### Review and run the analysis

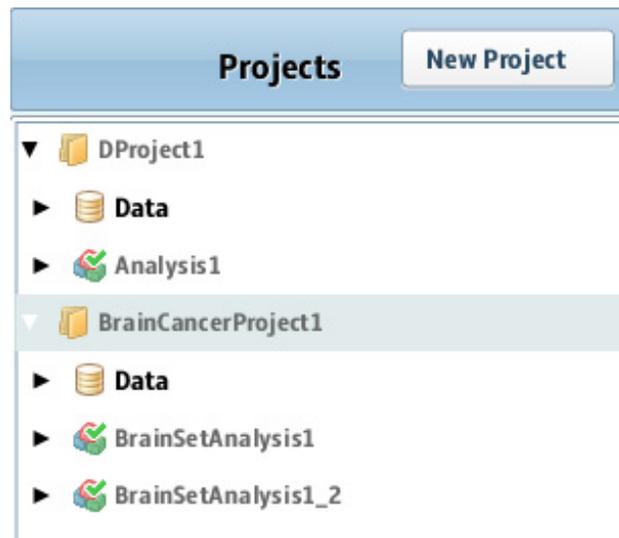
**Note:** For a description of genomic resequencing parameters, see [“Genomic resequencing analysis parameters” on page 165](#).

The Review Analysis view in the Run Analysis window includes two tabs: Parameters and Data. In the Parameters tab, click the  , next to parameter categories to show or hide the parameters.

1. Review the parameters. To edit the parameters, click **Edit**.
2. To review the data that will be analyzed, click the **Data** tab.
3. If you are ready to run the analysis, click **Start Analysis**.  
The window closes.

### Checking Analysis Status

1. In the Projects organizer (shown [on page 75](#)), click the project to check the status of the analysis run.



2. Click the analysis name to show details about the analysis in the status overview (shown on page 75).
3. Click the Status tab to view the Progress column, which shows the percentage of completion for the Analysis.

**LiverCancerProject1\_042511**

Overview **Status**

Analysis Runs

| name                    | XSQ ID                    | Analysis          | Run Start Time        | Secondary Analysis | Secondary Progress | Te |
|-------------------------|---------------------------|-------------------|-----------------------|--------------------|--------------------|----|
| en_20100402_2_10103F... | helen_20100402_2_10103... | BrainSetAnalysis1 | 2011-02-09 17:00:00.0 | [saet]             | 100%               |    |

When analysis has been completed, a green check mark appears on the Analysis name.

4. Click the Analysis icon to see the overview of the results.
5. Click the **View Results in the Secondary Progress** box to open the View Results window.
6. Click each module step to see results in the tab sections.
7. Click each tab to view various results.
8. Close the View Results window to return to the Home view.

## View analysis results

See [Chapter 9, “View Analysis Results” on page 103](#) for instructions on how to review analysis results.

### View results in a genome browser

Output files generated by Genomic Resequencing runs are compatible with third-party browser such as the Integrative Genomics Viewer (IGV) available from the Broad Institute and the UCSC Genome Browser. See [“Pairing information in a BAM file” on page 348](#) for information on using LifeScope™ Software output files with genome browsers.

## Genomic resequencing analysis output files

See the following chapters for output files generated by a genomic resequencing analysis:

- [Chapter 18, “Perform Human Copy Number Variation Analysis” on page 263](#)
- [Chapter 20, “Perform SNP Finding Analysis” on page 289](#)
- [Chapter 23, “Perform Small Indel Analysis” on page 307](#)

## 13

# Perform Small RNA Analysis

This chapter covers:

|                                                |     |
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| ■ Small RNA analysis library types . . . . .   | 183 |
| ■ Small RNA analysis input files . . . . .     | 183 |
| ■ Small RNA analysis modules . . . . .         | 186 |
| ■ Small RNA analysis parameters . . . . .      | 187 |
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| ■ Small RNA output files . . . . .             | 195 |

## Introduction to Small RNA analysis

LifeScope™ Genomic Analysis Software small RNA analysis modules are used to analyze high throughput small RNA sequencing raw data. These modules encapsulate a workflow to map reads to the target genome, to detect small RNAs in the genome, and to determine the expression of the detected small RNAs.

## Small RNA analysis library types

Small RNA analysis supports the fragment library type.

## Small RNA analysis input files

Small RNA analysis requires input for the Mapping, Coverage, and Counts modules:

### Mapping input files

The small RNA mapping module requires the following input:

- One or more XSQ files containing sequencing data.
- A Genome Coordinates General Feature Format (GFF) file containing precursor sequences with genomic locations. This file is available at:  
<http://www.mirbase.org/ftp>
- A single multi-fasta reference file for the filter mapping step.
- A single multi-fasta reference file for the genome mapping step.

The mapping modules accepts as input one or more input XSQ files.

## RNA-Seq reads

The RNA-Seq reads used in the small RNA modules are different from the genomic reads. For RNA-Seq reads:

- Only transcribed sequences are measured by the system.
- Genome coverage is non-uniform due to variation in transcriptional intensity.
- A large subset of reads originate from uninteresting sequences such as ribosomal RNA (rRNA).
- A subset of the reads originates from splice junctions and cannot align contiguously on the genome.

## Reads input specification

Plan your analysis input carefully. The way you define your reads input affects the behavior of your analysis. The following factors control how your input data is analyzed:

- **Index (barcode) IDs** – Using an index ID restricts your input to the reads data of one or more indices.
- **Grouping of reads** – Each group of reads is analyzed together as one specimen. The output data for a group is combined into one set of results files.
- **Multiple sample runs** – Unrelated reads can be processed together in one run of LifeScope™, but analyzed separately as separate input data.

See [“Define input data” on page 71](#) for more information on designing how to add input data to your analysis.

Mapping one tag of paired data is not supported.

## Legacy data

If the data you want to process with LifeScope™ is in CSFASTA and QUAL files, these files must be converted to the XSQ file format. The LifeScope™ graphical user interface automatically converts these files to the XSQ format before mapping data.

## Coverage input files

The small RNA coverage module requires one or more BAM files containing mapped data. The following describe the alignment input accepted. This module accepts:

- Only fragment data for RNA libraries.
- Multiple BAM files as input.
- BAM files with different read lengths.
- Both color space and base space files, and a combination of color and base space data.

## Counts input files

The small RNA counts module uses the following input:

- One or more BAM files containing mapped data.
- A Genome Coordinates GFF file containing precursor sequences with genomic locations.
- *(Optional)* A file containing mature form sequences with locations relative to precursor sequences. If the mature sequences file is not provided as input, the module compute counts per precursor sequence.

These files are described in the following sections: alignment files, precursor sequences, and mature form sequences.

### BAM file metadata

The following table lists BAM file metadata used by the small RNA counts module.

| Field name    | Field in BAM header                                                                                    | Requirement                                                                                                                                                      |
|---------------|--------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Library Type  | The LT field in the @CO line for every @RG line. (Every @RG read group line has one @CO comment line.) | @RG lines must be present. Every @CO line must have an LT field, and the LT field must be set to the string "Fragment".                                          |
| Sequence Name | The SN field in each @SQ header line.                                                                  | Only contigs with an @SQ line with an SN field are analyzed. If a contig does not have an @SQ line in the header, the alignments in that contig are not counted. |
| Sort Order    | The SO field in the HD header line.                                                                    | The SO field must be present and must be set to the string "Chromosome".                                                                                         |

### Precursor sequences

This file is a Genome Coordinates GFF file containing precursor sequences with genomic locations. You must use the same file that was used during the small RNA mapping step. This file is available the following link:

<http://www.mirbase.org/ftp>

### Mature form sequences

The mature form sequences file provides locations relative to precursor sequences. This tab-separated file is available the following link:

<ftp://mirbase.org/pub/mirbase/CURRENT/miRNA.xls>

Only this specific mature forms file is supported.

If the mature sequences file is not provided as input, the module compute counts per precursor sequence.

## Small RNA analysis modules

Small RNA analysis includes the following modules:

| Secondary Analysis Module | Used for Library Type |
|---------------------------|-----------------------|
| Small RNA Mapping         | Fragment              |
| BAMStats                  |                       |

| Tertiary Analysis Module      | Used for Library Type |
|-------------------------------|-----------------------|
| Small RNA Mapping             | Fragment              |
| Small RNA Filtered BAM Counts |                       |
| Small RNA Counts              |                       |
| Coverage                      |                       |

### Small RNA Mapping

The small RNA mapping module maps small RNA (also known as microRNA or miRNA) reads using the mapping tool mapReads. This module maps the small RNA reads to three different references in three steps:

- In the first step, the given set of reads are mapped to filter sequences in order to eliminate the reads generated from irrelevant sources (such as tRNA, adaptor sequences, or others).
- In the second step, the remaining reads from the first step are mapped to the list of known small RNA precursor sequences (miRBase annotations) downloaded from Sanger's web site.
- In the third step, the unmapped reads from the second step are mapped to the genome reference sequence, in order to find novel small RNAs in the sample. The mapped reads outputs from the second and third steps are merged, and the merged file is the primary output of the small RNA mapping module.

The major components of this module are:

1. Filter reference mapping
2. miRBase reference mapping
3. Genome reference mapping
4. Merging of mapped reads output (MA files)

Parameters are provided to skip any of the three mapping steps.

Usual read lengths from small RNA sequencing are 35–50 bases, and the length of the miRNA fragments is only 18–28 bp. The reads contain both the miRNA sequence and a P2 adaptor sequence at the end. The mapReads phase does an extra step of extension onto adaptor, to correctly remove the adaptor sequence from the read and to report only small RNA sequences in the BAM file.

After the miRBase mapping step, the mapping output file (a MA file) has alignments with respect to the smaller miRBase reference locations. Before merging the MA files, the program converts these miRBase alignments to genomic reference locations, using the input GFF file (described in [“.gtf file format” on page 196](#)).

The mapping output files from miRBase mapping and genome mapping do not contain any common reads (common bead ids).

**Coverage** The small RNA coverage module calculates read coverage per position.

**Counts** The LifeScope™ small RNA counts module in calculates counts per precursor or mature sequence, where counts are the number of reads mapped. This module is similar to the WT counttags module, which computes counts per exon.

## Small RNA analysis parameters

Parameters for small RNA analysis include:

- General parameters
- **Secondary analysis:** Small RNA Mapping, BAMStats
- **Tertiary analysis:** Small RNA Mapping, Small RNA Filtered BAM Counts, Small RNA Counts, and Coverage

**General** General parameters include:

| Parameter name                  | Default value                                                          | Description                                                                                                                                                           |
|---------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| analysis.assembly.name          | hg19                                                                   | Name of the genome assembly used in current analysis. Examples are hg18 and hg19.                                                                                     |
| analysis.mirbase.precursor.file | /data/results/referenceData/lifetech/hg19/mirbase/hsa.gff              | File containing precursor microRNA database annotations. Set this parameter to the miRBase GFF file for your organism of choice.                                      |
| analysis.mirbase.mature.file    | /data/results/referenceData/external/hg19/mirbase/miRNA.txt            | File containing mature form microRNA database annotations. This file is a tab-delimited text file version of miRBase's mirRNA.xls ( <i>not</i> miRBase's mirRNA.txt). |
| analysis.filter.reference       | /data/results/referenceData/lifetech/hg19/human_filter_reference.fasta | File containing a collection of sequences that are used to filter out commonly-occurring motifs and contaminating sequences prior to mapping.                         |
| analysis.sample.name            | —                                                                      | User-defined sample name.                                                                                                                                             |
| analysis.platform               | SOLID                                                                  | Always specify “SOLID”. Other values are reserved for future use.                                                                                                     |

**Small RNA Mapping** There are no Main parameters for the Small RNA Mapping module. Advanced parameters include:

| Parameter name                                | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-----------------------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>01 Mapping</b>                             |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Mapping in base space                         | False         | Include base space data in the mapping.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Include base space data in mapping.</li> <li>• <b>False:</b> Do not include base space data in mapping.</li> </ul>                                                                                                                                                                                                                                                              |
| Filter mapping                                | True          | Turn filter mapping on or off.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Turn on filter mapping.</li> <li>• <b>False:</b> Turn off filter mapping.</li> </ul>                                                                                                                                                                                                                                                                                                     |
| miRBase mapping                               | True          | Turn miRBase mapping on or off.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Turn on miRBase mapping.</li> <li>• <b>False:</b> Turn off miRBase mapping.</li> </ul>                                                                                                                                                                                                                                                                                                  |
| Genome mapping                                | True          | Turn genome mapping on or off.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Turn on genome mapping.</li> <li>• <b>False:</b> Turn off genome mapping.</li> </ul>                                                                                                                                                                                                                                                                                                     |
| <b>02 BAM Conversion</b>                      |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Reference correction base filter QV threshold | 10            | Bases with a quality value below the value of this parameter are replaced with 'N'.<br>Allowed values: Integers 0–255.                                                                                                                                                                                                                                                                                                                                                                           |
| Reference weight                              | 8             | This parameter is used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. This parameter adds weight (in terms of a Phred score) to the signals that are compatible with the reference. Color combinations that result in a variant are considered compatible with the reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation.<br>Allowed values: Integers 0–100. |
| Create unmapped BAM files                     | False         | Create or do not create BAM files with unmapped reads.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Create.</li> <li>• <b>False:</b> Do not create.</li> </ul>                                                                                                                                                                                                                                                                                                       |
| BAM generation mapping QV threshold           | 0             | Provide control over the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer.<br>Allowed values: Integers 0–100.                                                                                                                                                                                                                                                             |

| Parameter name                                            | Default value | Description                                                                                                                                                                               |
|-----------------------------------------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| BAM generation reference correction<br>add color sequence | True          | Add or do not add color sequence in BAM records. <ul style="list-style-type: none"> <li>• <b>True:</b> Add color sequence.</li> <li>• <b>False:</b> Do not add color sequence.</li> </ul> |

**Small RNA Counts** There are no Main parameters for the Small RNA Counts module. Advanced parameters include:

| Parameter name          | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                               |
|-------------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Counts min quality      | 2             | The mapping quality value threshold for selecting the alignments. Alignments with a mapping quality value lower than this threshold are not written to output.<br>Allowed values: Integers 0–100.                                                                                                                                                                                                                         |
| Counts per feature      | True          | Controls the aggregated count output. The primary key in the output GTF file is: {Feature, start, end}.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Reads of different starts and ends are counted separately for every feature and are output on separate lines.</li> <li>• <b>False:</b> The aggregated count of all the reads mapped to a feature are output on the same line.</li> </ul> |
| Counts primary only     | True          | Use only primary alignments or use all the alignments from the input BAM file as input.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Consider only primary alignments (both gapped and ungapped).</li> <li>• <b>False:</b> Consider all alignments.</li> </ul>                                                                                                                                |
| Coverage overflow limit | 3             | The maximum allowed offset on either side of an alignment or feature, when looking for overlap between an alignment and a feature. If this limit is set to 0, then the start and end coordinates of the alignment record and feature must exactly match.<br>Allowed values: Integers 0–20.                                                                                                                                |

**Coverage** There are no Main parameters for the Coverage module. Advanced Coverage parameters include:

| Parameter name       | Default value | Description                                                                                                                                                                                                         |
|----------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Coverage min quality | 2             | <i>[Optional]</i> The mapping quality value threshold for selecting the alignments. Alignments with a mapping quality value lower than this threshold are not written to output.<br>Allowed values: Integers 0–100. |

| Parameter name          | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                  |
|-------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Coverage per chromosome | True          | <i>(Optional)</i> Whether coverage output is generated as one file per every chromosome per strand, or as a single file with coverage of all chromosomes per strand.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Generate one file per every chromosome per strand.</li> <li>• <b>False:</b> Generate a single file with coverage of all chromosomes per strand.</li> </ul> |
| Coverage primary only   | True          | <i>(Optional)</i> Whether only primary alignments or all the alignments from the input BAM file are used as input.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Consider only primary alignments (both gapped and ungapped).</li> <li>• <b>False:</b> Consider all alignments.</li> </ul>                                                                                    |
| Coverage min value      | 0             | <i>(Optional)</i> The mapping quality threshold value for selecting alignments from the input BAM. In order to be included in the output, a position's coverage must be greater than or equal to this value.<br><br>Allowed values: Integers $\geq 0$ .                                                                                                                                                      |

### Small RNA Filtered BAM Counts

There are no Main parameters for the Small RNA Filtered BAM Counts module. Advanced parameters include:

| Parameter name      | Default value | Description                                                                                                                                                                                                                                         |
|---------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Counts primary only | True          | <i>(Optional)</i> Count only primary alignments from the BAM file or count all alignments from the input BAM file as input.<br><br><b>True:</b> Count only primary alignments (both gapped and ungapped).<br><b>False:</b> Consider all alignments. |

### Small RNA Filtered BAMStats

There are two categories of Small RNA Filtered BAMStats parameters: Main and Advanced.

#### Main

| Parameter name   | Default value | Description                                                                                                                                                                                                            |
|------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Maximum coverage | 10,000        | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br><br>Allowed values: Integers $\geq 0$ . |

| Parameter name                                                           | Default value                              | Description                                                                                                                                                                                                                                                                                                     |
|--------------------------------------------------------------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Whether to combine data from both the strands for coverage in WIG format | 1                                          | Combine or do not combine data from both strands for coverage in WIG format.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: Do not generate wig files.</li> <li>• <b>1</b>: Generate the combined coverage file.</li> </ul>                                                          |
| <b>Advanced</b>                                                          |                                            |                                                                                                                                                                                                                                                                                                                 |
| Parameter name                                                           | Default value                              | Description                                                                                                                                                                                                                                                                                                     |
| Input directory for BAMStats                                             | `\${analysis.output.dir}/fragment.mapping` | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample.                                                                                                                                                                                            |
| Output directory                                                         | `\${task.output.dir}`                      | The path to the output directory where BAMStats will write its chart (.cht) files.                                                                                                                                                                                                                              |
| Primary alignments only for coverage in WIG file format                  | 1                                          | Use only primary alignments for coverage in WIG file format.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: Do not restrict coverage in WIG file format to only primary alignments.</li> <li>• <b>1</b>: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Bin size for coverage in WIG file format                                 | 100                                        | The bin size for coverage in WIG file format.<br><br>Allowed values: Integers 1-100,000.                                                                                                                                                                                                                        |

## Perform Small RNA analysis

### (Optional) Import data

You can optionally import data to be analyzed. For instructions on how to import data, see “Import Data” [on page 81](#).

### Create or select a project

1. In the Home view (shown [on page 75](#)), either click **Create a New Project** (described [on page 80](#)) or select a project in the Projects organizer (shown [on page 75](#)).
2. If you create a project:
  - a. Type a name and description in the Enter Project Name view.  
**Note:** The name cannot have spaces or special characters.
  - b. Click **Create New Project**.
  - c. In the Projects lists, select the new project.
3. In the Task Wizards section (shown [on page 75](#)), click **Add Data to Project** to choose a data type.

**Add data to a project**

Add data from the read repository to a project by choosing a data type and finding data. You can also optionally group data.

**Note:** Multiple files cannot have the same name. Make sure that the names of the files that you add are distinct.

**Note:** If the LifeScope™ Software administrator has changed the path to the read repository since your last login, restart LifeScope™ Software to update the repository to the new path.

1. In the Add Data to Project window, select **Raw unmapped (XSQ) data**, then click **Next** to find data.
2. In the Read Repository Filter table, select the read-sets you want to map and analyze.  
If you want to group data, click **Next**. If you do not want to group data, skip [step 3](#) and proceed to [step 4](#).
3. (*Optional*) In the Read-sets in Project table, click the checkbox of the data files you want to group, then click **Add Group to Project**. The files appear in the Groups in Project table.  
To rename a group, click the checkbox of the group in the Groups in Project table, then click **Edit**. In the Edit Group window, enter a new name.  
To remove a data file from a group, click the checkbox of the data file, then click **Delete**.
4. Click **Add Analysis** to proceed, or
  - Click **Cancel** to refrain from adding data and close the Add Data to Project window, or
  - Click **Finish** to add the data and close the Add Data to Project window.

**Create an analysis**

1. In the Choose Data view of the Create Analysis window, select data files from the Available Data in Project table, then click **Next** to choose an analysis.
2. In the Choose Analysis view, enter a name for the analysis. You can optionally describe your project.  
If you are re-using an Old Analysis, select **Reuse Old Analysis** and select the name of the analysis you want to use.  
**Note:** The name cannot have spaces or special characters.
3. Select **Small RNA**, then click **Next** to choose references.

4. In the Data To Be Analyzed table, click **Select the reference for the reads** to open the repository file browser.
5. In the Browse for Reference File window, navigate to the location of the reference genome of your sample. Open the folder with your .fasta file, for example:  
data ▶ referenceData ▶ lifetech ▶ hg18 ▶ reference ▶ human\_hg18.fasta  
Select the file, then click **OK**.  
The file name appears in the Reference column.

**Note:** To change the reference file, click on a button in the Select the reference for the reads column, then select another reference file.

6. After you have created the analysis:
  - Click **Edit** to proceed, or
  - Click **Cancel** to refrain from choosing references and close the Create Analysis window, or
  - Click **Finish** to complete analysis creation and close the window.

## Edit the analysis

1. In the Edit Analysis window, accept the Secondary Analysis default settings.
2. In the Tertiary Analysis section, you can include an analysis module by selecting it in the Available Modules column, then clicking the > button. To include all modules, click the **All>>** button.  
To exclude a module, select it in the Include column, then click the < button. To exclude all modules, click the **All<<** button.
3. Click **Next** to set module parameters.

## Set module parameters

This section describes the procedures for setting general parameters and parameters for mapping, see [“Genomic resequencing analysis parameters” on page 165](#).

You can restore the default settings of parameters by clicking the **Reset to Defaults** button.

To view descriptions of parameters, place your mouse cursor over a  button.

## Set general parameters

1. Enter an analysis assembly name. and analysis sample name.
2. Set the parameters if necessary. The fields will be automatically populated if the structure of the reference directories is correctly set.
  - analysis.mirbase.precursor.file
  - analysis.mirbase.mature.file
  - analysis.filter.reference

Click the  buttons to browse directories and choose files.

3. Always leave the default analysis.platform setting, which is “SOLID”.
4. If you want to accept the default parameters for all modules, click **Review**. If you want to edit module parameters, click **Next** to set Small RNA Mapping parameters.

## Set Small RNA Mapping parameters

1. There are two categories of Small RNA Mapping parameters: Main and Advanced. Accept the default settings or click the Main and Advanced tabs to edit the settings.
2. Click **Next** to edit Small RNA Counts parameters.

Set Small RNA Counts parameters

1. Accept the default settings of the Advanced parameters or edit the settings. There are no Main parameters.
2. Click **Next** to edit Coverage parameters.

Set Coverage parameters

1. There are two categories of Coverage parameters: Main and Advanced. Accept the default settings or edit the settings.
2. Click **Next** to edit Small RNA Filter Mapping parameters.

Set Small RNA Filter Mapping parameters

1. There are two categories of Small RNA Filter Mapping parameters: Main and Advanced. Accept the default settings or click the Main and Advanced tabs to edit the settings.
2. Click **Next** to edit Small RNA Filtered BAMStats parameters.

Set Small RNA Filtered BAMStats parameters

1. There are two categories of Small RNA Filtered BAMStats parameters: Main and Advanced. Accept the default settings or click the Main and Advanced tabs to edit the settings.
2. After you have edited module parameters:
  - Click **Review** to proceed, or
  - Click **Cancel** to erase your edits and close the Edit Analysis window, or
  - Click **Finish** to save your edits and close the Edit Analysis window.

## Review and run the analysis

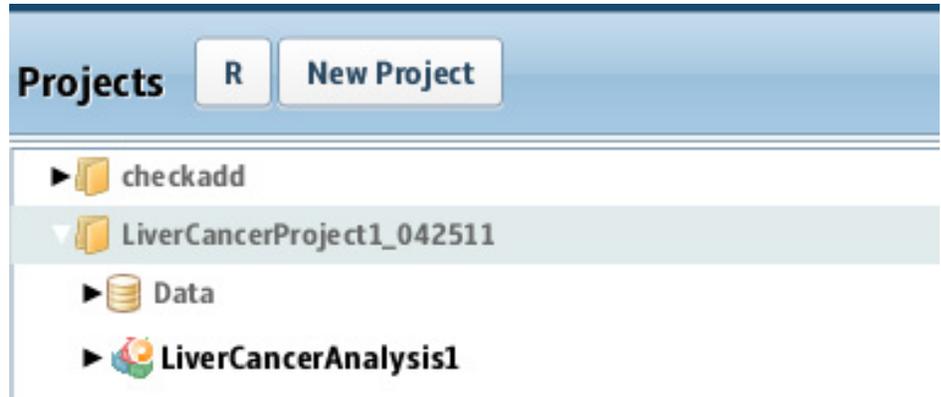
**Note:** For a description of genomic resequencing parameters, see [“Genomic resequencing analysis parameters” on page 165](#).

The Review Analysis view in the Run Analysis window includes two tabs: Parameters and Data. In the Parameters tab, click the  , next to parameter categories to show or hide the parameters.

1. Review the parameters. To edit the parameters, click **Edit**.
2. To review the data that will be analyzed, click the **Data** tab.
3. If you are ready to run the analysis, click **Start Analysis**.  
The window closes.

## Checking Analysis Status

1. In the Projects organizer (shown [on page 75](#)), click the project to check the status of the analysis run.



2. Click the analysis name to show details about the analysis in the status overview (shown on page 75).
3. Click the Status tab to view the Progress column, which shows the percentage of completion for the Analysis.

When analysis has been completed, a green check mark appears on the Analysis name.

4. Click the Analysis icon to see the overview of the results.
5. Click the **View Results in the Secondary Progress** box to open the View Results window.
6. Click each module step to see results in the tab sections.
7. Click each tab to view various results.
8. Close the View Results window to return to the Home view.

## View analysis results

See [Chapter 9, “View Analysis Results” on page 103](#) for instructions on how to review analysis results.

### View results in a genome browser

Output files are compatible with third-party browser such as the Integrative Genomics Viewer (IGV) available from the Broad Institute and the UCSC Genome Browser. See [“Pairing information in a BAM file” on page 348](#) for information on using LifeScope™ Software output files with genome browsers.

## Small RNA output files

### Mapping output files

The output of Small RNA mapping is a BAM file.

## Coverage output files

The small RNA coverage module's output files are in the wiggle (\*.wig) file format. Wiggle files are visualized in genomics browsers such as the Integrative Genomics Viewer (IGV). You can download the IGV browser from the Broad Institute web site:

[www.broadinstitute.org/igv](http://www.broadinstitute.org/igv)

The wiggle file specification is available from this site:

<http://genome.ucsc.edu/goldenPath/help/wiggle.html>

If the parameter `RNA.coverage.per.chromosome` is set to `false`, the small RNA coverage module generates two output files with coverage of all chromosomes per strand:

- `coverage_positive.wig`
- `coverage_negative.wig`

If the parameter `RNA.coverage.per.chromosome` is set to `true`, the small RNA coverage module generates one coverage file per chromosome per strand:

- `coverage_chr**_positive.wig`
- `coverage_chr**_negative.wig`

## Counts output files

The Small RNA counts module creates .gtf files containing the counts per feature, in precursor or mature form.

This is example content from the counts.gtf file:

```
chr1    mirBase hsa-miR-4251 3044539 3044599 0 - .
Precursor_ID "hsa-mir-4251"; RPM "0.00";
```

### .gtf file format

The following table provides descriptions of the Small RNA Counts General Features Format (GFF) output file format.

| Column name | Description                                                                         | Examples                                                  |
|-------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------|
| ##          | Header comment lines.                                                               | —                                                         |
| #           | Header of the results.                                                              | —                                                         |
| seqname     | The name of the sequence. Must be a chromosome or a scaffold.                       | chr1                                                      |
| source      | The program that generated this feature.                                            | miRBase                                                   |
| feature     | The name of this type of feature.                                                   | start_codon<br>stop_codon<br>exon<br>has-mir-<br>has-miR- |
| start       | The starting position of the feature in the sequence. The first base is numbered 1. | 3044539                                                   |
| end         | The ending position of the feature (inclusive).                                     | 3044599                                                   |
| score       | The tag count for the feature.                                                      | 0                                                         |

| Column name | Description                                                                                                                                                                                                                                                                                                          | Examples                                    |
|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| strand      | The strand containing the feature. Allowed values: <ul style="list-style-type: none"> <li>'+' : The feature is on the 3' strand.</li> <li>'-' : The feature is on the 5' strand.</li> <li>':' : Unknown or not required.</li> </ul>                                                                                  | -                                           |
| frame       | If the feature is a coding exon, frame is a number representing the reading frame of the first base. If the feature is not a coding exon, the value is ':'. Allowed values: <ul style="list-style-type: none"> <li>0, 1, 2: The feature is a coding exon.</li> <li>':': The feature is not a coding exon.</li> </ul> | .                                           |
| attributes  | A list of attributes delimited by semi-colons. Each attribute is a type/value pair, with type and value separated by a single space character. Type is a string matching the pattern [A-Za-z1-9_]+. Value is a number or a double-quoted string.                                                                     | Precursor_ID "hsa-mir-4251";<br>RPM "0.00"; |

Additional output includes "Reads Per Million RPM" for every feature defined as:  
 $RPM \text{ of a feature} = \#count \text{ of feature} * \#Total \text{ reads aligned to Sanger GFF file} / 1000000.$

## Mapping statistics output files

This section describes the mapping statistics files generated by the BAMStats module. When statistics reports are separated by tag type, the report's file name includes the tag in the file name. For small RNA analyses, the tag name is F3.

The output files generated by BAMStats include the name of the BAM file. The file name pattern is:

*BAMFileName-fileID-barcodeID.StatisticsReportName.tag.extension*

This string is referred to by *prefix* in the following table, the mapping statistics output table. In the description of mapping statistics output file names in the following table, the string *tag* is used to refer to the tags in the following table.

| Report                               | Description, axis information, filename                                                                                                                                                                                                                                                     |
|--------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Alignment Length Distribution</b> |                                                                                                                                                                                                                                                                                             |
|                                      | A bar plot giving the distribution of alignment lengths found in various tags used in alignment. The report is separated by tag type to provide visibility into the accuracy of individual tags.<br>Only the primary alignment for each bead is considered in calculating the distribution. |
|                                      | Y axis: Frequency<br>X axis: Alignment length (from 0 to the maximum read length)                                                                                                                                                                                                           |
|                                      | Output file name: <i>prefix</i> .Alignment.Length.Distribution. <i>tag</i> .cht                                                                                                                                                                                                             |
| <b>Base Mismatch Distribution</b>    |                                                                                                                                                                                                                                                                                             |

| Report                                                                   | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                          | <p>A bar plot giving the distribution of total number of mismatches found in various tags used in alignment. The bins are summed over all alignment lengths.</p> <p>Only the primary alignment for each bead is considered in calculating the distribution.</p> <p>Y axis: Frequency<br/>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)</p> <p>Output file name: <i>prefix.BaseMismatch.Distribution.tag.cht</i></p>                                                                                                                                                                                                                                                                                                                                          |
| <b>Distribution of Alignment Length and Number of Mismatches in Tags</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                          | <p>A report providing a simultaneous picture of the alignment length and number of mismatches distributions in various tags used in alignment. Only primary alignments for each tag are considered in calculating this distribution. The bins range from 0 to the maximum read length, and from 0 to the maximum number of mismatches allowed.</p> <p><b>Note:</b> This report is located in the <code>Misc</code> folder, and is not displayed in the UI.</p> <p>Y axis: Alignment length (from 0 to the max read length)<br/>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)<br/>Z axis: Frequency</p> <p>Output file name: <i>prefix.AlignmentLength.Mismatch.tag.csv</i></p>                                                                               |
| <b>Distribution of Mismatches by Position</b>                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                          | <p>A bar plot providing a distribution of errors (mismatches to reference) by position within the read. Only the primary alignments for each bead are used to generate this distribution.</p> <p>The base-space report is generated on every run.</p> <p>Y axis: Frequency<br/>X axis: Position (from 0 to maximum read length)</p> <p>Output file name: <i>prefix.Mismatches.By.Position.BaseSpace.tag.cht</i>, and (only if color-space data is present in the BAM file) <i>prefix.Mismatches.By.Position.ColorSpace.tag.cht</i></p>                                                                                                                                                                                                                                                 |
| <b>Color Mismatch Distribution</b>                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                          | <p>A bar plot providing a distribution of the total number of color mismatches in various tags used in alignment. Only the primary alignments for each bead are used to generate this distribution. This report is only generated if mapping is done in color space.</p> <p>Y axis: Frequency<br/>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)</p> <p>Output file name: <i>prefix.ColorMismatches.Distribution.tag.cht</i></p>                                                                                                                                                                                                                                                                                                                              |
| <b>Distribution of Alignment Length and Color Mismatches</b>             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                          | <p>A report providing a simultaneous picture of the alignment length and number of color mismatches distributions in various tags used in alignment. Only primary alignments for each tag are considered in calculating this distribution. The bins range from 0 to the maximum read length, and from 0 to the maximum number of mismatches allowed. This report is only generated if mapping is done in color space.</p> <p><b>Note:</b> This report is located in the <code>Misc</code> folder, and is not displayed in the UI.</p> <p>Y axis: Alignment length (from 0 to the max read length)<br/>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)<br/>Z axis: Frequency</p> <p>Output file name: <i>prefix.AlignmentLength.ColorMismatches.tag.csv</i></p> |
| <b>Base QV Distribution</b>                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

| Report                                                         | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                | <p>A bar plot providing a distribution of base quality values generated using the reference assisted error correction/base conversion algorithm. The base QV distributions are separated for each tag as quality of individual tags could be very different.</p> <p>Y axis: Frequency<br/>X axis: Base QV (from 0 to the maximum QV)</p> <p>Output file name: <i>prefix.BaseQV.tag.cht</i></p>                                                                                                                                                                                                                         |
| <b>Base QVs by Position</b>                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A report providing a distribution of base quality values by individual base positions. This report identifies if certain base positions (particularly towards the end of the read) have poor base quality values. All the tags (F3/R3/F5-P2) used in the alignment are combined into a single bin.</p> <p>Y axis: Base QV<br/>X axis: Base position (from 0 to read length)<br/>Z axis: Frequency</p> <p>Output file name: <i>prefix.BaseQV.by.Position.csv</i></p>                                                                                                                                                 |
| <b>Distribution of Mismatches by Base QV</b>                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A line plot providing a distribution of errors (mismatches to reference) by base quality values bins.</p> <p>Y axis: Percentage of total mismatches<br/>X axis: Base QV (from 0 to maximum QV)</p> <p><b>Note:</b> This report gives, for each QV, a measure of the raw mismatches at positions with that QV. This information does not measure whether the base QVs generated are well calibrated to the probability of error in that particular base position.</p> <p>Output file name: <i>prefix.Mismatches.By.BaseQV.tag.cht</i></p>                                                                            |
| <b>Distribution by Mapping QVs by Tag</b>                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A bar plot providing a distribution of mapping quality values for individual tags (F3/R3/F5-P2). Only the primary alignment for each bead is used in calculating this distribution.</p> <p>Y axis: Frequency<br/>X axis: Mapping QV (from 0 to maximum mapping QV)</p> <p>Output file name: <i>prefix.MappingQV.tag.cht</i></p>                                                                                                                                                                                                                                                                                     |
| <b>Coverage Report</b>                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A line plot providing a distribution of coverage obtained after mapping/pairing. Only the primary alignment for each bead is used in this calculation.</p> <p>Y axis: Frequency<br/>X axis: Coverage (from 0 to number of reads)</p> <p>Output file name: <i>prefix.Coverage.cht</i></p>                                                                                                                                                                                                                                                                                                                            |
| <b>Coverage Report by Chromosome (Contig) and Base Windows</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A line plot providing a distribution of coverage within each reference window. The coverage is calculated within each window along a reference chromosome. The window size can theoretically be anywhere from a single base to the contig length. However the calculations of base level coverage are computationally expensive and less interpretable as the window size increases. Only the primary alignment for each bead is used in this calculation.</p> <p>Y axis: Frequency<br/>X axis: Coverage (from 0 to number of reads)</p> <p>Output file name: <i>prefix.Coverage.By.Chromosome.contignn.cht</i></p> |

| Report                        | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Coverage by Strand</b>     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                               | A line plot providing the distribution of coverage within each reference window separated by reference strand (+/-). Only the primary alignment for each bead is used in this calculation.                                                                                                                                                                                                                                                                                   |
|                               | Y axis: Frequency<br>X axis: Coverage (from 0 to number of reads)                                                                                                                                                                                                                                                                                                                                                                                                            |
|                               | Output file name: <i>prefix.Coverage.By.Strand.Positive.cht</i> , <i>prefix.Coverage.By.Strand.Negative.cht</i>                                                                                                                                                                                                                                                                                                                                                              |
| <b>Coverage files</b>         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                               | Coverage reports in wiggle format. For each genome position, reports the number of reads that cover (map to or span) the position. Because reporting coverage for each position results in very large files, coverage is reported for bins, with each bin spanning a user-defined number of bases. For each bin, the mean coverage of all the positions in that bin is reported. The parameter <code>bamstats.wig.binsize</code> controls the size of the bins in this file. |
|                               | Each coverage file includes a header as the first line. The header lines follow this pattern:<br>track type=wiggle_0 name=<chrname> description=<coverage from positive/negative/both strand><br>visibility=full color=0,0,255 fixedStep chrom=<chrname> start=<startpos> step=<binsize> span=<binsize>                                                                                                                                                                      |
|                               | See also <a href="#">"Coverage files" on page 201</a> .                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                               | Output file name: <i>coverage_chrnn.pos.wig</i> , <i>coverage_chrnn.neg.wig</i>                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Unique Start Positions</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                               | This report lists the following: <ul style="list-style-type: none"> <li>• The total number of unique start positions.</li> <li>• The average number of unique start positions per reference position.</li> </ul> See also <a href="#">"Unique Start Position" on page 201</a> .                                                                                                                                                                                              |
|                               | Output file name: <i>BAMfilename-summary.txt</i> , <i>Samplename.-summary.txt</i>                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Summary Report</b>         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                               | The summary file provides statistics for each BAM file in the sample. See <a href="#">"Summary file"</a> .                                                                                                                                                                                                                                                                                                                                                                   |
|                               | Output file name: <i>filename.-summary.tbl</i>                                                                                                                                                                                                                                                                                                                                                                                                                               |

## Example of mapping statistics output

This section provides example output of some mapping statistics output files.

### Summary file

The summary file provides statistics for each BAM file in the sample. This list describes labels used in the summary file:

- **NumUnFilteredBeads** – The total number of beads, before any filtering on the instrument. This value is also the fragment count in the input XSQ file.
- **NumFilteredBeads** – The number of beads that pass filtering on the instrument.
- **NumMapped** – The number of reads with primary alignment.
- **% filtered mapped** – The number of primary reads divided by the number of beads passing instrument filtering ( $\text{NumMapped} / \text{NumFilteredBeads}$ ).
- **% total mapped** – The number of primary reads divided by the number of total beads ( $\text{NumMapped} / \text{NumUnFilteredBeads}$ ).
- **Megabases of coverage** – The sum of alignment lengths, for primary alignments only.

The number of beads filtered out in the instrument is found by subtracting the number of beads that pass filtering from the total number of beads:

$$\text{NumUnFilteredBeads} - \text{NumFilteredBeads}$$

The following is example contents for a summary file.

```
BamFileName, IsColorInBam, IsBaseInXSQ, IsECC, LibraryType,
ReadLength, Megabases of Coverage,
NumFragmentsPassingFilters, NumFragmentsTotal,
Tag1-NumMapped,
Tag1- % total Mapped,
Tag1- % filtered mapped,
(Tag1-AlignmentLength;Min;Max;Avg;Median;StdDev) ,
(Tag1-NumBaseMismatches;Min;Max;Avg;Median;StdDev) ,
(Tag1-MappingQV;Min;Max;Avg;Median;StdDev) ,
(Tag1-BaseQV;Min;Max;Avg;Median;StdDev) ,
(Tag1-NumColorMismatches;Min;Max;Avg;Median;StdDev) ,
(Coverage;Min;Max;Avg;Median;StdDev)
```

The following is an example line of output in the summary file:

```
example.bam Y N N FRAG 50 2184653 100000 100000 51536 51.54
51.54 (F3;25;50;42.39;45;6.48) (F3;0;50;2.98;0;9.24)
(F3;0;100;65.43;72;25.31) (F3;1;41;35.09;41;11.65)
(F3;0;9;1.20;1;1.39) (0;399;0.43;0;5.96)
```

### Unique Start Position

The following is an example of a Unique Start Position report, for fragment data:

```
Unique Start Positions F3
Total Number of Unique Start Positions: 16536 (16.043154 %
of primary alignments)
Average Number of Unique Start Positions per Reference
Position: 0.001630
```

Unique Start Position reports have the following naming patterns:

- *BAMfilename*-summary.txt
- *Samplename*-summary.txt

### Coverage files

Example contents for the file `coverage_chr1_positive.wig` are:

```
browser position chr1:1-200000000
browser hide all
browser pack refGene encodeRegions
# minimumMapq=25, minimumCoverage=1,
alignmentFilteringMode=PRIMARY, filterOrphanedMates=false,
track name="BAM Coverage positive strand" description="BAM
Coverage positive strand" visibility="full color 0,0,255"
priority=10 yLineMark=0 type=wiggle_0 yLineOnOff=on
variableStep chrom=chr1 span=1
336171
```

```
336181
336191
336201
336211
336221
336231
336241
336251
336261
336271
...
```

Example contents for the file `coverage_chr1_negative.wig` are:

```
browser position chr1:1-200000000
browser hide all
browser pack refGene encodeRegions
# minimumMapq=25, minimumCoverage=1,
alignmentFilteringMode=PRIMARY, filterOrphanedMates=false,
track name="BAM Coverage negative strand" description="BAM
Coverage negative strand" visibility="full color 0,0,255"
priority=10 yLineMark=0 type=wiggle_0 yLineOnOff=on
variableStep chrom=chr1 span=1
57432
57442
57452
57463
57473
57483
57493
57503
57513
57523
57533
...
```

## 14

# Perform Whole Transcriptome Analysis

This chapter covers:

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## Introduction to whole transcriptome analysis

This chapter has these purposes:

- To provide instructions for running whole transcriptome analysis single-read and paired-end modules using LifeScope™ Software.
- To list and describe configurable LifeScope™ Software parameters.

You can use the 5500 Series SOLiD™ Sequencer to sequence RNA prepared with RNA-Seq sample preparation kits. RNA sequencing produces high-depth, short-read sequencing data that can be used to measure RNA expression. Like microarray analysis, RNA-Seq measures expression intensity across many genomic features. Unlike microarray analysis, RNA-Seq can be used to identify novel transcriptome features in a sample.

Like other SOLiD® applications, RNA-Seq produces short reads in the form of XSQ files. Using LifeScope™ Software, whole transcriptome analysis modules take these reads as input, and perform the following steps:

1. **Align reads** — The aligning reads step identifies the alignments between the reads and the reference genome sequence and reports the alignments as a BAM file. Whole transcriptome analysis mapping also makes use of gene annotations, which define exons, genes, and transcripts, in order to improve alignment.
2. **Count known exons** — The counting known exons step identifies the number of reads that align within genomic features.

3. **Calculate coverage** — The calculating coverage step reports the read coverage at each genomic position.
4. **Find junctions** — The finding junctions step identifies splice junctions of various types, including fusion junctions from paired-end reads.

The 5500 Series SOLiD™ Sequencer™ System produces RNA-Seq reads in both single-read and paired-end configurations. While there is overlap in the analytical techniques, the analysis of single-read and paired-end data is performed with two distinct pipelines. The single-read pipeline consists of alignment, counting, and coverage steps. The paired-end pipeline extends single-read analysis with an alternative alignment step and an additional junction finding step.

## Whole transcriptome analysis library types

Whole transcriptome analysis supports data from the following library types of data:

- Fragment
- Paired-end

## Whole transcriptome analysis input files

The whole transcriptome analysis mapping module accepts as input one or more input XSQ files. The input reads can have different read lengths but they must be of the same library type. The XSQ files processed in a single analysis can not have different library types. In one mapping analysis, the input must be of only one library type, either fragment or paired-end.

Fragment mapping module supports mapping one tag of a paired-end read-set, but tertiary analysis does not run on BAM files generated from such processing.

### RNA-Seq reads

The RNA-Seq reads used in whole transcriptome analysis are different from the genomic reads. For RNA-Seq reads:

- Only transcribed sequences are measured by the system.
- Genome coverage is non-uniform due to variation in transcriptional intensity.
- A large subset of reads originate from uninteresting sequences such as ribosomal RNA (rRNA).
- A subset of the reads originates from splice junctions and cannot align contiguously on the genome.

### Multiple samples

LifeScope™ can perform alignment from multiple reads files (in XSQ format) for the same sample. If a sample is sequenced on the instrument in different runs or on different lanes, several XSQ files are generated for that sample. The reads from these XSQ files should be analyzed together. LifeScope™ facilitates the analysis by grouping the reads into read-sets.

## Reference input files

WTA uses several files defining reference information: filter-reference, genome-reference and annotation files. The filter-reference file is a FASTA-formatted file defining uninteresting reference sequences such as Ribosomal RNA, tRNA, or vector sequences. To increase the speed of analysis, reads that align to sequences in the filter reference are excluded from downstream alignment steps. Many of the entries in the filter reference are species-specific. The filter reference file must be appropriate for the species being studied.

The principal output of alignment analysis is a set of alignments between the reads and the genome-reference in BAM format. The genome-reference file is a FASTA-formatted file defining sequences in the reference genome.

The annotation file is a GTF-formatted file defining known genes, transcripts, and exons in the genome reference. See “[Annotations input files](#)”.

Junction-reference and exon-reference files are generated internally by the software. These files are entirely derived from the GTF and genome-reference files. A user is not required to provide these references. The junction-reference file is a FASTA-formatted file with an entry for every potential intragenic splice-junction defined in the annotation file. The entry contains the position of the junction and the associated flanking sequence. The exon-reference is a FASTA-formatted file with an entry for every exon defined in the annotation file.

## Annotations input files

While genomic resequencing relies only on alignment to a reference sequence, whole transcriptome analysis also makes use of gene annotations. Gene annotations define the exons, genes, and transcripts used to improve alignment.

Whole transcriptome analysis modules use genome annotation files in GTF format. Go to the following URLs to see the GTF format explained in detail:

[genes.cse.wustl.edu/GTF2.html](http://genes.cse.wustl.edu/GTF2.html)

[genome.ucsc.edu/FAQ/FAQformat.html#format4](http://genome.ucsc.edu/FAQ/FAQformat.html#format4)

The GTF file must match the genome reference to ensure that the whole transcriptome analysis modules work correctly.

See the *LifeScope™ Genomic Analysis Software Command Shell User Guide* for more information.

### UCSC genome annotations

#### Format UCSC Genome Browser Database annotations for WT analyses

The UCSC Genome Browser Database has genome annotations available for many assemblies at

[hgdownload.cse.ucsc.edu/goldenPath/](http://hgdownload.cse.ucsc.edu/goldenPath/)

The GTF-formatted annotations available for download are not properly normalized by gene ID. The required content is present for each assembly in the file export of the refGene database table `database/refGene/txt/gz`.

For example, annotation for human genome build 18 is available at:

[hgdownload.cse.ucsc.edu/goldenPath/hg18/database/refGene.txt.gz](http://hgdownload.cse.ucsc.edu/goldenPath/hg18/database/refGene.txt.gz)

**Note:** The GTF-formatted annotation is not in GTF format. You must convert the annotation before using it in whole transcriptome analysis.

### Convert the refGene.txt.gz file

Run the script `bin/refgene2gff.sh` to convert the `refGene.txt.gz` file:

```
gunzip refGene.txt.gz
refgene2gff.sh -i refGene.txt -o refGene.gff
```

Genome annotations that are downloaded from the UCSC Genome Browser and converted by the annotation conversion script are optimal because they contain Human Genome Organization (HUGO)-style gene names. HUGO-style gene names allow interpretation when using a genome browser or reading reports.

The annotation conversion script works with the latest format of `refGene.txt` files. Assemblies, such as the rat genome, use an alternative format for the `refGene.txt` file. The `refgene2gff.sh` script does not convert alternative formats.

## ENSEMBL GTF files Format ENSEMBL GTF files for whole transcriptome analysis pipelines

The ENSEMBL website [ensembl.org/](http://ensembl.org/) has GTF-formatted genome annotations available for many popular assemblies. Unlike the GTF files directly downloadable from UCSC (see “Whole transcriptome analysis output file formats” on page 218), ENSEMBL GTF files are properly normalized by gene and transcript IDs. Visit the following site to download an ENSEMBL GTF file:

<http://www.ensembl.org/>

ENSEMBL GTF files use gene accession numbers instead of HUGO-style gene names. ENSEMBL GTF files also use unprefixed sequence identifiers, such as 1,2,3...X,Y,MT. The ENSEMBL GTF files are incompatible with genome reference FASTA files that have UCSC-style sequence IDs with the prefix “chr”, for example, chr1, chr2, chr3...chrX, chrY, chrM.

### Reformat the ENSEMBL GTF file

To reformat the ENSEMBL GTF file to use UCSC-style gene IDs, run the `reformat_ensembl_gtf.pl` script, and redirect the output to a new file with `.reformatted` in the file name.

```
For Example:
reformat_ensembl_gtf.pl Homo_sapiens.GRCh37.63.gtf > \
Homo_sapiens.GRCh37.63.reformatted.gtf
```

The input GTF file name typically includes the NCBI build number and the Ensembl release number in its name, according to the following pattern:

```
Homo_sapiens.<reference_ncbi_build_no>.<Ensembl_release_no> .
.gtf
```

## Whole transcriptome analysis modules

Whole transcriptome analysis includes the following modules:

| Secondary Analysis Module                     | Used for Library Type |
|-----------------------------------------------|-----------------------|
| Mapping                                       | Fragment, paired-end  |
| Whole transcriptome splice junction extractor | Paired-end            |
| Tertiary Analysis Module                      | Used for Library Type |
| Whole transcriptome exon sequence extractor   | Paired-end            |
| Coverage                                      | Fragment, paired-end  |
| Whole transcriptome count features            |                       |
| Splice finding                                |                       |

### Whole Transcriptome Fragment Mapping

Whole transcriptome fragment mapping uses eXtensible SeQUence (.xsq) files to create a \*.bam file.

### Whole Transcriptome Exon Sequence Extractor

Extract sequences of exons to prepare a special reference for mapping and tertiary algorithms.

### Coverage

The whole transcriptome coverage module calculates read coverage per position.

### Whole Transcriptome Count Features

Filter, count, and normalize the reads that align on exons and genes, to generate easy-to-use expression output.

### Splice Finding

Detect and quantify two types of evidences for splicing junctions, powered by the SASR (Suffix Array Single Read) aligner, to reconstruct transcript graphs and discover new fusion transcripts.

## Whole transcriptome analysis parameters

Parameters for whole transcriptome analysis include:

- General parameters
- **Secondary analysis:** Mapping (including optional BAMStats)
- **Tertiary analysis:** Whole Transcriptome Fragment Mapping, Coverage, Whole Transcriptome Count Features, and Whole Transcriptome Exon Sequence Extractor, and Whole Transcriptome Splice Junction.

**General**

General parameters include:

| Parameter                 | Default value | Description                                                                                                                                                                                               |
|---------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| analysis.assembly.name    | —             | The name of the genome assembly used in current analysis. Examples: hg18, hg19.                                                                                                                           |
| analysis.filter.reference | —             | The file containing a collection of sequences that are used to filter out commonly-occurring motifs and contaminating sequences prior to mapping.                                                         |
| annotation.gtf.file       | —             | The file containing gene and exon annotations corresponding to the genome assembly used in the analysis.                                                                                                  |
| analysis.space            | Base          | Your preference of whether analysis should be done in base space or in color space. This setting should be the same for all modules in your run. See <a href="#">"Choose analysis space" on page 98</a> . |
| analysis.platform         | SOLID         | Always specify "SOLID". Other values are reserved for future use.                                                                                                                                         |

Use the  button to open the File Chooser and search for input files.**Whole Transcriptome Fragment Mapping**

There are two categories of mapping parameters: Main and Advanced.

**Main**

| Parameter               | Default value | Description                                                                                                                                                                                                                                                                                                                |
|-------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Enable filter mapping   | True          | Perform filter mapping.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Perform filter mapping.</li> <li>• <b>false</b>: Do not perform filter mapping.</li> </ul>                                                                                                                                |
| Enable junction mapping | True          | Perform junction mapping.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Perform junction mapping.</li> <li>• <b>false</b>: Do not perform junction mapping.</li> </ul>                                                                                                                          |
| Map in base space       | False         | Map in base space. Set to true if input data has base space available.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Map in base space.</li> <li>• <b>false</b>: Map in color space.</li> </ul> If only color space is available, then the module fails when base space mapping is turned on.   |
| Add color sequence      | True          | Map in color space. Set to true if input data has color space available.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Map in base space.</li> <li>• <b>false</b>: Map in color space.</li> </ul> If only color space is available, then the module fails when base space mapping is turned on. |

### Advanced

| Parameter                 | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Min junction overhang     | 8             | Alignments to junctions are not reported if they have fewer than this number of bases on aligned on either side of the splice.<br>Allowed values: Integers 0 - 100.                                                                                                                                                                                                                                                                                                                       |
| Make unmapped BAM files   | False         | Create an additional BAM output file containing unmapped reads. Allowed values: <ul style="list-style-type: none"> <li><b>true</b>: Create a BAM output file containing the unmapped reads.</li> <li><b>false</b>: Do not create the unmapped reads output file.</li> </ul>                                                                                                                                                                                                               |
| Mapping quality threshold | 0             | Minimum mapping quality for an alignment to be reported in a BAM file.<br>Allowed values:<br>Integers 0 - 255                                                                                                                                                                                                                                                                                                                                                                             |
| Refcor base qv filter     | 2             | Bases with a quality value below the value of this parameter are replaced with "N".<br>Allowed values:<br>Integers 0 - 255                                                                                                                                                                                                                                                                                                                                                                |
| Reference weight          | 8             | This parameter is used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. This parameter adds weight (in terms of a Phred score) to the signals that are compatible with reference. Color combinations that result in a variant are considered compatible with reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation.<br>Allowed values: Integers 0 - 100 |

### Whole Transcriptome

| Parameter   | Default value | Description |
|-------------|---------------|-------------|
| Read length | 75            | Read length |

### BAMStats

| Parameter                    | Default value                       | Description                                                                                                          |
|------------------------------|-------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| Input directory for BAMStats | \${analysis.output.dir}/wt.frag.map | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample. |
| Output directory             | \${task.output.dir}                 | The path to the output directory where BAMStats will write its chart (.cht) files.                                   |

| Parameter                                                                | Default value | Description                                                                                                                                                                                                                                                                                                             |
|--------------------------------------------------------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Maximum Coverage                                                         | 10000         | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br>Allowed values: Integers 1 - 10,000.                                                                                                     |
| Maximum insert size                                                      | 100000        | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br>Allowed values: Integers 1 - 100000.                                                                      |
| Insert bin size                                                          | 1000          | Bin size for insert range distribution.<br>Allowed values: Integers $\geq$ 1.                                                                                                                                                                                                                                           |
| Whether to combine data from both the strands for coverage in WIG format | 1             | Combine or do not combine data from both strands for coverage in WIG format.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: Do not generate wig files.</li> <li>• <b>1</b>: Generate the combined coverage file.</li> </ul>                                                                      |
| Primary alignments only for coverage in WIG file format                  | 1             | Use only primary alignments for coverage in WIG file format.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: Do not restrict coverage in WIG file format to only primary alignments.<sup>2</sup></li> <li>• <b>1</b>: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Bin size for coverage in WIG file format                                 | 100           | The bin size for coverage in WIG file format.<br>Allowed values: Integers $>$ 1.                                                                                                                                                                                                                                        |

### Whole Transcriptome Exon Sequence Extractor

There are no Whole Transcriptome Exon Sequence Extractor parameters to review or edit.

### Coverage

You can optionally edit Advanced Coverage parameters.

| Parameter          | Default value | Description                                                                                                                                                                                         |
|--------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Coverage min value | 0             | The mapping quality value threshold for selecting the alignments. Alignments with a mapping quality value lower than this threshold are not written to output.<br>Allowed values: Integers 0 to 100 |

| Parameter               | Default value | Description                                                                                                                                                                                                                                                                                                                                                                |
|-------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Coverage min quality    | 2             | The mapping quality threshold value for selecting alignments from the input BAM. In order to be included in the output, a position's coverage must be greater than or equal to this value.<br>Allowed values: Integers $\geq 0$ .                                                                                                                                          |
| Coverage per chromosome | True          | Generate coverage output as one file per every chromosome per strand, or as a single file with coverage of all chromosomes per strand.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Generate one file per every chromosome per strand.</li> <li>• <b>false</b>: Generate a single file with coverage of all chromosomes per strand.</li> </ul> |
| Coverage primary only   | True          | Use only primary alignments or all the alignments from the input BAM file as input.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Consider only primary alignments (both gapped and ungapped).</li> <li>• <b>false</b>: Consider all alignments.</li> </ul>                                                                                     |

## Whole Transcriptome Count Features

There are two categories of Whole Transcriptome Count Features parameters: Main and Advanced.

### Main parameters

| Parameter     | Default value           | Description                                 |
|---------------|-------------------------|---------------------------------------------|
| <b>Global</b> |                         |                                             |
| *GTF file     | \$(annotation.gtf.file) | The GTF file used to create the gene model. |

### Advanced parameters

| Parameter           | Default value | Description                                                                                                                                                                                                                                                                                |
|---------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Generate gene count | True          | Generate gene count.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Generate gene count.</li> <li>• <b>false</b>: Do not generate gene count.</li> </ul>                                                                                                         |
| Counts primary      | True          | Use only primary alignments or use all the alignments from the input BAM file as input.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Consider only primary alignments (both gapped and ungapped).</li> <li>• <b>false</b>: Consider all alignments.</li> </ul> |

| Parameter             | Default value | Description                                                                                                                                                                                                                                                                                 |
|-----------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mapping quality value | 10            | Minimum mapping quality value for the alignment.<br>Allowed values: Integers 0–255.                                                                                                                                                                                                         |
| Overflow limit        | 3             | The maximum allowed offset on either side of an alignment or feature, when looking for overlap between an alignment and a feature. If this limit is set to 0, then the start and end coordinates of the alignment record and feature must match exactly.<br>Allowed values: Integers 0–100. |

**Splice Finding** You can optionally use the Splice Finding analysis module. There are two categories of Splice Finding parameters: Main and Advanced.

#### Main

| Parameter                                                 | Default value            | Description                                                                                        |
|-----------------------------------------------------------|--------------------------|----------------------------------------------------------------------------------------------------|
| <b>Global</b>                                             |                          |                                                                                                    |
| *GTF file                                                 | \$(annotation.gitf.file) | The GTF file used to create the gene model.                                                        |
| <b>General</b>                                            |                          |                                                                                                    |
| First read max read length                                | 50                       | Maximum read length of the first read.<br>Allowed values: 25–150                                   |
| Second read max read length                               | 25                       | Maximum read length of the second read.<br>Allowed values: 25–150                                  |
| Minimum single read evidence for junction                 | 1                        | Minimum single read evidence for junction.<br>Minimum value: 0<br>Maximum value: 1                 |
| Minimum paired end read evidence for junction             | 1                        | Minimum paired end read evidence for junction.<br>Minimum value: 0<br>Maximum value: 1             |
| Minimum combined evidence for junction                    | 2                        | Minimum combined evidence for junction.<br>Minimum value: 0<br>Maximum value: 1                    |
| Minimum single read evidence for alternative splicing     | 1                        | Minimum single read evidence for alternative splicing.<br>Minimum value: 0<br>Maximum value: 1     |
| Minimum paired-end read evidence for alternative splicing | 1                        | Minimum paired-end read evidence for alternative splicing.<br>Minimum value: 0<br>Maximum value: 1 |

| Parameter                                          | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|----------------------------------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Minimum combined evidence for alternative splicing | 2             | Minimum combined evidence for alternative splicing.<br>Minimum value: 0<br>Maximum value: 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Minimum split read evidence for fusion             | 2             | Minimum split read evidence for fusion.<br>Minimum value: 0<br>Maximum value: 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Minimum paired end evidence for fusion             | 2             | Minimum paired end evidence for fusion.<br>Minimum value: 0<br>Maximum value: 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Minimum combined evidence for fusion               | 4             | Minimum combined evidence for fusion.<br>Minimum value: 0<br>Maximum value: 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Single Read</b>                                 |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Single read                                        | 1             | Run single read fusion finding.<br>Allowed values: <ul style="list-style-type: none"> <li>• 0: Do not run single read fusion finding.</li> <li>• 1: Run single read fusion finding</li> </ul>                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Paired End</b>                                  |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Paired read                                        | 0             | Run paired-end fusion finding.<br>Allowed values: <ul style="list-style-type: none"> <li>• 0: Do not run paired-end fusion finding.</li> <li>• 1: Run paired-end fusion finding.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                       |
| Paired read min mapq                               | 10            | A read-pair that has mapping quality lower than this value is inadmissible as evidence. <ul style="list-style-type: none"> <li>• Minimum value: 0</li> <li>• Maximum value: 255</li> </ul>                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Output</b>                                      |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Output format                                      | 4             | Output format parameter. Allowed values: <ul style="list-style-type: none"> <li>• 1 = tabular output format</li> <li>• 2 = BED output format</li> <li>• 3 = BED, tabular, and SEQ output formats</li> <li>• 4 = all formats, including Circos</li> </ul> <p>With option 3, tabular, BED and also additional "seq" files are separately created. Seq files contain 50 base pairs of sequences from each end of a junction exon and are useful for validation.</p> <ul style="list-style-type: none"> <li>• Minimum value: 1</li> <li>• Maximum value: 4</li> </ul> |

## Perform whole transcriptome analysis

### (Optional) Import data

You can optionally import data to be analyzed. For instructions on how to import data, see “Import Data” [on page 81](#).

### Log in to LifeScope™

1. Navigate to LifeScope™ Software at a url given to you by your network administrator.
2. In the Login screen, enter your username and password, then either click **Login** or press **Enter** to open the LifeScope™ home view (shown [on page 75](#)).

### Create or select a project

1. In the home view (shown [on page 75](#)), either click **Create a New Project** (described [on page 80](#)) or select a project in the Projects organizer (shown [on page 75](#)).
2. If you create a project:
  - a. type a name and description in the Enter Project Name view.  
**Note:** The name cannot have spaces or special characters.
  - b. Click **Create New Project**.
  - c. In the Projects lists, select the new project.
3. In the Task Wizards section (shown [on page 75](#)), click **Add Data to Project** to choose a data type.

### Add data to a project

Add data from the read repository to a project by choosing a data type and finding data. You can also optionally group data.

**Note:** Multiple files cannot have the same name. Make sure that the names of the files that you add are distinct.

**Note:** If the LifeScope™ Software administrator has changed the path to the read repository since your last login, restart LifeScope™ Software to update the repository to the new path.

1. In the Add Data to Project window, select **Raw unmapped (XSQ) data**, then click **Next** to find data.
2. In the Read Repository Filter table, select the read-sets you want to map and analyze.  
If you want to group data, click **Next**. If you do not want to group data, skip [step 3](#) and proceed to [step 4](#).
3. (Optional) In the Read-sets in Project table, click the checkbox of the data files you want to group, then click **Add Group to Project**. The files appear in the Groups in Project table.

To rename a group, click the checkbox of the group in the Groups in Project table, then click **Edit**. In the Edit Group window, enter a new name.

To remove a data file from a group, click the checkbox of the data file, then click **Delete**.

4. Click **Add Analysis** to proceed, or
  - Click **Cancel** to refrain from adding data and close the Add Data to Project window, or
  - Click **Finish** to add the data and close the Add Data to Project window.

## Create an analysis

1. In the Choose Data view of the Create Analysis window, select data files from the Available Data in Project table, then click **Next** to choose an analysis.

2. In the Choose Analysis view, enter a name for the analysis. You can optionally describe your project.

**Note:** The name cannot have spaces or special characters.

**Note:** Note: If you are reusing an old analysis, select **Reuse Old Analysis** and select the name of your desire analysis.

3. Select **Whole Transcriptome** then click **Next** to choose references.



4. Click **Select the reference for the reads**.
5. In the Browse for Reference File window, navigate to the location of the reference genome of your sample. Open the folder with your .fasta file, for example:
  - data ▶ results ▶ referenceData ▶ lifetech ▶ hg18 ▶ reference ▶ human\_hg18.fasta

Select the file, then click **OK**.

The file name appears in the Reference column.

**Note:** To change the reference file, click **Select the reference for the reads**.

6. After you have created the analysis:
  - Click **Edit** to proceed, or
  - Click **Cancel** to refrain from creating the analysis and close the Create Analysis window, or
  - Click **Finish** to complete analysis creation and close the window.

## Edit the analysis

1. In the Edit Analysis window, select the type of analysis modules to run during and after secondary analysis mapping.

If you want to exclude BAMStats, click **Customize**. In the Customize Mapping window, uncheck BAMStats, then click **OK**.

If you do not want to pre-process data, uncheck the box.

2. In the Tertiary Analysis section, you can include an analysis module by selecting it in the Available Modules column, then clicking the > button. To include all modules, click the **All>>** button.

To exclude a module, select it in the Include column, then click the < button. To exclude all modules, click the **All<<** button.

3. Click **Next** to set module parameters.

## Set module parameters

This section describes the procedures for setting general parameters and parameters for the Whole Transcriptome Fragment Mapping, Coverage, Whole Transcriptome Count Features, and Whole Transcriptome Count Exon Sequence Extractor modules. For descriptions of module parameters, see [“Whole transcriptome analysis parameters” on page 207](#).

You can restore the default settings of parameters by clicking the **Reset to Defaults** button.

To view descriptions of parameters, place your mouse cursor over a  button.

### Set general parameters

1. Enter an analysis assembly name.
2. Set the `analysis.fliter.reference` and `annotation.gtf.file` parameters.  
Click the  buttons to open the File Chooser, navigate to reference files, and choose files.  
**Note:** Verify that the file path to the `annotation.gtf` file is correct. An incorrect file path will result in a failed analysis.
3. Set `analysis.space` to your preference of whether analysis should be done in base space or in color space. This setting should be the same for all modules in your run. For more information, see [“Choose analysis space” on page 98](#).
4. Always leave the default `analysis.platform` setting, which is “SOLID”.
5. If you want to accept the default parameters for all modules, click **Review**. If you want to edit module parameters, click **Next**.

### Set Whole Transcriptome Fragment Mapping parameters

1. There are four categories of mapping parameters: Main, Advanced, Whole Transcriptome and BAMStats. Accept the default settings or click the tabs to edit the settings.
2. Click **Next** to edit Coverage parameters.

### Set Coverage parameters

1. There is one category of Coverage parameters: Advanced. Accept the default settings or edit the settings.
2. Click **Next** to edit Whole Transcriptome Count Features parameters.

### Set Whole Transcriptome Count Features parameters

1. There are two categories of Whole Transcriptome Count Features parameters: Main and Advanced. Accept the default settings or edit the settings.

There are no Whole Transcriptome Exon Sequence Extractor parameters.

2. If you are ready to start the analysis, click **Review** to proceed.
3. If you want to erase your edits, click **Cancel** to close the Edit Analysis window.

- If you want to start the analysis later, click **Finish** to save your edits and close the Edit Analysis window.

## Review and run the analysis

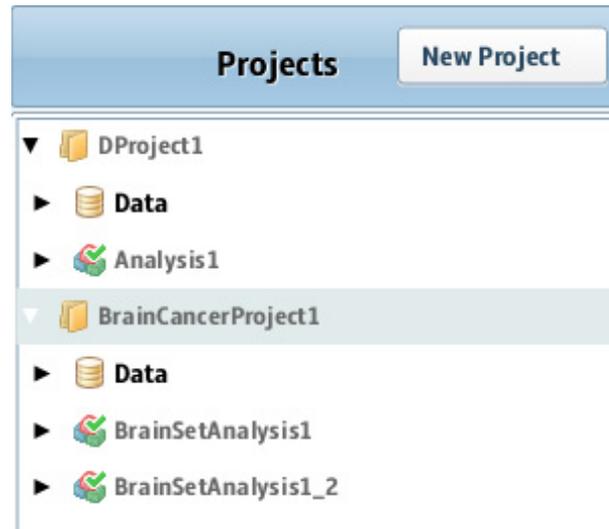
**Note:** For a description of whole transcriptome parameters, see [“Whole transcriptome analysis parameters” on page 207](#).

The Review Analysis view in the Run Analysis window includes two tabs: Parameters and Data. In the Parameters tab, click the  next to parameter categories to show or hide the parameters.

- Review the parameters. To edit the parameters, click **Edit**.
- To review the data that will be analyzed, click the **Data** tab.
- If you are ready to run the analysis, click **Start Analysis**.  
The Run Analysis window closes.

## Check Analysis Status

- In the Projects organizer (shown [on page 75](#)), click the project to check the status of the analysis run.



- Click the analysis name to show and show details about the analysis in the status overview (shown [on page 75](#)).  
To see analysis results in the status overview, click the Results icon  in the Projects organizer.
- Click the Status tab to view the Progress column, which shows the percentage of completion for the analysis.  
  
When analysis has been completed, a green check mark appears on the analysis module icon in the Projects organizer.
- Click the Analysis icon to see the overview of the results. In the status overview, click the Status tab to see the progress of the analysis run.
- Click **View Results** in a progress column to open the View Results window. In a progress column, a failed analysis is indicated by Fail!
- Click each module to see results in the Overview and Status tabs.

7. Click each tab to view various results.
8. Close the View Results window to return to the Home view.

## View analysis results

See [Chapter 9, “View Analysis Results” on page 103](#) for instructions on how to review analysis results.

### View results in a genome browser

Output files are compatible with third-party browser such as the Integrative Genomics Viewer (IGV) available from the Broad Institute and the UCSC Genome Browser. See [“Pairing information in a BAM file” on page 348](#) for information on using LifeScope™ Software output files with genome browsers.

## Whole transcriptome analysis output files

| Input file type(s) | Output file type             |
|--------------------|------------------------------|
| *.xsq              | *.bam                        |
| *.bam              | *.gff.3, *.gtf, *.txt, *.wig |
| *.bam              | position error               |

## Whole transcriptome analysis output file formats

### BAM files

The whole transcriptome analysis paired-end pipeline produces BAM files that are identical to those produced by the resequencing pipeline [samtools.sourceforge.net/SAM1.pdf](http://samtools.sourceforge.net/SAM1.pdf). However, the BAM files from the whole transcriptome analysis single-read pipeline differ from BAM files produced elsewhere in LifeScope™ Software.

### Alignment report

WT mapping runs produce an alignment report, the `alignmentReport.txt` file in the results directory.

Below is an example of the alignment report produced by the WT pipeline (the frequency lists have been truncated).

The 100.0% value reported for the “Reads mapped, not filtered” line should be interpreted as “Reads mapped, not filtered” comprising 100% of the category described in its section, not that the category “Reads mapped, not filtered” comprises 100% of the total reads.

```

-----
ALIGNMENT REPORT
-----

Counts:
Total reads:                102,837,343  (100.0%)
Reads mapped:               78,065,351   ( 75.9%)
Reads filtered:             12,029,269   ( 11.7%)

```

```

-----
Reads mapped, not filtered                68,780,034  (100.0%)
Reads with too many mappings (N >= 10):   5,897,833  (  8.6%)
Reads with number of mappings in proper range (N < 10):
62,882,201    ( 91.4%)
Reads uniquely aligned (score.clear.zone = 3):
53,417,152    ( 77.7%)
Reads uniquely aligned to junctions       4,816,207  (  7.0%)
Reads uniquely aligned to known junctions 4,773,948  (  6.9%)

```

ABSOLUTE FREQUENCIES

```

Alignment_Score  All_Read_Alignments_(N=_936)
Primary_Read_Alignments_(N=_935)
Unique_Read_Alignments_(N=_866)
0.0      0      0      0
1.0      0      0      0
2.0      0      0      0
36.0     0      0      0
37.0     87     87     87
38.0     0      0      0
39.0     0      0      0
47.0     0      0      0
48.0     0      0      0
49.0    849    848    779
50.0     0      0      0
Total    936    935    866

```

RELATIVE FREQUENCIES

```

Alignment_Score  All_Read_Alignments_(N=_936)
Primary_Read_Alignments_(N=_935)
Unique_Read_Alignments_(N=_866)
0.0      0.0%    0.0%    0.0%
1.0      0.0%    0.0%    0.0%
2.0      0.0%    0.0%    0.0%
36.0     0.0%    0.0%    0.0%
37.0     9.3%    9.3%    10.0%
38.0     0.0%    0.0%    0.0%
39.0     0.0%    0.0%    0.0%
40.0     0.0%    0.0%    0.0%
47.0     0.0%    0.0%    0.0%
48.0     0.0%    0.0%    0.0%
49.0    90.7%    90.7%    90.0%
50.0     0.0%    0.0%    0.0%
Total   100.0%  100.0%  100.0%

```

### WT filtering stats

An example whole transcriptome human filter reference is provided under the `<examplesdir>/demos/WholeTranscriptome/references` folder in the optional LifeScope™ Software examples distribution. This example reference fasta includes contigs from barcode primers, human ribosomal RNAs, tRNAs, and other known targets for filtering. The filter reference may be expanded or removed with more csfasta reference records at the discretion of the user.

The stats for filtered reads are generated after a LifeScope™ Software run. First two lines of the stats file states the number of reads processed, and the number of reads mapped to the filtered reference followed by its percentage. Each following line reports a contig name and the count of reads that aligned to that contig. Paired-end filtering stats may be found in the Intermediate folder. The following is a truncated example of filtering stats output:

```
countOfReadsProcessed: 175,839,941
countOfReadsMapping:   18,783,220 (10.7%)
...
Barcode-047-3-end reverse   83
Barcode-048-3-end reverse  2,350
gi|124517659|ref|NR_003286.1| Homo sapiens 18S ribosomal RNA
(LOC100008588)   5,175,899
gi|142372596|ref|NR_003285.2| Homo sapiens 5.8S ribosomal RNA
(LOC100008587)   78,936
gi|124517661|ref|NR_003287.1| Homo sapiens 28S ribosomal RNA
(LOC100008589)   7,616,212
chr6.trna95-AlaAGC (58249908-58249836) Ala (AGC) 73 bp Sc:
42.26   5
chr6.trna25-AlaAGC (26859897-26859969) Ala (AGC) 73 bp Sc:
46.89   2
chr6.trna94-AlaAGC (58250620-58250548) Ala (AGC) 73 bp Sc:
54.62   4
chr6.trna160-AlaAGC (26881822-26881750) Ala (AGC) 73 bp Sc:
54.69   35
```

## 15

# Perform Whole Transcriptome Mapping

This chapter contains:

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## Introduction to whole transcriptome mapping

This chapter provides background information about secondary analysis (mapping and pairing, also known as aligning reads) and tertiary analysis (data visualization and analysis, including counting tags, determining coverage, and finding junctions). The information in this chapter includes descriptions of parameters you can configure to customize your analysis.

## Examples of running the whole transcriptome mapping module

The following standard workflows provide examples of running the whole transcriptome analysis mapping module:

- Fragment whole transcriptome
- Pair-end whole transcriptome

## Reads input files

The whole transcriptome analysis mapping module accepts as input one or more input XSQ files. The input reads can have different read lengths but they must be of the same library type. For example, paired-end read-sets of length 50 and 60 can be processed together. The XSQ files processed in a single analysis can not have different library types. In one mapping analysis, the input must be of only one library type, either fragment or paired-end.

Fragment mapping module supports mapping one tag of a paired-end read-set, but tertiary analysis does not run on BAM files generated from such processing.

If the data you want to process with LifeScope™ is in CSFASTA and QUAL files, these files must be converted to the XSQ file format. The LifeScope™ graphical user interface automatically converts these files to the XSQ format before mapping data.

### RNA-Seq reads

The RNA-Seq reads used in whole transcriptome analysis are different from the genomic reads. For RNA-Seq reads:

- Only transcribed sequences are measured by the system.
- Genome coverage is non-uniform due to variation in transcriptional intensity.
- A large subset of reads originate from uninteresting sequences such as ribosomal RNA (rRNA).
- A subset of the reads originates from splice junctions and cannot align contiguously on the genome.

#### Multiple samples

LifeScope™ can perform alignment from multiple reads files (in XSQ format) for the same sample. If a sample is sequenced on the instrument in different runs or on different lanes, several XSQ files are generated for that sample. The reads from these XSQ files should be analyzed together. LifeScope™ facilitates the analysis by grouping the reads into read-sets.

## Annotations input files

While genomic resequencing relies only on alignment to a reference sequence, whole transcriptome analysis also makes use of gene annotations. Gene annotations define the exons, genes, and transcripts used to improve alignment.

whole transcriptome analysis modules use genome annotation files in GTF format. Go to the following URLs to see the GTF format explained in detail:

[genes.cse.wustl.edu/GTF2.html](http://genes.cse.wustl.edu/GTF2.html)

[genome.ucsc.edu/FAQ/FAQformat.html#format4](http://genome.ucsc.edu/FAQ/FAQformat.html#format4)

The GTF file must match the genome reference to ensure that the whole transcriptome analysis modules work correctly.

---

**IMPORTANT!** Make sure the GTF file is for the same genome assembly as the FASTA file, and that matching sequence identifiers are used. Gene and transcript identifiers in the GTF file must be properly normalized. Identifier normalization is a known issue in GTF files from the UCSC Genome Browser, which is a popular source of GTF-formatted annotation. The UCSC GTF files always report the same value for gene and transcript IDs.

---

**Note:** LifeScope™ Software includes a command line script (`refgene2gff.sh`) to transform a GTF file into the format required for use with whole transcriptome analysis pipelines. For additional information, see the *LifeScope™ Genomic Analysis Software Command Shell User Guide* (Part no. 4471875).

## UCSC genome annotations

### Format UCSC Genome Browser Database annotations for whole transcriptome analyses

The UCSC Genome Browser Database has genome annotations available for many assemblies at

[hgdownload.cse.ucsc.edu/goldenPath/](http://hgdownload.cse.ucsc.edu/goldenPath/)

The GTF-formatted annotations available for download are not properly normalized by gene ID. The required content is present for each assembly in the file export of the refGene database table `database/refGene/txt/gz`.

For example, annotation for human genome build 18 is available at:

[hgdownload.cse.ucsc.edu/goldenPath/hg18/database/refGene.txt.gz](http://hgdownload.cse.ucsc.edu/goldenPath/hg18/database/refGene.txt.gz)

**Note:** The GTF-formatted annotation is not in GTF format. You must convert the annotation before using it in whole transcriptome analysis.

## Reference input files

WTA uses several files defining reference information: filter-reference, genome-reference and annotation files. The filter-reference file is a FASTA-formatted file defining uninteresting reference sequences such as Ribosomal RNA, tRNA, or vector sequences. To increase the speed of analysis, reads that align to sequences in the filter reference are excluded from downstream alignment steps. Many of the entries in the filter reference are species-specific. The filter reference file must be appropriate for the species being studied.

The principal output of alignment analysis is a set of alignments between the reads and the genome-reference in BAM format. The genome-reference file is a FASTA-formatted file defining sequences in the reference genome.

The annotation file is a GTF-formatted file defining known genes, transcripts, and exons in the genome reference. See [“Annotations input files” on page 222](#).

Junction-reference and exon-reference files are generated internally by the software. These files are entirely derived from the GTF and genome-reference files. A user is not required to provide these references. The junction-reference file is a FASTA-formatted file with an entry for every potential intragenic splice-junction defined in the annotation file. The entry contains the position of the junction and the associated flanking sequence. The exon-reference is a FASTA-formatted file with an entry for every exon defined in the annotation file.

## Mapping parameters

There are three categories of mapping parameters:

| Mapping Parameter | Used in analysis . . . |
|-------------------|------------------------|
| Fragment          | ChIP-Seq               |
|                   | Genomic resequencing   |
|                   | MethylMiner™           |
|                   | Targeted resequencing  |
|                   | Whole transcriptome    |
| Paired-end        | Genomic resequencing   |
|                   | MethylMiner™           |
|                   | Targeted resequencing  |
|                   | Whole transcriptome    |
| Mate Pair         | Genomic resequencing   |

### Fragment Mapping

There are four categories of fragment mapping parameters: Main, Advanced, Whole Transcriptome, and BAMStats.

#### Main

| Parameter               | Default value | Description                                                                                                                                                                                                                                                                                   |
|-------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Enable filter mapping   | True          | <ul style="list-style-type: none"> <li>• <b>True:</b> Perform filter mapping.</li> <li>• <b>False:</b> Do not perform filter mapping.</li> </ul>                                                                                                                                              |
| Enable junction mapping | False         | <ul style="list-style-type: none"> <li>• <b>True:</b> Perform junction mapping.</li> <li>• <b>False:</b> Do not perform junction mapping.</li> </ul>                                                                                                                                          |
| Add color sequence      | True          | Add color sequence to BAM records: Values: <ul style="list-style-type: none"> <li>• True: Add color.</li> <li>• False: Do not add color.</li> </ul>                                                                                                                                           |
| Map in base space       | False         | Allow mapping in base space if input data has base space available. If only color space is available, then mapping will fail when base space mapping is turned on. Values: <ul style="list-style-type: none"> <li>• True: Map in base space.</li> <li>• False: Map in color space.</li> </ul> |

## Advanced

| Parameter                 | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|---------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Min junction overhang     | 8             | Alignments to junctions are not reported if they have fewer than this number of bases unaligned on either side of the splice.<br>Allowed values: Integers 0–100.                                                                                                                                                                                                                                                                                      |
| Make unmapped BAM files   | 0             | Create BAM files containing unmapped reads.<br>Values: <ul style="list-style-type: none"> <li>• <b>True:</b> Create.</li> <li>• <b>False:</b> Do not create.</li> </ul>                                                                                                                                                                                                                                                                               |
| Mapping quality threshold | 0             | Provide control the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer.<br>Allowed values: Integers 0–255.                                                                                                                                                                                                                       |
| refcor base QV filter     | 10            | Resulting base calls with quality less than this value will be replaced with "N."<br>Allowed values: Integers 0–255.                                                                                                                                                                                                                                                                                                                                  |
| Reference weight          | 8             | Used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. Adds weight (in terms of Phred score) to the signals that are compatible with reference. Color combinations that result in a variant are considered compatible with reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation.<br>Allowed values: Integers 0–100. |

## Whole Transcriptome

| Parameter   | Default value | Description                                                                                                                                                                                               |
|-------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Read length | 75            | Position in the read at which the error rate inflates, for instance, 35–40 for 50bp long reads. (If set to 0, then the value is equal to $0.8 * \text{readLength}$ ).<br>Allowed values: Integers 25–100. |

## BAMStats

| Parameter                    | Default value                                         | Description                                                                                                          |
|------------------------------|-------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| Input directory for BAMStats | <code>\${analysis.output.dir}/fragment.mapping</code> | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample. |
| Output directory             | <code>\${task.output.dir}</code>                      | The path to the output directory where BAMStats will write its chart (.cht) files.                                   |

|                                                                          |         |                                                                                                                                                                                                                                                                                                             |
|--------------------------------------------------------------------------|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Maximum Coverage                                                         | 10,000  | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br>Allowed values: Integers $\geq 0$ .                                                                                          |
| Maximum insert size                                                      | 100,000 | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br>Allowed values: Integers 0–100000.                                                            |
| Insert bin size                                                          | 100     | Bin size for insert range distribution.<br>Allowed values: Integers 1–100000                                                                                                                                                                                                                                |
| Whether to combine data from both the strands for coverage in WIG format | 1       | Combine or do not combine data from both strands for coverage in WIG format.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: Do not generate wig files.</li> <li>• <b>1</b>: Generate the combined coverage file.</li> </ul>                                                          |
| Primary alignments only for coverage in WIG file format                  | 1       | Use only primary alignments for coverage in WIG file format.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: Do not restrict coverage in WIG file format to only primary alignments.</li> <li>• <b>1</b>: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Bin size for coverage in WIG file format                                 | 100     | The bin size for coverage in WIG file format.<br>Allowed values: Integers 1–100,000.                                                                                                                                                                                                                        |

**Paired-end**

There are five categories of paired-end parameters: Main, Advanced, Whole Transcriptome Splice Junction Extractor, and BAMStats. There are no parameters for the Whole Transcriptome Exon Sequence Extractor analysis module.

**Main**

| Parameter               | Default value | Description                                                                                                                                                                    |
|-------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Enable filter mapping   | True          | <ul style="list-style-type: none"> <li>• <b>True</b>: Perform filter mapping.</li> <li>• <b>False</b>: Do not perform filter mapping.</li> </ul>                               |
| Enable junction mapping | True          | <ul style="list-style-type: none"> <li>• <b>True</b>: Perform junction mapping.</li> <li>• <b>False</b>: Do not perform junction mapping.</li> </ul>                           |
| Enable exon mapping     | True          | <ul style="list-style-type: none"> <li>• <b>True</b>: Perform exon mapping.</li> <li>• <b>False</b>: Do not perform exon mapping.</li> </ul>                                   |
| F3 rescue enabled       | True          | Attempt or do not attempt rescue of F3 reads. <ul style="list-style-type: none"> <li>• <b>True</b>: Attempt rescue.</li> <li>• <b>False</b>: Do not attempt rescue.</li> </ul> |

| Parameter           | Default value | Description                                                                                                                                                                                                                                                                                   |
|---------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| F5 rescue enabled   | True          | Attempt or do not attempt rescue of F5 reads. <ul style="list-style-type: none"> <li>• <b>True:</b> Attempt rescue.</li> <li>• <b>False:</b> Do not attempt rescue.</li> </ul>                                                                                                                |
| Map in base space   | False         | Allow mapping in base space if input data has base space available. If only color space is available, then mapping will fail when base space mapping is turned on. Values: <ul style="list-style-type: none"> <li>• True: Map in base space.</li> <li>• False: Map in color space.</li> </ul> |
| Add color sequence  | True          | Add color sequence to BAM records: Values: <ul style="list-style-type: none"> <li>• True: Add color.</li> <li>• False: Do not add color.</li> </ul>                                                                                                                                           |
| Average insert size | 120           | The average the insert size. Used to compute the rescue distance: $\text{rescueDistance} = \text{avgInsertSize} + (3 * \text{stdInsertSize})$ .<br>Allowed values: Integers 0–100000.                                                                                                         |
| Stdev insert size   | 60            | Standard deviation of the insert size.<br>Allowed values: Integers 1–100000.                                                                                                                                                                                                                  |

### Advanced

There are three categories of Advanced parameters: general, Rescue, and BAM Generation.

#### General

| Parameter             | Default value | Description                                                                                                                                                      |
|-----------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Min junction overhang | 8             | Alignments to junctions are not reported if they have fewer than this number of bases unaligned on either side of the splice.<br>Allowed values: Integers 0–100. |

#### Rescue

| Parameter                               | Default value | Description                                                                                       |
|-----------------------------------------|---------------|---------------------------------------------------------------------------------------------------|
| Max number of allowed mismatches for F3 | 8             | Maximum number of mismatches allowed in a rescued F3 alignment.<br>Allowed values: Integers 0–15. |
| Max number of allowed mismatches for F5 | 6             | Maximum number of mismatches allowed in a rescued F5 alignment.<br>Allowed values: Integers 0–10. |

## BAM Generation

|                           |    |                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|---------------------------|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Create unmapped BAM files | 0  | Create BAM files containing unmapped reads.<br>Values:<br><ul style="list-style-type: none"> <li>• <b>True:</b> Create.</li> <li>• <b>False:</b> Do not create.</li> </ul>                                                                                                                                                                                                                                                                            |
| Mapping quality threshold | 0  | Provide control the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer.<br>Allowed values: Integers 0–255.                                                                                                                                                                                                                       |
| refcor base QV filter     | 10 | Resulting base calls with quality less than this value will be replaced with "N."<br>Allowed values: Integers 0–255.                                                                                                                                                                                                                                                                                                                                  |
| Reference weight          | 8  | Used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. Adds weight (in terms of Phred score) to the signals that are compatible with reference. Color combinations that result in a variant are considered compatible with reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation.<br>Allowed values: Integers 0–100. |

## Whole Transcriptome

| Parameter   | Default value | Description                                                                                                                                                                                   |
|-------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Read length | 75            | Position in the read at which the error rate inflates, for instance, 35-40 for 50bp long reads. (If set to 0, then the value is equal to 0.8*readLength).<br>Allowed values: Integers 25–100. |

## BAMStats

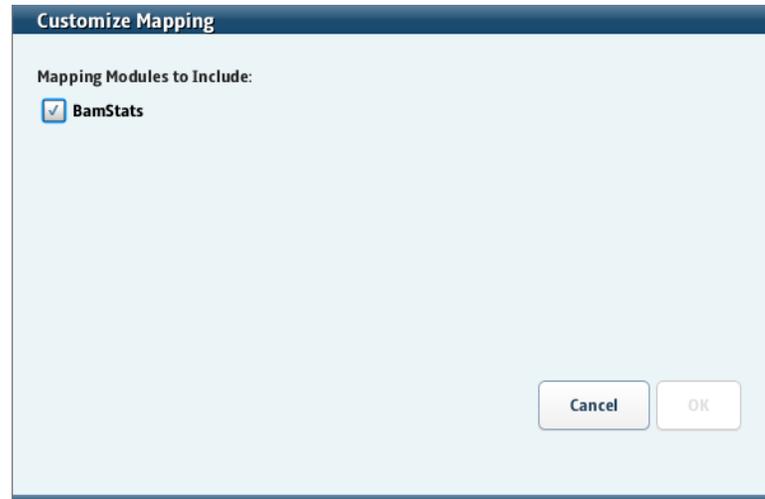
| Parameter                    | Default value                              | Description                                                                                                                                                                                                      |
|------------------------------|--------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Input directory for BAMStats | `\${analysis.output.dir}/fragment.mapping` | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample.                                                                                             |
| Output directory             | `\${task.output.dir}`                      | The path to the output directory where BAMStats will write its chart (.cht) files.                                                                                                                               |
| Maximum coverage             | 10,000                                     | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br>Allowed values: Integers 0–10000. |

| Parameter                                                                | Default value | Description                                                                                                                                                                                                                                                                                |
|--------------------------------------------------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Maximum insert size                                                      | 100,000       | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br>Allowed values: Integers 0–100000.                                           |
| Insert bin size                                                          | 100           | Bin size for insert range distribution.<br>Allowed values: Integers $\geq 1$ .                                                                                                                                                                                                             |
| Whether to combine data from both the strands for coverage in WIG format | 1             | Combine or do not combine data from both strands for coverage in WIG format.<br>Allowed values: <ul style="list-style-type: none"><li>• 0: Do not combine data.</li><li>• 1: Combine data.</li></ul>                                                                                       |
| Primary alignments only for coverage in WIG file format                  | 1             | Use only primary alignments for coverage in WIG file format.<br>Allowed values: <ul style="list-style-type: none"><li>• 0: Do not restrict coverage in WIG file format to only primary alignments.</li><li>• 1: Restrict coverage in WIG file format to only primary alignments.</li></ul> |
| Bin size for coverage in WIG file format                                 | 100           | The bin size for coverage in WIG file format.<br>Allowed values: Integers $> 1$ .                                                                                                                                                                                                          |

## Map data

Mapping data is automatic in LifeScope™. To map data:

1. In the Projects organizer (shown on page 75), select a project.
2. Click an analysis icon , then click **Edit Analysis**.
3. In the Edit Analysis window, accept the default settings for mapping and pre-processing data in the Secondary Analysis section.  
If you want to exclude BAMStats, click **Customize**. In the Customize Mapping window, uncheck BamStats, then click **OK**.



If you do not want to pre-process data, uncheck the box.

## Mapping output files

### Overview

The mapping module generates a BAM file containing alignments in coordinate order. For information about the BAM file, see [Appendix A, “File Format Descriptions and Data Uses”](#) on page 341.

The number of BAM files generated depends on the input XSQ files. One BAM file is generated per read-set (a read-set is defined as data from one barcode, in one XSQ file). If the input data is an XSQ file containing 96 barcodes, then the mapping module generates at least 96 output BAM files. Beads that are unclassified in any barcode are output into a separate additional BAM file.

The filenames for the output BAM files are created using information from the input XSQ file, including: file base name, file id, index name, and index id. The BAM files are named according to the following patterns:

- Non-indexed BAM files: *xsqname-fileID-1.bam*
- Indexed BAM files: *xsqname-fileID-idx\_bcIndex-bcID.bam*

The fields in the filenames are:

- **xsqname** – The XSQ file base name, without the .xsq extension.
- **fileID** – The file ID for internal XSQ file tracking.
- **bcIndex** – The barcode index.
- **bcID** – The barcode identifier for internal barcode tracking.

The mapping output files are used by the BAMStats mapping statistics module and by the whole transcriptome tertiary analysis modules: whole transcriptome analysis counts, whole transcriptome analysis coverage, and whole transcriptome analysis splice junctions.

### BAM file differences

This section describes two types of differences seen in WT BAM files, compared to other LifeScope™ BAM files.

### Single-read optional fields

A BAM file produced by the WT paired-end pipeline is identical to that produced by the resequencing pipeline. However, the BAM format from the WT single-read pipeline differs from BAM files produced elsewhere in LifeScope™ Software. The single-read pipeline produces separate BAM files for filtered, unmapped, and mapped reads.

## Mapping statistics parameters

For descriptions of mapping statistics parameters, see the BAMStats table [on page 228](#).

## Summary of mapping statistics output

### File formats

For every input BAM file, a set of statistics files are generated. These files are in CHT, CSV, TXT, and WIG formats. Each CHT file corresponds to one displayed chart. A CHT file specifies the type of chart, the displayed range of each axis, and the data points, without using external references.

The CHT file format is an internal file format based on the CSV file format, with addition header information included. CHT header information is the following:

```
# name:  
# type: scatter2d | pie | vbar | line  
# title:  
# xaxisname:  
# yaxisname:  
# xrange: <min>:<tickinterval>:<max>  
# yrange: <min>:<tickinterval>:<max>  
XAXISNAME, SERIES1NAME, SERIES2NAME, ...  
x1, y1.1, y1.2, ...  
x2, y2.1, y2.2, ...  
x3, y3.1, y3.2, ...
```

The wiggle format (.wig) is a public format typically used for coverage. Visit their site for more information:

[hgdownload.cse.ucsc.edu/goldenPath/help/wiggle.html](http://hgdownload.cse.ucsc.edu/goldenPath/help/wiggle.html)

A genome browser such as the Integrative Genomics Viewer (IGV) can be used to visualize the coverage. For information is available from their site:

[www.broadinstitute.org/igv/](http://www.broadinstitute.org/igv/)

For a collection of input BAM files that belong to a sample, a set of cumulative statistics files are generated. The cumulative statistics files are also in CHT, CSV, TXT, and WIG formats. The cumulative statistics can be visualized in the LifeScope™ Software UI.

## Summary information

A tab-separated summary file (*BAMfilename*-summary.tbl) is generated that summarizes key mapping quality statistics per input BAM file. This file contains one row for each input BAM file in the sample. The summary file is displayed in the LifeScope™ Software UI. See [“Summary file” on page 236](#).

## Directory structure

The output of the BAMStats module has the following directory structure:

```
bamstats/
  <sampleName>/*.cht, *.tbl
  <sampleName>/<bam>/.*cht
  <sampleName>/<bam>/Misc/*.csv, *.txt, *.wig
  <sampleName>/Misc/*.csv, *.txt
```

The position error files and probe errors files are created in the BAM file directories.

## Overview

The summary report contains a snapshot of statistics in all BAM files present in this sample. This report is displayed in the LifeScope™ Software UI.

```
<sampleName1>/BAMfilename-summary.tbl
```

Following directories and reports contain the cumulative statistics from all BAM files that belong to this sample. The Misc folder is not displayed in UI.

```
<sampleName1>/*.cht
<sampleName1>/Misc/*.csv, *.wig, *.txt
```

Following statistics are generated per BAM file in the mapping directory. These reports are not displayed in the UI.

```
<sampleName1>/<BAMfilename>/.*cht
<sampleName1>/<BAMfilename>/Misc/*.csv, *.txt, *.wig
```

In a sample, some of the BAM files possibly represent unhealthy DNA or RNA, causing the cumulative statistics to look poor. The summary tbl file is the unified location for examining the quality of data of all BAM files in the sample.

If the data for a particular BAM file is not as expected, look at the directory-level reports for that BAM file, for details.

# Mapping statistics output files

This section describes the mapping statistics files generated by the BAMStats module. When statistics reports are separated by tag type, the report's file name includes the tag in the file name. The tags used in file names are:

| Library type | Tag |
|--------------|-----|
| Fragment     | F3  |
| Mate-pair    | F3  |
|              | R3  |

| Library type | Tag   |
|--------------|-------|
| Paired-end   | F3    |
|              | F5-P2 |

The output files generated by BAMStats include the name of the BAM file. The file name pattern is:

*BAMFileName-fileID-barcodeID.StatisticsReportName.tag.extension*

This string is referred to by *prefix* in the following table, the mapping statistics output table. In the description of mapping statistics output file names in the following table, the string *tag* is used to refer to the tags in the following table.

| Report                                                                   | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Alignment Length Distribution</b>                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                          | <p>A bar plot giving the distribution of alignment lengths found in various tags used in alignment. The report is separated by tag type to provide visibility into the accuracy of individual tags.</p> <p>Only the primary alignment for each bead is considered in calculating the distribution.</p> <p>Y axis: Frequency<br/>X axis: Alignment length (from 0 to the maximum read length)</p> <p>Output file name: <i>prefix.Alignment.Length.Distribution.tag.cht</i></p>                                                                                                                                                                                                                            |
| <b>Base Mismatch Distribution</b>                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                          | <p>A bar plot giving the distribution of total number of mismatches found in various tags used in alignment. The bins are summed over all alignment lengths.</p> <p>Only the primary alignment for each bead is considered in calculating the distribution.</p> <p>Y axis: Frequency<br/>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)</p> <p>Output file name: <i>prefix.BaseMismatch.Distribution.tag.cht</i></p>                                                                                                                                                                                                                                                            |
| <b>Distribution of Alignment Length and Number of Mismatches in Tags</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                          | <p>A report providing a simultaneous picture of the alignment length and number of mismatches distributions in various tags used in alignment. Only primary alignments for each tag are considered in calculating this distribution. The bins range from 0 to the maximum read length, and from 0 to the maximum number of mismatches allowed.</p> <p><b>Note:</b> This report is located in the <code>Misc</code> folder, and is not displayed in the UI.</p> <p>Y axis: Alignment length (from 0 to the max read length)<br/>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)<br/>Z axis: Frequency</p> <p>Output file name: <i>prefix.AlignmentLength.Mismatch.tag.csv</i></p> |
| <b>Distribution of Mismatches by Position</b>                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                          | <p>A bar plot providing a distribution of errors (mismatches to reference) by position within the read. Only the primary alignments for each bead are used to generate this distribution.</p> <p>The base-space report is generated on every run.</p> <p>Y axis: Frequency<br/>X axis: Position (from 0 to maximum read length)</p> <p>Output file name: <i>prefix.Mismatches.By.Position.BaseSpace.tag.cht</i>, and (only if color-space data is present in the BAM file) <i>prefix.Mismatches.By.Position.ColorSpace.tag.cht</i></p>                                                                                                                                                                   |

| Report                                                       | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Color Mismatch Distribution</b>                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                              | A bar plot providing a distribution of the total number of color mismatches in various tags used in alignment. Only the primary alignments for each bead are used to generate this distribution. This report is only generated if mapping is done in color space.                                                                                                                                                                                                                                                              |
|                                                              | Y axis: Frequency<br>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                              | Output file name: <i>prefix.ColorMismatches.Distribution.tag.cht</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Distribution of Alignment Length and Color Mismatches</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                              | A report providing a simultaneous picture of the alignment length and number of color mismatches distributions in various tags used in alignment. Only primary alignments for each tag are considered in calculating this distribution. The bins range from 0 to the maximum read length, and from 0 to the maximum number of mismatches allowed. This report is only generated if mapping is done in color space.<br><br><b>Note:</b> This report is located in the <code>Misc</code> folder, and is not displayed in the UI. |
|                                                              | Y axis: Alignment length (from 0 to the max read length)<br>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)<br>Z axis: Frequency                                                                                                                                                                                                                                                                                                                                                                       |
|                                                              | Output file name: <i>prefix.AlignmentLength.ColorMismatches.tag.csv</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Base QV Distribution</b>                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                              | A bar plot providing a distribution of base quality values generated using the reference assisted error correction/base conversion algorithm. The base QV distributions are separated for each tag as quality of individual tags could be very different.                                                                                                                                                                                                                                                                      |
|                                                              | Y axis: Frequency<br>X axis: Base QV (from 0 to the maximum QV)                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                              | Output file name: <i>prefix.BaseQV.tag.cht</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Base QVs by Position</b>                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                              | A report providing a distribution of base quality values by individual base positions. This report identifies if certain base positions (particularly towards the end of the read) have poor base quality values. All the tags (F3/R3/F5-P2) used in the alignment are combined into a single bin.                                                                                                                                                                                                                             |
|                                                              | Y axis: Base QV<br>X axis: Base position (from 0 to read length)<br>Z axis: Frequency                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                              | Output file name: <i>prefix.BaseQV.by.Position.csv</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Distribution of Mismatches by Base QV</b>                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                              | A line plot providing a distribution of errors (mismatches to reference) by base quality values bins.                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                              | Y axis: Percentage of total mismatches<br>X axis: Base QV (from 0 to maximum QV)<br><br><b>Note:</b> This report gives, for each QV, a measure of the raw mismatches at positions with that QV. This information does not measure whether the base QVs generated are well calibrated to the probability of error in that particular base position.                                                                                                                                                                             |
|                                                              | Output file name: <i>prefix.Mismatches.By.BaseQV.tag.cht</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Distribution by Mapping QVs by Tag</b>                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

| Report                                                         | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|----------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                | <p>A bar plot providing a distribution of mapping quality values for individual tags (F3/R3/F5-P2). Only the primary alignment for each bead is used in calculating this distribution.</p> <p>Y axis: Frequency<br/>X axis: Mapping QV (from 0 to maximum mapping QV)</p> <p>Output file name: <i>prefix.MappingQV.tag.cht</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Distribution by Pairing QVs</b>                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                | <p>A bar plot providing a distribution of pairing quality values. Only the primary alignment for each bead is used in calculating this distribution.</p> <p>Y axis: Frequency<br/>X axis: Pairing QV (from 0 to maximum pairing QV)</p> <p>Output file name: <i>prefix.PairingQVs.cht</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Coverage Report</b>                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                | <p>A line plot providing a distribution of coverage obtained after mapping/pairing. Only the primary alignment for each bead is used in this calculation.</p> <p>Y axis: Frequency<br/>X axis: Coverage (from 0 to number of reads)</p> <p>Output file name: <i>prefix.Coverage.cht</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Coverage Report by Chromosome (Contig) and Base Windows</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                | <p>A line plot providing a distribution of coverage within each reference window. The coverage is calculated within each window along a reference chromosome. The window size can theoretically be anywhere from a single base to the contig length. However the calculations of base level coverage are computationally expensive and less interpretable as the window size increases. Only the primary alignment for each bead is used in this calculation.</p> <p>Y axis: Frequency<br/>X axis: Coverage (from 0 to number of reads)</p> <p>Output file name: <i>prefix.Coverage.By.Chromosome.contignn.cht</i></p>                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Coverage by Strand</b>                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                | <p>A line plot providing the distribution of coverage within each reference window separated by reference strand (+/-). Only the primary alignment for each bead is used in this calculation.</p> <p>Y axis: Frequency<br/>X axis: Coverage (from 0 to number of reads)</p> <p>Output file name: <i>prefix.Coverage.By.Strand.Positive.cht</i>, <i>prefix.Coverage.By.Strand.Negative.cht</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Coverage files</b>                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                | <p>Coverage reports in wiggle format. For each genome position, reports the number of reads that cover (map to or span) the position. Because reporting coverage for each position results in very large files, coverage is reported for bins, with each bin spanning a user-defined number of bases. For each bin, the mean coverage of all the positions in that bin is reported. The parameter <code>bamstats.wig.binsize</code> controls the size of the bins in this file.</p> <p>Each coverage file includes a header as the first line. The header lines follow this pattern:<br/> <code>track type=wiggle_0 name=&lt;chrname&gt; description=&lt;coverage from positive/negative/both strand&gt;<br/> visibility=full color=0,0,255 fixedStep chrom=&lt;chrname&gt; start=&lt;startpos&gt; step=&lt;binsize&gt; span=&lt;binsize&gt;</code></p> <p>See also <a href="#">"Coverage files" on page 238</a>.</p> <p>Output file name: <i>coverage_chrnn.pos.wig</i>, <i>coverage_chrnn.neg.wig</i></p> |

| Report                                        | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                     |
|-----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><i>Insert Range Distribution</i></b>       |                                                                                                                                                                                                                                                                                                                                                                             |
|                                               | A line plot providing the distribution of insert sizes for paired data (PE and LMP library types).                                                                                                                                                                                                                                                                          |
|                                               | Y axis: Frequency<br>X axis: Insert Range bins                                                                                                                                                                                                                                                                                                                              |
|                                               | Output file name: <i>prefix.Insert.Range.Distribution.cht</i>                                                                                                                                                                                                                                                                                                               |
| <b><i>Distribution of Read Pair Types</i></b> |                                                                                                                                                                                                                                                                                                                                                                             |
|                                               | For paired data, this report calculates the distribution of read pair types (AAA, AAB, C**, etc.).                                                                                                                                                                                                                                                                          |
|                                               | Y axis: Frequency<br>X axis: Read pair type                                                                                                                                                                                                                                                                                                                                 |
|                                               | Output file name: <i>prefix.ReadPair.Type.cht</i>                                                                                                                                                                                                                                                                                                                           |
| <b><i>Pairing Statistics</i></b>              |                                                                                                                                                                                                                                                                                                                                                                             |
|                                               | A pie chart showing the percentages of the following F3, R3 (or F5) combinations, for paired data: <ul style="list-style-type: none"> <li>• Mapped, Mapped</li> <li>• Mapped, Unmapped</li> <li>• Unmapped, Mapped</li> <li>• Unmapped, Unmapped</li> <li>• Mapped, Missing</li> <li>• Missing, Mapped</li> <li>• Unmapped, Missing</li> <li>• Missing, Unmapped</li> </ul> |
|                                               | Output file name: <i>prefix.Pairing.Stats.cht</i>                                                                                                                                                                                                                                                                                                                           |
| <b><i>Unique Start Positions</i></b>          |                                                                                                                                                                                                                                                                                                                                                                             |
|                                               | This report lists the following: <ul style="list-style-type: none"> <li>• The total number of unique start positions.</li> <li>• The average number of unique start positions per reference position.</li> </ul> See also <a href="#">“Unique Start Position” on page 237</a> .                                                                                             |
|                                               | Output file name: <i>BAMfilename-summary.txt, Samplename.-summary.txt</i>                                                                                                                                                                                                                                                                                                   |
| <b><i>Summary Report</i></b>                  |                                                                                                                                                                                                                                                                                                                                                                             |
|                                               | The summary file provides statistics for each BAM file in the sample. See <a href="#">“Summary file”</a> .                                                                                                                                                                                                                                                                  |
|                                               | Output file name: <i>filename.-summary.tbl</i>                                                                                                                                                                                                                                                                                                                              |

### Example of mapping statistics output

This section provides example output of some mapping statistics output files.

#### Summary file

The summary file provides statistics for each BAM file in the sample. This list describes labels used in the summary file:

- **NumUnFilteredBeads** – The total number of beads, before any filtering on the instrument. This value is also the fragment count in the input XSQ file.
- **NumFilteredBeads** – The number of beads that pass filtering on the instrument.
- **NumMapped** – The number of reads with primary alignment.

- **% filtered mapped** – The number of primary reads divided by the number of beads passing instrument filtering ( $\text{NumMapped} / \text{NumFilteredBeads}$ ).
- **% total mapped** – The number of primary reads divided by the number of total beads ( $\text{NumMapped} / \text{NumUnFilteredBeads}$ ).
- **Megabases of coverage** – The sum of alignment lengths, for primary alignments only.

The number of beads filtered out in the instrument is found by subtracting the number of beads that pass filtering from the total number of beads:

$$\text{NumUnFilteredBeads} - \text{NumFilteredBeads}$$

The following is example contents for a summary file. The PredictedInsertSize and Tag2 fields only are present with paired data.

```
BamFileName, IsColorInBam, IsBaseInXSQ, IsECC, LibraryType,
ReadLength, Megabases of Coverage, PredictedInsertSize,
NumFragmentsPassingFilters, NumFragmentsTotal,
Tag1-NumMapped,
Tag1- % total Mapped,
Tag1- % filtered mapped,
Tag2-NumMapped,
Tag2- % total Mapped,
Tag2- % filtered mapped,
(Tag1-AlignmentLength;Min;Max;Avg;Median;StdDev) ,
(Tag1-NumBaseMismatches;Min;Max;Avg;Median;StdDev) ,
(Tag1-MappingQV;Min;Max;Avg;Median;StdDev) ,
(Tag1-BaseQV;Min;Max;Avg;Median;StdDev) ,
(Tag1-NumColorMismatches;Min;Max;Avg;Median;StdDev) ,
(Tag2-AlignmentLength;Min;Max;Avg;Median;StdDev) ,
(Tag2-NumBaseMismatches;Min;Max;Avg;Median;StdDev) ,
(Tag2-MappingQV;Min;Max;Avg;Median;StdDev) ,
(Tag2-BaseQV;Min;Max;Avg;Median;StdDev) ,
(Tag2-NumColorMismatches;Min;Max;Avg;Median;StdDev)
(Coverage;Min;Max;Avg;Median;StdDev)
```

The following is an example line of output in the summary file:

```
example.bam Y N N FRAG 50 2184653 100000 100000 51536 51.54
51.54 (F3;25;50;42.39;45;6.48) (F3;0;50;2.98;0;9.24)
(F3;0;100;65.43;72;25.31) (F3;1;41;35.09;41;11.65)
(F3;0;9;1.20;1;1.39) (0;399;0.43;0;5.96)
```

### Unique Start Position

The following is an example of a Unique Start Position report, for fragment data:

```
Unique Start Positions F3
Total Number of Unique Start Positions: 16536 (16.043154 %
of primary alignments)
Average Number of Unique Start Positions per Reference
Position: 0.001630
```

Unique Start Position reports have the following naming patterns:

- *BAMfilename*-summary.txt
- *Samplename*-summary.txt

## Coverage files

Example contents for the file `coverage_chr1_positive.wig` are:

```
browser position chr1:1-200000000
browser hide all
browser pack refGene encodeRegions
# minimumMapq=25, minimumCoverage=1,
alignmentFilteringMode=PRIMARY, filterOrphanedMates=false,
track name="BAM Coverage positive strand" description="BAM
Coverage positive strand" visibility="full color 0,0,255"
priority=10 yLineMark=0 type=wiggle_0 yLineOnOff=on
variableStep chrom=chr1 span=1
336171
336181
336191
336201
336211
336221
336231
336241
336251
336261
336271
...
```

Example contents for the file `coverage_chr1_negative.wig` are:

```
browser position chr1:1-200000000
browser hide all
browser pack refGene encodeRegions
# minimumMapq=25, minimumCoverage=1,
alignmentFilteringMode=PRIMARY, filterOrphanedMates=false,
track name="BAM Coverage negative strand" description="BAM
Coverage negative strand" visibility="full color 0,0,255"
priority=10 yLineMark=0 type=wiggle_0 yLineOnOff=on
variableStep chrom=chr1 span=1
57432
57442
57452
57463
57473
57483
57493
57503
57513
```

57523  
57533  
...

## Whole transcriptome analysis output file formats

### Alignment report

Whole transcriptome analysis mapping runs produce an alignment report, the alignmentReport.txt file in the results directory.

Below is an example of the alignment report produced by the whole transcriptome analysis pipeline (the frequency lists have been truncated).

The 100.0% value reported for the “Reads mapped, not filtered” line should be interpreted as “Reads mapped, not filtered” comprising 100% of the category described in its section, not that the category “Reads mapped, not filtered” comprises 100% of the total reads.

```
-----
ALIGNMENT REPORT
-----

Counts:
Total reads:                102,837,343  (100.0%)
Reads mapped:               78,065,351  ( 75.9%)
Reads filtered:             12,029,269  ( 11.7%)
-----
Reads mapped, not filtered          68,780,034  (100.0%)
Reads with too many mappings (N >= 10):  5,897,833  (  8.6%)
Reads with number of mappings in proper range (N < 10):
62,882,201    ( 91.4%)
Reads uniquely aligned (score.clear.zone = 3):
53,417,152    ( 77.7%)
Reads uniquely aligned to junctions          4,816,207  (  7.0%)
Reads uniquely aligned to known junctions    4,773,948  (  6.9%)
```

```
ABSOLUTE FREQUENCIES
Alignment_Score  All_Read_Alignments_(N=_936)
Primary_Read_Alignments_(N=_935)
Unique_Read_Alignments_(N=_866)
0.0      0      0      0
1.0      0      0      0
2.0      0      0      0
36.0     0      0      0
37.0     87     87     87
38.0     0      0      0
39.0     0      0      0
47.0     0      0      0
48.0     0      0      0
49.0    849    848    779
50.0     0      0      0
Total    936    935    866
```

RELATIVE FREQUENCIES

```

Alignment_Score      All_Read_Alignments_(N=_936)
Primary_Read_Alignments_(N=_935)
Unique_Read_Alignments_(N=_866)
0.0      0.0%      0.0%      0.0%
1.0      0.0%      0.0%      0.0%
2.0      0.0%      0.0%      0.0%
36.0     0.0%      0.0%      0.0%
37.0     9.3%      9.3%      10.0%
38.0     0.0%      0.0%      0.0%
39.0     0.0%      0.0%      0.0%
40.0     0.0%      0.0%      0.0%
47.0     0.0%      0.0%      0.0%
48.0     0.0%      0.0%      0.0%
49.0     90.7%     90.7%     90.0%
50.0     0.0%      0.0%      0.0%
Total    100.0%     100.0%     100.0%

```

### Whole transcriptome analysis filtering stats

An example whole transcriptome human filter reference is provided under the `<examplesdir>/demos/WholeTranscriptome/references` folder in the optional LifeScope™ Software examples distribution. This example reference fasta includes contigs from barcode primers, human ribosomal RNAs, tRNAs, and other known targets for filtering. The filter reference may be expanded or removed with more csfasta reference records at the discretion of the user.

The stats for filtered reads are generated after a LifeScope™ Software run. First two lines of the stats file states the number of reads processed, and the number of reads mapped to the filtered reference followed by its percentage. Each following line reports a contig name and the count of reads that aligned to that contig. Paired-end filtering stats may be found in the Intermediate folder. The following is a truncated example of filtering stats output:

```

countOfReadsProcessed: 175,839,941
countOfReadsMapping:   18,783,220 (10.7%)
...
Barcode-047-3-end reverse   83
Barcode-048-3-end reverse  2,350
gi|124517659|ref|NR_003286.1| Homo sapiens 18S ribosomal RNA
(LOC100008588)   5,175,899
gi|142372596|ref|NR_003285.2| Homo sapiens 5.8S ribosomal RNA
(LOC100008587)   78,936
gi|124517661|ref|NR_003287.1| Homo sapiens 28S ribosomal RNA
(LOC100008589)   7,616,212
chr6.trna95-AlaAGC (58249908-58249836) Ala (AGC) 73 bp Sc:
42.26   5
chr6.trna25-AlaAGC (26859897-26859969) Ala (AGC) 73 bp Sc:
46.89   2
chr6.trna94-AlaAGC (58250620-58250548) Ala (AGC) 73 bp Sc:
54.62   4
chr6.trna160-AlaAGC (26881822-26881750) Ala (AGC) 73 bp Sc:
54.69   35

```

## 16

# Perform MethylMiner™ Mapping

This chapter covers:

- Introduction to MethylMiner™ mapping ..... 241
- MethylMiner™ mapping library types ..... 242
- MethylMiner™ mapping input files. .... 242
- MethylMiner™ mapping parameters ..... 243
- Perform MethylMiner™ mapping ..... 245
- View MethylMiner™ mapping results ..... 249
- MethylMiner™ mapping output files ..... 249
- Further analysis of MethylMiner™ mapping results ..... 250

## Introduction to MethylMiner™ mapping

DNA methylation is an epigenetic modification crucial for organism development and normal gene regulation. Life Technologies have introduced a versatile methyl-CpG binding protein-based system, the MethylMiner™ Kit, for the enrichment of methylated sequences from genomic DNA, that, with the use of SOLiD® System sequencing, allows for focused evaluation of methylation patterns in genome-wide studies. The enriched reads can also be bisulfite-converted, which would additionally allow determination of methylation status of individual cytosines.

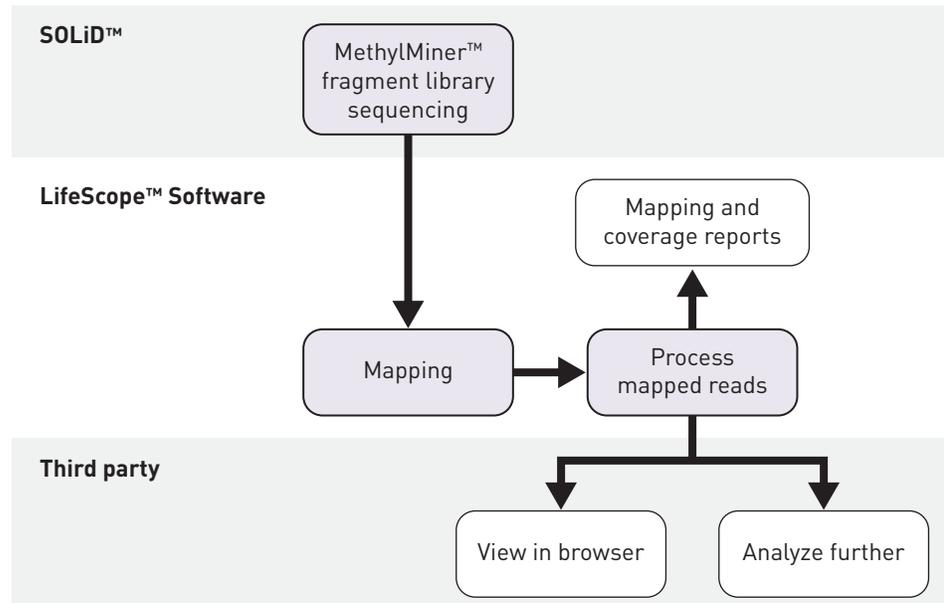
SOLiD® System sequencing can also, of course, be used to study methylation in unenriched, whole-genome bisulfite-converted read data.

The purpose of LifeScope™ Genomic Analysis Software MethylMiner™ tools is to provide a data analysis workflow for mapping and analyzing MethylMiner™ enriched and unenriched fractions of genomic DNA as well as bisulfite-converted and unconverted reads sequenced on the SOLiD® System.

The MethylMiner™ analysis workflow functionality currently includes:

- Mapping of unconverted and bisulfite-converted reads.
- Mapping statistics and statistics on read coverage and depth.
- Mapped reads output in BAM-format files.
- Visualization of mapped reads on publicly available genome browsers.

The following illustration shows the MethylMiner™ analysis workflow with SOLiD® software and third-party browsers.



## MethylMiner™ mapping library types

Genomic resequencing analysis supports data from the following library types:

- Fragment
- Paired-end

## MethylMiner™ mapping input files

| Input File                   | File Extension |
|------------------------------|----------------|
| (Optional) Color space FASTA | *.csfasta      |
| FASTA                        | *.fa, *.fasta  |
| (Optional) Quality Value     | *qual, *qv     |
| Extensible sequence          | *.xsq          |

## MethylMiner™ mapping parameters

### General parameters

General parameters include:

| Parameter              | Default value | Description                                                       |
|------------------------|---------------|-------------------------------------------------------------------|
| analysis.assembly.name | —             | Default: unknown                                                  |
| analysis.regions.file  | —             | Default: unknown                                                  |
| analysis.platform      | SOLID         | Always specify "SOLID". Other values are reserved for future use. |

Use the  button to open the File Chooser and search for input files.

### Fragment mapping parameters

There are three categories of parameters for mapping: Main, Advanced, and BAMStats

#### Main

| Parameter          | Default value | Description                                                                                                                                                                                                                                                                                   |
|--------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Add color sequence | True          | Add color sequence to BAM records: Values: <ul style="list-style-type: none"> <li>• True: Add color.</li> <li>• False: Do not add color.</li> </ul>                                                                                                                                           |
| Map in base space  | False         | Allow mapping in base space if input data has base space available. If only color space is available, then mapping will fail when base space mapping is turned on. Values: <ul style="list-style-type: none"> <li>• True: Map in base space.</li> <li>• False: Map in color space.</li> </ul> |

#### Advanced

| Parameter                     | Default value | Description                                                                                                                                                                                                                       |
|-------------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| BAM soft clip                 | False         | Modify an unaligned portion of a read that needs to be presented in the BAM record. Values: <ul style="list-style-type: none"> <li>• True: Soft clip.</li> <li>• False: Do not soft clip.</li> </ul>                              |
| Base quality filter threshold | 0             | Replace resulting base-calls with a quality value less than the specified value.                                                                                                                                                  |
| Create unmapped BAM files     | False         | Create BAM files containing unmapped reads. Values: <ul style="list-style-type: none"> <li>True: Create.</li> <li>False: Do not create.</li> </ul>                                                                                |
| Mapping QV/threshold          | 0             | Provide control the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer. Allowed values: Integers 0 to 1,000. |

| Parameter                        | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reference weight                 | 15            | Used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. Adds weight (in terms of Phred score) to the signals that are compatible with reference. Color combinations that result in a variant are considered compatible with reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation.<br><br>Allowed values: Integers 0 - 100. |
| Second map gapped algorithm type | GLOBAL        | Do indel finding, and control the behavior of indel finding.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• NONE: Turn off indel finding.</li> <li>• GLOBAL: Mapping reports global alignment up to one indel.</li> <li>• LOCAL: Mapping reports local alignment up to one indel.</li> </ul>                                                                                                                                               |

### BAMStats

| Parameter                                                                | Default value               | Description                                                                                                                                                                                                                                            |
|--------------------------------------------------------------------------|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Input directory for BAMStats                                             | \${analysis.output.dir}/bam | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample.                                                                                                                                   |
| Output directory                                                         | \${task.output.dir}         | The path to the output directory where BamStats will write its chart (.cht) files.                                                                                                                                                                     |
| Maximum Coverage                                                         | 10,000                      | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br><br>Allowed values: Integers 1 - 10,000.                                |
| Maximum insert size                                                      | 100,000                     | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br><br>Allowed values: Integers 1 - 100000. |
| Insert bin size                                                          | 100                         | Bin size for insert range distribution.<br><br>Allowed values: Integers >= 0-1.                                                                                                                                                                        |
| Whether to combine data from both the strands for coverage in WIG format | 1                           | Combine or do not combine data from both strands for coverage in WIG format.<br><br>Allowed values: <ul style="list-style-type: none"> <li>• 0: Do not generate wig files.</li> <li>• 1: Generate the combined coverage file.</li> </ul>               |

|                                                         |     |                                                                                                                                                                                                                                                                                                  |
|---------------------------------------------------------|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Primary alignments only for coverage in WIG file format | 1   | Use only primary alignments for coverage in WIG file format.<br>Allowed values:<br><ul style="list-style-type: none"> <li>• 0: Do not restrict coverage in WIG file format to only primary alignments.</li> <li>• 1: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Bin size for coverage in WIG file format                | 100 | The bin size for coverage in WIG file format.<br>Allowed values: Integers > 1-100000                                                                                                                                                                                                             |

## Perform MethylMiner™ mapping

Unconverted MethylMiner™ reads must be mapped to a regular (unconverted) reference genome sequence. Bisulfite-converted reads must be mapped to a pair of appropriately converted reference sequences (forward and reverse conversions), as recommended below.

Bisulfite-converted reads must be mapped to a pair of appropriately converted reference sequences (forward and reverse conversions), as recommended below.

**Note:** MethylMiner™ supports fragment and paired-end libraries. Do not use this module with mate-pair libraries.

Follow these recommendations to map MethylMiner™ bisulfite-converted reads:

1. For mapping bisulfite-converted reads, the converted reference sequence pairs below are recommended. Use either Pair 1 or Pair 2 below.
  - Pair 1:
    - Reference with all non-CpG C's converted to T's (the forward conversion)
    - Reference with all non-CpG G's converted to A's (the reverse conversion)

We have found that Pair 1 is most appropriate for bisulfite-converted reads from MethylMiner™ samples.

- Pair 2:
  - Reference with all C's converted to T's
  - Reference with all G's converted to A's

Pair 2 is most appropriate for bisulfite-converted reads from whole-genome data (not MethylMiner™ enriched).

2. The two sets of reference sequences within a given pair must be saved as separate reference files, and the mapping must be performed separately with each of them.

**Note:** After mapping, the additional consolidation of the results from the forward and reverse conversions must be done by the user, and further analysis can be done with third-party tools.

### (Optional) Import data

You can optionally import data to be analyzed. For instructions on how to import data, see "Import Data" on page 81.

**Log in to  
LifeScope™**

1. Navigate to LifeScope™ Software at a designated url, given to you by your network administrator.  
http://<IP address>:<port number>/LifeScope.html  
where *IP address* is the address of the system or head node and *port number* is the number of the port used by the server.
2. In the Login screen, enter your username and password, then either click **Login** or press **Enter** to open the LifeScope™ home view (shown on page 75).

**Create or select a  
project**

1. In the home view (shown on page 75), either click **Create a New Project** (described on page 80) or select a project in the Projects organizer (shown on page 75).
2. If you create a project:
  - a. Type a name and description in the Enter Project Name view.  
**Note:** The name cannot have spaces or special characters.
  - b. Click **Create New Project**.
  - c. In the Projects lists, select the new project.
3. In the Task Wizards section (shown on page 75), click **Add Data to Project** to choose a data type.

**Add data to a  
project**

Add data from the read repository to a project by choosing a data type and finding data. You can also optionally group data.

**Note:** Multiple files cannot have the same name. Make sure that the names of the files that you add are distinct.

**Note:** If the LifeScope™ Software administrator has changed the path to the read repository since your last login, restart LifeScope™ Software to update the repository to the new path.

1. In the Add Data to Project window, select **Raw unmapped (XSQ) data**, then click **Next** to find data.
2. In the Read Repository Filter table, select the read-sets you want to map and analyze.  
If you want to group data, click **Next**. If you do not want to group data, skip [step 3](#) and proceed to [step 4](#).
3. (Optional) In the Read-sets in Project table, click the checkbox of the data files you want to group, then click **Add Group to Project**. The files appear in the Groups in Project table.  
To rename a group, click the checkbox of the group in the Groups in Project table, then click **Edit**. In the Edit Group window, enter a new name.  
To remove a data file from a group, click the checkbox of the data file, then click **Delete**.
4. Click **Add Analysis** to proceed, or
  - Click **Cancel** to refrain from adding data and close the Add Data to Project window, or
  - Click **Finish** to add the data and close the Add Data to Project window.

## Create an analysis

1. In the Choose Data view of the Create Analysis window, select data files from the Available Data in Project table, then click **Next** to choose an analysis.
2. In the Choose Analysis view, enter a name for the analysis and a description. If you are re-using an Old Analysis, select **Reuse Old Analysis** and select the name of the analysis you want to use.  
**Note:** The name cannot have spaces or special characters.
3. Select **MethyMiner™**, then click **Next** to choose references.



To see the type of data mapped by the MethyMiner™ module, place your cursor over the .

4. In the Data To Be Analyzed table, click **Select the reference for the reads** to open the repository file browser.
5. In the Browse for Reference File window, navigate to the location of the reference genome of your sample. Open the folder with your .fasta file, for example:  
data ▶ referenceData ▶ lifetech ▶ hg18 ▶ reference ▶ human\_hg18.fasta  
Select the file, then click **OK**.  
The file name appears in the Reference column.  
**Note:** To change the reference file, click on **Select the reference for the reads**, then select another reference file.
6. After you have created the analysis:
  - Click **Edit** to proceed, or
  - Click **Cancel** to refrain from choosing references and close the Create Analysis window, or
  - Click **Finish** to complete analysis creation and close the window.

## Edit the analysis

In the Choose Modules view of the Edit Analysis window, accept the default settings for secondary and tertiary analyses, then click **Next** to set module parameters.

### Set module parameters

This section describes the procedures for setting general parameters and fragment mapping parameters. For descriptions of module parameters, see [“MethyMiner™ mapping parameters” on page 243](#).

You can restore the default settings of parameters by clicking the **Reset to Defaults** button.

To view descriptions of parameters, place your mouse cursor over a  button.

### Set general parameters

1. Enter an analysis assembly name, if necessary.
2. Always leave the default analysis.platform setting, which is “SOLID”.
3. Click **Next** to set fragment mapping parameters.

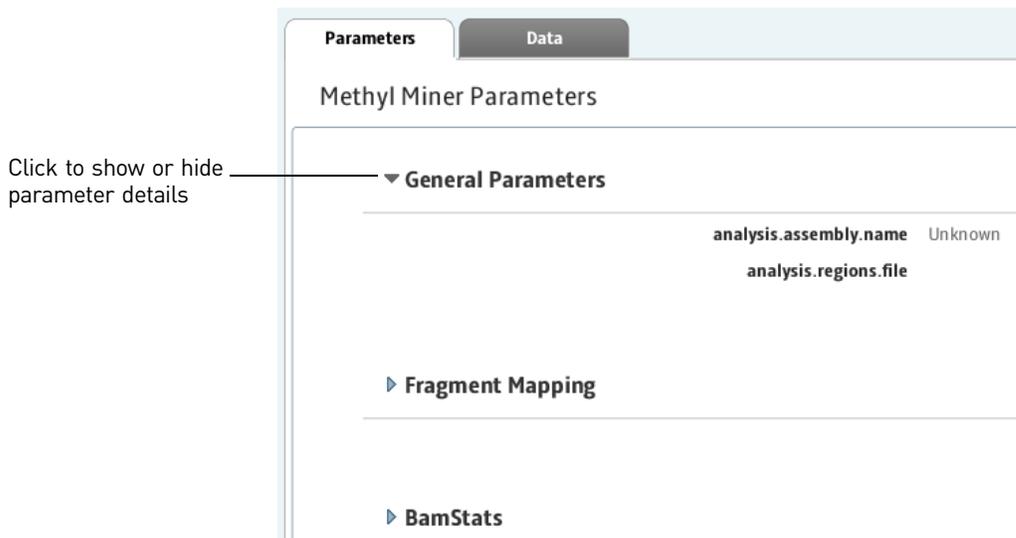
Set fragment mapping parameters

1. There are three categories of fragment mapping parameters: Main, Advanced, and BAMStats. Accept the default settings or click the Main, Advanced, and BAMStats tabs to edit the settings.
2. After you have edited an analysis:
  - Save your edits and proceed by clicking **Review**, or
  - Save your edits and close the window by clicking **Finish**, or
  - Erase your edits and close the Edit Analysis window by clicking **Cancel**.

## Review and run the analysis

**Note:** For a description of MethylMiner™ parameters, see [“MethylMiner™ mapping parameters”](#) on page 243.

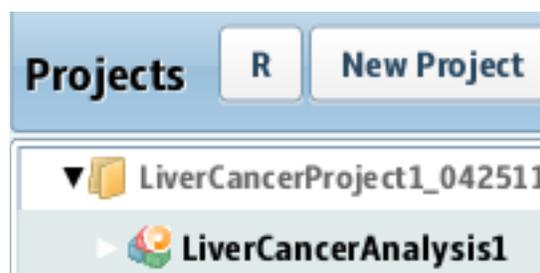
The Review Analysis view in the Run Analysis window includes two tabs: Parameters and Data. In the Parameters tab, click the  , next to the parameter categories to show or hide the parameters.



1. Review the parameters. To edit the parameters, click **Edit**.
2. To review the data that will be analyzed, click the **Data** tab.
3. If you are ready to run the analysis, click **Start Analysis**.  
The window closes.

## Check Analysis Status

1. In the Projects organizer (shown [on page 75](#)), click the project to check the status of data mapping.



2. Click the analysis name to show and show details about the analysis in the status overview (shown on page 75).
3. Click the Status tab to view the Progress column, which shows the percentage of completion for data mapping.

LiverCancerProject1... • LiverCancerAnalysis1

Overview **Status** Parameters

Analysis Runs

| Name             | XSQ ID          | Analysis             | Secondary Analysis         | Secondary Progress | Tertiary Analysis | Tertiary Progress |
|------------------|-----------------|----------------------|----------------------------|--------------------|-------------------|-------------------|
| DH10B_Test4_None | DH10B_Test4.xsq | LiverCancerAnalysis1 | [Fragment Mapping, BamS... | 9%                 |                   |                   |

After MethylMiner™ mapping has been completed, you can view mapping results.

## View MethylMiner™ mapping results

MethylMiner™ mapping results include additional files and log files.

LiverCancerProject1\_042511 • LiverCancerAnalysis1 • Mapping • solid0054\_20110102\_PE\_LFD\_RD\_SetA\_1\_HuRef100\_F3.colorRemake\_DefaultLibrary

Details Statistics **Additional Files** Logs

Additional Files:

| Directory                                                                                   | File                                                                  | Size      | Type |
|---------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|-----------|------|
| fragment.mapping/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary | solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake-1.bam     | 545208 KB | bam  |
| fragment.mapping/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary | solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake-1.bam.bai | 90 KB     | bai  |

LiverCancerProject1\_042511 • LiverCancerAnalysis1 • Mapping • solid0054\_20110102\_PE\_LFD\_RD\_SetA\_1\_HuRef100\_F3.colorRemake\_DefaultLibrary

Details Statistics Additional Files **Logs**

Log Files:

| Directory        | File                                                                                    | Size  |
|------------------|-----------------------------------------------------------------------------------------|-------|
| fragment.mapping | refcor.secondary-Homo_sapiens.NCBI36.54.dna.chr22-fragment.mapping.1.run.log            | 10 KB |
| fragment.mapping | refcor.secondary-Homo_sapiens.NCBI36.54.dna.chr22-fragment.mapping.10.run.log           | 10 KB |
| fragment.mapping | refcor.secondary-Homo_sapiens.NCBI36.54.dna.chr22-fragment.mapping.9.run.log            | 9 KB  |
| fragment.mapping | secondary-Homo_sapiens.NCBI36.54.dna.chr22-fragment.mapping.4.run.20110425193421974.log | 28 KB |
| fragment.mapping | refcor.secondary-Homo_sapiens.NCBI36.54.dna.chr22-fragment.mapping.2.run.log            | 10 KB |

See Chapter 9, “View Analysis Results” on page 103 for instructions on how to review mapping results.

## MethylMiner™ mapping output files

The output of MethylMiner™ mapping is BAM files.

The output mapping statistics reports generated by MethylMiner™ mapping include:

- The number and percent of all reads mapped and of reads mapped uniquely, in the file mapping-stats.txt and also in files ending in F3.stats and F5-P2.stats.
- Genome coverage tables for at least read depths 0X to 50X, in output files ending in coverage-histogram-F3.txt and coverage-histogram-F5-P2.txt.

**View output files in a genome browser**

Output files generated by mapping runs are compatible with third-party browser such as the Integrative Genomics Viewer (IGV) available from the Broad Institute and the UCSC Genome Browser.

## Further analysis of MethylMiner™ mapping results

Besides viewing mapped reads in genome browsers, you can further analyze mapped reads outside of LifeScope™ Genomic Analysis Software with software available in the SOLiD® development community or with other third-party tools.

MethylMiner™ unconverted mapped reads can be processed with peak-finding programs like MACS to identify genome regions of significant methylation.

Similarly, MethylMiner™ bisulfite-converted mapped reads can be processed with peak-finding programs to identify regions of significant methylation. These reads can also be processed at nucleotide resolution to report the methylation status of individual C bases, for bases covered at sufficient read depth.

## 17

# Perform ChIP-Seq Mapping

This chapter covers:

|                                              |     |
|----------------------------------------------|-----|
| ■ Introduction to ChIP-Seq mapping . . . . . | 251 |
| ■ ChIP-Seq library types . . . . .           | 251 |
| ■ ChIP-Seq input files . . . . .             | 252 |
| ■ ChIP-Seq parameters . . . . .              | 252 |
| ■ Perform ChIP-Seq mapping . . . . .         | 254 |
| ■ View ChIP-Seq mapping results. . . . .     | 258 |
| ■ ChIP-Seq output files . . . . .            | 258 |

## Introduction to ChIP-Seq mapping

LifeScope™ Genomic Analysis Software provides the ability to map data and create an output file type compatible with a variety of third-party Chromatin Immunoprecipitation Sequencing (ChIP-Seq) data analysis tools. The ChIP-Seq application has publicly available analysis software that can be used with LifeScope™ Software output.

Examples of such software tools include:

- SAMTools and BEDTools for generic manipulation of SAM/BAM and BED files, respectively
- MACS, QuEST, and USeq for peak-finding
- browsers like the UCSC genome browser, or IGV for viewing mapped reads and peaks relative to other genomic annotations

The ChIP assay is a method for analyzing epigenetic modifications and genomic DNA sequences bound to specific regulatory proteins. ChIP-Seq is a combined assay and sequencing technique for identifying and characterizing elements in protein-DNA interactions. It typically examines transcription factors (TF) bound to DNA and finds DNA sequence motifs common to binding sites.

Using the MAGnify™ ChIP-Seq kit with the SOLiD® sequencing system enables you to generate sequence read data from a ChIP-Seq experimental approach. LifeScope™ Software gives you the option to map the read data.

## ChIP-Seq library types

ChIP-Seq mapping supports data from the fragment library type.

## ChIP-Seq input files

| Input File                   | File Extension |
|------------------------------|----------------|
| (Optional) Color space FASTA | *.csfasta      |
| (Optional) FAST-All (FASTA)  | *.fa, *.fasta  |
| (Optional) Quality Value     | *qual, *qv     |
| Extensible sequence          | *.xsq          |

## ChIP-Seq parameters

Parameters for ChIP-Seq mapping include general parameters and fragment mapping.

### General

General parameters include:

| Parameter              | Default value | Description                                                                                                                                                                                               |
|------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| analysis.assembly.name | hg19          | The name of the genome assembly used in current analysis. Examples are hg18 and hg19.                                                                                                                     |
| analysis.space         | Base          | Your preference of whether analysis should be done in base space or in color space. This setting should be the same for all modules in your run. See <a href="#">"Choose analysis space" on page 98</a> . |

Use the  button to open the File Chooser and search for input files.

### Fragment mapping

There are three categories of parameters for mapping: Main, Advanced, and BAMStats.

#### Main

| Parameter          | Default value | Description                                                                                                                                                                                                                                                                                   |
|--------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Add color sequence | True          | Add color sequence to BAM records: Values: <ul style="list-style-type: none"> <li>• True: Add color.</li> <li>• False: Do not add color.</li> </ul>                                                                                                                                           |
| Map in base space  | False         | Allow mapping in base space if input data has base space available. If only color space is available, then mapping will fail when base space mapping is turned on. Values: <ul style="list-style-type: none"> <li>• True: Map in base space.</li> <li>• False: Map in color space.</li> </ul> |

## Advanced

| Parameter                        | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|----------------------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| BAM soft clip                    | False         | Modify an unaligned portion of a read that needs to be presented in the BAM record. Values: <ul style="list-style-type: none"> <li>• True: Soft clip.</li> <li>• False: Do not soft clip.</li> </ul>                                                                                                                                                                                                                                                 |
| Base quality filter threshold    | 0             | Replace resulting base-calls with a quality value less than the specified value.                                                                                                                                                                                                                                                                                                                                                                     |
| Create unmapped BAM files        | False         | Create BAM files containing unmapped reads. Values: <ul style="list-style-type: none"> <li>True: Create.</li> <li>False: Do not create.</li> </ul>                                                                                                                                                                                                                                                                                                   |
| Mapping QV/threshold             | 0             | Provide control the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer. Allowed values: Integers 0 to 1,000.                                                                                                                                                                                                                    |
| Reference weight                 | 15            | Used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. Adds weight (in terms of Phred score) to the signals that are compatible with reference. Color combinations that result in a variant are considered compatible with reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation. Allowed values: Integers 0 - 100. |
| Second map gapped algorithm type | GLOBAL        | Do indel finding, and control the behavior of indel finding. Allowed values: <ul style="list-style-type: none"> <li>• NONE: Turn off indel finding.</li> <li>• GLOBAL: Mapping reports global alignment up to one indel.</li> <li>• LOCAL: Mapping reports local alignment up to one indel.</li> </ul>                                                                                                                                               |

## BAMStats

| Parameter                    | Default value                              | Description                                                                                                          |
|------------------------------|--------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| Input directory for BAMStats | `\${analysis.output.dir}/fragment.mapping` | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample. |
| Output directory             | `\${task.output.dir}`                      | The path to the output directory where BamStats will write its chart (.cht) files.                                   |

|                                                                          |         |                                                                                                                                                                                                                                                                                               |
|--------------------------------------------------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Maximum Coverage                                                         | 10,000  | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br>Allowed values: Integers 1 - 10,000.                                                                           |
| Maximum insert size                                                      | 100,000 | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br>Allowed values: Integers 1 - 100000.                                            |
| Insert bin size                                                          | 100     | Bin size for insert range distribution.<br>Allowed values: Integers >= 0-1.                                                                                                                                                                                                                   |
| Whether to combine data from both the strands for coverage in WIG format | 1       | Combine or do not combine data from both strands for coverage in WIG format.<br>Allowed values: <ul style="list-style-type: none"> <li>• 0: Do not generate wig files.</li> <li>• 1: Generate the combined coverage file.</li> </ul>                                                          |
| Primary alignments only for coverage in WIG file format                  | 1       | Use only primary alignments for coverage in WIG file format.<br>Allowed values: <ul style="list-style-type: none"> <li>• 0: Do not restrict coverage in WIG file format to only primary alignments.</li> <li>• 1: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Bin size for coverage in WIG file format                                 | 100     | The bin size for coverage in WIG file format.<br>Allowed values: Integers > 1-100000.                                                                                                                                                                                                         |

### Mark Duplicates

| Parameter                                      | Default value                                         | Description                                                                                  |
|------------------------------------------------|-------------------------------------------------------|----------------------------------------------------------------------------------------------|
| Input directory with structured mapping output | <code>\${analysis.output.dir}/fragment.mapping</code> | Folder with one group per sequencing indexing run, and named [Group]/[File]-[number].bam.    |
| Process one bam file at a time                 | True                                                  | Set to false if your data is on multiple barcodes from the same sample run on the same lane. |

## Perform ChIP-Seq mapping

### (Optional) Import data

You can optionally import data to be analyzed. For instructions on how to import data, see “Import Data” on page 81.

### Log in to LifeScope™

1. Navigate to LifeScope™ Software at `http://<IP address>:<port number>/LifeScope.html`

where *IP address* is the address of the system or head node and *port number* is the number of the port used by the server.

2. In the Login screen, enter your username and password, then either click **Login** or press **Enter** to open the LifeScope™ home view (shown on page 75).

### Create or select a project

1. In the home view (shown on page 75), either click **Create a New Project** (described on page 80) or select a project in the Projects organizer (shown on page 80).
2. If you create a project:
  - a. Type a name and description in the Enter Project Name view.  
**Note:** The name cannot have spaces or special characters.
  - b. Click **Create New Project**.
  - c. In the Projects lists, select the new project.
3. In the Task Wizards section (shown on page 75), click **Add Data to Project** to choose a data type.

### Add data to a project

Add data from the read repository to a project by choosing a data type and finding data. You can also optionally group data.

**Note:** Multiple files cannot have the same name. Make sure that the names of the files that you add are distinct.

**Note:** If the LifeScope™ Software administrator has changed the path to the read repository since your last login, restart LifeScope™ Software to update the repository to the new path.

1. In the Add Data to Project window, select **Raw unmapped (XSQ) data**, then click **Next** to find data.
2. In the Read Repository Filter table, select the read-sets you want to map and analyze.  
If you want to group data, click **Next**. If you do not want to group data, skip [step 3](#) and proceed to [step 4](#).
3. (*Optional*) In the Read-sets in Project table, click the checkbox of the data files you want to group, then click **Add Group to Project**. The files appear in the Groups in Project table.  
To rename a group, click the checkbox of the group in the Groups in Project table, then click **Edit**. In the Edit Group window, enter a new name.  
To remove a data file from a group, click the checkbox of the data file, then click **Delete**.
4. Click **Add Analysis** to proceed, or
  - Click **Cancel** to refrain from adding data and close the Add Data to Project window, or
  - Click **Finish** to add the data and close the Add Data to Project window.

### Create an analysis

1. In the Choose Data view of the Create Analysis window, select data files from the Available Data in Project table, then click **Next** to choose an analysis.

- In the Choose Analysis view, enter a name for the analysis and a description.  
If you are re-using an Old Analysis, select **Reuse Old Analysis** and select the name of the analysis you want to use.

**Note:** The name cannot have spaces or special characters.

- Select **ChIP-Seq**, then click **Next** to choose references.

\* Analysis Type:  Genomic Resequencing  Methyl Miner  ChIP-Seq  Small RNA

To see the type of data mapped by the ChIP-Seq module, place your cursor over the button.

- In the Data To Be Analyzed table, click **Select the reference for the reads** to open the repository file browser.

- In the Browse for Reference File window, navigate to the location of the reference genome of your sample. Open the folder with your .fasta file, for example:

data ▶ referenceData ▶ lifetech ▶ hg18 ▶ reference ▶ human\_hg18.fasta

Select the file, then click **OK**.

The file name appears in the Reference column.

**Note:** To change the reference file, click **Select the reference for the reads**, then select another reference file.

- After you have created the analysis:
  - Click **Edit** to proceed, or
  - Click **Cancel** to refrain from choosing references and close the Create Analysis window, or
  - Click **Finish** to complete analysis creation and close the window.

## Edit the analysis

In the Choose Modules view of the Edit Analysis window, accept the default settings for secondary and tertiary analyses, then click **Next** to set module parameters.

### Set module parameters

This section describes the procedures for setting general parameters and fragment mapping parameters. For descriptions of module parameters, see [“ChIP-Seq parameters” on page 252](#).

You can restore the default settings of parameters by clicking the **Reset to Defaults** button.

To view descriptions of parameters, place your mouse cursor over a button.

### Set general parameters

- Enter an analysis assembly name.
- Set the analysis.regions.file parameter.  
Click the button to choose a file.
- Always leave the default analysis.platform setting, which is “SOLID”.

4. Click **Next** to set mapping parameters.

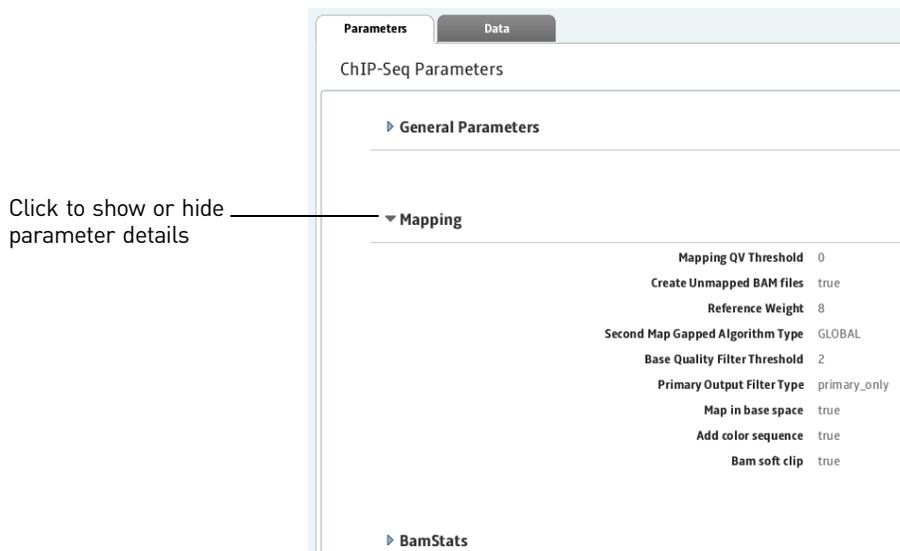
Set mapping parameters

1. There are four categories of mapping parameters: Main, Advanced, BamStats, and Mark Duplicates. Accept the default settings or click the Main, Advanced, BAMStats, and Mark Duplicates tabs to edit the settings.
2. After you have edited an analysis:
  - Save your edits and proceed by clicking **Review**, or
  - Save your edits and close the window by clicking **Finish**, or
  - Erase your edits and close the Edit Analysis window by clicking **Cancel**.

### Review and run the analysis

**Note:** For a description of ChIP-Seq parameters, see [“ChIP-Seq parameters” on page 252](#).

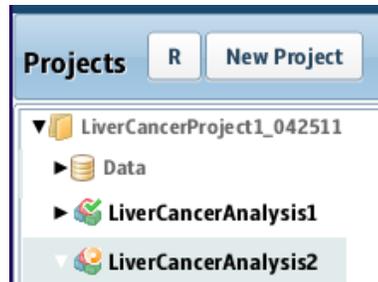
The Review Analysis view in the Run Analysis window includes two tabs: Parameters and Data. In the Parameters tab, click the  , next to the parameter categories to show or hide the parameters.



1. Review the parameters. To edit the parameters, click **Edit**.
2. To review the data that will be analyzed, click the **Data** tab.
3. If you are ready to run the analysis, click **Start Analysis**.  
The window closes.

### Check Analysis Status

1. In the Projects organizer (shown [on page 75](#)), click the project to check the status of data mapping.



2. Click the analysis name to show and show details about the analysis in the status overview (shown on page 75).
3. Click the Status tab to view the Progress column, which shows the percentage of completion for data mapping.

LiverCancerProject1... • LiverCancerAnalysis2

Overview Status Parameters

Analysis Runs

| Name                         | X5Q ID                       | Analysis             | Secondary Analysis | Secondary Progress | Tertiary Analysis | Tertiary Progress |
|------------------------------|------------------------------|----------------------|--------------------|--------------------|-------------------|-------------------|
| solid0054_20110102_PE_LFD... | solid0054_20110102_PE_LFD... | LiverCancerAnalysis2 | [Mapping_BamStats] | 13%                |                   |                   |

After ChIP-Seq mapping has been completed, you can view mapping results.

## View ChIP-Seq mapping results

ChIP-Seq mapping results include additional files and log files.

LiverCancerProject1\_042511 • LiverCancerAnalysis2 • Mapping • solid0054\_20110102\_PE\_LFD\_RD\_SetA\_1\_HuRef100\_F3.colorRemake\_DefaultLibrary

Details Statistics Additional Files Logs

Additional Files:

| Directory                                                                                   | File                                                                  | Size      | Type |
|---------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|-----------|------|
| fragment.mapping/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary | solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake-1.bam.bai | 90 KB     | bai  |
| fragment.mapping/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake_DefaultLibrary | solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake-1.bam     | 646173 KB | bam  |

LiverCancerProject1\_042511 • LiverCancerAnalysis2 • Mapping • solid0054\_20110102\_PE\_LFD\_RD\_SetA\_1\_HuRef100\_F3.colorRemake\_DefaultLibrary

Details Statistics Additional Files Logs

Log Files:

| Directory        | File                                                                                           | Size  |
|------------------|------------------------------------------------------------------------------------------------|-------|
| fragment.mapping | secondary-Homo_sapiens.NCB136.54.dna.chr22-fragment.mapping.main.20110426163010482.log         | 71 KB |
| fragment.mapping | secondary-Homo_sapiens.NCB136.54.dna.chr22-fragment.mapping.10.run.20110426163028605.log       | 29 KB |
| fragment.mapping | secondary-Homo_sapiens.NCB136.54.dna.chr22-fragment.mapping.11.gather-00.20110426164729990.log | 10 KB |
| fragment.mapping | secondary-Homo_sapiens.NCB136.54.dna.chr22-fragment.mapping.8.run.20110426163028444.log        | 28 KB |

See Chapter 9, “View Analysis Results” on page 103 for instructions on how to review mapping results.

## ChIP-Seq output files

The outputs of ChIP-Seq mapping are a BAM file (.bam) and BAM file index (.bai). The .bai file is required to view the associated BAM file in some genomic browsers.

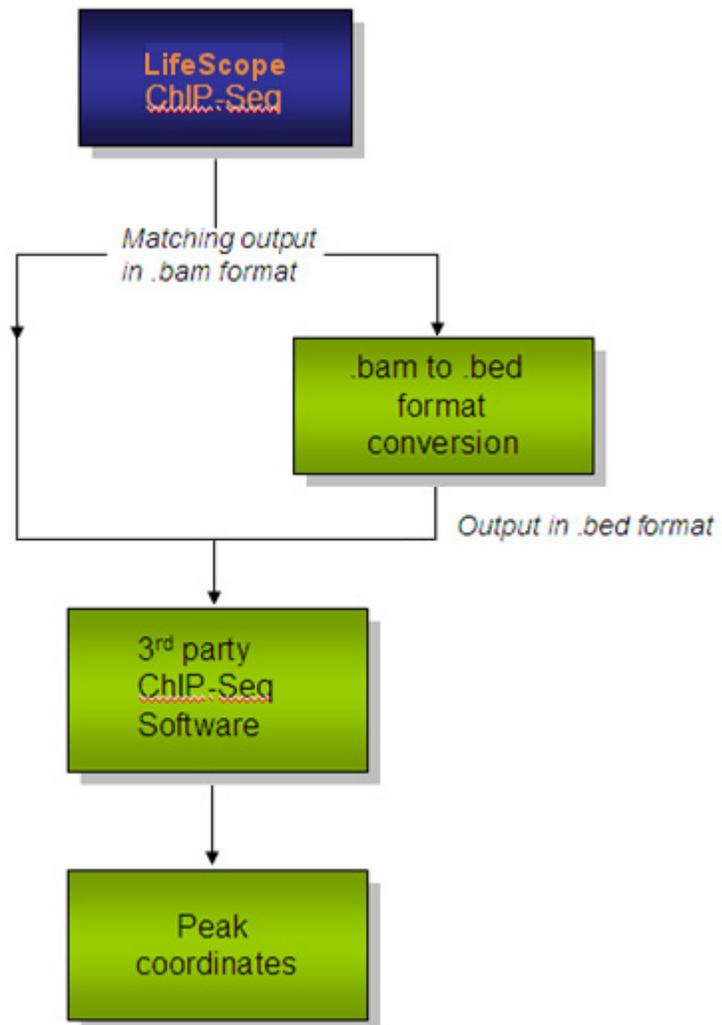
After ChIP-Seq mapping has been completed, the resulting BAM file can be used with compatible third-party commercial and academic ChIP-Seq analysis software tools (see the illustration on page 259).

Examples of such software tools include:

- SAMTools and BEDTools for generic manipulation of SAM/BAM and BED files, respectively
- MACS, QuEST, and USeq for peak-finding
- browsers like the UCSC genome browser, or IGV for viewing mapped reads and peaks relative to other genomic annotations

You can download a BAM-to-BED format converter from third-party tools sites, for example:

<http://sourceforge.net/projects/bedtools>





PART V  
Analysis Modules



## 18

# Perform Human Copy Number Variation Analysis

This chapter covers:

- Introduction to Human Copy Number Variation analysis ..... 263
- Human Copy Number Variation analysis parameters ..... 263
- Perform Human Copy Number Variation analysis ..... 266
- View Human CNV analysis output ..... 266

## Introduction to Human Copy Number Variation analysis

Human Copy Number Variation (Human CNV) analysis in LifeScope™ Genomic Analysis Software detects copy number variations in a data sample that is mapped to the human reference sequence hg18. LifeScope™ Software Human CNV analysis currently supports only humans, because normalization is species-specific.

By default, analysis does not call CNVs that are within 1 MBase of the centromeres and telomeres of the chromosomes. A centromere is a region of DNA typically found near the middle of a chromosome where two identical sister chromatids come in contact. The centromere is involved in cell division as the point of mitotic spindle. A telomere is a region of repetitive DNA at the end of a chromosome. The telomere protects the end of the chromosome from deterioration.

## Human Copy Number Variation analysis parameters

Categories of CNV parameters include Advanced and (if you selected Annotation for CNV output) Annotation. There are no Main parameters.

**Note:** To revert parameters to their default settings, click **Reset to Defaults**.

### Advanced

There are two categories of Advanced parameters: general and `cnv.stringency.setting`.

| Parameter     | Default Value | Description                                                                                                          |
|---------------|---------------|----------------------------------------------------------------------------------------------------------------------|
| Window size   | 5000          | Size of the window block to be considered as a region.<br>Allowed values: Integers $\geq 100$ .                      |
| Trim distance | 1000          | Distance in kilo bases to be trimmed from the extreme ends of the chromosome arms. Allowed values: Integers 0-100000 |
| Min quality   | 0             | Minimum quality value of the alignments. Allowed values: Integers 0-99                                               |
| Ploidy        | 2             | General ploidy of the genome. Allowed values: Integers $\geq 1$ .                                                    |

|                      |       |                                                                                                                                                                                                                                                               |
|----------------------|-------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ploidy exception     | None  | List of all the contigs whose ploidy is different to the general ploidy of the genome. Entries in the list are in the format {contig id: ploidy of the contig}, and are separated by commas. Use the string "None" to indicate no entries.                    |
| Local normalization  | False | Whether or not genome-wide normalization or local normalization should be performed. <ul style="list-style-type: none"> <li>• <b>True:</b> Perform chromosome-arm local normalization.</li> <li>• <b>False:</b> Perform genome-wide normalization.</li> </ul> |
| Write coverage       | False | Create coverage output files <ul style="list-style-type: none"> <li>• <b>True:</b> Create coverage output files, in WIG format.</li> <li>• <b>False:</b> Do not create.</li> </ul>                                                                            |
| Coverage window size | 1000  | Size of the window block to be considered as a region for writing coverage output. The mean coverage of all bases in each of these windows is output. Allowed values: Integers: 1–100000.                                                                     |

#### cnv.stringency.setting parameters

| Parameter                  | Default Value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Deletions min mappability  | 50            | Minimum mappability percentage for regions to be shown as copy number deletions. Allowed values: 0.0000 - <100.0000<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this parameter to 25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 0.</li> </ul>       |
| Insertions min mappability | 10            | Minimum mappability percentage for the regions to be shown as copy number insertions. Allowed values: 0.0000 - <100.0000.<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this parameter to 25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 0.</li> </ul> |
| Deletion min windows       | 2             | Minimum number of windows for the regions to be shown as copy number deletions. Allowed values: Integers ≥0.<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this parameter to 4.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 1.</li> </ul>               |
| Insertion min windows      | 2             | Minimum number of windows for the regions to be shown as copy number insertions. Allowed values: Integers ≥0.<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this parameter to 4.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 1.</li> </ul>              |

|                       |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-----------------------|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Deletion max p-value  | 1.0                                       | Maximum p-value for regions to be shown as copy number deletions.<br>Allowed values: >0.0000 - 1.0000.<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this to 0.25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 1.0.</li> </ul>                                            |
| Insertion max p-value | 1.0                                       | Maximum p-value for regions to be shown as copy number insertions.<br>Allowed values: >0.0000 - 1.0000.<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then LifeScope™ sets this to 0.25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then LifeScope™ sets this parameter to 1.0.</li> </ul>                                           |
| Stringency setting    | Medium                                    | Allowed values:<br>High: Recommend when a very low false positive tolerance is allowed<br>Medium: Default values<br>Low: Aggressive CNV calling<br>Lower settings result in more CNV calls, but with more false positives.<br>Higher settings result in fewer CNV calls, but with fewer false positives.                                                                                                                                                      |
| Deletion max ratio    | 0.5                                       | Maximum ratio between the coverage of the region and the expected coverage, for a region to be called as CNV deletion.<br>Allowed values: >0.000-<1.0000.<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then this parameter is set to 0.25.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then this parameter is set to 0.7.</li> </ul> |
| Insert min ratio      | 1.25                                      | Minimum ratio between the coverage of the region and the expected coverage, for a region to be called as CNVs insertion.<br>Allowed values: Floats ≥1.0.<br>If this parameter is not specified, and <ul style="list-style-type: none"> <li>• <a href="#">Stringency setting</a> is set to <i>High</i>, then this parameter is set to 1.75.</li> <li>• <a href="#">Stringency setting</a> is set to <i>Low</i>, then this parameter is set to 1.25.</li> </ul> |
| Gender                | Male                                      | Set the ploidy of all chromosomes for Human.                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Mappability directory | <code>\${analysis.mappability.dir}</code> | Path to the directory of mappability files.                                                                                                                                                                                                                                                                                                                                                                                                                   |

**(Optional)  
Annotation**

You can optionally annotate the mapped output of Human Copy Number Variation analysis. For descriptions of the Annotation parameters, see [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on page 317.

## Perform Human Copy Number Variation analysis

The Human CNV analysis module detects CNV in SOLiD® System data that originates from a single human sample. Slide(s) from this sample must be mapped to the hg18 reference to facilitate correct normalization.

Use the Human CNV module to perform tertiary analysis. To use the module:

1. Select a project in the Projects organizer (shown on [page 75](#)).
2. Create an analysis or edit an analysis that has not yet been run:  
**Create an analysis:** Click either:
  - **Analysis** in the top menu, then **Create**, or
  - **Create Analysis** in the Task Wizards section (shown [page 75](#)).**Edit an analysis:** Click **Edit Analysis** in the Task Wizards section.
3. In the Edit Analysis window, select **Human Copy Number Variation** in the Available Modules pane, click the > button to move it to the Include pane. You can optionally annotate the analysis output.  
Click **Next** to set the general parameters.
4. Set the General Parameters:
  - analysis.assembly.name
  - annotation.dbsnp.file
  - analysis.regions.file
  - annotation.gtf.fileClick **Next**.
5. Review and edit the **Pair End Mapping** parameters, described in “[Paired-end](#)” on [page 274](#), then click **Next**.
6. Review and edit the Human Copy Number Variation analysis parameters, described in “[Human Copy Number Variation analysis parameters](#)” on [page 263](#), then click **Next**.
7. If you selected Annotation in [step 3](#), review and edit the Annotation parameters, described in [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on [page 317](#).
8. Run the analysis.

### View analysis status

In the Projects organizer (shown [on page 75](#)), click the **Human Copy Number Variation** analysis module, then click the **Status** tab in the overview section (shown [on page 75](#)).

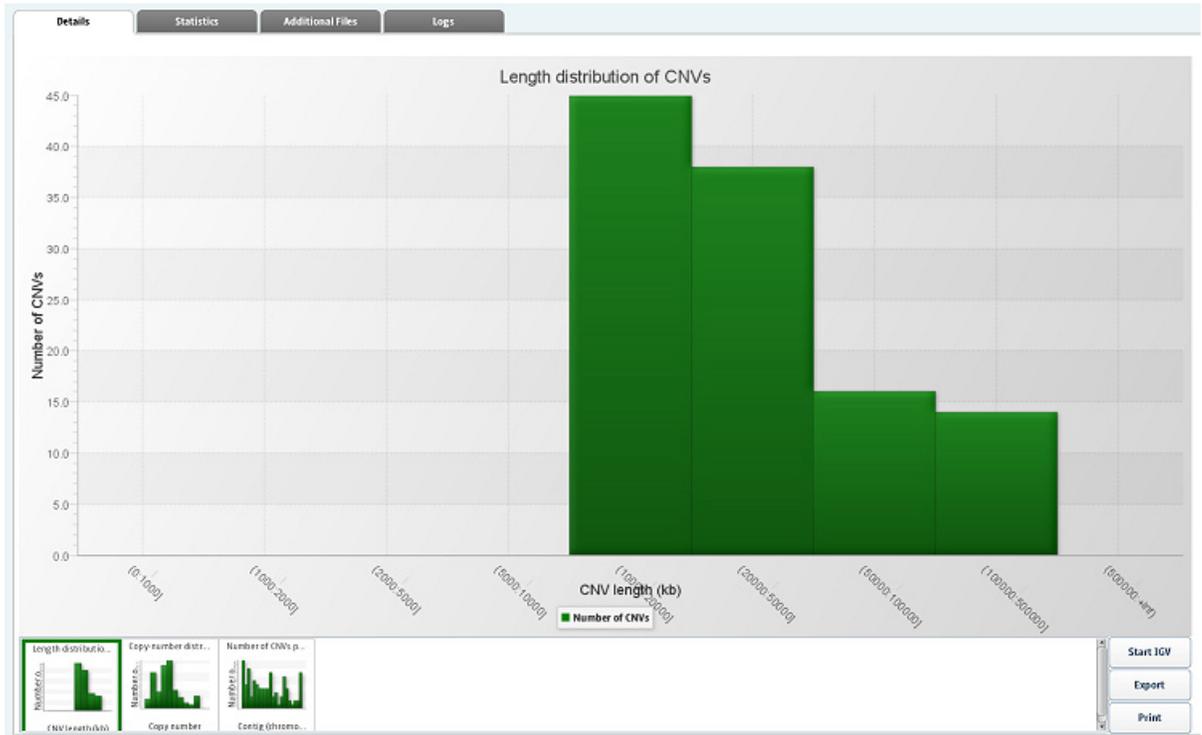
## View Human CNV analysis output

If the analysis was successful, click **View Results** in the Progress column.

In the View Results window, you can view details, statistics, additional files, and logs. For more information about viewing analysis results, see [Chapter 9, “View Analysis Results”](#) on [page 103](#).

## Details

The Details tab shows analysis results in the form of bar charts and pie charts. The following illustration is an example of details for the Human CNV analysis module.



## Statistics

The Statistics tab shows a LifeScope™ Software report of statistics that you can view, export, and print. You cannot edit the statistics. The following illustration is an example of the statistics in a Human CNV analysis.

```

*****
LIFESCOPE REPORT
*****
Input File:
/datapod1/sitest1/results/projects/lifescopel/GRIMP/GR_IMP_COLORS/SPACE_NONBARCODE/outputs/cnv/solid0049_20090724_HuReseqIMP_1_F3R3_None/outputC
NVs.gtf
Date: Apr 22, 2011 4:18:18 AM
Annotation file, dbSNP: /panasas/lifescopel/analysis/referenceData/lifetech/hg18/dbSNP/dbSNP_b130.tab
Annotation file, Genes and Exons, GTF: /panasas/lifescopel/analysis/referenceData/lifetech/hg18/refGene/refGene.hg18.20090513.gtf
*****
Statistics Overview
*****
-- Basic Statistics -----
Number of variants                               113
-- Variant-Specific Statistics (CNV) -----
Distribution of CNV copy number
Copy_Number      Number
0                 5
1                20
2                 9
3                24
4                27
5                10
6                 6
7                 3
8                 2
9                 7
>9                0
Distribution of CNV length
Length (bases)      Number
(0:1000]            0
(1000:2000]        0
(2000:5000]        0
(5000:10000]       0
(10000:20000]      45
(20000:50000]      38
(50000:100000]     16
    
```

### Additional files

The following table is an example of additional files for the Human CNV analysis module.

| Directory                                     | Files                        | File Type |
|-----------------------------------------------|------------------------------|-----------|
| cnv/solid0049_20090724_HuReseqLMP_1_F3R3_None | dbSnpDeployed_annotated.gff3 | .gff3     |
|                                               | dbSnpDeployed_genes.tab      | .tab      |
|                                               | ALLCNVs.out                  | .out      |
|                                               | dbSnpDeployed_genes.bed      | .bed      |

### Logs

The following table is an example of logs for the Human CNV analysis module.

| Directory | Files                                                               | File Type |
|-----------|---------------------------------------------------------------------|-----------|
| cnv       | cnv-solid0049_20090724_HuReseqLMP_1_F3R3_None.20110422034750570.log | .log      |

## 19

# Perform Resequencing Mapping

This chapter covers:

|                                             |     |
|---------------------------------------------|-----|
| ■ Introduction to resequencing mapping..... | 269 |
| ■ Input files.....                          | 270 |
| ■ Mapping parameters.....                   | 270 |
| ■ Map data.....                             | 277 |
| ■ Mapping output files.....                 | 278 |
| ■ Mapping statistics output files.....      | 280 |

## Introduction to resequencing mapping

This chapter describes how to perform resequencing mapping. The mapping analysis results files are required as input for tertiary analysis modules.

During secondary analysis LifeScope™ Genomic Analysis Software performs the following steps:

- Maps or aligns reads to a reference genome.
- Accepts one or more input XSQ (eXtensible SeQuence) files to generate one or more sorted BAM files containing aligned reads.
- For mate-pair or paired-end libraries, pairs alignments that have the same bead ID.
- *(Optional)* Generates mapping statistics.

The 5500 Series SOLiD™ Sequencer generates XSQ files, optionally with ECC (Exact Call Chemistry) reads. If an ECC primer round has been performed, the XSQ output also includes the sequence information in base space, in addition to color space. The ECC primer round is recommended during sequencing, for significant increases in sequencing accuracy.

XSQ is an extensible binary file format for storing sequence data, and supports multiple independent reads at the same position in a fragment. For information on the XSQ file format refer to this site:

<http://solidsoftwaretools.com/gf/project/xsq>

## Input files

The mapping module accepts as input one or more input XSQ files. The input reads can have different read lengths but they must be of the same library type. For example, LMP read-sets of length 50 and 60 can be processed together. The XSQ files processed in a single analysis can not have different library types. In one mapping analysis, the input must be of only one library type, either Fragment, LMP, or PE.

### Plan your input read-sets

Plan your analysis input carefully. The way you define your reads input affects the behavior of your analysis. The following factors control how your input data is analyzed:

- **Index (barcode) IDs** – Using an index ID restricts your input to the reads data of one or more indices.
- **Grouping of reads** – Each group of reads is analysed together as one specimen. The output data for a group is combined into one set of results files.
- **Multiple sample runs** – Unrelated reads can be processed together in one run of LifeScope™, but analyzed separately as separate input data.

See [“Define input data” on page 71](#) for more information on designing adding input data to your analysis.

Mapping one tag of paired data is not supported.

### Legacy data

If the data you want to process with LifeScope™ is in CSFASTA and QUAL files. LifeScope™ automatically converts these files to the XSQ format before mapping.

## Mapping parameters

There are three categories of mapping parameters:

| Mapping Parameter | Used in analysis . . . |
|-------------------|------------------------|
| Fragment          | ChIP-Seq               |
|                   | Genomic resequencing   |
|                   | MethylMiner™           |
|                   | Targeted resequencing  |
|                   | Low frequency variant  |
|                   | Whole transcriptome    |
| Paired-end        | Genomic resequencing   |
|                   | MethylMiner™           |
|                   | Targeted resequencing  |
|                   | Low frequency variant  |
|                   | Whole transcriptome    |
| Mate Pair         | Genomic resequencing   |

**Fragment Mapping** There are four categories of fragment mapping parameters: Main, Advanced, SAET, and BAMStats.

**Main**

| Parameter          | Default value | Description                                                                                                                                                                                                                                                                                   |
|--------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Add color sequence | True          | Add color sequence to BAM records: Values: <ul style="list-style-type: none"> <li>• True: Add color.</li> <li>• False: Do not add color.</li> </ul>                                                                                                                                           |
| Map in base space  | False         | Allow mapping in base space if input data has base space available. If only color space is available, then mapping will fail when base space mapping is turned on. Values: <ul style="list-style-type: none"> <li>• True: Map in base space.</li> <li>• False: Map in color space.</li> </ul> |

**Advanced**

| Parameter                     | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-------------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| BAM soft clip                 | False         | Modify an unaligned portion of a read that needs to be presented in the BAM record. Values: <ul style="list-style-type: none"> <li>• True: Soft clip.</li> <li>• False: Do not soft clip.</li> </ul>                                                                                                                                                                                                                                               |
| Base quality filter threshold | 0             | Replace resulting base-calls with a quality value less than the specified value.                                                                                                                                                                                                                                                                                                                                                                   |
| Create unmapped BAM files     | False         | Create BAM files containing unmapped reads. Values:<br>True: Create.<br>False: Do not create.                                                                                                                                                                                                                                                                                                                                                      |
| Mapping QV/threshold          | 0             | Provide control the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer. Allowed values: Integers 0–1,000.                                                                                                                                                                                                                     |
| Primary output filter type    | Primary_only  |                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Reference weight              | 15            | Used during base translation. In the read reconstruction process, multiple signals are combined to generate the final base call. Adds weight (in terms of Phred score) to the signals that are compatible with reference. Color combinations that result in a variant are considered compatible with reference. Additional weight helps to eliminate base errors caused by color error(s) during base translation. Allowed values: Integers 0–100. |

| Parameter                        | Default value | Description                                                                                                                                                                                                                                                                                         |
|----------------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Second map gapped algorithm type | GLOBAL        | Do indel finding, and control the behavior of indel finding.<br>Allowed values: <ul style="list-style-type: none"> <li>NONE: Turn off indel finding.</li> <li>GLOBAL: Mapping reports global alignment up to one indel.</li> <li>LOCAL: Mapping reports local alignment up to one indel.</li> </ul> |

### SAET

The SAET tab is accessible only if you select SAET pre-processing when you choose modules.

| Parameter                    | Default value | Description                                                                                                                                                                                                                                            |
|------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genome length                | 1000000       | Expected length of sequenced (or enriched) DNA region. For example, 4,600,000 for the E.Coli 4.6 MB genome or 30,000,000 for the entire Human Transcriptome.<br>Allowed values: Integers $\geq 200$ .                                                  |
| On target ratio              | 0.5           | The expected ratio of reads that come from the targeted region.<br>Allowed values: Floats 0.0–1.0.                                                                                                                                                     |
| Update quality values        | True          | Update quality value of modified calls.<br>Allowed values:<br>True: Update the QV of modified calls.<br>False: Do not update the qv for modified calls.                                                                                                |
| Trusted quality value        | 25            | Correction is applied to calls with a quality value below the value of this parameter.<br>Allowed values: Integers $\geq 1$ .                                                                                                                          |
| Support votes                | 2             | The minimum number of k-mer votes required make a correction.<br>Allowed values: Integers $\geq 1$ .                                                                                                                                                   |
| Trusted frequency            | 0             | The lowest multiplicity of the seed to be included in the spectrum. (If set to 0, then the value is computed internally.)<br>Allowed values: Integers $\geq 0$ .                                                                                       |
| Maximum corrections per read | 0             | Maximum number of allowed corrections per read. (If set to 0, then the value is set to $\lceil \text{readLength}/8 \rceil$ ). Reduce if over-corrections are observed, or increase if under-corrections are observed.<br>Allowed values: Integers 0–9. |

| Parameter                              | Default value | Description                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Number of recursive runs               | 1             | The error correction step is repeated the provided number of times. Reduce if over-corrections are observed, or increase if under-corrections are observed.<br><br>Allowed values: 1, 2, or 3.                                                                                                                                                               |
| Position of error inflation point      | 0             | Position in the read at which the error rate inflates, for instance, 35-40 for 50bp long reads. (If set to 0, then the value is equal to 0.8*readLength).<br><br>Allowed values: Integers.                                                                                                                                                                   |
| Disable random sampling for large data | False         | Disables random sampling in spectrum building. If set to 0, then for large datasets (coverage > 300x), a subset of reads is used in spectrum building. Allowed values: <ul style="list-style-type: none"> <li>• True: Disables random sampling in spectrum building.</li> <li>• False: Do not disable random sampling in spectrum building. true?</li> </ul> |
| K-mer size                             | 0             | Size of k-mer (>5) used in spectrum construction and error correction. (If set to 0, then the value is computed internally.)<br><br>Allowed values:<br>Integers 0-28.                                                                                                                                                                                        |

### BAMStats

| Parameter                    | Default value                            | Description                                                                                                                                                                                                                                           |
|------------------------------|------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Input directory for BAMStats | \${analysis.output.dir}/fragment.mapping | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample.                                                                                                                                  |
| Output directory             | \${task.output.dir}                      | The path to the output directory where BAMStats will write its chart (.cht) files.                                                                                                                                                                    |
| Maximum Coverage             | 10,000                                   | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br><br>Allowed values: Integers >= 0.                                     |
| Maximum insert size          | 100,000                                  | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br><br>Allowed values: Integers 0-100,000. |
| Insert bin size              | 100                                      | Bin size for insert range distribution.<br><br>Allowed values: Integers 1-100,000                                                                                                                                                                     |

|                                                                          |     |                                                                                                                                                                                                                                                                                                             |
|--------------------------------------------------------------------------|-----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Whether to combine data from both the strands for coverage in WIG format | 1   | Whether to generate a WIG format coverage file with combined data from both the strands.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: Do not generate wig files.</li> <li>• <b>1</b>: Generate the combined coverage file.</li> </ul>                                              |
| Primary alignments only for coverage in WIG file format                  | 1   | Use only primary alignments for coverage in WIG file format.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>0</b>: Do not restrict coverage in WIG file format to only primary alignments.</li> <li>• <b>1</b>: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Bin size for coverage in WIG file format                                 | 100 | The bin size for coverage in WIG file format.<br>Allowed values: Integers 1-100,000.                                                                                                                                                                                                                        |

**Paired-end**

There are four categories of paired-end parameters: Main, Advanced, SAET, BAMStats, and Mark Duplicates

**Main**

| Parameter                    | Default value | Description                                                                                                                                                                                                                                                                                                                |
|------------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Map in base space            | False         | Map in base space. Set to true if input data has base space available.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Map in base space.</li> <li>• <b>false</b>: Map in color space.</li> </ul> If only color space is available, then the module fails when base space mapping is turned on.   |
| Add color sequence           | True          | Map in color space. Set to true if input data has color space available.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>true</b>: Map in base space.</li> <li>• <b>false</b>: Map in color space.</li> </ul> If only color space is available, then the module fails when base space mapping is turned on. |
| Minimum insert size estimate | 0             | 0-100000                                                                                                                                                                                                                                                                                                                   |
| Maximum insert size estimate | 20,000        | 0-100000                                                                                                                                                                                                                                                                                                                   |

**Advanced**

| Parameter     | Default value | Description                                                                                                                                                                                          |
|---------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| BAM soft clip | False         | Modify an unaligned portion of a read that needs to be presented in the BAM record. Values: <ul style="list-style-type: none"> <li>• True: Soft clip.</li> <li>• False: Do not soft clip.</li> </ul> |

| Parameter                     | Default value | Description                                                                                                                                                                                                                       |
|-------------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Base quality filter threshold | 0             | Replace resulting base-calls with a quality value less than the specified value.                                                                                                                                                  |
| Create unmapped BAM files     | False         | Create BAM files containing unmapped reads.<br>Values:<br>True: Create.<br>False: Do not create.                                                                                                                                  |
| Mapping QV/threshold          | 0             | Provide control the contents written to the output BAM file depending on the quality value of the alignment. To preserve only high quality alignments, set this value to a positive integer.<br>Allowed values: Integers 0–1,000. |

SAET

| Parameter                    | Default value | Description                                                                                                                                                                                                                                            |
|------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genome length                | 1000000       | Expected length of sequenced (or enriched) DNA region. For example, 4,600,000 for the E.Coli 4.6 MB genome or 30,000,000 for the entire Human Transcriptome.<br>Allowed values: Integers $\geq 200$ .                                                  |
| On target ratio              | 0.5           | The expected ratio of reads that come from the targeted region.<br>Allowed values: Floats 0.0–1.0.                                                                                                                                                     |
| Update quality values        | True          | Update quality value of modified calls.<br>Allowed values:<br>True: Update the QV of modified calls.<br>False: Do not update the qv for modified calls.                                                                                                |
| Trusted quality value        | 25            | Correction is applied to calls with a quality value below the value of this parameter.<br>Allowed values: Integers $\geq 1$ .                                                                                                                          |
| Support votes                | 2             | The minimum number of k-mer votes required make a correction.<br>Allowed values: Integers $\geq 1$ .                                                                                                                                                   |
| Trusted frequency            | 0             | The lowest multiplicity of the seed to be included in the spectrum. (If set to 0, then the value is computed internally.)<br>Allowed values: Integers $\geq 0$ .                                                                                       |
| Maximum corrections per read | 0             | Maximum number of allowed corrections per read. (If set to 0, then the value is set to $\lceil \text{readLength}/8 \rceil$ ). Reduce if over-corrections are observed, or increase if under-corrections are observed.<br>Allowed values: Integers 0–9. |

| Parameter                              | Default value | Description                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Number of recursive runs               | 2             | The error correction step is repeated the provided number of times. Reduce if over-corrections are observed, or increase if under-corrections are observed.<br><br>Allowed values: 1, 2, or 3.                                                                                                                                                               |
| Position of error inflation point      | 0             | Position in the read at which the error rate inflates, for instance, 35-40 for 50bp long reads. (If set to 0, then the value is equal to $0.8 * \text{readLength}$ ).<br><br>Allowed values: Integers.                                                                                                                                                       |
| Disable random sampling for large data | False         | Disables random sampling in spectrum building. If set to 0, then for large datasets (coverage > 300x), a subset of reads is used in spectrum building. Allowed values: <ul style="list-style-type: none"> <li>• True: Disables random sampling in spectrum building.</li> <li>• False: Do not disable random sampling in spectrum building. true?</li> </ul> |
| K-mer size                             | 0             | Size of k-mer (>5) used in spectrum construction and error correction. (If set to 0, then the value is computed internally.)<br><br>Allowed values:<br>Integers 0–28.                                                                                                                                                                                        |

### BAMStats

| Parameter                    | Default value                                         | Description                                                                                                                                                                                                                                          |
|------------------------------|-------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Input directory for BAMStats | <code>\${analysis.output.dir}/fragment.mapping</code> | The input directory for BAMStats. There should be one directory per sample containing the BAM files for that sample.                                                                                                                                 |
| Output directory             | <code>\${task.output.dir}</code>                      | The path to the output directory where BAMStats will write its chart (.cht) files.                                                                                                                                                                   |
| Maximum Coverage             | 10000                                                 | Defines the maximum coverage allowed for locations in the reference. Locations with coverage more than the maximum coverage value are ignored during coverage calculations.<br><br>Allowed values: Integers 0–10,000.                                |
| Maximum insert size          | 100000                                                | Defines the maximum insert size allowed for mate pair and paired-end libraries. Reads with an insert size greater than the maximum insert size value are ignored for the Insert Range Report calculations.<br><br>Allowed values: Integers 0–100000. |
| Insert bin size              | 1000                                                  | Bin size for insert range distribution.<br><br>Allowed values: Integers $\geq 1$ .                                                                                                                                                                   |

| Parameter                                                                | Default value | Description                                                                                                                                                                                                                                                                                                    |
|--------------------------------------------------------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Whether to combine data from both the strands for coverage in WIG format | 1             | Combine or do not combine data from both strands for coverage in WIG format.<br>Allowed values:<br><ul style="list-style-type: none"> <li>• <b>0</b>: Do not generate wig files.</li> <li>• <b>1</b>: Generate the combined coverage file.</li> </ul>                                                          |
| Primary alignments only for coverage in WIG file format                  | 1             | Use only primary alignments for coverage in WIG file format.<br>Allowed values:<br><ul style="list-style-type: none"> <li>• <b>0</b>: Do not restrict coverage in WIG file format to only primary alignments.</li> <li>• <b>1</b>: Restrict coverage in WIG file format to only primary alignments.</li> </ul> |
| Bin size for coverage in WIG file format                                 | 100           | The bin size for coverage in WIG file format.<br>Allowed values: Integers > 1.                                                                                                                                                                                                                                 |

### Mark Duplicates

| Parameter                                      | Default value                         | Description                                                                                                                     |
|------------------------------------------------|---------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| Input directory with structured mapping output | `\${analysis.output.dir}/pair.mapping | Folder with one group per Sequencing Indexing run, and named [Group]/[File]-[number].bam                                        |
| Process one bam file at a time                 | True                                  | Set to false if your data is on multiple barcodes that are all from the same sample and run on the same lane on the instrument. |

## Map data

Mapping data is automatic in LifeScope™ Software. To map data:

1. In the Projects organizer (shown [on page 75](#)), select a project.
2. Click an analysis icon , then click **Edit Analysis**.
3. In the Edit Analysis window, accept the default settings for mapping and pre-processing data in the Secondary Analysis section.  
If you want to exclude BAMStats, click **Customize**. In the Customize Mapping window, uncheck BAMStats, then click **OK**.



If you do not want to pre-process data, uncheck the box.

## Mapping output files

For every input BAM file, a set of statistics files are generated. These files are in chart (.cht), comma-separated values (.csv), text (.txt), and wiggle (.wig) formats. Each CHT file corresponds to one displayed chart. A CHT file specifies the type of chart, the displayed range of each axis, and the data points, without using external references.

The .cht file format is an internal file format based on the .csv file format, with additional header information. This is an example of .cht header information:

```
# name:
# type: scatter2d | pie | vbar | line
# title:
# xaxisname:
# yaxisname:
# xrange: <min>:<tickinterval>:<max>
# yrange: <min>:<tickinterval>:<max>
XAXISNAME, SERIES1NAME, SERIES2NAME, ...
x1, y1.1, y1.2, ...
x2, y2.1, y2.2, ...
x3, y3.1, y3.2, ...
```

The wiggle format (.wig) is a public format typically used for coverage. Visit their site for more information:

[hgdownload.cse.ucsc.edu/goldenPath/help/wiggle.html](http://hgdownload.cse.ucsc.edu/goldenPath/help/wiggle.html)

A genome browser such as the Integrative Genomics Viewer (IGV) can be used to visualize the coverage. For information is available from their site:

[www.broadinstitute.org/igv/](http://www.broadinstitute.org/igv/)

For a collection of input BAM files that belong to a sample, a set of cumulative statistics files are generated. The cumulative statistics files are also in CHT, CSV, TXT, and WIG formats. The cumulative statistics can be visualized in the LifeScope™ Software UI.

**Mapping output files**

**Details**

There are no details for the paired-end mapping analysis module.

**Statistics**

There are no statistics for the paired-end mapping analysis module.

**Additional files**

The following table is an example of additional files for the paired-end mapping analysis module.

| Directory                     | Files                   | File Type |
|-------------------------------|-------------------------|-----------|
| pair.mapping/WT_chr17_PE_None | WT_chr17_PE-1-1.bam.bai | .bai      |
|                               | WT_chr17_PE-1-1.bam     | .bam      |

**Logs**

The following table is an example of logs for the paired-end mapping analysis module.

| Directory    | Files                                                                   | File Type |
|--------------|-------------------------------------------------------------------------|-----------|
| pair.mapping | secondary-hg18_validated-pair.mapping.2.gather-00.20110425225114531.log | .log      |
|              | refcor.secondary-hg18_validated-pair.mapping.1.run.log                  |           |

**Additional files**

The following table is an example of additional files for the BAMStats analysis module.

| Directory                                                     | Files                                            | File Type |
|---------------------------------------------------------------|--------------------------------------------------|-----------|
| PairEndMapping.BamStats/WT_chr17_PE_None/WT_chr17_PE-1-1/Misc | WT_chr17_PE-1-1.bam_chr2.NEG.wig                 | .wig      |
| PairEndMapping.BamStats/WT_chr17_PE_None/Misc                 | WT_chr17_PE_None.Mismatches.by.ReadPair.Type.csv | .csv      |

**Logs**

The following table is an example of logs for the BAMStats analysis module.

| Directory               | Files                                                                               | File Type |
|-------------------------|-------------------------------------------------------------------------------------|-----------|
| PairEndMapping.BamStats | secondary-hg18_validated-PairEndMapping.BamStats.1.convertRef.20110425225121545.log | .log      |

## Mapping statistics output files

This section describes the mapping statistics files generated by the BAMStats module. When statistics reports are separated by tag type, the report's file name includes the tag in the file name. The tags used in file names are:

| Library type | Tag   |
|--------------|-------|
| Fragment     | F3    |
| Mate-pair    | F3    |
|              | R3    |
| Paired-end   | F3    |
|              | F5-P2 |

The output files generated by BAMStats include the name of the BAM file. The file name pattern is:

*BAMFileName-fileID-barcodeID.StatisticsReportName.tag.extension*

This string is referred to by *prefix* in the following table, the mapping statistics output table. In the description of mapping statistics output file names in the following table, the string *tag* is used to refer to the tags in the following table.

| Report                                                                   | Description, axis information, filename                                                                                                                                                                                                                                                     |
|--------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Alignment Length Distribution</b>                                     |                                                                                                                                                                                                                                                                                             |
|                                                                          | A bar plot giving the distribution of alignment lengths found in various tags used in alignment. The report is separated by tag type to provide visibility into the accuracy of individual tags.<br>Only the primary alignment for each bead is considered in calculating the distribution. |
|                                                                          | Y axis: Frequency<br>X axis: Alignment length (from 0 to the maximum read length)                                                                                                                                                                                                           |
|                                                                          | Output file name: <i>prefix</i> .Alignment.Length.Distribution. <i>tag</i> .cht                                                                                                                                                                                                             |
| <b>Base Mismatch Distribution</b>                                        |                                                                                                                                                                                                                                                                                             |
|                                                                          | A bar plot giving the distribution of total number of mismatches found in various tags used in alignment. The bins are summed over all alignment lengths.<br>Only the primary alignment for each bead is considered in calculating the distribution.                                        |
|                                                                          | Y axis: Frequency<br>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)                                                                                                                                                                                                |
|                                                                          | Output file name: <i>prefix</i> .BaseMismatch.Distribution. <i>tag</i> .cht                                                                                                                                                                                                                 |
| <b>Distribution of Alignment Length and Number of Mismatches in Tags</b> |                                                                                                                                                                                                                                                                                             |

| Report                                                       | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|--------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                              | <p>A report providing a simultaneous picture of the alignment length and number of mismatches distributions in various tags used in alignment. Only primary alignments for each tag are considered in calculating this distribution. The bins range from 0 to the maximum read length, and from 0 to the maximum number of mismatches allowed.</p> <p><b>Note:</b> This report is located in the <code>Misc</code> folder, and is not displayed in the UI.</p> <p>Y axis: Alignment length (from 0 to the max read length)<br/>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)<br/>Z axis: Frequency</p> <p>Output file name: <code>prefix.AlignmentLength.Mismatch.tag.csv</code></p>                                                                               |
| <b>Distribution of Mismatches by Position</b>                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                                              | <p>A bar plot providing a distribution of errors (mismatches to reference) by position within the read. Only the primary alignments for each bead are used to generate this distribution.</p> <p>The base-space report is generated on every run.</p> <p>Y axis: Frequency<br/>X axis: Position (from 0 to maximum read length)</p> <p>Output file name: <code>prefix.Mismatches.By.Position.BaseSpace.tag.cht</code>, and (only if color-space data is present in the BAM file) <code>prefix.Mismatches.By.Position.ColorSpace.tag.cht</code></p>                                                                                                                                                                                                                                           |
| <b>Color Mismatch Distribution</b>                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                                              | <p>A bar plot providing a distribution of the total number of color mismatches in various tags used in alignment. Only the primary alignments for each bead are used to generate this distribution. This report is only generated if mapping is done in color space.</p> <p>Y axis: Frequency<br/>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)</p> <p>Output file name: <code>prefix.ColorMismatches.Distribution.tag.cht</code></p>                                                                                                                                                                                                                                                                                                                              |
| <b>Distribution of Alignment Length and Color Mismatches</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                                              | <p>A report providing a simultaneous picture of the alignment length and number of color mismatches distributions in various tags used in alignment. Only primary alignments for each tag are considered in calculating this distribution. The bins range from 0 to the maximum read length, and from 0 to the maximum number of mismatches allowed. This report is only generated if mapping is done in color space.</p> <p><b>Note:</b> This report is located in the <code>Misc</code> folder, and is not displayed in the UI.</p> <p>Y axis: Alignment length (from 0 to the max read length)<br/>X axis: Number of mismatches (from 0 to the maximum mismatches allowed)<br/>Z axis: Frequency</p> <p>Output file name: <code>prefix.AlignmentLength.ColorMismatches.tag.csv</code></p> |
| <b>Base QV Distribution</b>                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                                              | <p>A bar plot providing a distribution of base quality values generated using the reference assisted error correction/base conversion algorithm. The base QV distributions are separated for each tag as quality of individual tags could be very different.</p> <p>Y axis: Frequency<br/>X axis: Base QV (from 0 to the maximum QV)</p> <p>Output file name: <code>prefix.BaseQV.tag.cht</code></p>                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Base QVs by Position</b>                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

| Report                                                         | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                | <p>A report providing a distribution of base quality values by individual base positions. This report identifies if certain base positions (particularly towards the end of the read) have poor base quality values. All the tags (F3/R3/F5-P2) used in the alignment are combined into a single bin.</p> <p>Y axis: Base QV<br/>X axis: Base position (from 0 to read length)<br/>Z axis: Frequency</p> <p>Output file name: <i>prefix.BaseQV.by.Position.csv</i></p>                                                                                                                                                 |
| <b>Distribution of Mismatches by Base QV</b>                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A line plot providing a distribution of errors (mismatches to reference) by base quality values bins.</p> <p>Y axis: Percentage of total mismatches<br/>X axis: Base QV (from 0 to maximum QV)</p> <p><b>Note:</b> This report gives, for each QV, a measure of the raw mismatches at positions with that QV. This information does not measure whether the base QVs generated are well calibrated to the probability of error in that particular base position.</p> <p>Output file name: <i>prefix.Mismatches.By.BaseQV.tag.cht</i></p>                                                                            |
| <b>Distribution by Mapping QVs by Tag</b>                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A bar plot providing a distribution of mapping quality values for individual tags (F3/R3/F5-P2). Only the primary alignment for each bead is used in calculating this distribution.</p> <p>Y axis: Frequency<br/>X axis: Mapping QV (from 0 to maximum mapping QV)</p> <p>Output file name: <i>prefix.MappingQV.tag.cht</i></p>                                                                                                                                                                                                                                                                                     |
| <b>Distribution by Pairing QVs</b>                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A bar plot providing a distribution of pairing quality values. Only the primary alignment for each bead is used in calculating this distribution.</p> <p>Y axis: Frequency<br/>X axis: Pairing QV (from 0 to maximum pairing QV)</p> <p>Output file name: <i>prefix.PairingQVs.cht</i></p>                                                                                                                                                                                                                                                                                                                          |
| <b>Coverage Report</b>                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A line plot providing a distribution of coverage obtained after mapping/pairing. Only the primary alignment for each bead is used in this calculation.</p> <p>Y axis: Frequency<br/>X axis: Coverage (from 0 to number of reads)</p> <p>Output file name: <i>prefix.Coverage.cht</i></p>                                                                                                                                                                                                                                                                                                                            |
| <b>Coverage Report by Chromosome (Contig) and Base Windows</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                | <p>A line plot providing a distribution of coverage within each reference window. The coverage is calculated within each window along a reference chromosome. The window size can theoretically be anywhere from a single base to the contig length. However the calculations of base level coverage are computationally expensive and less interpretable as the window size increases. Only the primary alignment for each bead is used in this calculation.</p> <p>Y axis: Frequency<br/>X axis: Coverage (from 0 to number of reads)</p> <p>Output file name: <i>prefix.Coverage.By.Chromosome.contignn.cht</i></p> |
| <b>Coverage by Strand</b>                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

| Report                                 | Description, axis information, filename                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                        | <p>A line plot providing the distribution of coverage within each reference window separated by reference strand (+/-). Only the primary alignment for each bead is used in this calculation.</p> <p>Y axis: Frequency<br/>X axis: Coverage (from 0 to number of reads)</p> <p>Output file name: <i>prefix.Coverage.By.Strand.Positive.cht</i>, <i>prefix.Coverage.By.Strand.Negative.cht</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Coverage files</b>                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                        | <p>Coverage reports in wiggle format. For each genome position, reports the number of reads that cover (map to or span) the position. Because reporting coverage for each position results in very large files, coverage is reported for bins, with each bin spanning a user-defined number of bases. For each bin, the mean coverage of all the positions in that bin is reported. The parameter <code>bamstats.wig.binsize</code> controls the size of the bins in this file.</p> <p>Each coverage file includes a header as the first line. The header lines follow this pattern:<br/>track type=wiggle_0 name=&lt;chrname&gt; description=&lt;coverage from positive/negative/both strand&gt;<br/>visibility=full color=0,0,255 fixedStep chrom=&lt;chrname&gt; start=&lt;startpos&gt; step=&lt;binsize&gt; span=&lt;binsize&gt;</p> <p>See also <a href="#">"Coverage files" on page 285</a>.</p> <p>Output file name: <i>coverage_chrnn.pos.wig</i>, <i>coverage_chrnn.neg.wig</i></p> |
| <b>Insert Range Distribution</b>       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                        | <p>A line plot providing the distribution of insert sizes for paired data (PE and LMP library types).</p> <p>Y axis: Frequency<br/>X axis: Insert Range bins</p> <p>Output file name: <i>prefix.Insert.Range.Distribution.cht</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Distribution of Read Pair Types</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                        | <p>For paired data, this report calculates the distribution of read pair types (AAA, AAB, C**, etc.).</p> <p>Y axis: Frequency<br/>X axis: Read pair type</p> <p>Output file name: <i>prefix.ReadPair.Type.cht</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Pairing Statistics</b>              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                        | <p>A pie chart showing the percentages of the following F3, R3 (or F5) combinations, for paired data:</p> <ul style="list-style-type: none"> <li>• Mapped, Mapped</li> <li>• Mapped, Unmapped</li> <li>• Unmapped, Mapped</li> <li>• Unmapped, Unmapped</li> <li>• Mapped, Missing</li> <li>• Missing, Mapped</li> <li>• Unmapped, Missing</li> <li>• Missing, Unmapped</li> </ul> <p>Output file name: <i>prefix.Pairing.Stats.cht</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Unique Start Positions</b>          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

| Report                | Description, axis information, filename                                                                                                                                                                                                                                                      |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                       | <p>This report lists the following:</p> <ul style="list-style-type: none"> <li>• The total number of unique start positions.</li> <li>• The average number of unique start positions per reference position.</li> </ul> <p>See also <a href="#">“Unique Start Position” on page 285</a>.</p> |
|                       | Output file name: <i>BAMfilename</i> -summary.txt, <i>Samplename</i> .-summary.txt                                                                                                                                                                                                           |
| <b>Summary Report</b> |                                                                                                                                                                                                                                                                                              |
|                       | The summary file provides statistics for each BAM file in the sample. See <a href="#">“Summary file”</a> .                                                                                                                                                                                   |
|                       | Output file name: <i>filename</i> .-summary.tbl                                                                                                                                                                                                                                              |

### Example of mapping statistics output

This section provides example output of some mapping statistics output files.

#### Summary file

The summary file provides statistics for each BAM file in the sample. This list describes labels used in the summary file:

- **NumUnFilteredBeads** – The total number of beads, before any filtering on the instrument. This value is also the fragment count in the input XSQ file.
- **NumFilteredBeads** – The number of beads that pass filtering on the instrument.
- **NumMapped** – The number of reads with primary alignment.
- **% filtered mapped** – The number of primary reads divided by the number of beads passing instrument filtering ( $\text{NumMapped} / \text{NumFilteredBeads}$ ).
- **% total mapped** – The number of primary reads divided by the number of total beads ( $\text{NumMapped} / \text{NumUnFilteredBeads}$ ).
- **Megabases of coverage** – The sum of alignment lengths, for primary alignments only.

The number of beads filtered out in the instrument is found by subtracting the number of beads that pass filtering from the total number of beads:

$$\text{NumUnFilteredBeads} - \text{NumFilteredBeads}$$

The following is example contents for a summary file. The `PredictedInsertSize` and `Tag2` fields only are present with paired data.

```
BamFileName, IsColorInBam, IsBaseInXSQ, IsECC, LibraryType,
ReadLength, Megabases of Coverage, PredictedInsertSize,
NumFragmentsPassingFilters, NumFragmentsTotal,
Tag1-NumMapped,
Tag1- % total Mapped,
Tag1- % filtered mapped,
Tag2-NumMapped,
Tag2- % total Mapped,
Tag2- % filtered mapped,
(Tag1-AlignmentLength;Min;Max;Avg;Median;StdDev) ,
(Tag1-NumBaseMismatches;Min;Max;Avg;Median;StdDev) ,
(Tag1-MappingQV;Min;Max;Avg;Median;StdDev) ,
(Tag1-BaseQV;Min;Max;Avg;Median;StdDev) ,
(Tag1-NumColorMismatches;Min;Max;Avg;Median;StdDev) ,
(Tag2-AlignmentLength;Min;Max;Avg;Median;StdDev) ,
```

```
(Tag2-NumBaseMismatches;Min;Max;Avg;Median;StdDev) ,
(Tag2-MappingQV;Min;Max;Avg;Median;StdDev) ,
(Tag2-BaseQV;Min;Max;Avg;Median;StdDev) ,
(Tag2-NumColorMismatches;Min;Max;Avg;Median;StdDev)
(Coverage;Min;Max;Avg;Median;StdDev)
```

The following is an example line of output in the summary file:

```
example.bam Y N N FRAG 50 2184653 100000 100000 51536 51.54
51.54 (F3;25;50;42.39;45;6.48) (F3;0;50;2.98;0;9.24)
(F3;0;100;65.43;72;25.31) (F3;1;41;35.09;41;11.65)
(F3;0;9;1.20;1;1.39) (0;399;0.43;0;5.96)
```

### Unique Start Position

The following is an example of a Unique Start Position report, for fragment data:

```
Unique Start Positions F3
Total Number of Unique Start Positions: 16536 (16.043154 %
of primary alignments)
Average Number of Unique Start Positions per Reference
Position: 0.001630
```

Unique Start Position reports have the following naming patterns:

- *BAMfilename*-summary.txt
- *Samplename*-summary.txt

### Coverage files

Example contents for the file `coverage_chr1_positive.wig` are:

```
browser position chr1:1-200000000
browser hide all
browser pack refGene encodeRegions
# minimumMapq=25, minimumCoverage=1,
alignmentFilteringMode=PRIMARY, filterOrphanedMates=false,
track name="BAM Coverage positive strand" description="BAM
Coverage positive strand" visibility="full color 0,0,255"
priority=10 yLineMark=0 type=wiggle_0 yLineOnOff=on
variableStep chrom=chr1 span=1
336171
336181
336191
336201
336211
336221
336231
336241
336251
336261
336271
```

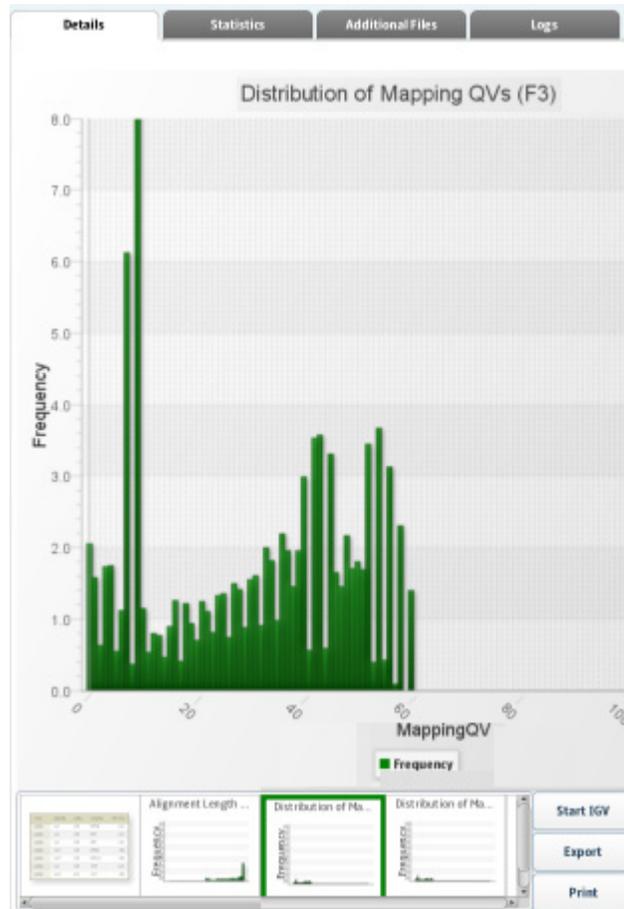
...

Example contents for the file `coverage_chr1_negative.wig` are:

```
browser position chr1:1-200000000
browser hide all
browser pack refGene encodeRegions
# minimumMapq=25, minimumCoverage=1,
alignmentFilteringMode=PRIMARY, filterOrphanedMates=false,
track name="BAM Coverage negative strand" description="BAM
Coverage negative strand" visibility="full color 0,0,255"
priority=10 yLineMark=0 type=wiggle_0 yLineOnOff=on
variableStep chrom=chr1 span=1
57432
57442
57452
57463
57473
57483
57493
57503
57513
57523
57533
...
```

## BAMStats charts

Most BAMStats reports can be displayed as charts in LifeScope™ Software. The following illustration is an example of chart output from the BAMStats analysis module.





# Perform SNP Finding Analysis

This chapter covers:

- Introduction to SNP Finding analysis . . . . . 289
- SNP analysis input files . . . . . 289
- SNP Finding analysis parameters . . . . . 290
- Perform SNP Finding analysis . . . . . 292
- View SNP Finding analysis output . . . . . 293

## Introduction to SNP Finding analysis

The LifeScope™ Genomic Analysis Software SNP Finding analysis module uses the diBayes algorithm to find Single Nucleotide Polymorphisms (SNPs). The diBayes package performs independent SNP analysis at each position in the reference, using either a Bayesian or Frequentist algorithm.

The SNP Finding module is used to call SNPs from mapped and processed SOLiD® System reads. The module takes the reads, quality values, the reference sequence, and error information on each SOLiD® System slide as its input, and calls SNPs.

The SNP Finding module creates these results files:

- A list of SNPs.
- A quartile file with coverage as well as color and base quality value distribution.
- (Optional) A consensus FASTA file with the same number of bases as the reference sequence.
- (Optional) A consensus calls file with a list of all covered positions.
- (Optional) A collection of annotated files.

The consensus calls file and the quartile file are each generated as one file per contig. The list of SNPs and the consensus FASTA file are each generated both as one file per contig and also as a consolidated file for the entire run. See [“View SNP Finding analysis output” on page 293](#).

## SNP analysis input files

The SNP input files include the reference file, the BAM file of the mapping module, and position error and probe error file output of the BAMStats mapping statistics module.

| Input File                     | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reference genome               | <p>The sequence to which the reads are aligned (mapped). The required format for the reference file is the *.fasta format, for example, chr20.validated.fasta.</p> <p><b>Note:</b> When you run the SNP Finding tool, you must use the same reference that was used for mapping.</p> <p>The reference sequence might have multiple chromosomes or contigs, and it might contain IUB codes at positions of known SNPs. A heterozygote is called with fewer reads providing evidence at these positions if the reference sequence contains IUB codes, because the prior probability of a heterozygote existing at this position is higher.</p>                                   |
| F3 (R3/F5) position error file | <p>F3 (R3/F5) position error files are tab-delimited text files created during secondary analysis. The files record the frequencies of dicolor mismatches between reads and the reference at different positions in a read. For fragment runs, SNP Finding only requires an F3 position error file. Both F3 and R3(F5) position error files are created for mate-pair or paired-end runs, and both are required for running SNP Finding. Position error files are generated during mapping.</p>                                                                                                                                                                                |
| F3 (R3/F5) probe error file    | <p>F3 (R3/F5) probe error files are tab-delimited text files that record the frequencies of dicolor mismatches between the reads and the reference as a function of different 6-mer probes. LifeScope™ Software calculates the probe error files.</p> <p>Reads only have the F3 tag for fragment runs. Thus, SNP Finding requires only an F3 probe error file. Both F3 and R3/F5 probe error files are created for mate-pair (paired-end) runs, and both are required to run SNP Finding on paired data. Different SOLiD® system runs of the same sample might generate different F3 (R3/F5) probe error files for each run, because of the random nature of probe errors.</p> |
| *.bam file                     | <p>The *.bam input file is generated by LifeScope™ Software at the end of mapping or pairing.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

## SNP Finding analysis parameters

Certain parameters affect only the module's base-space or color-space algorithm. These parameters are noted in the table. Changes to a base-space parameter's setting have no effect if the module runs its color-space algorithm. Similarly, changes to a color-space parameter's setting have no effect if the module runs its base-space algorithm.

There are three categories of SNP Finding parameters: Main, Advanced, and Annotation.

**Note:** To revert parameters to their default settings, click **Reset to Defaults**.

### Main

| Parameter name                     | Default value | Description                        |
|------------------------------------|---------------|------------------------------------|
| Call stringency                    | medium        | Call stringency.                   |
| Skip high coverage positions (Het) | 1             | Skip high coverage positions (Het) |
| Minimum mapping quality value      | 8             | Minimum mapping quality value.     |

**Advanced**

There are six categories of Advanced parameters: general, Read filter, General position filter, Heterozygous position filter, Homozygous position filter, and Output file processing.

| Parameter name                                                       | Default value | Description                                                           |
|----------------------------------------------------------------------|---------------|-----------------------------------------------------------------------|
| Detect adjacent SNPs                                                 | 0             | Detect adjacent SNPs.                                                 |
| Polymorphism rate                                                    | 0.001         | Polymorphism rate.                                                    |
| <b>Read filter</b>                                                   |               |                                                                       |
| Include reads with unmapped mate                                     | 0             | Include reads with unmapped mate.                                     |
| Exclude reads with indels                                            | True          | Exclude reads with indels.                                            |
| Require only uniquely mapped reads                                   | 0             | Require only uniquely mapped reads                                    |
| Ignore reads with a higher mismatch count to alignment length ratio  | 1.0           | Ignore reads with a higher mismatch count to alignment length ratio.  |
| Ignore reads with a lower alignment length to read length ratio      | 1.0           | Ignore reads with a lower alignment length to read length ratio.      |
| <b>General position filter</b>                                       |               |                                                                       |
| Require alleles to be present in both strands                        | False         | Require alleles to be present in both strands                         |
| Minimum base quality value for a position                            | 14            | Minimum base quality value for a position.                            |
| Minimum base quality value of the non-reference allele of a position | 14            | Minimum base quality value of the non-reference allele of a position. |
| <b>Heterozygous position filter</b>                                  |               |                                                                       |
| Minimum allele ratio (Het)                                           | 0.15          | Minimum allele ratio (Het).                                           |
| Minimum coverage (Het)                                               | 2             | Minimum coverage (Het).                                               |
| Minimum unique start position (Het)                                  | 2             | Minimum unique start position (Het).                                  |
| Minimum non-reference color QV (Het)                                 | 7             | Minimum non-reference color QV (Het).                                 |
| Minimum non-reference base QV (Het)                                  | 14            | Minimum non-reference base QV (Het).                                  |
| Minimum ratio of valid reads (Het)                                   | 0.65          | Minimum ratio of valid reads (Het).                                   |
| Minimum valid tricolor counts (Het)                                  | 2             | Minimum valid tricolor counts (Het).                                  |
| <b>Homozygous position filter</b>                                    |               |                                                                       |
| Minimum coverage (Hom)                                               | 1             | Minimum coverage (Hom).                                               |
| Minimum count of the non-reference allele (Hom)                      | 2             | Minimum count of the non-reference allele (Hom).                      |
| Minimum average non-reference base QV (hom)                          | 14            | Minimum average non-reference base QV (Hom).                          |

| Parameter name                                                  | Default value | Description                                                                                                                                                                                                |
|-----------------------------------------------------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Minimum average non-reference color QV (hom)                    | 7             | Minimum average non-reference color QV (Hom)                                                                                                                                                               |
| Minimum unique start position of the non-reference allele (Hom) | 2             | Minimum unique start position of the non-reference allele (Hom).                                                                                                                                           |
| <b>Output file processing</b>                                   |               |                                                                                                                                                                                                            |
| Output fasta file                                               | True          | Output or do not output the FASTA file. <ul style="list-style-type: none"> <li>• <b>True:</b> Output the FASTA file.</li> <li>• <b>False:</b> Do not output the FASTA file.</li> </ul>                     |
| Output consensus file                                           | True          | Output or do not output the consensus file. <ul style="list-style-type: none"> <li>• <b>True:</b> Output the consensus file.</li> <li>• <b>False:</b> Do not output the consensus file.</li> </ul>         |
| Compress the consensus file                                     | False         | Compress or do not compress the consensus file. <ul style="list-style-type: none"> <li>• <b>True:</b> Compress the consensus file.</li> <li>• <b>False:</b> Do not compress the consensus file.</li> </ul> |

**(Optional)  
Annotation**

You can optionally annotate the mapped output of SNP Finding analysis. For descriptions of the Annotation parameters, see [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on page 317.

## Perform SNP Finding analysis

Use the SNP Finding module to perform tertiary analysis. To use the module:

1. Select a project in the Projects organizer (shown on [page 75](#)).
2. Create an analysis or edit an analysis that has not yet been run:
  - Create an analysis:** Click either:
    - **Analysis** in the top menu, then **Create**, or
    - **Create Analysis** in the Task Wizards section (shown [page 75](#)).
  - Edit an analysis:** Click **Edit Analysis** in the Task Wizards section.
3. In the Edit Analysis window, select **SNP Finding** in the Available Modules pane, click the > button to move it to the Include pane. You can optionally annotate the analysis output.

Click **Next** to set the general parameters.
4. Set the General Parameters:
  - analysis.assembly.name
  - annotation.dbsnp.file
  - analysis.regions.file

**Note:** The analysis.regions.file is required with the following workflows and analyses:

- Low frequency variant workflows
- Targeted resequencing workflows

– Enrichment analyses

- annotation.gtf.file
- analysis.space

**Note:** Use the analysis.space parameter to record your preference of whether analysis should be done in base space or in color space. This setting should be the same for all modules in your run. The actual analysis space used depends on others factors such as whether the preferred space is available in the input files. See [“Choose analysis space” on page 98](#).

- analysis.platform

Always leave the default analysis.platform setting, which is “SOLID”  
Click **Next**.

5. Review and edit all mapping parameters, described in [“SNP analysis input files” on page 289](#), then click **Next**.
6. Review and edit the SNP Finding analysis parameters, described in [“SNP Finding analysis parameters” on page 290](#), then click **Next**.
7. If you selected Annotation in [step 3](#), review and edit the Annotation parameters, described in [Chapter 24, “Add Genomic Annotations to Analysis Results” on page 317](#).
8. Run the analysis.

### View analysis status

In the Projects organizer (shown [on page 75](#)), click the **SNP Finding** analysis module, then click the **Status** tab in the overview section (shown [on page 75](#)).

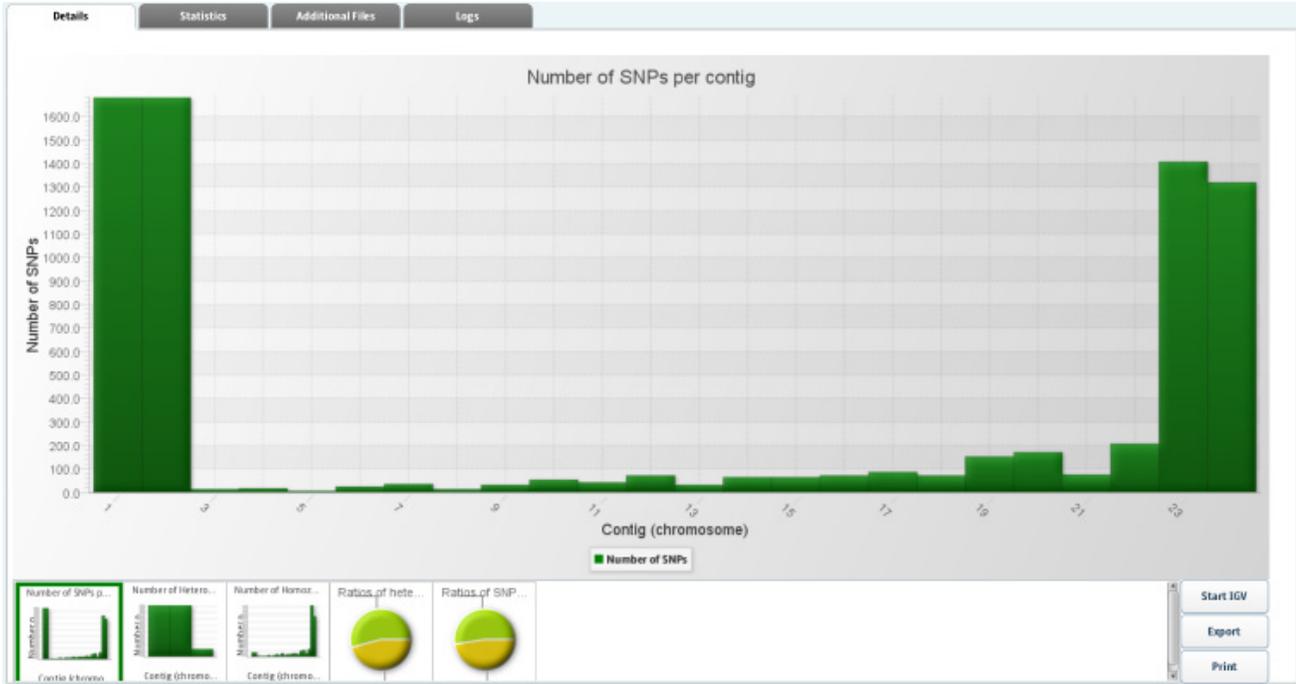
## View SNP Finding analysis output

If the analysis was successful, click **View Results** in the Progress column.

In the View Results window, you can view details, statistics, additional files, and logs. For more information about viewing analysis results, see [Chapter 20, “Perform SNP Finding Analysis” on page 289](#).

### Details

The Details tab shows analysis results in the form of bar charts and pie charts. The following illustration is an example of details for the SNP Finding analysis module.



## Statistics

The Statistics tab shows a LifeScope™ Software report of statistics that you can view, export, and print. You cannot edit the statistics. The following illustration is an example of the statistics in a SNP Finding analysis.

```

*****
LIFESCOPE REPORT
*****
Input File: /datapod1/sitest1/results/projects/lifescop/BrianCancerProject1_050411/BrainCancerAnalysis1/outputs/di
genome/hg19_simulated_TR_ECC_BC16_Frag50_320M11_BC1/BrainCancerAnalysis1_SNP.gff3
Date: May 4, 2011 9:55:13 PM
Annotation file, dbSNP: /panasas/lifescop20/analysis/referenceData/lifetech/hg19/dbSNP/00-All.vcf
Annotation file, Genes and Exons, GTF: /panasas/lifescop20/analysis/referenceData/lifetech/hg19/refGene/refGene.hg19.201
*****
Statistics Overview
*****

-- Basic Statistics -----
Number of variants                7388
Number of heterozygous variants   3375
Number of homozygous variants    4013

-- Variant-Specific Statistics (SNP) -----
Number of transition SNPs        3508
Number of transition heterozygous SNPs 1214
Number of transition homozygous SNPs 2294
Number of transversion SNPs     3880
Number of transversion heterozygous SNPs 2161
Number of transversion homozygous SNPs 1719
Transition:Transversion ratio    1.000 : 1.106

-- dbSNP Annotation Statistics -----
Number of SNPs in dbSNP         140

```

## Additional files

Additional files for SNP Finding analysis output include .bed, .gff3, .fasta, and .txt files.

The following table is an example of additional files for the SNP Finding analysis module.

| Directory                                                                    | Files                                          | File Type |
|------------------------------------------------------------------------------|------------------------------------------------|-----------|
| dibayes.genome/<br>hg19_Simulated_TR_ECC_BC16_Frag50_320Mil_<br>BC1/contig1  | BrainCancerAnalysis1_Consensus_Calls.txt       | .txt      |
| dibayes.genome/<br>hg19_Simulated_TR_ECC_BC16_Frag50_320Mil_<br>BC1/contig11 | BrainCancerAnalysis1_Consensus_Basespace.fasta | .fasta    |
| dibayes.genome/<br>hg19_Simulated_TR_ECC_BC16_Frag50_320Mil_<br>BC1          | BrainCancerAnalysis1_SNP_genes.bed             | .bed      |
| dibayes.genome/<br>hg19_Simulated_TR_ECC_BC16_Frag50_320Mil_<br>BC1/contig17 | BrainCancerAnalysis1_SNP.gff3                  | .gff3     |

## Logs

The following table is an example of logs for the SNP Finding analysis module.

| Directory      | Files                                                                                 | File Type |
|----------------|---------------------------------------------------------------------------------------|-----------|
| dibayes.genome | dibayes.genome-hg19_Simulated_TR_ECC_BC16_Frag50_320Mil_<br>BC1.20110504200517354.log | .log      |



## 21

# Perform Inversion Analysis

This chapter covers:

|                                            |     |
|--------------------------------------------|-----|
| ■ Introduction to inversion analysis ..... | 297 |
| ■ Inversion analysis input files .....     | 297 |
| ■ Inversion analysis parameters .....      | 298 |
| ■ Perform inversion analysis .....         | 299 |
| ■ View inversion analysis output .....     | 300 |

## Introduction to inversion analysis

An inversion is defined by its two breakpoints. The numbers of mate-pairs supporting occurrence of the starting and ending inversion breakpoints are counted for each base pair. The genomic ranges corresponding to local peaks of these counts, if above a score threshold, are called as candidate breakpoint ranges. To define an inversion, its starting and ending breakpoints are paired up only if they are the reciprocal nearest neighbor of each other in the correct order. The score for the inversion is the harmonic mean of its two breakpoints. Each breakpoint range can be scanned for coverage of normal (AAA) mate pairs to identify a sub-range with the lowest normal mate pair coverage as the most probable location of a breakpoint, and to differentiate homozygous inversions from heterozygous ones. The supporting evidence of all inversions can be visually inspected using a genomic browser.

To better resolve inversion breakpoints, new references are constructed for the inversions identified and inverted with respect to flanking regions with redundant breakpoint ranges. SOLiD<sup>®</sup> System reads are then matched and paired against the references, in which process reads split across boundaries of breakpoint ranges are parsed and clustered into candidate inversion breakpoints.

## Inversion analysis input files

The inversion module takes the BAM file output of the mapping module as input. Because the inversion module specifically selects for pairs that are in the incorrect orientation, it is important to know the correct orientation. As a result, either the library type information in the LB field or the `library.type` parameter must be properly set.

Libraries in the input file or files must be either:

- All mate-pair
- All paired-end

When different library types are provided as input on the same run, the inversion module fails with an error.

## Read lengths

Input BAM files may contain multiple read lengths.

## Inversion analysis parameters

**Note:** To revert parameters to their default settings, click **Reset to Defaults**.

Review and edit the Main parameters for the Inversion analysis module. There are no Advanced parameters.

### Main

| Parameters                      | Default value | Description                                                                                                                                                                                                                                                                                      |
|---------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Library type                    | Mate-pair     | Specifies the library type.<br>Allowed values: <ul style="list-style-type: none"> <li>• matepair</li> <li>• pairedend</li> </ul>                                                                                                                                                                 |
| Calculate MP coverage           | False         | Whether to calculate normal mate-pair coverage around inversion breakpoints. Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Use this calculation.</li> <li>• <b>False:</b> Do not use this calculation.</li> </ul>                                                        |
| Number of chromosomes           | 25            | The number of chromosomes used to distribute jobs on a cluster (one job per chromosome).<br>Allowed values: Integers $\geq 0$ .                                                                                                                                                                  |
| ABX score                       | 0             | The ABX score. ABX is ABA/ABB/ABC, which are mates with both tags on the correct strands but in reverse order.<br>Allowed values: $\geq 0.0000$ – $\leq 1.000$                                                                                                                                   |
| Force update intermediate files | False         | Whether to force updating of all intermediate files.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Force updating of all intermediate files.</li> <li>• <b>False:</b> Do not force updating of all intermediate files.</li> </ul>                                     |
| Downweight MP mismatches        | False         | Whether to down-weight mate-pairs with mismatches exponentially.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Down-weight mate-pairs with mismatches exponentially.</li> <li>• <b>False:</b> Do not down-weight mate-pairs with mismatches exponentially.</li> </ul> |
| Map BXXX MP length              | 3000000       | The maximal mapped length of BXX mate-pairs.<br>Allowed values: Integers $\geq 0$ .                                                                                                                                                                                                              |

| Parameters                  | Default value | Description                                                                                                                                                                                                              |
|-----------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Max inversion length        | 100000        | The maximum mapped length of inversions.<br>Allowed values: Integers $\geq 0$ .                                                                                                                                          |
| Min pairing quality         | 10            | Minimum pairing quality threshold.<br>Allowed values: Integers 0–100                                                                                                                                                     |
| Score run individually      | True          | Whether to score every run individually.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True</b>: Score every run individually.</li> <li>• <b>False</b>: Do not score every run individually.</li> </ul> |
| Breakpoint rescue           | False         | Whether to pair breakpoints with rescue.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True</b>: Pair breakpoints with rescue.</li> <li>• <b>False</b>: Do not pair breakpoints with rescue.</li> </ul> |
| Recover small inversions    | False         | Whether to recover small inversions.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True</b>: Recover small inversions.</li> <li>• <b>False</b>: Do not recover small inversions.</li> </ul>             |
| Max length small inversions | 0             | The maximum mapped length of small inversions.<br>Allowed values: Integers $\geq 0$ .                                                                                                                                    |
| Breakpoint score threshold  | 4             | The break point score threshold.<br>Allowed values: Integers 0–100.                                                                                                                                                      |
| Output score threshold      | 0             | The output score threshold.<br>Allowed values: Integers 0–100.                                                                                                                                                           |
| Breakpoint peak width       | 100           | The break point peak width.<br>Allowed values: Integers 0–100.                                                                                                                                                           |

## Perform inversion analysis

To perform inversion analysis:

1. Select a project in the Projects organizer (shown [on page 75](#)).
2. Create an analysis or edit an analysis that has not yet been run:
  - Create an analysis:** Click either:
    - **Analysis** in the top menu, then **Create**, or
    - **Create Analysis** in the Task Wizards section (shown [on page 75](#)).
  - Edit an analysis:** Click **Edit Analysis** in the Task Wizards section.
3. In the Edit Analysis window, select **Inversion** in the Available Modules pane, click the > button to move it to the Include pane. You can optionally annotate the analysis output.  
Click **Next**.

4. Set the general parameters, described in the following table:

| Parameter                | Description                                                                                                                                                                                               |
|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| analysis.assembly.name   | The name of the genome assembly used in current analysis. Examples are hg18 and hg19.                                                                                                                     |
| annotation.dbsnp.file    | The path to the file used to annotate SNPs and small InDels. LifeTech-provided files are dbSNP_b130.tab(hg18) and 00-All.vcf(hg19).                                                                       |
| analysis.mappability.dir | The path to the directory containing binary mappability files used in CNV module.                                                                                                                         |
| analysis.regions.file    | The path to the file containing genomic regions, such as a .bed format file containing exome targets. Used with enrichment analyses, including targeted resequencing and low frequency variant workflows. |
| annotation.gtf.file      | The path to the file containing gene and exon annotations corresponding to the genome assembly used in the analysis.                                                                                      |
| analysis.space           | Your preference of whether analysis should be done in base space or in color space. This setting should be the same for all modules in your run. See <a href="#">“Choose analysis space” on page 98</a> . |

- Review and edit the mapping parameters, then click **Next**.
- Review and edit the Inversion analysis parameters, described in [“Inversion analysis parameters” on page 298](#), then click **Next**.
- If you selected Annotation in [step 3](#), review and edit the Annotation parameters, described in [Chapter 24, “Add Genomic Annotations to Analysis Results” on page 317](#).
- Run the analysis.

### View analysis status

In the Projects organizer (shown [on page 75](#)), click the **Inversion** analysis module, then click the **Status** tab in the overview section (shown [on page 75](#)).

## View inversion analysis output

If the analysis was successful, click **View Results** in the Progress column.

In the View Results window, you can view details, statistics, additional files, and logs. For more information about viewing analysis results, see [Chapter 9, “View Analysis Results” on page 103](#).

### Details

There are no details of the output for the Inversion analysis module.

### Statistics

There are no statistics of the output for the Inversion analysis module.

### Additional files

Additional files for large indel analysis output include count files (for example, 1, 2, 3), all and chromosome (chr) files, .gff and .gff3 files, .o5MR files, .orphan files, .txt files, and .w100 files.

The following table is an example of additional files for the Inversion analysis module.

| Directory                                                              | Files                          | File Type |
|------------------------------------------------------------------------|--------------------------------|-----------|
| inversion/solid0049_20090724_HuReseqLMP_1_F3R3_None/starts/<br>start.1 | all 24                         | 24        |
| inversion/solid0049_20090724_HuReseqLMP_1_F3R3_None/gff                | chr14.gff                      | .gff      |
| inversion/solid0049_20090724_HuReseqLMP_1_F3R3_None                    | inversions.s4.w100.100000.GFF3 | .GFF3     |
| inversion/solid0049_20090724_HuReseqLMP_1_F3R3_None/BXX                | chr                            | chr       |
| inversion/solid0049_20090724_HuReseqLMP_1_F3R3_None                    | pair.txt                       | .txt      |
| inversion/solid0049_20090724_HuReseqLMP_1_F3R3_None                    | pair.orphan                    | orphan    |

### Logs

The following table is an example of logs for the Inversion analysis module.

| Directory | Files                                                                              | File Type |
|-----------|------------------------------------------------------------------------------------|-----------|
| inversion | inversion-solid0049_20090724_HuReseqLMP_1_F3R3_None.20110422034750573.log          | .log      |
|           | tertiary-solid0049_20090724_HuReseqLMP_1_F3R3_None-inversion.20110422034715217.log |           |



## 22

# Perform Large Indel Analysis

This chapter covers:

- Introduction to large indel analysis . . . . . 303
- Large indel analysis input files . . . . . 303
- Large indel analysis parameters . . . . . 304
- Perform large indel analysis . . . . . 305
- View large indel analysis output . . . . . 306

## Introduction to large indel analysis

The large indel analysis module identifies deviations in clone insert size. These deviations indicate intrachromosomal structural variations compared to a reference genome. Insertions and deletions (indels) up to 100 kB are inferred by identifying positions in the genome in which the pairing distance between mapped mate-pairs is deviates significantly from what is expected at the given level of clone coverage.

The module creates a look-up table of standard deviations at each level of clone coverage. The table produces an asymptotic curve in which the minimum size of detectable indels at a given level of significance drops rapidly as the clone coverage increases. The look-up table is used to determine the significance of the deviation in average insert size at each position in the genome.

Regions of the genome that are significantly deviated are selected as candidate indels, and hierarchical clustering is used to segregate the clones into groups in which the difference in the sizes of all clones in a group is less than the specified range. Clusters with too few clones, specified by you, are removed and the candidates are assessed to determine if a homozygous or heterozygous population of deviated insert sizes remains. All clones deviated by  $\geq 100$  kB are discarded. Clones from various libraries with various insert sizes contribute to a single indel call by combining the probabilities associated with the clones from each library.

## Large indel analysis input files

The Large Indel analysis module requires one or more Binary Alignment sequence Map (BAM) files containing mapped data and an hg18 or hg19 reference file as input.

## Large indel analysis parameters

**Note:** To revert parameters to their default settings, click **Reset to Defaults**.

There are three categories for the Large Indel analysis module: Main, Advanced and (if you selected Annotation for Small Indel output) Annotation.

### Main

| Parameters         | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|--------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Library type       | Mate-pair     | Specifies the library type.<br>Allowed values: <ul style="list-style-type: none"> <li>• matepair</li> <li>• pairedend</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Max clone coverage | 1000          | The maximum physical (clone) coverage for analysis. Loci with clone coverage above this threshold are not analyzed.<br>Allowed values: Integers $\geq 3$ .<br>You can use this parameter in combination with the parameter <a href="#">High coverage</a> to reduce false positives in high density genomes, for example, bacteria.                                                                                                                                                                                                                      |
| Min coverage       | 3             | The minimum physical (clone) coverage allowed. Loci with clone coverage below this threshold are not analyzed.<br>Allowed values: Integers $\geq 1$ .                                                                                                                                                                                                                                                                                                                                                                                                   |
| Ploidy             | 2             | General ploidy of the genome.<br>Allowed values: Integers $\geq 1$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Max insert size    | 100000        | Maximum insert size, in base pairs.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Call stringency    | medium        | Specifies the large indel call stringency. Automates parameter adjustment to the desired stringency level.<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>highest</b>: Recommend when a very low false positive tolerance is allowed.</li> <li>• <b>high</b>: Increased filtering.</li> <li>• <b>medium</b>: Default values.</li> <li>• <b>low</b>: Very aggressive.</li> </ul> Lower settings results in more indel calls, but with more false positives. Higher settings result in fewer indel calls, but with fewer false positives. |

## Advanced

| Parameters          | Default value                             | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|---------------------|-------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| BAS file            | —                                         | The BAS file contains metadata information about the data source(s) and relevant mapping/pairing statistics. The BAS format is a pseudo-standard file format for storing BAM file metadata. For details see this site:<br><a href="ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/pilot_data/README.bas">ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/pilot_data/README.bas</a>                                                                                                                                                                        |
| Min pairing quality | 25 (for mate-pair)<br>10 (for paired-end) | Paired reads below this threshold are ignored.<br>Allowed values: Integers 0–100.                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| p Value             | 1e-10                                     | P-value threshold, that is, the raw probability of committing a Type 1 error incorrectly identifying a large indel.<br>Allowed values: Floats 0.0–1.0.                                                                                                                                                                                                                                                                                                                                                                                               |
| High coverage       | False                                     | Eliminate clone coverage weighting. This option significantly reduces the number of false positives when analyzing very high coverage genomic data. (Very high coverage genomic data is typically greater than 1000x read coverage, and is common for bacterial genomes.)<br>Allowed values: <ul style="list-style-type: none"> <li>• <b>True:</b> Eliminate clone coverage weighting. The algorithm ignores coverage when calculating the structural significance of candidate indels.</li> <li>• <b>False:</b> No effect on the module.</li> </ul> |

**(Optional) Annotation**

You can optionally annotate the mapped output of Small Indel analysis. For descriptions of the Annotation parameters, see [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on page 317.

## Perform large indel analysis

To perform large indel analysis:

1. Select a project in the Projects organizer (shown [on page 75](#)).
2. Create an analysis or edit an analysis that has not yet been run:
  - Create an analysis:** Click either:
    - **Analysis** in the top menu, then **Create**, or
    - **Create Analysis** in the Task Wizards section (shown [on page 75](#)).
  - Edit an analysis:** Click **Edit Analysis** in the Task Wizards section.
3. In the Edit Analysis window, select **Large Indel** in the Available Modules pane, click the > button to move it to the Include pane. You can optionally annotate the analysis output.

Click **Next** to set the general parameters.

4. Review and edit the general parameters, then click **Next**.  
If you chose raw, unmapped data (XSQ), go to [step 5](#). If you chose already mapped data (BAM), skip to [step 6](#).
5. Review and edit the mapping parameters, then click **Next**.
6. Review and edit the Large Indel analysis parameters, described in “[Large indel analysis parameters](#)” on page 304, then click **Next**.
7. If you selected Annotation in [step 3](#), review and edit the Annotation parameters, described in [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on page 317.
8. Run the analysis.

### View analysis status

In the Projects organizer (shown [on page 75](#)), click the **Small Indel** analysis module, then click the **Status** tab in the overview section (shown [on page 75](#)).

## View large indel analysis output

If the analysis was successful, click **View Results** in the Progress column.

In the View Results window, you can view details, statistics, additional files, and logs. For more information about viewing analysis results, see [Chapter 9, “View Analysis Results”](#) on page 103.

### Details

There are no details of the output for large indel analysis.

### Statistics

There are no statistics of the output for large indel analysis.

### Additional files

Additional files for large indel analysis output include .bam and .bai files.

The following table is an example of additional files for the Large Indel analysis module.

| Directory            | Files                                                  | File Type |
|----------------------|--------------------------------------------------------|-----------|
| pair.mapping/Group_1 | solid0064_20101210_MP_2X60_T3_1_F3R3.mixed-1-1.bam     | .bam      |
|                      | solid0064_20101210_MP_2X60_T3_1_F3R3.mixed-1-1.bam.bai | .bai      |

### Logs

The following table is an example of logs for the Large Indel analysis module.

| Directory    | Files                                                   | File Type |
|--------------|---------------------------------------------------------|-----------|
| pair.mapping | secondary-hg18-pair.mapping.2.run.20110502143247523.log | .log      |

## 23

# Perform Small Indel Analysis

This chapter covers:

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- Small indel analysis input files ..... 307
- Small indel analysis parameters ..... 307
- Perform small indel analysis ..... 312
- View small indel analysis output ..... 313

## Introduction to small indel analysis

Small indel analysis is a tertiary module in LifeScope™ Genomic Analysis Software. BAM file output from the mapping and/or pairing modules that contain gapped and ungapped alignments serve as direct inputs for small indel discovery. The gapped alignments in the BAM files are referred to as the indel evidence.

The LifeScope™ Software Small Indel module allows flexible processing of these indel evidences. LifeScope™ Software takes these gapped alignments and combines them (based on their proximity from each other) to form candidates. After these combined results are formed, small indel analysis allows for the combination of several runs together, and for filtering based on the average read position of the indel and number of non-redundant reads. The small indel module produces the candidates in a text format that is easy for you to analyze and that is also useful for importing into databases and in a public general feature format, version 3, (GFF3) format.

When an indel occurs in a sequence, and that sequence is measured using color-space, the color-space sequence has a gap the same size as the indel. The color-space sequence also leaves a signature that can indicate whether there is a measurement error within the gap, thereby reducing false-positive small indel calls.

## Small indel analysis input files

The Small Indel analysis module requires one or more Binary Alignment sequence Map (BAM) files containing mapped data and an hg18 or hg19 reference file as input.

## Small indel analysis parameters

**Note:** To revert parameters to their default settings, click **Reset to Defaults**.

Categories for the Small Indel analysis module include Advanced and (if you selected Annotation for Small Indel output) Annotation. There are no Main parameters.

**Advanced**

There are five categories of Advanced parameters: General Options, Pileup, Mapping Quality Filtering, Heuristic Filtering, and Indel Size Filtering.

## General Options

| Parameters                      | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                        |
|---------------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Detail level                    | 0             | For BAM file inputs, the level of detail in output: <ul style="list-style-type: none"> <li>• <b>0</b>: Keeps only position information about the anchor read and no information for the ungapped alignment.</li> <li>• <b>1-8</b>: Keeps only some of the alignment's anchor alignment but none of the ungapped alignment.</li> <li>• <b>9</b>: Is most detailed, but also the slowest.</li> </ul> |
| Zygoty profile name             | max-mapping   | Zygoty profile name. <ul style="list-style-type: none"> <li>• <b>classic</b> Run classic mapping.</li> <li>• <b>max-mapping</b>: Run max mapping.</li> <li>• <b>max-mapping-v2</b>: Run max mapping version 2.</li> <li>• <b>gap-align-only</b> and <b>no-calls</b>: Force all zygoty calls to be homozygous calls.</li> </ul>                                                                     |
| Genomic region                  | -             | Names a specific genomic region to be selected from the BAM file. Only full chromosomes are guaranteed not to alter results. Specifying partial chromosomes is allowed but may result in the loss of indels near the edges of that region. For example, chr1:2945-9659 causes a reduction of coverage for approximately a read length after position 2945.                                         |
| Display base QVs                | False         | Display base QV scores in the GFF file. Allowed values: <p><b>True</b>: Display the FASTQ base QV scores for all of the reads used for each indel in the GFF file. FASTQ strings contain semi-colons, so adding these strings may produce a GFF file that is not compatible with certain applications.</p> <p><b>False</b>: Do not display QV scores in the output file.</p>                       |
| Number of alignments per pileup | 1000          | For pileups with more than this number of reads, set the expected number of alignments per pileup. Allowed values: Integers $\geq 0$ .                                                                                                                                                                                                                                                             |
| Random seed                     | 94404         | The random seed value used to determine which pseudo random set of reads to use when there are greater than 1000 reads in a pileup. The random number generator used is the Mersenne Twister MT19937 algorithm. Allowed values: Integers $\geq 0$ .                                                                                                                                                |

Pileup

| Parameters   | Default value | Description                                                                                                                                                                                                                                                                                                      |
|--------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Min num evid | 2             | Minimum number of evidences required for an indel call. A value higher than the average coverage level in most cases has a significant reduction in sensitivity.<br><br>Allowed values: Integers.                                                                                                                |
| Max num evid | -1            | Maximum number of evidences. Allowed values: Integers. Use -1 for no maximum.<br>Setting this value to some multiple of the average coverage could remove indels found in abnormally high coverage areas.                                                                                                        |
| ConsGroup    | 1             | Indel grouping method. Allowed values:<br><b>1:</b> Conservative grouping of indels with 5bp max between consecutive evidences.<br><b>2:</b> Lax grouping. Groups indels that are at maximum the higher of 15 or 7 times the indel size.<br><b>9:</b> No grouping. Makes every indel evidence a separate pileup. |

Mapping Quality Filtering

| Parameters                 | Default value | Description                                                                                                                                                                                                                                                              |
|----------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Max reported alignments    | -1            | Only uses those alignments where the NH field (the number of reported alignments, from the BAM record) is this value or lower. A value of -1 is to have no upper limit. The range where this is effective depends on the input BAM file's range of values of the NH tag. |
| Min mapping quality        | 8             | Keeps only reads that have this or higher pairing qualities. For paired tags, mapping quality is for the pair (pairing quality), and for fragment, it is the single tag's map quality.<br><br>Reads that are lower than this value are filtered out.                     |
| Min best mapping quality   | 10            | For a particular indel called with a set of reads, at least one pairing quality in this set must be higher than this value. Allows for supporting evidences to have a lower mapping quality threshold than the best read.                                                |
| Min anchor mapping quality | -1            | Minimum mapping quality for a non-indel (anchor) tag. Effective only for paired reads, for the number of anchors queried as defined by <code>small.indel.detail.level</code> .                                                                                           |

| Parameters               | Default value | Description                                                                                                                                                                                                                                                                                                                                      |
|--------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ungapped BAM flag filter | ProperPair    | For ungapped alignments, specifies the BAM flag properties that a read must have to be included. Allowed values: A comma-separated string of one or more of these values: <ul style="list-style-type: none"> <li>• ProperPair</li> <li>• UniqueHit</li> <li>• NoOptDup</li> <li>• Primary</li> <li>• None</li> </ul> None turns off all filters. |
| Gapped BAM flag filter   | Primary       | For gapped alignments, specifies the BAM flag properties that a read must have to be included. Allowed values: A comma-separated string of one or more of these values: <ul style="list-style-type: none"> <li>• ProperPair</li> <li>• UniqueHit</li> <li>• NoOptDup</li> <li>• Primary</li> <li>• None</li> </ul> None turns off all filters.   |
| Edge length deletions    | 0             | Gap alignments that do not have this minimum length on either side of the indel will not be considered. Allowed values: Integers $\geq 0$ .                                                                                                                                                                                                      |
| Edge length insertions   | 0             | Gap alignments that do not have this minimum length on either side of the indel will not be considered. Allowed values: Integers $\geq 0$                                                                                                                                                                                                        |

#### Heuristic Filtering

| Parameters        | Default value | Description                                                                                                                                                                                                                                                                                                                           |
|-------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Perform filtering | True          | Whether or not to perform filtering in each pileup. Allowed values: <ul style="list-style-type: none"> <li>• <b>True</b>: Perform filtering on pileups.</li> <li>• <b>False</b>: Do not perform filtering on pileups.</li> </ul> Parameters that change the makeup of pileups, such as <a href="#">Min num evid</a> are still active. |

| Parameters                      | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|---------------------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Indel size distribution allowed | can-cluster   | <p>Indel sizes in a pileup are allowed to have certain indel size distributions.</p> <p>Allowed values:</p> <ul style="list-style-type: none"> <li>• <b>similar-size</b>: 75% of the reads of a pileup must have exactly the same size.</li> <li>• <b>similar-size-any-large-deletions</b>: Any pileups with at least 2 large deletion alignments, the other pileups must have similar sizes.</li> <li>• <b>can-cluster</b>: Allowed if at least one cluster of any indel size is found.</li> <li>• <b>can-cluster-any-large-deletions</b>: Any pileups with at least 2 large deletion alignments; other pileups must be able to cluster (will have indels with two more reads with larger deletions, even if they don't form good clusters).</li> <li>• <b>any</b>: Can have any size distribution (same as <code>small.indel.require.called.indel.size=false</code>).</li> </ul> |
| Remove singletons               | True          | <p>Remove the singletons that occur when different alignment methods are combined based on identical bead ids and read sequence. Allowed values:</p> <ul style="list-style-type: none"> <li>• <b>True</b>: Remove singletons.</li> <li>• <b>False</b>: Do not remove the singletons.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Alignment compatibility filter  | 1             | <p>Alignment compatibility level. Checks color space compatibility around the gap. Allowed values:</p> <ul style="list-style-type: none"> <li>• <b>0</b>: No alignment compatibility filtering.</li> <li>• <b>1</b>: The small indel module determines whether the data contains base-space or color-space sequence.</li> <li>• <b>2</b>: Force the use of base-space sequence, if present.</li> <li>• <b>3</b>: Force the use of color-space sequence, if present.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                     |
| Max coverage ratio              | 12            | <p>Maximum clipped coverage/# non-redundant support ratio.</p> <p>Use -1 for no limit (no coverage ratio filtering).</p> <p>Allowed values: Integers.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Max nonreds 4Fit                | 2             | <p>Maximum number of non-redundant reads where read position filtering is applied.</p> <p>Allowed values: Integers.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Min from end pos                | 9.1           | <p>Minimum average number of base pairs from the end of the read required of the pileup, when there are at most a certain number of reads defined by <a href="#">Max nonreds 4Fit</a>.</p> <p>Allowed values: Floats.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

## Indel Size Filtering

| Parameters          | Default value | Description                                                     |
|---------------------|---------------|-----------------------------------------------------------------|
| Min insertions size | 0             | Minimum insertion size to include.<br>Allowed values: Integers. |
| Min deletions size  | 0             | Minimum deletion size to include.<br>Allowed values: Integers.  |
| Max insertions size | 1000000000    | Maximum insertion size to include.<br>Allowed values: Integers. |
| Max deletions size  | 1000000000    | Maximum deletion size to include.<br>Allowed values: Integers.  |

**(Optional) Annotation**

You can optionally annotate the mapped output of Small Indel analysis. For descriptions of the Annotation parameters, see [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on page 317.

## Perform small indel analysis

The Small Indel analysis module detects small indels in SOLiD® System data that originates from a single human sample. Slide(s) from this sample must be mapped to the hg18 or hg19 reference to facilitate correct normalization. Use the Small Indel module to perform tertiary analysis.

To perform small indel analysis:

1. Select a project in the Projects organizer (shown [on page 75](#)).
2. Create an analysis or edit an analysis that has not yet been run:
 

**Create an analysis:** Click either:

  - **Analysis** in the top menu, then **Create**, or
  - **Create Analysis** in the Task Wizards section (shown [on page 75](#)).

**Edit an analysis:** Click **Edit Analysis** in the Task Wizards section.
3. In the Edit Analysis window, select **Small Indel** in the Available Modules pane, click the > button to move it to the Include pane. You can optionally annotate the analysis output.  
Click **Next** to set the general parameters.
4. Review and edit the general parameters, then click **Next**.  
If you chose raw, unmapped data (XSQ), go to [step 5](#). If you chose already mapped data (BAM), skip to [step 6](#).
5. Review and edit the mapping parameters, then click **Next**.
6. Review and edit the Small Indel analysis parameters, described in [“Small indel analysis parameters”](#) on page 307, then click **Next**.

7. If you selected Annotation in [step 3](#), review and edit the Annotation parameters, described in [Chapter 24, “Add Genomic Annotations to Analysis Results”](#) on [page 317](#).
8. Run the analysis.

### View analysis status

In the Projects organizer (shown [on page 75](#)), click the **Small Indel** analysis module, then click the **Status** tab in the overview section (shown [on page 75](#)).

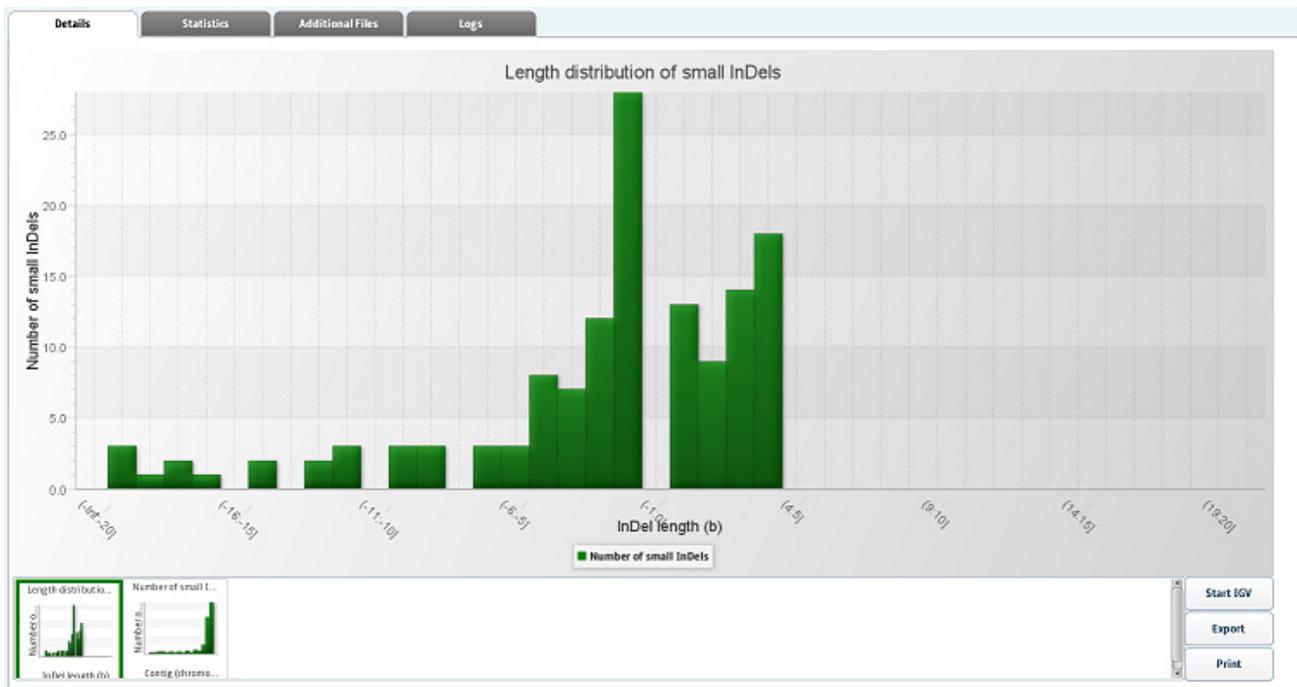
## View small indel analysis output

If the analysis was successful, click **View Results** in the Progress column.

In the View Results window, you can view details, statistics, additional files, and logs. For more information about viewing analysis results, see [“View analysis results”](#) on [page 103](#).

### Details

The Details tab shows analysis results in the form of bar charts and pie charts. The illustration [on page 313](#) is an example of details for the Small Indel analysis module.



### Statistics

The Statistics tab shows a LifeScope™ Software report of statistics that you can view, export, and print. You cannot edit the statistics. The following illustration is an example of the statistics in a small indel analysis.

```

*****
LIFESCOPE REPORT
*****
Input File:      /panasas/lifescopetesttempl/results/projects/co
indel/solid0054_20110102_PE_LFD_RD_SetA_1_HuRef100_F3.colorRemake.
Date:    Apr 21, 2011 10:05:52 PM
Annotation file, dbSNP: /panasas/lifescopetest/analysis/referenceDa
Annotation file, Genes and Exons, GTF: /panasas/lifescopetest/analys
*****
Statistics Overview
*****

-- Basic Statistics -----
Number of variants                186
Number of heterozygous variants   127
Number of homozygous variants     59

-- Variant-Specific Statistics (InDel) -----
Distribution of InDel length

Length(bases)      Number
(-Inf:-20]         0
(-20:-19]          3
(-19:-18]          8
(-18:-17]          1
(-17:-16]          5
(-16:-15]          1
(-15:-14]          4
(-14:-13]          2
(-13:-12]          1
(-12:-11]          4
(-11:-10]          3
(-10:-9]           1
(-9:-8]            6
(-8:-7]            1
(-7:-6]            3
(-6:-5]            4
(-5:-4]            5
(-4:-3]            10
(-3:-2]            11
(-2:-1]            61

```

**Additional files**

Additional files for small indel analysis output include .align, .bed, .gff3, .pas, sql, .sum, .tab, .txt, and .ungapped files.

The following table is an example of additional files for the Small Indel analysis module.

| Directory                                                        | Files                               | File Type |
|------------------------------------------------------------------|-------------------------------------|-----------|
| small.indel/<br>hg19_Simulated_TR_ECC_BC16_Frag50_320Mil_<br>BC1 | BrainCancerAnalysis1.pas            | .pas      |
|                                                                  | BrainCancerAnalysis1_genes.bed      | .bed      |
|                                                                  | BrainCancerAnalysis1.ungapped       | .ungap... |
|                                                                  | BrainCancerAnalysis1.gff3           | .gff3     |
|                                                                  | BrainCancerAnalysis1_annotated.gff3 |           |
|                                                                  | BrainCancerAnalysis1.txt            | .txt      |
|                                                                  | BrainCancerAnalysis1_genes.tab      | .tab      |
|                                                                  | BrainCancerAnalysis1.align          | .align    |
| BrainCancerAnalysis1.sql                                         | .sql                                |           |

**Logs**

The following table is an example of logs for the Small Indel analysis module.

| Directory   | Files                                                                                   | File Type |
|-------------|-----------------------------------------------------------------------------------------|-----------|
| small.indel | small.indel-hg19_Simulated_TR_ECC_BC16_Frag50_320Mil_BC1.20110504200216106.log          | .log      |
|             | tertiary-hg19_Simulated_TR_ECC_BC16_Frag50_320Mil_BC1-small.indel.20110504200214855.log |           |



## 24

# Add Genomic Annotations to Analysis Results

This chapter covers:

|                                                  |     |
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| ■ Annotation sources .....                       | 320 |
| ■ Annotation parameters .....                    | 321 |
| ■ About annotations and LifeScope™ modules ..... | 337 |
| ■ Examples of annotation output files .....      | 338 |

## Overview

The annotations module is an optional post-processing step available with select LifeScope™ Genomic Analysis Software analysis modules. The annotations processing makes a copy of the analysis module's GFF file and adds new attributes to the GFF entries in the copied file. The new attributes are taken from information in publicly available sources about the variants in the input, about features intersecting the variants in the input file, or about any biological function potentially changed by the variants.

Annotations processing does not modify the original LifeScope™ analysis module's output file.

Several output files are potentially generated by the annotations processing, depending on the analysis modules involved. One annotations output file contains all of the content of the GFF file generated by the modules, with added attributes for annotations. Another annotations output file is a subset of the previous file, and it contains only those entries that pass filtering requirements that you specified. Annotation processing also produces output files containing only mutated genes, or only SNPs. Several statistics files are also produced.

Two sources of annotation are used:

- A UCSC or ENSEMBL GTF file, used to determine whether a variant overlaps a gene or exon.
- The National Center for Biotechnology Information (NCBI) dbSNP database, which contains information on SNPs and indels already found by other studies. We use the following fields of the of the dbSNP entries:
  - The reference SNP identifier (rsID)
  - The functional code
  - The corresponding locus ID (the gene)

If a conflict is found between the dbSNP data and the GTF data, both annotations are reported. The annotation module does not attempt to resolve these conflicts.

Annotation sources can be either the refGene GTF file with respect to the human hg18 reference build and the dbSNP 130 build or the human hg19 reference build and the dbSNP 132 build (both available with LifeScope™ Software), or a source that you provide. Only GTF files and dbSNP data can be used as sources. The data from these three tables in dbSNP is required:

- SNPChrPosOnRef
- SNPContigLoc
- SNPContigLocusId

Annotations processing supports these annotation types:

- DNA features: genes and protein-coding features
- Verified variants in existing database

Post-processing types include:

- Annotate genomic variants with the annotation types that apply
- Filter based on annotation
- Report a list of gene and protein-coding annotations involved the variants
- Report statistics

The annotations module is available with the following modules:

- Resequencing:
  - SNPs
  - Small indels
  - Large indels
  - Human CNV
  - Inversions
- Targeted resequencing:
  - SNPs
  - Small indels
- Low frequency variant:
  - SNPs

### Memory requirement

For hg18 or hg19 sources, 15gb is required.

### Input file handling

Annotations processing uses the GFF output of an analysis modules, but does not modify the GFF file. Annotations are added to a copy of the GFF file.

### Filters

Annotation processing optionally generates an output file containing only those entries that fulfill *all* the conditions you specify with annotation filtering options. Annotation filters are described in the following table.

| Filtering option                                 | Default |
|--------------------------------------------------|---------|
| Report <i>only</i> variants in exons             | Off     |
| Report <i>only</i> variants in genes             | Off     |
| Report <i>only</i> variants that appear in dbSNP | Off     |

| Filtering option                                                | Default |
|-----------------------------------------------------------------|---------|
| Report <i>only</i> variants that do not appear in dbSNP         | Off     |
| Report <i>all</i> variants, whether or not they appear in dbSNP | On      |

The following table shows the annotation filtering supported in select LifeScope™ modules.

| Annotation type | SNP | CNV | Small indel | Large indel | Inversion |
|-----------------|-----|-----|-------------|-------------|-----------|
| geneID          | Yes | Yes | Yes         | Yes         | Yes       |
| exonID          | Yes | Yes | Yes         | Yes         | Yes       |
| rsID            | Yes | —   | Yes         | —           | —         |
| functionCode    | Yes | —   | —           | —           | —         |

## Statistics

The following table lists the statistics included in the annotations output files and the LifeScope™ Genomic Analysis Software modules that support these annotations. See the following table for information about SNPs transitions and transversions.

| Analysis type                                 | Statistics                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| All supported modules<br>(SNPs, CNVs, Indels) | Number of variants<br>Number of variants per chromosome<br>Number of heterozygous variants<br>Number of homozygous variants<br>Number of heterozygous SNPs per chromosome<br>Number of homozygous SNPs per chromosome                                                                                                                                                                                             |
| SNPs                                          | Number of heterozygous SNPs that are transitions, transversions<br>Number of homozygous SNPs that are transitions, transversions (compared to the reference)                                                                                                                                                                                                                                                      |
| Indels                                        | Indel variant length distribution (negative for deletion, positive for insertion)                                                                                                                                                                                                                                                                                                                                 |
| CNVs                                          | Copy number distribution<br>CNV length distribution                                                                                                                                                                                                                                                                                                                                                               |
| Annotations from dbSNPs                       | Number of SNPs or indels in dbSNP<br>Number of homozygous SNPs or indels in dbSNP<br>Number of heterozygous SNPs or indels in dbSNP<br>Overall dbSNP concordance (percentage of SNPs or indels in dbSNP)<br>Heterozygous dbSNP concordance (the percentage of heterozygous SNPs or indels found in dbSNP)<br>Homozygous dbSNP concordance (the percentage of homozygous SNPs or indels found in dbSNP)            |
| Annotations from GTF file content             | Number of variants in exons, and the percentage of exons that are variant<br>Number of heterozygous variants in exons, and their percentage<br>Number of homozygous variants in exons, and their percentage<br>Number of variants in genes, and the percentage that are variant<br>Number of heterozygous variants in genes, and their percentage<br>Number of homozygous variants in genes, and their percentage |

The following table describes how transitions and transversions are determined based on the reference allele and the SNP Finding output genotype.

| SNP Finding genotype | Reference allele   second allele | A  | C  | G  | T  | Other |
|----------------------|----------------------------------|----|----|----|----|-------|
| A                    | A                                | 0  | TV | TS | TV | 0     |
| C                    | C                                | TV | 0  | TV | TS | 0     |
| G                    | G                                | TS | TV | 0  | TV | 0     |
| T                    | T                                | TV | TS | TV | 0  | 0     |
| M                    | A C                              | TV | TV | TV | TV | TV    |
| R                    | A G                              | TS | TS | TS | TS | TS    |
| W                    | A T                              | TV | TV | TV | TV | TV    |
| S                    | C G                              | TV | TV | TV | TV | TV    |
| Y                    | C T                              | TS | TS | TS | TS | TS    |
| K                    | T G                              | TV | TV | TV | TV | TV    |
|                      | Other                            | 0  | 0  | 0  | 0  | 0     |

## Workflows

Annotation is integrated into the resequencing, targeted resequencing, and low frequency variant standard workflows. The table shows the modules with integrated annotation support, in these workflows.

| Workflow              | Library type | SNP | CNV | Small indel | Large indel |
|-----------------------|--------------|-----|-----|-------------|-------------|
| Genomic resequencing  | Fragment     | Yes | Yes | Yes         | —           |
| Genomic resequencing  | Mate-pair    | Yes | Yes | Yes         | Yes         |
| Genomic resequencing  | Paired-end   | Yes | Yes | Yes         | Yes         |
| Targeted resequencing | Fragment     | Yes | —   | Yes         | —           |
| Targeted resequencing | Paired-end   | Yes | —   | Yes         | —           |
| Low frequency variant | Fragment     | Yes | —   | —           | —           |
| Low frequency variant | Paired-end   | Yes | —   | —           | —           |

## Annotation sources

The table lists common annotation sources supported by LifeScope™ Software. Files required to support certain annotations are available with LifeScope™ Software. These files support the following:

- Annotations based on the hg18 and the dbSNP 130 build
- Annotations based on the hg19 and the dbSNP 132 build

**Note:** The dbSNP 132 file used is a VCF file containing 1000 Genome data.

| Type of annotation                                                                                                               | Location and notes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|----------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Variants in existing databases                                                                                                   | NCBI Variation dbSNP resources: includes single nucleotide polymorphisms, microsatellites, and small-scale insertions and deletions. dbSNP contains population-specific frequency and genotype data, experimental conditions, molecular context, and mapping information for both neutral polymorphisms and clinical mutations. (This text is taken from NCBI website <a href="http://www.ncbi.nlm.nih.gov/guide/variation">http://www.ncbi.nlm.nih.gov/guide/variation</a> .)<br><a href="http://www.ncbi.nlm.nih.gov/snp">http://www.ncbi.nlm.nih.gov/snp</a> |
| Genes and protein-coding features<br><b>Note:</b> Supported by LifeScope™ Software only if the data is downloaded in GTF format. | RefGene data from UCSC:<br><a href="ftp://hgdownload.cse.ucsc.edu/goldenPath/hg18/database/refGene.txt.gz">ftp://hgdownload.cse.ucsc.edu/goldenPath/hg18/database/refGene.txt.gz</a><br>The European Bioinformatics Institute's Alternative Splicing Database Project:<br><a href="http://www.ebi.ac.uk/asd">http://www.ebi.ac.uk/asd</a>                                                                                                                                                                                                                       |

**Note:** While every effort is made to provide correct web addresses, Applied Biosystems is not responsible for the changes to these addresses, nor for the data provided by these locations.

## Annotation parameters

This section describes the annotation parameters used with LifeScope™ analyses. There are three categories of Annotation Statistics and Reporting options: General, Sources, and Filtering.

### Annotation Statistics and Reporting General Options

| Parameters       | Default value                                                                                              | Description                                                                                                                                                                                                                                                                                                                                                                     |
|------------------|------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Input GFF3 file  | <code>\${analysis.output.dir}/small.indel/<br/>\${analysis.sample.name}/<br/>\${analysis.name}.gff3</code> | The input variant GFF file, generated by a LifeScope™ analysis module (see “ <a href="#">Show only variants in genes</a> ” on page 323).<br>Expected file extensions: .gff, .gff3.<br>Example: /data//HuRef_chr22/DB_out/<br>HuRef_rmm13_SNP.gff3<br>Must be a valid path, and the file must be readable.<br>This file may be the output of an earlier version of the software. |
| Output directory | <code>\${analysis.output.dir}/small.indel/<br/>\${analysis.sample.name}</code>                             | Path to the output directory. ASF writes all output files to this directory.                                                                                                                                                                                                                                                                                                    |

## Annotation Statistics and Reporting Sources Options

| Parameters                         | Default value | Description                                                                                                                                                                                                                                                                    |
|------------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| dBSNP concordance for dbSNP SNPs   | False         | Annotate variants with SNP entries from the dbSNP file. <ul style="list-style-type: none"> <li>• <b>True:</b> Do not annotate variants with SNP entries from the dbSNP file.</li> <li>• <b>False:</b> Annotate variants with SNP entries from the dbSNP file.</li> </ul>       |
| dBSNP concordance for dbSNP indels | True          | Annotate variants with indel entries from the dbSNP file. <ul style="list-style-type: none"> <li>• <b>True:</b> Do not annotate variants with indel entries from the dbSNP file.</li> <li>• <b>False:</b> Annotate variants with indel entries from the dbSNP file.</li> </ul> |
| dBSNP indel border slack           | 5             | An indel is considered to be matched to a dbSNP indel if the two overlap or if the distance between them is less than or equal to the value of <code>annotation.indel.border.slack</code> .<br>Allowed values: Integers $\geq 0$                                               |

Annotation Statistics and Reporting Filtering Options

| Parameters                                  | Default value | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|---------------------------------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Show only coding variants                   | False         | When set to true, filters the annotated output: restricts the Filtered Variant annotation output file to only the variants that overlap any exon. <ul style="list-style-type: none"> <li>• <b>True:</b> Restrict output to variants that overlap any exon.</li> <li>• <b>False:</b> Do not filter the output for variants that overlap any exon.</li> </ul> <b>Note:</b> If set to true, then the setting for the parameter <a href="#">Show only variants in genes</a> is ignored.                                                |
| Show only variants in genes <sup>‡</sup>    |               | When True, filters the annotated output: restricts the Filtered Variant annotation output file to only the variants that overlap any gene, even if it does not overlap any exon. <ul style="list-style-type: none"> <li>• <b>True:</b> Restrict output to variants that overlap any gene.</li> <li>• <b>False:</b> Do not filter the output for variants that overlap any gene.</li> </ul> <b>Note:</b> If the parameter <a href="#">Show only coding variants</a> is set to true, then the setting for this parameter is ignored. |
| Show only variants in dbSNP <sup>‡</sup>    |               | When set to true, filters the annotated output: restricts the Filtered Variant annotation output file to only SNPs or small indels that exist in dbSNP. <ul style="list-style-type: none"> <li>• <b>True:</b> Restrict output to SNPs or small indels variants that <i>appear</i> in dbSNP.</li> <li>• <b>False:</b> Do not filter the output for variants that <i>appear</i> in dbSNP.</li> </ul>                                                                                                                                 |
| Show only variant not in dbSNP <sup>‡</sup> |               | When set to true, filters the annotated output: restricts the Filtered Variant annotation output file to only SNPs or small indels that do not exist in dbSNP. <ul style="list-style-type: none"> <li>• <b>True:</b> Restrict output to SNPs or small indels variants that <i>do not appear</i> in dbSNP.</li> <li>• <b>False:</b> Do not filter the output variants that <i>do not appear</i> in dbSNP.</li> </ul>                                                                                                                |

<sup>‡</sup> Do not use for CNV analysis.

The following table describes function codes for refSNPs in gene features. This table is taken from the dbSNP website:

<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=handbook&part=ch5>

| Functional class | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Database code |
|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| Locus region     | Variation is within 2 kbp 5' or 500 bp 3' of a gene feature (on either strand), but the variation is not in the transcript for the gene. This class is indicated with an "L" in graphical summaries. <p><b>Note:</b> As of build 127, function code 1 has been modified into a <i>two-digit code</i> that more precisely indicates the location of a SNP. The two-digit code has function code 1 as the first digit, which keeps the meaning as described above, and 3 or 5 as the second digit, which indicates whether the SNP is 3' or 5' to the region of interest. See function codes 13 and 15 in this table.</p> | 1             |

| Functional class | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Database code |
|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| Coding           | Variation is in the coding region of the gene. This class is assigned if the allele-specific class is unknown. This class is indicated with a 'C' in graphical summaries.<br><b>Note:</b> This code was <i>retired</i> as of dbSNP build 127.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 2             |
| Coding-synon     | The variation allele is synonymous with the contig codon in a gene. An allele receives this class when substitution and translation of the allele into the codon makes no change to the amino acid specified by the reference sequence. A variation is a synonymous substitution if all alleles are classified as contig reference or coding-synon. This class is indicated with a 'C' in graphical summaries.                                                                                                                                                                                                                                                                                                                                                                                                            | 3             |
| Coding-nonsynon  | The variation allele is nonsynonymous for the contig codon in a gene. An allele receives this class when substitution and translation of the allele into the codon changes the amino acid specified by the reference sequence. A variation is a nonsynonymous substitution if any alleles are classified as coding-nonsynon. This class is indicated with a "C" or "N" in graphical summaries.<br><b>Note:</b> As of build 128, function code 4 has been modified into a <i>two-digit code</i> that more precisely indicates the nonsynonymous nature of the SNP. The two-digit code has function code 4 as the first digit, which keeps its original meaning, and 1, 2, or 4 as the second digit, which indicates whether the SNP is nonsense, missense, or frameshift. See function codes 41, 42, and 44 in this table. | 4             |
| mRNA-UTR         | The variation is in the transcript of a gene but not in the coding region of the transcript. This class is indicated by a "T" in graphical summaries.<br><b>Note:</b> As of build 127, function code 5 has been modified into a <i>two-digit code</i> that more precisely indicates the location of a SNP. The two digit code has function code 5 as the first digit, which keeps its original meaning, and 3 or 5 as the second digit, which indicates whether the SNP is 3' or 5' to the region of interest. See function codes 53 and 55 in this table.                                                                                                                                                                                                                                                                | 5             |
| Intron           | The variation is in the intron of a gene but not in the first two or last two bases of the intron. This class is indicated by an "L" in graphical summaries.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 6             |
| Splice-site      | The variation is in the first two or last two bases of the intron. This class is indicated by a "T" in graphical summaries.<br><b>Note:</b> As of build 127, function code 7 has been modified into a <i>two-digit code</i> that more precisely indicates the location of a SNP. The two-digit code has function codes 7 as the first digit, which keeps its original meaning, and 3 or 5 as the second digit, which indicates whether the SNP is 3' or 5' to the region of interest. See function codes 73 and 75 in this table.                                                                                                                                                                                                                                                                                         | 7             |
| Contig-reference | The variation allele is identical to the contig nucleotide. Typically, one allele of a variation is the same as the reference genome. The letter used to indicate the variation is a "C" or "N", depending on the state of the alternative allele for the variation.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 8             |
| Coding-exception | The variation is in the coding region of a gene, but the precise location cannot be resolved because of an error in the alignment of the exon. The class is indicated by a "C" in graphical summaries.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 9             |
| NearGene-3       | Function Code 13, where:<br>1: locus region (see function code 1 in this table)<br>3: SNP is 3' to and 0.5 kbp away from gene                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 13            |

| Functional class                | Description                                                                                                                                                                                    | Database code |
|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| NearGene-5                      | Function Code 15, where:<br>1: locus region (see function code 1 in this table)<br>3: SNP is 5' to and 0.5 kbp away from gene                                                                  | 15            |
| Coding-nonsynonymous sense      | Function Code 41, where:<br>4: Coding-nonsynonymous (see function code 4 in this table)<br>1: Nonsense (changes to the Stop codon)                                                             | 41            |
| Coding-nonsynonymous missense   | Function Code 42, where:<br>4: Coding-nonsynonymous (see function code 4 in this table)<br>2: missense (alters codon to make an altered amino acid in the protein product)                     | 42            |
| Coding-nonsynonymous frameshift | Function Code 44, where:<br>4: Coding-nonsynonymous (see function code 4 in this table)<br>4 (as the second digit): frameshift (alters codon to make an altered amino acid in protein product) | 44            |
| UTR-3                           | Function code 53, where:<br>5: UTR (untranslated region: see function code 5 in this table)<br>3: SNP located in the 3' untranslated region                                                    | 53            |
| UTR-5                           | Function code 55, where:<br>5: UTR (untranslated region: see function code 5 in this table)<br>5 (as the second digit): SNP located in the 5' untranslated region                              | 55            |
| Splice-3                        | Function code 73, where:<br>7: splice site (see function code 7 in this table)<br>3: 3' acceptor dinucleotide                                                                                  | 73            |
| Splice-5                        | Function code 75, where:<br>7: splice site (see function code 7 in this table)<br>5: 5' donor dinucleotide                                                                                     | 75            |

**Variant Statistics output file for SNPs**

A Variant Statistics file is a text file containing the statistics about the variants in the input GFF file.

The following is an example of the content of a Variant Statistics file for SNPs:

```
*****
LIFESCOPE REPORT
*****

Input File:/data/swdev/minita_test/
solid0064_20101210_MP_2x60_T3_1/2.0/color_lmp/outputs/
SNP.Finding/color_lmp_SNP.gff3
Date:Apr 14, 2011 2:17:54 PM
Annotation file, dbSNP:/data/analysis/LifeScope_resources/
lifetech/hg18/dbSNP/dbSNP_b130.tab
```

```

Annotation file, Genes and Exons, GTF:/data/analysis/
LifeScope_resources//lifetech/hg18/GTF/refGene.20090513.gtf

*****
Statistics Overview
*****

-- Basic Statistics -----

Number of variants                3072881
Number of heterozygous variants   1639649
Number of homozygous variants    1433232

-- Variant-Specific Statistics (SNP) -----

Number of transition SNPs        2070418
Number of transition heterozygous SNPs 1103589
Number of transition homozygous SNPs  966829
Number of transversion SNPs       1002463
Number of transversion heterozygous SNPs 536060
Number of transversion homozygous SNPs  466403
Transition:Transversion ratio      2.065 : 1.000

-- dbSNP Annotation Statistics -----

Number of SNPs in dbSNP          2913081
Number of heterozygous SNPs in dbSNP 1506740
Number of homozygous SNPs in dbSNP  1406341
dbSNP concordance                 94.80%
dbSNP heterozygous concordance     91.89%
dbSNP homozygous concordance       98.12%

-- Per-Chromosome Statistics -----

nVar: Number of variants
nHetVar: Number of heterozygous variants
nHomVar: Number of homozygous variants

contig      nVar      nHetVar      nHomVar
chr1        233502     125860       107642
chr2        247682     132717       114965
chr3        210137     115661       94476
chr4        225996     118326       107670
chr5        185137     102825       82312
chr6        191793     104138       87655
chr7        170500     94092        76408
chr8        160126     86553        73573
chr9        128205     70291        57914
chr10       157345     85408        71937
chr11       154271     80792        73479
chr12       139569     72915        66654
chr13       118842     60095        58747
chr14       93526      48742        44784

```

|       |       |       |       |
|-------|-------|-------|-------|
| chr15 | 90141 | 46673 | 43468 |
| chr16 | 95819 | 53400 | 42419 |
| chr17 | 79447 | 44311 | 35136 |
| chr18 | 85824 | 43117 | 42707 |
| chr19 | 59142 | 31904 | 27238 |
| chr20 | 65136 | 38340 | 26796 |
| chr21 | 45656 | 26098 | 19558 |
| chr22 | 36633 | 20355 | 16278 |
| chrX  | 76085 | 18909 | 57176 |
| chrY  | 22328 | 18127 | 4201  |
| chrM  | 39    | 0     | 39    |

**Variant Statistics  
output file for  
small indels**

A Variant Statistics file is a text file containing the statistics about the variants in the input GFF file.

The following is an example of the content of a Variant Statistics file for small indels:

```
*****
LIFESCOPE REPORT
*****

Input File:/data/swdev/minita_test/
solid0064_20101210_MP_2x60_T3_1/2.0/color_lmp/outputs/
small.indel/color_lmp.gff3
Date:Apr 14, 2011 10:01:01 AM
Annotation file, dbSNP:/data/analysis/LifeScope_resources/
lifetech/hg18/dbSNP/dbSNP_b130.tab
Annotation file, Genes and Exons, GTF:/data/analysis/
LifeScope_resources//lifetech/hg18/GTF/refGene.20090513.gtf

*****
Statistics Overview
*****

-- Basic Statistics -----

Number of variants                296183
Number of heterozygous variants   194752
Number of homozygous variants     100934

-- Variant-Specific Statistics (InDel) -----
Distribution of InDel length

Length(bases)                    Number
(-Inf:-500]                      0
(-500:-490]                      14
(-490:-480]                      8
(-480:-470]                      13
(-470:-460]                      21
(-460:-450]                      14
(-450:-440]                      18
(-440:-430]                      17
(-430:-420]                      18
```

|             |      |
|-------------|------|
| (-420:-410] | 18   |
| (-410:-400] | 15   |
| (-400:-390] | 24   |
| (-390:-380] | 21   |
| (-380:-370] | 24   |
| (-370:-360] | 30   |
| (-360:-350] | 39   |
| (-350:-340] | 62   |
| (-340:-330] | 115  |
| (-330:-320] | 202  |
| (-320:-310] | 181  |
| (-310:-300] | 80   |
| (-300:-290] | 43   |
| (-290:-280] | 52   |
| (-280:-270] | 42   |
| (-270:-260] | 56   |
| (-260:-250] | 49   |
| (-250:-240] | 35   |
| (-240:-230] | 39   |
| (-230:-220] | 35   |
| (-220:-210] | 33   |
| (-210:-200] | 37   |
| (-200:-190] | 34   |
| (-190:-180] | 30   |
| (-180:-170] | 40   |
| (-170:-160] | 49   |
| (-160:-150] | 42   |
| (-150:-140] | 55   |
| (-140:-130] | 78   |
| (-130:-120] | 55   |
| (-120:-110] | 68   |
| (-110:-100] | 51   |
| (-100:-90]  | 60   |
| (-90:-80]   | 61   |
| (-80:-70]   | 94   |
| (-70:-60]   | 107  |
| (-60:-50]   | 115  |
| (-50:-40]   | 229  |
| (-40:-30]   | 332  |
| (-30:-20]   | 1064 |
| (-20:-19]   | 220  |
| (-19:-18]   | 392  |
| (-18:-17]   | 268  |
| (-17:-16]   | 568  |
| (-16:-15]   | 436  |
| (-15:-14]   | 624  |
| (-14:-13]   | 541  |
| (-13:-12]   | 1126 |
| (-12:-11]   | 1009 |
| (-11:-10]   | 1636 |
| (-10:-9]    | 1276 |
| (-9:-8]     | 2367 |
| (-8:-7]     | 1566 |
| (-7:-6]     | 3498 |

|           |       |
|-----------|-------|
| (-6:-5]   | 5458  |
| (-5:-4]   | 15589 |
| (-4:-3]   | 11836 |
| (-3:-2]   | 22210 |
| (-2:-1]   | 77896 |
| (-1:0]    | 0     |
| (0:1]     | 65663 |
| (1:2]     | 19317 |
| (2:3]     | 14044 |
| (3:4]     | 25082 |
| (4:5]     | 3749  |
| (5:6]     | 2199  |
| (6:7]     | 1011  |
| (7:8]     | 1596  |
| (8:9]     | 892   |
| (9:10]    | 1169  |
| (10:11]   | 705   |
| (11:12]   | 1289  |
| (12:13]   | 803   |
| (13:14]   | 1264  |
| (14:15]   | 3547  |
| (15:16]   | 192   |
| (16:17]   | 164   |
| (17:18]   | 123   |
| (18:19]   | 141   |
| (19:20]   | 268   |
| (20:30]   | 530   |
| (30:+Inf) | 0     |

-- dbSNP Annotation Statistics -----

|                                        |        |
|----------------------------------------|--------|
| Number of InDels in dbSNP              | 246619 |
| Number of heterozygous InDels in dbSNP | 150203 |
| Number of homozygous InDels in dbSNP   | 96105  |
| dbSNP concordance                      | 83.27% |
| dbSNP heterozygous concordance         | 77.13% |
| dbSNP homozygous concordance           | 95.22% |

-- Per-Chromosome Statistics -----

nVar: Number of variants  
nHetVar: Number of heterozygous variants  
nHomVar: Number of homozygous variants

| contig | nVar  | nHetVar | nHomVar |
|--------|-------|---------|---------|
| chr1   | 22753 | 15323   | 7385    |
| chr2   | 24215 | 16211   | 7975    |
| chr3   | 20327 | 13613   | 6686    |
| chr4   | 22322 | 14798   | 7485    |
| chr5   | 18224 | 12212   | 5989    |
| chr6   | 19046 | 12807   | 6213    |
| chr7   | 16887 | 11032   | 5824    |
| chr8   | 14530 | 9625    | 4889    |

|       |       |      |      |
|-------|-------|------|------|
| chr9  | 11539 | 7926 | 3601 |
| chr10 | 14864 | 9836 | 5000 |
| chr11 | 14951 | 9502 | 5428 |
| chr12 | 14000 | 9180 | 4793 |
| chr13 | 11885 | 7685 | 4184 |
| chr14 | 9439  | 6105 | 3323 |
| chr15 | 8945  | 5912 | 3022 |
| chr16 | 7705  | 5304 | 2382 |
| chr17 | 7849  | 5323 | 2508 |
| chr18 | 8605  | 5530 | 3066 |
| chr19 | 5866  | 3825 | 2026 |
| chr20 | 6107  | 4120 | 1977 |
| chr21 | 5067  | 2977 | 2072 |
| chr22 | 3587  | 2301 | 1280 |
| chrX  | 6351  | 2650 | 3675 |
| chrY  | 1118  | 954  | 151  |
| chrM  | 1     | 1    | 0    |

### Variant Statistics output file for large indels

A Variant Statistics file is a text file containing the statistics about the variants in the input GFF file.

The following is an example of the content of a Variant Statistics file for large indels:

```
*****
LIFESCOPE REPORT
*****

Input File:/data/swdev/minita_test/
solid0064_20101210_MP_2x60_T3_1/2.0/color_lmp/outputs/
large.indel/large-indels.gff3
Date:Apr 14, 2011 10:00:05 AM
Annotation file, dbSNP:/data/analysis/LifeScope_resources/
lifetech/hg18/dbSNP/dbSNP_b130.tab
Annotation file, Genes and Exons, GTF:/data/analysis/
LifeScope_resources//lifetech/hg18/GTF/refGene.20090513.gtf

*****
Statistics Overview
*****

-- Basic Statistics -----

Number of variants                21
Number of heterozygous variants   2
Number of homozygous variants    19

-- Variant-Specific Statistics (InDel) -----
Distribution of InDel length

Length(bases)          Number
(-Inf:-500000]         0
(-500000:-100000]     0
(-100000:-50000]      0
```

```
(-50000:-20000]          0
(-20000:-10000]         0
(-10000:-5000]          0
(-5000:-1000]           0
(-1000:-500]            0
(-500:-200]             7
(-200:-100]             5
(-100:0]                2
(0:100]                 2
(100:200]               4
(200:500]               1
(500:1000]              0
(1000:5000]             0
(5000:10000]            0
(10000:20000]           0
(20000:50000]           0
(50000:100000]          0
(100000:500000]         0
(500000:+Inf)           0
```

-- dbSNP Annotation Statistics -----

```
Number of InDels in dbSNP          0
Number of heterozygous InDels in dbSNP 0
Number of homozygous InDels in dbSNP 0
dbSNP concordance                   0.00%
dbSNP heterozygous concordance      0.00%
dbSNP homozygous concordance        0.00%
```

-- Per-Chromosome Statistics -----

```
nVar: Number of variants
nHetVar: Number of heterozygous variants
nHomVar: Number of homozygous variants
```

| contig | nVar | nHetVar | nHomVar |
|--------|------|---------|---------|
| chr2   | 1    | 0       | 1       |
| chr9   | 1    | 0       | 1       |
| chr10  | 7    | 2       | 5       |
| chr18  | 4    | 0       | 4       |
| chr19  | 2    | 0       | 2       |
| chrY   | 4    | 0       | 4       |
| chrM   | 2    | 0       | 2       |

**Variant Statistics output file for CNVs**

A Variant Statistics file is a text file containing the statistics about the variants in the input GFF file.

The following is an example of the content of a Variant Statistics file for CNVs:

```
*****
LIFESCOPE REPORT
*****
```

```

Input File:/data/swdev/minita_test/
solid0064_20101210_MP_2x60_T3_1/2.0/color_lmp/outputs/cnv/
OutputCNVs.gff3
Date:Apr 14, 2011 9:58:12 AM
Annotation file, dbSNP:/data/analysis/LifeScope_resources/
lifetech/hg18/dbSNP/dbSNP_b130.tab
Annotation file, Genes and Exons, GTF:/data/analysis/
LifeScope_resources//lifetech/hg18/GTF/refGene.20090513.gtf

*****
Statistics Overview
*****

-- Basic Statistics -----

Number of variants                                     120

-- Variant-Specific Statistics (CNV) -----

Distribution of CNV copy number
Copy_Number      Number
      0              5
      1             20
      2             23
      3             20
      4             22
      5              8
      6              9
      7              1
      8              2
      9             10
     >9              0

Distribution of CNV length
Length(bases)      Number
(0:1000]            0
(1000:2000]         0
(2000:5000]         0
(5000:10000]        0
(10000:20000]       41
(20000:50000]       54
(50000:100000]      11
(100000:500000]     10
(500000:+Inf)       4

-- Per-Chromosome Statistics -----

nVar: Number of variants

contig      nVar
chr1         7
chr2         5

```

|       |    |
|-------|----|
| chr3  | 11 |
| chr4  | 4  |
| chr5  | 7  |
| chr6  | 11 |
| chr7  | 4  |
| chr8  | 5  |
| chr9  | 3  |
| chr10 | 3  |
| chr11 | 8  |
| chr12 | 4  |
| chr13 | 3  |
| chr14 | 2  |
| chr15 | 4  |
| chr16 | 2  |
| chr17 | 4  |
| chr19 | 4  |
| chr20 | 1  |
| chr22 | 2  |
| chrX  | 17 |
| chrY  | 9  |

**Mutated Genes output file**

The Mutated Genes output file contains the list of genes whose coding regions were modified by at least one of the variants in the input file. The Mutated Genes file is a text file in tab-separated format. The file contains an entry per row, and one gene per entry. The first 12 columns are the same as the columns in the BED file (*genes.bed*). Additional columns are added by the annotations processing.

The following table lists the information included in the Mutated Genes output file.

| Field number | Field name        | Description                                                                                                                                                                                                                                                                     |
|--------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1            | Chromosome/Contig | The contig of the gene.                                                                                                                                                                                                                                                         |
| 2            | Start             | The left-most coordinate of the gene.                                                                                                                                                                                                                                           |
| 3            | End               | The right-most coordinate of the gene.                                                                                                                                                                                                                                          |
| 4            | GeneID            | The gene identifier as provided by the GTF file.                                                                                                                                                                                                                                |
| 5            | Score             | A score between 0 and 1000. The score is a normalized value for the number of coding variants such that all numbers scale between 0 and 1000. The score is equal to the number of coding variants for this entry divided by the maximum number of coding variants for any gene. |
| 6            | Strand            | The strand of the gene.                                                                                                                                                                                                                                                         |
| 7            | Start             | The left-most coordinate of the gene.                                                                                                                                                                                                                                           |
| 8            | End               | The right-most coordinate of the gene.                                                                                                                                                                                                                                          |
| 9            | Variant color     | The color depends on the type of the variant that overlaps this gene:<br>Red: SNP<br>Green: Indel (small indels, large indels)<br>Cyan: CNV                                                                                                                                     |
| 10           | Number of exons   | The number of exons of the gene.                                                                                                                                                                                                                                                |

| Field number | Field name                | Description                                                                                                                                    |
|--------------|---------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| 11           | Exon start position list  | A comma-separated list of start-coordinates for exons in the gene. All coordinates are relative to the start position of the gene.             |
| 12           | Exon length list          | A comma-separated list of the lengths of the exons in the gene.                                                                                |
| 13           | Total exon length         | The sum the length of all exons.                                                                                                               |
| 14           | Number of coding variants | The number of variants overlapping at least one exon.                                                                                          |
| 15           | Number of variants        | The number of variants overlapping the gene.                                                                                                   |
| 16           | Variant type              | The type of the variant. (One type is listed per line.)                                                                                        |
| 17           | Variant start list        | A comma-separated list for start coordinates of variants overlapping the gene. All coordinates are relative to the start position of the gene. |
| 18           | Variant length list       | A comma-separated list of lengths of the variants overlapping the gene.                                                                        |

### Potential uses of a Mutated Genes file

You can use a spreadsheet application or scripts to remove unwanted columns. First save a backup of your output file. The following are examples of uses for a Mutated Genes file:

- The first 12 columns provide information for visualization of modified genes and exons.
- Column 4, the GeneID, provides a list of modified genes. This column provides a gene list to focus on for enrichment analysis.
- A combination of the first 10 and the last 2 fields provides information useful for visualizing variants that overlap the gene.
- Fields 14 and 15 provide the number of variants that overlap exons and the number of variants that overlap the gene. With these columns you can filter by the number of variants overlapping the exons in the gene. With the GeneID in column 4, this information identifies genes to focus on for further analysis.

### SNP Finding tab-delimited output file

The first eight columns of this output file are the same as the input GFF file. The ninth column in the input file is replaced by individual columns for each attribute, as shown in the table [on page 334](#). This output file is generated only on SNPs module runs.

| Attribute | Description                                                                             |
|-----------|-----------------------------------------------------------------------------------------|
| Seqid     | The string ID of the sequence to which the start and end coordinates refer.             |
| Source    | The source of the data.                                                                 |
| Type      | Sequence ontology derived type for this variation. For SNP Finding, this is always SNP. |
| Start     | Start position of the SNP.                                                              |
| End       | End position of the SNP.                                                                |
| Score     | Calculated p-value of the SNP.                                                          |
| Strand    | Not used.                                                                               |
| Phase     | Not used.                                                                               |

| Attribute                    | Description                                                                                                                                                  |
|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genotype                     | Genotype in the form of IUB codes for bases observed in all the reads. The base of the reference sequence at the current position.                           |
| Coverage                     | The number of the reads that cover the current position.                                                                                                     |
| refAlleleCounts              | The number of reads of the reference allele at the current position.                                                                                         |
| refAlleleStarts              | The number of different start positions of reads having the reference allele at the current position.                                                        |
| refAlleleMeanQV              | The mean of quality values of all reference allele reads at the current position.                                                                            |
| novelAlleleCount             | The number of reads of the most abundant non-reference allele at the current position.                                                                       |
| novelAlleleStarts            | The number of different start positions of reads having the most abundant non-reference allele at the current position.                                      |
| novelAlleleMeanQV            | The mean of quality values of all novel allele reads.                                                                                                        |
| mostAbundantAlleleDiColor2   | The most abundant allele in the reads (not necessarily the reference allele) in dicolor encoding (for example, 00, 01, ... 32, 33), of 16 possible dicolors. |
| secondAbundantAlleleDiColor3 | The second most abundant allele in the reads.                                                                                                                |
| Het                          | Heterozygosity flag. Allowed values: 0,1 <ul style="list-style-type: none"> <li>• <b>0</b>: Homozygous SNP</li> <li>• <b>1</b>: Heterozygous SNP</li> </ul>  |

The following is a truncated example of SNP Finding tab-delimited output file content:

```
Seqid Source Type Start End Score Strand Phase genotype
reference coverage refAlleleCounts refAlleleStarts
refAlleleMeanQV novelAlleleCounts novelAlleleStarts
novelAlleleMeanQV mostAbundantAlleleDiColor2
secondAbundantAlleleDiColor3 het
chr1 SOLiD_SNP Finding SNP 10007 10007 0.806886 . . A
G 31 1 16 2 2 25 21 21 0
chr1 SOLiD_SNP Finding SNP 224472 224472 0.879526 . . C
T 3 1 1 26 2 2 24 10 10 0
chr1 SOLiD_SNP Finding SNP 553488 553488 0.0625 . . C
T 3 0 0 0 2 2 25 20 20 0
chr1 SOLiD_SNP Finding SNP 556955 556955 0.095335 . . Y
T 10 7 6 14 2 2 26 13 31 1
chr1 SOLiD_SNP Finding SNP 558326 558326 0.093729 . . R
A 8 6 6 26 2 2 22 03 21 1
chr1 SOLiD_SNP Finding SNP 558554 558554 0.0761 . . Y
T 1411 9 20 3 3 22 03 21 1
```

### SNP Finding annotated tab-delimited output file

This output file is the same as the SNP Finding tab-delimited output file with four additional columns added. As with the SNP Finding tab-delimited output file, the first eight columns of this output file are the same as the input file. The ninth column in the input file is replaced by individual columns for each attribute, as shown in the following table. This output file is generated only on SNPs module runs.

| Field  | Description                              |
|--------|------------------------------------------|
| geneID | The gene id as provided by the GTF file. |

| Field        | Description                                                                                                                                                                                                                          |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| exonID       | The exon id formed by concatenating the GeneID and the exon index number in the list of exons of the gene sorted in transcription order. Transcription order ties are broken listing by the shortest exon first (<GeneID>-<exon #>). |
| rsID         | The dbSNP id of the SNP.                                                                                                                                                                                                             |
| functionCode | The dbSNP functional code.                                                                                                                                                                                                           |

The following is a truncated example of SNP Finding annotated tab-delimited output file content:

```
Seqid Source Type Start End Score Strand Phase genotype
reference coverage refAlleleCounts refAlleleStarts
refAlleleMeanQV novelAlleleCounts novelAlleleStarts
novelAlleleMeanQV mostAbundantAlleleDiColor2
secondAbundantAlleleDiColor3 het geneID exonID rsID functionCode
chr1 SOLiD_SNP Finding SNP 10007 10007 0.806886 . . A
G 3 1 1 16 2 2 25 21 21 0 WASH5P
chr1 SOLiD_SNP Finding SNP 224472 224472 0.879526 . . C
T 3 1 1 26 2 2 24 10 10 0
chr1 SOLiD_SNP Finding SNP 553488 553488 0.0625 . . C
T 3 0 0 0 2 2 25 20 20 0
chr1 SOLiD_SNP Finding SNP 556955 556955 0.095335 . . Y
T 10 7 6 14 2 2 26 13 31 1 9326622
chr1 SOLiD_SNP Finding SNP 558326 558326 0.093729 . . R
A 8 6 6 26 2 2 22 03 21 1 2153587
chr1 SOLiD_SNP Finding SNP 558554 558554 0.0761 . . Y
T 14 11 9 20 3 3 22 03 21 1 8179256
chr1 SOLiD_SNP Finding SNP 658055 658055 0.0625 . . T
C 3 0 0 0 2 2 22 20 20 0
```

### SNP Finding filtered annotated tab-delimited output file

This file is the same as the SNP Finding annotated tab-delimited output file, except that it contains only those entries that fulfill all the conditions set by the your annotation filtering settings (described in [“Annotation parameters” on page 321](#)).

This output file is generated only on SNPs module runs.

The following is a truncated example of SNP Finding filtered annotated tab-delimited output file content:

```
Seqid Source Type Start End Score Strand Phase genotype
reference coverage refAlleleCounts refAlleleStarts
refAlleleMeanQV novelAlleleCounts novelAlleleStarts
novelAlleleMeanQV mostAbundantAlleleDiColor2
secondAbundantAlleleDiColor3 het geneID exonID rsID functionCode
chr1 SOLiD_SNP Finding SNP 556955 556955 0.095335 . . Y
T 10 7 6 14 2 2 26 13 31 1 9326622
chr1 SOLiD_SNP Finding SNP 558326 558326 0.093729 . . R
A 8 6 6 26 2 2 22 03 21 1 2153587
chr1 SOLiD_SNP Finding SNP 558554 558554 0.0761 . . Y
T 14 11 9 20 3 3 22 03 21 1 8179256
chr1 SOLiD_SNP Finding SNP 708249 708249 0.003906 . . G
A 5 0 0 0 3 3 23 30 30 0 10900602
chr1 SOLiD_SNP Finding SNP 708418 708418 0.0625 . . C
T 2 0 0 0 2 2 15 13 13 0 10751453
```

```
chr1 SOLiD_SNP Finding SNP 710103 710103 0.003906 . . C
T 3 0 0 0 3 3 17 01 01 0 3121393
chr1 SOLiD_SNP Finding SNP 713754 713754 0.003906 . . C
G 4 0 0 0 3 3 21 33 33 0 2977670
```

## About annotations and LifeScope™ modules

This section describes which annotation functionality is available with the various LifeScope™ Software modules.

The following table shows the LifeScope™ Software modules that support the annotation attributes.

| Label          | SNPs | CNV | Small indel | Large indel |
|----------------|------|-----|-------------|-------------|
| geneID, exonID | Yes  | Yes | Yes         | Yes         |
| rsID - SNPs    | Yes  | —   | —           | —           |
| rsID - Indels  | —    | —   | Yes         | —           |
| functionCode   | Yes  | —   | Yes         | —           |

The following table lists the LifeScope™ modules that support annotation filtering.

| Label                    | SNPs | CNV | Small indel | Large indel |
|--------------------------|------|-----|-------------|-------------|
| Only in exons            | Yes  | Yes | Yes         | Yes         |
| Only in genes            | Yes  | Yes | Yes         | Yes         |
| Only <i>not</i> in dbSNP | Yes  | —   | Yes         | —           |
| Only in dbSNP            | Yes  | —   | Yes         | —           |

The following table lists the LifeScope™ modules that support for the various annotation statistics. Heterozygous and homozygous statistics are included for applicable variants.

| Label                                         | SNPs | CNV | Small indel | Large indel |
|-----------------------------------------------|------|-----|-------------|-------------|
| Number of variants (total and per chromosome) | Yes  | Yes | Yes         | Yes         |
| Transitions and transversions                 | Yes  | —   | —           | —           |
| Variant length distribution                   | —    | Yes | Yes         | Yes         |
| Copy number distribution                      | —    | Yes | —           | —           |
| dbSNP concordance                             | Yes  | —   | Yes         | —           |
| Overlapping exons (number and percent)        | Yes  | Yes | Yes         | Yes         |
| Overlapping genes (number and percent)        | Yes  | Yes | Yes         | Yes         |

## Examples of annotation output files

If the analysis was successful, click **View Results** in the Progress column.

In the View Results window, you can view additional file and logs of annotation output. For more information about viewing analysis results, see [Chapter 9, “View Analysis Results”](#) on page 103.

Annotation output includes only text files (.txt) and log files (.log). There are neither details nor statistics for annotation output.

### Additional files

The following table is an example of a text file listed in Additional Files.

| Directory                                                               | Files      | File Type |
|-------------------------------------------------------------------------|------------|-----------|
| SmallIndel.Annotation/<br>hg19_Simulated_TR_CS_BC96_PE50X25_320Mil_BC55 | README.txt | .txt      |

You can download the README.txt file, open it, and view the contents, for example:

```
Outputs generated at '/datapod1/sitest1/results/projects/lifescopelife/TRPEBCCS/TRPEBCCS/outputs/small.indel/hg19_Simulated_TR_CS_BC96_PE50X25_320Mil_BC55'
```

### Logs

The following table is an example of a log file listed in Log Files.

| Directory              | Files                                                                                              | File Type |
|------------------------|----------------------------------------------------------------------------------------------------|-----------|
| Small Indel Annotation | tertiary-hg19_Simulated_TR_CS_BC96_PE50X25_320Mil_BC55-SmallIndel.Annotation.20110503204415163.log | .log      |

You can download the .log file, save and open it to view details about the analysis run.

# PART VI

## Appendices



## A

# File Format Descriptions and Data Uses

This appendix covers:

|                                               |     |
|-----------------------------------------------|-----|
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## Introduction

This appendix describes file formats used with LifeScope™ Genomic Analysis Software 2.0. It also describes how data is used in files.

Before reading the section about the SOLiD® System BAM file contents, you should be familiar with the general SAM specification and with the SAM specification field definitions. You can see the SAM specification at:

<http://samtools.sourceforge.net>

LifeScope™ Software secondary analysis (mapping and pairing) now produces a BAM file as the main alignment format. Mate-pair and paired-end analysis directly produces a BAM file, while a single file conversion is needed for fragment libraries. Depending on the output filter selected, unmapped and secondary alignments can be included.

LifeScope™ Software supports the XSQ sequence data file format introduced with the 5500 Series Genetic Analysis Systems.

## XSQ file format

XSQ (eXtensible SeQuence) is an extensible file format for storing sequence data. The XSQ format supports multiple independent reads at the same position in a fragment. XSQ is a binary format based on the open HDF format.

Sequencing run data are automatically exported from the 5500 Series Genetic Analysis Systems in XSQ binary file format. If an ECC primer round has been performed, the XSQ output also includes the sequence information in base space, in addition to color space.

### XSQ file content overview

#### 5500 Series Genetic Analysis Systems data

XSQ files generated by the 5500 Series Genetic Analysis Systems contain:

- **Base-space data** – Reference-free base-space data from primary analysis.
- **Color-space data** – Referred to as 2+4 color, as follows:
  - The 2BE (Base Encoding) data is the data for first 5 primer rounds of regular 2BE probes. This data is in the same format as a data created by a SOLiD<sup>®</sup> 4 System non-ECC run.
  - The 4BE data is the data for the 6th primer round, the optional ECC round.

Data for a maximum of one optional ECC primer round is supported in an XSQ file.

Paired-end libraries do not support ECC data. Fragment and mate-pair libraries support ECC data on both tags.

#### SOLiD<sup>®</sup> 4 System data

SOLiD<sup>®</sup> 4 System CSFASTA and QUAL files can be processed by LifeScope<sup>™</sup> Software, but first must be converted to XSQ format. Converted XSQ files contain only:

- Color-space 2BE data
- Color for both tags (one tag for fragment and both tags for mate-pair and paired-end data).

### XSQ file format properties

For a description of XSQ file format properties, see the XSQ file format specification, available on the SOLiD<sup>®</sup> Software Community website:

<http://solidsoftwaretools.com/gf/project/xsq/>

## BAM headers in LifeScope<sup>™</sup>

The BAM file generates all of the header information required by the SAM format specification, including @HD, @SQ, and @RG lines.

To view the content of the BAM file header, use the following command:

```
samtools view -H <BAMfilename>
```

### Sequence dictionary (@SQ)

Sequence header lines include the reference file URL, for example, `file:///share/reference/genomes/hg18.fa` in the optional UR field of the reference file. This value might become invalid if you relocate files.

**Read group (@RG)**

Read groups receive an arbitrary ID and sample name. The library field (LB) contains information that is important to downstream algorithms that use pairing information. A library name, which is specified by the tool parameter `library.name`, and the library type, are separated by a dash in the LB field. The library type is a structured value that details the nominal length of the two tags and the protocol used. as shown in the following syntax example:

```
l1 (x12) [F|MP|RR|RRBC]
```

In the syntax example, *l1* is the nominal length of the first read and *l2* is the nominal length of the second read. There will only be one number for fragment libraries. The library types correspond to fragment (*F*), mate-pair (*MP*), reverse read (*RR*), and reverse read-bar coded (*RRBC*).

Detecting structural variations, particularly large insertions and deletions, depends on the statistically likely range of pairing insert (PI) sizes. The pairing tool generates the information about PI sizes. The information has been used in legacy file formats to define the three-letter pairing “category”, specifically the third letter. The PI field in the read group captures the range of pairing insert sizes with a range of the form shown in the following example:

```
PI;low-high
```

In the PI example, *low* is the lower bound of the pairing range, and *high* is the upper bound.

**Header (@HD) sort order**

The LifeScope™ SNPs module requires that BAM headers of its input files contain an @HD line with a SO sort-order field, even if the BAM file is sorted properly. The required header line is:

```
@HD VN:1.0 GO:none. SO:coordinate
```

This header contains the information that the BAM file is sorted by genomic coordinates. BFAST mapping, for example, by default sorts the BAM file by coordinates, but does not update the header to reflect the sort order.

This section describes how to update the @HD SO field. If your BAM file is sorted properly but does not contain the SO sort-order field, follow these instructions before using a BAM file generated outside of LifeScope™ with the SNPs module:

1. Write the BAM file header to a file
 

```
samtools view -H input.bam > input_header.sam
```
2. Edit the BAM header file (`input_header.sam` created in the previous step). Insert the following as the first line of the header:
 

```
@HD VN:1.0 GO:none. SO:coordinate
```

 Make sure that the fields are tab-separated.
3. Update your BAM file with this new header. This command requires `samtools-0.1.8` or later.
 

```
samtools reheader input_header.sam input.bam
```

These instructions are not required with BAM files generated by LifeScope™ mapping modules.

## XSQ metadata in BAM headers

LifeScope™ secondary analysis modules write metadata contained in XSQ reads files to their mapping output files, as metadata in BAM header comment (@CO) lines. The new metadata is not in the BAM specification, but BAM files with this metadata conform to the BAM specification. The metadata table on [page 344](#) lists the XSQ file fields with both the new @CO field names and the existing BAM header fields that contain the XSQ metadata.

### References to existing BAM header lines

Each @CO metadata line with metadata information references one of the following types of BAM header lines:

- **Program group** – @PG lines capture information about the program that generated the data originally. The sequencing instrument and primary analysis software are considered to be the initial program. An example @PG line is:  

```
@PG ID:Mordor_201103161921_0_3 {...}
```
- **Read group** – @RG lines contain information on library type and read length. (See “[Read group \(@RG\)](#)” on [page 343](#) for more information.) An example @RG line is:  

```
@RG ID:1 SM:Stooges LB:Larry DS:"75x35PE" PI:225 {...}
```
- **General header** – @HD lines are general header lines.

All the attributes on the @CO metadata line then apply to referenced line.

The following example shows how @CO lines containing BAM metadata information refer to read group and program group lines. Except for @CO HD lines, the second field of the @CO metadata lines tie its attributes to either @RG or @PG lines. In the example, the RG:1 fields tie the metadata in those @CO lines to the @RG read group with an ID of 1. The PG:Mordor\_201012161921\_0\_3 field ties the metadata in that @CO line to the @PG program group with an ID of Mordor\_201012161921\_0\_3.

```
@CO RG:1 IA:215 IS:5.232 IN:200 IM:250
@CO RG:1 TN:50 TX:75 TB:0 TC:1
@CO RG:1 UN:25 UX:35 TB:0 TC:1
@CO RG:1 BX:0 EC:0
@CO PG:Mordor_201012161921_0_3 AS:"primary5500 1.0.7"
@CO PG:Mordor_201012161921_0_3 \
    CL:"/usr/bin/primary5500 <with full arguments>"
@CO HD UF:"f47ac10b-58cc-4372-a567-0e02b2c3d479"
```

### Metadata table

The following table lists the existing BAM header fields that contain the XSQ metadata, the new @CO metadata field names, and the XSQ file fields. See also “[Input read count fields](#)” on [page 346](#).

| Field name | Public BAM 1.3 spec field | New field in @CO line | XSQ field name                                                | Optional or mandatory |
|------------|---------------------------|-----------------------|---------------------------------------------------------------|-----------------------|
| Reference  | @SQ SP                    | —                     | Species<br>{ 'Homo sapiens'   'Mus musculus'   'other'   ...} | Mandatory             |
| Assembly   | @SQ AS                    | —                     | Assembly { 'hg18'   'hg19'   'mm9'   ...}                     | Mandatory             |
| —          | —                         | @CO HD UF             | FileUUID                                                      | Optional              |

| Field name                                   | Public BAM 1.3 spec field | New field in @CO line | XSQ field name                                                                                                                                                                              | Optional or mandatory |
|----------------------------------------------|---------------------------|-----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Program                                      | @PG ID                    | —                     | InstrumentName_Date_FlowcellAssignment_LaneNumber                                                                                                                                           | Mandatory             |
| Analysis Software                            | —                         | @CO PG:x AS           | AnalysisSoftware                                                                                                                                                                            | Optional              |
| InstrumentSerial                             | —                         | @CO PG:x PS           | InstrumentSerial                                                                                                                                                                            | Optional              |
| InstrumentName                               | —                         | @CO PG:x PN           | InstrumentName                                                                                                                                                                              | Optional              |
| InstrumentVendor                             | —                         | @CO PG:x PV           | InstrumentVendor                                                                                                                                                                            | Optional              |
| InstrumentModel                              | —                         | @CO PG:x PM           | InstrumentModel                                                                                                                                                                             | Optional              |
| ReadGroupID                                  | @RG ID                    | —                     | LibraryName or LibraryName_IndexName (if present)                                                                                                                                           | Mandatory             |
| —                                            | —                         | @CO RG:x IX           | IndexName (if present)                                                                                                                                                                      | Optional              |
| —                                            | —                         | @CO RG:x II           | IndexID (if present)                                                                                                                                                                        | Optional              |
| Sequencing Center                            | @RG CN                    | —                     | SequencingCenter                                                                                                                                                                            |                       |
| Description                                  | @RG DS                    | —                     | l1(xl2)[F MP RR RRBC]                                                                                                                                                                       |                       |
| Library Description                          | —                         | @CO RG:x LD           | LibraryDetails.Description                                                                                                                                                                  | —                     |
| Library Type                                 | —                         | @CO RG:x LT           | LibraryType { 'MatePair'   'PairedEnd'   'Fragment' }                                                                                                                                       | Mandatory             |
| Application Type                             | —                         | @CO RG:x AT           | ApplicationType { 'Whole Genome Resequencing'   'Targeted Resequencing'   'Whole Transcriptome (Fragment)'   'Whole Transcriptome (PairedEnd)'   'Small RNA'   'ChIP-Seq'   'Methylation' } | Mandatory             |
| —                                            | @RG DT                    | —                     | RunStartDate                                                                                                                                                                                | Optional              |
| —                                            | —                         | @CO RG:x DE           | RunEndTime                                                                                                                                                                                  | Optional              |
| Library                                      | @RG LB                    | —                     | LibraryName                                                                                                                                                                                 | Mandatory             |
| Predicted Insert Size                        | @RG PI                    | —                     | (LibraryInsertSizeMin + LibraryInsertSizeMax) / 2                                                                                                                                           | Conditional           |
| Calculated Average Insert Size               | —                         | @CO RG:x IA           | <calculated>                                                                                                                                                                                | Mandatory             |
| Calculated Insert Size Std Dev               | —                         | @CO RG:x IS           | <calculated>                                                                                                                                                                                | Mandatory             |
| Min Insert Size from User Input              | —                         | @CO RG:x IN           | LibraryInsertSizeMinimum                                                                                                                                                                    | Conditional           |
| Max Insert Size from User Input              | —                         | @CO RG:x IM           | LibraryInsertSizeMaximum                                                                                                                                                                    | Conditional           |
| Input Read Count Passing All Filtering Steps | —                         | @CO RG:x CU           | The sum across all ImageUnits of <i>ImageUnitID.Fragments.NumFragmentsPassed</i> (passes all filtering steps)                                                                               | Optional              |
| Input Read Count Total                       | —                         | @CO RG:x CT           | The sum across all ImageUnits of <i>ImageUnitID.FragmentCount</i> (filtered and unfiltered)<br>Not used in data converted from pre-5500 systems. Mandatory for 5500 data.                   | Mandatory             |
| Specimen                                     |                           | @CO RG:x SP           | SampleIdentifier                                                                                                                                                                            | Optional              |

| Field name                   | Public BAM 1.3 spec field | New field in @CO line | XSQ field name                           | Optional or mandatory                          |
|------------------------------|---------------------------|-----------------------|------------------------------------------|------------------------------------------------|
| Platform name                | @RG PL                    | —                     | "SOLID"                                  | Mandatory                                      |
| Lane Id                      | @RG PU                    | —                     | FlowcellAssignment_LaneNumber            | Optional                                       |
| Sample (Pool)                | @RG SM                    | —                     | SequencingSampleName                     | Optional                                       |
| —                            | —                         | @CO RG:x SD           | SequencingSampleDescription              | Optional                                       |
| Tag1 Was Base Present In XSQ | —                         | @CO RG:x BX           | Tag1.IsBasePresent                       | Mandatory                                      |
| Tag1 Min Read Len            | —                         | @CO RG:x TN           | Tag1.MinTrimmedReadLength                | Mandatory                                      |
| Tag1 Max Read Len            | —                         | @CO RG:x TX           | Tag1.NumColorCalls or Tag1.NumBaseCalls  | Mandatory                                      |
| Tag2 Was Base Present In XSQ | —                         | @CO RG:x BY           | Tag2.IsBasePresent                       | Mandatory for MP, PE.<br>Optional for fragment |
| Tag2 Min Read Len            | —                         | @CO RG:x UN           | Tag2.MinTrimmedReadLength                |                                                |
| Tag2 Max Read Len            | —                         | @CO RG:x UX           | Tag2.NumColorCalls or Tag2.NumBaseCalls  |                                                |
| ECC run                      | —                         | @CO RG:x EC           | <whether this was an ECC run (inferred)> | Mandatory                                      |
| ERCC                         | —                         | @CO RG:x ER           | ERCC                                     | Optional                                       |
| —                            | —                         | @CO RG:x CO           | Operator                                 | Optional                                       |
| —                            | —                         | @CO RG:x UU           | LibraryIndexUUID                         | Optional                                       |
| —                            | —                         | @CO RG:x PN           | Application                              | Optional                                       |
| —                            | —                         | @CO RG:x PJ           | ProjectName                              | Optional                                       |
| —                            | —                         | @CO RG:x SO           | SampleOwner                              | Optional                                       |

### Input read count fields

This section describes aspects of the Input Read Count fields from the viewpoint of the sequencing instrument and the viewpoint of secondary analysis.

The Input Read Counts fields are:

- **CU** – Input Read Count Passing All Filtering Steps, @CO RG:x CU
- **CT** – Input Read Count Total, @CO RG:x CT

The number of *total beads*, before any filtering on the instrument, is given by these fields:

- **NumUnfilteredBeads** – In mapping statistics output
- **FragmentCount** – In an XSQ file
- **CT** – In BAM header metadata

The number of *beads that pass filtering* on the instrument is given by these fields:

- **NumFilteredBeads** – In mapping statistics output
- **NumFragmentsPassed** – In an XSQ file
- **CU** – In BAM header metadata

The number of *beads filtered out* on the instrument is given by:

$$\text{NumUnfilteredBeads} - \text{NumFilteredBeads}$$

### Example of @CO metadata in a BAM header

The following is an example of a BAM header with an @CO comment line containing metadata information. The @CO line applies to the @RG ID:12345678 line below it. (The @SQ lines for chromosomes 2–22 are deleted.)

```

@HD VN:1.0 GO:noneSO:coordinate
@SQ SN:chr1 LN:247249719 UR:file:/ref/hg18.fasta
    SP:Homosapiens AS:hg19
    ...
@SQ SN:chrX LN:154913754 UR:file:/ref/hg18.fasta
    SP:Homosapiens AS:hg19
@SQ SN:chrY LN:57772954 UR:file:/ref/hg18.fasta
    SP:Homosapiens AS:hg19
@SQ SN:chrM LN:16571 UR:file:/ref/hg18.fasta
    SP:Homosapiens AS:hg19
@PG ID:SOLiD_12321_1
@CO RG:12345678 LT:MatePair AT:WholeGenomeResequencing
    LN:<50X50MP> PI:1500 IA:1500 IS:300 IN:1200 IM:1800
    PU:1234 SM:sample BX:0 TN:50 TX:50 BY:1 UN:50 UX:60 EC:0
@RG ID:12345678 DS:50x50MP LB:MP PI:1500 PU:1234 SM:sample

```

### @CO syntax and rules

This section describes the syntax rules and conventions for @CO metadata lines. The @CO metadata rules are:

- The second field of each metadata @CO line either notes that the line is a general header (HD), or refers to a single @RG ID or @PG ID.
- Each metadata @CO line contains one or more tab-separated attributes.
- For a metadata @CO line that refers to either a @RG or @PG line, all of the @CO attributes must apply to the same @RG or @PG.
- The beginning of metadata @CO lines must match the regular expression “^@CO\t[PR]G:” and contain no other non-metadata text. LifeScope™ ignores and does not change @CO lines that do not match the regular expression.
- The @CO PG:*id* line describing the instrument is required and must refer to a valid @PG ID:*id* program group line. This @CO line contains information about the primary analysis done on the instrument.

According to the BAM specification, the @HD line is optional. If present, the @HD line is required to be the first line of the header. The order of all other header lines is not defined. The following order of @CO metadata lines is preferred when possible (but not required):

- Place the @CO HD:UF comment immediately after the @HD line.
- Place @CO RG: and @CO PG: comment lines before @CO lines for ease of visibility.
- Place @CO RG: and @CO PG: comment lines before the @RG and @PG blocks that the comment lines refer to.

## Color-space attributes

The SAM format specification includes the attribute tags CS, CQ and CM. All BAM files support color-space reads (see the following table).

| Attribute tag | Description                                                                                                                                                                                                   |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CS            | Color-space (CS) read. The CS field contains the original color-space read, which includes the primer base, in the orientation of the CSFASTA file. CS entries are not manipulated to be top-strand relative. |
| CQ            | Color qualities. Color qualities are encoded according to the ascii-33 scheme used for the QUAL field. The orientation is the same as the orientation used for the CSFASTA file.                              |
| CM            | The number of color-space mismatches.                                                                                                                                                                         |

## Pairing information in a BAM file

The BAM file that is produced by the pairing tool supports both mate-pair and paired-end protocols using the standard SAM format fields, in particular the ISIZE and FLAG fields.

### Calculation of tag names

The paired libraries use tag names to refer to members of the pair. The mate-pair libraries use F3 and R3 as the tag names. The paired-end libraries use F3 and F5 as the tag names. Use the FLAG field and information from the LB field of the read group to recapitulate tag names (see the following table).

| FLAG bit                       | Library type | Tag name |
|--------------------------------|--------------|----------|
| 0x0040 (first read in a pair)  | MP           | F3       |
| 0x0080 (second read in a pair) | MP           | R3       |
| 0x0040                         | RR           | F3       |
| 0x0080                         | RR           | F5       |
| 0x040                          | F            | F3       |

### Proper pairs

Legacy file formats, such as \*.mates, and GFF, described pairs using a three-letter category. Pairs in the AAA category correspond to the “proper pair” concept in the SAM format. The pairs reflect pairings that are not altered by a structural variation such as an inversion or deletion (see the illustration [on page 349](#)). The BAM file field values for proper pairs are different for mate-pair and paired-end libraries:

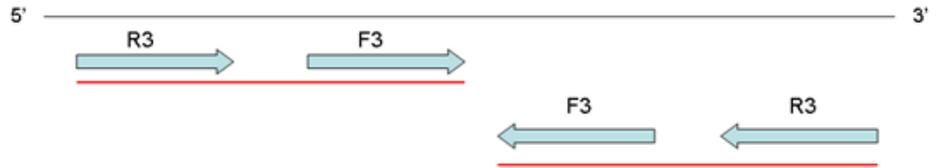
#### Mate-pair libraries

- Strand flag is equal for both mates (both 0 or both 1).
- ISIZE is between the lower and upper limit of the insert range.
- For forward strand hits R3 POS < F3 POS.
- For reverse strand hits F3 POS < R3 POS.

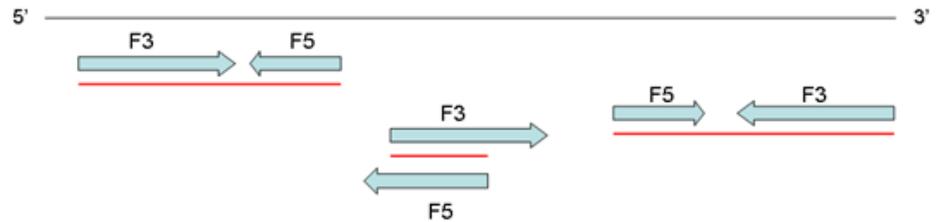
**Paired-end**

- Strand flag is opposite for the mates.
- ISIZE is between the lower and upper limit of the insert range. In the case of paired-end libraries, the ISIZE might be smaller than the sum of the alignment lengths.
- F3 POS < F5 POS if F3 is on the forward strand.
- F5 POS < F3 POS if F5 is on the forward strand.

**Mate Pairs**



**Paired End**



**Single read mapping quality**

As described in the SAM format specification, the MAPQ field for paired results contains a pairing quality value. Under some circumstances, it is valuable to include the original single-read alignment quality value. The original single-read alignment value is maintained in the SM:i attribute in BAM files.

**Hard clipping of incomplete extensions**

LifeScope™ mapping uses a seed-extend algorithm. The algorithm increases mapping throughput by matching a seed, usually 25 bp, and extending the alignment until mismatches drive down the alignment score. Many alignments do not completely cover the color-space read. Because the base-space sequence of color reads cannot be precisely known in the absence of alignment, incomplete extensions are represented as a hard-clip (H) operation in the BAM CIGAR string (see the following illustration).

```

T33232030301212311201322311232302131021221120112222
AAGGCCTCTGAACCCACTCAGGTACTTAGCTGTAGATGGACATCAGTTAATTCGATGAC
22221102112212013120323211322310211321210303023233T
* * * _____
8H42M
    
```

The above illustration shows the read in normal orientation (see the top section) and aligned in reverse orientation to the reference top strand (see the middle section). The lines below the alignment show the extent of the seed (top horizontal line) and extension (bottom horizontal line) phases of mapping. The extension only results in 42 bases of alignment. The remaining portion of the color alignment has a number of mismatches that prevent extension. These are coded as hard-clipped regions. The CIGAR field in the BAM file is top-strand relative, so even though the hard clipping is on the end of the reversed color read, it is on the beginning of the CIGAR string.

## Visualize BAM output

You can use third-party software visualization tools to view BAM files in a browser.

### Integrative Genomics View (IGV)

The Integrative Genomics Viewer (IGV) available from the Broad Institute is a visualization tool for interactive exploration of large, integrated datasets. The IGV reads BAM files directly, which allows for easy viewing and inspection of alignments against the genome (see the illustration [on page 349](#)).

For more information, go to the following site:

[www.broadinstitute.org/igv/](http://www.broadinstitute.org/igv/)

If you use IGV to visualize the BAM files, verify that the BAI file is present. The BAI file is the index that is built for BAM files and is a standard part of the public SAM specification. If the pairing and MaToBam tools do not automatically create the BAI file:

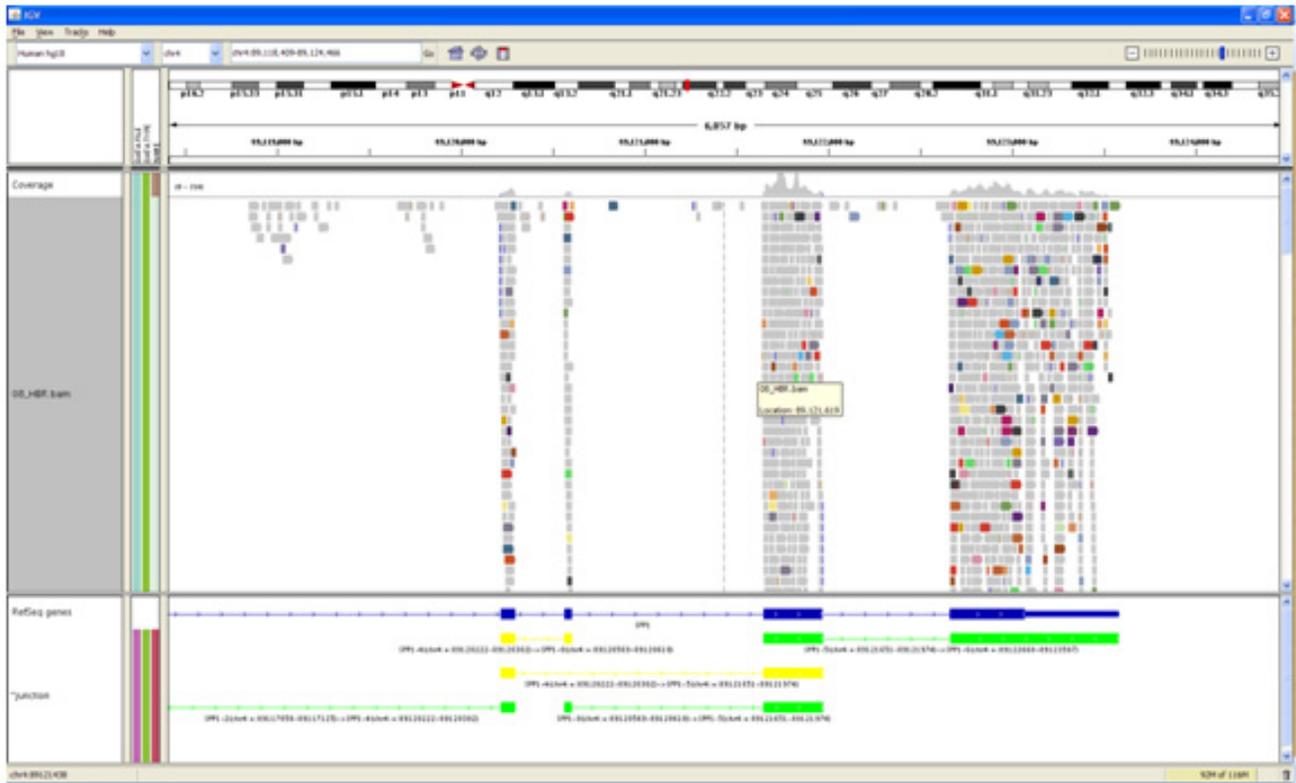
1. Log in to the LifeScope™ cluster.
2. At a command prompt, enter:  

```
samtools index <bam file name>.bam
```

Indexing only works if the file is sorted in coordinate order. If the file is not sorted in coordinate order, at a command prompt, run the following command to sort the file in coordinate order:

```
samtools sort <unsorted bam name>.bam <sorted bam name>
```

The following illustration is an example of BAM file visualized in the IGV.



**UC Santa Cruz (UCSC) genome browser**

The UCSC Genome Browser serves as an interactive web-based microscope that allows researchers to view all 23 chromosomes of the human genome at any scale, from a full chromosome down to an individual nucleotide.

For more information, go to the Genome Browser website:

[www.cbse.ucsc.edu/research/browser](http://www.cbse.ucsc.edu/research/browser)

**BED file format**

The Browser Extensible Display (BED) format was developed to extend the UCSC Genome Browser with user-defined tracks. BED is used to visualize the splice and fusion junctions in the UCSC Genome browser and in the IGV browser (see the illustration on page 352). For general documentation about the BED format, including information about all of the BED fields, go to:

[genome.ucsc.edu/FAQ/FAQformat.html](http://genome.ucsc.edu/FAQ/FAQformat.html)

For information about visualization software, see “Pairing information in a BAM file” on page 348.

Each line in the track defines a junction where `chromStart` is the smaller of the coordinates and `chromEnd` is the greater.

There are two blocks because a junction typically contains two exons. `BlockSizes` are the lengths of the exons. The block starts the beginning of the exons. When fusions on different strands or chromosomes, two lines are added to the output, with each line representing one chromosome. Different colors are used to color-code different types:

The illustration of the IGV on page 352 shows the Upstream Hypersensitive Region (UHR) gene region displayed with the Integrative Genomics Viewer (IGV) for positions 3,530,193 to 3,548,355 of Human Chr-1. The following sections describe the tracks in the illustration on page 352.

**WIG (x2) tracks**

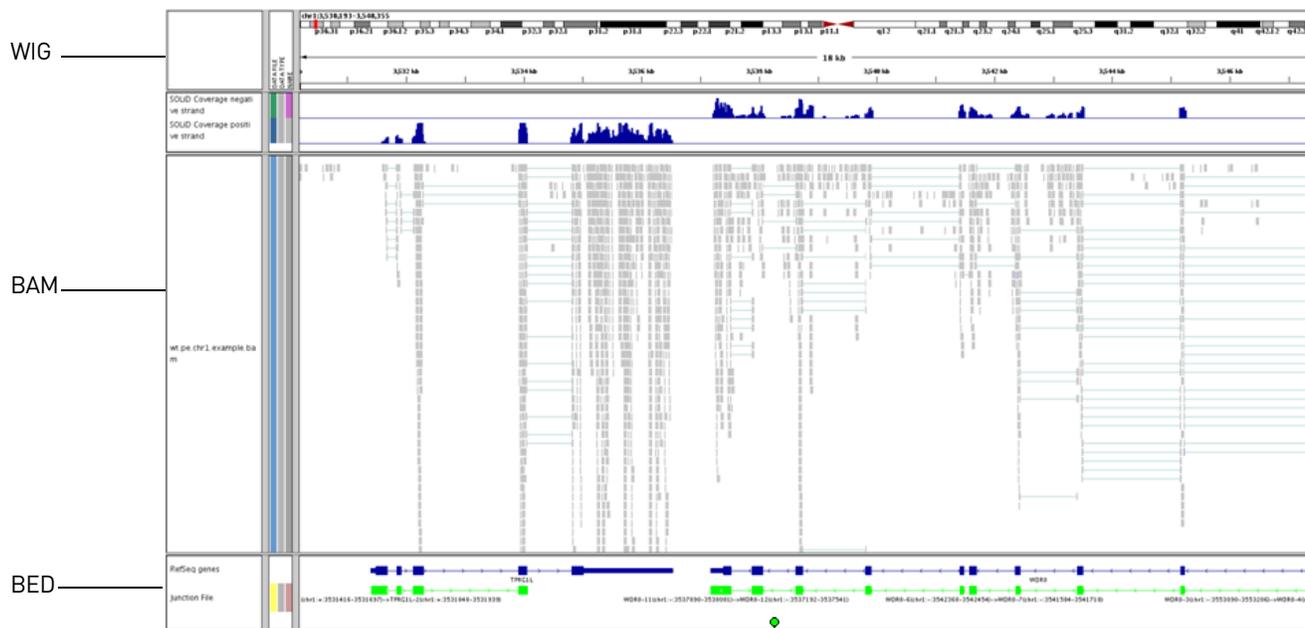
The top two tracks show the genomic coverage using the negative strand and positive strand generated by the Bam2Wig tool (Max: 100 coverage).

**BAM track**

The middle track shows the alignments from the BAM file. For display purposes, reads are filtered with MAPQ threshold of 45 (a stringent filter). Bases with quality value 5 to 20 are shaded.

**BED track**

The fifth track shows the junctions detected by the Junction Finder (BED file). As shown in the illustration on page 352, all junctions detected are “known” and so are shaded in green.



**BEDGRAPH file format**

The following table describes the BEDGRAPH format fields. These are tab-separated text fields.

| Field name               | Example content                        | Description                                         |
|--------------------------|----------------------------------------|-----------------------------------------------------|
| Track annotation headers | track type=bedGraph<br>name="Coverage" | Annotations used for UCSC browser display purposes. |

| Field name    | Example content | Description                                                                                                                                                                                       |
|---------------|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Contig name   | chr1            | Contig name of the feature. This value comes from the sequence name (SN) in the BAM header. However, in order to be viewed in the UCSC browser, these values must be of the form chr1, chr2, etc. |
| Feature start | 148             | Start location of the feature. Zero-based, inclusive.                                                                                                                                             |
| Feature end   | 150             | End location of the feature. Zero-based, exclusive (half-open ranges).                                                                                                                            |
| Coverage      | 1               | Depth of coverage over the given (start-end) range.                                                                                                                                               |

## GFF3 file format

LifeScope™ Software creates the generic feature format, version 3, (GFF3) file as an output file containing the detected CNV Regions. You can visualize a CNVs.gff file in a browser such as the Integrative Genomics Viewer (IGV), which is available from the Broad Institute [www.broadinstitute.org/igv/](http://www.broadinstitute.org/igv/).

The file format for \*.gff files is

`<cnv.output.prefix>_CNVs.gff3`

where `<cnv.output.prefix>` is the value defined for the output prefix.

## Reference file data overview

Nearly all LifeScope™ Software tools use reference sequence data and, in some cases, the tools also use reference annotations and metadata.

Reference data takes a number of forms, which are described in the following sections.

### Contig multi-fasta file

A single, multiple-entry FASTA file where each entry corresponds to a genomic contig or chromosome.

### Single contig FASTA file

A single-entry FASTA file containing one contig or chromosome.

### GTF file

A genome annotation file. This file describes gene models used by the whole transcriptome and small RNA modules. It is similar in form to the GTF files downloaded from the UCSC website, but includes some changes for WT or small RNA processing.

Genomic reference files downloaded from UCSC; for example, you can download hg19 from:

<http://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/chromFa.tar.gz>

The file can be used as a basis for the reference sequence data in LifeScope™ Software tools. A few small transformations are required to prepare the GTF files for use by LifeScope™ applications.

A GTF file is an ASCII text file composed of lines of text separated by line feed characters (UNIX-style new lines). Each line of text represents a comment or a tab delimited content about a genomic feature. A comment line always starts with a “#” character. GTF does not specify any format of structured content in comments. Comments containing parsable metadata should precede any lines containing genomic features. For more detail about the format, refer to the following site:

<http://genome.ucsc.edu/FAQ/FAQformat.html#format4>

### Reference sequence data validation

The `reference_validation.pl` script provided with LifeScope™ Software checks and corrects common issues with FASTA sequence files that can cause errors in LifeScope™ Software runs. Examples of errors generated by an uncorrected GTF file can include extra spaces and mixed case.

### Concatenation

The contig multi-FASTA reference file can be generated by concatenating the individual chromosome files together.

### Select a reference file

Be sure to use the correct reference file type for your requirements. The identifiers in the FASTA definition line are carried through many tools, including the BAM file generated by mapping and pairing, and the GFF files typically written by tertiary analysis tools.

For some downstream viewing and integration tools, for example, the UCSC genome browser, the identifiers can be used to connect sequence references with other sources of annotation.

## Read-set file format

An RRS (Read Range Specifier) file, also called a read-set file, is an internal file that defines which reads data is processed in a mapping run. LifeScope™ automatically creates the RRS file for your mapping analysis. This format is considered an internal file format, and the specification for this file format can change in future versions of LifeScope™ Software.

An RRS file allows a mapping analysis to process, in a single run:

- Data from a single XSQ reads file.
- Data from multiple XSQ reads files.
- Data for a range of barcodes (a subset of XSQ file data).
- Data for a range of panels within a barcode (also a subset of XSQ file data).

The RRS file is a tab-separated file with three columns which describe the input XSQ file, the sample name, and optionally a barcode range and panel range (see the following table in this section). The following rules apply to RRS files:

- One read-set (barcode) cannot be part of two samples.
- For a single analysis, mapping can be in color or base. If the mapping is in color, all XSQ files in the RRS file must have color available. If the mapping is in base, all XSQ files in the RRS file must have base available. Mapping fails if these restrictions are not met.
- For barcoded XSQ files, the RRS file can specify a range of barcodes. If some of specified barcodes are missing, mapping will log a warning for the missing barcode IDs.

- One read-set is specified per content line.
- At least one of the read-sets needs to be valid for mapping module to complete.

The requirements for RRS fields are described in the following table. The read-set file also accepts comments, which contain a '#' character at the beginning of the line.

| Column name        | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample name        | <p>Multiple read-sets with the same sample name are treated as a single sample (and are processed together).</p> <p>Allowed values: A string of alpha-numeric characters up to 255 chars long. Spaces are not allowed.</p> <p>Examples: Human_101, Bob, Sample19</p> <p>(This sample name does not need to match any information provided on the instrument.)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| File id            | <p>A positive integer that distinguishes between XSQ files according to these rules:</p> <ul style="list-style-type: none"> <li>• Two different XSQ files must have different file IDs.</li> <li>• Two identical XSQ files must have same file ID.</li> </ul> <p>(For read-set files created with the UI, LifeScope™ assigns this number.)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| read-set range URL | <p>A string specifying one or more XSQ data files and optionally also barcode and panel specifiers. The format is a URI scheme:</p> <p><i>fileName?start=barcodeID.panelstart&amp;end=barcodeID.panelend</i></p> <p><i>fileName</i>: either a read in the reads repository or the absolute path to an XSQ file on the Linux file system (and that file must be a valid XSQ file for which the user has read permission).</p> <p><i>barcodeID</i>: integers from 1–96.</p> <p><i>panelstart</i>, <i>panelend</i>: integers from 1–4000. These fields correspond to panels on the sequencing instrument.</p> <p>The start= section must appear before the end= section. Start <i>barcodeID</i> value must be &lt;= the end <i>barcodeID</i> value. The <i>panelstart</i> value must be &lt;= the <i>panelend</i> value.</p> |

### Example RRS file

The following is a simple example of an RRS file which defines an entire XSQ file as the read-set. The XSQ file in this example has been imported into the reads repository, so an absolute path to the local file system is not required.

```
#SampleName FileID ReadSets
Huref 1 reads/solid0054_20110102_RD_HuRef100_F3.xsq
```

## VCF file

The Variant Call Format, in a text format. The file contains meta-information lines, a header line, and data lines that each contain information about a position in the genome. This file format was developed as part of the 1000 Genomes project, and is described here:

<http://vcftools.sourceforge.net/specs.html>

Recent releases of dbSNP are available in as VCF files. An example is:

[ftp://ftp.ncbi.nih.gov/snp/organisms/human\\_9606/VCF/v4.0/00-All.vcf.gz](ftp://ftp.ncbi.nih.gov/snp/organisms/human_9606/VCF/v4.0/00-All.vcf.gz)

## B

# Legacy Formats

This appendix covers:

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- GFF to BAM mapping ..... 357

## Introduction

This appendix describes how header fields and alignment data in General Feature Format (GFF) files correspond to similar fields in the Binary Alignment sequence Map (BAM) file format.

## GFF to BAM mapping

The following table shows the GFF file field name mapped to the corresponding BAM file field name for header fields.

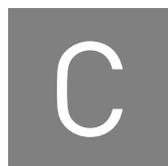
| GFF field            | Corresponding BAM field | Comments                                                                                                                                                                                                |
|----------------------|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ##reference-name     | @SQ UR field            | This header field contains the reference file name in the UR field.                                                                                                                                     |
| ##history            | —                       | —                                                                                                                                                                                                       |
| ##color-code         | —                       | Only a single color encoding is used.                                                                                                                                                                   |
| ##primer-base        | —                       | This field is not needed. The primer base is stored with the color read attribute.                                                                                                                      |
| ##max-num-mismatches | —                       | —                                                                                                                                                                                                       |
| ##max-read-length    | @RG LB field            | The read lengths in the file are determined from the library type information in the LB field of the read group.                                                                                        |
| ##line-order         | @HD SO field            | The sort order field in the BAM header indicates the line order. Valid values are: <ul style="list-style-type: none"> <li>• <b>None</b></li> <li>• <b>Coordinate</b></li> <li>• <b>Qname</b></li> </ul> |

The following table shows the GFF file field name mapped to the corresponding BAM file field name for alignment data.

| GFF field                                  | Corresponding BAM field                                                    | Comments                                                                                                                                                                                                                                                                                                              |
|--------------------------------------------|----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| seqid                                      | RNAME                                                                      | GFF files use an ordinal number to indicate the reference contig. The ordinal number could be translated to names by a header table. The BAM file uses the @SQ lines to perform a similar lookup.                                                                                                                     |
| source                                     | @RG PL                                                                     | The read group platform (@RG PL field) is used in a manner similar to the SOLiD® GFF source.                                                                                                                                                                                                                          |
| type                                       | —                                                                          | All records are alignments.                                                                                                                                                                                                                                                                                           |
| start                                      | POS                                                                        | The 1-based, left-most, top-strand position of the alignment.                                                                                                                                                                                                                                                         |
| end                                        | Calculated from CIGAR                                                      | The BAM file does not support an explicit end point. An explicit end point can be calculated from the CIGAR string.                                                                                                                                                                                                   |
| score                                      | —                                                                          | A read quality is not stored in the BAM alignment record. Mapping and pairing quality is stored in the BAM alignment record.                                                                                                                                                                                          |
| strand                                     | 5th FLAG bit (query strand)                                                | The fifth bit of the FLAG field indicates the strand of the alignment.                                                                                                                                                                                                                                                |
| <b>GFF attributes</b>                      |                                                                            |                                                                                                                                                                                                                                                                                                                       |
| aID ( <i>bead id</i> )                     | QNAME                                                                      | The bead id.                                                                                                                                                                                                                                                                                                          |
| at ( <i>adaptor type</i> )                 | Calculated value                                                           | The adaptor type or primer set field can be calculated as described in <a href="#">“Calculation of tag names” on page 348</a> .                                                                                                                                                                                       |
| b ( <i>bases</i> )                         | SEQ                                                                        | Base space representation of the aligned read. Bases are all uppercase. Equal signs “=” are not used.                                                                                                                                                                                                                 |
| c ( <i>category</i> )                      | 2nd FLAG bit (proper pair) and calculation                                 | The most commonly-used category value, AAA, corresponds to the proper pair bit in the FLAG field. Use POS, MPOS, and strand bits in the FLAG field to calculate other categories. You can find C** pairs (different contigs) by interrogating the mate reference (MRNM). The D** is indicated by the fourth FLAG bit. |
| g ( <i>color-space read</i> )              | CS attribute                                                               | The color read is stored in the CS attribute. However, unlike the 'g' GFF attribute, the color read is stored in an unaltered CSFASTA form that includes the last primer base.                                                                                                                                        |
| mq (mq [mapping quality])                  | MAPQ (or SM for pairs)                                                     | The mapping quality is stored in the MAPQ field for fragment libraries and in the SM attribute for paired results.                                                                                                                                                                                                    |
| o ( <i>offset</i> )                        | CIGAR (hard-clipping)                                                      | The offset for an alignment can be computed by checking the number of hard clip (H) operations on the CIGAR string.                                                                                                                                                                                                   |
| p ( <i>mappability ambiguity</i> )         | —                                                                          | Use mapping quality for uniqueness determinations.                                                                                                                                                                                                                                                                    |
| pq ( <i>pairing quality</i> )              | MAPQ (for pairs)                                                           | The pairing quality is stored in the MAPQ field for paired results.                                                                                                                                                                                                                                                   |
| q ( <i>color qualities</i> )               | CQ                                                                         | The CQ attribute stores color quality. The values are not represented as integers, but are represented by ascii-33 encoding.                                                                                                                                                                                          |
| r ( <i>reference color at mismatches</i> ) | Calculate reference color at mismatches using the samtools fillmd command. | CIGAR strings do not distinguish between matches and mismatches. Calculate this information using the samtools fillmd command, though in a form that is different from the rb GFF attribute.                                                                                                                          |
| s ( <i>color annotations</i> )             | —                                                                          | —                                                                                                                                                                                                                                                                                                                     |

| <b>GFF field</b>                      | <b>Corresponding BAM field</b>                       | <b>Comments</b>                                                                                                                                                                                                                  |
|---------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>u (accumulated mismatch count)</i> | Per-alignment mismatches supported with CM attribute | The CM attribute specifies the number of color mismatches for the current alignment. You can use a combination of the number of color mismatches across alignments in the BAM file to reconstruct values like the 'u' attribute. |





# Run the SOLiD<sup>®</sup> Accuracy Enhancement Tool

This chapter covers:

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| ■ SAET output files .....       | 364 |

## Overview

The SOLiD<sup>®</sup> Accuracy Enhancement Tool (SAET) improves color call accuracy prior to mapping. This method implements a proprietary spectral alignment error correction algorithm that uses quality values and properties of color space. Significantly improving its performance over conventional error correction methods, the use of SAET produces more accurate mapping with both an increased number of reads with zero miscalls and an increased number of mapped reads. The SNP calling on error-corrected data results in an increase in true positive calls and a decrease in the number of false positive calls.

SAET performance was tested on various datasets, including a large spectrum of genome sizes and complexities, coverages, read lengths, and experiment types. SAET shows similar performance on datasets sequenced from enriched regions or genomes of 1 kbp to 3 Gbp, with coverage from 10 to 4000x and a read length of 25 to 75 bp.

On average, SAET requires up to 5 GB RAM for each 1 Gbp of sequenced region. SAET can be applied for diploid organisms. SAET should not be used for rare variant detection datasets where the frequency of rare variant may be less than twice that of the color calling error rate.

For more information about SAET, see [Chapter 10, “Perform Targeted Resequencing and Enrichment Analyses”](#) on page 111.

## SAET usage guidelines

SAET is recommended for use with targeted resequencing and is on by default in targeted resequencing standard workflows. SAET has not been validated with other experiment types and may have insufficient memory to run. If you do choose to run SAET with other experiment types, you can do the following:

- Run SAET before you run the resequencing or whole transcriptome mapping and pairing tools.

- Use the SAET output with resequencing and whole transcriptome mapping and pairing.
- Use updated quality values file for SNP calling.
- Avoid the generation of the updated quality values, if the downstream analysis modules in your workflow do not use quality values. Your SAET runtime is reduced approximately by half if you do not update quality values.

## SAET in analysis modules

The following analysis modules apply SAET in targeted resequencing of the fragment and paired-end library types:

- SAET
- Mapping
- Enrichment
- SNPs
- Small indels
- Annotations

SAET corrects errors in reads before mapping. This correction significantly improves mapping quality and the quality of variant calls.

## SAET input files

By default, SAET takes an input file of SOLiD® system reads in the eXtensible SeQuence (XSQ) format, and an expected length of the sequenced region, such as the:

- Genome size for whole genome sequencing
- Size of enriched region in targeting resequencing
- Size of transcribed region in whole transcriptome sequencing

The required input file is in XSQ format, which is typically generated by 5500 Series SOLiD™ Sequencer. The file must contain reads in color-space that require correction. SAET constructs a spectrum of all k-mers from the set of all reads. Then each read is corrected individually if necessary.

In targeted resequencing, the SAET module accepts as an input the BED, GFF, or TXT file with segments of target regions.



## SAET parameters

SAET is designed to reduce the error rate in the reads generated by the SOLiD® System. Reducing the error rate increases the number of mapped reads for resequencing analyses, which can for example lead to an increase in true positive SNP-calls and a decrease in false positive SNP-calls.

The following table describes the SAET parameters.

| Parameter                    | Default value | Description                                                                                                                                                                                                                                            |
|------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genome length                | 1000000       | Expected length of sequenced (or enriched) DNA region. For example, 4,600,000 for the E.Coli 4.6 MB genome or 30,000,000 for the entire Human Transcriptome.<br>Allowed values: Integers $\geq 200$ .                                                  |
| On target ratio              | 0.5           | The expected ratio of reads that come from the targeted region.<br>Allowed values: Floats 0.0–1.0.                                                                                                                                                     |
| Update quality values        | True          | Update quality value of modified calls.<br>Allowed values:<br>True: Update the qv of modified calls.<br>False: Do not update the qv for modified calls.                                                                                                |
| Trusted quality value        | 25            | Correction is applied to calls with a quality value below the value of this parameter.<br>Allowed values: Integers $\geq 1$ .                                                                                                                          |
| Support votes                | 2             | The minimum number of k-mer votes required make a correction.<br>Allowed values: Integers $\geq 1$ .                                                                                                                                                   |
| Trusted frequency            | 0             | The lowest multiplicity of the seed to be included in the spectrum. (If set to 0, then the value is computed internally.)<br>Allowed values: Integers $\geq 0$ .                                                                                       |
| Maximum corrections per read | 0             | Maximum number of allowed corrections per read. (If set to 0, then the value is set to $\lceil \text{readLength}/8 \rceil$ ). Reduce if over-corrections are observed, or increase if under-corrections are observed.<br>Allowed values: Integers 0–9. |
| Number of recursive runs     | 1             | The error correction step is repeated the provided number of times. Reduce if over-corrections are observed, or increase if undercorrections are observed.<br>Allowed values: 1, 2, or 3.                                                              |

| Parameter                              | Default value | Description                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Position of error inflation point      | 0             | Position in the read at which the error rate inflates, for instance, 35-40 for 50bp long reads. (If set to 0, then the value is equal to $0.8 * \text{readLength}$ ).<br>Allowed values: Integers.                                                                                                                                                           |
| Disable random sampling for large data | False         | Disables random sampling in spectrum building. If set to 0, then for large datasets (coverage > 300x), a subset of reads is used in spectrum building. Allowed values: <ul style="list-style-type: none"> <li>• True: Disables random sampling in spectrum building.</li> <li>• False: Do not disable random sampling in spectrum building. true?</li> </ul> |
| K-mer size                             | 0             | Size of k-mer (>5) used in spectrum construction and error correction. (If set to 0, then the value is computed internally.)<br>Allowed values:<br>Integers 0–28.                                                                                                                                                                                            |

## SAET output files

SAET writes the corrected reads and quality values into new XSQ files.

The SAET output files are used as input to mapping analyses.



# Administration

This appendix covers:

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| ■ Administrate users.....      | 368 |
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## Introduction

As an administrator, you can search for users, add users, deactivate user accounts, and configure licenses and user setups and profiles.

The GUI Admin Portal is the main administration utility for LifeScope™ Software.

## Admin tasks

This section describes typical admin tasks such as user management, scratch space cleanup, reference repository management, and resetting the admin password.

### User management

User management tasks vary with the authentication realm used for your installation:

- **LifeScope** – Users are created in the LifeScope™ Software user repository. User account management (including account creation) is performed with the LifeScope™ Admin Portal (included in this installation). There is no relationship between the LifeScope™ Software users accounts and Linux® host system users or LDAP users.
- **LDAP** – User accounts on LDAP-compliant authentication server (OpenLDAP, Active Directory, etc.) are eligible to be LifeScope™ Software users. User account management is performed with the LifeScope™ Admin Portal (included in this installation). As admin, you select the LDAP users to be allowed LifeScope™ Software access. You do not create user accounts.
- **Host** – Local (on the installation machine or cluster) Linux users are all LifeScope™ Software users. You do not create user accounts or select LifeScope™ Software users.

As admin, in the Admin Portal you can disable a LifeScope™ Software user and later re-enable the user.

See the following user management topics in this chapter:

- [“Administrate users” on page 368](#)
- [“Configure user accounts” on page 370](#)

### Scratch space cleanup

Under normal operation, LifeScope™ Software removes the files and directories it created in scratch space. However, as admin you must periodically check the scratch directory and manually delete old files that LifeScope™ Software did not remove.

The scratch directory is setting during installation. The default is `/scratch/solid/lifescopes`.

The files and directories to delete have various names, but contain the name of an analysis module and end in a time stamp. Example names are:

- `wholeTranscriptomeFrag_secondary-human_chr17_6-wt.frag.map.main.20110430091312676`
- `test45_secondary-hg18-saet.main.20110702013417478`
- `test52_20110707030127_secondary-hg18-saet.main.20110707100009799`
- `saet_secondary-hg18-saet.main.20110427011039729`

**Note:** Be careful to not remove files or directories for a currently-running LifeScope™ Software process.

## Reference file conflicts

Depending on the local file permissions, the LifeScope admin might be responsible for adding the reference files to the reference repository. In order to avoid conflicts with other reference files in the reference repository, always add new reference files to the correct assembly subfolder under the `internal` directory. Refer to the Reference Repository appendix in *LifeScope™ Genomic Analysis Software Command Shell User Guide* (Part no. 4471875).

## Reset the admin password

The `resetpwd.sh` script is provided as an emergency way to reset the administrator's password to its default value. To reset the admin password, follow these steps (at a Linux prompt):

1. Stop the LifeScope™ server:  
`lscope-server.sh stop`
2. Reset the admin password:  
`resetpwd.sh lifescape`
3. Restart the LifeScope™ server.
4. In the LifeScope™ UI, change the admin password to a secure password, according to your local policy.

The use of the `resetpwd.sh` script is not recommended for any user account except the LifeScope™ admin. Passwords reset for other users may become out of sync the authenticating realm.

## Read-set repository path: notify users

If you change the path to the read-set repository, notify users to restart LifeScope™ Software so that the repository can be updated to the new path.

## License management

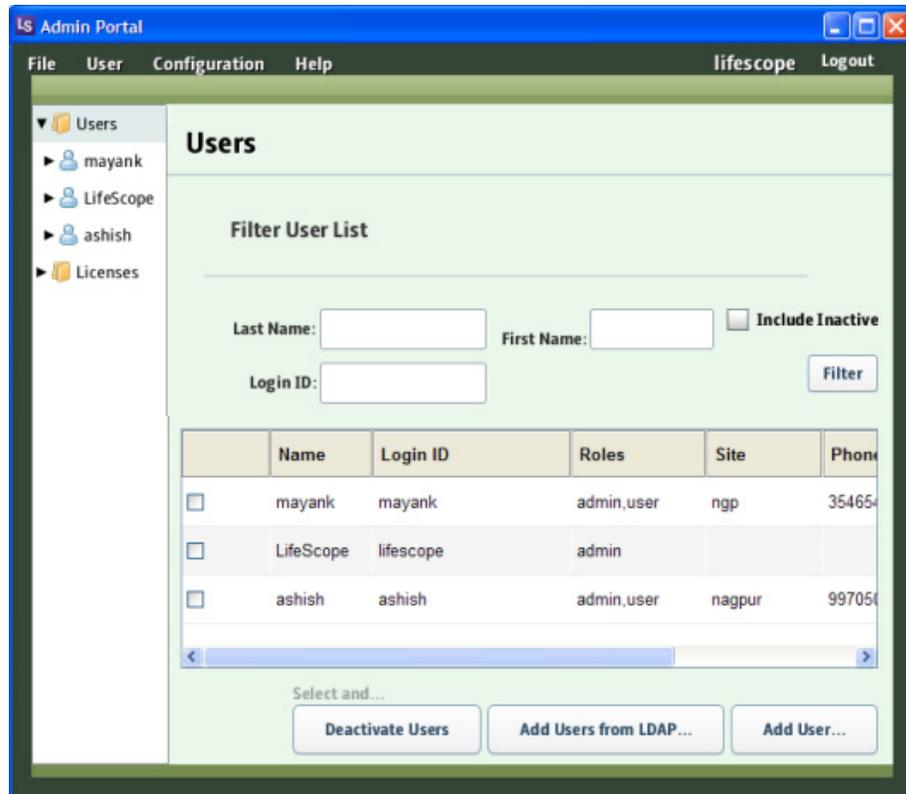
Licenses are released during normal exit of the client software. If the client software exits abnormally, the LifeScope™ Software server may not be able to release the license. The only way to free that unreleased license is through stopping and restarting the LifeScope™ Software server module.

## Log in

In the Admin window, enter your username and password, then click **Login** to open the Admin Portal shown on .

If you cannot remember your password, click the **Forgot Password** button, then follow the prompts.

If you need assistance, click **Login Help**.



## Administrate users

Follow these steps to access and work within the Admin tool window:

1. If you are not already logged in as an administrator, do so.  
Log in as “admin” to display the Admin tool window.  
The left panel in the Admin tool window shows folders for Users and Licenses.
2. Click the arrow next to any folder to display a list of its contents.
3. Click any folder to open it and show detailed information about the contents in the right panel of the Admin window.
4. Click on a user name in the left panel to show a profile of the user in the right pane.

### Search for users

There are two ways to search for users:

- Click **Users** in the top drop-down menu, then select **Search**.
- In the right (details) pane, a user’s Last Name, First Name, Login ID, or Email address in the data entry boxes, then click the **Filter** button. To include inactive users in your search, click that checkbox.

### Add users

You can add a single user or you can add multiple users from the Lightweight Directory Access Protocol (LDAP).

To add a single user:

1. Click:

- **Users** in the top menu, then select **Add User**, or
- Click the **Add User** button in the Users section of the Admin tool.

After users are added you can search for a specific user by entering the user's last name, first name, login ID, or e-mail address, and then clicking the **Filter** button.

2. In the Add User window, provide the following information:

- Name  
Name can be input as "last name, first name" or as "first name<space>last name".
- To search for a user, click the **Browse** button.
- Username
- Password
- Notes about the user
- Select whether the user is an administrator or a biologist.
- Email address
- Phone number
- Office
- Site

3. Click **OK** to add the user.

To add multiple users:

1. Click **Users** in the top menu, then select **Add User** and repeat the steps explained in Step 2 [on page 368](#)

or

2. Click the **Add Users from LDAP** button in the Users section of the Admin tool, select the users to add, and click **Add**.

To add multiple users (LDAP only):

1. In the Add Users from LDAP window, select the users to add.

2. After you have selected users to add, click the **Add** button.

## Deactivate users

To deactivate a user:

1. Click the checkbox next to the user's name in Users section of the Admin tool.
2. Select click the **X Deactivate Users** button.
3. In the Deactivate Users window, click **OK** to deactivate the user.

## Delete users

Once added, users cannot be deleted; they can only be deactivated.

## Reactivate users

To reactivate a user:

1. Click the checkbox next to the user's name in the left column list of users.
2. Click the **Active** box at the bottom of the User Profile page.
3. Click **Save Changes**.

## Configure user accounts

This section describes how to configure licenses and a user's setup and profile.

**Configure licenses** To configure a user's license for sites using named licenses:

1. Click **Configuration** in the top menu, then select **License**.
2. In the License section, click the checkbox in the License column for users who have software licenses. If a user's license has expired, click the License checkbox to erase the check mark.

To apply your changes, click **Save Changes**.

**Configure a user's setup** To configure a user's setup:

1. Click **Configuration** in the top menu, then select **Setup**.
2. In the Edit Setup window, use the **Browse** buttons to search for the following repositories:
  - Read-Sets
  - References
  - Projects

**Note:** If you change the path to the read-set repository, notify users to restart LifeScope™ Software so that the repository can be updated to the new path.

3. In the data entry box, edit the repository locations.
4. To edit network services, use the Browse buttons to search for the following services:
  - SMTP
  - LDAP
  - Licenses
5. In the data entry box, edit the network services.
6. To apply your changes, click **OK**.

**Configure a user's profile** To configure a user's profile:

In the left panel of the Admin tool, click the **Users** folder, then click a user's name to view and edit the user's profile.

To apply your changes, click **Save Changes**.

## Read-set repository path: notify users

If you change the path to the read-set repository, notify users to restart LifeScope™ Software so that the repository can be updated to the new path.

## Troubleshooting

If power was lost, causing the LifeScope™ server to shut down, and the server generates the message “read-only db connection,” do the following procedure:

**Note:** This is a non-recoverable action.

1. Stop the server:

```
lscope-server.sh stop
```

2. Delete the locked user database file:

```
rm /share/apps/lifescopeserver/UserDB/db*.lck
```

3. Restart the LifeScope™:

```
lscope-server.sh start
```

If the problem persists, or if the error message is different (indicating a corrupt database), do the following procedure to delete the user database (UserDB), which will remove all the current users in the system. Then recreate the users.

1. Stop the server:

```
lscope-server.sh stop
```

2. Delete the user database:

```
rm -rf /share/apps/lifescopeserver/UserDB
```

3. Restart the LifeScope™:

```
lscope-server.sh start
```

## Help

The Help option in the top menu contains instructions on how to use the Admin tool. Within the Help menu, select **User's Guide** for an Adobe Acrobat PDF copy of this document, **Tutorial** for step-by-step instructions, or **About** for the version of LifeScope™ Software and copyright information.





# Convert 1.3 BAM Files

This appendix covers:

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- Convert a 1.3 BAM file..... 374

## Introduction

This appendix describes how to convert a 1.3 BAM file to a 2.x BAM file that can be used as input to LifeScope™ Software tertiary processing. This step is required before a 1.3 BAM file can be used with LifeScope™ Software.

BAM file conversion is available in the GUI interface.

**Note:** The conversion does not support 1.3 BAM files containing fragment libraries. The conversion is not supported in the command shell interface.

## Convert a 1.3 BAM file

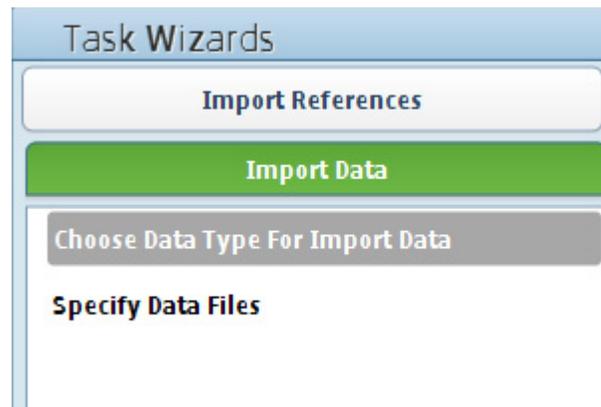
This section describes instructions to convert a 1.3 BAM file for use with LifeScope™ Software 2.x.

### Open the conversion screen

The conversion screen open when you import a 1.3 BAM file into the reads repository.

These instructions assume that you have created a project in the LifeScope™ Software GUI. Follow these steps to import and convert a 1.3 BAM file:

1. Open your project in the LifeScope™ Software GUI.
2. Click the Import Data tab in the left Task Wizards pane.



3. In the Import Data • Choose Data Type for Imported Data screen, in the “Already mapped data” section, click the **LifeScope/BioScope BAM** button to select it.

### Import Data • Choose Data Type For Import Data

Type of Data to be Added:

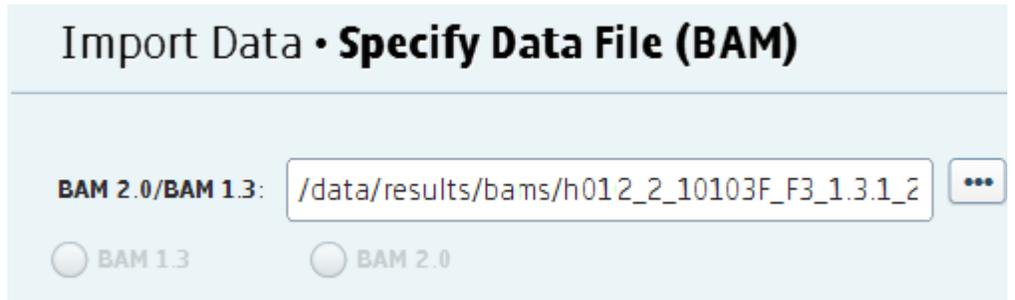
| Raw unmapped data                                                                         | Already mapped data                                                                                            |
|-------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|
| <input type="radio"/> XSQ <input style="float: right;" type="button" value="?"/>          | <input checked="" type="radio"/> LifeScope/BioScope BAM <input style="float: right;" type="button" value="?"/> |
| <input type="radio"/> CSFASTA/QUAL <input style="float: right;" type="button" value="?"/> |                                                                                                                |

Click Next >>.

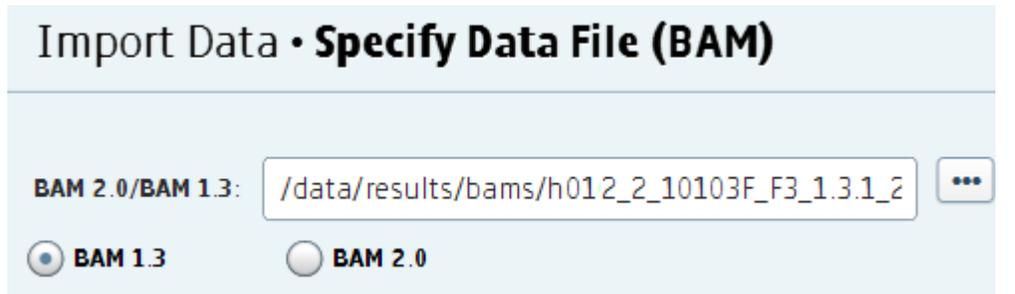
4. The Specify Data File (BAM) screen appears.



Click the ellipsis  button on the right, and the Browse to BAM file picker opens. Browse to the BAM file that you want to convert, select it, and click **OK**. The full path name of the BAM file is displayed in the Bam 2.0/BAM 1.3 field.



5. Click the **BAM 1.3** button.



Clicking the **BAM 1.3** button opens the Convert OLD BAM File conversion screen.

See [“Specify conversion information”](#) for instructions on entering the conversion information.

### Specify conversion information

Required fields are marked with a red asterisk  next to the field label. The conversion utility populates fields if possible.

Use information in the following table to fill out the conversion required fields.

| Field                                  | Comments                                                                                                                                                                                                                                                                    | XSQ field   |
|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| library.type                           | The library type of the BAM data. Fragment is not supported. Supported values are: <ul style="list-style-type: none"> <li>• MatePair</li> <li>• PairedEnd</li> </ul> <b>Note:</b> Values are case-sensitive, and must <i>exactly</i> match one of the above allowed values. | @CO RG:x LT |
| num.color.calls.for.first.tag          | The maximum untrimmed read length across all reads for the first tag (F3).                                                                                                                                                                                                  | @CO RG:x TX |
| num.color.calls.for.second.tag         | The maximum untrimmed read length across all reads for the second tag.                                                                                                                                                                                                      | @CO RG:x UX |
| is.base.present.for.first.tag          | Enter 0.                                                                                                                                                                                                                                                                    | @CO RG:x BX |
| is.base.present.for.second.tag         | Enter 0.                                                                                                                                                                                                                                                                    | @CO RG:x BY |
| trimmed.read.min.length.for.first.tag  | The minimum trimmed read length across all unfiltered reads for the first tag. <p><b>Note:</b> <i>Unfiltered reads</i> refers to reads that have not been filtered out.</p> <p>If the length is unknown, enter the value of num.color.calls.for.first.tag.</p>              | @CO RG:x TN |
| trimmed.read.min.length.for.second.tag | The minimum trimmed read length across all unfiltered reads for the second tag. <p>If the length is unknown, enter the value of num.color.calls.for.second.tag.</p>                                                                                                         | @CO RG:x UN |
| run.end.time                           | Enter the instrument run end time in the format YYYY-MM-DD. If the run time is not known, enter a past date of your choice, for example, 2011-04-01.                                                                                                                        | @CO RG:x DE |

| Field                       | Comments                                                                                                                                                                                                                                                                                                                                                                                                                    | XSQ field   |
|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| library.insert.size.minimum | The expected minimum insert size, in nucleotides.<br>If the size is unknown, enter 300 for MatePair data, or 30 for PairedEnd data.                                                                                                                                                                                                                                                                                         | @CO RG:x IN |
| library.insert.size.maximum | The expected maximum insert size, in nucleotides.<br>If the size is unknown, enter 3000 for MatePair data, or 300 for PairedEnd data.                                                                                                                                                                                                                                                                                       | @CO RG:x IM |
| is.ecc.run                  | Enter 0.                                                                                                                                                                                                                                                                                                                                                                                                                    | @CO RG:x EC |
| application.type            | The application intended to process this data. Supported values are: <ul style="list-style-type: none"> <li>• Whole Genome Resequencing</li> <li>• Targeted Resequencing</li> <li>• Whole Transcriptome (Fragment)</li> <li>• Whole Transcriptome (PairedEnd)</li> <li>• Small RNA</li> <li>• ChIP-Seq</li> <li>• Methylation</li> <li>• &lt;arbitrary string&gt;</li> </ul> If unknown, enter "Whole Genome Resequencing". | @CO RG:x AT |

The following table describes the optional conversion fields.

| Field                             | Comments                                                                                                                                                                                       | XSQ field   |
|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| library.insert.size.std.deviation | Leave blank. The program calculates this value.                                                                                                                                                | @CO RG:x IS |
| library.insert.size.average       | Leave blank. The program calculates this value.                                                                                                                                                | @CO RG:x IA |
| application                       | Leave blank. List of programs that have processed the BAM file.                                                                                                                                | @CO RG:x PN |
| index.id                          | Leave blank. Not used in 1.3 BAM files.                                                                                                                                                        | @CO RG:x II |
| index.name                        | Leave blank. Not used in 1.3 BAM files.                                                                                                                                                        | @CO RG:x IX |
| is.ercc.control.in.run            | Leave blank. Not used in 1.3 BAM files.                                                                                                                                                        | @CO RG:x ER |
| library.description               | A description of the sample.                                                                                                                                                                   | @CO RG:x LD |
| library.index.UUID                | Leave blank. Not used in 1.3 BAM files.                                                                                                                                                        | @CO RG:x UU |
| operator                          | The name or ID of the person setting up the instrument run.                                                                                                                                    | @CO RG:x CO |
| project.owner                     | Project name: the name of the lab project or the research project.                                                                                                                             | @CO RG:x PJ |
| sequencing.sample.description     | Description of the sample on the instrument (description of the sample that is eventually named in the BAM header @RG SM: field). The sample on the instrument can contain multiple libraries. | @CO RG:x SD |
| sample.id                         | Sample (specimen on the instrument) identifier. Must be a unique name.                                                                                                                         | @CO RG:x SP |
| sample.owner                      | The owner of the sample.                                                                                                                                                                       | @CO RG:x SO |

Follow these steps to complete the Convert Old BAM File screen:

1. Use the information in the table of required conversion fields ([page 376](#)) to enter the correct information in the required fields.  
Use the scroll bar to enter all required fields. Required fields are marked with a red asterisk .
2. When you have entered the required fields, click **OK**.

When the conversion is finished, the newly-converted BAM file is added to the bams repository.

The converted BAM is then available for use in LifeScope™ Software analyses. When a user opens the Add Data to Project • Find Data (BAM) screen, the converted BAM file appears in the BAM Data In Project window.



# LifeScope™ Genomic Analysis Software v2

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v1.0



# Glossary

|                             |                                                                                                                                                                                                                                                           |
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| alignment                   | The process of mapping sequencing reads to a reference genome or sequence.                                                                                                                                                                                |
| alignment browser           | An interactive software in which to view alignments of sequencing reads with the reference genome or sequence.                                                                                                                                            |
| alignment score             | Matching score; an optimal alignment is an alignment giving the highest score of matches.                                                                                                                                                                 |
| allele                      | One of two or more alternative nucleotide sequences at the same location on homologous chromosomes.                                                                                                                                                       |
| analysis                    | A data analysis run in LifeScope™ Software; may include secondary analysis on data from multiple sequencer runs.                                                                                                                                          |
| analysis type               | An <i>analysis</i> based on common genetic-analysis experiment types. LifeScope™ Software provides: Genomic Resequencing Analysis, Targeted Resequencing Analysis, Low Frequency Variant Detection, Small RNA Analysis, and Whole Transcriptome Analysis. |
| annotated gene              | Within one of several reference databases, a gene sequence that has biological attributes attached that describe structure or function, such as coding regions or biochemical function.                                                                   |
| annotation                  | Biological attributes or metadata that are attached to sequence data or files. Examples include: genes and protein-coding features, and verified variants.                                                                                                |
| BAM file                    | The compressed binary version of the Sequence Alignment/Map (SAM) format, a compact and index-able representation of nucleotide sequence alignments. The BAM file is generated from the mapping step and has special attributes to support SOLiD® data.   |
| barcode                     | A short, unique sequence that is incorporated into a library that enables identification of the library during multiplex sequencing.                                                                                                                      |
| barcode group, barcode pool | The conceptual grouping used during data analysis if more than one barcode is used during libration preparation from a single biological specimen.                                                                                                        |
| barcoded library            | A library that has a unique barcode sequence incorporated that enables identification of the library during multiplex sequencing.                                                                                                                         |
| base-space analysis         | Analysis of SOLiD® sequencing data that has been converted from color space to a nucleotide sequence.                                                                                                                                                     |

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| Bayesian                | An algorithm that is used to evaluate the probability of the existence of a heterozygote or a non-reference homozygote when the coverage at the position is not high.                                                                                                                                                                                                                                                |
| bead                    | The substrate to which the individual strands of DNA molecules in a SOLiD <sup>®</sup> library are attached. The covalently attached strand serves as the template for SOLiD <sup>®</sup> sequencing after the bead is immobilized on the flowchip surface.                                                                                                                                                          |
| BED file                | BED format provides a flexible way to define the data lines that are displayed in an annotation track. The required BED fields are: <ul style="list-style-type: none"><li>• chrom - name of the chromosome</li><li>• chromStart - starting position of the feature in the chromosome (the first base in a chromosome is numbered 0).</li><li>• chromEnd - ending position of the feature in the chromosome</li></ul> |
| BEDGRAPH                | In targeted resequencing, a text file in BEDGRAPH format that describes the depth of coverage within targets by on-target alignments.                                                                                                                                                                                                                                                                                |
| bisulfite read          | A sequence read of bisulfite-converted DNA, in which unmethylated cytosine residues have been converted to uracil. Used in methylation analysis of genomic DNA.                                                                                                                                                                                                                                                      |
| BLAST                   | In bioinformatics, Basic Local Alignment Search Tool, or BLAST, is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences.                                                                                                                                                                                   |
| call stringency         | The criteria for calling a nucleic acid species or genetic variant present in the biological sample.                                                                                                                                                                                                                                                                                                                 |
| calling threshold       | The criteria for calling a nucleic acid species or genetic variant present in the biological sample.                                                                                                                                                                                                                                                                                                                 |
| ChIP-Seq                | Sequence analysis of genomic regions associated with DNA-binding proteins.                                                                                                                                                                                                                                                                                                                                           |
| classic mapping         | An algorithm that searches for matches at the full read length, allowing up to a user-specified number of mismatches. Now called global mapping.                                                                                                                                                                                                                                                                     |
| clear zone              | The threshold to decide whether a read is mapped uniquely in the reference. Clear zones are used in previous versions of the software, but are not used with LifeScope <sup>™</sup> Software.                                                                                                                                                                                                                        |
| clipping, hard clipping | An operation that codes a portion of a color alignment with a number of mismatches that prevent seed or anchor extension.                                                                                                                                                                                                                                                                                            |
| clone                   | A single bead with a clonal population of templates for sequencing, generated during templated bead preparation/emulsion PCR.                                                                                                                                                                                                                                                                                        |
| color balance           | The relative proportion of beads in a given sequencing cycle that are called as each of the four colors.                                                                                                                                                                                                                                                                                                             |
| color call              | For each SOLiD <sup>®</sup> chemistry cycle, the color that is determined for each bead.                                                                                                                                                                                                                                                                                                                             |

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| color space, color-space analysis | Color space data is a sequence of colors obtained from 2-base encoded probes in SOLiD <sup>®</sup> System sequencing, representing a series of overlapping dinucleotides. Color space data is converted to a nucleotide sequence by aligning to a reference.                                                                                                                        |
| complexity                        | The size of the genome that is composed of unique sequences. With respect to SOLiD <sup>®</sup> libraries, the number of independent molecules derived from the original DNA input, not from amplification.                                                                                                                                                                         |
| consensus calls                   | A file that lists the SNPs called with the SNP Finding algorithm, and provides general information about each position.                                                                                                                                                                                                                                                             |
| contig                            | A nucleotide sequence that has been assembled from shorter, overlapping sequences.                                                                                                                                                                                                                                                                                                  |
| core                              | A single core is equivalent to a processor on a traditional computer. Multiple cores increase the performance and efficiency of a computer that is running multiple programs.                                                                                                                                                                                                       |
| counting                          | Expression analysis that focuses on relative or absolute quantification of RNA molecules in a biological specimen.                                                                                                                                                                                                                                                                  |
| copy number variation (CNV)       | A variation in the number of copies of DNA segments among individuals in a population. The length of the segment can vary, and it is typically more than a few base pairs. Some use a broader definition that includes any insertion, deletion, or duplication longer than a few base pairs.                                                                                        |
| CountTags                         | A module to count the number of reads that align within genomic features.                                                                                                                                                                                                                                                                                                           |
| counting                          | Sequence analysis that generates read or tag counts for annotated regions of a reference sequence.                                                                                                                                                                                                                                                                                  |
| coverage                          | <ul style="list-style-type: none"> <li>• In genomic analysis, the number of aligned (or mapped) sequencing reads that span a position in the reference genome.</li> <li>• In RNA analysis, this term is sometimes used to describe the fraction of the reference sequence that has sequencing reads aligned (or mapped), at a certain <a href="#">calling threshold</a>.</li> </ul> |
| csfasta file                      | The SOLiD <sup>®</sup> System produces color-space sequence reads in a fasta-format labeled as CSFASTA. These reads can be retained and analyzed in color space.                                                                                                                                                                                                                    |
| dbSNP                             | A Single Nucleotide Polymorphism database repository for single nucleotide polymorphisms and short insertion and deletion polymorphisms, hosted by the NCBI.                                                                                                                                                                                                                        |
| <i>de novo</i> sequencing         | The initial generation of the primary genetic sequence of a particular organism, without use of a reference genome or sequence.                                                                                                                                                                                                                                                     |
| deletion                          | A gap in a nucleotide sequence with respect to a reference genome or sequence.                                                                                                                                                                                                                                                                                                      |
| dibayes                           | Analysis module to identify SNPs from mapped and processed SOLiD <sup>®</sup> System color space reads. Also called diBayes and SNP Finding.                                                                                                                                                                                                                                        |

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| discordant                     | In large indel analysis, mate-paired reads that have been mapped to a reference genome, and whose inter-read distance deviates significantly from the expected insert size of the mate-paired library as a whole.<br><br>Discordant pairs have insert sizes that deviate significantly from the expected value. Discordant pairs containing a putative deletion appear larger when mapped to the reference. |
| EBI                            | European Bioinformatics Institute.                                                                                                                                                                                                                                                                                                                                                                          |
| enriched data                  | Sequencing data that is associated with a region of interest in a targeted resequencing experiment or in a low frequency variant detection workflow.                                                                                                                                                                                                                                                        |
| enrichment statistics          | Calculated results derived from the targeted resequencing enrichment module in LifeScope™ Software. Also generated in low frequency variant detection workflows.                                                                                                                                                                                                                                            |
| ENSEMBL                        | A genome browser. <a href="http://www.ensembl.org">www.ensembl.org</a> .                                                                                                                                                                                                                                                                                                                                    |
| error correction               | (SAET) This step takes each read in the input file and attempts to correct it if any of its k-mers are not present in the spectrum. Use of multi-threaded versioning may be recommended for speed of process.                                                                                                                                                                                               |
| Error expectation metric (EEM) | A whole transcriptome pipeline, a parameter that is used in the calculation of the Junction Confidence Value metric, to determine the statistical significance of detected variant <a href="#">fusion transcripts</a> .                                                                                                                                                                                     |
| evidence graph                 | A visual representation of the data structure of a candidate fusion transcript.                                                                                                                                                                                                                                                                                                                             |
| exon                           | In eukaryotic organisms, a segment of a gene that encodes part or all of a protein. Exons may be separated by introns that are spliced out of the primary transcript to produce a mature mRNA for translation into protein.                                                                                                                                                                                 |
| exon mapping                   | A whole transcriptome pipeline, a step in which reads are mapped to the set of exon sequences defined by the genome annotation.                                                                                                                                                                                                                                                                             |
| F3 tag                         | Sequencing data derived from the P1 end of the template in the SOLiD® templated bead, using forward ligation chemistry. The F3 tag is generated using the SOLiD™ FWD1 Seq. Primers or the SOLiD™ Small RNA Seq. Primers. See the <i>5500 Series SOLiD® Sequencers: Reagents and Consumables Ordering Guide</i> (Part no. 4465650) for an illustration.                                                      |
| F5 tag                         | Sequencing data derived from the P2 end of the template in the SOLiD® templated bead, using reverse ligation chemistry. The F5 tag is generated using the SOLiD® REV1 (DNA) Seq. Primers or the SOLiD® REV1 (RNA) Seq. Primers. See the <i>5500 Series SOLiD® Sequencers: Reagents and Consumables Ordering Guide</i> (Part no. 4465650) for an illustration.                                               |
| false positive                 | Sequence variants that are present in the output data from a sequencing run but are not present in the biological source.                                                                                                                                                                                                                                                                                   |

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| filter                       | A reference sequence of interest that is used to select reads that align with or map to the reference sequence for further analysis.                                                                                                                                                                                                                                                                                             |
| filter mapping               | Alignment of sequencing reads to a reference sequence of interest, to select reads for further analysis.                                                                                                                                                                                                                                                                                                                         |
| Frequentist                  | An algorithm for finding <a href="#">SNPs</a> (alternative to SNP Finding algorithm).                                                                                                                                                                                                                                                                                                                                            |
| function code (dbSNPs)       | A code in the dbSNP database that indicates the consequence, if any, of a SNP to the transcript in which it is located.                                                                                                                                                                                                                                                                                                          |
| fusion transcript            | An RNA molecule that results from transcription of a gene fusion. See also <a href="#">gene fusion</a>                                                                                                                                                                                                                                                                                                                           |
| gapped alignment             | A read alignment to the reference sequence that indicates an insertion or deletion. The pairing algorithm searches for gapped alignments (indels) when one of the <a href="#">tags</a> (F3/R3/F5-P2) maps to the reference genome and the other tag does not map to the genome within the insert-size range. Small indel analysis calls indels from a consensus of gapped alignments, using the BAM file as input.               |
| gene fusion                  | A section of the genome that maps to an exon from one gene followed by an exon from another gene. It can occur as the result of a translocation, deletion, or chromosomal inversion. A gene fusion junction excludes exon-to-exon boundaries that arise from alternative splicing of a transcript.                                                                                                                               |
| genomic classification table | For mate-paired or paired-end reads, a code that describes the strandedness, distance, and orientation of the two reads.                                                                                                                                                                                                                                                                                                         |
| genomic mapping              | See <a href="#">mapping</a> .                                                                                                                                                                                                                                                                                                                                                                                                    |
| genomics                     | Global analysis of the genome to discern elements involved in regulation of gene activity or expression, with an emphasis on genetic variation such as single nucleotide polymorphisms, small and large insertions and deletions, and other structural variants such as translocations and inversions. Some use the term genomics to as an umbrella term that includes transcriptomics, epigenomics, and analysis of the genome. |
| group                        | A collection of read-sets that are required to be analyzed together, in groups that you define. Reads that are grouped together are treated as one specimen, even if the reads are in different *.xsq input files.                                                                                                                                                                                                               |
| hemizygous                   | A small indels tool, a parameter that indicates that the reference allele and the small indel are both present.                                                                                                                                                                                                                                                                                                                  |
| hg18, hg19                   | Reference sequence assemblies of the human genome using the nomenclature used by the UCSC Genome Browser.                                                                                                                                                                                                                                                                                                                        |
| highly expressed junctions   | A whole transcriptome pipeline, high-count sequencing reads that span an exon-exon junction.                                                                                                                                                                                                                                                                                                                                     |
| homozygous                   | In diploid organisms, having two identical alleles in the corresponding genes.                                                                                                                                                                                                                                                                                                                                                   |

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| HUGO-style gene names                  | Gene names following the Human Genome Organization style.                                                                                                                                                                                                                                                                                                                                                                |
| HuRef                                  | A diploid human genome sequence of one individual (J. Craig Venter).                                                                                                                                                                                                                                                                                                                                                     |
| indel                                  | A difference in sequence due to either an insertion or a deletion event; especially used when the evolutionary direction of the change is unspecified.                                                                                                                                                                                                                                                                   |
| insert size                            | The physical size of the genomic DNA segments or RNA molecules represented in a SOLiD <sup>®</sup> library. <ul style="list-style-type: none"> <li>• Fragment libraries: the size of the sheared DNA fragments.</li> <li>• Mate-paired libraries: the length of the genomic DNA segment spanned by the corresponding mate-pair tags.</li> <li>• Whole transcriptome libraries: the size of the RNA fragments.</li> </ul> |
| insertion                              | An insertion of nucleotide sequence with respect to the reference genome or sequence.                                                                                                                                                                                                                                                                                                                                    |
| intron                                 | The genomic sequence between two exons that is spliced out of a primary transcript prior to translation. See <i>exon</i> .                                                                                                                                                                                                                                                                                               |
| inversion                              | A segment of DNA that is in its native location but is in the reverse orientation.                                                                                                                                                                                                                                                                                                                                       |
| junction                               | A place where two regions that are not contiguous in the genomic sequence are joined in a single sequenced region under consideration.                                                                                                                                                                                                                                                                                   |
| Junction Confidence Value (JCV) metric | Whole transcriptome pipeline, a statistical confidence metric to detect false positives and assign a confidence level to a detected junction.                                                                                                                                                                                                                                                                            |
| junction mapping                       | Whole transcriptome pipeline, a mapping step that incorporates exon, gene, and transcript definitions in the genome annotation for that region.                                                                                                                                                                                                                                                                          |
| junk seed                              | An initial alignment in a seed-and-extend algorithm that is not aligned with the correct sequence                                                                                                                                                                                                                                                                                                                        |
| large indel                            | An insertion or deletion, with respect to a reference sequence, of more than 200 bp.                                                                                                                                                                                                                                                                                                                                     |
| LB field                               | A field in a BAM file that contains library type information.                                                                                                                                                                                                                                                                                                                                                            |
| library                                | A set of DNA or cDNA molecules prepared from the same biological specimen and prepared for sequencing on the SOLiD <sup>®</sup> System.                                                                                                                                                                                                                                                                                  |
| local mapping                          | The initial alignment step that starts with locating short matches between a read and the reference sequence.                                                                                                                                                                                                                                                                                                            |
| locus-spanning                         | Pairs of reads derived from mate pairs or paired-end reads that span a relatively large distance in the reference sequence.                                                                                                                                                                                                                                                                                              |

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| Map Fragment data tool | Tool that maps sequence data from fragment libraries.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| mappability            | In the CNVs analysis module, the fraction of mappable bases in the candidate CNV region.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| mapping                | The process of aligning sequencing reads to a reference genome or sequence.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| mate alignment         | The process of mapping paired-end or mate-paired reads to the reference sequence, taking into account the proximity of the reads on a template clone.                                                                                                                                                                                                                                                                                                                                                                                                                        |
| mate-paired library    | Library consisting of two DNA segments that reside a known distance apart in the genome, linked by an internal adaptor, and with P1 and P2 Adaptors ligated to the 5' and 3' ends of the <a href="#">template strand</a> , respectively.                                                                                                                                                                                                                                                                                                                                     |
| mates                  | Sequencing reads that are linked through their origin in a mate-paired library or a paired-end sequencing run.                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| mer                    | The number of nucleotides in a sequencing read.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| merging (WTA)          | Algorithm parameter in paired-end WTA analysis that enables the merging of genomic and junction mappings.                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| methylation analysis   | The study of how methylation of nucleic acids is involved in DNA structure and control of gene expression.                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| module                 | A virtual piece of LifeScope™, such as mapping, SNP Finding, or CNV.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| NCBI                   | National Center for Biotechnology Information.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| node                   | A node is a single computer that is connected to the network. A multi-node cluster has several nodes (computers).                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| normalization          | In general, the process of comparing an experimental measurement to a reference measurement.<br><br>In the CNV analysis module, the process of comparing the relative coverage of a region of interest to the global coverage, based on the human reference genome.<br><br>In targeted resequencing, the process of adjusting the amplicon amounts after PCR enrichment and before library preparation, so that amplicons are represented equally in the library.<br><br>In the whole transcriptome pipeline, the parameter RPKM is a normalized measure of gene expression. |
| paired-end sequencing  | Sequencing runs that acquire sequence from each end of the insert in a DNA fragment or whole transcriptome library, using both forward and reverse reads.                                                                                                                                                                                                                                                                                                                                                                                                                    |
| pairing                | An algorithm that occurs after mapping in the LifeScope™ Software workflow                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

|                       |                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pairing distances     | Pairing distances (sometimes called insert sizes) for each pair are assigned during the mapping/pairing pipelines and subsequently used by the large indel tool to determine indel candidacy.                                                                                                                                                                                                                                      |
| parallelization       | A tertiary analysis tool by which you can split spectrum generation into multiple jobs, where each job generates a subspectrum from the subset of reads.                                                                                                                                                                                                                                                                           |
| pipeline              | Sets of steps or modules that are used in sequence for different data analysis goals, such as whole transcriptome analysis or methylation analysis.                                                                                                                                                                                                                                                                                |
| ploidy                | The number of chromosome sets in a cell.                                                                                                                                                                                                                                                                                                                                                                                           |
| polyploidy            | The number of chromosome sets in a cell.                                                                                                                                                                                                                                                                                                                                                                                           |
| polymorphism          | A genetic variant in a population of individuals that may or not may be associated with an observable (phenotypic) trait.                                                                                                                                                                                                                                                                                                          |
| primer set            | In the SOLiD <sup>®</sup> System, the set of primers that are used sequentially to initiate ligation sequence chemistry.                                                                                                                                                                                                                                                                                                           |
| project               | A project is a container for your analysis runs. You create your projects. Within each project, you create one or more analyses. Each project is private to an individual LifeScope <sup>™</sup> user.                                                                                                                                                                                                                             |
| p-value               | The statistical significance of an alignment score is frequently assessed by its p-value, which is the probability that this score or a higher one can occur simply by chance, given the probabilistic models for the sequences.                                                                                                                                                                                                   |
| quality value         | An empirically defined value based on a phred-like score equating to the confidence that the color called for that cycle is the correct one. In general, the brighter the bead, the greater the difference in signal between the primary and secondary colors, the higher the quality value (QV).                                                                                                                                  |
| R3 tag                | The R3 tag applies only to mate-paired libraries; sequencing data derived from the mate-pair tag closer to the P2 end of the SOLiD <sup>®</sup> templated bead, using forward ligation chemistry. The R3 tag initiates in the IA sequence using the SOLiD <sup>™</sup> FWD2 Seq. Primers. See the <i>5500 Series SOLiD<sup>®</sup> Sequencers: Reagents and Consumables Ordering Guide</i> (Part no. 4465650) for an illustration. |
| raw reads             | A format in which the nucleotide sequence appears without headers or comments.                                                                                                                                                                                                                                                                                                                                                     |
| read, sequencing read | Sequencing data from a single bead with a single primer set.                                                                                                                                                                                                                                                                                                                                                                       |
| read-set              | A group of reads belonging either to one *.xsq file (in the case of non-indexed data) or to one barcode from one *.xsq file (in the case of indexed data).                                                                                                                                                                                                                                                                         |
| read-set group        | A collection of similar read-sets designated by a user.                                                                                                                                                                                                                                                                                                                                                                            |

|                                                 |                                                                                                                                                                                                                                                                                                                                                                                  |
|-------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| read-set repository                             | A storage place in LifeScope™ Software for instrument data intended to be input data for LifeScope™ Software analyses.                                                                                                                                                                                                                                                           |
| reference, reference genome, reference sequence | A sequence against which sequencing reads are aligned before further bioinformatics analysis.                                                                                                                                                                                                                                                                                    |
| reference file                                  | A file containing the reference sequence information, usually in the *.fasta format.                                                                                                                                                                                                                                                                                             |
| RefSeq                                          | A multi-organism database archive of DNA, RNA, and protein sequences, hosted by the NCBI.                                                                                                                                                                                                                                                                                        |
| repository                                      | A virtual container with your reference files, reads files, and projects. Repositories include: <ul style="list-style-type: none"> <li>• Projects, which contains your projects.</li> <li>• Reads (read-sets), which contains your *.xsq data files.</li> <li>• References, which contains the reference genomes used in your analyses.</li> </ul>                               |
| rescue                                          | An alignment method that is applied to read pairs that have at least one alignment, but no pair of alignments occurring within an expected range. Use the rescue method to find additional alignments.                                                                                                                                                                           |
| rescue distance                                 | The maximum distance $x$ for a pair considered to be properly paired (SAM flag 0x2) is calculated by solving equation $\Phi((x-\mu)/\sigma)=x/L*p_0$ , where $\mu$ is the mean, $\sigma$ is the standard error of the insert size distribution, $L$ is the length of the genome, $p_0$ is prior of anomalous pair and $\Phi()$ is the standard cumulative distribution function. |
| resequencing                                    | The process of genomic sequencing in cases where a reference sequence already exists, and the new sequence is compared to the reference.                                                                                                                                                                                                                                         |
| resource manager                                | Software for executing batch and interactive jobs on a cluster of networked computers.                                                                                                                                                                                                                                                                                           |
| RNA-Seq                                         | Gene expression analysis using sequence-based approaches. RNA-Seq can include whole transcriptome analysis, small RNA analysis, and SOLiD® SAGE™ analysis.                                                                                                                                                                                                                       |
| RPKM                                            | The number of reads mapping to a transcript per kilobase of transcript length per million mappable reads. RPKM is used to set a threshold for calling a transcript or new RNA species or isoform “present.” 1 RPKM is equivalent to 20 reads mapping to a 1 kb transcript per $20 \times 10^6$ mappable reads.                                                                   |
| SAET                                            | SOLiD® Accuracy Enhancer Tool for correcting spectral-alignment errors in raw data; reduces color-calling error rate without alignment to reference genome. SAET takes as input a file with SOLiD® reads in .csfasta format and outputs corrected reads and quality values into new .csfasta and .qual files, respectively.                                                      |

|                         |                                                                                                                                                                                                                                                                                                                                                       |
|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SAGE™ analysis          | (Serial Analysis of Gene Expression) Nucleotide sequence analysis seeking to find specific gene expression information using short stretches of cDNA (also known as <i>tags</i> ) from the 3' ends of RNA molecules. In the SOLiD® System, the SAGE tag is 25–27 bp in length.                                                                        |
| sample, barcoded sample | In the 5500 Series Genetic Analysis Systems, the set of templated beads that will be sequenced in a single flowchip lane. A barcoded sample contains templated beads from up to 96 barcoded libraries.                                                                                                                                                |
| SAMtools                | SAMtools provide various utilities for manipulating alignments in the SAM format, including sorting, merging, indexing and generating alignments in a per-position format. SAMtools is hosted by SourceForge.net.                                                                                                                                     |
| Satay plot              | A cross-axis graph displaying the spectral purity and signal intensity of beads in a sequencing run that meet defined thresholds in each parameter ( <i>on-axis beads</i> ). <i>Best beads</i> meet the highest stringency thresholds; <i>good beads</i> meet less stringent thresholds.                                                              |
| secondary analysis      | Sequence alignment and mapping to a reference sequence.                                                                                                                                                                                                                                                                                               |
| seed                    | A field: size of seed used in spectrum construction. Seeding: Using a heuristic method, finding homologous sequences, not by comparing either sequence in its entirety, but rather by locating short matches between two sequences.                                                                                                                   |
| seed-and-extend         | Seed-and-extend algorithms map reads to a reference genome. Certain softwares can map reads with any number of differences or mismatches using the observation that for an $r$ bp read to align to the reference with at most $k$ differences, the alignment must have a region of length $s=r/k+1$ called a seed that exactly matches the reference. |
| sense strand            | The strand of DNA with the same nucleotide sequence as that of the corresponding mRNA. Also called the <i>coding strand</i> .                                                                                                                                                                                                                         |
| sequence trimming       | See <a href="#">trimming</a> .                                                                                                                                                                                                                                                                                                                        |
| sequencing sample       | A physical pooling of one or more library preparations before templated bead preparation. The sequencing sample can be used on one or more lanes on a flowchip.                                                                                                                                                                                       |
| single read             | Forward-only sequence data that generates the F3 <a href="#">tag</a> .                                                                                                                                                                                                                                                                                |
| small indel             | An insertion or deletion, with respect to a reference sequence, of less than 10 base pairs in length.                                                                                                                                                                                                                                                 |
| small RNA analysis      | Global sequence analysis of the small RNA population of a cellular RNA sample; small RNA includes microRNAs (miRNAs), short interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs), and repeat-associated siRNAs (rasiRNAs).                                                                                                                       |
| small RNA library       | A SOLiD® System-compatible library that is prepared from the small RNA fraction of a total RNA sample.                                                                                                                                                                                                                                                |
| SNP                     | Single Nucleotide Polymorphisms (SNPs); single base pair variants in genomic DNA or the corresponding RNA transcript.                                                                                                                                                                                                                                 |

|                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SNP Finding           | An analysis module to identify <a href="#">SNPs</a> from mapped and processed SOLiD® System color space reads. Also called diBayes.                                                                                                                                                                                                                                                                                                                                                                                                      |
| spectrum building     | (SAET) When a temporary file with spectrum constructed from all reads generates in the output directory. This process is fast, resource less, and creates temporary swap files when memory usage is above 2GB.                                                                                                                                                                                                                                                                                                                           |
| splice junction       | Exon-to-exon boundaries that arise from alternative splicing of a transcript. See also <a href="#">gene fusion</a> .                                                                                                                                                                                                                                                                                                                                                                                                                     |
| splicing              | The process whereby introns are removed from a primary mRNA, resulting in a mature mRNA that is ready for translation into protein.                                                                                                                                                                                                                                                                                                                                                                                                      |
| strandedness          | The polarity or orientation of a nucleic acid strand with respect to being sense or antisense. Libraries prepared using the SOLiD® Total RNA-Seq Kit preserve the strandedness of the original RNA molecule such that <a href="#">F3 tag</a> reads align to the sense strand and <a href="#">F5 tag</a> reads align to the antisense strand.                                                                                                                                                                                             |
| stringency            | Stringency is one criterion used to filter out junk reads in ReadBuilder stage. Specifically, in the SNP Finding tool, the stringency parameter (call.stringency) defines the criteria by which to report SNPs in analyzing genomic data. There are several main empirical stringency settings (low_frequency, low, medium, high, highest).                                                                                                                                                                                              |
| tag                   | A tag is: <ul style="list-style-type: none"> <li>• Sequencing data from a single bead with a single primer set; sometimes used interchangeably with <a href="#">read</a>, <a href="#">sequencing read</a>.</li> <li>• A length of DNA or cDNA to be sequenced; especially, a relatively short stretch of DNA or cDNA that is used to infer information about the longer native molecule from which it is derived, such as in <a href="#">mate-paired library</a> sequencing and <a href="#">SAGE™ analysis</a>, respectively.</li> </ul> |
| tags: BC, F3, F5, R3  | Sequencing data derived from specific locations on the SOLiD® templated bead. See the <i>5500 Series SOLiD® Sequencers: Reagents and Consumables Ordering Guide</i> (Part no. 4465650) for an illustration.                                                                                                                                                                                                                                                                                                                              |
| targeted resequencing | Comparative sequence analysis of selected candidate genes or regions to discover genetic variants and mutations.                                                                                                                                                                                                                                                                                                                                                                                                                         |
| template strand       | The strand of each DNA molecule that is covalently attached to SOLiD® P1 beads during templated bead preparation and that serves as a template for SOLiD® System sequencing.                                                                                                                                                                                                                                                                                                                                                             |
| tertiary analysis     | Data analysis that takes place after mapping and alignment.                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| threading             | The ability of an operating system to execute different parts of a program, called threads, simultaneously. A multi-threaded software program is designed so that all the threads can execute at the same time without interfering with each other.                                                                                                                                                                                                                                                                                      |

|                              |                                                                                                                                                                                                                                                                                                                   |
|------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| transcript rescue distance   | See <a href="#">rescue distance</a> .                                                                                                                                                                                                                                                                             |
| transcriptome                | The compilation of all transcribed sequences from a genome, both coding and non-coding.                                                                                                                                                                                                                           |
| transition                   | A single nucleotide (point) mutation that changes a purine nucleotide to another purine nucleotide (for example, A to G), or a pyrimidine nucleotide to another pyrimidine nucleotide (for example T to C).                                                                                                       |
| translocation                | A mutation in which a chromosomal segment changes position, usually moving from one chromosome to a different, nonhomologous chromosome.                                                                                                                                                                          |
| transversion                 | A single nucleotide (point) mutation that changes a purine nucleotide to a pyrimidine nucleotide (for example, A to C), or a pyrimidine nucleotide to a purine nucleotide.                                                                                                                                        |
| trimming                     | Filtering noise algorithmically which removes 5' overhangs and shortens 3' overhangs, typically to 4–5 bases. Trimming reads requires both modifying the .csfasta reads file to replace the color to be trimmed with a dot (“.”), and modifying the mapping parameters to account for the alignment change.       |
| trusted frequency (SAET)     | (SAET) Use this developer option (-trustfreq freq) to overwrite estimated frequency cutoff of trusted seeds. All seeds with frequency < “freq” are filtered out of spectrum.                                                                                                                                      |
| trusted seed                 | (SAET) If globally computed cutoff for frequency of trusted seeds does not meet your purpose, e.g., it is too low and too many junk seeds are considered correct or it is too high and many correct but low frequency seeds are filtered out, then use -trustfreq option to overwrite estimated frequency cutoff. |
| ungapped alignment           | See <a href="#">gapped alignment</a> .                                                                                                                                                                                                                                                                            |
| uniquely placed reads        | A read that is mapped only once in a genome with a given number of mismatches.                                                                                                                                                                                                                                    |
| variant                      | A difference in the nucleotide sequence of interest, with respect to the reference sequence.                                                                                                                                                                                                                      |
| visualization                | Viewing mapped reads on any number of publicly available genome browsers.                                                                                                                                                                                                                                         |
| weighting                    | A weighting scheme can outperform a simple binary scheme traditionally applied in genomic analysis, independent of the organism, and used to improve the quality of the data.                                                                                                                                     |
| whole transcriptome analysis | Global sequence analysis of coding and non-coding RNA transcripts along their entire length.                                                                                                                                                                                                                      |
| whole transcriptome library  | A SOLiD® System-compatible library that is prepared from total or poly(A) RNA that enables sequence analysis of the transcripts along their entire length.                                                                                                                                                        |

|             |                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| workflow    | Configuration files that are used for common end-to-end analysis runs, including multiple <a href="#">modules</a> . LifeScope™ Software runs these common workflows: resequencing, targeted resequencing, low frequency variant, whole transcriptome, small RNA, Methyl Miner, and ChIP-Seq.                                                                                                                          |
| WTA         | See <a href="#">whole transcriptome analysis</a> .                                                                                                                                                                                                                                                                                                                                                                    |
| *.xsq       | eXtensible SeQuence, an extensible file format for storing sequence data. A binary sequence output file generated by the instrument. This file contains primary analysis results for a single lane in a flowchip.                                                                                                                                                                                                     |
| z-normalize | Parameters called moving statistics, such as the number of locus-spanning pairs, and the sample average insert size, are calculated from a subset of pairs at each genomic position, yielding the absolute insert size deviation between the sample and the population in units of standard deviation. This normalization step allows multiple libraries with variable insert sizes to be combined into one analysis. |
| zygosity    | The combination of <i>alleles</i> at a site in a nucleotide sequence; for example, homozygous reference allele, homozygous non-reference allele, or heterozygous.                                                                                                                                                                                                                                                     |



# Documentation

## Related documentation

For the latest documentation on LifeScope™ Genomic Analysis Software, go to:

[http://www3.appliedbiosystems.com/AB\\_Home/Support/index.htm](http://www3.appliedbiosystems.com/AB_Home/Support/index.htm)

| Document                                                             | Part number | Description                                                                                            |
|----------------------------------------------------------------------|-------------|--------------------------------------------------------------------------------------------------------|
| <i>LifeScope™ Genomic Analysis Software Command Shell User Guide</i> | 4471875     | Describes how to use the software's command shell interface, for secondary and tertiary data analysis. |

## Obtaining support

For the latest services and support information for all locations, go to:

[www.appliedbiosystems.com](http://www.appliedbiosystems.com)

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.



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