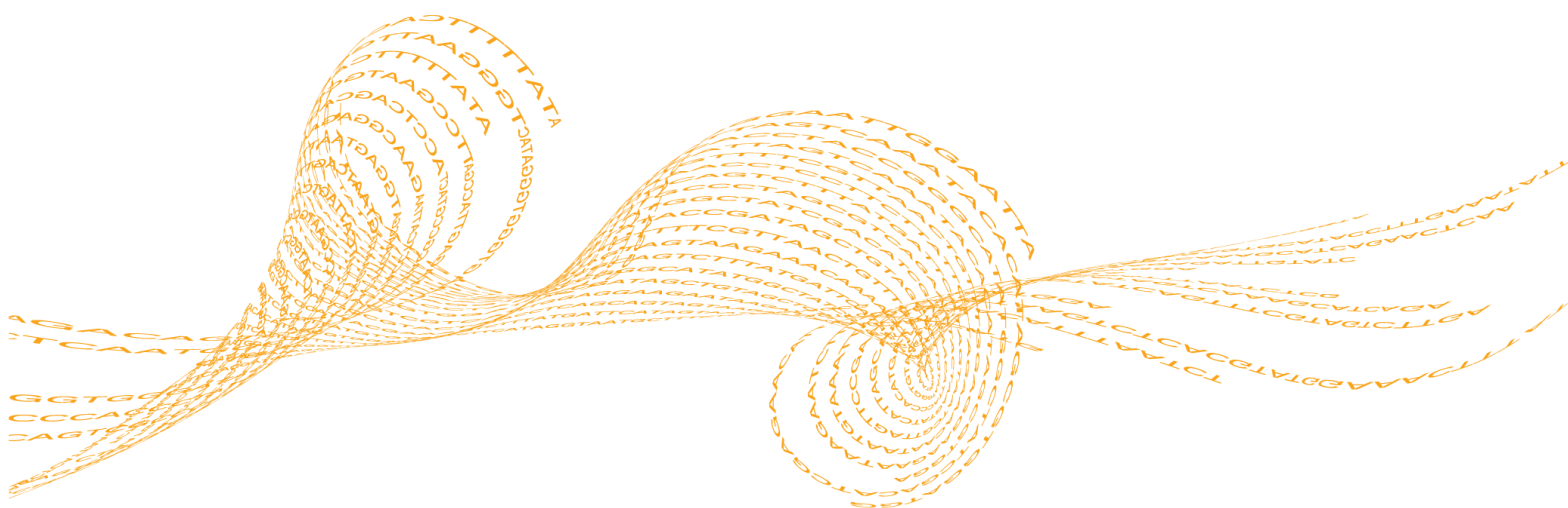


Sequence Genotyper Reference Guide

For Research Use Only. Not for use in diagnostic procedures.

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Introduction

Sequence Genotyper analysis software performs genotype interpretations using Bovine Parentage designated target markers and genome VCF files.

Use Sequence Genotyper to create projects, import sample and target files, and generate reports.

Installation

Downloading the Software

You can download the Sequence Genotyper software from the Illumina website at support.illumina.com/. From the Sequence Genotyper Support page, select **Downloads**. A MyIllumina account is required.

Installing the Software

To start the installation, double-select the Setup.exe file. The installation wizard opens. Click **Next** to accept all default settings.

Starting the Software

To start the software, go to **Start | All Programs | Illumina | Sequence Genotyper**, or select the Sequence Genotyper icon on your desktop.



Requirements

Computing Requirements

- ▶ 1 GHz or faster processor
- ▶ 4 GB RAM
- ▶ 1 GB free hard disk space for install
- ▶ 50–100 GB hard disk space for data (more is recommended)

Software Requirements

- ▶ Windows 7
- ▶ Microsoft .NET 4.5 or higher



NOTE
Sequence Genotyper is not supported on virtual machines.

Dashboard Overview

The Dashboard is the Sequence Genotyper home screen. The Dashboard displays the following information:

- ▶ Number of Projects, Targets, Samples, and Reports
- ▶ Disk Usage
- ▶ Call Rate (Last 20 Samples)

The dashboard includes a Projects Table that lists the Projects in Sequence Genotyper. Each project listed includes the ID, name, description, and the date that the project was last modified.




- ▶ **Action**—Project file management options. To change the name or description, click Edit. To delete the project and associated reports, click Delete.
- ▶ **Project ID**—Lists the project ID.
- ▶ **Project**—Lists the project name.
- ▶ **Description**—Lists the project description.
- ▶ **Last Updated**—Lists the date and time that the project was last modified.
- ▶ **Reports**—Report file management options. To view a summary of the reports in the project, click View. To add a report, click Add.
- ▶ **Samples**—To view a list of the samples in the project, click Samples.

Common Elements





There are some common UI elements that are shared among Sequence Genotyper pages, and which enable general tasks:

- ▶ Header
- ▶ Toolbar

Header Buttons

Icon	Element	Description
	Home	The Home button opens the Dashboard.
	Alerts	The Alerts button provides information about software updates.
	Information	The Information button displays the End User License Agreement.


Toolbar Buttons

Icon	Element	Description
	Projects	See <i>Projects</i> on page 6.
	Targets	See <i>Targets</i> on page 7.
	Samples	See <i>Samples</i> on page 9.
	Reports	See <i>Reports</i> on page 12.


Projects

Use Projects to collect and organize your reports, source data, and target files.

Create a Project

- 1 Use 1 of the following methods to create a project:
 - ▶ Click the Projects toolbar icon , and then click **Create Project**.
 - ▶ From the Dashboard, click **Create Project**.
 - ▶ From the Dashboard, click **View Projects**, and then click **Create Project**.
The Create Project dialog opens.
- 2 In the Project Name field, enter the name of your project.
- 3 In the Description field, enter a description for your project.
- 4 Click **Create**.
The project is created and the Create Reports screen opens. See *Create a Report* on page 12.

View Projects

- 1 Use 1 of the following methods to view projects.
 - ▶ Click the Projects toolbar icon .
 - ▶ From the Dashboard, click **View Projects**.
- 2 [Optional] Search for a project.
- 3 Select a project.
A summary of the project and associated reports appears in the side pane.

Edit a Project

- 1 In the Projects Table on the Dashboard, locate the project you want to edit, and then click **Edit**.
The Edit Project dialog opens.
- 2 Edit the project information as needed, and then click **Edit**.

Delete a Project

- 1 In the Projects table on the Dashboard, locate the project you want to delete, and then click **Delete**.
A confirmation dialog lists the associated reports that will be deleted.
- 2 [Optional] Click **View** to view a report.
The project is opened in the View Reports screen.



NOTE

Sequence Genotyper cancels the project deletion when the report is opened. If you still wish to delete the project after viewing the report, click the Home button to return to the dashboard and reselect the project.


- 3 Click **Delete**.

Targets

Sequence Genotyper includes a target file (*.csv) that provides coverage for ISAG Core, ISAG Additional, and Illumina Custom SNPs.

You can add your own custom target files to use in your projects. For information about creating and importing custom targets, see *Add a Custom Target File* on page 8.

View a Target File

- Use 1 of the following methods to view a target file.
 - Click the Targets toolbar icon .
 - From the Dashboard, click **View Targets**.
- [Optional] Search for a target file.
- Select a target file.
A summary of the file appears in the side pane.
- Click **View Targets**.
A table of target details is displayed.
- [Optional] Click a column name to sort in ascending or descending order.
- [Optional] Search for a specific target.



Target Table

Target tables contain the following information.

Column Heading	Description
Target ID	The target ID number
Target Name	The target name
Chr	The chromosome of the reference genome.
Position	The genomic coordinate of the variant on the reference chromosome.
Reference Allele	The reference allele.
Alternate Allele	The alternate allele.
Plus/Minus Strand	The genomic Plus/Minus strand.
TOP/BOT Strand	The TOP/BOT designation of the reference and alternate alleles. Refer to the Illumina TOP/BOT technote for information about this designation.
Type	SNP panel type. Types include the following: <ul style="list-style-type: none"> • ISAG Core—100 ISAG essential SNPs • ISAG Additional—100 ISAG additional SNPs • Custom—Additional disease-associated and inheritance SNPs

Add a Custom Target File

Sequence Genotyper includes a target template file that you can modify with your own data and import for use in your projects.

- 1 Open the target files template.
 - a Click the Targets toolbar icon .
 - b Click  **Open Target File Template**.

The template file opens.

- 2 Create your custom target file using the following steps.
 - a Add your sample data to the file. Do not add, delete, or move columns.
 - b Select **File | Save As** and save the *.csv file.
- 3 Click **Import Target File**.
- 4 Choose the strand type used in your target file.
 - ▶ Plus/Minus
 - ▶ Top/Bot
 - ▶ Both
- 5 Click **Browse**, select the target file you created, and then click **Open**.

The target file is added and is available to use in reports.

Samples

Samples are genome VCF files (*.genome.vcf) generated on a MiSeq. You can add multiple sample files to a project and generate a single report that includes all the samples in the project.

For information about generating sample files, refer to the documentation for your instrument and library kit .

To view the samples in your project, see *View Samples by Project* on page 10 and *View Samples by Report* on page 10.

Genome VCF Files




Genome VCF (gVCF) files are VCF v4.1 files that follow a set of conventions for representing all sites within the genome in a reasonably compact format. The gVCF files include all sites within the region of interest in a single file for each sample.

The gVCF file shows no-calls at positions with low coverage, or where a low-frequency variant (< 3%) occurs often enough (> 1%) that the position cannot be called to the reference. A genotype (GT) tag of *.* indicates a no-call.

For more information, see sites.google.com/site/gvcftools/home/about-gvcf.

Add Samples

Add sample files in the Create Reports screen. See *Create a Report* on page 12.

- 1 Click the Reports toolbar icon . The Projects dialog opens.
- 2 Select a project. Information about the selected project appears in the side pane.
- 3 Click **Add New Report**. The Create Reports screen opens.
- 4 Select sample files to upload using either of the following methods. Genome VCF files from the control sample will have a 0 call rate and should not be included.
 - ▶ Drag the file to the drag and drop area.
 - ▶ Click , browse to the file, and click **Open**.
- 5 [Optional] Click  **Refresh** to update the Previously Uploaded Samples list.



NOTE


You do not need to create a report immediately after adding samples. Uploaded samples are permanently added to the project and are automatically included in all future project reports.

View Samples


There are 2 ways to view samples.

- ▶ To view samples by project, see *View Samples by Project* on page 10.
- ▶ To view samples by report, see *View Samples by Report* on page 10.

View Samples by Project

- 1 Use 1 of the following methods to view samples:
 - ▶ Click the Samples toolbar icon .
 - ▶ From the Dashboard, click **View Samples**.
- 2 Select **Project** for the View Samples By option.
- 3 [Optional] Search a project name.
- 4 Select a project.
A summary of the project and associated reports appears in the side pane.
- 5 In the project summary, click **View Samples**.
- 6 [Optional] Search a sample name to filter the results.
- 7 [Optional] Click **Report ID** to view samples for the report.

View Samples by Report

- 1 Use 1 of the following methods to view samples:
 - ▶ Click the Samples toolbar icon .
 - ▶ From the Dashboard, click **View Samples**.
- 2 Select **Report** for the View Samples By option.
- 3 [Optional] Search a report name.
- 4 Select a report.
A summary of the report appears in the side pane.
- 5 In the report summary, click **View Samples**.
- 6 [Optional] Search a sample name to filter the results.

Samples Table

Heading	Description
Sample ID	The sample ID number.
Project ID	The project ID number.
Report ID	The report ID number. This column is only available when viewing samples by report.
Sample Name	The sample name.
SNP Count	The total number of targets in the target file. This column is only available when viewing samples by report.
GT Call Count	The total number of targets successfully called. This column is only available when viewing samples by report.
GT Call Rate	The percentage of targets successfully called. This column is only available when viewing samples by report.






Heading	Description
File Size	The sample file size.
Date	The date the sample file was last modified.

Reports


Sequence Genotyper compares one or more sample (*.genome.vcf) files against a target file and converts the results into a report of genotype calls.


You can view the data in Sequence Genotyper or in a report file (*.csv) that is saved locally when the data are generated.

Create a Report

- 1 Click the Reports toolbar icon .
The Projects dialog opens.
- 2 Select a project.
Information about the selected project appears in the side pane.
- 3 Click **Add New Report**.
The Create Reports screen opens.
- 4 Select a target from the Select Target list. Sequence Genotyper includes a target for Bovine Parentage. For information about creating additional target files, see *Add a Custom Target File* on page 8.
- 5 [Optional] Click **Open** to view the selected target in CSV format.
- 6 Select sample files to upload using either of the following methods. Genome VCF files from the control sample will have a 0 call rate and should not be included.
 - ▶ Drag the file to the drag and drop area.
 - ▶ Click , browse to the file, and click **Open**.
- 7 [Optional] Click  **Refresh** to update the Previously Uploaded Samples list.
- 8 After all samples have been added, click .
- 9 In the Report Name field, enter the report name.
- 10 Select a Strand type from the list.
- 11 Click  **Generate**.
Sequence Genotyper generates the report and opens the View Report screen. See *Report Table* on page 13.

View a Report

- 1 Use 1 of the following methods to view a report:
 - ▶ Click the Reports toolbar icon .
 - ▶ From the Dashboard, click **View Reports**.
- 2 [Optional] Search for a report.
- 3 Select a report.
Summary information about the selected report appears in the side pane.
- 4 Click **View Report**.
The View Report screen opens.
- 5 [Optional] Click the navigation arrows at the top of the list to scroll through pages.
- 6 [Optional] Sort and filter report content.

- ▶ To sort a column in ascending or descending order, click the column heading.
- ▶ To filter by calls, click the Filter icon  and select a filter.
 - ▶ **No Calls**—Data for this variant did not meet quality expectations. No call could be made.
 - ▶ **Unexpected Calls**—The nucleotide found at this location did not match the Reference or Alternate Allele
 - ▶ **Clear filter**—Clears the filters
- ▶ To filter by target, enter any part of the Target name in the Search field. Clear the search field to remove the filter.

Report Summary

The Report Summary lists information about each report.

- ▶ **Report ID**—The report ID number.
- ▶ **Report Name**—The name of the report.
- ▶ **Strand Type**—The strand type (Top/Bot or Plus/Minus) used in the report.
- ▶ **Sample Count**—The number of samples in the report.
- ▶ **Target Count**—The number of targets in the report.
- ▶ **Last Modified Date**—The date the report was last modified.
- ▶ **Total Failed Calls**—(Number of failed calls)/(Total number of calls).
- ▶ **Call Rate**—The percentage of targets successfully called.

Report Table

The Sequence Genotyper Report lists the genotypes and call rates for each sample in the project.

Below the table, the call rate % by sample bar chart shows the call rate for each sample. The precise call rate is displayed by mousing over the bar chart, and is listed in the report table.

Column Heading	Description
Target	Target name.
Chr	The chromosome of the reference genome.
Coordinate	The genomic coordinate of the variant on the reference chromosome.
Ref Allele	The reference allele.
Alternate Allele	The alternate allele.
Call Rate	The percent of samples with successful genotype calls for the given target.
Sample	The Sample column displays the following: <ul style="list-style-type: none"> • Sample name • Detected genotype call, no calls, or unexpected calls

Open a Report File

Sequence Genotyper creates 2 report (*.csv) files that are saved locally when the report is generated.

- 1 In the View Reports window, click **Open Report**.

- 2 Select a report type.
 - ▶ **Matrix Report**—The matrix report includes additional variant call QC metrics that originate from the genome vcf files.
 - ▶ **Simple Report**—Includes only Allele1 and Allele2 for each sample and target.
- 3 Click **Open**.
The selected report (*.csv) file opens.

Matrix Report

The matrix report includes variant, chromosome, position, reference allele, and alt allele columns. Additional variant call metrics originating from the genome vcf files are included for each sample.

Table 1 Matrix Report Additional Fields

Heading	Description
GenoType	The genotype report in reference to either the Plus strand or the Top strand.
Original Genotype	The genotype reported in genome VCF files.
Filters	If all filters are passed, PASS is written in the filter column. <ul style="list-style-type: none"> • LowGQX—The genotyping quality (GQ) is below a cutoff. • LowQual—The variant quality (QUAL) is below a cutoff. • LowVariantFreq—The variant frequency is less than the given threshold.
DP	The depth (number of base calls aligned to a position and used in variant calling).
VF	The variant frequency (percentage of reads supporting the alternate allele).
GQX	Genotype quality value.

Revision History

Document	Date	Description of Change
Document # 1000000012396 v00	July 2016	Initial release.

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 2 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 3 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com