VEROGEN

Universal Analysis Software Imagen Module

Version 2

Reference Guide

Revision History

Document #	Date	Description of Change
VD2022014 Rev. A	August 2022	Initial release of the Universal Analysis Software – Imagen Module

Table of Contents

Software Overview

Introduction

Universal Analysis Software (UAS v2) is a forensic genomics solution for run setup, sample management, data analysis, and reporting. Pre-installed on a dedicated server, the software integrates with the MiSeq FGx® Sequencing System to automatically generate FASTQ files and perform alignment from run data.

This reference guide provides details on the analysis of data generated from the ForenSeq Imagen kit using the Imagen and Imagen Geo analysis methods.

Supported Assays and Analysis Methods

The ForenSeq Imagen kit supports two distinct assay types, each with a corresponding analysis method in UAS:

- **ForenSeq Imagen** — Capable of predicting externally visible phenotypes.
- **ForenSeq Imagen Geo** — Capable of predicting externally visible phenotypes and estimating biogeographical ancestry.

Analysis Workflow

The following diagram outlines the analysis workflow, which includes UAS and two applications that run on the MiSeq FGx System: MiSeq FGx Control Software (MFCS) and Real-Time Analysis (RTA). For more information on these applications, see the *MiSeq FGx Sequencing System Reference Guide (document # VD2018006)*.

Figure 1   Overview of data analysis steps

1 Sign In or Create Account
The user enters a user name and password to access the software.

2 Set Up a Run
The user manually inputs sample information or imports a .txt file.

3 Sequence Libraries
The control software images the flow cell and RTA performs image analysis and base calling.

4 Analyze Data
The software uses data from RTA to align reads and make variant calls with quality indicators. The user reviews the results in the software.

5 Generate Reports
The user generates reports on the analysis results in a variety of formats.

UAS v2   MiSeq FGx System

Access the Software

UAS is a browser-based application that does not require an internet connection. Open a web browser (Chrome is recommended) on a computer with access to the same network as the MiSeq FGx System. Set screen resolution to at least 992 pixels for optimal display.

To access the UAS user interface, enter the server address in the web browser address bar. If the Domain Name System (DNS) does not list the server, enter the server IP address for the network.

Create an Account

Before using UAS, you must create an account. The first user to create an account automatically becomes the system administrator and is assigned administrative privileges. The administrator is responsible for approving new accounts. For instructions, see *Enable and Disable Accounts* (page 47).

Lost passwords are not recoverable, so creating a second administrator account is highly recommended. If the administrator password is lost and only one administrator account exists, the software must be uninstalled and reinstalled.

1. Access the network server through a web browser.
2. On the Login page, select **Register as a new user**.
3. On the Register page, complete the following fields:
   - **Email** — Enter an email address to be the username for the account.
   - **Password** — Enter a password for the account.
   - **Confirm password** — Reenter the password for the account.
4. Select **Register** to submit your account for approval from the system administrator.

   A confirmation message appears. You can access the software after approval.

Sign In or Sign Out

1. Sign in as follows.
   a. Access the network server through a web browser.
   b. Enter your email address (username) and password.
   c. Select **Login**.

   The software opens to the Home page.
2. When ready to sign out, select 🧑 **Account**, and then select **Logout**.

Change a Password

1. On the navigation rail, select 🧑 **Account**.
2. Select **Change Password**.
3. In the Current Password field, enter your password.
4. In the New Password field, enter a new password.
5. In the Confirm Password field, repeat the new password.
6. Select **Save** to apply the new password.

Primary Workspaces and Navigation

The following pages comprise the primary workspaces in UAS. For a detailed overview of each workspace, see Chapter 2. Workspaces.

- **Home** — View and create runs and projects. This page functions as a system dashboard.
- ▦ **Runs** — Set up and manage runs before and during a run.
- ▦ **Projects** — View and manage projects and samples after a run and generate reports.
- **Results** — Review data on a sample-by-sample basis after a run.

A vertical navigation rail appears on the left side of each page in the software. Icons on the navigation rail allow you to open other workspaces, manage settings, or search for a run, project, or sample by name or date.

The software also includes commenting, history, and other functions that supplement run setup, data analysis, and reporting. For instructions on using these supplementary features, see Chapter 6. Run, Project, and Sample Management.

Software Status

The software displays a state for runs and analyses. The state determines which modifications are permitted and are also useful for general status updates.

Run State

After a run is created and saved, you can view the run state by selecting the ⓘ Run Info icon in the ▣ Runs workspace. The following table describes each possible state.

Table 1   Run states

State	Description
Created	The run information is saved in UAS.
Sequencing	The run is in process on the MiSeq FGx System.
Completed	The run completed successfully.
Errored	An error prevented the run from finishing, or it was manually stopped.
Paused	A user suspended the run.
Stopped	A user ended the run due to incorrect setup, poor data quality, or other issue.

Analysis State

After a sample is added to a run, you can view the analysis state by selecting the ⓘ Sample Info icon on the Results page. The following table describes each possible state.

Table 2   Analysis states

Symbol	Retain Status	Description
▶	Created	UAS has not started analysis.
🕐	Queued	The analysis is in line to start.
🔄	Processing	Analysis or reanalysis is in progress.
✔	Completed	Analysis has successfully completed.
⚠	Errored	Analysis has unsuccessfully completed.

Glossary

Term	Description
Amplicon	The product of PCR amplification of a targeted region of interest in a genome.
Amplicon end position	The last base position of an amplicon, including the PCR primer binding sequence.

Term	Description
Amplicon start position	The first base position of an amplicon, including the PCR primer binding sequence.
Analysis method	Settings and thresholds that inform the analysis of sequencing data to generate allele calls and QC indicators.
Analytical threshold (AT)	The percentage that a read count must reach for the software to call a base or type an allele. Signal below the AT might be visible but is not typed.
Assay type	The library prep kit used to process samples for sequencing and analysis.
Call	The reported base for a position (coordinate) based on the analysis method.
Cluster density	The number of thousands of clusters on a flow cell per square millimeter (K/mm²).
Clusters	A clonal group of DNA bound to the surface of a flow cell that produces one read. One template DNA strand seeds each cluster and is clonally amplified using bridge amplification until the cluster has about 1000 copies.
Clusters passing filter	The percentage of clusters that pass quality filters. For more information, see *Clusters Passing Filter* (page 55).
Coverage	The average number of reads for a locus or allele in a sample that align to or cover known reference bases. Sequencing coverage level (referred to as *read depth*, *read count*, or *intensity* in the software) can help determine whether an allele call is made with confidence.
Cycle	A chemistry step and an imaging step to call one base in a sequencing run. For cycle numbers, see *Cycles per Read* (page 57).
Effective Analytical	The number of reads (intensity) needed to meet the analytical threshold.
Effective Interpretation	The number of reads (intensity) needed to meet the interpretation threshold.
Flow cell	A glass slide coated with oligos complementary to the library adapter sequences, allowing the library to adhere for a run.
Forward primer length	The number of bases in the forward PCR primer (oligonucleotide).
Forward primer start coordinate	The starting base at the 5' end of the forward PCR primer. The base depends on the amplicon and analysis method.
Human Sequencing Control (HSC)	A pre-amplified and tagged positive control added to a library pool to facilitate troubleshooting of instrument and sequencing reagent issues.
Index adapter sequence	A short stretch of oligonucleotides added to each sample during library prep for identification purposes. The software uses the oligonucleotide sequences to demultiplex pooled libraries.
Intensity	The software reports signal intensity as the number of reads.
Intralocus balance	The balance of read counts between typed alleles at a heterozygous locus. The balance is measured as the intensity of the minimum intensity typed allele divided by the intensity of the maximum intensity typed allele. When intensities are identical, the intralocus balance is 100%.
Interpretation threshold (IT)	The percentage that determines the read count that must be reached for the software to call a base or type an allele. Signal below the IT but greater than or equal to the AT can be manually typed.

Term	Description
Library	A DNA sample that uses primers and adapters to litigate specific regions of the genome for sequencing.
Minimum read count	The minimum read count a position must reach for a base to be called.
N indicator	The quality indicator for reagent blanks and negative amplification controls.
Negative amplification control	A sample that does not contain DNA as a template for amplification.
No call	The call assigned when a position lacks read counts for a specific base.
Original call	An unmodified allele call based on the run analysis method.
P indicator	The quality indicator for positive amplification controls that use control DNA.
Phasing	The percentage of bases in a cluster that fall behind the current cycle in Read 1. For details, see *Phasing and Pre-phasing* (page 55).
Plexity	The number of libraries combined in a pool for sequencing. For example, if eight libraries are combined in a pool, the plexity is eight.
Positive amplification control	A known sequence of DNA provided in the library prep kit and prepared with forensic and reference samples to ensure that library prep and sequencing reagents are functional.
Prephasing	The percentage of bases in a cluster that jump ahead of the current cycle in Read 1. For details, see *Phasing and Pre-phasing* (page 55).
Project	A collection of analyzed results for at least one sample, and the primary workspace for viewing and modifying results and creating reports.
Read	The sequence string of A, T, C, and G bases corresponding to an amplicon. A run can simultaneously generate millions of reads from multiple loci and multiple samples.
Reagent blank	An extraction control without DNA that is carried through the entire sample processing.
Read 1 (i5) primer sequence	The sequence of the PCR primer that is tagged with the Index 2 (i5) adapter sequence. Index 2 is part of the adapter sequence, as is the binding site for the Read 1 sequencing primer.
Read 2 (i7) primer sequence	The sequence of the PCR primer that is tagged with the Index 1 (i7) adapter sequence. Index 1 is part of the adapter sequence, as is the binding site for the Read 2 sequencing primer.
Reagent cartridge kit lot	The batch code to identify the batch or lot that a component of the MiSeq FGx Reagent Kit or MiSeq FGx Reagent Micro Kit was manufactured in.
Reanalysis	The process of reanalyzing a sample with another analysis method, after the original run analysis method.
Reference SNP ID number (rsID)	An identification tag the National Center for Biotechnology Information (NCBI) assigns to a group of SNPs that map to the same location.
Reverse primer length	The number of bases in the reverse PCR primer (oligonucleotide).
Reverse primer start coordinate	The starting base at the 5' end of the reverse PCR primer. The base depends on the amplicon and analysis method.
Run	The process of clustering and sequencing libraries to generate base calls.

Term	Description
Run analysis method	The analysis method assigned to a sample for the initial run analysis.
Sample	The DNA input for a library prep kit, before the addition of primers and adapters.
Sample plate	The 96-well plate that contains libraries for sequencing.
Sample representation	The number of reads per sample for a run. These data are available after sequencing and analysis are complete.
Sample type	The function of a sample: forensic or reference sample, positive amplification control (NA24385), negative amplification control, and reagent blank.
Sample well position	The column and row position in a 96-well plate containing samples or libraries. For example, position A1 indicates a well at the intersection of row A and column 1.
Single-nucleotide polymorphism (SNP)	Variation of one base pair in a DNA sequence relative to a known DNA reference sequence.
System event	An action the software automatically performs and records.
Typed	An allele call that contributes to the locus genotype.
Unique Dual Index (UDI)	Premixed Index 1 (i7) and Index 2 (i5) adapters for dual index combinations that have no redundant sequences.
Untyped	A no-call allele that does not contribute to the locus genotype.
User action	A user interaction with the software, such as adding a sample to a run.
User-modified call	A base call that a user changed.

Workspaces

Home Page

The **Home** page displays information and functions organized into runs and projects. Selecting a run opens the
Runs workspace, and selecting a project opens the ■ **Projects** workspace. Color-coded Quality Metrics icons next
to each run provide an overall view of run performance; hovering over an icon displays detailed metrics.

Figure 2   Overview of the Home page
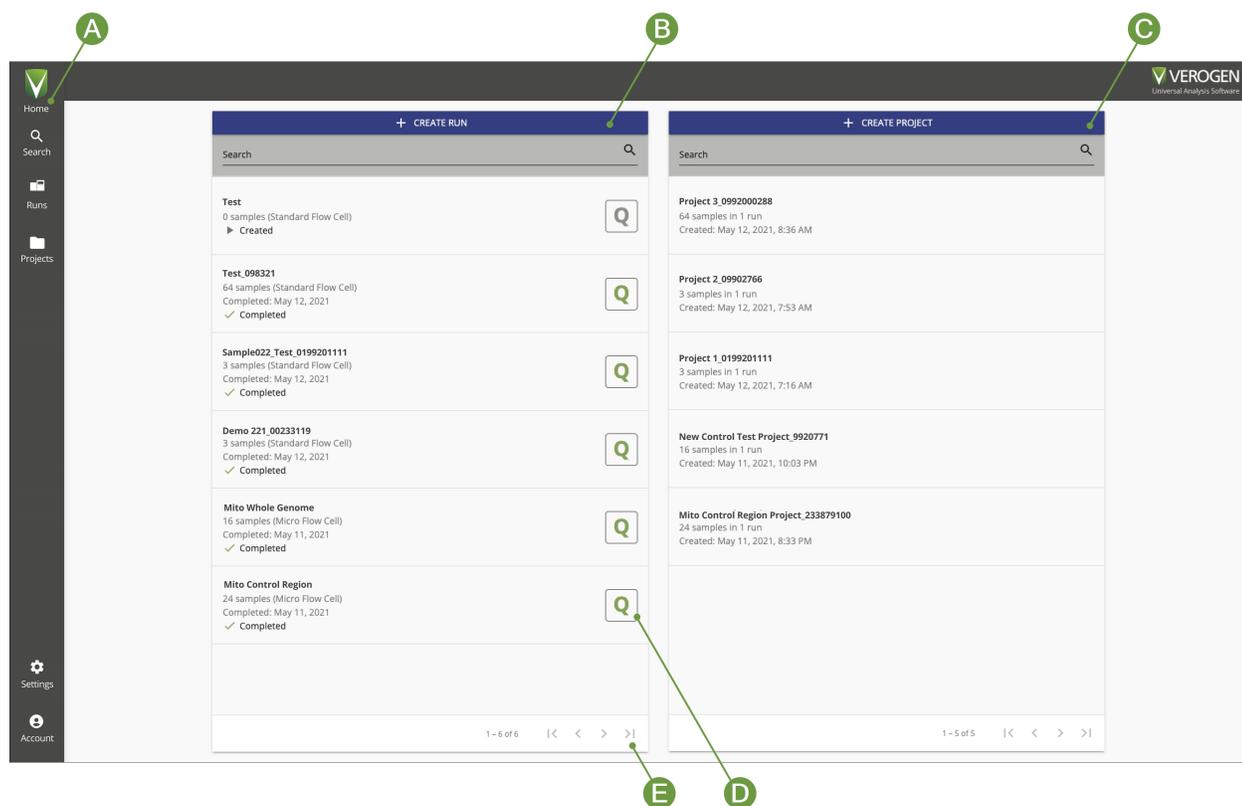
A   **Navigation rail — Icons to open workspaces, search, or manage settings**
B   **Runs — List of runs with quality metrics and options to search and create**
C   **Projects — List of projects with options to search and create**
D   **Quality Metrics icon — Quality metrics for a completed run**
E   **Scroll arrows — Move through all runs or projects**

Runs

The Runs workspace provides tools to create and monitor sequencing runs, along with tools to input and organize sample information.

Selecting ▮▯ **Runs** on the navigation rail opens the Runs navigation drawer, from which you can create a new run or open an existing run. Runs are listed from newest to oldest, with the run state, the number of samples in the run, the flow cell type, and a color-coded Quality Metrics icon; hovering over an icon displays detailed metrics. Use the search bar to find a run by name, or use the arrows at the bottom of the drawer to scroll through the complete list of runs.

Creating or opening a run opens the full Runs workspace, which includes a header bar, a Run Metrics page, and a Sample List page. (By default, a run opens to the Sample List page.)

Figure 3   Overview of the Runs workspace
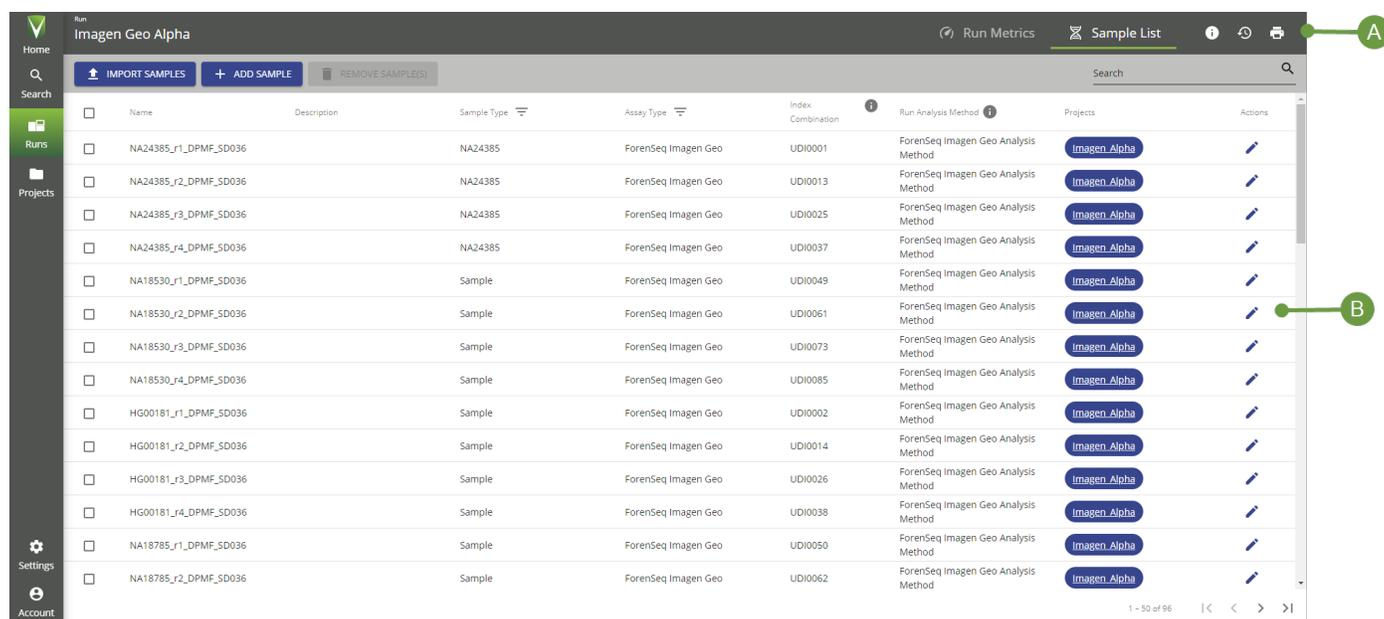
A   **Header bar — Includes icons to navigate to the Run Metrics and Sample List pages, view Run Info or Run Activity, or convert run details to a printable format.**
B   **Main workspace — Displays contents of the currently open page. (Here, the Sample List page is shown.)**

The following sections provide detailed information about each of these features. For more information about runs, see Chapter 3. Run Setup and Chapter 6. Run, Project, and Sample Management.

> Throughout the software, selecting a run name hyperlink takes you to the Sample List page for that run.

Runs Header Bar

The ▮▯ **Runs** header bar includes the following icons:

Table 3   Runs header bar icons

Icon	Name	Function
	Run Metrics	Open the Run Metrics page, from which you can view quality metrics and sample representation data for a run. See *Run Metrics Page (Runs)* (below).
	Sample List	Open the Sample List page for a run. See *Sample List Page (Runs)* (below).
	Run Info	Edit the run and view the run state, cycles per read, and other run information.
	Run Activity	See system events and user actions for the run and add comments. This icon is visible when the history setting is enabled. See *System Settings* (page 48).
	Print	Print the Sample List and Run Metrics pages for a run.

Run Metrics Page (Runs)

This section describes the Run Metrics page as it appears in the ▉▉ **Runs** workspace. A Run Metrics page with additional features is also available in the ▉ **Projects** workspace.

The Run Metrics page displays run quality metrics and sample representation data, providing an overall view of run performance. Quality metrics are not available until sequencing begins; sample representation data are not available until a run completes analysis.

For more information about these metrics, see Appendix A. Run Metrics.

Sample List Page (Runs)

This section describes the Sample List page as it appears in the ▉▉ **Runs** workspace. A Sample List page with different features is also available in the ▉ **Projects** workspace.

The Sample List page displays information in a table about each sample in a selected run. Functions for importing, adding, searching, and deleting samples appear above the table.

You can sort the table of samples by name, description, or index combination, and you can filter samples by sample type or assay type. To see filter options and apply or close filters, select the ═ **Filter** icon next to a column name. Use the arrows at the bottom of the table to scroll through the complete list of samples.

To edit sample details, select the ✎ **Edit** icon in the Actions column. Not all fields can be edited.

Printing

The 🖶 **Print** icon in the header bar converts the Sample List page and Run Metrics page for a run into a printable format.

1. Open a run from the ▉▉ **Runs** navigation drawer, then select 🖶 **Print**.
    The software displays the Sample List and Run Metrics pages in a combined, printable view.
2. Select **Print** to open the Print window.

3. Choose printing options and print.
4. Select **Back to Run**.

Projects

The Projects workspace is the post-sequencing workspace for creating and managing runs and samples, accessing analysis results, and generating reports.

Selecting ▣ **Projects** on the navigation rail opens the Projects navigation drawer, from which you can create a new project or open an existing project. Projects are listed from newest to oldest. Use the search bar to find a project by name, or use the arrows at the bottom of the drawer to scroll through the complete list of projects.

Creating or opening a project opens the full Projects workspace, which includes a header bar, a sidebar, a Run Metrics page, a Sample List page, and a Reports page. (By default, a project opens to the Run Metrics page.)

Figure 4 Overview of the Projects workspace
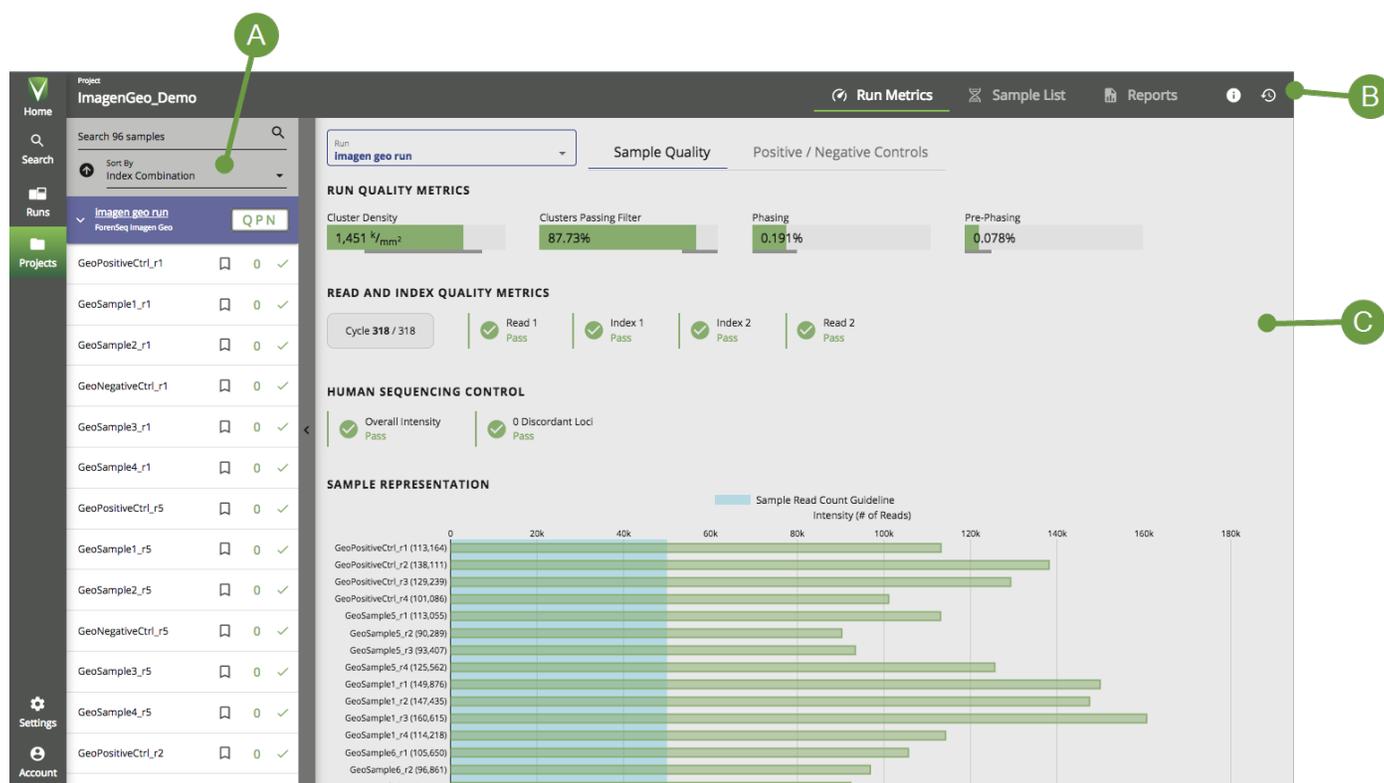
A **Sidebar** — Lists all samples in the project, grouped by run. Can be collapsed.
B **Header bar** — Includes icons to navigate to the Run Metrics, Sample List, and Reports pages, or view Project Info or Activity.
C **Main workspace** — Displays contents of the currently open page. (Here, the Run Metrics page is shown.)

The following sections provide detailed information about each of these features. For more information about projects, see Chapter 6. Run, Project, and Sample Management.

Projects Header Bar

The ▪ **Projects** header bar includes the following icons:

Table 4   Projects header bar icons

Icon	Name	Function
	Run Metrics	Open the Run Metrics page, from which you can view quality metrics, sample representation data, and positive and negative control data for runs in the project. See *Run Metrics Page (Projects)* (below).
	Sample List	Open the Sample List page for the project. See *Sample List Page (Projects)* (next page).
	Reports	Open the Reports page for the project. See *Reports Page* (page 20).
	Project Info	Edit the project name and description, and view other information about the project.
	Activity	See system events and user actions for the project and add comments. This icon is visible when the history setting is enabled. See *System Settings* (page 48).

Projects Sidebar

The ▪ **Projects** sidebar lists all samples in the selected project, grouped by run. To show or hide the samples in a run, select the arrow next to the run name. Selecting a run name opens the Sample List page for that run in the ▪ **Runs** workspace.

> A color-coded **QPN** icon next to each run provides an overall view of run performance; selecting the icon opens the Run Metrics page for the run. For more information, see Appendix A. Run Metrics.

Each listed sample shows the sample name, a button to bookmark the sample, the number of QC indicators flagged, and the analysis state. A checkmark indicates the analysis has completed; hover over checkmark to see the completion date.

To navigate through the list of samples:

· Use the search bar to find a sample by name.
· By default, samples are sorted by index combination. Use the **Sort By** drop-down menu to sort samples by a different characteristic. For more information about these sample characteristics, see *Sorting and Filtering Samples* (next page).

The sidebar is collapsible. To close or reopen the sidebar, select the vertical button located to the right of the sidebar.

Run Metrics Page (Projects)

> This section describes the Run Metrics page as it appears in the ▪ **Projects** workspace. A Run Metrics page with more limited features is also available in the ▪ **Runs** workspace.

The Run Metrics page displays metrics that provide an overall view of run performance. To access the page, select the icon on the Projects header bar, or select the **QPN** button next to a run in the Projects sidebar.

The page is divided into two tabs: **Sample Quality**, which includes quality metrics and sample representation data, and **Positive / Negative Controls**. To view metrics for a different run, select a run from the **Run** drop-down menu or the Projects sidebar.

For more information about these metrics, see Appendix A. Run Metrics.

Sample List Page (Projects)

This section describes the Sample List page as it appears in the █ **Projects** workspace. A Sample List page with different features is also available in the ▆ **Runs** workspace.

The Sample List page displays information in a table about each sample in a selected project. Functions for adding, copying, reanalyzing, and searching samples, as well as generating reports (see Chapter 5. Reports), appear above the table. A bookmarking function is available within the table.

The table includes the following information about each sample:

Table 5   Sample characteristics

Column name	Definition
Sample / Run	The name of the sample, followed by the run the sample is from. You can sort by sample name, or filter by run.
Bookmark	User-applied bookmarks to mark specific samples. All samples are either bookmarked or unbookmarked. See Bookmarking Samples (next page).
Analysis Method	The analysis method applied to the sample. By default, previous analyses are hidden; to view samples from all previous analyses, close this filter option.
Sample Type	The function of the sample: forensic or reference sample, positive amplification control, negative amplification control, or reagent blank.
Assay Type	The library prep kit used to process the sample for sequencing and analysis.
QC Indicators	The number of quality control (QC) indicators for a sample. Samples with QC indicators may require further review. Hovering over a QC number reveals a pop-up window showing the number of QC indicators of each type that were flagged. See Quality Control Indicators (page 23).
Biological Sex	The biological sex of the sample contributor, as estimated by Y-SNPs. Possible values are *XX*, *XY*, or *inconclusive*. See Biological Sex Estimation for Samples (page 63).
Contributor Status	Whether the sample comes from one contributor or multiple contributors. Possible values are *single-source*, *mixture*, or *inconclusive*. See Contributor Status of Samples (page 63).

Sorting and Filtering Samples

You can sort or filter the table of samples by any of the characteristics listed above.

· To sort by a particular column, select that column name. An arrow appears indicating whether the sort is ascending or descending.

· To see filter options and apply or close filters, select the ▾ **Filter** icon next to a column name.

- To close all currently applied filters, select **Reset Filters** at the top of the sample list.

Use the arrows at the bottom of the table to scroll through the complete list of samples.

Bookmarking Samples

Bookmarks can be added by a user to designate specific samples of interest. Once you have bookmarked one or more samples, you can easily apply an action to that group of samples on the Sample List page, such as creating a custom project report, reanalyzing the samples, or copying the samples to another project.

A bookmark can be added from the Sample List page, the sample sidebar, or the Results page for a specific sample. When you bookmark a sample, it moves to the top of the Sample List page. All bookmarks remain marked until they are manually unselected, even if you navigate away and later return to a project.

Reports Page

The Reports page lists all previously generated reports for a project, organized by creation date starting with the most recent report. To further organize results, you can sort by the Date Created and File Name columns or filter the Report Type column.

To download one or more reports, select the checkboxes for those reports, then select **Download**. If you selected multiple reports, the software downloads those reports as a zipped file.

For more information about reports, see Chapter 5. Reports.

Results Page

Selecting a sample within a project opens the Results page. Each Results page is a sample-specific workspace for in-depth analysis of results.

The Results page for Imagen samples includes the following features:

Figure 5 Overview of the Results page
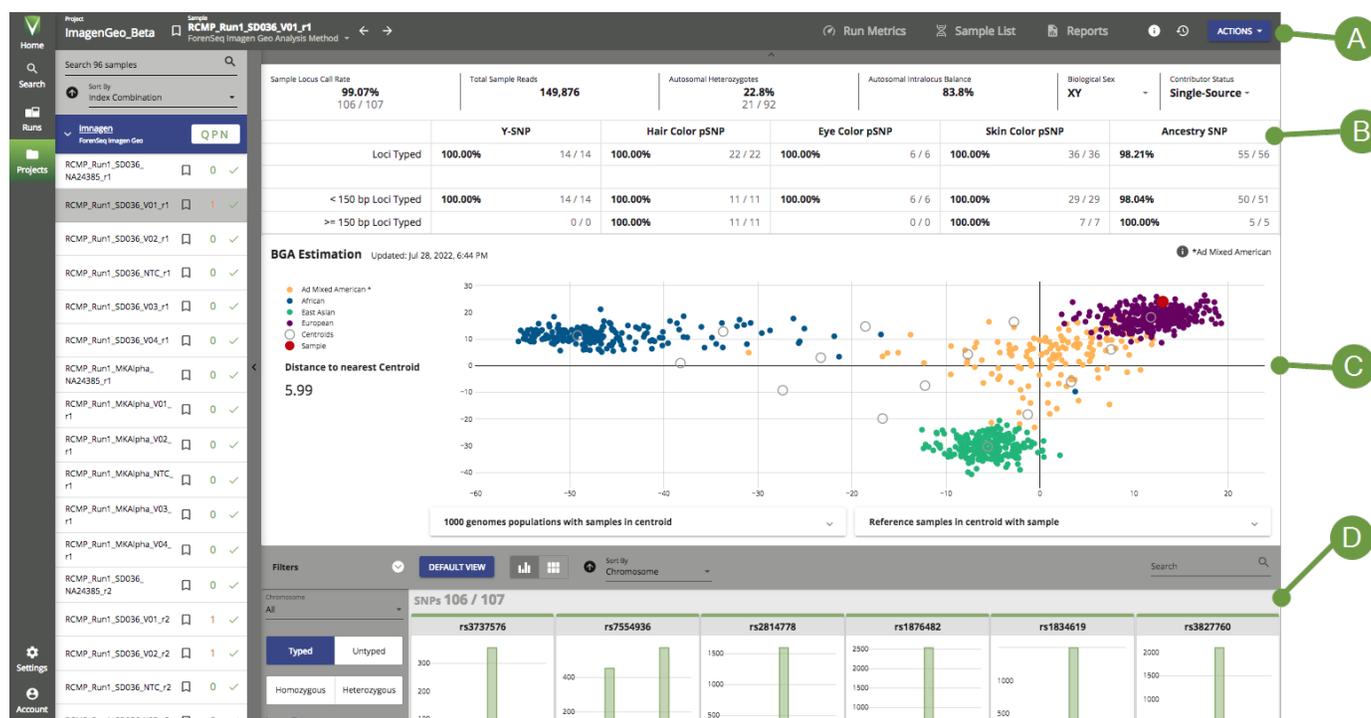
A **Header bar** — Similar to the Projects header bar, with additional features.
B **Sample Overview** — Basic information about the sample.
C **BGA Estimation** — A plot showing estimates of biogeographical ancestry. (Appears only for ForenSeq Imagen Geo samples.)
D **Sample Details** — Detailed information about each locus in the sample.

The following sections provide detailed information about each of these features. For more information about results, see *Review Results* (page 30) and *Modify Results* (page 31) in Chapter 4. Data Analysis.

Results Header Bar

The header bar of a Results page is similar to the Projects header bar, with the following additional or modified features. (See *Projects Header Bar* (page 18) for comparison.)

- In addition to the project name, the header bar shows the sample name and the analysis method applied to the sample. Use the arrows to navigate through the sample list and view results for other samples in the project.

> For samples that have been analyzed multiple times, you may view results from a different analysis by selecting it from the drop-down menu next to the analysis method name. Analyses are listed in chronological order, with the most recent first. See *Reanalyze Samples* (page 32).

- Selecting the ▢ Bookmark icon applies a bookmark to the sample. See *Bookmarking Samples* (previous page).
- The ⓘ Sample Info dialog box displays information such as sample type, analysis state, and analysis folder path.
- The 🕘 Activity dialog box displays system events and user actions for the sample, as well as the project.

- A blue **Actions** button provides functions for generating reports, reanalyzing the sample, or copying the sample to another project.

Sample Overview

The Sample Overview section of an Imagen Results page displays basic information about the sample, including the locus call rate, total number of reads, intralocus balance metrics, biological sex, and contributor status. You may edit the biological sex or contributor status of the sample. Under these data, a table displays the percentage and total number of typed loci relative to base pair length for each SNP type.

The overview section is collapsible. To close or reopen the section, select the horizontal button located above the section.

BGA Estimation

> This section of the Results page appears only for samples prepared with the ForenSeq Imagen Geo library prep method and sequenced using the Imagen Geo workflow in MiSeq FGx®.

The BGA Estimation section of an Imagen Results page displays a plot showing estimates of the biogeographical ancestry of the sample based on aSNP and pSNP data. An identical plot appears in the BGA Estimation report. Tables below the plot can be expanded to show detailed information about samples included in the centroid.

For more information, see *BGA Estimation Report* (page 37) and *Biogeographical Ancestry Estimation* (page 62).

Sample Details

The Sample Details section of an Imagen Results page displays detailed information about each locus in the sample.

Loci are displayed in a grid. You may toggle between two view options:

- In Histogram view, each locus appears as a histogram showing the intensity of each allele relative to the analytical threshold.
- In Card view, each locus appears as a card showing the intensity of each allele as a number, along with the intralocus balance ("ILB"). Alleles are color-coded: A is green, T is red, C is blue, and G is gray.

Imagen sample results include data for the following SNP types:

Table 6   Imagen SNP types

SNP Type	Description
Y-SNP	Informs lineage and estimates biological sex.
Ancestry SNP (aSNP)	Estimates biogeographical ancestry.
Eye Color pSNP	Estimates eye color.
Hair Color pSNP	Estimates hair color.
Skin Color pSNP	Estimates skin color.

Locus Details

Selecting a locus card or histogram opens the Locus Details pane, providing additional information about the locus:

- Thresholds and intralocus balance appear at the bottom of the pane.
- A toggle button indicates whether each allele is typed.
- Any quality control (QC) indicators, if applicable, appear above the expanded histogram to the left of the pane. See *Quality Control Indicators* (below).
- A User Actions tab lists system events and user actions performed on the locus, from newest to oldest. You may also add comments to the locus.

The **Previous Locus** and **Next Locus** arrows allow you to scroll through the Locus Details panes for other loci. To close the Locus Details pane, select ✖ **Close**.

Sorting and Filtering Loci

You can sort and filter loci using the options at the top of the Sample Details section and in the Filters sidebar. For more information about these options, see *Review Results* (page 30). The Filters sidebar is collapsible.

Use the search bar to find a locus by name. Select **Default View** to clear selected sort, filter, and search options and restore defaults.

Quality Control Indicators

Each locus card or histogram is color-coded to denote quality control (QC) indicators. A horizontal green bar (or turquoise in Dark Mode) denotes a locus with no QC indicators, while an orange bar denotes a locus with at least one QC indicator. Any applicable indicators are shown above the enlarged histogram next to the Locus Details pane. Any applicable indicators are displayed on the card.

Possible QC indicators include the following:

Table 7   QC indicators

Symbol	Name	Description
	User Modified	Someone edited the typed status of an allele at the locus.
	Allele Count	The locus has more typed alleles than expected for a single-source sample of the indicated biological sex.
	Unexpected Allele	The locus has an unexpected allele (non-reference or alternate) that is greater than or equal to the AT.
	Imbalanced	The locus is not homozygous, not hemizygous, and falls below the intralocus balance setting.
	Analytical Threshold	The locus has expected allele signal below the AT and no alleles greater than or equal to the IT.
	Interpretation Threshold	The locus has expected allele signal greater than or equal to the AT, but below IT.
	Not Detected	The software did not detect signal for the locus.
	Detected	An unexpected locus was typed, e.g., an NTC with typed loci or a XX (Female) sample types Y STRs above the threshold.

Run Setup

Create a Run

1. On the Home page, select **Create Run**.
2. In the Create Run dialog box, complete the following fields. (Required fields are indicated on-screen with an asterisk *.)
   - **Run Name** — Enter a preferred name to identify the run.
   - **Description** — Enter a description of the run.
   - **Flow Cell Type** — Select **Standard** or **Micro**, depending on your reagent kit.
   - **Reagent Cartridge Kit Lot #** — Enter the LOT number printed on the reagent cartridge packaging.
3. Select **Save** to create the run.
   The Sample List page opens.

Edit a Run

1. On the navigation rail, select ▊ **Runs**. Then select a run.
2. Select ⓘ **Information**, and then select ✏ **Edit**.
3. In the Edit Run dialog box:
   - Edit the run name.
   - Add or modify the optional run description.
   - Add or modify the optional reagent cartridge kit lot number.
   Editing the flow cell type requires a run state of Created.
4. Select **Save**.

Add Samples to a Run

Add samples and sample information to a run either by importing a sample sheet or by adding individual samples. After samples are added, the Sample List page in the ▊ **Runs** workspace lists all samples included in the run.

All samples in a run must have the same assay type.

When adding samples to a run, you associate each sample with at least one project. If you enter the name of a new project, the software creates the project. Because a sample can be assigned to multiple projects, a run can include samples from multiple projects.

Import a Sample Sheet

1. On the navigation rail, select ▊▊ **Runs**. Then select a run.
2. Select **Import Samples**.
3. [Optional] Select **Sample Sheet Template** to download a .txt file that provides examples of Sample Sheet formats for each Assay format.
4. Select **Drop Files Here**, and then navigate to the sample sheet location.
5. Select the sample sheet (.txt file), and then select **Open**.

   The sample sheet appears under the file upload area. Uploading another .txt file overrides the current file because the software accepts only one sample sheet per run.
6. Select **Next**.
7. Review the information from the uploaded sample sheet to ensure accuracy. Scroll to see all entries. If utilizing the template, make sure to delete the sections pertaining to assay types that are not utilized in the current analysis.
8. Select **Import** to add the samples to the run.

Assigning Unique Dual Indexes

The sample sheet template includes two columns for entering index adapter names: Index 1 (i7 index) and Index 2 (i5 index).

Enter the Unique Dual Index (UDI) name in the Index 1 column or both columns.

Assigning Projects

The Initial Project List column of a sample sheet specifies which project a sample is assigned to. To assign multiple projects to a sample, enter each project name separated by a comma followed by a space and put quotes around the entry. For example:

```
"Project 1, Project 2, Project 3"
```

Add Individual Samples

1. On the navigation rail, select ▊▊ **Runs**. Then select a run.
2. Select **Add Sample**.
3. In the Add Sample dialog box, complete the following fields (Required fields are indicated on-screen with an asterisk *.)

Field	Instruction
Sample Name	Enter a unique identifier for the sample. Each sample name must be unique in UAS, regardless of sample type.
Description	Enter a preferred description of the sample.
Assay Type*	Select the library prep method: • **ForenSeq Imagen** and **ForenSeq Imagen Geo** — Libraries prepared with the ForenSeq Imagen Kit.

Field	Instruction
Sample Type	Select a sample type: • **Sample** — A forensic or reference sample. • **NA24385** — The positive control. • **Negative Amplification Control** — The negative control (water). • **Reagent Blank** — The extraction control.
Index Combination	Select the name of a premixed i7 and i5 index adapter (UDI).
Run Analysis Method	Select a method for analyzing samples in the run.
Initial Projects	Enter the name of a project to assign the sample to. To assign the sample to multiple projects, enter multiple project names.
Sample Plate	Enter a preferred name for the 96-well plate.
Sample Well Position	Enter the position of the plate well the sample occupies.

* Because the software allows one assay type per run, the assay type assigned to the first sample is automatically assigned to the others.

4. Select **Add** to add the sample to the run.

Manage Samples in a Run

After adding samples to a run, you can edit sample information, change project assignments, or remove samples from the run. Use filters, sorting, and the search function to quickly find samples.

Find a Sample

1. On the navigation rail, select **Runs**. Then select a run.
2. To search for samples, enter any of the following information in the Search field:
    - Sample name
    - Description
    - Index adapter name
    - Run analysis method
3. To filter the samples, do as follows.
    a. In the Sample Type or Assay Type column, select **Filter**.
    b. Select at least one checkbox.
    c. Reselect **Filter** to apply the selected filters.
4. To sort the samples, select a column heading.
    The Name, Description, and Index Combination columns are sortable.

Edit Sample Information

1. On the navigation rail, select **Runs**. Then select a run.
2. In the Actions column, select **Edit**.
3. In the Edit Sample dialog box, modify the desired fields.

Field	Instruction
Sample Name	Edit the unique identifier for the sample.
Description	Enter or edit a preferred description of the sample.
Assay Type*	Select a different library prep method: • **ForenSeq Imagen** and **ForenSeq Imagen Geo** — Libraries prepared with the ForenSeq Imagen Kit.
Sample Type	Select a different sample type: • **Sample** — A forensic or reference sample. • **NA24385** — The positive control. • **Negative Amplification Control** — The negative control (water). • **Reagent Blank** — The extraction control.
Index Combination*	Select the name of a different premixed i7 and i5 index adapter.
Run Analysis Method*	Select a different run analysis method.
Initial Projects*	Enter additional projects or remove current projects. For detailed instructions, see *Modify Project Assignments* (below).
Sample Plate	Edit or enter a preferred name for the 96-well plate.
Sample Well Position	Edit or enter the position of the well the sample occupies.

* Editing these fields requires a run state of Created. To edit them after run completion, remove the sample from the run, make the necessary edits, and add the sample to the run again.

4. Select **Save** to apply the changes.

Modify Project Assignments

When the run state is Created, you can change which projects a sample is assigned to or assign the sample to additional projects.

1. On the navigation rail, select **Runs**. Then select a run.
2. In the Actions column, select **Edit Sample Details** for the applicable sample.
3. In the Edit Sample dialog box, edit the Initial Projects field as follows.
   - To remove a sample from a project, select **X** next to the project name.
   - To add a sample to a project, enter the project name.
   - To add a sample to multiple projects, enter multiple project names.
   - To create a project for the sample, enter a new project name.
4. Select **Save**.

Remove Samples from a Run

1. On the navigation rail, select **Runs**. Then select a run.
2. Select the checkbox of each sample you want to remove from the run.
3. Select **Remove Samples**.
4. In the Remove Samples dialog box, select **Confirm**.

Monitor a Run

During a run, the Run Metrics page indicates which cycle the run is on and displays metrics so you can monitor run status and performance. Green indicates passing metrics and orange indicates metrics that require further evaluation. These metrics are a subset of the run metrics used to evaluate performance after sequencing is complete.

1. On the navigation rail, select ▣ **Runs**. Then select a run.
2. Select ⌒ **Run Metrics** to review quality metrics and sample representation.
   - Review the values for cluster density and clusters passing filter.
   - Review the phasing and prephasing values for Read 1.
   - Check Read 1, Index 1, Index 2, and Read 2 to confirm that all four reads passed.
   - Check Overall Intensity and Discordant Loci to confirm that the HSC passed.
   - Compare the samples to the sample read count guideline to confirm performance.
   - Review the number of reads in parentheses next to the sample name or hover over each sample.

   For more information, see *Quality Metrics* (page 54) and *Sample Representation* (page 58).
3. Select ⌫ **Run Activity** to review the run activity.
4. Select **Close** to return to the run.

Data Analysis

Review Run Metrics

Run metrics provide run quality, results for positive and negative controls, and sample representation for an overall view of run performance. Green indicates passing metrics and orange indicates metrics that require further evaluation.

For help with metrics requiring further evaluation, see *Troubleshoot a Run* (page 49). For detailed descriptions of each metric, see Appendix A. Run Metrics.

1. On the navigation rail, select ▬ **Projects**.
2. Select a project. The Run Metrics page opens.
3. On the Run Metrics page, do as follows.
   - Review the values for cluster density and clusters passing filter.
   - Review the phasing and prephasing values for Read 1.
   - Check Read 1, Index 1, Index 2, and Read 2 to confirm that all four reads passed.
   - Check Overall Intensity and Discordant Loci to confirm that the HSC passed.
   - Compare the samples to the sample read count guideline to confirm performance.
   - Review the number of reads in parentheses next to the sample name or hover over each sample.
4. Select **Positive & Negative Controls**.
   - Under NA24385:
     — Expand **Discordance** to see the Discordance table.
     — Use the scroll arrows to move through all positions in the table.

     > The Discordance table only appears if there are discordant loci within samples in the run.

   - Under Reagent Blanks, review the number of SNPs typed for each reagent blank.
   - Under Negative Amplification Controls, review the number of SNPs typed.
   - Select a sample name to go to the Results page.
5. If any metrics do not meet the minimum requirements, see *Troubleshoot a Run* (page 49) for troubleshooting guidance. For additional assistance, contact Verogen Technical Support. Collect the following items from D:\Illumina\MiSeqFGxOutput to help Verogen with troubleshooting:
   - InterOp folder
   - runInfo.xml file
   - runParameters.xml file

Review Results

The Results page for each Imagen sample includes filters and sortable categories to organize and locate loci for review. The following sections describe how to use these features to review results.

- For information on the Results page, see *Results Page* (page 20).
- For information on how the software estimates biological sex and contributor status, see Appendix B. Supporting Information.

By default, the Results page displays results with the Typed filter applied, and **Sort By** is set to ascending Chromosome.

Filter Loci

1. On the navigation rail, select ▪ **Projects**.
2. Select a project, and then select a sample to open the Results page.
3. In the Sample Details section, select any of the following filters to view only the desired loci:

Filter	Effect
Chromosome	Display loci located on the selected chromosome only.
Typed	Display only loci that are typed.
Untyped	Display only loci that are untyped.
Homozygous	Display only loci that have the same allele.
Heterozygous	Display only loci that have different alleles.
Locus Type	Displays only loci that belong to the selected SNP types.
QC Indicators	Displays only loci flagged with the selected QC indicators.

4. To remove filters, select the following options:
   - **Clear Selection** — Clear all filters in a section.
   - **Default View** — Clear selected filters and restore the default filters.

Sort Loci

1. On the navigation rail, select ▪ **Projects**.
2. Select a project, and then select a sample to open the Results page.

3. In the Sample Details section, select the Sort By list, and then select one of the following options:

Sort Option	Effect
None	Do not apply any sorting.
Intensity	Sort loci by intensity value.
ILB	Sort loci by intralocus balance percentage.
Amplicon Size	Sort loci by amplicon size in bp.
Chromosome	Sort loci by chromosome number or letter.
SNP Type	Sort loci by SNP category.
SNP Name	Sort loci by rsID.
Allele Count	Sort loci by the number of alleles.

4. To further sort loci into ascending or descending order, select the arrow next to each sort list.
5. To clear your sorting selections and restore the defaults, select **Default View**.

Find a Locus

1. On the navigation rail, select ▨ **Projects**.
2. Select a project, and then select a sample to open the Results page.
3. In the Search field, enter the whole or partial name of the locus you want to find, and then press **Enter**.

   The Results page displays loci matching the search term.

Modify Results

You can modify Imagen results by typing and untyping alleles, changing the biological sex of a sample, or changing the contributor status of a sample. You can also add comments to loci explaining any changes. For instructions, see *Comment on a Locus* (page 43).

Change the Biological Sex of a Sample

1. On the navigation rail, select ▨ **Projects**.
2. Select a project, and then select a sample to open the Results page.
3. In the Sample Overview section, select the Biological Sex drop-down menu, and then select the applicable chromosomes:
   - **XX** — The sample is from a female contributor.
   - **XY** — The sample is from a male contributor.
   - **Inconclusive** — The biological sex of the contributor is unknown.

Change the Contributor Status of a Sample

1. On the navigation rail, select ▨ **Projects**.
2. Select a project, and then select a sample to open the Results page.
3. In the Sample Overview section, select the Contributor Status drop-down menu, and then select a status:
   - **Single-Source** — The sample is from one contributor.

- **Mixture** — The sample is from multiple contributors.
- **Inconclusive** — Whether the sample is from one contributor or multiple contributors is unknown.

Type or Untype an Allele

A green toggle button and green histogram bar indicate a typed allele. A gray toggle button and bar indicate an untyped allele. An orange toggle button indicates a flagged allele. In Dark Mode, turquoise indicates a typed allele, gray indicates an untyped allele and orange indicates a flagged allele.

1. On the navigation rail, select **Projects**.
2. Select a project, and then select a sample to open the Results page.
3. In the Sample Details section of the page, select a locus card or histogram to open the Locus Details pane.
4. Type or untype an allele by selecting the toggle button associated with the allele.
   The User Modified QC indicator appears. Reverting the call removes the indicator.

Add Analyzed Samples to a Project

You can add analyzed samples in a project to other projects. After adding a sample to another project, sample modifications are **not** applied in the newly assigned project.

1. On the navigation rail, select **Projects**. Then select a project.
2. On the Sample List page, select **Add Samples**.
3. In the Add Samples dialog box, in the **Search** field, enter a sample name.
4. In the search results, select the checkbox of each sample you want to add to the project.
   Each selection appears in a summary list with the corresponding analysis method.
5. To remove a sample, clear the checkbox or select **X** in the summary.
6. Select **Add** to add the samples.

Reanalyze Samples

Reanalyze samples to apply an analysis method with user-defined parameters. The software preserves results from all analysis methods.

1. On the navigation rail, select **Projects**.
2. Select a project.
3. On the Sample List page, select the checkbox of each sample you want to reanalyze.
4. Select **Reanalyze**.
5. In the Reanalyze Samples dialog box, choose one of the following options:

Reanalysis Option	Instruction
Assign the same analysis method to all samples selected for reanalysis.	Select a method from the Analysis Methods list, and then select **Apply to All**.
Assign an analysis method to each sample selected for reanalysis.	Select a method from each list in the New Analysis Method column.

The software lists only analysis methods that are compatible with the assay used to prepare the sample.

6. Select **Next**.

7. Review the sample names, previous analysis methods, and new analysis methods.

8. To make changes, select **Previous**.

9. Select **Save** to initiate reanalysis.

   In the Projects sidebar, an icon appears next to each sample undergoing reanalysis.

10. When reanalysis is complete, select a sample to view results.

    The Results page displays results for the most recent analysis.

Switch Analysis Methods

A Results page shows results for the analysis method displayed in the Select an Analysis Method list. For reanalyzed samples, the list includes all analysis methods applied to the sample with the most recent listed first. Select different analysis methods to see other results.

1. On the navigation rail, select **Projects**.

2. Select a project, and then select a sample to open the Results page.

3. On the Results header bar, under the sample name, select the arrow next to the name of the current analysis method to choose another method.

   The Results page refreshes to show results for the selected method.

Reports

Report Types

The software compiles Imagen results and other information into the following reports:

- **Sample** — Results for one sample.
- **Project** — Results for all samples in a project.
- **BGA Estimation** — Estimates of biogeographical ancestry based on aSNP and pSNP data.

  > This report type is available only for samples prepared with the ForenSeq Imagen Geo library prep method and sequenced using the Imagen Geo workflow in MiSeq FGx®.

- **HIrisPlex-S** — Typed SNPs for hair color, eye color, and skin color. Results are output as a .csv file that can be used with the HIrisPlex-S online tool to generate phenotype estimates.

Generating Reports

The following sections describe how to generate each type of report. Once generated, reports are automatically downloaded and saved to your local machine based on your browser settings. Previously generated reports can also be accessed from the Reports page (see *Reports Page* (page 20)).

For report contents, see *File Formats for Reports* (page 36).

> This chapter describes how to create reports from the Sample List page. All report types except project reports can also be created from the Results page for an individual sample.

Create a Sample Report

You can create one sample report at a time or simultaneously generate multiple sample reports, one for each selected sample in a project.

1. On the navigation rail, select ▇ **Projects**.
2. Select a project, then select ⧖ **Sample List** to open the Sample List page.
3. Select the sample or samples for which you want to create reports.

   > To quickly select all samples, select the checkbox at the top of the sample list. For information on sorting and filtering samples, see *Sample List Page (Projects)* (page 19).

4. Select the blue **Actions** button, then select **Sample** from the drop-down menu.
5. Confirm the samples selected in the dialog box, then select **Create**.

The software generates the report or reports. Based on your browser settings, the report is automatically downloaded and saved to your local machine. If you selected multiple samples, the software downloads those reports as a zipped file.

All generated reports can also be accessed from the Reports page. See *Reports Page* (page 20).

Create a Project Report

If the software is currently sequencing samples, project report creation is disabled for those samples and a notification is displayed. You may still create a project report for any samples (in the same project or another) whose sequencing is complete.

1. On the navigation rail, select ▭ **Projects**.
2. Select a project, then select ⵝ **Sample List** to open the Sample List page.
3. Select the samples you want to include in your report.

To quickly select all samples, select the checkbox at the top of the sample list. For information on sorting and filtering samples, see *Sample List Page (Projects)* (page 19).

4. Select the blue **Actions** button, then select **Project** from the drop-down menu.
5. Confirm the samples selected in the dialog box, then select **Create**.

The software generates the report. Based on your browser settings, the report is immediately downloaded and saved to your local machine.

If a project includes samples with different assay types, the software generates one project report per assay. Multiple reports are downloaded as a zipped file.

All generated reports can also be accessed from the Reports page. See *Reports Page* (page 20).

Create a BGA Estimation Report

This report type is available only for samples prepared with the ForenSeq Imagen Geo library prep method and sequenced using the Imagen Geo workflow in MiSeq FGx®.

You can create one BGA Estimation report at a time or simultaneously generate multiple BGA Estimation reports, one for each selected sample in a project.

1. On the navigation rail, select ▭ **Projects**.
2. Select a project, then select ⵝ **Sample List** to open the Sample List page.
3. Select the sample or samples for which you want to create reports.

To quickly select all samples, select the checkbox at the top of the sample list. For information on sorting and filtering samples, see *Sample List Page (Projects)* (page 19).

4. Select the blue **Actions** button, then select **BGA Estimation Report** from the drop-down menu.

5. Confirm the samples selected in the dialog box, then select **Create**.

The software generates the report or reports. Based on your browser settings, the report is automatically downloaded and saved to your local machine. If you selected multiple samples, the software downloads those reports as a zipped file.

> All generated reports can also be accessed from the Reports page. See *Reports Page* (page 20).

Create a HIrisPlex-S Report

You can create one HIrisPlex-S report at a time or simultaneously generate multiple HIrisPlex-S reports, one for each selected sample in a project.

1. On the navigation rail, select ▮▮ **Projects**.
2. Select a project, then select ⧖ **Sample List** to open the Sample List page.
3. Select the sample or samples for which you want to create reports.

> To quickly select all samples, select the checkbox at the top of the sample list. For information on sorting and filtering samples, see *Sample List Page (Projects)* (page 19).

4. Select the blue **Actions** button, then select **HIrisPlex-S Report** from the drop-down menu.
5. Confirm the samples selected in the dialog box, then select **Create**.

The software generates the report or reports. Based on your browser settings, the report is automatically downloaded and saved to your local machine. If you selected multiple samples, the software downloads those reports as a zipped file.

> All generated reports can also be accessed from the Reports page. See *Reports Page* (page 20).

File Formats for Reports

The following sections describe the format and contents of each type of report the software generates for Imagen samples. In the reports, rsID numbers identify individual SNPs. For instructions on creating reports, see *Generating Reports* (page 34).

Sample Report

An Imagen sample report is an Excel workbook with one worksheet per SNP type: Y-, ancestry, and phenotype. The workbook also includes a Settings worksheet and, if applicable, a Sample History worksheet. The Settings worksheet lists AT, IT, and intralocus balance for each locus. The Sample History worksheet includes administrative details for the sample and a sample history log listing time-stamped system events and user actions.

The header section of each SNP worksheet lists the analysis method, biological sex, contributor status, project creation date and time, user who generated the report, and the sample, project, and run names. Coverage information appears under the header, followed by a table listing information for each locus:

- Locus
- Genotype with allele name
- Whether the allele is typed
- Reads

- Quality control indicators

Project Report

An Imagen project report is an Excel workbook. A header section lists the project name, project creation date and time, and the user who generated the report. A Samples table lists each sample you selected in the report with the following information:

- Sample name
- Analysis method
- Date and time completed
- Sample type
- Locus call rate
- Typed loci
- Sample reads
- Biological sex
- Contributor status
- QC indicators
- Description

BGA Estimation Report

This report type is available only for samples prepared with the ForenSeq Imagen Geo library prep method and sequenced using the Imagen Geo workflow in MiSeq FGx®.

A BGA Estimation report is an Excel workbook detailing estimates of biogeographical ancestry based on aSNP and pSNP data. The workbook is composed of four worksheets containing the following information:

Table 8   BGA Estimation Report contents

Worksheet	Contents
Estimation	• A plot of the biogeographical ancestry results, indicating the distance from the sample to the nearest centroid • Population information for samples in the centroid (taken from the 1000 Genomes Project): population names and abbreviations, the number of samples in each population ("Count"), and the number of samples used to train the model
SNP Data	• *Coverage Information* — For each locus, the allele name, whether the locus is typed, and the number of reads • *Phenotypic SNPs*, *Common SNPs*, and *Biogeographical Ancestry SNPs* — For each SNP, the number of loci typed, the genotype, and any QC indicators. (The number of loci typed is the number of aSNP and pSNP loci identified out of the total targeted aSNP and pSNP loci.)
SNP Balance Figure	• A filter to exclude alleles that are typed or untyped • For each SNP, the number of reads per base, with grand totals provided at the bottom of the table
Settings	• *SNP Thresholds* — The AT, IT, and intralocus balance for each locus

Additionally, in the Estimation, SNP Data, and Settings worksheets, a header section lists the sample name, project name, analysis method, run name, the biological sex and contributor status of the sample, the date and time the project was created, and the user who generated the report. The Settings worksheet also lists the software version.

For more information about how the software computes estimates of biogeographical ancestry, see *Biogeographical Ancestry Estimation* (page 62) in Appendix B. Supporting Information.

Generating SNP Data

The SNP Data worksheet provides data for aSNPs and pSNPs. Estimating hair and eye color requires genotype results for hair color, eye color, and common SNPs. Common SNPs are SNPs that estimate hair color, eye color, and biogeographical ancestry.

If one of these SNPs is not typed, the software does not generate a result. In contrast, generating a result for biogeographical ancestry requires that only one biogeographical ancestry SNP be typed. The absence of SNPs in the biogeographical ancestry estimation diminishes the accuracy of the estimation. With each execution, the estimation model is retrained using only the aSNPs with a multilocus genotype.

HIrisPlex-S Report

A HIrisPlex-S report is a `.csv` file that includes typed SNPs for hair color, eye color, and skin color. The file can be used with HIrisPlex-S, a multinomal logistic regression model, to generate phenotype estimates. For more information about HIrisPlex-S, see *Phenotype Estimation* (page 62) and https://hirisplex.erasmusmc.nl/.

Verogen provides an additional tool, the Imagen-HIrisPlex Analysis Tool (I-HAT), to further process phenotype estimates output from HIrisPlex-S. I-HAT is a Microsoft Excel macro, and comes packaged with your installation of UAS. To obtain phenotype estimates from a HIrisPlex-S report, first upload the file to HIrisPlex-S, then run I-HAT on the results.

Using HIrisPlex-S

Once you have generated a HIrisPlex-S report (see *Create a HIrisPlex-S Report* (page 36)), use HIrisPlex-S as follows:

1. In your browser, navigate to https://hirisplex.erasmusmc.nl/.
2. Under the heading *The HIrisPlex-S System*, select **Browse…**.
3. Locate the HIrisPlex-S report `.csv` file on your local machine, then select **Open**.
   The file is uploaded to HIrisPlex-S for processing.
4. Once HIrisPlex-S has finished processing your report, you will be prompted to save the resulting file (`Result.csv`) to your machine. Select **Save**.

Using I-HAT

Once you have a results file from HIrisPlex-S, use I-HAT as follows:

1. Open the file `I-HATmod06012022.xlsm` with Microsoft Excel. If prompted to enable macros, select **Enable Macros**.
2. Open the `.csv` file output by HIrisPlex-S.
3. On the Developer ribbon, select **Macros**.
4. On the resulting dialog box, do the following:
   a. From the *Macro name* list, select **'I-HATmod06012022.xlsm'!HIrisPlexMacros**.

b. Set *Macros in* to **All Open Workbooks**.

c. Select **Run**.

The workbook is renamed "HIrisPlexS Results", and a new worksheet labeled "HIrisPlexS Probabilities" is added to the workbook. This worksheet contains the final phenotype estimates, categorized into results for eye color, hair color, and skin color. The workbook is automatically saved as an `.xlsx` file to the same directory as the original HIrisPlex-S output file, with "Probabilities" appended to the filename.

Run, Project, and Sample Management

Create a Project

Create a project to group a selection of samples. For example: create a project of controls to compare controls from multiple projects, or group samples in a comparison to generate a project report of compared samples.

> Although possible, combining samples with different assay types in a project is not recommended.

1. On the navigation rail, select ▪▪ **Projects**.
2. Select **Create Project**.
3. In the Create Project dialog box, complete the following fields:
   - **Project Name** — Enter a preferred name to identify the project.
   - **Description** — Optionally enter a description of the project.
4. Select **Save** to create the project.
   The new project opens to the Run Metrics page.
5. Open the Sample List page and select **Add Samples**.
   You can add any samples in the software to the project, analyzed or not.
6. In the Add Samples dialog box, in the **Search** field, enter a sample name.
7. In the search results, select the checkbox of each sample you want to add to the project.
   Each selection appears in a summary list with the corresponding analysis method.
8. To remove a sample, clear the checkbox or select **X** in the summary.
9. Select **Add** to add the samples.
   The Sample List page lists the added samples. Runs the samples belong to appear in the Project sidebar.

Edit a Project

1. On the navigation rail, select ▪▪ **Projects**.
2. Select a project, and then select ⓘ **Project Info**. On the resulting pop-up, select ✎ **Edit**.
3. In the Edit Project dialog box, do as follows:
   - Edit the project name.
   - Add a project description or modify the existing description.
4. Select **Save** to apply the changes.

Obtain the Analysis Folder Path

The analysis folder resides on the ForenSeq Universal Analysis Server and contains the Variant Call Format (VCF) and Binary Alignment Map (BAM) files for the analysis.

1. On the navigation rail, select ▮ **Projects**.
2. Select a project, and then select a sample to open the Results page.
3. Select the Information icon.

   The path appears in a pop-up window.
4. The path is displayed under **Analysis Folder Path**.

History

When the history setting is enabled, you can review system events and user actions recorded for a run, project, or sample and add comments. For example, you can comment on a sample to explain a call modification. For instructions on enabling the history setting, see *System Settings* (page 48).

The software also records system events and user actions for loci in samples and allows commenting. Loci history and commenting is always available, regardless of whether the history setting is enabled.

Review Run Activity

1. On the navigation rail, select ▮ **Runs**.
2. Select a run, and then select ↺ **Run Activity**.
3. In the Run Activity dialog box, review system events and user actions for the run.

   The dialog box lists system events and user actions from newest to oldest.
4. Navigate using the scroll bar and arrows. Narrow results using the following fields:
   - **Search** — Search the current history.
   - **Start Date and End Date** — View the history within a specified time frame.
   - **Show System Events** — See system events and user actions for the history or see user actions only.
5. When finished, select **Close**.

Review Project Activity

1. On the navigation rail, select ▮ **Projects**.
2. Select a project, and then select ↺ **Activity**.
3. In the Project Activity dialog box, review system events and user actions for the project.

   The dialog box lists system events and user actions from newest to oldest.
4. Navigate using the scroll bar and arrows. Narrow results using the following fields:
   - **Search** — Search the current history.
   - **Start Date and End Date** — View the history within a specified time frame.
   - **Show System Events** — See system events and user actions for the history or see user actions only.
5. When finished, select **Close**.

Review Sample Activity

1. On the navigation rail, select **Projects**.
2. Select a project, select a sample, and then select **Activity**.
3. In the Sample Activity dialog box, review system events and user actions for the sample.
   The dialog box lists system events and user actions from newest to oldest.
4. Navigate using the scroll bar and arrows. Narrow results using the following fields:
   - **Search** — Search the current history.
   - **Start Date and End Date** — View the history within a specified time frame.
   - **Show System Events** — See system events and user actions for the history or see user actions only.
5. When finished, select **Close**.

Review Locus Activity

1. On the navigation rail, select **Projects**.
2. Select a project, and then select a sample to open the Results page.
3. In the Sample Details section of the page, select a locus card or histogram to open the Locus Details pane.
4. Select the **User Actions** tab.
   The tab lists system events and user actions from newest to oldest.
5. Review system events and user actions for the locus.
6. When finished, select **Close**.

Comment on a Run

1. On the navigation rail, select **Runs**.
2. Select a run, and then select **Run Activity**.
   The Run Activity dialog box opens.
3. To add a comment to the history:
   a. In the Add New Comment field, type a comment.
   b. Select **Add** to save the comment.
   The comment appears as an event at the top of the history.
4. To comment on an entry in the history:
   a. Select the system event or user action. Use the fields and scrolling to help locate the entry, if necessary.
   b. In the Edit Comment box, type a comment.
   c. Select **Save** to add the comment to the system event or user action.
   The comment appears as the last item for the entry.
5. When finished, select **Close**.

Comment on a Project

1. On the navigation rail, select **Projects**.

2. Select a project, and then select ⟲ **Activity**.
   The Project Activity dialog box opens.
3. To add a comment to the history:
   a. In the Add New Comment field, type a comment.
   b. Select **Add** to save the comment.
   The comment appears as an event at the top of the history.
4. To comment on an entry in the history:
   a. Select the system event or user action. Use the fields and scrolling to help locate the entry, if necessary.
   b. In the Edit Comment box, type a comment.
   c. Select **Save** to add the comment to the system event or user action.
   The comment appears as the last item for the entry.
5. When finished, select **Close**.

Comment on a Sample

1. On the navigation rail, select ▬ **Projects**.
2. Select a project, select a sample, and then select ⟲ **Activity**.
   The Sample Activity dialog box opens.
3. To add a comment to the history:
   a. In the Add New Comment field, type a comment.
   b. Select **Add** to save the comment.
   The comment appears as an event at the top of the history.
4. To comment on an entry in the history:
   a. Select the system event or user action. Use the fields and scrolling to help locate the entry, if necessary.
   b. In the Edit Comment box, type a comment.
   c. Select **Save** to add the comment to the system event or user action.
   The comment appears as the last item for the entry.
5. When finished, select **Close**.

Comment on a Locus

Add a comment to a locus to, for example, explain why you changed an allele from untyped to typed. You can also edit or delete existing comments.

1. On the navigation rail, select ▬ **Projects**.
2. Select a project, and then select a sample to open the Results page.
3. In the Sample Details section of the page, select a locus card or histogram to open the Locus Details pane.
4. Select the **User Actions** tab.
5. In the Add New Comment field, type a comment.
6. Select **Comment** to add the comment to the locus.
   • To edit the comment, select it, modify it as needed in the Edit Comment field, and select **Save**.

- To delete the comment, select it, and then select 🗑 Delete Comment .

Software Settings

Settings Overview

Use the following settings, which are available from the **Settings** icon on the navigation rail, to configure the software. All settings except Dark Mode are visible to administrators only.

- **Analysis Methods** — Create and manage analysis methods.
- **User Management** — Add users and assign permissions.
- **System Settings** — Set visibility into system events and user actions.
- **System Information** — View software version numbers for various bio-informatics analysis methods.
- **Dark Mode** — Change the color scheme of the user interface so it is color-blind compatible.

Analysis Methods

An analysis method is a collection of settings that inform how the software analyzes sequencing data and performs allele calling. The software ships with one or more default Verogen analysis methods.

- ForenSeq Imagen — Verogen Imagen Analysis Method
- ForenSeq Imagen Geo — Verogen Imagen Geo Analysis Method

Create an Analysis Method

A default Verogen analysis method cannot be modified, but you can create an analysis method with the desired settings in one of two ways: copy an existing analysis method and modify its settings, or start with a blank analysis method.

> Performance is supported when using the default analysis methods. Perform internal validation studies as needed to develop settings for new analysis methods.

For information on how Verogen determined the default settings in the Kintelligence analysis method and help determining alternative settings, see *SNP Typing in Universal Analysis Software and Kinship Estimation with GEDmatch PRO Technical Note (document # VD2020058).*

Copy an Analysis Method

1. On the navigation rail, select ⚙ **Settings**.
2. Select **Analysis Methods**.
3. Select **Create Analysis Method**.

The New Analysis Method dialog box opens.

4. In the New Analysis Method Options list, select **Copy an Existing Analysis Method**.

5. In the Source Analysis Method list, select an analysis method to use as a template.

6. In the New Analysis Method Name field, enter a unique name for the new analysis method.

7. Select **Save** to create the analysis method.

   The new method appears with the default settings from the copied method.

8. Modify the default settings as desired.

   For instructions, see *Modify Analysis Settings* (below).

Use a Blank Analysis Method

1. On the navigation rail, select ⚙ **Settings**.

2. Select **Analysis Methods**.

3. Select **Create Analysis Method**.

   The New Analysis Method dialog box opens.

4. In the New Analysis Method Options list, select **New Blank Analysis Method**.

5. In the New Analysis Method Name field, enter a unique name for the new analysis method.

6. Select **Save** to create the analysis method.

   The new method appears with blank settings.

7. Enter the desired settings for the analysis method.

   For instructions, see *Modify Analysis Settings* (below).

Modify Analysis Settings

For a user-created Imagen analysis method, you can modify the AT, IT, and intralocus balance and specify which loci to include in the analysis.

Edit an Analysis Method

1. Open the analysis method you want to edit:

   a. On the navigation rail, select ⚙ **Settings**.

   b. Select **Analysis Methods**.

   c. In the Manage Analysis Methods list, select the analysis method you want to edit. (Default Verogen analysis methods cannot be modified.)

2. To rename the analysis method:

   a. In the header bar, select the ✏ **Edit** icon. A field appears.

   b. Enter a new name for the analysis method.

   c. Select ✔ to apply the name change, or ✖ to cancel.

3. To change the loci included in the analysis method or their settings:

   a. In the list of loci, select the checkbox of one or more loci. (You can filter the list by SNP type, sort the list by column, or search for a locus by name. Scroll to see all loci.)

   b. Select **Edit X Rows**, where X is the number of loci selected.

      The Edit Analysis Method dialog box opens.

    c.   To add or remove loci from the analysis method, select the **Include** toggle button.

    d.   To modify analysis thresholds, enter a value from 0 to 100 in the appropriate fields. (Values represent percentages. The IT value must be greater than or equal to the AT value.)

    e.   Select **Save X Loci** to apply the new settings, where X is the number of loci selected.

> The software applies any new settings to all selected loci. If you want to apply different settings to different loci, select one locus at a time.

Delete an Analysis Method

You can delete any user-created analysis method. The default Verogen analysis methods cannot be deleted.

1. On the navigation rail, select ⚙ **Settings**.
2. Select **Analysis Methods**.
3. In the Select an Assay Type list, select the assay that corresponds to the analysis method you want to delete.
4. Select the applicable analysis method.
5. In the upper-right corner of the analysis method, select **Delete Analysis Method**.
6. Select **OK** to confirm the deletion.

Change the Default Analysis Method

1. On the navigation rail, select ⚙ **Settings**.
2. Select **Analysis Methods**.
3. In the Select an Assay Type list, select the kit that corresponds to the analysis method you want to set as the default.
4. Select the applicable analysis method.

    A green star identifies the current default.

5. Set the selected analysis method as the default for the assay type by selecting the empty star next to the analysis method name:

User Management

The Admin Settings page lists each account by username. On this page, an administrator can manage permissions for each account, including granting access to new accounts and assigning access levels.

Enable and Disable Accounts

New accounts are disabled by default and must be enabled by an administrator.

1. On the navigation rail, select ⚙ **Settings**.
2. Select **User Management**.
3. Select **Enabled** to enable or disable an account.

    Blue indicates an enabled account.

Assign Access Levels

1. On the navigation rail, select ⚙ **Settings**.

2. Select **User Management**.
3. Select **Administrator** to enable or disable administrator access for an account.

   Blue indicates an administrator account.

System Settings

The System Settings page hosts the history setting. When enabled, this setting lets you view system events and user actions for runs, projects, and samples and includes these activities in project and sample reports.

By default, the history setting is enabled. However, the software *always records activity*, regardless of whether the setting is enabled. When enabling the setting, you can choose whether to store or delete activities that occurred since the last disabling.

Enable Run, Project, and Sample History

1. On the navigation rail, select ⚙ Settings.
2. Select **System Settings**.
3. Select **Enable or disable visibility of system events and user actions recorded by the software**.
4. In the dialog box, select whether to store or delete previous system events and user actions:
   - **Permanently Delete All Previous History** — Start recording and delete all previous activity. You can access only activities recorded from this point forward.
   - **Store Previous History** — Continue recording and grant access to all activity starting from the creation of a run, project, or sample.

   An 🕘 Activity icon appears on the 🗔 **Runs** header bar, 📁 **Projects** header bar, and Results page. Selecting the icon opens the history.

Disable Run, Project, and Sample History

1. On the navigation rail, select ⚙ Settings.
2. Select **System Settings**.
3. Select **Enable or disable visibility of system events and user actions recorded by the software**.

   The 🕘 Activity icon disappears from the the 🗔 **Runs** header bar, 📁 **Projects** header bar, and Results page.

Enable or Disable Dark Mode

1. On the navigation rail, select ⚙ Settings.
2. Select **Dark Mode** to darken or brighten the user interface.

Display Software Version

1. On the navigation rail, select ⚙ Settings.
2. Select **System Information**.

Troubleshooting

Troubleshoot a Run

The following sections provide recommendations for troubleshooting run problems. Most recommendations include library prep steps, which are documented in the library prep kit reference guides. Visit verogen.com/documentation to download the guide for your kit.

Low Intensity

Low intensity (number of reads) can indicate a problem with sample quantity or quality.

- Make sure that DNA concentrations are not below the targeted amount.
- Depending on the source, purify or dilute the DNA, prepare libraries, and resequence the sample.
- Resequence with fewer samples.

High Cluster Density

Review run metrics: If cluster density is high, but sample representation shows that reads are above the sample read count guideline, proceed with analysis. The following factors can result in insufficient data for analysis.

- During normalization, library from the Purified Library Plate were overpipetted.
  — If the sample has a high number of alleles with quality control indicators, be sure to use correct volumes and reagents for normalization and resequence the sample.
  — If the sample volume is correct, further dilute libraries in the Pooled Normalized Libraries tube.
  — If normalization volumes or reagents were incorrect, repeat normalization and resequence.
- Too much adapter dimer in library prep.
  — If your laboratory has a Bioanalyzer or Fragment Analyzer, analyze 1–2 µl library from the Purified Library Plate to check the amount of primer dimer.
    - If the checked library has > 5% adapter dimer, repeat library purification using the 30 µl library remaining in each well of the Purified Library Plate. Adjust purification to use 30 µl Sample Purification Beads 2 (SPB2), add 32.5 µl Resuspension Buffer (RSB), and transfer 30 µl to a new 96-well PCR plate. Proceed with normalization.
    - For questions, contact Verogen Technical Support.
- An insufficient volume of Hybridization Buffer (HT1) was added, or too much normalized library was added to HT1.
  — If the sample has a high number of alleles with quality control indicators, resequence the sample. Make sure the normalized library is sufficiently diluted.

Low Cluster Density

Review run metrics: If cluster density for the run is low, but sample representation shows that reads are above the sample read count guideline, proceed with analysis. If the sample has an insufficient number of reads due to low density, the problem might be due to the following factors.

- DNA input was too low or too degraded, or the DNA dilution was incorrect. If possible, repeat library prep with more DNA.
- A critical reagent was not added during library prep. Repeat the prep.
- A larger volume than expected of HT1 was pipetted during library prep. Repeat sequencing using the correct volume.
- Excessive sample was lost during purification. Repeat the library prep using best practices for bead handling. Ensure that the ethanol made for SPRI washing is made at the correct ratio.
- The library was not otherwise denatured properly.
  — Make sure that the final library pool is denatured with freshly diluted 0.2 N NaOH (HP3) that has a pH > 12.5.
- MiSeq FGx Maintenance Status:
  — Perform a maintenance wash and repeat sequencing.
  — Refill the wash tray and bottle for every wash.
  — Make all wash solutions daily.

Low Percentage of Clusters Passing Filter

If the percentage of clusters passing filter is too low, the problem might be due to the following factors.

- The cluster density is too high; see *High Cluster Density* (previous page). However, if sample representation shows that reads are above the sample count guideline, proceed with analysis.
- MiSeq FGx Maintenance Status:
  — Perform a maintenance wash and repeat sequencing.
  — Refill the wash tray and bottle for every wash.
  — Make all wash solutions daily.
- If the problem remains unresolved, reagents might not be performing as expected. Contact Verogen Technical Support.

High Phasing and Pre-phasing

The following factors can cause high phasing or pre-phasing.

- The cluster density is too high. See *High Cluster Density* (previous page).
- The ambient temperature is too high.
  — Verify the appropriate temperature in the *MiSeq FGx Sequencing System Site Prep Guide (document # VD2018012)*.
  — Check the MiSeq FGx System temperature logs to confirm whether the ambient temperature is too high. Contact Verogen Technical Support for help locating the logs.
- The reagent chiller temperature is too low or too high. The system displays an error.
- MiSeq FGx Maintenance Status:

— Perform a maintenance wash and repeat sequencing.

— Refill the wash tray and bottle for every wash.

— Make all wash solutions daily.

- If the problem remains unresolved, reagents might not be performing as expected. Contact Verogen Technical Support.

Incomplete Run

The following factors can cause a run to stop prematurely.

- The HSC was not added during library prep. Add HSC per denature and dilute instructions and repeat the run.
- Cluster density is too low. See *Low Cluster Density* (previous page).
- If an error message is displayed, contact Verogen Technical Support.

Low Quality Scores for Read 1 or Read 2

The following factors can cause low quality scores for Read 1 and Read 2.

- Phasing and pre-phasing are too high. See *High Phasing and Pre-phasing* (previous page).
- The cluster density is too high. See *High Cluster Density* (page 49).
- If the problem remains unresolved, reagents might not be performing as expected. Contact Verogen Technical Support.

Low Quality Scores for Index 1 Read or Index 2 Read

The following factors can cause low quality scores for the Index 1 Read or the Index 2 Read.

- Phasing and pre-phasing are too high. See *Phasing and Pre-phasing* (page 55).
- The cluster density is too high. See *High Cluster Density* (page 49).
- Not enough samples were sequenced, so diversity in the index reads was low.
- If the problem remains unresolved, reagents might not be performing as expected. Contact Verogen Technical Support.

Low Reads per Sample

Review sample representation: If the samples exceed the sample read count (intensity) guideline, proceed with analysis. If the samples are below the guideline, the following factors are the likely cause.

- DNA input was too low or the DNA dilution was incorrect. If possible, repeat library prep with more DNA.
- A critical reagent was not added during library prep. Repeat the prep.
- The libraries were not properly normalized. Make sure that the correct volumes of RSB and libraries from the Purified Library Plate were mixed and at concentration of 0.75 ng/μL.
- HT1 was overpipetted during library prep. Repeat library normalization.

Low Reads per Sample for the HSC

Review sample representation: If the samples exceed the sample read count guideline, proceed with analysis. If the samples are below the guideline, the following factors are the likely cause:

- HSC was not added during library prep. Add HSC per denature and dilute instructions and repeat the run.

- HT1 was overpipetted during library prep. Repeat library normalization.
- HSC was not denatured with freshly diluted 0.2 N NaOH. Make sure HSC is denatured with freshly diluted 0.2 N NaOH before adding it to the library, and then repeat the run.

Troubleshoot Analysis Errors

The following sections provide recommendations for troubleshooting potential analysis problems.

Analysis Error Message

Samples will error during analysis if there are no reads for the index combination.

1. Ensure that the correct index combination was used for the sample.
2. If the index combination is incorrect, follow steps in *Remove Samples from a Run* (page 27).
3. Add the sample back to the run with the correct index combination, following the steps in *Add Samples to a Run* (page 24).

    The sample will automatically analyze.

If further errors occur, contact Verogen Technical Support

1. Navigate to the run folder, following the steps for obtaining the analysis folder path in *Review Run Activity* (page 41).
2. Navigate to the Log folder for that sample analysis.
3. Copy the log text file.
4. Navigate to D:\verogen\fuas\logs and copy the most recent log text file.

Quality Metrics Icons Are Unavailable

When all Quality Metrics icons are not available, the system might be unable to locate the data or the network where data are stored is disconnected. Use the following steps to troubleshoot.

1. Navigate to the run folder, following the steps for obtaining the analysis folder path in *Review Run Activity* (page 41).
2. Navigate up to the run folder (designated by a run number).
3. Ensure that the InterOp folder, the runInfo.xml file, and the runParameters.xml file are present.
    — If the folder is not present, copy this folder with the runInfo.xml and runParameters.xml files from the MiSeq, following the steps in *Review Run Metrics* (page 29).

Perform a Status Check

A status log in UAS aggregates information from a run, sample, or installation failure and a history of actions. Use the following instructions to export this information to send to Verogen when troubleshooting.

1. On the navigation rail, select ▣ **Runs**. Then select a run.
2. On the header bar, select ⓘ **Run Info**, and then select **Run Status Check**.
3. In the Status Log Report dialog box, select **Copy**.

    The copy command copies the content displayed under System Status and Most Recent Log to your clipboard.
4. Paste the copied content to an external file.

5. When finished, select **Close**.

Run Metrics

Accessing Run Metrics

Available in ▮ **Projects** after sequencing, run metrics provide data about run quality, sample representation, and positive and negative amplification control results, for an overall view of run performance.

To view run metrics, select the Run Metrics tab on the Projects header bar or the **QPN** icon next to a sample name in the Projects sidebar. A subset of these metrics, run quality and sample representation, is also available from the Run Metrics page in the ▮▮ **Runs** workspace.

Quality Metrics

Quality metrics monitor run quality during sequencing. After sequencing, the software preserves the quality metrics and makes them available in both ▮▮ **Runs** and ▮ **Projects** on the Run Metrics page.

Color-coded bars indicate results for each metric and show optimum ranges for cluster density, clusters passing filter, phasing, and pre-phasing. Short, vertical bars with corresponding icons indicate the quality of each read and the HSC.

Figure 6 Quality metrics overview

A   Color-coded horizontal bars and values indicate run quality metrics.
B   Thinner horizontal bars in dark gray indicate optimum ranges.
C   Color-coded vertical bars and icons indicate the quality of each read and the HSC.

Run Quality Metrics

Run quality metrics include cluster density, clusters passing filter, phasing, and pre-phasing values. The following colors indicate overall quality.

Table 9   Colors for run quality metrics

Color	Indication
	Values are within the target range.
	Values require further evaluation of run quality.
	The target range for the metric.

Cluster Density

Cluster density is the number of clusters per square millimeter (K/mm²). For ForenSeq libraries, the target cluster density is 400–1650 K/mm². Values outside this range can still produce results sufficient for analysis. However, substantial deviations from the target range can impact other quality metrics and decrease the amount of usable data.

Clusters Passing Filter

Clusters passing filter is the percentage of clusters that passed the quality filter. This metric is based on the Illumina chastity filter, which measures quality and can detect low-quality base calls. Data appear after cycle 25.

> The chastity of a base call is the ratio of the intensity of the greatest signal divided by the sum of the two greatest signals. If multiple base calls have a chastity value < 0.6 in the first 25 cycles, reads do not pass filter.

For ForenSeq libraries, the target value for a cluster passing filter is ≥ 80%. Values < 80% can still produce results that are sufficient for analysis. However, substantial deviations from the target can impact other quality metrics and decrease the amount of data.

Phasing and Pre-phasing

For both phasing and pre-phasing, lower percentages indicate higher quality run statistics. Phasing and pre-phasing values outside the target ranges can still produce results that are sufficient for analysis.

- Phasing shows the percentage of molecules in a cluster that fall behind the current cycle in Read 1. For ForenSeq libraries, the target phasing value is ≤ 0.25%.
- Pre-phasing shows the percentage of molecules in a cluster that jump ahead of the current cycle in Read 1. For ForenSeq libraries, the target pre-phasing value is ≤ 0.15%.

Read and Index Quality Metrics

The read and index quality metrics use the following colors and icons to indicate the status of each read and overall quality.

Table 10   Colors for read and index quality metrics

Color	Indication
🟩	The average quality of assessed reads is within the target range.
🟧	The average quality of assessed reads is outside the target range.
⬜	The read has not yet occurred.

Table 11   Icons for read and index quality metrics

Icon	Indication
✅	The average quality of assessed reads is within the target range.
⚠️	The average quality of assessed reads is outside the target range.
⚫	The read has not yet occurred.

Reads in a Run

A sequencing run completes up to four reads. Read 1 and Read 2 sequence the DNA template strands, and the Index 1 Read and Index 2 Read sequence the index adapters.

- **Read 1** — Read 1 sequencing primer is annealed to the template strand during cluster generation. RTA evaluates the first 50 cycles for quality.
- **Index 1 Read** — The Read 1 product is removed and the Index 1 sequencing primer is annealed to the same template strand as in Read 1. After index read preparation, the Index 1 Read is performed. RTA evaluates all eight cycles for quality.
- **Index 2 Read** — The Index 1 Read product is removed and the template anneals to the P5 primer grafted to the flow cell surface. The run proceeds through seven chemistry-only cycles without any imaging, followed by eight cycles of sequencing. RTA evaluates all eight cycles for quality.
- **Read 2** — The Index 2 Read product is extended to copy the original template strand. The original template strand is then removed and the Read 2 sequencing primer is annealed.

Cycles per Read

Quality metrics for each read appear after the read is complete. The number of cycles in each read depends on the assay.

Assay Type	Read	Cycles	Quality Metrics Appear
ForenSeq Imagen or ForenSeq Imagen Geo	Read 1	1–151	Cycle 152
	Index 1 Read	152–159	Cycle 160
	Index 2 Read	160–167	Cycle 168
	Read 2	168–318	After cycle 318

Human Sequencing Control

The software assesses the HSC and uses the following colors indicate the overall result.

Table 12   Colors for HSC results

Color	Indication
🟩	The control meets or exceeds the criteria.
🟧	The control does not meet at least one criterion.

When the HSC is orange, evaluate the run quality metrics to help determine whether to repeat the run. If the run quality metrics are green, indicating that values are within target ranges, you can interpret the sample with caution.

Loci

The HSC includes the loci in the following table. The software lists any loci that do not meet intensity or genotype concordance criteria.

Loci	151 or 201 Cycles	Loci	151 or 201 Cycles
D3S1358	✓	PentaE	✓
D5S818	✓	TPOX	✓
D7S820	✓	DYS391	✓
D8S1179	✓	DYS392	✓
D13S317	✓	DYS460	✓
D16S539	✓	DXS7423	✓
D18S51	✓	DXS10103	✓
FGA	✓		
PentaD	✓		

Sample Representation

Sample representation is a metric that provides quantitative sample and run information. After sequencing, the software preserves sample representation data and makes it available in both 🗔 Runs and 📁 Projects on the Run Metrics page.

A bar chart shows the number of reads (intensity) and read distribution for each sample in a run, along with the sample read count guideline for the run. Hovering over a bar displays the exact number of reads for that sample, and selecting a bar takes you to the Results page for that sample. Hovering over the chart reveals buttons that allow you to zoom, pan, or autoscale the chart, or download the chart as a `.png` file.

Figure 7 Sample Representation bar chart

A Sample name and exact number of reads for the sample
B Threshold of the read count guideline
C Bar indicating number of reads for the sample

Sample Read Count Guideline

The sample read count guideline varies by assay type. As guidelines, these values are intended to help with quality reviews of the run and samples. Samples with fewer reads can still demonstrate complete coverage and provide enough data for interpretation. Review quality metrics and sample data to help make a determination.

Assay Type	Sample Read Count Guideline
ForenSeq Imagen	50,000 reads per sample

The following colors indicate how sample read counts compare to the applicable sample read count guideline.

Table 13 Colors for sample read counts

Color	Indication
	The sample read count guideline.
	The sample meets or exceeds the guideline.
	The sample does not meet the guideline and requires careful interpretation.

Positive and Negative Controls

The software assesses the same positive and negative control metrics for every run, regardless of assay type. These post-sequencing metrics are available in [folder] **Projects** on the Run Metrics page.

Table 14 Control assessments

Assay Type	Positive Amplification Control	Value
ForenSeq Imagen	NA24385 Positive Amplification Control DNA	SNPs typed

Positive Amplification Control

Positive amplification control metrics provide data for each sample identified as NA24385. Every sample is analyzed and compared to this control. The software then lists each sample with values for how many reads passed filter and SNPs typed called. Colors indicate overall success. If applicable, a table displays discordant positions or loci.

SNPs Typed

SNPs typed shows the number of loci with a base call out of the total number of loci for NA24385. A short, vertical bar displays the following colors to indicate call success and concordance with NA24385.

Table 15   Colors for loci for NA24385

Color	Indication
	All loci have calls and are concordant.
	At least one locus does not have a call or contains a discordant call.

Discordance Table

When a sample has calls that are discordant with the NA24385 calls, the positive control metrics include a discordance table. For each discordant call, the table displays the position number or locus where the discordance occurred, the call observed for the sample, and the expected call.

The table displays up to 50 loci. When the number of discordant loci exceeds 50, use the scroll arrows to move through the table.

Pass Filter Reads

Pass filter reads displays the following colors to indicate whether the number of reads that passed filter meet the guideline of 15 million reads for ForenSeq Imagen libraries. Reads below the guideline might still be usable. Review quality metrics and sample data to help make a determination.

Table 16   Colors for pass filter reads

Color	Indication
	The total number of pass filter reads exceeds the guideline.
	The total number of pass filter reads is below the guideline.
	The guideline for the number of reads that pass filter.

Negative Amplification Control

Negative control metrics provide data for each sample identified as a negative amplification control or reagent blank. The software lists each of these samples with the number of SNPs typed called and a color-coded indicator of overall success.

SNPs Typed

SNPs typed shows how many loci a base call was made for. The short vertical bar uses the following colors to indicate call success.

Table 17   Colors for base calls of loci

Color	Indication
▇	No loci have a base call.
▇	At least one locus has a base call.

Supporting Information

Biogeographical Ancestry Estimation

The software uses principal component analysis (PCA) to determine estimates of biogeographical ancestry. Data from Phase I of the 1000 Genomes Project (1000genomes.org) was used to train the model on the following super populations: European, East Asian, and African (excepting African Ancestry in Southwest USA [ASW]). For context, the unknown sample is projected with the Ad-Mixed Americans super population onto the first two pretrained components based on aSNP genotype calls.[4]

On the Estimation worksheet of the BGA Estimation report (see *BGA Estimation Report* (page 37)), the distance to the nearest centroid indicates how related a sample is to the general grouping for the centroid. For comparison, the worksheet displays the distance for 1000 Genome Project samples that contribute to the centroid. The plot on the Estimation worksheet includes one centroid for each of the major ancestries and orientational centroids at the one-quarter intervals between these groups. Centroids contextualize results and logically group populations.

Figure 8   Sample PCA plot
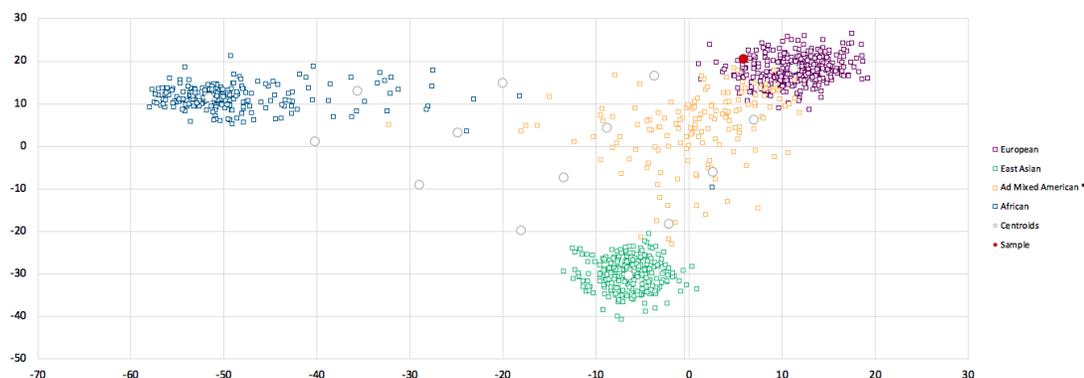
[4] Abecasis, Gonçalo R., David Altshuler, Adam Auton, Lisa D. Brooks, Richard M. Durbin, Richard A. Gibbs, Matt E. Hurles, et al., "A map of human genome variation from population-scale sequencing," *Nature* 467 (October 2010): 1061–1073, https://doi.org/10.1038/nature09534.

Phenotype Estimation

The software uses HIrisPlex[1–3], a multinomial logistic regression model, to determine the probabilities for hair, eye and skin color.

[1] Walsh, Susan, Lakshmi Chaitanya, Lindy Clarisse, Laura Wirken, Jolanta Draus-Barini, Leda Kovatsi, Hitoshi Maeda, et al., "Developmental validation of the HIrisPlex system: DNA-based eye and hair colour prediction for forensic and

anthropological usage,"*Forensic Science International: Genetics* 9 (March 2014): 150–161, https://doi.org/10.1016/j.fsigen.2013.12.006.

[2] Liu, Fan, Katevan Duijn, Johannes R. Vingerling, Albert Hofman, André G. Uitterlinden, A. Cecile J.W. Janssens, and Manfred Kayser, "Eye color and the prediction of complex phenotypes from genotypes," *Current Biology* 10, no. 5 (March 2009): R192–R193, https://doi.org/10.1016/j.cub.2009.01.027.

[3] Walsh, Susan, Fan Liu, Kaye N. Ballantyne, Mannis van Oven, Oscar Lao, and Manfred Kayser, "IrisPlex: A sensitive DNA tool for accurate prediction of blue and brown eye colour in the absence of ancestry information," *Forensic Science International: Genetics* 5, no. 3 (June 2011): 170–180, https://doi.org/10.1016/j.fsigen.2010.02.004.

Biological Sex Estimation for Samples

The software applies the following logic to estimate the biological sex of an Imagen sample.

- If the sample has ≥ 3 Y-SNP loci typed, the biological sex is male (XY). (Exception: If all autosomal loci are typed and the call rate is ≥ 50%, then > 10 typed Y-SNP loci are required to identify the sample as XY.)
- If the sample has zero Y-SNP loci typed and the call rate is ≥ 50%, the biological sex is female (XX).
- If the sample is a negative amplification control, the contributor status is mixture, or the scenarios for male and female do not apply, the biological sex is inconclusive.

Contributor Status of Samples

The software applies the following logic to determine the contributor status of an Imagen sample.

- Any of the following scenarios indicate a mixture:
  — The sample has ≥ 3 Y-SNPs with two alleles.
  — The sample has ≤ 45% auSNP intralocus balance.
  — The sample has ≤ 80% auSNP intralocus balance and ≥ 45% auSNP heterozygosity.
  — All auSNPs are typed, and the sample has ≥ 2 and ≤ 10 Y-SNP loci.
- Either of the following scenarios indicate a single source:
  — The sample has zero Y-SNP loci with 2 alleles, ≥ 65% auSNP intralocus balance, and < 45% auSNP heterozygosity.
  — The sample has zero Y-SNP loci with 2 alleles, > 80% auSNP intralocus balance, and ≤ 65% auSNP heterozygosity.
- If the sample is a negative amplification control, the call rate is < 50%, or the scenarios for mixture and single-source do not apply, the contributor status is inconclusive.

To adjust heterozygosity percentages for call rate, the observed heterozygosity is divided by the sample call rate.

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Technical Support

For technical assistance, contact Verogen Technical Support.

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Safety data sheets (SDS)—Available for download from verogen.com/product-documentation.

Product documentation—Available for download from verogen.com/product-documentation.

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