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Universal Analysis Software for ForenSeq® Signature Plus Module User Guide

Bioinformatics solution for the analysis of forensic genomic aSTRs, X STRs, Y STRs, and SNPs

Sample to Insight

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1. Software Overview

1.1. 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 comprehensive instructions for using UAS, including details about the analysis of data generated from the ForenSeq Signature Plus Kit and the ForenSeq DNA Signature Prep Kit using the default QIAGEN ForenSeq analysis methods.

Note: This guide describes version 2.7 of UAS.

1.2. Analysis workflow

Table 1 outlines the analysis workflow, which includes UAS and 2 applications that run on the MiSeq FGx System: MiSeq FGx Control Software (MFCS) and Real-Time Analysis (RTA).

Table 1. Overview of analysis steps

No.	Step	Description	Further information
1	Sign In or Create Account	The user enters identifying information to access UAS or creates a new account.	Accessing UAS ("Analysis methods" below)
2	Set Up a Run	The user imports sample information as a .txt file, or manually adds sample information.	Run Setup ("Run setup – Create a Run" on page 9)
3	Sequence Libraries	MFCS images the flow cell, and RTA performs image analysis and base calling. UAS communicates with the MiSeq FGx System to provide the user with updated run information during sequencing.	*MiSeq FGx Sequencing System Reference Guide* (document # VD2018006)
4	Analyze Data	When a run has finished, UAS automatically begins analyzing the resulting data. The user reviews these results.	Data Analysis ("Data Analysis – Sample Results" on page 19)
5	Generate Reports	The user generates reports on the analysis results in a variety of formats.	Reports ("Generating Reports" on page 26)

1.3. 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 the following default QIAGEN analysis method options:

- ForenSeq DNA Signature Prep— ForenSeq DNA Signature Prep Analysis Method Mix A
- ForenSeq Signature Plus — ForenSeq Signature Plus Analysis Method Mix A
- ForenSeq DNA Signature Prep — ForenSeq DNA Signature Prep Analysis Method Mix B
- ForenSeq Signature Plus — ForenSeq Signature Plus Analysis Method Mix B

In addition to the default QIAGEN ForenSeq analysis methods, you may create user-defined analysis methods with custom settings. (Default analysis methods cannot be modified.) For more information, see “Managing Analysis Methods”, section 8 on page 46.

1.3.1. Accessing UAS

UAS is a browser-based application that does not require an internet connection. The software can be accessed through a web browser on a computer with access to the same on-site local network as your MiSeq FGx System.

To access the UAS user interface, enter the network IP address of your UAS server in the browser's address bar.

Note: Accessing UAS through your on-site local network requires proper configuration of your UAS server and other computers on the network. New UAS servers come pre-configured with the software; local network configuration and software updates are facilitated by QIAGEN Technical Support.

If you cannot access UAS, contact QIAGEN Technical Support.

Supported browsers

QIAGEN recommends using Google Chrome™ to access the UAS web application. Comparable performance of the software on other web browsers is not guaranteed.

Set the screen resolution of your computer to at least 992 pixels for optimal display.

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”, section 8.7.1 on page 55.

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.

Important: 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.

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.

1.4. General features

The following features are available throughout UAS. For information about other supplemental software features, including commenting, history, and archiving, see "Run, Project, and Sample Management", section 7 on page 37, and "Software Settings", section 8 on page 46.

1.4.1. Sorting and filtering tables

Throughout the software, tables are used to display various information. Tables can be sorted and filtered as follows:

- To sort a table by a certain attribute, select that column name. The column name becomes bold, and an arrow appears to the right of the column name, indicating whether the sort is ascending or descending. Selecting the column name again toggles through the sort options: ascending, descending, or none.
- To filter a table by a certain attribute, select the **Filter** icon next to a column name. A drop-down menu appears, listing the possible values for that attribute. Select the checkboxes to include or exclude particular values from the table.

  Note: Some columns cannot be filtered.
- To reset filters to their default values, select **Reset Filters** in the toolbar above the table. Use the **X** in the individual filter chip to remove one filter at a time.
- To search for items in a table by name or other value, use the **Search** field.

1.4.2. Customizing tables

All tables throughout the software can be customized. Select **Customize Table** in the toolbar above any table to reveal a drop-down menu with a customizable list of the table's columns.

- To change the order of columns in the table, select and hold the handle icons next to each column name and drag them up or down.
- To hide a column from the table altogether, toggle the checkbox for that column.
- To reset all columns to their default order and visibility, select **Reset**.

1.4.3. Sample bookmarks

Throughout the software, bookmarks can be added by a user to designate specific samples of interest. These bookmarks can then be used to sort or filter the tables of samples displayed in the **Run** and **Project** workspaces.

Bookmarks are a sample-specific attribute that is shared across locations; if a user applies a bookmark on one page, it will appear on others as well. Bookmarks remain marked until they are unselected.

2. Workspaces

The software includes these primary workspaces:

- Home Page and Navigation – See "Home page and Navigation" below.
- Runs — Set up runs and manage runs during sequencing. See section 3 on the facing page
- Projects — View sequencing results, analyze data, and generate reports.
- Reports — See "Reports" on page 26.
- Database — Perform kinship analysis of antemortem and postmortem samples.

  Note: This workspace applies only to the Kintelligence HT module of UAS.

2.1. Home page and Navigation

The Home page functions as a system dashboard. Two panes list the most recent runs and projects, with options to create a new run or project or search for an existing run or project. Selecting a run or project opens that item in the Runs or Projects workspace, respectively. Color-coded Quality Metrics icons next to each run provide an at-a-glance view of run performance; hovering over that icon reveals detailed metrics.

Throughout the software, a vertical navigation rail appears on the left side of each page. The navigation rail provides buttons to open other workspaces, access software, and account settings, or return to the Home page. The Search button allows you to quickly search for a sample, run, or project by name or date.

Figure 1. Overview of the Home page. [A] Navigation rail — Buttons to open workspaces, search for a sample, run, or project, access settings, or return to the Home page. **[B]** Search — Search for a sample, run, or project by name or date. **[C]** Workspaces — Open one of the primary workspaces. **[D]** Settings — Access software or account settings. **[E]** Runs — A list of the most recent runs, with options to create a new run or search for an existing run. **[F]** Projects — A list of the most recent projects, with options to create a new project or search for an existing project. **[G]** Quality Metrics icon—This icon displays color—coded quality metrics for a completed run; hovering over it reveals detailed metrics.

3. Run Page: Overview and Functions

The Runs workspace provides tools to create and monitor sequencing runs, including tools to input and organize sample information. This workspace includes the following features:

- Metrics Page — View quality metrics and sample representation data for a run.
- Samples Page — View and edit samples in a run or add new samples.
- Supplemental Features for Runs — Use the Run Info, Run Activity, and Print buttons to perform additional functions.

Selecting Runs on the navigation rail opens a navigation drawer from which you can create a new run or open an existing run. Runs are listed from newest to oldest. A color-coded Quality Metrics icon next to each run provides an at-a-glance view of run performance; hovering over that icon reveals detailed metrics. Selecting a run opens the Samples page for that run.

Note: Throughout the software, selecting a hyperlink with the name of a run takes you to the Samples page for that run.

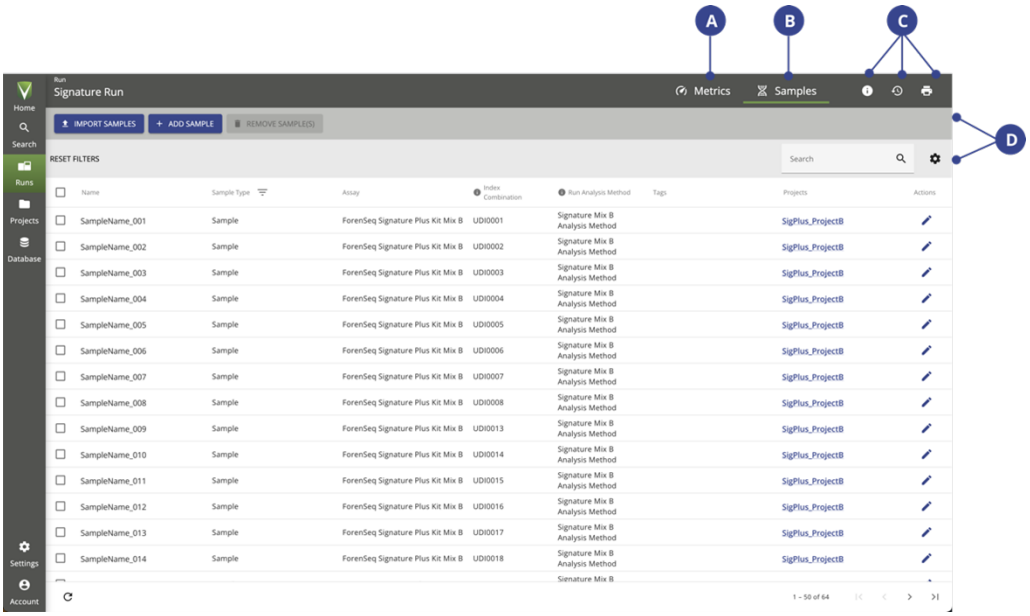


Figure 2. Overview of the Runs workspace. [A] Metrics page — Displays quality metrics and sample representation data for a run. (Jump to page here) **[B]** Samples page — Displays all samples in a run, with options to add new samples or edit sample information. **[C]** Supplemental features — Run Info, Run Activity, and Print buttons provide additional functions. **[D]** Toolbar — Available actions and options to sort or filter the Samples table.

3.1. 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 Samples page opens.

3.1.1. 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 Samples page in the Runs workspace lists all samples included in the run.

When adding samples to a run, each sample must be associated 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.

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

3.1.2. 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 view all entries. If using 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.

3.1.3. 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.

3.1.4. Assigning projects

The **InitialProjectList** column of a sample sheet specifies which project a sample is assigned to. To assign a sample to multiple projects, enter project names separated by a comma followed by a space, and surround the entire list of project names with quotation marks (e.g., “Project 1, Project 2, Project 3”).

3.1.5. 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.
Tags	Tags are recommended for the ForenSeq Kintelligence HT workflow. Leave this input blank for 2.7 release.
Description	Enter a preferred description of the sample.
Assay Type*	Select the library prep method: • ForenSeq Signature Plus — Libraries prepared with the ForenSeq Signature Plus Kit Mix A • ForenSeq Signature Plus Mix B • ForenSeq DNA Signature Prep Mix A • ForenSeq DNA Signature Prep Mix B
Sample Type	Select a sample type: • Sample • The positive amplification control (ForenSeq DNA Signature Prep) • The positive amplification control (ForenSeq Signature Plus) • The negative amplification control (nuclease-free water). • Reagent Blank — The extraction control.
Index Combination	• ForenSeq DNA Signature Prep select i7 and i5 index adapater • ForenSeq Signature Plus select the name of a premixed i7 and i5 index adapater (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.

3.1.6. Edit sample information

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

   Note: 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.

3.1.7. Run state

After a run is created and saved, you can view its state by opening the run in the Runs workspace and then selecting the **Run Info** button. The following table describes each possible state.

Table 2. 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.

3.2. Run metrics page

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

The 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" on page 62.

Monitor a Run

During a run, the 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 **Metrics** to review quality metrics and sample representation.
    a. Review the values for cluster density and clusters passing filter.
    b. Review the phasing and prephasing values for Read 1.
    c. Check Read 1, Index 1, Index 2, and Read 2 to confirm that all 4 reads passed.
    d. Check Overall Intensity and Discordant Loci to confirm that the HSC passed.
    e. Compare the samples to the sample read count guideline to confirm performance.
    f. Review the number of reads in parentheses next to the sample name or hover over each sample. For more information, see "Quality Metrics" on page 62 and "Sample representation" on page 66.
3. Select **Run Activity** to review the run activity.
4. Select **Close** to return to the run.

3.3. Samples page

Note: This section describes the Samples page as it appears in the Runs workspace. A Samples page with different features is also available in the Projects workspace.

The Samples page lists all samples in a selected run as a table. This page offers the following functions:

- Import Samples — Add multiple samples to the run using a .txt file.
- Add Sample — Manually add an individual sample
- Edit Sample Details — In the Actions column, select this icon to edit information for a particular sample. (Not all attributes can be edited.)
- Search — Search the table by sample name, run name, or index combination.
- Filter or sort the table by specific attributes.

3.4. Supplemental features for Runs

The following additional functions are available in the Runs header bar:

- Run Info — Edit the run name and view the run state, cycles per read, and other run information. See “Run State”, section 3.1.7 on page 11, and “Edit a Run”, section 7.1.1 on page 37.
- Run Activity — View system events and user actions for the run and add comments. See “Review Run Activity”, section 7.4.1 on page 40, and “Comment on a Run”, section 7.4.5 on page 41.

  Note: This icon is visible only when the history setting is enabled. See “System Settings”, section 8.8 on page 55.
- Print — Print the Samples and Metrics pages for a run. See “Print a Run”, section 7.1.2 on page 37.

4. Project Page: Overview and Functions

The Projects workspace provides tools to view sequencing results, analyze data, and generate reports. This workspace includes the following features:

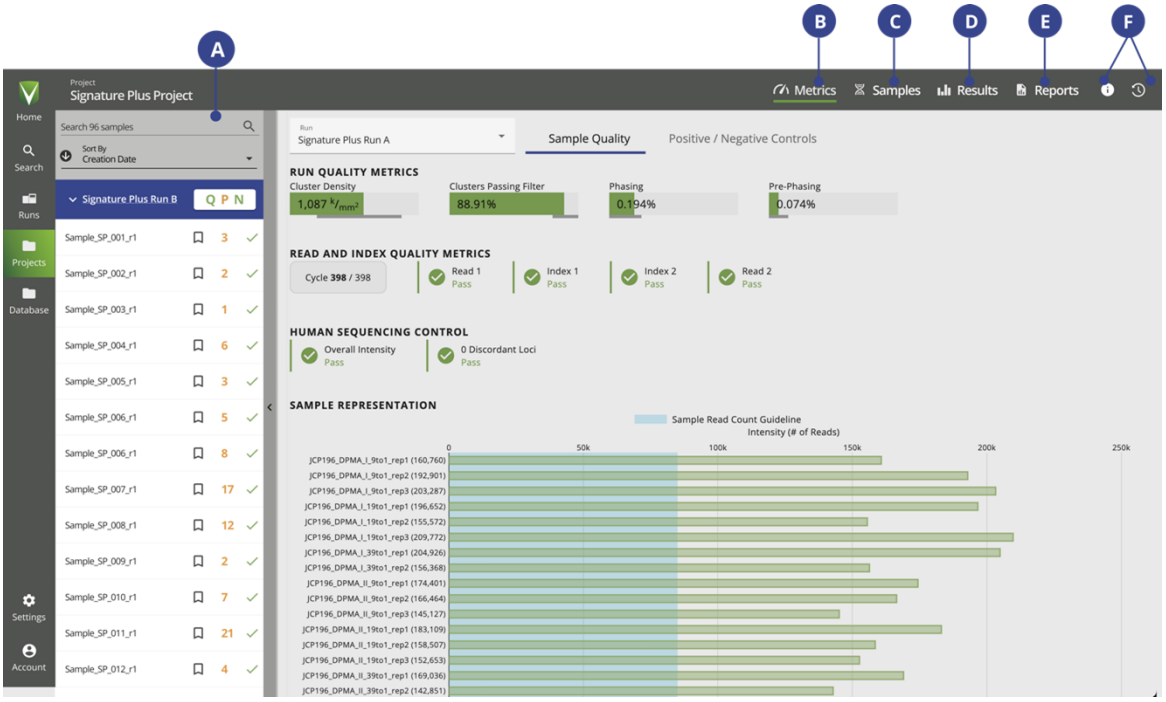


Figure 3. Overview of the Project Workspace. [A] Projects sidebar — Lists all samples in the project, grouped by run. Select a sample to view its results. **[B]** Metrics page — Displays metrics that provide an overall view of run performance. **[C]** Samples page — Displays all samples in the project as a table. **[D]** Sample Results page — A sample-specific page that displays in-depth analysis results for a particular sample. **[E]** Reports page — View and download previously generated reports. **[F]** Supplemental features — Project Info and Activity buttons provide additional functions.

Selecting **Projects** on the navigation rail opens a navigation drawer from which you can create a new project or open an existing project. Projects are listed from newest to oldest. By default, a project opens to the Metrics page.

For more information about the use of the Projects workspace, see “Data Analysis”, section 5 on page 19, and “Reports”, section 6 on page 26.

4.1. Samples page

Note: This section describes the Samples page as it appears in the Projects workspace. A Samples page with different features is also available in the Runs workspace.

The Samples page lists all samples in the project as a table. This page offers the following functions:

- Actions — A drop-down menu with available actions. (Select the checkboxes for one or more samples to apply an action to them.)

- New Report — Create a report using the selected samples. See "Reports", section 6 on page 26.
- Reanalyze — Analyze the selected samples again using a different analysis method. See "Reanalyze Samples", section 4.1.3 on the next page.
- Copy to... — Copy the selected samples to another project, or create a new project with the selected samples. See "Add Analyzed Samples to a Project", section 4.1.2 below.
- Create archive... — Create an archive for the selected samples. See "Archiving and Importing Data", section 7.5 on page 42.

- Add Samples — Search for samples in other projects to add to the current project.
- Search — Search the table by sample name, run name, or index combination.
- Filter or sort the table by specific attributes. See "Sorting and Filtering Tables", section 1.4.1 on page 6.

  Note: By default, a filter is applied to hide previous analyses from the table.

4.1.1. Samples table

The Samples table lists the following information about each sample in the project.

Table 3. Samples table details

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. See "Sample Bookmarks", section 1.4.3 on page 6.
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: *Sample*, *Negative Amplification Control*, or *Reagent Blank*, *HL60*, *2800M*, *NA24385* (positive amplification control), *Antemortem Sample*, *Postmortem Sample*.
Assay Type	The ForenSeq kit used to prepare the sample for sequencing and analysis.
QC Indicators	The number of quality control (QC) indicators that were flagged for the sample. Hovering over this number reveals a tooltip showing the number of QC indicators by type. Samples with QC indicators may require further review. See "Quality Control indicators", section 5.4.1 on page 22.
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" on page 70.
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" on page 70.

4.1.2. 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 Samples page, select **Add Samples**.
3. In the Add Samples dialog box, do the following:

a. In the Search field, enter a sample name.

b. 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.

c. To remove a sample, clear the checkbox or select **X** in the summary.

d. Select **Add** to add the samples.

4.1.3. 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 Samples 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.

Note: The software lists only analysis methods 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.

    Note: Go to "Switch Analysis Methods", section 5.0.2 on page 19, to learn how to switch and review the different analyses.

4.2. Sample Results page

The **Sample Results** page (labeled Results in the software) is a sample-specific page where you can view analysis results for a particular sample. Selecting a sample from the Samples page or the Projects sidebar opens its Sample Results page.

The Sample Results page offers different features depending on the assay type of the selected sample. For a detailed overview of each pane within the Sample Results page for Signature Plus samples, see "Data Analysis", section 5 on page 19.

4.2.1. Reports page

Note: This section describes the Reports page as it appears in the Projects workspace.

The Reports page allows you to view and download previously generated reports. (Reports are created from the Samples page or the Sample Results page.)

To download one or more reports, select the checkboxes for those reports, and then select **Download**. If you selected multiple reports, the software downloads those reports as a zipped file. For more information about reports, see "Reports", section 6 on page 26.

4.2.2. Supplemental features for projects

The following additional functions are available in the Projects header bar:

- Project Info — View information about the project or the currently selected sample, or edit the project name and description. See "Edit a Project", section 7.2.2 on page 38.
- Activity — View system events and user actions for the project or the currently selected sample, or add comments to the project or currently selected sample. See "History", section 7.4 on page 39.

  Note: This icon is visible only when the history setting is enabled. See "System Settings", section 8.8 on page 55.

Sample analysis state

After a sample is added to a run, you can view its analysis state by opening the sample's Sample Results page in the Projects workspace, and then selecting the **Project Info** button. The following table describes each possible state.

Table 4. Sample analysis states

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.

4.3. Projects sidebar

The Projects sidebar allows you to quickly locate samples within a project. Samples are grouped by run; selecting a sample opens the Sample Results page for that sample. By default, samples are sorted by index combination; use the **Sort By** drop-down menu to sort samples by a different attribute. The sidebar is collapsible.

Three icons appear next to each sample:

- Bookmark — A button to toggle a bookmark for the sample.
- Quality Control indicators — An orange number indicates the total number of quality control (QC) indicators that were

flagged for the sample. Hovering over this number reveals a tooltip showing the number of QC indicators by type.

- Analysis state — The analysis state of the sample.

A color-coded QPN icon next to each run in the sidebar provides an overall view of run performance. Selecting this icon opens the Metrics page for the run.

4.4. Project Metrics page

Note: This section describes the Metrics page as it appears in the Projects workspace. A Metrics page with more limited features is also available in the Runs workspace.

The Metrics page displays metrics that provide an overall view of run performance. The page is divided into 2 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” on page 62.

5. Data Analysis – Sample Results

Before generating reports, review results on a sample-by-sample basis using the Sample Results page in the Projects workspace.

Review quality control (QC) indicators and overall sample metrics. See "Locus quality indicators", section 5.4.1 on page 22.

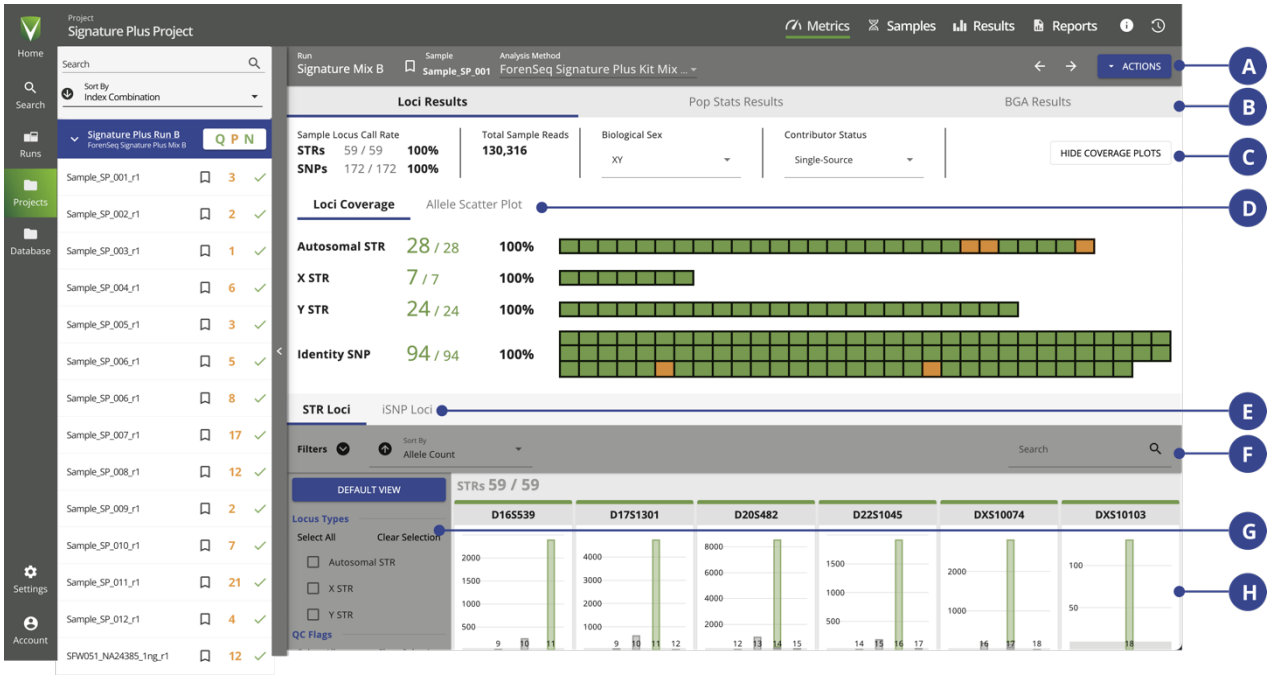


Figure 4. Overview of the Samples Results page. **[A]** Toolbar - Displays general information about the sample and available actions. **[B]** Sample Results - Section Tabs (Loci Results, Pop Stat Results, BGA Results). Select each tab to view results for Loci and coverage results, Population Statistics, and Biogeographical Ancestry (Mix B only) **[C]** Sample Overview - Key metrics for the sample and options to change the sample's biological sex or contributor status. **[D]** Coverage Plots - The coverage views display coverage by loci or alleles within the Loci Results tab. **[E]** Sample Details - Displays the histogram section that provides options to sort or filter by autosomal, X, and Y STRs, and identity SNPs. **[F]** Loci Sort Options - Sort loci by attribute and search for a locus by name. **[G]** Loci Filter Options - Filter loci by type or quality control (QC) flags. **[H]** Loci Details - Selecting a histogram reveals additional information about the locus.

5.0.1. Toolbar

A toolbar on the Sample Results page displays the following information and functions:

- Run and Sample — The run name and sample name.
- Bookmark — Apply a bookmark to the sample. (See "Sample Bookmarks", section 1.4.3 on page 6.)
- Analysis Method — The analysis method is applied to the sample.
- Arrow keys allow you to scroll through results for other samples.
- Actions — Provides options to generate reports, reanalyze the sample, or copy the sample to another project.

5.0.2. Switch Analysis Methods

A Results page displays the results for the selected analysis method from the drop-down 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 view

other results.

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

   Note: If a sample has been analyzed multiple times, you can select a different analysis method using the drop-down menu to see the results from that analysis. Analyses are listed in chronological order, with the most recent first. See "Reanalyze Samples", section 4.1.3 on page 16.

5.1. Loci Results tab

Change the results view by selecting the top tabs under the toolbar and above the overview section. The Locus Results will be selected and opened as the default view. The other tabs will show Population Statistics Results and Biogeographical Ancestry Results.

Note: The Biogeographical Ancestry Results section of the Results page appears only for samples prepared with the ForenSeq Signature Plus Mix B and the ForenSeq DNA Signature Prep Mix B library prep methods.

5.2. Sample overview

The Sample Overview section of the Sample Results page displays basic information about the sample, including the locus call rate, total reads, biological sex, and contributor status. You can modify both the biological sex and contributor status of the sample.

5.2.1. Change the biological sex of a sample

1. On the navigation rail, select **Projects**.
2. Select a project, then select a sample to open the Sample 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.

5.2.2. Change the contributor status of a sample

1. On the navigation rail, select **Projects**.
2. Select a project, then select a sample to open the **Sample 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.

5.3. Coverage plots view

The coverage views display coverage by loci or alleles. By default, loci coverage is displayed and depicted as a heatmap. Divided into aSTRs, X-STRs, Y-STRs, and iSNPs, the heatmap shows the number of loci typed out of the total number of possible loci. Hovering over a green or orange segment displays the locus name, intensity, and amplicon length in base pairs (bp). Gray segments do not contain these data.

Switching the view to alleles opens a scatter diagram that plots amplicon length along the X-axis and intensity along the Y-axis. Each dot represents a typed allele in the sample: yellow dots indicate Autosomal STRs, purple dots indicate X-STRs, light blue dots indicate Y-STRs, and green dots indicate Identify SNPs. Hovering over a dot reveals the STR type, locus name and allele number, intensity value, and amplicon length. Selecting the portion of the allele scatterplot using a screen select allows a user to zoom into this visualization section.

Clicking on a selected locus square on the heatmap or an individual dot on the allele scatter diagram takes you directly to that locus in the histogram.

5.4. Sample Details

The Sample Details or histogram view displays intensity values and thresholds for each locus in the sample. Loci are separated into tabs by type, STRs, or SNPs. All loci are displayed by default with the ability to filter, sort, and search options to refine results. Each locus is depicted as a histogram that shows a bar chart with one bar for each allele. Hovering over a bar reveals the intensity. Effective analytical thresholds appear as gray shading so you can confirm whether each allele meets the thresholds. When reviewing SNPs, an additional card view shows 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.

Selecting a locus card or histogram opens a pane with further information and options:

- Details — View thresholds and intralocus balance metrics, and type or untype specific alleles. (See "Type or Untype an Allele", section 5.6.1 on page 24.)
- User Actions — View user actions for the locus or add a comment to the locus. (See "Review Locus Activity", section 7.4.4 on page 41 and "Comment on a Locus", section 7.4.8 on page 42.)
- Any quality control (QC) flags, if applicable, appear above the expanded histogram.
- Use the Previous Locus and Next Locus arrows to scroll between loci. To close the locus details pane, select **Close**.

Note: Coverage plots can be hidden by clicking the **Hide Coverage Plots** button on the top right corner in the Sample Overview section. Hiding the coverage plot scales the histogram view to the larger screen area. Collapsing the filter bar or the project sidebar by selecting the gray bar with the arrow also scales up the histogram view.

5.4.1. Locus quality 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. Possible QC indicators include the following:

Table 5. QC indicators

Symbol	Name	Description
	Analytical Threshold	The locus has an expected allele signal below the AT and no alleles greater than or equal to the IT.
	Interpretation Threshold	The locus has an expected allele signal greater than or equal to the AT, but below the IT.
	Not Detected	The software did not detect a signal for the locus.
	User Modified	A user typed or untyped an allele at the locus.
	Imbalanced	The locus is not homozygous, not hemizygous, and falls below the intralocus balance setting.
	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.
	Detected	An unexpected locus was typed (e.g., an NTC with typed loci, or an XX (Female) sample types Y SNPs above the threshold).
	Stutter†	At least one sequence exceeds the stutter filter percentage at a stutter position of a possible parent allele.

* SNPs only.

† STRs only.

5.5. Loci sorting and filtering

The following sort options and other functions appear in the horizontal bar above the grid of loci:

- Filter Bar — Expand or collapse the Filters sidebar.
- Search — Find a locus by name.
- Selecting Default View in the Filters sidebar clears selected sort, view, and search options, and restores defaults.
- Toggle between view options (SNP Tab only):
    - Histogram view — Each locus appears as a histogram showing the intensity of each allele relative to the analytical threshold.

- Card view — Each locus appears as a card showing the intensity of each allele as a number, along with the intralocus balance (ILB).

5.5.1. Sort loci

Sort loci by one or more of the following attributes. Select the arrow next to each drop-down menu to toggle the sort between ascending and descending.

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
Allele count	Sort loci by the number of alleles.
Intensity	Sort loci by intensity value.
Intralocus Balance	Sort loci by intralocus balance percentage.
Amplicon Size	Sort loci by amplicon size in bp.
Chromosome	Sort loci by chromosome number or letter.
Locus Type	Sort loci by category type.
Locus Name	Sort loci by rsID.

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**.

5.5.2. Filter loci

The Filters sidebar provides options to filter loci by chromosome, typed vs. untyped, or homozygous vs. heterozygous. More granular filter options are also available by type of QC Indicator.

Filter	Effect
Locus Type	Displays only loci that belong to the selected loci types.
QC Indicators	Displays only loci flagged with the selected QC indicators. (See list of locus quality indicators)

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, by default, the STRs loci tab is open, or select the SNP tab to view iSNP filter and sorting options. Then select any of the Locus type or QC Indicator filters to view only the desired loci.

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.

5.5.3. 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, then press Enter. The Results page displays loci matching the search term.

5.6. Locus Details

Selecting a histogram or a card opens Locus Details, which enlarges the bar chart and shows additional data for each allele, including intralocus balance, thresholds, intensity, stutter, sequences, and QC indicators.

Toggle keys indicate whether alleles are typed.

- The Previous Locus and Next Locus arrows move through details for each locus.
- The **X** icon closes locus details.

5.6.1. Type or untype an allele

Note: A green toggle histogram bar indicates 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, then select a sample to open the Sample Results page.
3. In the Sample Details section of the page, select a histogram or locus card 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.

5.7. Population Statistic Results tab

Change the results view by selecting the **Pop Stat Results** tab under the toolbar and above the overview section.

Note: Pop stats are generated automatically. To turn on or off specific pop stats or to upload a custom group, you must be an admin and go to **Settings** > **Population Group Settings** to manage system defaults (for more information, see section 8.5 on page 49).

1. On the navigation rail, select **Projects**.
2. Select a project, then select a sample to open the Results page.
3. There are 3 tabs above the Sample Overview section. Select the tab labeled **Pop Stat Results**.
4. Population statistics are displayed in horizontal bars.
5. Option to filter and sort results:

a. To filter results, select the button tabs labeled **iSNPs** or **Autosomal STRs**. Click it again to return to view all results unfiltered.

b. Sort by selecting the arrow in the dropdown and selecting between organizing results by Probability or Pop. Group Name. Select the arrow to change the list from ascending to descending order.

For more information, see section 8.5 on page 49.

5.8. Biogeographical Ancestry Results Tab

The BGA Results Tab displays a plot showing estimates of the biogeographical ancestry of the sample based on Ancestry SNPs and Phenotype SNP data. An identical plot appears in the BGA Estimation report. The tables below the plot can be expanded to show detailed information about samples included in the centroid.

Note: This section of the Results page appears only for samples prepared with the ForenSeq Signature Plus Mix B library prep method. For more information, see "Biogeographical Ancestry Estimation" on page 71.

6. Reports

6.1. Report types

The software can compile Signature Plus results and other information into the following reports:

- Sample — Results for one sample.
- Project — Results for all samples in a project.
- CODIS — Variants from the selected samples for upload to CODIS.
- Flanking Region — Results from variants in the flanking region of the STR amplicon.

Note: The below report types are available only for samples prepared with the ForenSeq Signature Plus Mix B library prep method and sequenced using the Signature Plus workflow in MiSeq FGx®.

- BGA Estimation — Estimates of biogeographical ancestry based on aSNP and pSNP data.
- HIrisPlex-S — Typed SNPs for hair and eye color. Results are output as a ***.csv** file that can be used with the HIrisPlex-S online tool to generate phenotype estimates. For more information, see "HIrisPlex-S report", section 6.8 on page 34.

6.2. 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", section 4.2.1 on page 17).

Note: This chapter describes how to create reports from the Project Samples page. All report types except project reports can also be created from the Results page for an individual sample.

6.3. Sample report

A Signature Plus sample report is an Excel workbook with 2 worksheets each for aSTRs, Y-STRs, and iSNPs, and a settings worksheet that lists analysis thresholds for each locus.

- The Autosomal STRs and Y STRs worksheets include the following sample information.
  - A header section lists the following information for the sample:
    - Sample name
    - Project name
    - Analysis method
    - Run name
    - Biological sex
    - Contributor status

        - Project creation date and time
        - User who generated the report
    - Locus information is formatted in a table that lists each locus with genotypes and any QC indicators. A fraction indicates how many loci are typed out of the total possible number of loci.
    - Coverage information is formatted in a table that lists the following information for each locus:
        - Locus name and each allele name
        - Whether each allele is typed
        - Number of reads for each allele
        - Bracketed and repeat sequence of each allele
    - The Autosomal STR Figure and Y STR Figure worksheets indicate the total number of reads for the sample with a breakdown of how many reads each allele received.
        - This information is presented in both a table and a bar chart.
        - Filters for locus name and typed or untyped alleles are available at the top of the table.
    - The Settings worksheet displays the analysis

The SNP worksheet report is an Excel workbook with one worksheet per SNP type: identity (Mix A), ancestry, and phenotype (Mix B Only). 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

6.3.1. 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, and then select **Samples** to open the Sample List page.
3. Select the sample or samples for which you want to create reports.

   Note: To quickly select all samples, select the checkbox at the top of the Samples table. For information on sorting and filtering samples, see “Samples Page”, section 3.3 on page 12.

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.

Note: All generated reports can also be accessed from the Reports page. See "Reports Page", section 4.2.1 on page 17.

6.4. Project Report

A Signature Plus 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:

- Analysis method
- Contributor status
- Biological sex
- Locus call rate
- Sample name and optional description
- Total sample reads

6.4.1. Create a Project Report

Note: 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, and then select **Samples** to open the Sample List page.
3. Select the samples you want to include in your report.

   Note: To quickly select all samples, select the checkbox at the top of the Samples table. For information on sorting and filtering samples, see "Samples Page", section 3.3 on page 12.
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, which is immediately downloaded and saved to your local machine based on your browser settings.

Note: 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.

Note: All generated reports can also be accessed from the Reports page. See "Reports Page", section 4.2.1 on page 17.

6.5. CODIS report

Create a CODIS report for one sample or multiple samples. Creating a report for one sample lets you specify extra specimen information and choose which positions to include in the report. When creating a report for multiple samples, the software automatically includes all positions for each sample.

A CODIS report is a ***.cmf** file with a CMF header, reference sequence, and specimen profile. The specimen profile provides the following information for each sample in the report:

- Specimen ID
- Specimen category
- Source ID

6.5.1. Create a CODIS report for one sample

1. On the navigation rail, select **Projects**.
2. Select a project, then select a Sample to open the Sample's Results page.
3. Select the blue **Actions** button, then select **CODIS Report**. The Create CODIS Report dialog box opens.

   Note: Add pre-populated CODIS defaults by going to **Settings**, then **CODIS Report Defaults**. For more information, see "CODIS Report Defaults", section 8.3 on page 48.
4. Edit the following CMF Header fields if you have not added the default values in Settings:
   - Destination Laboratory ORI — Identifier for the agency that processes the CODIS report.
   - Source Laboratory ORI — Identifier for the agency that processes the specimen.
   - Submission User ID — Identification of the person who generates the CODIS report.
   - Batch ID — Identifier that tracks where a DNA profile originated. (Optional)
5. To include the name of the library prep kit in the report, toggle **Include Kit Name in Report**.
6. Select **Next**.
7. On step 2, Specimen Information, edit the Specimen ID field: enter a unique identifier for the specimen or accept the default sample name.
8. In the Specimen Category list, select a preconfigured category.
9. **Optional**: Select **More Options**.
   a. Complete the following fields:
      - Source ID — Specify whether the identity of the specimen contributor is known.
      - NCIC Number — Enter the unique number for storage in the NCIC system.
      - ViCAP number — Enter the unique number for storage in the ViCAP system.
      - Case ID — Enter the law enforcement identifier associated with the specimen.
      - Specimen Comment — Enter any additional information about the specimen.

b. If the specimen has possible no call regions or other conditions requiring additional information to help with match resolution, select **Partial Profile**.

10. Select **Next**.
11. Select the toggle key for each position you want to remove from the report. By default, all positions are included.
12. Select **Create** to generate the report.
13. The software then generates the report. Based on your browser settings, the report is automatically downloaded and saved to your local machine.

    Note: All generated reports can also be accessed from the Reports page.

6.5.2. Create a CODIS report for multiple samples

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

   The Create CODIS Report dialog box opens.
4. Select the checkbox of each sample you want to include in the report.
   a. Scroll and use the arrows to move through the complete list of samples in the project.
   b. Under **Summary**, select **Delete** to remove samples from the report.
5. Select **Next**.

   Note: Add pre-populated CODIS defaults by going to Settings, CODIS Report Defaults. For more information, see "CODIS Report Defaults", section 8.3 on page 48.
6. Edit the following CMF Header fields if you have not added the default values in Settings:
   - Destination Laboratory ORI — Identifier for the agency that processes the CODIS report.
   - Source Laboratory ORI — Identifier for the agency that processes the specimen.
   - Submission User ID — Identification of the person who generates the CODIS report.
   - Batch ID — Identifier that tracks where a DNA profile originated. (Optional)
7. To include the name of the library prep kit in the report, select **Include Kit Name in the Report**.
8. Select **Next**.
9. In the Specimen ID field for each sample, enter a unique identifier for the specimen cited in the report.
10. Assign specimen categories per the following table.

Option	Instruction
Assign the same category to all	In the Specimen Category list at the top of the dialog box, select a category, and then select **Apply to All Specimens**.
Assign a different category to each sample	In the Specimen Category list for each sample, select the applicable category.

11. Select **Create** to generate the report.

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

    Note: All generated reports can also be accessed from the Reports page.

6.6. Flanking Region Report

A Signature Plus Flanking Regions report is a sample-level Excel workbook with worksheets each for Autosomal STRs, X and Y-STRs, and iSNPs. In addition, a settings worksheet lists more information about each flanking region's analysis thresholds for each locus.

- The Autosomal STRs, X and Y STRs, and iSNPs worksheets include the following sample information for each unique flank sequence per locus per STR length
  - A header section lists the following information
    - The Sample Name is prepended to the words "Flanking Region Report"
    - The name of the Project from which the report was generated
    - The name of the Sample for which the report was generated.
    - The name of the Analysis Method for which the report was generated
    - The date, time, and time zone on the system when the report was generated.
    - The user who generated the report
  - Coverage information is formatted in a table that lists the following information for each STR locus:
    - Locus name
    - STR Length
    - Number of reads supporting the STR sequence
    - Bracketed sequence representing the amplicon
    - The full sequence representing the amplicon and the flanking regions
    - GRCh38 Position Start and GRCh38 Position End
    - Reference Bases added to Upstream Flank
    - Reference Bases added to the downstream Flank
    - Reported strand has typed allele (above Interpretation threshold)
  - Nucleotide differences between two or more sequences of the same length at the same locus are displayed below
    - The base difference within the repeat region is highlighted in bold, black text
    - The base difference within the flank region is underlined and highlighted in bold, blue text
  - Coverage information is formatted in a table that list the following for each iSNP locus:
    - Locus name
    - Chromosome
    - iSNP & Variant Reference SNP

- iSNP & Variant GRCh38 Position
- Detected Bases

  Note: Bases detected at the ForenSeq iSNP positions are presented in bold, black text. Bases detected for the predefined potential positions of variation outside of the target iSNP position are presented in bold, blue text.
- Read
- SNP location with Flanking Region Sequence

6.6.1. Create a Flanking Region Report for One Sample

1. On the navigation rail, select **Projects**.
2. Select a project, then select a Sample to open the Sample's Results page.
3. Select the blue **Actions** button, then select **Flanking Region Report** to generate the report.
4. Confirm the sample 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.

Note: All generated reports can also be accessed from the Reports page. See "Reports Page", section 4.2.1 on page 17.

6.6.2. Create a Flanking Region Report for Multiple Samples

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

   Note: To quickly select all samples, select the checkbox at the top of the Samples table. For information on sorting and filtering samples, see the "Samples Page", section 3.3 on page 12.
4. Select the blue **Actions** button, then select **Flanking Region Report** from the drop-down menu.
5. Confirm the samples selected in the dialog box, then select **Create**.

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

Note: 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", section 4.2.1 on page 17.

6.7. Phenotype and Ancestry Estimation Report

The Phenotype and Ancestry Estimation report is an Excel workbook detailing estimates of Hair and Eye color and biogeographical ancestry based on aSNP and pSNP data. The workbook is composed of 4 worksheets containing the following information:

Table 6. Phenotype and Ancestry Estimation Report contents

Worksheet	Contents
Estimation	Table for hair and eye color estimation values • 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.

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 (1).

On the Estimation worksheet, 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 chart 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.

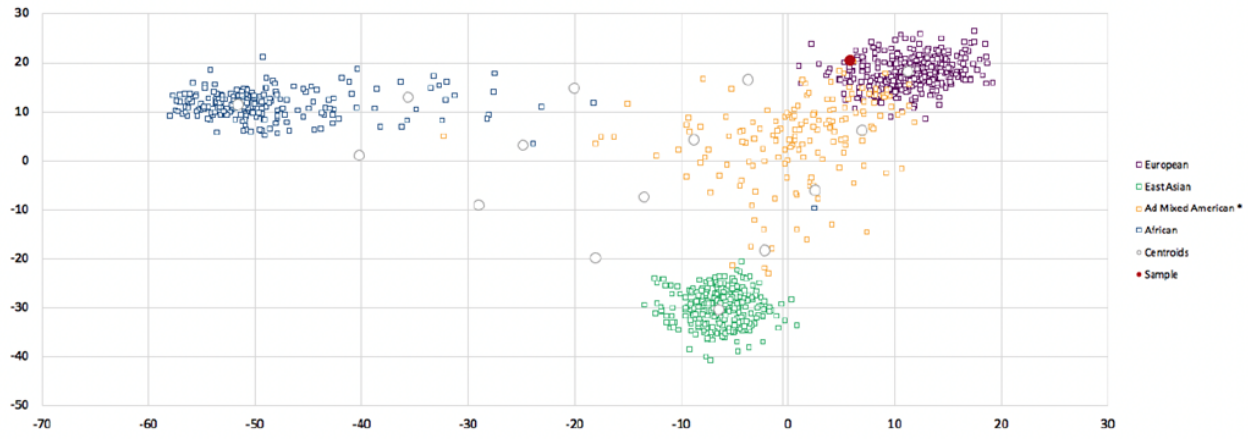


Figure 5. Sample PCA plot.

For more information about how the software computes estimates of biogeographical ancestry, see "Biogeographical Ancestry Estimation" on page 71.

6.7.1. 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.

The software does not generate a result if one of these SNPs is not typed. 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 multi-locus genotype.

6.7.2. Create a Phenotype Ancestry Estimation Report

Note: This report type is available only for samples prepared with the ForenSeq DNA Signature Prep and ForenSeq Signature Plus library prep method and sequenced using the ForenSeq DNA Signature Prep or ForenSeq Signature Plus workflow in MiSeq FGx.

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

1. On the navigation rail, select **Projects**.
2. Select a project, then select **Samples** to open the Sample List page.
3. Select the sample or samples you want to create reports for. Select the checkbox at the top of the sample list to quickly select all samples. For information on sorting and filtering samples, see “Sample List Page”, section 4.2 on page 16.
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**.

Note: 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 select 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”, section 4.2.1 on page 17.

6.8. HIrisPlex-S report

A HIrisPlex-S report is a ***.csv** file that includes typed SNPs for hair color and eye color. The file can be used with HIrisPlex-S, a multinomial logistic regression model, to generate phenotype estimates. For more information about HIrisPlex-S, see "References" on page 61 and **hirisplex.erasmusmc.nl**.

QIAGEN 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, upload first the file to HIrisPlex-S, and then run I-HAT on the results.

6.8.1. 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, and then select **Samples** to open the Sample List page.
3. Select the sample or samples you want to create reports for. Select the checkbox at the top of the sample list to quickly select all samples.
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**.

Note: The software generates the report or reports. Based on your browser settings, the report is automatically downloaded and saved to your local machine. The software downloads those reports as a zipped file if you select multiple samples. All generated reports can also be accessed from the Reports page. See "Reports Page", section 4.2.1 on page 17.

6.8.2. Using HIrisPlex-S

Once you have generated a HIrisPlex-S Report (see above), use HIrisPlex-S as follows:

1. In your browser, navigate to **hirisplex.erasmusmc.nl**.
2. Scroll down. Under the heading The HIrisPlex System, select **Choose File**.

   Note: When scrolling to upload your report, use the HIrisPlex System for results. If you use the HIrisPlex-S the skin results will not be accurate. The Signature Plus assay does not include all valid skin SNPs.
3. Locate the HIrisPlex-S report ***.csv** file on your local machine, then select **Upload File**. The file is uploaded to HIrisPlex for processing.
4. Once HIrisPlex has finished processing your report, the ***.csv** file will be automatically stored to your selected folder for Downloads.

6.8.3. Using I-HAT

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

1. Open the file **I-HAT.xlsm** with Microsoft Excel. If prompted to enable macros, select **Enable Macros**.

   Note: Locate the **I-HAT.xlsm** file by going to your **Verogen Server** > **FUAS** > **Toolbox** > **Tools**.

   If the prompt to **Enable Macros** does not appear, ensure your Developer tab is turned on. Depending on which version of Excel you use, you can access the Developer tab by going to **File** > **Options** > **Customize Ribbon** and checking the Developer box. In 2024 Excel, go to **Excel Preferences**, select **Ribbon & Toolbar**, go to **Main Tabs**, and check **Developer** section.
2. Open the ***.csv** file output by HIrisPlex.
3. Select the **Developer** tab, then select **Macros**.
4. On the resulting dialog box, do the following:
   a. From the Macro name list, select **'I-HAT.xlsm'!HIrisPlexSMacro**.
   b. Set **Macros** in the dropdown menu to **I-HAT.xlsm**.
   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 and hair 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.

7. Run, Project, and Sample Management

7.1. Managing Runs

7.1.1. Edit a Run

1. On the navigation rail, select **Runs**. Then select a run.
2. Select **Run Info**, 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.
4. Select **Save**.

7.1.2. Print a Run

The Print icon in the header bar converts the Samples page and 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 Samples and 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** to return to the Metrics page.

7.2. Managing Projects

7.2.1. 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.

Note: Although permitted by the software, 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 Metrics page.
5. Open the Samples page and select **Add Samples**.

Note: You can add any samples in the software to the project, analyzed or not.

6. In the Add Samples dialog box, do the following:
    a. In the Search field, enter a sample name.
    b. 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.
    c. To remove a sample, clear the checkbox or select **X** in the summary.
    d. Select **Add** to add the samples.

The Samples page lists the added samples. Runs the samples belong to appear in the Projects sidebar.

7.2.2. 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:
    a. Edit the project name.
    b. Add a project description or modify the existing description.
4. Select **Save** to apply the changes.

7.2.3. 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**.

7.2.4. Obtain the Analysis Folder Path

The analysis folder resides on your UAS 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, then select a sample to open the Sample Results page.

3. Select the **Information** icon. The path appears in a pop-up window.
4. The path is displayed under Analysis Folder Path.

7.3. Managing Samples

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.

7.3.1. 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 the **Filter** to apply the selected filters.
4. To sort the samples, select a column heading. The Name, Description, and Index Combination columns are sortable.

7.3.2. 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**.

7.4. 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. Instructions on enabling the history setting are on “System Settings”, section 8.8 on page 55.

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

7.4.1. 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 the results down 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**.

7.4.2. 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 the results down 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**.

7.4.3. 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 the results down 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**.

7.4.4. Review Locus Activity

1. On the navigation rail, select **Projects**.
2. Select a project, then select a sample to open the Sample 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**.

7.4.5. 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**.

7.4.6. 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**.

7.4.7. 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**.

7.4.8. 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, then select a sample to open the Sample 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**.

7.5. Archiving and Importing Data

Note: This feature is available only for administrators.

You may archive (i.e., export) data pertaining to samples, runs, and projects from UAS in order to transfer the data to another UAS installation or free up space on your UAS server. The data is exported in a packaged ***.zip** file. Once exported, the data may be seamlessly imported back into UAS from the packaged file.

The software offers 2 different archive functions:

- Archive a Sample — Archives data pertaining to one or more samples as FASTQ files. Data are copied but **not** deleted from UAS.
- Archive a Run or Project — Archives data pertaining to a run or project. You may choose to leave the original data in UAS **or** delete the original data from UAS once the archive has been created.

Note: UAS will not delete any data or files that are still associated with another run or project. For instance, if you choose to archive and delete a project that shares a run with another project, then all data for that project except that specific run will be deleted.

Archive ***.zip** files are saved to the folder `D:\Verogen\FUAS\Archives\` on your UAS server. Previously generated archive files are listed on the Data Management page, accessible through the Settings menu on the navigation rail.

7.5.1. Archive Contents

An archive of one or more samples consists of FASTQ files for those samples, including their associated analyses, overrides, run metrics, and other data.

By default, a run or project archive includes FASTQ files for all associated samples. You may optionally choose to create a "complete" archive of a run or project, which additionally includes the original sequencing files (BCLs). Choosing to create a complete archive will significantly increase the size of the packaged ***.zip** file.

A complete archive of a run or project may include the following files:

- All FASTQ files for associated samples
- config.xml
- ***.locs** files
- ***.bcl** files
- ***.stats** files
- ***.filter** files
- For a run, all project analyses and sample database data related to that run
- For associated samples, sample comparisons and related statistics, Project Reports, Sample Reports, CODIS
- Reports, phenotype estimations, and corresponding reports

7.5.2. Create an Archive for One or More Samples

Note: Creating a sample archive copies the associated data but does not remove the original data from UAS.

1. On the navigation rail, select **Projects**.
2. Select a project from the navigation drawer.
3. Select **Samples** to open the Samples page.
4. Within the Samples table, select the checkboxes for the sample or samples you wish to archive.

Note: As a tip, you may sort or filter the Samples table to locate samples more quickly. See "Sorting and Filtering Tables", section 1.4.1 on page 6.

5. Select **Actions**. Under **Options**, select **Create Archive...**. The Archive Samples window opens.
6. Review the selected sample or samples, and then select **Next**.
7. **Optional**: Enter a new name for the run, and then select **Next**.
8. Enter a name for the archive ***.zip** file, and then select Archive. UAS begins to process the archive file.

The Data Management page, accessible through the Settings menu on the navigation rail, will list the archive file and its status (Processing or Completed). Once completed, the archive ***.zip** file is saved to the folder `D:\Verogen\FUAS\Archives\` on your UAS server.

7.5.3. Create an Archive for a Run or Project

1. On the navigation rail, select **Settings**.
2. Select **Data Management**. The Data Management page opens.
3. Select **Archive**. The Create an Archive window opens.
4. Select the run or project you wish to archive, then select **Next**.
5. At the Archive Options & Rename step, do the following:

   a. Enter a name for the archive ***.zip** file.

   b. **Optional**: Select the options **Complete archive with all run files** or **Delete data after archive is complete** as desired.

   c. **Optional**: Enter a new name for the run or project.

   When finished, select **Next**.
6. Review the options you selected, and then select **Archive**. UAS begins to process the archive file.

Note: Depending on the size of the run or project you are archiving, UAS may take 10 min or more to process the file.

The Data Management page will now list the archive file and its status (Processing or Completed). Once completed, the archive **.zip** file is saved to the folder `D:\Verogen\FUAS\Archives\` on your UAS server.

7.5.4. Import an Archive

1. **Optional**: If the archive file you wish to import is not already in the folder `D:\Verogen\FUAS\Archives\` on your UAS server, copy the file into the folder.

   Note: Only archive files that were originally created by an installation of UAS version 2.6 or later can be imported.
2. On the navigation rail, select **Settings**.
3. Select **Data Management**. The Data Management page opens.
4. Select **Import**. The Import Archive window opens.
5. Select an archive file, and then select **Next**.

Note: If you choose to import an archive of a run or project whose original files were never deleted from UAS, then you will be creating a duplicate version of that run or project in UAS. If the name of the imported archive file is identical to the name of an existing run or project, UAS will append a number to the imported file's name.

6. Review the archive file you chose to import, then select **Import**.

8. Software Settings

8.1. Settings overview

Use the following settings, which are available from the Settings menu on the navigation rail, to configure the software. Some settings are available only for administrators.

- Analysis Methods — Create and manage analysis methods
- Data Management — Archive data from UAS and import previously created archive files
- CODIS Report Defaults — Set CODIS report inputs and defaults
- Population Group Settings — Manage population settings and choose loci
- 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 bioinformatics analysis methods
- Dark Mode — Change the user interface color scheme to be color-blind compatible

8.2. Managing analysis methods

The default QIAGEN analysis methods cannot be modified, but you may create user-defined analysis methods with custom settings.

Note: The performance of UAS is guaranteed only when using the default analysis methods. Perform internal validation studies as needed to develop settings for custom analysis methods.

Note: Whenever QIAGEN updates the default analysis methods, any user-created analysis methods will not automatically update. You must re-copy the default analysis methods if you wish to incorporate any changes in those analysis methods into your custom analysis methods.

8.2.1. Create an analysis method

You can create an analysis method with the desired settings in one of 2 ways: copy an existing analysis method and modify its settings, or start with a blank analysis method.

8.2.2. 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).

8.2.3. 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, section 8.2.4 below.

8.2.4. Modify analysis settings

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

8.2.5. 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 QIAGEN 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 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 view 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.

8.2.6. Delete an analysis method

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

1. On the navigation rail, select **Settings**.
2. Select **Analysis Methods**.
3. In the 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.

8.2.7. Change the default analysis method

1. On the navigation rail, select **Settings**.
2. Select **Analysis Methods**.
3. In the Assay Type list, open and scroll down to the kit that corresponds to the analysis method you want to set as the default.
4. A green star identifies the current default. Select the empty star next to the analysis method name to set it as the new default.

8.3. Data Management

Note: This feature is available only for administrators.

The Data Management page provides functions for archiving data from UAS and importing previously generated archive files. Existing archive files are listed in a table, along with their state (Processing or Completed).

For more information about these functions, see “Archiving and Importing Data”, section 7.5 on page 42.

8.4. CODIS Report Defaults

To expedite report creation, define default values that appear in the CMF header of every CODIS report. You can override these values on a per-report basis.

8.4.1. Define CODIS Report Defaults

1. On the navigation rail, select **Settings**.
2. Select **CODIS Report Defaults**.
3. Select **CODIS Report for STR samples**.
4. In each of the following fields, enter a default value:

- Destination Laboratory ORI — Identifier for the agency that processes the CODIS report.
- Source Laboratory ORI — Identifier for the agency that processes the specimen.
- Submission User ID — Identification of the person who generates the CODIS report.

5. Select **Save** to apply the default values.

8.4.2. Set Specimen Categories

The Specimen Categories setting determines which specimen categories are available to assign to samples when creating a CODIS report. By default, all categories are available.

1. On the navigation rail, select **Settings**.
2. Select **CODIS Report Defaults**.
3. Select **CODIS Report for STR samples**.
4. Select the **Specimen Categories** tab. This tab lists all specimen categories, divided into CODIS and Custom columns.
5. Select a specimen category to make it available or unavailable for reports. A blue toggle key indicates an available category.

8.4.3. Add Custom Specimen Categories

To supplement the predefined categories, the software can be updated with custom specimen categories. Custom categories can be renamed, enabled, disabled, and deleted.

1. On the navigation rail, select **Settings**.
2. Select **CODIS Report Defaults**.
3. Select **CODIS Report for STR samples**.
4. Select the **Specimen Categories** tab.
5. In the New Specimen Category Name field, enter a name of your choice.
6. Select **Add**. The new category appears in the Custom column and is enabled by default.

8.5. Population Group Settings

Population statistics calculations by the software require 2 components: at least 1 population group database to reference and a method of calculation. The software comes installed with several population group databases ready for use. You can use population databases included with the software or upload your own. Although random match probability is the default calculation method, you can select likelihood ratio instead.

The software also supports the ability to define source attribution thresholds. Source attributions are relative to guidelines from Budowle et al. (2000).

Population group database selection, method of population statistics calculation, and source attribution thresholds are controlled on the Population Group Settings page. The Population Group Settings page is launched from the Maintenance Page.

8.5.1. Defining the Statistics Calculation Method

The ForenSeq Universal Analysis Software supports the calculation of population statistics by either random match probability or likelihood ratio methods, using the $2p$ or $2p - p^2$ rule. When selected, the likelihood ratio method creates the inverse of the result retrieved from the random match probability method. By default, the software uses random match probability and the $2p - p^2$ setting for population statistics calculations. If you select a different calculation method, this method is used for all new calculations in the system going forward. Existing results remain unchanged.

8.5.2. Changing the calculation method for Population Statistics

1. On the navigation rail, select **Settings**. The Population System Defaults page opens.
2. Under the Method section, select **RMP** (Random Match Probability) or **LR** (Likelihood Ratio).

   Note: If you select **Likelihood Ratio**, the software automatically inverts the source attribution threshold setting. See "Changing the Source Attribution Threshold", section 8.5.4 on the facing page.
3. Under the Population Statistics Calculation, select **Use** $2p$ or **Use** $2p - p^2$.
4. Click **Save**.

8.5.3. Applying the $2p$ Rule to a Homozygous Locus

When the genotype of a locus is homozygous (diploid), the formula for population statistics calculation is

$$P(A)^2 + P(A) \times (1 - P(A)) \times \theta$$

where

- $P(A)$ is the effective frequency of allele A in use for the reference population, and
- θ is the population structure correction factor.

The calculation method for a homozygous locus can be changed by applying the $2p$ rule in the locus detail box. The results of applying the $2p$ rule to a homozygous locus in the locus detail box is dependent on the population statistics calculation

setting on the Population Group Settings page. When the setting is Use $2p$, and the $2p$ rule is applied in the locus detail box, the calculation formula becomes $2 \times P(A)$. In contrast, when the population statistics calculation setting is Use $2p - p^2$, and the $2p$ rule is applied in the locus detail box, the calculation formula becomes $2 \times P(A) - P(A)^2$.

8.5.4. Changing the Source Attribution Threshold

The source attribution threshold is defined to facilitate visualization of population group statistics in a display bar. Population statistics that are less than the frequency of the source attribution threshold are colored on the results bar in orange. The threshold is visible on the display as a gray bar below the result.

There are 2 source attribution threshold settings, each corresponding with a particular population group statistics calculation method. The software calculates population statistics as random match probability or likelihood ratio methods. The likelihood ratio method creates the inverse of the result retrieved from the random match probability method. By default, as corresponds with the random match probability method, the source attribution threshold is set to >1.

Note: If you select **Random Match Probability**, the software automatically inverts the source attribution threshold setting. See "Modify the Source Attribution Threshold" below.

8.5.5. Modify the Source Attribution Threshold

1. On the navigation rail, select **Settings**.
2. Select **Population Group Settings**. The Population System Defaults page opens.
3. Under the section Source Attribution Threshold, enter in the following options:
    - If random match probability is used for population statistics calculations, enter a value greater than 1 in the Source Attribution Threshold field.
    - If the likelihood ratio is in use, enter a value less than 1.
4. Click **Save**, or click **Cancel** to return to the source attribution threshold back to the default settings.

8.5.6. Custom Population Group Requirements

To add a custom population group successfully, the software requires that the file conform to a particular format.

Note: Types of loci that are not supported for custom population group files include X STRs, Y STRs, aSNPs, pSNPs, and loci with 3 alleles.

Table 7. Requirements for Custom Population Group Files

Type of requirement	Details
File Type	File contents are text tab-delimited (***.txt**).
Type of STR Loci Supported	Autosomal STRs with genotype entries that contain numbers
Type of SNP Loci Supported	iSNPs with genotype entries that contain A, C, T, or G. Each genotype must contain exactly 2 alleles.
Homozygotes	Entry contains the allele 2 times. For example, entry A A, or 12 12.
Number of Subjects in the Population Group	>3 and ≤2500

Table 7. Requirements for Custom Population Group Files (continued)

Type of requirement	Details
Locus Names	Match the name of the locus in the software.
Table Rows	The first row of the table contains the loci being defined. In the rows that follow, each subject identifier is unique to a row and is not repeated in other rows.
Table Columns	The first column of the table contains the subject identifiers for each row. Each subject must contain a genotype for each locus in the file.

8.5.7. Custom Population Group File Examples

There are 2 options for the entry of genotypes in custom population files. Make sure that the file is consistent, with each sample in the file entered in the table in the same way. In the first table option, each allele is in an independent cell, so that each locus has 2 columns of data.

SampleCode	CFS1PO	CFS1PO	D10S1248	D10S1248	DS12S391	DS12S391
STRSample1	11	12	14	14	17	21
STRSample2	9	11	12	13	16	16
STRSample3	10	12	14	15	16	20
STRSample4	11	12	11	14	16	19
STRSample5	8	12	14	14	15	18

In the second table option, each locus is in a single column, with alleles separated by a comma.

SampleCode	rs1005533	rs10092491	rs1015250	rs1024116	rs1028528	rs1031825
SNPSample1	A,G	T,C	C,G	A,A	A,G	C,C
SNPSample2	G,A	T,T	G,C	A,A	G,A	A,C
SNPSample3	G,G	T,C	G,G	G,G	G,A	A,A
SNPSample4	G,A	C,T	C,G	A,G	A,G	C,C
SNPSample5	A,A	T,T	C,G	G,A	G,A	A,A

8.5.8. Adding Custom Population Groups

In addition to the population groups installed with the software, you can add custom population groups of your own to use in calculating population statistics.

1. On the navigation rail, select **Settings**.
2. Select **Population Group Settings**.

   The Population System Defaults page opens.
3. In the section labeled Custom, select **Upload Custom Pop Group**.
4. In the Upload Populations dialog box, select the locus file type that will be uploaded: iSNPs or Autosomal STRs.
5. Select **Drop File Here**, and then navigate to the population group file.

6. Select the pop group file (***.txt**), then select **Open**. The pop group file appears under the file upload area. Uploading another pop group ***.txt** file overrides the current file because the software accepts only one file upload at a time.
7. A message will appear if the file upload was successful, then click **Next**.

   Note: If your pop group file has any issues, a message listing the errors will display. To continue, hit **Cancel** and go back to adjust that file's errors or upload a new file.
8. Enter a name for the population group in the Database Name field. A default value is displayed in the Minimum Allele Frequency and Theta fields. The default value is calculated from the population size using the equation

$$\text{Minimum Allele Frequency} = \frac{5}{2}N$$

   where N is the number of subjects in the group.

   Select **Next**.
9. Confirm all information is correct and then select **Upload**.

8.6. Selecting Population Groups

Population groups installed with the software and any population groups you defined for calculating population statistics are listed on the Population Group Settings page. The minimum allele frequency and Theta value are next to each population group. All population groups installed with the software are available for population statistics calculations by default.

8.6.1. Upload Default Population Groups

Note: If the Default Population groups were not installed, follow the steps below.

1. On the navigation rail, select **Settings**.
2. Select **Population Group Settings**. The Population System Defaults page opens.
3. In the section labeled Custom, select **Upload Custom Pop Group**.
4. In the Upload Populations dialog box, select the locus file type that will be uploaded: iSNPs or Autosomal STRs.
5. Select **Drop File Here**, then navigate to the system folders: `/fuas/pop-groups`.
6. Select the pop group file (*.txt), then select **Open**. The pop group file appears under the file upload area. Uploading another pop group *.txt file overrides the current file because the software accepts only one file upload at a time.
7. A message will appear if the file upload was successful, then click **Next**.

   Note: If your pop group file has any issues, a message listing the errors will display. To continue, hit **Cancel** and go back to adjust that file's errors or upload a new file.
8. Enter a name for the population group in the Database Name field. A default value is displayed in the Minimum Allele Frequency and Theta fields. The default value is calculated from the population size using the equation

$$\text{Minimum Allele Frequency} = \frac{5}{2}N$$

   where N is the number of subjects in the group.

Select **Next**.

9. Confirm all information is correct and then select **Upload**.

8.6.2. Turn Default or Custom Population Groups Off / On

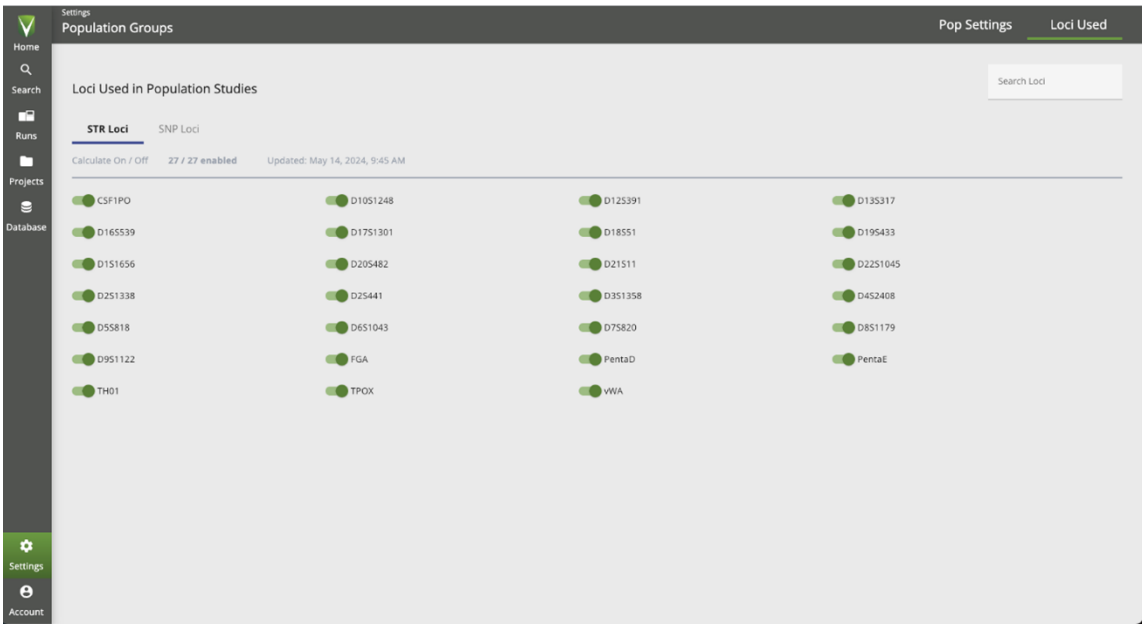
1. On the navigation rail, select **Settings**.
2. Select **Population Group Settings**. The Population System Defaults page opens.
3. Select a toggle to turn a population group on or off. On is indicated in green, and off is displayed in gray. The system will automatically update; no further action is required.

8.6.3. Define Loci for Population Studies

By default, the ForenSeq Universal Analysis Software uses autosomal STR and iSNP loci typed with the ForenSeq DNA Signature Plus Kit to calculate population statistics. The inclusion of loci for calculating population statistics can be defined by an administrator. Deselected loci are not used to calculate population statistics.

8.6.4. Define loci used in population statistics

1. On the navigation rail, select **Settings**.
2. Select **Population Group Settings**. The Population System Defaults page opens.
3. Select the tab at the top labeled **Loci Used**. The Loci Used in Population Studies page opens with the STR Loci tab selected as default.
4. Switch to the SNP list by selecting the tab next to STR Loci labeled **SNP Loci**.
5. To find a specific locus, start typing in the Search feature.
6. To deselect a locus, click the toggle next to the locus name. An active locus is green, and a deactivated locus is gray. The system will automatically update; no further action is required.



8.7. User Management

Note: This feature is available only for administrators.

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.

8.7.1. 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.

8.7.2. 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.

8.8. System Settings

Note: This feature is available only for administrators.

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.

8.8.1. 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.

8.8.2. 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 Runs header bar, Projects header bar, and Results page.

8.8.3. System Information

The System Information page displays version numbers for your UAS installation and each installed assay module and contact information for QIAGEN Technical Support.

8.8.4. Dark Mode

Toggle the Dark Mode setting in the Settings menu to change the color scheme of the user interface to be colorblind compatible.

9. Troubleshooting

9.1. 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 **qiagen.com** to download the guide for your kit.

9.1.1. 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.

9.1.2. 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, the library from the Purified Library Plate were over pipetted.
    - 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.
- 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.

9.1.3. 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 washing of purification beads 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.

9.1.4. 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", section 9 on the previous page. However, if sample representation shows that reads are above the sample count guideline, proceed with analysis.
- Phasing or prehasing is too high:
  - 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 QIAGEN Technical Support.

9.1.5. 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", section 9 on the 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 QIAGEN Technical Support for help locating the logs.
- The reagent chiller temperature is too low or too high. The system displays an error.
- Instrument blockage
  - 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 QIAGEN Technical Support.

9.1.6. 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”, section 9.1.3 on page 57.

If an error message is displayed, contact QIAGEN Technical Support.

9.1.7. 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” (on the previous page).
- The cluster density is too high. See “High Cluster Density”, section 9 on page 57.
- If the problem remains unresolved, reagents might not be performing as expected. Contact QIAGEN Technical Support.

9.1.8. 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 “High phasing and pre-phasing”, section9.1.5 on the previous page.
- The cluster density is too high. See “High Cluster Density”, section 9 on page 57.
- 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 QIAGEN Technical Support.

9.1.9. 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 a concentration of 0.75 ng/µL.
- HT1 was overpipetted during library prep. Repeat library normalization.

9.1.10. 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.

9.2. Troubleshoot Analysis Errors

The following sections provide recommendations for troubleshooting potential analysis problems.

9.2.1. 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", section 7.3.2 on page 39.
3. Add the sample back to the run with the correct index combination, following the steps in "Add Samples to a Run", section 3.1.1 on page 10. The sample will automatically analyze. If further errors occur, contact QIAGEN Technical Support.
4. Navigate to the run folder, following the steps for obtaining the analysis folder path in "Review Run Activity", section 7.4.1 on page 40.
5. Navigate to the Log folder for that sample analysis.
6. Copy the log text file.
7. Navigate to `D:\verogen\fuas\logs` and copy the most recent log text file.

9.2.2. 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 "Review Run Activity", section 7.4.1 on page 40.
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.

Note: If the folder is not present, copy this folder with the runInfo.xml and runParameters.xml files from the MiSeq, follow the "Review Run Activity", section 7.4.1 on page 40.

9.2.3. Perform a Status Check

A status log in UAS aggregates information from a run, sample, or installation failure and a history of actions. When troubleshooting, use the following instructions to export this information to QIAGEN:

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**.

References

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4. Bär, W., Brinkmann, B., Budowle, B., Carracedo, A., Gill, P., Lincoln, P., ... & Olaisen, B. (1997). DNA recommendations: further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. *International Journal of Legal Medicine*, *110*, 175-176.
5. Parson, W., Ballard, D., Budowle, B., Butler, J. M., Gettings, K. B., Gill, P., ... & Phillips, C. (2016). Massively parallel sequencing of forensic STRs: considerations of the DNA commission of the International Society for Forensic Genetics (ISFG) on minimal nomenclature requirements. *Forensic Science International: Genetics*, *22*, 54-63.

Appendix A: 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 **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 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 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 8. 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.

Note: The chastity of a base call is the ratio of the intensity of the greatest signal divided by the sum of the 2 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 9. 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 10. 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 4 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 8 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 7 chemistry-only cycles without any imaging, followed by 8 cycles of sequencing. RTA evaluates all 8 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 DNA Signature Prep	Read 1	1–351	Cycle 352
	Index 1 Read	352–359	Cycle 360
	Index 2 Read	360–367	Cycle 368
	Read 2	368–398	After cycle 398
ForenSeq Signature Plus	Read 1	1–351	Cycle 352
	Index 1 Read	352–359	Cycle 360
	Index 2 Read	360–367	Cycle 368
	Read 2	368–398	After cycle 398

Human Sequencing Control

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

Table 11. 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	351 cycles
D3S1358	✓
D5S818	✓
D7S820	✓
D8S1179	✓
D13S317	✓
D16S539	✓

Loci	351 cycles
D18S51	✓
FGA	✓
PentaD	✓
PentaE	✓
TPOX	✓
DYS391	✓
DYS392	✓
DYS460	✓
DXS7423	✓
DXS10103	✓

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 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.

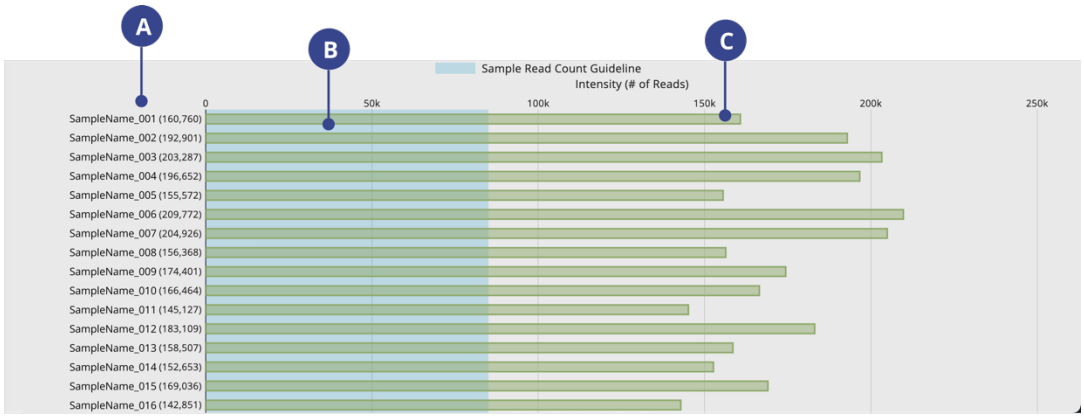

Figure 7. Sample Representation bar chart. [A] Sample name and exact number of reads for the sample. **[B]** Threshold of the read count guideline. **[C]** Bar indicating number of reads for the sample.

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 DNA Signature Prep 85,000 reads per sample
- ForenSeq Signature Plus 85,000 reads per sample

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

Table 12. 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.

Table 13. Control assessments

Assay type	Positive amplification control	Value
ForenSeq DNA Signature Prep	2800M Positive Amplification Control DNA	STRs typed
ForenSeq Signature Plus	NA24385 Positive Amplification Control DNA	STRs typed

Positive amplification control

Positive amplification control metrics provide data for each sample identified as 2800M for the ForenSeq DNA Signature Prep Kit or NA24385 for the ForenSeq Signature Plus Kit. Every sample is analyzed and compared to this control. The software then lists each sample with values for how many reads passed filter and STRs 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 2800M or NA24385. A short, vertical bar displays the following colors to indicate call success and concordance with 2800M or NA24385.

Table 14. 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 2800M or 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 85,000 reads for the ForenSeq DNA Signature Prep Kit and ForenSeq Signature Plus Kit. Reads below the guideline might still be usable. Review quality metrics and sample data to help make a determination.

Table 15. 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 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 16. Colors for base calls of loci

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

Appendix B: Supporting Information

Biological sex estimation for samples

The software applies the following logic to estimate the biological sex of a Kintelligence HT sample.

- If the sample has ≥10 Y-SNP loci typed, the biological sex is male (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 a Kintelligence HT sample.

Any of the following scenarios indicate a mixture:

- The sample has ≥10 Y-SNPs with 2 alleles.
- The sample has ≥10 Y-SNP loci typed and ≥10 X-SNPs with 2 alleles.
- The sample has zero Y-SNP loci typed with ≥60% autosomal SNP (auSNP) heterozygosity and ≥50% X-SNP heterozygosity.

If the sample has ≤50% auSNP heterozygosity, ≤40% X-SNP heterozygosity, ≥65% auSNP intralocus balance, and zero Y-SNPs with 2 alleles, the sample is single-source.

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.

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

Recommendations for sample quality

The kinship analysis algorithm used by UAS is sensitive to the SNP Overlap value (the number of typed SNPs in common between 2 samples). If the SNP Overlap for 2 samples is low (≤6000), only relationships of up to 3 degrees can be identified. If the SNP Overlap is high (>6000), relationships of higher degrees (fourth and fifth) are more likely to be identified (3).

Therefore, it is imperative to maximize the SNP Overlap by ensuring that each sample included in kinship queries has a high number of typed SNPs, especially if the samples are distantly related. This is because for distant relationships, allele sharing

is expected to be low, and if there is a high level of locus dropout, there will be fewer loci that have shared alleles between 2 samples.

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 (excluding African Ancestry in Southwest USA [ASW]). For context, the unknown sample is projected with the Ad-Mixed Americans super population onto the first 2 pre-trained components based on aSNP genotype calls (1).

On the Estimation worksheet of the BGA Estimation report, the distance to the nearest centroid indicates how related a sample is to the general grouping for the centroid. The worksheet compares the distance for 1000 Genome Project samples contributing to the centroid. The plot on the Estimation worksheet includes one centroid for each of the significant ancestries and orientational centroids at the one-quarter intervals between these groups. Centroids contextualize results and logically group populations.

Strand information

Sequencing of DNA Signature Plus libraries consists of one long sequencing read of 351 cycles to allow coverage of the longest possible STR repeat regions, followed by two 8-cycle index sequencing reads for demultiplexing libraries, and a 31-cycle read to confirm the last 31 nucleotides of each amplicon. This sequencing configuration only allows one strand of the amplicon to be sequenced. The direction of the sequencing is driven by the sequencing adapters. This direction was selected based on sequencing quality. Most STR amplicons are sequenced on the forward strand; however, the STR loci in Table 17 were sequencing on the reverse strand.

All allele calls are reported in the forward strand. However, the information may not have been captured during forward sequencing.

The amplicons for the following loci are sequenced on the reverse strand.

- D22S1045
- D2S441
- DXS10103
- DXS7132
- DXS7423
- DXS8378
- HPRTB

In 1997, STR nomenclature guidelines recommended reporting the repeat sequence of the coding region strand (4). In 2016, the DNA commission of the International Society for Forensic Genetics (ISFG) proposed updating the nomenclature guidelines to report the repeat sequences and locations on the forward or plus strand of the most recent genome assembly

GRCh38 (5). Earlier versions of the ForenSeq Universal Analysis Software were launched commercially prior to 2016 and reported repeat sequences on the coding strand. With the transition to the Universal Analysis Software 2.x, the STR sequence reporting is now consistent with the recommendations proposed in 2016 (5). The loci listed in Table 17 were reported on the coding strand in the UAS v1.3 software and are now reported on the forward strand in UAS 2.7 (and newer) version of the software. The repeat sequence for these loci for the Control DNA NA24385 are shown for the two versions of the software.

Table 17a. Repeat sequences for UAS v1.3 and UAS v2.6 (and older versions)

Loci	CE equivalent	Repeat sequences
D1S1656	13	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGGTGTGTGTG TG
	14	AGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGGTGTGT GTGTG
D2S1338	22	TGCCTGCCTGCCTGCCTGCCTGCCTGCCTTCCTTCCTTCCTTCCTTCCTTCCTTCC TTCCTTCCTTCCTTCCGTCCTTCCTTCC
	24	TGCCTGCCTGCCTGCCTGCCTGCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCC TTCCTTCCTTCCTTCCTTCCTTCCGTCCTTCCTTCC
FGA	20	TTTCTTTCTTTCTTTTTTCTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTT CTTTCTTTCTCCTTCCTTCC
	23	TTTCTTTCTTTCTTTTTTCTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTT CTTTCTTTCTTTCTTTCTTTCTCCTTCCTTCC
D5S818	11	AGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGAG
	12	AGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGAT
CSF1PO	10	AGATAGATAGATAGATAGATAGATAGATAGATAGAT
	12	AGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGAT
SE33*	17	AAAGAAAGAGAAAGAAAGAAAGAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAG AAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGGAAAGAAAGAAAGAG
	24.2	AAAGAAAGAGAAAGAAAGAAAGAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAG AAAGAAAGAAAGAAAGAAAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAA AGGAAGGAAAGAAAGAG
D6S1043	11	AGATAGATAGATAGATAGATAGATAGATAGATAGATAGAT
	14	AGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGAT
D7S820	11	GATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGATTGATAGTTT
	12	GATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGATTGATA GTTT
vWA	16	TCTATCTGTCTGTCTGTCTGTCTATCTATCTATCTATCTATCTATCTATCTATCTA TCTATCCATCTA
	18	TCTATCTGTCTGTCTGTCTGTCTATCTATCTATCTATCTATCTATCTATCTATCTA TCTATCTATCTATCCATCTA
PentaE	10	AAAGAAAAGAAAAGAAAAGAAAAGAAAAGAAAAGAAAAGAAAAGAAAAGA
	18	AAAGAAAAGAAAAGAAAAGAAAAGAAAAGAAAAGAAAAGAAAAGAAAAGAAAAGA AAAGAAAAGAAAAGAAAAGAAAAGAAAAGA
D19S433	14	AAGGAAAGAAGGTAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGG AAGGAGAGAGGAAGAAAGAGAG
	16.2	AAGGAAAAGGTAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAA GGAAGGAAGGAAGGAGAGAGGAAGAAAGAGAG

Table 17a. Repeat sequences for UAS v1.3 and UAS v2.6 (and older versions) (continued)

Loci	CE equivalent	Repeat sequences
DYS19	14	TAGATAGATAGATAGGTAGATAGATAGATAGATAGATAGATAGATAGATAGATAGA
DYS635	21	TCTATCTATCTATCTATGTATGTATCTATCTATGTATGTATCTATCTATCTATCTATCTA TCTATCTATCTATCTATCTATCTA
DYS389I	13	TCTGTCTGTCTGTCTATCTATCTATCTATCTATCTATCTATCTATCTATCTA
DYS389II	29	TCTGTCTGTCTGTCTGTCTGTCTATCTATCTATCTATCTATCTATCTATCTATCTA TCTATCATTATACCTACTTCTGTATCCAACTCTCATCTGTATTATCTATGTATCTGTCTG TCTGTCTATCTATCTATCTATCTATCTATCTATCTATCTATCTA
DYS390	25	TCTATCTATCTGTCTGTCTGTCTGTCTGTCTGTCTGTCTATCTATCTATCTATCTA TCTATCTATCTATCTATCTATCTATCTGTCTATCTATCTATCTA
Y-GATA-H4	10	AGATAGATAGATAGATCTATAGATAGATAGGTAGGTAGGTAGATAGATAGATAGATAGAT AGATAGATAGATAGATAGAATGGATAGATTAGATGGATGAATAGATAGATAGATAGATAC ATAGATAG
DYS385a	16	AAGGAAGGAAGGAAGGAGAAAGAAAGTAAAAAAGAAAGAAAGAGAAAAAGAGAAAAAGAA AGAAAGAGAAGAAAGAGAAAGAGGAAAGAGAAAGAAAGGAAGGAAGGAAGGAAGGAAGGG AAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAG AAAGAGAAAAAGAAA
DYS385b	18	AAGGAAGGAAGGAAGGAGAAAGAAAGTAAAAAAGAAAGAAAGAGAAAAAGAGAAAAAGAA AGAAAGAGAAGAAAGAGAAAGAGGAAAGAGAAAGAAAGGAAGGAAGGAAGGAAGGAAGGG AAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAG AAAGAAAGAAAGAGAAAAAGAAA
DYS460	11	ATAGATAGATAGATAGATAGATAGATAGATAGATAGATAG
DYS392	13	TATTATTATTATTATTATTATTATTATTATTATTAT
DXS8378	12	CTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTAT
DXS7132	13	TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGA
HPRTB	13	AGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGAT
DXS7423	15	TCCATCCATCCATCTGTCCTTCCATCCATCCATCCATCCATCCATCCATCCATCCA TCCATCCA

* Locus present in ForenSeq MainstAY SE Kit only.

Table 17b. Repeat sequences for UAS v2.7

Loci	CE equivalent	Repeat sequences
D1S1656	13	ACACACACACACCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTAT CTA
	14	ACACACACACACCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTAT CTATCTA
D2S1338	22	GGAAGGAAGGACGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAA GGCAGGCAGGCAGGCAGGCAGGCA
	24	GGAAGGAAGGACGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAA GGAAGGAAGGAAGGCAGGCAGGCAGGCAGGCA
FGA	20	AAAGGAAGGAAGGAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGA AAGAGAAAAAAGAAAGAAAGAAA

Table 17b. Repeat sequences for UAS v2.7 (continued)

Loci	CE equivalent	Repeat sequences
	23	AAAGGAAGGAAGGAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGA AAGAAAGAAAGAAAGAGAAAAAAGAAAGAAAGAAA
D5S818	11	CTCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCT
	12	ATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCT
CSF1PO	10	ATCTATCTATCTATCTATCTATCTATCTATCTATCTATA
	12	ATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATA
SE33*	17	CTCTTTCTTTCTTTCCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTC TTTCTTTCTTTCTTTCTTTCTTTCTCTTTCTTTCTTTCTCTTTCTTT
	24.2	CTCTTTCTTTCCTTCCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTT TCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTCTTTC TTTCTTTCTCTTTCTTT
D6S1043	11	CTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCT
	14	CTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCT
D7S820	11	AAACTATCAATCTGTCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATC
	12	AAACTATCAATCTGTCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATC TATC
vWA	16	GGATAGATGGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGAC AGACAGACAGATAGAT
	18	GGATAGATGGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGAT AGACAGACAGACAGACAGATAGAT
PentaE	10	TTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTT
	18	TTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTC TTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTT
D19S433	14	CTCTCTTTCTTCCTCTCTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCC TTCCTTCCTACCTTCTTTCCTT
	16.2	CTCTCTTTCTTCCTCTCTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCC TTCCTTCCTTCCTTCCTTCCTACCTTTTCCTT
DYS19	14	TCTATCTATCTATCTATCTATCTATCTATCTATCTATCTACCTATCTATCTATCTA
DYS635	21	GATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATACATACATAGATA GATACATACATAGATAGATAGATAGA
DYS389I	13	ATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGACAGACAGA
DYS389II	29	ATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGACAGACAGACAGATACATAG ATAATACAGATGAGAGTTGGATACAGAAGTAGGTATAATGATAGATAGATAGATAGATAG ATAGATAGATAGATAGATAGATAGACAGACAGACAGACAGACAGA
DYS390	25	GATAGATAGATAGATAGACAGATAGATAGATAGATAGATAGATAGATAGATAGATA GATAGATAGACAGACAGACAGACAGACAGACAGACAGACAGATAGATAGA
Y-GATA-H4	10	CTATCTATGTATCTATCTATCTATCTATTCATCCATCTAATCTATCCATTCTATCTATCT ATCTATCTATCTATCTATCTATCTATCTACCTACCTACCTATCTATCTATAGATCTATCT ATCTATCT

Table 17b. Repeat sequences for UAS v2.7 (continued)

Loci	CE equivalent	Repeat sequences
DYS385a	16	CTTTCTTTTTCTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTT CTTTCTTTCTTTCTTTCCCTTCCTTCCTTCCTTCCTTCCTTTCTTTCTCTTTCCTCTTTC TCTTTCTTCTCTTTCTTTCTTTTTCTCTTTTTCTCTTTCTTTCTTTTTTACTTTCTTTCT CCTTCCTTCCTTCCTT
DYS385b	18	CTTTCTTTTTCTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTT CTTTCTTTCTTTCTTTCTTTCTTTCCCTTCCTTCCTTCCTTCCTTCCTTTCTTTCTCTTT CCTCTTTCTCTTTCTTCTCTTTCTTTCTTTTTCTCTTTTTCTCTTTCTTTCTTTTTTACT TTCTTTCTCCTTCCTTCCTTCCTT
DYS460	11	TATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTAT
DYS392	13	AATAATAATAATAATAATAATAATAATAATAATAATAATA
DXS8378	12	ATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAG
DXS7132	13	GATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGA
HPRTB	13	TCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTA
DXS7423	15	TGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGAAGGACAGATGGA TGGATGGA

* Locus present in ForenSeq MainstAY SE Kit only.

Document Revision History

Date	Description
10/2024	Initial release

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