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# Universal Analysis Software User Guide for Kintelligence HT Module

Bioinformatics solution for analysis and management of forensic genomic data

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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<sup>®</sup> 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 Kintelligence HT kit using the default analysis methods.

**Note:** This guide describes version 2.6 of UAS.

### 1.1.1. 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 (chapter 1.2 on the facing page)
2	Set Up a Run	The user imports sample information as a .txt file, or manually adds sample information.	Run Setup (chapter 3 on page 21)
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.	<i>MiSeq FGx Sequencing System Reference Guide</i> (document # VD2018006)
4	Analyze Data	When a run has finished, UAS automatically begins analyzing the resulting data. The user reviews these results. For Kintelligence HT samples, the user assesses sample quality and toggles each sample's database status accordingly.	Data Analysis (chapter 4 on page 25)
5	Analyze Kinship	The user runs queries on Kintelligence HT samples in the database to identify kinship results, assigns pedigrees to potential relationships, and reviews likelihood ratios and other data.	Kinship Analysis (chapter 5 on page 29)
6	Generate Reports	The user generates reports on the analysis results in a variety of formats.	Reports (chapter 6 on page 39)

\* MiSeq FGx System

### 1.1.2. 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 analysis methods:

- ForenSeq Kintelligence HT — ForenSeq Kintelligence HT Analysis Method

In addition to the default 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 8.2 on page 55.

## 1.2. 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 are unable to access UAS, refer to the *Universal Analysis Software Server Support Reference Guide* (document # VD2023010) for troubleshooting instructions or contact QIAGEN Technical Support.

### 1.2.1. 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.

### 1.2.2. 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 8.4.1 on page 58).

**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.

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.


### 1.2.3. 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**.

### 1.2.4. 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.

## 1.3. 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 — Color-coded quality metrics for a completed run; hovering over the icon reveals detailed metrics.

## 1.4. Workspaces

The software includes 3 primary workspaces:

-  Runs — Set up runs and manage runs during sequencing.
-  Projects — View sequencing results, analyze data, and generate reports.
-  Database — Perform kinship analysis of antemortem and postmortem samples.

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

For a detailed overview of each of these workspaces, see "Workspaces", section 2 on page 9.


## 1.5. 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", chapter 7 on page 45 and "Software Settings", chapter 8 on page 55.


### 1.5.1. 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.
- To search for items in a table by name or other value, use the  **Search** field.

### 1.5.2. 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 corresponding checkbox.
- To reset all columns to their default order and visibility, select **Reset**.

### 1.5.3. 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  **Projects** and  **Database** 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.

### 1.5.4. Sample tags

Sample tags are user-defined labels that allow you to identify connected samples and group them together. Tags may be used to sort and filter the tables of samples displayed in the  **Runs** and  **Database** workspaces, and may be edited within those tables or added when importing sample information for a run.

Verogen recommends that you use sample tags to facilitate quick look-up of connected samples in your database when creating queries. For more information, see "Kinship Analysis", chapter 5 on page 29.

**Note:** Tags are not case-sensitive.

Resource	Description
<i>ForenSeq Kintelligence HT Kit Handbook (3563)</i>	Describes the protocol for using the ForenSeq Kintelligence HT Kit to prepare libraries for sequencing.
<i>Universal Analysis Software Server Support Reference Guide (document # VD2023010)</i>	Provides comprehensive technical information to support the operation of servers hosting UAS, including technical specifications and troubleshooting instructions.




## 2. Workspaces

### 2.1. Runs

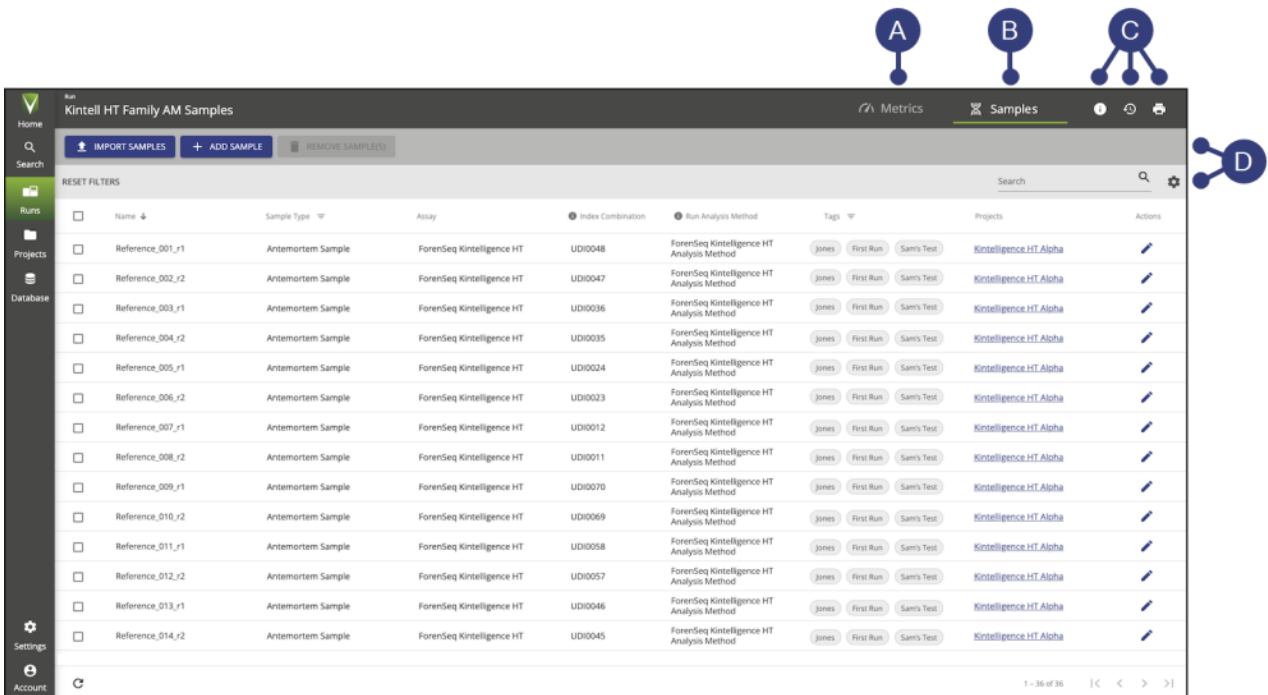
The **Runs** workspace provides tools to create and monitor sequencing runs, including tools to input and organize sample information. This workspace includes the following features:

- Metrics page (2.1.1 on the next page) — View quality metrics and sample representation data for a run.
- Samples page (2.1.2 on the next page) — View and edit samples in a run, or add new samples.
- Supplemental features for runs (2.1.3 on the next page) — 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.

For more information about the use of the **Runs** workspace, see "Run Setup", chapter 3 on page 21.

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





The screenshot displays the 'Runs' workspace interface. At the top, there are navigation tabs for 'Metrics' and 'Samples'. Below the navigation is a search bar and a 'RESET FILTERS' button. The main area contains a table of runs with columns for Name, Sample Type, Assay, Index Combination, Run Analysis Method, Tags, Projects, and Actions. Callout A points to the 'Metrics' tab, B to the 'Samples' tab, C to the 'Run Info', 'Run Activity', and 'Print' buttons, and D to the search and filter icons in the top right.

Name	Sample Type	Assay	Index Combination	Run Analysis Method	Tags	Projects	Actions
Reference_001_r1	Antemortem Sample	ForenSeq Kintelligence HT	UDI0048	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_002_r2	Antemortem Sample	ForenSeq Kintelligence HT	UDI0047	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_003_r1	Antemortem Sample	ForenSeq Kintelligence HT	UDI0036	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_004_r2	Antemortem Sample	ForenSeq Kintelligence HT	UDI0035	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_005_r1	Antemortem Sample	ForenSeq Kintelligence HT	UDI0024	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_006_r2	Antemortem Sample	ForenSeq Kintelligence HT	UDI0023	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_007_r1	Antemortem Sample	ForenSeq Kintelligence HT	UDI0012	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_008_r2	Antemortem Sample	ForenSeq Kintelligence HT	UDI0011	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_009_r1	Antemortem Sample	ForenSeq Kintelligence HT	UDI0070	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_010_r2	Antemortem Sample	ForenSeq Kintelligence HT	UDI0069	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_011_r1	Antemortem Sample	ForenSeq Kintelligence HT	UDI0058	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_012_r2	Antemortem Sample	ForenSeq Kintelligence HT	UDI0057	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_013_r1	Antemortem Sample	ForenSeq Kintelligence HT	UDI0046	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	
Reference_014_r2	Antemortem Sample	ForenSeq Kintelligence HT	UDI0045	ForenSeq Kintelligence HT Analysis Method	Jones First Run Sam's Text	Kintelligence HT Aloha	

**Figure 2. Overview of the Runs workspace.** **A.** Metrics page — Displays quality metrics and sample representation data for a run. **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.



### 2.1.1. 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 "Run Metrics", chapter 10 on page 65.


### 2.1.2. 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. See "Import a sample sheet" on page 21.
- **Add Sample** — Manually add an individual sample. See "Add individual samples" on page 22.
-  **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. See "Sorting and filtering tables" on page 7.

### 2.1.3. Supplemental features for runs

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

-  **Run Info** — Edit the run and view the run state, cycles per read, and other run information. See "Run state", section 3.3.1 on page 24, and "Edit a run", section 7.1.1 on page 45.
-  **Run Activity** — View system events and user actions for the run and add comments. See "Review run activity", section 7.4.1 on page 49, and "Comment on a run" 7.4.5 on page 50.  
**Note:** This icon is visible only when the history setting is enabled. See "System settings", section 8.5 on page 58.
-  **Print** — Print the Samples and Metrics pages for a run. See "Print a run", section 7.1.2 on page 45.

## 2.2. Projects

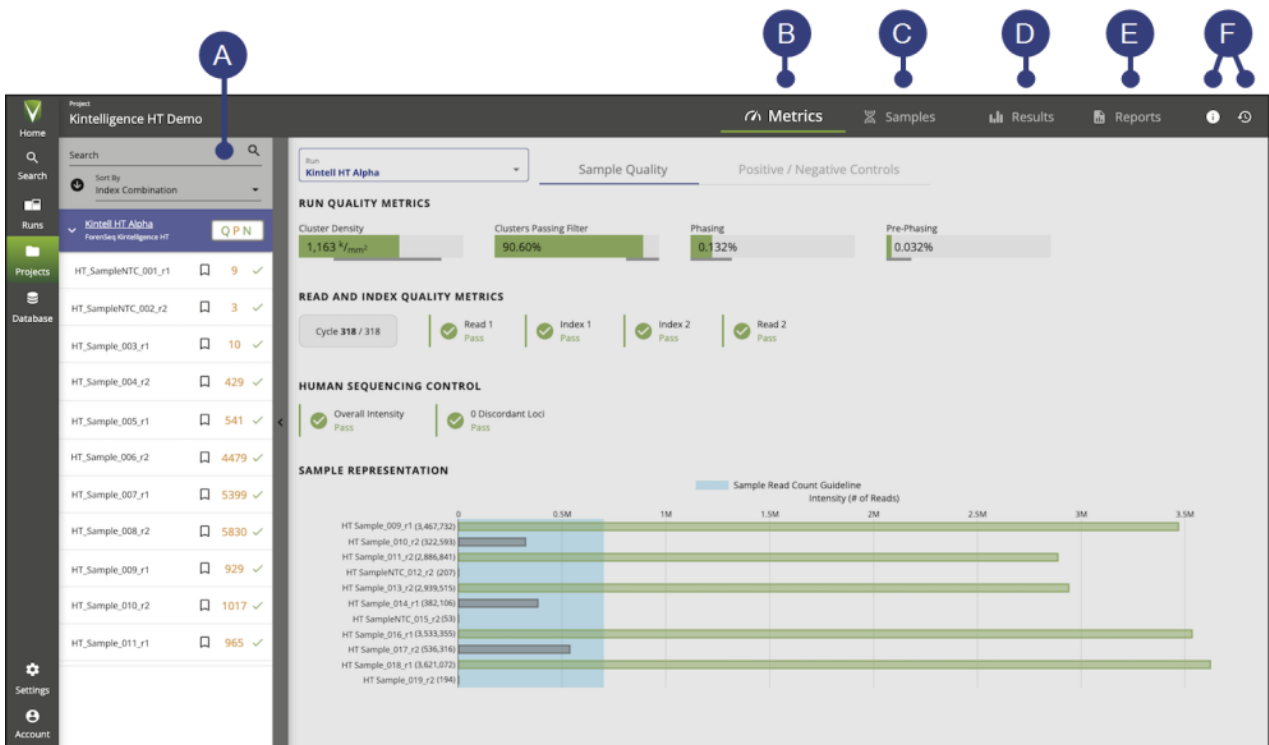
The **Projects** workspace provides tools to view sequencing results, analyze data, and generate reports. This workspace includes the following features:

- "Projects sidebar" (section 2.2.1 on the facing page) — Quickly locate a sample within the project.
- "Metrics page" (section 2.1.1 above) — View metrics that provide an overall view of run performance.

- "Samples page" (section 2.2.3 on the next page) — View all samples in the project as a table.
- "Sample results" (section 2.2.4 on page 13) — View in-depth analysis results for a specific sample.
- "Reports page" (section 2.2.5 on page 13) — View and download previously generated reports.
- "Supplemental features for projects" (section 2.2.6 on page 13) — Use the **Project Info** and **Activity** buttons to perform 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", chapter 4 on page 25, and "Reports", chapter 6 on page 39.




**Figure 3. Overview of the Projects 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.

### 2.2.1. 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. (See "Sample bookmarks", section 1.5.3 on page 8.)
- 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. (See "Quality control indicators" on page 18.)
- Analysis state — The analysis state of the sample. (See "Sample analysis state", section 3.3.2 on page 24.)

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.



### 2.2.2. 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", chapter 10 on page 65.

### 2.2.3. 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", chapter 6 on page 39.)
  - **Reanalyze** — Analyze the selected samples again using a different analysis method. (See "Reanalyze samples", section 4.5 on page 27.)
  - **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 4.4 on page 27.)
  - **Create archive...** — Create an archive for the selected samples. See "Archiving and importing data", section 7.5 on page 52.
- 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 1.5.1 on page 7.)

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


### Samples table

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

**Table 2. 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" on page 8.
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: Antemortem Sample, Postmortem Sample, NA24385 (positive amplification control), Negative Amplification Control, or Reagent Blank.
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" on page 18.
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", section 11.1 on page 72.
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", section 11.2 on page 72.

### 2.2.4. 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 Kintelligence HT samples, see "Overview of sample results", section 2.4 on page 16.

For more information about interpreting sample results, see "Review sample results", section 4.2 on page 25, and "Modify sample results", section 4.3 on page 26.

### 2.2.5. Reports page

**Note:** This section describes the Reports page as it appears in the  Projects workspace. See Kinship Reports page for the similar page in the  Database 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", chapter 6 on page 39.

### 2.2.6. 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 46.
- **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 49.

**Note:** This icon is visible only when the history setting is enabled. See "System settings", section 8.5 on page 58.

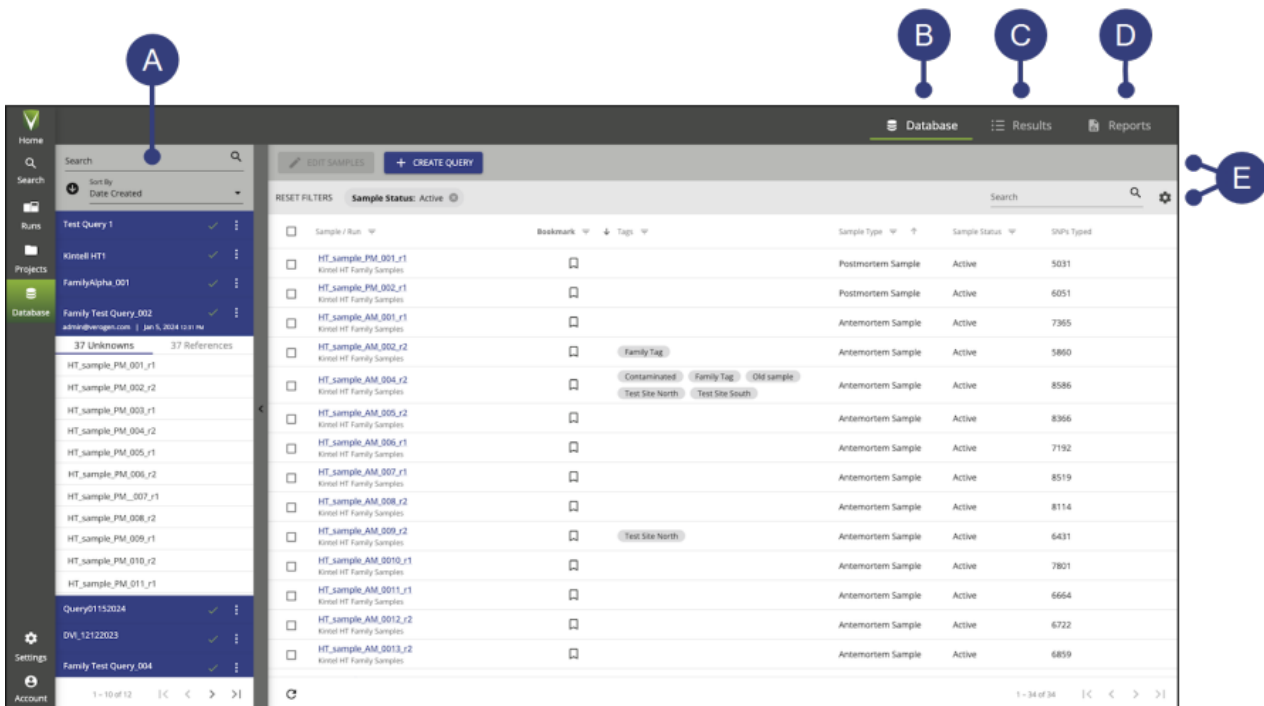
## 2.3. Database

The Database workspace is the post-sequencing workspace for identifying and analyzing potential relationships between Kintelligence HT samples. Selecting **Database** on the navigation rail opens the Database workspace.

This workspace includes the following features:


- Database Sidebar (section 2.3.1 on the facing page) — Create and manage kinship queries, open query results, and generate Kinship Query Reports.
- Database Page (section 2.3.2 on the facing page) — View all Kintelligence HT samples in your local database in a table. (This table may be sorted and filtered by tag, sample type, or other sample attribute.)
- Kinship Results Page (section 2.3.3 on the facing page) — View the results of kinship queries, assign and visualize pedigrees, and calculate pedigree likelihood ratios.
- Kinship Reports Page (section 2.3.4 on page 16) — View and download previously generated Kinship Query Reports.


For detailed information about the use of the Database workspace, see "Kinship Analysis", chapter 5 on page 29.



**Figure 4. Overview of the Database workspace.** **A.** Database sidebar — Lists all previously-run kinship queries. Select an Unknown sample within a query to view its results. **B.** Database page — Displays all Kintelligence HT samples in your local database as a table. **C.** Kinship Results page — Displays the results of a particular query as a table of potential relationships. **D.** Kinship Reports page — View and download previously-generated Kinship Query Reports. **E.** Toolbars — Available actions and options to sort or filter the Database table.

### 2.3.1. Database sidebar


The  **Database** sidebar allows you to create and manage database queries, open query results, and generate Kinship Query Reports. The sidebar is collapsible.

Previously generated queries appear in a list in the sidebar. By default, queries are sorted by creation date. Use the  **Search** bar to find a query by name.


Selecting a query opens a drop-down pane showing the Unknown and Reference samples included in the query. Selecting an Unknown sample then opens the Kinship Results page for that sample.

For more information about queries, see "Using queries", section 5.4 on page 31.

### 2.3.2. Database page


The Database page lists all Kintelligence HT samples in your local database as a table. By default, the  **Database** workspace opens to the Database page.

This page offers the following functions:

- Edit Samples — Edit the tags or sample type associated with one or more samples. See "Review sample information", section 5.3.2 on page 30.
- Create Query — Create a new query. See "Create a query", section 5.4.1 on page 32.
-  **Search** — Search the table for a specific sample or run by name.
- Filter or sort the table by specific attributes. (See "Sorting and filtering tables", section 1.5.1 on page 7.)

For more information about using this page, see "Database management", section 5.3 on page 29.

### 2.3.3. Kinship results page

The Kinship Results page (labeled  **Results** in the software) displays the results of a specific kinship query. Selecting an Unknown sample from within a query in the **Database** sidebar opens this page.

This page includes the following features:

- A table listing all potential relationships between the selected Unknown sample and the Reference samples included in the query, along with likelihood ratios and other data.



**Note:** Likelihood ratios for each potential relationship will not appear until the user has assigned a pedigree. See "Assign a pedigree", section 5.5.3 on page 35.


- Pedigree Tree — A visual tree illustrating the potential relationship between 2 samples.

**Note:** The Pedigree Tree for a potential relationship will not appear until the user has assigned a pedigree.

For more information about the use of this page, see "Review kinship results", section 5.5 on page 33.

### 2.3.4. Kinship reports page


**Note:** This section describes the Reports page as it appears in the  **Database** workspace. See Reports page for the similar page in the  **Projects** workspace.

The Kinship Reports page (labeled  **Reports** in the software) allows you to view and download previously generated Kinship Query Reports. (Kinship Query Reports are created by selecting **Create Report** from the drop-down menu next to a query in the **Database** sidebar.)

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, see "Kinship query reports", section 5.6 on page 38.

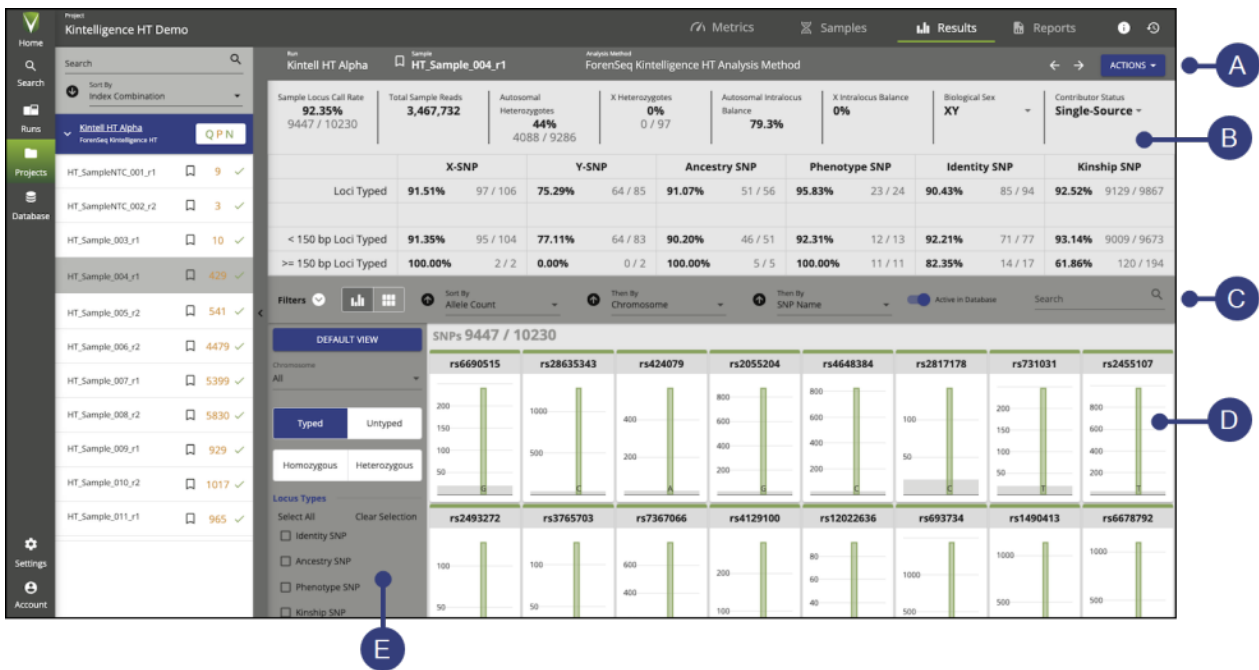
## 2.4. Overview of sample results

The Sample Results page in the  **Projects** workspace offers different features depending on the assay type of the selected sample. For Kintelligence HT samples, this page includes the following features:

- **Toolbar** (section 2.4.1 on the facing page) — Displays general information about the sample and available actions.
- **Sample Overview** (section 2.4.2 on the facing page) — View key metrics for the sample, or change the biological sex or contributor status of the sample.
- **Locus Details** (section 2.4.3 on page 18) — View results for particular loci, and type or untype alleles.
  - **Quality Control Indicators** — View any quality control (QC) indicators that were flagged for each locus.
  - **Locus Sort Options** — Sort loci by attribute, search for a locus by name, or toggle between view options (Card or Histogram). For Kintelligence HT samples, toggle the sample's database status (Active or Inactive).
  - **Locus Filter Options** — Filter loci by type or quality control (QC) flags.

For more information about the use of this page, see "Data Analysis", chapter 4 on page 25.





**Figure 5. Overview of the Sample Results page.** **A.** Toolbar — Displays general information about the sample and available actions. **B.** Sample overview — Key metrics for the sample, and options to change the sample's biological sex or contributor status. **C.** Locus sort options — Options to sort loci by attribute, search for a locus by name, toggle between view options (Card or Histogram), and toggle the sample's database status (Active or Inactive). **D.** Locus details — Results for each locus in the sample, represented as cards or histograms. Selecting a card or histogram reveals additional information about the locus. **E.** Locus filter options — Options to filter loci by type or quality control (QC) flags.

### 2.4.1. 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 1.5.3 on page 8.)
- **Analysis Method** — The analysis method applied to the sample.

**Note:** If a sample has been analyzed multiple times, you can select a different analysis method using the drop-down menu to see results from that analysis. Analyses are listed in chronological order, with the most recent first. See "Reanalyze samples", section 4.5 on page 27.

- 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.

### 2.4.2. Sample overview


The sample overview section of the Sample Results page for a Kintelligence HT sample displays basic information about the sample, including the locus call rate, total number of reads, intralocus balance metrics, biological sex, and contributor status. You may change the biological sex or contributor status of the sample by selecting the applicable drop-down menu.

Under these data, a table displays the percentage and total number of typed loci relative to base pair length for each SNP type.

### 2.4.3. Locus details

The locus details section of the Sample Results page displays analysis results for each locus in the sample. Loci are displayed in a grid, as either cards or histograms.

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 4.3.3 on page 26.)
- 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 50, and "Comment on a locus", section 7.4.8 on page 52.)
- Any quality control (QC) flags, if applicable, appear above the expanded histogram. (See "Quality control indicators", next section.)
- Use the **Previous Locus** and **Next Locus** arrows to scroll between loci. To close the locus details pane, select  **Close**.

### Quality control indicators

Each locus card or histogram is color-coded to denote quality control (QC) indicators. A horizontal green bar denotes a locus with no QC indicators, while an orange bar denotes a locus with at least one QC indicator.

When you select a locus, any specific applicable QC indicators appear above the enlarged histogram. Table 3 lists the possible QC indicators.

Table 3. 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).


### Locus sort options

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

- **Filters** — Expand or collapse the Filters sidebar.
- **Toggle between view options:**
  - **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).
- **Sort By** — 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.

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

- **Active in Database** — Toggle the status of the sample in your local database.
 

**Note:** The Active in Database toggle appears only for antemortem and postmortem Kintelligence HT samples. By default, every Kintelligence HT sample is marked Active. For information about assessing the quality of Kintelligence HT samples, see Assessing Sample Quality.
-  **Search** — Find a locus by name.

Selecting **Default View** in the **Filters** sidebar clears selected sort, view, and search options and restores defaults.

### Locus filter options

The **Filters** sidebar provides options to filter loci by chromosome, typed vs. untyped, or homozygous vs. heterozygous. More granular filter options are also available:

- Locus Type — Display only loci with the selected SNP types.

SNP Type	Description
Identity SNP (iSNP)	Informs on source attribution.
Ancestry SNP (aSNP)	Estimates biogeographical ancestry.
Phenotype SNP (pSNP)	Estimates hair and eye color.
Kinship SNP (kSNP)	Informs on potential biological associations (relatives).
X-SNP	Informs lineage and estimates biological sex in combination with Y-SNPs.
Y-SNP	Informs lineage and estimates biological sex in combination with X-SNPs.

- QC Flags — Display only loci flagged with the selected quality control (QC) indicators, or loci with no QC indicators. For information about QC indicators, see "Quality control indicators" on page 18.


Selecting **Default View** clears selected filters and restores defaults.

## 3. Run Setup

### 3.1. 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.

### 3.2. 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.

#### 3.2.1. Import a sample sheet

To reduce the number of corrections that may be needed later in the workflow, you may add sample types (antemortem or postmortem) and any useful sample tags at this stage.

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.

### Assigning unique dual indexes

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

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

### Assigning projects

The 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").

### Assigning sample tags

The SampleTagList column of a sample sheet allows you to assign custom tags to Kintelligence HT samples. For each sample, enter any tags separated by a comma followed by a space, and surround the entire list of tags with quotation marks (e.g., "Tag 1, Tag 2, Tag 3").

Sample tags are optional. For more information about using sample tags, see "Sample tags" on page 8.

### 3.2.2. 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	Enter any sample tags. Previously created tags appear in a drop-down menu. For more information about using sample tags, see "Sample tags" on page 8.
Description	Enter a preferred description of the sample.
Assay Type*	Select the library prep method: <ul style="list-style-type: none"> <li>• ForenSeq Kintelligence HT — Libraries prepared with the ForenSeq Kintelligence HT Kit.</li> </ul>
Sample Type	Select a sample type: <ul style="list-style-type: none"> <li>• Antemortem Sample — An antemortem sample.</li> <li>• Postmortem Sample — A postmortem sample.</li> <li>• NA24385 — The positive control.</li> <li>• Negative Amplification Control — The negative control (nuclease-free water).</li> <li>• Reagent Blank — The extraction control.</li> </ul>
Index Combination	Select the name of a premixed i7 and i5 index adapter (UDI).
Run Analysis Method	Select a method for analyzing samples in the run.
Initial Projects	Enter the name of a project to assign the sample to. To assign the sample to multiple projects, enter multiple project names.
Sample Plate	Enter a preferred name for the 96-well plate.
Sample Well Position	Enter the position of the plate well the sample occupies.

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


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

### 3.3. 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.
  - Review the values for cluster density and clusters passing filter.
  - Review the phasing and prephasing values for Read 1.
  - Check Read 1, Index 1, Index 2, and Read 2 to confirm that all 4 reads passed.
  - Check Overall Intensity and Discordant Loci to confirm that the HSC passed.
  - Compare the samples to the sample read count guideline to confirm performance.
  - Review the number of reads in parentheses next to the sample name or hover over each sample.

For more information, see "Quality metrics" in section 10.2 on page 65, and "Sample representation" in section 10.3 on page 69.

3. Select  **Run Activity** to review the run activity.
4. Select **Close** to return to the run.



### 3.3.1. 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 4. 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.

### 3.3.2. 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 5. Sample analysis states**

Symbol	Retain status	Description
	Created	UAS has not started analysis.
	Queued	The analysis is in line to start.
	Processing	Analysis or reanalysis is in progress.
	Completed	Analysis has successfully completed.
	Errored	Analysis has unsuccessfully completed.




## 4. Data Analysis

### 4.1. Review run metrics


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

For help with metrics requiring further evaluation, see "Troubleshoot a run", section 9.1 on page 60. For detailed descriptions of each metric, see Appendix A "Run Metrics", chapter 10 on page 65.

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

**Note:** The Discordance table only appears if there are discordant loci.
  - Under Reagent Blanks, review the number of SNPs typed for each reagent blank.
  - Under Negative Amplification Controls, review the number of SNPs typed.
  - Select a sample name to go to the Results page.
5. If any metrics do not meet the minimum requirements, see "Troubleshoot a run", section 9.1 on page 60 for troubleshooting guidance. For additional assistance, contact QIAGEN Technical Support. Collect the following items from D:\Illumina\MiSeqFGXoutput to help QIAGEN with troubleshooting:
  - InterOp folder
  - runInfo.xml file
  - runParameters.xml file

### 4.2. Review sample results

Before proceeding to kinship analysis or generating reports, review results on a sample-by-sample basis using the Sample Results page in the  **Projects** workspace. For information about the features available on this page, including how to

locate specific loci, see "Overview of sample results", section 2.4 on page 16.

For each Kintelligence HT sample:


- Review quality control (QC) indicators and overall sample metrics. See "Quality control indicators", section on page 18.
- Assess sample quality, and then set the sample's database status (Active or Inactive) as appropriate. See "Assessing sample quality", section 5.2 on page 29.

**Note:** By default, all antemortem and postmortem Kintelligence HT samples are marked Active in the database.


## 4.3. Modify sample results

You can modify Kintelligence HT results by typing and untyping alleles, changing the biological sex of a sample, changing the contributor status of a sample, or changing the database status of a sample. You can also add comments to loci explaining any changes. For instructions, see "Comment on a locus", section 7.4.8 on page 52.

### 4.3.1. Change the biological sex of a sample

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

### 4.3.2. Change the contributor status of a sample

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

### 4.3.3. Type or untype an allele


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

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

#### 4.3.4. Change the database status of a sample


1. On the navigation rail, select  **Projects**.
2. Select a project, and then select a sample to open the Sample Results page.
3. In the Locus Details section of the page, toggle **Active in Database** on or off as appropriate.

**Note:**

By default, all antemortem and postmortem Kintelligence HT samples are marked Active in the database.


#### 4.4. 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.

#### 4.5. 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 that are compatible with the assay used to prepare the sample.

6. Select **Next**.
7. Review the sample names, previous analysis methods, and new analysis methods.
8. To make changes, select **Previous**.
9. Select **Save** to initiate reanalysis.


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

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

The Results page displays results for the most recent analysis.

#### 4.5.1. Switch analysis methods

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

1. On the navigation rail, select  **Projects**.
2. Select a project, and then select a sample to open the Sample Results page.
3. On the Results header bar, under the sample name, select the arrow next to the name of the current analysis method to choose another method.

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

# 5. Kinship Analysis

## 5.1. Kinship analysis workflow

Once a Kintelligence HT run has finished sequencing and you have reviewed overall run metrics, follow the steps in Table 6 to identify and analyze potential relationships between samples.


To reduce the number of corrections that may be needed later in the workflow, you may add sample types (antemortem or postmortem) and any useful sample tags during run setup. See "Add samples to a run", section 3.2 on page 21.

**Table 6. Overview of kinship analysis steps**

No.	Step	Description	Further information
1	Assess Sample Quality and Set Database Status	The user assesses the quality of each antemortem or postmortem sample in the run and assigns its status in the database (Active or Inactive) accordingly.	"Assessing sample quality" below
2	Manage Samples in Database	The user reviews samples on the Database page and edits sample tags or sample types as needed.	"Database management" below
3	Create a Query	The user creates a query comparing one or more Unknown samples and one or more Reference samples in the database.	"Using queries" on page 31
4	Assign Pedigrees and Review Kinship Results	For each identified potential relationship, the user assigns a pedigree, and then reviews likelihood ratios and other data.	"Review kinship results", section 5.5 on page 33
5	Generate Kinship Query Reports	The user generates Kinship Query Reports as desired.	"Kinship query reports", section 5.6 on page 38

## 5.2. Assessing 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) when searching for distant relationships. 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.

By default, all Kintelligence HT antemortem and postmortem samples are marked Active in the database, regardless of their number of typed SNPs. Before proceeding to kinship analysis, you should review each sample individually using its Sample Results page in the  **Projects** workspace and assess whether its number of typed SNPs will be sufficient for your particular needs, then toggle the sample's database status (Active or Inactive) accordingly.

For further information about the levels of typed SNPs needed to identify relationships of various degrees, see "Recommendations for sample quality", section 11.3 on page 72.


## 5.3. Database management

This section describes how to view and manage Kintelligence HT samples in your local database. Properly managing your database will streamline the rest of the kinship analysis workflow.

### 5.3.1. Database table

The Database page consists of a table listing the following information about each Kintelligence HT sample in your local database.

**Table 7. Database table details**


Column name	Definition
Sample / Run	The name of the sample, followed by the run the sample is from. Selecting a sample name opens that sample's Sample Results page in the  Projects workspace.
Bookmark	User-applied bookmarks to mark specific samples of interest. See "Sample bookmarks", section 1.5.3 on page 8.
Tags	User-defined labels to identify connected samples and group them together. See "Sample tags", section 1.5.4 on page 8.
Sample Type	The type of sample (Antemortem or Postmortem).
Sample Status	The sample's status (Active or Inactive) in the database. (Only Active samples can be added to queries.)
SNPs Typed	The sample's total number of SNPs typed.

### 5.3.2. Review sample information


Review samples in the Database table and edit their attributes as needed.

- Tags — Add or edit sample tags as desired.

As a tip, use sample tags to facilitate quick look-up of connected samples in your database when creating queries.


- Sample Type — Samples should be assigned a type (Antemortem Sample or Postmortem Sample) during run setup. If you assigned the incorrect type to any samples at that stage, edit their type in the Database table accordingly.
- Sample Status — By default, the Database table shows only samples marked Active. To show Inactive samples as well, remove the default filter in the toolbar above the table, or select the  **Filter** icon next to the Sample Status column and select the Inactive checkbox.

**Note:** Only Active samples can be added to queries.

**Note:** You can toggle the database status of a Kintelligence HT sample from its Sample Results page in the  Projects workspace. (Selecting a sample name in the Database table opens its Sample Results page.) You cannot change the database status of samples from within the Database table.

#### Add or edit sample tags

**Note:** You may also edit tags from the Samples page in the  Runs workspace.

1. On the navigation rail, select  **Database**.
2. In the Database table, select the checkboxes for one or more samples.
3. Select **Edit Samples**.

The Edit Samples window appears.


4. In the Tags field, add, edit, or remove tags as desired.
  - To add a tag, type the name of the tag, and then press **Enter**. (Existing tags also appear in a drop-down menu.)
  - To remove a tag, select **X** next to the name of the tag.


- To edit the name of an already assigned tag, first remove the tag, and then add a new tag with the desired name.

**Note:** Any existing tags that have been applied to all of the selected samples will appear in the Tags field. Tags that only apply to some of the selected samples will not appear here. If you want to edit or remove tags that only apply to some of your selected samples, you must exit the Edit Samples window and select only those samples in the Database table, or edit samples individually.

5. When you have finished modifying tags, select **Save**.

### Edit sample type

**Note:** If you wish to change the type of a sample to anything other than Antemortem Sample or Postmortem Sample, you must do so from the Samples page in the  Runs workspace.

1. On the navigation rail, select  **Database**.
2. In the Database table, select the checkboxes for one or more samples.
3. Select **Edit Samples**.

The Edit Samples window appears.

4. Select the **Sample Type** tab.
5. Select **Antemortem Sample** or **Postmortem Sample**, and then select **Save**.

### 5.3.3. Refreshing the database

Whenever any of the following events occur, the software must update the database before a new query can be run:

- One or more new Kintelligence HT samples finish sequencing. (Once sequenced, every Kintelligence HT sample is marked "Active in Database" by default.)
- A user changes the database status of a sample (Inactive vs. Active).
- A user changes an allele call (typed vs. untyped) in a locus associated with a sample in the database.
- A user changes the biological sex of a sample in the database.
- A user deletes a sample in the database from a run.

An alert will appear at the top of the Database page indicating that there are pending updates to the database, along with the total number of samples that have been added, modified, or deleted. The software automatically pulls these pending updates into the database the next time a user runs a query.

**Note:** If samples in the database are changed during or after a query run, you may want to re-run the query with a refreshed database. A query cannot be re-run directly, but you can select **Copy to New Query** from the drop-down menu next to the name of a query to copy its samples to a new query and then run the (identical) new query.

## 5.4. Using queries

Queries allow you to compare Kintelligence HT samples within your local database and identify possible kinship matches. To perform a search, each query must include at least one Unknown sample and at least one Reference sample.

**Note:** Both antemortem and postmortem samples may be added to queries as Unknowns or References, depending on your needs.


### 5.4.1. Create a query

**Note:** If you navigate away from the Database workspace to another workspace while in the process of creating a query, the new query will remain pending until you return. However, closing or refreshing the UAS web application will remove the pending query.

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

2. Select **Create Query**.

The Database sidebar opens. A new query appears in the sidebar (highlighted in green).

3. Enter a name for the new query, and then select the checkmark .


**Note:** You must enter a name before you will be able to start the query.

4. Using the Database table, add samples to the query:

a. Select the checkboxes for one or more Unknown samples, and then select + **Unknown**.

b. Select the checkboxes one or more Reference samples, and then select + **Reference**.


Sort or filter the Database table to quickly locate samples of interest. See "Sorting and filtering tables", section 1.5.1 on page 7.

**Note:** To remove a sample from the query, select  next to the name of the sample.

5. When you have finished selecting samples, select **Start Search**.

**Note:** Before starting the query search, the software automatically refreshes the database with any pending updates. See "Refreshing the database", section 5.3.3 on the previous page.

### 5.4.2. Managing queries

The Database sidebar lists all previously created queries. By default, queries are sorted by creation date. Use the  **Search** bar to find a query by name.

Selecting  next to the name of a query opens a menu with available actions:

- Create Report — Generate a Kinship Query Report. See "Kinship query reports", section 5.6 on page 38.
- Copy to New Query — Copy the Unknown and Reference samples included in this query to a new query.
- Delete Query — Permanently delete a query.

**Note:** QIAGEN recommends regularly deleting queries that are no longer needed, in order to keep the list of queries manageable. Once deleted, a query cannot be recovered, but an identical query can still be created as long as the included samples are still in the database.



## Query state

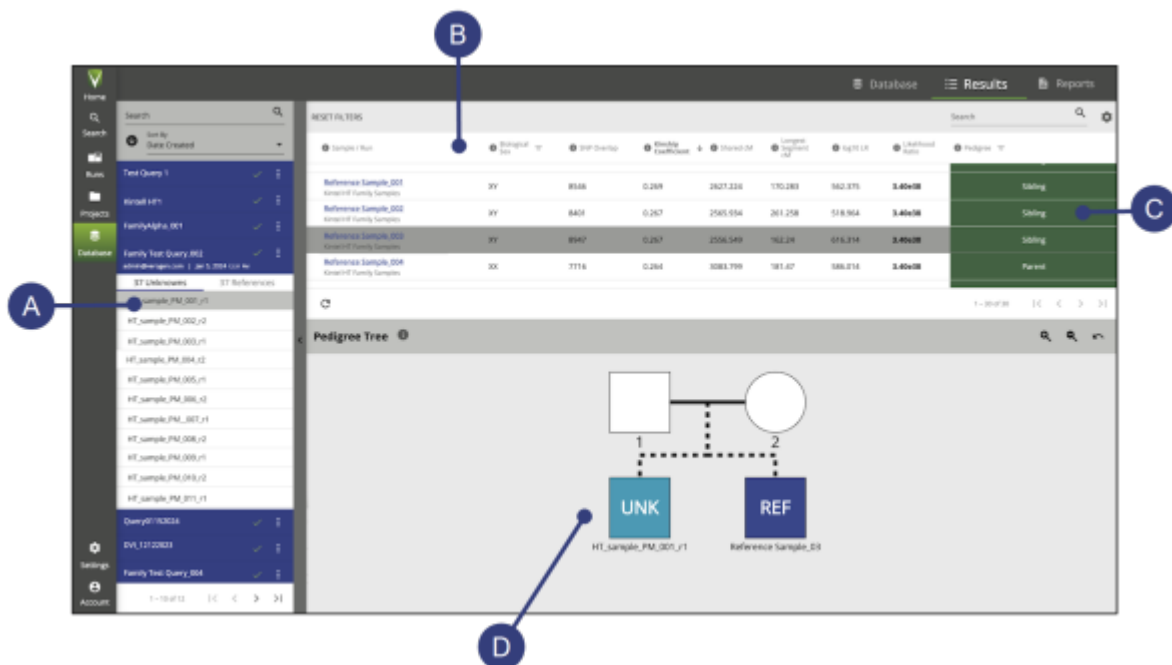
An icon next to each query in the Database sidebar indicates the current state of the query. Table 8 ("Kinship Analysis" on page 29) describes each possible query state.

**Note:** Depending on the number of samples in your local database, searches may complete so quickly that the Queued and Processing states do not appear.

## 5.5. Review kinship results

When a query has completed its search, you can view its results by selecting the query in the Database sidebar, and then selecting an Unknown sample within the query. The Kinship Results page for that Unknown sample opens, listing all potential relationships between the selected Unknown sample and the Reference samples as a table.

By default, the pedigree for each potential relationship is Unknown. Once you select a pedigree, the software calculates likelihood ratios for that potential relationship and displays a visual Pedigree Tree below the table.



**Figure 6. Overview of the Kinship Results page.** A. Select a sample — Select an Unknown sample from a query in the Database sidebar to open the Kinship Results page for that sample. B. Kinship Results table — Lists all potential relationships between the selected Unknown sample and the Reference samples included in the query, with various data. Likelihood ratios will not appear until the user has assigned a pedigree. C. Assign a pedigree — Select a pedigree for a potential relationship to calculate likelihood ratios and create a visual Pedigree Tree. (By default, the pedigree for each potential relationship is Unknown.) D. Pedigree Tree — A visual representation of the potential relationship between the selected Unknown and Reference samples. The Pedigree Tree will not appear until the user has assigned a pedigree.

### 5.5.1. Kinship results table

Each row in the Kinship Results table represents a potential relationship between the selected Unknown sample and a Reference sample. By default, the closest relationship (as measured by Kinship Coefficient) appears at the top of the table.

Table 9 describes the information listed in the Kinship Results table for each potential relationship. For information about using and interpreting these values, see "Assign a pedigree", section 5.5.3 on the facing page, and "Interpreting likelihood ratios", same section.

**Note:** A query may produce zero results, in which case the Kinship Results table will be empty.

**Table 8. Kinship Results table details**

Column name	Definition
Sample / Run	The name of the Reference sample that was identified as potentially related to the selected Unknown sample, followed by the run the Reference sample is from.
Biological Sex	The biological sex of the Reference sample contributor, as estimated by Y-SNPs or user-defined. Possible values are XX, XY, or inconclusive. See Biological Sex Estimation for Samples (on page 87).
SNP Overlap	The number of typed SNPs in common between the Unknown and Reference samples.
Kinship Coefficient	A value indicating the degree of relatedness between the Unknown and Reference samples.
Shared cM	The total amount of shared DNA between the Unknown and Reference samples, in centimorgans.
Longest Segment cM	The longest stretch of shared DNA between the Unknown and Reference samples, in centimorgans.
log <sub>10</sub> LR	The base-10 logarithm of the Likelihood Ratio (see below). This value will not appear until a pedigree has been selected in the Pedigree column.
Likelihood Ratio	The probability that the Unknown and Reference samples are related as postulated by the pedigree selected in the Pedigree column. This value will not appear until a pedigree has been selected.
Pedigree	The user-selected pedigree relationship between the Unknown and Reference samples. By default, the pedigree for each potential relationship is Unknown.

### 5.5.2. Identifying potential relationships

When a query is run, the software compares each Unknown sample to each Reference sample and applies the logic described in Table 9 to identify potential relationships. Results with values that do not fall within these ranges will not appear in the Kinship Results table.

The SNP Overlap value for a given pair of Unknown and Reference samples indicates the number of typed SNPs in common between those samples. For sample pairs with a low SNP Overlap ( $\leq 6,000$ ), the Kinship Coefficient must be above 0.031 for the samples to be considered potentially related. For sample pairs with a high SNP Overlap ( $> 6,000$ ), instead the Shared cM and Longest Segment cM values must be above the minimum thresholds listed below.

**Note:** This table also appears in the software as a tooltip above the Kinship Results table for easy reference.

**Table 9. Criteria used to determine whether a potential relationship appears in the Kinship Results table**

SNP overlap	Minimum shared cM	Minimum longest segment cM	Minimum kinship coefficient
>9000	120	30	n/a
>8000	140	30	n/a
>6000	160	30	n/a
$\leq 6000$	n/a	n/a	0.031

These thresholds were generated to ensure high specificity and sensitivity, and are identical to those used by GEDmatch PRO (5, 6).

### 5.5.3. Assign a pedigree

By default, the pedigree for each potential relationship is Unknown. Once the user has assigned a pedigree, the software will calculate likelihood ratios for that potential relationship, and a visual Pedigree Tree will appear below the Kinship Results table.

#### Estimating pedigrees

If you are unsure about the pedigree relationship between 2 samples, the Kinship Coefficient and Shared cM values can be used to help estimate the degree of relationship. Refer to Table 10 and Table 11 for the expected ranges for each degree of relationship (9).

For potential parent-child relationships, refer also to "Recommendations for parent-child pedigrees", section 11.4 on page 73, for supporting information.

**Note:** These tables also appear in the software as tooltips above the Kinship Results table for easy reference.


**Table 10. Kinship coefficient ranges expected for each degree of relationship**

Relationship degree	Minimum kinship coefficient	Maximum kinship coefficient
Self	0.484	n/a
1st degree	0.196	0.31
2nd degree	0.076	0.17
3rd degree	0.023	0.11
4th degree	0.064	0.076
5th degree	-0.0009	0.025

**Table 11. Shared cM ranges expected for each degree of relationship**

Relationship degree	Minimum shared cM	Maximum shared cM
Self	6200	n/a
1st degree	2328	3065
2nd degree	1165	2021
3rd degree	448	1081
4th degree	78	665
5th degree	0	319

#### Add or Edit Pedigree

1. On the navigation rail, select  **Database**.
2. In the Database sidebar, select a completed query, and then select an Unknown sample within that query. The Kinship Results page for that sample opens.

3. In the Kinship Results table, select the Pedigree box for a potential relationship.

**Note:** Each row in the table represents a potential relationship to a Reference sample. By default, the pedigree for each potential relationship is Unknown.

4. A window opens displaying a color-coded chart of possible pedigree relationships. Select a relationship, and then select **Save**.

**Note:** For half-pedigree relationships, select **M** for the maternal side or **P** for the paternal side.

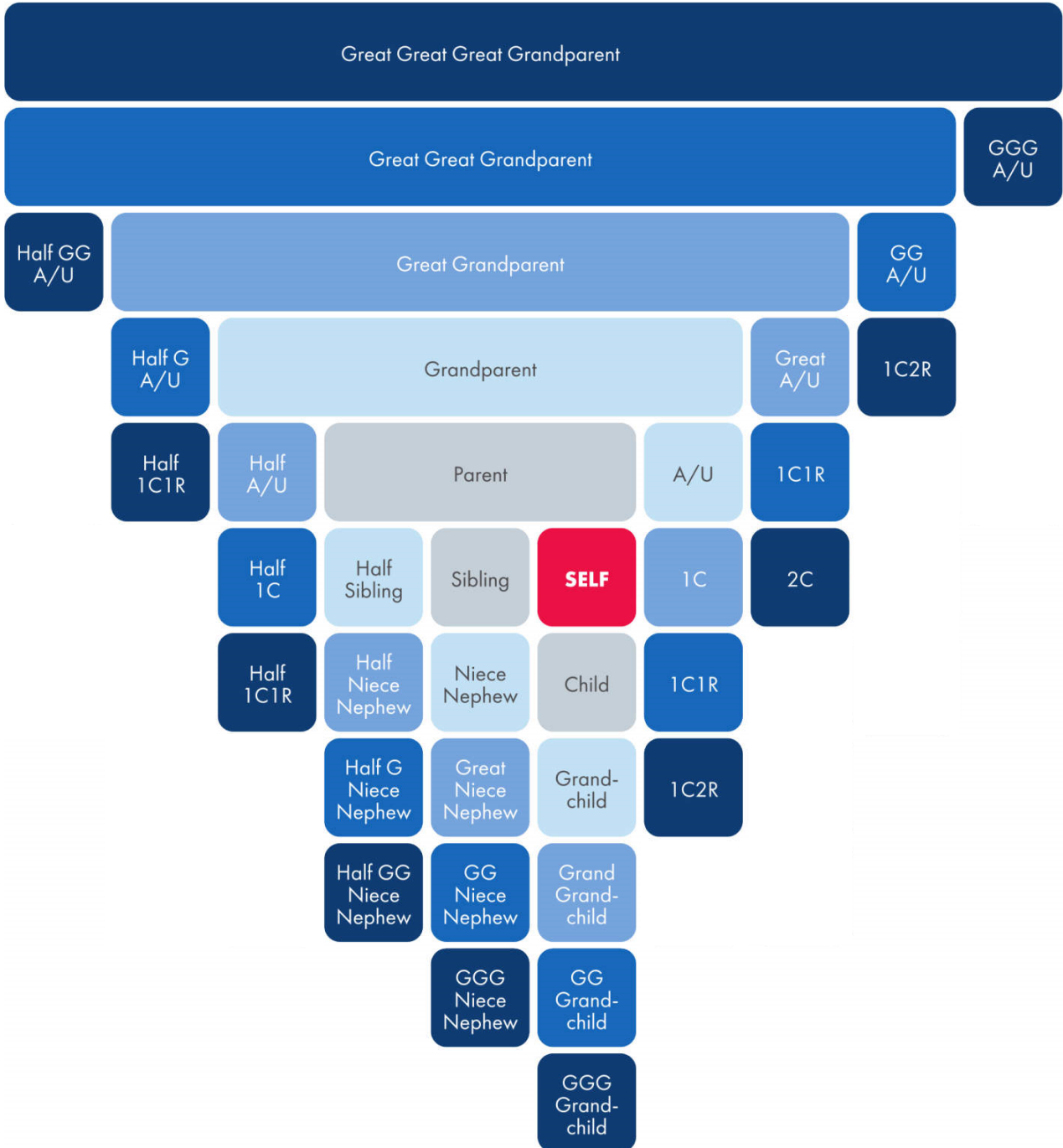


Figure 7. Generation chart.

A Pedigree Tree illustrating the postulated relationship appears below the Kinship Results table, and the software calculates likelihood ratios for the relationship.

### Pedigree tree functions

Within a Pedigree Tree, the following functions are available:

- Right-clicking a node in the tree opens a menu with available actions:
  - Toggle as Deceased — Mark the node as deceased.
  - Change Sex — Change the biological sex of the node.  
**Note:** If you change the biological sex of a node, the software recalculates the Likelihood Ratio and log<sub>10</sub> LR values for the potential relationship.
  - Edit Label — Edit the label that appears below the node in the tree.
- Zoom in or out by using the buttons to the upper-right of the tree, or using the scroll function of your mouse or trackpad. Double-clicking the Pedigree Tree bar resets the tree to its default size.

## Interpreting likelihood ratios

The software calculates 2 likelihood ratio values:

- Likelihood Ratio — The probability that 2 samples are related as postulated by the assigned pedigree, as opposed to being unrelated.
- log<sub>10</sub> LR — The base-10 logarithm of the Likelihood Ratio value.

The largest Likelihood Ratio value that UAS can display is  $3.40 \times 10^{38}$ ; larger ratios are replaced by this number. In these cases, the log<sub>10</sub> LR value will be more useful for interpretation.

For potential parent-child relationships, see "Recommendations for parent-child pedigrees", section 11.4 on page 73 for further essential information about interpreting likelihood ratios.


## 5.6. Kinship query reports

Once you have reviewed results from a query and assigned pedigrees as desired, you can download that data as a Kinship Query Report. The report is an Excel workbook containing the data shown in the Kinship Results table for all Unknown samples included in that query, as well as general attributes of the Unknown and Reference samples included in the query.

View and download previously generated Kinship Query Reports on the Reports page.

**Note:** Kinship Query Reports do not include Pedigree Trees. If you wish to add a Pedigree Tree to a report, you must take a screenshot of the tree manually.

### 5.6.1. Create a kinship query report

1. On the navigation rail, select  **Database**.
2. In the Database sidebar, locate a query, and then open the drop-down menu next to the name of the query.
3. Select **Create Report**. The software generates the report. Based on your browser settings, the report is automatically downloaded and saved to your local machine.

# 6. Reports

## 6.1. Report types

The software can compile Kintelligence HT results and other information into the following reports:

- Sample — Results for one sample.
- Project — Results for all samples in a project.
- GEDmatch PRO — SNP genotype calls reported on the plus strand for upload to GEDmatch PRO.
- Phenotype and Ancestry — Estimates of hair color, eye color, and biogeographical ancestry based on aSNP and pSNP data.
- Kinship Query Report — Kinship results from a query, with data about each potential relationship between the Unknown and Reference samples included in the query.

## 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.

For report contents, see "File Formats for Reports", section 6.3 on page 41.

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

### 6.2.1. 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 Samples 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 2.2.3 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 2.2.5 on page 13.

## 6.2.2. 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 Samples 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 2.2.3 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. Based on your browser settings, the report is immediately downloaded and saved to your local machine.

**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 2.2.5 on page 13.

## 6.2.3. Create a GEDmatch PRO report

You can create one GEDmatch PRO report at a time or simultaneously generate multiple GEDmatch PRO 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 Samples 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".

4. Select the blue **Actions** button, then select GEDmatch PRO 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 2.2.5 on page 13.



### 6.2.4. Create a Phenotype and Ancestry report

You can create one Phenotype and Ancestry report at a time or simultaneously generate multiple Phenotype and Ancestry 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 Samples 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 2.2.3 on page 12.

4. Select the blue **Actions** button, then select **Phenotype and Ancestry** 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 2.2.5 on page 13.

### 6.2.5. Create a kinship query report

Unlike other report types, Kinship Query Reports are created from within the  Database sidebar. Previously generated Kinship Query Reports are accessible from the Reports page in the  Database workspace, rather than the similar page in the  Projects workspace.

For more information, see "Kinship query reports", section 5.6 on page 38.

## 6.3. File formats for reports

The following sections describe the format and contents of each type of report the software generates for Kintelligence HT samples. In the reports, rsID numbers identify individual SNPs. For instructions on creating reports, see "Generating reports", section 6.2 on page 39.

### 6.3.1. Sample report

A Kintelligence HT sample report is an Excel workbook with one worksheet per SNP type: X-, Y-, ancestry, phenotype, identity, and kinship. 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

### 6.3.2. Project report

A Kintelligence HT 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

### 6.3.3. GEDmatch PRO report

A GEDmatch PRO report is a **\*.txt** file. Using human genome assembly GRCh37 (hg19) as the reference genome, the report lists each SNP locus call by rsID with the following information:

- Chromosome and position
- Allele 1 and allele 2 typed calls reported on the plus strand

### 6.3.4. Phenotype and Ancestry report

A phenotype and ancestry report is an Excel workbook comprised of four worksheets that detail the estimates of hair color, eye color, and biogeographical ancestry: Estimation, SNP Data, SNP Balance Figure, and Settings. In the Estimation and SNP Data worksheets, a header section lists the software version, analysis method, sample biological sex, project creation date and time, the user who generated the report, and sample, project, and run names.

- Estimation
  - Hair color results: brown, red, black, and blonde
  - Eye color results: intermediate, brown, blue
  - Biogeographical ancestry results (plot): the sample with distance to the nearest centroid
  - Population information for samples in the centroid: 1000 Genomes population and abbreviation, count, and data used to train models

- SNP Data
  - Allele name, whether the locus is typed, and the number of reads for each locus
  - Number of loci typed, genotype, and QC indicators for hair and eye color SNPs, common SNPs, and biogeographical ancestry SNPs

**Note:** Loci typed is the number of aSNP and pSNP loci identified out of total targeted aSNP and pSNP loci.
- SNP Balance Figure
  - Whether the allele is typed
  - Number of reads per base for each SNP with a grand total
- Settings
  - Software version
  - AT, IT, and intralocus balance for each locus

### Estimating phenotype

The software uses HlrPlex, a multinomial logistic regression model, to determine the probabilities for hair, eye, and skin color. The software publishes these probabilities to the Estimation worksheet (1–3).

### Estimating biogeographical ancestry

The software uses principal component analysis (PCA) to determine estimates of biogeographical ancestry. Data from Phase I of the 1000 Genomes Project ([1000genomes.org](http://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 2 pretrained components based on aSNP genotype calls (4).

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.

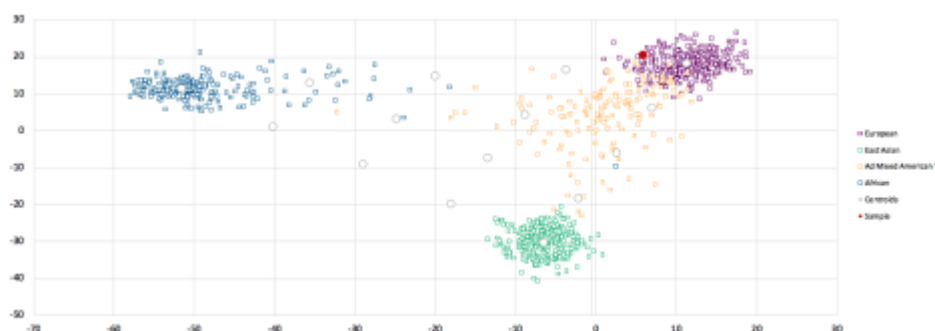


Figure 8. Sample PCA plot.

## Generating SNP data




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

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

# 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.

7. To remove a sample, clear the checkbox or select **X** in the summary.
8. 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:
  - Edit the project name.
  - Add a project description or modify the existing description.
4. Select **Save** to apply the changes.

### 7.2.3. 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, and 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 **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. 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.


Field	Instruction
Sample Name	Edit the unique identifier for the sample.
Tags	Add, edit, or remove sample tags. Previously created tags appear in a drop-down menu.
Description	Enter or edit a preferred description of the sample.
Assay Type*	Select a different library prep method: <ul style="list-style-type: none"> <li>ForenSeq Kintelligence HT — Libraries prepared with the ForenSeq Kintelligence HT Kit.</li> </ul>
Sample Type	Select a different sample type: <ul style="list-style-type: none"> <li>Antemortem Sample — An antemortem sample.</li> <li>Postmortem Sample — A postmortem sample.</li> <li>NA24385 — The positive control.</li> <li>Negative Amplification Control — The negative control (nuclease-free water).</li> <li>Reagent Blank — The extraction control.</li> </ul>
Index Combination*	Select the name of a different premixed i7 and i5 index adapter.
Run Analysis Method*	Select a different run analysis method.
Initial Projects*	Enter additional projects or remove current projects. For detailed instructions, see "Edit sample information", section 7.3.2 on the previous page.
Sample Plate	Edit or enter a preferred name for the 96-well plate.
Sample Well Position	Edit or enter the position of the well the sample occupies.

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

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


### 7.3.3. 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.

- On the navigation rail, select  **Runs**. Then select a run.
- In the Actions column, select **Edit Sample Details** for the applicable sample.
- 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.
- Select **Save**.



### 7.3.4. 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. For instructions on enabling the history setting, see "System settings" on page 58.

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



### 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 results using the following fields:
  - Search — Search the current history.
  - Start Date and End Date — View the history within a specified time frame.
  - Show System Events — See system events and user actions for the history or see user actions only.
5. When finished, select **Close**.

### 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 results using the following fields:
  - Search — Search the current history.
  - Start Date and End Date — View the history within a specified time frame.
  - Show System Events — See system events and user actions for the history or see user actions only.
5. When finished, select **Close**.


### 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 results using the following fields:
  - Search — Search the current history.
  - Start Date and End Date — View the history within a specified time frame.
  - Show System Events — See system events and user actions for the history or see user actions only.
5. When finished, select **Close**.



### 7.4.4. Review locus activity

1. On the navigation rail, select  **Projects**.
2. Select a project, and 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, and 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 are not deleted from UAS.
- **Archive a Run or Project** — Archives data pertaining to a run or project. You may choose either to leave the original data in UAS, or to 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, an archive for a run or project 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, and 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.


As a tip, sort or filter the Samples table to locate samples more quickly. See "Sorting and filtering tables", section 1.5.1 on page 7.

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, and 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 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 minutes 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 may be imported.

2. On the navigation rail, select  **Settings**.
3. Select **Data Management**.

The Data Management page opens.

4. Select **Import**.

The Import an 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 effectively be creating a duplicate version of that run or project in UAS.

**Note:** 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 name of the imported file.

6. Review the archive file you have chosen to import, and 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.
- 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 color scheme of the user interface to be color-blind compatible.

## 8.2. Managing analysis methods

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

**Note:** 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.

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

### 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.

#### 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", section 8.2.2 below.

### Use a Blank Analysis Method

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

2. Select **Analysis Methods**.

3. Select **Create Analysis Method**.

The New Analysis Method dialog box opens.

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

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

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

The new method appears with blank settings.

7. Enter the desired settings for the analysis method.

For instructions, see "Modify analysis settings", section 8.2.2 below.

### 8.2.2. Modify analysis settings

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

#### Edit an analysis method

1. Open the analysis method you want to edit:

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



- b. Select **Analysis Methods**.

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

2. To rename the analysis method:

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

- b. Enter a new name for the analysis method.

- c. Select  to apply the name change, or  to cancel.

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



- a. In the list of loci, select the checkbox of one or more loci. (You can filter the list by SNP type, sort the list by column, or search for a locus by name. Scroll to view all loci.)
- b. Select **Edit X Rows**, where X is the number of loci selected.


The Edit Analysis Method dialog box opens.

3. To add or remove loci from the analysis method, select the **Include** toggle button.
4. 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.)
5. 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.

### 8.2.3. Delete an analysis method

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

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

### 8.2.4. Change the default analysis method

1. On the navigation rail, select  **Settings**.
2. Select **Analysis Methods**.
3. In the Select an Assay Type list, select the kit that corresponds to the analysis method you want to set as the default.
4. Select the applicable analysis method.  
A green star identifies the current default.
5. Set the selected analysis method as the default for the assay type by selecting the empty star next to the analysis method name:

## 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 52.


## 8.4. 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.


### 8.4.1. 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.

### 8.4.2. 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.


## 8.5. 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.


### 8.5.1. 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.

### 8.5.2. 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 the  Runs header bar,  Projects header bar, and Results page.

## 8.6. System information

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

## 8.7. Dark mode

Toggle the Dark Mode setting in the  **Settings** menu to change the color scheme of the user interface to be color-blind 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 the product page to download relevant literature.

#### 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, library from the Purified Library Plate were overpipetted.
  - If the sample has a high number of alleles with quality control indicators, be sure to use correct volumes and reagents for normalization and resequence the sample.
  - If the sample volume is correct, further dilute libraries in the Pooled Normalized Libraries tube.
  - If normalization volumes or reagents were incorrect, repeat normalization and resequence.
- 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 than expected volume of HT1 was pipetted during library prep. Repeat sequencing using the correct volume.
- Excessive sample was lost during purification. Repeat the library prep using best practices for bead handling. Ensure that the ethanol made for SPRI washing is made at the correct ratio.

- The library was not otherwise denatured properly.
  - Make sure that the final library pool is denatured with freshly diluted 0.2 N NaOH (HP3) that has a pH >12.5.
- MiSeq FGx Maintenance Status:
  - Perform a maintenance wash and repeat sequencing.
  - Refill the wash tray and bottle for every wash.
  - Make all wash solutions daily.

#### 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 9.1.2 on the previous page. However, if sample representation shows that reads are above the sample count guideline, proceed with analysis.
- MiSeq FGx Maintenance Status:
  - Perform a maintenance wash and repeat sequencing.
  - Refill the wash tray and bottle for every wash.
  - Make all wash solutions daily.
- If the problem remains unresolved, reagents might not be performing as expected. Contact 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 9.1.2 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 in locating the logs.
- The reagent chiller temperature is too low or too high. The system displays an error.
- MiSeq FGx Maintenance Status:
  - Perform a maintenance wash and repeat sequencing.
  - Refill the wash tray and bottle for every wash.
  - Make all wash solutions daily.
- If the problem remains unresolved, reagents might not be performing as expected. Contact 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 60.
- 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", section 9.1.5 on the previous page.
- The cluster density is too high. See "High cluster density", section 9.1.2 on page 60.
- 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 "Phasing and pre-phasing" on page 66.
- The cluster density is too high. See "High cluster density", section 9.1.2 on page 60.
- 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 concentration of 0.75 ng/ $\mu$ L.
- 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 7.3.4 on page 49.
3. Add the sample back to the run with the correct index combination, following the steps in "Add samples to a run", section 3.2 on page 21.

The sample will automatically analyze.

If further errors occur, contact QIAGEN Technical Support.

1. Navigate to the run folder, following the steps for obtaining the analysis folder path in "Review run activity" on page 49.
2. Navigate to the Log folder for that sample analysis.
3. Copy the log text file.
4. Navigate to D:\verogen\fuas\logs and copy the most recent log text file.

### 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 in "Review run activity", section 7.4.1 on page 49.
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, following the steps in "Review run metrics", section 4.1 on page 25.

## 9.3. Perform a status check

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

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


The copy command copies the content displayed under System Status and Most Recent Log to your clipboard.


4. Paste the copied content to an external file.
5. When finished, select **Close**.





# 10. Appendix A. Run Metrics

## 10.1. 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.

## 10.2. 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 9. 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.

### 10.2.1. Run quality metrics

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

Table 12. 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/mm<sup>2</sup>). For ForenSeq libraries, the target cluster density is 400–1650 K/mm<sup>2</sup>. 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%.




### 10.2.2. 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 13. 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 14. 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 Kintelligence HT	Read 1	1–151	Cycle 152
	Index 1 Read	152–159	Cycle 160
	Index 2 Read	160–167	Cycle 168
	Read 2	168–318	After cycle 318

### 10.2.3. Human sequencing control

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

**Table 15. Colors for HSC results**

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

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

#### Loci

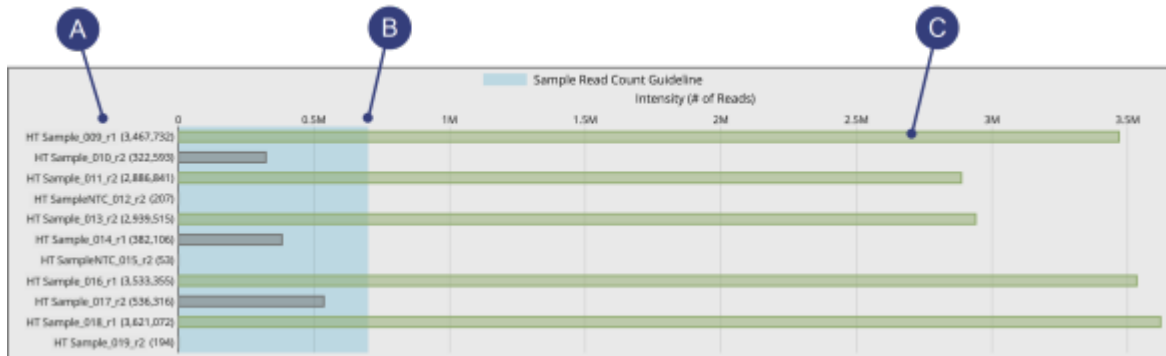
The HSC includes the loci in the following table. The software lists any loci that do not meet intensity or genotype concordance criteria.

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

## 10.3. 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 10. 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.




### 10.3.1. 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 Kintelligence HT	700,000 reads per sample

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

**Table 16. 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.

## 10.4. Positive and negative controls

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

**Table 17. Control assessments**

Assay Type	Positive amplification control	Value
ForenSeq Kintelligence HT	NA24385 Positive Amplification Control DNA	SNPs typed



### 10.4.1. Positive amplification control

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

#### SNPs typed

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

**Table 18. Colors for loci for NA24385**

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

#### Discordance table




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

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

#### Pass filter reads

Pass filter reads displays the following colors to indicate whether the number of reads that passed filter meet the guideline of 700,000 reads for Kintelligence HT libraries. Reads below the guideline might still be usable. Review quality metrics and sample data to help make a determination.

**Table 19. 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.



### 10.4.2. 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 20. Colors for base calls of loci**

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

# 11. Appendix B. Supporting Information

## 11.1. 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  $\geq 10$  Y-SNP loci typed, the biological sex is male (XY).
- If the sample has zero Y-SNP loci typed and the call rate is  $\geq 50\%$ , 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.

## 11.2. 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  $\geq 10$  Y-SNPs with 2 alleles.
  - The sample has  $\geq 10$  Y-SNP loci typed and  $\geq 10$  X-SNPs with 2 alleles.
  - The sample has zero Y-SNP loci typed with  $\geq 60\%$  autosomal SNP (auSNP) heterozygosity and  $\geq 50\%$  X-SNP heterozygosity.
- If the sample has  $\leq 50\%$  auSNP heterozygosity,  $\leq 40\%$  X-SNP heterozygosity,  $\geq 65\%$  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.

## 11.3. 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 ( $\leq 6,000$ ), only relationships of up to 3 degrees can be identified. If the SNP Overlap is high ( $> 6,000$ ), relationships of higher degrees (fourth and fifth) are more likely to be identified (5).

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.

Postmortem samples are likely to have lower numbers of typed SNPs due to the often high levels of degradation and low DNA inputs (6); however, antemortem samples are typically of high quality and quantity. Therefore, maximizing the number of typed SNPs in antemortem samples is essential to achieving a high overall SNP Overlap between antemortem and postmortem samples.



In order to ensure a high SNP Overlap and identify relationships of up to 3 degrees, QIAGEN recommends the following minimum thresholds:

- For postmortem samples, the recommended minimum number of typed SNPs is 2000.
- For antemortem samples, the recommended minimum number of typed SNPs is 9000.

These minimum thresholds are general recommendations; different thresholds may be needed depending on the degree of relationship between 2 samples. If a sample has fewer typed SNPs than the minimums listed above, identification of self, first-, and second-degree relationships may still be possible (6).

For further information about determining the appropriate numbers of typed SNPs and the kinship algorithm used by UAS, refer to Snedecor et al. (2022) (5) and Radecke et al. (2023) (6).

## 11.4. Recommendations for Parent-Child Pedigrees

Data obtained from Kintelligence HT samples is prone to sister allele dropout due to the increased plexity (number of samples per sequencing run) and the high degradation often observed in DVI samples (6). Sister allele dropout results in lowered heterozygosity, and can affect the calculation of likelihood ratios for parent-child pedigrees. For this reason, if you select a parent-child pedigree for a potential relationship, QIAGEN recommends that you review the heterozygosity of the samples (particularly the postmortem sample).

Note the following:

- The loss of sister alleles across loci leads to higher numbers of homozygous genotypes and can result in opposite homozygous genotypes between 2 samples. For example, at a given locus, if the parent sample was genotyped as homozygous reference (0/0) and the child was genotyped as homozygous alternative (1/1), these 2 samples cannot be related as parent-child given the genotypes because we would expect one allele to be shared between a parent and child.

Due to the elevated levels of sister allele dropout observed in degraded and/or low-DNA-input samples (6), this phenomenon is more likely to occur in Kintelligence HT samples.

- The scenario described above would result in a calculated locus likelihood ratio (LR) of zero. To ensure that a locus LR is not zero in these cases, the software applies the following equation for parent-child pedigrees with opposing homozygous genotypes:

$$LR_{\text{locus}} = \frac{g}{p^2 q^2}$$

where  $g$  represents a constant genotype error rate value (7, 8) and is set to 0.001 in UAS,  $p$  is the reference allele frequency in the population, and  $q$  is the alternative allele frequency in the population.

This equation, although resolving a locus LR of zero, may overestimate the locus LR. If opposing homozygous genotypes are prevalent and the user selects a parent-child pedigree, this may result in an overestimation of the overall Likelihood Ratio for the potential relationship. An increase in opposing homozygous genotypes is correlated with low heterozygosity (<30%).

**Note:** This problem affects only parent-child pedigrees. Opposing homozygous genotypes are possible and expected for all other pedigrees, even siblings.

See Snedecor et al. (5) for further details about how the loss of sister alleles and resulting lower heterozygosity affects kinship calculations, and other studies (6–8) for details about why the value 0.001 was chosen to be the constant in these cases.

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# Document Revision History

Date	Description
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