Universal Analysis Software Kintelligence Module

Version 2

Reference Guide

VEROGEN PROPRIETARY
Document # VD2022002 Rev. A
June 2022

Revision History

Document #	Date	Description of Change
VD2022002 Rev. A	June 2022	Initial release of the Universal Analysis Software – Kintelligence Module
VD2019002 Rev. E	October 2021	• Updated Dark Mode to include color deficiency accessibility • Updated Icons for Run Status and added new status for Analysis Status • Added instructions for creating Flanking Regions report and the report format for ForenSeq MainstAY analysis • Added ability to find SW version number in system settings • Updated calculation for total Alleles in MainstAY sample summary, for female samples • Included instructions to identify software version number
VD2019002 Rev. D	May 2021	Updated software descriptions to Universal Analysis Software v2.3, which supports the ForenSeq MainstAY Kit with NA24385 as the positive control and refreshes the user interface. • Added the ForenSeq MainstAY assay, Analysis Method, and STR reports. • Added run metrics for ForenSeq MainstAY libraries. • Added instructions for performing a status check for troubleshooting. • Updated instructions for creating CODIS reports, including the addition of options for batch IDs, case IDs, kit names, and partial profiles. • Updated ForenSeq Positive Amplification Control options for ForenSeq mtDNA Control Region and ForenSeq mtDNA Whole Genome to HL60 and ForenSeq Positive Amplification Control options for ForenSeq Kintelligence and ForenSeq MainstAY to NA24385 Updated the safety data sheet (SDS) link to verogen.com/product-documentation. Renamed the software to Universal Analysis Software and this guide to *Universal Analysis Software v2.0 Reference Guide*. Added the term stutter to the glossary. Corrected target run metrics for ForenSeq Kintelligence libraries. Replaced kinship with Kintelligence.

Document #	Date	Description of Change
VD2019002 Rev. C	February 2021	Updated software descriptions to ForenSeq Universal Analysis Software v2.2, which supports the ForenSeq Kintelligence Kit with NA24385 as the positive control. • Added the ForenSeq Kintelligence assay, Verogen Kintelligence Analysis Method, and Kintelligence reports. • Added run metrics for ForenSeq Kintelligence libraries. • Added instructions for assigning Unique Dual Index adapters, accessing the analysis folder, switching analysis methods, and printing. Updated the ForenSeq trademark to a registered trademark. Refreshed the format of the guide: • Updated the layout and formatting. • Updated fonts, table styles, and other design elements. Added guidance for failed run metrics. Added a link to the Verogen Documentation web page. Added the glossary terms clusters, intensity, intralocus balance, positions called, reference SNP ID number, short tandem repeat, typed and untyped, and Unique Dual Index. Added ambient temperature and reagent chiller temperature as potential sources of high phasing or prephasing values. Reorganized information on primary workspaces, reports, analysis methods, and reviewing sample data. Renamed the Sample Details page to Results and updated other user interface terminology as needed. Clarified that Dark Mode is the only software setting available to non-administrators. Corrected flow cell compatibility for the ForenSeq mtDNA Control Region Kit.

Document #	Date	Description of Change
VD2019002 Rev. B	August 2020	Updated software descriptions for ForenSeq Universal Analysis Software v2.1, which provides the following enhancements: • Support for the ForenSeq mtDNA Whole Genome Kit • A Run, Project, and Sample History setting in System Settings • Project History and Sample History worksheets for Project and Sample Reports Updated guide format and organization, including adding an index and consolidating workspace descriptions. Revised step-by-step instructions to be more succinct and include field descriptions. Updated telephone numbers for Verogen contact information. Updated glossary term definitions and added the terms coverage plot, indel, library, mtDNA navigator, plexity, position viewer, system event, and user action. Updated figures to include callouts highlighting user interface elements. Added information on strand bias and lost passwords. Added instructions for creating projects, assigning a sample to multiple projects, modifying user-created analysis methods, and enabling and disabling dark mode. Added another possible cause of low cluster density: belatedly combining bead-based normalized libraries and Hybridization Buffer (HT1). Noted that the analysis folder contains VCF and BAM files. Noted that the software follows SWGDAM nomenclature rules. Noted the conditions for displaying the Call or No Call filter. Clarified that each sample name must be unique in the software. Clarified that phasing and prephasing values are for Read 1 only.
VD2019002 Rev. B	August 2020	Distributed troubleshooting information among individual sections. Moved information on analysis method settings to *Analysis Defaults for ForenSeq Universal Analysis Software v2 (Pub. No. VD20200045)*. Corrected duplicate entries of D7S820 and D16S539 in the Human Sequencing Control. Corrected the list of information included in project and sample reports. Corrected citations that appear in the appendix.
VD2019002 Rev. A	August 2019	Initial release

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Software Overview

Introduction

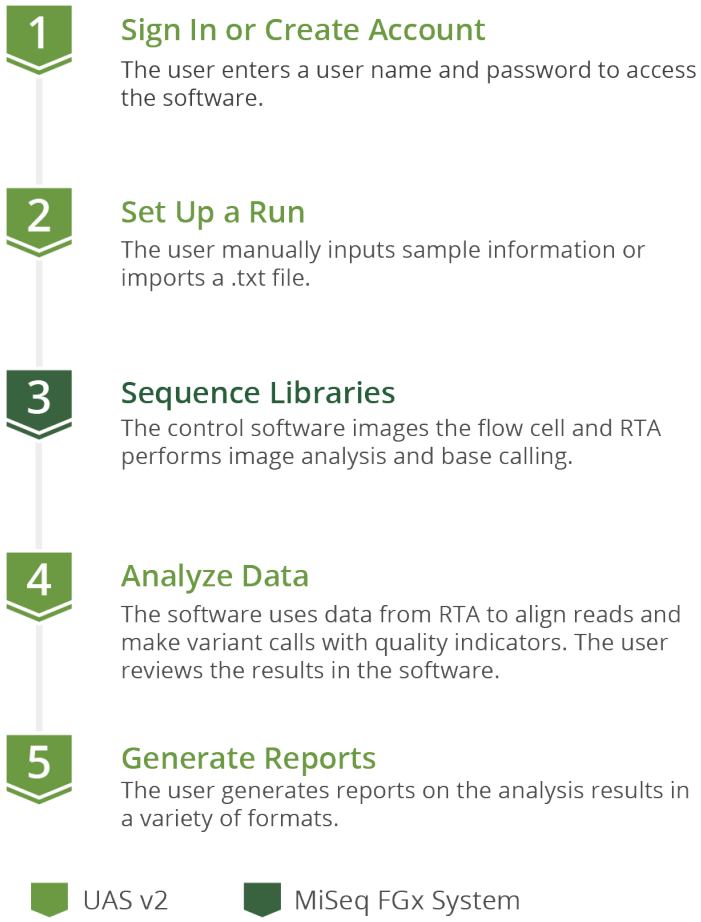
Universal Analysis Software (UAS v2) is a forensic genomics solution for run setup, sample management, data analysis, and reporting. Pre-installed on a dedicated server, the software integrates with the MiSeq FGx® Sequencing System to automatically generate FASTQ files and perform alignment from run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

This reference guide provides details on the analysis of data generated from the ForenSeq Kintelligence kit using the Kintelligence analysis method.

Analysis Workflow

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

Figure 1 Overview of data analysis steps



Access the Software

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

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

Create an Account

Before using UAS, you must create an account. The first user to create an account is automatically assigned administrator privileges. The administrator is responsible for approving new accounts. For instructions, see *Enable and Disable Accounts* (page 42).

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

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

Sign In or Sign Out

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

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

Change a Password

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

Primary Workspaces

The following pages comprise the primary workspaces in UAS:

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

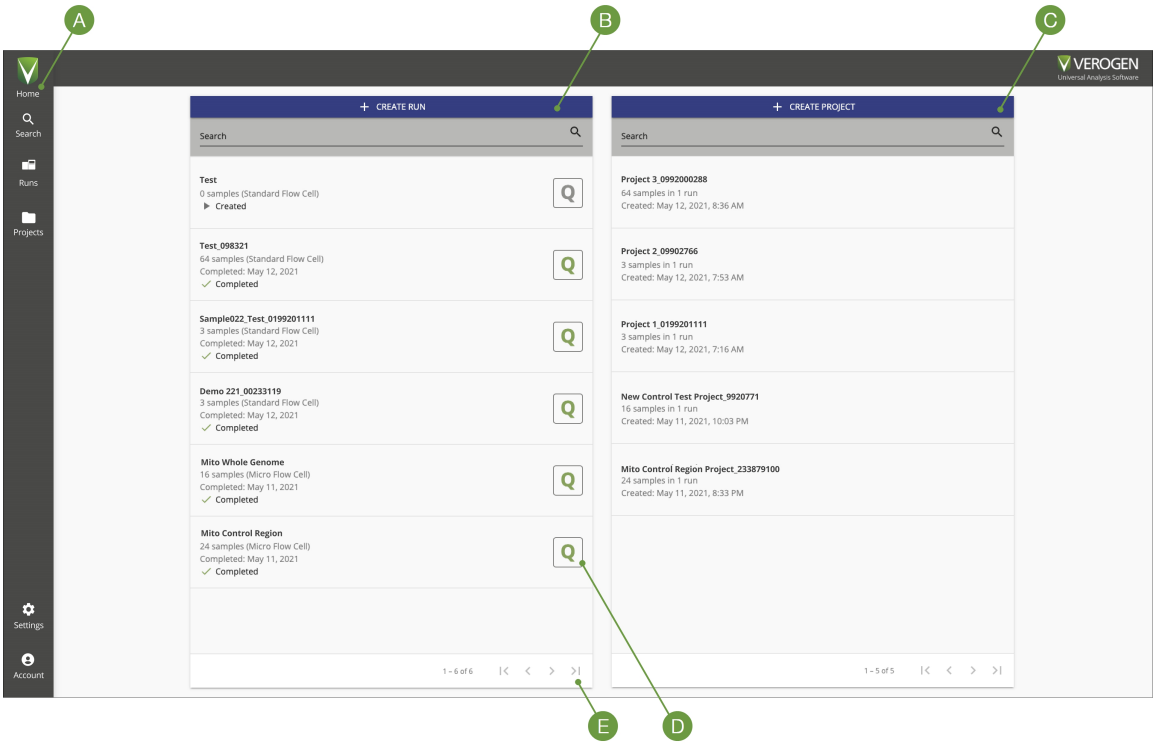
The software also includes printing, commenting, and other functions that supplement run setup, data analysis, and reporting. For instructions on using these supplementary features, see Chapter 5 Run, Project, and Sample Management.

Home Page and Navigation

The Home page displays information and functions organized into runs and projects. Selecting a run opens the Run Details page, and selecting a project opens the Project Details page. Color-coded Quality Metrics icons provide an overall view of run performance. Hovering over an icon displays more detailed metrics.

The navigation rail vertically arranges icons and puts key destinations within easy reach. It appears on the left side of each page so you can go to runs, projects, settings, and your account or can return to the Home page.

Figure 2 Overview of the Home page



- **A Navigation rail** — Icons that go to key destinations
- **B Runs** — List of runs with quality metrics and options to search and create
- **C Projects** — List of projects with options to search and create
- **D Quality Metrics icon** — Quality metrics for a completed run
- **E Scroll arrows** — Move through all runs or projects

Runs

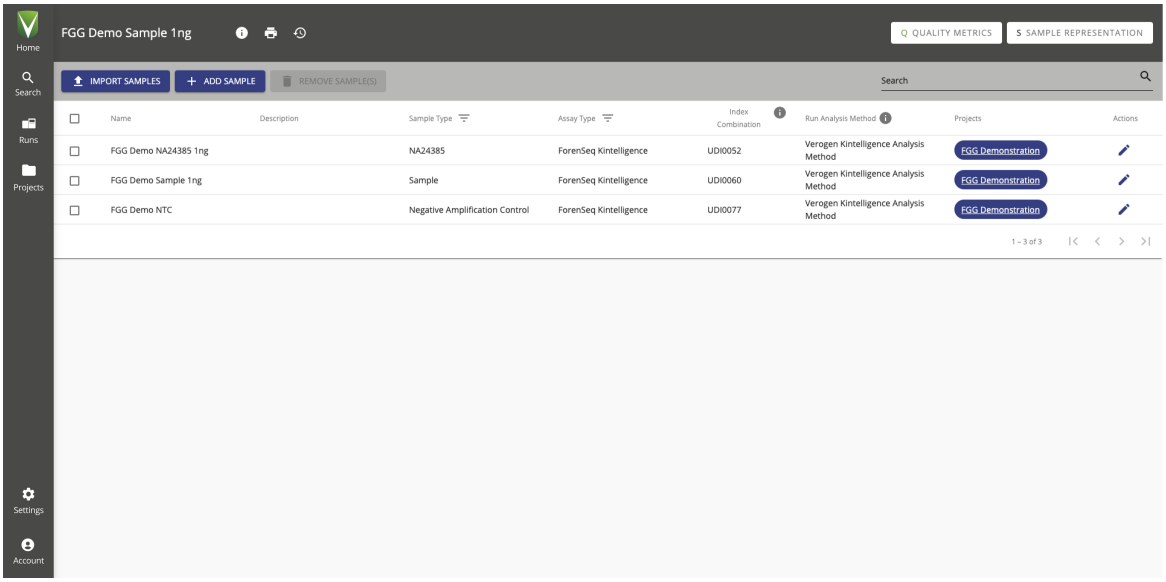
Runs is the sequencing workspace for creating and monitoring runs and the input and organization of sample information. Selecting Runs on the navigation rail opens the Runs navigation drawer, which provides functions for creating and searching runs.

The Runs navigation drawer also lists every run in the software with the run state, number of samples in the run, flow cell type, and color-coded Quality Metrics icon. Runs are listed from newest to oldest. Use the scroll arrows to move through the complete list.

Run Details Page

Selecting a run from the Runs navigation drawer opens the Run Details page. Each Run Details page is a run-specific workspace for managing the run and samples in the run. Throughout the software, selecting a run name hyperlink goes to the Run Details page.

Figure 3 Run Details Page on the Universal Analysis Software user interface



A table displays all samples in the run with information about each sample and options for sorting and filtering. Functions for importing, adding, searching, and deleting samples appear above the table. The header section of a Run Details page displays the run name, quality metrics, sample representation, and the following icons.

Table 1 Run Details page icons

Icon	Name	Function
	Run Details	Edit the run and view the run state, cycles per read, and other run information.
	Run Activity	See system events and user actions for the run and add comments. This icon is visible when the history setting is enabled. See *System Settings* (page 43).
	Print	Print the Run Details page.

Projects

Projects is the post-sequencing workspace for creating and managing projects, accessing analysis results, and generating reports. Selecting Projects on the navigation rail opens the Projects navigation drawer, which provides functions for creating and searching projects.

The Projects navigation drawer also lists every project in the software with the number of samples and runs in the project and the date and time of project creation. Projects are listed from newest to oldest. Use the scroll arrows to move through the complete list.

Project Side Sheet

Selecting a project from the Projects navigation drawer opens the Project side sheet and the Samples page. The Project side sheet lists all samples in the project, grouped by run. Expand the runs to see which samples are included. A green checkmark indicates an analyzed sample.

The Project side sheet provides the following icons for accessing additional pages and project information.

Table 2 Project side sheet icons

Icon	Name	Function
	Edit	Change the project name and add or change the optional description.
	Samples	Open the Samples page for the project.
	Reports	Open the Reports page for the project.
	Project Activity	See system events and user actions for the project and add comments. This icon is visible when the history setting is enabled. See *System Settings* (page 43).
	Print	Print the Samples page.
	QPN	Open the QPN page for each run in the project. Each icon letter is color-coded to provide high-level status. See Appendix A Run Metrics.

Samples Page

The Samples page lists information for each sample in a selected project with functions for adding, reanalyzing, and searching samples. Filters narrow results by sample or assay type and sort by sample name, run name, and analysis method.

A QC Indicators column displays the number of quality control (QC) indicators for a sample. The column displays 0 for a sample with zero QC indicators. Samples with QC indicators may require further review.

Reports Page

The Reports page lists reports in a project by creation date, starting with the most recent report. On this page, you can create reports or find and download existing reports. Sort by the Date Created and File Name columns and filter the Report Type column to organize results.

QPN Page

The QPN page is divided into three tabs: Quality Metrics, Positive and Negative Controls, and Sample Representation. Together, these tabs provide an overall view of run performance. For details, see Appendix A Run Metrics.

Results Page

Selecting a Kintelligence sample from a project opens the Results page. Each Results page is a sample-specific workspace for in-depth analysis of results. A header section displays the sample name, analysis method applied to the sample, button to create reports, and the following icons.

Table 3 Results page icons

Icon	Name	Function
	Sample Details	View the sample type and analysis state, analysis folder path, and other analysis information.

Icon	Name	Function
	Sample Activity	See system events and user actions for the sample and add comments. This icon is visible when the history setting is enabled. See *System Settings* (page 43).
	Print	Print the Sample Overview section of the Results page.

In addition to the header, the Results page for a Kintelligence sample includes a Sample Overview section and a Sample Details section.

Sample Overview Section

The Sample Overview section displays locus call rate, total reads, biological sex, contributor status, and heterozygote and intralocus balance metrics. Under these data, a table displays the percentage and total number of typed loci relative to base pair length per SNP type. The entire section is collapsible, and you can edit both biological sex and contributor status.

Sample Details Section

The Sample Details section displays data for the SNP types listed in the following table. A card represents each locus, displaying the locus name, chromosome, allele, intensity, and intralocus balance. Each allele is color-coded: A is green, T is red, C is blue, and G is gray.

Table 4 SNP types

SNP Type	Description
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.
Ancestry SNP (aSNP)	Estimates biogeographical ancestry.
Phenotype SNP (pSNP)	Estimates hair and eye color.
Identity SNP (iSNP)	Informs on source attribution.
Kinship SNP (kSNP)	Informs on potential biological associations (relatives).

Locus Details

Selecting a card in the Sample Details section opens locus details, which include allele intensity, thresholds, intralocus balance, and quality control (QC) indicators. Under Typed, a toggle key indicates whether the allele is typed.

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

Locus Quality Indicators

Each card displays a color-coded horizontal bar: gray indicates a locus with no QC indicators and orange indicates a locus with at least one QC indicator. Any applicable indicators are displayed on the card.

Table 5 QC indicators

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

Software Status

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

Run State

After a run is created and saved, you can view the run state by selecting the Information icon on the Run Details page displays the run state. The following table describes each possible state.

Table 6 Run states

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

Analysis State

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

Table 7 Analysis states

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

Glossary

Term	Description
Amplicon	The product of PCR amplification of a targeted region of interest in a genome.
Amplicon end position	The last base position of an amplicon, including the PCR primer binding sequence.
Amplicon start position	The first base position of an amplicon, including the PCR primer binding sequence.
Analysis method	Settings and thresholds that inform the analysis of sequencing data to generate allele calls and QC indicators.
Analytical threshold (AT)	The percentage that a read count must reach for the software to call a base or type an allele. Signal below the AT might be visible but is not typed.
Assay type	The library prep kit used to process samples for sequencing and analysis.
Call	The reported base for a position (coordinate) based on the analysis method.
Cluster density	The number of thousands of clusters on a flow cell per square millimeter (K/mm^2).
Clusters	A clonal group of DNA bound to the surface of a flow cell that produces one read. One template DNA strand seeds each cluster and is clonally amplified using bridge amplification until the cluster has about 1000 copies.
Clusters passing filter	The percentage of clusters that pass quality filters. For more information, see *Clusters Passing Filter* (page 49).
Coverage	The average number of reads for a locus or allele in a sample that align to or cover known reference bases. Sequencing coverage level (referred to as *read depth*, *read count*, or *intensity* in the software) can help determine whether an allele call is made with confidence.
Cycle	A chemistry step and an imaging step to call one base in a sequencing run. For cycle numbers, see *Cycles per Read* (page 51).
Flow cell	A glass slide coated with oligos complementary to the library adapter sequences, allowing the library to adhere for a run.
Forward primer length	The number of bases in the forward PCR primer (oligonucleotide).

Term	Description
Forward primer start coordinate	The starting base at the 5' end of the forward PCR primer. The base depends on the amplicon and analysis method.
Human Sequencing Control (HSC)	A pre-amplified and tagged positive control added to a library pool to facilitate troubleshooting of instrument and sequencing reagent issues.
Index adapter sequence	A short stretch of oligonucleotides added to each sample during library prep for identification purposes. The software uses the oligonucleotide sequences to demultiplex pooled libraries.
Index CV	The coefficient of variation for the number of read counts across all indexes.
Intensity	The software reports signal intensity as the number of reads.
Intralocus balance	The balance of read counts between typed alleles at a heterozygous locus. The balance is measured as the intensity of the minimum intensity typed allele divided by the intensity of the maximum intensity typed allele. When intensities are identical, the intralocus balance is 100%.
Interpretation threshold (IT)	The percentage that determines the read count that must be reached for the software to call a base or type an allele. Signal below the IT but greater than or equal to the AT can be manually typed.
Library	A DNA sample that uses primers and adapters to litigate specific regions of the genome for sequencing.
Minimum read count	The minimum read count a position must reach for a base to be called.
N indicator	The quality indicator for reagent blanks and negative amplification controls.
Negative amplification control	A sample that does not contain DNA as a template for amplification.
No call	The call assigned when a position lacks read counts for a specific base.
Original call	An unmodified allele call based on the run analysis method.
P indicator	The quality indicator for positive amplification controls that use control DNA.
Phasing	The percentage of bases in a cluster that fall behind the current cycle in Read 1. For details, see *Phasing and Prephasing* (page 49).
Plexity	The number of libraries combined in a pool for sequencing. For example, if eight libraries are combined in a pool, the plexity is eight.
Positive amplification control	A known sequence of DNA provided in the library prep kit and prepared with forensic and reference samples to ensure that library prep and sequencing reagents are functional.
Prephasing	The percentage of bases in a cluster that jump ahead of the current cycle in Read 1. For details, see *Phasing and Prephasing* (page 49).
Project	A collection of analyzed results for at least one sample, and the primary workspace for viewing and modifying results and creating reports.
Read	The sequence string of A, T, C, and G bases corresponding to an amplicon. A run can simultaneously generate millions of reads from multiple loci and multiple samples.

Term	Description
Reagent blank	An extraction control without DNA that is carried through the entire sample processing.
Read 1 (i5) primer sequence	The sequence of the PCR primer that is tagged with the Index 2 (i5) adapter sequence. Index 2 is part of the adapter sequence, as is the binding site for the Read 1 sequencing primer.
Read 2 (i7) primer sequence	The sequence of the PCR primer that is tagged with the Index 1 (i7) adapter sequence. Index 1 is part of the adapter sequence, as is the binding site for the Read 2 sequencing primer.
Reagent cartridge kit lot	The batch code to identify the batch or lot that a component of the MiSeq FGx Reagent Kit or MiSeq FGx Reagent Micro Kit was manufactured in.
Reanalysis	The process of reanalyzing a sample with another analysis method, after the original run analysis method.
Reference SNP ID number (rsID)	An identification tag the National Center for Biotechnology Information (NCBI) assigns to a group of SNPs that map to the same location.
Reverse primer length	The number of bases in the reverse PCR primer (oligonucleotide).
Reverse primer start coordinate	The starting base at the 5' end of the reverse PCR primer. The base depends on the amplicon and analysis method.
Run	The process of clustering and sequencing libraries to generate base calls.
Run analysis method	The analysis method assigned to a sample for the initial run analysis.
Sample	The DNA input for a library prep kit, before the addition of primers and adapters.
Sample plate	The 96-well plate that contains libraries for sequencing.
Sample representation	The number of reads per sample for a run. These data are available after sequencing and analysis are complete.
Sample type	The function of a sample: forensic or reference sample, positive amplification control, negative amplification control, and reagent blank.
Sample well position	The column and row position in a 96-well plate containing samples or libraries. For example, position A1 indicates a well at the intersection of row A and column 1.
Single-nucleotide polymorphism (SNP)	Variation of one base pair in a DNA sequence relative to a known DNA reference sequence.
System event	An action the software automatically performs and records.
Typed	An allele call that contributes to the locus genotype.
Unique Dual Index (UDI)	Premixed Index 1 (i7) and Index 2 (i5) adapters for dual index combinations that have no redundant sequences.
Untyped	A no-call allele that does not contribute to the locus genotype.
User action	A user interaction with the software, such as adding a sample to a run.
User-modified call	A base call that a user changed.

Run Setup

Create a Run

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

Edit a Run

1. On the navigation rail, select Runs. Then select a run.
2. Select Information, and then select Edit.
3. In the Edit Run dialog box:
   - Edit the run name.
   - Add or modify the optional run description.
   - Add or modify the optional reagent cartridge kit lot number.

   Editing the flow cell type requires a run state of Created.
4. Select Save.

Add Samples to a Run

Add samples and sample information to a run either by importing a sample sheet or by adding individual samples. After samples are added, the Run Details page lists all samples included in the run.

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

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

Import a Sample Sheet

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

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

Assigning Unique Dual Indexes

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

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

Assigning Projects

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

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

Add Individual Samples

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

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

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

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

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

Manage Samples in a Run

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

Find a Sample

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

Edit Sample Information

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

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

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

4. Select Save to apply the changes.

Modify Project Assignments

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

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

Remove Samples from a Run

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

Monitor a Run

During a run, the Run Details 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 Quality Metrics.
   - Review the values for cluster density and clusters passing filter.
   - Review the phasing and prephasing values for Read 1.
   - Check Read 1, Index 1, Index 2, and Read 2 to confirm that all four reads passed.
   - Check Overall Intensity and 0 Discordant Loci to confirm that the HSC passed.

   For more information, see *Quality Metrics* (page 48).
3. Select Run Activity.
   - Review the run activity.
   - Select Close to return to the run.
4. Select Sample Representation.
   - 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 *Sample Representation* (page 54).
5. Select Close to return to the run.

Data Analysis

Review Run Metrics

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

For help with metrics requiring further evaluation, see *Troubleshoot a Run* (page 44). For detailed descriptions of each metric, see Appendix A Run Metrics.

1. On the navigation rail, select Projects.
2. Select a project, and then select QPN for a run in the project.
3. On the Quality Metrics page, do as follows.
   - Review the values for cluster density and clusters passing filter.
   - Review the phasing and prephasing values for Read 1.
   - Check Read 1, Index 1, Index 2, and Read 2 to confirm that all four reads passed.
   - Check Overall Intensity and 0 Discordant Loci to confirm that the HSC passed.
4. Select Positive & Negative Controls.
   - Under NA24385:
     - Expand Discordance to see the Discordance table.
     - Use the scroll arrows to move through all positions in the table.
   - 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. Select Sample Representation.
   - 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.
6. If any metrics do not meet the minimum requirements, see *Troubleshoot a Run* (page 44) for troubleshooting guidance. For additional assistance, contact Verogen Technical Support. Collect the following items from D:\Illumina\MiSeqFGxOutput to help Verogen with troubleshooting:
   - InterOp folder
   - runInfo.xml file
   - runParameters.xml file

Review Results

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

- For information on the Results page, see *Results Page* (page 13).
- For information on how the software estimates biological sex and contributor status, see Appendix B Supporting Information.

By default, the Results page displays results with the Typed filter and all SNP Type filters applied except X-SNP and Y-SNP. Sort 1 is set to descending Allele Count, Sort 2 is set to ascending Chromosome, and Sort 3 is set to SNP Name.

Filter Loci

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

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

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

Sort Loci

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

3. Under Sample Details, select the Sort 1, Sort 2, or Sort 3 list, and then select one of the following options:

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

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

Find a Locus

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

Modify Results

You can modify Kintelligence results by typing and untyping alleles, changing the sample biological sex, and changing the contributor status.

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

Type or Untype an Allele

1. On the navigation rail, select **Projects**.
2. Select a project, and then select a Kintelligence sample.
3. On the Results page, under Sample Details, select a locus.
   The locus details appear.
4. Type or untype an allele using one of the following methods:
   - Under Typed, select a toggle key.
   - On the histogram, select a bar.

   The User Modified icon appears, indicating the modification. Reverting the call removes the icon.

Change the Contributor Status

1. On the navigation rail, select Projects.
2. Select a project, and then select a sample to open the Results page.
3. In the Sample Overview section, select the Contributor Status list, 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.

Change the Sample Biological Sex

1. On the navigation rail, select Projects.
2. Select a project, and then select a sample to open the Results page.
3. In the Sample Overview section, select the Biological Sex list, 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.

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, in the Search field, enter a sample name.
4. In the search results, select the checkbox of each sample you want to add to the project.
   Each selection appears in a summary list with the corresponding analysis method.
5. To remove a sample, clear the checkbox or select X in the summary.
6. Select Add to add the samples.

Reanalyze Samples

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

1. On the navigation rail, select Projects.
2. Select a project.
3. On the 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.

   The software lists only analysis methods that are compatible with the assay used to prepare the sample.
6. Select Next.
7. Review the sample names, previous analysis methods, and new analysis methods.
8. To make changes, select Previous.
9. Select Save to initiate reanalysis.
   In the Projects sidebar, an icon appears next to each sample undergoing reanalysis.
10. When reanalysis is complete, select a sample to view results.
    The Results page displays results for the most recent analysis.

Switch Analysis Methods

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

1. On the navigation rail, select Projects.
2. Select a project, and then select a sample to open the Results page.
3. In the Select an Analysis Method list, select an analysis method.
   The Results page is refreshed to show results for the selected method.

Reports

Generating Reports

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

- **Phenotype and Ancestry** — Estimates of hair color, eye color, and biogeographical ancestry based on aSNP and pSNP data.
- **GEDmatch PRO** — SNP genotype calls reported on the plus strand for upload to GEDmatch PRO.
- **Project** — Results for all samples in a project.
- **Sample** — Results for one sample.

The following sections describe how to generate each type of report. For report contents, see *File Formats for Reports* (page 31).

Create a Phenotype and Ancestry Report

1. On the navigation rail, select Projects.
2. Select a project, and then select a sample to open the Results page.
3. Select Create Report, and then select Phenotype & Ancestry to generate the report.
4. Select Reports to open the Reports page.
   The Reports page lists the new report on top.
5. In the Actions column, select Download to download the report as an .xmlx file.

Create a GEDmatch PRO Report

1. On the navigation rail, select Projects.
2. Select a project, and then select a sample to open the Results page.
3. Select Reports, and then select GEDmatch PRO to generate the report.
4. Select Reports to open the Reports page.
   The Reports page lists the new report on top.
5. In the Actions column, select Download to download the report as a .txt file.

Create a Project Report

1. On the navigation rail, select Projects.
2. Select a project, and then select Reports to open the Reports page.
3. Select Create Report, and then select Project.
   If the software is analyzing samples, project report creation is disabled and a notification is displayed.
4. When prompted, select Yes to create the report.

The software generates the report and returns to the Reports page, where the new report appears at the top of the list.

> If a project includes samples with different assay types, the software generates one project report per assay.

5. In the Actions column, select **Download** to download a report as an .xmlx file.

Create Sample Reports

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

Create One Sample Report

1. On the navigation rail, select **Projects**.
2. Select a project, and then select a sample to open the Results page.
3. Select **Reports**, and then select **Sample Report** to generate the report.
4. Select **Reports** to open the Reports page.
   The Reports page lists the new report on top.
5. In the Actions column, select **Download** to download the report as an .xmlx file.

Create Multiple Sample Reports

1. On the navigation rail, select **Projects**.
2. Select a project, and then select **Reports** to open the Reports page.
3. Select **Create Report**, and then select **Sample**.
4. In the Create Sample Report dialog box, select the checkbox of each sample you want to create a report for.
   - Scroll and use the arrows to move through the complete list of samples in the project.
   - Use the Delete icons under Summary to remove samples from the report.
5. Select **Confirm** to generate the reports.
   The software returns to the Reports page, where the new reports appear at the top of the list.
6. In the Actions column, select **Download** to download a report as an .xmlx file.

Create One Sample Report from Reports

1. On the navigation rail, select **Projects**.
2. Select a project, and then select **Reports** to open the Reports page.
3. Select **Create Report**, and then select **Sample**.
4. In the Create Sample Report dialog box, select the checkbox of the sample you want to create a report for. Scroll and use the arrows to see all samples.
5. Select **Confirm** to generate the report.
   The software returns to the Reports page, where the new report appears at the top of the list.
6. In the Actions column, select **Download** to download a report as an .xmlx file.

File Formats for Reports

The following sections describe the format and contents of each type of report the software generates for Kintelligence samples. In the reports, rsID numbers identify individual SNPs. For instructions on creating Kintelligence reports, see *Generating Reports* (page 29).

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

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

[1] Walsh, Susan, Lakshmi Chaitanya, Lindy Clarisse, Laura Wirken, Jolanta Draus-Barini, Leda Kovatsi, Hitoshi Maeda, et al., "Developmental validation of the HIrisPlex system: DNA-based eye and hair colour prediction for forensic and anthropological usage,"***Forensic Science International: Genetics*** 9 (March 2014): 150–161, https://doi.org/10.1016/j.fsigen.2013.12.006.

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

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

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) was used to train the model on the following super populations: European, East Asian, and African (excepting African Ancestry in Southwest USA [ASW]). For context, the unknown sample is projected with the Ad-Mixed Americans super population onto the first two pretrained components based on aSNP genotype calls.[4]

On the Estimation worksheet, 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.

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

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.

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

Project Report

A Kintelligence 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 in the report with the following information:

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

Sample Report

A Kintelligence 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

Run, Project, and Sample Management

Create a Project

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

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

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

Edit a Project

1. On the navigation rail, select Projects.
2. Select a project, and then select Edit.
3. In the Edit Project dialog box, do as follows.
   - Edit the project name.
   - Add or modify the optional project description.
4. Select Save to apply the changes.

Obtain the Analysis Folder Path

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

1. On the navigation rail, select Projects.
2. Select a project, and then select a sample to open the Results page.
3. Select the Information icon.
4. The path is displayed under Analysis Folder Path.

Printing

The Print icon converts the following pages into a printable format:

- **Run Details** — Prints the header of a Run Details page, the table listing samples in the run, run quality metrics, and sample representation.
- **Samples** — Prints the Samples page of a project.
- **Results** — Prints the header of a Results page and the Sample Overview section.

Print a Run Details Page

1. On the navigation rail, select Runs. Then select a run.
2. On the Run Details page, select Print.
   The software displays a printable view of the Run Details page.
3. Select Print to open the Print dialog box.
4. Choose printing options and print.
5. Select Back to Run.

Print a Samples Page

1. On the navigation rail, select Projects. Then select a project.
2. On the Project sidebar, select Print.
   The software displays a printable view of the Samples page.
3. Select Print to open the Print dialog box.
4. Choose printing options and print.
5. Select Back to Project.

Print a Sample Overview Section

1. On the navigation rail, select Projects.
2. Select a project, and then select a Kintelligence sample.
3. On the Results page, select Print Sample Results.
   The software displays a printable view of the Sample Overview section.
4. Select Print to open the Print dialog box.

5. Choose printing options and print.
6. Select Back to Project.

History

When the history setting is enabled, you can review system events and user actions recoded for a run, project, or sample and add comments. For example, you can comment on a sample to explain a call modification. For instructions on enabling the history setting, see *System Settings* (page 43).

Review Run Activity

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

Review Project Activity

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

Review Sample Activity

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

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

Review Locus Activity

1. On the navigation rail, select **Projects**.
2. Select a project, and then select a sample.
   The Results page opens.
3. Under Histogram View, select **Select All**, **Autosomal STRs**, or **Y STRs**.
4. Select a card to open the locus details.
5. Select the **User Actions** tab.
   The tab lists system events and user actions from oldest to newest.
6. Review system events and user actions for the locus.
7. Navigate using the 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 or see user actions only.
8. When finished, select **Close**.

Comment on a Run

1. On the navigation rail, select **Runs**.
2. Select a run, and then select **Run Activity**.
   The Run Activity dialog box opens.
3. To add a comment to the history:
   1. **a.** In the Add New Comment field, type a comment.
   2. **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:
   1. **a.** Select the system event or user action. Use the fields and scrolling to help locate the entry, if necessary.
   2. **b.** In the Edit Comment box, type a comment.
   3. **c.** Select **Save** to add the comment to the system event or user action.

   The comment appears as the last item for the entry.
5. When finished, select **Close**.

Comment on a Project

1. On the navigation rail, select **Projects**.
2. Select a project, and then select **Project Activity**.
   The Project Activity dialog box opens.
3. To add a comment to the history:

1. **a.** In the Add New Comment field, type a comment.
2. **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:
    1. **a.** Select the system event or user action. Use the fields and scrolling to help locate the entry, if necessary.
    2. **b.** In the Edit Comment box, type a comment.
    3. **c.** Select **Save** to add the comment to the system event or user action.

    The comment appears as the last item for the entry.
5. When finished, select **Close**.

Comment on a Sample

1. On the navigation rail, select **Projects**.
2. Select a project, select a sample, and then select **Sample Activity**.
   The Sample Activity dialog box opens.
3. To add a comment to the history:
    1. **a.** In the Add New Comment field, type a comment.
    2. **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:
    1. **a.** Select the system event or user action. Use the fields and scrolling to help locate the entry, if necessary.
    2. **b.** In the Edit Comment box, type a comment.
    3. **c.** Select **Save** to add the comment to the system event or user action.

    The comment appears as the last item for the entry.
5. When finished, select **Close**.

Comment on a Locus

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

1. On the navigation rail, select **Projects**.
2. Select a project, and then select a sample.
   The Results page opens.
3. Under Histogram View, select **Select All**, **Autosomal STRs**, or **Y STRs**.
4. Select a card to open the locus details.
5. Select the **User Actions** tab.
6. In the Add New Comment field, type a comment.
7. Select **Add** to add the comment to the locus.

- To edit the comment, select it, modify it as needed in the Edit Comment field, and select **Save**.
- To delete the comment, select it, and then select **Delete Comment**.

Software Settings

Settings Overview

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

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

Analysis Methods

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

- ForenSeq Kintelligence — Verogen Kintelligence Analysis Method

Create an Analysis Method

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

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

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

Copy an Analysis Method

1. On the navigation rail, select Settings.
2. Select Analysis Methods.
3. Select Create Analysis Method.
   The New Analysis Method dialog box opens.

4. In the New Analysis Method Options list, select **Copy an Existing Analysis Method**.
5. In the Source Analysis Method list, select an analysis method to use as a template.
6. In the New Analysis Method Name field, enter a unique name for the new analysis method.
7. Select **Save** to create the analysis method.
   The new method appears with the default settings from the copied method.
8. Modify the default settings as desired.
   For instructions, see *Modify Analysis Settings* (below).

Use a Blank Analysis Method

1. On the navigation rail, select **Settings**.
2. Select **Analysis Methods**.
3. Select **Create Analysis Method**.
   The New Analysis Method dialog box opens.
4. In the New Analysis Method Options list, select **New Blank Analysis Method**.
5. In the New Analysis Method Name field, enter a unique name for the new analysis method.
6. Select **Save** to create the analysis method.
   The new method appears with blank settings.
7. Enter the desired settings for the analysis method.
   For instructions, see *Modify Analysis Settings* (below).

Modify Analysis Settings

For a user-created Kintelligence 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. If necessary, navigate to the analysis method you want to edit:
   1. **a.** On the navigation rail, select **Settings**.
   2. **b.** Select **Analysis Methods**.
   3. **c.** In the Select an Assay Type list, select the assay that corresponds to the applicable analysis method.
   4. **d.** Select the applicable analysis method.
2. To rename the analysis method, do as follows.
   1. **a.** Select **Edit** to open the Edit Analysis Info dialog box.
   2. **b.** In the Analysis Method Name field, enter a new name.
   3. **c.** Select **Save** to apply the name change and close the dialog box.
3. Select the checkbox of at least one locus to edit.
   - Enter a whole or partial locus name in the Search Locus Name field to find a locus.
   - Select the checkbox of applicable SNP types to filter the list of loci.
   - Select any column heading to sort the column.

- Scroll to see all loci.

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

4. Select **Edit Selected Loci**.

   The Edit Analysis Method dialog box opens.

5. To add or remove the loci from the analysis, select **Include**.

   Green indicates that the loci are included. Gray indicates exclusion.

6. To modify the analysis thresholds, enter a value **0**–**100** in the applicable fields: Analytical Threshold, Interpretation Threshold, and Intralocus Balance.

   The IT value must be greater than or equal to the AT value.

7. Select **Save** to apply the new settings.

Delete an Analysis Method

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

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

Change the Default Analysis Method

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

   A green star identifies the current default.

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

User Management

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

Enable and Disable Accounts

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

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

Blue indicates an enabled account.

Assign Access Levels

1. On the navigation rail, select Settings.
2. Select User Management.
3. Select Administrator to enable or disable administrator access for an account.
   Blue indicates an administrator account.

System Settings

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

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

Enable Run, Project, and Sample History

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

   An Activity icon appears on the Run Details page, Project sidebar, and Results page. Selecting the icon opens the history.

Disable Run, Project, and Sample History

1. On the navigation rail, select Settings.
2. Select System Settings.
3. Select Enable or disable visibility of system events and user actions recorded by the software.
   The History icon disappears from the Run Details page, Project sidebar, and Results page.

Enable or Disable Dark Mode

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

Display Software Version

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

Troubleshooting

Troubleshoot a Run

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

Low Intensity

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

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

High Cluster Density

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

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

Low Cluster Density

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

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

Low Percentage of Clusters Passing Filter

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

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

High Phasing and Prephasing

The following factors can cause high phasing or prephasing.

- The cluster density is too high. See *High Cluster Density* (previous page).
- The ambient temperature is too high.
  - Verify the appropriate temperature in the ***MiSeq FGx Sequencing System Site Prep Guide (document # VD2018012)***.
  - Check the MiSeq FGx System temperature logs to confirm whether the ambient temperature is too high. Contact Verogen Technical Support for help locating the logs.
- The reagent chiller temperature is too low or too high. The system displays an error.
- MiSeq FGx Maintenance Status:
  - Perform a maintenance wash and repeat sequencing.
  - Refill the wash tray and bottle for every wash.
  - Make all wash solutions daily.
- If the problem remains unresolved, reagents might not be performing as expected. Contact Verogen Technical Support.

Incomplete Run

The following factors can cause a run to stop prematurely.

- The HSC was not added during library prep. Add HSC per denature and dilute instructions and repeat the run.
- Cluster density is too low. See *Low Cluster Density* (page 44).
- If an error message is displayed, contact Verogen Technical Support.

Low Quality Scores for Read 1 or Read 2

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

- Phasing and prephasing are too high. See *High Phasing and Prephasing* (previous page).
- The cluster density is too high. See *High Cluster Density* (page 44).
- If the problem remains unresolved, reagents might not be performing as expected. Contact Verogen Technical Support.

Low Quality Scores for Index 1 Read or Index 2 Read

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

- Phasing and prephasing are too high. See *Phasing and Prephasing* (page 49).
- The cluster density is too high. See *High Cluster Density* (page 44).
- Not enough samples were sequenced, so diversity in the index reads was low.
- If the problem remains unresolved, reagents might not be performing as expected. Contact Verogen Technical Support.

Low Reads per Sample

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

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

Low Reads per Sample for the HSC

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

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

Troubleshoot Analysis Errors

The following sections provide recommendations for troubleshooting potential analysis problems.

Analysis Error Message

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

1. Ensure that the correct index combination was used for the sample.
2. If the index combination is incorrect, follow steps in *Remove Samples from a Run* (page 22).
3. Add the sample back to the run with the correct index combination, following the steps in *Add Samples to a Run* (page 19).

   The sample will automatically analyze.

If further errors occur, contact Verogen Technical Support

1. Navigate to the run folder, following the steps for obtaining the analysis folder path in *Review Run Activity* (page 36).
2. Navigate to the Log folder for that sample analysis.
3. Copy the log text file.
4. Navigate to D:\verogen\fuas\logs and copy the most recent log text file.

Quality Metrics Icons Are Unavailable

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

1. Navigate to the run folder, following the steps for obtaining the analysis folder path in *Review Run Activity* (page 36).
2. Navigate up to the run folder (designated by a run number).
3. Ensure that the InterOp folder, the runInfo.xml file, and the runParameters.xml file are present.
   - If the folder is not present, copy this folder with the runInfo.xml and runParameters.xml files from the MiSeq, following the steps in *Review Run Metrics* (page 24).

Perform a Status Check

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

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

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

Run Metrics

Accessing Run Metrics

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

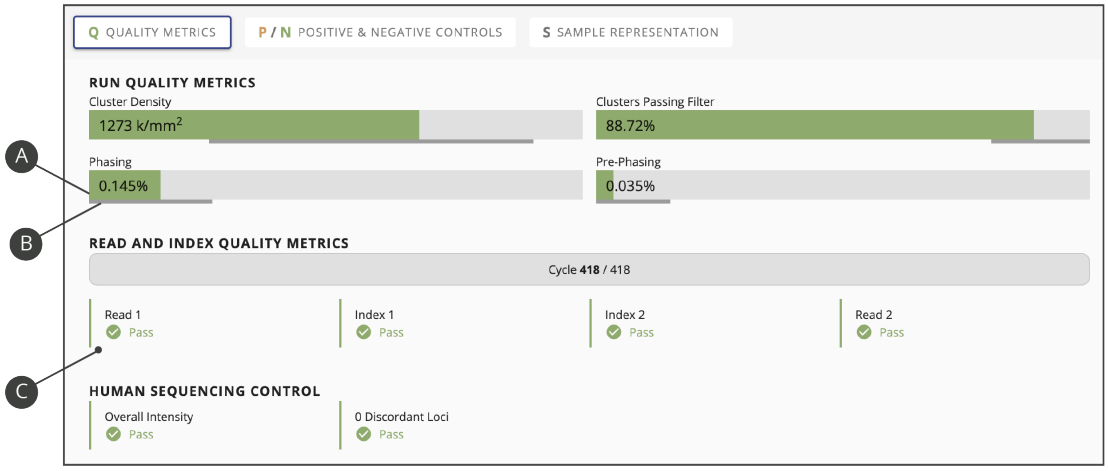
To open run metrics, select a QPN icon on the Project side sheet. A subset of these metrics, run quality and sample representation, are also available on Run Details pages.

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. In Runs, the quality metrics appear in a pop-up window. In Projects, they appear on a page.

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

Figure 4 Quality metrics overview



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

Run Quality Metrics

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

Table 8 Colors for run quality metrics

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

Cluster Density

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

Clusters Passing Filter

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

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

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

Phasing and Prephasing

For both phasing and prephasing, lower percentages indicate higher quality run statistics. Phasing and prephasing 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%.
- Prephasing shows the percentage of molecules in a cluster that jump ahead of the current cycle in Read 1. For ForenSeq libraries, the target prephasing value is ≤ 0.15%.

Read and Index Quality Metrics

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

Table 9 Colors for read and index quality metrics

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

Table 10 Icons for read and index quality metrics

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

Reads in a Run

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

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

Cycles per Read

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

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

Human Sequencing Control

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

Table 11 Colors for HSC results

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

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

Loci

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

Loci	151 or 201 Cycles
D3S1358	✓
D5S818	✓
D7S820	✓
D8S1179	✓
D13S317	✓
D16S539	✓
D18S51	✓
FGA	✓
PentaD	✓

Loci	151 or 201 Cycles
PentaE	✓
TPOX	✓
DYS391	✓
DYS392	✓
DYS460	✓
DXS7423	✓
DXS10103	✓

Positive and Negative Controls

The software assesses the same positive and negative control metrics for every run, regardless of assay type.

Table 12 Control assessments

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

Positive Amplification Control

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

SNPs Typed

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

Table 13 Colors for loci for NA24385

Color	Indication
	All loci have calls and are concordant.

Color	Indication
	At least one locus does not have a call or contains a discordant call.

Discordance Table

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

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

Pass Filter Reads

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

Table 14 Colors for pass filter reads

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

Negative Amplification Control

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

SNPs Typed

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

Table 15 Colors for base calls of loci

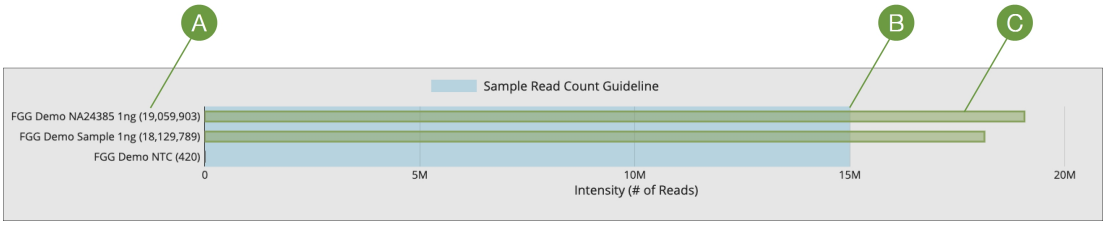
Color	Indication
	No loci have a base call.
	At least one locus has a base call.

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. In Runs, sample representation data appear in a pop-up window. In Projects, these data appear on a page.

Sample representation shows an index CV percentage for the run and the number of reads (intensity) and read distribution for each sample. These data are presented in a bar chart that also shows the sample read count guideline. Hovering over a bar displays the exact number of reads for the sample.

Figure 5 Sample Representation bar chart

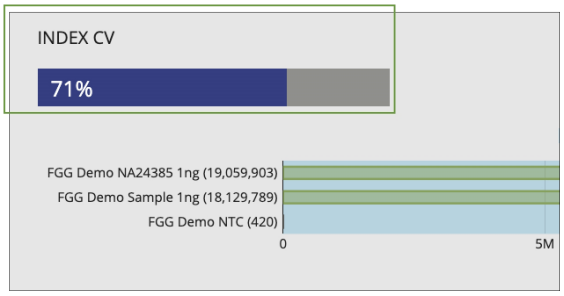


A **Sample name and exact number of reads for the sample**
B **Threshold of the read count guideline**
C **Bar indicating number of reads for the sample**

Index CV

Above the sample representation bar chart, Index CV shows a percentage that represents the distribution of read counts for all samples in the run. CV is the coefficient of variation for the number of read counts across all indexes.

Figure 6 Index CV percentage



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	15 million 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.

Supporting Information

Contributor Status of Samples

The software applies the following logic to determine the contributor status of a Kintelligence sample.

- Any of the following scenarios indicate a mixture:
  - The sample has ≥ 10 Y-SNPs with two alleles.
  - The sample has ≥ 10 Y-SNP loci typed and ≥ 10 X-SNPs with two alleles.
  - The sample has zero Y-SNP loci typed with ≥ 60% autosomal SNP (auSNP) heterozygosity and ≥ 50% X-SNP heterozygosity.
- If the sample has ≤ 50% auSNP heterozygosity, ≤ 40% X-SNP heterozygosity, ≥ 65% auSNP intralocus balance, and zero Y-SNPs with two 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.

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

Biological Sex Estimation for Samples

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

- If the sample has ≥ 10 Y-SNP loci typed, the biological sex is male (XY).
- If the sample has zero Y-SNP loci typed and the call rate is ≥ 50%, the biological sex is female (XX).
- If the sample is a negative amplification control, the contributor status is mixture, or the scenarios for male and female do not apply, the biological sex is inconclusive.

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Technical Support

For technical assistance, contact Verogen Technical Support.

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

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

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