

Universal Analysis Software

MainstAY Product Line

Module

Version 2

Reference Guide

VEROGEN PROPRIETARY

Document # VD2022001 Rev. A

June 2022

Revision History

Document #	Date	Description of Change
VD2022001Rev. A	June 2022	Initial release of the Universal Analysis Software – MainstAY Module
VD2019002 Rev. E	October 2021	<ul style="list-style-type: none"> • 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	<p>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.</p> <ul style="list-style-type: none"> • 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 <p>Updated the safety data sheet (SDS) link to verogen.com/product-documentation. Renamed the software to Universal Analysis Software and this guide to <i>Universal Analysis Software v2.0 Reference Guide</i>. Added the term stutter to the glossary. Corrected target run metrics for ForenSeq Kintelligence libraries. Replaced kinship with Kintelligence.</p>

Universal Analysis Software – MainstAY Product Line Module Reference Guide

Document #	Date	Description of Change
VD2019002 Rev. C	February 2021	<p>Updated software descriptions to ForenSeq Universal Analysis Software v2.2, which supports the ForenSeq Kintelligence Kit with NA24385 as the positive control.</p> <ul style="list-style-type: none">• 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. <p>Updated the ForenSeq trademark to a registered trademark. Refreshed the format of the guide:</p> <ul style="list-style-type: none">• Updated the layout and formatting.• Updated fonts, table styles, and other design elements. <p>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.</p>

Universal Analysis Software – MainstAY Product Line Module Reference Guide

Document #	Date	Description of Change
VD2019002 Rev. B	August 2020	<p>Updated software descriptions for ForenSeq Universal Analysis Software v2.1, which provides the following enhancements:</p> <ul style="list-style-type: none"> • 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 <p>Updated guide format and organization, including adding an index and consolidating workspace descriptions.</p> <p>Revised step-by-step instructions to be more succinct and include field descriptions.</p> <p>Updated telephone numbers for Verogen contact information.</p> <p>Updated glossary term definitions and added the terms coverage plot, indel, library, mtDNA navigator, plexity, position viewer, system event, and user action.</p> <p>Updated figures to include callouts highlighting user interface elements.</p> <p>Added information on strand bias and lost passwords.</p> <p>Added instructions for creating projects, assigning a sample to multiple projects, modifying user-created analysis methods, and enabling and disabling dark mode.</p> <p>Added another possible cause of low cluster density: belatedly combining bead-based normalized libraries and Hybridization Buffer (HT1).</p> <p>Noted that the analysis folder contains VCF and BAM files.</p> <p>Noted that the software follows SWGDAM nomenclature rules.</p> <p>Noted the conditions for displaying the Call or No Call filter.</p> <p>Clarified that each sample name must be unique in the software.</p> <p>Clarified that phasing and prephasing values are for Read 1 only.</p>
VD2019002 Rev. B	August 2020	<p>Distributed troubleshooting information among individual sections.</p> <p>Moved information on analysis method settings to <i>Analysis Defaults for ForenSeq Universal Analysis Software v2 (Pub. No. VD20200045)</i>.</p> <p>Corrected duplicate entries of D7S820 and D16S539 in the Human Sequencing Control.</p> <p>Corrected the list of information included in project and sample reports.</p> <p>Corrected citations that appear in the appendix.</p>
VD2019002 Rev. A	August 2019	Initial release

Table of Contents

Revision History	2
Table of Contents	5
Chapter 1 Software Overview	8
Introduction	8
Access the Software	9
Primary Workspaces	10
Software Status	15
Glossary	16
Chapter 2 Run Setup	19
Create a Run	19
Edit a Run	19
Add Samples to a Run	19
Manage Samples in a Run	21
Monitor a Run	23
Chapter 3 Data Analysis	24
Review Run Metrics	24
Review Results	25
Modify Results	26
Add Analyzed Samples to a Project	27
Reanalyze Samples	27
Chapter 4 Reports	29
Generating Reports	29
File Formats for Reports	32
Chapter 5 Run, Project, and Sample Management	36
Create a Project	36
Edit a Project	36
Obtain the Analysis Folder Path	37
Printing	37

History	37
Chapter 6 Software Settings	41
Settings Overview	41
Analysis Methods	41
CODIS Report Defaults	44
User Management	45
System Settings	45
Enable or Disable Dark Mode	46
Display Software Version	46
Chapter 7 Troubleshooting	47
Troubleshoot a Run	47
Perform a Status Check	51
Appendix A Run Metrics	52
Accessing Run Metrics	52
Quality Metrics	52
Positive and Negative Controls	56
Sample Representation	58
Appendix B Supporting Information	60
Contributor Status for Samples	60
Biological Sex Estimation for Samples	60
Stutter Filtering for Samples	60
Reporting Flanking Regions with Samples	67
Index	68
Technical Support	71

Universal Analysis Software – MainstAY Product Line Module Reference Guide

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Software Overview

Introduction	8
Access the Software	9
Primary Workspaces	10
Software Status	15
Glossary	16

Introduction

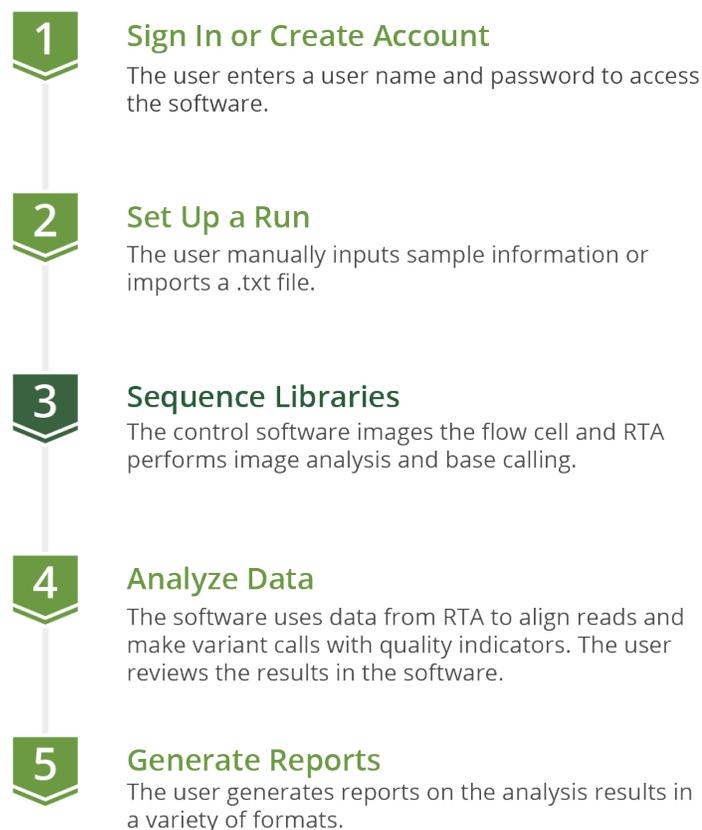
Universal Analysis Software (UAS v2) is a forensic genomics solution for run setup, sample management, data analysis, and reporting. Pre-installed on a dedicated server, the software integrates with the MiSeq FGx[®] Sequencing System to automatically generate FASTQ files and perform alignment from run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

This reference guide provides details on the analysis of data generated from the ForenSeq MainstAY kit using the MainstAY 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



 UAS v2

 MiSeq FGx System

Access the Software

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

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

Create an Account

Before using UAS, you must create an account. The first user to create an account is automatically assigned administrator privileges. The administrator is responsible for approving new accounts. For instructions, see [Enable and Disable Accounts \(page 45\)](#).

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.
 1. **a.** Access the network server through a web browser.
 2. **b.** Enter your email address (username) and password.
 3. **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.

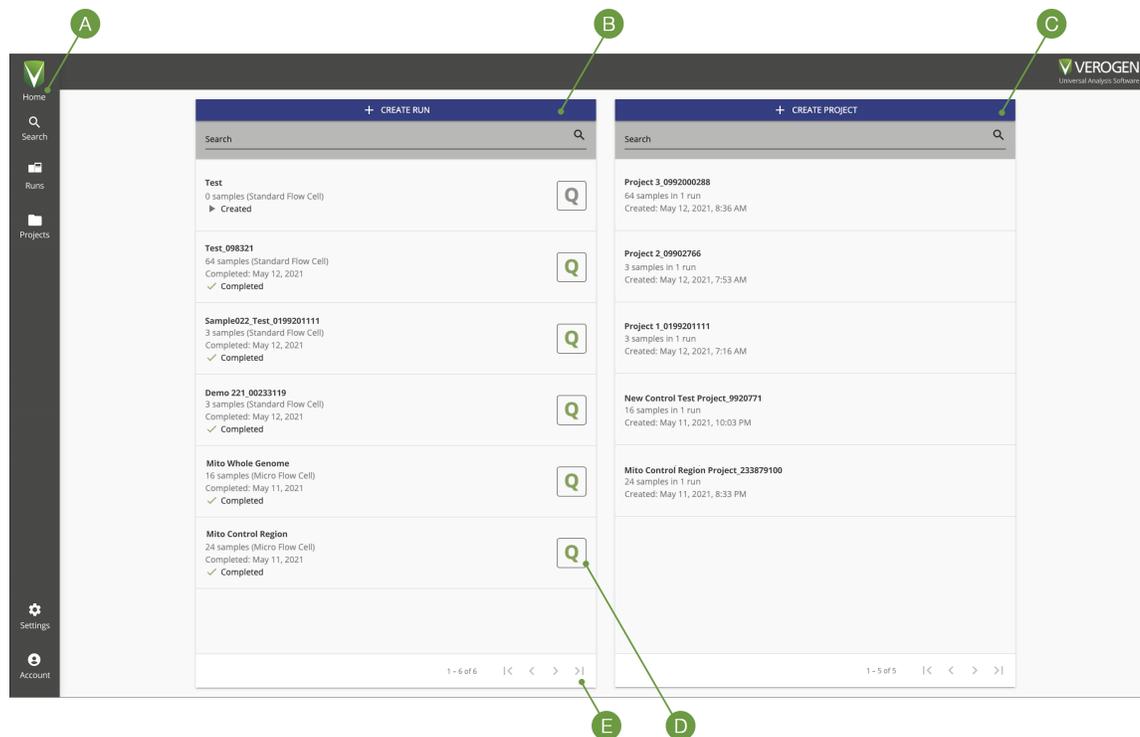
The software also includes printing, commenting, and other functions that supplement run setup, data analysis, and reporting. For instructions on using these supplementary features, see [Chapter 5 Run, Project, and Sample Management](#).

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

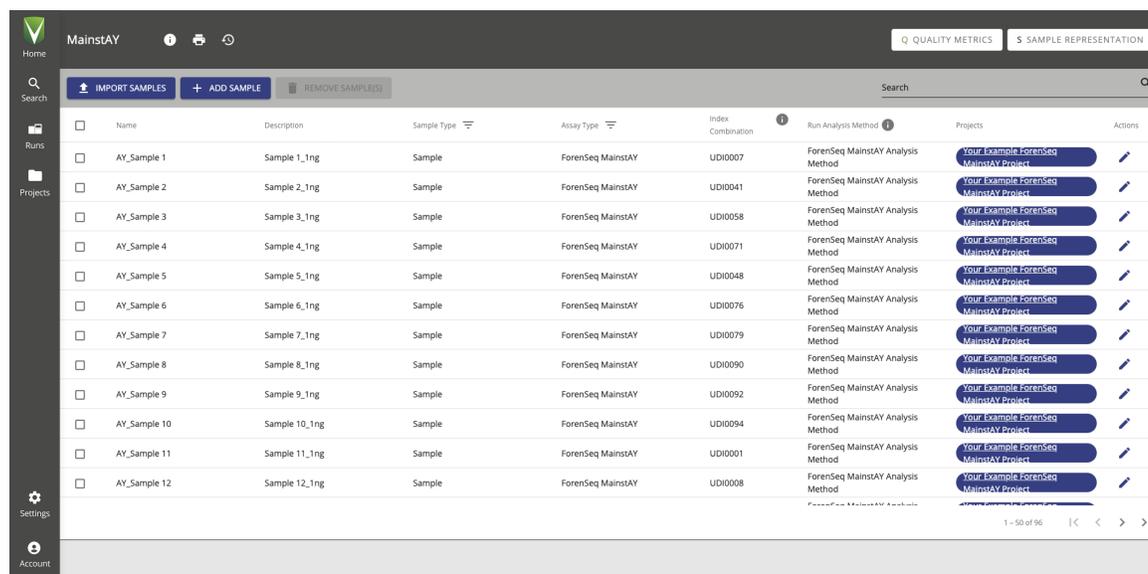
Runs is the sequencing workspace for creating and monitoring runs and the input and organization of sample information. Selecting Runs on the navigation rail opens the Runs navigation drawer, which provides functions for creating and searching runs.

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 45) .
	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 45) .
	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 MainstAY 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 45) .

In addition to the header, the Results page for a MainstAY sample includes a Sample Overview section and detailed coverage and histogram views.

Sample Overview Section

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

Coverage View

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

Switching the view to alleles opens a scatter diagram that plots amplicon length along the X-axis and intensity along the Y-axis. Each dot represents a typed allele in the sample: yellow dots indicate aSTRs and blue dots indicate Y-STRs. Hovering over a dot reveals the STR type, locus name and allele number, amplicon length, and intensity value. Selecting the portion of the allele scatterplot using a screen-select allows a user to zoom into this section of the visualization. Clicking on a selected locus square on the heatmap or on an individual dot on the allele scatter diagram takes you directly to that locus in the histogram.

Histogram View

The histogram view displays intensity values and thresholds for each locus in the sample. All loci are displayed by default, but you can switch to viewing only aSTR loci or only Y-STR loci. You can also use the filtering, sort, and search options to refine results.

Coverage plots can be hidden by clicking the **Hide Coverage Plots** button on the top right corner of the user interface. Hiding the coverage plot scales the histogram view to the larger screen area. Collapsing the filter bar or the project side sheet by selecting the gray bar with the arrow also scales up the histogram view. Each locus is depicted as a card that displays a bar chart with one bar for each allele. Hovering over a bar reveals the intensity. Effective analytical thresholds appear as gray shading so you can confirm whether each allele meets the thresholds.

Locus Details

Selecting a card opens locus details, which enlarges the bar chart and shows additional data for each allele, including intralocus balance, thresholds, intensity, stutter, sequences, and QC indicators. Toggle keys indicate whether alleles are typed.

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

Locus Quality Indicators

Each card displays a color-coded horizontal bar: green indicates a locus with no QC indicators and orange indicates a locus with at least one QC indicator.

Table 4 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.
	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.
	Stutter	At least one sequence exceeds the stutter filter percentage at a stutter position of a possible parent allele.

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 5 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 6 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 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.
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 53) .
Coverage	The average number of reads for an allele in a sample that align to or cover known reference bases. Sequencing coverage level (referred to as <i>read depth</i> , <i>read count</i> , or <i>intensity</i> 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 55) .
Differences indicator	A software feature that highlights different allele calls in the same sample.
Flanking Region	The regions of the sequence that are adjacent to the STR repeat motif.
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).

Universal Analysis Software – MainstAY Product Line Module Reference Guide

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 percentage of reads assigned to each library, representing the distribution of read counts of the libraries in the run. CV is the coefficient of variation for the number of read counts across all indexes.
Indel	The insertion or deletion of bases in a genome.
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 ligate specific regions of the genome for sequencing.
Library type	The function of a library, either primer-directed sequencing or non-directed sequencing, for custom assays and analysis.
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.
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 53) .
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 53) .
Project	A collection of analyzed results for at least one sample, and the primary workspace for viewing and modifying results and creating reports.

Universal Analysis Software – MainstAY Product Line Module Reference Guide

Term	Description
Read	The sequence string of A, T, C, and G bases corresponding to an amplicon. A run can simultaneously generate millions of reads from multiple loci and multiple samples.
Reagent blank	An extraction control without DNA that is carried through the entire sample processing.
Read 1 (i5) primer sequence	The sequence of the PCR primer that is tagged with the Index 2 (i5) adapter sequence. Index 2 is part of the adapter sequence, as is the binding site for the Read 1 sequencing primer.
Read 2 (i7) primer sequence	The sequence of the PCR primer that is tagged with the Index 1 (i7) adapter sequence. Index 1 is part of the adapter sequence, as is the binding site for the Read 2 sequencing primer.
Reagent cartridge kit lot	The batch code to identify the batch or lot that a component of the MiSeq FGx Reagent Kit or MiSeq FGx Reagent Micro Kit was manufactured in.
Reanalysis	The process of reanalyzing a sample with another analysis method, after the original run analysis method.
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.
Short tandem repeat (STR)	Areas of the genome where short sequences of DNA (2–6 bp) are repeated, such as (GATA) <i>n</i> .
Stutter	Polymerase slippage that can occur during PCR amplification of repetitive DNA sequences, library prep, cluster generation, or sequencing. The slippage can create DNA amplification products that are less than or greater than the size of the parent allele.
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	19
Edit a Run	19
Add Samples to a Run	19
Manage Samples in a Run	21
Monitor a Run	23

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: <ul style="list-style-type: none"> • ForeSeq MainstAY — Libraries prepared with the ForeSeq MainstAY kit. • ForeSeq MainstAY SE — Libraries prepared with the ForeSeq MainstAY kit.

Field	Instruction
Sample Type	Select a sample type: <ul style="list-style-type: none"> • Sample — A forensic or reference sample. • NA24385 — The positive control. • Negative Amplification Control — The negative control (water). • Reagent Blank — The extraction control.
Index Combination	Select the name of a premixed i7 and i5 index adapter (UDI).
Index 1	Select the name of an i7 index adapter.
Index 2	Select the name of an i5 index adapter.
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.

- In the Actions column, select  **Edit**.
- 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: <ul style="list-style-type: none"> • ForenSeq MainstAY — Libraries prepared with a ForenSeq MainstAY kit. • ForenSeq MainstAY SE — Libraries prepared with a ForenSeq MainstAY kit.
Sample Type	Select a different sample type: <ul style="list-style-type: none"> • Sample — A forensic or reference sample. • NA24385 — The positive control. • Negative Amplification Control — The negative control (water). • Reagent Blank — The extraction control.
Index 1*	Select the name of a different i7 index adapter.
Index 2*	Select the name of a different i5 index adapter.
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.

- 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.

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

Remove Samples from a Run

- 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 \(page 52\)](#).

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 \(page 58\)](#).
5. Select **Close** to return to the run.

Data Analysis

Review Run Metrics	24
Review Results	25
Modify Results	26
Add Analyzed Samples to a Project	27
Reanalyze Samples	27

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 \(page 47\)](#). 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 STRs typed for each reagent blank.
 - Under Negative Amplification Controls, review the number of STRs typed for each negative control.
 - 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 \(page 47\)](#) 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 MainstAY sample includes filters and sorting to organize and locate loci for review. By default, the histogram view sorts loci in ascending order by chromosome without any filters applied. The following sections describe how to use these features to review the results. For feature descriptions, see [Results Page \(page 13\)](#).

Filter Loci

1. On the navigation rail, select  Projects.
2. Select a project, and then select a sample to open the Results page.
3. In the Histogram View, on the filter pane, select **Select All**, **Autosomal STRs**, or **Y STRs**.
4. Under the **QC Indicators** section, select the check box associated with the filter that needs to be applied.
 - To apply all filters except No QC Indicators, select **Select All**.
 - To clear all filters, select **Clear Selection**.

Filter	Effect
Allele Count	Filter to display loci by the number of alleles.
Imbalanced	Filter to display loci that are not balanced.
Analytical Threshold	Filter to display loci that are below the AT.
Interpretation Threshold	Filter to display loci that are below the AT.
Not Detected	Filters to display loci that do not show expected results (e.g., Male samples that don't type Y STRs).
Detected	Filters to display loci with unexpected results (e.g., Female samples with Y STRs).
User Modified	Filter to display loci that were modified by the User
Stutter	Filter to display loci that show stutter
No QC Indicators	Filter to display loci that do not have a QC flagged

The software displays only the loci flagged with the selected indicators. For indicator descriptions, see [Locus Quality Indicators \(page 14\)](#).

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 Histogram View, select **All**, **Autosomal STRs**, or **Y STRs**.
4. Select **Sort By**, and then select one of the following options:

Sort Option	Effect
Allele Count	Sort loci by the number of alleles.
Amplicon Size	Sort loci by amplicon size in bp.

Sort Option	Effect
Chromosome	Sort loci by chromosome number or letter.
Intralocus Balance	Sort loci by intralocus balance percentage.
Intensity	Sort loci by intensity value.
STR Name	Sort loci by locus name.
STR Type	Sort loci by aSTRs and Y-STRs.

5. To further sort loci into ascending or descending order, select the arrow to the left of the Sort By list.

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. Under Histogram View, select **All, Autosomal STRs, or Y STRs**.
4. In the Search field, enter the whole or partial name of the locus you want to find.
The Results page displays loci matching the search term.
5. To return to the full view, clear the Search field.

Modify Results

You can modify MainstAY results by typing and untyping alleles, changing the sample biological sex, and changing the contributor status. You can also add comments to loci explaining any changes. For instructions, see [Comment on a Locus \(page 40\)](#).

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 an STR sample to open the Results page.
3. Under Histogram View, select **All, Autosomal STRs, or Y STRs**.
4. Select the locus you want to edit.
The locus details appear.
5. 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.

Universal Analysis Software – MainstAY Product Line Module Reference Guide

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	29
File Formats for Reports	32

Generating Reports

The software compiles STR results into the following reports:

- **CODIS** — Variants from the selected samples for upload to CODIS.
- **Flanking Region** — Results from variants in the flanking region of the STR amplicon.
- **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 \(page 32\)](#).

Create a CODIS Report

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

Create a CODIS Report for One Sample

1. On the navigation rail, select  **Projects**.
2. Select a project, and then select a sample.
3. Select  **Reports**, and then select **CODIS Report**.
The Create CODIS Report dialog box opens.
4. [Optional] Select **CODIS Report Defaults** to edit default report settings.
For more information, see [CODIS Report Defaults \(page 44\)](#).
5. Edit the following CMF header fields or accept the default values.
 - **Destination Laboratory ORI** — Identifier for the agency that processes the CODIS report.
 - **Source Laboratory ORI** — Identifier for the agency that processes the specimen.
 - **Submission User ID** — Identification of the person who generates the CODIS report.
 - **Batch ID** — Identifier that tracks where a DNA profile originated.
6. To include the name of the library prep kit in the report, select **Include Kit Name in Report**.
7. Select **Next**.
8. In the Specimen ID field, enter a unique identifier for the specimen or accept the default sample name.
9. In the Specimen Category list, select a preconfigured category or select **Custom** and enter the category name.
10. [Optional] Select **More Options**.
 1. **a.** Complete the following fields:

- • **Source ID** — Specify whether the identity of the specimen contributor is known.
 - • **NCIC Number** — Enter the unique number for storage in the NCIC system.
 - • **ViCAP number** — Enter the unique number for storage in the ViCAP system.
 - • **Case ID** — Enter the unique identifier for the case.
 - • **Case ID** — Enter the law enforcement identifier associated with the specimen.
 - • **Specimen Comment** — Enter any additional information about the specimen.
2. **b.** If the specimen has possible no call regions or other conditions requiring additional information to help with match resolution, select **Partial Profile**.
11. Select **Next**.
 12. Select the toggle key for each position you want to remove from the report.
By default, all positions are included.
 13. Select **Create** to generate the report.
 14. Select  **Reports** to open the Reports page.
The Reports page lists all reports for the project with the new report on top.
 15. In the Actions column, select **Download Report** to download the report as a .cmf file.

Create a CODIS Report for Multiple Samples

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 **CODIS Report**.
The Create CODIS Report dialog box opens.
4. Select the checkbox of each sample you want to include in the report.
 - Scroll and use the arrows to move through the complete list of samples in the project.
 - Under Summary, select **Delete** to remove samples from the report.
5. Select **Next**.
6. [Optional] Select **CODIS Report Defaults** to edit default report settings.
For more information, see [CODIS Report Defaults \(page 44\)](#).
7. Edit the following CMF header fields or accept the default values:
 - **Destination Laboratory ORI** — Identifier for the agency that processes the CODIS report.
 - **Source Laboratory ORI** — Identifier for the agency that processes the specimen.
 - **Submission User ID** — Identification of the person who generates the CODIS report.
 - **Batch ID** — Identifier that tracks where a DNA profile originated.
8. To include the name of the library prep kit in the report, select **Include Kit Name in Report**.
9. Select **Next**.
10. In the Specimen ID field for each sample, enter a unique identifier for the specimen cited in the report.
11. Assign specimen categories per the following table. If you select Custom, enter a name for the custom specimen category.

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

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

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

13. In the Actions column, select **Download Report** to download the report as a .cmf 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 One Sample Report from Results

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 a Flanking Region Report

Create a MainstAY Flanking Region report for one sample or multiple samples.

Create a Flanking Region Report for One Sample

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 **MainstAY Flanking Region 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 a report as an .xmlx file.

Create a Flanking Region Report for Multiple Samples

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 **MainstAY Flanking Region Report**.
4. In the Create MainstAY Flanking Region 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.

File Formats for Reports

The following sections describe the format and contents of each type of report the software generates for MainstAY samples. For instructions on creating reports, see [Generating Reports \(page 29\)](#).

CODIS Report

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

- Specimen ID
- Specimen category
- Source ID
- Fragments

Project Report

A MainstAY project report is an Excel workbook with two worksheets each for aSTRs and Y-STRs. All four worksheets include a header section that lists the project name, project creation date and time, and user who generated the report.

- The Autosomal STRs and Y STRs worksheets provide genotype information formatted in a table that lists the following information for each sample in the project.
 - Sample name and optional description
 - Analysis method
 - Contributor status
 - Biological sex
 - Typed alleles for each locus in the sample
- The Autosomal STR Coverage and Y STR Coverage worksheets provide coverage information formatted in a table that lists the following information for each sample in the project.
 - Sample name
 - Analysis method
 - Locus name and each allele name
 - Whether each allele is typed
 - Number of reads for each allele
 - Repeat sequence of each allele

Sample Report

A MainstAY sample report is an Excel workbook with two worksheets each for aSTRs and Y-STRs and a settings worksheet that lists analysis thresholds for each locus.

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

Universal Analysis Software – MainstAY Product Line Module Reference Guide

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

Flanking Region Report

A MainstAY Flanking Regions report is a sample level Excel workbook with two worksheets each for aSTRs and Y-STRs and a settings worksheet that lists additional information flanking region. about the analysis thresholds for each locus.

- The Autosomal STRs and Y STRs worksheets include the following sample information for each unique flank sequence per locus per STR length
 - A header section lists the following information
 - The Sample Name prepended to the words “Flanking Region Report”
 - The name of the Project from which the report was generated
 - The name of the Sample for which the report was generated.
 - The name of the Analysis Method for which the report was generated
 - The date, time, and time zone on the system when the report was generated.
 - User who generated the report
 - Coverage information is formatted in a table that lists the following information for each locus:
 - Locus name
 - STR Length
 - Number of reads supporting the STR sequence
 - The full sequence representing the amplicon and the flanking regions
 - Standing direction for every unique sequence of the reported loci
 - Nucleotide differences between two or more sequences of the same length at the same locus are displayed as below
 - Base difference within the repeat region are highlighted in bold, black text
 - Base difference within the flank region are underlined and highlighted in bold, blue text

Universal Analysis Software – MainstAY Product Line Module Reference Guide

- The Settings worksheet includes table for each unique flank sequence per locus per STR length. It displays the locus name, STR length, total reported length in bases, forward and reverse read sequence of the flanking region including the repeat region, and the total read count for each sequence.

Run, Project, and Sample Management

Create a Project	36
Edit a Project	36
Obtain the Analysis Folder Path	37
Printing	37
History	37

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.

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**.

History

When the history setting is enabled, you can review system events and user actions recorded for a run, project, or sample and add comments. For example, you can comment on a sample to explain a call modification. For instructions on enabling the history setting, see [System Settings \(page 45\)](#).

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

Review Run Activity

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

Review Project Activity

1. On the navigation rail, select  **Projects**.
2. Select a project, and then select  **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	41
Analysis Methods	41
CODIS Report Defaults	44
User Management	45
System Settings	45
Enable or Disable Dark Mode	46
Display Software Version	46

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.
- **CODIS Report Defaults** — Set default values to prepopulate CODIS reports.
- **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 MainstAY — ForenSeq MainstAY Analysis Method
- ForenSeq MainstAYSE — ForenSeq MainstAY SE 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 MainstAY analysis method and help determining alternative settings, see *Robust thresholds and filters for reliable implementation of the ForenSeq MainstAY kit. (document # VD2021030).*

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 MainstAY analysis method, you can modify the AT, IT, stutter filter, 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.

- To rename the analysis method, do as follows.
 - a.** Select  **Edit** to open the Edit Analysis Info dialog box.
 - b.** In the Analysis Method Name field, enter a new name.
 - c.** Select **Save** to apply the name change and close the dialog box.
- 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 STR 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.
- Select **Edit Selected Loci**.

The Edit Analysis Method dialog box opens.
- To add or remove the loci from the analysis, select **Include**.

Green indicates that the loci are included. Gray indicates exclusion.
- To modify the analysis thresholds, enter a value **0–100** in the applicable fields: Analytical Threshold, Interpretation Threshold, Stutter Filter, and Intralocus Balance.

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

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

Change the Default Analysis Method

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

A green star identifies the current default.
- Set the selected analysis method as the default for the assay type by selecting the empty star next to the analysis method name:

CODIS Report Defaults

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

Define CODIS Report Defaults

1. On the navigation rail, select  **Settings**.
2. Select **CODIS Report Defaults**.
3. Select **CODIS Report** for STR samples.
4. In each of the following fields, enter a default value:
 - **Destination Laboratory ORI** — Identifier for the agency that processes the CODIS report.
 - **Source Laboratory ORI** — Identifier for the agency that processes the specimen.
 - **Submission User ID** — Identification of the person who generates the CODIS report.
5. Select **Save** to apply the default values.

Set Specimen Categories

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

1. On the navigation rail, select  **Settings**.
2. Select **CODIS Report Defaults**.
3. Select **CODIS Report** for STR samples.
4. Select the **Specimen Categories** tab.

This tab lists all specimen categories, divided into CODIS and Custom columns.
5. Select a specimen category to make it available or unavailable for reports.

A blue toggle key indicates an available category.

Add Custom Specimen Categories

Add custom specimen categories to the software to supplement the predefined categories. Custom categories can be renamed, enabled or disabled, and deleted.

1. On the navigation rail, select  **Settings**.
2. Select **CODIS Report Defaults**.
3. Select **CODIS Report** for STR samples.
4. Select the **Specimen Categories** tab.
5. In the **New Specimen Category Name** field, enter a name of your choice.
6. Select **Add**.

The new category appears in the Custom column and is enabled by default.

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	47
Perform a Status Check	51

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, maintaining a minimum of eight samples per run.

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 or the Normalization Storage (LNB1) 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.
- LNB1 was insufficiently pipetted during normalization. Consider diluting libraries in the Pooled Normalized Libraries tube less.
- 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.
- After heating, the bead-based normalized library pool was not immediately added to HT1. If possible, repeat the sequencing run following the protocol.
- The library was not otherwise denatured properly.
 - Make sure that the final library pool is denatured with freshly diluted 2 N NaOH (HP3) that has a pH > 12.5, if applicable.
 - Make sure that the final library pool is heat-denatured, if applicable.
- 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.

Universal Analysis Software – MainstAY Product Line Module Reference Guide

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

Incomplete Run

The following factors can cause a run to stop prematurely.

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

Low Quality Scores for Read 1 or Read 2

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

- Phasing and prephasing are too high. See [High Phasing and Prephasing \(previous page\)](#).
- The cluster density is too high. See [High Cluster Density \(page 47\)](#).
- 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 \(page 53\)](#).
- The cluster density is too high. See [High Cluster Density \(page 47\)](#).
- 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.
- LNB1 was insufficiently pipetted during normalization.
 - If the sample has a high number of alleles with quality control indicators, resequence the sample.

Universal Analysis Software – MainstAY Product Line Module Reference Guide

- If the sample has a low number of alleles with quality control indicators, consider diluting the libraries in the Pooled Normalized Libraries tube less and resequence the sample.
- If the run does not pass, repeat library normalization.
- HT1 was overpipetted during library prep. Repeat library normalization.
- The libraries were not heat-denatured during library prep. Perform the heat-denature step in the Diluted Normalized Libraries tube and repeat the run.

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.
- The libraries were not heat-denatured during library prep. Perform the heat-denature step and repeat the run.
- HSC was not denatured with freshly diluted HP3. Make sure HSC is denatured with freshly diluted HP3 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 \(page 38\)](#).
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 \(page 38\)](#).
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 52
 Quality Metrics 52
 Positive and Negative Controls 56
 Sample Representation 58

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.

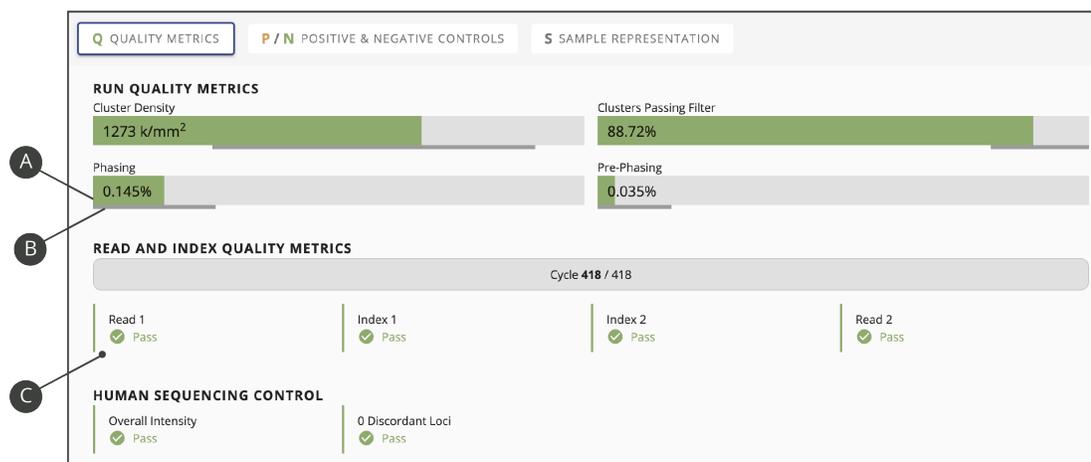
To open run metrics, select a QPN icon on the Project side sheet. A subset of these metrics, run quality and sample representation, are also available on Run Details pages.

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 7 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 8 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 9 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 MainstAY	Read 1	1–351	Cycle 352
	Index 1 Read	352–359	Cycle 360
	Index 2 Read	360–367	Cycle 368
	Read 2	368–398	After cycle 398
ForenSeq MainstAY SE	Read 1	1–351	Cycle 352
	Index 1 Read	352–359	Cycle 360
	Index 2 Read	360–367	Cycle 368
	Read 2	368–398	After cycle 398

Human Sequencing Control

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

Table 10 Colors for HSC results

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

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

Loci

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

Loci	351 Cycles	Loci	351 Cycles
D3S1358	✓	PentaE	✓
D5S818	✓	TPOX	✓
D7S820	✓	DYS391	✓
D8S1179	✓	DYS392	✓
D13S317	✓	DYS460	✓
D16S539	✓	DXS7423	✓
D18S51	✓	DXS10103	✓
FGA	✓		
PentaD	✓		

Positive and Negative Controls

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

Table 11 Control assessments

Assay Type	Positive Amplification Control	Value
ForenSeq MainstAY	NA24385 Positive Amplification Control DNA	STRs typed
ForenSeq MainstAY SE	NA24385 Positive Amplification Control DNA	STRs 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 STRs typed called. Colors indicate overall success. If applicable, a table displays discordant positions or loci.

STRs Typed

STRs 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 12 Colors for loci for NA24385

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

Discordance Table

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

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

Pass Filter Reads

Pass filter reads displays the following colors to indicate whether the number of reads that passed filter meet the guideline of $\geq 15,000$ reads for ForenSeq MainstAY libraries. Reads below the guideline might still be usable. Review quality metrics and sample data to help make a determination.

Table 13 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 STRs typed called and a color-coded indicator of overall success.

STRs Typed

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

Table 14 Colors for base calls of loci

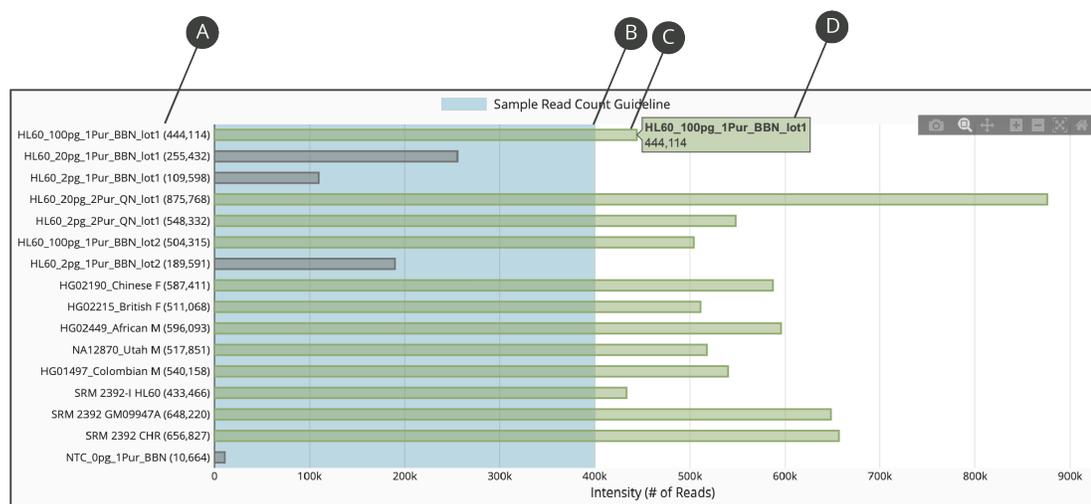
Color	Indication
■	No loci have a base call.
■	At least one locus has a base call.

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

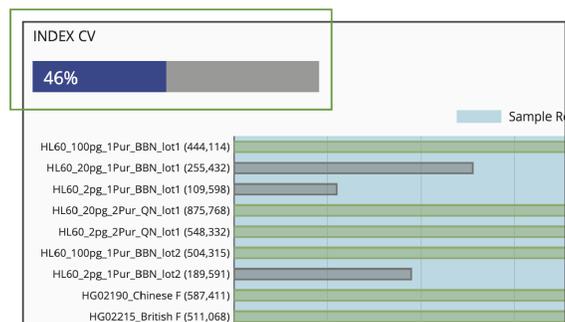


- A** Sample name and exact number of reads for the sample
- B** Threshold of the read count guideline
- C** Bar indicating number of reads for the sample
- D** Exact number of reads for the sample (hovering)

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 MainstAY	15,000 reads per sample
ForenSeq MainstAY SE	15,000 reads per sample

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

Table 15 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 for Samples	60
Biological Sex Estimation for Samples	60
Stutter Filtering for Samples	60
Reporting Flanking Regions with Samples	67

Contributor Status for Samples

The software applies the following logic to determine the contributor status of a sample processed with ForenSeq MainstAY.

- If the sample is not a negative amplification control, and no loci have more alleles called than possible for that locus the contributor status is single-source.
- If the sample is not a negative amplification control and at least 3 loci have more alleles than possible for the locus the contributor status is a mixture.
- If the sample is a negative amplification control or 1–2 of the loci have more alleles called than is possible for that locus, the contributor status is inconclusive.

Biological Sex Estimation for Samples

The software applies the following logic to estimate the biological sex of a sample processed with the ForenSeq MainstAY kit. Biological sex is estimated prior to estimating determining Contributor Status.

- If the sample is not a negative control, at least 50% of the autosomal STRs have signal above the analytical threshold and no Y-STR loci have signal greater than the analytical threshold, the biological sex is female (XX).
- If the sample is not a negative control and at least 5 Y-STR loci have signal above the analytical threshold, the biological sex is male (XY).
- If the sample is a negative amplification control, the contributor status is a mixture or if 1–4 Y-STRs have an allele above the analytical threshold the biological sex is inconclusive. In addition, if less than 50% of the autosomal STRs have signal above the analytical threshold and no Y-STR loci are typed, the biological sex is inconclusive.

Stutter Filtering for Samples

A sequence is considered stutter, with offset (n) if the sequences differ in length by k times the reference repeat unit length, the number of repeat units in the potential stutter are identical to an adjacent unit of length of the reference repeat unit and there are no other mismatches between the sequences. The software assesses differences in the sequences for k = 2, -1 and +1 repeat units. The stutter filter setting, which depends on the differences in repeat units is applied as is for the n-4 and n-5 positions. The stutter filter setting is squared at the n-8 or n-10 and n+4 or n+5 positions. The n+1 repeat stutter filter is adjustable by users. The expected stutter intensity is the product of the stutter filter setting and the called allele intensity at a repeat length. The stutter quality control indicator for elevated stutter is displayed under the following conditions.

- Uncalled read intensity, with sequence of potential stutter of a called allele.
- Uncalled read intensity is greater than the maximum expected stutter (%) if the potential parent allele.

Table 16 Default stutter filters for AUTOSOMAL STRs in the ForenSeq MainstAY analysis method

AUTOSOMAL STRs			
Locus	Stutter	Threshold	Method
CSF1PO	-1	11%	max
	1	4%	max
	-2	2%	max
TH01	-1	13%	mean + 3SD
	1	2%	mean + 3SD
	-2	1.50%	min stutter
PentaE	-1	11%	max
	1	4%	mean + 3SD
	-2	1.50%	min stutter
PentaD	-1	9%	mean + 3SD
	1	1.50%	min stutter
	-2	1.50%	min stutter
FGA	-1	26%	max
	1	3%	mean + 3SD
	-2	5%	max
D22S1045	-1	16%	max
	1	8%	max
	-2	3%	max
D21S11	-1	16%	mean + 3SD
	1	3%	max
	-2	2%	mean + 3SD
D20S482	-1	19%	max
	1	4%	max
	-2	3%	max
D19S433	-1	19%	max
	1	3%	mean + 3SD
	-2	2%	mean + 3SD

AUTOSOMAL STRs			
Locus	Stutter	Threshold	Method
D18S51	-1	23%	max
	1	3%	max
	-2	4%	max
D17S1301	-1	24%	max
	1	2%	max
	-2	3%	mean + 3SD
D16S539	-1	20%	max
	1	3%	mean + 3SD
	-2	2%	mean + 3SD
TPOX	-1	9%	max
	1	1.50%	min stutter
	-2	1.50%	min stutter
D13S317	-1	9%	mean + 3SD
	1	4%	max
	-2	1.50%	min stutter
D10S1248	-1	20%	max
	1	2%	max
	-2	3%	mean + 3SD
D9S1122	-1	18%	max
	1	8%	max
	-2	3%	mean + 3SD
D8S1179	-1	25%	mean + 3SD
	1	7%	max
	-2	4%	mean + 3SD
D7S820	-1	12%	max
	1	3%	max
	-2	2%	mean + 3SD
D6S1043	-1	16%	max
	1	3%	mean + 3SD
	-2	3%	mean + 3SD

AUTOSOMAL STRs			
Locus	Stutter	Threshold	Method
D5S818	-1	11%	max
	1	6%	max
	-2	2%	mean + 3SD
D4S2408	-1	10%	max
	1	3%	mean + 3SD
	-2	1.50%	min stutter
D3S1358	-1	15%	max
	1	3%	max
	-2	2%	mean + 3SD
D2S1338	-1	25%	max
	1	2%	mean + 3SD
	-2	3%	mean + 3SD
D2S441	-1	9%	max
	1	2%	mean + 3SD
	-2	1.50%	min stutter
D1S1656	-1	24%	max
	1	4%	max
	-2	3%	mean + 3SD
D12S391	-1	31%	max
	1	5%	max
	-2	4%	mean + 3SD
vWA	-1	19%	max
	1	2%	mean + 3SD
	-2	4%	max

Table 17 Default stutter filters for Y-STRs in the ForenSeq MainstAY analysis method

Y-STRs			
Locus	Stutter	Threshold	Method
DYS19	-1	20%	max
	1	5%	mean + 3SD
	-2	2%	mean + 3SD
DYS635	-1	14%	max
	1	3%	max
	-2	3%	max
DYS612	-1	38%	max
	1	10%	max
	-2	10%	mean + 3SD
DYS576	-1	19%	max
	1	6%	max
	-2	2%	mean + 3SD
DYS570	-1	18%	mean + 3SD
	1	4%	max
	-2	3%	max
DYS549	-1	10%	max
	1	3%	max
	-2	1.50%	min stutter
DYS533	-1	11%	mean + 3SD
	1	3%	max
	-2	1.50%	min stutter
DYS522	-1	16%	mean + 3SD
	1	6%	mean + 3SD
	-2	1.50%	min stutter
DYS505	-1	13%	max
	1	2%	mean + 3SD
	-2	3%	mean + 3SD

Universal Analysis Software – MainstAY Product Line Module Reference Guide

Y-STRs			
Locus	Stutter	Threshold	Method
DYS481	-1	47%	mean +
	1	7%	3SD
	-2	16%	max max
DYS460	-1	13%	max
	1	4%	max
	-2	2%	mean + 3SD
DYS643	-1	11%	max
	1	2%	mean +
	-2	1.50%	3SD min stutter
DYS448	-1	3%	mean +
	1	1.50%	3SD
	-2	1.50%	min stutter min stutter
DYS438	-1	7%	max
	1	1.50%	min
	-2	1.50%	stutter min stutter
DYS393	-1	12%	max
	1	7%	max
	-2	1.50%	min stutter
DYS437	-1	16%	max
	1	2%	mean +
	-2	2%	3SD mean + 3SD
DYS392	-1	17%	mean +
	1	3%	3SD
	-2	3%	max max
DYS391	-1	17%	max
	1	2%	max
	-2	3%	max

Y-STRs			
Locus	Stutter	Threshold	Method
DYS390	-1	15%	mean +
	1	2%	3SD
	-2	2%	mean + 3SD mean + 3SD
DYS389II	-1	19%	max
	1	2%	mean +
	-2	5%	3SD max
DYS389I	-1	18%	max
	1	2%	max
	-2	2%	mean + 3SD
DYF387S1	-1	24%	max
	1	3%	mean +
	-2	3%	3SD mean + 3SD
DYS385a-b	-1	26%	mean +
	1	4%	3SD
	-2	5%	mean + 3SD max
DYS439	-1	10%	max
	1	3%	max
	-2	1.50%	min stutter
Y-GATA-H4	-1	17%	max
	1	3%	mean +
	-2	4%	3SD max

Table 18 Default stutter filters for AUTOSOMAL STRs in a custom ForenSeq MainstAY analysis method with ePCR1 buffer system

AUTOSOMAL STRs			
Locus	Stutter	Threshold	Method
CSF1PO	-1	11%	max
	1	4%	max
	-2	2%	max

Universal Analysis Software – MainstAY Product Line Module Reference Guide

AUTOSOMAL STRs			
Locus	Stutter	Threshold	Method
TH01	-1	13%	mean +
	1	2%	3SD
	-2	2.00%	mean + 3SD min stutter
PentaE	-1	14%	max
	1	4%	mean +
	-2	1.50%	3SD min stutter
PentaD	-1	9%	mean +
	1	1.50%	3SD
	-2	1.50%	min stutter min stutter
FGA	-1	26%	max
	1	3%	mean +
	-2	6%	3SD max
D22S1045	-1	26%	max
	1	8%	max
	-2	6%	max
D21S11	-1	16%	mean +
	1	3%	3SD
	-2	2%	max mean + 3SD
D20S482	-1	19%	max
	1	4%	max
	-2	3%	max
D19S433	-1	19%	max
	1	7%	mean +
	-2	2%	3SD mean + 3SD
D18S51	-1	23%	max
	1	14%	max
	-2	5%	max
D17S1301	-1	24%	max
	1	2%	max
	-2	4%	mean + 3SD

AUTOSOMAL STRs			
Locus	Stutter	Threshold	Method
D16S539	-1	22%	max
	1	3%	mean +
	-2	2%	3SD mean + 3SD
TPOX	-1	9%	max
	1	1.50%	min
	-2	1.50%	stutter min stutter
D13S317	-1	9%	mean +
	1	4%	3SD
	-2	1.50%	max min stutter
D10S1248	-1	21%	max
	1	2%	max
	-2	3%	mean + 3SD
D9S1122	-1	21%	max
	1	8%	max
	-2	3%	mean + 3SD
D8S1179	-1	29%	mean +
	1	7%	3SD
	-2	5%	max mean + 3SD
D7S820	-1	12%	max
	1	3%	max
	-2	2%	mean + 3SD
D6S1043	-1	16%	max
	1	4%	mean +
	-2	6%	3SD mean + 3SD
D5S818	-1	11%	max
	1	6%	max
	-2	2%	mean + 3SD

AUTOSOMAL STRs			
Locus	Stutter	Threshold	Method
D4S2408	-1	10%	max
	1	3%	mean +
	-2	1.50%	3SD min stutter
D3S1358	-1	15%	max
	1	4%	max
	-2	3%	mean + 3SD
D2S1338	-1	25%	max
	1	3%	mean +
	-2	4%	3SD mean + 3SD
D2S441	-1	9%	max
	1	2%	mean +
	-2	1.50%	3SD min stutter
D1S1656	-1	32%	max
	1	4%	max
	-2	4%	mean + 3SD
D12S391	-1	38%	max
	1	5%	max
	-2	9%	mean + 3SD
vWA	-1	21%	max
	1	2%	mean +
	-2	4%	3SD max

Table 19 Default stutter filters for Y-STRs in a custom ForenSeq MainstAY analysis method with ePCR1 buffer system

Y-STRs			
Locus	Stutter	Threshold	Method
DYS19	-1	20%	max
	1	5%	mean +
	-2	2%	3SD mean + 3SD

Y-STRs			
Locus	Stutter	Threshold	Method
DYS635	-1	14%	max
	1	3%	max
	-2	3%	max
DYS612	-1	45%	max
	1	13%	max
	-2	13%	mean + 3SD
DYS576	-1	19%	max
	1	6%	max
	-2	2%	mean + 3SD
DYS570	-1	21%	mean +
	1	4%	3SD
	-2	3%	max max
DYS549	-1	10%	max
	1	3%	max
	-2	1.50%	min stutter
DYS533	-1	11%	mean +
	1	3%	3SD
	-2	1.50%	max min stutter
DYS522	-1	16%	mean +
	1	6%	3SD
	-2	1.50%	mean + 3SD min stutter
DYS505	-1	21%	max
	1	2%	mean +
	-2	3%	3SD mean + 3SD
DYS481	-1	51%	mean +
	1	8%	3SD
	-2	16%	max max
DYS460	-1	13%	max
	1	4%	max
	-2	2%	mean + 3SD

Universal Analysis Software – MainstAY Product Line Module Reference Guide

Y-STRs			
Locus	Stutter	Threshold	Method
DYS643	-1	11%	max
	1	2%	mean +
	-2	1.50%	3SD min stutter
DYS448	-1	3%	mean +
	1	1.50%	3SD
	-2	1.50%	min stutter min stutter
DYS438	-1	7%	max
	1	1.50%	min
	-2	1.50%	stutter min stutter
DYS393	-1	12%	max
	1	7%	max
	-2	1.50%	min stutter
DYS437	-1	16%	max
	1	2%	mean +
	-2	2%	3SD mean + 3SD
DYS392	-1	27%	mean +
	1	5%	3SD
	-2	3%	max max
DYS391	-1	17%	max
	1	3%	max
	-2	3%	max
DYS390	-1	15%	mean +
	1	2%	3SD
	-2	2%	mean + 3SD mean + 3SD
DYS389II	-1	23%	max
	1	16%	mean +
	-2	5%	3SD max

Y-STRs			
Locus	Stutter	Threshold	Method
DYS389I	-1	18%	max
	1	2%	max
	-2	3%	mean + 3SD
DYF387S1	-1	24%	max
	1	3%	mean +
	-2	4%	3SD mean + 3SD
DYS385a-b	-1	33%	mean +
	1	4%	3SD
	-2	6%	mean + 3SD max
DYS439	-1	10%	max
	1	5%	max
	-2	1.50%	min stutter
Y-GATA-H4	-1	27%	max
	1	3%	mean +
	-2	4%	3SD max

Reporting Flanking Regions with Samples

Sequence Data from amplicon flanking regions may be used to further classify samples processed with the ForenSeq MainstAY Kit. Flanking region sequence data are not depicted in the user interface of the Universal Analysis Software and do not affect genotyping results in the software. These data are provided for informational use and genotypes from data within an amplicon’s flanking sequences are not declared.

Analysis setting thresholds such as analytical thresholds, interpretation thresholds, and stutter filters, are applied by the Universal Analysis Software only during genotyping of the MainstAY loci and are not applied for flanking region data analysis.

The following are applied when reporting flanking region sequence data:

- The reported amplicon sequence includes the sequence data from Read 1 for nucleotides that exist between the PCR primers.
- Unique sequences for a locus present at greater than 10 reads are reported.
- Sequence data downstream of the flanking regions are not reported for DYS389II, DYS439, or DYS570.
- Flanking regions for nine amplicons might be truncated to ensure sequencing data integrity. The maximum number of bases that will be displayed is tabulated below.

Table 20 STR loci with potentially truncated flanking sequences

STR Locus	Maximum Reported Amplicon Length (Bases)
Penta E	197
DXS10135	258
DXS8378	168
DYS19	237
DYS390	254
DYS392	177
DYS448	199
DYS460	233
DYS522	253

Index

-
- A**
- activity setting 12, 45
 - adapter dimer 47
 - administrators
 - access 45
 - accounts 9
 - privileges 41, 45
 - settings 45
 - allele calls 41
 - alleles 26, 47, 49
 - modifying 14
 - amplicons
 - lengths 14
 - modifying 42
 - analysis files 37
 - analysis methods 20, 27
 - default settings 41
 - editing 42
 - favorites 43
 - filtering 43
 - sorting 43
 - viewing results 28
 - analysis parameters 41, 59
 - analysis service 50
 - analysis status 15
 - analytical thresholds 42
 - applications 8
 - assay types 20, 41-42
 - AT 33
- B**
- BAM files 37
 - base pairs 14
 - biological sex 14, 26-27, 60
- C**
- call rates 60
 - calls 14
 - chastity filters 53
 - Chrome 9
 - chromosomes 27
 - cluster density 47-49, 52-53
 - clusters passing filter 48, 52-53
 - CMF headers 29-30, 33, 44
 - CODIS reports 29
- D**
- color coding 10, 14
 - colors 23-24
 - commenting 26, 37, 40
 - comments
 - deleting 40
 - editing 40
 - configuration, software 41
 - contact information, Verogen 71
 - contributor status 60
 - contributors 14, 26, 29-30, 33
 - control software 8
 - controls 20, 24, 52
 - comparing 36
 - metrics 56
 - types 56
 - coverage 33
 - custom assays 20
 - customer support 71
 - cycle numbers 55
- D**
- dark mode 41
 - dashboard 10
 - data recording 45
 - database reports 29
 - default analysis settings 42
 - discordance 24, 57
 - diversity 49
 - DNA inputs 47-49
 - DNS 9
 - documentation 71
 - Domain Name System 9
 - downloading reports 13, 29-32
- E**
- EMPOP reports 29
 - error messages 49
 - Excel workbooks 33
- F**
- female samples 60
 - filtering
 - analysis methods 43
 - reports 13
 - filters 13-14, 21, 42, 60-63, 65
- G**
- flow cells 19
 - editing type 19
 - ForenSeq Analysis service 50
- G**
- GEDmatch PRO reports 29
 - genotype concordance 55-56
 - genotypes 33
- H**
- heatmaps 14
 - help, technical 71
 - History icon 45
 - history setting 11-12, 37, 45
 - home-brew assays 20, 42
 - home page 10
 - HSC 49-50
 - loci 56
 - quality metrics 23, 52
 - results 55
 - hyperlink run names 10
 - hyperlink sample names 10
- I**
- i5 read 54
 - i7 read 54
 - icons 10, 12, 14-15, 54
 - importing samples 20
 - inconclusive biological sex 60
 - Index 1 and Index 2 Reads, quality 54
 - index adapters 20, 54
 - index CV 58
 - Information icon 37
 - installing 9
 - intensities 14
 - intensity 14, 49, 53, 55-56, 58
 - internet requirements 9
 - InterOp folder 24
 - interpretation thresholds 42
 - intralocus balance 14, 33
 - IT 33
- K**
- K/mm² 53

-
- L**
library prep kits 20
 reference guides 47
library types 42
loci 56
 searching 43
 user actions 40
locus call rates 14
locus calls 14
logging in 10
logging out 10
lost passwords 9
- M**
male samples 60
metrics, colors 23-24
minimum quality score 42
minimum read count 42
MiSeq FGx Control Software 8
MiSeq FGx System 8, 15
mixtures 14, 26, 60
- N**
navigation rails 10
negative amplification controls 13, 57, 60
networks
 requirements 9
 troubleshooting 50
non-directed sequencing 42
number of reads 47
- O**
outputs 32
- P**
pass filter reads 24, 58-59
passwords, lost 9
paused runs 15
permissions 45
phasing and prephasing 24, 49, 52-53
plexities 49
positions
 selecting 29
positions called 24
positive amplification controls 13, 56
primer-directed sequencing 42
primers 54
- Print icon 37
printing 12
privileges, administrator 45
project summary, printing 37
projects 10
 assigning samples 20, 22
 creating 19-20, 22
 descriptions 36
 icons 12
 naming 12, 36
 summaries 10
- Q**
Q icon 10
QC indicators 13-14
QPN 12, 52
QPN icons 13
quality control 47, 59
quality filters 53
quality metrics 10-11, 13, 23, 55
- R**
rails 10
read counts 59
read cycles 55
read definitions 54
read distributions 58
Reads 1 and 2, quality 54
reagent blanks 57
reagent kits 19
reagent performance 48-50
Real-Time Analysis 8
reanalyzing samples 15, 28
recording data 45
reference sequences 33
reports
 contents 32
 downloading 13, 29-32
 filtering 13
 finding 13
 types 29
 worksheets 33
resolution 9
results 13
Results page 10
 printing 37
RTA 54
run cycles 55
- run details 10
run metrics 48, 55
run monitoring 11, 23, 52, 58
run status 15
runs 10
 general information 11
 metrics 47, 55
 performance 11, 23
 quality 24, 52
 status 23
- S**
safety data sheets 71
sample comparisons, reporting 36
sample information 13, 19, 29, 33
Sample Overview section, printing 37
sample read count guideline 24, 47-50, 58
sample representation 11, 13, 23-24, 47-50, 52
sample results 13
sample sheets 19-20
 entering unique dual index (UDI) adapters 20
samples
 adding information 19
 assigning to projects 19-20, 22, 27, 36
 importing 20
 intensity 47
 locating 21
 modifying 22
 reanalyzing 28
 removing from runs 23
 searching 21
 status 15
Samples page
 printing 37
scatterplots 14
screen resolution 9
SDS 71
searching
 loci 43
 reports 13
 samples 21
sequencing processes 54
servers 8, 37

sex chromosomes 27
single-source samples 14, 26
software reset 9
sorting 13, 21, 43
specimens 29-30, 33
star icons 43
stopped runs 15
stutter 14, 33
stutter filters 42-43, 60-63, 65
support, library prep 47
system dashboard 10
system events, definition 45

T

technical assistance 24, 71
technical notes 41
third-party assays 20, 42
thresholds 33, 41-42, 59
total reads 14
troubleshooting 50
troubleshooting files 24
typing alleles 14

U

uncalled read intensity 60
uninstalling 9
unique dual index (UDI) adapters
 definition 18
 sample sheets 20
untyping alleles 14
user access 45
user actions, definition 45

V

validations 42
VCF files 37

W

washes 48
web browser 9

X

XX biological sex 60
XY biological sex 60

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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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