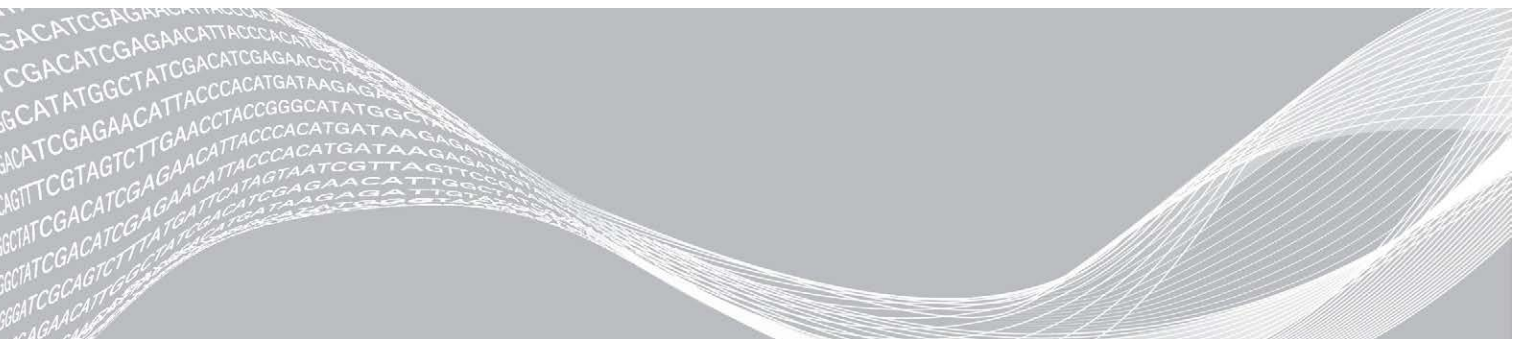


VeriSeq NIPT Solution v2

Software Guide



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Revision History

Document	Date	Description of Change
Document # 1000000067940 v02	April 2019	Added detail to NIPT and Supplementary reports to align with training materials.
Document # 1000000067940 v01	February 2019	Release of the VeriSeq NIPT Solution v2 Software Guide for customer usage.
Document # 1000000067940 v00	November 2018	Initial release for internal usage only.

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Chapter 1 VeriSeq NIPT Solution v2

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System Architecture	2

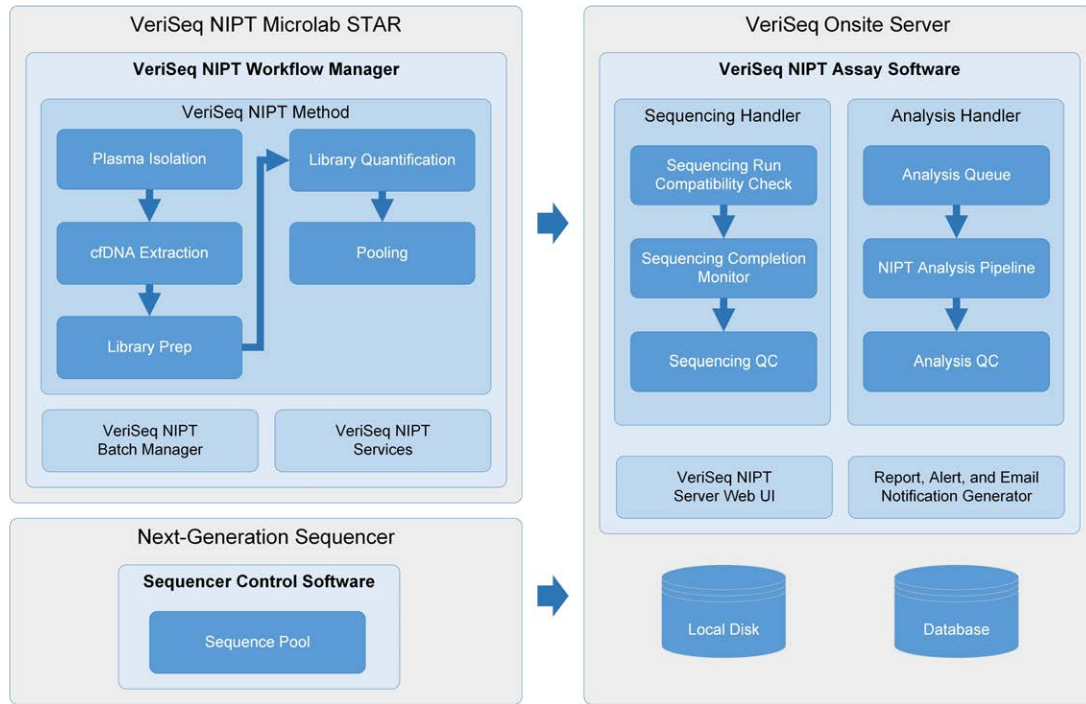
Introduction

The VeriSeq NIPT Solution v2 is an *in vitro* diagnostic test intended for sequencing-based screening for the detection of fetal aneuploidies from maternal peripheral whole blood samples in pregnant women of at least 10 weeks gestation. The test offers two options for types of screening: basic and genomewide. The basic screen provides information on the aneuploidy status for chromosomes 21, 18, 13, X, and Y only. Genomewide screens provide partial deletions and duplications for all autosomes and aneuploidy status for all chromosomes. Both screening types offer an option to request the reporting of sex chromosome aneuploidy (SCA). With either screening type, this product must not be used as the sole basis for diagnosis or other pregnancy management decisions.

The VeriSeq NIPT Solution v2 system architecture comprises:

- ▶ **VeriSeq NIPT Microlab STAR (ML STAR)**—An automated liquid handling instrument that uses the VeriSeq NIPT Workflow Manager and VeriSeq NIPT Sample Prep Kits to prepare and track library samples. The ML STAR prepares samples intended for analysis using the VeriSeq NIPT Assay Software v2 according to the Instructions for Use found in the *VeriSeq NIPT Solution v2 Package Insert (document # 1000000078751)*.
- ▶ **Next-Generation Sequencer (NGS)**—A genomewide sequencing instrument that provides on-instrument cluster generation and sequencing. The sequencer control software provides the steps to set up a sequencing run and generates sequencing reads for all samples in the quantified library pool.
- ▶ **VeriSeq Onsite Server v2**—A server that hosts the VeriSeq NIPT Assay Software v2 and stores data to analyze paired-end sequencing data. The Assay Software continuously monitors and analyzes sequencing data and produces sample results, process reports, and notifications.

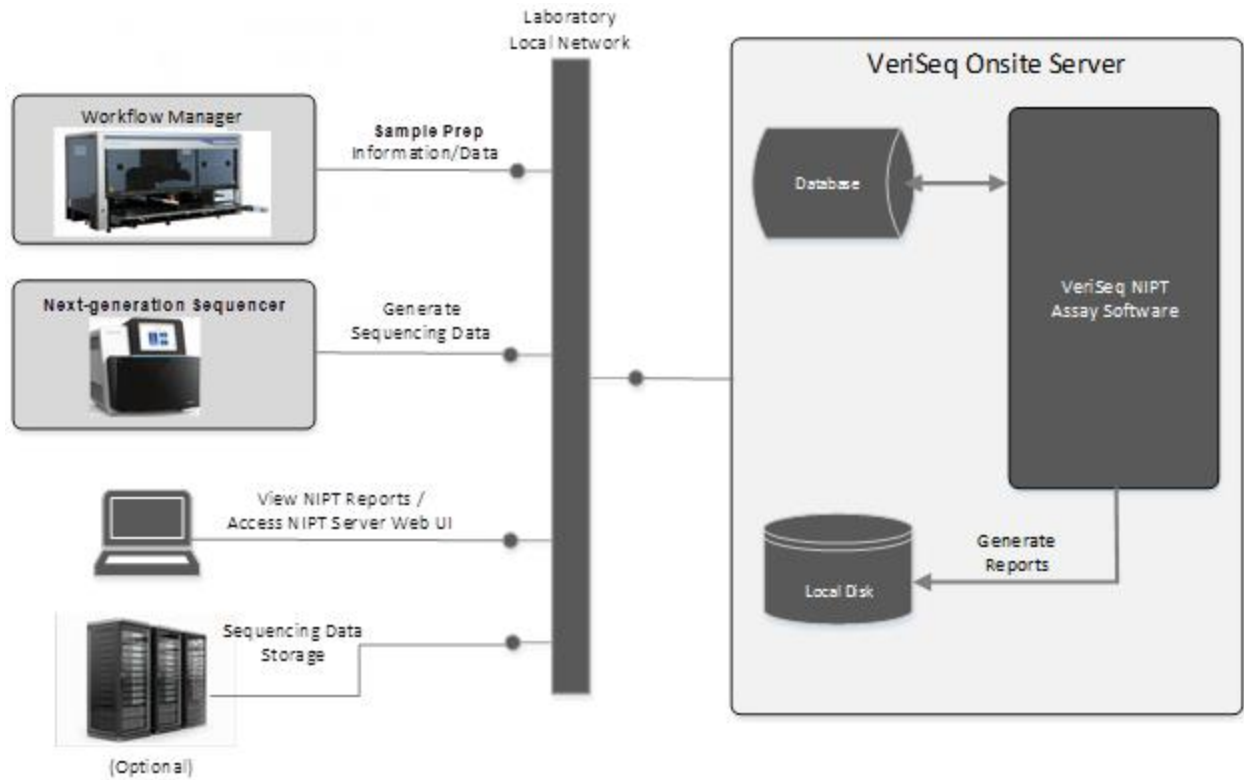
Figure 1 VeriSeq NIPT Solution v2 Components



System Architecture

The VeriSeq NIPT Solution v2 uses the laboratory local area network (LAN) to connect all system equipment using the same subnet. Using the LAN provides flexible equipment positioning and expandable throughput by connecting additional sequencers and/or ML STAR workstations. [Figure 2](#) provides an overview.

Figure 2 VeriSeq NIPT Solution v2 Overview



Chapter 2 VeriSeq NIPT Workflow Manager

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Introduction

The VeriSeq NIPT Workflow Manager is installed on the ML STAR and provides a simple and intuitive graphical user interface that automates the preparation of blood samples according to the VeriSeq NIPT Solution v2. The Workflow Manager maintains a data connection to the VeriSeq Onsite Server v2 for the purposes of data processing, storage, sample tracking, and enforcement of the workflow logic.

The Workflow Manager provides access to three different software modules, also known as methods:

- ▶ VeriSeq NIPT Method
- ▶ VeriSeq NIPT Batch Manager
- ▶ VeriSeq NIPT Services

VeriSeq NIPT Method

The VeriSeq NIPT Method (Method) directs the automated processing of samples on the ML STAR. The method accomplishes the following processing steps:

- ▶ **Plasma Isolation**—Transfers 1 ml of isolated plasma from a blood collection tube. The process logic creates a batch with the Assay Software. Each batch contains sample data, including the sample barcode, sample type, screen type, well position, and sex-reporting flag.
- ▶ **cfDNA Extraction**—Purifies cfDNA from 900 µl of plasma.
- ▶ **Library Preparation**—Creates libraries from purified cfDNA that are ready for sequencing. The libraries include unique indexes for each sample in the batch.
- ▶ **Library Quantification**—Determines cfDNA concentration using an intercalating fluorescent dye in a 384-well microplate format. The plate includes a labeled DNA standard curve and duplicates of each sample in the batch. The system uses the raw fluorescence readings from the microplate reader and calculates the sample concentrations based on the standard curve.
- ▶ **Pooling and Normalization**—Combines libraries into single pools for sequencing. The system uses the previously determined concentrations to calculate the appropriate transfer volumes for each sample in the sequencer-ready pool.

VeriSeq NIPT Batch Manager

The VeriSeq NIPT Batch Manager manages the status of samples, batches, and pools through the user interface. The system allows sample tracking across multiple liquid handling systems and sequencers, and through the analysis pipeline. For more information about sample processing procedures, see the *VeriSeq NIPT Solution v2 Package Insert (document # 1000000078751)*.

You can manage samples within the workflow through three different categories, referred to as objects:

Object	Description
Sample	Result of a one-time draw of 1 ml of plasma from a single blood tube. Samples are associated with the barcode of the blood tube (the sample barcode) and the batch.
Batch	Plate of 24, 48, or 96 samples processed through the cfDNA Extraction and Library Prep process.
Pool	Normalized and diluted volume of sequencer-ready, dual-index libraries. Each pool contains up to 48 samples.

During processing, the following actions can be applied to objects:

Action	Object	Report Generated	Description
Invalidation	Sample	Sample Invalidation	Sample flagged by the user as no longer valid for processing. No test result is generated for invalidated samples. Example: Visible blood cell carryover during Plasma Isolation.
	Batch	Batch Invalidation	Batch flagged by the user as no longer valid. If batch invalidation occurs before pool generation, all samples are invalidated. Example: Dropped or otherwise improperly handled plate.
	Pool	Pool Invalidation	Pool flagged by the user as no longer valid. After two pool invalidations, all samples within the pool are invalidated. Example: Entire pool volume used during two sequencing failures.
QC Failure	Sample	Sample Invalidation	VeriSeq NIPT Solution v2 automatically flagged sample as invalid due to the failure of a specified QC metric or from a system-detected liquid handling failure.
	Batch	Batch Invalidation	VeriSeq NIPT Solution v2 automatically flagged entire batch as invalid. Example: System failure during liquid handling.
Cancelation	Sample	Sample Cancelation	Laboratory management flagged sample as canceled. No test result is generated.
Edit Sample Attributes	Sample	Sex Reporting	Sex reporting flagged by the user as Yes, No, or SCA. For Sample sex reporting designated as Yes, the sex of the sample is generated. For sample sex reporting designated as No, the sex of the sample is not generated. For sample sex reporting designated as SCA, only sex chromosome aneuploidies are reported.
	Sample	Sample Type	Sample type flagged by the user as Singleton, Twin, Control, or No Template Control (NTC). The designation of the sample sex type directly affects the analysis of the test. To ensure accurate test results, the sample type is required to be accurate.
	Sample	Screen Type	Screen type flagged by the user as basic (21, 18, 13, X, and Y only) or genomewide (all chromosomes).

After an invalidation, QC failure, or cancelation action, the object is not processed further. Laboratory information management systems (LIMS) can use Sample Invalidation reports to indicate sample reprocessing from the blood collection tube.

Sample Sheet Input

The input sample sheet provides patient-related sample information including the sample type and sex chromosomes reporting status. The system requires complete sample information before sequencing pools can be generated.

**CAUTION**

To avoid errors, do not include any sample information for NTCs in the sample sheet. Leave them out entirely and do not include rows for them. The system automatically applies barcodes, screen type, sample type and sex reporting for NTCs.

The input sample sheet must be a tab-delimited text file, *.txt. The header column names in the file must match the header column names exactly as they appear in the following table.

Header Column	Data Type	Requirement	Description
batch_name	String/Blank	Required	Indicates the batch name of the sample. Must match the batch name entered into the calling method (Workflow Manager) to confirm that the input sample sheet is associated with the correct batch. There is a 26-character maximum. Column may be left blank. NOTE Sample sheets without a batch_name column will not be accepted.
sample_barcode	String	Required	Barcodes on blood sample tubes loaded onto the ML STAR. If an integer value is used as sample barcode, it should not exceed 15 digits. An alphanumeric sample barcode can be a maximum of 32 characters. Use only numbers, letters, dashes (-), and underscores (_).
sample_type	String	Required	Indicates the sample type for analysis. Allowed values are "Singleton", "Twin", "Control", and "NTC".
sex_chromosomes	String	Required	Indicates the fetal sex chromosomes reporting. Allowed values are "yes" (report), "no" (do not report), and "sca" (report only for sex chromosome aneuploidies).
screen_type	String	Required	Indicates the type of screening for analysis. Allowed values are "basic" and "genomewide".

The input sample sheet is uploaded during Plasma Isolation or Pooling and can be uploaded using the Batch Manager. Sample information is confirmed during the sample uploading process. Samples uploaded during Plasma Isolation can include a complete sample list or a subset of samples. During Pooling, the system requests any missing sample information not uploaded during Plasma Isolation.

The user has control of sample loading for either all samples in the batch (for batches generated by the customer LIMS) or for retests (with the remaining open positions filled with available samples).

Choose from three ways of using sample sheets:

- ▶ Predefined batches (LIMS-created batches)
- ▶ Ad-hoc batching (Workflow Manager created batches)
- ▶ Hybrid batching (padded LIMS priority samples)

Predefined Batches—LIMS-Created Batches

Batches can be created by the customer LIMS before the start of sample processing. In predefined batches, all samples are already associated with a batch before they are loaded onto the ML STAR. The sample sheet uploaded during plasma isolation includes every sample in the batch along with all sample information. Sample sheets for LIMS-created batches must include the batch ID column to make sure that the correct batch ID name was manually entered into the Workflow Manager at the start of processing.

This approach has the advantage of locking down the exact samples loaded because the system requires that all samples in the sample sheet be in the batch. No further information is required and the lab can proceed to the final report without additional data input.

- ▶ **Advantages**—Allows for complete control of batch contents. Prevents the loading of unwanted samples.
- ▶ **Disadvantages**—Requires a system for creating batches from inventory (advanced LIMS). Can require lab personnel to retrieve the correct samples from storage or require an advanced sample storage system.

Ad-hoc Batching—Created Batches

Batches can be created in the lab by physically collecting sample tubes and loading them onto the ML STAR during plasma isolation. No prior sample-to-batch association is required, and the user in the lab determines which samples to include in the batch.

When prompted, the user selects **No Sample Sheet** during plasma isolation. The Workflow Manager associates the loaded samples with the manually entered batch ID and generates a batch initiation report. The report can be sent to the lab LIMS system to indicate that the batch has been created and to provide a list of the associated samples.

- ▶ **Advantages**—No LIMS or sample sheet are required. Users can modify the batch initiation report with information on sample type, screen type, and sex reporting for upload during pooling. Flexible, any sample can be added at any time.
- ▶ **Disadvantages**—No automated control over which samples are included in the batch. The user can load an unwanted sample. Sample data must be uploaded during pooling.

Hybrid Batching—LIMS Priority Samples

The customer LIMS system can create a batch with a subset of the samples predefined. The remaining samples in the batch are filled in by the Workflow Manager from the samples loaded. In this case, the user uploads a partial sample sheet during plasma isolation. Again, labs are encouraged to fill out the Batch ID column if they want to predefine their batch names. Sometimes, a user may choose to upload an empty sample sheet that only contains the batch name to double-check the manually entered batch name. This strategy works well for prioritizing high-value samples, such as retests, to make sure that these high-value samples are included in the run. The decision on which samples to include in the batch is determined partially by the LIMS and partially by the user in the lab.

- ▶ **Advantages**—Maintains the flexibility of the Ad-hoc Batching strategy while retaining the ability to dictate the batch name and some of the samples in the batch (ie, retests).
- ▶ **Disadvantages**—Does not fully dictate the samples; therefore, an unwanted sample can still be loaded. This strategy still requires information on some samples during pooling.

Edit Sample Attributes

At any point before starting a sequencing run, you can use the VeriSeq NIPT Batch Manager to change individual sample sex chromosome reporting, screen type, and sample type attributes.

- 1 Access the Batch Manager.
- 2 Enter the Batch ID and User Name or operator initials, and select **OK**.
- 3 On the batch plate diagram, select the well position associated with the desired sample.
- 4 Confirm that the correct sample is displayed, and then select a Sample Type attribute from the Sample Type drop-down list.
- 5 Select a Sex Reporting attribute from the Sex Reporting drop-down list.
- 6 Select a Screen Type attribute from the Screen Type drop-down list.
- 7 Select **Edit**.

Sample, Batch, and Pool Invalidation

Depending on the step in sample processing, the user can invalidate an individual sample, a batch, or a sample pool. After invalidation, the sample, batch, or pool is no longer processed.

At any point before generating a test report, use either the VeriSeq NIPT Method or Batch Manager to invalidate one or more samples.

Invalidation Using VeriSeq NIPT Method

- 1 During sample processing, in the Well Comments window at the end of each Workflow Manager process, select the individual wells to fail, and select **OK**.
- 2 Select at least one annotation from the drop-down menus, or select the **Other** checkbox and enter a comment.
- 3 Select the **Fail Sample** checkbox, and select **OK**.
- 4 Confirm that the system is to fail the sample.

Invalidation Using Batch Manager

Use Batch Manager to invalidate:

- ▶ A sample
- ▶ A batch before the Pool step is complete.
- ▶ A sample pool after the Pool step is complete and before generating a test report.



NOTE

Exit any currently running methods before running the Batch Manager.

Access Batch Manager

- 1 To access Batch Manager, do one of the following:
 - ▶ On the App Launcher, select **VeriSeq NIPT Batch Manager**.
 - ▶ On a computer connected to the network, browse to `C:\Program Files (x86)\HAMILTON\Methods\VeriSeqNIPT` and open the Batch Manager method file (`VeriSeqNIPT_Batch_Manager.med`) with the Hamilton Run Controller.

Sample Invalidation

- 1 Access the Batch Manager.
- 2 Enter the Batch ID and User Name or operator initials, and select **OK**.
- 3 On the batch plate diagram, select the well position associated with the failed sample.
- 4 Confirm that the correct sample is displayed, and select **Invalidate Sample**.
- 5 Enter a reason for failure, and select **Invalidate**.
On the batch plate diagram, the invalidated sample changes from green to red, and the status label changes from valid to failed.

Batch Invalidation

- 1 Access the Batch Manager.

- 2 Enter the Batch ID and User Name or operator initials, and select **OK**.
- 3 On the batch plate diagram, select **Invalidate Batch**.
- 4 Enter a reason for failure, and select **Invalidate**.
On the batch plate diagram, if no valid pools exist for the batch, all samples change from green to red. Valid pools within the batch remain valid.

Pool Invalidation

- 1 Access the Batch Manager.
- 2 Enter the Batch ID and User Name or operator initials, and select **Pool Manager**.
- 3 Scan the barcode of the pool.
- 4 Enter User Name or operator initials, and select **OK**.
- 5 Enter a reason for failure, and select **Invalidate**.

Upload Sample Sheet

The user can upload a sample sheet containing sample information through the Batch Manager. Using this feature, the user can, for example, upload or change sample information in large sets.

- 1 Access the Batch Manager.
- 2 Enter the Batch ID and User Name or operator initials, and select **OK**.
- 3 Select the **Upload New Sample Sheet**.
- 4 Browse to and select the desired sample sheet, and then select **OK**.

Sample Cancelation

- 1 Access the Batch Manager.
- 2 Enter the Batch ID and User Name or operator initials, and select **OK**.
- 3 On the batch plate diagram, select the well position associated with the canceled sample.
- 4 Confirm that the correct sample is displayed, and select **Cancel Sample**.
- 5 Enter a reason for failure, and select **Cancel**.
On the batch plate diagram, the canceled sample changes from green to red.

VeriSeq NIPT Services

VeriSeq NIPT Services (Services) include several tools that are used for configuration and verification of both the ML STAR and Workflow Manager. These tools are not required for normal operation of the system, but may be required to assist Illumina or Hamilton Technical Support while troubleshooting the system. These tools are also used to adjust system parameters due to a drift in cluster density.

Launch VeriSeq NIPT Services



NOTE

Close all running methods before executing Services.

To access VeriSeq NIPT Services, do one of the following:

- ▶ On the App Launcher, select **VeriSeq NIPT Services**.
- ▶ On a computer connected to the network, browse to **C:\Program Files (x86)\HAMILTON\Methods\VeriSeqNIPT** and open the VeriSeq NIPT Services method file (**VeriSeqNIPT_Service.med**) with the Hamilton Run Controller.

The Services tools enable two types of tests:

- ▶ **Individual Tests**—Component tests used for troubleshooting the ML STAR hardware.
- ▶ **Service Tools**—Tools used to configure the Workflow Manager.

Individual Tests

To help troubleshoot hardware problems encountered on the Workflow Manager, the following system tests may be required.

System Test	Description
Barcode/Autoload	Tests for proper configuration of the system deck, AutoLoader, and barcode scanning functionality.
CPAC	Tests functioning of the CPAC on-deck heater systems. Also checks for proper wiring of individual units to the control box.
BVS Vacuum	Tests functioning of the on-deck basic vacuum system (BVS) to confirm that the vacuum can engage and achieve operational pressures.
Independent Channel	Tests functioning of the independent pipette channels. Performs liquid retention test to detect dripping of pipette channels and consistency of delivery volumes.
iSwap	Tests iSwap robotic arm functioning and confirms gross deck teaching positions.
96-Head	Tests functioning of the CO-RE 96 pipette head. Performs liquid retention test to detect dripping of pipette channels and consistency of delivery volumes.

To execute individual tests:

- 1 Select specific test to be executed.



NOTE

Full IOQ Execution runs all six tests sequentially.

- 2 Follow the on-screen instructions, making note of observations for equipment function and any system errors encountered.
- 3 When finished, select **Abort** to exit method.
- 4 If asked to provide system trace logs generated during test, the logs are available at **C:\Program Files (x86)\HAMILTON\LogFiles** and begin with **VeriSeqNIPT_Services**.

Service Tools

The Services Tools allow for the configuration of the Workflow Manager and some assay parameters.

System Test	Description
Server Configuration	Configures and tests the connection between the VeriSeq NIPT Workflow Manager and the Assay Software. Proper communication between these systems is required for execution of the Workflow Manager.
Assay Configuration	Used to reset the default library concentration.
Deck Teach Tool	Used to export and import deck teach positions from a file.

Server Configuration

If the network address for the VeriSeq Onsite Server v2 changes, direct the Workflow Manager to the new address.

- 1 From the Services Tools menu, select **Server Configuration**.
- 2 Update the URL with the new address of the Onsite Server.
- 3 Select **Test Connection** to send a test message.
If this message is not received, contact Illumina Technical Support.
- 4 From the System Configuration screen, select **OK**, and select **Apply** to save the new address.

Assay Configuration

You can adjust the concentration of the sequencer libraries in the sequencer pools within the Workflow Manager on a run-by-run basis during the pooling process (see the *VeriSeq NIPT Solution v2 Package Insert (document # 1000000078751)*). The Assay Configuration tool can also be used to change the default value for this concentration.

You can also use the Assay Configuration tool to change the values of other parameters. You can set the Default Sex Chromosome Reporting parameter to Yes or No. This setting determines what attribute to assign to samples when the Use Default button is selected during sample preparation. The Screen Type parameter can be set to Basic or Genomewide and determines the screen type for a sample.

- 1 Select **Assay Configuration**.
- 2 Update the Target Library Concentration (pg/μl) box to the desired value.
- 3 Update Default Sex Chromosome Reporting to the desired value.
- 4 Update Screen Type to the desired value.
- 5 Select **Apply**.

Deck Teach Tool

During troubleshooting, it may be necessary to export the taught position values. Use the Deck Teach Tool to generate a list of the positions along with their values.

- 1 Select **Deck Teach Tool**.
- 2 Select **Export**.
- 3 Select an output location for the text file containing the taught deck positions.
- 4 Select **OK**.

The Deck Teach Tool saves a text file containing the values for all the taught labware positions for the Workflow Manager installation.

- 5 Select **Cancel** to return to the Method Selection screen.

Chapter 3 Next-Generation Sequencer

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Introduction

A next-generation sequencing system generates sequencing reads for all samples in the quantified library pool and integrates with the VeriSeq NIPT Solution v2 via the Onsite Server. Sequencing data are evaluated by the Analysis Handler of the Assay Software.

Consider the following when integrating a next-generation sequencing system with the VeriSeq NIPT Solution v2.

- ▶ Data storage integration.
- ▶ Analysis throughput capacity.
- ▶ Network traffic constraints.

Sequence Pool

The Assay Software requires a next-generation sequencer capable of generating sequencing data on the prepared library pool according to the following specifications:

- ▶ Production of 2 x 36 paired end reads.
- ▶ Compatible with index adapters in the VeriSeq NIPT Sample Prep Kit.
- ▶ Two channel chemistry.
- ▶ Automatic production of .BCL files.

Data Storage Integration

A typical sequencing run for the VeriSeq NIPT Solution v2 requires 25–30 GB for next-generation sequencing system data. The actual data size may vary based on the final cluster density. The Onsite Server provides more than 7.5 TB of storage space, which is enough space for about 300 sequencing runs ($7,500 / 25 = 300$).

For data storage purposes, map the next-generation sequencing system to the Onsite Server for one of the following methods:

- ▶ Use the Onsite Server as the data repository. In this configuration, the sequencer is mapped directly to the server and persists data on the local drive.
- ▶ For a high throughput lab, use network-attached storage (NAS). Configure the next-generation sequencing system to persist the sequencing data directly to a specific location on the NAS. In this setup, configure the Onsite Server to monitor the specific NAS location that enables the server to monitor upcoming sequencing runs. Multiple next-generation sequencing systems can be added to increase sample throughput. For more information on how to map the server to the NAS, see *Managing a Shared Network Drive* on page 23.

For more information on how to map the next-generation sequencing systems to the server or to the NAS, see the system's user guide.

Analysis Throughput Capacity

The VeriSeq NIPT Analysis Pipeline typically processes data for a single sequencing run in approximately 5 hours. When expanding the lab for sample throughput, consider that a single server is able to process a maximum of four runs per day, which totals to 48 samples x 4 = 192 samples per day. Contact Illumina Support for additional throughput solutions.

Network Traffic Constraints

The VeriSeq NIPT Solution v2 uses the lab Local Area Network (LAN) for data throughput between the next-generation sequencing system, Onsite Server, and NAS (if configured). When expanding for sample throughput, consider the following IT infrastructure traffic constraints:

- ▶ The average data traffic of approximately 25 GB generated over approximately 10 hours is about 0.7 MB/sec per sequencer.
- ▶ The lab infrastructure may also support other sources of traffic that must be factored in.

VeriSeq NIPT Local Run Manager

If you use a next-generation sequencing system that has the VeriSeq NIPT Local Run Manager (LRM) module, prepare for sequencing by completing the following:

- 1 In Local Run Manager, select **Create Run**.
- 2 In the dropdown menu, select **VeriSeq NIPT**.
- 3 Complete the following fields.
 - ▶ Run Name
 - ▶ Run Description (optional)
 - ▶ Pool Barcode



CAUTION

The Pool Barcode entered in the LRM module must match the Pool Barcode entered in the Workflow Manager. Incorrect run configurations are rejected by the Assay Software and may require resequencing.

- 4 Select **Save Run**.

After you finish setting up your run, you can initiate the run using the instrument software.

Chapter 4 VeriSeq NIPT Assay Software v2

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Introduction

The VeriSeq NIPT Assay Software v2 generates statistics to evaluate the chromosome copy number of the tested samples and provides a determination of aneuploidy on the chromosomes selected for analysis. The selection of chromosomes for analysis depends on the screening type you choose: basic (chromosomes 21, 18, 13, X, and Y) or genomewide (all chromosomes). When you select the genomewide option, the software also tests for the presence of sub-chromosomal regions of copy number gain or loss within the autosome. A next-generation sequencing instrument generates analysis input in the form of 36-base paired-end reads.

The VeriSeq NIPT Assay Software v2 operates on the VeriSeq Onsite Server v2. The Onsite Server is a central component of the VeriSeq NIPT Solution v2 and acts as a connection point between the VeriSeq NIPT Workflow Manager, the next-generation sequencing system, and the user.

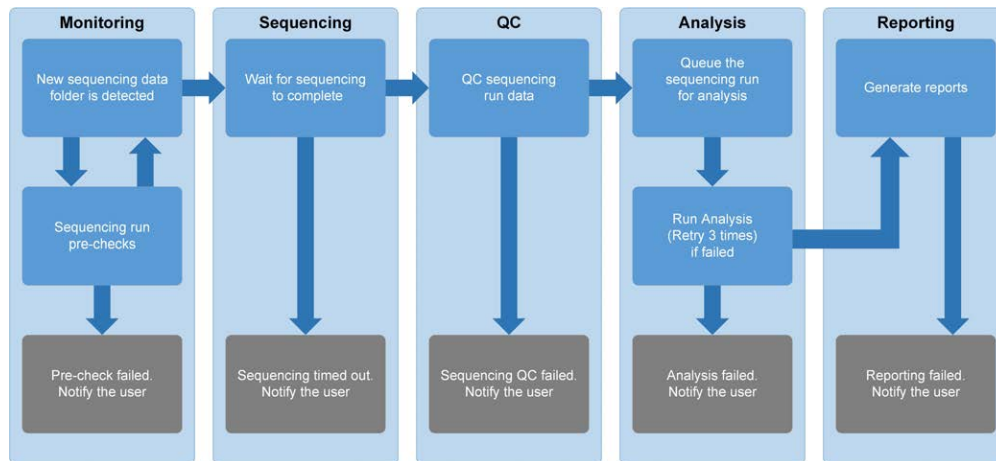
The Assay Software aligns the reads against the reference human genome and performs analysis on reads that align to a unique location or site in the genome. The Assay Software excludes duplicate reads and sites that are associated with high variation in coverage across euploid samples. Sequencing data are normalized for nucleotide content and to correct for batch effects and other sources of unwanted variability. Information about the cfDNA fragment length is derived from the paired-end sequencing reads. The Assay Software also assesses sequencing coverage statistics on regions known to be enriched for either fetal or maternal cfDNA. Data generated from fragment length and coverage analysis are used to estimate fetal fraction (FF) for each sample.

For each screening option selected for a sample from the test menu, the Assay Software reports whether or not an anomaly was detected. In the basic screen, all anomalies are aneuploidies. For the genomewide screen, an anomaly can be an aneuploidy or a partial deletion or duplication.

Assay Software Components

The Assay Software continuously runs and monitors new sequencing data as it is added to the Input folder on the Onsite Server. When a new sequencing run is identified, the following flow is triggered.

Figure 3 Data Flow Diagram



- 1 **Monitoring**—Prechecks the validity of the new sequencing run. The validity check includes checks for the run parameters compatibility (do the values match the expected values), association of the flow cell with a known existing pool tube, and making sure that results have not been reported previously for the same samples in that pool (rerun). If any of these checks fail, the user is notified through the email notification system and through the Alerts log on the Web UI.
- 2 **Sequencing**—Continuously monitors for the completion of the sequencing run. A timer is set that defines a timeout for the run to complete. If the timeout expired, the user is notified through the email notification system and through the Alerts log on the Web UI.
- 3 **QC**—Examines the InterOp QC files generated by the sequencer. The Assay Software checks the total number of clusters, cluster density, and the reads quality scores. If the QC criteria fails, the user is notified through the email notification system and through the Alerts log on the Web UI.
- 4 **Analysis**—Manages the analysis queue for multiple sequencing runs generated by various instruments configured with the server. The server processes a single analysis job at a time based on the First In, First Out (FIFO) principle. After the analysis has completed successfully, the next scheduled analysis in the queue is launched. If an analysis run fails or times out, the Assay Software automatically relaunches the analysis up to three times. After each failure, the user is notified through the email notification system and through the Alerts log on the Web UI.
- 5 **Reporting**—Generates the report that contains the final results after the analysis completes. If a failure occurs and reports are not generated, the user is notified through the email notification system and through the Alerts log on the Web UI.

Assay Software Tasks

The Assay Software performs both automated and user-initiated tasks.

Automated Tasks

The Assay Software completes the following automated tasks:

- ▶ **Sample preparation log collation and storage**—Produces a set of output files at the end of each step and stores them in the ProcessLogs folder located in the Output folder. For more information, see *Reports File Structure* on page 33 for an overview and *Process Reports* on page 47 for details.

- ▶ **Alert, email, and report notification generation**—Monitors the validity status of the batch, pool, and sample during the Sample Preparation steps and QC of the sequencing data and analysis results per sample. Based on these validation checks, the Assay Software determines whether to continue with the process and if the results are to be reported. The Assay Software terminates the process when a batch or a pool is invalidated based on QC results. An email notification is sent to the user, a report is generated, and an alert is logged on the Web UI.
- ▶ **Sequence data analysis**—Analyzes the raw sequence data for each sample multiplexed in the pool using the integrated NIPT Analysis Software. The Assay Software determines the aneuploidy results for each sample. The system does not report results for samples invalidated or canceled by the user. For samples that fail the QC criteria, an explicit rationale for the failure is provided; however, results for the failed sample are suppressed. For more information, see *NIPT Report on page 37*.
- ▶ **Results file generation**—Provides sample results in a tab-separated value file format, which is saved to the Output folder. For more information, see *NIPT Report on page 37*.
- ▶ **Report generation**— The Assay Software generates supplementary result information, notifications, and process reports. For more information, see *System Reports on page 33*.
- ▶ **Sample, pool, and batch invalidation**—
 - ▶ **Sample invalidation**—The Assay Software marks individual samples as invalid when the user:
 - ▶ Explicitly invalidates the sample.
 - ▶ Invalidates the whole plate during library preparation before the pools are created.
 When a sample is marked as invalid, a Sample Invalidation Report is automatically generated, see *Sample Invalidation Report on page 46*.
 - ▶ **Pool and batch invalidation report generation**—Pools and batches can only be invalidated by the user. Invalidated pools are not processed by the system. Pools that had already been created from an invalid batch are not automatically invalidated and can be further processed by the system. However, new pools cannot be created from an invalidated batch. When a pool is invalidated, the system issues a Pool Retest Request Report under the following conditions:
 - ▶ The batch is valid.
 - ▶ There are no more available pools for this batch.
 - ▶ The number of allowed pools from the batch has not been exhausted.
 For more information, see *Pool Retest Request Report on page 46*.
- ▶ **Retest management**—
 - ▶ **Pool failures**—Failed pools are typically pools that failed the Sequencing QC metrics. The Assay Software does not proceed with processing failed pools if the run is terminated. Resequencing using a second pool aliquot.
 - ▶ **Sample failures**—The software allows failed samples to be retested if needed. Failed samples need to be incorporated into a new batch and reprocessed through assay steps.
 - ▶ **Reruns**—The system does not reanalyze pools with samples that had previously been processed and reported successfully. Rerunning a sample can be done by replating the sample on a new batch.

User Tasks

The VeriSeq NIPT Solution v2 enables users to perform the following tasks:

Using the Workflow Manager:

- ▶ Mark as invalid an individual sample, all samples within a batch, or all samples associated with a pool.

- ▶ Mark a given sample as canceled. The Assay Software then marks the result as canceled in the final results report.

Using the Assay Software:

- ▶ Configure software to be installed and integrated into the laboratory network infrastructure.
- ▶ Change configuration settings such as network settings, shared folder locations, and user account management.
- ▶ View system and batch status, result and batch processing reports, activity and audit logs, and assay results.



NOTE

Users can perform certain tasks depending on user permissions. For more information, see [Assigning User Roles on page 22](#).

Sequencing Handler

The Assay Software manages the sequencing runs generated by the sequencing instruments via the Sequencing Handler. It identifies new sequencing runs, validates run parameters, and correlates the pool barcode with a known pool created during the library prep process. If an association cannot be made, a notification to the user is generated and the processing of the sequencing run is stopped.

After the validation is completed successfully, the Assay Software continues to monitor the sequencing runs for completion. Completed sequencing runs are queued to be processed by the Analytic Pipeline Handler (see [Analytic Pipeline Handler on page 18](#)).

Sequencing Run Compatibility

The server only analyzes sequencing runs that are compatible with the cfDNA analytical workflow.

Use only compatible sequencing methods and software versions to generate base calls.



NOTE

Regularly monitor sequencing data performance metrics to make sure that the quality of the data is within specification.

The VeriSeq NIPT Local Run Manager module configures sequencing using the following read parameters:

- ▶ Paired-end run with 2 x 36 cycle reads.
- ▶ Dual indexing with two 8-cycle index reads.

Analytic Pipeline Handler

The analytic pipeline handler launches the analysis pipeline for aneuploidy detection. The pipeline processes one sequencing run at a time at an average duration of less than 5 hours per pool. If the analysis fails to process the pool, or does not complete the analysis due to power failure or timeout, the Analytic Pipeline Handler automatically requeues the run. If processing the pool fails consecutively 3 times, the run is tagged as failed and the user is notified.

A successful analysis run triggers the NIPT report generation. For more information, see [NIPT Report on page 37](#).

Workflow Timeout and Storage Requirements

The cfDNA analytical workflow is subject to the following timeout and storage limitations.

Parameter	Default Value
Maximum Sequencing Time	20 hours
Maximum Analysis Time	10 hours
Minimum Scratch Space Storage	900 GB

Web User Interface

The Assay Software hosts a local Web UI that allows easy access to the Onsite Server from anywhere on the network. The Web UI provides the following functions:


- ▶ **View recent activities**—Identifies the steps completed during the assay execution. The user is alerted to many of these activities by the email notification system. For more information, see [Assay Software Notifications on page 55](#).
- ▶ **View errors and alerts**—Identifies problems that might prevent the assay from proceeding further. Error messages and alerts are sent to the user through the email notification system. For more information, see [Assay Software Notifications on page 55](#).
- ▶ **Configure the server network settings**—Illumina personnel typically configure the network during system installation. Modifications may be required if the local network requires IT changes. For more information, see [Changing Network and Server Settings on page 25](#).
- ▶ **Manage server access**—The Onsite Server allows Administrator and Operator level access. These access levels control viewing of the activity, alert, and error logs and modification of the networking and data mapping settings. For more information, see [Managing Users on page 22](#).
- ▶ **Configure sequencing data folder**—By default, the server stores sequencing data. However, a central NAS can be added to expand storage capacity. For more information, see [Mapping Server Drives on page 30](#).
- ▶ **Configure email notification subscribers list**—Manages a list of subscribers to receive email notifications comprising error messages and assay process alerts. For more information, see [Configuring System Email Notifications on page 26](#).
- ▶ **Reboot or shutdown the server**—Restarts or shuts down the server, if needed. A reboot or shutdown can be required for a configuration setting to take effect or as a remedy for a server failure. For more information, see [Rebooting the Server on page 30](#) and [Shutting Down the Server on page 31](#).

End User License Agreement

On the first login to the Web UI, you are requested to accept the End User License Agreement (EULA). By selecting **Download EULA**, you can download the license agreement to your computer. The software requires you to accept the EULA before you can continue working with the Web UI.

After you have accepted the EULA, you can return to the EULA page and download the document if needed.

Configuring the Web UI

Select the Settings icon  for a drop-down list of configuration settings. Settings appear based on user role and associated permissions. For more information, see [Assigning User Roles on page 22](#).



NOTE

A Technician does not have access to any of these functions.

Setting	Description
User Management	Add, activate/deactivate, and edit user credentials. Service Engineers and Administrators only.
Email Configuration	Edit subscribers list for email notifications.
Change Shared Folder Password	Change the sbsuser password for accessing the Onsite Server's shared folders.
Reboot Server	Service Engineers or Administrators only.
Shut Down Server	Service Engineers or Administrators only.

Logging in to the Web UI

To access the Assay Software interface and login:

- On a computer connected to the same network as the Onsite Server, open one of the following web browsers:
 - ▶ Chrome v69 or later
 - ▶ Firefox v62 or later
 - ▶ Internet Explorer v11 or later
- Enter the server IP address or the server name provided by Illumina at installation, equivalent to \\<VeriSeq Onsite Server v2 IP address>\login.
For example, \\10.10.10.10\login.
- If a browser security warning appears, add a security exception to proceed to the login screen.
- In the login screen, enter the case-sensitive user name and password provided by Illumina, and select **Log In**.



NOTE

After 10 minutes of inactivity, the Assay Software automatically logs out the current user.

Using the Dashboard

The VeriSeq NIPT Assay Software v2 Dashboard appears after logging in and is the main navigation window. To return to the Dashboard at any time, select the **Dashboard** menu option.

The dashboard always shows the recent 50 activities that were logged (if there are less than 50 it only shows the ones that are logged). You can fetch the previous 50 and browse through the activity history by selecting **Previous** in the lower-right corner of the activity table.

Figure 4 VeriSeq NIPT Assay Software Dashboard

WHEN	USER	SUBSYSTEM	DETAILS	LEVEL
2016-07-29 09:17 PDT		Assay	Aneuploidy Detection Report generated for '160728_NB551043_0005_AHCLWJBGXY'	Activity
2016-07-29 09:17 PDT		Assay	Analysis started for '160728_NS500411_0171_AHCLJBGXY'	Activity
2016-07-29 05:23 PDT		Assay	Sequencing QC passed for '160728_NS500411_0171_AHCLJBGXY'	Activity
2016-07-29 05:14 PDT		Assay	Analysis started for '160728_NB551043_0005_AHCLWJBGXY'	Activity
2016-07-29 05:14 PDT		Assay	Sequencing QC passed for '160728_NB551043_0005_AHCLWJBGXY'	Activity
2016-07-28 19:56 PDT		Assay	Sequencing started for '160728_NB551043_0005_AHCLWJBGXY'	Activity
2016-07-28 19:55 PDT		Assay	Sequencing started for '160728_NS500411_0171_AHCLJBGXY'	Activity
2016-07-28 17:18 PDT		Assay	Batch 'DVT0151_PL02_1': pool 'PT2008505' created	Activity
2016-07-28 17:18 PDT		Assay	Batch 'DVT0151_PL02_1': pool 'PT2008521' created	Activity
2016-07-28 16:14 PDT		Assay	Batch 'DVT0151_PL02_1' completed library	Activity
2016-07-28 10:30 PDT		Assay	Batch 'DVT0151_PL02_1' initiated	Activity

Viewing Recent Activities

The Recent Activities tab contains a brief description of recent Assay Software and Onsite Server activities.

Name	Description
When	Activity date and time
User	If applicable, identifies the user who performed the activity
Subsystem	Entity or process that performed the activity such as user, assay, or configuration
Details	Activity description
Level	Level assigned to the activity from the following options: <ul style="list-style-type: none"> • Activity—Indicates an activity within the server such as system reboot or user login/logout. • Notice—Indicates an unsuccessfully executed step. For example, sample invalidation or QC failure. • Warning—Indicates that an error occurred during normal execution and proper hardware function. For example, unrecognized run parameters or failed analysis.

Viewing Recent Errors

The Recent Errors tab contains a brief description of recent software and server errors.

Name	Description
When	Activity date and time
User	If applicable, identifies the user who performed the activity
Subsystem	Entity or process that performed the activity such as user, assay, or configuration
Details	Activity description
Level	Level assigned to the activity from the following options: <ul style="list-style-type: none"> • Urgent—Major hardware error that compromises system operation. Contact Illumina Technical Support. • Alert—Error in normal operation. For example, a disk corruption, space or configuration issue that prohibits report generation or email notifications. • Error—System or server error during normal operation. For example, a configuration file issue or hardware failure.

Viewing System Status and Alerts

To view the server status summary, from the Dashboard, select the **Server Status** tab.

The summary displays the following information:

- ▶ **Date**—Current date and time
- ▶ **Time zone**—Time zone configured for the server; used for email, alerts, and report date and time
- ▶ **Hostname**—System name consists of the network hostname and the DNS domain name
- ▶ **Disk space usage**—Percentage of disk space currently in use for storing data
- ▶ **Software**—Software regulatory configuration (eg, CE-IVD)
- ▶ **Version**— VeriSeq NIPT Assay Software v2 version

The summary may also display a **Server alarm** button that silences the RAID controller alarm. This button only appears for Administrators. If you press this button, contact Illumina Technical Support for additional assistance.

Managing Users



NOTE

Only Service Engineers and Administrators have permission to add, edit, or delete permissions for Technicians and other users at their level.

Assigning User Roles

User roles define user access and rights to perform certain tasks.

Role	Description
Service	An Illumina Field Service Engineer who performs initial installation and system setup (including creation of the Administrator). Also troubleshoots, performs server repair, sets up and changes configuration settings, and provides ongoing software support.
Administrator	A Laboratory Administrator who sets up and maintains configuration settings, administrates users, defines email subscribers list, changes shared folder password, and reboots and shuts down the server.
Technician	A Laboratory Technician who views system status and alerts.

Adding Users

At initial installation, an Illumina Field Service Engineer adds the Administrator user.

To add a user:

- 1 From the User Management screen, select **Add New User**.



NOTE

All fields are required.

- 2 Enter the user name.



NOTE

The acceptable characters for the user name are case-insensitive, alphanumeric characters (eg, a–z, and 0–9), ‘_’ (underscore), and ‘-’ (hyphen) only. User names must be 4–20 characters and contain at least one numerical character. The first character of the user name cannot be numerical.

The Assay Software uses user names to identify the persons involved in the different aspects of assay processing and interactions with the Assay Software.

- 3 Enter the full name of the user. The full name is shown only in the user profile.
- 4 Enter and confirm the password.



NOTE

Passwords must be 8–20 characters and contain at least one uppercase letter, one lowercase letter, and one numerical character.

- 5 Enter an email address for the user.
A unique email address is required for each user.
- 6 Select the desired user role from the drop-down list.
- 7 Select the **Active** box to activate the user immediately or deselect the box to activate the user later (ie, after training).
- 8 Select **Save** twice to save and confirm changes.
The new user now appears on the User Management screen.

Editing Users

To edit user information:

- 1 From the User Management screen, select the user name for the desired user.
- 2 Edit the information for the user as needed, and select **Save** when done.
- 3 Select **Save** again when the dialog box appears to confirm changes.
The changes to the user now appear on the User Management screen.

Deactivating Users

To deactivate a user:

- 1 From the User Management screen, select the desired user name.
- 2 Clear the **Activate** checkbox, and select **Save**.
- 3 On the confirmation message, select **Save**.
The user status changes to Disabled in the User Management screen.

Managing a Shared Network Drive



NOTE

Only Service Engineers or Administrators have permission to add, edit, or delete shared folder locations.

Adding a Shared Network Drive

Configure the system to store sequencing data on a dedicated NAS rather than on the server connected to the sequencing system. An NAS can provide larger capacity for storage and continuous data backup.

- 1 From the Dashboard, select **Folders**.
- 2 Select **Add folder**.
- 3 Enter the following information provided by the IT administrator:
 - ▶ **Location**—Full path to the NAS location including the folder where the data are stored.

- ▶ **Username**—User name designated for the Onsite Server when it accesses the NAS.
 - ▶ **Password**—Password designated for the Onsite Server when it accesses the NAS.
- 4 Select **Save**.
 - 5 Select **Test** to test the NAS connection.
If the connection fails, confirm the server name, location name, user name, and password with the IT administrator.
 - 6 Restart the server to apply the changes.

**NOTE**

A shared network drive configuration can support only one sequencing data folder.

Editing a Shared Network Drive

- 1 From the Dashboard, select **Folders**.
- 2 Edit the Location path, and select **Save**.
- 3 Select **Test** to test the NAS connection.
If the connection fails, confirm the server name, location name, user name, and password with the IT administrator.

Deleting a Shared Network Drive

- 1 From the Dashboard, select **Folders**.
- 2 Select the Location path to modify.
- 3 Select **Delete** to remove the external sequencing folder.

Configuring Network and Certificate Settings

An Illumina Field Service Engineer uses the Network Configuration screen to configure network and certificate settings during initial installation.

**NOTE**

Only Service Engineers and Administrators have permission to change network and certificate settings.

- 1 From the Dashboard, select **Configuration**.
- 2 Select the **Network Configuration** tab, and configure the network settings as appropriate.
- 3 Select the **Certification Configuration** tab to generate the SSL certificate.

Changing Certificate Settings

A secure socket layer (SSL) certificate is a data file that allows a secure connection from the Onsite Server to a browser.

- 1 Use the Certificate Configuration tab to add or change SSL certificate settings.
 - ▶ **Laboratory Email**—Contact email at the testing laboratory (requires a valid email address format).
 - ▶ **Organization Unit**—Department.
 - ▶ **Organization**—Name of testing laboratory.
 - ▶ **Location**—Street address of testing laboratory.
 - ▶ **State**—State location of testing laboratory (auto populates based on email address).
 - ▶ **Country**—Country location of testing laboratory (auto populates based on email address).

- ▶ **Certificate Thumbprint (SHA1)**— Certification identification number.
The Certificate Thumbprint (SHA1) makes sure that users do not get certificate warnings when accessing the VeriSeq NIPT Assay Software v2. The SHA1 appears after generating or regenerating a certificate. See *Regenerating a Certificate* on page 25 for more information.

- 2 Select **Save** to implement any changes made.

Changing Network and Server Settings



NOTE

Coordinate all network and server setting changes with the IT administrator to avoid server connection errors.

- 1 Use the Network Configuration tab to set up or change the network and Onsite Server settings.
 - ▶ **Static IP Address**—IP address designated for the Onsite Server.
 - ▶ **Subnet Mask**—Local network subnet mask.
 - ▶ **Default Gateway Address**—Default router IP address.
 - ▶ **Hostname**—Designated name to reference the Onsite Server on the network (defined as localhost by default).
 - ▶ **DNS Suffix**—Designated DNS suffix.
 - ▶ **Nameserver 1 and 2**— DNS server IP addresses or names.
 - ▶ **NTP Time Server 1 and 2**—Servers for NTP time synchronization.
 - ▶ **MAC Address**—Server networking MAC address (read only).
 - ▶ **Timezone**—Server local time zone.
- 2 Confirm that the entries are correct, and select **Save** to reboot the server and implement any changes made.



CAUTION

Incorrect settings can disrupt the connection with the server.

Downloading and Installing a Certificate

To download and install an SSL certificate:

- 1 From the Dashboard, select **Configuration**.
- 2 Select the **Certification Configuration** tab.
- 3 Select **Download Certificate** from the Network Configuration screen.
- 4 Open the downloaded file, and select **Install Certificate**.
- 5 Follow the prompts in the import wizard to install the certificate.
- 6 Select **OK** in the dialog boxes to close them.

Regenerating a Certificate



NOTE

Only Service Engineers and Administrators have permission to regenerate certificates and reboot the system.

To regenerate a certificate after network or certificate settings have changed:

- 1 Select **Regenerate Certificate** from the Network Configuration screen.

- 2 Select **Regenerate Certificate and Reboot** to proceed, or select **Cancel** to exit.

Configuring System Email Notifications

The VeriSeq NIPT Assay Software v2 communicates with users by sending email notifications indicating the assay progress and alerts for errors or required user action. *Assay Software Notifications on page 55* describes the various email notifications sent by the system.




NOTE

Make sure that the email spam settings allow email notifications from the server. Email notifications are sent from an account named **VeriSeq@<customer email domain>**, where the **<customer email domain>** is specified by the local IT team when the server is installed.

Creating an Email Subscriber List

Email notifications are sent to a list of specified subscribers that can be defined in the following steps.

To specify a subscriber list:

- 1 From the Dashboard, select the Settings icon .
- 2 Select **Email Configuration**.
- 3 In the Subscribers field, enter email addresses separated by commas.
Verify that the email addresses are entered correctly. The software does not validate email address format.
- 4 Select **Send test message** to generate a test email to the subscribers list.
Check your email inbox to verify that the email was sent.
- 5 Select **Save**.

Logging Out

- ▶ Select the user profile icon in the upper right-hand corner of the screen, and select **Log Out**.

Analysis and Reporting

After sequencing data are collected, they are demultiplexed, converted to a FASTQ format, aligned to a reference genome, and analyzed for aneuploidy detection. Various metrics, as described below, are determined to qualify the final answer for any given sample.

Demultiplexing and FASTQ Generation

Sequencing data stored in BCL format are processed through the bcl2fastq conversion software, which demultiplexes data and converts BCL files to standard FASTQ file formats for downstream analysis. For each sequencing run, the Assay Software creates a sample sheet (SampleSheet.csv). This file contains sample information provided to the software during the sample prep process (using the software API). These sample sheets contain a header with information about the run and descriptors for the samples processed in a particular flow cell.

The following table provides sample sheet data details.



CAUTION

Do NOT modify or edit this sample sheet file. It is system generated and modifications can cause adverse effects downstream, including incorrect results or analysis failure.

Column Name	Description
SampleID	Sample identification.
SampleName	Sample name. Default: same as SampleID.
Sample_Plate	Plate identification for a given sample. Default: blank.
Sample_Well	Well identification on the plate for a given sample.
I7_Index_ID	Identification of the first index adapter.
index	Nucleotide sequence of the first adapter.
I5_Index_ID	Identification of the second adapter.
index2	Nucleotide sequence of the second adapter.
Sample_Project	Project identification for a given sample. Default: blank.
SexChromosomes	Analysis pertaining to sex chromosomes. One of the following: <ul style="list-style-type: none"> • Yes—Sex chromosome aneuploidy and sex reporting requested. • No—Neither sex chromosome aneuploidy nor sex reporting requested. • SCA—Sex chromosome aneuploidy reporting requested, sex reporting not requested.
SampleType	Sample type. One of the following: <ul style="list-style-type: none"> • Singleton—Single embryo pregnancy. • Twin—Multiple embryo pregnancy. • Control—Control sample of known sex and aneuploidy classification. • NTC—No template control sample (no DNA).

Sequencing QC

Sequencing QC metrics identify flow cells that are likely to fail analysis with high probability. The cluster density, percent reads passing filter (PF), prephasing, and phasing metrics describe the general sequencing data quality and are common to many next-generation sequencing applications. The predicted aligned reads metric estimates the flow cell level of the sequencing depth. If low-quality data fails the predicted aligned reads metric, processing the run is terminated. For more information, see [Sequencing QC Metrics and Boundaries on page 32](#).

Fetal Fraction Estimates

Fetal fraction refers to the percent of cell-free, circulating DNA in a maternal blood sample that is derived from the placenta. The Assay Software uses information from both the cfDNA fragment size distribution and the differences in genomic coverage between maternal and fetal cfDNA to calculate a fetal fraction estimate.¹

Statistics Used in Final Scoring

For all chromosomes, paired-end sequencing data are aligned with the reference genome (HG19). Unique nonduplicated aligned reads are aggregated into 100 kb bins. The corresponding bin counts are adjusted for GC bias and according to previously established region-specific genomic coverage. Using such normalized bin counts, statistical scores are derived for each autosome by comparing the coverage regions that can be affected by aneuploidy with the rest of the autosomes. A log likelihood ratio (LLR) is computed for each sample by taking into account these coverage-based scores and the estimated fetal fraction. The LLR is the probability of a sample being affected given the observed coverage and fetal fraction versus the probability of a sample being unaffected given the same observed coverage. The calculation of this ratio also takes into

¹Kim, S.K., et al, Determination of fetal DNA fraction from the plasma of pregnant women using sequence read counts, *Prenatal Diagnosis* Aug 2015; 35(8):810-5. doi: 10.1002/pd.4615

account the estimated uncertainty in fetal fraction. For subsequent calculations, the natural logarithm of the ratio is used. The Assay software assesses the LLR for each target chromosome and each sample to provide a determination of aneuploidy.

Statistics for chromosomes X and Y are different from the statistics used for autosomes. For fetuses identified as female, SCA calls require classification agreement by LLR and by normalized chromosomal value.¹ Specific LLR scores are calculated for [45,X] (Turner syndrome) and for [47,XXX]. For fetuses identified as male, SCA calls for either [47,XXY] (Klinefelter syndrome) or [47,XYY] can be based on the relationship between the normalized chromosomal values for chromosomes X and Y (NCV_X and NCV_Y). Samples pertaining to male fetuses for which NCV_X is in the range observed for euploid female samples can be called [47,XXY]. Samples pertaining to male samples for which NCV_X is in the range observed for euploid male samples but for which chromosome Y is over represented can be called [47,XYY].

Some values of NCV_Y and NCV_X fall outside of the system's ability to make a determination of SCA. These samples produce a Not Reportable result for XY classification. Autosomal results are still provided for these samples if all other QC metrics are passed.

Analysis QC

Analytical QC metrics are metrics that are computed during analysis and are used to detect samples that deviate too far from expected behavior. Data for samples that fail these metrics are deemed to be unreliable and are marked as failed. When samples produce results outside the expected ranges for these metrics, the NIPT Report provides a QC reason as a warning or a cause of failure. Refer to *QC Reason Messages on page 41* for more information about these QC reasons.

NTC Samples QC

The VeriSeq NIPT Solution allows the addition of NTC samples as part of your run. The ML STAR can generate up to 2 NTCs per run for 24-sample and 48-sample batches and up to 4 NTCs for 96-sample batches. Regardless of how many NTC samples are added, the software checks for a minimum average of 4,000,000 unique mapped fragments per sample per pool. For this reason, do not add more than 2 NTC samples per pool. For more information, see *Sequencing QC Metrics and Boundaries on page 32*.

QC statuses for NTC samples are:

- ▶ **NTC sample processing**—When processing an NTC sample, the software applies a PASS QC result when coverage for the sample is low, as expected for NTC.
- ▶ **Patient sample as NTC**—When a patient sample, marked as NTC, is processed, high coverage is detected. Because the sample is marked as NTC, the software flags the sample QC status as FAIL with the following reason: NTC SAMPLE WITH HIGH COVERAGE.

VeriSeq Onsite Server v2

The VeriSeq Onsite Server v2 runs a Linux-based operating system and provides about 7.5 TB storage capacity for data. Assuming 25 GB data size per sequencing run, the server can store up to 300 runs. An automated notification is issued when the minimum storage capacity is not available. The server is installed on the Local Area Network.

¹Bianchi D, Platt L, Goldberg J et al. Genome Wide Fetal Aneuploidy Detection by Maternal Plasma DNA Sequencing. *Obstet Gynecol.* 2012;119(5):890–901. doi:10.1097/aog.0b013e31824fb482.

Local Disk

The Assay Software makes specific folders on the Onsite Server available to the user. These folders can be mapped using a Samba share protocol to any workstation or laptop on the local network.

Folder Name	Description	Access
Input	Contains sequencing data generated by the next-generation sequencing system mapped to the server.	Read and write.
Output	Contains all software-generated reports.	Read only.
Backup	Contains database backups.	Read only.



NOTE

Mapping the local disk is based on Server Message Block (SMB) protocol. The software currently supports SMB1 and SMB2 versions. The server requires SMB signing. Enable these versions on the equipment (laptop/workstation) you are mapping.

Local Database

The Assay Software maintains a local database where the library information, sequencing run information, and analysis results are persisted. The database is an integral part of the Assay Software and is not accessible to the user. The system maintains an automatic mechanism for database backup on the Onsite Server. In addition to the following database processes, users are encouraged to back up the database regularly to an external location.

- ▶ **Database backup**—A snapshot of the database is automatically saved on an hourly, a daily, a weekly, and a monthly basis. Hourly backups are removed after a daily backup is created. Likewise, the daily backups are removed when the weekly backup is ready. The weekly backups are removed after a monthly backup is created, and only 1 monthly backup is kept. The recommended practice is to create an automated script that can persist the backup folder on a local NAS.
- ▶ **Database restore**—The database can be restored from any given backup snapshot. Restores are done by Illumina Field Service Engineers only.
- ▶ **Data backup**—Although the Onsite Server can be used as the main storage point for sequencing runs, it can only store approximately 300 runs. Illumina recommends setting up an automated data backup that runs on a continuous basis to another long-term storage device or an NAS.
- ▶ **Maintenance**—Other than data backup, the Onsite Server does not require the user to perform any maintenance. Updates for the Assay Software or the Onsite Server itself are provided by Illumina Technical Support.

Archiving Data

Consult your local IT site archiving policy to determine how to archive the input and output directories. The Assay Software monitors the remaining disk space in the input directory and notifies users by email when the remaining storage capacity goes below 1 TB.

Do not use the Onsite Server for data storage. Transfer data to the Onsite Server and archive on a regular schedule.

A typical sequencing run that is compatible with the cfDNA analysis workflow requires 25–30 GB for next-generation sequencer runs. The actual run folder size depends on final cluster density.

Only archive data when the system is idle and no analysis or sequencing runs are in progress.

Mapping Server Drives

The Onsite Server has 3 folders that can be individually mapped to any computer with Microsoft Windows:

- ▶ **input**—Maps to the sequencing data folders. Mount on the computer connected to the sequencing system. Configure the sequencing system to stream data to the input folder.
- ▶ **output**—Maps to the server analysis reports and assay process reports.
- ▶ **backup**—Maps to the database backup files.

To map each folder:

- 1 Log in to the computer within the Onsite Server subnetwork.
- 2 Right-click **Computer**, and select **Map network drive**.
- 3 Select a letter from the Drive drop-down list.
- 4 In the Folder field, enter \\<VeriSeq Onsite Server v2 IP address>\<folder name>. For example: \\10.50.132.92\input.
- 5 Enter the user name and password.
Successfully mapped folders appear mounted on the computer.



NOTE

Mapping the local disk is based on Server Message Block (SMB) protocol. The software currently supports SMB1 and SMB2 versions. The server requires SMB signing. Enable these versions on the equipment (laptop/workstation) you are mapping.

Rebooting the Server



NOTE

Only Service Engineers and Administrators have permission to reboot the server.

To reboot the server:

- 1 On the **Settings** drop-down list, select **Reboot Server**.
- 2 Select **Reboot** to reboot the system, or **Cancel** to exit without rebooting.
- 3 Enter a reason for shutting down the server.
The reason is logged for troubleshooting purposes.



NOTE

Rebooting the system can take several minutes.

Shutting Down the Server



NOTE

Only Service Engineers and Administrators have permission to shut down the server.

To shut down the Onsite Server server:

- 1 On the **Settings** drop-down list, select **Shut Down Server**.
- 2 Select **Shut Down** to shut down the Onsite Server, or select **Cancel** to exit without shutting down.
- 3 Enter a reason for shutting down the Onsite Server.
The reason is logged for troubleshooting purposes.

Recovering from Unexpected Shutdown

In the event of a power outage or accidental shutdown by the user during an analysis run, the system:

- ▶ Automatically restarts the Assay Software upon reboot.
- ▶ Recognizes that the analysis run failed and resubmits the run to the queue for processing.
- ▶ Generates output when analysis successfully completes.



NOTE

If analysis fails, the Assay Software allows the system to resubmit the run for analysis up to 3 times.

Appendix A QC Metrics

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Quantitation QC Metrics and Boundaries

Metric	Description	Lower Bound	Upper Bound	Rationale
standard_r_squared	R-squared value of the standards curve model.	0.980	N/A	Standards curve models showing poor linearity in log-log space are not good predictors of true sample concentrations.
standard_slope	Slope of the standards curve model.	0.95	1.15	Standards curve models that slope outside expected performance bands indicate an unreliable model.
ccn_library_pg_ul	Maximum allowable sample concentration.	N/A	1000 pg/μl	Samples with calculated DNA concentrations that exceed specifications indicate excess genomic DNA contamination.
median_ccn_pg_ul	Median calculated concentration value for all samples in batch.	16 pg/μl	N/A	A sequencing pool of appropriate volume cannot have an excessive number of overly dilute samples. Batches with high numbers of dilute samples indicate sample prep process failure.

Sequencing QC Metrics and Boundaries

Metric	Description	Lower Bound	Upper Bound	Rationale
cluster_density	Sequencing cluster density.	152,000 per mm ²	338,000 per mm ²	Flow cell with low cluster density does not generate enough reads. Over clustered flow cells usually produce sequencing data of low quality.
pct_pf	Percent reads passing chastity filter.	≥50%	N/A	Flow cells with extremely low %PF can have abnormal base representation and are likely to indicate problems with PF reads.
prephasing	Fraction of prephasing.	N/A	≤0.003	Empirically optimized recommendations for the VeriSeq NIPT Solution v2.
phasing	Fraction of phasing.	N/A	≤0.004	Empirically optimized recommendations for the VeriSeq NIPT Solution v2.
predicted_aligned_reads	Estimated average number of uniquely mapped fragments per sample.	≥4,000,000	N/A	Determined as minimal observed NES across normal population.

Appendix B System Reports

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Introduction

The Assay Software generates two categories of reports:

- ▶ Results and notifications reports.
- ▶ Process reports.

There are also two report types:

- ▶ **Informational**—Process-related report that provides information on the assay progress and can be used to confirm the completion of a specific step. The report also provides information such as QC results and ID numbers.
- ▶ **Actionable**—Asynchronous report triggered by a system event or user action that requires attention by the user.

This section describes each report and provides the report details for LIMS integration.

Output Files

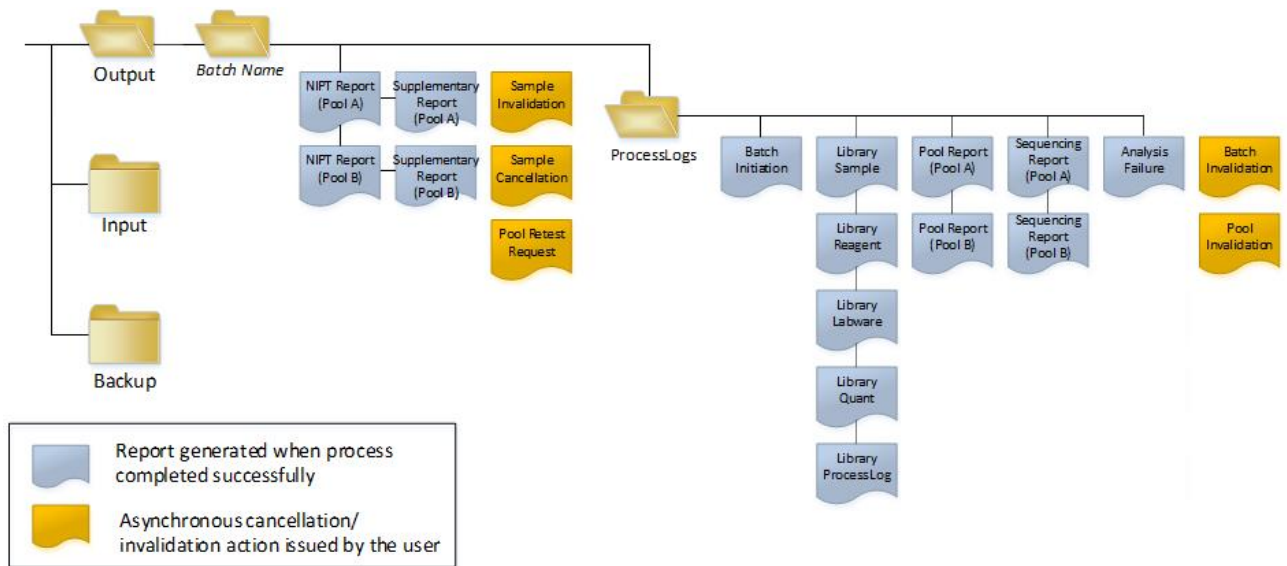
The Assay Software reports are generated on the Onsite Server internal hard drive mapped to the user drive as a read-only Output folder. Each report is generated with a corresponding standard MD5 checksum file, which is used to verify that the file has not been modified.

All reports are plain text formatted as tab delimited. The reports can be opened with any text editor or with a tabulated data program, such as Microsoft Excel.

Reports File Structure

The Assay Software saves reports in a specific structure under the Output folder.

Figure 5 Assay Software Reports Folder Structure



The Assay Software saves reports to the *Batch Name* folder with the following organization:

- ▶ **Main folder (Batch Name folder)**—Contains reports that provide results or are associated with LIMS-generated email notifications. For details, see *Results and Notifications Reports* on page 37.
- ▶ **ProcessLogs folder**—Contains reports related to process. For details, see *Process Reports* on page 47

A list of all reports is provided in *System Reports Summary* on page 34.

System Reports Summary

Report Name	Report Type	Report Entity	Report File Name Format
<i>NIPT Report</i>	Actionable	Pool/ flow cell	<batch_name>_<pool_type>_<pool_barcode>_<flowcell>_nipt_report_<YYYYMMDD_hhmmss>.tab
<i>Supplementary Report</i>	Informational	Pool/flow cell	<batch_name>_<pool_type>_<pool_barcode>_<flowcell>_supplementary_report_<YYYYMMDD_hhmmss>.tab
<i>Sample Invalidation Report</i>	Actionable	Sample	<batch_name>_<sample_barcode>_sample_invalidation_report_<YYYYMMDD_hhmmss>.tab
<i>Sample Cancellation Report</i>	Actionable	Sample	<batch_name>_<sample_barcode>_sample_cancellation_report_<YYYYMMDD_hhmmss>.tab
<i>Pool Retest Request Report</i>	Actionable	Pool	<batch_name>_<pool_type>_pool_retest_request_<YYYYMMDD_hhmmss>.tab
<i>Batch Initiation Report</i>	Informational	Batch	ProcessLogs/<batch_name>_batch_initiation_report_<YYYYMMDD_hhmmss>.tab
<i>Batch Invalidation Report</i>	Informational	Batch	ProcessLogs/<batch_name>_batch_invalidation_report_<YYYYMMDD_hhmmss>.tab

Report Name	Report Type	Report Entity	Report File Name Format
<i>Library Sample Report</i>	Informational	Batch	ProcessLogs/<batch_name>_library_sample_report_<YYYYMMDD_hhmmss>.tab
<i>Library Reagent Report</i>	Informational	Batch	ProcessLogs/<batch_name>_library_reagent_report_<YYYYMMDD_hhmmss>.tab
<i>Library Labware Report</i>	Informational	Batch	ProcessLogs/<batch_name>_library_labware_report_<YYYYMMDD_hhmmss>.tab
<i>Library Quant Report</i>	Informational	Batch	ProcessLogs/<batch_name>_library_quant_report_<YYYYMMDD_hhmmss>.tab
<i>Library Process Log</i>	Informational	Batch	ProcessLogs/<batch_name>_library_process_log.tab
<i>Pool Report</i>	Informational	Pool	ProcessLogs/<batch_name>_<pool_barcode>_pool_report_<YYYYMMDD_hhmmss>.tab
<i>Pool Invalidation Report</i>	Informational	Pool	ProcessLogs/<batch_name>_<pool_barcode>_pool_invalidation_report_<YYYYMMDD_hhmmss>.tab
<i>Sequencing Report</i>	Informational	Pool/ flow cell	ProcessLogs/<batch_name>_<pool_type>_<pool_barcode>_<flowcell>_sequencing_report_<YYYYMMDD_hhmmss>.tab
<i>Analysis Failure Report</i>	Informational	Pool/ flow cell	ProcessLogs/<batch_name>_<pool_barcode>_analysis_failure_report_<YYYYMMDD_hhmmss>.tab

Report Generation Events

Report	Description	Generation Event
NIPT Report	Contains the final results of a successful analysis run.	<ul style="list-style-type: none"> Sequencing run analysis completes.
Supplementary Report	Contains supplementary results for a successful analysis run.	<ul style="list-style-type: none"> Sequencing run analysis and NIPT report both complete.
Sample Invalidation	Contains information about an invalidated sample.	<ul style="list-style-type: none"> User invalidates a sample.
Sample Cancellation	Contains information about a canceled sample.	<ul style="list-style-type: none"> User cancels a sample.
Pool Retest Request	Indicates that a second pool can be generated from an existing batch. Contains information about the pool retest status. ¹	<ul style="list-style-type: none"> User invalidates a pool.
Batch Initiation	Indicates a new batch processing start.	<ul style="list-style-type: none"> User initiates a new batch.
Batch Invalidation	Contains information about a user-initiated invalidated batch.	<ul style="list-style-type: none"> Batch is invalidated.
Library Sample	Lists all samples in the batch.	<ul style="list-style-type: none"> Batch is invalidated. Library Preparation method completes. Batch fails quantification.
Library Reagent	Contains library processing reagent information.	<ul style="list-style-type: none"> Batch is invalidated. Library Preparation method completes. Batch fails quantification.

Report	Description	Generation Event
Library Labware	Contains library processing labware information.	<ul style="list-style-type: none"> • Batch is invalidated. • Library Preparation method completes. • Batch fails quantification.
Library Quant	Contains library quantification test results.	<ul style="list-style-type: none"> • Batch is invalidated. • Library Preparation method completes. • Batch fails quantification.
Library Process Log	Contains steps executed during library processing.	<ul style="list-style-type: none"> • Batch is invalidated. • Library Preparation method completes. • Batch fails quantification. • Batch process completes.
Pool	Contains sample pooling volumes.	<ul style="list-style-type: none"> • Pooling method completes.
Pool Invalidation	Contains information about a user-initiated invalidated pool.	<ul style="list-style-type: none"> • User invalidates a pool.
Sequencing	Contains sequencing QC results.	<ul style="list-style-type: none"> • Sequencing QC passes . • Sequencing fails. • Sequencing times out.
Analysis Failure	Contains analysis information for a failed pool.	<ul style="list-style-type: none"> • Sequencing run analysis fails.

¹ User invalidates a pool from a valid batch that has not exceeded the maximum number of pools.

Results and Notifications Reports

NIPT Report

The NIPT Report for the VeriSeq NIPT Assay Software v2 contains the chromosome classification results formatted as one sample per row for each sample in the pool.

Column	Description	Preset Value Options	Type	Regex
batch_name	Batch name.	Not applicable.	text	^[a-zA-Z0-9_-]{1,36}\$
sample_barcode	Unique sample barcode.	Not applicable.	text	^[a-zA-Z0-9_-]{1,36}\$
sample_type	Sample type information provided from point of collection or lab user. Determines aneuploidy classification presentation.	One of the following: <ul style="list-style-type: none"> • Singleton—Single embryo pregnancy. • Twin—Multiple embryo pregnancy. • Control—Control sample of known sex and aneuploidy classification. • NTC—No template control sample (no DNA). • Not specified—A sample type was not provided for this sample. 	enum	<i>Values specified in Preset Value Options.</i>
sex_chrom	Sex chromosome analysis requested. Determines the presentation of aneuploidy classification and sex chromosome information.	One of the following: <ul style="list-style-type: none"> • Yes—Sex chromosome aneuploidy and sex reporting requested. • No—Neither sex chromosome aneuploidy nor sex reporting requested. • SCA—Sex chromosome aneuploidy reporting requested, sex reporting not requested. • Not specified—A sex chromosome reporting option was not provided for this sample. 	enum	<i>Values specified in Preset Value Options.</i>
screen_type	Screening type.	One of the following: <ul style="list-style-type: none"> • Basic— Screen chromosomes 13, 18, or 21. • Genomewide— Screen entire genome. • Not specified— A screen type was not provided for this sample. 	text	<i>Values specified in Preset Value Options.</i>
flowcell	Sequencing flow cell barcode.	Not applicable.	text	^[a-zA-Z0-9_-]{1,36}\$

Column	Description	Preset Value Options	Type	Regex
class_sx	Sex chromosome aneuploidy classification.	One of the following depending on sample type and sex chromosome reporting options selected: <ul style="list-style-type: none"> • ANOMALY DETECTED – XO— Monosomy X. • ANOMALY DETECTED – XXX— Trisomy X. • ANOMALY DETECTED – XXY— 2 X chromosomes in a male fetus. • ANOMALY DETECTED – XYY— 2 Y chromosomes. • NO ANOMALY DETECTED— Negative sample and sex not reported. • NO ANOMALY DETECTED – XX— Negative sample with a female fetus. • NO ANOMALY DETECTED – XY— Negative sample with male fetus. • NOT REPORTABLE— Software could not report sex chromosome. • NO CHR Y PRESENT— Twin pregnancy with no Y chromosome detected. • CHR Y PRESENT— Twin pregnancy with Y chromosome detected. • CANCELLED— Sample canceled by the user. • INVALIDATED— Sample has failed QC or invalidated by the user. • NOT TESTED— Sex chromosome was not tested. • NA— Category is not applicable to sample. 	class_sx	<i>Values specified in Preset Value Options.</i>
class_auto	Classification for aneuploidies in autosomes. Reported as ANOMALY DETECTED if an anomaly within the selected screen type was detected for the sample.	One of the following: <ul style="list-style-type: none"> • ANOMALY DETECTED— Autosomal chromosomal anomaly detected. • NO ANOMALY DETECTED— No autosomal anomaly detected. • CANCELLED— Sample canceled by the user. • INVALIDATED— Sample has failed QC or invalidated by the user. • NA— Category is not applicable to sample. 	text	<i>Values specified in Preset Value Options.</i>
anomaly_description	ISCN-style string that describes all reportable anomalies. Multiple anomalies are separated by semi-colons.	DETECTED: followed by semicolon-separated strings concatenating the following formats, in chromosome order: (\+ -)[12]?[0-9] (del dup)\{[12]?[0-9]\}\(((p q)[0-9]{1,2}\.[0-9]{1,2})?)?{2}\) XO XXX XXY XYY or NO ANOMALY DETECTED NA INVALIDATED CANCELLED .	text	<i>Semicolon-separated strings and other values described in the Anomaly Description Rules section.</i>

Column	Description	Preset Value Options	Type	Regex
qc_flag	QC analysis results. Only the qc_flag values of WARNING and PASS report results. All other values do not.	One of the following: <ul style="list-style-type: none"> • PASS • WARNING • FAIL • CANCELLED • INVALIDATED • NTC_PASS 	enum	<i>Values specified in Preset Value Options.</i>
qc_reason	QC failure or warning information.	One of the following: <ul style="list-style-type: none"> • NONE (QC status = PASS) • MULTIPLE ANOMALIES DETECTED (QC status = WARNING) • FAILED IFACT • DATA OUTSIDE OF EXPECTED RANGE • FRAGMENT SIZE DISTRIBUTION OUTSIDE OF EXPECTED RANGE • FLOWCELL DATA OUTSIDE OF EXPECTED RANGE • FAILED TO ESTIMATE FETAL FRACTION • SEQUENCING DATA OUTSIDE OF EXPECTED RANGE • UNEXPECTED DATA • NTC SAMPLE WITH HIGH COVERAGE • CANCELLED • INVALIDATED 	text	<i>Values specified in Preset Value Options.</i>
ff	Estimated fetal fraction.	Percent sample cfDNA from fetus rounded to the nearest integer. Results less than 1% are presented as < 1%.	text	<i>Not applicable.</i>

Anomaly Description Rules

If the VeriSeq NIPT Assay Software v2 analysis identifies an anomaly, the `anomaly_description` field in the NIPT Report displays the value DETECTED followed by a string of text. This text describes all reportable anomalies based on the International Standing Committee on Cytogenetic Nomenclature (ISCN) style. The string contains multiple elements separated by semicolons. Each element represents a trisomy or monosomy in an autosome, a sex chromosome aneuploidy, or a partial deletion or duplication.

Trisomy and monosomy elements are noted as `+<chr>` and `-<chr>`, respectively, where `<chr>` is the chromosome number.

For example, a sample with a trisomy on chromosome 5 appears as follows:

+5

A sample with a monosomy on chromosome 6 appears as follows:

-6

Sex chromosome aneuploidies use standard notation, with four possible values:

- ▶ XO - for monosomy on chromosome X.
- ▶ XXX - for trisomy on chromosome X.
- ▶ XXY - for 2 X chromosomes in males.
- ▶ XYY - for 2 Y chromosomes in males.

Partial deletions or duplications only report for autosomes and only appear in genomewide screenings. The syntax of a partial deletion or duplication is `<type>(<chr>(<start band><end band>))`, where:

- ▶ `<type>` is the event type, either `del` for deletion or `dup` for duplication.
- ▶ `<chr>` is the chromosome number.
- ▶ `<start band>` is the cyto band containing the start of the event.
- ▶ `<end band>` is the cyto band containing the end of the event.

For example, a partial deletion or duplication in which the cytoband between p14 and q15 on chromosome 22 has a duplication appears as follows:

dup (22) (p14q15)

The `anomaly_description` field follows three ordering rules:

- 1 Elements are ordered by the chromosome number, regardless of whether an entire chromosome or a partial deletion or duplication. A sex chromosome aneuploidy, if present, appears last.
- 2 For partial deletions or duplications within the same chromosome, deletions come before duplications.
- 3 Partial deletions or duplications of the same type within the same chromosome are ordered by the starting base, which appears in the Supplementary Report.

QC Reason Messages

The qc_reason column in the NIPT Report displays a QC failure or warning when analysis results fall outside the expected range for an analytical QC metric. QC failures result in the full results suppression for chromosome aneuploidy, sex classification, supplementary report results, and estimated fetal fraction, which correspond to the following NIPT Report fields: class_auto, class_sx, anomaly_description, and ff.

QC Reason Message	Description	Recommended Action
FAILED iFACT	individual Fetal Aneuploidy Confidence Test (iFACT)—QC metric that combines fetal fraction estimation with run metrics associated with coverage to determine whether the system has statistical confidence to make a call on a given sample.	Reprocess sample.
DATA OUTSIDE OF EXPECTED RANGE	The average deviation from euploid coverage is not consistent with the trained data distribution. Possibly caused by contamination or incorrect sample processing.	Reprocess sample.
FRAGMENT SIZE DISTRIBUTION OUTSIDE OF EXPECTED RANGE	The fragment size distribution is not consistent with the trained data distribution. Possibly caused by contamination or incorrect sample processing.	Reprocess sample.
FLOWCELL DATA OUTSIDE OF EXPECTED RANGE	Flowcell data is not consistent with the trained data distribution. Possibly caused by error in flowcell setup.	Reprocess sample.
FAILED TO ESTIMATE FETAL FRACTION	Unable to produce a valid fetal fraction estimate.	Reprocess sample.
SEQUENCING DATA OUTSIDE OF EXPECTED RANGE	Input sequencing data is not consistent with the trained data distribution. Possibly caused by contamination or incorrect sample processing.	Resequence flowcell.
UNEXPECTED DATA	The report generates a QC concern that does not correspond to any of the other QC reasons listed in this table.	Contact Illumina Technical Support.
MULTIPLE ANOMALIES DETECTED	Two or more reportable anomalies (including whole chromosome aneuploidies and partial deletions or duplications) are detected in the sample. The detection of multiple anomalies may indicate a mishandling of the sample or a more rare event, such as maternal malignancy. This message is a warning. It does not represent a QC failure. Results are reported so you can see the detected anomalies. However, you may need to reprocess the sample.	Reprocess sample.

QC Reason Message	Description	Recommended Action
NTC SAMPLE WITH HIGH COVERAGE	High coverage detected for an NTC sample (no DNA material expected). Possibly caused by contamination or incorrect sample processing.	Reprocess sample.
CANCELLED	Sample was canceled by a user.	Not applicable.
INVALIDATED	Sample was invalidated by a user.	Not applicable.

Supplementary Report

The Supplementary Report contains data for additional metrics based on a batch, sample, or region. In this report, each row represents a metric. Multiple metrics apply for the same batch, sample, or region.

The tab-separated file has six columns, as described in the table below.

Column	Description	Type	Regex
flowcell	Barcode for the flowcell.	text	^[a-zA-Z0-9_]{1,36}\$
batch_name	Name of the relevant batch.	text	^[a-zA-Z0-9_]{1,36}\$
sample_barcode	Barcode for the sample.	text	NA (not applicable) for per-batch metrics. ^[a-zA-Z0-9_]{1,36}\$
region	Either the whole chromosome or a description of the region of the partial deletion or duplication.	text	NA (not applicable) – for per-batch or per-sample metrics. chr[12]?[0-9X] – for whole chromosome region metrics. (del dup)\{[12]?[0-9X]\}\(((p q)[0-9]{1,2}(\.[0-9]{1,2})?)\{2\}\) – for partial deletion or duplication region metrics.
metric_name	Name of the metric described.	text	^[a-zA-Z0-9_]{1,36}\$
metric_value	Value of the metric.	varies	<i>Refer to Supplementary Report Metrics.</i>

Supplementary Report Metrics

The Supplementary Report contains data for the following metrics. Each metric appears on a per batch, per sample, or per region basis.

Metrics for Chromosome X only appear if you select the sex chromosome options Yes or SCA.

Value ranges appear as Minimum Value, Maximum Value surrounded by either parenthesis or brackets. Parenthesis indicate that an edge value is excluded from the range, while brackets indicate that an edge value is included in the range. Inf is an abbreviation for infinity.

Metric Name	Frequency	Description	Type	Regex or Value Range
genome_assembly	Per batch	The system of coordinates for alignment of the sequencing data and report region coordinates. Always GRCh37 for VeriSeq NIPT Solution v2.	text	^GRCh37\$
frag_size_dist	Per sample	Standard deviation of the differences between actual and expected cumulative fragment size distributions.	float	(0, Inf)
fetal_fraction	Per sample	Reported fetal fraction.	float	(0, 1)

Metric Name	Frequency	Description	Type	Regex or Value Range
NCV_X	Per sample	Normalized chromosome value for X chromosome. Only appears if the sex chromosome reporting option allows for it. Otherwise, this metric appears as NOT TESTED.	float	(-Inf, Inf)
NCV_Y	Per sample	Normalized chromosome value for Y chromosome. Only appears if the sex chromosome reporting option allows for it. Otherwise, this metric appears as NOT TESTED.	float	(-Inf, Inf)
number_of_cnv_events	Per sample	The number of partial deletion or duplication regions detected in the sample.	integer	(0, Inf)
non_excluded_sites	Per sample	The number of reads remaining after filtering which are counted for analysis.	integer	(0, Inf)
region_classification	Per region	Classification of the region by the system in the same format as the anomaly_description field in the NIPT Report. For chromosome X, if no reportable sex chromosome anomaly was detected, the region classification will match the value of class_sx in the NIPT Report. Value options (regex): DETECTED: (\+ -)[12]?[0-9] DETECTED: (del dup)\{[12]?[0-9]\}\(((p q)[0-9]{1,2}(\.[0-9]{1,2})?)?{2}\) NO ANOMALY DETECTED DETECTED: (XO XXX XXY XYY) NO ANOMALY DETECTED - XX NO ANOMALY DETECTED - XY NOT REPORTABLE CHR Y PRESENT CHR Y NOT PRESENT	text	<i>Values specified in Description.</i>
chromosome	Per region	The chromosome symbol.	text	chr[12]?[0-9X]
start_base	Per region	First base included in the region.	integer	[1, Inf)
end_base	Per region	Last base included in the region.	integer	[1, Inf)
start_cytoband	Per region	Cytogenetic band of the first base included in the region.	text	(p q)[0-9]{1,2}(\.[0-9]{1,2})?
end_cytoband	Per region	Cytogenetic band of the last base included in the region.	text	(p q)[0-9]{1,2}(\.[0-9]{1,2})?
region_size_mb	Per region	The size of the region in megabases.	float	(0, Inf)

Metric Name	Frequency	Description	Type	Regex or Value Range
region_llr_trisomy	Per region	The LLR (Log-Likelihood Ratio) score for trisomy for the region. Indicates the evidence for trisomy compared to the evidence for no change (disomy). A trisomy is called if this LLR score exceeds a predetermined threshold. For partial deletions or duplications, this metric only appears if the type is a gain (dup). Otherwise, this metric appears as NA (not applicable).	float	(-Inf, Inf)
region_llr_monosomy	Per region	The LLR score for monosomy for the region. Indicates the evidence for monosomy compared to the evidence for no change (disomy). A monosomy is called if this LLR score exceeds a predetermined threshold. For partial deletions or duplications, this metric only appears if the type is a loss (del). Otherwise, this metric appears as NA (not applicable). This metric appears as NOT TESTED if you choose to perform the basic screening type.	float	(-Inf, Inf)
region_t_stat_long_reads	Per region	The t-statistic for the region. The t-statistic is the difference in coverage between the region and the rest of the genome, compared to the variation in the sample. This is a signal-to-noise metric that captures the detectability of any shift in coverage in the region. "long_reads" indicates that the coverage used for this t-statistic includes the full range of fragment sizes used in analysis. The t-statistic is combined with the fetal fraction estimated for the sample to generate LLR scores.	float	(-Inf, Inf)
region_mosaic_ratio	Per region	The proportion of the fetal material that is aneuploid. This metric is based on the ratio of the fetal fraction inferred from the coverage of the region to the fetal fraction for the sample. In samples with fetal fractions close to zero, mosaic ratios can take negative values due to variability in the estimate of the sample fetal fraction used in their calculation.	float	(-Inf, Inf)
region_mosaic_llr_trisomy	Per region	The LLR score for trisomy calculated using the fetal fraction inferred from the coverage in the region instead of the fetal fraction for the sample. For partial deletions or duplications, this metric only appears if the type is a gain (dup). Otherwise, this metric appears as NA (not applicable).	float	(-Inf, Inf)
region_mosaic_llr_monosomy	Per region	The LLR score for monosomy calculated using the fetal fraction inferred from the coverage in the region instead of the fetal fraction for the sample. For partial deletions or duplications, this metric only appears if the type is a loss (del). Otherwise, this metric appears as NA (not applicable). This metric appears as NOT TESTED if you choose to perform the basic screening type.	float	(-Inf, Inf)

Sample Invalidation Report

The system generates a Sample Invalidation Report for each sample invalidated or failed.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
sample_barcode	Unique barcode of the invalidated sample.	text	^[a-zA-Z0-9_-]{1,36}\$
reason	User-provided reason for sample invalidation.	text	^[a-zA-Z0-9_-]{1,36}\$
operator	Username of the operator who invalidated or failed the sample.	text	^[a-zA-Z0-9_-]{1,36}\$
timestamp	Date and time of sample invalidation.	ISO 8601 timestamp	

Sample Cancellation Report

The system generates a Sample Cancellation Report for each sample canceled.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
sample_barcode	Unique barcode of the canceled sample.	text	^[a-zA-Z0-9_-]{1,36}\$
reason	User-provided reason for sample cancellation.	text	^[a-zA-Z0-9_-]{1,36}\$
operator	Username of the operator who canceled the sample.	text	^[a-zA-Z0-9_-]{1,36}\$
timestamp	Date and time of sample cancellation.	ISO 8601 timestamp	

Pool Retest Request Report

The Pool Retest Request Report indicates that an invalidated pool can be repooled. The system generates a Pool Retest Request Report when the first of two possible sequence runs (pools) for that pool type is invalidated.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
pool_type	Type of the pool.	enum	A B C E
reason	User-provided reason for invalidating the previous pool.	text	^[a-zA-Z0-9_-]{1,36}\$
timestamp	Date and time of request.	ISO 8601 timestamp	

Process Reports

Batch Initiation Report

The system generates a Batch Initiation Report when a batch is initiated and validated successfully before plasma isolation.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
sample_barcode	Unique sample barcode.	text	^[a-zA-Z0-9_-]{1,36}\$
sample_type	Sample type of the sample barcode.	enum	singleton control twin ntc
well	Well associated with a sample.	text	^[a-zA-Z]{1,1}[0-9]{1,2}\$
assay	Assay name.	text	^[a-zA-Z0-9_-]{1,100}\$
method_version	Assay automation method version.	text	VeriSeq v2 NIPT Assay
workflow_manager_version	Workflow manager version associated with the batch.	text	^[a-zA-Z0-9_-]{1,100}\$

Batch Invalidation Report

The system generates a Batch Invalidation Report when the batch is invalidated or failed.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
reason	User-provided reason for batch invalidation.	text	^[a-zA-Z0-9_-]{1,36}\$
operator	Initials of the operator invalidating the batch.	text	^[a-zA-Z0-9_-]{1,36}\$
timestamp	Date and time of batch invalidation.	ISO 8601 timestamp	

Library Sample Report

The system generates a Library Sample Report at batch failure or invalidation, at successful library completion, and at successful quantification completion.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
sample_barcode	Unique sample barcode.	text	^[a-zA-Z0-9_-]{1,36}\$
qc_status	Sample status after completion of the assay steps.	enum	pass fail
qc_reason	Reason for QC status.	text	^[a-zA-Z0-9_-]{1,36}\$
starting_volume	Initial volume of blood collection tube in ml at the time of plasma isolation.	float	
index	Index associated with a sample.	text	^[a-zA-Z0-9_-]{1,36}\$
ccn_library_pg_ul	Library concentration in pg/μl.	float	
plasma_isolation_comments	User comments when performing plasma isolation (free text).	text	^[a-zA-Z0-9_-]{1,512}\$
cfdna_extraction_comments	User comments when performing cfDNA extraction (free text).	text	^[a-zA-Z0-9_-]{1,512}\$
library_prep_comments	User comments when performing library preparation (free text).	text	^[a-zA-Z0-9_-]{1,512}\$
quantitation_comments	User comments when performing quantification (free text).	text	^[a-zA-Z0-9_-]{1,512}\$

Library Reagent Report

The system generates a Library Reagent Report at batch failure or invalidation, at successful library completion, and at successful quantification completion.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
process	Process name, in the format of PROCESS:sub-process. Value options: <ul style="list-style-type: none"> • ISOLATION—batch_validation, prespin, postspin, data_transact. • EXTRACTION—setup, chemistry, data_transact. • LIBRARY—setup, chemistry, data_transact, complete. • QUANT—setup, build_standards, build_384, analysis, data_transact. • POOLING—analysis, setup, pooling, data_transact, complete. 	text	^[A-Z]{1,36}:[a-z0-9_]{1,36}\$
reagent_name	Reagent name.	text	^[a-zA-Z0-9_-]{1,36}\$
lot	Reagent barcode.	text	^[a-zA-Z0-9_-]{1,36}\$
expiration_date	Expiration date in manufacturer format.	text	^[a-zA-Z0-9:/_ -]{1,100}\$
operator	Username of the operator.	text	^[a-zA-Z0-9_-]{1,36}\$
initiated	Initiation timestamp associated with reagent.	ISO 8601 timestamp	

Library Labware Report

The system generates a Library Labware Report at batch failure or invalidation, at successful library completion, and at successful quantification completion.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
labware_name	Labware name.	text	^[a-zA-Z0-9_-]{1,36}\$
labware_barcode	Labware barcode.	text	^[a-zA-Z0-9_-]{1,36}\$
initiated	Initiation timestamp associated with labware.	ISO 8601 timestamp	

Library Quant Report

The system generates a Library Quant Report at successful quantification completion.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
quant_id	Numerical identification.	long	
instrument	Quantification instrument name (free text).	text	^[a-zA-Z0-9_-]{1,36}\$
standard_r_squared	R-squared.	float	
standard_intercept	Intercept.	float	
standard_slope	Slope.	float	
median_ccn_pg_ul	Median sample concentration.	float	
qc_status	Quantification QC status.	enum	pass fail
qc_reason	Description of failure reason, if any.	text	^[a-zA-Z0-9_-]{1,36}\$
initiated	Initiation timestamp associated with quantification.	ISO 8601 timestamp	

Library Process Log

The system generates a Library Process Log at the start and completion or failure of each batch process; at batch failure or invalidation; and at analysis completion (generated per pool).

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
process	Batch process name, in the format of PROCESS:sub-process. Value options: ISOLATION —batch_validation, prespin, postspin, data_transact. EXTRACTION —setup, chemistry, data_transact. LIBRARY —setup, chemistry, data_transact, complete. QUANT —setup, build_standards, build_384, analysis, data_transact. POOLING —analysis, setup, pooling, data_transact, complete.	text	^[A-Z]{1,36}:^[a-z0-9_-]{1,36}\$
operator	Operator initials.	text	^[a-zA-Z0-9_-]{1,36}\$
instrument	Instrument name.	text	^[a-zA-Z0-9_-]{1,36}\$
started	Date and time of batch process start.	ISO 8601 timestamp	
finished	Date and time of batch process completion or failure.	ISO 8601 timestamp	
status	Current batch.	enum	completed failed started aborted

Pool Report

The system generates a Pool Report at successful library completion, at batch failure and at batch invalidation if the event occurs after pooling has started.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
sample_barcode	Unique sample barcode.	text	^[a-zA-Z0-9_-]{1,36}\$
pool_barcode	Pool barcode associated with a sample.	text	^[a-zA-Z0-9_-]{1,36}\$
pool_type	Pool type associated with a sample.	enum	A B C E
pooling_volume_ul	Pooling volume in μ l.	float	
pooling_comments	User comments when performing pooling (free text).	text	^[a-zA-Z0-9_-]{1,512}\$

Pool Invalidation Report

The system generates a Pool Invalidation Report when the pool is invalidated.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
pool_barcode	Pool barcode of the invalidated pool.	text	^[a-zA-Z0-9_-]{1,36}\$
reason	User-provided reason for pool invalidation.	text	^[a-zA-Z0-9_-]{1,36}\$
operator	Initials of the operator who invalidated the pool.	text	^[a-zA-Z0-9_-]{1,36}\$
timestamp	Date and time of pool invalidation.	ISO 8601 timestamp	

Sequencing Report

The system generates a Sequencing Report for the sequencing run when sequencing completes or sequencing times out.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
pool_barcode	Pool barcode associated with sequencing run.	text	^[a-zA-Z0-9_-]{1,36}\$
instrument	Sequencer serial number.	text	^[a-zA-Z0-9_-]{1,36}\$
flowcell	Flow cell associated with sequencing run.	text	^[a-zA-Z0-9_-]{1,36}\$
software_version	Concatenation of software application/version used to generate the data on the sequencer.	text	
run_folder	Sequencing run folder name.	text	^[a-zA-Z0-9_-]+\$
sequencing_status	Sequencing run status.	enum	completed timed out failed
qc_status	QC status of sequencing run.	enum	pass fail error
qc_reason	QC reasons for QC failure, semicolon-separated values.	text	^[a-zA-Z0-9_-]{1,36}\$
cluster_density	Cluster density (median per flow cell across tiles).	float	
pct_q30	Percent bases above Q30.	float	
pct_pf	Percent reads passing filter.	float	
phasing	Phasing.	float	
prephasing	Prephasing.	float	
predicted_aligned_reads	Predicted aligned reads.	long	
started	Timestamp associated with sequencing start.	ISO 8601 timestamp	
completed	Timestamp associated with sequencing completion.	ISO 8601 timestamp	

Analysis Failure Report

The system generates an Analysis Failure Report when the maximum number of analysis attempts fail for the sequencing run.

Column	Description	Type	Regex
batch_name	Batch name.	text	^[a-zA-Z0-9_-]{1,36}\$
pool_barcode	Pool barcode associated with failed analysis.	text	^[a-zA-Z0-9_-]{1,36}\$
flowcell	Flow cell barcode associated with failed analysis.	text	^[a-zA-Z0-9_-]{1,36}\$

Column	Description	Type	Regex
sequencing_run_folder	Sequencing Run folder associated with failed analysis.	text	^[a-zA-Z0-9_]+\$
analysis_run_status	Sequencing Run status associated with failed analysis.	text	^[a-zA-Z0-9_]+\$
timestamped	Timestamp associated with analysis start.	ISO 8601 timestamp	
timefinished	Timestamp associated with analysis failed.	ISO 8601 timestamp	

Appendix C Troubleshooting

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Introduction

VeriSeq NIPT Solution v2 troubleshooting assistance comprises:

- ▶ Assay Software and system notifications.
- ▶ Recommended actions for system issues.
- ▶ Instructions for performing preventive and failure analyses using preinstalled test data.

Assay Software Notifications

This section describes the Assay Software notifications:

Progress Notifications

Progress notifications indicate the normal progress of the assay execution. These notifications are logged as “Activities” and do not require any user actions.

Notification	Step	When	Alert Level	Email	Recommended Action
Batch initiation	Library Prep	User created a new batch.	Activity	Yes	Not applicable.
Batch Library Complete	Library Prep	Library completed for the current batch.	Activity	No	Not applicable.
Pool Complete	Library Prep	Pool has been generated from a batch.	Activity	No	Not applicable.
Sequencing Started	Sequencing	The system detected a new sequencing data folder.	Activity	No	Not applicable.
Sequencing QC passed	Sequencing	The sequencing run has completed and the sequencing QC check passed.	Activity	No	Not applicable.
Sequencing Run Associated With Pool	Sequencing	The sequencing run has been successfully associated with a known pool.	Activity	No	Not applicable.
Analysis Started	Analysis	Analysis started for the specified sequencing run.	Activity	Yes	Not applicable.
Analysis Completed NIPT Report Generated	Post Analysis	The analysis has completed and reports generated.	Activity	Yes	Not applicable.

Invalidation Notifications

Invalidation notifications indicate events that occur in the system due to the user invalidating a batch or a pool through the Workflow Manager. These notifications are logged as “Notices” and do not require any user actions.

Notification	Step	When	Alert Level	Email	Recommended Action
Batch Invalidation	Library Prep	User invalidated a batch.	Notice	Yes	Not applicable.
Pool Invalidation – Repool	Library Prep	User invalidated the first possible pool (of a certain type) for the batch.	Notice	Yes	Not applicable.
Pool Invalidation – Use second aliquot	Library Prep	User invalidated the first possible pool (of a certain type) for the batch.	Notice	Yes	Not applicable.

Notification	Step	When	Alert Level	Email	Recommended Action
Sequencing Completed Pool Invalidated	Sequencing	The sequencing run has completed but the pool was invalidated by the user.	Notice	Yes	Not applicable.
Sequencing QC passed – All samples are invalid	Sequencing QC	The sequencing run QC check has completed, but all samples are invalid.	Notice	Yes	Not applicable.
Analysis Completed Pool Invalidated	Post Analysis	The analysis has completed but the pool was invalidated by the user.	Notice	Yes	Not applicable.

Recoverable Error Notifications

Recoverable errors are conditions from which the VeriSeq NIPT Assay Software can recover when the user follows the recommended action. If the issue persists, email Illumina Technical Support.

Notification	Step	When	Alert Level	Email	Recommended Action
Missing Instrument Path	Sequencing	The system cannot locate/connect to an external sequencing folder.	Alert	Yes	<ul style="list-style-type: none"> If using a NAS, check the network connection. See Recommended Action Procedures on page 60 Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Insufficient Disk Space for Sequencing	Sequencing	The system detected a new sequencing data folder, but is estimating that there is not enough disk space for the data.	Alert	Yes	<ol style="list-style-type: none"> Check available disk space. See Recommended Action Procedures on page 60. Clear disk space or backup data. See Recommended Action Procedures on page 60.
Sequencing Run Invalid Folder	Sequencing	Invalid characters in the Sequencing Run folder.	Warning	Yes	The sequencing run folder was renamed incorrectly. Rename the run to a valid name.
Sequencing Started but Pool Barcode File Missing	Sequencing	The software did not detect the file containing the pool barcode for 30 minutes after the sequencing started.	Warning	Yes	Possible sequencer or NAS failure. Check the sequencer configuration and the network connection. The system will continue to look for the pool barcode file until sequencing is completed.
Cannot Verify Sequencing Run Completion	Sequencing	The software was not able to read the run completion status file in the sequencing folder.	Warning	Yes	Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.

Notification	Step	When	Alert Level	Email	Recommended Action
Missing Sample Attributes	Pre-Analysis	The software could not find a definition for sample type, sex chromosome option, or screen type for some of the samples.	Notice	Yes	One or more sample attributes were not provided for the specified sample. Enter the missing sample attributes in the Workflow Manager or invalidate the sample to enable the software to proceed.
Sample Sheet Generation failed	Pre-Analysis	The software failed to generate sample sheet.	Alert	Yes	<ul style="list-style-type: none"> • Check available disk space. See <i>Recommended Action Procedures on page 60</i>. If space is low, clear disk space or backup data. See <i>Recommended Action Procedures on page 60</i>. • If using a NAS, check the network connection. See <i>Recommended Action Procedures on page 60</i>. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Unable to check disk space	Pre-Analysis	The software could not check disk space.	Alert	Yes	<ul style="list-style-type: none"> • If using a NAS, check the network connection. See <i>Recommended Action Procedures Action ID 2 on page 60</i>. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Insufficient Disk Space for Analysis	Pre-Analysis	The software detected that there is not enough disk space for starting a new analysis run.	Alert	Yes	Clear disk space or backup data. See <i>Recommended Action Procedures Action ID 3 on page 60</i> .
Unable to launch Analysis Pipeline	Pre-Analysis	The software was unable to start an analysis run for the given sequencing folder.	Alert	Yes	Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Sequencing folder Read/Write permission failed	Pre-Analysis	The software test that checks Read/Write permission to the sequencing run folder failed.	Warning	Yes	<ul style="list-style-type: none"> • If using a NAS, check the network connection. See <i>Recommended Action Procedures on page 60</i>. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Analysis Failed - Retry	Analysis	Analysis has failed. Retrying.	Notice	Yes	None
Results Already Reported	System	The software determined that an NIPT report was already generated for the current pool type.	Activity	Yes	None

Notification	Step	When	Alert Level	Email	Recommended Action
Unable to deliver email notifications	System	The system is unable to deliver email notifications.	Warning	NA	<ol style="list-style-type: none"> 1. Check email configuration defined on system for validity. See instructions in <i>Configuring System Email Notifications</i> on page 26. 2. Send a test email. See instructions in <i>Configuring System Email Notifications</i> on page 26. 3. Reboot the server. If the problem persists, email Illumina Technical Support.
Time Skew Detected	Library prep	The software detected a time skew of over 1 minute between the timestamp provided by the Workflow Manager and the server local time.	Warning	No	<ol style="list-style-type: none"> 1. Check local time on the Workflow Manager machine. 2. Check the Onsite Server local time reported on the Web UI (Server Status tab).

Unrecoverable Error Notifications

Unrecoverable errors are conditions that reach a terminal state where no further action can resume the assay execution.

Notification	Step	When	Alert Level	Email	Recommended Action
Batch Failure	Library Prep	Batch QC failed.	Notice	Yes	Restart library plating.
Report Generating Failure	Reporting	The system failed to generate a report.	Alert	Yes	<ul style="list-style-type: none"> • Check available disk space. See <i>Recommended Action Procedures</i> on page 60. If space is low, clear disk space or backup data. See <i>Recommended Action Procedures</i> on page 60. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Failed to Parse Run Parameters file	Sequencing	The system was not able to open/parse the RunParameters.xml file.	Warning	Yes	The RunParameters.xml file is corrupted. Check the sequencer configuration and resequence the pool.
Unrecognized Run Parameters	Sequencing	The software read Run Parameters that are not compatible.	Warning	Yes	The software is was unable to construct sequencing run parameters from the sequencer configuration file. Check the sequencer configuration and resequence the pool.
Invalid Run Parameters	Sequencing	The software read required Run Parameters that are not compatible with the assay.	Warning	Yes	The software compatibility check failed. Check the sequencer configuration and resequence the pool.
No Pool Barcode found	Sequencing	The software was not able to associate the flow cell for the sequencing run with a known pool barcode.	Warning	Yes	Possible incorrect pool barcode entry. Resequence the pool.

Notification	Step	When	Alert Level	Email	Recommended Action
Sequencing Completed but Pool Barcode File Missing	Sequencing	The sequencing run was completed but the file containing the pool barcode was not detected.	Alert	Yes	Possible sequencer failure. Contact Illumina Technical Support for assistance.
Unable to read Pool Barcode File	Sequencing	The file containing the pool barcode is corrupted.	Alert	Yes	Possible sequencer or network failure. Contact Illumina Technical Support for assistance.
Pool Barcode File Mismatch	Sequencing	The detected pool barcode file references a different flowcell ID than the one associated with the sequencing run.	Alert	Yes	Possible sequencer failure. Contact Illumina Technical Support for assistance.
Sequencing Timed Out	Sequencing	The sequencing run has not completed in a given time frame.	Warning	Yes	Check the sequencer and the network connection. Resequence the pool.
Sequencing QC files generation failed	Sequencing QC	The sequencing run has completed but the interop QC files are corrupted.	Alert	Yes	Check the sequencer, and the network connection. Resequence the pool.
Sequencing QC failed	Sequencing QC	The sequencing run has completed and the sequencing QC check failed.	Notice	Yes	Resequence the pool.
Analysis Failed for Maximum number of attempts	Analysis	All Analysis attempts have failed. Will not retry.	Warning	Yes	Resequence the second pool.
Analysis Post-Processing Failed	Post-Analysis	The software failed to post process the analysis results.	Alert	Yes	<ul style="list-style-type: none"> • If using a NAS, check the network connection. See Recommended Action Procedures on page 60. • Possible hardware failure. Reboot the server. If problem persists, email Illumina Technical Support.
Analysis Upload Failed	Post-Analysis	The software failed to upload the analysis results to the database.	Alert	Yes	<ul style="list-style-type: none"> • If using a NAS, check the network connection. See Recommended Action Procedures on page 60. • Possible hardware failure. Reboot the server. If problem persists, email Illumina Technical Support.

Recommended Action Procedures

Action ID	Recommended Action	Steps
1	Check the network connection	<p>Make sure that the remote storage NAS and the local machine are on the same network.</p> <ol style="list-style-type: none"> From a Windows command line (cmd), type the following command: ping <Server IP> If using a NAS, also check the connection with the NAS. Make sure that there are no lost packets. If there are lost packets, contact the IT Administrator. Test the connection: <ol style="list-style-type: none"> Log in to the Onsite Server Web UI. From the Dashboard menu, select Folder. Select Test, and determine if the test is successful. If the test fails, see Editing a Shared Network Drive on page 24 and make sure that all settings are configured correctly.
2	Check available disk space	<p>Make sure that the Windows machine maps to the Onsite Server Input folder. For more information, see Mapping Server Drives on page 30.</p> <p>Right-click the drive that maps to the Input folder. Select Properties, and view the free space information.</p>
3	Clear disk space / Backup data	<p>Illumina recommends a periodic data backup and/or storing sequencing data on the server side. For more information, see Managing a Shared Network Drive on page 23.</p> <ol style="list-style-type: none"> For data stored locally on the Onsite Server: <p>Make sure that the Windows machine maps to the Onsite Server Input folder. For more information, see Mapping Server Drives on page 30.</p> <ol style="list-style-type: none"> Double-click the Input folder, and enter the credentials to access it. Sequencing run data are listed with folder names matching sequencing run names. Delete or backup the processed sequencing folders. For data stored on a remote NAS: <p>Make sure that the remote storage NAS and the local machine are on the same network. Obtain access to the folder on the remote drive. Access credentials from the IT Administrator are required.</p> <ol style="list-style-type: none"> Sequencing run data are listed with folder names matching sequencing runs names. Delete or backup the processed sequencing folders.

System Issues

Issue	Recommended Action
Software fails to start.	If errors are detected when starting the Assay Software, a summary of all errors appears instead of the Log In screen. Contact Illumina Technical Support to report the errors listed.
Database restore required.	If a backup restore of a database is required, contact an Illumina Field Service Engineer.
System drift detected.	When a system drift is detected, the Assay Software no longer processes communication from other system components. An administrator can reset the system back to normal operation after it has entered the drift detection state.
RAID controller alarm activates.	An administrator can select the Server alarm button in the Server Status tab of the Assay SoftwareDashboard to silence the RAID controller alarm. If you press this button, contact Illumina Technical Support for additional assistance.

Data Processing Tests

Preinstalled data sets on the Onsite Server enable operational testing of the server and the analysis engine.

Testing the Server

This test simulates a sequencing run while simulating an analysis results generation, without actually launching the Analysis Pipeline. Run this test to make sure that the Onsite Server is functioning correctly and that reports and email notifications are generated. Duration: Approximately 3–4 minutes.

Procedure

- 1 Open the mounted input directory, and then open the TestingData folder.
- 2 Make a copy of one of the following folders, which can be found in the TestingData folder:
 - ▶ For NextSeq data: 170725_NS500110_0382_AHT3MYBGX2_Copy_Analysis_Workflow.
 - ▶ For NextSeqDx data: 180911_NDX550152_0014_XXXXXXXXDX_Copy_Analysis_Workflow.
- 3 Rename the copy to a folder with an _XXX suffix. The _XXX represents a sequential count of the test run. For example, if _002 exists in the folder, rename the new copy to _003.
- 4 Move the renamed folder to the input folder.
- 5 Wait for 3–5 min for the run to complete. Make sure that the following email notifications have been received:
 - a Sequencing Run Analysis Started
 - b NIPT Report generated for Sequencing Run

Associate both reports with the sequencing name assigned to the folder.
- 6 In the output folder, open the TestData_NS_CopyWorkflow or TestData_NDx_CopyWorkflow folder, and check for one of the following reports:
 - ▶ For NextSeq: TestData_NS_CopyWorkflow_C_TestData_NS_CopyWorkflow_PoolC_HT3MYBGX2_nipt_report_YYYYMMDD_HHMMSS.tab.
 - ▶ For NextSeqDx: TestData_NDx_CopyWorkflow_C_TestData_NDx_CopyWorkflow_PoolC_XXXXXXXXDX_nipt_report_YYYYMMDD_HHMMSS.tab.

The expected file size is approximately 7 Kb.

- 7 Move the test sequencing run back to the TestingData folder. This practice helps manage the number of times the sequencing test executes.

**NOTE**

You can delete old copies of test files to create space.

Running Full Analysis Test Data

This test executes a full analysis run. Run this test if the server fails to process/analyze data or times out. Duration: Approximately 4–5 hours.

Procedure

- 1 Open the mounted input directory, and open the TestingData folder.
- 2 Rename the following folder by adding the _000 suffix: 180911_NDX550152_0014_XXXXXXXXDX_FullRun.
The suffix creates a unique name for each sequencing run. If the run has a suffix already, rename the folder by incrementing the suffix numerical value by 1.
- 3 Move the renamed folder to the input folder.
- 4 Wait for about 4–5 hours for the analysis to complete. Make sure that the following email notifications have been received:
 - a Sequencing Run Analysis Started
 - b NIPT Report generated for Sequencing Run

Associate both reports with the sequencing name assigned to the folder.

- 5 In the output folder, open the TestData_NDx_FullRun folder, and check for the following report: TestData_NDx_FullRun_C_TestData_NDx_FullRun_PoolC_XXXXXXXXDX_nipt_report_YYYYMMDD_HHMMSS.tab.
The expected file size is approximately 7 Kb.
- 6 Move the test sequencing run back to the TestingData folder.

Appendix D Additional Resources

The following documentation is available for download from the Illumina website.

Resource	Description
VeriSeq NIPT Solution v2 Package Insert (document # 1000000078751)	Defines the product and intended use, and provides instructions for use and troubleshooting procedures.
<i>Microlab® STAR Line Operator's Manual</i> , Hamilton Doc ID 624668	Provides operation and maintenance information and technical specifications for the Hamilton Microlab STAR automated liquid handling instrument.

Visit the VeriSeq NIPT Solution v2 [support pages](#) on the Illumina website for access to documentation, software downloads, online training, and frequently asked questions.

Appendix E Acronyms

Acronym	Definition
BCL	Base Call File
CE-IVD	European Conformity marking for <i>in vitro</i> diagnostic product.
cfDNA	Cell-Free DNA
DNA	Deoxyribonucleic Acid
DNS	Domain Name System
FASTQ	Text-based file format for storing the output of sequencing instruments.
FF	Fetal Fraction
FIFO	First In, First Out
iFACT	individual Fetal Aneuploidy Confidence Test
IP	Internet Protocol
LIMS	Laboratory Information Management System
LIS	Laboratory Information System
LLR	Log Likelihood Ratios
MAC	Media Access Control
NAS	Network-Attached Storage
NES	Non Excluded Sites
NGS	Next-Generation Sequencing
NIPT	Non-Invasive Prenatal Testing
NTC	No Template Control
NTP	Network Time Protocol
PF	Passing Filter
PQ	Process Qualification
QC	Quality Control
Regex	Regular Expression. A sequence of characters which can be used by string matching algorithms for data validation.
RTA	Real-Time Analysis
RUO	Research Use Only
SCA	Sex Chromosome Aneuploidy
SDS	Safety Data Sheets
SHA1	Secure Hash Algorithm 1
SSL	Secure Sockets Layer

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
Email: techsupport@illumina.com

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
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Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
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Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

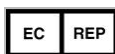
Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



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