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VeriSeq NIPT Solution v2

Software Guide

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Revision History

Document	Date	Description of Change
Document # 1000000067940 v10	August 2026	Updated the following: • Web User Interface introduction. • View System Status and Alerts in the Dashboard. • Local Disk folder name list. • Map Server Drives for the Onsite Server. Added the following: • Update the Server.

Document	Date	Description of Change
Document # 1000000067940 v09	May 2025	Updated the following: • Callout text in Architecture Overview graphic • Description of the sample object of batch management. • Instructions for samples uploaded during plasma isolation. • Caution instructions to include barcode and pooling reuse. Added the following: • Clarification that sample order is not checked by the Workflow Manager. • Requirements for the Run Name field in the Local Run Manager instructions. • Reference to Sequencing Analysis Viewer Software User Guide for QC metric interpretation guidance. • Instructions for adjustments required for retesting after a pool failure. • Explanation and troubleshooting information for the new Plate Level Contamination error. • Instructions for power cycling the system. • Clarification regarding environmental considerations. • Information in the supplementary report metrics about NES upper and lower limits with regards to iFACT failure. Replaced all instances of sequencer with next-generation sequencing system or sequencing system.
Document # 1000000067940 v08	June 2023	Removed descriptions of sample sheets for hybrid batches to align with software functionality.

Document	Date	Description of Change
Document # 1000000067940 v07	February 2023	Modified server configuration options to enhance security. Changing automation password on ML-STAR requires field visit from Illumina field service personnel. Clarified the guidelines for adding barcode information to input sample sheets, and for uploading sample sheets for hybrid batches. Updated the guidelines for creating a user name. Removed the reference to the Network Password field from server configuration instructions. Updated the example provided for partial deletion or duplication anomaly. Added ordering rule for anomaly_description field. For anomalies within the same chromosome, whole chromosome aneuploidies come before partial deletions or duplications. Added Type and Regex columns to Results and Notifications and Process reports. Updated wording throughout document to improve clarity.
Document # 1000000067940 v06	August 2021	Updated EU Authorized Representative address.

Document	Date	Description of Change
Document # 1000000067940 v05	September 2020	• Added instructions for new Backup Encryption and Network Password features. • Updated Downloading and Installing a Certificate section with more detailed instructions. • Added step to enter Network Password and a reminder to generate a certificate to the Server Configuration section for Workflow Manager. • Updated Mapping Server Drives to indicate administrator only user permissions and updated SMB version compatibility. • Added reference to Backup Encryption to Archiving Data section for the Onsite Server. • Added note to Assay Software Web UI introduction indicating the software cannot be accessed via mobile devices. • Added clarifying notes about the capitalization of outputs in the NIPT Report. • Updated the presentation of value option information for human readability in the Results and Notifications Reports section. • Updated the naming convention for Workflow Manager to display full software name of VeriSeq NIPT Workflow Manager consistently.
Document # 1000000067940 v04	February 2020	• Updated Sample Sheet Input and Upload Sample Sheet topics to clarify functionality limitation of the sample sheet upload. • Updated Australian Sponsor and Illumina Netherlands addresses.

Document	Date	Description of Change
Document # 1000000067940 v03	October 2019	• Added an Environmental Considerations section for the VeriSeq Onsite Server v2. • Updated the presentation of sex chromosomal anomaly results in the Results and Notifications Reports section of Appendix B to match the presentation shown in the NIPT Report.
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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VeriSeq NIPT Solution v2

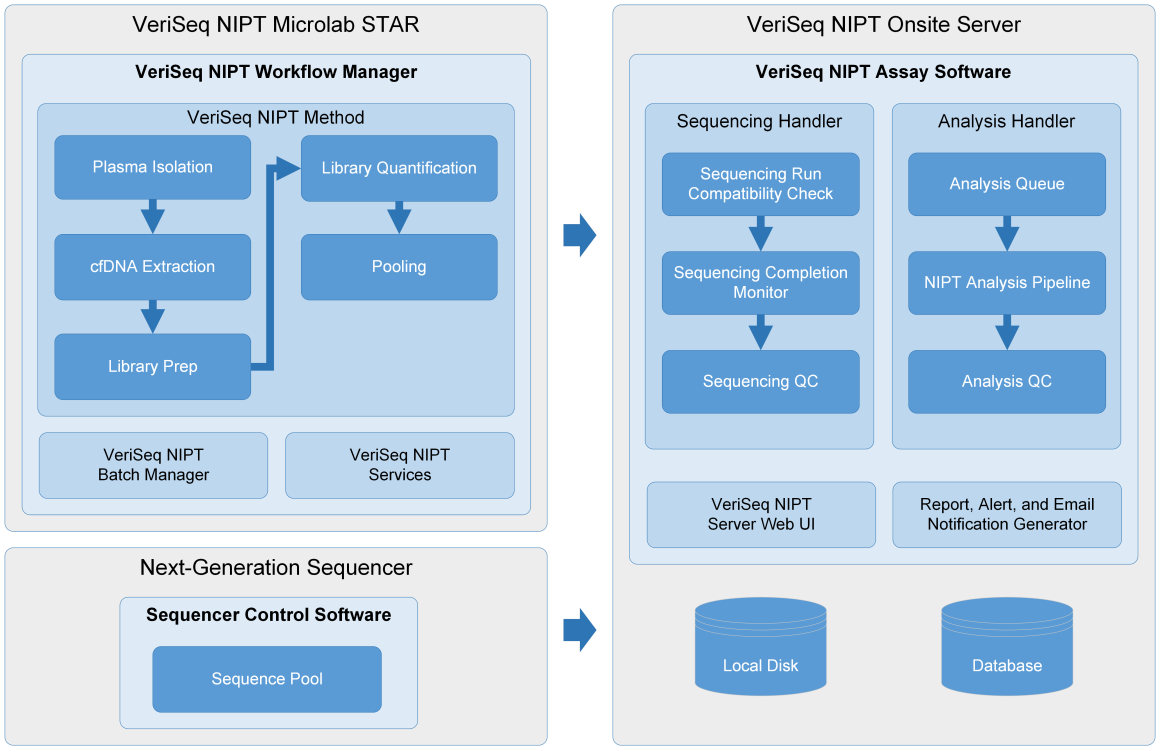
Introduction

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 screening provides information on the aneuploidy status for chromosomes 21, 18, 13, X, and Y only. Genomewide screenings 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 the following components:

- **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 uses the VeriSeq NIPT Assay Software v2 to prepare samples intended for analysis, according to the Instructions for Use provided in the *VeriSeq NIPT Solution v2 Package Insert (document # 1000000078751)*.
- **Next Generation Sequencing (NGS) Instrument**—A genomewide sequencing instrument that provides on-instrument cluster generation and sequencing. The 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 VeriSeq NIPT Assay Software continuously monitors and analyzes sequencing data and produces sample results, process reports, and notifications.

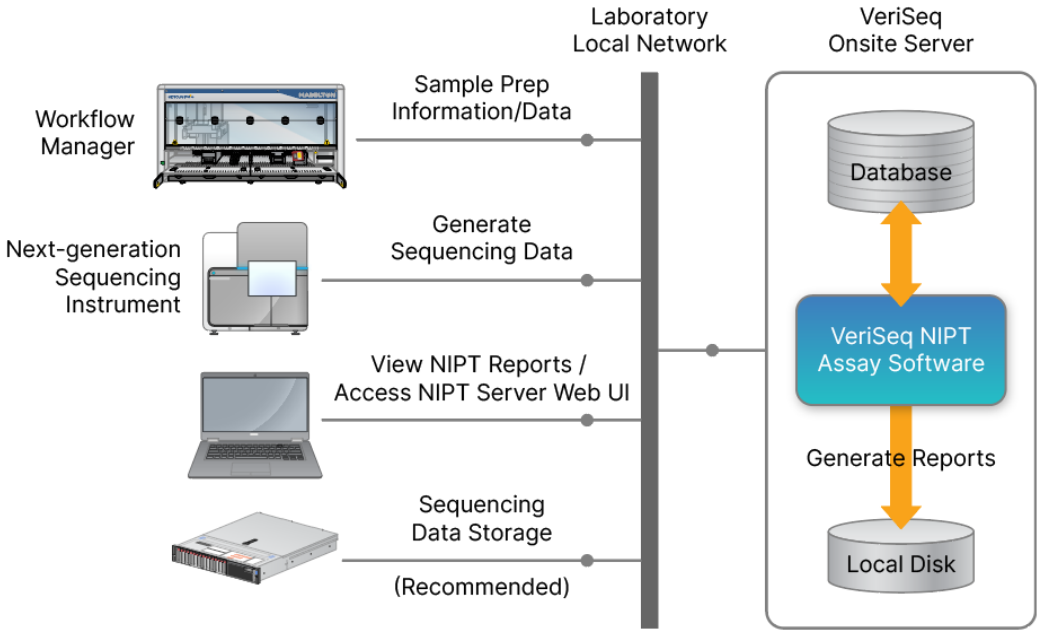
Figure 1 VeriSeq NIPT Solution v2 Components



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 instruments and/or ML STAR workstations. The following illustration provides an overview of the system architecture.

Figure 2 VeriSeq NIPT Solution v2 Architecture Overview



VeriSeq NIPT Workflow Manager

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 VeriSeq NIPT 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 VeriSeq NIPT Workflow Manager provides access to the following 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 VeriSeq NIPT Assay Software. Each batch contains sample data, including the sample barcode, sample type, screen type, well position, and sex-reporting flag.
- **Cell-Free DNA (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 for each sample into the pool tube. The pool tube is then ready for sequencing.

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 sequencing instruments, and through the analysis pipeline. For more information about sample processing procedures, refer to the *VeriSeq NIPT Solution v2 Package Insert (document # 1000000078751)*.

You can manage samples within the workflow through three different categories, referred to as objects. These objects are described in the following table.

Object	Description
Sample	Result from the processing of 1 ml of plasma sample 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 instrument-ready, dual-index libraries. Each pool contains up to 48 samples.

The following table describes the actions that can be applied to objects during processing.

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.

Action	Object	Report Generated	Description
QC Failure	Sample	Sample Invalidation	VeriSeq NIPT Solution v2 automatically flagged sample as invalid due to the failure of a specified Quality Control (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.
Cancellation	Sample	Sample Cancellation	Laboratory management flagged sample as cancelled. No test result is generated.
Edit Sample Attributes	Sample	Sex Reporting	Sex reporting flagged by the user as Yes, No, or SCA. • Yes—The sex of the sample is generated. • No—The sex of the sample is not generated. • 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 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 cancellation 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.

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. 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 (_). The sample barcode is not case-sensitive. Case-sensitive barcodes are not considered unique. Sample barcode must be unique and must not differ only in capitalization. For example, sample names Sample01 and sample01 are not unique.
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. The system automatically applies barcodes, screen type, sample type, and sex reporting for NTCs. Different information is required based on if the sample sheet is uploaded during plasma isolation or pooling. Sample information is confirmed during the sample uploading process. The

Workflow Manager does not check the sample order. Samples uploaded during plasma isolation must include all samples in the batch except NTC. During pooling, the system requests any missing sample information not uploaded during plasma isolation, even for NTCs (ie, sex chromosome and screen type).

CAUTION

To avoid errors, do not include sample information or rows for NTCs in the sample sheet during the plasma isolation step.

You can control sample loading for all samples in a batch generated by the LIMS, or for specific samples that require retesting. If loading samples for retest purposes, fill the remaining open positions with available samples.

Choose from the following strategies for using sample sheets:

- Predefined batches (LIMS created batches)
- Ad hoc batches (VeriSeq NIPT Workflow Manager created batches)

Predefined Batches

You can use the LIMS to create batches before sample processing begins. 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 batches created in a LIMS must include values in the Batch ID column. Including the batch ID helps to make sure that the correct batch ID name was manually entered into the Workflow Manager at the start of processing.

The predefined batch approach locks 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. The lab can proceed to the final report without additional data input.

Features and requirements of the predefined batches approach are as follows.

- Allows for complete control of batch contents.
- Prevents the loading of unwanted samples.
- Requires a system for creating batches from inventory (advanced LIMS).
- Can require lab personnel to retrieve the correct samples from storage. Alternatively, requires an advanced sample storage system.

Ad Hoc Batches

You can create batches 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. You determine which samples to include in the batch.

When prompted by the Workflow Manager, select **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.

Features and requirements of the ad hoc batching approach are as follows.

- No LIMS or sample sheet are required.
- You can modify the batch initiation report with information on sample type, screen type, and sex reporting for upload during pooling. You can add samples at any time.
- There is no automated control over which samples are included in the batch. You can load an unwanted sample.
- Sample data must be uploaded during pooling.

Edit Sample Attributes

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. For details, refer to *Access Batch Manager* on page 10.
2. Enter the batch ID and user name or operator initials, and then select **OK**.
3. On the batch plate diagram, select the well position associated with a 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 sample processing step, you 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

To invalidate samples, complete the following steps during sample processing.

1. In the Well Comments window at the end of each Workflow Manager process, select the individual wells to fail, and then 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 the following:

- 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 Before running the Batch Manager, exit any currently running methods.

Access Batch Manager

To access Batch Manager, use one of the following actions:

- 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

Upload a sample sheet containing sample information through the Batch Manager. Use this feature to 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**.

For details on the information to include in the sample sheet, refer to *Sample Sheet Input* on page 6.

Sample Cancellation

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 cancelled 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 cancelled 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

Close all running methods before executing Services.

Access VeriSeq NIPT Services using one of the following methods:

- 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 the following:

- **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.

Execute individual tests as follows.

1. Select specific test to be executed.

   NOTE Full IOQ Execution runs all six tests sequentially.

2. Follow the onscreen 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 VeriSeq NIPT 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 as follows:

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 then select **Apply** to save the new address.

When you update the network address, you also must update the secure sockets layer (SSL) certificate for the PC running Workflow Manager. Access the VeriSeq NIPT Assay Software v2 through this PC and refer to *Download and Install a Certificate* on page 31.

Only Illumina Field Service Engineers can update the automation password for the ML STAR. Before you change the password stored on the server, via the web interface, make sure a member of the Illumina Field Service team has visited your site and updated the ML STAR password. If you update the password in the server web interface without updating it on the ML STAR, you will render the system unusable.

Assay Configuration

You can use the Assay Configuration tool to set the values of the following parameters:

- **Target Library Concentration**—Sets the default concentration value of the libraries in the sequencing system pools within the Workflow Manager. Concentration values are applied on a run-by-run basis during the pooling process.For more information, refer to the *VeriSeq NIPT Solution v2 Package Insert (document # 1000000078751)*.
- **Default Sex Chromosome Reporting**—Determines the attribute to assign to samples when the Use Default button is selected during sample preparation. Set this parameter to Yes or No.

- **Screen Type**—Determines the screen type for a sample. Set this parameter to Basic or Genomewide.

Configure assay parameters as follows.

1. Select **Assay Configuration** and configure the parameters as needed.
   - Update the Target Library Concentration (pg/µl) box to the required value.
   - Update Default Sex Chromosome Reporting to the required value.
   - Update Screen Type to the required value.
2. Select **Apply**.

Deck Teach Tool

During troubleshooting, you might need 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. The output location defaults to the location listed. Accept the default location, or 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.

Next-Generation Sequencing System

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 VeriSeq NIPT 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 VeriSeq NIPT Assay Software requires a next-generation sequencing system 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 base call (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 a temporary data repository. In this configuration, the instrument 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, refer to *Manage a Shared Network Drive* on page 29.

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

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 Technical 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 sequencing system.
- 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 module, prepare for sequencing as follows.

1. In VeriSeq NIPT Local Run Manager, select **Create Run**.
2. From the drop-down menu, select **VeriSeq NIPT**.
3. Complete the following fields:
   - Run Name (must be new and unique)
   - Run Description (optional)
   - Pool Barcode

CAUTION

The pool barcode entered in the Local Run Manager module must match the pool barcode entered in the Workflow Manager. Incorrect run configurations are rejected by the VeriSeq NIPT Assay Software and may require resequencing. Pool barcodes must be new and unique. Previously scanned barcodes cannot be reused, even if the pooling event has not occurred. Re-initiated batches require a clean, unregistered tube. Analysis fails if the barcode is associated with a previously analyzed batch or any aborted pooling events.

4. Select **Save Run**.
   After you finish setting up the run, you can initiate the run using the instrument software.

VeriSeq NIPT Assay Software v2

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.

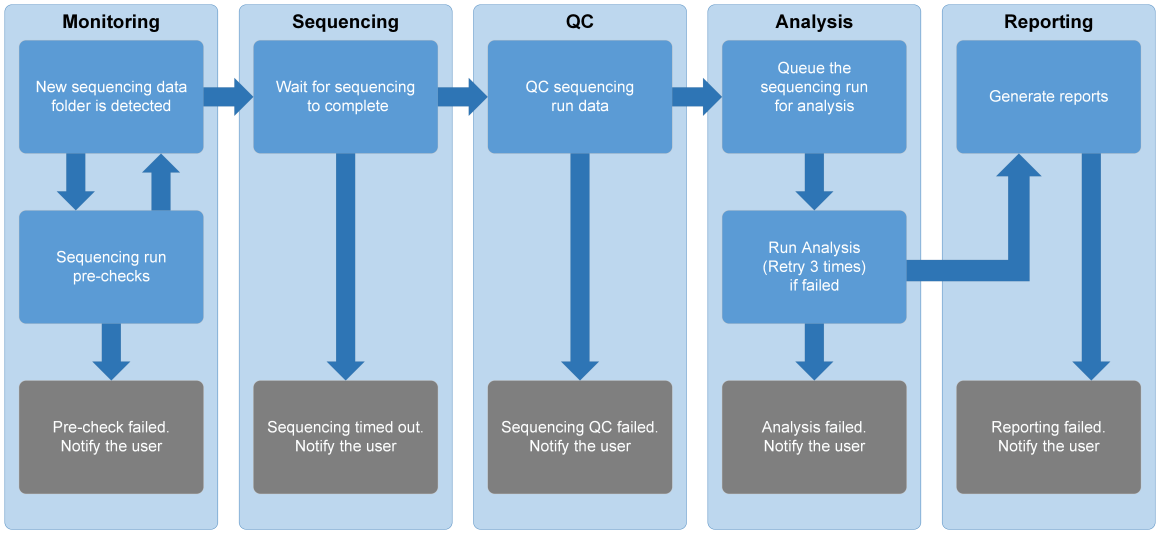
The VeriSeq NIPT Assay Software aligns the reads against the reference human genome and performs analysis on reads that align to a unique location or site in the genome. The VeriSeq NIPT Assay Software excludes duplicate reads and sites that are associated with high variation in coverage across euploid 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 VeriSeq NIPT 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 VeriSeq NIPT Assay Software reports whether or not an anomaly was detected. In the basic screen, all anomalies are aneuploidies. For the genomwide screen, an anomaly can be an aneuploidy or a partial deletion or duplication.

VeriSeq NIPT Assay Software Components

The VeriSeq NIPT 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 flow is triggered as follows.

Figure 3 Data Flow Diagram



1. **Monitoring**—Prechecks the validity of the new sequencing run. When the software detects a new sequencing run, the following validity checks are conducted:
   a. Checks that the run parameters are compatible with the expected values.
   b. Associates the flow cell with a known existing pool tube.
   c. Confirms that the pool has not been processed previously. The system does not allow re-runs.

   If any check fails, the user is notified through the email notification system and though the Alerts log on the Web user interface (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 sequencing system. The VeriSeq NIPT Assay Software checks the total number of clusters, cluster density, and the reads quality scores. For guidance on interpreting InterOp QC metrics, refer to the *Sequencing Analysis Viewer Software User Guide (document # 15020619)*. 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 VeriSeq NIPT 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.

VeriSeq NIPT Assay Software Tasks

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

Automated Tasks

The VeriSeq NIPT 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. Refer to *Reports File Structure* on page 46 for an overview and *Process Reports* on page 68 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 VeriSeq NIPT Assay Software determines whether to continue with the process and if the results are to be reported. The VeriSeq NIPT 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 VeriSeq NIPT Assay Software determines the aneuploidy score for each sample. The system does not report results for samples invalidated or cancelled 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, refer to *NIPT Report* on page 52.
- **Results file generation**—Provides sample results in a tab-separated value file format, which is saved to the Output folder. For more information, refer to *NIPT Report* on page 52.
- **Report generation**— The VeriSeq NIPT Assay Software generates supplementary result information, notifications, and process reports. For more information, refer to *System Reports* on page 46.

- **Sample, pool, and batch invalidation**
  - **Sample invalidation**—The VeriSeq NIPT 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, refer to *Sample Invalidation Report* on page 67.
  - **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 already 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, refer to *Pool Retest Request Report* on page 68.
- **Retest management**
  - **Pool failures**—Failed pools are typically pools that failed the Sequencing QC metrics. The VeriSeq NIPT Assay Software does not proceed with processing failed pools if the run is terminated. Resequence using a second pool aliquot with adjustments to the ratio of pool to HT1, the pool concentration, or both.
  - **Sample failures**—The software allows failed samples to be retested if needed. Failed samples must be incorporated into a new batch and reprocessed through assay steps.
  - **Reruns**—The system does not reanalyze pools with samples that have been processed previously and reported successfully. Rerun a sample by replating the sample on a new batch.

User Tasks

The VeriSeq NIPT Solution v2 enables users to perform tasks as follows.

Using the Workflow Manager:

- Mark the following as invalid:
  - An individual sample.
  - All samples within a batch.
  - All samples associated with a pool.
- Mark a given sample as cancelled. The VeriSeq NIPT Assay Software then marks the result as cancelled in the final results report.

Using the VeriSeq NIPT 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 The ability to perform tasks depends on user permissions. For more information, refer to *Assign User Roles* on page 27.

Sequencing Handler

The VeriSeq NIPT 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 VeriSeq NIPT Assay Software continues to monitor the sequencing runs for completion. Completed sequencing runs are queued to be processed by the Analytic Pipeline Handler (for more information, refer to *Analytic Pipeline Handler* on page 22).

Sequencing Run Compatibility

The VeriSeq NIPT Assay Software 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 three times, the Analytic Pipeline Handler tags the run as failed and generates an error message.

A successful analysis run triggers the NIPT report generation. For more information, refer to *NIPT Report* on page 52.

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

NOTE The VeriSeq NIPT Assay Software Web UI does not support the use of mobile devices.

- **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, refer to *Assay Software Notifications* on page 78.
- **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, refer to *Assay Software Notifications* on page 78.
- **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, refer to *Configure Network and Server Settings* on page 30.
- **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, refer to *Manage Users* on page 27.
- **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, refer to *Map Server Drives* on page 40.

- **Configure email notification subscribers list**—Manages a list of subscribers to receive email notifications comprising error messages and assay process alerts. For more information, refer to *Configure System Email Notifications* on page 32.
- **Update the server**—Updates the Onsite Server operating system, if needed. Update packages can be downloaded from Illumina and applied to the server. For more information, refer to *Update the Server* on page 40.
- **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, refer to *Reboot the Server* on page 41 and *Shut Down the Server* on page 42.
- **Configure database backup encryption**—Enables encryption and setting an encryption password for database backups of the server. This feature also allows the generation of a temporary, unencrypted backup. For more information, refer to *Configure Backup Encryption* on page 33.
- **Configure network passwords**—Set network passwords for communication between the server and both sequencing systems and VeriSeq NIPT Microlab STAR instruments. For more information, refer to *Configure Network Passwords* on page 34.

End User License Agreement

When you first log in to the web UI, you are requested to accept the End User License Agreement (EULA). To download the license agreement to your computer, select **Download EULA**. 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.

Configure the Web UI

Select the Settings icon to access a drop-down list of configuration settings. Settings display based on user role and associated permissions. For more information, refer to *Assign User Roles* on page 27.

NOTE Technicians do not have access to 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.

Setting	Description
Change Shared Folder Password	Change the sbsuser password for accessing the Onsite Server shared folders. The password can only contain alphanumeric characters.
Reporting Settings	Service Engineers or Administrators only.
Reboot Server	Service Engineers or Administrators only.
Shut Down Server	Service Engineers or Administrators only.

Log in to the Web UI

Log in to the VeriSeq NIPT Assay Software interface as follows.

1. 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
2. Enter the server IP address or the server name provided by Illumina at installation, equivalent to `https://<Onsite Server IP address>/login`. (eg, `https://10.10.10.10/login`).
3. If a browser security warning appears, add a security exception to proceed to the login screen. The security warning indicates the computer does not have the secure sockets layer (SSL) certificate installed. Follow the instructions in *Download and Install a Certificate* on page 31 to install this certificate.
4. In the login screen, enter the case-sensitive user name and password provided by Illumina, and then select **Log In**.

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

The Dashboard

After logging in, the VeriSeq NIPT Assay Software v2 Dashboard displays. The Dashboard 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 those that are logged). To fetch the previous 50 activities and browse through the activity history, select **Previous** in the lower-right corner of the activity table.

View Recent Activities

The Recent Activities tab contains a brief description of recent VeriSeq NIPT 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: • **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.

View 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: • **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.

View System Status and Alerts

The **Server Status** tab displays the following information.

- **Date**—Current date and time.
- **Time zone**—Time zone configured for the server. The time zone information is used for email, alerts, and report date and time.
- **Hostname**—System name consists of the network hostname and the domain name system (DNS) 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.
- **Latest Cybersecurity Patch Version**—Latest update package installed on the server.

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.

Manage Users

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

Assign 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.

Add Users

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

Add a user as follows.

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

   NOTE All fields are required.

2. Enter the user name. Requirements are as follows.
   - Lowercase alphanumeric (a–z, and 0–9) characters only.
   - Must be 4–20 characters and contain at least one numerical character.
   - The first character cannot be numerical.

   NOTE The user name is not case-sensitive.

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

Edit Users

Edit user information as follows.

1. From the User Management screen, select the user name.
2. Edit the information for the user, and then select **Save**.
3. Select **Save** again to confirm changes.
   The changes to the user now appear on the User Management screen.

Deactivate Users

Deactivate a user as follows.

1. From the User Management screen, select the user name.
2. Clear the **Activate** checkbox, and then select **Save**.

3. On the confirmation message, select **Save**.
   The user status changes to Disabled in the User Management screen.

Manage a Shared Network Drive

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

Add 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.

Edit 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.

Delete 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.

Configure 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 Secure Sockets Layer (SSL) certificate.

Configure Certificate Settings

A Secure Sockets 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 configure the following 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.
   - **Country**—Country location of testing laboratory.
   - **Certificate Thumbprint (SHA1)**—Certification identification number.
     The 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. For more information, refer to *Regenerate a Certificate* on page 32.
2. Select **Save** to implement the changes made.

Configure 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 configure the following 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 Network Time Protocol (NTP) time synchronization.
- **MAC Address**—Server networking MAC address (read only).
- **Timezone**—Server local time zone.

2. Confirm that the entries are correct, and then select **Save** to reboot the server and implement the changes made.

CAUTION

Incorrect settings can disrupt the connection with the server.

Download and Install a Certificate

To download and install an SSL certificate for the VeriSeq NIPT Assay Software v2:

1. From the Dashboard, select **Configuration**.
2. Select the **Certification Configuration** tab.
3. Select **Download Certificate** from the Network Configuration screen.
   The root_cert.der certificate file downloads.

NOTE If you are prompted to save the file, choose a memorable location. If not, identify the default download location. Some browsers automatically save the file to a Downloads folder.

4. Navigate to the folder on your computer where you saved the file.
5. Right-click the **root_cert.der** file and select **Install Certificate**.
6. If a Security Warning window displays, select **Open** to open the file.
   The Certificate Import Wizard opens.
7. In the Welcome window for the Certificate Import Wizard, select **Local Machine** for the Store Location, and then select **Next**.
8. Select the option to **Place all certificates in the following store**, and then select the **Browse...** button.
9. In the Select Certificate Store window, select **Trusted Root Certification Authorities**, and then select **OK**.

10. Make sure the Certificate Store field displays Trusted Root Certification Authorities, and then select **Next**.
11. In the Completing the Certificate Import Wizard window, select **Finish**.
12. If a Security Warning window displays, select **Yes** to install the certificate.
13. In the successful import dialog box, select **OK** to exit the Wizard.

Regenerate 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. From the Network Configuration screen, select **Regenerate Certificate**.
2. Select **Regenerate Certificate and Reboot** to proceed, or select **Cancel** to exit.

Configure 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. For information about the email notifications sent by the system, refer to *Assay Software Notifications* on page 78.

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.

Create an Email Subscriber List

Email notifications are sent to a list of specified subscribers.

Specify a subscriber list as follows.

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 **Save**.
5. Select **Send test message** to generate a test email to the subscribers list.
   Check your email inbox to verify that the email was sent.

NOTE Make sure you select the **Save** button before you send a test message. Sending a test message before you save discards any changes.

Configure Backup Encryption

The VeriSeq NIPT Assay Software v2 allows administrators to enable or disable backup encryption. Administrators can also set or update the encryption password for database backups. This password is necessary to restore a database backup. Make sure to store the password in a secure location for future reference.

NOTE Only Administrators have permission to set up database backup encryption.

Set up backup encryption as follows.

1. From the Dashboard, select the Settings icon.
2. Select **Backup Encryption**.
3. Select the **Encrypt Backups** checkbox.
4. In the **Encryption Password** field, enter the preferred encryption password.
5. Enter the same password in the **Confirm Password** field.
6. Select **Save**.

Generate an Unencrypted Backup

The VeriSeq NIPT Assay Software enables administrators to generate an unencrypted backup file that can be used by Illumina Technical Support. The unencrypted backup file only exists for 24 hours before it is automatically deleted.

NOTE Only Administrators have permission to generate an unencrypted backup.

Create an unencrypted backup as follows.

1. From the Dashboard, select the Settings icon.
2. Select **Backup Encryption**.
3. Select **Generate Unencrypted Backup**.
4. Select **Yes** in the confirmation window.
   A prompt displays confirming the unencrypted backup request.
5. Select **OK**.

You can confirm the creation of an unencrypted backup by returning to the VeriSeq NIPT Assay Software Dashboard and viewing the Recent Activities table. A new activity should confirm the successful creation of an unencrypted backup.

Configure Network Passwords

An Administrator or Illumina Field Service Engineer can use the Network Passwords page to configure passwords for communication between the Onsite Server and VeriSeq NIPT Solution v2 components.

CAUTION

Only Service Engineers and Administrators have permission to change network passwords.

Configure network passwords as follows.

1. From the Dashboard, select the Settings icon.
2. Select **Network Passwords**.
3. In the **Sequencer Password** field, enter a password for the sequencing instruments.
4. Reenter the password in the **Confirm Password** field.

   CAUTION

   Updating the sequencer password while a sequencing run is in progress can cause data loss.

5. Select **Save Sequencer Password**.
   The server stores the password for the sequencing instrument. Update any instruments connected to the server to make sure they use this password.
6. In the **Automation Password** field, enter a password for the VeriSeq NIPT Microlab STAR.

   CAUTION

   Updating the automation password while sample preparation is in progress can cause data loss.

   Only Illumina Field Service Engineers can update the automation password for the ML STAR. Before you change the password stored on the server, via the web interface, make sure a member of the Illumina Field Service team has visited your site and updated the ML STAR password. If you update the password in the server web interface without updating it on the ML STAR, you will render the system unusable.

7. Reenter the password for ML STAR in the **Confirm Password** field.
8. Select **Save Automation Password**.
   The server stores the password for the ML STAR. Update any ML STAR instruments already connected to the server to make sure they use this password.

Log Out

- In the upper right corner of the screen, select the user profile icon, and then 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. This section describes the various metrics that are determined for any given sample.

Demultiplexing and FASTQ Generation

Sequencing data stored in BCL format are processed through the bcl2fastq conversion software. The bcl2fastq conversion software demultiplexes data and converts BCL files to standard FASTQ file formats for downstream analysis. For each sequencing run, the VeriSeq NIPT 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: • **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.

Column Name	Description
SampleType	Sample type. One of the following: • **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, refer to *Sequencing QC Metrics and Boundaries* on page 45.

Fetal Fraction Estimates

Fetal fraction (FF) refers to the percent of cell-free, circulating DNA in a maternal blood sample that is derived from the placenta. The VeriSeq NIPT 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 FF 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 FF. The LLR is the probability of a sample being affected given the observed coverage and FF versus the probability of a sample being unaffected given the same observed coverage. The calculation of this ratio also takes into account the estimated uncertainty in FF. 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.

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

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 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 59 for more information about these QC reasons.

NTC Samples QC

The VeriSeq NIPT Solution allows the addition of NTC samples as part of the 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, refer to *Sequencing QC Metrics and Boundaries* on page 45.

QC statuses for NTC samples are as follows.

- **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.

[1]Bianchi 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.

Plate Level Contamination

Plate level contamination is detected in the analysis results by identifying the presence of chromosome Y for every valid, non-NTC sample in a pool that has passed QC.

Invalid samples are excluded because their results cannot be relied upon to give an accurate indication of the presence of chromosome Y. NTCs are excluded because any detected readings for these samples indicate non-plate level contamination. The exclusions are indicated separately in the NIPT report.

If plate level contamination is detected for a pool, the user is notified through the email notification system and through the Alerts log on the Web UI. The run will not process any further, and the NIPT and Supplementary reports will not be generated.

VeriSeq Onsite Server v2

The VeriSeq Onsite Server v2 runs a Linux-based operating system and provides approximately 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.

Local Disk

The VeriSeq NIPT 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.
Update	Location for update package files used for patching the server.	Read and write.

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

Local Database

The VeriSeq NIPT 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 VeriSeq NIPT 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 one monthly backup is kept. The recommended practice is to create an automated script that can persist the backup folder on a local NAS. These backups do not include the input and output folders.

  NOTE The VeriSeq NIPT Assay Software v2 provides an encryption option for database backup. Refer to *Configure Backup Encryption* on page 33 for more information.

- **Database restore**—The database can be restored from any given backup snapshot. Restores are done by Illumina Field Service Engineers only. The encryption password must be supplied to restore an encrypted backup. This password must be the password in effect at the time of the backup.
- **Data backup**—Although the Onsite Server can be used as the main storage point for sequencing runs, it can only store approximately 300 runs. You can set 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 VeriSeq NIPT Assay Software or the Onsite Server itself are provided by Illumina Technical Support.

Archive Data

Consult your local IT site archiving policy to determine how to archive the input and output directories. The VeriSeq NIPT 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 sequencing system 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.

Map Server Drives

The Onsite Server has four 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.
- **update**—Maps to the system update package files.

NOTE Only active Service Engineers and Administrators have permission to map server drives.

Map each folder as follows.

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 (as an active administrator) of the VeriSeq NIPT Assay Software v2. Successfully mapped folders appear mounted on the computer. If the role, active status, or the password of the administrator changes, the active connection of the mapped server is terminated. 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 SMB2 and higher versions. The server requires SMB signing. Enable these versions on the equipment (laptop/workstation) you are mapping.

Update the Server

NOTE Only Service Engineers and Administrators have permission to update the server.

The VeriSeq Onsite Server operating system is updated by installing update package files. These packages are downloaded from Illumina and copied to a designated folder on the Onsite Server's internal hard drive, which is mapped as the read/write **update** folder on the user drive. After the files are copied, the update packages are installed through the Web UI.

NOTE Update package files are cumulative – Installing the latest update package applies all applicable updates from previous update packages.

To update the server:

1. Make sure that an up-to-date database backup is available and stored externally to the server.
2. Download the latest update package file from the Illumina Product Security Portal.
3. Copy the downloaded package file to the mapped **update** folder.
4. On the **Settings** drop-down list, select **Update Server**. A list of available package files displays, along with the status of each of the files.
5. Identify the required update package in the list and select **Install** to start the installation process.
6. Enter a reason for updating the server. The reason is logged for troubleshooting purposes.
7. Select **Install Update Package**. The server goes offline to install the update. When installation is complete, the server resumes normal operation. The version of the update package is shown on the Server Status tab of the Dashboard under Latest Cybersecurity Patch Version.

CAUTION

No sequencing run or sample preparation should be active during the reboot. Doing so can lead to losing data. Rebooting the system can take several minutes. Plan your lab activity around the reboot.

Reboot 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.

CAUTION

No sequencing run or sample preparation should be active during the reboot. Doing so can lead to losing data. Rebooting the system can take several minutes. Plan your lab activity around the reboot.

Power Cycling

For the ML STAR and its peripherals, for example PC, power cycling is an important maintenance step to ensure smooth operation and prevent system errors. It is also a crucial step at the end of the workflow to turn off peripherals like the pump or CPAC systems. To avoid unnecessary power usage and potential issues, do not leave the system switched on overnight after use.

Shut 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.

CAUTION

No sequencing run or sample preparation should be active while shutting down the server. Doing so can result in losing data.

Recover from Unexpected Shutdown

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

- Automatically restarts the VeriSeq NIPT 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 VeriSeq NIPT Assay Software allows the system to resubmit the run for analysis up to three times.

Environmental Considerations

The following table provides ambient temperature considerations for the Onsite Server. These considerations do not apply to the ML STAR.

Altitude	Operational Ambient Temperature	Non-Operational Ambient Temperature
Sea Level	10°C to 40°C	0°C to 60°C
+10,000 feet	0°C to 30°C	-10°C to 50°C

Information on the disposal of electronic equipment under the Waste Electrical and Electronic Equipment (WEEE) Directive and regulations is provided on the Illumina website at https://support.illumina.com/weee-recycling.html.

QC Metrics

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.

System Reports

Introduction

The VeriSeq NIPT Assay Software generates the following categories of reports:

- Results and notifications reports.
- Process reports.

A report can be informational or actionable.

- **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

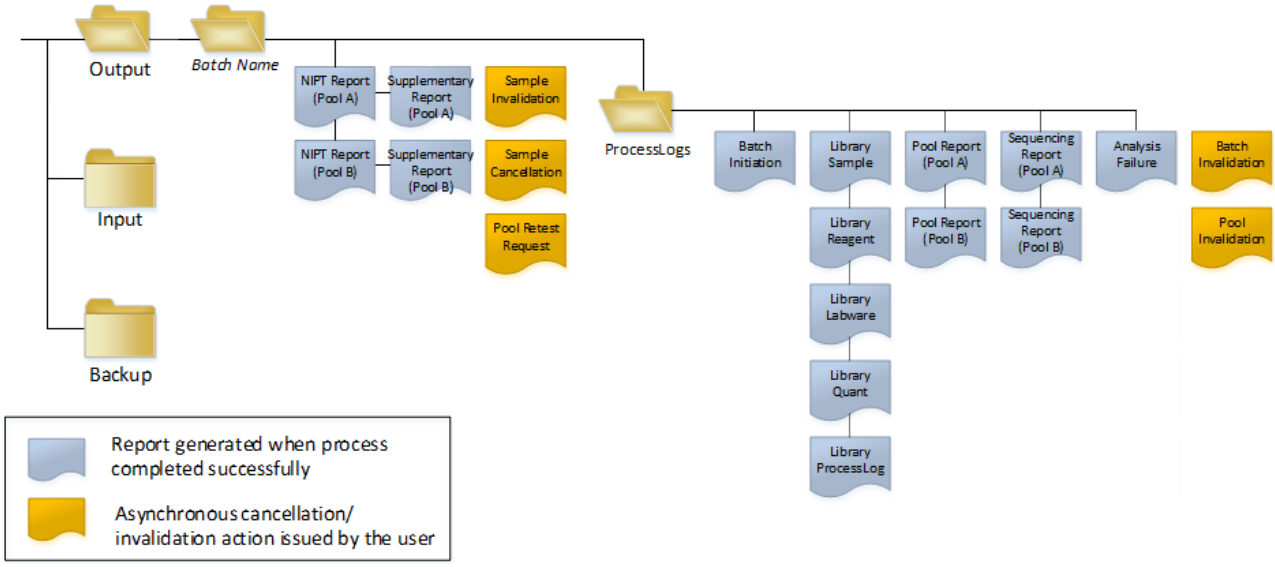
The VeriSeq NIPT Assay Software reports are generated on the Onsite Server internal hard drive mapped to the user drive as a read-only Output folder. Each report is generated with a corresponding standard MD5 checksum file, which is used to verify that the file has not been modified.

All reports are plain text formatted as tab delimited. You can open the reports with any text editor or with a tabulated data program, such as Microsoft Excel®.

Reports File Structure

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

Figure 4 VeriSeq NIPT Assay Software Reports Folder Structure



The VeriSeq NIPT 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, refer to *Results and Notifications Reports* on page 52.
- **ProcessLogs folder**—Contains reports related to process. For details, refer to *Process Reports* on page 68.

A list of all reports is provided in *System Reports Summary* on page 48.

System Reports Summary

Report Name	Report Type	Report Entity	Report File Name Format
NIPT Report on page 52	Actionable	Pool/ flow cell	<batch_name>_<pool_type>_<pool_barcode>_<flowcell>_nipt_report_<YYYYMMDD_hhmmss>.tab
Supplementary Report on page 62	Actionable	Pool/flow cell	<batch_name>_<pool_type>_<pool_barcode>_<flowcell>_supplementary_report_<YYYYMMDD_hhmmss>.tab
Sample Invalidation Report on page 67	Actionable	Sample	<batch_name>_<sample_barcode>_sample_invalidation_report_<YYYYMMDD_hhmmss>.tab
Sample Cancellation Report on page 68	Actionable	Sample	<batch_name>_<sample_barcode>_sample_cancellation_report_<YYYYMMDD_hhmmss>.tab
Pool Retest Request Report on page 68	Actionable	Pool	<batch_name>_<pool_type>_pool_retest_request_<YYYYMMDD_hhmmss>.tab
Batch Initiation Report on page 69	Informational	Batch	ProcessLogs/<batch_name>_batch_initiation_report_<YYYYMMDD_hhmmss>.tab
Batch Invalidation Report on page 69	Informational	Batch	ProcessLogs/<batch_name>_batch_invalidation_report_<YYYYMMDD_hhmmss>.tab
Library Sample Report on page 70	Informational	Batch	ProcessLogs/<batch_name>_library_sample_report_<YYYYMMDD_hhmmss>.tab
Library Reagent Report on page 71	Informational	Batch	ProcessLogs/<batch_name>_library_reagent_report_<YYYYMMDD_hhmmss>.tab
Library Labware Report on page 72	Informational	Batch	ProcessLogs/<batch_name>_library_labware_report_<YYYYMMDD_hhmmss>.tab

Report Name	Report Type	Report Entity	Report File Name Format
Library Quant Report on page 73	Informational	Batch	ProcessLogs/<batch_name>_library_quant_report_<YYYYMMDD_hhmmss>.tab
Library Process Log on page 73	Informational	Batch	ProcessLogs/<batch_name>_library_process_log.tab
Pool Report on page 75	Informational	Pool	ProcessLogs/<batch_name>_<pool_barcode>_pool_report_<YYYYMMDD_hhmmss>.tab
Pool Invalidation Report on page 75	Informational	Pool	ProcessLogs/<batch_name>_<pool_barcode>_pool_invalidation_report_<YYYYMMDD_hhmmss>.tab
Sequencing Report on page 76	Informational	Pool/ flow cell	ProcessLogs/<batch_name>_<pool_type>_<pool_barcode>_<flowcell>_sequencing_report_<YYYYMMDD_hhmmss>.tab
Analysis Failure Report on page 77	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.	• Sequencing run analysis completes.
Supplementary Report	Contains supplementary results for a successful analysis run.	• Sequencing run analysis and NIPT report both complete.
Sample Invalidation	Contains information about an invalidated sample.	• User invalidates a sample.
Sample Cancellation	Contains information about a cancelled sample.	• 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]	• User invalidates a pool.
Batch Initiation	Indicates a new batch processing start.	• User initiates a new batch.
Batch Invalidation	Contains information about a user-initiated invalidated batch.	• Batch is invalidated.
Library Sample	Lists all samples in the batch.	• Batch is invalidated. • Library Preparation method completes. • Batch fails quantification.
Library Reagent	Contains library processing reagent information.	• Batch is invalidated. • Library Preparation method completes. • Batch fails quantification.

Report	Description	Generation Event
Library Labware	Contains library processing labware information.	• Batch is invalidated. • Library Preparation method completes. • Batch fails quantification.
Library Quant	Contains library quantification test results.	• Batch is invalidated. • Library Preparation method completes. • Batch fails quantification.
Library Process Log	Contains steps executed during library processing.	• Batch is invalidated. • Library Preparation method completes. • Batch fails quantification. • Batch process completes.
Pool	Contains sample pooling volumes.	• Pooling method completes.
Pool Invalidation	Contains information about a user-initiated invalidated pool.	• User invalidates a pool.
Sequencing	Contains sequencing QC results.	• Sequencing QC passes. • Sequencing fails. • Sequencing times out.
Analysis Failure	Contains analysis information for a failed pool.	• 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, aneuploidy reporting, and QC criteria.	One of the following: • **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	*Values specified in Preset Value Options.*

Column	Description	Preset Value Options	Type	Regex
sex_chrom	Sex chromosome analysis requested. Determines the presentation of aneuploidy classification and sex chromosome information.	One of the following: • **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. The NIPT Report displays the values of yes, no, and sca as all lowercase.	enum	*Values specified in Preset Value Options.*
screen_type	Screening type.	One of the following: • **Basic**-Screen chromosomes 13, 18, or 21. • **Genomewide**-Screen entire genome. • **Not Specified**–A screen type was not provided for this sample. The NIPT Report displays the values of basic and genomewide as all lowercase.	text	*Values specified in Preset Value Options.*
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 option selected: • **ANOMALY DETECTED**—View the anomaly_ description for anomaly specifics. • **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 cancelled by the user. • **INVALIDATED**—Sample has failed QC or invalidated by the user. • **NOT TESTED**—Sex chromosome was not tested. • **Not applicable**— Category is not applicable to sample.	class_ sx	*Values specified in Preset Value Options.*

Column	Description	Preset Value Options	Type	Regex
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: • **ANOMALY DETECTED**—Autosomal chromosomal anomaly detected. • **NO ANOMALY DETECTED**—No autosomal anomaly detected. • **CANCELLED**—Sample cancelled by the user. • **INVALIDATED**—Sample has failed QC or invalidated by the user. • **Not applicable**— Category is not applicable to sample.	text	*Values specified in Preset Value Options.*
anomaly_ description	ISCN-style string that describes all reportable anomalies. Multiple anomalies are separated by semicolons.	**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** | **not applicable** | **INVALIDATED** | **CANCELLED**.	text	*Semicolon-separated strings and other values described in the Anomaly Description Rules on page 57 section.*
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: • **PASS** • **WARNING** • **FAIL** • **CANCELLED** • **INVALIDATED** • **NTC_PASS**	enum	*Values specified in Preset Value Options.*

Column	Description	Preset Value Options	Type	Regex
qc_reason	QC failure or warning information.	One of the following: • **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	*Values specified in Preset Value Options.*
ff	Estimated fetal fraction.	Percent sample cfDNA from fetus rounded to the nearest integer. Results less than 1% are presented as < 1%.	text	*Not applicable.*

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 the following 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 the following is true:

- <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 at p13 on chromosome 19 has a duplication appears as follows:

```
dup(19)(p13.3,p13.2)
```

The anomaly_description field follows four 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 anomalies within the same chromosome, whole chromosome aneuploidies come before partial deletions or duplications.
3. For partial deletions or duplications within the same chromosome, deletions come before duplications.

4. Partial deletions or duplications of the same type within the same chromosome are ordered by the starting base, which appears in the Supplementary Report.

NOTE For the genomewide screen, the software can report an aneuploidy and a partial deletion or duplication as affecting the same chromosome. If this result occurs, consult the supplementary report for additional metrics to aid interpretation.

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, 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.

QC Reason Message	Description	Recommended Action
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 are 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 are 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.

QC Reason Message	Description	Recommended Action
MULTIPLE ANOMALIES DETECTED	Two or more reportable anomalies (including whole chromosome aneuploidies and CNV events) 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.
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 cancelled 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 following table.

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	*Refer to Supplementary Report Metrics* on page 62.

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. Parentheses indicate that an edge value is excluded from the range. 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)
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	Number of partial deletion or duplication regions detected in the sample.	integer	(0, Inf)
non_ excluded_ sites	Per sample	Number of reads remaining after filtering, which are counted for analysis. For samples with ≤ 2 million or ≥ 60 million reads, analysis QC fails and a FAILED iFACT message displays. NES is one of several specific metrics used to calculate iFACT QC and is not the sole determinant for pass or fail results.	integer	(0, Inf)

Metric Name	Frequency	Description	Type	Regex or Value Range
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 matches 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	*Values specified in Description.*
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). If this LLR score exceeds a predetermined threshold, a trisomy is called. For partial deletions or duplications, this metric only appears if the type is a gain (dup). Otherwise, this metric appears as not applicable (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 not applicable (not applicable). This metric appears as NOT TESTED if you choose to perform the basic screening type.	float	(-Inf, Inf)

Metric Name	Frequency	Description	Type	Regex or Value Range
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 not applicable (not applicable).	float	(-Inf, Inf)

Metric Name	Frequency	Description	Type	Regex or Value Range
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 not applicable (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

This section provides details on the process reports generated by the VeriSeq NIPT Assay Software.

Batch Initiation Report

The system generates a Batch Initiation Report when a batch is initiated and validated successfully before plasma isolation. The report can be sent to the LIMS to indicate that the batch has been created and to provide a list of associated samples.

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 NIPT v2 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:subprocess. 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}$
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}$

Column	Description	Type	Regex
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 µl.	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	Sequencing system 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 sequencing system.	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}$
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_]+$
timestarted	Timestamp associated with analysis start.	ISO 8601 timestamp	
timefinished	Timestamp associated with analysis failed.	ISO 8601 timestamp	

Troubleshooting

Introduction

VeriSeq NIPT Solution v2 troubleshooting assistance comprises the following features:

- VeriSeq NIPT 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 VeriSeq NIPT 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.

Notification	Step	When	Alert Level	Email	Recommended Action
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.
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, contact 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	• If using a NAS, check the network connection. Refer to *Recommended Action Procedures* on page 88 • 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	1. Check available disk space. Refer to *Recommended Action Procedures* on page 88. 2. Clear disk space or backup data. Refer to *Recommended Action Procedures* on page 88.
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 instrument or NAS failure. Check the instrument configuration and the network connection. The system will continue to look for the pool barcode file until sequencing is completed.

Notification	Step	When	Alert Level	Email	Recommended Action
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.
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	• Check available disk space. Refer to *Recommended Action Procedures* on page 88. If space is low, clear disk space or backup data. Refer to *Recommended Action Procedures* on page 88. • If using a NAS, check the network connection. Refer to *Recommended Action Procedures* on page 88. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.

Notification	Step	When	Alert Level	Email	Recommended Action
Unable to check disk space	Pre-Analysis	The software could not check disk space.	Alert	Yes	• If using a NAS, check the network connection. Refer to *Recommended Action Procedures* on page 88 Action ID *2* on page 88. • 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. Refer to *Recommended Action Procedures* on page 88 Action ID *3* on page 89.
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	• If using a NAS, check the network connection. Refer to *Recommended Action Procedures* on page 88. • 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	1. Check email configuration defined on system for validity. Refer to *Configure System Email Notifications* on page 32. 2. Send a test email. Refer to *Configure System Email Notifications* on page 32. 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	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	• Check available disk space. Refer to *Recommended Action Procedures* on page 88. If space is low, clear disk space or back up data. Refer to *Recommended Action Procedures* on page 88. • 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 instrument 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 instrument configuration file. Check the instrument configuration and resequence the pool.

Notification	Step	When	Alert Level	Email	Recommended Action
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 instrument 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.
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 sequencing system failure. Contact IlluminaTechnical Support for assistance.
Unable to read Pool Barcode File	Sequencing	The file containing the pool barcode is corrupted.	Alert	Yes	Possible sequencing system 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 sequencing system 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 sequencing system and the network connection. Resequence the pool.

Notification	Step	When	Alert Level	Email	Recommended Action
Sequencing QC files generation failed	Sequencing QC	The sequencing run has completed but the interop QC files are corrupted.	Alert	Yes	Check the sequencing system 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	• If using a NAS, check the network connection. Refer to *Recommended Action Procedures* on page 88. • 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	• If using a NAS, check the network connection. Refer to *Recommended Action Procedures* on page 88. • Possible hardware failure. Reboot the server. If problem persists, email Illumina Technical Support.
Plate Level Contamination Detected	Post-Analysis	Chromosome Y has been detected for all samples that passed QC in the pool.	Alert	Yes	Restart library plating.

Recommended Action Procedures

Action ID	Recommended Action	Steps
1	Check the network connection	Make sure that the remote storage NAS and the local machine are on the same network. 1. From a Windows command line (cmd), type the following command: **ping <Server IP>** If using a NAS, also check the connection with the NAS. 2. Make sure that there are no lost packets. If there are lost packets, contact the IT Administrator. 3. Test the connection as follows: a. Log in to the Onsite Server Web UI. b. From the Dashboard menu, select **Folder**. c. Select **Test**, and determine if the test is successful. If the test fails, refer to *Edit a Shared Network Drive* on page 29 and make sure that all settings are configured correctly.
2	Check available disk space	Make sure that the Windows machine maps to the Onsite Server Input folder. For more information, refer to *Map Server Drives* on page 40. Right-click the drive that maps to the Input folder. Select **Properties** and view the free space information.

Action ID	Recommended Action	Steps
3	Clear disk space / Back up data	Illumina recommends a periodic data backup and/or storing sequencing data on the server side. For more information, refer to *Manage a Shared Network Drive* on page 29. 1. For data stored locally on the Onsite Server: Make sure that the Windows machine maps to the Onsite Server Input folder. For more information, refer to *Map Server Drives* on page 40. a. Double-click the Input folder, and enter the credentials to access it. b. Sequencing run data are listed with folder names matching sequencing run names. c. Delete or back up the processed sequencing folders. 2. For data stored on a remote NAS: 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. a. Sequencing run data are listed with folder names matching sequencing runs names. b. Delete or back up the processed sequencing folders.

System Issues

Issue	Recommended Action
Software fails to start.	If errors are detected when starting the VeriSeq NIPT 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 VeriSeq NIPT 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 VeriSeq NIPT Assay Software Dashboard 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.

Test 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_NB551052_0252_AH5KGJBGX9_Copy_Analysis_Workflow.
   - For NextSeqDx data: 180911_NDX550152_0014_AXXXXXXXDX_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.
6. Associate reports with the sequencing name assigned to the folder.
7. 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_H5KGJBGX9_nipt_report_YYYYMMDD_HHMMSS.tab.
   - For NextSeqDx: TestData_NDx_CopyWorkflow_C_TestData_NDx_CopyWorkflow_PoolC_XXXXXXXDX_nipt_report_YYYYMMDD_HHMMSS.tab.

   The expected file size is approximately 7.1 Kb.
8. 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.

Run 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_AXXXXXXXDX_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.
5. Associate reports with the sequencing name assigned to the folder.

6. In the output folder, open the TestData_NDx_FullRun folder, and check for the following report: TestData_NDx_FullRun_C_TestData_NDx_FullRun_PoolC_XXXXXXXDX_nipt_report_YYYYMMDD_HHMMSS.tab.
   The expected file size is approximately 7.1 Kb.
7. Move the test sequencing run back to the TestingData folder.

Resources and References

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.
Microlab® STAR Line Operator's Manual, Hamilton Doc ID 624668	Provides operation and maintenance information and technical specifications for the Hamilton Mircolab 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.

Acronyms

Acronym	Definition
BCL	Base Call File
CE-IVD	European Conformity marking for *in vitro* 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
LLR	Log Likelihood Ratios
MAC	Media Access Control
NAS	Network-Attached Storage
NES	Non Excluded Sites

Acronym	Definition
NGS	Next Generation Sequencing
NIPT	Non Invasive Prenatal Testing
NTC	No Template Control
NTP	Network Time Protocol
PF	Passing Filter
QC	Quality Control
Regex	Regular Expression. A sequence of characters that can be used by string matching algorithms for data validation.
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

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

Product documentation—Available for download from support.illumina.com.

Illumina, Inc.
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com

CE
2797

IVD

EC REP
Illumina Netherlands B.V.
Steenoven 19
5626 DK Eindhoven
The Netherlands

Australian Sponsor
Illumina Australia Pty Ltd
Nursing Association Building
Level 3, 535 Elizabeth Street
Melbourne, VIC 3000
Australia

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