

# VeriSeq NIPT Solution

## Software Guide



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## Revision History

Document	Date	Description of Change
Document # 1000000001949 v04	August 2021	Updated EU Authorized Representative address.
Document # 1000000001949 v03	December 2019	Updated back cover with new Notified Body number and addresses. Updated Non-Excluded Site and NCD information and metrics in the Analysis QC sections. Fixed error in VeriSeq Onsite Server section heading and product name and corrected typo in server size in the Local Database section.
Document # 1000000001949 v02	April 2018	Updated the following in the VeriSeq NIPT Workflow Manager chapter: <ul style="list-style-type: none"> <li>• Added Edit Sample Attributes row to table in VeriSeq NIPT Batch Manager section.</li> <li>• Added batch_name and sample_barcode information in Sample Sheet Input table.</li> <li>• Added Edit Sample Attributes task.</li> <li>• Revised steps in Sample Invalidation, Batch Invalidation, and Pool Invalidation tasks.</li> <li>• Added Upload Sample Sheet section.</li> <li>• Added Default Sex Chromosome Reporting parameters information in Assay Configuration section.</li> </ul> Revised the following sections in the VeriSeq NIPT Assay Software chapter: <ul style="list-style-type: none"> <li>• Revised information about NTCs per run in NTC Samples QC section.</li> <li>• Added note about Server Message Box (SMB) protocol in Local Disk and Mapping Server Drives sections.</li> </ul>
Document # 1000000001949 v01	June 2017	Updated back cover with information for Australia.
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# VeriSeq NIPT Solution

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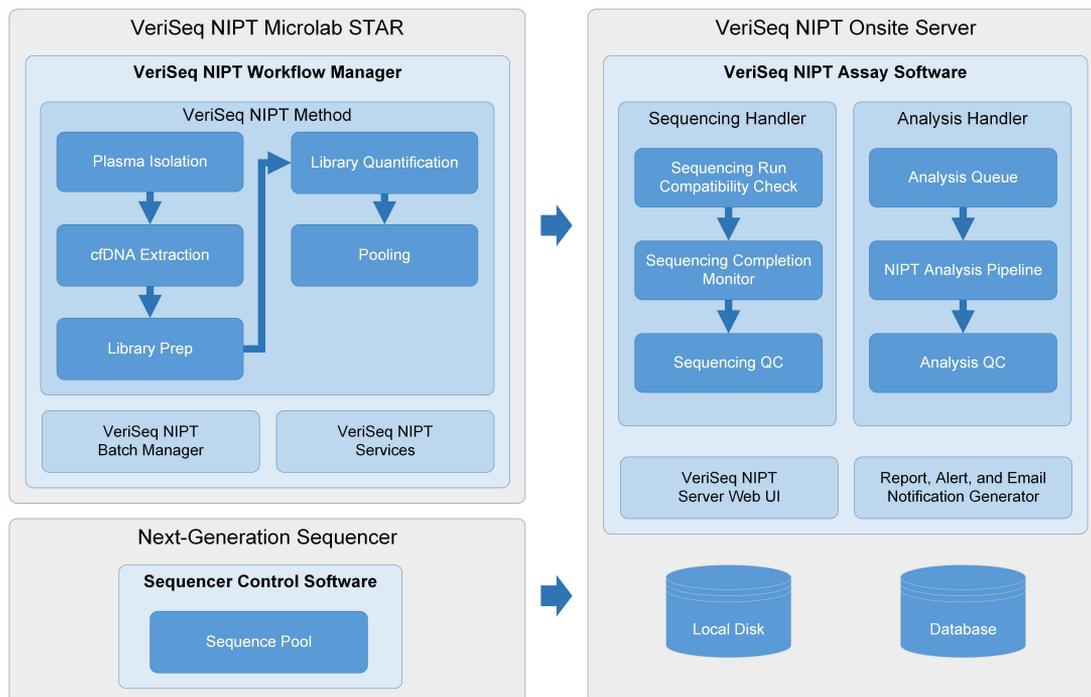
## Introduction

The VeriSeq NIPT Solution is an *in vitro* diagnostic test intended for use as a sequencing based screening test for the detection of fetal aneuploidies from maternal peripheral whole blood samples in pregnant women of at least 10 weeks gestation. VeriSeq NIPT provides information regarding aneuploidy status for chromosomes: 21, 18, 13, X, and Y. This product must not be used as the sole basis for diagnosis or other pregnancy management decisions.

The VeriSeq NIPT Solution system architecture comprises:

- ▶ **VeriSeq NIPT Microlab STAR (ML STAR)**—An automated liquid handling instrument that uses the VeriSeq NIPT Workflow Manager and VeriSeq NIPT Sample Prep Kits to prepare and track library samples. The ML STAR prepares samples intended for analysis using the VeriSeq NIPT Assay Software according to the Instructions for Use found in the *VeriSeq NIPT Solution Package Insert (document # 1000000001856)*.
- ▶ **Next-Generation Sequencer (NGS)**—A genome-sequencing instrument that provides on-instrument cluster generation and sequencing. The sequencer control software provides the steps to set up a sequencing run and generates sequencing reads for all samples in the quantified library pool. Sequencing data are evaluated by the Analysis Handler of the Assay Software.
- ▶ **VeriSeq Onsite Server**—A standalone server that uses the Assay Software to analyze paired-end sequencing data. The Assay Software provides various statistics, including log likelihood ratios, that can be used to assess over or under chromosomal representation in each individual sample.

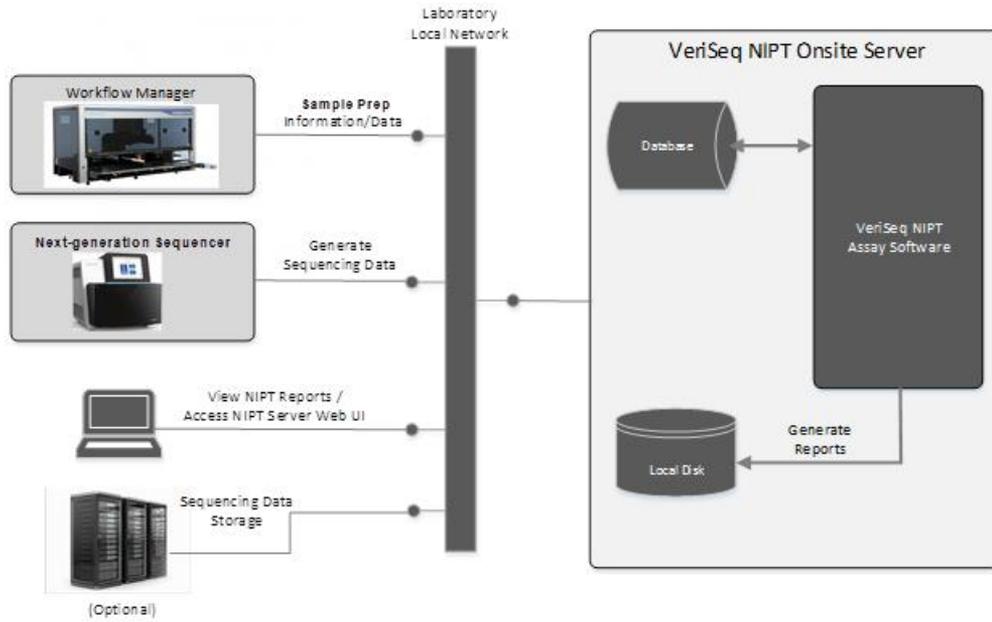
Figure 1 VeriSeq NIPT Solution Components



## System Architecture

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

Figure 2 VeriSeq NIPT Solution Overview



# VeriSeq NIPT Workflow Manager

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## Introduction

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

The Workflow Manager provides access to 3 methods:

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

## VeriSeq NIPT Method

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

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

## VeriSeq NIPT Batch Manager

The VeriSeq NIPT Batch Manager (Batch Manager) manages the status of samples, batches, and pools using the same interface used to execute the automated procedures. The system allows sample tracking across multiple liquid handling systems and sequencers, and through the analysis pipeline. For more information about sample processing procedures, see the *VeriSeq NIPT Solution Package Insert (document # 1000000001856)*.

Samples are managed within the workflow through the following objects:

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

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

Action	Object	Report Generated	Description
Invalidation	Sample	Sample Invalidation	Sample flagged by the user as no longer valid for processing. No test result is generated for invalidated samples. Example: Visible blood cell carryover during Plasma Isolation.
	Batch	Batch Invalidation	Batch flagged by the user as no longer valid. If batch invalidation occurs before pool generation, all samples are invalidated. Example: Dropped or otherwise improperly handled plate.
	Pool	Pool Invalidation	Pool flagged by the user as no longer valid. After 2 pool invalidations, all samples within the pool are invalidated. Example: Entire pool volume used during 2 sequencing failures.
QC Failure	Sample	Sample Invalidation	VeriSeq NIPT Solution automatically flagged sample as invalid due to the failure of a specified QC metric or from a system-detected liquid handling failure.
	Batch	Batch Invalidation	VeriSeq NIPT Solution automatically flagged entire batch as invalid. Example: System failure during liquid handling.
Cancelation	Sample	Sample Cancelation	Laboratory management flagged sample as canceled. No test result is generated.
Edit Sample Attributes	Sample	Sample Sex Reporting	Sex reporting flagged by the user as Yes, No, or SCA. For Sample sex reporting designated as Yes, the sex of the sample is generated. For sample sex reporting designated as No, the sex of the sample is not generated. For sample sex reporting designated as SCA, only sex chromosome aneuploidies are reported.
	Sample	Sample Sex Type	Sex type flagged by the user as Singleton, Twin, NTC, or Control. The designation of the sample sex type directly affects the analysis of the test. To ensure accurate test results, the sample sex type is required to be accurate.

After an invalidation, QC failure, or cancelation action, the object is not processed further. Laboratory information management systems (LIS/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. NOTE Sample sheets without a batch_name column will not be accepted.
sample_barcode	String	Required	Barcodes on blood sample tubes loaded onto the ML STAR. If an integer value is used as sample barcode, it should not exceed 15 digits. An alphanumeric sample barcode can be a maximum of 32 characters. Use only numbers, letters, dashes (-), and underscores (_).
sample_type	String	Required	Indicates the sample type for analysis. Allowed values are "Singleton", "Twin", "Control", and "NTC".
sex_chromosomes	String	Required	Indicates the fetal sex chromosomes reporting. Allowed values are "yes" (report), "no" (do not report), and "sca" (report only for sex chromosome aneuploidies).

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

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

Choose from 3 ways of using sample sheets:

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

## Predefined Batches—LIMS-Created Batches

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

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

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

## Ad-hoc Batching—Created Batches

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

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

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

## Hybrid Batching—LIMS Priority Samples

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

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

## Edit Sample Attributes

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

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

## Sample, Batch, and Pool Invalidation

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

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

## Sample Invalidation Using VeriSeq NIPT Method

- 1 During sample processing, in the Well Comments window at the end of each Workflow Manager process, select the individual wells to fail, and click **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 click **OK**.
- 4 Confirm that the system is to fail the sample.

## Sample Invalidation Using Batch Manager

Use Batch Manager to invalidate:

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



### NOTE

Exit any currently running methods before running the Batch Manager.

## Access Batch Manager

- 1 To access Batch Manager, do one of the following:
  - ▶ On the **App Launcher**, click **VeriSeq NIPT Batch Manager**.
  - ▶ On a computer connected to the network, browse to C:\Program Files (x86)\HAMILTON\Methods\VeriSeqNIPT. 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 click **OK**.
- 3 On the batch plate diagram, click the well position associated with the failed sample.
- 4 Confirm that the correct sample is displayed, and click **Invalidate Sample**.
- 5 Enter a reason for failure, and click **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 click **OK**.
- 3 On the batch plate diagram, click **Invalidate Batch**.
- 4 Enter a reason for failure, and click **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 click **Pool Manager**.
- 3 Scan the barcode of the pool.
- 4 Enter User Name or operator initials, and click **OK**.
- 5 Enter a reason for failure, and click **Invalidate**.

## Upload Sample Sheet

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

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

## Sample Cancelation

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

## VeriSeq NIPT Services

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

## Launch VeriSeq NIPT Services



### NOTE

Close all running methods before executing Services.

Open the Hamilton Run Controller, and then open the method file:

- ▶ Open VeriSeq NIPT Services from the Hamilton AppLauncher.  
OR
- ▶ "C:\Program Files (x86)\HAMILTON\Methods\VeriSeqNIPT\VeriSeqNIPT\_Service.med"

The Services tools enable 2 types of tests:

- ▶ **Individual Tests**—Component tests used for troubleshooting the ML STAR hardware.

- ▶ **Service Tools**—Tools used to configure the Workflow Manager.

## Individual Tests

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

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

To execute individual tests:

- 1 Select specific test to be executed.



### NOTE

Full IOQ Execution runs all 6 tests sequentially.

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

## Service Tools

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

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

## Server Configuration

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

- 1 From the Services Tools menu, select **Server Configuration**.
- 2 Update the URL with the new address of the Onsite Server.

- 3 Click **Test Connection**.  
If this message is not received, contact Illumina Technical Support.
- 4 From the System Configuration screen, click **OK**, and click **Apply** to save the new value.

## Assay Configuration

The concentration of the sequencer libraries in the sequencer pools can be adjusted within the Workflow Manager on a run-by-run basis during the pooling process (see the *VeriSeq NIPT Solution Package Insert (document # 1000000001856)*). The Assay Configuration tool can also be used to change the default value for this concentration.

The Default Sex Chromosome Reporting parameters can be set to Yes or No. This parameter determines what attribute to assign to samples when the Use Default button is selected during sample preparation. The Assay Configuration tool can be used to change this value.

- 1 Click **Assay Configuration**.
- 2 Update the Target Library Concentration (pg/μl) box to the desired value.
- 3 Update Default Sex Chromosome Reporting to the desired value.
- 4 Click **Apply**.  
If no errors are encountered, the default system target library concentration resets.

## Deck Teach Tool

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

- 1 Click **Deck Teach Tool**.
- 2 Click **Export**.
- 3 Select an output location for the text file containing the taught deck positions.
- 4 Click **OK**.  
The Deck Teach Tool saves a text file containing the values for all the taught labware positions for the Workflow Manager installation.
- 5 Click **Cancel** to return to the Method Selection screen.

# Next-Generation Sequencer

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## Introduction

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

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

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

## Sequence Pool

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

- ▶ Production of 2x36 paired end reads
- ▶ Compatible with index adapters in the VeriSeq NIPT Sample Prep Kit
- ▶ 2-dye based chemistry
- ▶ Automatic production of .BCL files

## Data Storage Integration

A typical sequencing run for the VeriSeq NIPT Solution 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 1 of the following methods:

- ▶ Use the Onsite Server as the data repository. In this configuration, the sequencer is mapped directly to the server and persists data on the local drive.
- ▶ For a high throughput lab, use network-attached storage (NAS). Configure the next-generation sequencing system to persist the sequencing data directly to a specific location on the NAS. In this setup, configure the Onsite Server to monitor the specific NAS location that enables the server to monitor upcoming sequencing runs. Multiple next-generation sequencing systems can be added to increase sample throughput. For more information on how to map the server to the NAS, see *Managing a Shared Network Drive* on page 21.

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

## Analysis Throughput Capacity

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

## Network Traffic Constraints

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

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

# VeriSeq NIPT Assay Software

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## Introduction

The Onsite Server is a central component of the VeriSeq NIPT Solution and acts as a connection point between the Workflow Manager, the next-generation sequencer, and the user. The Onsite Server hosts the Assay Software that runs continuously as a background service.

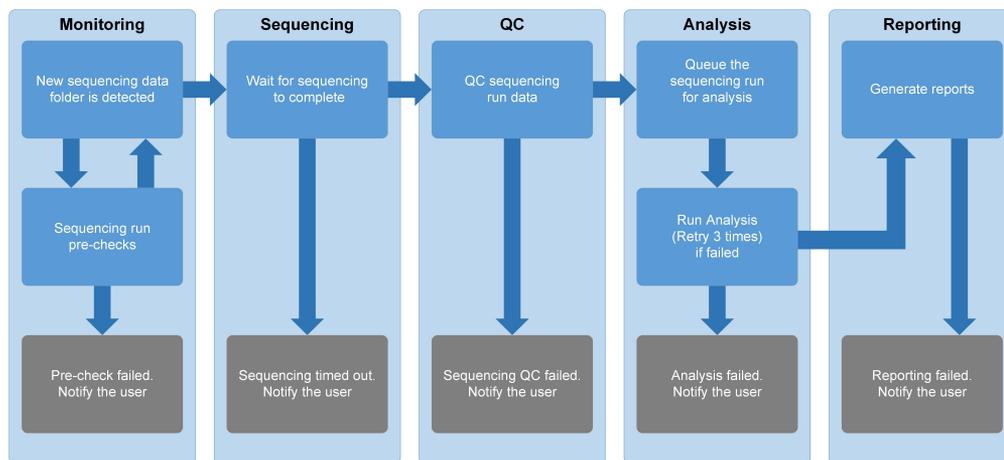
The Assay Software generates statistics to evaluate the chromosome copy number of the tested samples and provides a determination of aneuploidy on target chromosomes 21, 18, 13, X, and Y. A next-generation sequencing instrument generates analysis input in the form of 36-base paired-end reads. The Assay Software aligns the reads against the reference human genome and performs analysis on reads that align to a unique location or site in the genome. The Assay Software excludes duplicate reads and sites that are associated with high variation in coverage across euploid samples. Sequencing data are normalized for nucleotide content and to correct for batch effects and other sources of unwanted variability. Information from cfDNA fragment length is derived from the paired-end sequencing reads. The Assay Software also assesses sequencing coverage statistics on regions known to be enriched for either fetal or maternal cfDNA. Data generated from fragment length and coverage analysis are used to estimate fetal fraction for each sample. Using this information, the VeriSeq NIPT Assay Software generates statistics for each test chromosome in each sample and provides a determination of aneuploidy by comparing:

- ▶ Probability of a sample being affected given the normalized sequencing data on a region
- ▶ Estimated fetal fraction to the probability of a sample being unaffected given the same information

## Assay Software

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

Figure 3 Data Flow Diagram



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

## Assay SoftwareTasks

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

### Automated Tasks

The Assay Software completes the following automated tasks:

- ▶ **Sample preparation log collation and storage**—Produces a set of output files at the end of each step and stores them in the ProcessLog folder located in the Output folder. For more information, see *Reports File Structure on page 34* for an overview and *Process Reports on page 40* for details.
- ▶ **Alert, email, and report notification generation**—Monitors the validity status of the batch, pool, and sample during the Sample Preparation steps and QC of the sequencing data and analysis results per sample. Based on these validation checks, the Assay Software determines whether to continue with the process and if the results are to be reported. The Assay Software terminates the process when a sample 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 informatics pipeline algorithm. The Assay Software determines the aneuploidy results for each sample. The system does not report results for samples invalidated or canceled by the user. For samples that fail the QC criteria, an explicit rationale for the failure is provided; however, results for the failed sample are suppressed. For more information, see *NIPT Report on page 37*.
- ▶ **Results file generation**—Provides sample results in a tab-separated value file format, which is saved to the Output folder. For more information, see *Reports File Structure on page 34*.
- ▶ **Report generation**— The Assay Software generates results, notifications, and process reports. For more information, see *on page 34*.
- ▶ **Sample, pool, and batch invalidation**—

- ▶ **Sample invalidation**—The Assay Software marks individual samples as invalid when the user:
  - ▶ Explicitly invalidates the sample.
  - ▶ Invalidates the whole plate during library preparation before the pools are created.
 When a sample is marked as invalid, a Sample Invalidation Report is automatically generated, see [Sample Invalidation Report on page 39](#).
- ▶ **Pool and batch invalidation report generation**—Pools and batches can only be invalidated by the user. Invalidated pools are not processed by the system. Pools that had already been created from an invalid batch are not automatically invalidated and can be further processed by the system. However, new pools cannot be created from an invalidated batch. When a pool is invalidated, the system issues a Pool Retest Request Report under the following conditions:
  - ▶ The batch is valid.
  - ▶ There are no more available pools for this batch.
  - ▶ The number of allowed pools from the batch has not been exhausted.
 For more information, see [Pool Retest Request Report on page 40](#).
- ▶ **Retest management**—
  - ▶ **Pool failures**—Failed pools are typically pools that failed the Sequencing QC metrics. The Assay Software does not proceed with processing failed pools if the run is terminated. Resequence using a second pool aliquot.
  - ▶ **Sample failures**—The software allows failed samples to be retested if needed. Failed samples need to be incorporated into a new batch and reprocessed through assay steps.
  - ▶ **Reruns**—The system does not reanalyze pools with samples that had previously been processed and reported successfully. Rerunning a sample can be done by replating the sample on a new batch.

## User Tasks

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

Using the Workflow Manager:

- ▶ Mark as invalid an individual sample, all samples within a batch, or all samples associated with a pool.
- ▶ Mark a given sample as canceled. The Assay Software then marks the result as canceled in the final results report.

Using the Assay Software:

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



### NOTE

Users can perform certain tasks depending on user permissions. For more information, see [Assigning User Roles on page 19](#).

## Sequencing Handler

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

After the validation is completed successfully, the Assay Software continues to monitor the sequencing runs for completion. Completed sequencing runs are queued to be processed by the Analytic Pipeline Handler (see *Analytic Pipeline Handler* on page 16).

## Sequencing Run Compatibility

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

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



### NOTE

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

Configure sequencing using compatible read parameters.

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

## Analytic Pipeline Handler

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

A successful analysis run triggers the NIPT report generation. For more information, see *NIPT Report* on page 37.

## Workflow Timeout and Storage Requirements

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

Parameter	Default Value
Maximum Run Parameters Wait Time	4 hours
Maximum Sequencing Time	20 hours
Maximum Analysis Time	10 hours
Minimum Scratch Space Storage	2 TB

## Web User Interface

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

- ▶ **View recent activities**—Identifies the steps completed during the assay execution. The user is alerted to many of these activities by the email notification system. For more information, see [Assay Software Notifications on page 48](#).
- ▶ **View errors and alerts**—Identifies problems that might prevent the assay from proceeding further. Error messages and alerts are sent to the user through the email notification system. For more information, see [Assay Software Notifications on page 48](#).
- ▶ **Configure the server network settings**—Illumina personnel typically configure the network during system installation. Modifications may be required if the local network requires IT changes. For more information, see [Changing Network and Server Settings on page 22](#).
- ▶ **Manage server access**—The Onsite Server allows Administrator and Operator level access. These access levels control viewing of the activity, alert, and error logs and modification of the networking and data mapping settings. For more information, see [Managing Users on page 19](#).
- ▶ **Configure sequencing data folder**—By default, the server stores sequencing data. However, a central NAS can be added to expand storage capacity. For more information, see [Mapping Server Drives on page 28](#).
- ▶ **Configure email notification subscribers list**—Manages a list of subscribers to receive email notifications comprising error messages and assay process alerts. For more information, see [Configuring System Email Notifications on page 23](#).
- ▶ **Reboot or shutdown the server**—Restarts the server, if needed. A reboot or shutdown can be required for a configuration setting to take effect or as a remedy for a server failure. For more information, see [Rebooting the Server on page 28](#).

## End User License Agreement

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

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

## Configuring the Web UI

Select the Settings icon  for a drop-down list of configuration settings. Settings appear based on user role and associated permissions. For more information, see [Assigning User Roles on page 19](#).



### NOTE

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

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

## Logging in to the Web UI

To access the Assay Software interface and login:

- 1 On a computer connected to the same network as the Onsite Server, open 1 of the following web browsers:
  - ▶ Chrome v33 or later
  - ▶ Firefox v27 or later
  - ▶ Internet Explorer v11 or later
- 2 Enter the server IP address or the server name provided by Illumina at installation, equivalent to \\<VeriSeq Onsite Server IP address>\login.  
For example, \\10.10.10.10\login.
- 3 If a browser security warning appears, add a security exception to proceed to the login screen.
- 4 In the login screen, enter the case-sensitive user name and password provided by Illumina, and click **Log In**.



### NOTE

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

## Using the Dashboard

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

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

**Figure 4** VeriSeq NIPT Assay Software Dashboard

WHEN	USER	SUBSYSTEM	DETAILS	LEVEL
2016-07-29 09:17 PDT		Assay	Aneuploidy Detection Report generated for '160728_NB551043_0005_AHCLWJBGXY'	Activity
2016-07-29 09:17 PDT		Assay	Analysis started for '160728_NS500411_0171_AHCLGJBGXY'	Activity
2016-07-29 05:23 PDT		Assay	Sequencing QC passed for '160728_NS500411_0171_AHCLGJBGXY'	Activity
2016-07-29 05:14 PDT		Assay	Analysis started for '160728_NB551043_0005_AHCLWJBGXY'	Activity
2016-07-29 05:14 PDT		Assay	Sequencing QC passed for '160728_NB551043_0005_AHCLWJBGXY'	Activity
2016-07-28 19:56 PDT		Assay	Sequencing started for '160728_NB551043_0005_AHCLWJBGXY'	Activity
2016-07-28 19:55 PDT		Assay	Sequencing started for '160728_NS500411_0171_AHCLGJBGXY'	Activity
2016-07-28 17:18 PDT		Assay	Batch 'DVT0151_PL02_1':pool 'PT2008505' created	Activity
2016-07-28 17:18 PDT		Assay	Batch 'DVT0151_PL02_1':pool 'PT2008521' created	Activity
2016-07-28 16:14 PDT		Assay	Batch 'DVT0151_PL02_1' completed library	Activity
2016-07-28 10:30 PDT		Assay	Batch 'DVT0151_PL02_1' Initiated	Activity

## Viewing Recent Activities

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

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

## Viewing Recent Errors

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

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

## Viewing System Status and Alerts

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

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

## Managing Users



### NOTE

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

## Assigning User Roles

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

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

## Adding Users

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

To add a user:

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



### NOTE

All fields are required.

- 2 Enter the user name.



### NOTE

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

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

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

### Note

Passwords must be 8–20 characters and contain at least one uppercase letter, one lowercase letter, and one numerical character.
- 5 Enter an email address for the user.  
A unique email address is required for each user.
- 6 Select the desired user role from the drop-down list.
- 7 Select the **Active** box to activate the user immediately or deselect the box to activate the user later (ie, after training).
- 8 Click **Save** twice to save and confirm changes.  
The new user now appears on the User Management screen.

## Editing Users

To edit user information:

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

## Deactivating Users

To deactivate a user:

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

## Managing a Shared Network Drive



### NOTE

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

## Adding a Shared Network Drive

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

- 1 From the Dashboard, select **Folders**.
- 2 Click **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 Click **Save**.
- 5 Click **Test** to test the NAS connection.  
If the connection fails, confirm the server name, location name, user name, and password with the IT administrator.
- 6 Restart the server to apply the changes.



### NOTE

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

## Editing a Shared Network Drive

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

## Deleting a Shared Network Drive

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

## Configuring Network and Certificate Settings

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



### NOTE

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

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

## Changing Certificate Settings

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

- 1 Use the Certificate Configuration tab to add or change SSL certificate settings.
  - ▶ **Laboratory Email**—Contact email at the testing laboratory (requires a valid email address format)
  - ▶ **Organization Unit**—Department
  - ▶ **Organization**—Name of testing laboratory
  - ▶ **Location**—Street address of testing laboratory
  - ▶ **State**—State location of testing laboratory (auto populates based on email address)
  - ▶ **Country**—Country location of testing laboratory (auto populates based on email address)
  - ▶ **Certificate Thumbprint (SHA1)**—Certification identification number



### NOTE

The Certificate Thumbprint (SHA1) appears after generating or regenerating a certificate. See [Regenerating a Certificate on page 23](#) for more information.

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



### NOTE

The SHA1 makes sure that users do not get certificate warnings when accessing the VeriSeq NIPT Assay Software.

## Changing Network and Server Settings



### NOTE

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

- 1 Use the Network Configuration tab to set up or change the network and Onsite Server settings.
  - ▶ **Static IP Address**—IP address designated for the Onsite Server
  - ▶ **Subnet Mask**—Local network subnet mask
  - ▶ **Default Gateway Address**—Default router IP address
  - ▶ **Hostname**—Designated name to reference the Onsite Server on the network (defined as localhost by default)
  - ▶ **DNS Suffix**—Designated DNS suffix
  - ▶ **Nameserver 1 and 2**— IP address or DNS server name for Network Time Protocol (NTP) time synchronization servers

- ▶ **NTP Time Server 1 and 2**—Servers for NTP time synchronization
  - ▶ **MAC Address**—Server networking MAC address (read only)
  - ▶ **Timezone**—Server local time zone
- 2 Confirm that the entries are correct, and click **Save** to reboot the server and implement any changes made.

**CAUTION**

Incorrect settings can disrupt the connection with the server.

## Downloading and Installing a Certificate

To download and install an SSL certificate:

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

## Regenerating a Certificate

**NOTE**

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

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

- 1 Select **Regenerate Certificate** from the Network Configuration screen.
- 2 Click **Regenerate Certificate and Reboot** to proceed, or click **Cancel** to exit.

## Configuring System Email Notifications

The VeriSeq NIPT Assay Software communicates with users by sending email notifications indicating the assay progress and alerts for errors or required user action. *Assay Software Notifications* on page 48 describes the various email notifications sent by the system.

**NOTE**

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

## Creating an Email Subscriber List

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

To specify a subscriber list:

- 1 From the Dashboard, click 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 Click **Send test message** to generate a test email to the subscribers list. Check your email inbox to verify that the email was sent.
- 5 Click **Save**.

## Analysis and Reporting

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

### Demultiplexing and FASTQ Generation

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



#### NOTE

Users are highly encouraged NOT to modify or edit this sample sheet file as it is system generated and can cause adverse effects downstream including analysis failure.

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

## Sequencing QC

Sequencing QC metrics identify flow cells that are likely to fail analysis with high probability. The cluster density, percent reads passing filter (PF), prephasing, and phasing metrics describe the general sequencing data quality and are common to many next-generation sequencing applications. The predicted aligned reads metric estimates the flow cell level of the sequencing depth. If low-quality data fails the predicted aligned reads metric, processing the run is terminated. For more information, see *Sequencing QC Metrics and Boundaries* on page 31.

## Fetal Fraction Estimates

Fetal fraction refers to the percent of cell-free, circulating DNA in a maternal blood sample that is derived from the placenta. The Assay Software calculates the fetal fraction estimate through a predetermined weighted average of 2 values, 1 based on the cfDNA fragment size distribution and 1 based on differences in genomic coverage between maternal and fetal cfDNA.<sup>1</sup>

## Statistics Used in Final Scoring

For autosomes, 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 by comparing the coverage regions that can be affected by aneuploidy with the rest of the autosomes. A log likelihood ratio (LLR) is computed for each sample by taking into account these coverage-based scores and the estimated fetal fraction. The LLR is the probability of a sample being affected given the observed coverage and fetal fraction versus the probability of a sample being unaffected given the same observed coverage. The calculation of this ratio also takes into account the estimated uncertainty in fetal fraction. For subsequent calculations, the natural logarithm of the LLR is used. The Assay software assesses the LLR for each target chromosome and each sample to provide a determination of aneuploidy.

Statistics for chromosomes X and Y are different from the statistics used for autosomes. For fetuses identified as female, SCA calls require classification agreement by LLR and by normalized chromosomal value.<sup>2</sup> 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).<sup>\*</sup> 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.

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

<sup>2</sup>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.

## Analysis QC

Analytical QC metrics are metrics that are computed during analysis and are used to detect samples that deviate too far from expected behavior. Data for samples that fail these metrics are deemed to be unreliable and are marked as failed. Analytical QC metrics and the associated cutoffs or acceptable ranges are listed in *Analytical QC Metrics and Boundaries on page 32*. The following table describes the metrics.

Category	Metric	Description
Counting QC	Clusters	Indicates low (more likely) or high (highly unlikely) cluster density.
Counting QC	NonExcludedSites (aligned_reads)	Indicates the minimum sequencing depth required for overall aneuploidy detection.
Likelihood Score for Chromosome Denominators	NCD_13 NCD_18 NCD_21 NCD_X NCD_Y	Indicates the uniformity of coverage for the whole-genome sequencing, relative to the expected behavior. Samples that fail this QC metric can either have strong genomic abnormalities (outside of the regions of interest for aneuploidy detection) or the libraries for these samples are not biased.
Fragment Size Distribution	FragSizeDist (frag_size_dist)	Indicates the distribution of cfDNA fragment size distribution, relative to the expected behavior. For example, sheared genomic DNA has a different distribution of fragment size than cfDNA and will fail this metric.
Coverage Relative to Fetal Fraction	NES_FF_QC	Indicates the sufficiency of sequencing depth given the estimated fetal fraction for any given sample. Detection of aneuploidy in samples with high fetal fraction at a specified level of confidence can be accomplished at a lower sequencing depth than in samples with lower fetal fraction.
Coverage Relative to Fetal Fraction	iFACT	Indicates whether a sufficient sequencing depth has been observed, given the estimated fetal fraction for any given sample. Detection of aneuploidy in samples with high fetal fraction at a specified level of confidence can be accomplished at a lower sequencing depth than in samples with lower fetal fraction.

## NTC Samples QC

The VeriSeq NIPT Solution allows the addition of NTC samples as part of your run. The ML STAR can generate up to 2 NTCs per run for 48-sample batches and up to 4 NTCs for 96-sample batches. Regardless of how many NTC samples are added, the software checks for a minimum average of 4,000,000 unique mapped fragments per sample per pool. For this reason, do not add more than 2 NTC samples per pool. For more information, see *Sequencing QC Metrics and Boundaries on page 31*.

QC statuses for NTC samples are:

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

## VeriSeq Onsite Server

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

## Archiving Data

Illumina recommends archiving the /data01/runs and /data01/analysis\_output directories in agreement with local IT site archiving policy. The Assay Software monitors the remaining disk space in the /data01/runs 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 analysis server and archive on a regular schedule.

A typical sequencing run that is compatible with the cfDNA analysis workflow requires 25–30 GB for next-generation sequencer runs. The actual run folder size depends on final cluster density. The server provides more than 7.5 TB of storage space, which is enough space for about 300 sequencing runs.

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

## Local Disk

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

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



### NOTE

Mapping the local disk is based on Server Message Block (SMB) protocol. The software currently supports SMB1 and SMB2 versions. Make sure these are enabled on the equipment (laptop/workstation) that you are mapping.

## Local Database

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

- ▶ **Database backup**—A snapshot of the database is automatically saved on an hourly, a daily, and a monthly basis. Hourly backups are removed after a daily backup is created. Likewise, the daily backups are removed when the weekly backup is ready. The weekly backups are removed after a monthly backup is created, and only 1 monthly backup is kept. The recommended practice is to create an automated script that can persist the backup folder on a local NAS.
- ▶ **Database restore**—The database can be restored from any given backup snapshot. Restores are done by Illumina Field Service Engineers only.
- ▶ **Data backup**—Although the Onsite Server can be used as the main storage point for sequencing runs, it can only store approximately 300 runs. Illumina recommends setting up an automated data backup that runs on a continuous basis to another long-term storage device or an NAS.

- ▶ **Maintenance**—Other than data backup, the Onsite Server does not require the user to perform any maintenance. Updates for the Assay Software or the Onsite Server itself are provided by Illumina Technical Support.

## Mapping Server Drives

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

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

To map each folder:

- 1 Log in to the computer within the Onsite Server subnet.
- 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 IP address>\<folder name>. For example: \\10.50.132.92\input.
- 5 Enter the user name and password.  
Successfully mapped folders appear mounted on the computer.



### NOTE

Mapping the local disk is based on Server Message Block (SMB) protocol. The software currently supports SMB1 and SMB2 versions. Make sure these are enabled on the equipment (laptop/workstation) that you are mapping.

## Logging Out

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

## Rebooting the Server



### NOTE

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

To reboot the server:

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



### NOTE

Rebooting the system can take several minutes.

## Shutting Down the Server



### NOTE

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

To shut down the Onsite Server server:

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

## Recovering from Unexpected Shutdown

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

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



### NOTE

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

# QC Metrics

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## Quantitation QC Metrics and Boundaries

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

## Sequencing QC Metrics and Boundaries

Metric	Description	Lower Bound	Upper Bound	Rationale
cluster_density	Sequencing cluster density	152,000 per mm <sup>2</sup>	338,000 per mm <sup>2</sup>	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.
phasing	Fraction of phasing	N/A	≤0.004	Empirically optimized recommendations for the VeriSeq NIPT Solution.
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.

## Analytic QC Metrics and Boundaries

Category	Metric	Lower Bound	Upper Bound	Failure Message	Expected failure rate	Potential Causes
Counting QC	NonExcludedSites (aligned_reads)	2,000,000	60,000,000	FAILED iFACT	<1%	Poor library or incorrect library quantification; low cluster numbers; possibly recoverable upon rerun from plasma.
Likelihood Score for Chromosome Denominators	<ul style="list-style-type: none"> <li>• NCD_13</li> <li>• NCD_18</li> <li>• NCD_21</li> <li>• NCD_X</li> <li>• NCD_Y</li> </ul>	<ul style="list-style-type: none"> <li>• -17.61</li> <li>• -16.40</li> <li>• -17.20</li> <li>• -14.89</li> <li>• -42.97</li> </ul>	10,000 (for all)	DATA OUTSIDE OF EXPECTED RANGE	<0.2%	Unexpected chromosomal representation somewhere in the genome; unlikely to get resolved by rerunning the sample. Possible reason: data outside of expected range.
Fragment Size Distribution	FragSizeDist (frag_size_dist)	0	0.07	FRAGMENT SIZE DISTRIBUTION OUTSIDE OF EXPECTED RANGE	<1%	Unexpected distribution of fragment sizes. Possible reasons: failure of size selection process, low coverage, compromised sample.
Coverage Relative to Fetal Fraction	NES_FF_QC	0	1.5	FAILED iFACT	approx. 1.2%	Insufficient coverage relative to fetal fraction.

## NTC Samples QC

The VeriSeq NIPT Solution allows the addition of NTC samples as part of your run. The ML STAR can generate up to 2 NTCs per run for 48-sample batches and up to 4 NTCs for 96-sample batches. Regardless of how many NTC samples are added, the software checks for a minimum average of 4,000,000 unique mapped fragments per sample per pool. For this reason, do not add more than 2 NTC samples per pool. For more information, see *Sequencing QC Metrics and Boundaries on page 31*.

QC statuses for NTC samples are:

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

# System Reports

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## Introduction

The Assay Software generates 2 categories of reports:

- ▶ Results and notifications reports
- ▶ Process reports

There are also 2 report types:

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

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

## Output Files

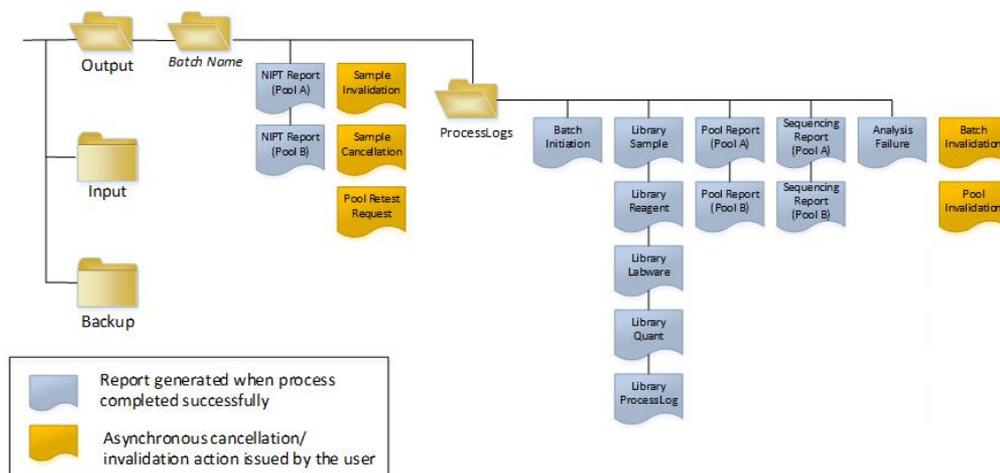
The Assay Software reports are generated on the Onsite Server internal hard drive mapped to the user drive as a read-only Output folder. Each report is generated with a corresponding standard MD5 checksum file, which is used to verify that the file has not been modified.

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

## Reports File Structure

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

Figure 5 Assay Software Reports Folder Structure



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

- ▶ **Main folder (Batch Name folder)**—Contains reports that provide results or are associated with LIMS-generated email notifications. For details, see *Results and Notifications Reports on page 37*.
- ▶ **ProcessLog folder**—Contains reports related to process. For details, see *Process Reports on page 40*

A list of all reports is provided in *System Reports Summary on page 35*.

## System Reports Summary

Report Name	Report Type	Report Entity	Report File Name Format
<i>NIPT Report</i>	Actionable	Pool/ flow cell	<batch_name>_A_<pool_barcode>_<flowcell>_nipt_report_20150528_163503.tab
<i>Sample Invalidation Report</i>	Actionable	Sample	<batch_name>_<sample_barcode>_sample_invalidation_report_20150528_163503.tab
<i>Sample Cancellation Report</i>	Actionable	Sample	<batch_name>_<sample_barcode>_sample_cancellation_report_20150528_163503.tab
<i>Pool Retest Request Report</i>	Actionable	Pool	<batch_name>_<pool_type>_pool_retest_request_20150528_163503.tab
<i>Batch Initiation Report</i>	Informational	Batch	ProcessLogs/<batch_name>_batch_initiation_report_20150528_163503.tab
<i>Batch Invalidation Report</i>	Informational	Batch	ProcessLogs/<batch_name>_batch_invalidation_report_20150528_163503.tab
<i>Library Sample Report</i>	Informational	Batch	ProcessLogs/<batch_name>_library_sample_report_20150529_083503.tab
<i>Library Reagent Report</i>	Informational	Batch	ProcessLogs/<batch_name>_library_reagent_report_20150529_163503.tab
<i>Library Labware Report</i>	Informational	Batch	ProcessLogs/<batch_name>_library_labware_report_20150518_163503.tab
<i>Library Quant Report</i>	Informational	Batch	ProcessLogs/<batch_name>_library_quant_report_20150518_163503.tab
<i>Library Process Log</i>	Informational	Batch	ProcessLogs/<batch_name>_library_process_log.tab
<i>Pool Report</i>	Informational	Pool	ProcessLogs/<batch_name>_<pool_barcode>_pool_report_20150528_163503.tab
<i>Pool Invalidation Report</i>	Informational	Pool	ProcessLogs/<batch_name>_<pool_barcode>_pool_invalidation_report_20150528_163503.tab

Report Name	Report Type	Report Entity	Report File Name Format
<i>Sequencing Report</i>	Informational	Pool/ flow cell	ProcessLogs/<batch_name>_A_<pool_barcode>_<flowcell>_sequencing_report_20150528_163503.tab ProcessLogs/<batch_name>_B_<pool_barcode>_<flowcell>_sequencing_report_20150528_163503.tab
<i>Analysis Failure Report</i>	Informational	Pool/ flow cell	ProcessLogs/<batch_name>_<pool_barcode>_analysis_failure_report_20150528_163503.tab

## Report Generation Events

Report	Description	Generation Event
NIPT	Contains the final results of a successful analysis run	<ul style="list-style-type: none"> <li>Sequencing run analysis completes</li> </ul>
Sample Invalidation	Contains information about an invalidated sample	<ul style="list-style-type: none"> <li>User invalidates a sample</li> </ul>
Sample Cancelation	Contains information about a canceled sample	<ul style="list-style-type: none"> <li>User cancels a sample</li> </ul>
Pool Retest Request	Indicates that a second pool can be generated from an existing batch. Contains information about the pool retest status. <sup>1</sup>	<ul style="list-style-type: none"> <li>User invalidates a pool</li> </ul>
Batch Initiation	Indicates a new batch processing start	<ul style="list-style-type: none"> <li>User initiates a new batch</li> </ul>
Batch Invalidation	Contains information about a user-initiated invalidated batch	<ul style="list-style-type: none"> <li>Batch is invalidated</li> </ul>
Library Sample	Lists all samples in the batch	<ul style="list-style-type: none"> <li>Batch is invalidated</li> <li>Library Preparation method completes</li> <li>Batch fails quantification</li> </ul>
Library Reagent	Contains library processing reagent information	<ul style="list-style-type: none"> <li>Batch is invalidated</li> <li>Library Preparation method completes</li> <li>Batch fails quantification</li> </ul>
Library Labware	Contains library processing labware information	<ul style="list-style-type: none"> <li>Batch is invalidated</li> <li>Library Preparation method completes</li> <li>Batch fails quantification</li> </ul>
Library Quant	Contains library quantification test results	<ul style="list-style-type: none"> <li>Batch is invalidated</li> <li>Library Preparation method completes</li> <li>Batch fails quantification</li> </ul>
Library Process Log	Contains steps executed during library processing	<ul style="list-style-type: none"> <li>Batch is invalidated</li> <li>Library Preparation method completes</li> <li>Batch fails quantification</li> <li>Batch process completes</li> </ul>
Pool	Contains sample pooling volumes	<ul style="list-style-type: none"> <li>Pooling method completes</li> </ul>
Pool Invalidation	Contains information about a user-initiated invalidated pool	<ul style="list-style-type: none"> <li>User invalidates a pool</li> </ul>
Sequencing	Contains sequencing QC results	<ul style="list-style-type: none"> <li>Sequencing QC passes</li> <li>Sequencing times out (fails)</li> </ul>
Analysis Failure	Contains analysis information for a failed pool	<ul style="list-style-type: none"> <li>Sequencing run analysis fails</li> </ul>

<sup>1</sup> 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 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	N/A	text	^[a-zA-Z0-9_]{1,36}\$
sample_barcode	Unique sample barcode	N/A	text	^[a-zA-Z0-9_]{1,36}\$
sample_type	Sample type information provided from point of collection. Determines aneuploidy classification presentation.	One of the following: <ul style="list-style-type: none"> <li>• <b>Singleton</b>—Single embryo pregnancy</li> <li>• <b>Twin</b>—Multiple embryo pregnancy</li> <li>• <b>Control</b>—Control sample of known sex and aneuploidy classification</li> <li>• <b>NTC</b>—No template control sample (no DNA)</li> </ul>	enum	Values specified in Preset Value Options
sex_chrom	Sex chromosome analysis requested. Determines aneuploidy classification presentation.	One of the following: <ul style="list-style-type: none"> <li>• <b>yes</b>—Sex chromosome aneuploidy and sex reporting requested</li> <li>• <b>no</b>—Neither sex chromosome aneuploidy nor sex reporting requested</li> <li>• <b>sca</b>—Sex chromosome aneuploidy reporting requested, sex reporting not requested</li> </ul>	enum	Values specified in Preset Value Options
flowcell	Sequencing flow cell barcode	N/A	text	NA
class_13	Aneuploidy classification results for chromosome 13	One of the following: <ul style="list-style-type: none"> <li>• <b>ANEUPLOIDY DETECTED</b></li> <li>• <b>NO ANEUPLOIDY DETECTED</b></li> <li>• <b>CANCELLED</b></li> <li>• <b>INVALIDATED</b></li> <li>• <b>NA</b></li> </ul>	class_13	Aneuploidy classification results for chromosome 13
class_18	Aneuploidy classification results for chromosome 18	One of the following: <ul style="list-style-type: none"> <li>• <b>ANEUPLOIDY DETECTED</b></li> <li>• <b>NO ANEUPLOIDY DETECTED</b></li> <li>• <b>CANCELLED</b></li> <li>• <b>INVALIDATED</b></li> <li>• <b>NA</b></li> </ul>	class_18	Aneuploidy classification results for chromosome 18

Column	Description	Preset Value Options	Type	Regex
class_21	Aneuploidy classification results for chromosome 21	One of the following: <ul style="list-style-type: none"> <li>• ANEUPLOIDY DETECTED</li> <li>• NO ANEUPLOIDY DETECTED</li> <li>• CANCELLED</li> <li>• INVALIDATED</li> <li>• NA</li> </ul>	class_21	Aneuploidy classification results for chromosome 21
class_sx	Sex chromosome aneuploidy classification	One of the following: <ul style="list-style-type: none"> <li>• ANEUPLOIDY DETECTED – XO – Monosomy X</li> <li>• ANEUPLOIDY DETECTED – XXX – Trisomy X</li> <li>• ANEUPLOIDY DETECTED – XXY – 2 X chromosomes in males</li> <li>• ANEUPLOIDY DETECTED – XYY – 2 Y chromosomes</li> <li>• NO ANEUPLOIDY DETECTED – Negative sample and sex not reported</li> <li>• NO ANEUPLOIDY DETECTED – XX – Negative sample with a female fetus</li> <li>• NO ANEUPLOIDY DETECTED – XY – Negative sample with male fetus</li> <li>• SEX CHROMOSOMES NOT REPORTABLE – Software could not report sex chromosome</li> <li>• NO CHR Y PRESENT – Twin pregnancy with no Y chromosome detected</li> <li>• CHR Y PRESENT – Twin pregnancy with Y chromosome detected</li> <li>• CANCELLED – Sample cancelled by the user</li> <li>• INVALIDATED – Sample has failed QC or invalidated by the user</li> <li>• NOT TESTED – Sex chromosome was not tested</li> </ul>	class_sx	Sex chromosome aneuploidy classification
qc_flag	QC analysis results	One of the following: <ul style="list-style-type: none"> <li>• CANCELLED</li> <li>• INVALIDATED</li> <li>• PASS</li> <li>• NTC_PASS</li> <li>• FAIL</li> </ul>	enum	Values specified in Preset Value Options

Column	Description	Preset Value Options	Type	Regex
qc_failure	QC failure information	One of the following: <ul style="list-style-type: none"> <li>• FAILED iFACT</li> <li>• DATA OUTSIDE OF EXPECTED RANGE</li> <li>• FRAGMENT SIZE DISTRIBUTION OUTSIDE OF EXPECTED RANGE</li> <li>• NTC SAMPLE WITH HIGH COVERAGE</li> <li>• CANCELLED</li> <li>• INVALIDATED</li> <li>• NONE (QC status = Pass)</li> </ul>	text	Values specified in Preset Value Options
ff	Estimated fetal fraction	Percent sample of DNA from fetus rounded to the nearest integer. Results less than 1% are presented as < 1%.	text	NA

## QC Failure Messages

Analysis QC failure results in full results suppression for chromosome aneuploidy, sex classification, and estimated fetal fraction, which correspond to the following NIPT Report fields: class\_13, class\_18, class\_21, class\_sx, and ff.

QC Failure 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	Deviation from euploid coverage on nontarget chromosomes Possibly associated with trisomy or monosomy of any target chromosome or nonspecific large copy number variants across chromosomes	Reprocess sample
FRAGMENT SIZE DISTRIBUTION OUTSIDE OF EXPECTED RANGE	The data distribution is not consistent with the trained data distribution. Possibly caused by contamination or incorrect sample processing.	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 the users	NA
INVALIDATED	Sample was invalidated by the users	

## 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	ISO 8601 timestamp

## Sample Cancelation Report

The system generates a Sample Cancelation 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 cancelation	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 cancelation	ISO 8601 timestamp	ISO 8601 timestamp

## Pool Retest Request Report

The Pool Retest Request Report indicates that either Pool A or Pool B can be repooled. The system generates a Pool Retest Request Report when the first of 2 possible sequence runs (pools) for Pool A or Pool B is invalidated.

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

## Process Reports

### Batch Initiation Report

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

Column	Description	Type	Regex
batch_name	Batch name	text	^[a-zA-Z0-9_-]{1,36}\$
sample_barcode	Unique sample barcode	text	^[a-zA-Z0-9_-]{1,36}\$
sample_type	Sample type of the sample barcode Value options: Singleton, Control, Twin, NTC	enum	Value specified in Description
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	^[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	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 Value options: pass, fail	text	^[a-zA-Z0-9_-]{1,36}\$
starting_volume	Initial volume of blood collection tube 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/ $\mu$ l	float	
plasma_isolation_comments	User comments when performing plasma isolation (free text)	text	^[a-zA-Z0-9_-]{1,36}\$
cfdna_extraction_comments	User comments when performing cfDNA extraction (free text)	text	^[a-zA-Z0-9_-]{1,36}\$
library_prep_comments	User comments when performing library preparation (free text)	text	^[a-zA-Z0-9_-]{1,36}\$
quantitation_comments	User comments when performing quantification (free text)	text	^[a-zA-Z0-9_-]{1,36}\$

## 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. Value options: <ul style="list-style-type: none"> <li>• <b>ISOLATION</b>—batch_validation, prespin, postspin, data_transact</li> <li>• <b>EXTRACTION</b>—setup, chemistry, data_transact</li> <li>• <b>LIBRARY</b>—setup, chemistry, data_transact, complete</li> <li>• <b>QUANT</b>—setup, build_standards, build_384, analysis, data_transact</li> <li>• <b>POOLING</b>—analysis, setup, pooling, data_transact, complete</li> </ul>	text	^[a-zA-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	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	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	ISO 8601 timestamp

## Library Process Log

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

Column	Description	Type	Regex
batch_name	Batch name	text	^[a-zA-Z0-9_-]{1,36}\$
process	Batch process name. Value options: <b>ISOLATION</b> —batch_validation, prespin, postspin, data_transact <b>EXTRACTION</b> —setup, chemistry, data_transact <b>LIBRARY</b> —setup, chemistry, data_transact, complete <b>QUANT</b> —setup, build_standards, build_384, analysis, data_transact <b>POOLING</b> —analysis, setup, pooling, data_transact, complete	text	^[a-zA-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	ISO 8601 timestamp
finished	Date and time of batch process completion or failure	ISO 8601 timestamp	ISO 8601 timestamp
status	Current batch Value options: completed, failed, started, aborted	enum	Values specified in Description

## 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 Value options: A, B, C	enum	Values specified in Description
pooling_volume_ul	Pooling volume in µl	float	
pooling_comments	User comments when performing pooling (free text)	text	^[a-zA-Z0-9_-]{1,36}\$

## 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	ISO 8601 timestamp

## Sequencing Report

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

Column	Description	Type	Regex
batch_name	Batch name	text	^[a-zA-Z0-9_-]{1,36}\$
pool_barcode	Pool barcode associated with sequencing run	text	^[a-zA-Z0-9_-]{1,36}\$
instrument	Sequencer serial number	text	^[a-zA-Z0-9_-]{1,36}\$
flowcell	Flow cell associated with sequencing run	text	NA
software_version	Concatenation of software application/version used to analyze the data on the instrument	text	
run_folder	Sequencing run folder name	text	
sequencing_status	Sequencing run status Value options: completed, timed out	enum	Values specified in Description
qc_status	QC status of sequencing run Value options: pass, fail	enum	Values specified in Description
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	float	
started	Timestamp associated with sequencing start	ISO 8601 timestamp	ISO 8601 timestamp
completed	Timestamp associated with sequencing completion	ISO 8601 timestamp	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}\$

Column	Description	Type	Regex
flowcell	Flow cell barcode associated with failed analysis	text	NA
sequencing_run_folder	Sequencing Run status associated with failed analysis	text	
analysis_run_status	Sequencing Run status associated with failed analysis Value options: failed_max_analysis_attempts	text	Values specified in Description
timestarted	Timestamp associated with analysis start	ISO 8601 timestamp	ISO 8601 timestamp
timefinished	Timestamp associated with analysis failed	ISO 8601 timestamp	ISO 8601 timestamp

# Troubleshooting

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## Introduction

VeriSeq NIPT Solution troubleshooting assistance comprises:

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

## Assay Software Notifications

This section describes the Assay Software notifications:

## Progress Notifications

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

Notification	Step	When	Alert Level	Email	Recommended Action
Batch initiation	Library Prep	User created a new batch	Activity	Yes	NA
Batch Library Complete	Library Prep	Library completed for the current batch	Activity	No	NA
Pool Complete	Library Prep	Pool has been generated from a batch	Activity	No	NA
Sequencing Started	Sequencing	The system detected a new sequencing data folder	Activity	No	NA
Sequencing QC passed	Sequencing	The sequencing run has completed and the sequencing QC check passed	Activity	No	NA
Analysis Started	Analysis	Analysis started for the specified sequencing run	Activity	Yes	NA
Analysis Completed NIPT Report Generated	Post Analysis	The analysis has completed and reports generated	Activity	Yes	NA

## 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	NA
Pool Invalidation – Repool	Library Prep	User invalidated the first possible pool (of a certain type) for the batch	Notice	Yes	NA
Pool Invalidation – Use second aliquot	Library Prep	User invalidated the first possible pool (of a certain type) for the batch	Notice	Yes	NA
Sequencing Completed Pool Invalidated	Sequencing	The sequencing run has completed while the pool was invalidated by the user	Notice	Yes	NA
Sequencing QC passed – All samples are invalid	Sequencing QC	The sequencing run QC check has completed, but all samples are invalid	Notice	Yes	NA
Analysis Completed Pool Invalidated	Post Analysis	The analysis has completed while the pool was invalidated by the user	Notice	Yes	NA

## Recoverable Error Notifications

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

Notification	Step	When	Alert Level	Email	Recommended Action
Missing Instrument Path	Sequencing	The system cannot locate/connect to an external sequencing folder	Alert	Yes	<ul style="list-style-type: none"> <li>If using a NAS, check the network connection. See <i>Recommended Action Procedures</i> Action ID 1 on page 54.</li> <li>Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.</li> </ul>
Insufficient Disk Space for Sequencing	Sequencing	The system detected a new sequencing data folder, but is estimating that there is not enough disk space for the data	Alert	Yes	<ol style="list-style-type: none"> <li>Check available disk space. See <i>Recommended Action Procedures</i> Action ID 2 on page 54.</li> <li>Clear disk space or backup data. See <i>Recommended Action Procedures</i> Action ID 3 on page 55</li> </ol>
Sequencing Run Invalid Folder	Sequencing	Invalid characters in the Sequencing Run folder	Alert	Yes	The sequencing run folder was renamed incorrectly. Rename the run to a valid name.
RTA Complete is not accessible	Sequencing	The software was not able to read the RTAComplete file in the sequencing folder	Warning	Yes	Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Missing Sample Type	Pre-Analysis	The software could not find definition for sample type to some of the samples	Notice	Yes	Sample type attribute was not provided for the specified sample. Invalidate the sample to enable the software to proceed.
Missing Sex Chromosome	Pre-Analysis	The software could not find definition for sex chromosomes to some of the samples	Notice	Yes	Sex chromosome attribute was not provided for the specified sample. Invalidate the sample to enable the software to proceed.
Missing Sample Type and Sex Chromosome	Pre-Analysis	The software could not find definitions for sample types and sex chromosome to some of the samples	Notice	Yes	Sample type and sex chromosome attribute were not provided for the specified sample. Invalidate the sample to enable the software to proceed.

Notification	Step	When	Alert Level	Email	Recommended Action
Sample Sheet Generation failed	Pre-Analysis	The software failed to generate sample sheet	Alert	Yes	<ul style="list-style-type: none"> <li>Check available disk space. See <i>Recommended Action Procedures</i> Action ID 2 on page 54. If space is low, clear disk space or backup data. See <i>Recommended Action Procedures</i> Action ID 3 on page 55.</li> <li>If using a NAS, check the network connection. See <i>Recommended Action Procedures</i> Action ID 1 on page 54.</li> <li>Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.</li> </ul>
Unable to check disk space	Pre-Analysis	The software could not check disk space	Alert	Yes	<ul style="list-style-type: none"> <li>If using a NAS, check the network connection. See <i>Recommended Action Procedures</i> Action ID 2 on page 54.</li> <li>Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.</li> </ul>
Insufficient Disk Space for Analysis	Pre-Analysis	The software detected that there is not enough disk space for starting a new analysis run	Alert	Yes	Clear disk space or backup data. See <i>Recommended Action Procedures</i> Action ID 3 on page 55.
Unable to launch Analysis Pipeline	Pre-Analysis	The software was unable to start an analysis run for the given sequencing folder	Alert	Yes	Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Sequencing folder Read/Write permission failed	Pre-Analysis	The software test that checks Read/Write permission to the sequencing run folder failed	Warning	Yes	<ul style="list-style-type: none"> <li>If using a NAS, check the network connection. See <i>Recommended Action Procedures</i> Action ID 1 on page 54.</li> <li>Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.</li> </ul>
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
Unable to deliver email notifications	System	The system is unable to deliver email notifications.	Warning	NA	<ol style="list-style-type: none"> <li>Check email configuration defined on system for validity. See instructions in <i>Configuring System Email Notifications</i> on page 23.</li> <li>Send a test email. See instructions in <i>Configuring System Email Notifications</i> on page 23.</li> <li>Reboot the server. If the problem persists, email Illumina Technical Support.</li> </ol>

Notification	Step	When	Alert Level	Email	Recommended Action
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	<ul style="list-style-type: none"> <li>Check available disk space. See <i>Recommended Action Procedures</i> Action ID 2 on page 54. If space is low, clear disk space or backup data. See <i>Recommended Action Procedures</i> Action ID 3 on page 55.</li> <li>Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.</li> </ul>
Failed to Parse Run Parameters file	Sequencing	The system was not able to open/parse the RunParameters.xml file	Warning	Yes	The RunParameters.xml file is corrupted. Check the sequencer configuration and resequence the pool.
Unrecognized Run Parameters	Sequencing	The software read Run Parameters that are not compatible	Warning	Yes	The software is was unable to construct sequencing run parameters from the sequencer configuration file. Check the sequencer configuration and resequence the pool.
Invalid Run Parameters	Sequencing	The software read required Run Parameters that are not compatible with the assay	Warning	Yes	The software compatibility check failed. Check the sequencer configuration and resequence the pool.
No Pool Barcode found	Sequencing	The software was not able to associate the flow cell for the sequencing run with a known pool barcode	Warning	Yes	Possible incorrect pool barcode entry. Resequencing the pool.
Sequencing Timed Out	Sequencing	The sequencing run has not completed in a given time frame	Warning	Yes	Check the sequencer and the network connection. Resequencing the pool.
Sequencing QC files generation failed	Sequencing QC	The sequencing run has completed but the interop QC files are corrupted	Alert	Yes	Check the sequencer, and the network connection. Resequencing the pool.

Notification	Step	When	Alert Level	Email	Recommended Action
Sequencing QC files corrupted	Sequencing QC	The sequencing run has completed and the sequencing QC check are corrupted	Warning	Yes	Check the sequencer and the network connection. Resequence the pool.
Sequencing QC failed	Sequencing QC	The sequencing run has completed and the sequencing QC check failed	Notice	Yes	Resequence the pool.
Analysis Failed for Maximum number of attempts	Analysis	All Analysis attempts have failed. Will not retry.	Warning	Yes	Resequence the second pool.
Analysis Post-Processing Failed	Post-Analysis	The software failed to post process the analysis results	Alert	Yes	<ul style="list-style-type: none"> <li>If using a NAS, check the network connection. See <a href="#">Recommended Action Procedures</a> Action ID 1 on page 54.</li> <li>Possible hardware failure. Reboot the server. If problem persists, email Illumina Technical Support.</li> </ul>
Analysis Upload Failed	Post-Analysis	The software failed to upload the analysis results to the database	Alert	Yes	<ul style="list-style-type: none"> <li>If using a NAS, check the network connection. See <a href="#">Recommended Action Procedures</a> Action ID 1 on page 54.</li> <li>Possible hardware failure. Reboot the server. If problem persists, email Illumina Technical Support.</li> </ul>

## Recommended Action Procedures

Action ID	Recommended Action	Steps
1	Check the network connection	<p>NOTE Make sure that the remote storage NAS and the local machine are on the same network.</p> <ol style="list-style-type: none"> <li>From a Windows command line (cmd), type the following command: <b>ping &lt;Server IP&gt;</b> NOTE If using a NAS, also check the connection with the NAS.</li> <li>Make sure that there are no lost packets. NOTE If there are lost packets, contact the IT Administrator.</li> <li>Test the connection: <ol style="list-style-type: none"> <li>Log in to the Onsite Server Web UI.</li> <li>From the Dashboard menu, select <b>Folder</b>.</li> <li>Click <b>Test</b>, and determine if the test is successful. If the test fails, see <a href="#">Editing a Shared Network Drive</a> on page 21 and make sure that all settings are configured correctly.</li> </ol> </li> </ol>
2	Check available disk space	<p>NOTE Make sure that the Onsite Server Input folder is mapped to the Windows machine. For more information, see <a href="#">Mapping Server Drives</a> on page 28.</p> <p>Right-click the drive that maps to the Input folder. Select <b>Properties</b>, and view the free space information.</p>

Action ID	Recommended Action	Steps
3	Clear disk space / Backup data	<p>NOTE Illumina recommends a periodic data backup and/or storing sequencing data on the server side. For more information, see <a href="#">Managing a Shared Network Drive on page 21</a>.</p> <ol style="list-style-type: none"> <li>For data stored locally on the Onsite Server:           <p>NOTE Make sure that the Onsite Server Input folder is mapped to the Windows machine. For more information, see <a href="#">Mapping Server Drives on page 28</a>.</p> <ol style="list-style-type: none"> <li>Double-click the Input folder, and enter the credentials to access it.</li> <li>Sequencing run data are listed with folder names matching sequencing run names.</li> <li>Delete or backup the processed sequencing folders.</li> </ol> </li> <li>For data stored on a remote NAS:           <p>NOTE Make sure that the remote storage NAS and the local machine are on the same network.</p> <p>NOTE Obtain access to the folder on the remote drive. Access credentials from the IT Administrator are required.</p> <ol style="list-style-type: none"> <li>Sequencing run data are listed with folder names matching sequencing runs names.</li> <li>Delete or backup the processed sequencing folders.</li> </ol> </li> </ol>

## System Issues

Issue	Recommended Action
Software fails to start	If errors are detected when starting the Assay Software, a summary of all errors appears instead of the Log In screen. Contact Illumina Technical Support to report the errors listed.
Database restore required	If a backup restore of a database is required, contact an Illumina Field Service Engineer.
System drift detected	When a system drift is detected, the Assay Software no longer processes communication from other system components. An administrator can reset the system back to normal operation after it has entered the drift detection state.

## Data Processing Tests

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

### Testing the Server

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

#### Procedure

- 1 Open the mounted input directory, and then open the TestingData folder.
- 2 Make a copy of the following folder, which can be found in the TestingData folder: 150824\_NS500404\_0121\_AHGKH5BGXX\_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 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 Aneuploidy Detection Report generated for Sequencing Run



#### NOTE

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

- 5 In the output folder, open the SampleTestRun folder, and check for the following report: SampleTestRun\_C\_SampleTestRun\_PoolA\_HGKH5BGXX\_nipt\_report\_YYYYMMDD\_HHMMSS.tab. The expected file size is approximately 5.9 Kb.
- 6 Move the test sequencing run back to the TestingData folder. This practice helps manage the number of times the sequencing test executes.

### Running Full Analysis Test Data

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

#### Procedure

- 1 Open the mounted input directory, and open the TestingData folder.
- 2 Rename the following folder by adding the \_000 suffix: 150528\_NB500886\_0002\_AH7MHHBGXX\_FullIRun.  
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 Aneuploidy Detection Report generated for Sequencing Run
- 5 In the output folder, open the SampleTestRun folder, and check for the following report:  
SampleTestRun2\_C\_SampleTestRun2\_PoolA\_H7MHHBGXX\_nipt\_report\_20151105\_162434.tab.  
The expected file size is approximately 7.1 Kb.
  - 6 Move the test sequencing run back to the TestingData folder.



**NOTE**

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

# Additional Resources

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

Resource	Description
<i>VeriSeq NIPT Solution Package Insert</i> (document # 1000000001856)	Defines the product and intended use, and provides instructions for use and troubleshooting procedures.
<i>Microlab® STAR Line Operator's Manual</i> , Hamilton Doc ID 624668	Provides operation and maintenance information and technical specifications for the Hamilton Mircolab STAR automated liquid handling instrument.

Visit the VeriSeq NIPT Solution [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 <i>in vitro</i> diagnostic product
cfDNA	Cell-Free DNA
DNA	Deoxyribonucleic Acid
DNS	Domain Name System
FASTQ	Text-based file format for storing the output of sequencing instruments
FF	Fetal Fraction
FIFO	First In, First Out
iFACT	individual Fetal Aneuploidy Confidence Test
IP	Internet Protocol
LIMS	Laboratory Information Management System
LIS	Laboratory Information System
LLR	Log Likelihood Ratios
MAC	Media Access Control
NAS	Network-Attached Storage
NES	Non Excluded Sites
NGS	Next-Generation Sequencing
NIPT	Non-Invasive Prenatal Testing
NTC	No Template Control
NTP	Network Time Protocol
PF	Passing Filter
PQ	Process Qualification
QC	Quality Control
RTA	Real-Time Analysis
RUO	Research Use Only
SCA	Sex Chromosome Aneuploidy
SDS	Safety Data Sheets
SHA1	Secure Hash Algorithm 1
SSL	Secure Sockets Layer

# Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: [www.illumina.com](http://www.illumina.com)  
Email: [techsupport@illumina.com](mailto:techsupport@illumina.com)

## Illumina Customer Support Telephone Numbers

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Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
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United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

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

Product documentation—Available for download in PDF from the Illumina website. Go to [support.illumina.com](http://support.illumina.com), select a product, then select **Documentation & Literature**.



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