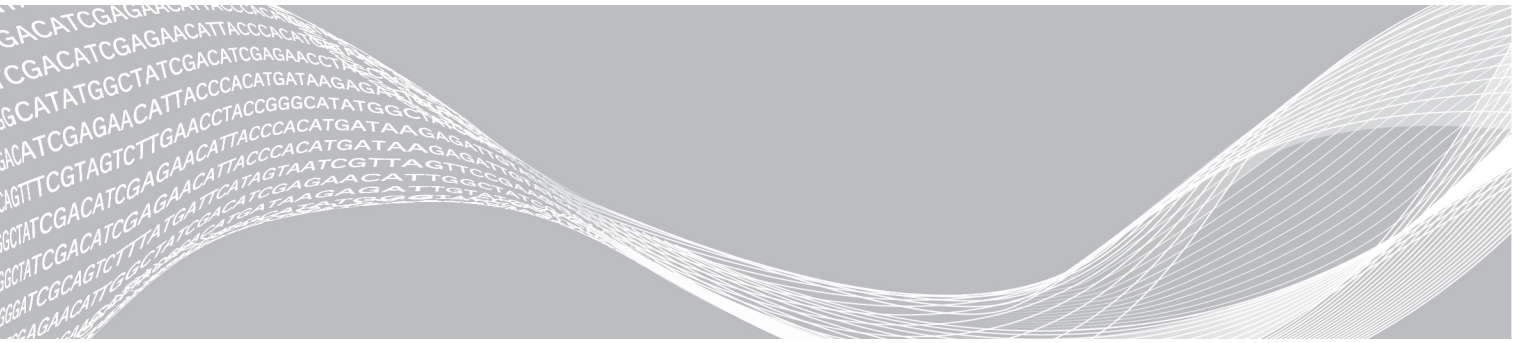
illumina®

VeriSeq NIPT Analysis Software (48 Samples)

User Guide



Document # 1000000026777 v04
August 2021

FOR IN VITRO DIAGNOSTIC USE

Revision History

Document	Date	Description of Change
Document # 1000000026777 v04	August 2021	Updated EU Authorized Representative address.
Document # 1000000026777 v03	February 2020	Updated EU Authorized Representative address.
Document # 1000000026777 v02	July 2018	Added Limitations of the Procedure and Appendix B Method Comparison Study.
Document # 1000000026777 v01	March 2017	Corrected report name in first sentence of Library Sample Report section, corrected document number in page footers.
Document # 1000000026777 v00	January 2017	Initial release

Table of Contents

Introduction

Overview

The VeriSeq NIPT Analysis Software (48 Samples) is provided pre-installed on the VeriSeq NIPT Analysis Server (48 Samples), Illumina Part Number 20016240. The server and pre-installed software provide analytical capability for analysis of compatible Next Generation Sequencing (NGS) data generated from sequencing of cfDNA libraries for detection of fetal aneuploidies based on chromosomal representation. The VeriSeq NIPT Analysis Software (48 Samples) uses a software API (Application Programming Interface) to receive and persist batch, pool, and sample prep information. This software, once installed and configured, runs as a background service with minimum to no intervention from the user.

The Analysis Software generates statistics to evaluate the chromosome copy number of the tested samples. A next-generation sequencing instrument generates analysis input in the form of 36-base paired-end reads. The Analysis 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 Analysis 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 Analysis 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. Log likelihood ratios (LLR) are calculated for each test chromosome in each sample 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

Using the methods described:

- LLR scores are reported for chromosomes 13, 18, and 21
- Normalized chromosomal values (NCV) are reported for chromosomes X and Y
- Specialized LLR scores are reported for under and over representation of chromosome X

The VeriSeq NIPT Assay Software utilizes the individualized Fetal Aneuploidy Confidence Test (iFACT), a dynamic threshold metric that indicates whether the system has generated sufficient sequencing coverage, given the fetal fraction estimate for each sample. The system provides analysis results only if a sample meets the iFACT threshold. If a sample fails to achieve this threshold, the QC assessment displays FAILED iFACT and the system does not generate a result. The iFACT assessment is applied to all samples. In addition to iFACT, the VeriSeq NIPT Assay Software assesses several other QC metrics during analysis. The QC assessment displays either a QC flag or a QC failure for any metrics outside of the acceptable range. In the case of QC failure, the system does not generate a result for the sample.

The Analysis Software does not generate aneuploidy calls directly, but rather provides LLR and NCV scores as described above. The threshold for calling samples as unaffected or affected based on these scores is determined by users from their own clinical validation study.

Intended Use

The VeriSeq NIPT Analysis Software (48 Samples) generates quantitative scores to aid in the detection and differentiation of fetal aneuploidy status for chromosomes 21, 18, 13, X, and Y by analyzing sequencing data generated from cell free DNA (cfDNA) fragments isolated from maternal peripheral whole blood specimens in pregnant women of at least 10 weeks gestation.

The quantitative scores are log likelihood ratio scores associated with under-or-over representation of a target chromosome relative to an expectation for a diploid genome.

Limitations of the Procedure

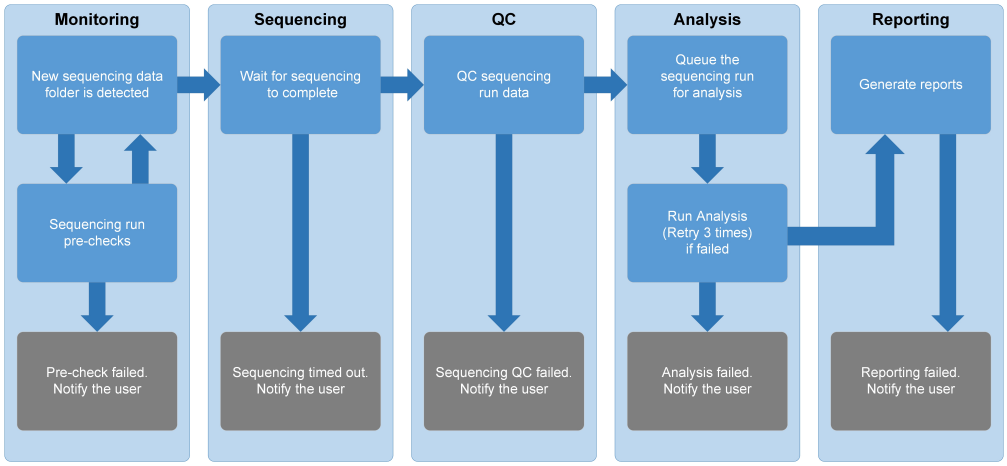
- The VeriSeq NIPT Analysis Software (48 Samples) is designed to be used as part of a screening test, which should not be considered in isolation from other clinical findings and test results. User defined cutoffs applied to the data outputs of this software should consider the relative benefits of increasing sensitivity at the cost of specificity and vice versa. No single cutoff achieves concurrent 100% sensitivity and 100% specificity. While rare, samples with a relatively low FF for the sequencing depth at which they have been processed can have data outputs near the threshold and may have lower accuracy.
- The VeriSeq NIPT Analysis Software (48 Samples) outputs data for use in reporting on the following:
  - Over representation of chromosomes 21, 18, and 13
  - The following sex chromosomal aneuploidies: XO, XXX, XXY, and XYY
- The VeriSeq NIPT Analysis Software (48 Samples) is not intended for use in reporting polyploidy.
- The algorithms used in the VeriSeq NIPT Analysis Software (48 Samples) can be confounded by certain maternal and fetal factors including, but not limited to, the following:
  - Recent maternal blood transfusion
  - Maternal organ transplant
  - Maternal surgical procedure
  - Maternal immunotherapy or stem cell therapy
  - Maternal malignancy
  - Maternal mosaicism
  - Confined placental mosaicism
  - Fetal demise
  - Disappearing twin
  - Fetal partial trisomy or partial monosomy
  - Fetal mosaicism

VeriSeq NIPT Analysis Software (48 Samples)

Analysis Software

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

Figure 1 Data Flow Diagram



Monitoring—Pre-checks the validity of the new sequencing run. The following validity checks are conducted once the software detects a new sequencing run:

1. Checks that the run parameters are compatible with expected values.
2. Makes an association between the pool barcode being sequenced with pool information recoded during sample prep process using the software API.
3. Confirms that the pool had not been processed previously (system doesn't allow re-runs).

1. 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.
2. QC—Examines the InterOp QC files generated by the sequencer. The Analysis 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.
3. 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 Analysis 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.

4 **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.

Analysis SoftwareTasks

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

Automated Tasks

The Analysis 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 19 for an overview and *Process Reports* on page 27 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 Analysis Software determines whether to continue with the process and if the results are to be reported. The Analysis 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 Analysis Software determines the LLR score for each target chromosome 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 23.
- **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 19.
- **Report generation**— The Analysis Software generates results, notifications, and process reports. For more information, see on page 19.
- **Sample, pool, and batch invalidation**—
  - **Sample invalidation**—The Analysis 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 26.
  - **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 26.

- Retest management—
  - Pool failures—Failed pools are typically pools that failed the Sequencing QC metrics. The Analysis 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 Analysis Software (48 Samples) enables users to perform the following tasks:

Using the software API, the following commands can be issued to the Analysis Software:

- 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 Analysis Software then marks the result as canceled in the final results report.

Using the Analysis 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 9.

Sequencing Handler

The Analysis 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 Analysis 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 6).

Sequencing Run Compatibility

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

Use only compatible sequencing methods 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 to generate the chromosomal aneuploidy LLR score. 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 23.

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

Email Notifier

The Analysis Software sends notifications containing progress information and alerts during the assay execution. Email notifications that contain ACTION REQUIRED in the subject line provide detailed steps about how to resolve the issue. For more information, see *Results and Notifications Reports* on page 23.

The notifier sends emails to the subscribers list, which is defined using the Web UI. For more information, see*Web User Interface* on page 6.

Web User Interface

The Analysis Software hosts a local Web UI that allows easy access to the 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 *VeriSeq NIPT Analysis Software (48 Samples) Notifications* on page 42.
- **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 *VeriSeq NIPT Analysis Software (48 Samples) Notifications* on page 42.
- **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 12.
- **Manage server access**—The 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 9.

- **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 17.
- **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 13.
- **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 17.

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 9.

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 Analysis Software interface and login:

1. On a computer connected to the same network as the 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 NIPT Analysis Server (48 Samples) 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

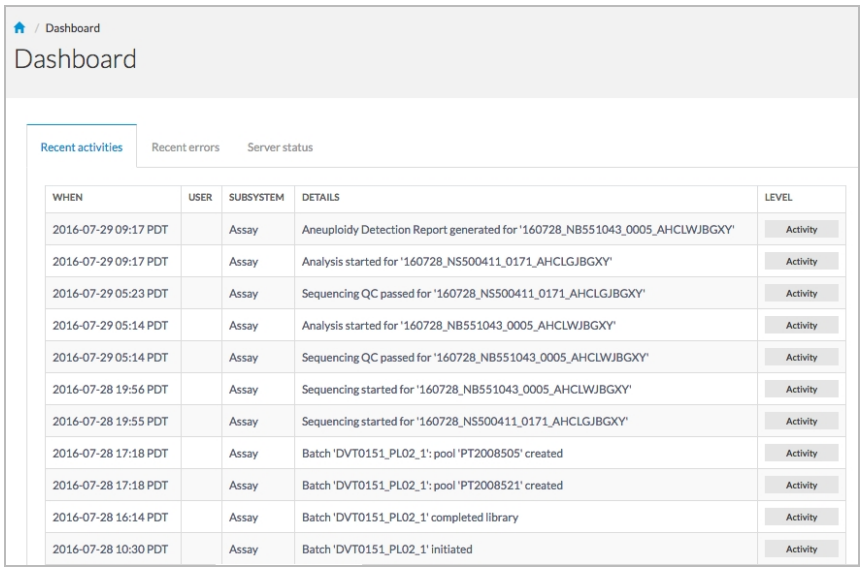
After 10 minutes of inactivity, the Analysis Software automatically logs out the current user.

Using the Dashboard

The VeriSeq NIPT Analysis Software (48 Samples) 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 thorough the activity history by clicking Previous in the lower-right corner of the activity table.

Figure 2 VeriSeq NIPT Analysis Software Dashboard



Viewing Recent Activities

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

Name	Description
When	Activity date and time
User	If applicable, identifies the user who performed the activity
Subsystem	Entity or process that performed the activity such as user, assay, or configuration
Details	Activity description
Level	Level assigned to the activity from the following options: • **Activity**—Indicates an activity within the server such as system reboot or user login/logout. • **Notice**—Indicates an unsuccessfully executed step. For example, sample invalidation or QC failure. • **Warning**—Indicates that an error occurred during normal execution and proper hardware function. For example, unrecognized run parameters or failed analysis.

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

Name	Description
Level	Level assigned to the activity from the following options: • **Urgent**—Major hardware error that compromises system operation. Contact Illumina Technical Support. • **Alert**—Error in normal operation. For example, a disk corruption, space or configuration issue that prohibits report generation or email notifications. • **Error**—System or server error during normal operation. For example, a configuration file issue or hardware failure.

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 Analysis Software (48 Samples) 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 Analysis Software uses user names to identify the persons involved in the different aspects of assay processing and interactions with the Analysis 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 Server when it accesses the NAS
- **Password**—Password designated for the 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 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 13 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 Analysis Software (48 Samples).

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 Server settings.
- **Static IP Address**—IP address designated for the Server
- **Subnet Mask**—Local network subnet mask
- **Default Gateway Address**—Default router IP address
- **Hostname**—Designated name to reference the 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 Analysis Software (48 Samples) communicates with users by sending email notifications indicating the assay progress and alerts for errors or required user action. *VeriSeq NIPT Analysis Software (48 Samples) Notifications* on page 42 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.

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. Analysis Reports are described in Chapter 3.

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 Analysis 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

Column Name	Description
Sample_Well	Well identification on the plate for a given sample
I7_Index_ID	Identification of the first index adapter
index	Nucleotide sequence of the first adapter
I5_Index_ID	Identification of the second adapter
index2	Nucleotide sequence of the second adapter
Sample_Project	Project identification for a given sample; default: blank
SexChromosomes	Analysis pertaining to sex chromosomes. One of the following: • **yes**–Sex chromosome aneuploidy and sex reporting requested • **no**–Neither sex chromosome aneuploidy nor sex reporting requested • **sca**–Sex chromosome aneuploidy reporting requested, sex reporting not requested
SampleType	Sample type. One of the following: • **Singleton**–Single embryo pregnancy • **Twin**–Multiple embryo pregnancy • **Control**–Control sample of known sex and aneuploidy LLR score • **NTC**–No template control sample (no DNA)

Sequencing QC

Sequencing QC metrics identify flow cells that are likely to fail analysis with high probability. The cluster density, percent reads passing filter (PF), prephasing, and phasing metrics describe the general sequencing data quality and are common to many next-generation sequencing applications. The predicted aligned reads metric estimates the flow cell level of the sequencing depth. If low-quality data fails the predicted aligned reads metric, processing the run is terminated. For more information, see *Sequencing QC Metrics and Boundaries* on page 36.

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 Analysis 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.[1]

Statistical Output

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.

[1] 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

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

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 *Analytic QC Metrics and Boundaries* on page 36. 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_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. High LLR score 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. High LLR score 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.

Instrument Short

The Instrument Short 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.

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

Archiving Data

Illumina recommends archiving the /data01/runs and /data01/analysis_output directories in agreement with local IT site archiving policy. The Analysis 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 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 Analysis Software makes specific folders on the 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 Sequencer 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 Analysis 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 Analysis Software and is not accessible to the user. The system maintains an automatic mechanism for database backup on the 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 Server can be used as the main storage point for sequencing runs, it can only store approximately 400 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 Server does not require the user to perform any maintenance. Updates for the Analysis Software or the Server itself are provided by Illumina Technical Support.

Mapping Server Drives

The 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 Server subnetwork.
2. Right-click **Computer**, and select **Map network drive**.
3. Select a letter from the Drive drop-down list.
4. In the Folder field, enter \\<VeriSeq NIPT Analysis Server (48 Samples) 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 Server server:

1 On the **Settings** drop-down list, select **Shut Down Server**.

2 Select **Shut Down** to shut down the Server, or select **Cancel** to exit without shutting down.

3 Enter a reason for shutting down the 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 Analysis 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 Analysis Software allows the system to resubmit the run for analysis up to 3 times.

System Reports

Introduction

The Analysis 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

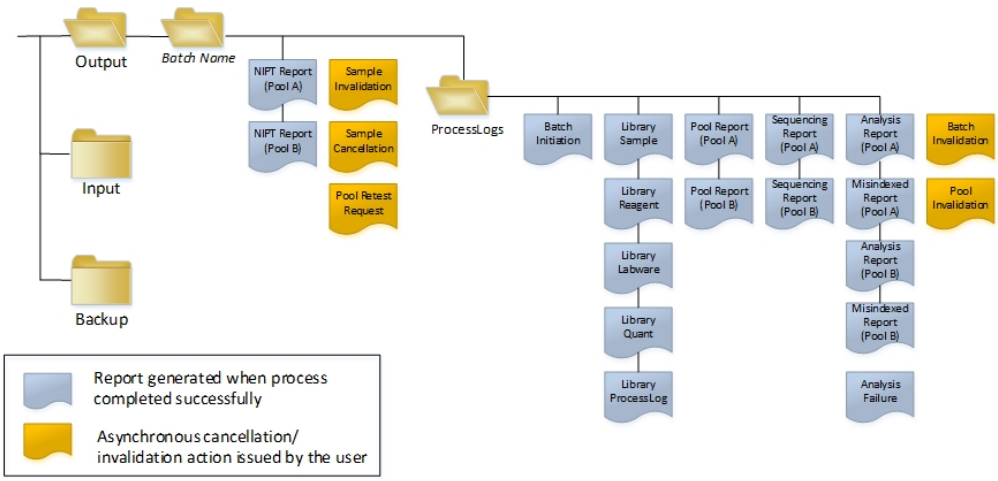
The Analysis Software reports are generated on the Server internal hard drive mapped to the user drive as a read-only Output folder. Each report is generated with a corresponding standard MD5 checksum file, which is used to verify that the file has not been modified.

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

Figure 3 Analysis Software Reports Folder Structure



The Analysis 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 23.
- **ProcessLog folder**—Contains reports related to process. For details, see *Process Reports* on page 27

A list of all reports is provided in *System Reports Summary* on page 21.

System Reports Summary

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

Report Name	Report Type	Report Entity	Report File Name Format
Pool Invalidation Report	Informational	Pool	ProcessLogs/<batch_name>_<pool_barcode>_pool_invalidation_report_20150528_163503.tab
Sequencing Report	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
Analysis Report	Informational	Pool/ flow cell	ProcessLogs/<batch_name>_A_<pool_barcode>_<flowcell>_analysis_report_20150528_163503.tab
Misindexed Report	Informational	Pool/ flow cell	ProcessLogs/<batch_name>_A_<pool_barcode>_<flowcell>_misindexed_report_20150528_163503.tab
Analysis Failure Report	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	• Sequencing run analysis completes
Sample Invalidation	Contains information about an invalidated sample	• User invalidates a sample
Sample Cancelation	Contains information about a canceled sample	• User cancels a sample
Pool Retest Request	Indicates that a second pool can be generated from an existing batch. Contains information about the pool retest status. [1]	• User invalidates a pool
Batch Initiation	Indicates a new batch processing start	• User initiates a new batch
Batch Invalidation	Contains information about a user-initiated invalidated batch	• Batch is invalidated
Library Sample	Lists all samples in the batch	• Batch is invalidated • Library Preparation method completes • Batch fails quantification
Library Reagent	Contains library processing reagent information	• Batch is invalidated • Library Preparation method completes • Batch fails quantification

Report	Description	Generation Event
Library Labware	Contains library processing labware information	• Batch is invalidated • Library Preparation method completes • Batch fails quantification
Library Quant	Contains library quantification test results	• Batch is invalidated • Library Preparation method completes • Batch fails quantification
Library Process Log	Contains steps executed during library processing	• Batch is invalidated • Library Preparation method completes • Batch fails quantification • Batch process completes
Pool	Contains sample pooling volumes	• Pooling method completes
Pool Invalidation	Contains information about a user-initiated invalidated pool	• User invalidates a pool
Sequencing	Contains sequencing QC results	• Sequencing QC passes • Sequencing times out (fails)
Analysis	Contains the additional analytic data of a successful run	• Sequencing run analysis completes
Misindexed	Contains information about misindexed reads	• Sequencing run analysis completes
Analysis Failure	Contains analysis information for a failed pool	• Sequencing run analysis fails

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

Results and Notifications Reports

NIPT Report

The NIPT Report contains the statistical LLR 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}$

Column	Description	Preset Value Options	Type	Regex
sample_type	Sample type information provided from point of collection.	One of the following: • **Singleton**–Single embryo pregnancy • **Twin**–Multiple embryo pregnancy • **Control**–Control sample of known sex and aneuploidy score • **NTC**–No template control sample (no DNA)	enum	Values specified in Preset Value Options
sex_chrom	Sex chromosome analysis requested.	One of the following: • **yes**–Sex chromosome score and sex reporting requested • **no**–Neither sex chromosome score nor sex reporting requested • **sca**–Sex chromosome score reporting requested, sex reporting not requested	enum	Values specified in Preset Value Options
flowcell	Sequencing flow cell barcode	N/A	text	NA
score_t13	Likelihood ratio score for evidence of trisomy on chr 13	Numeric	Floating point	| x | < 500.00
score_t18	Likelihood ratio score for evidence of trisomy on chr 18	Numeric	Floating point	| x | < 500.00
score_t21	Likelihood ratio score for evidence of trisomy on chr 21	Numeric	Floating point	| x | < 500.00
score_tx	Likelihood ratio score for evidence of trisomy on chr X	Numeric	Floating point	| x | < 500.00
score_mx	Likelihood ratio score for evidence of monosomy on chr X	Numeric	Floating point	| x | < 500.00
ncv_x	Normalized chromosomal value for chr X	Numeric	Floating point	| x | < 500.00
ncv_y	Normalized chromosomal value for chr Y	Numeric	Floating point	| x | < 500.00

Column	Description	Preset Value Options	Type	Regex
qc_flag	QC analysis results	One of the following: • CANCELLED • INVALIDATED • PASS • NTC_PASS • FAIL	enum	Values specified in Preset Value Options
qc_failure	QC failure information	One of the following: • FAILED iFACT • DATA OUTSIDE OF EXPECTED RANGE • FRAGMENT SIZE DISTRIBUTION OUTSIDE OF EXPECTED RANGE • NTC SAMPLE WITH HIGH COVERAGE • CANCELLED • INVALIDATED • NONE (QC status = Pass)	text	Values specified in Preset Value Options
ff	Estimated fetal fraction	Percent sample cfDNA from fetus rounded to the nearest integer. Results less than 1% are presented as < 1%.	text	NA

QC Failure Messages

Analysis QC failure results in full suppression for results, sex score, and estimated fetal fraction, which correspond to the following NIPT Report fields: score_t13, score_t18, score_t21, score_tx, score_mx, ncv_x, ncv_y, 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/µl	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: • ISOLATION—batch_validation, prespin, postspin, data_transact • EXTRACTION—setup, chemistry, data_transact • LIBRARY—setup, chemistry, data_transact, complete • QUANT—setup, build_standards, build_384, analysis, data_transact • POOLING—analysis, setup, pooling, data_transact, complete	text	^[a-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: ISOLATION—batch_validation, prespin, postspin, data_transact EXTRACTION—setup, chemistry, data_transact LIBRARY—setup, chemistry, data_transact, complete QUANT—setup, build_standards, build_384, analysis, data_transact POOLING—analysis, setup, pooling, data_transact, complete	text	^[a-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 Report

The system generates an Analysis Report for a sequencing run when analysis completes successfully.

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}$

Column	Description	Type	Regex
sample_type	Sample type Value options: Singleton, Twin, Control, NTC	enum	Values specified in Description
sex_chrom	Sex chromosome reporting option Value options: yes, no, sca	enum	Values specified in Description
flowcell	Flow cell barcode	text	NA
index	Sample index	text	^[a-zA-Z0-9_-]{1,36}$
well	Plate well location	text	^[a-zA-Z0-9_-]{1,36}$
qc_flag	QC disposition based on analysis results Value options: PASS, FAIL	enum	Values specified in Description
qc_failure	Concatenation of reasons for failure	text	See *QC Failure Messages* on page 25
ff	Estimated FF	numeric	
aligned_reads	Total number of aligned reads per sample	numeric	
indexing_rate	Fraction of all reads indexed to an individual sample	float	
alignment_rate	Fraction of all aligned reads to indexed reads for a given sample	float	
euploid_coverage	Log-likelihood score for evidence of euploid coverage on non-target chromosomes	numeric	
frag_size_dist	Deviation from expected fragment size distribution	numeric	
max_misindexed_rate	Fraction of reads assigned to indexes not present on the flow cell	numeric	
score_t13	Likelihood ratio score for evidence of trisomy on chr 13	numeric	
score_t18	Likelihood ratio score for evidence of trisomy on chr 18	numeric	
score_t21	Likelihood ratio score for evidence of trisomy on chr 21	numeric	
score_tx	Likelihood ratio score for evidence of trisomy on chr X	numeric	
score_mx	Likelihood ratio score for evidence of monosomy on chr X	numeric	
ncv_x	Normalized chromosomal value for chr X	numeric	
ncv_y	Normalized chromosomal value for chr Y	numeric	
chr1_coverage to chr22_coverage, chrX_coverage, chrY_coverage	Normalized chromosomal coverage for each of the 24 chr	numeric	

Misindexed Report

The system generates a Misindexed Report for a sequencing run when analysis completes successfully.

Column	Description	Type	Regex
batch_name	Batch name	text	^[a-zA-Z0-9_-]{1,36}$
pool_type	Pool type associated with the pool barcode Value options: A, B, C	enum	Values specified in Description
pool_barcode	Pool barcode associated with a sample	text	^[a-zA-Z0-9_-]{1,36}$
flowcell	Flow cell barcode	text	NA
index	Index associated with a given number of reads	text	^[a-zA-Z0-9_-]{1,36}$
indexedreads	Number of reads mapped to the index		

Analysis Failure Report

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

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

QC Metrics

Sequencing QC Metrics and Boundaries

Metric	Description	Lower Bound	Upper Bound	Rationale
cluster_ density	Sequencing cluster density	152,000 per mm^2	338,000 per mm^2	Flow cell with low cluster density does not generate enough reads. Over clustered flow cells usually produce sequencing data of low quality.
pct_pf	Percent reads passing chastity filter	≥50%	N/A	Flow cells with extremely low %PF can have abnormal base representation and are likely to indicate problems with PF reads.
prephasing	Fraction of prephasing	N/A	≤0.003	Empirically optimized recommendations for the VeriSeq NIPT Analysis Software (48 Samples).
phasing	Fraction of phasing	N/A	≤0.004	Empirically optimized recommendations for the VeriSeq NIPT Analysis Software (48 Samples).
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)	1,000,000	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	NCD_Y	-200	10,000	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.

Method Comparison Study

Method Comparison Data

Remaining plasma aliquots of 461 samples that were previously run on the Verifi® test were processed with the VeriSeq NIPT assay and sequence data was analyzed with theVeriSeq NIPT Analysis Software (48 Samples). This set of samples included unaffected (“euploid”) and trisomy 21 (T21) samples from male and female fetuses. This method comparison study did not include trisomy 13 (T13) nor trisomy 18 (T18) samples as T21 is the most difficult to detect since it is the smallest of these three chromosomes. T21 and fetal sex calls for VeriSeq NIPT were based on specific cutoffs (LLR=1.5 for T21 calling and a fetal fraction-adjusted cutoff for fetal sex). A matrix of the 461 Verifi andVeriSeq NIPT classification calls are shown in the table below. With respect to T21 classification, 82/87 (94.3%) and 374/374 (100%) were classified concordantly between the two tests as T21 and Euploid, respectively. 460/461 (99.8%) were classified concordantly with respect to fetal sex classification. The % negative agreement with Verifi for XXX, XXY, XYY and Monosomy X was 99.9%, as there was one sample that was classified as XX by Verifi and XXX by VeriSeq NIPT.

	T21 (XX)	T21 (XY)	Euploid (XX)	Euploid (XY)	Euploid (XXX)	Total
T21 (XX)	45	0	4	0	0	49
Verifi, T21 (XY)	1	36	0	1	0	38
Euploid (XX)	0	0	188	0	1	189
Euploid (XY)	0	0	0	185	0	185

There were in total 7 discrepant outcomes, 1 for fetal sex, 5 for T21 and one for Trisomy X. The one sample for which fetal sex calling was discordant between the two assays was called T21 by both assays. No clinical outcome information was available for the samples in this method comparison study including the samples with discrepant results. A plot of the samples comparing NCV_21 and estimated fetal fraction (data derived from the VeriSeq NIPT Analysis Software (48 Samples)) is shown in Figure 4. The discrepant samples yielded NCV scores at or near the Verifi decision boundary. The VeriSeq NIPT Analysis Software (48 Samples) combines both NCV and fetal fraction to derive a new score called log likelihood ratio (LLR). Figure 5 shows samples plotted comparing LLR vs fetal fraction. Generally, this method of scoring requires concordance between estimated fetal fraction and chromosomal representation for a sample to be classified as positive. Preliminary studies have shown calls based on LLR scoring can improve overall specificity of the NIPT test. Varying LLR cutoffs leads to different positive and negative agreement rates, as shown in Figure 6.

Figure 4 NCV versus fetal fraction for Chromosome 2, horizontal line corresponds to an NCV cutoff of 4

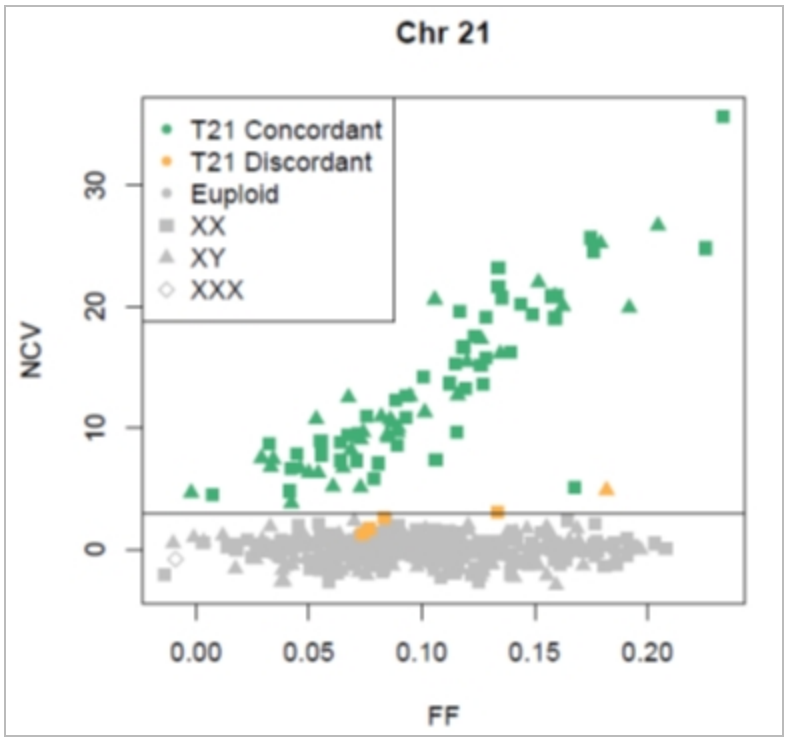


Figure 5 LLR versus fetal fraction for chromosome 2, horizontal line corresponds to the LLR cutoff of 1.5

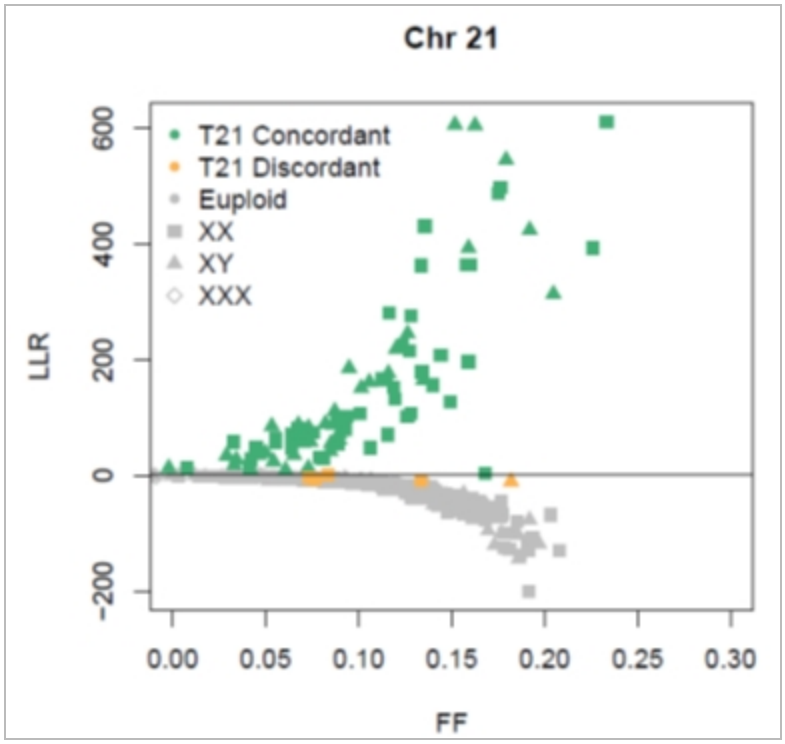
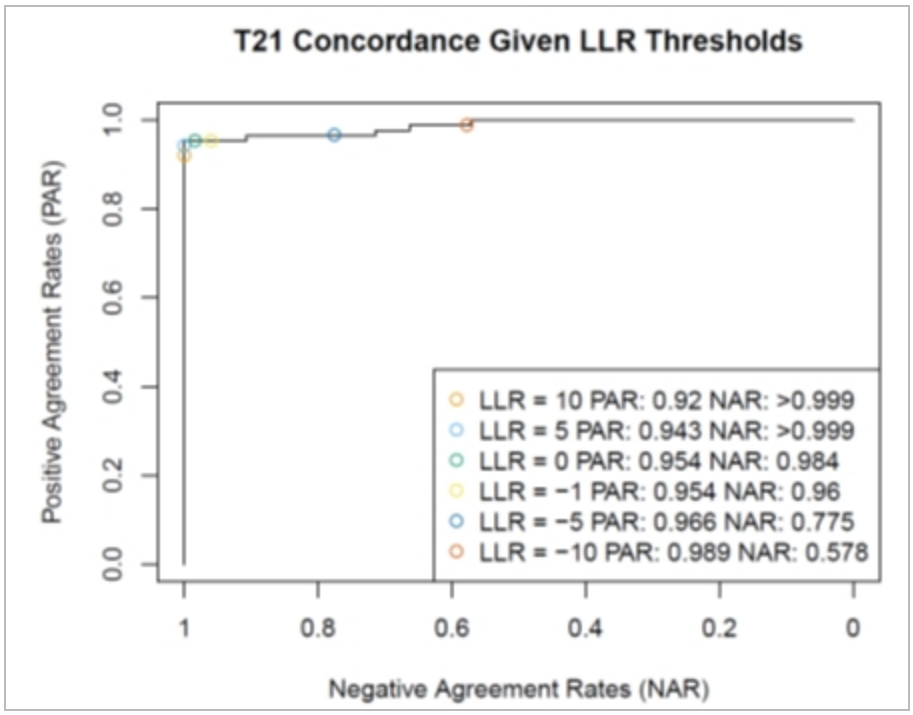


Figure 6 Positive agreement versus negative agreement rates for varying LLR cutoffs for chromosome 21



Connecting a Compatible Next Generation Sequencer

Introduction

A Next Generation Sequencer generates sequencing reads for all samples in the quantified library pool and integrates with the VeriSeq NIPT Analysis Software (48 Samples) via the Server. Sequencing data are evaluated by the Analysis Handler of the Analysis Software.

Consider the following when integrating a Next Generation Sequencer with the VeriSeq NIPT Analysis Software (48 Samples).

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

Sequence Pool

The Analysis 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 Sample Prep Long
- 2-dye based chemistry
- Automatic production of .BCL files

Data Storage Integration

A typical sequencing run intended for analysis by the VeriSeq NIPT Analysis Software (48 Samples) requires 25–30 GB for Next Generation Sequencer data. The actual data size may vary based on the final cluster density. The 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 Sequencer to the Server for 1 of the following methods:

- Use the 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 Sequencer to persist the sequencing data directly to a specific location on the NAS.
  In this setup, configure the Server to monitor the specific NAS location that enables the server to monitor upcoming sequencing runs. Multiple Next Generation Sequencer 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 10.

For more information on how to map the Next Generation Sequencer 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.

Network Traffic Constraints

The VeriSeq NIPT Analysis Software (48 Samples) uses the lab Local Area Network (LAN) for data throughput between the Next Generation Sequencer, 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.

Troubleshooting

Introduction

VeriSeq NIPT Analysis Software (48 Samples) troubleshooting assistance comprises:

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

VeriSeq NIPT Analysis Software (48 Samples) Notifications

This section describes the Analysis 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 API. These actions are communicated to the Analysis Software using the software API.

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 [[[Undefined variable Software.Software_Primary]]] 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	• If using a NAS, check the network connection. See *Recommended Action Procedures*Action ID *1* on page 48. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Insufficient Disk Space for Sequencing	Sequencing	The system detected a new sequencing data folder, but is estimating that there is not enough disk space for the data	Alert	Yes	1. Check available disk space. See *Recommended Action Procedures* Action ID *2* on page 48. 2. Clear disk space or backup data. See *Recommended Action Procedures* Action ID *3* on page 48
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.
Sample Sheet Generation failed	Pre-Analysis	The software failed to generate sample sheet	Alert	Yes	• Check available disk space. See *Recommended Action Procedures* Action ID *2* on page 48. If space is low, clear disk space or backup data. See *Recommended Action Procedures* Action ID *3* on page 48. • If using a NAS, check the network connection. See *Recommended Action Procedures* Action ID *1* on page 48. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.

Notification	Step	When	Alert Level	Email	Recommended Action
Unable to check disk space	Pre-Analysis	The software could not check disk space	Alert	Yes	• If using a NAS, check the network connection. See *Recommended Action Procedures* Action ID *2* on page 48. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Insufficient Disk Space for Analysis	Pre-Analysis	The software detected that there is not enough disk space for starting a new analysis run	Alert	Yes	Clear disk space or backup data. See *Recommended Action Procedures* Action ID *3* on page 48.
Unable to launch Analysis Pipeline	Pre-Analysis	The software was unable to start an analysis run for the given sequencing folder	Alert	Yes	Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Sequencing folder Read/Write permission failed	Pre-Analysis	The software test that checks Read/Write permission to the sequencing run folder failed	Warning	Yes	• If using a NAS, check the network connection. See *Recommended Action Procedures* Action ID *1* on page 48. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Analysis Failed - Retry	Analysis	Analysis has failed. Retrying.	Notice	Yes	None
Results Already Reported	System	The software determined that an NIPT report was already generated for the current pool type	Activity	Yes	None
Unable to deliver email notifications	System	The system is unable to deliver email notifications.	Warning	NA	1. Check email configuration defined on system for validity. See instructions in *Configuring System Email Notifications* on page 13. 2. Send a test email. See instructions in *Configuring System Email Notifications* on page 13. 3. Reboot the server. If the problem persists, email Illumina Technical Support.
Time Skew Detected	Library prep	The software detected a time skew of over 1 minute between the timestamp provided by the API and the server local time	Warning	No	1. Check local time provided via the API. 2. Check the Server local time reported on the Web UI (Server Status tab).

Unrecoverable Error Notifications

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

Notification	Step	When	Alert Level	Email	Recommended Action
Batch Failure	Library Prep	Batch QC failed	Notice	Yes	Restart library plating.
Report Generating Failure	Reporting	The system failed to generate a report	Alert	Yes	• Check available disk space. See *Recommended Action Procedures* Action ID *2* on page 48. If space is low, clear disk space or backup data. See *Recommended Action Procedures* Action ID *3* on page 48. • Possible hardware failure. Reboot the server. If the problem persists, email Illumina Technical Support.
Failed to Parse Run Parameters file	Sequencing	The system was not able to open/parse the RunParameters.xml file	Warning	Yes	The RunParameters.xml file is corrupted. Check the sequencer configuration and resequence the pool.
Unrecognized Run Parameters	Sequencing	The software read Run Parameters that are not compatible	Warning	Yes	The software is was unable to construct sequencing run parameters from the sequencer configuration file. Check the sequencer configuration and resequence the pool.
Invalid Run Parameters	Sequencing	The software read required Run Parameters that are not compatible with the assay	Warning	Yes	The software compatibility check failed. Check the sequencer configuration and resequence the pool.
No Pool Barcode found	Sequencing	The software was not able to associate the flow cell for the sequencing run with a known pool barcode	Warning	Yes	Possible incorrect pool barcode entry. Resequence the pool.
Sequencing Timed Out	Sequencing	The sequencing run has not completed in a given time frame	Warning	Yes	Check the sequencer and the network connection. Resequence the pool.
Sequencing QC files generation failed	Sequencing QC	The sequencing run has completed but the interop QC files are corrupted	Alert	Yes	Check the sequencer, and the network connection. Resequence the pool.
Sequencing QC 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.

Notification	Step	When	Alert Level	Email	Recommended Action
Analysis Post-Processing Failed	Post-Analysis	The software failed to post process the analysis results	Alert	Yes	• If using a NAS, check the network connection. See *Recommended Action Procedures*Action ID *1* on page 48. • Possible hardware failure. Reboot the server. If problem persists, email Illumina Technical Support.
Analysis Upload Failed	Post-Analysis	The software failed to upload the analysis results to the database	Alert	Yes	• If using a NAS, check the network connection. See *Recommended Action Procedures*Action ID *1* on page 48. • Possible hardware failure. Reboot the server. If problem persists, email Illumina Technical Support.

Recommended Action Procedures

Action ID	Recommended Action	Steps
1	Check the network connection	NOTE Make sure that the remote storage NAS and the local machine are on the same network. 1. From a Windows command line (cmd), type the following command: **ping <Server IP>** NOTE If using a NAS, also check the connection with the NAS. 2. Make sure that there are no lost packets. NOTE If there are lost packets, contact the IT Administrator. 3. Test the connection: a. Log in to the Server Web UI. b. From the Dashboard menu, select **Folder**. c. Click **Test**, and determine if the test is successful. If the test fails, see *Editing a Shared Network Drive* on page 11 and make sure that all settings are configured correctly.
2	Check available disk space	NOTE Make sure that the Server Input folder is mapped to the Windows machine. For more information, see *Mapping Server Drives* on page 17. Right-click the drive that maps to the Input folder. Select **Properties**, and view the free space information.
3	Clear disk space / Backup data	NOTE Illumina recommends a periodic data backup and/or storing sequencing data on the server side. For more information, see *Managing a Shared Network Drive* on page 10. 1. For data stored locally on the Server: NOTE Make sure that the Server Input folder is mapped to the Windows machine. For more information, see *Mapping Server Drives* on page 17. a. Double-click the Input folder, and enter the credentials to access it. b. Sequencing run data are listed with folder names matching sequencing run names. c. Delete or backup the processed sequencing folders. 2. For data stored on a remote NAS: NOTE Make sure that the remote storage NAS and the local machine are on the same network. NOTE Obtain access to the folder on the remote drive. Access credentials from the IT Administrator are required. a. Sequencing run data are listed with folder names matching sequencing runs names. b. Delete or backup the processed sequencing folders.

System Issues

Issue	Recommended Action
Software fails to start	If errors are detected when starting the Analysis 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 Analysis 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 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 lunching the Analysis Pipeline. Run this test to make sure that the 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 NIPT 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_FullTRun.
The suffix creates a unique name for each sequencing run. If the run has a suffix already, rename the folder by incrementing the suffix numerical value by 1.

3 Move the renamed folder to the input folder.

4 Wait for about 4–5 hours for the analysis to complete. Make sure that the following email notifications have been received:

a Sequencing Run Analysis Started
b NIPT Report generated for Sequencing Run

5 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.

Acronyms

Acronym	Definition
BCL	Base Call File
CE-IVD	European Conformity marking for *in vitro* diagnostic product
cfDNA	Cell-Free DNA
DNA	Deoxyribonucleic Acid
DNS	Domain Name System
FASTQ	Text-based file format for storing the output of sequencing instruments
FF	Fetal Fraction
FIFO	First In, First Out
iFACT	individual Fetal Aneuploidy Confidence Test
IP	Internet Protocol
LIMS	Laboratory Information Management System
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
Email: techsupport@illumina.com

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.066.5835	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

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

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

Document # 1000000026777 v04

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

CE 2797

IVD

EC REP

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

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

FOR IN VITRO DIAGNOSTIC USE

illumina®