illumina®

NovaSeq X Plus

Product Documentation

ILLUMINA PROPRIETARY
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For Research Use Only. Not for use in diagnostic procedures (except as specifically noted).

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Safety and Compliance

This section provides important safety information pertaining to the installation, servicing, and operation of the sequencing system. This section includes product compliance and regulatory statements. Read this section before performing any procedures on the system.

The country of origin and date of manufacture of the system are printed on the instrument label.

Safety Considerations and Markings

This section identifies potential hazards associated with installing, servicing, and operating the instrument. Do not operate or interact with the instrument in a manner that exposes you to any of these dangers.

General Safety Warnings

Make sure that all personnel are trained in the correct operation of the instrument and any potential safety considerations.

Follow all operating instructions when working in areas marked with this label to minimize risk to personnel or the instrument.

Laser Safety Warning

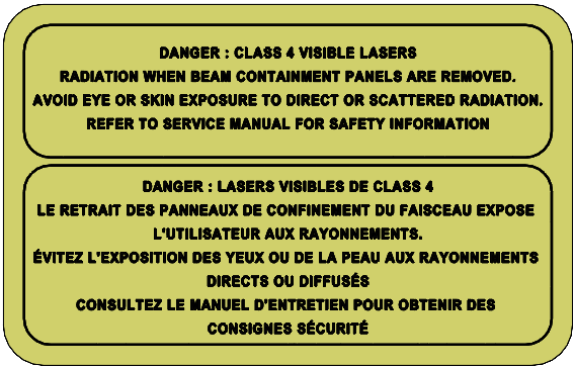
The NovaSeq X Series are Class 1 laser products that contain two Class 4 lasers.

Class 4 lasers present an eye hazard from direct and diffuse reflections. Avoid eye or skin exposure to direct or reflected Class 4 laser radiation. Class 4 lasers can cause combustion of flammable materials and produce serious skin burns and injury from direct exposure.

When the flow cell compartment is open or beam seal interlock removed, safety interlock switches block the laser beam.

Figure 1 Class 4 Laser Warnings

Figure 2 Illumina field service engineer laser warning label



Protective Earth

The instrument has a connection to protective earth through the enclosure. The safety ground on the power cord returns protective earth to a safe reference. The protective earth connection on the power cord must be in good working condition when using this device.

Hot Surface Safety Warning

Do not operate the instrument with any of the panels removed.

Do not touch the temperature station in the flow cell compartment. The heater used in this area is normally controlled between ambient room temperature (22°C) and 60°C. Exposure to temperatures at the upper end of this range can result in burns.

Heavy Object Safety Warning

The instrument weighs approximately 722 kg (1591 lb) shipped and approximately 588 kg (1296 lb) installed and can cause serious injury if dropped or mishandled.

Do not operate the instrument with any of the panels removed. Do not touch moving parts within the instrument.

Product Compliance and Regulatory Markings

Simplified Declaration of Conformity

Illumina, Inc. hereby declares that the NovaSeq X Series is in compliance with the following Directives:

- EMC Directive [2014/30/EU]
- Low Voltage Directive [2014/35/EU]
- RED Directive [2014/53/EU]

Illumina, Inc. hereby declares that the Compute Server is in compliance with the following Directives:

- RoHS Directive [2011/65/EU] as amended by EU 2015/863

The full text of the EU Declaration of Conformity is available at the following internet address: support.illumina.com/certificates.html.

Restriction of Hazardous Substances (RoHS)

This label indicates that the instrument meets the WEEE Directive for waste.

Visit support.illumina.com/weee-recycling.html for guidance on recycling your equipment.

Human Exposure to Radio Frequency

This equipment complies with maximum permissible exposure (MPE) limits for the general population per Title 47 CFR § 1.1310 Table 1.

This equipment complies with the limitation of human exposure to electromagnetic fields (EMFs) for devices operating within the frequency range 0 Hz to 10 GHz, used in radio frequency identification (RFID) in an occupational or professional environment. (EN 50364:2010 sections 4.0.)

For information on RFID compliance, refer to the *RFID Reader Compliance Guide (document # 1000000002699)*.

EMC Considerations

This equipment has been designed and tested to the CISPR 11 Class A standard. In a domestic environment, it might cause radio interference. If radio interference occurs, you might need to mitigate it.

Do not use the device in close proximity to sources of strong electromagnetic radiation, which can interfere with proper operation.

Regulatory and Compliance Statements

FCC Compliance

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions:

1. This device may not cause harmful interference.
2. This device must accept any interference received, including interference that may cause undesired operation.

Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.

This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instrumentation manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case users will be required to correct the interference at their own expense.

FCC Shielded Cables

Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.

IC Compliance

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

This device complies with Industry Canada license-exempt RSS standards. Operation is subject to the following two conditions:

1. This device may not cause interference.
2. This device must accept any interference, including interference that may cause undesired operation of the device.

Korea Compliance

해당무선설비는운용중전파혼신가능성이있음.

A급기기(업무용방송통신기자재)

이기기는업무용(A급)으로전자파적합로서판매자또는사용자는이점을주의

하시기바라며, 가정외의지역에서사용하는것을목적으로합니다.

Thailand Compliance

This telecommunication equipment conforms to NTC/NBTC technical requirements.

Nigeria Compliance

Connection and use of this communications equipment is permitted by the Nigerian Communications Commission.

Taiwan NCC Compliance

低功率電波輻射性電機管理辦法 第十二條 經型式認證合格之低功率射頻電機，非經許可，公司、商號 或使用者均不得擅自變更頻率、加大功率或變更原設計之特 性及功能。 第十四條 低功率射頻電機之使用不得影響飛航安全及干擾合法通信； 經發現有干擾現象時，應立即停用，並改善至無干擾時方得 繼續使用。 前項合法通信，指依電信法規定作業之無線電通信。 低功率射頻電機須忍受合法通信或工業、科學及醫療用電波 輻射性電機設備之干擾。

Japan Compliance

型式指定を取得した高周波利用設備が内蔵されています。

System Overview

This section provides an overview of the NovaSeq™ X Plus system, including information on hardware, software, data analysis, and run management. For detailed specifications, data sheets, applications, and related products, refer to the NovaSeq X Series support site page.

Features

- High throughput and accuracy
  - Delivers meaningful genomic insights at scale using high throughput next-generation sequencing (NGS).
  - Uses XLEAP-SBS chemistry, an update to Illumina SBS chemistry.
  - Enables comprehensive and efficient analysis of NGS data using data analysis pipelines powered by the Illumina DRAGEN Bio-IT Platform.
- Productivity
  - Allows multiple flow cell configurations to enable ~165 Gb to 16 Tb of sequencing data and up to 104 billion paired-end reads per run. Output is adjustable to match sequencing workflow needs.
  - Provides integrated onboard and cloud-based data analysis workflows and lossless data compression powered by the Illumina DRAGEN Bio-IT Platform.
- Sustainability
  - Ships at ambient temperatures (no ice packs or dry ice) by using lyophilized reagents, which reduces cartridge volume, packaging weight, and the amount of shipping materials used.
  - Reduces the plastic mass of the instrument by providing recyclable plastics and buffer cartridges made with plant-based biopolymers.

Sequencing Overview

The following information includes additional details on NovaSeq X Series sequencing workflow.

Cluster Generation

During cluster generation, single DNA molecules are bound to the surface of the flow cell[1] and simultaneously amplified to form clusters.

[1] A glass slide with physically separated lanes. The lanes are coated with oligos complementary to the library adapter sequences, allowing the library to adhere for a sequencing run.

Sequencing

Clusters are imaged using two-channel chemistry, one green channel and one blue channel, to encode data for the four nucleotides. After one tile on the flow cell is imaged, the next tile is imaged. The process is repeated for each cycle of sequencing (~5 minutes per cycle).

Primary Analysis

Following image analysis, Real-Time Analysis (RTA4) software performs base calling[1], filtering, and quality scoring[2]. As the run progresses, the NovaSeq X Series Control Software automatically transfers base call files[3] (*.CBCL) to the specified output location for data analysis. You can view quality metrics generated by RTA4 in real-time using the NovaSeq X Series Control Software, Sequencing Analysis Viewer (SAV), or BaseSpace Sequence Hub.

Secondary analysis begins after sequencing is complete. The method of secondary data analysis depends on your application and system configuration.

Secondary Analysis

BaseSpace Sequence Hub and Illumina Connected Analytics (ICA) are the Illumina cloud computing environments for data analysis, storage, and run monitoring. Run monitoring is only visible in BaseSpace Sequence Hub. BaseSpace Sequence Hub hosts DRAGEN and BaseSpace Sequence Hub apps, which support common analysis methods for sequencing. Illumina Connected Analytics hosts the DRAGEN for ICA pipelines. You can use pre-built ICA pipelines or create custom pipelines using your sequencing and analysis data.

If analyzing sequencing data in the cloud, CBCL data are uploaded automatically to the cloud and are available in BaseSpace Sequence Hub and ICA. Analysis automatically begins after the data upload completes.

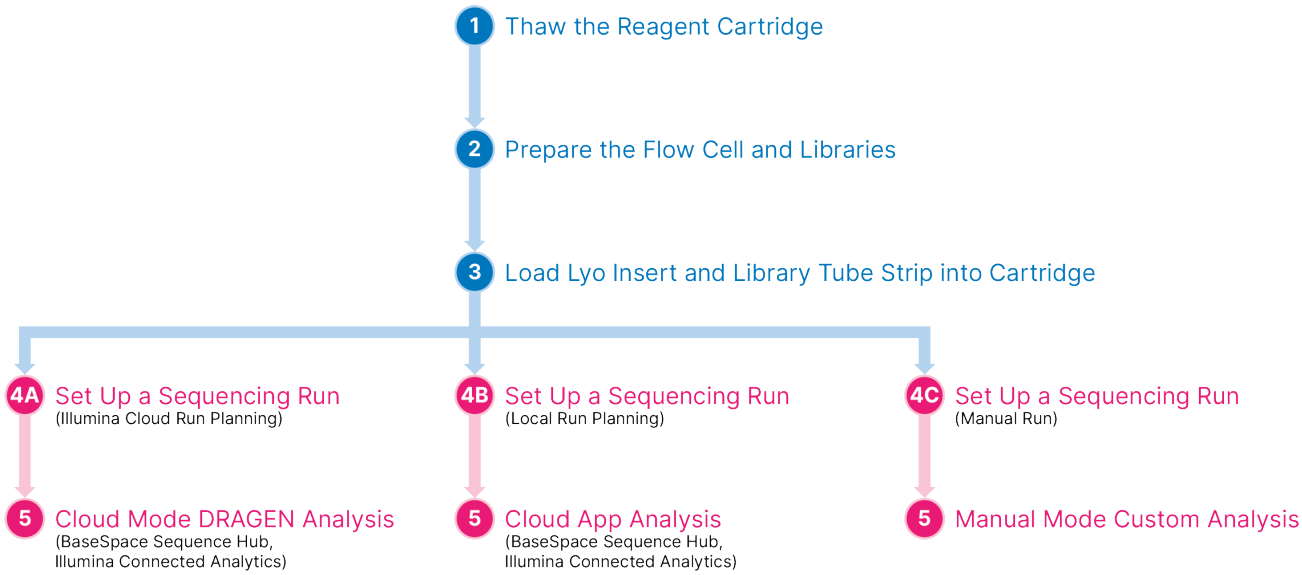
If analyzing sequencing data locally, DRAGEN secondary analysis is performed on-instrument and output files are stored in a selected output folder.

- For more information on BaseSpace Sequence Hub, refer to BaseSpace Sequence Hub support page.
- For more information on Illumina DRAGEN Bio-IT Platform, refer to DRAGEN Bio-IT Platform support page.
- For more information on Illumina Connected Analytics, refer to Illumina Connected Analytics support page.
- For an overview of all apps, refer to BaseSpace Sequence Hub Apps.

[1]Determining a base (A, C, G, or T) for every cluster in a tile at a specific cycle.
[2]Calculates a set of quality predictors for each base call, and then uses the predictor value to look up the Q-score.
[3]Contains the base call and associated quality score for every cluster of each sequencing cycle.

Sequencing Workflow

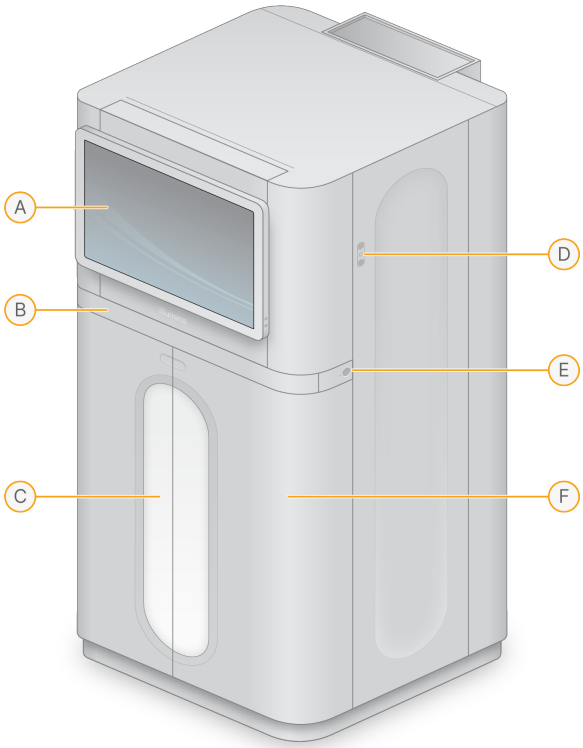
The following diagram illustrates the sequencing protocol using the NovaSeq X Plus.



Instrument Components

The NovaSeq X Plus comprises a touch screen monitor, a status bar, a power button with adjacent USB ports, and consumables compartments.

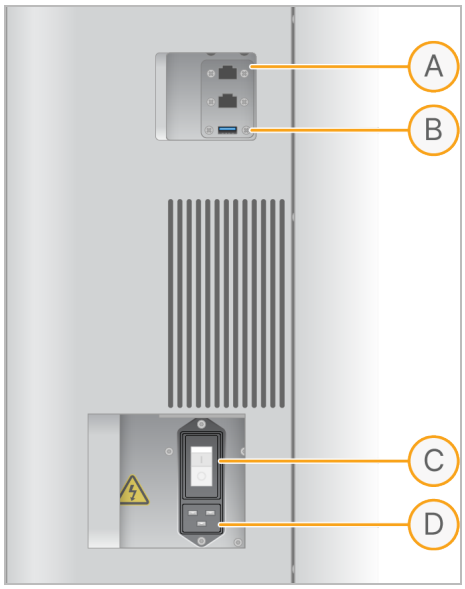
External Components



A. **Touch screen monitor**—Enables on-instrument configuration and setup using the NovaSeq X Series Control Software interface. You can adjust the touch screen monitor height using the buttons on the side of the monitor.
B. **Keyboard and trackpad tray**—Extendable tray for keyboard and trackpad. Push the tray in to open.
C. **Status bar**—Light color progresses as the system moves through its workflow. Blue indicates consumable loading, blue and purple indicate pre-run checks, and multicolor indicates sequencing. Solid red indicates critical errors. Red and white indicate other errors.
D. **USB 3.0 ports (3)**—Provides access to USB connections for peripheral components.
E. **Power button**—Controls instrument power and indicates whether the system is on (glows), off (dark), or off but with AC power (pulses).
F. **Liquids compartment**—Contains reagent and buffer cartridges, and bottles for used reagents.

Power and Auxiliary Connections

The back of the instrument has two Ethernet ports for an Ethernet connection and the switch and inlet that controls power to the instrument. Use the USB 2.0 port to connect a UPS.



A. **Ethernet ports (2)**—Ethernet cable connection.
B. **USB 2.0 port**—USB connection for the UPS.
C. **Toggle switch**—Turns instrument on and off.
D. **Power inlet**—Power cord connection.

Flow Cell Compartment

The flow cell compartment contains the flow cell stage, which holds flow cell A on the left and flow cell B on the right. The compartment is located behind the instrument monitor. When loading the flow cell, the control software automatically raises the monitor.

An optical alignment target mounted on the flow cell stage diagnoses and corrects optical problems. When prompted by the control software, the optical alignment target realigns the system and adjusts camera focus to improve sequencing results.

The control software controls the opening and closing of the flow cell compartment door. The door opens automatically to load a flow cell. After loading, the software closes the compartment door, moves the flow cell into position, and vacuum seals and engages the clamps. Sensors verify the presence and compatibility of the flow cell.

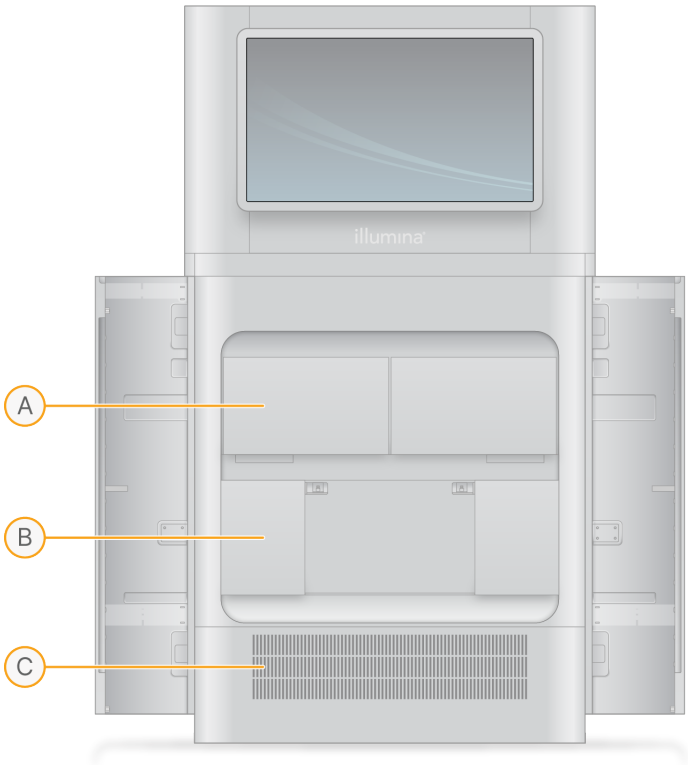
Liquids Compartment

Starting a run requires accessing the liquids compartment to load reagents and buffer and empty used reagent bottles. The two doors enclose the liquids compartment, which is divided into two matching sides for side A and side B.

The air filter covers the fan in the bottom drawer in the front of the instrument. Keep the front of the instrument clear of obstructions and floor clean to avoid clogging the air filter. Refer to *Site Preparation* on page 17 for more information on placement.

The drawers have interior lights that signal when to remove the used cartridges and bottles. The lights turn off after the drawers are closed.

The instrument doors unlock automatically when loading sequencing or wash consumables during run or maintenance wash setup. When the instrument is not sequencing, you can manually unlock the instrument doors to change the air filter or to empty reagent waste outside of run setup.



A. **Reagents drawers**—Holds the reagent and buffer cartridges.
B. **Used reagents drawer**—Holds the large and small used reagents bottles.
C. **Air filter compartment**—Provides access to the replaceable air filter.

Used Reagents

The fluidics system is designed to route the reagent cartridge reagents, which are potentially hazardous, to the small used reagent bottle. Reagents from the buffer cartridge are routed to the large used reagent bottle. However, cross-contamination between used reagent streams can occur. For safety, assume that both used reagent bottles contain potentially hazardous chemicals. The safety data sheet (SDS) provides detailed chemistry information.

Integrated Software

The system software suite includes integrated applications that perform sequencing runs and analysis.

- **NovaSeq X Series Control Software**—Controls instrument operation and provides an interface for configuring the system, setting up a sequencing run, monitoring run statistics as sequencing progresses, and viewing DRAGEN data. You can access the control software on-instrument or through a computer connected to a local network.
- **Real-Time Analysis (RTA4)**—Performs image analysis and base calling during the run. For more information, refer to *Real-Time Analysis* on page 79.
- **Universal Copy Service (UCS)**—Copies output files to the output folder throughout a run. If applicable, the service also transfers data to BaseSpace Sequence Hub or Illumina Connected Analytics (ICA).
- **Illumina DRAGEN Bio-IT Platform**—Performs hardware accelerated secondary analysis for a select menu of applications.

The NovaSeq X Series Control Software is interactive and runs automated background processes. RTA4 and UCS run as background processes only.

System Information

In the NovaSeq X Series Control Software, select the instrument icon to open the global navigation menu. Select the Settings icon, and then select About to view the Illumina contact information and the following system information:

- NovaSeq X Series Control Software version
- Computer name
- OS image version
- Instrument serial number
- Total run count

Notifications and Alerts

You can view all system notifications by selecting the instrument icon to open the global navigation menu, and then selecting Notifications. The Notifications screen contains two tabs: Notifications and Error and warning history. Select the Error and warning history tab to view a list of current or historic notifications, errors, and warnings. Select the Notifications tab to view a list of current notifications.

When an error or warning occurs, the NovaSeq X Series Control Software alerts you during the action.

- Critical system errors require immediate attention to shut down the instrument and contact Illumina Technical Support for assistance.
- Non-critical system errors require action before starting or proceeding with the run. Depending on the error, the NovaSeq X Series Control Software provides the appropriate action to resolve the error.

- Warnings do not require action before starting or proceeding with the run. When a warning occurs, the NovaSeq X Series Control Software provides the appropriate action to resolve the warning.
- Notifications provide information regarding events that are not related to the current action. The numbers of current notifications are displayed on the Notifications icon in the global navigation menu. You can dismiss notifications or resolve the notification on the Notifications tab.

Minimize or Exit the Control Software

Minimize or exit the control software to access other applications. For example, to access other applications on the instrument to perform operations outside of the control software (eg, locate the sample sheet in file explorer).

1. In the NovaSeq X Series Control Software, select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Minimize software** to minimize the control software or **Exit software** to close the control software.
   If you exit the control software, you need to sign in again when reopening.
3. To maximize or open the control software, select **NovaSeq X Series Control Software** from the toolbar.

Use the Remote NovaSeq X Series Control Software

You can access the NovaSeq X Series Control Software off-instrument using a computer connected to the same local network used with your sequencing system.

Access the Remote NovaSeq X Series Control Software

To access the control software remotely, use the IP address and host name information provided by your Illumina representative. Compatible browsers are Chrome/Chromium, Edge, Firefox, and Safari.

If your computer uses Mac or Linux operating systems, you need to install a root certificate to access the NovaSeq X Series Control Software remotely. The root certificate is provided by your Illumina representative. To install the root certificate, use the documentation provided by the manufacturer of your operating system.

Navigate the Remote NovaSeq X Series Control Software

After signing into the NovaSeq X Series Control Software from a computer connected to the local network, the Runs screen opens automatically.

To access additional features, open the Menu drop-down in the upper-left corner. You can navigate back to the Runs screen by selecting **Exit** on any screen.

The following features are available. Refer to *User Permissions* on page 40 for information on the permissions available to each user group.

- **Runs**—Perform any of the following actions:
  - Plan new sequencing runs. Refer to *Plan a Local Sequencing Run* on page 53 for more information.
  - Monitor active run progress. Refer to *Monitor Run Progress* on page 72 for more information.
  - Edit and delete locally planned runs. Refer to *Run Management* on page 14 for more information.
- **Users**—Add and manage users. Refer to *User Accounts* on page 40 for more information.
- **Password policy**—View and edit password settings. Refer to *Edit Password Settings* on page 43 for more information.
- **Applications**—View and manage DRAGEN applications. Refer to *Manage DRAGEN Applications* on page 46 for more information.
- **Resources**—Import and manage genomes and reference files. Refer to *Import Resource Files* on page 48 for more information.
- **DRAGEN**—Update DRAGEN license and perform a DRAGEN self-test. Refer to *Install DRAGEN License* on page 123and *Perform DRAGEN Self Test* on page 123 for more information.
- **Custom kits**—Add and manage custom index adapter and library prep kits. Refer to *Import Custom Library Prep and Index Adapter Kits* on page 49 for more information.
- **Audit log**—Review the audit log. Refer to *Review Audit Log* on page 124 for more information.
- **Cloud settings**—Configure cloud settings and external storage options. Refer to *Configure Cloud Settings and Proactive Support* on page 43 and *Specify the Default Output Folder Location* on page 45 for more information.
- **About**—View Illumina contact and system information. Refer to *System Information* on page 12.

Run Management

The Runs screen displays the list of planned runs, active runs, and completed runs. Each run is identified by the run name. You can search for a run using the run name, library tube strip ID, and the first DRAGEN application added to the run. You can also view the amount of instrument data storage consumed by all runs and the amount of storage space still available. Refer to *Clear Hard Drive Space* on page 113 for information on deleting runs.

In the remote control software, you can export the sample sheet of a run. Select the run name, and then select **Sample Sheet**. Select **Save as** to save the sample sheet.

Planned Runs

The Planned tab displays runs planned locally or in the cloud. You can plan runs locally on the NovaSeq X Plus instrument or a networked computer. To plan runs in the cloud, you can use BaseSpace Sequence Hub.

You can edit or delete locally planned runs on the Planned tab. To edit a planned run, select the run on the Planned tab. To delete a planned run, select the ellipsis icon in the Actions column.

The Planned tab displays the following information:

- **Status**—The status of the sequencing run. Planned runs can exist in one of the following statuses:
  - **Planned**—Run is available to select for sequencing.
  - **Draft**—Run is not available to select for sequencing.
  - **Needs attention**—Run is not available due to an error (eg, cloud connection has been disrupted). You can review the error in the Run details screen.
- **Run name**—The name of the run.
- **Application**—The DRAGEN secondary analysis applications associated with the run. For more information on installing applications, refer to *Manage DRAGEN Applications* on page 46.
- **Last modified**—The date and time the run was last edited.

Active Runs

The Active tab displays any in-progress runs, including planned runs and runs created manually. The Active tab includes the date sequencing began, instrument side, sequencing status, and the % ≥ Q30, yield, and total reads PF metrics.

In the Active tab, you can cancel data upload to your storage location or cancel secondary analysis. Refer to *End a Run* on page 121 for more information. Canceling a run during sequencing is not available on the Active tab.

Select the run name to navigate to the Run details page and view additional details about the run. Select the drop-down next to the run to view additional details on the sequencing status and associated DRAGEN applications.

For more information on run metrics and run status, refer to *Monitor Run Progress* on page 72.

Completed Runs

The Completed tab displays runs that have completed sequencing and analysis, were canceled, or failed to complete sequencing or analysis. You view the location of the sequencing and analysis output data, sequencing metrics, and the amount of instrument data storage consumed by the run. You can view the DRAGEN applications associated with the run, the location the planned run was created in, the Avg %Q30, and the data output amount. When sequencing data are transferred off the instrument, the data output amount metric is no longer available.

To view additional run results, such as detailed sequencing and secondary analysis metrics, select the run.

To delete a run, select the ellipsis icon in the Action column. You can delete the run or delete only the run data. Deleting run data removes sequencing and analysis folders generated by the run, but retains basic run details and does not remove the run from the Completed tab. Deleting the run removes the run entirely.

Site Preparation

This section provides specifications and guidelines for preparing your site for installation and operation of NovaSeq X Series.

Delivery and Installation

An Illumina representative delivers the system, uncrates components, and places the instrument. Make sure that the lab space is ready before delivery.

The uncrated instrument requires at least 87 cm (34 in) doorway and elevator clearance. If your doorway or elevator clearance is less than 87 cm, contact your Illumina representative.

Floor loading risks related to instrument installation must be evaluated and addressed by building facility personnel.

Only Illumina authorized personnel can uncrate, install, or move the instrument. Mishandling of the instrument can affect the alignment or damage instrument components.

An Illumina representative installs and prepares the instrument. When connecting the instrument to a data management system or remote network location, the path for data storage must be selected before the date of installation. The Illumina representative can test the data transfer process during installation.

After your Illumina representative has installed and prepared the instrument, ***do not*** relocate it. Moving the instrument improperly can affect the optical alignment and compromise data integrity. If you must relocate the instrument, contact your Illumina representative.

Crate Dimensions and Content

The sequencing system and components are shipped in two crates. Use the following dimensions to determine the minimum door width required to accommodate the shipping crates.

For Crate #1, the forklift access points are on the depth side of the crate. Make sure to check doorway and elevator clearance when transporting the instrument in the crate.

Table 1 Crate Dimensions

Measurement	Crate #1	Crate #2
Height	175.90 cm (69.25 in)	121.92 cm (48 in)
Width	109.22 cm (43 in)	91. 44 cm (36 in)
Depth	154.76 cm (60.93 in)	101.6 (40 in)
Weight	722 kg (1591 lb)	238 kg (525 lb)

The following contents are included in each crate.

Crate #1 contains the instrument.

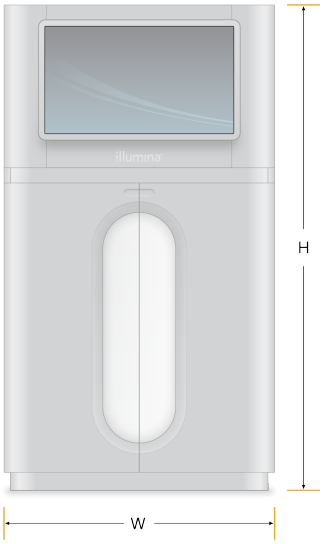
Crate #2 contains five boxes with the following contents.

- Box—Uninterruptible power supply (UPS), weight 46 kg (100 lb)
- Box—Kit accessories enclosure, weight 12 kg (26 lb)
- Box—Power and display accessories, weight 13 kg (30 lb):
  - Monitor
  - Monitor cable carrier
  - Ethernet cable
  - Region-specific power cord
  - Keyboard
- Box—Accessories, weight 10 kg (22 lb):
  - Chimney adapter
  - Air filter replacements (4)
  - Tubing kit assembly
  - Wash reagent cartridge
  - Wash flow cell
  - Coolants (3)
  - Waste caps (4)
  - Library tube strip adapter (3)
  - External used reagent tubes
  - Web sling
  - Carabiner clip

Laboratory Requirements

Use the specifications and requirements provided in this section to set up your lab space.

Instrument Dimensions



Measurement	Instrument Dimensions
Height	158.8 cm (62.5 in)
Width	86.4 cm (34 in)
Depth	93.3 cm (36.7 in)
Weight	588 kg (1296 lb)*

* Total instrument weight after installation, including UPS and consumables.

Placement Requirements

Position the instrument to allow proper ventilation, access for servicing the instrument, and access to the power switch, power outlet, and power cord.

- Position the instrument so that personnel can reach around the right side of the instrument to turn on or turn off the power switch. This switch is on the back panel adjacent to the power cord.
- Position the instrument so that personnel can quickly disconnect the power cord from the outlet.
- Make sure that the instrument is accessible from all sides using the following minimum clearance dimensions.
- Keep the front of the instrument clear of obstructions and floor clean to avoid clogging the air filter.
- Place the UPS on either side of the instrument. The UPS can be placed within the minimum clearance range of the instrument sides.

Access	Minimum Clearance
Front	Allow at least 152.4 cm (60 in) in front of the instrument for opening the front doors and to provide general lab access for movement of personnel around the lab.
Sides	Allow at least 70 cm (27.5 in) on each side of the instrument for access and clearance around the instrument. Instruments placed side-by-side only require 70 cm (27.5 in) total between two instruments.
Rear	Allow at least 30.5 cm (12 in) behind the instrument placed next to a wall for ventilation and access. Allow at least 61 cm (24 in) between two instruments placed back-to-back.
Top	Make sure that shelving and other obstructions are not above the instrument.

Incorrect placement can reduce ventilation. Reduced ventilation increases heat output and noise output, which compromises data integrity and personnel safety.

Vibration Guidelines

Keep the vibration level of the lab floor at the VC-A standard of 50 µm/s for ⅓ octave band frequencies of 8–80 Hz, or lower. This level is typical for labs. Do not exceed the ISO Operating Room (baseline) standard of 100 µm/s for ⅓ octave band frequencies of 8–80 Hz.

During sequencing runs, use the following best practices to minimize vibrations and ensure optimal performance:

- Place the instrument on a flat hard floor and keep the clearance area free of clutter.
- Do not place keyboards, used consumables, or other objects on top of the instrument.
- Do not install the instrument near sources of vibration that exceed the ISO Operating Room standard. For example:
  - Motors, pumps, freezers, centrifuges, shake testers, drop testers, and heavy air flows in the lab.
  - Floors directly above or below HVAC fans, and controllers, and helipads.
  - Construction or repair work on the same floor as the instrument.
  - Areas with high foot traffic.
- Keep sources of vibration such as dropped items and movement of heavy equipment at least 100 cm (39.4 in) from the instrument.
- Use only the touch screen, keyboard, and trackpad to interact with the instrument. Do not directly impact the instrument surfaces during operation.

Storage Requirements for Reagent Kits

Use the following specifications to determine storage requirements.

Reagents are shipped at ambient temperature.

Storage Temperatures and Dimensions

A single flow cell run requires one of each of the following items. A dual flow cell run requires two of each item. When you receive your kit, promptly store components at the indicated temperature to ensure proper performance.

Item	Storage Temperature	Dimensions
Reagent cartridge	-25°C to -15°C	9.8 cm x 23.7 cm x 31.2 cm (3.9 in x 9.4 in x 31.1 in)
Lyo insert*	-25°C to -15°C	5.1 cm x 14.3 cm x 19.1 cm (2.0 in x 5.6 in x 7.5 in)
Custom primer buffer	-25°C to -15°C	12.6 cm x 3.8 cm x 4.9 cm (5.0 in x 1.5 in x 1.9 in)
Pre-load buffer	-25°C to -15°C	10.1 cm x 3.8 cm x 3.8 cm (4.0 in x 1.5 in x 1.5 in)
Flow cell	2°C to 8°C	20.0 cm x 2.54 cm x 15.1 cm (7.9 in x 1.0 in x 5.9 in)
Buffer cartridge	15°C to 30°C	14.0 cm x 8.3 cm x 31.3 cm (5.5 in x 3.3 in x 12.3 in)
Library tube strip	15°C to 30°C	4.9 cm x 3.8 cm x 9.5 cm (1.9 in x 1.50 in x 3.8 in)

*Avoid stacking lyo inserts when storing.

Light Sensitivity

The reagent cartridge, buffer cartridge, and lyo insert contain reagents that are sensitive to light. Keep the items packaged until use and store in the dark with no sources of light.

Lab Setup for PCR Procedures

Some library prep methods require the polymerase chain reaction (PCR) process.

Establish dedicated areas and lab procedures to prevent PCR product contamination before you begin work in the lab. PCR products can contaminate reagents, instruments, and samples, delaying normal operations and causing inaccurate results.

Pre-PCR and Post-PCR Areas

Use the following guidelines to avoid cross-contamination.

- Establish a pre-PCR area for pre-PCR processes.

- Establish a post-PCR area for processing PCR products.
- Do not use the same sink to wash pre-PCR and post-PCR materials.
- Do not use the same water purification system for pre-PCR and post-PCR areas.
- Store supplies used for pre-PCR protocols in the pre-PCR area. Transfer them to the post-PCR area as needed.

Dedicate Equipment and Supplies

- Do not share equipment and supplies between pre-PCR and post-PCR processes. Dedicate a separate set of equipment and supplies in each area.
- Establish dedicated storage areas for consumables used in each area.

Electrical Requirements

Do not remove the outer panels from the instrument. There are no user-serviceable components inside. Operating the instrument with any of the panels removed creates potential exposure to line voltage and DC voltages.

Table 2 Power Specifications

Type	Specification
Line Voltage	200–240 VAC at 50/60 Hz
Peak Power Consumption	2700 Watts

Receptacles

Your facility must be wired with a minimum 15 amp grounded line with proper voltage. Requirements can vary depending on your region. Refer to *Power Cords* on page 22 for country-specific power requirements. An electrical ground is required. If the voltage fluctuates more than 10%, a power line regulator is required.

The instrument must be connected to a dedicated circuit that must not be shared with any other equipment.

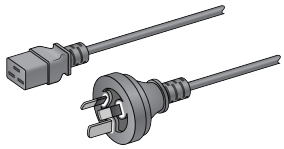
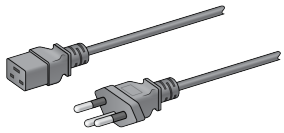
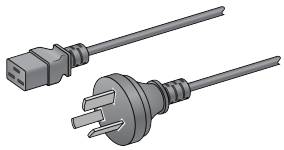
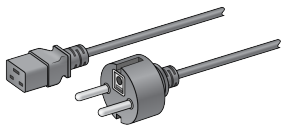
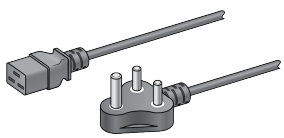
Power Cords

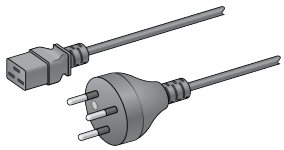
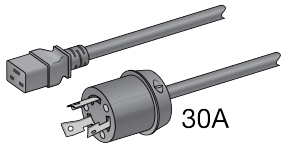
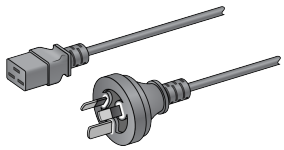
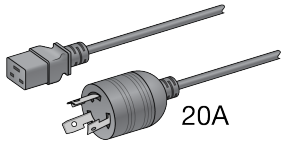
The instrument comes with an international standard IEC 60320 C20 receptacle, and is shipped with a region-specific power cord. To obtain equivalent receptacles or power cords that comply with local standards, consult a third-party supplier such as Interpower Corporation (`www.interpower.com`). All power cords are 2.5 m (8 ft) in length.

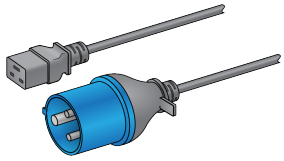
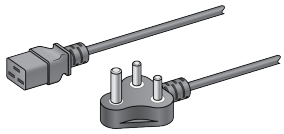
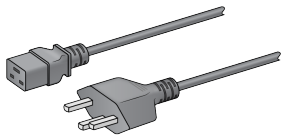
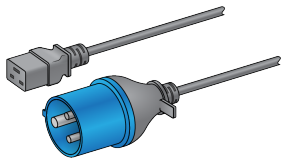
Hazardous voltages are removed from the instrument only when the power cord is disconnected from the AC power source.

Never use an extension cord to connect the instrument to a power supply.

Table 3 Power Cord Requirements of Selected Regions

Region	Shipped Power Cord	Electrical Supply	Socket
Australia	AS 3112 SAA Male to C19, 15 amps 	Line voltage: 230 VAC Minimum amp: 15 amps	15 Amp Type I
Brazil	NBR14136 Plug to C19, 16 amps 	Line voltage: 220 VAC Minimum amp: 16 amps	NBR 14136 Type N
China	GB2099 to C19, 16 amps 	Line voltage: 220 VAC Minimum amp: 16 amps	GB 1002, GB 2099, Type I
European Union[1]	Schuko CEE 7 (EU1-16p) to C19, 16 amps 	Line voltage: 220–240 VAC Minimum amp: 16 amps	Schuko CEE 7/3
India	IS1293 to C19, 16 amps 	Line voltage: 230 VAC Minimum amp: 16 amps	BS546A Type M

Region	Shipped Power Cord	Electrical Supply	Socket
Israel	IEC 60320 C19, 16 amps 	Line voltage: 230 VAC Minimum amp: 16 amps	SI 3216 Amp Type H
Japan	NEMA L6-30P, 30 amps 	Line voltage: 200 VAC Minimum amp: 30 amps	NEMA L6-30R
New Zealand	AS 3112 SAA Male to C19, 15 amps 	Line voltage: 230 VAC Minimum amp: 15 amps	Dedicated 15 Amp Type I
North America	NEMA L6-20P to C19, 20 amps 	Line voltage: < 220 VAC Minimum amp: 20 amps Line voltage: ≥ 220 VAC Minimum amp: 16 amps	NEMA L6-20R

Region	Shipped Power Cord	Electrical Supply	Socket
Singapore	IEC60309 316P6 to C19, 16 amps 	Line voltage: 230–250 VAC Minimum amp: 16 amps	IEC60309 316C6
South Africa	SANS 164-1 to C19, 16 amps 	Line voltage: 230 VAC Minimum amp: 16 amps	BS546A Type M
Switzerland	SEV 1011 Type 23 Plug J, 16 amps 	Line voltage: 230 VAC Minimum amp: 16 amps	SEV 1011 Type 23 J socket
United Kingdom	IEC60309 316P6 to C19, 16 amps 	Line voltage: 240 VAC Minimum amp: 16 amps	IEC60309 316C6

[1] Excepting Switzerland and the United Kingdom.

Alternatively, all regions can use IEC 60309.

Uninterruptible Power Supply

The following specifications apply to the worldwide UPS that ships with the instrument.

For countries that require a different model of UPS and battery, and alternatives, refer to *Country-Specific Uninterruptible Power Supply* on page 26.

- **UPS**—APC Smart-UPS X 3000 Rack/Tower LCD 200-240V, Model # SMX3000RMHV2U

Specification	UPS
Maximum output power	2700 Watts*/ 3000 VA
Input voltage (nominal)	200–240 VAC
Input frequency	50/60 Hz
Input connection	IEC-60320 C20
Typical run time (Average power of 2200 Watts)	9 minutes
Typical run time (Peak Power of 2700 Watts)	6 minutes
Thermal output	184 BTU/h
Weight	37.3 kg (82 lb)
Dimensions (Tower format: H × W × D)	43.2 cm × 66.7 cm × 17 cm (17 in × 26.26 in × 6.7 in)

* The UPS requires up to a maximum of 245 Watts to charge batteries and perform other internal functions. 2700 Watts is available for output during this time.

Country-Specific Uninterruptible Power Supply

Illumina supplies the following country-specific UPS.

Country	UPS Model #
Columbia	SRT3000RMXLW-IEC
India	UPS model #: SUA3000UXI Battery model #: SUA48XLBP
Japan	SRT5KXLJ
Mexico	SRT3000RMXLW-IEC
South Korea	SRT3000RMXLW-IEC
Thailand	SRT3000RMXLW-IEC

For additional specification information, refer to the APC website (`www.apc.com`).

Exact UPS and battery options are subject to availability and can change without notice.

Environmental Considerations

Table 4 Instrument Environmental Specifications

Element	Specification
Temperature*	Maintain a lab temperature of 15°C to 30°C. During a run, do not allow the ambient temperature to vary more than ±2°C. Failure to operate the instrument within the temperature range can degrade performance or cause a run to fail.
Humidity*	Maintain a noncondensing relative humidity between 20–80%.
Elevation	Locate the instrument at an elevation below 2000 m (6500 ft).
Air Quality	Operate the instrument in an indoor environment with air particulate cleanliness levels per ISO 9 (ordinary room air), or better. Keep the instrument away from sources of dust.
Vibration	Limit the continuous vibration of the lab floor to ISO operating room level (baseline), or better. During a sequencing run, limit intermittent disturbances or shocks to the floor near the instrument. Do not exceed ISO operating room level.

*Avoid a combination of high temperature and high humidity. For example, 30°C and 80% relative humidity.

Table 5 Noise Output

Noise Output	Distance From Instrument
< 75 dB	1 m (3.3 ft)

Table 6 Heat Output

Power Consumption	Thermal Output
Maximum: 2700 Watts Average: 2200 Watts	Maximum: 9200 BTU/h* Average: 7507 BTU/h

*Excludes thermal output from UPS.

Venting

A 10 inch, round, vertical chimney vents 80% of instrument heat output. You can vent to the room or connect the chimney to a user-supplied duct.

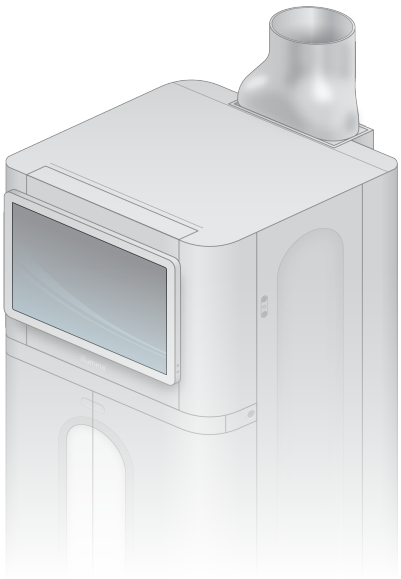
Use the following guidelines for venting ducts.

- Flexible ducting is preferred.
- Avoid bending flexible ducts where possible. Keep bends in flexible ducts to a minimum.
- Flexible ducts with bends must maintain the 10 inch diameter of the chimney at all points.
- Remove kinks or other restrictions to the airflow.
- Rigid ducting can be used. Use of rigid ducting can require Illumina personnel to move the instrument for service.
- Use the shortest length of ducting possible.
- Route to a space with sufficient ventilation to prevent airflow restriction or backup into the instrument.

Failure to follow these guidelines can impact instrument performance and can cause run failures.

Chimney airflow is up to 450 CFM. Chimney air temperature is up to 12°C higher than ambient temperature.

Figure 3 Chimney Placement for Venting



Bulk Used Reagent Handling

The NovaSeq X Series systems are equipped to dispense used reagent buffer to a customer-supplied bulk container for separate processing or handling. The supplied external used reagent tubes included in the accessory kit are 5 meters long, and connect to the left rear of the instrument.

Illumina only supports external used reagent collection with the supplied tubes. Each tube contains the buffer waste from a single flow cell position, and must be routed individually to the bulk container.

The container must be placed within 5 meters of the instrument. The aperture must be at a height of 1000 mm or less from the floor.

Network Connections

Illumina systems are designed to stream data at a regular cadence during the sequencing activity. Depending on the off-load rate, this data transmission could persist for some time after the completion of sequencing. Illumina instruments assume a mostly-up network. Network outages could impact data transmission. If a network outage occurs, the instruments are designed to cache all data locally; however, such caching could delay the start of the next sequencing run, depending on storage space on instrument. The instruments are designed to reinitiate data transfer upon restoration of the network.

Review network maintenance activities for potential compatibility risks with the NovaSeq X Series.

For information on the data storage requirements for each file type, refer to the Illumina Instrument Control Computer Security and Networking.

Use the following guidelines to install and configure a network connection:

- Use a dedicated connection between the instrument and data management system using the RJ-45 cable included with the instrument. Make this connection directly or through a network switch.
  - A 10 gigabit (Gb) intranet connection (instrument to network storage and boundary firewall) is required to maintain data transfer times. Lower connection speeds result in reduced instrument availability, increased data transfer times, and might impact sequencing run performance.
- Managed switches are recommended.
- Calculate the total capacity of the workload on each network switch. The number of connected instruments and ancillary equipment, such as a printer, can impact capacity.
- If possible, isolate sequencing traffic from other network traffic.
- A CAT-6 cable or better is recommended. An unshielded network cable that is 3 m (9.8 ft) long is provided with the instrument for network connections. A CAT-6A cable is recommended for cables longer than 50 m (164 ft).

Use the following recommended network bandwidth per instrument for connections based on 85–90% network efficiency. Primary analysis files include RTA4 and BCL sequencing output files. Secondary analysis files include on-instrument DRAGEN output files.

  - 800 megabits per second (Mb/s) (primary only) or ~3.5 gigabits per second (Gb/s) (primary and secondary) sustained network bandwidth for storing data locally.

- 800 Mb/s network bandwidth for uploading primary analysis data to the cloud.
- 15 Mb/s network bandwidth for run monitoring or Illumina Proactive Support only.

The NovaSeq X Plus uses a > 3 Gb/s network connection between the instrument and network storage. Using a 1 Gb/s connection may result in longer copy times or delay the start of subsequent sequencing runs.

Internal Connections

Connection	Value	Purpose
Host	https://127.0.0.1	GDS via the Kubernetes Client library
Port	6443	GDS via the Kubernetes Client library

Outbound Connections

Connection	Value	Purpose
Port	80	Off-instrument control software UI, BaseSpace Sequence Hub, or Illumina Proactive configuration
Port	443	Off-instrument control software UI or UCS
Port	8080	BaseSpace Sequence Hub or Illumina Proactive configuration
URL	lus.edicogenome.com	DRAGEN Licensing Server

Inbound Connections

Connection	Value	Purpose
Port	80	Off-instrument control software (certificate)
Port	443	Off-instrument control software (UI)

Consumables & Equipment

This section lists all components included in the reagent kit with storage conditions. This section also details the ancillary consumables and equipment that you must purchase to complete the protocol and perform maintenance and troubleshooting procedures.

Sequencing Consumables

The NovaSeq X Series 10B Reagent Kit is available in three configurations (100 cycle, 200 cycle, 300 cycle), and the catalog # contains the following components. Each component uses radio-frequency identification (RFID) for accurate consumable tracking and compatibility. The NovaSeq X Series 10B Reagent Kit is single-use only and required for sequencing.

- Reagent cartridge
- Buffer cartridge
- Flow cell
- Library tube strip
- Lyo insert
- Pre-load buffer
- Custom primers buffer

When you receive your kit, visually inspect each component and promptly store components at the indicated temperature to ensure proper performance.

All kit components are shipped at room temperature.

Item	Storage Temperature	Dimensions
Reagent cartridge	-25°C to -15°C	9.8 cm x 23.7 cm x 31.2 cm (3.9 in x 9.4 in x 31.1 in)
Lyo insert[1]	-25°C to -15°C	5.1 cm x 14.3 cm x 19.1 cm (2.0 in x 5.6 in x 7.5 in)
Custom primer buffer[2]	-25°C to -15°C	12.6 cm x 3.8 cm x 4.9 cm (5.0 in x 1.5 in x 1.9 in)
Pre-load buffer[2]	-25°C to -15°C	10.1 cm x 3.8 cm x 3.8 cm (4.0 in x 1.5 in x 1.5 in)
Flow cell	2°C to 8°C	20.0 cm x 2.54 cm x 15.1 cm (7.9 in x 1.0 in x 5.9 in)

Item	Storage Temperature	Dimensions
Buffer cartridge	Room temperature	14.0 cm x 8.3 cm x 31.3 cm (5.5 in x 3.3 in x 12.3 in)
Library tube strip	Room temperature	4.9 cm x 3.8 cm x 9.5 cm (1.9 in x 1.50 in x 3.8 in)

[1]Avoid stacking lyo inserts when storing.
[2]Store the pre-load and custom primer buffer vertically and in the packaging to prevent leaks.

Avoid dropping cartridges. Injury could occur if dropped. Skin irritation could occur if reagents leak from cartridges. Inspect cartridges for cracks before use.

Consumables Details

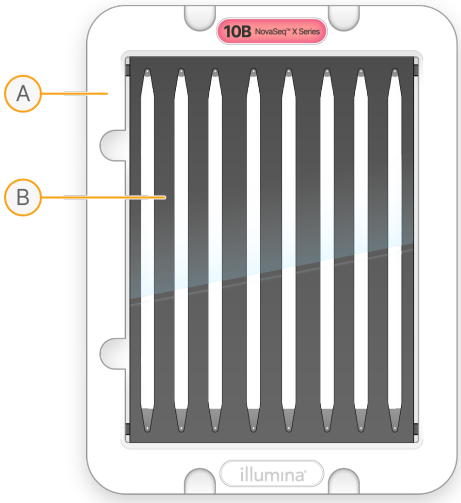
This section includes additional information on the supplied consumables and the library tube strip adapter.

Flow Cell

The 10B Flow Cell is a patterned flow cell encased in a plastic cartridge. The flow cell is a glass-based substrate containing billions of nanowells in an ordered arrangement, which increases the number of output reads and sequencing data. Clusters are generated in the nanowells from which sequencing is then performed.

The 10B Flow Cell has 8 lanes for sequencing pooled libraries. Each lane is imaged in multiple swaths. The software then divides the image of each swath into smaller portions called tiles. For more information, refer to *Real-Time Analysis* on page 79.

Flow Cell



A. Flow cell cartridge

B. 8-lane flow cell (10B)

The underside of the 10B Flow Cell contains one inlet gasket and eight outlet gaskets. Libraries and reagents enter the flow cell lanes through the gasket on the inlet end of the flow cell. Reagents are expelled from the lanes through the gaskets at the outlet end.

Avoid touching the gaskets when handling the flow cell.

Reagent Cartridge

The sequencing reagent cartridge is prefilled with reagents, buffers, and wash solution.

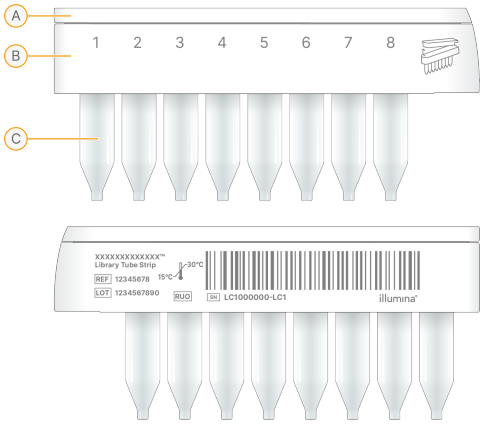
The cartridge contains all reagents for a run. The library tube strip and lyo insert are loaded into the thawed cartridge, which is then loaded onto the instrument. After the run begins, reagents and library are automatically transferred from the cartridge to the flow cell. When transporting, carry only one cartridge at a time and grip the cartridge by the sides.

Figure 4 Reagent Cartridge

Library Tube Strip

The library tube strip contains one sample tube for each flow cell lane. Each sample tube is numbered. During run planning, enter the sample tube number as the lane number.

Figure 5 Library Tube Strip

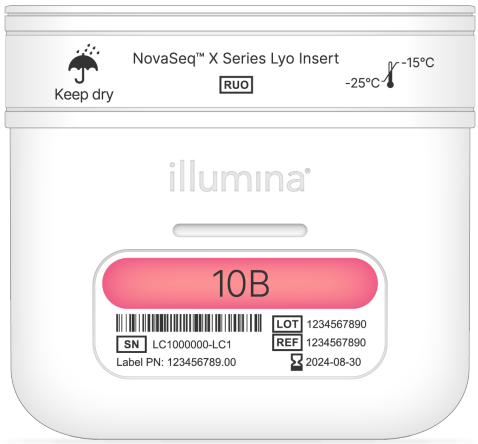


A. Library tube strip cap
B. Flow cell lane number
C. Sample tube

Lyo Insert

The lyo insert is prefilled with SBS and prepared ExAmp reagents. During sequencing, the instrument automatically rehydrates the ExAmp reagent, mixes the reagents with the library tube, and then transfers the mixture to the flow cell.

Figure 6 Lyo insert



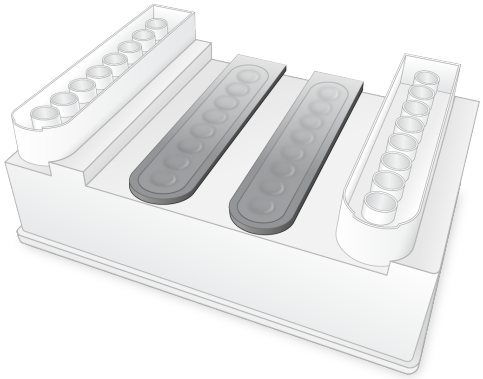
Buffer Cartridge

The buffer cartridge is prefilled with sequencing buffers and weighs up to 2.5 kg (5.5 lb). Indentations on the bottom allow buffer cartridges to be stacked. The buffer cartridge is loaded directly onto the instrument.

Figure 7 Buffer cartridge

Library Tube Strip Adapter

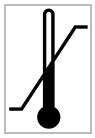
The NovaSeq X Plus includes an adapter to help with transporting, centrifuging, and storing the library tube strip. The adapter includes locations to insert the library tube strip sample tubes and cap. If sequencing on only one instrument side, make sure to balance the adapter before centrifuging by inserting an unused library tube strip.



Symbol Descriptions

The following table describes the symbols on the consumable or consumable packaging.

Symbol	Description
	The date the consumable expires. For best results, use the consumable before this date.
	Indicates the manufacturer (Illumina).
	Indicates the part number so that the consumable can be identified.
	Indicates the batch code to identify the batch or lot that the consumable was manufactured in.
	Indicates a health hazard.

Symbol	Description
	Storage temperature range in degrees Celsius. Store the consumable within the indicated range.

REF identifies the individual component, while LOT identifies the lot or batch the component belongs to.

User-Supplied Consumables & Equipment

The following section provides information on the required user-supplied consumables and equipment.

Consumables

Consumable	Supplier	Purpose
1 N NaOH	General lab supplier	Diluting to 0.2 N for denaturing libraries.
Air filter	Illumina, catalog # 20073109	Replacing air filter. NovaSeq X Plus ships with one air filter and four spares.
Disposable gloves, powder-free	General lab supplier	General purpose.
EZwaste HD 20 L, HDPE, 83 mm Cap, 4x 1/16", 3x 1/4" OD Tube Fittings and Filter	VWR, catalog #76018-560, or equivalent general lab supplier	Collecting bulk used reagent buffer waste.
Contec Polynit Heatseal wipes	VWR, catalog #68310-176 or equivalent general lab supplier	Cleaning and drying the flow cell and the flow cell stage.
Microcentrifuge tube, 1.5 ml	VWR, catalog # 20170-038, or equivalent	Combining volumes when diluting NaOH and library.
Reagent grade NaOCl, 5%	Sigma-Aldrich, catalog # 239305	Performing a maintenance wash.
Resuspension Buffer	General lab supplier	Diluting libraries to the required loading concentration.
Pipette tips, 20 µl	General lab supplier	Pipetting for diluting and loading libraries.

Consumable	Supplier	Purpose
Pipette tips, 200 µl	General lab supplier	Pipetting for diluting and loading libraries.
Pipette tips, 1000 µl	General lab supplier	Pipetting for diluting and loading libraries.
Reagent or spectrophotometric-grade isopropyl alcohol (70%), 100 ml bottle	General lab supplier	Cleaning the flow cell stage.
Water, laboratory-grade	General lab supplier	Diluting NaOH for denaturing libraries.
[Optional] PhiX Control v3	Illumina, catalog # FC-110-3001	Spiking in PhiX control.

Equipment

Item	Source
Freezer, -25°C to -15°C	General lab supplier
Graduated cylinder, 500 ml, sterile	General lab supplier
Ice bucket	General lab supplier
Pipette, 20 µl	General lab supplier
Pipette, 200 µl	General lab supplier
Pipette, 1000 µl	General lab supplier
Refrigerator, 2°C to 8°C	General lab supplier
Tub, water baths*	General lab supplier

* Use a tub that can accommodate the reagent cartridges and the appropriate water level.

Guidelines for Laboratory-Grade Water

Always use laboratory-grade water or deionized water to perform instrument procedures. Never use tap water. Use only the following grades of water or equivalents:

- Deionized water
- Illumina PW1
- 18 Megohms (MΩ) water
- Milli-Q water
- Super-Q water
- Molecular biology grade water

System Configuration

This section provides instructions for setting up and configuring your system. You can edit system settings on-instrument or on a networked computer.

For information on instrument control computer, Linux user accounts, networking, or security settings, refer to the Illumina Instrument Control Computer Security and Networking documentation.

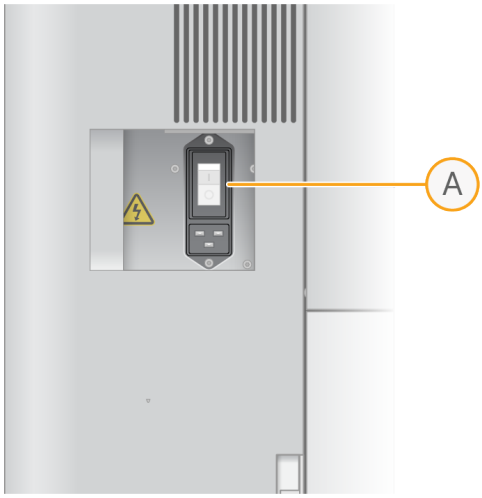
Start the Instrument

The first time the system is turned on, the NovaSeq X Plus operating system must initialize the NovaSeq X Series Control Software before launching the control software.

To turn on the instrument, do as follows.

1. Press the turn on (|) side of the toggle switch on the back of the instrument.

Figure 8 Power Toggle Switch Location



2. Press the power button on the right side of the instrument.

Figure 9 Power Button Location

3. Wait until the operating system finishes initialization (~35 minutes).
4. Enter the administrator user name and password provided by your Illumina representative at the time of installation.

User Accounts

The NovaSeq X Series Control Software has the following user groups:

- **Sequencer Operators**—Allows users to perform sequencing and access all sequencing features. To access the control software on-instrument, the user must be part of the sequencing operator group. Users are automatically added to the sequencer operator group.
- **Administrator**—Allows users to access all administrator functions in settings. You can assign the administrator group when adding a user.

User Permissions

The following permissions are available for each user group. To sign into the control software on-instrument, a user needs to be included in the Sequencer Operator group. Administrator permissions do not automatically include sequencer operator permissions. However, both groups can be selected when adding a user. For more information, refer to *Add Users* on page 41.

Permissions	Sequencer Operators	Administrators
Add custom index adapter kits and custom library prep kits		X
Manage resources		X
Manage sequencing runs	X	X

Permissions	Sequencer Operators	Administrators
Manage users, password policies, applications, external storage, and DRAGEN software		X
Minimize and exit control software	X	
Perform maintenance wash	X	
Perform sequencing	X	
Perform software updates and system checks		X
Plan sequencing runs	X	X
Replace air filter	X	X
Set cloud connectivity	X	X
Shut down instrument	X	
Unlock instrument doors	X	
View control software About screen	X	X
View audit log		X
View resources and applications	X	X

Add Users

You can add new users using the NovaSeq X Series Control Software. Only administrators can add users.

Cloud users are automatically created when they first sign into the instrument using their BaseSpace Sequence Hub credentials. After a cloud user is created, their user group is configured manually.

Add a User

To add a new user, do as follows.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Users**.
3. Select **Add user**.
4. Enter the following information:
   - User name
   - First name
   - Last name
5. Select the **User enabled** checkbox to set the user status as Active.

6. Enter a password.
   The user changes the password when they first sign in. Refer to *Password Requirements* on page 42 for password recommendations.
7. To add a user as an administrator, select the Administrators checkbox.
   To make sure that the administrator can access all permissions, leave the Sequencing Operators checkbox selected. Refer to *User Permissions* on page 40 for more information on group permissions.
8. Select **Save** when finished.

Password Requirements

When creating a user, use the following password recommendations.

Policy	Security Setting
Password length	8–64 characters
Minimum password character requirements	• 1 upper-case character • 1 lower-case character • 1 numerical character • 1 special character
Password history	Cannot match any of the previous 5 passwords

Manage Users

Administrators can manage users using the NovaSeq X Series Control Software. For more information on adding a user, refer to *User Accounts* on page 40.

Edit a User

To edit user information and user group, do as follows. You cannot edit the user name.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Users**.
3. Select the user to edit.
4. Edit user settings, and then select **Save**.

Remove Users

To remove users, do as follows.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Users**.
3. Select **Remove** for the user you would like to remove.

4. In the dialog box, select **Yes, remove**.
5. Repeat steps 3 and 4 for each user that you would like to remove.

Update Passwords

Administrators can reset passwords and update password settings.

Reset a Password

Administrators can reset passwords at any time. You can only reset your own password when you receive the password expiration notification. When an administrator resets a password, the user can sign in using the generated temporary password.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Users**.
3. Select the user to edit.
4. Select **Reset password**. Refer to *User Accounts* on page 40 for information on password restrictions.
   The user enters a new password the next time they sign in.
5. Select **Save** when finished.

Edit Password Settings

Administrators can edit how frequently passwords expire, the number of allowed sign-in attempts, and the time until automatic sign-out. When a password has expired, users receive a prompt to set a new password during sign in.

Password settings use the following defaults.

- Password expiration: 90 days
- Invalid sign-in attempts: Five attempts
- Automatic sign-out time: 30 minutes

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Password policy**.
3. Edit the password settings as desired.
   You can set the automatic sign-out time to a maximum of 60 minutes.
4. Select **Save**.

Configure Cloud Settings and Proactive Support

Use the following instructions to configure Proactive Support and BaseSpace Sequence Hub or ICA on your system. For more information on BaseSpace Sequence Hub, refer to the BaseSpace Sequence Hub support site page. For more information on ICA, refer to the Illumina Connected Analytics support site page.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Cloud settings**.
3. To enable a cloud connection, select the location of your BaseSpace Sequence Hub or ICA domain under the Hosting location drop-down.
4. If using BaseSpace Sequence Hub Enterprise or ICA, configure the following cloud option:
   - **Private domain name**—Enter your BaseSpace Sequence Hub or ICA domain name. Not required for BaseSpace Sequence Hub Professional or Basic accounts.
5. Select **Test configuration** to check your cloud connection.
   Make sure you have added the required endpoints to the allow list for your firewall. For a list of endpoints, refer to the *Illumina Control Computer Security and Networking*.
6. Select the following run settings. The selected run settings act as a default, but you can change the settings during run setup.
   - **Cloud run monitoring**—Select to enable remote run monitoring. Proactive support is automatically included. Run monitoring is only visible in BaseSpace Sequence Hub.
   - **Cloud run storage**—Store run data in the cloud and automatically launch analysis. Proactive support and run monitoring are automatically included.
7. To enable Proactive support only, select **Send instrument performance data to Illumina**.
8. Select **Save** to finish.

Set a Proxy Server

Only administrators can set a proxy server. When setting a proxy server, you can also view your IP configuration details.

1. If open, minimize or exit the NovaSeq X Series Control Software. Refer to *Minimize or Exit the Control Software* on page 13 for instructions.
2. Select the Instrument Network Configuration icon from the sidebar.
3. Sign in using your administrator credentials.
4. Select the Proxy Configuration tab, and then select **Enable proxy**.
5. Select **Enable proxy**, and then enter the server and port address.
6. **[Optional]** If the proxy server requires authentication, select the **Requires user name and password** checkbox, and then enter the user name and password.
7. Select **Update** to save and validate the proxy information.
8. To close the Instrument Network Configuration application, select the instrument icon, and then select **Minimize** or **Close**.

Update IP Configuration

If your IP information has changed, use the following instructions to update the IP configuration with your new information.

1. If open, minimize or exit the NovaSeq X Series Control Software. Refer to *Minimize or Exit the Control Software* on page 13 for instructions.
2. Select the Instrument Network Configuration icon from the sidebar.
3. Sign in using your administrator credentials.
4. Select the IP Configuration tab, and then enter a host name.
5. Select a network interface from the drop-down list.
6. If a transport layer security (TLS) certificate was uploaded during the initial instrument setup, select **Choose file**, and then select your certificate file.
   Make sure to upload the same certificate that was used during the initial setup.
7. Select **Update**.
8. To close the Instrument Network Configuration application, select the instrument icon, and then select **Minimize** or **Close**.

Specify the Default Output Folder Location

Use the instructions in this section to specify a default output folder or setup external storage. You can change the output folder for each run during run setup. The software saves CBCL files and other run data to the output folder.

An output folder is required unless cloud run storage is enabled. Only use a network drive as the default output folder. Using an on-instrument output folder negatively impacts sequencing run time.

Add a Network Drive

Use the following instructions to mount a persistent network drive and specify the default output folder location. Server Message Block (SMB) and Network File System (NFS) are the only supported methods for persistent mounting of a network drive on the NovaSeq X Plus.

Add a Network Drive to External Storage

To use your network drive as the output folder, you must first add it as an available external storage option.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **External storage**.
3. Select **Add network storage**.
4. Select the network drive type.
5. Enter the following information:

- Server location
- **[Optional]** Domain
- User name
- Password

6. If using SMB drive for network storage, select a file encryption option. Using encryption is recommended.
7. Select **Test configuration** to test the network storage connect.
8. After the test completes, select **Save**.

After saving, the network storage option becomes an available output folder location. For instructions on selecting the default output folder option, refer to *Specify External Storage as the Output Folder* on page 46.

If you would like to remove the network drive later, select **Remove volume** in the Actions column of the server on the External storage screen.

Specify External Storage as the Output Folder

If you would like to use an external storage option as your default output folder, select the external storage output folder as follows.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **External storage**.
3. Select a server location.
4. If an output folder has been added, select **Edit folders**, and then **Add folder**.
5. If an output folder has not been added, select **Add folder**.
6. Select a server location from the drop-down list, and then select one of the available folders.
7. Enter a folder nickname.
8. Enter a name for the output folder.
9. Select **Save**.
10. You can remove output folders by selecting **Remove** on the Edit folders screen.

Manage DRAGEN Applications

Administrators can manage DRAGEN applications. For more information on creating a planned run, refer to *Plan a Sequencing Run* on page 53.

Install Applications

Only administrators can install DRAGEN applications.

1. Download the application (*.zip) from the NovaSeq X Series support page. Save the installer to a network drive.
2. Select the instrument icon to open the global navigation menu.
3. Select **Settings**, and then select **Applications**.
4. Select **Install application**.
5. Navigate to the application file, and then select **Open**.
6. After the application finishes uploading, select **Install**.
   After the application installs, you can review the application settings. Refer to *View Application Settings* on page 47.

View Application Settings

The DRAGEN application provides a default library prep kit, index adapter kit, Read information, Index information, and permissions.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Applications**.
3. Select the application that you would like to view.
4. View any of the following information:
   - Library prep kits
   - Index adapter kits
   - Index Reads
   - Read Type
   - Index Lengths
   - Read Length
5. Select **Save** when finished.

Uninstall Applications

Administrators can uninstall applications.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Applications**.
3. Select the application that you would like to uninstall.
4. Select **Uninstall**.
5. Confirm to uninstall the application.

Import Resource Files

You can import reference genomes or reference files. You can remove existing reference genomes or reference files to clear hard drive space.

Import Reference Genomes

You can add and delete reference genomes on the Genomes tab in the Resources settings screen. The Genomes tab displays the genome name, if they are a standard or custom genome, the species, and the genome source.

1. Make sure that there are no sequencing runs or on-instrument secondary analyses in progress.
2. Select the instrument icon to open the global navigation menu.
3. Select **Settings**, and then select **Resources**.
4. On the Genomes tab, select **Import Genome**.
5. Navigate to the reference genome (*.tar.gz), and then select **Open**.
6. Select **Import**.

Import Reference Files

You can add and delete reference files on the Reference Files tab in the Resources settings screen. The Reference Files tab displays the reference file name, file type, file description, and the number of related reference genomes.

Generate Reference Files

If performing CNV calling, you can use an optional panel of normals file. The panel of normals file is a reference based normalization algorithm that uses externally supplied matched normal samples to determine a baseline level from which to call CNV events. These matched normal samples should be derived from the same sample type, library prep, sequencing workflow that is used for the case sample. The algorithm subtracts system level biases that are not sample specific.

You can use a panel of normals file for both somatic and germline variants.

If using the DRAGEN Enrichment secondary analysis, you can use a noise baseline file to filter out sequencing or systematic noise. You can download standard custom noise files from the Illumina Support Site or create a custom noise baseline file.

Use one of the following options to generate a panel of normals or noise baseline file. It is recommended to use ~50 samples.

- Use the DRAGEN server. Refer to the *DRAGEN Online Help* for instructions.
- Use DRAGEN Baseline Builder App on Illumina DRAGEN Bio-IT Platform, which accepts FASTQ, BAM, or CRAM. The Baseline Builder App generates a CNV Baseline file (*.combined.counts.txt.gz file).

Import Reference Files

1. Make sure that there are no sequencing runs or on-instrument secondary analyses in progress.
2. Select the instrument icon to open the global navigation menu.
3. Select **Settings**, and then select **Resources**.
4. On the Reference Files tab, select **Import reference file**.
5. Navigate to the reference file, and then select **Open**.
6. **[Optional]** Enter a description for the reference file.
7. Enter one of the following reference file types:
   - AuxCnvPanelOfNormalsFile
   - AuxNoiseBaselineFile
   - BedFile
   - RnaGeneAnnotationFile
8. Select the reference genomes related to the reference file.
9. Select **Save**.

Import Custom Library Prep and Index Adapter Kits

You can import custom library prep and index adapter kits to use in your sequencing runs. You must be an administrator to import custom kits. The custom index adapter kit is referenced by the custom library prep kit and must be imported first.

Import Custom Index Adapter Kit

You can import custom index adapter kits using the available template.

1. If the NovaSeq X Series Control Software is open on-instrument, minimize the control software. Refer to *Minimize or Exit the Control Software* on page 13.
2. Using a browser application on-instrument, access the remote NovaSeq X Series Control Software. For instructions, refer to *Use the Remote NovaSeq X Series Control Software* on page 13.
3. Open the Menu drop-down in the upper-left corner, and then select **Custom kits**.
4. To download the TSV template file, select **Download template**.
   The TSV template file is only available in the remote control software.
5. Edit the following sections:
   Fields must start with an alphanumeric character and can only contain alphanumeric characters, periods, dashes, and underscores.
   - **IndexKit**—Overview information for the index adapter kit, including name, version, description, and index strategy.

- **Resources**—Allows you to provide adapter sequences for Read 1 and Read 2. Based on the values in this section, the imported file sets the index kit type as one of the following options:
  - Standard layout (non-fixed)
  - Fixed layout (single plate)
  - Fixed plate layout (multi plate)
- **Indices**—A list of indexes, including name, adapter sequence, and whether the index is for Index 1 or Index 2.

6. Remove the template instructions included in the angle brackets (< >), and then save the TSV file.
7. Select **Import index adapter kit**, navigate to the custom index adapter kit (*.tsv), and select **Open**.
8. After successfully importing the custom index adapter kit, select the name to review and edit information.

Add Custom Library Prep Kit

Use the following instructions to upload custom library prep kits.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Custom kits**.
3. Select **Add library prep kit**, and enter the following information:
   - Library prep kit name
   - **[Optional]** Description
   - **[Optional]** Organization. The company or institution that owns the custom library prep kit. The organization cannot be Illumina.
   - Allowed read types
   - Default read type
   - Default read cycle
4. From the drop-down list, select at least one compatible index adapter kit.
5. Select **Save**.
6. After successfully adding the library prep kit, select the name to review and edit information.

Custom Primers

Using custom primers for a run requires two additional steps during run setup:

- Prepare and add the appropriate volume of each custom primer to the custom primer positions of the reagent cartridge.
- Download and select the custom primer recipe during run setup.

All other steps follow the run setup workflow. Refer to *Protocol* on page 53 for sequencing protocol instructions.

Custom Primers and PhiX

When custom primers are used for Read 1 or Read 2, the software directs the instrument to pull from the CP1 and CP2 wells. Therefore, Illumina primers are not used for the sequencing run. Illumina primers refer to primers already in the reagent cartridge.

If Illumina primers are not used for Read 1 or Read 2, the optional Illumina PhiX control is ***not*** sequenced. To use the PhiX control with custom primers, contact Illumina Technical Support for guidance.

Because PhiX is not indexed, sequencing data from the PhiX control is not generated for index reads regardless of which indexing primer is used.

Prepare and Add Custom Primers

Custom primers are prepared using TT1 and then added to the reagent cartridge. Make sure that the reagent cartridge is thawed and inspected before proceeding. TT1 is provided with the Custom Primer Buffer (catalog # 20065516).

Prepare Custom Primers

1. If frozen, thaw each custom primer to be used.
2. Use TT1 to dilute custom primers to yield the following volumes at 0.3 µM final concentration. When combining primers to make the custom Index Read or any custom read primer mixture, the total concentration of the mixture should be 0.3 µM and an equal volume should be assigned to each single primer in the mixture.

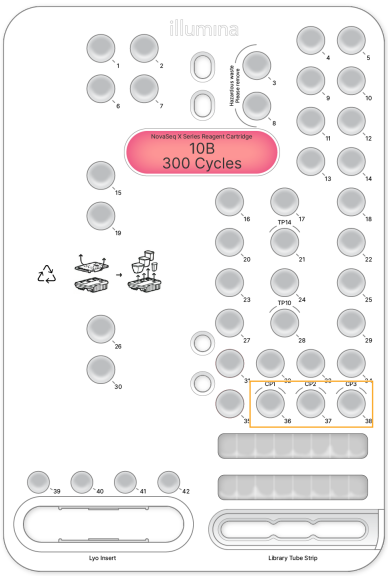
Custom Primer	Volume (ml)
Custom Read 1 primer	5.0
Custom Read 2 primer	5.0
Custom Index 1 and 2 primer mix	7.0

Add Custom Primers

1. Use a low-lint lab tissue to wipe clean the foil seal covering each custom primer position.

Position	Custom Primer
CP1	Custom Read 1 primer
CP2	Custom Read 2 primer
CP3	Custom Index 1 and 2 primer mix

Figure 10 Custom Primer Positions



2. Using a clean pipette tip, pierce the foil seal covering each custom primer position.
3. Add the following volumes of custom primer to the appropriate position on the reagent cartridge. Avoid touching the foil seal as you dispense the primer.

Position	Custom Primer	Volume (ml)
CP1	Custom Read 1 primer	5.0
CP2	Custom Read 2 primer	5.0
CP3	Custom Index 1 and 2 primer mix	7.0

Set Up a Run for Custom Primers

Using custom primers is specified during run setup. After selecting your run, you can add a custom recipe file to your run.

1. Download the custom recipe XML file from the NovaSeq X Series page on the Illumina support site.
2. In the Review run screen, select **Select file...** under Custom recipe.
3. Select the custom recipe file.
4. After you finish reviewing your run information, select **Load consumables** to proceed.

Protocol

This section provides step by step instructions on how to prepare consumables, dilute libraries, and set up a sequencing run.

When handling reagents and other chemicals, wear safety glasses, a lab coat, and powder-free gloves.

Make sure that you have the required consumables and equipment before starting a protocol. Refer to *Consumables & Equipment* on page 31.

Follow the protocols in the order shown, using the specified volumes, temperatures, and durations.

Plan a Sequencing Run

You can use one of the following options to plan a sequencing run for the NovaSeq X Plus. After setting up a run, your planned run is displayed on the Planned tab in the Runs screen and available for selection when initiating a sequencing run.

- To plan your run in the cloud, you can use the Run Planning tool in BaseSpace Sequence Hub. Before planning a run, make sure you have configured your cloud settings. Refer to *Configure Cloud Settings and Proactive Support* on page 43 for more information.
- To plan your run locally, you can use the NovaSeq X Series Control Software available on-instrument or via a networked computer.

Plan a Cloud Sequencing Run

You can use the Run Planning option in BaseSpace Sequence Hub to set up a sequencing run. After saving, the run is available on your instrument for sequencing. If your instrument is not connected to the cloud, you can use Run Planning to generate a sample sheet v2 file to upload for a new sequencing run.

Refer to Illumina Cloud Run Setup for instructions.

Plan a Local Sequencing Run

You can create a run locally on-instrument or using a networked computer. After sequencing, on-instrument analysis starts automatically. CBCL data and DRAGEN secondary analysis output files are stored in the selected output folder.

If using a networked computer, your computer must be connected to the same local network as your sequencing system and have a root certificate installed. To set up a run and perform sequencing without a network connection, refer to *Start a Manual Run* on page 67. For more information on how to access and use the NovaSeq X Series Control Software, refer to *Use the Remote NovaSeq X Series Control Software* on page 13.

Create a New Run

To create a new sequencing run locally, do as follows.

1. If creating a run on-instrument, select the instrument icon to open the global navigation menu.
2. If creating a run on a networked computer, open the Menu drop-down in the upper-left corner.
3. Select **Runs**, and then select the **Planned** tab.
4. If you are using the run planning interface to create a new run, select **Create run**.
5. If you are using a sample sheet v2 file to create a new run, select **Import run**, and then open your sample sheet v2 file. Use one of the following options to generate a sample sheet.
   - Navigate to the remote control software. Select the run name, and then select **Sample Sheet**. Select **Save as** to save the sample sheet.
   - Plan a sequencing run in Illumina Cloud Run Planning and use local storage. Export the sample sheet after you finish planning the run.
   - Use a sample sheet located in the output folder of a completed sequencing run.
6. In the Run name field, enter a name of your preference to identify the current run.
   The run name can contain a maximum of 255 alphanumeric characters, spaces, dashes, and underscores.
7. **[Optional]** Enter a description for the run.
   The run description cannot contain asterisks (*), brackets ([ ]), or commas (,).
8. Select the FASTQ file compression format.
9. Enter the number of cycles performed in each read:
   - **Read 1**—Enter up to 151 cycles.
   - **Index 1**—Enter the length of the index read for Index 1. For a PhiX-only run, enter 0 in both index fields.
   - **Index 2**—Enter the length of the index read for Index 2.
   - **Read 2**—Enter up to 151 cycles. This value is typically the same as the Read 1 value.
10. **[Optional]** Enter your library tube ID.
    The library tube ID is on the label of your library tube strip.
11. Select **Next**.
12. Select your analysis application.
13. **[Optional]** Enter a description for the configuration.
14. Select your library prep and index adapter kits.
    For information on adding custom library prep and index adapter kits, refer to *Import Custom Library Prep and Index Adapter Kits* on page 49.
15. Select **Next** to configure secondary analysis and add your sample information.
    For more information, refer to *Set up Secondary Analysis* on page 54.

Set up Secondary Analysis

NovaSeq X Series systems allow you to perform multiple DRAGEN analyzes in a single sequencing run. Before setting up secondary analysis, make sure you have installed the appropriate DRAGEN application on your instrument. For more information on installing DRAGEN applications, refer to *Install Applications on page 46*the NovaSeq X Series Product Documentation.

If storing data in the cloud, you can create up to seven analysis application and reference genome combinations with an additional BCL Convert-only application. If storing data locally, you You can create up to three analysis application and reference genome combinations with an additional BCL Convert-only application. For each combination, you can use up to eight configurations using a different library prep kit, index adapter kit, or configuration settings for an analysis application and reference genome combination already used.

The following combinations are included in the seven or three configuration limit:

- The same analysis application and application version with a different reference genome
- The same reference genome with a different application or application version
- A different application or application version with a different reference genome

DRAGEN BCL Convert

Use the following steps to configure DRAGEN BCL Convert analysis.

1. **[Optional]** Enter a description for the configuration.
2. Select your library prep and index adapter kits.
3. Enter the following optional settings.

Setting	Description
Adapter Read 1	Adapter sequence for Read 1. If using an Illumina library prep kit, you cannot modify this field.
Adapter Read 2	Adapter sequence for Read 2. If using an Illumina library prep kit, you cannot modify this field.
Override Cycles	Specify UMI cycles and mask out cycles of a read. The values are automatically populated according to your library prep and index adapter kit information.

4. Use one of the following options to enter your sample information for the samples used in DRAGEN BCL Convert analysis.
   - Enter sample information in a *.csv file by selecting **Download Template**. To import the edited sample template, select **Import Samples**, and then select the CSV file.

- Paste sample IDs and either index plate well positions or i7 and i5 indexes directly from an external file. Before pasting, enter the number of sample rows in the Rows field, and then select **+**. Sample IDs can contain up to 100 alphanumeric characters, hyphens, and underscores.

  Fixed-layout index plates require entries for well position. Indexes that do not have a fixed layout require entries for i7 and i5 indexes. i5 indexes must be entered in the forward orientation.

- Manually enter sample IDs and corresponding lane, well positions or indexes, barcode mismatches, and project. If Not Specified is selected for the library prep kit, enter Index 1 (i7) and Index 2 (i5) sequences in the forward orientation.

5. Select **Next**, and then review the run details.
6. **[Optional]** Perform any of the following actions:
   - To edit the run settings or configuration settings, select **Edit** next to the run or configuration.
   - To delete a configuration, select **Delete** next to the configuration, and then select **Yes, delete**.
   - To add another analysis configuration to the run, select **Add another configuration**.
7. To save the run, select one of the following options.
   - To edit the run details later, select **Save as draft**.
   - If storing data in the cloud, select Select **Save as planned** to finalize the run details and plan for sequencing.
   - If storing data locally, select **Export** to export a sample sheet v2 file.

DRAGEN Enrichment

Use the following steps to configure DRAGEN Enrichment analysis.

1. **[Optional]** Enter a description for the configuration.
2. Select your library prep and index adapter kits.
3. Select a reference genome.
   If possible, use a reference genome with alt mask. For instructions on importing custom reference genomes, refer to the BaseSpace Sequence Hub support site page*Import Reference Genomes* on page 48.
4. Select the germline or somatic variant type.
5. Select a variant calling workflow. If you select None, only alignment is performed. If you select All, the following variant calling workflows are performed.
   - Small variant caller
   - Structural variant caller
   - Copy number variant (CNV) caller (if panel of normals file is provided)

6. Select a *.bed file containing the regions you would like to target or upload a new custom file. A *bed file is only required if performing small or all variant calling.
   Make sure that the reference genome for the BED file matches the reference genome selected. For instructions on importing reference files, refer to *Import Reference Files* on page 48 the BaseSpace Sequence Hub support site page.
7. **[Optional]** If using the somatic variant type, select a noise baseline file.
   For instructions on importing noise baseline files, refer to *Import Reference Files* on page 48 the BaseSpace Sequence Hub support site page.
8. **[Optional]** If using the CNV caller, select a panel of normal files.
   For instructions on importing panel of normal files, refer to *Import Reference Files* on page 48 the BaseSpace Sequence Hub support site page.
9. Enter the following optional settings.

Setting	Description
Adapter Read 1	Adapter sequence for Read 1. If using an Illumina library prep kit, you cannot modify this field.
Adapter Read 2	Adapter sequence for Read 2. If using an Illumina library prep kit, you cannot modify this field.
Override Cycles	Specify UMI cycles and mask out cycles of a read. The values are automatically populated according to your library prep and index adapter kit information.

10. Use one of the following options to enter your sample information for the samples used in DRAGEN Enrichment analysis.
    - Enter sample information in a *.csv file by selecting **Download Template**. To import the edited sample template, select **Import Samples**, and then select the CSV file.
    - Paste sample IDs and either index plate well positions or i7 and i5 indexes directly from an external file. Before pasting, enter the number of sample rows in the Rows field, and then select **+**. Sample IDs can contain up to 100 alphanumeric characters, hyphens, and underscores.

      Fixed-layout index plates require entries for well position. Indexes that do not have a fixed layout require entries for i7 and i5 indexes. i5 indexes must be entered in the forward orientation.

    - Manually enter sample IDs and corresponding lane, well positions or indexes, barcode mismatches, and project. If Not Specified is selected for the library prep kit, enter Index 1 (i7) and Index 2 (i5) sequences in the forward orientation.

11. Select a map/align output format.
    The Analysis Settings section uses the settings in the first Enrichment configuration created for the sequencing run. To modify the settings, edit the first Enrichment configuration.
12. If storing data locally, selectSelect whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.
13. **[Optional]** Perform any of the following actions:
    - To edit the run settings or configuration settings, select **Edit** next to the run or configuration.
    - To delete a configuration, select **Delete** next to the configuration, and then select **Yes, delete**.
    - To add another analysis configuration to the run, select **Add another configuration**.
14. To save the run, select one of the following options.
    - To edit the run details later, select **Save as draft**.
    - If storing data in the cloud, select Select **Save as planned** to finalize the run details and plan for sequencing.
    - If storing data locally, select **Export** to export a sample sheet v2 file.

DRAGEN RNA

Use the following steps to configure DRAGEN RNA analysis.

1. **[Optional]** Enter a description for the configuration.
2. Select your library prep and index adapter kits.
3. Select a reference genome.
   If possible, use a reference genome with alt mask. For instructions on importing custom reference genomes, refer to the BaseSpace Sequence Hub support site page*Import Reference Genomes* on page 48.
4. **[Optional]** Select an RNA annotation file.
   For information on adding a new RNA annotation file, refer to *Import Reference Files* on page 48 the BaseSpace Sequence Hub support site page.
5. **[Optional]** Select **Yes** to enable down-sampling.
6. If down-sampling, select the number of reads to down-sample.
7. Enter the following optional settings.

Setting	Description
Adapter Read 1	Adapter sequence for Read 1. If using an Illumina library prep kit, you cannot modify this field.

Setting	Description
Adapter Read 2	Adapter sequence for Read 2. If using an Illumina library prep kit, you cannot modify this field.
Override Cycles	Specify UMI cycles and mask out cycles of a read. The values are automatically populated according to your library prep and index adapter kit information.

8. Use one of the following options to enter your sample information for the samples used in DRAGEN RNA analysis.
   - Enter sample information in a *.csv file by selecting **Download Template**. To import the edited sample template, select **Import Samples**, and then select the CSV file.
   - Paste sample IDs and either index plate well positions or i7 and i5 indexes directly from an external file. Before pasting, enter the number of sample rows in the Rows field, and then select **+**. Sample IDs can contain up to 100 alphanumeric characters, hyphens, and underscores.

   > Fixed-layout index plates require entries for well position. Indexes that do not have a fixed layout require entries for i7 and i5 indexes. i5 indexes must be entered in the forward orientation.

   - Manually enter sample IDs and corresponding lane, well positions or indexes, barcode mismatches, and project. If Not Specified is selected for the library prep kit, enter Index 1 (i7) and Index 2 (i5) sequences in the forward orientation.
9. Select a map/align output format.
   The Analysis Settings section uses the settings in the first RNA configuration created for the sequencing run. To modify the settings, edit the first RNA configuration.
10. If storing data locally, selectSelect whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.
11. Select the pipeline mode to perform. The pipeline mode determines the output files generated. The full pipeline output includes gene fusion detection and gene expression quantification.
12. If performing the full pipeline mode, select whether to enable differential expression.
13. If you enabled differential expression, select a control or comparison value for each sample. Manually select the information or download the import samples template, modify the analysis comparison group, and then import the edited template. Use the following guidelines when selecting analysis comparison values:
    - If the sample does not contain a control or comparison value, select NA as the value or leave the value blank.

- In each analysis group, any sample marked as control is compared with all samples marked as comparison.
- Each analysis group must have 2–15 control samples and 2–15 comparison samples.
- Analysis groups should not be reused between RNA analysis configurations with different reference genomes or RNA gene annotation files.

14. Select **Next**, and then review the run details.
15. **[Optional]** Perform any of the following actions:
    - To edit the run settings or configuration settings, select **Edit** next to the run or configuration.
    - To delete a configuration, select **Delete** next to the configuration, and then select **Yes, delete**.
    - To add another analysis configuration to the run, select **Add another configuration**.
16. To save the run, select one of the following options.
    - To edit the run details later, select **Save as draft**.
    - If storing data in the cloud, select Select **Save as planned** to finalize the run details and plan for sequencing.
    - If storing data locally, select **Export** to export a sample sheet v2 file.

DRAGEN Germline

Use the following steps to configure DRAGEN Germline analysis.

1. **[Optional]** Enter a description for the configuration.
2. Select your library prep and index adapter kits.
3. Select a reference genome.
   If possible, use a reference genome with alt mask. For instructions on importing custom reference genomes, refer to the BaseSpace Sequence Hub support site page*Import Reference Genomes* on page 48.
4. Select a variant calling workflow. If you select None, only alignment is performed. If you select All, the following variant calling workflows are performed.
   - Small variant caller
   - Structural variant caller
   - Copy number variant (CNV) caller
   - Repeat expansion detection
   - CYP2D6 caller
   - Regions of homozygosity (ROH) caller
5. Enter the following optional settings.

Setting	Description
Adapter Read 1	Adapter sequence for Read 1. If using an Illumina library prep kit, you cannot modify this field.
Adapter Read 2	Adapter sequence for Read 2. If using an Illumina library prep kit, you cannot modify this field.
Override Cycles	Specify UMI cycles and mask out cycles of a read. The values are automatically populated according to your library prep and index adapter kit information.

6. Use one of the following options to enter your sample information for the samples used in DRAGEN Germline analysis.
   - Enter sample information in a *.csv file by selecting **Download Template**. To import the edited sample template, select **Import Samples**, and then select the CSV file.
   - Paste sample IDs and either index plate well positions or i7 and i5 indexes directly from an external file. Before pasting, enter the number of sample rows in the Rows field, and then select **+**. Sample IDs can contain up to 100 alphanumeric characters, hyphens, and underscores.

     Fixed-layout index plates require entries for well position. Indexes that do not have a fixed layout require entries for i7 and i5 indexes. i5 indexes must be entered in the forward orientation.

   - Manually enter sample IDs and corresponding lane, well positions or indexes, barcode mismatches, and project. If Not Specified is selected for the library prep kit, enter Index 1 (i7) and Index 2 (i5) sequences in the forward orientation.
7. Select a map/align output format.
   The Analysis Settings section uses the settings in the first Germline configuration created for the sequencing run. To modify the settings, edit the first Germline configuration.
8. If storing data locally, selectSelect whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.
9. Select **Next**, and then review the run details.
10. **[Optional]** Perform any of the following actions:
    - To edit the run settings or configuration settings, select **Edit** next to the run or configuration.
    - To delete a configuration, select **Delete** next to the configuration, and then select **Yes, delete**.
    - To add another analysis configuration to the run, select **Add another configuration**.
11. To save the run, select one of the following options.

- To edit the run details later, select **Save as draft**.
- If storing data in the cloud, select Select **Save as planned** to finalize the run details and plan for sequencing.
- If storing data locally, select **Export** to export a sample sheet v2 file.

Thaw Consumables

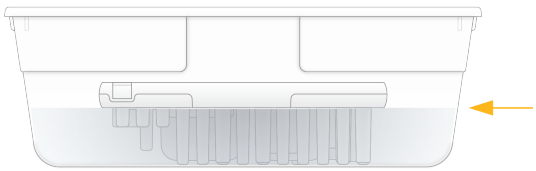
Use the following instructions to thaw consumables prior to sequencing.

Thaw Reagent Cartridge in a Controlled Water Bath

Use the following instructions to thaw the reagent cartridge in a room temperature water bath (15°C to 30°C).

Inserting the library tube strip or lyo insert into the reagent cartridge while thawing might cause reduced data quality or run failure.

1. Put on a new pair of powder-free gloves and remove the cartridge from -25°C to -15°C storage.
2. Remove the cartridge from the box, and then remove from the bag.
3. Submerge the reagent cartridge in a room temperature water bath until the water reaches the bottom of the cartridge cover.



Using hot water for thawing reagents might cause reduced data quality or run failure.

4. Thaw for 4 hours. Do not exceed 24 hours.
5. Inspect the position #12 well on the underside of the cartridge to make sure that the contents are free of ice, which indicates that the reagents are thawed.
6. Thoroughly dry the cartridge using paper towels. Dry between wells beneath the cartridge so that all water is removed.
7. Invert or gently the tap the bottom of the cartridge on the bench to remove excess water.
8. Inspect the foil seals for water.
9. If water is still present, blot dry with a lint-free tissue.
10. Gently tap the bottom of the cartridge on the bench to reduce air bubbles.

11. If reagents cannot be loaded into the instrument within 24 hours, store at 2°C to 8°C for up to 72 hours.

Thaw Reagent Cartridge in Refrigerator

Use the following instructions to thaw the reagent cartridge in a 2°C to 8°C refrigerator.

Do not insert the library tube strip or lyo insert into the reagent cartridge while thawing.

1. Put on a new pair of powder-free gloves and remove the cartridge from -25°C to -15°C storage.
2. Remove the cartridge from the box, and then remove from the bag.
3. Thaw in a 2°C to 8°C refrigerator for 48 hours.
4. If reagents cannot be loaded into the instrument within 24 hours, store at 2°C to 8°C for up to 72 hours.

Thaw Lyo Insert

1. Remove the lyo insert from -25°C to -15°C storage.
2. Thaw at room temperature for 10 minutes.
3. If lyo insert cannot be loaded within 24 hours, return to -25°C to -15°C storage.

Thaw Pre-load and Custom Primer Buffers

1. Remove pre-load and custom primer buffers insert from -25°C to -15°C storage.
2. Thaw at room temperature for 10 minutes, and then invert five times.
3. If pre-load and custom primer buffers cannot be loaded within 8 hours, return to -25°C to -15°C storage.

Thaw Flow Cell

1. Remove a new flow cell package from 2°C to 8°C storage.
2. Set the sealed flow cell package aside for 10–15 minutes to allow the flow cell to reach room temperature.
3. Leave flow cell in package until use. Use the flow cell within 2 hours of removing it from storage. If flow cell cannot be used within 2 hours, return to 2°C to 8°C storage and use within 24 hours.

Denature & Dilute Libraries

Use the following instructions to denature and dilute prepared libraries for sequencing on the NovaSeq X Plus.

Recommended Loading Concentrations

The optimal loading concentration depends on the library type and insert size. The following table provides the recommended final loading concentrations.

Library Type	Final Loading Concentration (pM)
Illumina DNA Prep with Enrichment	150
Illumina DNA PCR-Free	180
Illumina Stranded Total RNA with Ribo-Zero Plus	150
TruSeq DNA PCR-Free	90
TruSeq DNA Nano 350	160
TruSeq DNA Nano 550	160
PhiX	140

You may need to perform a titration of your library type to obtain optimal seeding concentration to yield the best %PF. When optimal loading concentration is determined, it should be applicable for identical library types.

Prepare NaOH

Prepare a fresh dilution of 0.2 N NaOH[1] to denature libraries for sequencing. Extra volume is prepared to prevent small pipetting errors from affecting the final NaOH concentration.

1. Combine the following volumes in a microcentrifuge tube.

Reagent	Volume for One Flow Cell (µl)	Volume for Two Flow Cells (µl)
Laboratory-grade water	90	180
Stock 2N NaOH	10	20

   These volumes result in 100 µl 0.2 N NaOH for one flow cell or 200 µl 0.2 N NaOH for two flow cells.
2. Vortex, and then centrifuge the tube several times to mix.

Dilute & Denature Libraries

Use the following instructions to prepare libraries for sequencing.

Reagent overage is included in the volumes.

Dilute Libraries and Add PhiX Control

1. Dilute libraries to 2 nM using RSB.

[1] sodium hydroxide

2. In a new 1.5 ml microcentrifuge tube, dilute libraries to the desired final loading concentration using RSB.
   The final volume should be 40 µl per sample well.

Final Loading Concentration (pM)	2 nM Library (µl)	RSB (µl)
90	9	31
100	10	30
110	11	29
120	12	28
130	13	27
140	14	26
150	15	25
160	16	24
170	17	23
180	18	22

3. **[Optional]** Spike in 1–2% nondenatured PhiX as follows.
   a. Dilute 10 nM PhiX to 1 nM using RSB.
   b. Add 1 µl PhiX to 40 µl nondenatured library diluted to the desired library concentration.

Denature Libraries

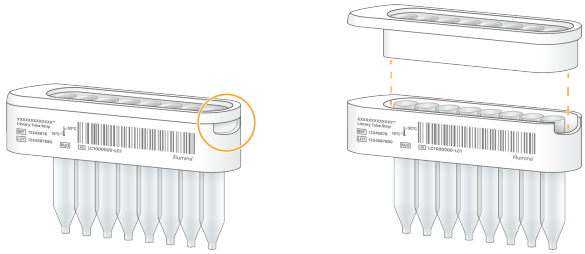
1. Add 10 µl 0.2 N NaOH to the nondenatured library tube and optional PhiX.
2. Cap and then vortex briefly.
3. Incubate at room temperature for 5 minutes to denature.
4. Add 150 µl Pre-load Buffer to neutralize.
5. Cap and then vortex briefly.
6. Centrifuge at 280 × g for up to 1 minute.
7. Store libraries on ice until transferred to the library tube strip (refer to *Load Lyo Insert and Library Tube Strip* on page 65).

Load Lyo Insert and Library Tube Strip

Before sequencing load the lyo insert and library tube strip into the reagent cartridge as follows.

1. Record the library tube ID on the library tube strip. The library tube ID is used when planning a sequencing run.
2. Uncap the library tube strip. Do not pierce the library tube strip foils.

Figure 11 Uncap Library Tube Strip



3. Dispense 160 µl denatured library or denatured library with PhiX into each sample tube.
4. Add 160 µl Pre-load Buffer to any unused sample tubes.
5. After dispensing libraries, cap the library tube strip.
   Make sure that no air gaps are present at the bottom of the tubes.
6. Centrifuge at 280 × g for 1 minute using the library tube strip adapter.
   Repeat centrifuge if libraries are not collected in the bottom of the tube.
7. Insert the library tube strip into the reagent cartridge and press down.
   An audible click indicates that the library tube strip is in place.
8. Insert the lyo insert into the reagent cartridge and press down.
   An audible click indicates that the lyo insert is in place.

Initiating a Sequencing Run

You can initiate a sequencing run by selecting a planned run or by creating a manual run. For information on planning a sequencing run, refer to *Plan a Sequencing Run* on page 53. If analyzing data in the cloud, secondary analysis begins automatically in BaseSpace Sequence Hub or ICA. If analyzing data locally, on-instrument analysis begins automatically and output files are stored in the selected output folder.

Start a Planned Run

Use the following instructions to start sequencing from a planned run. If using BaseSpace Sequence Hub or ICA, make sure you have configured your cloud settings. Refer to *Configure Cloud Settings and Proactive Support* on page 43 for more information.

1. If you are not signed in, follow the instructions provided in *Sign In and Sign Out* on page 73.
2. Navigate to the Start screen, and then select **Start**.
3. Select an instrument side(s) to perform the run on.
4. Select a run for each side from the list of planned runs.
   To create a manual run, select **Manual**. Refer to *Start a Manual Run* on page 67 for instructions.
5. Select **Review**, and then review your run information.
6. **[Optional]** Select a custom recipe file. If using custom primers, refer to *Custom Primers* on page 50 for more information.

If using the Illumina Stranded Total RNA Prep with Ribo-Zero Plus kit or the Illumina Stranded mRNA Prep kit, refer to *Dark Cycle Sequencing*.

7. After you finish reviewing your run information, select **Load consumables**.
   Refer to *Load Consumables* on page 67 for instructions.

Start a Manual Run

Use the following instructions to create a manual run.

1. If you are not signed in, enter your user name and password, and then select **Sign In**.
2. Select **Start**.
3. Select an instrument side to perform the run on.
4. At the top of the runs list, select **Manual** to switch to manual run entry.
   Planned is the default value.
5. Enter a run name.
   The run name can contain a maximum of 225 alphanumeric characters, spaces, dashes, and underscores.
6. Select single or paired-end for the read type.
7. Enter the number of cycles performed in each read:
   - **Read 1**—Enter up to 151 cycles.
   - **Index 1**—Enter the length of the index read for Index 1. For a PhiX-only run, enter 0 in both index fields.
   - **Index 2**—Enter the length of the index read for Index 2.
   - **Read 2**—Enter up to 151 cycles. This value is typically the same as the Read 1 value.
8. Select your output folder.
   You can change the default output folder in system settings. Refer to *Specify the Default Output Folder Location* on page 45 for more information.
9. **[Optional]** Select a custom recipe file.
10. **[Optional]** Select your sample sheet.

The selected sample sheet must be in v2 formatting. To create a sample sheet v2, download the generated sample sheet from Run Planning in

11. Select **Review**, and then review your run.
12. When finished, select **Load consumables**.

Load Consumables

Use the following instructions to load consumables.

If using a single run, the instrument side not selected is not editable and displays the Idle status instead.

Load the Flow Cell

Use the following instructions to load the flow cell onto the instrument.

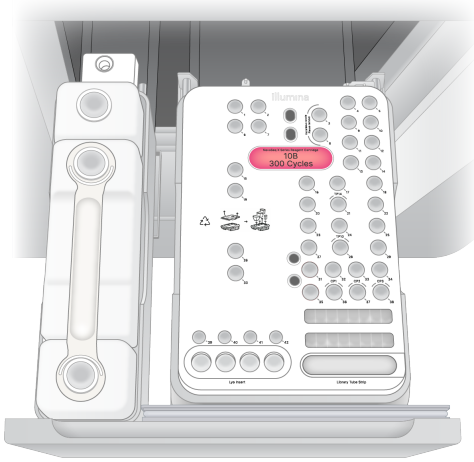
1. Make sure that the flow cell has reached room temperature before loading.
2. On the load consumables screen, select **Load flow cells**.
   After selecting, the display monitor raises and the flow cell door opens. The flow cell light indicates the instrument side sequencing is being performed on.
   Wait until the flow cell stage has fully extended before proceeding.
3. Remove and discard the used flow cell in accordance with the applicable standards for your region. The flow cell is not recyclable.
4. Inspect the flow cell stage for any contaminants (eg, particulates, lint, or dried reagent). If contaminants are visible, clean the flow cell stage as follows.
   a. Wet a polynit heatseal wipe with isopropyl alcohol (70%).
   b. Gently clean the applicable surface. Wipe in a lengthwise direction only.
      Unless you find contaminants on the manifolds, avoid touching them when wiping the flow cell stage.
   c. Repeat steps a and b until surfaces are clear of all contaminants.
   d. Dry with a new polynit heatseal wipe or an unused side of the used wipe to avoid contamination.
5. Put on a new pair of powder-free gloves to avoid contaminating the glass surface of the flow cell.
6. With the flow cell foil package over a flat surface, peel open the foil from the corner tab.
7. Remove the flow cell from the package. Grasp the flow cell by the sides to avoid touching the glass or the underside gaskets.
8. Inspect the flow cell for any contaminants (eg, particulates, lint, or dried reagent). If contaminants are visible, clean the flow cell as follows.
   a. Wet a polynit heatseal wipe with isopropyl alcohol (70%).
   b. Gently clean the applicable surface. Wipe in a lengthwise direction only.
   c. Repeat steps a and b until surfaces are clear of all contaminants.
   d. Dry with a new polynit heatseal wipe or an unused side of the used wipe to avoid contamination.
9. Discard the package appropriately.
10. Place the flow cell in the flow cell stage so that the top surface faces upward.
11. After flow cells are loaded, select **Close flow cell door**.
12. After the flow cell is confirmed, you can load the reagent and buffer cartridges. Refer to *Load Reagent and Buffer Cartridges* on page 69.
13. After all consumables are loaded, select **Verify**.
14. Review run details, and then select **Start Run**.

Load Reagent and Buffer Cartridges

Use the following instructions to load the reagent and buffer cartridges onto the instrument.

1. Make sure that the reagent cartridge was previously thawed and the lyo insert and library tube strip are inserted into the cartridge prior to loading.
2. On the load consumables screen, select **Load reagents and buffers**.
   The instrument doors unlock automatically and the system displays information on loading the reagent and buffer cartridges.
3. Remove the used reagent and buffer cartridges. Refer to *Recycle Used Consumables* on page 73 for instructions on recycling the reagent and buffer cartridges.
4. Inspect the buffer cartridge foils for any leaks.
5. Load the cartridges as follows.
   - Place the buffer cartridge in the left position.
   - Place the reagent cartridge in the right position so that the Illumina label faces you.

   Figure 12 Loaded Consumables



6. Empty the used reagent bottles. Refer to *Empty Used Reagent Bottles* on page 70 for more information.
7. Select **Confirm** after you have finished emptying the used reagent bottles.
8. Close the reagents and used reagents drawers, and then close the instrument doors.
9. After all consumables are loaded, select **Verify**.
10. Review run details, and then select **Start Run**.
    After starting a run, the instrument doors automatically lock.

Empty Used Reagent Bottles

Use the following instructions to empty the used reagent bottles before every sequencing run. For information on recycling the used reagent cartridge and buffer bottles, refer to *Recycle Used Consumables* on page 73.

> **This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations.** For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

Empty Used Small Bottle

1. Remove the small used reagent bottle from the back of the used reagents drawer. Grasp the bottle by the sides.
2. Remove the threaded cap from the cap holder behind the used reagent bottles.
3. Seal the bottle opening with the cap to prevent spills.
4. Keeping the contents separate from the contents of the large bottle, discard in accordance with the applicable standards for your region.
5. Return the uncapped bottle to the used reagents drawer. Store the cap on the cap holder.

Empty Used Large Bottle

1. Using the top handle, remove the large used reagent bottle from the front of the used reagents drawer.
2. Remove the threaded cap from the cap holder behind the used reagent waste bottles.
3. Seal the bottle opening with the cap to prevent spills.
4. Uncap the vent.
   Uncapping the vent helps minimize spillage along the sides of the bottle.
5. Discard the contents in accordance with the applicable standards for your region. Grip both handles when emptying.
6. Seal the vent with the cap after the bottle is empty.
7. Return the uncapped bottle with the vent capped to the waste drawer. Store the threaded cap on the cap holder.

8. Put on a new pair of powder-free gloves.

Pre-Run Checks

Pre-run checks include software system checks, instrument checks, alignment checks, and fluidics checks.

1. Wait about 35 minutes for pre-run checks to complete.
   After pre-run checks are completed, the run starts automatically.
2. To stop pre-run checks, select **Cancel**, and then **Yes, cancel checks** to confirm.

Consumables cannot be reused after the fluidics check begins.

3. If an error occurs, select **Retry** to redo the check.
4. If a fluidics error occurs, select one of the following. You cannot retry fluidics checks.
   - **Return to start**—Return to the Start screen.
   - **Back to consumables**—Return to the Load Consumables screen.

Monitor Run Progress

You can monitor run progress, cancel a run, or start a new run on the Sequencing screen. You can monitor run progress on-instrument or using a networked computer. If you have cloud run monitoring enabled, you can view run progress in BaseSpace Sequence Hub. To view additional run details and run status, refer to *Run Management* on page 14.

To view additional metrics and visualizations, you can use the Sequencing Analysis Viewer (SAV). For more information on SAV, refer to the Real-Time Analysis support site page.

If the run is canceled before post-run wash completes, perform a maintenance wash before beginning a new sequencing run. Refer to *Perform a Maintenance Wash* on page 116 for instructions.

1. Monitor run status on the Sequencing screen or the Active tab in the Runs screen.
   The Sequencing screen contains the estimated run completion time, which requires 10 previous runs to calculate accurate run completion time.
   The Runs screen contains additional information on the run status. The following run status information, including the time the process was started and finished, is available on the Active tab:
   - Analysis data transfer
   - Sequencing data transfer
   - External file transfer
   - Queued for analysis
   - Secondary analysis
2. Monitor the following metrics on the Sequencing or Runs screen.
   - **% ≥ Q30**—The average percentage of base calls with a Q-score ≥ 30.
   - **Yield**—The expected number of bases called for the run.
   - **Total reads PF**—The number of paired-ends (if applicable) reads passing filtering (in millions).
3. To review any additional run details, select the run name on the Sequencing screen or the Active tab in the Runs screen.
4. After the run completes, you can view additional run results by selecting the run name on the Sequencing screen or the Completed tab in the Runs screen.
5. Leave the consumables in the instrument. Do not remove until prompted during your next run setup.

Sign In and Sign Out

You are automatically signed out of the control software after 30 minutes of inactivity or the set sign-out time. Use the following instructions to sign in and manually sign out.

Sign In

After being signed out, click anywhere to sign in. Depending on your instrument configuration, your sign-in credentials can vary.

At the system level, only one user can be signed in at a time, but they can choose one user for each side to select a run.

If you are connected to the cloud, sign in with your BaseSpace Sequence Hub user name and password, and then select your workgroup. You can only select planned runs created by users in the selected workgroup. Alternatively, you can select **Sign in to local instrument** and sign in using your local account.

If you are not connected to the cloud, sign in with your local account user name and password.

If you are signing in for the first time, enter the temporary password created by your admin. You can change your password upon successful sign in.

Sign Out

Use the following instructions to sign out before automatic sign out occurs.

You can adjust the default sign-out time in the Password policy screen in Settings. Refer to *Edit Password Settings* on page 43 for instructions.

1. Select the instrument icon to open the global navigation menu.
2. Select **Profile** and then select **Sign out**.
3. After signing out, the control software closes the Profile menu and returns to the Sequencing screen.

Recycle Used Consumables

Use the following instructions to recycle the pre-load buffer, custom primer buffer, reagent cartridge, library tube strip, lyo insert, and buffer cartridge. The flow cell is not recyclable.

Recycle Buffers

Use the following instructions to recycle the pre-load buffer, custom primer buffer, and buffer cartridge.

Recycle the Pre-load and Custom Primer Buffer

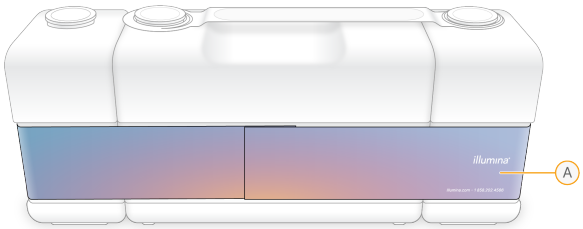
The pre-load and custom primer buffer tubes are composed of polypropylene plastic (PP). The pre-load and custom primer buffer caps are composed of high density polyethylene plastic (HDPE).

1. Rinse the pre-load and custom primer buffer tubes.
2. Recycle the tubes and caps in accordance with the applicable standards for your region.

Recycle the Buffer Cartridge

The buffer cartridge bottles and handle are composed of high density polyethylene plastic (HDPE). Use the following instructions to recycle the buffer cartridge.

1. Remove the buffer cartridge from the instrument.
2. Remove the foils, and then discard.
3. Empty the buffer cartridge in accordance with the applicable standards for your region.
4. Remove RFID label and RFID, and then discard.
5. Place the buffer cartridge on a flat surface, and then remove the retention band (A) to separate the three buffer bottles.



6. Rinse the buffer bottles, and then recycle in accordance with the applicable standards for your region.

Recycle Reagent Cartridge

After removing the reagent cartridge from the instrument, perform the following steps to recycle the library tube strip, lyo insert, and reagent cartridge.

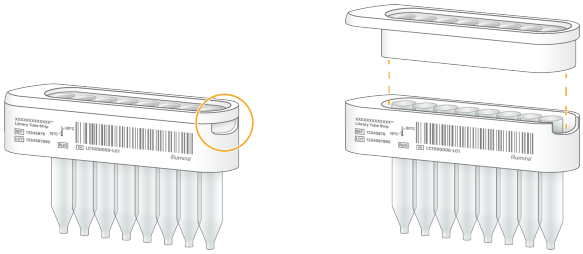
> **This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations.** For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

Recycle the Library Tube Strip and Adapter

The library tube strip is composed of polypropylene plastic (PP). The library tube strip adapter is composed of high density polyethylene plastic (HDPE).

1. Separate the library tube strip from the reagent cartridge by pushing the library tube strip upward.
2. Remove the library tube strip cap, and then discard.

Figure 13 Uncap Library Tube Strip

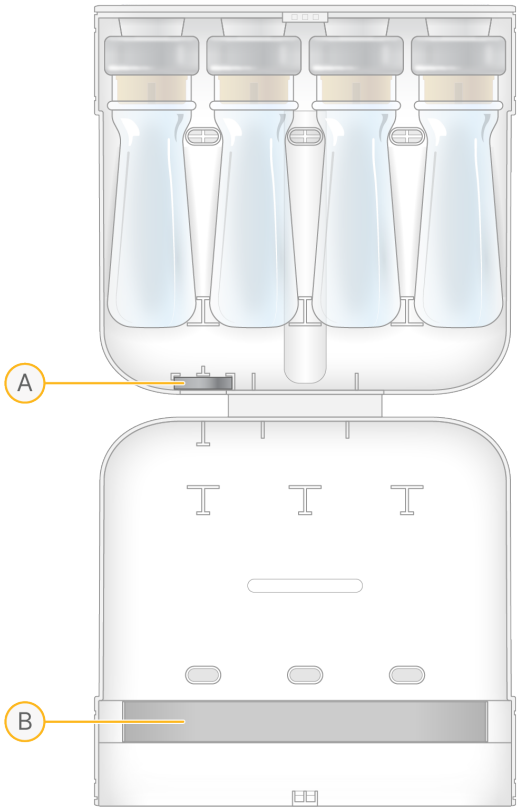


3. Remove the RFID label and RFID located underneath the library tube strip, and then discard.
4. Rinse the library tube strip, and then recycle in accordance with the applicable standards for your region.
5. Recycle the library tube strip adapter in accordance with the applicable standards for your region.

Recycle the Lyo Insert

The lyo insert cover is composed of polypropylene plastic (PP). The lyophilized reagent vials are not recyclable.

1. Separate the lyo insert from the reagent cartridge by pressing on the lyo insert label, and then pushing upward.
2. Remove the label at the top of the lyo insert, and then discard.
3. To open the lyo insert cover, compress the sides of the lyo insert.
4. Remove and dispose of the lyophilized reagent vials in accordance with the applicable standards for your region.
5. Remove and dispose of the RFID and foam strip.



A. RFID
B. Foam strip

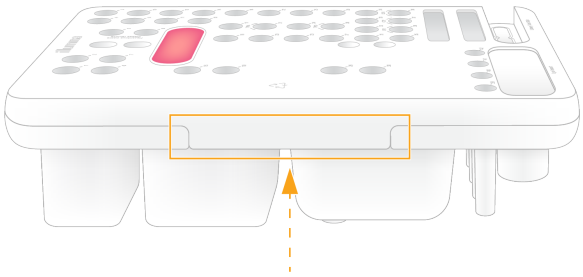
6. Recycle the lyo insert cover in accordance with the applicable standards for your region.

Recycle the Reagent Components

You can separate the position #3, #8, and the SBS wells from the reagent cartridge prior to recycling. The reagent cartridge cover, disassembled reagent cartridge, and position #3, #8, and SBS #3 wells are composed of polypropylene plastic (PP). SBS #1 and #2 wells are composed of polyethylene plastic (PET).

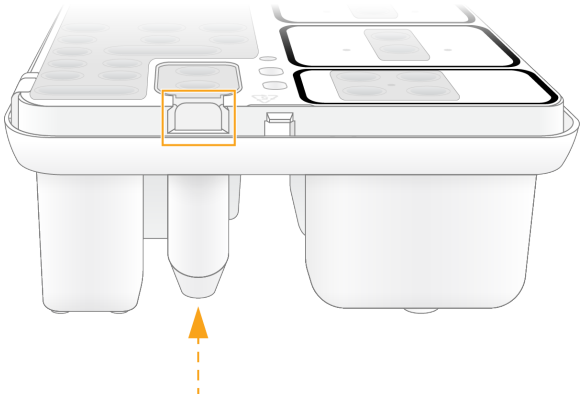
1. Remove the reagent cartridge cover by pulling the tabs on the sides of cover outward, and then lifting upward.
   An audible click indicates that the reagent cartridge cover is disconnected.

Figure 14 Reagent Cartridge Cover Tab Positions



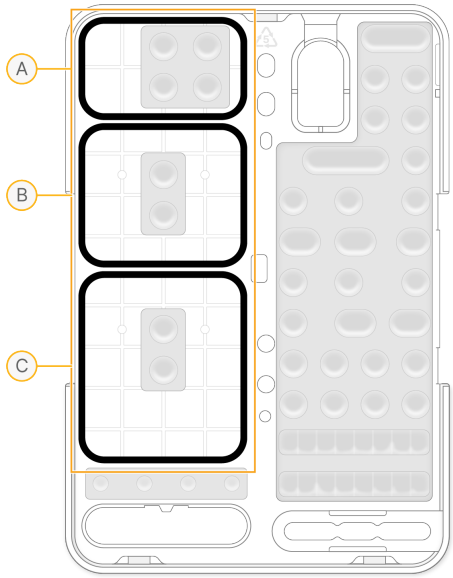
2. To detach the position #3 and #8 wells from the reagent cartridge, press the tab, and then push the well upward.

Figure 15 Remove Position #3 and #8 Wells



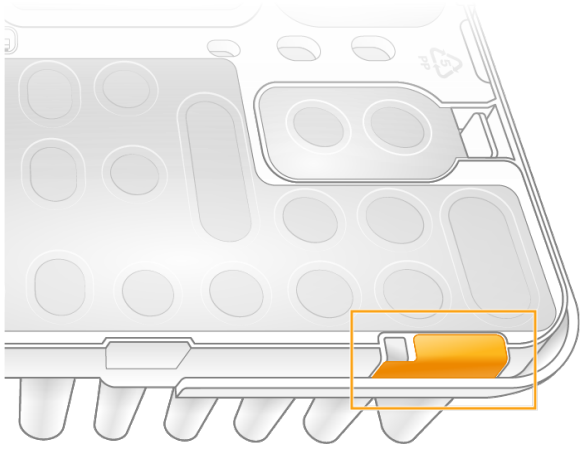
3. Recycle the position #3 and #8 wells in accordance with the applicable standards for your region.
4. To separate the three SBS wells from the reagent cartridge, compress the well sides, and then push the well upward.

Figure 16 SBS Well Locations



A. SBS #1 well—Polyethylene plastic (PET)
A. SBS #2 well—Polyethylene plastic (PET)
A. SBS #3 well—Polypropylene plastic (PP)

5. Recycle the SBS wells in accordance with the applicable standards for your region.
6. Remove all foils, the RFID label, and the RFID, and then discard.



7. Rinse the disassembled reagent cartridge and cover, and then recycle in accordance to the applicable standards for your region.

Sequencing Output

After initiating a sequencing run, Real-Time Analysis begins automatically. You can view RTA4 metrics on the Sequencing or Runs screen. To view sequencing and secondary analysis results, select the run name on the Completed tab of the Runs screen. The run results include detailed sequencing metrics, secondary analysis metrics, and DRAGEN application reports at the sample and run level.

You can also find output files in the specified default output folder location.

Real-Time Analysis

The NovaSeq X Series runs RTA4, an implementation of Real-Time Analysis software, on the instrument Compute Engine (CE). RTA4 extracts intensities from images received from the camera, performs base calling, assigns a quality score to base calls, aligns to PhiX, and reports data in InterOp files for viewing in the NovaSeq X Series Control Software.

To optimize processing time, RTA4 stores information in memory. If RTA4 is terminated, processing does not resume and any run data being processed in memory is lost.

RTA4 Inputs

RTA4 requires tile images contained in local system memory for processing. RTA4 receives run information and commands from the control software.

RTA4 Outputs

Images for each color channel are passed in memory to RTA4 as tiles. From these images, RTA4 outputs a set of quality-scored base call files and filter files. All other outputs are supporting output files.

File Type	Description
Base call files	Each tile that is analyzed is included in a concatenated base call (*.cbcl) file. Tiles from the same lane and surface are aggregated into 1 *.cbcl file for each lane and surface.
Filter files	Each tile produces a filter file (*.filter) that specifies whether a cluster passes filters.
Cluster location files	Cluster location (*.locs) files contain the X,Y coordinates for every cluster in a tile. A cluster location file is generated for each run.
InterOp files	Binary reporting files used for Sequencing Analysis Viewer. InterOp files are updated throughout the run.

Output files are used for downstream analysis.

Quality Scores

A quality score (Q-score) is a prediction of the probability of an incorrect base call. A higher Q-score implies that a base call is higher quality and more likely to be correct. After the Q-score is determined, results are recorded in base call (*.cbcl) files.

The Q-score succinctly communicates small error probabilities. Quality scores are represented as Q(X), where X is the score. The following table shows the relationship between a quality score and error probability.

Q-Score Q(X)	Error Probability
Q30	0.001 (1 in 1000)
Q20	0.01 (1 in 100)
Q10	0.1 (1 in 10)

Quality Scoring and Reporting

Quality scoring calculates a set of predictors for each base call, and then uses the predictor values to look up the Q-score in a quality table. Quality tables are created to provide optimally accurate quality predictions for runs generated by a specific configuration of sequencing platform and version of chemistry.

Quality scoring is based on a modified version of the Phred algorithm.

To generate the Q-table for the NovaSeq X Series, three groups of base calls were determined, based on the clustering of these specific predictive features. Following grouping of the base calls, the mean error rate was empirically calculated for each of the three groups and the corresponding Q-scores were recorded in the Q-table alongside the predictive features correlating to that group. As such, only three Q-scores are possible with RTA3 and these Q-scores represent the average error rate of the group. Overall, this results in simplified, yet highly accurate quality scoring. The three groups in the quality table correspond to marginal (< Q15), medium (~Q20), and high-quality (> Q30) base calls, and are assigned the specific scores of 12, 20 and 37 respectively. Additionally, a null score of 0 is assigned to any no-calls. This Q-score reporting model reduces storage space and bandwidth requirements without affecting accuracy or performance.

Sequencing Output Files

File Type	File Description, Location, and Name

Base call files	Each cluster analyzed is included in a base call file, aggregated in one file per cycle, lane, and surface. The aggregated file contains the base call and encoded quality score for every cluster.
	`Data\Intensities\BaseCalls\L001\C1.1`
	`L[lane]_[surface].cbcl`, for example `L001_1.cbcl`

Cluster location files	For each flow cell, a binary cluster location file contains the XY coordinates for clusters in a tile. A hexagonal layout that matches the nanowell layout of the flow cell predefines the coordinates.
	`Data\Intensities`
	`s_[lane].locs`

Filter files	The filter file specifies whether a cluster passed filters. Filter files are generated at cycle 26 using 25 cycles of data. For each tile, one filter file is generated.
	`Data\Intensities\BaseCalls\L001`
	`s_[lane]_[tile].filter`

Run information file	Lists the run name, number of cycles in each read, whether the read is an Index Read, and the number of swaths and tiles on the flow cell. The run info file is created at the beginning of the run. `[Root folder],RunInfo.xml`

Sequencing Output Folder Structure

By default, the NovaSeq X Plus generates output files in the output folder selected in the Settings tab. The `report.html` file includes a summary report for each DRAGEN application.

General Output Folder Structure

At a high level, the outputs are organized in the following structure:

`/usr/local/illumina/runs/<run_id>/`

- **Analysis (secondary analysis files)**
- **Data (primary analysis BCL files)**
- **InstrumentAnalyticsLogs**
- **InterOp**
- **Logs**
- `RTA.cfg`

- RTAComplete.txt
- CopyComplete.txt
- RunCompletionStatus.xml
- RunInfo.xml
- RunParameters.xml
- SampleSheet.csv

DRAGEN Output Folder Structure

For DRAGEN output files, refer to the following structure in the Analysis folder. These files can be found at `/usr/local/illumina/runs/<run_id>/Analysis/<no>/Data`. Depending on the modes of operation, there might be additional metric files included in the output.

Files generated for the Small Variant Calling workflow are generated when the user selects either SmallVariantCaller or All VariantCallers during run setup.

- **summary**
  - **<x.x.x>** (DRAGEN version)
    - highlevel_summary.json
    - detailed_summary.json
    - workflow_status.json
- **AggregateReports**
  - report.html
  - **report_files**
    - **reports**
      - **BCLConvert**
        - report.html
        - **report_files**
      - **Demux**
        - report.html
        - **report_files**
      - **DragenGermline**
        - report.html
        - **report_files**
      - **DragenEnrichment**

- report.html
- **report_files**

Demux

- **AggregateReports**
  - report.html
  - **report_files**
    - **samples**
      - **sample_name**
        - sample.html
  - Demultiplex_Stats.csv
  - Top_Unknown_Barcodes.csv
  - Index_Hopping_Counts.csv
  - IndexMetricsOut.bin

BCLConvert

- SampleSheet.csv
- **AggregateReports**
  - report.html
  - **report_files**
    - **samples**
      - **sample_name**
        - sample.html
- **fastq** or **ora_fastq**
  - <sample_ID>.S0_L00n_Rm_001.fastq.gz or *.fastq.ora (n=1-8, m=1-2)
  - **Reports**
    - Adapter_Metrics.csv
    - Quality_Metrics.csv
- **<sample_id>**
  - **fastqc**
  - **logs**
- **logs**

DragenGermline

- SampleSheet.csv
- **AggregateReports**
  - report.html
  - **report_files**
    - **samples**
      - **sample_name**
        - sample.html
- **fastq** or **ora_fastq**
  - <sample_ID>.S0_L00n_Rm_001.fastq.gz or *.fastq.ora (n=1-8, m=1-2)
  - **Reports**
    - Adapter_Metrics.csv
    - Quality_Metrics.csv
- **<sample_id>**
  - **germline_seq**
    - <sample_ID>.bam (or cram or not saved)
    - <sample_ID>.ploidy.vcf.gz
    - **[SmallVariantCaller or AllVariantCallers]**<sample_ID>.hard-filtered.vcf.gz
    - **[SmallVariantCaller or AllVariantCallers]**<sample_ID>.hard-filtered.gvcf.gz
    - **[AllVariantCallers]**<sample_ID>.sv.vcf.gz
    - **[AllVariantCallers]**<sample_ID>.cnv.vcf.gz
    - **[AllVariantCallers]**<sample_ID>.repeats.vcf.gz
    - **[AllVariantCallers]**<sample_ID>.cyp2d6.tsv
    - **[AllVariantCallers]**<sample_ID>.roh_metrics.csv
    - <sample_ID>.mapping_metrics.csv
    - <sample_ID>.fastqc_metrics.csv
    - report.html
  - **logs**
- **logs**
- **DragenEnrichment**
  - SampleSheet.csv

- `Bedfile.gz`
- **AggregateReports**
  - `report.html`
  - **report_files**
    - **samples**
      - **sample_name**
        - `sample.html`
- **fastq** or **ora_fastq**
  - `<sample_ID>.S0_L00n_Rm_001.fastq.gz` or `*.fastq.ora` (n=1-8, m=1-2)
  - **Reports**
    - `Adapter_Metrics.csv`
    - `Quality_Metrics.csv`
- **<sample_id>**
  - **enrichment_seq**
    - `<sample_ID>.bam` (or cram or not saved)
    - **[SmallVariantCaller or AllVariantCallers]**`<sample_ID>.hard-filtered.vcf.gz`
    - **[SmallVariantCaller or AllVariantCallers]**`<sample_ID>.hard-filtered.gvcf.gz` (only if GermlineOrSomatic = germline)
    - **[AllVariantCallers]**`<sample_ID>.sv.vcf.gz`
    - **[AllVariantCallers]**`<sample_ID>.cnv.vcf.gz` (only if AuxCnvPanelOfNormalsFile is provided)
    - **[AllVariantCallers]**`<sample_ID>.target.counts.gz` (only if AuxCnvPanelOfNormalsFile is provided)
    - **[AllVariantCallers]**`<sample_ID>.tn.tsv.gz` (only if AuxCnvPanelOfNormalsFile is provided)
    - **[AllVariantCallers]**`<sample_ID>.repeats.vcf.gz`
    - **[AllVariantCallers]**`<sample_ID>.roh_metrics.csv`
    - `<sample_ID>.mapping_metrics.csv`
    - `<sample_ID>.fastqc_metrics.csv`
    - `report.html`
  - **logs**

- **logs**

DragenRNA

- `SampleSheet.csv`
- **AggregateReports**
  - `report.html`
  - **report_files**
    - **samples**
      - **sample_name**
        - `sample.html`
- **fastq** or **ora_fastq**
  - `<sample_ID>.S0_L00n_Rm_001.fastq.gz` or `*.fastq.ora` (n=1-8, m=1-2)
  - **Reports**
    - `Adapter_Metrics.csv`
    - `Quality_Metrics.csv`
- **<sample_id>**
  - **rna_seq**
    - `<sample_ID>.bam` (or cram or not saved)
    - **[FullPipeline]**`<sample_ID>.fusion_candidates.vcf.gz`
    - **[FullPipeline]**`<sample_ID>.quant.genes.sf`
    - **[FullPipeline]**`<sample_ID>.quant.sf`
    - `<sample_ID>.mapping_metrics.csv`
    - `<sample_ID>.fastqc_metrics.csv`
    - `report.html`
  - **logs**
- **logs**
- **DifferentialExpression** (only if RnaDifferentialExpression is set to true)
  - **Comparison1**
  - **Comparison2**

RunInstrumentAnalyticsMetrics

`Secondary_Analysis_Complete.txt`

logs

NovaSeq X Plus Secondary Analysis Reports

From the Sequencing complete screen, select the run name to view run results. Navigate to the bottom of the Run details screen, and then select View DRAGEN report to view secondary analysis results. Alternatively, use the global menu to navigate to the Runs screen and select a completed run.

You can view DRAGEN report results at the following levels:

- Run—The run summary links to the workflow reports, including a demultiplex report, and provides an overview of the following information:
  - Version number
  - Number of total samples
  - Number of samples completed
  - Number of errors
- Workflow—Workflow reports aggregate data across all the samples included in that DRAGEN application and link to individual sample reports.
- Sample—Sample reports include detailed metrics for Read 1 and Read 2 of an individual sample.

The metrics that are available at the workflow and sample level vary depending on the report.

Demultiplex Report

A report with demultiplex metrics is automatically included in each run. The demultiplexing statistics report contains information on the number of passing filter reads that are assigned to each sample in the sample sheet. Any reads not clearly associated with a sample are classified as undetermined.

Report Tab	Description
Demultiplex Stats	Overview information and matching statistics for samples in the run: • **Lane**—The lane on the flow cell the sample was sequenced • **Sample ID**—The sample ID from the sample sheet. If a read does not correspond with a sample, the field displays `undetermined`. • **Index**—The concatenation of Index Read 1 and Index Read 2 from the sample sheet separated by a hyphen. If a read does not correspond to a sample, the field displays `undetermined` • **Reads**—Number of reads • **Matching**—Number of reads perfectly matching the index • **One Mismatch**—Number of reads matching the index with one mismatch • **Two Mismatches**—Number of reads matching the index with two mismatches • **% Reads**—Percentage of total reads assigned to the sample • **% Matching**—Percentage of sample reads with no mismatches in the index • **% One Mismatch**—Percentage of sample reads with one mismatch in the index • **%Two Mismatches**—Percentage of sample reads with two mismatches in the index
Top Unknown Barcodes	List of index adapter sequences detailing the number of reads, percentage of sample reads with unknown barcodes, and percentage of total reads assigned to a sample. Organized in descending order from indexes with the most number of reads to the least.
Index Hopping Counts	List of samples including number of reads, the percentage of sample reads with incorrect assignment, and percentage of total reads assigned to the sample.

DRAGEN BCL Convert Report

The DRAGEN BCL Convert report contains the following information:

Report Tab	Metric	Description
Summary	General Statistics	Overview information on each sample included in the workflow: • **Input Reads**—Number of input reads • **Read Length**—Estimated read length • **Q30 Bases R1**—Number of bases with a Phred quality score greater than or equal to 30 on Read 1 • **Q30 Bases R2**—Number of bases with a Phred quality score greater than or equal to 30 on Read 2
	Mean Base Quality by Position	The average Phred score at each read position across the samples.
	Read Length Distribution	The read count for each length (in base pairs) of the sequenced fragments in the run.
	Read Quality Distribution	The read counts for each Phred score assigned to bases that map to the samples in the run.
	%GC Content	The percentage of guanine or cytosine bases in the samples that were sequenced. In addition to data from each sample, the graph displays a theoretical %GC data.
	Ambiguous Base Content by Position	The percentage of bases at each read position that have ambiguous inheritance. This distribution should be as close to 0 as possible.
	Adapter Content by Position	The percentage of sequences for each base pair.

DRAGEN Enrichment Report

The DRAGEN Enrichment report contains the following information:

Report Tab	Metric	Description
Enrichment	Read Level Enrichment	Read enrichment information about each sample: • **Total Aligned Reads**—Total number of aligned reads • **Percent Aligned Reads**—Percent of aligned reads • **Targeted Aligned Reads**—Number of uniquely aligned reads • **Read Enrichment**—Percent of uniquely aligned reads • **Padded Target Aligned Reads**—Number of padded uniquely aligned reads • **Padded Read Enrichment**—Percent of padded uniquely aligned reads
	Base Level Enrichment	Base enrichment information about each sample including the following metrics: • **Total Aligned Bases**—Total number of aligned bases • **Percent Aligned Bases**—Percent of aligned bases • **Targeted Aligned Bases**—Number of uniquely aligned bases • **Base Enrichment**—Percent of uniquely aligned bases • **Padded Target Aligned Bases**—Number of padded uniquely aligned bases • **Padded Base Enrichment**—Percent of padded uniquely aligned bases
	Total Aligned Bases	Total number of aligned bases for each sample index.
Trimmer	Trimmed Reads	The total number of input reads and the total number of trimmed reads. Trimmed reads are also categorized in the following groups: • Fixed-length trimmed reads • Poly-g trimmed reads • Quality trimmed reads • Adapter trimmed reads • Bisulfite trimmed reads • N-base trimmed reads • Minimum-length trimmed reads • Cut-end trimmed reads The report value is N/A when trimmer is disabled and zero when trimmer is enabled but not applied.

Report Tab	Metric	Description
DRAGEN-FastQC	Mean Base Quality by Position	The average Phred score at each read position across the samples.
	Read Length Distribution	The read count for each length (in base pairs) of the sequenced fragments in the run.
	Read Quality Distribution	The read counts for each Phred score assigned to bases that map to the samples in the run.
	%GC Content	The percentage of guanine or cytosine bases in the samples that were sequenced. In addition to data from each sample, the graph displays a theoretical %GC data.
	Ambiguous Base Content by Position	The percentage of bases at each read position that have ambiguous inheritance. This distribution should be as close to 0 as possible.
	Adapter Content by Position	The percentage of sequences for each base pair.
	Mean Base Quality by Position	The average Phred score at each read position across the samples.

Report Tab	Metric	Description
QC	QC Summary	Quality control information for each sample including the following metrics: • **% Contam**—Estimated percentage of reads that might be from another human source • **Median Exon Coverage**—Median autosomal coverage over exon • **PCT Exon 1000x**—Percentage of exon with greater than 1000x coverage
	Extended QC Summary	Additional quality control information for each sample including the following metrics: • **Total Input Reads**—Total number of input reads • **PCT Chimeric Reads**—Percent of chimeric reads • **PCT Exon 500X**—Percentage of exon with greater than 500x coverage • **PCT Exon 1500X**—Percentage of exon with greater than 1500x coverage • **PCT Aligned Reads**—Percent of aligned reads • **Median Insert Size**—Median insert size
	Targeted Coverage QC Metrics	Target coverage quality control information for each sample including the following metrics: • **Median Target Coverage**—Median autosomal coverage • **Mean Target Coverage**—Average alignment coverage • **PCT Target 0.4X Mean**—Percentage of bases with read depth greater than 40% of the target region average • **PCT Target 500X**—Percentage of target region with at least 500x coverage • **PCT Target 1000X**—Percentage of target region with at least 1000x coverage • **PCT Target 1500X**—Percentage of target region with at least 1500x coverage

Report Tab	Metric	Description
Mapping	Fragment Length Summary	The mean and median insert sizes for fragments in each sample. Includes the standard deviation of insert sizes for each sample.
	Fragment Length Medians	The median fragment length for each sample index.
	Fragment Length	The length (in base pairs) of the sequenced fragments in the run.
	Duplicates	Number of duplicate aligned read and the percentage of total aligned reads that are duplicates.
Coverage	Coverage Summary	An overview of sample coverage including the following metrics: • **Mean Region Coverage Depth**—Average alignment coverage • **Uniformity of Coverage**—Percentage of bases with a read depth greater than 20% of the exon average • **Target Coverage at 1X**—Percentage of exon with at least 1x coverage • **Target Coverage at 10X**—Percentage of exon with at least 10x coverage • **Target Coverage at 20X**—Percentage of exon with at least 20x coverage • **Target Coverage at 50X**—Percentage of exon with at least 50x coverage
	Mean Region Coverage Depth	The average alignment coverage over QC coverage region for each sample index.
	Uniformity of Coverage	Uniformity of coverage (PCT > 0.2*mean) over QC coverage region for each sample index.
	Targeted Coverage Distribution	The depth and percentage of bases covered by a certain number of reads for each sample.
	Minimum Targeted Coverage Distribution	The depth and percentage of bases covered by at least a certain number of reads for each sample.

Report Tab	Metric	Description
Variants	SNVs	The following information for each sample: • **SNVs**—Total number of SNVs • **SNV Het/Hom Ratio**—Ratio of heterozygous to homozygous SNVs • **SNV Ts/Tv Ratio**—Ratio of transitions to transversions among SNVs
	Number of SNVs Passing	The number of SNVs passing for each sample index.
	Indels	The following information for each sample: • **Indels**—Number of indels • **Insertions (Het)**—Number of variants where both alleles are insertions but not homozygous • **Deletions (Het)**—Number of variants that contain homozygous deletions • **Indel Het/Hom Ratio**—Ratio of heterozygous to homozygous indels

DRAGEN Germline

The DRAGEN Germline report contains the following information:

Report Tab	Metric	Description
Summary	General Statistics	Overview information on each sample included in the workflow. • **Sex**—Sex of sample • **Depth**—Average alignment coverage over the genome • **% >20x**—Percentage of the genome with more than 20% coverage • **% Contam**—Estimated percent of reads that might be from another human source • **Variants**—Total number of small variants • **Input Reads**—Total number of input reads • **%Unmap**—Percentage of reads that are unmapped • **% Dup**—Percentage of duplicate marked reads • **% Prop Pair**—Percentage of reads properly paired • **Med IS**—Median insert size
	DRAGEN Modules	Indicates if QC and Trimming modules are enabled and the number of SNV, SV, and CNV for each sample.
	DRAGEN Specialized Callers	Indicates if the following specialized callers are enabled: • Aneuploidy • Zygosity • HLA • Repeat expansions • SMA caller • GBA caller • CYP2D6 caller
	DRAGEN Features	Indicates if the following DRAGEN features are enabled for each sample: • Target allele calling • Star allele calling for PGx

Report Tab	Metric	Description
Trimmer	Trimmed Reads	The total number of input reads and the total number of trimmed reads. Trimmed reads are also categorized in the following groups: • Fixed-length trimmed reads • Poly-g trimmed reads • Quality trimmed reads • Adapter trimmed reads • Bisulfite trimmed reads • N-base trimmed reads • Minimum-length trimmed reads • Cut-end trimmed reads The report value is N/A when trimmer is disabled and zero when trimmer is enabled but not applied.
DRAGEN-FastQC	Means Base Quality by Position	The average Phred score at each read position across the samples.
	Read Length Distribution	The read count for each length (in base pairs) of the sequenced fragments in the run.
	Read Quality Distribution	The read counts for each Phred score assigned to bases that map to the samples in the run.
	%GC Content	The percentage of guanine or cytosine bases in the samples that were sequenced. In addition to data from each sample, the graph displays a theoretical %GC data.
	Ambiguous Base Content by Position	The percentage of bases at each read position that have ambiguous inheritance. This distribution should be as close to 0 as possible.
	Adapter Content by Position	The percentage of sequences for each base pair.

Report Tab	Metric	Description
Mapping	Mapping Metrics	An overview of mapping metrics including the following information for each sample in the workflow: • **Total Input Reads**—Total number of input reads • **Number of Unique Reads**—Total number of unique reads • **QC-Failed Read**—Total number of runs failing one or more quality checks • **Mapped Reads**—Total number of mapped reads • **Total Bases**—Total number of input bases • **Mapped Bases R1**—Total number of input bases on Read 1 • **Q30 Bases**—Total number of bases with a Phred quality score of greater than or equal to 30 • **% Contam**—Estimated percentage of reads that might be from another human source
	Fragment Length	The length (in base pairs) of the sequenced fragments in the run.
	Mapped Coverage to Primary Contigs	Average coverage for each primary contig.
	Mapped Coverage to Unplaced and Alternate Contigs	Average coverage for each unplaced and alternate contig.

Report Tab	Metric	Description
Coverage	Coverage Metrics	An overview of coverage metrics including the following information for each sample in the workflow: • **Aligned Reads**—Total number of aligned reads • **Average Coverage**—Average alignment coverage over genome • **Uniformity of Coverage**—Percentage of bases with read depth greater than 20% of the genome average • **Mean/Median Ratio**—Ration of mean autosomal coverage to median autosomal coverage • **% >=1x**—Percentage of genome with at least 1x coverage • **% >=10x**—Percentage of genome with at least 10x coverage • **% >=20x**—Percentage of genome with at least 20x coverage • **% >=50x**—Percentage of genome with at least 50x coverage • **% >=100x**—Percentage of genome with at least 100x coverage
	WGS Coverage Distribution	Percent of bases covered by X reads mapped by depth for each sample.
	Minimum WGS Coverage Distribution	Percent of bases covered by at least X reads mapped by depth for each sample.
Aneuploidy	Aneuploidy Results (chr1-12)	Aneuploidy results for chromosomes 1–12.
	Aneuploidy Results (chr13-22,X,Y)	Aneuploidy results for chromosomes 13–22, X, and Y.

Report Tab	Metric	Description
Variants	Variant Statistics	Overview of the following variant information: • **Variants**—Total number of variants • **Multiallelic**—Total number of sites with three or more observed alleles • **SNP**—Total number of single nucleotide polymorphisms (SNPs) • **Ti/Tv**—Ratio of transitions to transversions among called variants • **Het/Him Ratio**—Ratio of heterozygous to homozygous variants • **Callability**—Percentage of autosomal non-N reference positions with a passing genotype call
	Structural Variant Metrics	Total number of structural variants (SVs), deletions, insertions, duplications, and breakend pairs.
	Copy Number Variant Metrics	Total coverage uniformity, total number of duplications, and total number of deletions. Coverage Uniformity is an autocorrelation metric calculated from coverage levels of adjacent regions. Values vary by library but should generally be < 0.4 for Germline WGS data.
Zygosity	Regions of Homozygosity (chr1-12)	The values provided in this section of report are ratios of the number of heterozygous to homozygous SNVs for chromosomes 1–12. Values less than 0.2 indicate a likely region of homozygosity.
	Regions of Homozygosity (chr13-22,X,Y)	The values provided in this section of report are ratios of the number of heterozygous to homozygous SNVs for chromosomes 13–22, X, and Y. Values less than 0.2 indicate a likely region of homozygosity.
HLA	HLA Typer Results	First and second results for the HLA-A, HLA-B, and HLA-C alleles. • **A1**—First allele result for HLA-A • **A2**—Second allele result for HLA-A • **B1**—First allele result for HLA-B • **B2**—Second allele result for HLA-B • **C1**—First allele result for HLA-C • **C2**—Second allele result for HLA-C

Report Tab	Metric	Description
Targeted Callers	Repeat Expansion	Identifies repeat expansions for long allele for each gene.

Report Tab	Metric	Description
	Targeted Caller Summary	The number of FXN repeats, the number of FMR1 repeats, and an indication of whether the SMN or GBA mutant was present. Values separated by a forward slash represent the number of repeats for both alleles.

Report Tab	Metric	Description
	Common Repeat Expansions	The number of repeat units for the specified motif in the given gene. Values separated by a forward slash represent the number of repeats for both alleles. • **AR**—Number of GCA repeats in AR • **ATN1**—Number of CAG repeats in ATN1 • **C9ORF72**—Number of GGGGCC repeats in C0ORF72 • **DMPK**—Number of CTG repeats in DMPK • **FMR1**—Number of CGG repeats in FMR1 • **FXN**—Number of GAA repeats in FXN • **FXN**—Number of alanine repeats in FXN • **HTT**—Number of CAG repeats in HTT • **HTT**—Number of CCG repeats in HTT

Report Tab	Metric	Description
	Ataxia Repeat Expansions	The number of repeat units for the specified motif in the given gene. Values separated by a forward slash represent the number of repeats for both alleles. • **ATXN1**—Number of CAG repeats in ATXN1 • **ATXN2**—Number of CAG repeats in ATXN2 • **ATXN3**—Number of CAG repeats in ATXN3 • **ATXN7**—Number of CAG repeats in ATXN7 • **ATXN7**—Number of GCC repeats in ATXN7 • **ATXN10**—Number of ATTCT repeats in ATXN10 • **ATXN8OS**—Number of CTG repeats in ATXN8OS
	Rare Repeat Expansions (A–M)	The number of repeat units for the specified motif in the given gene. Values separated by a forward slash represent the number of repeats for both alleles. • **AFF2**—Number of GCC repeats in AFF2 • **CACNA1A**—Number of CAG repeats in CACNA1A • **CBL**—Number of CGG repeats in CBL • **CNBP**—Number of CCTG repeats in CNBP • **CSTB**—Number of CCCCGCCCCGCG repeats in CSTB • **DIP2B**—Number of GGC repeats in DIP2B • **GLS**—Number of GCA repeats in GLS • **JPH3**—Number of CTG repeats in JPH3
	Rare Repeat Expansions (N–Z)	The number of repeat units for the specified motif in the given gene. Values separated by a forward slash represent the number of repeats for both alleles. • **NIPA1**—Number of alanine repeats in NIPA1 • **NOP56**—Number of GGCCTG repeats in NOP56 • **NOP56**—Number of CGCCTG repeats in NOP56 • **NOTCH2NL**—Number of GGC repeats in NOTCH2NL • **PABPN1**—Number of alanine repeats in PABPN1 • **PHOX2B**—Number of alanine repeats in PHOX2B • **PPP2R2B**—Number of CTG repeats in PPP2R2B • **RFC1**—Number of alanine repeats in RFC1 • **TBP**—Number of GCA repeats in TBP • **TCF4**—Number of CAG repeats in TCF4
PGx	Pharmacogenetics	The CYP2D6 genotype and if the CYP2D6 filter was PASS or FAIL for the sample.

DRAGEN RNA

The DRAGEN RNA report contains the following information:

Report Tab	Metric	Description
Summary	General Statistics	Overview information on each sample included in the workflow. • **Input Reads**—Total number of input reads • **Read Length**—Estimated read length • **Abundance**—Number of noninformative reads • **% Unmapped**—Percentage of reads that are unmapped • **CV Coverage**—The median coefficient of variation of coverage of the 1000 most highly expressed transcripts. The ideal value is zero. • **% Strand Match**—Percent of strand matched in expected orientation • **% Strand Mismatch**—Percent of strands mismatched in expected orientation
Trimmer	Trimmer Reads	The total number of input reads and the total number of trimmed reads. Trimmed reads are also categorized in the following groups: • Fixed-length trimmed reads • Poly-g trimmed reads • Quality trimmed reads • Adapter trimmed reads • Bisulfite trimmed reads • N-base trimmed reads • Minimum-length trimmed reads • Cut-end trimmed reads The report value is N/A when trimmer is disabled and zero when trimmer is enabled but not applied.

Report Tab	Metric	Description
DRAGEN-FastQC	Mean Base Quality by Position	The average Phred score at each read position across the samples.
	Run Length Distribution	The read counts for each length (in base pairs) of the sequenced fragments in the run.
	Read Quality Distribution	The read counts for each Phred score assigned to bases that map to the samples in the run.
	%GC Content	The percentage of guanine or cytosine bases in the samples that were sequenced. In addition to data from each sample, the graph displays a theoretical %GC data.
	Ambiguous Base Content by Position	The percentage of bases at each read position that have ambiguous inheritance. This distribution should be as close to 0 as possible.
	Adapter Content by Position	The percentage of sequences for each base pair.
Mapping	Mapping Metrics	An overview of mapping metrics including the following information for each sample in the workflow: • **Total Input Reads**—Total number of input reads • **Number of Unique Reads**—Total number of unique reads • **QC-Failed Read**—Total number of runs failing one or more quality checks • **Mapped Reads**—Total number of mapped reads • **Total Bases**—Total number of input bases • **Mapped Bases R1**—Total number of input bases on R1 • **Q30 Bases**—Total number of bases with a Phred quality score of greater than or equal to 30 • **% Contam**—Estimated percentage of reads that might be from another human source
	Insert Length Distribution	The length (in base pairs) of the sequenced fragments in the run.

Report Tab	Metric	Description
Regional Coverage	Coverage Uniformity Metrics	For each sample, this section of the report includes the following information: • **Median CV**—Coefficient of variation of coverage along transcripts • **Reads Aligned to Correct Strand**—Read pairs that match transcripts on the forward strand
Gene Coverage	Gene Coverage	Data on gene coverage for each sample organized in the following categories: • **>=1x**—Number of genes with at least 1x coverage • **>=10x**—Number of genes with at least 10x coverage • **>=30x**—Number of genes with at least 30x coverage • **>=100x**—Number of genes with at least 100x coverage
	Transcript Alignment	Percentages of fragments in the following categories: • **Transcript fragments**—Read pairs mapped to one or more annotated transcripts • **Unknown fragments**—Read pairs that overlap with an exon of a gene but do not match any transcript • **Intron fragments**—Read pairs that overlap with a gene but do not overlap with an exon • **Intergenic fragments**—Read pairs that do not overlap with any gene
	Transcript Coverage	Visualization mapping the coverage in relation to the normalized position along the transcript.

DRAGEN Secondary Analysis Output Files

This section provides information on each DRAGEN application, including output file information. In addition to generating files specific to each application, DRAGEN provides metrics from the analysis in a `<sample_name>.metrics.json` file and the reports described in *NovaSeq X Plus Secondary Analysis Reports* on page 87. For more information DRAGEN, refer to the Illumina DRAGEN Bio-IT Platform support site page.

All DRAGEN pipelines support the decompression of input BCL and compression of output BAM/CRAM files. BAM files will not be uploaded to Illumina DRAGEN Bio-IT Platform if Proactive, Run Monitoring and Storage is selected.

DRAGEN Enrichment

The DRAGEN Enrichment application supports the following features:

- Decompression of input BCL data

- FASTQ conversion
- FASTQ compression to ORA or Gzip formats
- Map/Alignment (includes sorting and duplicate marking)
- BAM/CRAM Compression (optional)
- Variant Calling

When the VariantCallingMode parameter is used, the pipeline supports algorithms for the following variant callers:

- None
- Small variant callers
- All variant callers:
  - Small
  - Structural
  - Copy number variants (for human, on-instrument reference genomes)

The following inputs are required:

- BCL data generated from the NovaSeq X Plus instrument
- Sample sheet
- BedFile (if Variant Calling Mode is not equal to none)
- GenomeOrSomatic

The following inputs are optional:

- AuxBaselineNoiseFile
- AuxCnvPanelOfNormalsFile (if Variant Calling Mode equals AllVariantCallers)

DRAGEN supports options to improve performance in somatic mode with the input of a noise baseline file. A Panel of Normals file is required for CNV.

DRAGEN Enrichment generates the following output files.

Component	Type	Output File Name
Mapping/aligning	BAM or CRAM	• `<sample_name>.bam`, or • `<sample_name>.cram`
Small variant calling	VCF and gVCF*	• `<sample_name>.hard-filtered.gvcf.gz` • `<sample_name>.hard-filtered.vcf.gz`
Structural variant calling	VCF	• `<sample_name>.sv.vcf.gz`
Copy number variants	VCF	• `<sample_name>.cnv.vcf.gz`

Component	Type	Output File Name
Metric generation	CSV	• <sample_name>.metrics.json • <sample_name>.qc_metrics.csv

* gVCF output files are only available for germline mode.

DRAGEN Germline

The DRAGEN Germline application supports the following features:

- Decompression of input BCL data
- FASTQ conversion
- FASTQ compression to ORA or Gzip formats
- Map/Alignment (includes sorting and duplicate marking)
- BAM/CRAM Compression (optional)
- Variant calling

The pipeline supports algorithms for the following variant callers:

- None
- Small variant callers
- All variant callers:
  - Structural
  - Copy number variants (for human, on-instrument reference genomes)
  - Repeat expansion (for human, on-instrument reference genomes)
  - Regions of Homozygosity (for human, on-instrument reference genomes)
  - CYP2D6 detection

The following inputs are required:

- BCL data generated from the NovaSeq X Plus instrument
- Sample sheet

DRAGEN Germline generates the following output files.

Component	Type	Output File Name	Requirements for Output
Mapping/aligning	BAM or CRAM	• <sample_name>.bam, or • <sample_name>.cram	N/A

Component	Type	Output File Name	Requirements for Output
Small variant calling	VCF and gVCF	• <sample_name>.hard-filtered.gvcf.gz • <sample_name>.hard-filtered.vcf.gz	N/A
Structural variant caller	VCF	• <sample_name>.sv.vcf.gz	Only generated for paired-end reads
Copy number variants	VCF	• <sample_name>.cnv.vcf.gz	Human genomes only
Repeat expansion	VCF	• <sample_name>.repeats.vcf.gz	Human genomes only
Regions of homozygosity	CSV and BED	• <sample_name>.roh_metrics.csv • <sample_name>.roh.bed	Human genomes only
CYP2D6 detection	TSV	• <sample_name>.cyp2d6.tsv	Human genomes only
Metric generation	CSV	• <sample_name>.metrics.json • <sample_name>.qc_metrics.csv	N/A

DRAGEN RNA

The DRAGEN RNA application supports the following features:

- Decompression of input BCL data
- FASTQ generation
- FASTQ compression (ORA or Gzip)
- Map/Alignment (includes sorting)
- **[Optional]** BAM/CRAM compression
- **[FullPipeline]** Gene Fusion detection
- **[FullPipeline]** Whole transcriptome gene expression (quantification of transcripts)

Differential Expression is also optionally supported.

The following inputs are required:

- BCL data generated from the NovaSeq X Plus instrument
- Sample sheet

The following input is optional:

- RnaGeneAnnotationFile

An RnaGeneAnnotationFile is packaged with each Illumina-provided human reference genome. If an RnaGeneAnnotationFile is provided by the customer on run set-up, it is used instead of the file packaged with the genome. If an RnaGeneAnnotationFile is not available either with the Illumina-provided genome or by the customer directly, then Gene Fusion and RNA quantification steps are skipped. If Differential Expression is enabled, an RnaGeneAnnotationFile must be provided.

DRAGEN RNA generates the following output files.

Component	Type	Output File Name	Description
Mapping/aligning	BAM or CRAM	• `<sample_name>.bam`, or • `<sample_name>.cram`	Alignment output meeting SAM specifications.
Gene fusion detection	Plain text	• `<sample_name>.fusion_candidates.preliminary` • `<sample_name>.fusion_candidates.final`	• Fusion candidates before filters are applied. • Fusion candidates after filters are applied.
Transcript quantification	Plain text	• `<sample_name>.quant.genes.sf` • `<sample_name>.quant.sf`	• Transcript quantification results at the gene level. • All transcript quantification results.
Metrics	JSON, CSV	• `<sample_name>.metrics.json` • `<sample_name>.qc_metrics.csv`	• Transcript quantification results at the gene level. • All transcript quantification results.
Differential expression	PNG	Refer to the following differential expression output files table.	To generate output files, a comparison must be set up in the sample sheet.

The following files are output when differential expression is enabled.

File Name	Description
`Control_vs_Comparison.genes.counts.csv`	Describes the number of reads mapped to each gene for each sample in the control and comparison groups.
`Control_vs_Comparison.genes.res.csv`	Contains DESeq2 results, describing the mean expression, log2 (fold change), standard error of log2 (fold change), p-value, adjusted p-value, and the expression status of each gene.
`Control_vs_Comparison.genes.rlog.csv`	Contains regularized log-transformed counts calculated by DESeq2
`Control_vs_Comparison.differential_expression_metrics.csv`	Contains differential expression analysis metrics.
`Control_vs_Comparison.genes.heatmap.png`	Plot showing a heat map of the expression of the differentially expressed genes with adjusted p-values < -0.05 for samples in the control and comparison groups. Only the top 30 differentially expressed genes are used if there are more than 30 differentially expressed genes. This file is not available when DESeq2 fails to converge or when there are no differentially expressed genes.
`Control_vs_Comparison.genes.ma.png`	Variation of gene expression ratios as a function of average signal intensity. The plot shows the differences between measurements taken in two samples, by transforming the data onto M (log ratio) and A (mean average) scales, then plotting these values. The MA plot shows the log2 fold changes attributable to a given variable over the mean of normalized counts for all the samples. Points are colored red if the adjusted p value is less than 0.1. Points that fall out of the window are plotted as open triangles pointing either up or down.
`Control_vs_Comparison.genes.pca.png`	Plot based on the first two principal components that explain the most variance.

DRAGEN BCL Convert

The DRAGEN BCL Convert application supports the following features:

- Decompression of input BCL data
- Sample demultiplexing

- UMI and adapter handling
- Per-sample settings
- Generation of FASTQ files
- Compression of FASTQ files to ORA or Gzip formats
- Generation of FASTQ QC metrics (only for the first 1024 samples)

The following inputs are required:

- BCL data generated from the NovaSeq X Plus instrument
- `RunInfo.xml`
- Sample sheet

DRAGEN BCL Convert generates the following outputs.

Component	Type	Output File Name
BclConvert	FASTQ	• `<Sample_ID>_Sm_L00n_Rp_001.fastq.gz` • m = surface number • n = lane number • p = read number from the set { 1, 2 }

The DRAGEN BCL Convert pipeline uses BCL data generated from your sequencing run and sample sheet information to output a FASTQ file. The FASTQ file name is `<Sample_ID>_Sm_L00n_Rp_001.fastq.gz`.

Demultiplexing Metrics

All DRAGEN pipelines generate the follow demultiplexing files by default. Aggregated files are stored in the `Demux` folder.

Component	Type	Output File Name
Demultiplexing	CSV	• `Demultiplex_Stats.csv`
Top unknown barcodes	CSV	• `Top_Unknown_Barcodes.csv`
Index hopping	CSV	• `Index_Hopping_Counts.csv`

Illumina DRAGEN QC Reports

For all pipelines, DRAGEN FastQC generates QC plots by default. Aggregated QC results are stored in the `AggregatedReports` folder.

Metrics are only generated if the number of samples is less than or equal to 1024.

For details on the QC plots provided, refer to *NovaSeq X Plus Secondary Analysis Reports* on page 87.

Maintenance

This section contains the procedures necessary for maintaining the NovaSeq X Plus.

Clear Hard Drive Space

If there is insufficient data storage space, a warning notification displays during pre-run checks. You can check the available space and the space consumed by runs or resources. Use the following steps to clear space by deleting completed runs and installed resources from an instrument run folder.

Delete a Run

Use the following instructions to delete a run. Only delete runs using the NovaSeq X Series Control Software rather than manually through the operating systems. Deleting runs manually can negatively impact the control software. You cannot requeue a deleted run.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Runs**.
3. For the run you want to delete, select the ellipsis icon in the Action column.
4. Select one of the following options.
    - **Delete run data**—Deletes the sequencing and analysis output folders, but does not remove the run from the Completed tab. You can view the run details, but cannot view the DRAGEN secondary analysis report.
    - **Delete run**—Deletes the run data and removes the run from the Completed tab.
5. In the dialog box, confirm run deletion.
6. Repeat steps 3 and 5 for each run that you want to delete.

Remove Resources

Use the following instructions to remove reference genomes or reference files.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Resources**.
3. Select the Genomes tab or Reference files tab.
4. For the genome or reference file you want to remove, select the delete icon.
5. In the dialog box, select **Yes, remove** to confirm.
6. Repeat steps 4 and 5 for each genome or reference file that you want to remove.

Software Updates

Updating the software makes sure that your system has the latest features and fixes. Software updates are bundled into a system suite, which includes the following software:

- NovaSeq X Series Control Software
- NovaSeq X Plus recipes
- Universal Copy Service
- Real-Time Analysis

You can manually download suite installers from the NovaSeq X Series support site page. Only administrators can install software updates.

1. When a software update is available, download the suite installer (*.run.gpg) from the NovaSeq X Series support page. Save the installer locally or to a network drive.
2. Make sure that there are no sequencing runs or on-instrument secondary analysis in progress.
3. Select the instrument icon to open the global navigation menu.
4. Select **Settings**, and then select **Software updates**.
5. Select **Select...** under browse for software updates.
6. Navigate to your installer file, and then select **Open**.
7. Select **Install updates**.
8. Enter your ilmnadmin password, and then select **Authenticate**.
9. After installed, select **Shut down**.
   Restarting the instrument is required to install the software update. Refer to *Shut Down or Restart the Instrument* on page 122.

Replace Air Filter

Use the following instructions to replace an expired air filter every 3 months. After replacing the air filter, you can reset the air filter expiration.

The air filter is single use and covers the fan in the bottom drawer in the front of the instrument. It ensures proper cooling and prevents debris from entering the system. The instrument is shipped with one air filter and four spares. Additional spares are included with a valid instrument service contract, or they can be purchased separately from Illumina.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Unlock doors**.
3. Select both sides, and then select **Unlock doors**.
   The instrument doors unlock, and you can the access the air filter drawer.
4. Press and hold the latch under the air filter drawer located at the bottom of the instrument.

5. Pull the filter drawer open.
6. Remove the used air filter.
7. Insert the new air filter so the label is facing towards you.
8. Close the filter drawer.
9. On the Unlock doors screen, select **Reset filter expiry**.
10. Make sure that the instrument doors are shut, and then select **Lock doors**.

Preventive Maintenance

Illumina recommends that you schedule a preventive maintenance service each year. If you are not under a service contract, contact your Territory Account Manager or Illumina Technical Support to arrange for a billable preventive maintenance service.

Perform a Maintenance Wash

A maintenance wash is required every 14 days or when a post-run wash fails or is incomplete.

The maintenance wash flushes the system with user-supplied dilutions of Tween 20 and NaOCl. The dilutions are pumped from the wash cartridges to the flow cell, used reagent bottles, and each cartridge reservoir to wash all sippers. Wash duration is about 3 hours.

A maintenance wash requires a wash reagent cartridge, a used buffer cartridge, and a new wash flow cell or a used 8-lane sequencing flow cell that has not been removed from the system.

Prepare Wash Solution

1. Add 400 ml laboratory-grade water to a 500 ml centrifuge bottle.
2. Add 0.2 ml 100% Tween 20 to result in at least 380 ml 0.05% Tween 20 wash solution.
   Using a freshly prepared dilution of Tween 20 limits the introduction of contaminants into the fluidics system.
3. Invert to mix.
4. Add wash solution to the wash solution well on the reagent cartridge.
5. Combine the following volumes in a 50 ml centrifuge tube to prepare 49 ml of 0.12% reagent grade NaOCl:
   - 5% reagent grade NaOCl (1.2 ml)
   - Deionized water (48 ml)

   Only use reagent grade NaOCl. Avoid general-purpose bleach products as these can contain ammonia compounds, which may lead to runs with low percentage passing filter reads.
6. Invert to mix.
7. Add 49 ml 0.12% reagent grade NaOCl to bleach well on the wash reagent cartridge.

Load Wash Consumables

Use the following instructions to load the maintenance wash consumables onto the instrument.

Start a Maintenance Wash

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Wash**.
3. Select an instrument side(s) to perform the wash on.
4. Proceed to *Load Wash Flow Cells* on page 117.

Load Wash Flow Cells

Perform a maintenance wash with either a used sequencing flow cell that is already loaded onto the instrument or a newly loaded wash flow cell. Use the following instructions as needed.

Used Sequencing Flow Cell

1. On the Wash screen, select **Unload wash flow cells**.
   After selecting, the display monitor raises and the flow cell door opens. The flow cell light indicates the instrument side that the wash is being performed on.
2. Confirm a used sequencing flow cell is already loaded into the system.
3. Select **Load wash flow cells**.
4. After all consumables are loaded, select **Start wash**.

New Wash Flow Cell

1. On the Wash screen, select **Unload wash flow cells**.
   After selecting, the display monitor raises and the flow cell door opens. The flow cell light indicates the instrument side that the wash is being performed on.
2. Inspect the flow cell stage for any contaminants (eg, particulates, lint, or dried reagent). If contaminants are visible, clean the flow cell stage as follows.
   a. Wet a polynit heatseal wipe with isopropyl alcohol (70%).
   b. Gently clean the applicable surface. Wipe in a lengthwise direction only.
      Unless you find contaminants on the manifolds, avoid touching them when wiping the flow cell stage.
   c. Repeat steps a and b until surfaces are clear of all contaminants.
   d. Dry with a new polynit heatseal wipe or an unused side of the used wipe to avoid contamination.
3. Put on a new pair of powder-free gloves to avoid contaminating the glass surface of the flow cell.
4. With the flow cell foil package over a flat surface, peel open the foil from the corner tab.
5. Remove the flow cell from the package. Grasp the flow cell by the sides to avoid touching the glass or the underside gaskets.
6. Inspect the wash flow cell for any contaminants (eg, particulates, lint, or dried reagent). If contaminants are visible, clean the wash flow cell as follows.
   a. Wet a polynit heatseal wipe with isopropyl alcohol (70%).
   b. Gently clean the applicable surface. Wipe in a lengthwise direction only.
   c. Repeat steps a and b until surfaces are clear of all contaminants.
   d. Dry with a new polynit heatseal wipe or an unused side of the used wipe to avoid contamination.
7. Discard the package appropriately.
8. Place the flow cell in the flow cell stage so that the top surface face upward.
9. After flow cells are loaded, select **Load wash flow cells**.

The control software displays information from the scanned flow cell after 1 minute.

10. After all consumables are loaded, select **Start wash**.

Load Wash Reagent Cartridge

Use the following instructions to load the wash reagent cartridge onto the instrument. You do not need to replace the buffer cartridge to perform a maintenance wash.

1. Rinse the wash reagent cartridge, and then dry the cartridge base using a paper towel.
   Do not dry inside the cartridge wells. Drying the reagent cartridge wash wells could impact the wash process.
2. On the Wash screen, select **Unload wash reagents**.
   The instrument doors unlock automatically and the system displays information on loading the wash reagent and buffer cartridges.
3. Remove the used sequencing reagent cartridge. Refer to *Recycle Used Consumables* on page 73 for instructions on recycling the reagent cartridges.
4. Load the wash reagent cartridge in the right position so that the Illumina label faces you.
5. Select **Load wash reagents**.
6. Empty the used reagent bottles. Refer to *Empty Used Reagent Bottles* on page 70 for more information.
7. Select **Confirm** after you have finished emptying the used reagent bottles.
8. Close the reagents drawers, and close the instrument doors.
9. After all consumables are loaded, select **Start wash**.
   After starting the wash, the instrument doors automatically lock. The doors unlock after the wash completes and returns you to the Start screen.

Empty Used Reagent Bottles

Use the following instructions to empty the used reagent bottles before every sequencing run. For information on recycling the used reagent cartridge and buffer bottles, refer to *Recycle Used Consumables* on page 73.

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

Empty Used Small Bottle

1. Remove the small used reagent bottle from the back of the used reagents drawer. Grasp the bottle by the sides.
2. Remove the threaded cap from the cap holder behind the used reagent bottles.
3. Seal the bottle opening with the cap to prevent spills.
4. Keeping the contents separate from the contents of the large bottle, discard in accordance with the applicable standards for your region.
5. Return the uncapped bottle to the used reagents drawer. Store the cap on the cap holder.

Empty Used Large Bottle

1. Using the top handle, remove the large used reagent bottle from the front of the used reagents drawer.
2. Remove the threaded cap from the cap holder behind the used reagent waste bottles.
3. Seal the bottle opening with the cap to prevent spills.
4. Uncap the vent.
   Uncapping the vent helps minimize spillage along the sides of the bottle.
5. Discard the contents in accordance with the applicable standards for your region. Grip both handles when emptying.
6. Seal the vent with the cap after the bottle is empty.

7. Return the uncapped bottle with the vent capped to the waste drawer. Store the threaded cap on the cap holder.

8. Put on a new pair of powder-free gloves.

Troubleshooting

This section provides step-by-step instructions for canceling a run, shutting down and restarting the instrument, and other troubleshooting procedures.

End a Run

You can end a sequencing run on-instrument. Ending a run on the NovaSeq X Plus is final. The software cannot resume the run or save sequencing data and consumables cannot be reused.

1. Navigate to the Sequencing screen.
2. Select **Cancel run** to end the run.
   Depending on the current state of the run, additional options to cancel data upload to your storage location or cancel analysis might be available.
3. Select **Yes, cancel run** to confirm ending the run.
   After you end the run, the run appears on the Completed tab with the Canceled status.

Requeue Secondary Analysis

During secondary analysis, you can requeue the run to perform on-instrument DRAGEN analysis again. You cannot requeue if there is a sequencing run in-progress or if the run data has been removed from the instrument.

To requeue analysis, do as follows.

1. Select the instrument icon to open the global navigation menu.
2. Select **Runs**, and then navigate to the Completed tab.
3. Select the run to requeue.
4. Under secondary analysis, select **Requeue analysis**.
5. If the requeue analysis is for a local run, select one of the following requeue options.
   - **Requeue analysis from this run**—Perform requeue using the files generated in the selected run. You can select a folder to output the requeued analysis files to.
   - **Requeue analysis from a sample sheet**—Perform requeue using a sample sheet. The requeue uses the analysis configurations specified in the sample sheet.
6. If the requeue analysis is for a cloud or manual run, select **Requeue analysis from sample sheet**.
7. Enter a sequencing data file path.
   This path determines the external storage location for the requeue analysis run.
8. Enter a description for the requeue in the Reason field.
9. Select **Requeue analysis** when finished.
10. Perform any of the following actions.

- To exclude a configuration from the requeue, select **Delete** next to the configuration name.
- To change configuration information, select **Edit** next to the configuration name.
- To include additional configurations, select **Add configuration**.

11. Select **Requeue analysis** when finished.

Shut Down or Restart the Instrument

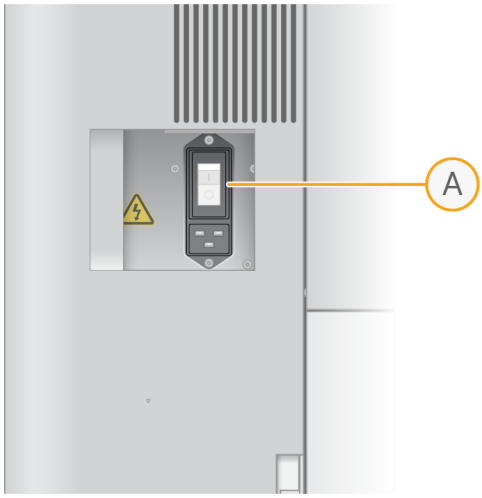
You can shut down the instrument safely when there are no sequencing runs or secondary analysis in-progress. Software messages indicate when to shut down and restart the instrument to resolve an error or warning.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Shut down**.
3. If the system does not shut down, press and hold the power button on the right side of the instrument for 5 seconds.

Figure 17 Power Button Location

4. Press the turn off (**O**) side of the toggle switch on the back of the instrument.

Figure 18 Power Toggle Switch Location



5. Wait 30 seconds.
6. Press the turn on (|) side of the toggle switch.
7. Press the power button on the right side of the instrument.
8. When the power button pulses, wait 30 seconds, and then press it.
   After the operating system has loaded, you can sign into the system.

Perform DRAGEN Self Test

You can perform a self test to identify if analysis errors are occurring as a result of one or more of your DRAGEN FPGA chips.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **DRAGEN**.
3. Select **Run self test**.

Install DRAGEN License

Your DRAGEN license is preinstalled on your instrument. You do not need to install your DRAGEN license unless troubleshooting license errors.

Only administrators can reinstall the DRAGEN license.

Use the following instructions to reinstall your DRAGEN license.

Install DRAGEN License Online

If the NovaSeq X Plus is connected to the internet, you can install DRAGEN licenses directly in the NovaSeq X Series Control Software.

1. In the control software, select the instrument icon to open the global navigation menu.

2. Select **Settings**, and then select **DRAGEN**.
3. Under Licenses select **Update from LUS**.
   The updated license is retrieved from the License Update Server.
4. After the new license is installed, select **Run Self-Test**.

Install DRAGEN License Offline

1. If your license file is corrupted, contact Illumina Technical Support to obtain a new license.
2. In the control software, select the instrument icon to open the global navigation menu.
3. Select **Settings**, and then select **DRAGEN**.
4. Under licenses select **Update**.
5. Navigate to your license *.zip file, and then select **Open**.
6. After the new license is installed, select **Run Self-Test**.

Review Audit Log

Administrators can review the instrument audit log on-instrument or on a networked computer. The audit log records all actions a user performs on the system.

Use the following instructions to review the audit log.

1. Select the instrument icon to open the global navigation menu.
2. Select **Settings**, and then select **Audit log**.
3. Use the following filters to refine audit log results.
   - Date—Filter actions by date range by selecting the calendar icon or manually entering dates in the From and To date fields in YYYY-MM-DD format.
   - Action type—Filter by the type of action performed by entering the action in the Type field.
   - User—Filter by the user that performed the action by entering the name of the user in the Who field.
   - Description—Filter by additional details by entering a description of the action in the Description field.
4. Select **Filter** to apply filters.
5. To export a PDF file of the audit log, select **Export log**.

Resources & References

The NovaSeq X Series support pages on the Illumina support site provide additional resources. Always check support pages for the latest versions.

Dark Cycle Sequencing

This section describes how to use dark cycle sequencing in the recipe.

Dark cycle sequencing is used to complete only the chemistry steps of a sequencing cycle. Check the Compatible Products page for your library prep kit on the Illumina Support Site to see if dark cycle sequencing is required.

Use the following steps for dark cycle sequencing.

1. Download the recipe XML file from the Illumina Support Site.
2. Edit the recipe XML file.
   a. Navigate to the `<ReadDefinitions>` section in the recipe file, and then identify the `<ReadDefinition Name="Read 1">` and the `<ReadDefinition Name="Read 2">` sections.
   b. In `<ReadDefinition Name="Read 1">`, add the following dark cycle step on a new line after `<ChemistryRef ChemistryName="FirstBase"/>`:

      ```
      <ChemistryRef ChemistryName="CompleteCycleReuse"/>.
      ```

   c. In `<ReadDefinition Name="Read 1">`, add the dark cycle step on a new line before `<ImagingRef ImagingName="Cycle1ReadImaging"/>`.
   d. In `<ReadDefinition Name="Read 2">`, add the dark cycle step on a new line before `<ImagingRef ImagingName="Cycle1ReadImaging"/>` and after `<ChemistryRef ChemistryName="FirstBase"/>`.
3. Save the recipe XML file.

The following is a sample recipe with the dark cycle step:

```
...
   <ReadDefinitions>
   <ReadDefinition Name="Read 1">
   <CycleStepCollection Name="Cycle1" Cycles="1">
   <ChemistryRef ChemistryName="FirstBase"/>
   <ChemistryRef ChemistryName="CompleteCycleReuse"/>
   <ImagingRef ImagingName="Cycle1Read1Imaging"/>
   </CycleStepCollection>
   <CycleStepCollection Name="CompleteCycle" Cycles="read1cycles-1">
   <ChemistryRef ChemistryName="CompleteCycleReuse"/>
   <ImagingRef ImagingName="CompleteCycleImaging" />
   </CycleStepCollection>
   </ReadDefinition>
   ...
   <ReadDefinition Name="Read 2">
   <CycleStepCollection Name="Cycle1" Cycles="1">
   <ChemistryRef ChemistryName="FirstBase"/>
   <ChemistryRef ChemistryName="CompleteCycleReuse"/>
   <ImagingRef ImagingName="CompleteCycleImaging" />
   </CycleStepCollection>
   <CycleStepCollection Name="CompleteCycle" Cycles="read2cycles-1">
   <ChemistryRef ChemistryName="CompleteCycleReuse"/>
   <ImagingRef ImagingName="CompleteCycleImaging" />
   </CycleStepCollection>
   </ReadDefinition>
   </ReadDefinitions>
   ...
```

Revision History

Document	Date	Description of Change
Document # 200027529 v01	February 2023	Added the following information for NovaSeq X Plus: • Protocol • Hardware components • Consumables and equipment • Software and system settings • Custom primers • Sequencing output files and structure • Maintenance and troubleshooting • Japan compliance Updated the following information: • Laser safety warnings • Crate contents and delivery requirements • Network connection requirements
Document # 200027529 v01	November 2022	• Added dimensions for sequencing consumables boxes. • Clarified RFID locations and instructions for opening the lyo insert cover. • Clarified instrument dimensions and network requirements.
Document # 200027529 v00	September 2022	Initial release.

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