illumina®

NovaSeq 6000Dx Instrument

Product Documentation

ILLUMINA PROPRIETARY
Document # 200010105 v02
August 2022

FOR IN VITRO DIAGNOSTIC USE

Revision History

Document	Date	Description of Change
Document # 200010105 v02	August 2022	Added safety information statement to system overview. Updated Safety and Compliance: • Added French laser warning and compliance statements for FCC, Canada, Japan, and Korea. • Consolidated EMC and safety information. Updated Site Preparation: • Added plug information for additional countries. • Removed plug information for China. Updated Consumables & Equipment: • Removed symbol key for consumables. • Updated part numbers from IUO to IVD. • Added 2 µl pipette. • Specified V2 wash cartridge. • Clarified consumables kit configurations. Updated Protocol: • Removed steps for preparation of NaOH. • Removed steps for denature and dilute. • Reordered sequencing run setup steps. • Specified that flow cell is boxed when it is removed from storage. • Provided temperature range for room temperature in flow cell preparation instructions. Updated Maintenance & Troubleshooting: • Clarified that staggered start of maintenance washes is not supported. • Removed reference to storing library tube from troubleshooting instructions.

Document	Date	Description of Change
Document # 200010105 v01	April 2022	Added Tris-HCl, pH 8.5 to user-supplied consumables. Specified temperature range for room temperature water bath. Corrected output size for S2 flow cells. Corrected catalog numbers for S2 and S4 buffer cartridges and library tubes. Corrected Tris-HCl, pH 7.0 to Tris-HCl, pH 8.0.
Document # 200010105 v00	March 2022	Initial release.

Table of Contents

System Overview

The Illumina® NovaSeq 6000Dx™ Instrument packages scalable throughput and flexible sequencing technology into a production-scale platform with the efficiency and cost-effectiveness of a benchtop system.

Features

- **Scalable sequencing**—The NovaSeq 6000Dx scales up to production-level sequencing with high-quality data for a broad range of applications.
- **Patterned flow cell**—A patterned flow cell generates tightly spaced clusters for high cluster density and data output.
- **Onboard ExAmp mixing**—The NovaSeq 6000Dx mixes the ExAmp reagents with the library, amplifies the library, and performs cluster generation for a streamlined sequencing workflow.
- **High-throughput line scanning**—The NovaSeq 6000Dx uses one camera with bidirectional scanning technology to quickly image the flow cell in two color channels simultaneously.
- **Dual mode**—The NovaSeq 6000Dx includes a single boot hard drive with separate *in vitro* diagnostic (IVD) and research use only (RUO) modes. The mode is selected using the toggle on the Sequencing, Runs, and Applications screens. After it is selected, the mode is clearly labeled on all screens.
- **Illumina DRAGEN Server for NovaSeq 6000Dx**—The included DRAGEN Server provides hardware accelerated data analysis.
- **Illumina Run Manager**—Plan runs, manage users, and set up analysis applications both on the NovaSeq 6000Dx and off instrument through a web browser using Illumina Run Manager.

Dual Mode Considerations

In vitro diagnostic (IVD) sequencing assays are executed in IVD mode. Only IVD sequencing reagents can be used in IVD mode. Always make sure that the correct mode is selected before beginning run planning.

This resource describes the use of the NovaSeq 6000Dx Instrument in IVD mode unless otherwise noted. Refer to the *NovaSeq 6000 Sequencing System Guide (document # 1000000019358)* for information on RUO features, including BaseSpace Sequence Hub integration.

Safety Considerations

Review *Safety & Compliance* on page 7 before performing any procedures on the system.

Sequencing Overview

Sequencing on the NovaSeq 6000Dx comprises cluster generation, sequencing, and base calling. Each step occurs automatically during a sequencing run. Secondary analysis is then performed on the Illumina DRAGEN Server for NovaSeq 6000Dx when the run is complete.

Cluster Generation

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

Sequencing

Clusters are imaged using two-channel chemistry, one green channel and one red channel, to encode data for the four nucleotides. The flow cell is scanned in multiple passes and each scan is analyzed as individually imaged tiles. The process is repeated for each cycle of sequencing.

Primary Analysis

During the sequencing run, Real-Time Analysis (RTA3) software performs base calling[1], filtering, and quality scoring.[2] As the run progresses, the control software automatically transfers concatenated base call files[3] (*.cbcl) to the specified output folder for data analysis.

Secondary Analysis

When sequencing and primary analysis are complete, secondary analysis begins. The method of secondary data analysis depends on your application and system configuration. Vvarious secondary analysis options are available for both RUO and IVD run types. If a sequencing run is created using an Illumina Run Manager application that uses the Illumina DRAGEN Server for NovaSeq 6000Dx to perform secondary analysis, sequencing data is sent to the server for analysis using the analysis app selected during run setup.

[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.

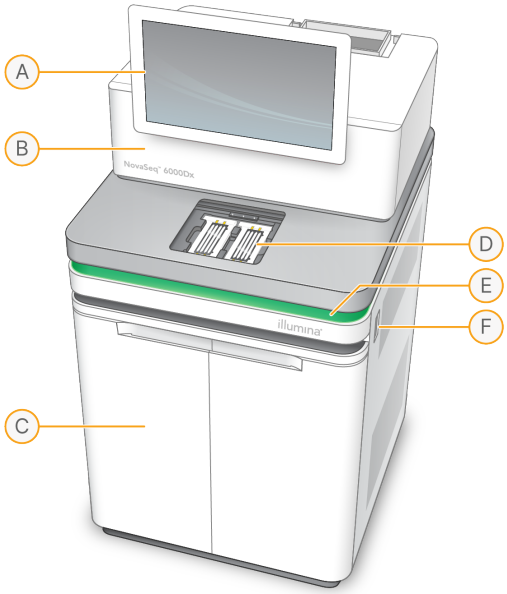
[3]Contains the base call and associated quality score for every cluster of each sequencing cycle.

Instrument Components

The NovaSeq 6000Dx Instrument comprises a touch screen monitor, a status bar, a power button with adjacent USB ports, and three compartments.

External Components

Figure 1 External Components



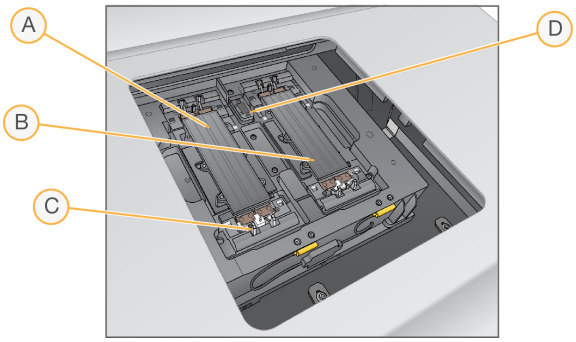
A. **Touch screen monitor**—Displays the instrument interface for system configuration and run setup and monitoring.
B. **Optics compartment**—Contains the optical components that enable dual surface imaging of flow cells.
C. **Liquids compartment**—Contains reagent and buffer cartridges, and bottles for used reagents.
D. **Flow cell compartment**—Holds the flow cells.
E. **Status bar**—Indicates flow cell status as ready to sequence (green), processing (blue), or needs attention (orange).
F. **Power and USB ports**—Provides access to the power button and USB connections for peripheral components.

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. Each side has four clamps that automatically position and secure the flow cell.

An optical alignment target mounted on the flow cell stage diagnoses and corrects optical problems. When prompted by the NovaSeq Operating Software (NVOS), the optical alignment target realigns the system and adjusts camera focus to improve sequencing results.

Figure 2 Flow Cell Stage Components



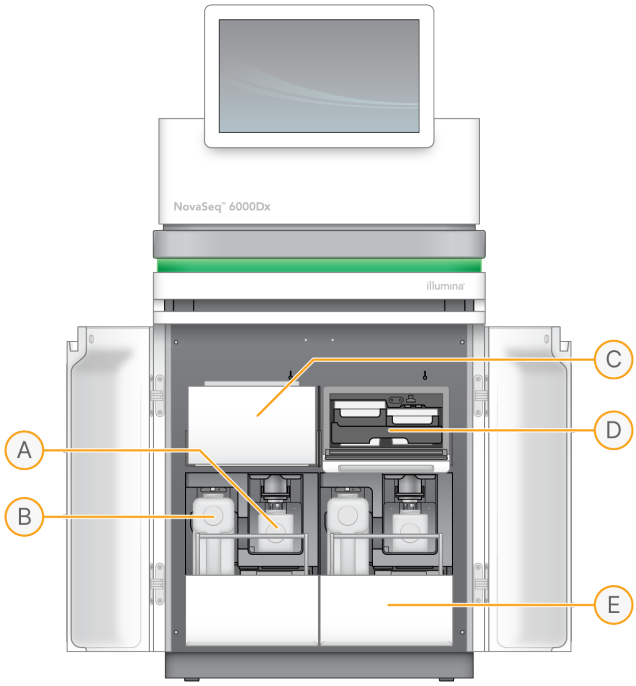
A. Side A flow cell holder
B. Side B flow cell holder
C. Flow cell clamp (one of four per side)
D. Optical alignment target

NVOS controls the opening and closing of the flow cell compartment door. The door opens automatically to load a flow cell for a run or maintenance wash. After loading, the software closes the compartment door, moves the flow cell into position, and engages the clamps and vacuum seal. Sensors verify the presence and compatibility of the flow cell.

Liquids Compartment

Setting up a run requires accessing the liquids compartment to load reagents and buffer, and empty used reagent bottles. Two doors enclose the liquids compartment, which is divided into two matching sides for flow cell A and flow cell B.

Figure 3 Liquids Compartment Components



A. **Small used reagent bottle**—Holds used reagents from the cluster cartridge, with a cap holder for easy cap storage.
B. **Large used reagent bottle**—Holds used reagents from the SBS and buffer cartridges, with a cap holder for easy cap storage.
C. **Reagent chiller**—Refrigerates the SBS and cluster cartridges.
D. **Reagent chiller drawer**—Color-coded positions hold the SBS cartridge on the left (gray label) and the cluster cartridge on the right (orange label).
E. **Buffer drawer**—Holds the large used reagent bottle on the left and the buffer cartridge on the right.

Used Reagents

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

If the system is configured to collect used reagents externally, the stream to the large used reagent bottle is routed externally. Cluster cartridge reagents go to the small used reagent bottle.

Instrument Software

The NovaSeq 6000Dx with DRAGEN Server includes integrated applications that perform sequencing runs, analysis on-instrument and on the server, and other related functions. For more information on configuring instrument software, refer to *System Configuration* on page 32.

- **NovaSeq Operating Software (NVOS)**—Guides you through loading procedures, controls instrument operations, and displays statistics as the run progresses. NVOS operates the flow cell stage, dispenses reagents, controls fluidics, sets temperatures, captures images of clusters on the flow cell, and provides a visual summary of quality statistics.
- **Real-Time Analysis (RTA)**—Performs image analysis and base calling during a run. NovaSeq 6000Dx uses RTA3, which incorporates architecture, security, and other feature enhancements to optimize performance.
- **Universal Copy Service (UCS)**—Copies output files from RTA3 and NVOS to the output folder and to the DRAGEN Server throughout a run. If the Universal Copy Service is interrupted during a run, the service makes multiple attempts to reconnect and automatically resume data transfer.
- **Illumina Run Manager**—Plan runs, view planned runs, and review run results on the NovaSeq 6000Dx or remotely using a web browser. Illumina Run Manager also controls user and application permissions.
- **Illumina DRAGEN Server for NovaSeq 6000Dx**— When sequencing on the NovaSeq 6000Dx is complete, the analysis begins on the DRAGEN Server. Analysis on the DRAGEN Server and sequencing on the instrument can run concurrently.

Safety & Compliance

This section provides important safety information pertaining to the installation, servicing, and operation of the NovaSeq 6000Dx Instrument, as well as product compliance and regulatory statements. Read this information 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.

CAUTION

If liquid is found on the floor near the instrument, avoid contact with the liquid and instrument and immediately restrict access to the area. Shut off power to the instrument using the power breaker. Immediately contact Illumina Technical Support.

General Safety Warnings

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

Flow Cell Safety Warnings

CAUTION

Use caution to keep fingers out of the flow cell door to avoid injury.

Heavy Object Safety Warning

The instrument weighs approximately 447 kg (985 lb) shipped and approximately 576 kg (1270 lb) installed and can cause serious injury if dropped or mishandled.

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.

Laser Safety Warning

The NovaSeq 6000Dx Instrument is a Class 1 laser product that contains two Class 4 lasers, one Class 3B laser, and one Class 3R laser.

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.

Class 3B lasers present an eye hazard. They can heat skin and materials, but are not a burn hazard.

Class 3R lasers present an eye hazard from direct eye exposure to the laser beam. Do not operate the instrument with any of the panels removed. When the flow cell door is open, safety interlock switches block the laser beam. If you operate the instrument with any panels removed, you risk exposure to direct or reflected laser light.

Figure 4 Class 4 and Class 3R Laser Warning (English)

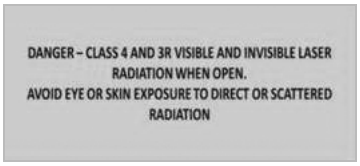
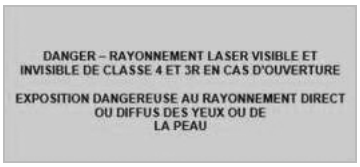


Figure 5 Class 4 and Class 3R Laser Warning (French)



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.

Product Compliance and Regulatory Statements

EMC Considerations

Evaluate the electromagnetic environment before operation of the device. 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.

This IVD medical equipment complies with the emissions and immunity requirements described in IEC 61326-2-6. This equipment is designed for use in a professional healthcare facility environment. It is likely to perform incorrectly if used in a home healthcare environment. If it is suspected that performance is affected by electromagnetic interference, correct operation may be restored by increasing distance between the equipment and the source of the interference. This equipment is not intended for use in residential environments and may not provide adequate protection to radio reception in such environments. Electromagnetic environment should be evaluated prior to operation of the device.

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

The intended use environment for the NovaSeq 6000Dx is limited to laboratory environments of professional healthcare facilities. The instrument is not intended to be used in any of the following environments: physician offices; intensive care units; emergency rooms or ambulatory centers; surgical or operating rooms; healthcare clinics; patient rooms; dental offices; limited care facilities; nursing homes; drugstores or pharmacies; first aid rooms; or near high sources of electromagnetic radiation (eg, MRI). Based on the intended use environment defined above, the NovaSeq 6000Dx is considered to be a CONTROLLED ELECTROMAGNETIC ENVIRONMENT with fixed electromagnetic sources and any malfunction of the NovaSeq 6000Dx will not directly cause harm, serious injury, or death of a patient when the NovaSeq 6000Dx is used as intended. Electromagnetic sources that might be used adjacent to NovaSeq 6000Dx include the following:

- Radio frequency identification (RFID) systems
- Wireless local area networks (WLAN)
- Handheld mobile radios (eg, TETRA, two-way radio)
- Paging systems
- Other wireless devices (including consumer devices)

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)*.

Simplified Declaration of Conformity

Illumina, Inc. hereby declares that the NovaSeq 6000Dx Instrument 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.

Waste Electrical and Electronic Equipment Regulation (WEEE)

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

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

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.

CAUTION

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.

NOTE 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.

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.

Japan Compliance

この装置は、クラスA機器です。この装置を住宅環境で使用すると電波妨害
を引き起こすことがあります。この場合には使用者が適切な対策を講ずるよう
要求されることがあります。VCCI - A

Korea Compliance

해당무선설비는운용중전파혼신가능성이있음.
A급기기(업무용방송통신기자재)
이기기는업무용(A급)으로전자파적합로서판매자또는사용자는이점을주의
하시기바라며, 가정외의지역에서사용하는것을목적으로합니다.

United Arab Emirates Compliance

- TRA Registered Number: ER0117765/13

- Dealer Number: DA0075306/11

Thailand Compliance

This telecommunication equipment conforms to the requirements of the National Telecommunications Commission.

Site Preparation

This section provides specifications and guidelines for preparing your site for installation and operation of the NovaSeq 6000Dx Instrument.

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.

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

CAUTION

Only 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, make sure that the path for data storage is selected before the date of installation. The Illumina representative can test the data transfer process during installation.

CAUTION

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.

DRAGEN Server Delivery

Refer to the Illumina DRAGEN Server for NovaSeq 6000Dx Product Documentation on the Illumina support site for information on DRAGEN Server delivery and installation information.

Crate Dimensions and Contents

The NovaSeq 6000Dx and components are shipped in one wooden crate (Crate #1) and one cardboard crate (Crate #2). Use the following dimensions to determine the minimum door width required to accommodate the shipping crates.

Measurement	Crate #1	Crate #2
Height	155 cm (61 in)	84 cm (33 in)
Width	104 cm (41 in)	122 cm (48 in)

Measurement	Crate #1	Crate #2
Depth	155 cm (61 in)	102 cm (40 in)
Crated Weight	628 kg (1385 lbs)	176 kg (388 lbs)

For Crate #1, the forklift access points are on the depth side of the crate. Take this into consideration for doorway and elevator clearance when transporting the instrument in the crate.

The weight of the UPS and external battery pack for the UPS contained in Crate #2 can vary based on the model shipped.

- Crate #1 contains the instrument.
- Crate #2 contains five boxes with the following contents:
  - Box—Uninterruptible power supply (UPS), weight 46 kg (100 lbs)
  - Box—External battery pack for the UPS, weight 64 kg (140 lbs)
  - Box—Accessories, total weight 31 kg (68 lbs)
    - Monitor
    - Large used reagents bottle and small used reagents bottle
    - Instrument leak tray
    - Wire thaw racks (4)
    - Wash flow cells (2)
    - SBS wash cartridges (2)
    - Cluster wash V2 cartridges (2)
    - Wireless keyboard and mouse, if appropriate for the region. In the absence of a wireless keyboard, use a wired keyboard.
  - Box—Additional components
    - Two buffer tray bottles that are individually packaged
    - Region-specific power cord
    - *IVD Instrument Doc Card (document # 200016882)*
  - Box—Chimney adapter

Laboratory Requirements

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

Instrument Placement

Figure 6 Instrument Dimensions

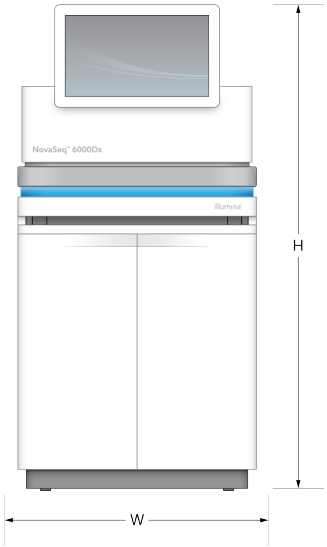


Table 1 Instrument Dimensions

Measurement	Instrument Dimensions*
Height	165.6 cm (65.2 in)
Width	80.0 cm (31.5 in)
Depth	94.5 cm (37.2 in)
Weight	481 kg (1059 lb)

* The UPS system is not included in this dimension, additional space must be allocated.

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.
- Place the UPS on either side of the instrument. The UPS can be placed within the minimum clearance range of the instrument sides.

Table 2 Instrument Clearance

Access	Minimum Clearance
Front	Allow at least 152.4 cm (60 in) in front of the instrument for opening the liquids compartment and to provide general lab access for movement of personnel around the lab.
Sides	Allow at least 76.2 cm (30 in) on each side of the instrument for access and clearance around the instrument. Instruments placed side by side only requires 76.2 cm (30 in) total between the 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.

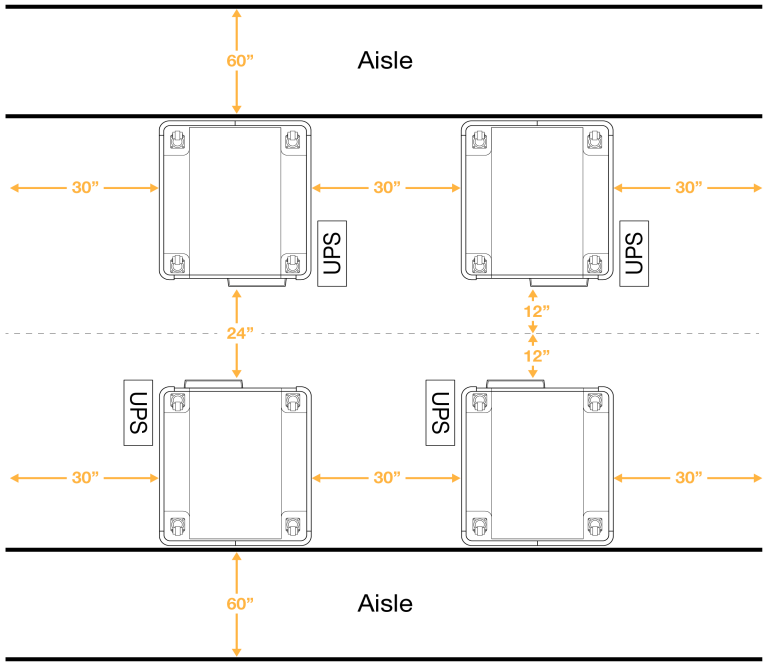
CAUTION

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

Multisystem Installation Layout

Refer to the diagram for an example layout for a multisystem installation, including minimum spacing requirements.

Figure 7 Multisystem Installation Layout



Environmental Considerations

Table 3 Instrument Environmental Specifications

Element	Specification
Air Quality	Operate the instrument in a Pollution Degree II environment or better. A Pollution Degree II environment is defined as an environment that normally includes only nonconductive pollutants.
Elevation	Locate the instrument at an altitude below 2000 meters (6500 feet).
Humidity	Transportation and Storage: Non-condensing humidity between 15–80%. Operating Conditions: Maintain a noncondensing relative humidity between 20–80%.
Location	Operate the instrument in indoors environments only.
Temperature	Transportation and Storage: -10°C to 50°C (14°F to 122°F). Operating Conditions: Maintain a lab temperature of 19°C to 25°C (22°C ±3°C). This temperature is the operating temperature of the instrument. During a run, do not allow the ambient temperature to vary more than ±2°C.
Ventilation	Consult your facilities department for ventilation requirements based on the instrument heat output specifications.

Element	Specification
Vibration	Limit the continuous vibration of the lab floor to ISO office level. During a sequencing run, do not exceed ISO operating room limits. Avoid intermittent shocks or disturbances near the instrument.

Table 4 Heat Output

Peak Power Consumption	Thermal Output
2500 Watts	Maximum 8530 BTU/h Average 6000 BTU/h

Table 5 Noise Output

Noise Output	Distance From Instrument
< 75 dB	1 meter (3.3 feet)

Bulk Used Reagent Handling

The NovaSeq 6000Dx is 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.

Venting

A 10 inch, round, vertical chimney vents 60% 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.

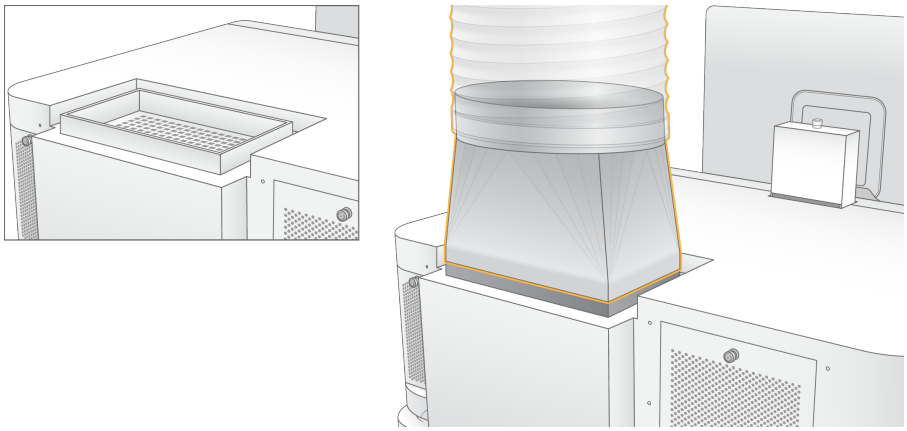
- Flexible ducting is preferred.
- Avoid bending flexible ducts where possible. Keep bends in flexible ducts to a minimum.
- Flexible ducts with bends must maintain the 10 inch diameter of the chimney at all points.
- Remove kinks or other restrictions to the airflow.
- Rigid ducting can be used. Use 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.

CAUTION

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

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

Figure 8 Chimney Placement for Venting



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.

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.
- 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 Considerations

Table 6 Power Specifications

Type	Specification
Line Voltage	200–240 VAC at 50/60 Hz
Peak Power Consumption	2500 Watts

For 200–240 Volts AC, your facility must be wired with a minimum 15 Amp grounded line with proper voltage. 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.

Fuses

The instrument contains no user-replaceable fuses.

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.

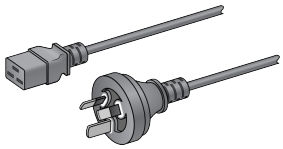
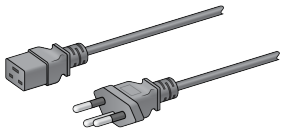
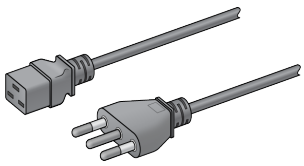
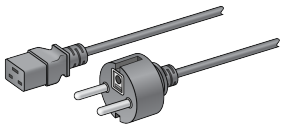
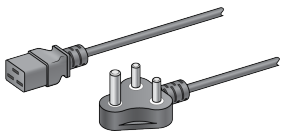
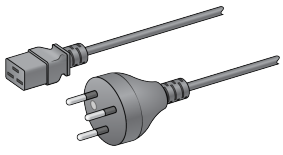
Hazardous voltages are removed from the instrument only when the power cord is disconnected from the AC power source.

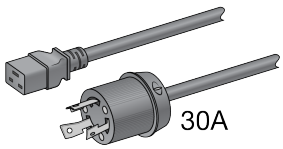
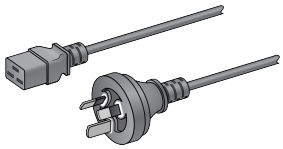
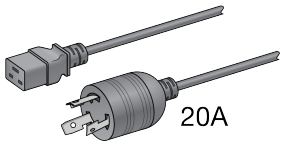
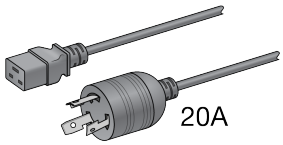
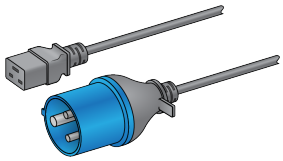
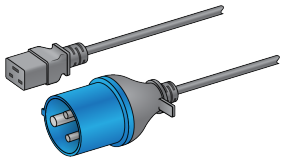
CAUTION

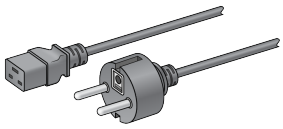
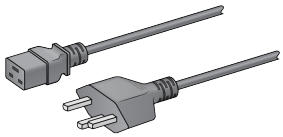
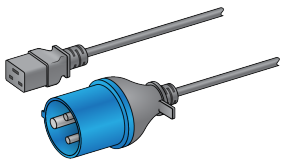
Never use an extension cord to connect the instrument to a power supply.

The following table lists the supported power supply for your region. Alternatively, all regions can use IEC 60309.

Table 7 Power Cord Requirements of Selected Regions

Region	Shipped Power Cord	Electrical Supply	Socket
Australia	AS 3112 SAA Male to C19, 15 Amps 	230 VAC, 15 Amps	15 Amp Type I
Brazil	NBR14136 Plug to C19, 16 Amps 	220 VAC, 16 Amps	NBR 14136 Type N
Chile	CEI 23-16 to C19, 16 Amps 	220 VAC, 16 Amps	CEI 23-16/VII, Type L
European Union[1] Serbia Ukraine	Schuko CEE 7 (EU1-16p) to C19, 16 Amps 	220–240 VAC, 16 Amps	Schuko CEE 7/3
India	IS1293 to C19, 16 Amps 	230 VAC, 16 Amps	BS546A Type M
Israel	IEC 60320 C19, 16 Amps 	230 VAC, 16 Amps	SI 3216 Amp Type H

Region	Shipped Power Cord	Electrical Supply	Socket
Japan	NEMA L6-30P, 30 Amps 	200 VAC, 30 Amps	NEMA L6-30R
New Zealand	AS 3112 SAA Male to C19, 15 Amps 	230 VAC, 15 Amps	Dedicated 15 Amp Type I
North America Colombia	NEMA L6-20P to C19, 20 Amps 	208 V, 16 Amps	NEMA L6-20R
Peru Philippines	NEMA L6-20P to C19, 20 Amps 	220 VAC, 16 Amps	NEMA L6-20R
Saudi Arabia	IEC60309 316P6 to C19, 16 Amps 	220 VAC, 16 Amps	IEC60309 316C6
Singapore	IEC60309 316P6 to C19, 16 Amps 	230–250 VAC, 16 Amps	IEC60309 316C6

Region	Shipped Power Cord	Electrical Supply	Socket
South Korea Thailand	Schuko CEE 7 (EU1-16p) to C19, 16 Amps 	220 VAC, 16 Amps	Schuko CEE 7/3
Switzerland	SEV 1011 Type 23 Plug J, 16 Amps 	230 VAC, 16 Amps	SEV 1011 Type 23 J socket
United Kingdom	IEC60309 316P6 to C19, 16 Amps 	230–250 VAC, 16 Amps	IEC60309 316C6

[1] Excepting Switzerland and the United Kingdom.

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 24.

- **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
Weight	95 kg (210 lb)
Dimensions (Tower format: H × W × D)	43.2 cm × 66.7 cm × 17 cm (17 in × 26.26 in × 6.72 in)

* The UPS requires up to a maximum of 330 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	SUA3000UXI
Japan	SRT5KXLJ
Mexico	SRT3000RMXLW-IEC
South Korea	SRT3000RMXLW-IEC
Thailand	SRT3000RMXLW-IEC

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

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

Consumables and Equipment

This section lists everything needed for a NovaSeq 6000Dx sequencing run. This includes Illumina supplied consumables and ancillary consumables and equipment that you must purchase from other suppliers. These items are required to complete the protocol and to perform maintenance and troubleshooting procedures.

For information on the symbols on consumables or consumable packaging, refer to *Illumina IVD Symbol Key (document # 1000000039141)*.

Sequencing Consumables

A NovaSeq 6000Dx run requires the following components:

- Buffer cartridge
- Cluster cartridge
- Flow cell
- Library tube
- SBS cartridge

NovaSeq 6000Dx consumables are packaged in the following configurations. Each component uses radio-frequency identification (RFID) for accurate consumable tracking and compatibility.

Table 8 Illumina-Supplied Consumables

Kit Name	Contents	Illumina Catalog Number
NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles)	S2 cluster cartridge S2 flow cell S2 SBS cartridge	20046931
NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles)	S4 cluster cartridge S4 flow cell S4 SBS cartridge	20046933
NovaSeq 6000Dx S2 Buffer Cartridge	S2 buffer cartridge	20062292
NovaSeq 6000Dx S4 Buffer Cartridge	S4 buffer cartridge	20062293
NovaSeq 6000Dx Library Tube	Single library tube	20062290
NovaSeq 6000Dx Library Tube, 24 Pack	24 library tubes	20062291

When you receive your consumables, promptly store components at the indicated temperature to ensure proper performance.

Table 9 NovaSeq 6000Dx Kit Storage

Consumable	Quantity	Storage Temperature	Length	Width	Height
Flow cell	1	2°C to 8°C	27.7 cm (10.9 in)	17 cm (6.7 in)	3.8 cm (1.5 in)
Cluster cartridge	1	-25°C to -15°C	29.5 cm (11.6 in)	13 cm (5.1 in)	9.4 cm (3.7 in)
SBS cartridge	1	-25°C to -15°C	30 cm (11.8 in)	12.4 cm (4.9 in)	11.2 cm (4.4 in)
Buffer cartridge	1	15°C to 30°C	42.2 cm (16.6 in)	20.6 cm (8.1 in)	21.1 cm (8.3 in)
Library tube	1	15°C to 30°C	4.1 cm (1.6 in)	2.3 cm (0.9 in)	12.4 (4.9 in)

Consumables Details

To identify compatible kit components, flow cells and cartridges are labeled with symbols that show the kit mode.

Table 10 Compatibility Labeling

Kit Mode	Marking on Label	Description
S2 kit components	S2	S2 flow cell generates up to 4.1 billion single reads passing filter with output up to 1000 Gb at 2 x 150 bp. The S2 flow cell provides fast sequencing for most high-throughput applications.
S4 kit components	S4	S4 flow cell generates up to 10 billion single reads passing filter with output up to 3000 Gb at 2 x 150 bp. The S4 flow cell is a four-lane version of the flow cell, designed for maximum output.

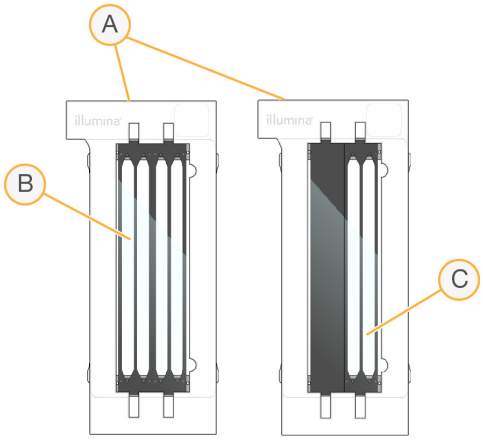
Flow Cell

The NovaSeq 6000Dx flow cell is a patterned flow cell encased in a cartridge. The flow cell is a glass-based substrate containing billions of nanowells in an ordered arrangement. Clusters are generated in the nanowells from which sequencing is then performed.

Each flow cell has multiple lanes for sequencing pooled libraries. The S2 flow cell has two lanes and the S4 flow cell has four. Each lane is imaged in multiple swaths, and the software then divides the image of each swath into smaller portions called tiles.

Some scratches and other minor cosmetic defects on the flow cell are normal and not expected to compromise data quality and yield. Illumina recommends using these flow cells as per normal.

Figure 9 Flow Cells



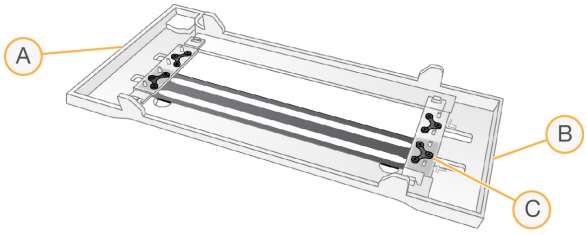
A. Flow cell cartridge
B. Four-lane flow cell (S4)
C. Two-lane flow cell (S2)

The underside of each flow cell has multiple gaskets. Libraries and reagents enter the flow cell lanes through the gaskets on the inlet end of the flow cell. Used reagents are expelled from the lanes through the gaskets at the outlet end.

CAUTION

Avoid touching the gaskets when handling the flow cell.

Figure 10 Inverted Flow Cell



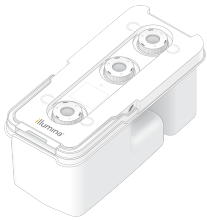
A. Outlet end
B. Inlet end
C. Gasket (one of four)

Buffer, Cluster, and SBS Cartridge Details

The NovaSeq 6000Dx buffer, cluster, and SBS cartridges have foil-seal reservoirs prefilled with reagents, buffers, and wash solution. Cluster and SBS cartridges are included with NovaSeq 6000Dx reagent kits. The buffer cartridge is sold separately.

The cartridges load directly onto the instrument and are color-coded and labeled to reduce loading errors. Guides in the reagent chiller and buffer drawers ensure proper orientation.

Table 11 NovaSeq 6000Dx Cartridges

Consumable	Description
Buffer cartridge 	Prefilled with sequencing buffers and weighs up to 6.8 kg (15 lb). A plastic handle facilitates carrying, loading, and unloading. The buffer cartridge contains reagents that are sensitive to light. Keep the buffer container packaged until use.
Cluster cartridge 	Prefilled with clustering, indexing, and paired-end reagents and wash solution. Includes a designated position for the library tube. Orange labeling distinguishes the cluster cartridge from the SBS cartridge. A denaturation reagent in position #30 contains formamide, which is an organic amide and reproductive toxin. To facilitate safe disposal of any unused reagent after the sequencing run, this reservoir is removable.
SBS cartridge 	Prefilled with sequencing reagents at volumes specific to the number of cycles the kit supports. Each of the three reagent positions has an adjacent position reserved for the automatic post-run wash. Gray labeling distinguishes the SBS cartridge from the cluster cartridge. The SBS cartridge contains reagents that are sensitive to light. Keep the SBS container packaged until use.

Reserved Cluster Cartridge Reservoirs

Three reservoirs are reserved for custom primers and an empty position is reserved for the library tube. For sample traceability, the library tube is loaded into the cluster cartridge during run setup and remains with the cartridge through the end of the run.

Figure 11 Numbered Reservoirs

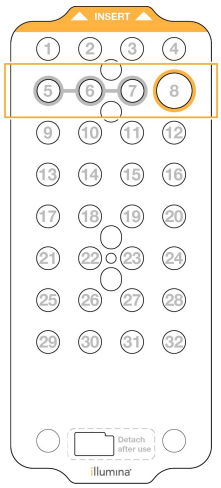


Table 12 Cluster Cartridge Reservoirs

Position	Reserved For
5, 6, and 7	Optional custom primers
8	Library tube

User-Supplied Consumables and Equipment

Table 13 Consumables

Consumable	Supplier	Purpose
Centrifuge bottle, 500 ml	General lab supplier	Diluting Tween 20 for a maintenance wash.
Centrifuge tube, 30 ml	General lab supplier	Diluting NaOCl for a maintenance wash.
Disposable gloves, powder-free	General lab supplier	General purpose.
Isopropyl alcohol wipes, 70% or Ethanol alcohol wipes, 70%	VWR, catalog # 95041-714, or equivalent General lab supplier	Cleaning components before a run and general purpose.
Lab tissue, low-lint	VWR, catalog # 21905-026, or equivalent	Drying the flow cell stage and general purpose.
Reagent grade NaOCl, 5%	Sigma-Aldrich, catalog # 239305	Performing a maintenance wash.

Consumable	Supplier	Purpose
Pipette tips, 2 µl	General lab supplier	Pipetting for diluting and loading libraries.
Pipette tips, 20 µl	General lab supplier	Pipetting for diluting and loading libraries.
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 (99%), 100 ml bottle	General lab supplier	Cleaning optics components periodically and support the objective cleaning cartridge.
Tween 20	Sigma-Aldrich, catalog # P7949	Performing a maintenance wash.
Water, laboratory-grade	General lab supplier	Diluting Tween 20 and sodium hypochlorite for a maintenance wash.

Table 14 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 two reagent cartridges and the appropriate water level. For example, (61 cm × 91.4 cm × 25.4 cm)(24 in × 36 in × 10 in).

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

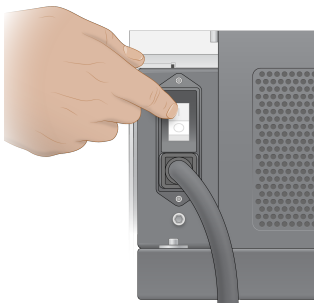
System Configuration

This section provides instructions for setting up the instrument, including descriptions of the instrument menus and the settings they contain.

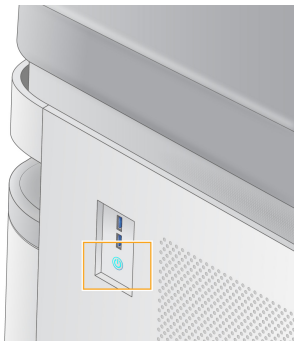
Starting the Instrument

The first time the system is turned on, NVOS is launched with a series of screens to guide you through first time setup. First time setup includes performing a system check to confirm instrument performance and configuring system settings.

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



2. Wait until the power button on the right side of the instrument glows blue, and then press the power button.



3. Wait until the operating system has finished loading. Use the NVOS icon to launch the control software. After the system initializes, a Sign In screen is displayed.
4. Enter the administrator user name and password provided by your Illumina representative at the time of installation.

Remote Access

The instrument interface can be accessed both on-instrument and remotely using a compatible browser. To access the instrument remotely, use the address and user account information provided by your Illumina representative. Compatible browsers are Chrome/Chromium, Edge, Firefox, and Safari.

Settings Menu

Access the following settings using the menu icon at the top left of any screen.

Setting	Description	On-Instrument Menu	Browser Menu
About DRAGEN	View information about DRAGEN Server, including: • Installed DRAGEN versions • Licensing information • FPGA serial number	X	X
About Instrument	View information about the instrument, including: • Instrument name • NVOS Version • Serial number • Available space • DRAGEN version	X	
Audit Log	View user logs, including: • User name • Type of action • Description of action • Date and time of action	X	X
DRAGEN	Edit DRAGEN Server settings. Refer to *DRAGEN Server Configuration* on page 38 for more information.	X	X
External Storage for Analysis	Configure external storage.	X	X
Instrument Pairing	Pair instrument with DRAGEN Server.	X	
Instrument Settings	View and edit RUO, IVD, and global settings.	X	
Process Management	Manage disk space.	X	

Setting	Description	On-Instrument Menu	Browser Menu
User Management	View and edit user information. Refer to *User Accounts* on page 35 for more information.	X	X

Process Management

The Process Management screen is accessible from the settings menu on the instrument. Use the screen to monitor run progress and manage disk space. Never delete files and folders directly from C:\.

Process Management displays available disk space, space used on CE and C:\, and the status of runs using disk space. Run Date and Name columns identify each run. For each run, Process Management lists the status of the following processes:

- **Run Status**—Based on the processing of CBCL files.
- **DRAGEN Server**—Based on file transfer to the Illumina DRAGEN Server for NovaSeq 6000Dx.
- **Network**—Based on file transfer using Universal Copy Service.

Table 15 Process Management Status Icons

Process	Icon	Description
Run Status	 Running	The run is in progress.
	 Complete	The run has completed sequencing or analysis.
DRAGEN Server	 Uploading	Files are being uploaded to DRAGEN Server.
	 Complete	All files are uploaded to DRAGEN Server.
Network	 Copying	Files are being copied to the output folder on the network.
	 Complete	All files are copied to the output folder on the network.
	N/A	Not applicable because the run is not configured to upload to a network output folder or the upload status is unknown.

For more information on process management troubleshooting, refer to *Troubleshooting* on page 70.

User Accounts

User account settings are found on the User Management screen, which is accessible through the Settings menu on the instrument and via browser. Only administrators can access the User Management screen. You must be signed in to use the instrument.

Application Permissions

You cannot use an application that has not been assigned to you.

Passwords

By default, passwords must be reset at least every 180 days. Admin users can configure settings to require more frequent password resets. Change your password on the User Management screen or by selecting your user icon at the top right of the interface.
Illumina does not store or maintain customer login credentials. Password security is the responsibility of the user.

User Roles

By default, new user accounts are assigned the role of user. Administrator and operator roles provide additional permissions.

Table 16 User Permissions

Permissions	Administrator	Operator	User
Grant access to administrator functions	X		
Configure app settings and permissions	X		
Pair instrument and server	X		
Initiate wash	X	X	
Set up and start sequencing runs	X	X	
View ongoing sequencing runs	X	X	X
Exit and minimize app	X		
Access Process Management screen	X	X	
Access instrument settings	X		

Permissions	Administrator	Operator	User
Turn off instrument	X	X	
Change forgotten passwords	X		
View instrument audit log	X		

Instrument Settings

The Instrument Settings screen is composed of three tabs: Global Settings, IVD Settings, and RUO Settings.

Global Settings

Global settings include the following options:

- **Instrument mode**— Control whether users can switch between IVD and RUO modes.
- **Proactive Support**—Toggle monitoring from Proactive Support.
- **User Idle Timeout**—Control the amount of time instrument can be idle before signing out users.

IVD Settings

IVD settings apply when the instrument is in IVD mode.

- **Run Setup**—Select run mode. Refer to *Configure Run Mode* on page 36 for more information.
- **Output Location**—Select server location for data output. Refer to *Data Output and Storage* on page 37 for more information on data output.

RUO Settings

The RUO settings include the following options:

- **Run Setup**— Select run mode. Refer to *Configure Run Mode* on page 36 for more information.
- **Default Workflow Type**—Control whether the NovaSeq Xp workflow is set as the default workflow type. NovaSeq Xp is available only in RUO mode.
- **Output Location**—Select server location for data output. Refer to *Data Output and Storage* on page 37 for more information on data output.
- **BaseSpace Sequence Hub Proactive Support**—Toggle monitoring from Proactive Support.

Configure Run Mode

The run mode is selected using the toggle on the Sequencing, Runs, and Applications screens. Select **Instrument Settings** from the main menu to set the run mode before planning or starting a run.

Illumina Run Manager

Plan a run on the DRAGEN Server.

1. From the Instrument Settings screen, navigate to either the RUO Settings or IVD Settings tab, depending on the desired mode.
2. Select the DRAGEN Server option.
3. Select **Save**.

Manual Run Mode

Create a run by manually entering run information in the instrument software. Manual run planning is available only in RUO mode.

1. From the Instrument Settings screen, navigate to the RUO Settings tab.
2. Select the manual run setup option.
3. Enter index settings and select **Save**.

Data Output and Storage

The following table provides file types and minimum storage requirements for a sequencing run and secondary analysis. The table lists requirements for a dual flow cell run by each flow cell type.

For single flow cell runs, the minimum space requirements are half of those listed in the table. Alternate run configurations have different storage requirements.

File Type	S2 300 Cycle (GB)	S4 300 Cycle (GB)
CBCL	930	2800
InterOp folder	2.3	7.0
FASTQ	1125	3387
BAM	1050	3160
gVCF and VCF	28	84

Map-mounted storage locations use the full UNC path. Do not use letters or symbolic links.

Example Data Usage

The following table provides an example for building an infrastructure that supports data generated with the NovaSeq 6000Dx Instrument. The table lists data storage options for whole genome sequencing analysis with BaseSpace Sequence Hub.

The examples assume that a dual flow cell 300 cycle run with S2 flow cells generates 2 TB of data at a usage rate of 10 runs per month. The S4 data points are extrapolated from the S2 assumptions.

- Adjust the numbers in the table for a lower rate of use. If you expect to perform repeat analysis of data sets, increase storage proportionately.
- Because actual data retention is subject to local policies, confirm conditions before calculating storage needs.
- Run sizes vary depending on multiple factors including length and the percentage of pass filter (PF). The numbers provided are intended to be a guide to the relative range of the data footprint.

File Type	Time Period	Number of Runs	S2 300 Cycle (TB)	S4 300 Cycle (TB)
BAM	Monthly	10 runs/1 month per system*	14	42
BAM	Annually	120 runs/1 year per system	168	504
VCF and gVCF	Monthly	10 runs/1 month per system	0.3	0.9
VCF and gVCF	Annually	120 runs/1 year per system	3.6	10.8

* Storage for data backup and archival is not included.

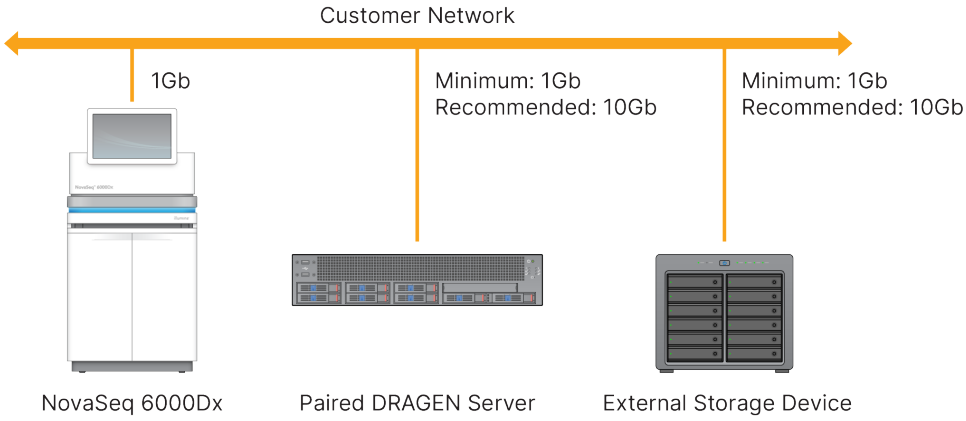
DRAGEN Server Configuration

The About DRAGEN Server screen contains information about the DRAGEN Server, including server details and license information. Select About DRAGEN Server from the Settings menu on the instrument or using a browser.

DRAGEN Server Networking Requirements

The NovaSeq 6000Dx requires a connection to the DRAGEN Server. The DRAGEN Server and NovaSeq 6000Dx are connected through the local network using the independent IP address for each. The minimum network connection required between the NovaSeq 6000Dx, DRAGEN Server, and external storage is one Gb. A 10 Gb connection for the DRAGEN Server and external storage is recommended for faster data transfer times. Illumina Run Manager allows for queuing of multiple analyses on the DRAGEN Server.

Figure 12 NovaSeq 6000Dx-DRAGEN Server Networking



The server requires a domain name to be assigned on the user's domain name system (DNS). It is recommended, but optional, to assign Transport Layer Security (TLS) certificates to the server domain name to ensure data encryption during transfer over the local network. If TLS certificates cannot be provided, the system will user self generated certificates.

Pair DRAGEN Server

An Illumina representative pairs the NovaSeq 6000Dx Instrument to the DRAGEN Server during initial setup. Use the following instructions if the server becomes disconnected from the instrument. An administrator user account is required to complete pairing.

1. From the Settings menu, select **Instrument Pairing**.
   The Instrument Pairing window opens.
2. Enter the server domain name.
3. Confirm the trusted certificate of the server and select **Log In**.
4. Log in with a valid administrator user account.
5. On the Confirm and Pair screen, select **Pair**.

Main Menu

The main menu is located on the left side of the user interface. The main menu is always visible, except on-instrument when run setup is in progress. The main menu contains icons that provide access to the following screens:

- **Sequencing**—Start sequencing or wash from the Sequencing screen. The Sequencing screen is visible to all users.
- **Runs**—View planned, active, and completed runs. Runs are visible to all users.
- **Applications**—View installed applications and assign user application permissions. Applications are visible to administrators via the instrument and browser.

Runs Screen

Planned Runs

Runs planned on the DRAGEN Server are displayed in the Planned tab of the Runs screen. To edit or delete a planned run, select the run and then select Edit or the trash can icon. Planned runs can exist in one of following states:

- **Draft**— Run has been created but is not available for sequencing.
- **Planned**— Run has been created and is available to begin sequencing
- **Needs Attention**— There are issues with the run that require user intervention. Select the run to either edit the run or dismiss the error. The status is changed to Planned.
- **Locked**— In the case that there is an issue with the instrument, it automatically locks the run. To unlock a run, select the run and then select **Unlock**.

Active Runs

Any in-progress run that has not completed all sequencing and analysis steps displays as Active. Select an active run to display more details about the status or cancel analysis.

Runs with errors that have prevented them from completing are also displayed in the Active tab. Select the run to view error messages and requeue analysis (if possible).

Completed Runs

Completed runs have finished all sequencing and analysis steps. Select a run to view run details or requeue analysis.

Applications

The Applications screen allows you to configure settings for installed applications and assign applications to users. The exact fields displayed on the Configuration screen vary depending on the application but may include the following:

- **Application name**
- **Application version**
- **DRAGEN version**
- **Library prep kits**—Select default library prep kits to be used with the application.
- **Index adapter kits**—Select default index adapter kits to be used with the application.
- **Read type**—Select a default read type.
- **Read lengths**—Select default read lengths.
- **Reference genome**—Upload and select a reference genome to be used with the application.
- **Output file formats**—Select the preferred output file formats.

- **Targeted regions list builder**—Upload and select one or more target regions files to be used with the application. At least one file must be enabled for each app.
- **Systematic noise file**—Upload and select one or more noise files to be used with the application. At least one file must be enabled for each app.

Application User Permissions

You can assign application permissions to users in User Management or by selecting users when setting up a new app.

Instrument Network and Security

Refer to Illumina Instrument Control Computer Security and Networking for further information on instrument security and network connections. The following sections include security and networking information specific to the NovaSeq 6000Dx.

Illumina does not install or provide technical support for network connections. Review network maintenance activities for potential compatibility risks with the NovaSeq 6000Dx Instrument.

Network Connections

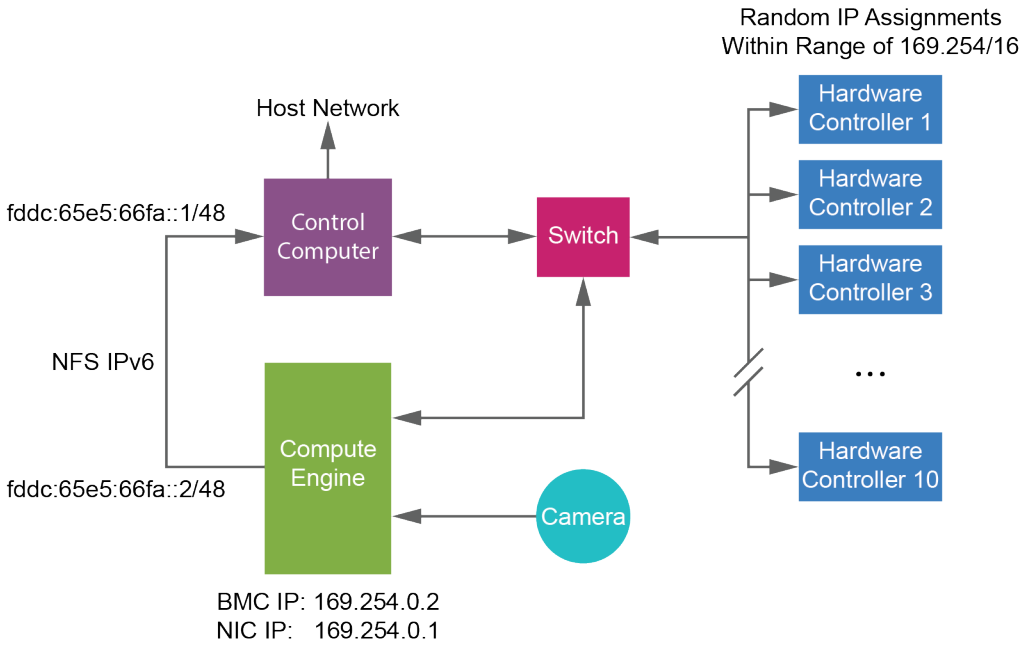
Use the following guidelines to install and configure a network connection:

- Use a dedicated 1 gigabit connection between the instrument and data management system. Make this connection directly or through a network switch.
- Required bandwidth for a connection is as follows.
  - 200 Mb/s/instrument for internal network uploads.
  - 200 Mb/s/instrument for BaseSpace Sequence Hub network uploads.
  - 5 Mb/s/instrument for Instrument Operational Data uploads.
- Switches must be managed.
- Network equipment such as switches must have a minimum capacity of 1 gigabit per second.
- 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.
- Illumina recommends the use of CAT-6 cables (minimum requirement is CAT-5e). A shielded network cable that is 3 meters (9.8 feet) long is provided with the instrument for network connections.

Control Computer Connections

For the system to function properly, reserve the IP ranges 169.254/16 and IPv6 fddc:65e5:66fa::*.

Figure 13 Network Illustration



NOTE The CE is not visible on the host network.

Internal Connections

Table 17 Internal Connections

Connection	Value	Purpose
Domain	localhost:*	All ports for localhost-to-localhost communication, which are needed for interprocess communication.

Connection	Value	Purpose
Port	5555	Hardware controller interface
	9030	Real-Time Analysis
	8080	NovaSeq Operating Software
	29644	Universal Copy Service
	22, 80, 111, 443, 623, 2049, 5900, 8889, 9980, fddc:65e5:66fa::1/48, fddc:65e5:66fa::2/48	Data transfer
	29000	Instrument orchestrator

Outbound Connections

Outbound connection information includes domain and IP address information for configuring access to BaseSpace Sequence Hub domains, Illumina Proactive, software updates, and uploads of run and performance data.

Table 18 IP Addresses and Ports

Component	TCP	UDP	IP
BMC	22,80,443,623,5900,8889	623	169.254.0.2
NFS	111,2049	111,2049	fddc:65e5:66fa::2/48 fddc:65e5:66fa::1/48
CE	22,9980	n/a	169.254.0.1
Hardware Controllers	n/a	n/a	169.254.x.x/16

Antivirus Software

An antivirus software of your choice can be used to protect the instrument control computer against viruses.

To avoid data loss or interruptions, configure the antivirus software as follows.

- Set for manual scans. Do not enable automatic scans.
- Perform manual scans only when the instrument is not in use.

- Set updates to download without user authorization, but ***not to install***.
  - Install the antivirus software only when the instrument is not in use and you can reboot the computer.
  - Do not allow the computer to reboot automatically after install.
- Exclude the application directory and data drives from any real-time file system protection.

Protocol

This section provides step by step instructions on how to prepare consumables and set up a sequencing run. Review all information in *Safety & Compliance* on page 7 before beginning a sequencing run.

Create a Sequencing Run

Use the following steps to create a run using Illumina Run Manager in either IVD or RUO mode. Alternatively, select **Import Run** on the Planned tab of the Runs page and import a sample sheet. Create new runs either on the instrument or by accessing Illumina Run Manager using a browser on a networked computer.

NOTE The exact information required by each analysis application differs, but the process to create a run includes the following steps.

1. From the Planned tab of the Runs screen, select **Create Run**.
2. Select an application, and then select **Next**.
3. Proceed through the settings screens. Depending on your application, the screens displayed can include the following:
   - **Run Settings**—Enter run parameters.
   - **Sample Data**—Enter sample data manually or by importing a CSV file containing sample information. Sample names must be unique.
   - **Analysis settings**—Enter settings for analysis.
4. On the Review screen, review run information and select **Save**.
   The run is added to the top of the runs list on the Planned tab.

Prepare Consumables

Thaw SBS and Cluster Cartridges

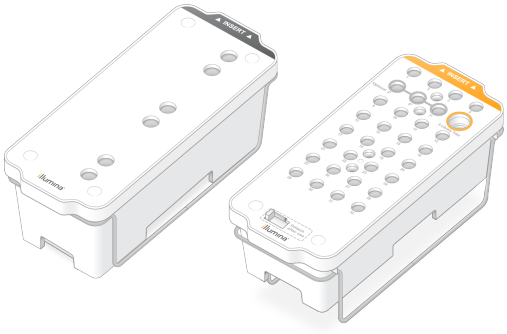
CAUTION

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

1. If a sequencing run is in progress, make sure that both sides of the instrument are available when the thaw is complete.
2. Remove the SBS and cluster cartridges from -25°C to -15°C storage.

3. Place each cartridge into a wire thaw rack.
   The racks are provided with the instrument and prevent capsizing in the water bath.

   Figure 14 Cartridges in Wire Thaw Racks



4. Use the following table to determine thaw duration.
   Thaw SBS and cluster cartridges in a room temperature (19°C to 25°C) water bath as follows. Submerge cartridges approximately halfway.

Cartridge	Duration of Thaw
S2 SBS cartridge	4 hours
S2 cluster cartridge	Up to 2 hours
S4 SBS cartridge	4 hours
S4 cluster cartridge	Up to 4 hours

   CAUTION

   Failure to start sequencing within four hours of thawing reagent cartridges can result in reduced data quality.

5. Thoroughly dry the cartridge bases using paper towels. Dry between the wells so that all water is removed.
6. Inspect the foil seals for water. If water is present, blot dry with a lint-free tissue.
7. Inspect the underside of each cartridge to make sure that the reservoirs are free of ice, which indicates that the reagents are thawed.
8. Invert each cartridge 10 times to mix reagents.

   CAUTION

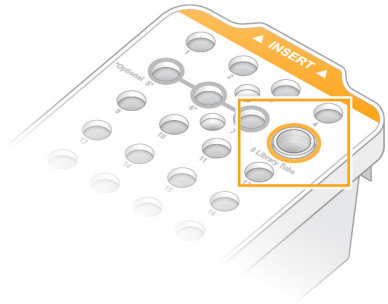
   Failure to thoroughly invert the cartridges can result in reduced data quality.

9. Gently tap the bottom of each cartridge on the bench to reduce air bubbles.

Load Library Tube

1. Without disturbing the library at the bottom, insert the uncapped library tube containing the denatured and diluted library pool into the **Library Tube** position (#8) of the cluster cartridge.
2. Insert the library tube into position #8 of the cluster cartridge.

Figure 15 Uncapped Library Tube Loaded Into Position #8

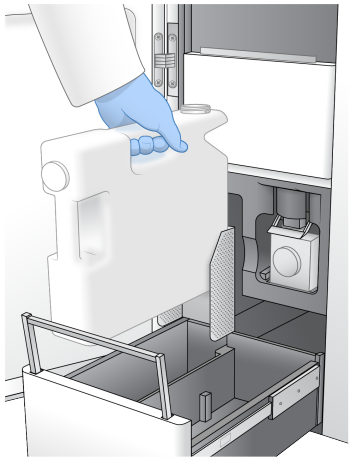


Empty Used Reagent Bottles

Use the following instructions to empty the used reagent bottles with ***every*** sequencing run. If your system is configured to route used reagents externally, the small bottle collects used reagents and must be emptied for each sequencing run. The large bottle must be in place.

1. Remove and empty the small used reagent bottle as follows.
   a. Raise the lever and remove the small used reagent bottle from the alcove. Grasp the bottle by the sides.
   b. Remove the threaded cap from the cap holder on the front of the bottle.
   c. Seal the bottle opening with the cap to prevent spills.
   d. Keeping the contents separate from the contents of the other bottle, discard in accordance with applicable standards for your region.
   e. Return the uncapped bottle to the alcove, and then lower the lever. Store the cap on the cap holder.
2. Remove and empty the large used reagent bottle as follows.
   a. Using the top handle, remove the large used reagent bottle from the left side of the buffer drawer.
   b. Remove the threaded cap from the cap holder on the front of the bottle.
   c. Seal the bottle opening with the cap to prevent spills.
   d. Discard the contents in accordance with applicable standards for your region. Grip both handles when emptying.
   e. Return the uncapped bottle to the buffer drawer. Store the cap on the cap holder.

Figure 16 Returning the Empty Bottle



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

> **CAUTION**
>
> Always put on a new pair of gloves after handling the used reagent bottle.

4. Close the buffer drawer, and then close the liquid compartment doors.

> **CAUTION**
>
> Failure to empty the used reagent bottles can result in a terminated run and overflow, which damages the instrument and poses a safety risk.

Prepare Flow Cell

1. Remove a new boxed flow cell package from 2°C to 8°C storage.
2. Set the sealed flow cell package aside at ambient temperature (19°C to 25°C) for 10–15 minutes. Use the flow cell within 12 hours of removing it from the package.

Load Consumables

Use the following instructions to start run setup and load consumables.

1. From the main menu, select **Sequence**, and then select a single or dual flow cell run as follows.
   - **A+B**—Set up a dual flow cell run.
   - **A**—Set up a single flow cell run on side A.
   - **B**—Set up a single flow cell run on side B.

   The system initiates run setup, starting with loading the flow cell.
2. Select **OK** to acknowledge the warning and open the flow cell door.

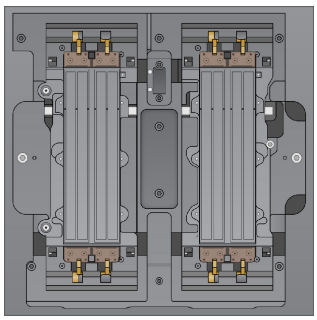
CAUTION

Keep the surface clear during the sequencing run and avoid leaning on the instrument. Pressure to the flow cell door can cause it to open, which stops the run. Stopped runs cannot be resumed.

Load the Flow Cell

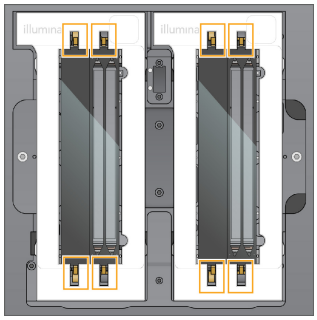
1. If present, remove the flow cell from the previous run.
2. If particulate is visible on the flow cell stage, clean the entire stage, including the fluidic interface and the glass surface of the optical alignment target, with an alcohol wipe. Dry with a lint-free tissue.

   Figure 17 Flow Cell Stage

3. Remove the flow cell from the packaging as follows.
   a. Put on a new pair of powder-free gloves to avoid contaminating the glass surface of the flow cell.
   b. With the package over a flat surface, peel open the foil from the corner tab.
   c. Remove the clear plastic retainer covering the flow cell.
   d. Remove the flow cell from the package. Grasp the flow cell by the sides to avoid touching the glass or the underside gaskets.
   e. If particulate is visible on either of the glass surfaces, clean the applicable surface with a lint-free alcohol wipe and dry with a low-lint lab tissue.
   f. Discard the package appropriately.
4. Align the flow cell over the four raised clamps and place it on the flow cell stage.

Figure 18 Loaded Flow Cells Aligned Over Clamps



5. Select **Close Flow Cell Door**.
   The flow cell door closes, the sensors and RFID are checked, and the flow cell ID appears on the screen.

Load the SBS and Cluster Cartridges

1. Open the liquid compartment doors, and then open the reagent chiller door.
2. Remove the used SBS and cluster cartridges, if present from a previous run.
   The used cartridges have pierced foil seals.
3. Dispose of unused contents in accordance with applicable standards.
   For safe disposal of position #30 of the cluster cartridge, refer to *Detach Position #30* on page 55.
4. Load the prepared cartridges into the reagent chiller drawer as follows, so that the Insert labels face the back of the instrument.
   - Place the SBS cartridge (gray label) into the left position.
   - Place the cluster cartridge (orange label) containing the uncapped library tube into the right position.

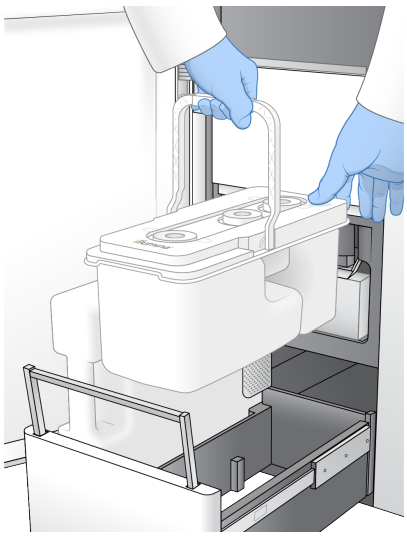
   Figure 19 Loaded Reagent Cartridges

5. Slide the drawer into the chiller, and then close the reagent chiller door.
   The sensors and RFIDs are checked. The IDs for the library tube and the two cartridges appear on the screen.

Load the Buffer Cartridge

1. Pull the metal handle to open the buffer drawer.
2. Remove the used buffer cartridge from the right side of the buffer drawer.
   The used buffer cartridge has pierced foil seals.
3. Place a new buffer cartridge into the buffer drawer so that the Illumina label faces the front of the drawer. Align the cartridge with the raised guides on the drawer floor and sides.
   When properly loaded, the buffer cartridge is evenly seated and the drawer can close.

   Figure 20 Load the Buffer Cartridge



4. If both used reagents bottles have been emptied, select the checkbox acknowledging that both used reagent bottles are empty.

   NOTE Failure to empty the used reagent bottles can result in a terminated run and overflow, which damages the instrument and poses a safety risk.

5. When consumables have been added, select **Run Selection** to proceed.

Select and Start Run

The instrument scans the library tube ID and searches for a matching planned run.

1. If a planned run matching the library tube ID is found for each side being used, run selection is skipped. Select **Review** to proceed.
2. If there is no matching run for one or either side, select **Run Selection**, and then select one or more planned runs.
   The same planned run cannot be selected on both sides.
3. When one or more runs are selected, select **Pre-Run Checks**.
4. Wait approximately 5 minutes for the pre-run check to complete.

The run starts automatically after successful completion.

NOTE To avoid overfilling the hard drive, do not copy any data to C:\ after the run starts.

Pre-Run Check Errors

Refer to *Troubleshooting* on page 70 for more information on pre-run check errors.

1. If pre-run checks fail due to a sensor error, such as flow cell not detected, you must exit and restart the workflow.
2. For other pre-run check failures, select **Retry** to restart the failed check or **Retry All** to restart all checks.
   Errors require resolution before the run can start.
3. Select the **Error** icon to see error details.
4. If the alignment check fails, resolve the error as follows.
   a. Select **Reload**, and then select **OK** to return to the Load screen.
   b. Remove any items from the top of the instrument, and then select **OK**. The flow cell door opens.
   c. Reload the flow cell, and then select **Run Setup**.
   d. Proceed through each screen to reread each RFID and return to the Pre-Run Checks screen.
   e. Redo the check.

Monitor Run Progress

The following details are displayed on the Sequencing screen while the run is in progress. The Sequencing screen is accessed via the main menu.

- **Status of individual run steps**
- **Time to completion**—The run completion date and time (yyyy-mm-dd hh:mm).
- **Run progress**—The current run step. The size of the progress bar is not proportional to the run rate of each step.
- **Q-scores**—The distribution of quality scores (Q-scores).
- **Intensity**—The value of cluster intensities of the 90th percentile for each tile. Plot colors indicate the red and green channels.
- **Clusters passing filter (%)**—The percentage of clusters passing filter.
- **Projected Total Yield (GB)**—The projected yield for the flow cell run. If the per lane metrics are selected (H), the numbers displayed are the current yield per lane and update per cycle throughout the run.
- **Q30**—The percentage of base calls for the run that have a Q-score of ≥ 30.

Status Icons

A status icon on the NVOS interface indicates run status. A number on the icon indicates the number of conditions for a status.

When a run status changes, the icon blinks. Select the icon to view a description of the condition. Select **Acknowledge** to clear the message, and then select **Close** to close the dialog box.

Status Icon	Status Name	Description
	Status okay	System is normal.
	Processing	System is processing.
	Warning	A warning has occurred and attention is required. Warnings do not stop a run or require action before proceeding.
	Error	An error has occurred. Errors require action before proceeding with the run.
	Information	A noncritical message is available.

Run Metrics

The software displays metrics generated during the run. Metrics appear in the form of plots, graphs, and tables based on data generated by RTA3 and written to InterOp files.

Clustering takes approximately 2 hours, then sequencing begins with cycle 1. Metrics are updated as sequencing progresses. Clusters passing filter, yield, and quality scores are available after cycle 26. Prior to cycle 26, no values are populated and are designated as not applicable.

Staggered Start of Runs

You can set up and start a run on the idle side of the instrument while a run is in progress on the other side. This setup is referred to as a staggered start. Staggered runs are set up at specific times during a run, as indicated by the following start countdown timer states.

- **Run Start: Available**—Staggered start is available. The date and time show when staggered start will become unavailable. Select **Sequence** to start a new staggered run after the current cycle is complete.

- **Run Start: Unavailable**—Staggered start is unavailable. The date and time show when staggered start will be available on the other side of the instrument.
- **Waiting...**—If a new run is attempted when staggered start is unavailable, the state changes to Waiting and the date and time show the approximate time that the instrument will be ready for the new run. The instrument proceeds to run setup when staggered start is available.

When you set up the new run, the software automatically pauses and resumes the run on the adjacent flow cell as needed. The system is placed in a safe state when paused.

Procedure

1. From the home screen, select **Sequence**, and then select **A** or **B**.
   The selected side must be the currently idle side.
2. Wait for the run on the adjacent flow cell to pause. To cancel the new run and prevent pausing, select **Cancel**.
   If the adjacent run is performing cluster generation, paired-end resynthesis, imaging, or washing, the software completes the current step before pausing.
3. When the adjacent run is paused and the flow cell door opens, set up the new run.
   After the new run is started, the paused run automatically resumes. and then the new run begins.

After Sequencing

The following sections provide instructions on steps that happen after sequencing has been completed.

Automatic Post-Run Wash

When sequencing is complete, the software initiates an automatic post-run wash that takes approximately 80 minutes. The system pumps 0.24% sodium hypochlorite (NaOCl) from position #17 and dilutes it to 0.12%. The 0.12% NaOCl is pumped to the ExAmp reagent and library positions, through the flow cell, and then to the used reagent bottles. The wash flushes template from the system to prevent cross-contamination.

When the wash is complete, the system is placed in a safe state and the Home button becomes active. Leave consumables in place until the next run. After the wash, the sippers remain in the SBS and cluster cartridges to prevent air from entering the system. The sippers in the buffer cartridge are raised so the used reagent bottles can be emptied. Wash buffer is then pumped through all lines to clear away NaOCl and reagents from the system.

NOTE If an error occurs during an automatic post-run wash, and the post-run wash is incomplete, a maintenance wash is required.

Detach Position #30

The reservoir in position #30 of the cluster cartridge contains formamide. It is removed from the used cluster cartridge and discarded separately.

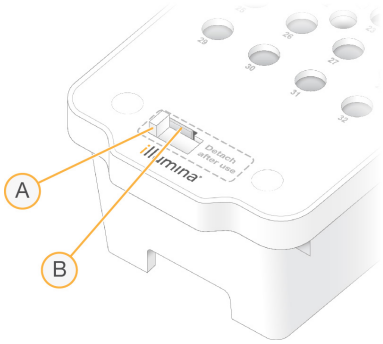
CAUTION

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.

1. While wearing gloves, push the white plastic tab labeled **Detach after use** to the right.
2. Place a hand or solid surface under the reservoir and press the clear plastic tab toward the Illumina label to release the reservoir from under the cluster cartridge.

NOTE Avoid stacking cluster cartridges when storing. Stacking might cause accidental detachment of the reservoir.

Figure 21 Removable Position #30



A. White plastic tab to detach
B. Clear plastic tab to release

3. Dispose of the reservoir in accordance with applicable standards.

Sequencing Output

During sequencing, data is automatically transferred from the NovaSeq 6000Dx Instrument to the DRAGEN Server. When primary analysis finishes, and transfer of data is complete, the secondary analysis on the DRAGEN Server can begin automatically using the analysis options defined by the application selected in Illumina Run Manager. The results produced depend on the options chosen during run setup. To view results from a run, select the desired run name in the Completed tab on the Runs screen. You can also find output files in the location specified on the Instrument Settings screen.

Real-Time Analysis

The NovaSeq 6000Dx Instrument runs RTA3, an implementation of Real-Time Analysis software, on the instrument Compute Engine (CE). RTA3 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.

To optimize processing time, RTA3 stores information in memory. If RTA3 is terminated, processing does not resume and any run data being processed in memory is lost.

RTA3 Inputs

RTA3 requires tile images contained in local system memory for processing. RTA3 receives run information and commands from the NVOS.

RTA3 Outputs

Images for each color channel are passed in memory to RTA3 as tiles. From these images, RTA3 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 one CBCL file for each lane and surface.
Filter files	Each tile produces a filter file (*.filter) that specifies whether a cluster passes filters.

RTA3 provides real-time metrics of run quality stored as InterOp files, which are a binary output containing tile, cycle, and read-level metrics.

Error Handling

RTA3 creates log files and writes them to the Logs folder. Errors are recorded in a text file in *.log file format.

The following log files are transferred to the final output destination at the end of processing:

- `info_00000.log` summarizes important run events.
- `error_00000.log` lists errors that occurred during a run.
- `warning_00000.log` lists warnings that occurred during a run.

Flow Cell Tiles

Tiles are small imaging areas on the flow cell. The camera takes one image of each swath, which the software divides into tiles for RTA3 processing. The total number of tiles depends on how many lanes, swaths, and surfaces are imaged on the flow cell.

- S2 flow cells have a total of 1408 tiles.
- S4 flow cells have a total of 3744 tiles.

Flow Cell Component	S2	S4	Description
Lanes	2	4	A lane is a physical channel with input and output ports.
Surfaces	2	2	The S2 and S4 flow cells are imaged on two surfaces: the top and bottom. The top surface of a tile is imaged first.
Swaths per lane	4	6	A swath is a column in a flow cell lane that the camera captures as one scanned image.
Tiles per swath	88	78	A tile is a portion of a swath and depicts an imaged area on the flow cell.
Total tiles generated	1408	3744	Lanes × surfaces × swaths × tiles per swath equals the total number of tiles.

The tile name is a five-digit number that represents the tile position on the flow cell. For example, tile name 1_1205 indicates lane 1, top surface, swath 2, tile 5.

- The first digit is the lane number:
  - 1 or 2 for an S2 flow cell.
  - 1, 2, 3, or 4 for an S4 flow cell.
- The second digit represents the surface: 1 for top or 2 for bottom.
- The third digit represents the swath number:
  - 1, 2, 3, or 4 for an S2 flow cell.

  - 1, 2, 3, 4, 5, or 6 for an S4 flow cell.
- The last two digits represent the tile number. The numbering starts with 01 at the outlet end of the flow cell through 88 or 78 at the inlet end.
  - 01 through 88 for an S2 flow cell.
  - 01 through 78 for an S4 flow cell.

Real-Time Analysis Workflow

Registration	Records the location of each cluster on the patterned flow cell.
↓	
Intensity extraction	Determines an intensity value for each cluster.
↓	
Phasing correction	Corrects the effects of phasing and prephasing.
↓	
Base calling	Determines a base call per cycle for every cluster.
↓	
Quality scoring	Assigns a quality score to every base call.

Registration

Registration aligns an image to the rotated square array of nanowells on the patterned flow cell. Because of the ordered arrangement of nanowells, the X and Y coordinates for each cluster in a tile are predetermined. Cluster positions are written to a cluster location (s.locs) file for each run.

If registration fails for any images in a cycle, no base calls are generated for that tile in that cycle.

Intensity Extraction

After registration, intensity extraction calculates an intensity value for each nanowell in a given image. If registration failed, the intensity for that tile cannot be extracted.

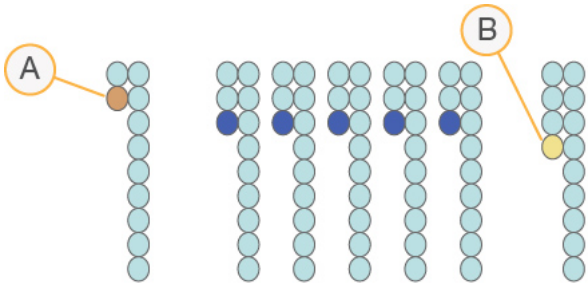
Phasing Correction

During the sequencing reaction, each DNA strand in a cluster extends by one base per cycle. Phasing and prephasing occurs when a strand becomes out of phase with the current incorporation cycle.

Phasing occurs when a base incorporation falls behind.

Prephasing occurs when a base incorporation jumps ahead.

Figure 22 Phasing and Prephasing



A. Read with a base that is phasing
B. Read with a base that is prephasing.

RTA3 corrects the effects of phasing and prephasing, which maximizes the data quality at every cycle throughout the run.

Base Calling

Base calling determines a base (A, C, G, or T) for every cluster of a given tile at a specific cycle. The NovaSeq 6000Dx Instrument uses two-channel sequencing, which requires only two images to encode the data for four DNA bases, one image from the green channel and one from the red channel.

A no call is identified as N. No calls occur when a cluster does not pass filter, registration fails, or a cluster is shifted off the image.

Intensities for each cluster are extracted from the red and green images and compared against each other, which results in four distinct populations. Each population corresponds to a base. The base calling process determines to which population each cluster belongs.

Figure 23 Visualization of Cluster Intensities

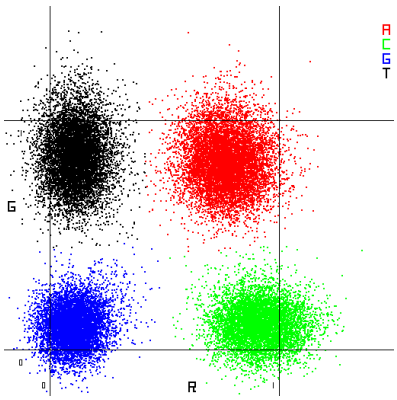


Table 19 Base Calls in 2-Channel Sequencing

Base	Red Channel	Green Channel	Result
A	1 (on)	1 (on)	Clusters that show intensity in both the red and green channels.
C	1 (on)	0 (off)	Clusters that show intensity in the red channel only.
G	0 (off)	0 (off)	Clusters that show no intensity at a known cluster location.
T	0 (off)	1 (on)	Clusters that show intensity in the green channel only.

Clusters Passing Filter

During the run, RTA3 filters raw data to remove reads that do not meet the data quality threshold. Overlapping and low-quality clusters are removed.

For two-channel analysis, RTA3 uses a population-based system to determine the chastity (intensity purity measurement) of a base call. Clusters pass filter (PF) when no more than one base call in the first 25 cycles has a chastity below a fixed threshold. When included, PhiX alignment is performed at cycle 26 on a subset of tiles for clusters that passed filter. Clusters that do not pass filter are not base called and not aligned.

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 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
Q40	0.0001 (1 in 10,000)
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 6000Dx Instrument, 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, 26, and 34 respectively. Additionally, a null score of 2 is assigned to any no-calls. This Q-score reporting model reduces storage space and bandwidth requirements without affecting accuracy or performance.

Figure 24 Simplified Q-Scoring with RTA3

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`
Thumbnail files	Thumbnail images for the first cycle of each sequencing read. `Thumbnail_Images\L001\C[X.1]`—Files are stored in a subfolder for each cycle. `s_[lane]_[tile]_[channel].jpg`—The thumbnail image includes the tile number.

Sequencing Output Folder Structure

The NVOS generates the output folder name automatically.

- **Config**—Configuration settings for the run.
- **Logs**—Log files describing operational steps, instrument analytics, and RTA3 events.
  - SampleSheet.csv—Sample sheet or other attached file, if applicable.
- **Data**
  - **Intensities**
    - **BaseCalls**
      - **L00[X]**—Base call files (*.cbcl) aggregated in one file per lane, surface, and cycle.

- s.locs—The cluster locations file for the run.

- **InterOp**—Binary files.
- **Recipe**—Run-specific recipe file.
- **Thumbnail Images**—Thumbnail images for every 10th tile.
- **LIMS**—The run setup file (*.json), if applicable.
- **Audit**
  - AuditInfo.xml
- RTA3.cfg
- RunInfo.xml
- RunParameters.xml
- RTAComplete.txt
- CopyComplete.txt
- SequenceComplete.txt
- IlluminaRunManagerCopyComplete.txt
- Manifest.tsv

Maintenance and Troubleshooting

These sections describe the maintenance and troubleshooting procedures for the NovaSeq 6000Dx.

For technical questions, visit the NovaSeq 6000Dx Instrument page on the Illumina support site. The support page provides access to documentation, downloads, and frequently asked questions. For access to support bulletins, sign in to your MyIllumina account.

For run quality or performance problems, contact Illumina Technical Support.

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.

V2 Maintenance Wash

The software prompts for a maintenance wash at the following times:

- When there has not been a maintenance wash within the last 14 days.
- 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 approximately 120 minutes.

A maintenance wash requires a used buffer cartridge and the following, which are provided with the instrument:

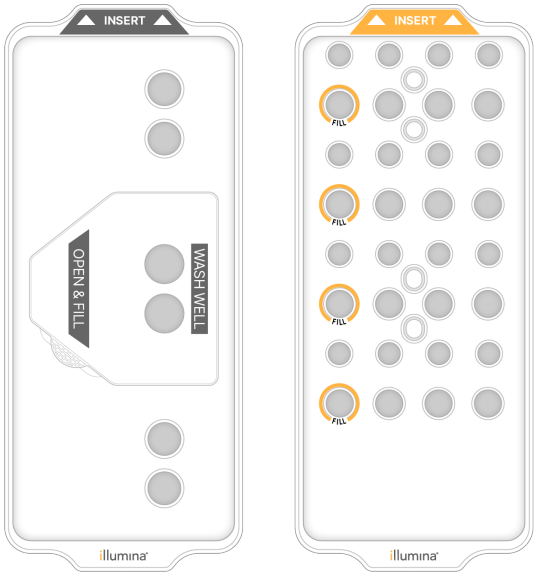
- SBS wash cartridge
- Cluster wash cartridge
- Four-lane wash flow cell

Like the reagent cartridges, the wash cartridges are color-coded to prevent loading errors. The SBS wash cartridge has a center well for the Tween 20 dilution. The NaOCl dilution is added to four reservoirs on the cluster wash cartridge.

CAUTION

Failure to empty the used reagent bottles can result in a terminated wash and overflow, which damages the instrument and poses a safety risk.

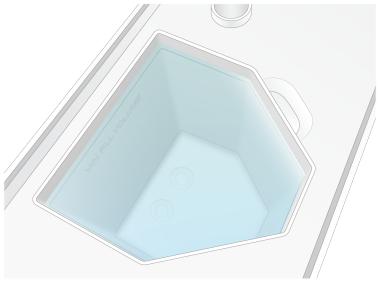
Figure 25 SBS Wash Cartridge (Left) and Cluster Wash V2 Cartridge (Right)



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 400 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. Remove the lid from the center well of the SBS wash cartridge.
5. Add wash solution to the center well. Fill to the fill line, which indicates the minimum required volume.
   The other reservoirs remain empty.

   Figure 26 Center Well Filled to Fill Line



6. In a 50 ml centrifuge tube, combine the following volumes to prepare 40 ml of 0.12% reagent grade NaOCl:
   - 5% reagent grade NaOCl (1 ml)

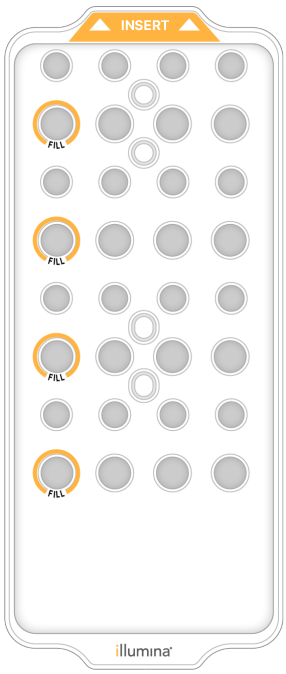
- Deionized water (39 ml)

CAUTION

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.

7. Invert to mix.
8. Add 4 ml 0.12% reagent grade NaOCl to the marked positions of the cluster wash V2 cartridge. The locations are marked Fill and are circled in orange. All other reservoirs remain empty.

Figure 27 Positions for 0.12% NaOCl



Load the Wash Flow Cell

1. Remove any items from the surface of the instrument.
   Keep the surface clear during the maintenance wash and avoid leaning on the instrument.

2. From the main menu, select **Sequencing**, select **Wash**, and then select which side to wash:
   - **A+B**—Wash both sides simultaneously.
   - **A**—Wash side A only.
   - **B**—Wash side B only.

   Staggered start of maintenance washes is not supported. The software initiates the series of wash screens.

   You can only start a maintenance wash for a single sidewhen the other side is either idle or performing SBS read cycles. The NVOS staggered start time indicates the instrument's availability for starting a new run or a wash. Refer to *Staggered Start of Runs* on page 53 for more information.
3. Select **OK** to acknowledge the warning and open the flow cell door.
4. Load a wash flow cell.
5. Select **Close Flow Cell Door**.
   The door closes, the sensors and RFID are checked, and the flow cell ID appears on the screen.

Load the Wash Cartridges

Wash cartridges are required for a maintenance wash. Do not use the used SBS and cluster cartridges.

1. Open the liquid compartment doors, and then open the reagent chiller door.
2. Remove the used SBS and cluster reagent cartridges. Dispose of unused contents in accordance with applicable standards for your region.
   For safe disposal of position #30 of the cluster cartridge, refer to *Detach Position #30*.
3. Load the wash cartridges into the reagent chiller drawer as follows, so that the **Insert** labels face the back of the instrument:
   - Place the SBS cartridge (gray label) into the left position.
   - Place the cluster wash V2 cartridge (orange label) into the right position.
4. Slide the drawer into the chiller, and then close the reagent chiller door.
   The sensors are checked and the RFID for each cartridge is scanned and displayed on the screen.
5. Open the buffer drawer.
6. If not already present, load a used buffer cartridge.

Empty Used Reagent Bottles

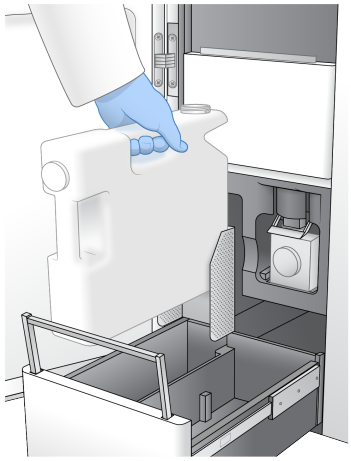
Use the following instructions to empty the used reagent bottles with ***every*** sequencing run. If your system is configured to route used reagents externally, the small bottle collects used reagents and must be emptied for each sequencing run. The large bottle must be in place.

CAUTION

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.

7. Remove and empty the small used reagent bottle as follows.
   a. Raise the lever and remove the small used reagent bottle from the alcove. Grasp the bottle by the sides.
   b. Remove the threaded cap from the cap holder on the front of the bottle.
   c. Seal the bottle opening with the cap to prevent spills.
   d. Keeping the contents separate from the contents of the other bottle, discard in accordance with applicable standards for your region.
   e. Return the uncapped bottle to the alcove, and then lower the lever. Store the cap on the cap holder.
8. Remove and empty the large used reagent bottle as follows.
   a. Using the top handle, remove the large used reagent bottle from the left side of the buffer drawer.
   b. Remove the threaded cap from the cap holder on the front of the bottle.
   c. Seal the bottle opening with the cap to prevent spills.
   d. Discard the contents in accordance with applicable standards for your region. Grip both handles when emptying.
   e. Return the uncapped bottle to the buffer drawer. Store the cap on the cap holder.

Figure 28 Returning the Empty Bottle



9. Put on a new pair of powder-free gloves.
10. Close the buffer drawer, and then close the liquid compartment doors.

CAUTION

Failure to empty the used reagent bottles can result in a terminated run and overflow, which damages the instrument and poses a safety risk.

Start the Wash

1. Select the checkbox to acknowledge that both used reagent bottles are empty, and then select **Start Wash**.
   The wash starts and the estimated time of wash completion is displayed.

CAUTION

Failure to empty the used reagent bottles can result in a terminated wash and overflow, which damages the instrument and poses a safety risk.

2. When the wash is complete, select **Home**.
3. Leave the consumables in place until the next run.
   The sippers remain in the SBS and cluster cartridges to prevent air from entering the system. The sippers in the buffer cartridge are raised so that the used reagent bottles can be emptied. Before the next maintenance wash, dispose of wash liquid remaining in the wash cartridge and rinse the reservoir with clean water. Allow the cartridges to dry fully between uses.

Troubleshooting

For technical questions, visit the NovaSeq 6000Dx Instrument page on the Illumina support site. The support site provides access to documentation, downloads, and frequently asked questions. For access to support bulletins, sign into your MyIllumina account.

For run quality or performance problems, contact Illumina Technical Support.

End a Run

Ending a run on the NovaSeq 6000Dx system is ***final***. The software cannot resume the run or save sequencing data, and consumables cannot be reused.

1. Select **End**, and then select **Yes** to confirm the command.
   If the run was ended after Read 1, the software initiates the automatic post-run wash.
2. If prompted, select from the following wash options:
   - **End Run Without Wash**—End the run and initiate a maintenance wash.
   - **End Run and Wash**—End the run and perform an automatic post-run wash.
   - **Cancel**—Continue with the current run.

   If the run is ended between clustering completion and Read 1 completion, the software displays the wash options. Otherwise, the software initiates the automatic post-run wash.
3. If you selected End Run Without Wash, follow the software prompts to set up a maintenance wash.

Leak Tray

A leak tray is built into the base of the instrument to collect leaked reagents or coolant and collect overflow from the used reagent bottles. Under normal conditions, the leak tray is dry. Leakage indicates a problem with the instrument, and overflow occurs when the used reagent bottles are not regularly emptied.

During the pre-run check, sensors detect whether the leak tray contains any liquids:

- If the leak tray contains liquid but is not full, the run can proceed but you must contact Illumina Technical Support.
- If the leak tray is full, the run cannot proceed and you must contact Illumina Technical Support.

CAUTION

Empty the used reagent bottles with ***every run***. Runs are stopped if either of the used reagent bottles is full. Overflow from either of the used reagent bottles damages the instrument, requires a site visit from an Illumina representative, and poses a safety risk.

Process Management Troubleshooting

The following table provides troubleshooting options for the N/A icon on the Process Management screen. The location of the icon depends on the run configuration.

- N/A icon displays in the BaseSpace Sequence Hub column when the run is configured to upload to BaseSpace Sequence Hub.
- N/A icon displays in the Network column when the run is configured to upload an output folder on the network.

Run Status	Troubleshooting Action
A run is in progress	Close the Process Management screen, wait approximately 5 minutes, and then reopen the screen.
A run is not in progress	Shut down and restart the instrument, and then reopen the Process Management screen.

If the N/A icon still displays after the troubleshooting action is complete, contact Illumina Technical Support.

Pre-Run Check Errors

If an error occurs during the pre-run checks, use the following actions to resolve the error. If you are setting up a dual flow cell run and one side fails, you can cancel the failed side and proceed with the side that passed.

When a pre-run check fails, the RFIDs for the flow cell, reagents, and buffers are not locked so you can use the consumables for a subsequent run. When the run is started, the sippers pierce the foil seals on the reagent cartridges and all RFIDs are locked.

System Check	Reason for Failure	Recommended Action
Sensors	A compartment door is open, a consumable is not properly loaded, or at least one sensor is not functional.	Select **Retry** and follow the onscreen prompts to resolve the error.
Disk Space	Disk space is insufficient because the specified location of the output folder is full.	Use the Process Management screen to clear disk space from the specified output folder location.
System Connectivity	The connection to RTA3, the fluidics system, or other connection has been interrupted.	Select **Retry** and follow the onscreen prompts to resolve the error.

System Check	Reason for Failure	Recommended Action
Alignment	The position of the flow cell prevents imaging.	Follow the onscreen prompts to reload the flow cell.

Reboot, Shut Down, or Power Cycle the Instrument

The NovaSeq 6000Dx can only be rebooted, shut down, or power cycled when the instrument is idle. If sequencing or analysis is in progress, a warning is displayed and there is no option to proceed.

- **Reboot**—Reboot restarts the instrument without fully shutting down.
  - To reboot the instrument, select **Reboot** from the Settings menu on the instrument.
- **Shut Down**—Shutting down the instrument safely shuts down all software and turns off instrument power. The status bar fades from green to white, indicating that the shutdown is in progress. Under normal circumstances, shutting down the instrument is unnecessary.
  - To shut down the instrument, select **Shut Down** from the Settings menu on the instrument or through a browser.
- **Power Cycle**—Power cycle fully shuts down and restarts the instrument. A power cycle should be performed any time a software crash event occurs.
  - To power cycle the instrument, select **Power Cycle** from the Settings menu on the instrument.

Requeue Analysis With No Changes

If no changes to run settings are made, a new run is created from the original run and reanalysis is started.

1. From the run results page, select **Requeue Analysis**.
   The Requeue Analysis window opens.
2. Select the option to requeue with no changes and provide a reason for requeue in the Reason field.
3. The new run appears on the Active Runs tab.

Requeue and Edit Settings

1. From the run results page, select **Requeue Analysis**.
2. On the Requeue Analysis window, select the option to edit run settings and requeue analysis. Provide a reason for requeue in the Reason field.
3. Edit the run description and select **Next**.
4. Edit samples or import a new sample sheet and select **Next**.
5. Edit analysis settings as desired and select **Requeue**.
   The run results for the original run are updated with a link to the requeued run.

Run Failure Before Clustering

If the software fails the run before clustering starts, you can save the reagent cartridges and library tube (including sample) for a new run. If reused immediately, you can also save the flow cell. When clustering starts, sippers pierce the foil seals and reagents are transferred to the library tube and flow cell, so the consumables and libraries cannot be used for another run.

You can use one of the following options for setting up a new run using the reagent cartridges, library tube, and flow cell saved from the failed run:

- **Set up a new run immediately**—Set up the new run within 4 hours of the failed run. The reagent cartridges, library tube, and flow cell remain loaded.
- **Set up a new run later**—Set up the new run within three weeks of the failed run. The reagent cartridges are unloaded from the instrument and stored. The saved consumables should be labeled with the date and stored under the original conditions. The flow cell cannot be reused and must be discarded.

Set Up a New Run Immediately

1. When the run fails and the other side of the instrument is idle, reboot the instrument. Otherwise, select **Home**.
2. Set up a new run.
3. Leave the current flow cell in place.
4. Open and close the reagent chiller door and the buffer drawer to prompt the NVOS to reread the reagent cartridge RFIDs.
   The cartridges, library tube, and flow cell can remain in the instrument for up to 4 hours after the failed run.
5. Empty the used reagent bottles, if needed, and return them to the instrument.
6. Proceed with run setup.

Troubleshooting Files

File	Folder	Description
Run information file (RunInfo.xml)	Root folder	Contains the following run information: • Number of cycles in the run • Number of reads in the run • Whether the read is indexed • Number of swaths and tiles on the flow cell

File	Folder	Description
Run parameters file (RunParameters.xml)	Root folder	Contains the run name and information about run parameters and run components, including the following RFID information: serial numbers, lot numbers, expiration dates, and catalog numbers.
InterOp files (*.bin)	InterOp	InterOp files are updated throughout the run.
Log files	Logs	Log files describe each step performed by the instrument for each cycle, including which reagent is used, and list software and firmware versions used with the run. The file named `[InstrumentName]_CurrentHardware.csv` lists the serial numbers of instrument components.

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

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Thailand	+66 1800 011 304	
United Kingdom	+44 800 012 6019	+44 20 7305 7197
United States	+1 800 809 4566	+1 858 202 4566
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Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

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

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