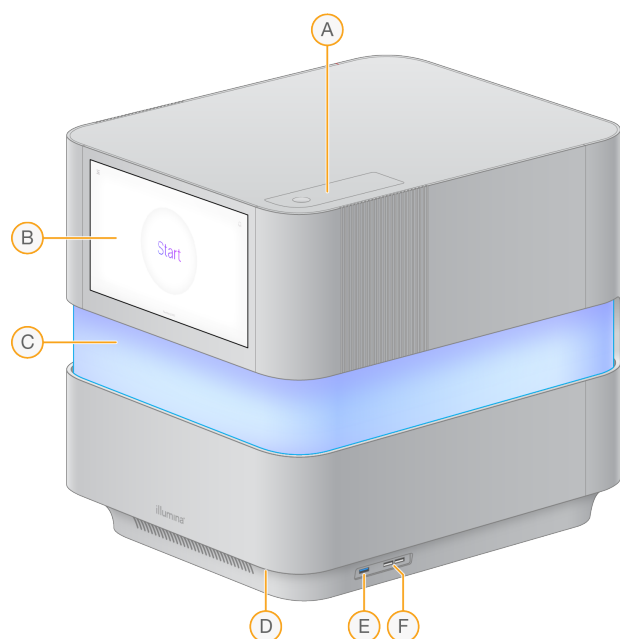


Figure 1 External System Components



- A. **Air filter compartment**—Provides access to replaceable air filter.
- B. **Touch-screen monitor**—Enables on-instrument configuration and setup using the control software interface.
- C. **Status bar**—Light color progresses as the system moves through its workflow. Blue and purple indicate interactivity (eg, pre-run checks) and multicolor indicates notable moments and data (eg, sequencing completion). Critical errors are indicated by a red light.
- D. **Power button**—Controls instrument power and indicates whether the system is on (glows), off (dark), or off but with AC power (pulses).
- E. **3.0 USB Port**—For connecting an external portable drive for data transfer.
- F. **2.0 USB Ports**—For connecting a mouse and keyboard.

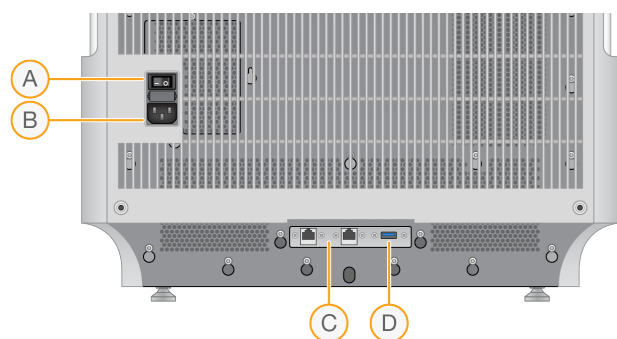
Power and Auxiliary Connections

You can gently move the instrument to access the power switch, USB port, and other auxiliary connections on the back of the instrument.

The back of the instrument has the switch and inlet that controls power to the instrument, and two Ethernet ports for an optional Ethernet connection. A 3.0 USB port provides the option to connect an external portable drive for data transfer (exFAT is not supported on this Linux-based platform).

NextSeq 1000 and NextSeq 2000 Sequencing Systems comes equipped with two Ethernet ports to expand system capability and flexibility. For example, one Ethernet port can be dedicated to communication with an internal network drive and the other port dedicated to external communication such as BaseSpace Sequence Hub or Proactive Support.

Figure 2 Rear Panel Components

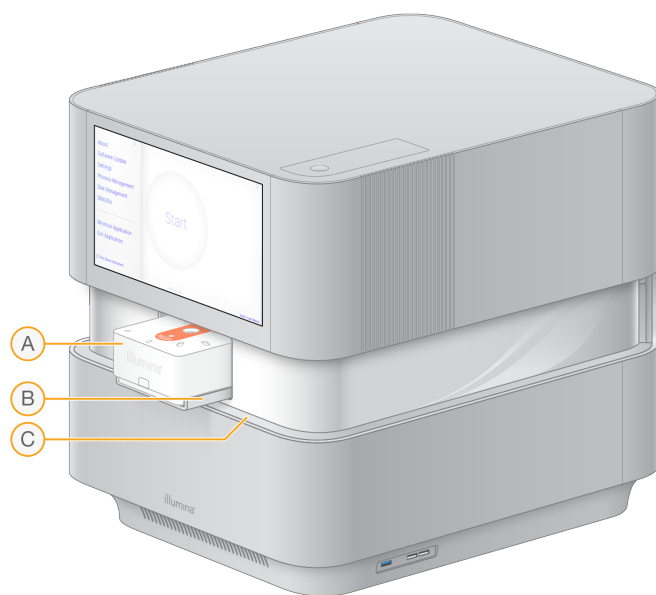


- A. **Toggle switch**—Turns instrument power on and off.
- B. **Power inlet**—Power cord connection.
- C. **Ethernet ports (2)**—Optional Ethernet cable connection.
- D. **3.0 USB port**—For connecting an external hard drive for data transfer.

Consumables Compartment

The consumables compartment contains the cartridge, including the flow cell and diluted library, for a sequencing run.

Figure 3 Loaded Consumables Compartment



- A. **Cartridge**—Contains the flow cell, library, and reagents, and collects used reagents during the run.
- B. **Tray**—Holds the cartridge during sequencing.
- C. **Visor**—Opens to provide access to the consumables compartment.

Integrated Software

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

- **NextSeq 1000/2000 Control Software**—Controls instrument operations and provides an interface for configuring the system, setting up a sequencing run, and monitoring run statistics as sequencing progresses.
- **Real-Time Analysis (RTA3)**—Performs image analysis and base calling during the run. For more information, see [Sequencing Output on page 55](#).
- **Universal Copy Service**—Copies sequencing output files from the run folder to BaseSpace Sequence Hub (if applicable) and the output folder, where you can access them.

The control software is interactive and runs automated background processes. Real-Time Analysis and Universal Copy Service run background processes only.

System Information

Select the control software menu in the upper left corner to open the About section. The About section contains Illumina contact information and the following system information:

- Instrument Serial Number
- Computer Name
- System Suite Version
- Image OS Version
- Total Run Count

Notifications and Alerts

The notification icon is located in the top-right corner. When a warning or error occurs, the right panel slides out to indicate notifications. Select the icon at any time to view a list of Current or Historic notifications for warnings and errors.

- Warnings require attention, but do not stop a run or require action other than acknowledgment.
- Errors require action before starting or proceeding with a run.

Minimize the Control Software

Minimize the control software to access other applications. For example, to browse to the output folder in File Explorer or find a sample sheet.

1. From the control software menu, select **Minimize Application**.
The control software is minimized.
2. To maximize the control software, Select **NextSeq 1000/2000 Control Software** from the toolbar.

Process Management

The Process Management screen displays temporary runs that are stored at `/usr/local/illumina/runs`. Each run is identified by run date, name, and ID. Information such as status of Run, Secondary Analysis, Output Folder, and Cloud are also shown for each run. Select the run to view additional information, including Workflow, Average % Q30, Total Reads PF, and Total Yield. To delete runs and clear space, see [Clear Hard Drive Space on page 75](#). To requeue on-instrument analysis, see [Requeue a Run on page 83](#).

Status of Run

This section displays the status of the sequencing run:

- **In Progress**—Sequencing run in progress.
- **Complete**—Sequencing run is complete.
- **Stopped**—Sequencing run was stopped.
- **Errored**—Sequencing run has an error.

Status of Secondary Analysis

This section displays the status of the on-instrument DRAGEN secondary analysis. This will display N/A if analysis is occurring in BaseSpace Sequence Hub.

- **Not Started**—DRAGEN analysis not yet started.
- **In Progress**—DRAGEN analysis in progress.
- **Stopped**—DRAGEN analysis has been stopped.
- **Errored**—DRAGEN analysis has an error.
- **Complete**—DRAGEN analysis is complete.

Status of Output Folder

This section displays the status of files being copied to the output folder:

- **In Progress**—Files are being copied to the output folder.
- **Complete**—Files have been successfully copied to the output folder.

Status of Cloud (BaseSpace Sequence Hub)

The section displays the status of files being uploaded to BaseSpace Sequence Hub via the cloud:

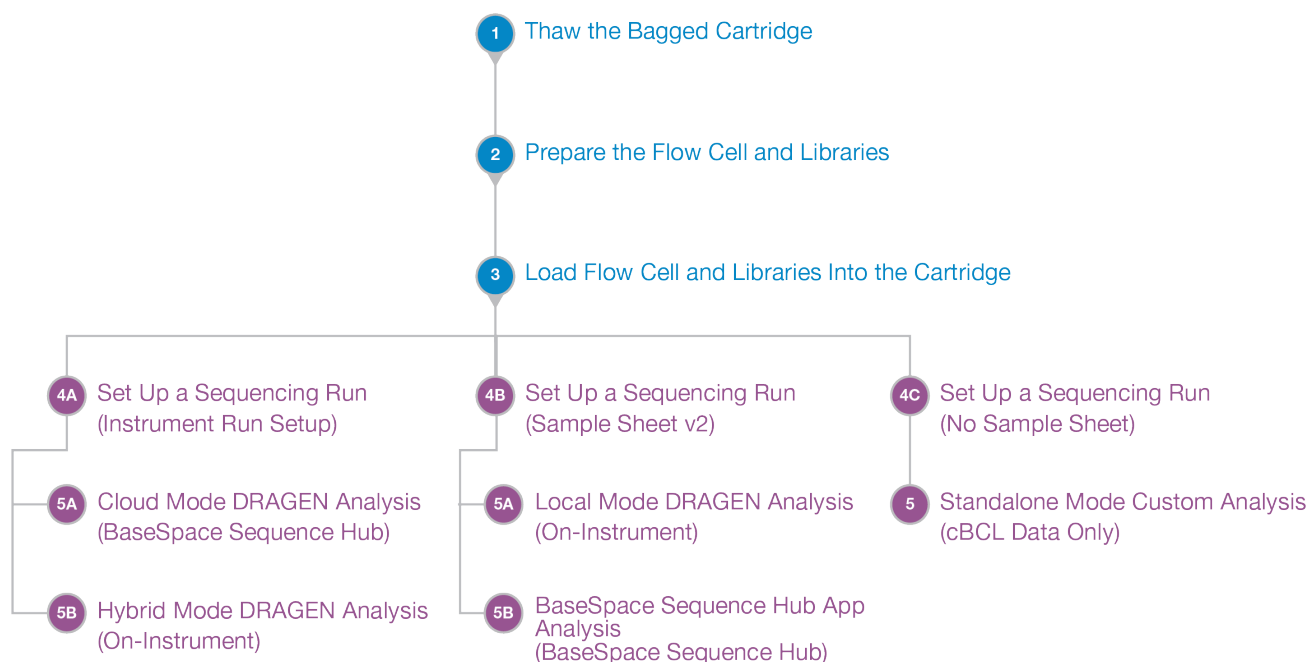
- **In Progress**—The control software is uploading files to BaseSpace Sequence Hub.
- **Complete**—Files have been successfully uploaded to BaseSpace Sequence Hub.

Troubleshooting a Status Issue

- If the run is in progress, close the Process Management screen, wait about five minutes, and then reopen.
- If the run is not in progress, power cycle the instrument, and then reopen the Process Management screen. See [Power Cycle the Instrument on page 83](#).

Sequencing Protocol Diagram

The following diagram illustrates the sequencing protocol using the NextSeq 1000/2000.



How Sequencing Works

Cluster generation, sequencing, and analysis comprise sequencing on the NextSeq 1000 and NextSeq 2000 Sequencing Systems. Each step occurs automatically during a sequencing run. Depending on system configuration, further analysis is performed off-instrument after the run is complete.

Cluster Generation

Library¹ is automatically denatured into single strands and further diluted onboard the instrument. During cluster generation, single DNA molecules are bound to the surface of the flow cell and amplified to form clusters². Cluster generation takes ~4 hours.

Sequencing

Clusters are imaged using two-channel chemistry, one green channel and one blue channel, to encode data for the four nucleotides. After one tile on the flow cell is imaged, the next tile is imaged. The process is repeated for each cycle of sequencing (~5 minutes per cycle). Following image analysis, Real-Time Analysis software performs base calling³, filtering, and quality scoring.⁴

Primary Analysis

As the run progresses, the control software automatically transfers base call files⁵ (*.cbcl) to the specified output folder for data analysis. During the sequencing run, real time analysis (RTA3) software performs image analysis, base calling, and demultiplexing⁶. When sequencing is complete secondary analysis begins. The method of secondary data analysis depends on your application and system configuration.

Secondary Analysis

BaseSpace Sequence Hub is the Illumina cloud computing environment for run monitoring, data analysis, storage, and collaboration. It hosts DRAGEN and BaseSpace Sequence Hub apps, which support common analysis methods for sequencing.

After initial sequencing analysis is complete, DRAGEN performs a secondary analysis by using one of the available analysis pipelines.

If using Cloud or Hybrid mode, DRAGEN retrieves sample sheet, reference genome, and run input files from the Instrument Run Setup in BaseSpace Sequence Hub. For Cloud mode, cBCL data are uploaded automatically to BaseSpace Sequence Hub, and BaseSpace Sequence Hub initiates DRAGEN secondary analysis. For Hybrid mode, DRAGEN secondary analysis is performed on-instrument, and output files can be stored in a selected folder or in the cloud.

¹A DNA or RNA sample that has adapters attached for sequencing. Preparation methods vary.

²A clonal group of DNA strands on a flow cell that produce one sequencing read. Each DNA strand on a flow cell seeds a template that is amplified until the cluster consists of hundreds or thousands of copies. For example, a flow cell with 10,000 clusters produces 10,000 single reads or 20,000 paired-end reads.

³Determining a base (A, C, G, or T) for every cluster in a tile at a specific cycle.

⁴Calculates a set of quality predictors for each base call, and then uses the predictor value to look up the Q-score.

⁵Contains the base call and associated quality score for every cluster of each sequencing cycle.

⁶An analysis process that differentiates reads for each library in a pool.

If using Local mode, DRAGEN retrieves the provided sample sheet, reference genome, and run input files from the NextSeq 1000 and NextSeq 2000 Sequencing Systems. DRAGEN secondary analysis is performed on-instrument and output files are stored in a selected output folder. If Proactive, Run Monitoring and Storage is selected, analysis can also be initiated through BaseSpace Sequence Hub apps after sequencing completion.

If using Standalone mode, set up a run without a sample sheet. This workflow is recommended for custom analysis workflows that start from cBCL data.

- For more information on BaseSpace Sequence Hub, see the [BaseSpace Sequence Hub Online Help](#).
- For more information on DRAGEN, see the [DRAGEN Bio-IT Platform support page](#).
- For an overview of all apps, see [BaseSpace Apps](#).