illumina®

NextSeq 1000 and 2000

Sequencing System Guide

Revision History

Document #	Date	Description of Change
1000000109376 v04	April 2021	Added instructions for importing baseline files. Added DRAGEN DNA Amplicon workflow. Added features for NextSeq 1000/2000 Control Software v1.3. Added information to select a proxy server. Updated RSB with Tween 20 shipping and storage temperature. Updated DRAGEN RNA workflow to include differential gene expression. Updated sequencing output folder structure. Updated sample sheet v2 formatting recommendations.
1000000109376 v03	November 2020	Corrected catalog numbers. Added information on adding new users.

Document #	Date	Description of Change
1000000109376 v02	October 2020	Added NextSeq 1000/2000 P3 Reagents kit. Added DRAGEN Single Cell RNA workflow. Added DRAGEN Enrichment workflow. Added FASTQ compression options. Added instructions for installing DRAGEN pipeline and license updates. Added instructions for importing custom reference genomes. Updated loading volume and concentrations for library types. Updated library dilution instructions. Added instructions to automatically purge reagent cartridge. Updated information on the support number of cycles. Updated instrument customization options. Updated Instrument Run Setup instructions. Updated DRAGEN sequencing output structure. Added information on DRAGEN QC reports. Added information on removing custom reference genomes from the hard drive. Added information on performing system checks. Updated sample sheet v2 settings.

Document #	Date	Description of Change
1000000109376 v01	June 2020	Updated software descriptions for NextSeq 1000/2000 Control Software. Clarified distinction between Cloud, Hybrid, Local, and Standalone mode throughout guide. Updated cartridge storage and thawing instructions. Updated information on supported number of cycles. Updated instructions for setting up secondary analysis. Updated reagent kit catalog numbers. Updated sequencing protocol diagram. Updated instructions for specifying a network drive as default output folder. Updated table of supported library types. Added instructions for importing a custom reference genome. Added instructions for setting up a run using a custom index kit and custom library prep kit. Updated user account and password requirements. Added details on DRAGEN output folder structure. Clarified instructions for draining used reagents from cartridge. Added background information on the Q-table. Updated instructions on installing control software updates. Added instructions on how to requeue a run. Added instructions on updating DRAGEN pipelines and license. Added instrument customization instructions. Updated illustrations to reflect new labeling. Changed door to visor throughout guide. Added description of the two Ethernet ports.
1000000109376 v00	March 2020	Initial release.

Table of Contents

System Overview

The Illumina® NextSeq™ 1000 Sequencing System and the Illumina® NextSeq™ 2000 Sequencing System provides a targeted approach to NGS[1]. This applications-focused system packages Illumina sequencing technology into a cost-effective desktop instrument that offers the following features:

- **Accessibility and reliability**—The NextSeq 1000/2000 has local DRAGEN analysis and onboard denature and dilution. An imaging module is built into the system and fluidics components are built into the consumable, simplifying instrument maintenance.

- **Single-step consumable loading**—A single-use cartridge is prefilled with all reagents required for a run. Library and flow cell load directly into the cartridge, which is then loaded onto the instrument. Integrated identification enables accurate tracking.

- **NextSeq 1000/2000 software**—A suite of integrated software controls instrument operations, processes images, and generates base calls.
  - **Cloud mode**—Plan your run with Instrument Run Setup on BaseSpace Sequence Hub. The selected analysis workflow is initiated automatically within the cloud. Run data and analysis results are also provided in the cloud.
  - **Hybrid mode**—Plan your run with Instrument Run Setup on BaseSpace Sequence Hub. The selected analysis workflow is then initiated through the on-instrument DRAGEN.
  - **Local mode**—Plan your run with a sample sheet v2 file format locally. The selected analysis workflow is initiated automatically through the on-instrument DRAGEN.
  - **Standalone mode**—Plan your run without a sample sheet.

This section provides an overview of the system, including information on hardware, software, and data analysis. It also assembles key concepts and terminology that are embedded throughout the documentation. For detailed specifications, data sheets, applications, and related products, see the NextSeq 1000 and NextSeq 2000 Sequencing Systems product page on the Illumina website.

Additional Resources

The NextSeq 1000 and NextSeq 2000 Sequencing Systems support pages on the Illumina website provide additional system resources. These resources include software, training, compatible products, and the following documentation. Always check support pages for the latest versions.

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[1]next-generation sequencing

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Resource	Description
Custom Protocol Selector	A tool for generating end-to-end instructions tailored to your library prep method, run parameters, and analysis method, with options to refine the level of detail.
NextSeq 1000 and NextSeq 2000 Sequencing Systems Safety and Compliance Guide (document # 1000000111928)	Provides information about operational safety considerations, compliance statements, and instrument labeling.
RFID Reader Module Compliance Guide (document # 1000000002699)	Provides information about the RFID reader in the instrument, compliance certifications, and safety considerations.
NextSeq 1000 and 2000 Denature and Dilute Libraries Guide (document # 1000000139235)	Provides instructions for manually denaturing and diluting prepared libraries for a sequencing run, and preparing optional PhiX control.
NextSeq 1000 and 2000 Custom Primers Guide (document # 1000000139569)	Provides information about replacing Illumina sequencing primers with custom sequencing primers.
NextSeq 2000 Sequencing System Site Prep Guide (document # 1000000109378)	Provides specifications for laboratory space, electrical requirements, and environmental and network considerations.
BaseSpace help (help.basespace.illumina.com)	Provides information about using BaseSpace™ Sequence Hub and available analysis options.
Index Adapters Pooling Guide (document # 1000000041074)	Provides pooling guidelines and dual indexing strategies.
Illumina Adapter Sequences (document # 1000000002694)	Provides lists of the adapter sequences for Illumina library prep kits.

Instrument Hardware

NextSeq 1000 and NextSeq 2000 Sequencing Systems comprises a power button, monitor, status bar, consumables compartment, and USB ports.

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Figure 1 External System Components
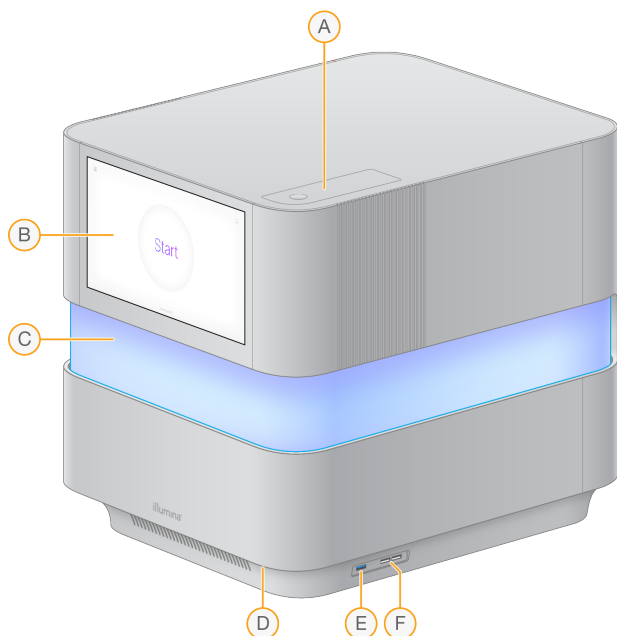
A. **Air filter compartment**—Provides access to replaceable air filter.
B. **Touch-screen monitor**—Enables on-instrument configuration and setup using the control software interface.
C. **Status bar**—Light color progresses as the system moves through its workflow. Blue and purple indicate interactivity (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
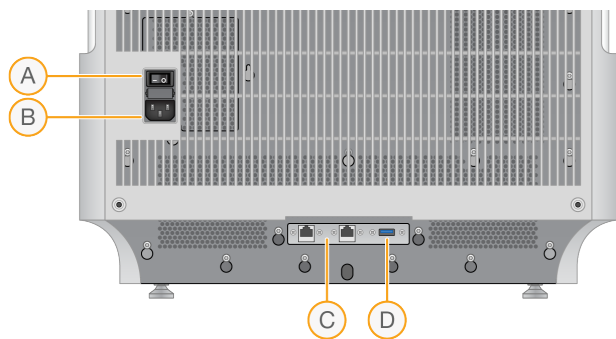
A. **Toggle switch**—Turns instrument power on and off.
B. **Power inlet**—Power cord connection.
C. **Ethernet ports (2)**—Optional Ethernet cable connection.
D. **3.0 USB port**—For connecting an external hard drive for data transfer.

Consumables Compartment

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

Figure 3 Loaded Consumables Compartment
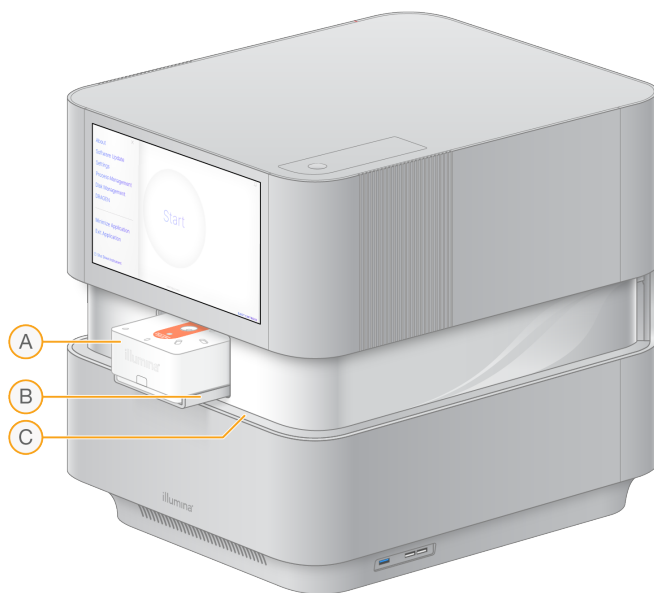
A. **Cartridge**—Contains the flow cell, library, and reagents, and collects used reagents during the run.
B. **Tray**—Holds the cartridge during sequencing.
C. **Visor**—Opens to provide access to the consumables compartment.

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 50.
- **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 70. To requeue on-instrument analysis, see *Requeue a Run* on page 78.

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 78.

Sequencing Protocol Diagram

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

```
1  Thaw the Bagged Cartridge

2  Prepare the Flow Cell and Libraries

3  Load Flow Cell and Libraries Into the Cartridge
```

4A Set Up a Sequencing Run (Instrument Run Setup)	4B Set Up a Sequencing Run (Sample Sheet v2)	4C Set Up a Sequencing Run (No Sample Sheet)
5A Cloud Mode DRAGEN Analysis (BaseSpace Sequence Hub)	5A Local Mode DRAGEN Analysis (On-Instrument)	5 Standalone Mode Custom Analysis (cBCL Data Only)
5B Hybrid Mode DRAGEN Analysis (On-Instrument)	5B BaseSpace Sequence Hub App Analysis (BaseSpace Sequence Hub)	

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

Library[1] is automatically denatured into single strands and further diluted onboard the instrument.

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

During cluster generation, single DNA molecules are bound to the surface of the flow cell and amplified to form clusters[1]. 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 calling[2], filtering, and quality scoring.[3]

Primary Analysis

As the run progresses, the control software automatically transfers base call files[4] (*.cbcl) to the specified output folder for data analysis. During the sequencing run, real time analysis (RTA3) software performs image analysis, base calling, and demultiplexing[5]. 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.

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

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

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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.

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System Configuration

This section provides instructions for setting up your system, including descriptions of software settings.

These instructions primarily describe the control software, with some information on configuring the network and operating system.

ℹ️ | Using Google Chrome on the instrument will prompt you to unlock your login keyring. You can safely ignore and cancel out of the prompt.

User Account Requirements

The Linux operating system has three accounts:

- root (super administrator)

- ilmnadmin (administrator)

- ilmnuser (user)

The administrator account is intended only for applying system updates, like updating NextSeq 1000/2000 Control Software, or for use by IT staff to mount a persistent network drive.

Perform all other functions, including sequencing, from the user account.

Password Requirements

The Field Service Engineer initiates a password change for all three accounts after completing instrument installation. Update each password every 180 days, when prompted.

Table 1 Default Password Policies

Policy	Setting
Enforce password history	Five passwords remembered
Lockout threshold	Ten invalid logon attempts
Minimum password length	Ten characters
Minimum character variety	Three each of: number, upper case letter, lower case letter, and symbol
Maximum repeating characters	Three characters

Policy	Setting
Password must meet complexity requirements	Disabled
Store passwords using reversible encryption	Disabled

Add a New User

1. Log in to ilmnadmin.
2. Select the power button, and then open the ilmnadmin drop-down.
3. Select **Account Settings**.
4. Select **Unlock**, and then enter the ilmnadmin password.
5. Select **Add User**.
6. Select the Standard account type, and then enter a new user name.
7. Select **Set password now**, and then enter a password.
8. Select **Add**.
   The new user is added to the Users list.
9. Grant the user access to the NextSeq 1000/2000 Control Software as follows.
   a. Open terminal.
   b. Enter the following:
   ```
   $ sudo usermod -a -G ilmnusers <new user name>
   ```
   c. If prompted, enter the ilmnadmin password.
10. To confirm the user permissions were set successfully, do as follows.
    a. Log in to the new user account.
    b. Navigate to the NextSeq 1000/2000 Control Software.
    c. From the control software menu, select **Settings**.
    d. Under Default Output Folder, make sure you can select and save the output folder path.
       If you can select and save the output folder path without any errors, the permissions are successfully set.

Reset Password

This section details how to reset the ilmnuser, ilmnadmin, or root password. Password recovery is not available. Resetting your password does not bypass account lockout after too many incorrect password attempts. You must wait 10 minutes before you can reset your password or try to log in.

Reset ilmnuser Password

You can reset the ilmnuser password if you know the ilmnadmin or root password.

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1. Log in to ilmnadmin.

2. Open terminal.

3. Enter `sudo passwd ilmnuser`.

4. Enter the ilmnadmin password at the prompt.

5. Enter a new ilmnuser password at the prompt.

6. Retype the new ilmnuser password at the prompt to confirm the new password.

Reset ilmnadmin Password

You can reset the ilmnadmin password if you know the root password.

1. Log in to root.

2. Open terminal.

3. Enter `passwd ilmnadmin` to change the ilmadmin password, or enter `passwd ilmnuser` to change the ilmnuser password.

4. Enter the new password at the prompt.

5. Retype the new password at the prompt to confirm the new password.

Reset Root Password

To reset the root password, use one of the following options:

- If you know the password from the time the last OS image was captured, restore to that saved image.

- If you do not remember the password, contact Illumina Tech Support.

Configure BaseSpace Sequence Hub and Proactive Support

Use the following instructions to configure BaseSpace Sequence Hub and Proactive Support on your system. To set up a BaseSpace Sequence Hub account, see the *BaseSpace Sequence Hub Online Help*.

1. From the control software menu, select **Settings**.

2. For BaseSpace Sequence Hub and Proactive Support Settings, select one of the following options:

Option	Description and Requirements
Proactive Support Only*	Send instrument performance data to Illumina for faster troubleshooting. Requires an internet connection.

Option	Description and Requirements
Proactive and Run Monitoring	Send InterOp and log files to BaseSpace Sequence Hub for remote run monitoring. This option is the default. Requires a BaseSpace Sequence Hub account and an internet connection.
Proactive, Run Monitoring and Storage	Send InterOp files, log files, and run data to BaseSpace Sequence Hub for remote monitoring and analysis. Requires a BaseSpace Sequence Hub account, an internet connection, and a sample sheet.
None	Disconnect runs from BaseSpace Sequence Hub accounts and do not send instrument performance data for Illumina Proactive Support.

* Depending on the control software version, the name of this setting on the software interface might differ from the name in this guide.

When any option except None is selected, Proactive Support is enabled. This is a free service that allows you to see your performance data on MyIllumina Customer Dashboard and allows Illumina's service teams to troubleshoot issues faster.

> ℹ️ | Proactive and Run Monitoring is turned on by default. To opt out of this service, select **None**.

3. If you selected None at step 2, select **Save** to finish. Otherwise, continue through step 6.

4. From the Hosting Location list, select the location of the BaseSpace Sequence Hub server where data are uploaded to.
   Make sure to use the Hosting Location in or closest to your region.

5. If you have an Enterprise subscription, enter the domain name (URL) used for your BaseSpace Sequence Hub account.
   For example: https://**yourlab**.basespace.illumina.com.

6. Select **Save**.

Specify the Default Output Folder Location

Use the instructions in this section to select a default output folder location. You can change the output folder for each run during run setup. The software saves cBCL files[1] and other run data to the output folder.

An output folder is required unless BaseSpace Sequence Hub is configured for Proactive, Run Monitoring and Storage. Only use an external or network drive as the default output folder. Using an on-instrument output folder negatively impacts your sequencing run.

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

Specify an External Drive Output Folder

Use the following instructions to select an external portable drive as the default output folder. A self-powered drive that is formatted to NFTS or GPT/EXTA is recommended.

1. Plug in an external portable drive using the 3.0 USB port on the side or back of the instrument. Make sure the external portable drive allows write permissions. If it is set to Read Only, the control software will not be able to save data to it.

2. Create a new folder on the external portable drive. This folder will become the default output folder location.
   NextSeq 1000/2000 Control Software requires at least two levels of nested folders to recognize the location as an external portable drive.

3. From the control software menu, select **Settings**.

4. Under Default Output Folder, select the existing folder path and navigate to the new folder on the external portable drive.

5. **[Optional]** If you have selected **Online Run Setup** under Run Mode, select an option from the Hosting Location drop-down menu.

6. Select **Save**.

Specify a Network Drive Default Output Folder

Use the following instructions to mount a persistent network drive and specify the default output folder location. Server Message Block (SMB)/Common Internet File Systems (CIFs) and Network File System (NFS) are the only supported methods for persistent mounting of a network drive on the NextSeq 1000/2000.

SMB/CIFS Mounting Instructions

1. If NextSeq 1000/2000 Control Software is open, select **Minimize Application**.

2. Log into ilmnadmin.

3. Select **Applications**.

4. Under Favorites, select **Terminal**.

5. Enter `sudo touch /root/.smbcreds` and then select **Enter**.

6. Enter the ilmnadmin password when prompted.
   The ilmnadmin password is required each time you use a `sudo` command.

7. Enter `sudo gedit /root/.smbcreds` and then select **Enter** to open the text file named smbcreds.

8. When the `.smbcreds` text file opens, enter your network log in credentials in the following format.

   ```
   username=<user name>
   password=<password>
   domain=<domain_name>
   ```

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Brackets are not required for username, password, and domain credentials. Domain credential is only required if the remote account is part of a domain.

9. Select **Save** and exit the file.

10. Identify the server name and share name for your SMB/CIFs server.
    The server name and share name cannot have spaces, for example:
    Server name: `192.168.500.100` or `Myserver-myinstitute-03`
    Share name: `/share1`

11. In the terminal, enter `sudo chmod 400 /root/.smbcreds` and then select **Enter** to grant read access to the `.smbcreds` text file.

12. Enter `sudo mkdir /mnt/<local name>`.
    `<local name>` is the name of the new directory in your network drive and can contain spaces. This is the directory that will appear on the instrument.

13. Select **Enter**.

14. Enter `sudo gedit /etc/fstab` and then select **Enter**.

15. When the fstab file opens, enter the following to the end of the file, and then select **Enter**.
    ```
    //<Server name>/<Share name> /mnt/<local name> cifs
    credentials=/root/.smbcreds,uid=ilmnadmin,gid=ilmnusers,dir_
    mode=0775,file_mode=0775,_netdev,x-systemd.automount,sec=ntlmssp 0 0
    ```

16. Select **Save** and exit the file.

17. In the terminal, enter `sudo mount -a -vvv` and then select **Enter**.
    The network drive is now mounted as `/mnt/<local name>`.

18. To confirm if the mount was successful, enter `<df | grep <local name>>` and then select **Enter**. The name of the fileshare should appear.

19. Enter `sudomkdir /mnt/<local name>/<output directory>` to create a subfolder within the local directory. The `<output directory>` represents your default output folder location.
    The NextSeq 1000/2000 Control Software requires at least two levels of nested folders to recognize the location as a mounted network drive.

20. Power cycle the instrument. See *Power Cycle the Instrument* on page 78.

21. Set the persistent mounted network drive as the default output folder. See *Specify the Persistent Network Drive as the Default Output Folder* on page 16.

NFS Mounting Instructions

1. If NextSeq 1000/2000 Control Software is open, select **Minimize Application**.

2. Log into ilmnadmin.

3. Identify the server name for your NFS server.
   The server name cannot have spaces, for example:
   Server name: `192.168.500.100` or `Myserver-myinstitute-03`

4. Select **Applications**.

5. Under Favorites, select **Terminal**.

6. Enter `sudo mkdir /mnt/<local name>` and then select **Enter**.
    `<local name>` is the name of the new directory in your network drive.

7. Enter `sudo gedit /etc/fstab` and then select **Enter**.

8. When the fstab file opens, enter the following, and then select **Enter**.
    `Server name:/share //mnt/<local name> nfs x-systemd.automount,defaults 0 0`

9. Select **Save** and exit the file.

10. In the terminal, enter `sudo mount -a -vvv` and then select **Enter**.
    The network drive is now mounted in the `/mnt/directory` within the `<local name>` folder.

11. Create a new `<sub folder>` within the `<local name>` folder. The subfolder represents your
    default output folder location.
    The NextSeq 1000/2000 Control Software requires at least two levels of nested folders to
    recognize the location as a mounted network drive.

12. Power cycle the instrument. See *Power Cycle the Instrument* on page 78.

13. Set the persistent mounted network drive as the default output folder. See *Specify the Persistent
    Network Drive as the Default Output Folder* on page 16.

Specify the Persistent Network Drive as the Default Output Folder

1. Login into ilmnuser.

2. From the NextSeq 1000/2000 Control Software menu, select **Settings**.

3. Under Default Output Folder, select the persistent network drive mount located at `/mnt/<local
    name>/<output directory>`.

4. **[Optional]** If you have selected **Online Run Setup** under Run Mode, select an option from the
    Hosting Location drop-down menu.

5. Select **Save**.

Import Custom Reference Genomes

New custom reference genomes can only be imported using the administrator account. For a list of all
compatible reference genomes, visit the NextSeq 1000/2000 Product Compatibility page.

1. Create a reference genome using the Reference Builder for Illumina Instruments BaseSpace
    Sequence Hub app. For more information, see *Reference Builder for Illumina Instruments v1.0.0
    App Online Help*.

2. Select the control software menu, and then select **Process Management**.

3. Make sure that there are no sequencing runs or on-instrument secondary analyses in progress.

4. From the control software menu, select **Minimize Application**.

5. Log into ilmnadmin.

6. Select the control software menu, and then select **DRAGEN**.

7. In the Genome section, select **View Installed Genomes** to view a list of all currently installed Illumina and custom genomes.

8. Close the modal.

9. Select **Choose** under Import New Reference Genomes, navigate to the reference genome file (*.tar.gz) on the portable or mounted network drive, and then select **Open**.

10. Select **Import**.

Import Noise Baseline Files

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

Generate a Custom Noise Baseline File

If using somatic mode, you can generate a custom noise baseline file. The noise baseline file is built using normal samples that do not match to the subject the samples are from. The recommended number of normal samples is 50.

To generate a custom noise baseline file, use one of the following methods:

- Use the DRAGEN Bio-IT Platform server. See the *DRAGEN Bio-IT Platform Online Help* for instructions.

- Use DRAGEN Baseline Builder App on BaseSpace Sequence Hub. Use the BCL Convert pipeline in BaseSpace Sequence Hub Instrument Run Setup to generate FASTQ files. After the sequencing run is complete and 50 samples are available, input the FASTQ files into the DRAGEN Baseline Builder App.

Import Baseline Files Using the User Interface

After importing the baseline file, you can set up your sequencing run using the DRAGEN Enrichment workflow in somatic mode.

1. Download a standard baseline file from the Illumina Support Site, or download the custom baseline file from the DRAGEN server or DRAGEN Baseline Builder App.

2. From the control software menu, select **Minimize Application**.

3. Log in to ilmnadmin.

4. Select **Applications**, and then select **Favorites**.

5. Select **+Other Locations**, and then select **Computer**.

6. Double-click **usr**, and then **local**.

7. Double-click **illumina**, and then **aux_files**.

8. Drag the noise baseline file to aux_files.

Import Baseline Files Using Terminal

After importing the baseline file, you can set up your sequencing run using the DRAGEN Enrichment workflow in somatic mode.

1. Download a standard baseline file from the Illumina Support Site, or download the custom baseline file from the DRAGEN server or DRAGEN Baseline Builder App.

2. From the control software menu, select **Minimize Application**.

3. Log in to ilmnadmin.

4. Select **Applications.**

5. Under Favorites, select **Terminal**.

6. Enter the following command.

```
cp [/path/to/baselinefile] /usr/local/illumina/aux_files
```

Configure Run Mode

The run mode applies to all runs and determines where to enter run parameters and how to analyze data.

Cloud or Hybrid Mode

1. From the control software menu, select **Settings**.

2. Select **Online Run Setup** under BaseSpace Sequence Hub Services & Proactive Support.

3. Configure additional settings appropriately by selecting the following:

    a. **Proactive and Run Monitoring** or **Proactive, Run Monitoring and Storage**.

    b. Dropdown menu for **Hosting Location**.

    c. **[Optional]** Enter a **Private Domain Name**.

4. Select **Save**.

Local or Standalone Mode

1. From the control software menu, select **Settings**.

2. Select **Local Run Setup** under BaseSpace Sequence Hub Services & Proactive Support.

3. Configure additional settings appropriately by selecting the following:

    a. **Proactive Support Only**, **Proactive and Run Monitoring**, **Proactive, Run Monitoring and Storage**, or **None**.

> ℹ │ BaseSpace Sequence Hub will only allow requeue functionality if **Proactive, Run Monitoring and Storage** is selected. In the event of an invalid sample sheet, this will allow you to make corrections to the sample sheet and requeue demultiplexing analysis. For on-instrument requeue functionality, see *Requeue a Run* on page 78.

    b. Dropdown menu for **Hosting Location**.

    c. **[Optional]** Enter a **Private Domain Name**.

4. Select **Save**.

Sample Sheet Considerations for Local or Standalone Mode

You must use the sample sheet v2 file format to analyze with DRAGEN. Sample sheet v2 file format is also compatible with BaseSpace Sequence Hub apps that are not DRAGEN enabled. For information on creating a sample sheet in the v2 file format, see *Sample Sheet v2 Settings* on page 81.

Instrument Customization

This section includes information on configuring the available customization settings. To set a default output folder, see *Specify the Default Output Folder Location*.

Name the Instrument

1. From the control software menu, select **Settings**.
2. Select Instrument Nickname, and enter a preferred name for the instrument.
   The name appears along the top of each screen.
3. Select **Save**.

Set Denature and Dilute Preferences

1. From the control software menu, select **Settings**.
2. Choose whether to automatically denature and dilute libraries onboard the instrument. The setting defaults to the option selected for your prior run.
   - To denature and dilute libraries automatically onboard the instrument, select the **Denature and Dilute On Board** checkbox.
   - To manually denature and dilute libraries, deselect the **Denature Dilute On Board** checkbox.
     See the *NextSeq 1000 and 2000 Denature and Dilute Libraries Guide (document # 1000000139235)* for instructions on denaturing and diluting libraries manually.

Set Automatic Reagent Purge Preference

1. From the control software menu, select **Settings**.

2. Choose whether the system automatically purges unused reagents to the spent reagents compartment after each run to streamline reagent waste disposal after run completion:

   - To purge automatically, select the **Purge Reagent Cartridge** checkbox.

   - To skip automatic purging, deselect the **Purge Reagent Cartridge** checkbox (this is the default setting).

   Purging unused reagents adds up to 2 hours to the workflow.

3. Select **Save**.

Configure Software Updates

1. From the control software menu, select **Settings**.

2. Choose whether the system automatically checks for software updates:

   - To check automatically, select the **Autocheck for software updates** checkbox.

   - To check manually, deselect the **Autocheck for software updates** checkbox.

   Checking for software updates automatically requires an internet connection. For more information on installing software updates, see *Software Updates* on page 70.

3. Select **Save**.

Change LCD Brightness

1. From the control software menu, select **Settings**.

2. Move the LCD Brightness slider to the desired percentage.

3. Select **Save**.

Set a Proxy Server

Proxy server support is only available in the NextSeq 1000/2000 Control Software v1.3.

1. From the control software menu, select **Settings**.

2. Select the current proxy settings to open the Proxy Settings screen.

3. Select the **Enable Proxy** checkbox, and then enter the server IP port address.

4. **[Optional]** If the proxy server requires authentication, select the **Requires Username and Password** checkbox, and then enter the user name and password.

5. Select **Save** to save and validate the proxy information.

6. Select one of the following options:

   - Select **Yes, I'm Finished** to restart the system and apply the new proxy settings.

   - Select **No, Take Me Back** to return to the Settings screen. The new proxy settings are saved, but are not applied until you restart the system.

Consumables & Equipment

This section lists everything that comes in the reagent kit with storage conditions. You can also see which ancillary consumables and equipment you must purchase to complete the protocol and perform maintenance and troubleshooting procedures.

Sequencing Consumables

Sequencing on the NextSeq 1000/2000 requires one single-use Illumina NextSeq 1000/2000 P2 Reagents kit or one single-use Illumina NextSeq 1000/2000 P3 Reagents kit. The NextSeq 1000/2000 P2 Reagent kit is available in three sizes (100-cycle, 200-cycle, 300-cycle) and the NextSeq 1000/2000 P3 Reagent kit is available in four sizes (50-cycle, 100-cycle, 200-cycle, 300-cycle).

The NextSeq 1000 Sequencing System is only compatible with Illumina NextSeq 1000/2000 P2 Reagents kit.

The reagent kit provides the cartridge and flow cell for sequencing. When you receive NextSeq 1000/2000 P2 Reagents or Illumina NextSeq 1000/2000 P3 Reagents kit:

- Promptly store components at the indicated temperatures to ensure proper performance.
- Do not open any silver foil bag until instructed to do so.
- Store cartridges in their box to avoid tearing or puncturing the foil bag.
- Store cartridges with the arrows pointing up.

⚠️ If cartridge label is not facing up, sequencing data will be negatively impacted.

Table 2 Kit Components

| Consumable	Quantity	Storage Temperature	Dimensions
Cartridge	1	-25°C to -15°C	29.2 cm × 17.8 cm × 12.7 cm (11.5 in × 7 in × 5 in)
Flow cell	1	2°C to 8°C*	21.6 cm × 12.7 cm × 1.9 cm (8.5 in × 5 in × 0.75 in)
RSB with Tween 20	1	-25°C to -15°C	4 cm x 6.6 cm x 5 cm) (1.6 in x 2.6 in x 2 in)

*Shipped at room temperature.

Both consumables have identifiers for tracking and ensuring compatibility. The cartridge and flow cell use RFID[1].

[1]radio-frequency identification

Flow Cell

The flow cell is a patterned, single-lane flow cell. A plastic cartridge encases the glass-based flow cell. A gray tab covers and protrudes from the flow cell to ensure safe handling.
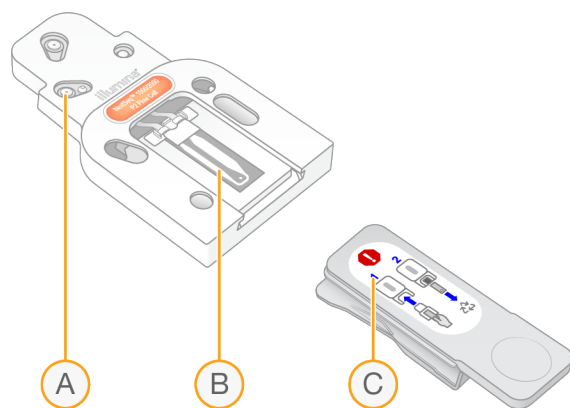

A. Plastic cartridge
B. Flow cell
C. Gray tab

Millions of nanowells cover the inner surface of the flow cell. Clusters are generated in the nanowells, from which the sequencing reaction is then performed. The patterned arrangement of the nanowells increases output reads and data.

Cartridge

The sequencing reagent cartridge is prefilled with clustering, sequencing, paired-end, and indexing reagents. A foil-sealed reservoir is reserved for libraries, and a slot on the front is reserved for the flow cell.
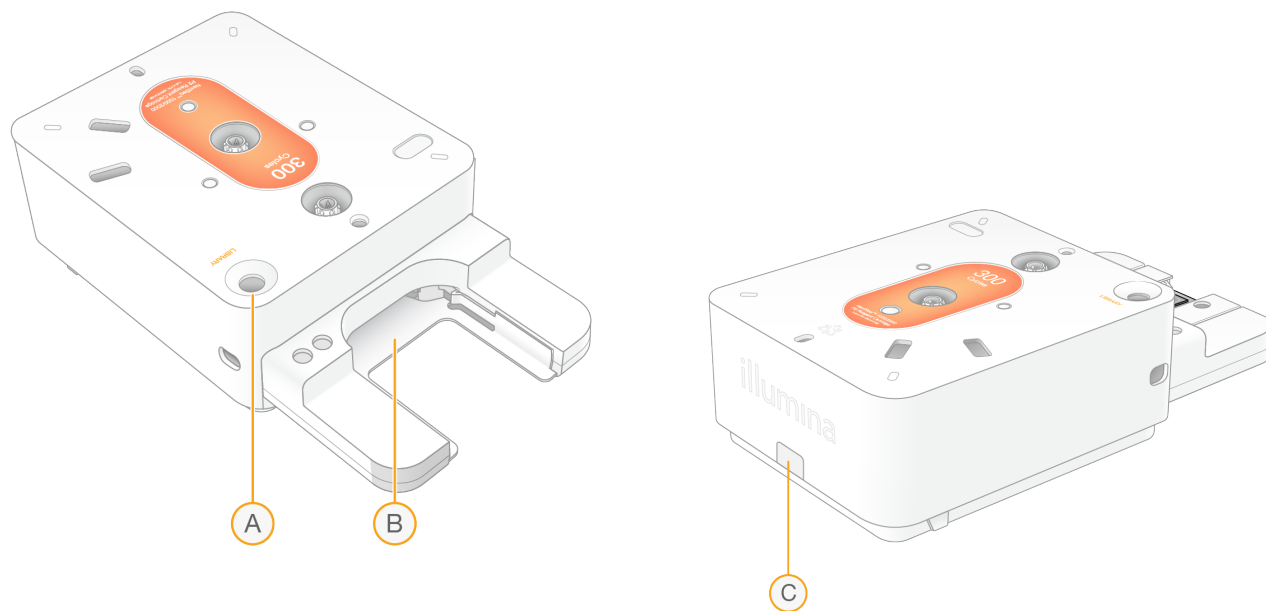
A. Library reservoir
B. Flow cell slot
C. Drainage plug

The cartridge contains all consumables for a run: reagents, library, and flow cell. The library and flow cell are loaded into the thawed cartridge, which is then loaded onto the instrument. After the run begins, reagents and library are automatically transferred from the cartridge to the flow cell.

The cartridge contains pumps, valves, and all fluidics for the system, including an underside reservoir to collect used reagents. The cartridge is discarded after a run, so instrument washes are not necessary.
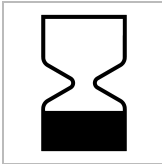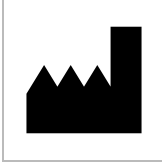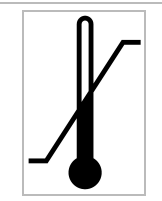
Supported Number of Cycles

The label on the cartridge indicates how many cycles are analyzed, not how many cycles are performed. The flow cell is compatible with any number of cycles and any read type.

All 100-cycle and 200-cycle cartridges include an extra 38 cycles. The 300-cycle cartridge includes an extra 27 cycles. For example, the 300-cycle cartridge provides sufficient reagents for up to 327 cycles of sequencing. For information on how many cycles to sequence, see *Number of Cycles in a Read* on page 29.

Symbol Descriptions

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

Symbol	Description
	The date the consumable expires. For best results, use the consumable before this date.
	Indicates the manufacturer (Illumina).
RUO	The intended use is Research Use Only (RUO).
REF	Indicates the part number so that the consumable can be identified.[1]
LOT	Indicates the batch code to identify the batch or lot that the consumable was manufactured in.[1]
	Indicates a health hazard.
	Storage temperature range in degrees Celsius. Store the consumable within the indicated range.[2]

Ancillary Consumables

Purchase the following consumables for sequencing and maintenance.

For Research Use Only. Not for use in diagnostic procedures.

Consumables for Sequencing

Table 3 Consumables for Sequencing

Consumable	Supplier	Purpose
Disposable gloves, powder-free	General lab supplier	General purpose.
NextSeq 1000/2000 P2 (v3) Reagents kit	Illumina: catalog # 20046811 (100 cycles) catalog # 20046812 (200 cycles) catalog # 20046813 (300 cycles)	Provides the reagent cartridge, flow cell, and NextSeq 1000/2000 RSB with Tween 20 for a single run. Compatible with NextSeq 1000 and NextSeq 2000.
NextSeq 2000 P3 Reagents kit	Illumina: catalog # 20046810 (50 cycles) catalog # 20040559 (100 cycles) catalog # 20040560 (200 cycles) catalog # 20040561 (300 cycles)	Provides the reagent cartridge, flow cell, and NextSeq 1000/2000 RSB with Tween 20 for a single run. Only compatible with the NextSeq 2000.
Microtubes, 1.5 ml	Fisher Scientific, catalog # 14-222-158, or equivalent low-bind tubes	Diluting libraries to the loading concentration.
Pipette tips, 10 µl	General lab supplier	Diluting libraries.
Pipette tips, 20 µl	General lab supplier	Diluting and loading libraries.
Pipette tips, 200 µl	General lab supplier	Diluting libraries.
Pipette tips, 1000 µl	General lab supplier	Piercing the library reservoir foil.
[Optional] PhiX Control v3	Illumina, catalog # FC-110-3001	Performing a PhiX-only run or spiking in a PhiX control.
[Optional] Paper towels	General lab supplier	Drying the cartridge after a water bath.

Consumables for Maintenance

Table 4 Consumables for Maintenance

Consumable	Supplier	Purpose
Disposable gloves, powder-free	General lab supplier	General purpose.
NextSeq 1000/2000 Air Filter Replacement*	Illumina, catalog # 20029759	Replacing the air filter every six months.

* The instrument ships with one installed and one spare. When not under warranty, replacements are user-supplied. Keep packaged until use.

Ancillary Equipment

Purchase the following equipment for sequencing purposes.

Item	Source	Purpose
Freezer, -25°C to -15°C	General lab supplier	Storing the cartridge.
Ice bucket	General lab supplier	Setting aside libraries until sequencing.
Pipette, 10 µl	General lab supplier	Diluting libraries to the loading concentration.
Pipette, 20 µl	General lab supplier	Diluting libraries to the loading concentration and loading libraries into the cartridge.
Pipette, 200 µl	General lab supplier	Diluting libraries to the loading concentration.
Refrigerator, 2°C to 8°C	General lab supplier	Storing the flow cell or thawing the cartridge.

Item	Source	Purpose
[Optional] One of the following temperature controlled water baths or equivalent that can hold at 25°C: • Thermo Scientific Precision 35L Circulating Water Bath (holds 5 cartridges simultaneously) • SHEL LAB 22L Digital Circulating Water Bath (holds 3 cartridges simultaneously)	• Thermo Fisher Scientific, catalog # TSCIR 35 • Shel Lab, catalog # SWBC22	Thawing the cartridge.

Protocol

This section provides step by step instructions on how to prepare consumables, dilute libraries, and set up a sequencing run in one of four run modes (Cloud, Hybrid, and Local mode use DRAGEN or BaseSpace Sequence Hub, while Standalone mode is a standalone run intended for generating cBCL data only for custom analysis workflows).

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

Make sure that you have the required consumables and equipment before starting a protocol. See *Consumables & Equipment* on page 21.

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

Sequencing Considerations

Before starting the protocol, review the following information to prepare for diluting libraries and setting up the run. Achieving the optimum loading concentration is critical for successful sequencing and analysis. Entering the correct number of cycles in a read helps ensure optimum data output.

Loading Volume and Concentrations

Loading volume is 20 µl. Loading concentration varies by library type:

Library Type	Loading Concentration (pM)
AmpliSeq™ for Illumina Library PLUS	750
Illumina DNA Prep	750
Illumina DNA Prep with Enrichment	1000
Illumina Stranded Total RNA with Ribo-Zero Plus	750
Illumina Stranded mRNA Prep	750
Illumina DNA PCR-Free	1000
100 % PhiX	650
TruSeq DNA Nano 350	1200
TruSeq DNA Nano 550	1500
TruSeq Stranded mRNA	1000

For other library types, 650 pM is the recommended starting loading concentration. Optimize this concentration over subsequent runs to identify a loading concentration that consistently yields data that meets specifications.

ℹ️ To optimize loading concentration, use the % Loading Concentration metric in the `PrimaryAnalysisMetrics.csv` output file available after run completion. If % Loading Concentration is < 95%, increase the loading concentration in 100 pM increments over subsequent runs.

Number of Cycles in a Read

For each read, entering a minimum of 26 cycles and a maximum of 151 cycles helps ensure data quality. The exact number of cycles depends on your experiment. NextSeq 1000/2000 Control Software requires at least 1 cycle for Read 1, but displays a warning when the number of cycles in Read 1 is less than 26.

The total number of cycles for Read 1, Index 1, Index 2, and Read 2 cannot be greater than the number of cycles supported by the kit plus 38 cycles for 100-cycle and 200-cycle kits and 27 cycles for P3 300-cycle kits. NextSeq 1000/2000 Control Software will display a warning when Index 1 and Index 2 are less than 6 cycles. The warning will not be shown if Index 1 or Index 2 is 0 cycles.

The minimum and maximum cycle numbers include an extra cycle. Always add one cycle to the desired read length to correct the effects of phasing and prephasing. Read length is the number of *sequencing* cycles in Read 1 and Read 2, which excludes extra cycles and index cycles. For more information, see Phasing Correction in *Real-Time Analysis Workflow* on page 53.

Example run setup:

- For a read length of 35 (single-read), enter **36** in the Read 1 field.
- For a read length of 150 per read (paired-end), enter **151** in the Read 1 field and **151** in the Read 2 field.

Plan a Sequencing Run in BaseSpace Sequence Hub

Use Instrument Run Setup in BaseSpace Sequence Hub to create and configure your run settings. If you are setting up a run in Cloud mode or Hybrid mode, submit the run configuration to your BaseSpace Sequence Hub account planned runs list in the Planned Runs tab. Runs available for sequencing on the NextSeq 1000 and NextSeq 2000 Sequencing Systems are displayed in the Planned Runs tab. If you are setting up a run in Local mode, use Instrument Run Setup to create and export your sample sheet in v2 file format. Alternatively, see *Sample Sheet v2 Settings* on page 81 to create a sample sheet without BaseSpace Sequence Hub using a provided template.

BaseSpace Sequence Hub Instrument Run Setup does not support more than 1536 samples.

Set up a Run

1. Navigate to BaseSpace Sequence Hub.

2. Enter your email address and BaseSpace Sequence Hub password, and then select **Sign in**.

3. Select the **Runs** tab, and then select the **New Run** drop-down.

4. Select **NextSeq 1000/2000**.

5. In the Run Name field, enter a unique name of your preference to identify the current run. The run name can contain a maximum of 225 alphanumeric characters, spaces, dashes, and underscores.

6. Select one of the following analysis locations.

   - **BaseSpace**—Analyze sequencing data in the cloud.

   - **Local**—Analyze sequencing data on-instrument or generate a Sample Sheet v2 for Local or Hybrid mode.

7. Select an analysis type and version.

   For more information about secondary analyses, see *DRAGEN Secondary Analysis Output Files* on page 58 or the BaseSpace Sequence Hub app documentation. If you selected DRAGEN Single Cell RNA analysis, see the NextSeq 1000/2000 Products Files page for information on third-party single cell RNA library prep kit compatibility.

   > ℹ️ For on-instrument analysis, the version selected must match the version of DRAGEN installed on the instrument. To confirm the version of DRAGEN installed on the instrument, see *DRAGEN Workflow and License Updates* on page 72.

8. **[Optional]** Set up custom index kits as follows.

   If you are using more than one library, the libraries must have the same index read lengths.

   a. Select **Add Custom Index Adapter Kit** under the Index Adapter Kit dropdown.

   b. Select a template type and enter the kit name, adapter sequences, index strategies, and index sequences.

      Make sure the second index (i5) adapter sequences are in forward orientation.

   c. Select **Create New Kit**.

9. **[Optional]** Set up custom library prep kit as follows.

   a. Select **Add Custom Library Prep Kit** under the Library Prep Kit dropdown.

   b. Enter the name, read types, default read cycles, and compatible index adapter kits for your custom library prep kit.

   c. Select **Create New Kit**.

10. Select the following instrument settings. Depending on the library prep kit, recommended options are automatically selected. Some library prep kits have hard-coded number of indexes reads and read types, which cannot be changed.

    - Library prep kit

- Index adapter kit

- Number of index reads

- Read type

- Number of sequencing cycles per read

> ℹ️ | If Not Specified is selected for library prep kit, number of index reads is not updated until index sequences are entered into the Sample Data section.

11. Enter sample information into the Sample Data spreadsheet using one of the following options. To group samples for data aggregation during downstream analysis, assign a name for the group in the Project column.

   - Select **Import Data**, and then select your sample sheet. Make sure that your sample sheet meets the formatting requirements. See *Sample Sheet v2 Settings* on page 81. Altering your sample sheet after initial download can result in analysis failure.

   - Paste sample IDs and either index plate well positions or i7 and i5 indexes directly from an external file. Before pasting, enter the number of sample rows in the Rows field, and then select **+**. Sample IDs can contain up to 20 alphanumeric characters, hyphens, and underscores.

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

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

12. Select **Next**.

Set up Secondary Analysis

Configure the settings for the analysis type selected for your run. For more information DRAGEN analysis workflows, see *DRAGEN Secondary Analysis Output Files* on page 58

Illumina DRAGEN BCL Convert

Use the following steps to configure Illumina DRAGEN BCL Convert analysis.

1. Enter the following optional settings.

Setting	Description
AdapterRead1	Adapter sequence for read 1. If using an Illumina library prep kit, leave the AdapterRead1 field empty.
AdapterRead2	Adapter sequence for read 2. If using an Illumina library prep kit, leave the AdapterRead2 field empty.

Setting	Description
BarcodeMismatchesIndex1	The number of allowed mismatches between the first index read and index sequence. The default value is 1. If a barcode is 6 bp, the recommended value is 0.
BarcodeMismatchesIndex2	The number of allowed mismatches between the second index read and index sequence. The default value is 1. If a barcode is 6 bp, the recommended value is 0.

OverrideCycles	String used to specify UMI cycles and mask out cycles of a read. The following values are allowed:
	• N—Specifies cycles to ignore.
	• Y—Specifies sequencing cycles.
	• I—Specifies index cycles.
	• U—Specifies UMI cycles to be trimmed.
	Each element is separated by semicolons. The following are examples of OverrideCycles input.
	`U8Y143;I8;I8;U8Y143`
	`N10Y66;I6;N10Y66`

2. Select whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.

3. Select one of the following FASTQ output format options:

    • **gzip**—Save the FASTQ files in gzip format.

    • **DRAGEN**—Save FASTQ files in ora format.

4. Complete the run configuration.

    • To send the run configuration to your BaseSpace Sequence Hub account, select **Submit Run**. Runs submitted to BaseSpace Sequence Hub appear in the planned runs list and are available for systems using Cloud mode or Hybrid mode.

    • To save the run configuration as a sample sheet in v2 file format, select **Export Sample Sheet** from the **Submit Run** drop-down list. The sample sheet is required to initiate runs on systems using Local mode. This option is only available if Local was selected for analysis location.

Illumina DRAGEN Enrichment

Use the following steps to configure Illumina DRAGEN Enrichment analysis.

1. Select a reference genome.
   If possible, use a reference genome with alt aware.

2. Select a *.bed file containing the regions you would like to target or upload a new custom file. Make sure the BED file's reference genome matches the reference genome selected in step 1. For a new custom BED file, use the following naming format: `name_of_panel_versionNumber.referencegenome.bed`.

   - **Local mode**—Select **Select Custom File (Local)** to upload for a single run or **Upload Custom File (BaseSpace)** for repeated use.

   - **Cloud or Hybrid mode**—Select **Upload Custom File (BaseSpace)**. The custom BED file is only available in the Workgroup it was uploaded to.

3. Select either the germline or somatic variant caller.

4. **[Optional]** If using the somatic variant caller, select a noise baseline file. See *Import Noise Baseline Files* on page 17 for more information.

5. Select a map/align output format.

6. Select whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.

7. Select one of the following FASTQ output format options:

   - **gzip**—Save the FASTQ files in gzip format.

   - **DRAGEN**—Save FASTQ files in ora format.

8. Complete the run configuration.

   - To send the run configuration to your BaseSpace Sequence Hub account, select **Submit Run**. Runs submitted to BaseSpace Sequence Hub appear in the planned runs list and are available for systems using Cloud mode or Hybrid mode.

   - To save the run configuration as a sample sheet in v2 file format, select **Export Sample Sheet** from the **Submit Run** drop-down list. The sample sheet and secondary analysis supporting files are downloaded in a *.zip folder and are required to initiate runs on systems using Local mode. This option is only available if Local was selected for analysis location.

Illumina DRAGEN Germline

Use the following steps to configure Illumina DRAGEN Germline analysis.

1. Select your reference genome.
   If possible, use a reference genome with alt aware.

2. Select a map/align output format.

3. Select whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.

4. Select one of the following FASTQ output format options:

   - **gzip**—Save the FASTQ files in gzip format.

   - **DRAGEN**—Save FASTQ files in ora format.

5. Complete the run configuration.

- To send the run configuration to your BaseSpace Sequence Hub account, select **Submit Run**. Runs submitted to BaseSpace Sequence Hub appear in the planned runs list and are available for systems using Cloud mode or Hybrid mode.

- To save the run configuration as a sample sheet in v2 file format, select **Export Sample Sheet** from the **Submit Run** drop-down list. The sample sheet and secondary analysis supporting files are downloaded in a *.zip folder and are required to initiate runs on systems using Local mode. This option is only available if Local was selected for analysis location.

Illumina DRAGEN RNA

Use the following steps to configure Illumina DRAGEN RNA analysis.

1. Select your reference genome.
   If possible, use a reference genome without alt aware.

2. Select your map/align output format.

3. Select whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.

4. Select one of the following FASTQ output format options:

   - **gzip**—Save the FASTQ files in gzip format.

   - **DRAGEN**—Save FASTQ files in ora format.

5. **[Optional]** Upload an RNA Annotation file in Gene Transfer Format (GTF).

   - **Local mode**—Select **Select Custom File (Local)** to upload for a single run or **Upload Custom File (BaseSpace)** for repeated use.

   - **Cloud or Hybrid mode**—Select **Upload Custom File (BaseSpace)**. The GTF file is only available in the Workgroup it was uploaded to.

   Once a GTF file has been uploaded to a BaseSpace Sequence Hub Workgroup, select the RNA Annotation file from the dropdown menu.

6. Select whether to enable differential expression.

7. If you enabled differential expression, select a control or comparison value for each sample.
   In each comparison group, any sample marked as control is compared with all samples marked as comparison. If the sample does not contain a control or comparison value, select **na** as the value.

8. Complete the run configuration.

   - To send the run configuration to your BaseSpace Sequence Hub account, select **Submit Run**. Runs submitted to BaseSpace Sequence Hub appear in the planned runs list and are available for systems using Cloud mode or Hybrid mode.

- To save the run configuration as a sample sheet in v2 file format, select **Export Sample Sheet** from the **Submit Run** drop-down list. The sample sheet and secondary analysis supporting files are downloaded in a *.zip folder if an optional GTF file has been provided and are required to initiate runs on systems using Local mode. This option is only available if Local was selected for analysis location.

Illumina DRAGEN Single Cell RNA

Use the following steps to configure Illumina DRAGEN Single Cell RNA analysis.

1. Select your reference genome.
   If possible, use a reference genome without alt aware.
2. **[Optional]** Upload an RNA Annotation file in Gene Transfer Format (GTF).
   - **Local mode**—Select **Select Custom File (Local)** to upload for a single run or **Upload Custom File (BaseSpace)** for repeated use.
   - **Cloud or Hybrid mode**—Select **Upload Custom File (BaseSpace)**. The GTF file is only available in the Workgroup it was uploaded to.

   Once a GTF file has been uploaded to a BaseSpace Sequence Hub Workgroup, select the RNA Annotation file from the dropdown menu.
3. Select your map/align output format.
4. Select whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.
5. Select one of the following FASTQ output format options:
   - **gzip**—Save the FASTQ files in gzip format.
   - **DRAGEN**—Save FASTQ files in ora format.
6. Select the configuration identical to your library prep kit type.
   For example, if you selected Single Cell RNA Library Kit 1 as your library prep kit, select Type 1 for the Configuration Type.
7. Select the barcode read.
8. **[Optional]** Edit the number of bases in the barcodes and the UMI. The values are automatically filled based on the library prep kit and configuration type you selected.
9. Select the strand orientation.
10. **[Optional]** Select a file containing your barcode sequences or upload a new custom file.
11. If using an Advanced/Custom configuration type, enter values for the number of override cycles, the barcode position, and the UMI position.
12. Complete the run configuration.

- To send the run configuration to your BaseSpace Sequence Hub account, select **Submit Run**. Runs submitted to BaseSpace Sequence Hub appear in the planned runs list and are available for systems using Cloud mode or Hybrid mode.

- To save the run configuration as a sample sheet in v2 file format, select **Export Sample Sheet** from the **Submit Run** drop-down list. The sample sheet and secondary analysis supporting files are downloaded in a *.zip folder if an optional GTF file has been provided and are required to initiate runs on systems using Local mode. This option is only available if Local was selected for analysis location.

Illumina DRAGEN Amplicon

Use the following steps to configure Illumina DRAGEN Amplicon analysis.

1. Select your reference genome.
2. Select a *.bed file containing the regions you would like to target, or upload a new custom file. Make sure the BED file's reference genome matches the reference genome selected in step 1. For a new custom BED file, use the following naming format: `name_of_panel_versionNumber.referencegenome.bed`.
    - **Cloud or Hybrid mode**—Select **Upload Custom File (BaseSpace)**. The custom BED file is only available in the Workgroup it was uploaded to.
    - **Local mode**—Select **Select Custom File (Local)** to upload for a single run or **Upload Custom File (BaseSpace)** for repeated use.
3. Select either the germline or somatic variant caller.
4. Select your map/align output format.
5. **[Local]** Select whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.
6. Select whether to save a copy of your FASTQ files. FASTQ files are only generated if you select to keep FASTQ files.
7. Select one of the following FASTQ output format options:
    - **gzip**—Save the FASTQ files in gzip format.
    - **DRAGEN**—Save FASTQ files in ora format.
8. Complete the run configuration.
    - To send the run configuration to your BaseSpace Sequence Hub account, select **Submit Run**. Runs submitted to BaseSpace Sequence Hub appear in the planned runs list and are available for systems using Cloud mode or Hybrid mode.
    - **[Local]** To save the run configuration as a sample sheet in v2 file format, select **Export Sample Sheet** from the **Submit Run** drop-down list. The sample sheet and secondary

analysis supporting files are downloaded in a *.zip folder and are required to initiate runs on systems using Local mode. This option is only available if Local was selected for analysis location.

Thaw the Bagged Cartridge and Flow Cell

This step thaws the cartridge *in the unopened bag* and prepares the flow cell. Thaw the bagged cartridge using one of three methods: controlled water bath, refrigerator, or room temperature air. Use the cartridge immediately after thawing, without refreezing. If you are unable to use the cartridge immediately after thawing, see *Return Consumables to Storage* on page 77.

Figure 4   Bagged Cartridge

Thaw Cartridge in a Controlled Water Bath

1. Put on a new pair of powder-free gloves and remove the cartridge from storage.
2. Remove cartridge from the box but *do not open the silver foil bag*.

⚠ | Thawing a torn or punctured bag in a water bath can result in sequencing failure. Thaw at room temperature or in a refrigerator instead.

3. Thaw the bagged cartridge in a controlled 25°C water bath for 6 hours:
   - Maintain a water depth of at least 9.5–10 cm regardless of how many cartridges you are thawing.
   - Set a temperature-controlled water bath to 25°C.
   - Face the bag label up and set in water bath without submerging.

   ⚠ | Do not attempt to weigh the cartridge down to submerge. If the bag label is not facing up

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⚠️ or the cartridge inverts during thawing, sequencing data will be negatively impacted.

- Do not exceed 8 hours in the water bath.
- Do not simultaneously thaw more cartridges than supported by the water bath. For compatible water baths, see *Ancillary Equipment* on page 26.
- Do not stack cartridges.

4. Remove cartridge from water bath and dry with paper towels.

Thaw Cartridge in a Refrigerator

1. Put on a new pair of powder-free gloves.
2. One day prior to anticipated run, remove the cartridge from -25°C to -15°C storage.
3. Remove cartridge from the box but *do not open the silver foil bag*.
4. Position the cartridge at room temperature so that the label faces up and air can circulate on sides and top.

   ⚠️ If the bag label is not facing up, sequencing data will be negatively impacted.

5. Thaw at room temperature for 6 hours.
6. Position the cartridge in a 2°C to 8°C refrigerator so that the label faces up and air can circulate on sides.

   ⚠️ If the bag label is not facing up, sequencing data will be negatively impacted.

7. Thaw in the refrigerator for 12 hours. Do not exceed 72 hours.

Thaw Cartridge at Room Temperature

1. Put on a new pair of powder-free gloves.
2. Remove the cartridge from -25°C to -15°C storage.
3. Remove cartridge from the box but *do not open the silver foil bag*.
4. Position the cartridge so that the label faces up and air can circulate on sides and top.

   ⚠️ If the bag label is not facing up, sequencing data will be negatively impacted.

5. Thaw at room temperature for 9 hours. Do not exceed 16 hours.

Prepare the Flow Cell and Cartridge

1. Prepare the flow cell as follows.
   a. Remove a new flow cell from 2°C to 8°C storage.

b. Set the unopened package aside at room temperature for 10–15 minutes to prevent condensation when removing the flow cell from the package. Preparing the flow cell now ensures that it reaches room temperature on time.

2. If using the refrigerator thaw method:

a. Remove thawed cartridge from 2°C to 8°C storage.

b. Set the unopened cartridge aside at room temperature for at least 15 minutes before sequencing. Do not exceed 1 hour.

Dilute Libraries

If using onboard denature and dilute, this step dilutes libraries to the applicable loading concentration. An optional 2% PhiX[1] spike-in provides additional metrics, base diversity, or a positive control. The PhiX spike-in percentage should be increased for libraries with lower base diversity.

If manually denaturing and diluting libraries, use the *NextSeq 1000 and 2000 Denature and Dilute Libraries Guide (document # 1000000139235)*. This step only applies to onboard denature and dilution.

Dilute Library to 2 nM

1. [Optional] Remove 10 nM PhiX stock from -25°C to -15°C storage.
   PhiX is needed only for an optional spike-in or a PhiX-only run.

2. [Optional] Thaw PhiX at room temperature for 5 minutes, and then quantify using a fluorescence-based method, such as Qubit, to confirm PhiX concentration.
   If quantification is not possible, proceed with 10 nM concentration.

3. Vortex library or PhiX briefly, and then centrifuge at 280 × g for 1 minute.

4. Using RSB with Tween 20 as diluent, prepare at least 24 µl 2 nM library in a low-bind microtube.
   For PhiX spike-in instructions, see *Add a PhiX Control (Optional)* on page 40.

5. Vortex briefly, and then centrifuge at 280 × g for 1 minute.

Dilute 2 nM Library to Loading Concentration

1. Combine the following volumes in a low-bind microtube to prepare 24 µl library diluted to the appropriate loading concentration:

---

[1]PhiX is a small, ready-to-use Illumina library with balanced nucleotide representation.

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Library Type*	Loading Concentration (pM)	2 nM Library Volume (µl)	RSB With Tween 20 Volume (µl)
Ampliseq for Illumina Library PLUS	750	9	15
Illumina DNA Prep	750	9	15
Illumina DNA Prep with Enrichment	1000	12	12
Illumina Stranded Total RNA with Ribo-Zero Plus	750	9	15
Illumina Stranded mRNA Prep	750	9	15
Illumina DNA PCR-Free	1000	12	12
TruSeq DNA Nano 350	1200	14.4	9.6
TruSeq DNA Nano 550	1500	18	6
TruSeq Stranded mRNA	1000	12	12
100 % PhiX	650	7.8	16.2

* For unlisted library types, start with a loading concentration of 650 pM and optimize over subsequent runs. This table provides example loading concentrations. The NextSeq 1000/2000 is compatible with all Illumina library preparation kits, but the optimal loading concentration can vary.

2. Vortex briefly, and then centrifuge at 280 × g for 1 minute.

3. Set aside diluted library on ice until ready for sequencing.
   Sequence libraries diluted to the loading concentration the same day they are diluted.

4. Proceed as follows.

   - If adding PhiX, see *Add a PhiX Control (Optional)* on page 40.

   - If not adding PhiX or are performing a PhiX-only run, see *Load Consumables Into the Cartridge* on page 41.

Add a PhiX Control (Optional)

1. Combine the following volumes in a low-bind microtube to prepare 20 µl 1 nM PhiX:

   - 10 nM PhiX (2 µl)

   - RSB with Tween 20 (18 µl)

2. Vortex briefly, and then centrifuge at 280 × g for 1 minute.

3. Add 1 µl 1 nM PhiX to 24 µl library diluted to the final loading concentration.

These volumes result in a ~2% PhiX spike-in. Actual percentage varies depending on library quality and quantity.

4. Set aside the library with PhiX spike-in on ice until ready for sequencing.
   Sequence libraries with PhiX spike-in the same day they are diluted.

Load Consumables Into the Cartridge

This step prepares the cartridge for sequencing by mixing the prefilled reagents and loading diluted libraries and the flow cell.

Prepare the Cartridge

1. Open the cartridge bag by tearing or cutting with scissors from the top notch on either side.

2. Remove the cartridge from the bag. Discard the bag and dessicant.

3. Invert the cartridge 10 times to mix reagents.
   Internal components can rattle during inversion, which is normal.
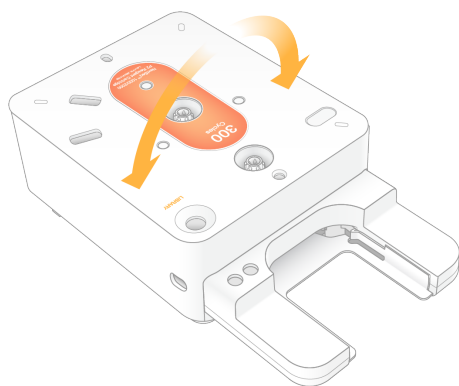
Load the Flow Cell

1. Open the silver foil package by tearing or cutting with scissors from the top slit on either side.
   If unable to use the flow cell immediately, see *Return Consumables to Storage* on page 77.

2. Pull the flow cell out of the package.

   Set aside the foil package and desiccant in case you need to return the flow cell to storage. The desiccant is contained in a pouch at the bottom of foil package. Discard them when sequencing begins.
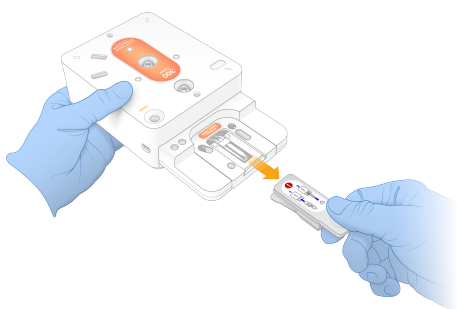
3. Hold the flow cell by the gray tab with the label on the tab facing up.

4. Push to insert the flow cell into the slot on the front of the cartridge.

   An audible click indicates that the flow cell is in place. When properly loaded, gray tab protrudes from the cartridge.
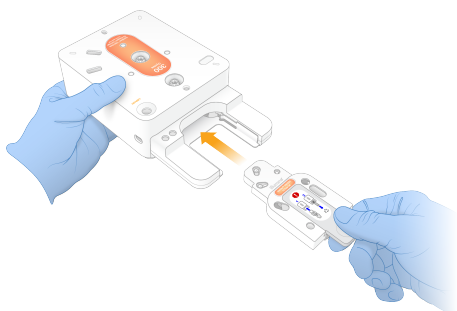
5. Pull back and remove the gray tab to expose the flow cell. Recycle the tab.

Load Libraries

1. Using a new P1000 pipette tip, pierce the Library reservoir and push the foil to the edges to enlarge the hole.

2. Discard the pipette tip to prevent contamination.

3. Add 20 μl diluted library to the *bottom* of the reservoir by slowly lowering the pipette tip to the bottom of reservoir before dispensing. Avoid touching the foil.
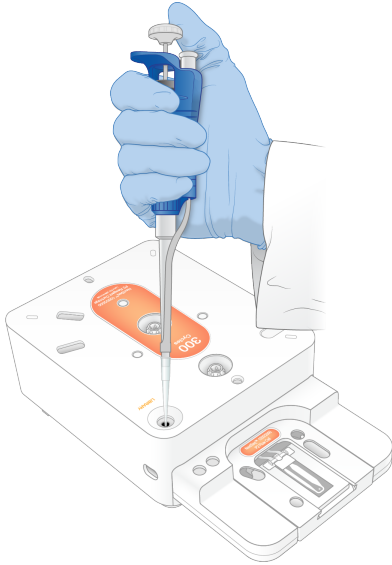
Initiating a Sequencing Run

This step initiates a sequencing run in one of four modes:

- **Cloud mode**—Run is selected from a list of planned runs in NextSeq 1000/2000 Control Software. During sequencing, cBCL data are uploaded to BaseSpace Sequence Hub. After sequencing, DRAGEN in BaseSpace Sequence Hub starts automatically.

- **Hybrid mode**—Run is selected from a list of planned runs in NextSeq 1000/2000 Control Software. After sequencing, on-instrument analysis starts automatically. cBCL data and DRAGEN secondary analysis output files are stored in the selected output folder.

- **Local mode**—A sample sheet in v2 file format is manually imported into NextSeq 1000/2000 Control Software. After sequencing, on-instrument analysis starts automatically. cBCL data and DRAGEN secondary analysis output files are stored in the selected output folder. If Proactive, Run Monitoring and Storage is selected, analysis can also be initiated through BaseSpace Sequence Hub apps after sequencing completion.

- **Standalone mode**—Set up a run, following the instructions in NextSeq 1000/2000 Control Software to generate cBCL data.

⚠️ | Opening the visor during the pre-run check or the run can cause run failure.

🔺 | Keep hands clear of instrument during visor opening and closing to avoid injury.

Initiating a Cloud or Hybrid Run

1. Configure the run mode, as described in *Configure Run Mode* on page 18.

2. Select **Start**.

3. Enter your BaseSpace Sequence Hub sign in credentials, and then select **Sign In**.

4. If you selected Proactive, Run Monitoring and Storage, select the Workgroup containing your run created in Instrument Run Setup in BaseSpace Sequence Hub.

   ⚠ A workgroup selection is required to avoid errors. Make sure you have selected a workgroup before proceeding.

5. Select **Next**.

6. Select your run.

7. Confirm the Analysis, Run Length, and Secondary Analysis version matches the correct run. Analysis displays Cloud_ to indicate that analysis occurs in BaseSpace Sequence Hub.

8. Select **Review**.

9. **[Optional]** Enter custom read primer and custom index primer locations.
   For information on preparing and adding custom primers, see the *NextSeq 1000 and 2000 Custom Primers Guide (document # 1000000139569)*. Make sure to visit the Compatible Products page for your library prep kit to check if Illumina custom primers are necessary.

10. **[Optional]** Select a custom recipe. For more information, see *Dark Cycle Sequencing* on page 94
    If using the NextSeq 1000/2000 Control Software v1.3 and the Illumina Stranded Total RNA Prep with Ribo-Zero Plus kit or the Illumina Stranded mRNA Prep kit, the custom recipe is automatically selected.

11. **[Optional]** To manually denature and dilute libraries, deselect the **Denature and Dilute On Board** checkbox. See the *NextSeq 1000 and 2000 Denature and Dilute Libraries Guide (document # 1000000139235)*.
    The default selection is configured in the NextSeq 1000/2000 Control Software settings.

12. **[Optional]** To change the output folder, select the Output Folder field and enter a new location.
    The Output Folder field is auto-populated from your default settings and is required unless **Proactive, Run Monitoring and Storage** is selected.
    If you selected Proactive, Run Monitoring and Storage, Save to BaseSpace Sequence Hub displays Enabled.
    If you selected Proactive and Run Monitoring, Save to BaseSpace Sequence Hub displays Disabled.

13. Review your run information, and then select **Prep**.

Initiating a Local Run

1. Configure the run mode, as described in *Configure Run Mode* on page 18.

2. Select **Start**.

3. If you selected Proactive, Run Monitoring and Storage or Proactive and Run Monitoring, enter your BaseSpace Sequence Hub sign in credentials, and then select **Sign In**.

4. If you selected Proactive, Run Monitoring and Storage, select the BaseSpace Sequence Hub Workgroup to save your run in, and then select **Next**.

⚠️ A workgroup selection is required to avoid errors. Make sure you have selected a workgroup before proceeding.

5. Select **Choose...** under Start With Sample Sheet, and navigate to the sample sheet in v2 formatting on the NextSeq 1000/2000 instrument, portable drive, or mounted network drive. Sample sheet file names cannot contain special characters.

   NextSeq 1000/2000 Control Software v1.3 automatically detects the DRAGEN version from the sample sheet and prompts you to switch versions, if needed. The DRAGEN version must be installed on the system. For installation information, see *Software Updates*.

   - **Instrument Run Setup Used**—Select the .zip folder containing the sample sheet v2 and supporting files if applicable. Otherwise, select the sample sheet v2.

   - **Instrument Run Setup Not Used**—Make sure the secondary analysis supporting file is located in the same directory as the sample sheet v2.

ℹ️ The selected sample sheet must be in v2 formatting. To create a sample sheet v2, download the generated sample sheet from Instrument Run Setup in BaseSpace Sequence Hub or edit a sample sheet v2 template provided on the NextSeq 1000/2000 support page. See *Sample Sheet v2 Settings* for more information on sample sheet v2 formatting and requirements. Make sure any files referenced in the sample sheet are located in the same folder as the sample sheet.

6. Select **Review**.

7. **[Optional]** Enter custom read primer and custom index primer locations.

   For information on preparing and adding custom primers, see the *NextSeq 1000 and 2000 Custom Primers Guide (document # 1000000139569)*. Make sure to visit the Compatible Products page for your library prep kit to check if Illumina custom primers are necessary.

8. **[Optional]** Select a custom recipe. For more information, see *Dark Cycle Sequencing* on page 94

   If using the NextSeq 1000/2000 Control Software v1.3 and the Illumina Stranded Total RNA Prep with Ribo-Zero Plus kit or the Illumina Stranded mRNA Prep kit, the custom recipe is automatically selected.

9. **[Optional]** To manually denature and dilute libraries, deselect the **Denature and Dilute On Board** checkbox. See the *NextSeq 1000 and 2000 Denature and Dilute Libraries Guide (document # 1000000139235)*.

   The default selection is configured in the NextSeq 1000/2000 Control Software settings.

10. **[Optional]** To change the output folder, select the Output Folder field and enter a new location.

    The Output Folder field is auto-populated from your default settings and is required unless Proactive, Run Monitoring and Storage is selected.

    If you selected Proactive, Run Monitoring and Storage, Save to BaseSpace Sequence Hub will display Enabled.

If you selected Proactive and Run Monitoring, Save to BaseSpace Sequence Hub displays Disabled.

11. Review your run information, and then select **Prep**.

Initiating a Standalone Run

1. Configure the run mode, as described in *Configure Run Mode* on page 18.

2. Select **Start**.

3. If you selected Proactive, Run Monitoring and Storage or Proactive and Run Monitoring, enter your BaseSpace Sequence Hub sign in credentials, and then select **Sign In**.

4. If you selected Proactive, Run Monitoring and Storage, select the BaseSpace Sequence Hub Workgroup to save your run in, and then select **Next**.

5. Select **Set Up New Run**.

6. In the Run Name field, enter a unique name of your preference to identify the current run.
   The run name can contain alphanumeric characters, dashes, hyphens, and underscores.

7. For Read Type, select how many sequencing reads to perform:

   - **Single Read**—Perform one read, which is the simpler, faster option.

   - **Paired End**—Perform two reads, the consensus of which generates higher-quality data and provides more accurate alignment.

8. Enter the number of cycles performed in each read:
   There is no maximum number of index cycles, but the sum of the read cycles and index cycles must be less than the number of cycles indicated on the cartridge label plus 27.

Read 1—Enter **1–151** cycles.

Index 1—Enter the number of cycles for the Index 1 (i7) primer. For a PhiX-only run, enter **0** in both index fields.

Index 2—Enter the number of cycles for the Index 2 (i5) primer.

Read 2—Enter up to **151** cycles. This value is typically the same as the Read 1 value.

9. If you selected Proactive, Run Monitoring and Storage, select **Choose...** to import a sample sheet. NextSeq 1000/2000 Control Software v1.3 automatically detects the DRAGEN version from the sample sheet and prompts you to switch versions, if needed. The DRAGEN version must be installed on the system. For installation information, see *Software Updates* on page 70.

> ℹ️ The selected sample sheet must be in v2 formatting. To create a sample sheet v2, download the generated sample sheet from Instrument Run Setup in BaseSpace Sequence Hub or edit a sample sheet v2 template provided on the NextSeq 1000/2000 support page. See *Sample Sheet v2 Settings* on page 81 for more information on sample sheet v2 formatting and requirements. Make sure any files referenced in the sample sheet are located in the same folder as the sample sheet.

10. **[Optional]** Enter custom read primer and custom index primer locations.

For information on preparing and adding custom primers, see the *NextSeq 1000 and 2000 Custom Primers Guide (document # 1000000139569)*. Make sure to visit the Compatible Products page for your library prep kit to check if Illumina custom primers are necessary.

11. **[Optional]** Select a custom recipe. For more information, see *Dark Cycle Sequencing* on page 94

12. **[Optional]** To manually denature and dilute libraries, deselect the **Denature and Dilute On Board** checkbox. See the *NextSeq 1000 and 2000 Denature and Dilute Libraries Guide (document # 1000000139235)*.
    The default selection is configured in the NextSeq 1000/2000 Control Software settings.

13. **[Optional]** To change the output folder, select the Output Folder field and enter a new location.
    The Output Folder field is auto-populated from your default settings and is required unless Proactive, Run Monitoring and Storage is selected.

14. Select **Prep**.

Load the Consumables Onto the Instrument

1. Make sure that the cartridge was previously thawed and inverted 10 times to mix prior to loading the flow cell (gray tab removed) and diluted library.

2. Select **Load**.
   The NextSeq 1000/2000 Control Software opens the visor and ejects the tray.

3. Place the cartridge onto the tray with the label facing up and the flow cell inside the instrument. Push cartridge in until it locks into seat.
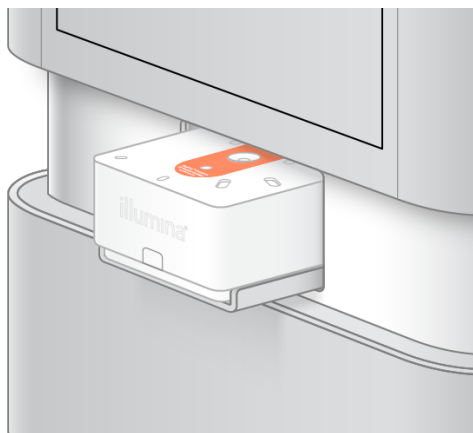
4. Select **Close** to retract the cartridge and close the visor.
   The NextSeq 1000/2000 Control Software displays information from the scanned consumables after ~3 minutes.

5. **[Optional]** Select **Eject Cartridge** to remove the cartridge.
   The visor opens after 1 minute and ejects the cartridge.

6. Select **Sequence**.

Pre-Run Checks

Pre-run checks include an instrument check followed by a fluidics check. The fluidics check pierces the cartridge seals, which will cause 3-4 popping sounds to emit from the instrument. This is expected. The reagent is now passed through the flow cell.

⚠ ❘ Consumables cannot be reused once the fluidics check starts.

1. Wait about 15 minutes for pre-run checks to complete.
   The run starts automatically after successful completion.
2. If an error occurs during the instrument check, select **Retry** to redo the check.
   When a check is in progress, the circle for that check is animated.
3. To troubleshoot recurring errors, see *Error Message Resolution*.

Monitor Run Progress

1. Monitor run progress and metrics as they appear on the Sequencing screen.
   - **Estimated run completion**—The approximate date and time of run completion. The estimated run completion metric requires 10 previous runs to calculate accurate run completion time.
   - **Average %Q30**—The average percentage of base calls with a Q-score ≥ 30.
   - **Projected Yield**—The expected number of bases called for the run.
   - **Total Reads PF**—The number of paired end (if applicable) clusters passing filtering (in millions).
   - **Real Time Demux**—Status of demultiplexing when initiated at the beginning of Read 2 following completion of Read 1, Index 1, and Index 2 cycles. Status will display Complete even if index cycles are not performed. Not available for Cloud mode runs.
   - **Real Time Alignment**—Status of Read 1 alignment when initiated at the beginning of Read 2 following completion of Read 1, Index 1, and Index 2 cycles. Not available for Cloud mode runs.
   Q30 and yield metrics appear after cycle 26 (~6 hours after starting the run).
2. To monitor run processes, select the control software menu, and then select **Process Management**.
3. To cancel a run, select **End Run**. See *Cancel a Run* on page 77 for more information on canceling runs.
4. Unload consumables from the instrument. Remove the cartridge from the instrument within 3 days.

Unload Consumables

1. When sequencing is complete, select **Eject Cartridge**.
   The software ejects the used cartridge from the instrument.
2. Remove the cartridge from the tray.
3. Remove the flow cell from the cartridge.
4. Dispose of the flow cell, which has electronic components, in accordance with applicable standards for your region.

5. [Optional] Remove the drainage plug under the Illumina logo on the side of the cartridge over an appropriate area (ie, sink or hazardous liquid waste receptacle) with the plug facing horizontal or downward away from your face. Drain used reagents in accordance with applicable standards for your region. Drain time depends on the cartridge size if automatic reagent purge is not enabled.

> ⚠️ **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, see the SDS at support.illumina.com/sds.html.

6. Dispose of the reagent cartridge.
   A post-run wash is not necessary because fluidics are discarded with the cartridge.
7. Select **Close Door** to reload the tray and return to the Home screen.
   The software automatically reloads the tray and sensors confirm cartridge removal.

Clean Cartridge Tray

Cleaning the cartridge tray is only necessary if the reagent has leaked onto the cartridge tray.

1. Remove the cartridge from the instrument.
2. Put on a new pair of powder-free gloves and any additional protective wear.
3. Spray 10% bleach solution onto a cloth.
4. Wipe the cartridge tray using the cloth, and then immediately remove the bleach solution using a heavy duty wipe.
   The bleach stains the cartridge tray if not removed immediately.
5. Spray 70% ethanol solution on the cartridge tray and immediately remove using a heavy duty wipe.
6. Set the cartridge tray back to load position.

Sequencing Output

This section describes the Real-Time Analysis software, which performs base calling, assigns quality scores, and outputs data. Learn about the different output file types and where to locate them after a run.

Real-Time Analysis Overview

The NextSeq 1000 and NextSeq 2000 Sequencing Systems 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 for viewing in the Instrument Control Software.

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 control software.

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 1 *.cbcl file for each lane and surface.
Filter files	Each tile produces a filter file (*.filter) that specifies whether a cluster passes filters.
Cluster location files	Cluster location (*.locs) files contain the X,Y coordinates for every cluster in a tile. A cluster location file is generated for each run.

Output files are used for downstream analysis in DRAGEN and BaseSpace Sequence Hub.

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 per tile.

The NextSeq 1000/2000 P2 Flow Cell has a total of 132 tiles. The NextSeq 1000/2000 P3 Flow Cell has a total of 264 tiles.

Table 5 Flow Cell Tiles

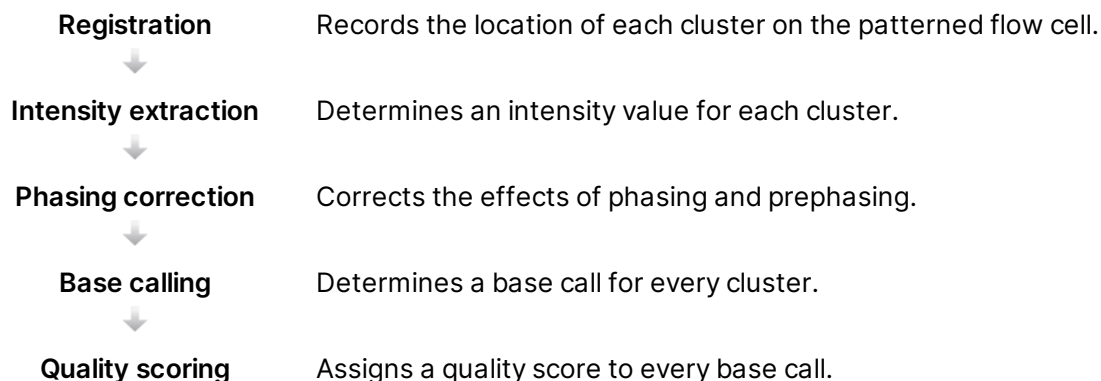| Flow Cell Component	NextSeq 1000/2000 P2 Flow Cell	NextSeq 1000/2000 P3 Flow Cell	Description
Lanes	1	2	Lanes are optically distinct, but not fluidically separate channels.
Surfaces	2	2	The P2 and P3 flow cells are imaged on two surfaces: the top and bottom. The top surface of a tile is imaged first.
Swaths per lane	6	6	A swath is a column in a flow cell lane.
Tiles per swath	11	11	A tile is a portion of a swath and depicts an imaged area on the flow cell.
Total tiles generated	132	264	Lanes × surfaces × swaths × tiles per swath equals the total number of tiles.

Tile Naming

The tile name is a four-digit number that represents the tile position on the flow cell. For example, tile name 1205 indicates top surface, swath 2, tile 05.

The first digit represents the surface: 1 for top or 2 for bottom.

The second digit represents the swath number: 1, 2, 3, 4, 5, or 6.

The last two digits represent the tile number. For swath numbers 1-4, the numbering starts with 01 at the outlet end of the flow cell through 11 at the inlet end. For swath numbers 5-6, the numbering starts with 01 at the inlet end and 11 at the outlet end.

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 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. Use Sequencing Analysis Viewer to identify which images failed registration.

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 falls behind.

Prephasing occurs when a base jumps ahead.

Figure 5 Phasing and Prephasing
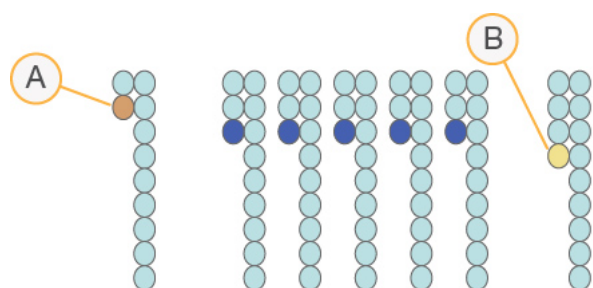
A. Read with a base that is phasing
B. Read with a base that is prephasing.

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 NextSeq 1000 and NextSeq 2000 Sequencing Systems uses two-channel sequencing, which requires only two images to encode the data for four DNA bases, one from the green channel and one from the blue 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 green and blue images and compared against each other, which results in four distinct populations. Each population corresponds to a base. The base calling process determines which population each cluster belongs to.

Table 6 Base Calls in 2-Channel Sequencing

Base	Green Channel	Blue Channel	Result
A	1 (present)	1 (present)	Clusters that show intensity in both the green and blue channels.
C	0 (not present)	1 (present)	Clusters that show intensity in the blue channel only.
G	0 (not present)	0 (not present)	Clusters that show no intensity at a known cluster location.

Base	Green Channel	Blue Channel	Result
T	1 (present)	0 (not present)	Clusters that show intensity in the green channel only.

Figure 6   Visualization of Cluster Intensities
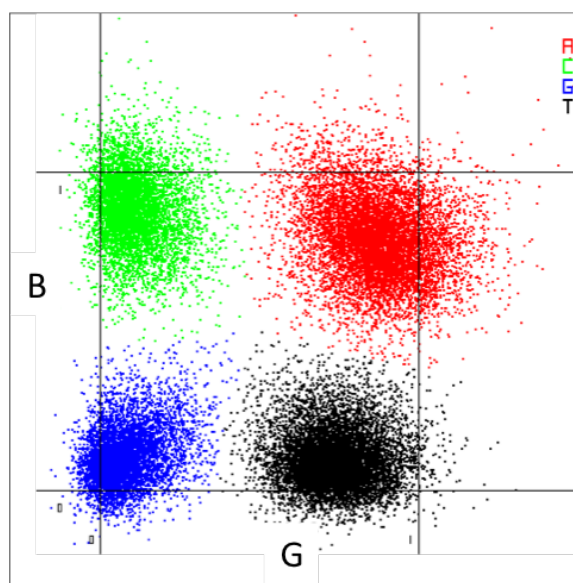
ℹ️ The color of each cluster correlates to the %Base plots in Sequence Analysis Viewer (SAV) and BaseSpace Sequence Hub Run Data by Cycle and are not meant to correlate with the green and blue channel.

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 base call (*.cbcl) files.

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

Q-Score Q(X)	Error Probability
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.

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

To generate the Q-table for the NextSeq 1000 and NextSeq 2000 Sequencing Systems, 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 (*Simplified Q-Scoring with RTA3* on page 57). 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, 23, and 37 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 7 Simplified Q-Scoring with RTA3

Sequencing Output Files

File Type	File Description, Location, and Name

Concatenated Base call files	Each cluster analyzed is included in a concatenated base call file, aggregated in one file per cycle, lane, and surface. The aggregated file contains the concatenated base call and encoded quality score for every cluster. The concatenated base call files are used by BaseSpace Sequence Hub or bcl2fastq2.
	`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`

File Type	File Description, Location, and Name
InterOp files	Binary reporting files can be viewed on-instrument with the Instrument Control Software or off-instrument in SAV or BaseSpace Sequence Hub. InterOp files are updated throughout the run. `InterOp` folder
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`

DRAGEN Secondary Analysis Output Files

The DRAGEN Bio-IT Platform analyzes your sequencing output on-instrument further by using one of the following analysis pipelines.

- BCL Convert
- Germline
- RNA
- Enrichment
- Single Cell RNA
- DNA Amplicon

This section provides information on each DRAGEN pipeline, including output file information. In addition to generating files specific to each pipeline, DRAGEN provides metrics from the analysis in a `<sample_name>.metrics.json` file and the reports described in *DRAGEN BCL Convert Pipeline* on page 63. For more information DRAGEN, see the DRAGEN Bio-IT Platform support site page.

All DRAGEN pipelines support the decompression of input BCL and compression of output BAM/CRAM files.

Output file considerations:

- For Germline, RNA, Enrichment, and DNA Amplicon pipelines running on-instrument analysis, BAM files will not be uploaded to BaseSpace Sequence Hub if Proactive, Run Monitoring and Storage is selected.

DRAGEN Enrichment Pipeline

The DRAGEN Enrichment pipeline supports the following features. If using DRAGEN 3.7 or later, both germline and somatic (tumor only) mode are supported.

- Sample demultiplexing
- Mapping and alignment, including sorting and duplicate marking

- Small variant calling
- Structural variant calling

To perform variant calling, a *.bed file must be included in the sample sheet or specified in the Instrument Run Setup on BaseSpace Sequence Hub. Structural variant calling is only generated for paired-end reads and germline mode.

If using DRAGEN Enrichment version 3.8 or later, you an input a noise baseline file to improve performance in somatic mode. See *Import Noise Baseline Files* on page 17.

The pipeline generates the following output files.

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

* gVCF output files are only available for germline mode.

DRAGEN Germline Pipeline

The DRAGEN Germline pipeline supports the following features:

- Sample demultiplexing
- Mapping and alignment, including sorting and duplicate marking
- Small variant calling
- Structural variant calling for paired-end reads
- Copy number variant calling for human genomes
- Repeat expansions for human genomes
- Regions of homozygosity for human genomes
- **[DRAGEN v3.8 or later]** CYP2D6 detection

Structural variant calling is only generated for paired-end reads.

The pipeline generates the following output files.

Component	Type	Output File Name
Mapping/aligning	BAM or CRAM	• <sample_name>.bam, or • <sample_name>.cram
Small variant calling	VCF and gVCF	• <sample_name>.hard-filtered.gvcf.gz • <sample_name>.hard-filtered.vcf.gz
Structural variant caller	VCF	• <sample_name>.sv.vcf.gz
Copy number variants	VCF	• <sample_name>.cnv.vcf.gz
Repeat expansion	VCF	• <sample_name>.repeats.vcf.gz
Regions of Homozygosity	CSV and BED	• <sample_name>.roh_metrics.csv • <sample_name>.roh.bed
CYP2D6 Detection	TSV	• <sample_name>.cyp2d6.tsv

DRAGEN DNA Amplicon Pipeline

The DRAGEN pipeline supports the following features:

- Sample demultiplexing

- Mapping and alignment, including sorting and duplicate marking

- Small variant calling in germline or somatic mode.

To perform variant calling, a *.bed file must be included in the sample sheet or specified in the Instrument Run Setup on BaseSpace Sequence Hub.

The pipeline generates the following output files.

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

*gVCF output files are only available in germline mode.

DRAGEN RNA Pipeline

The DRAGEN RNA pipeline supports the following features

- Sample demultiplexing

- Mapping and alignment, including sorting and duplicate marking

- Gene fusion detection

- Transcript quantification

- **[DRAGEN v3.8, or later]** Differential gene expression

To generate output files, specify a GTF file in the sample sheet or make sure the default `genes.gtf.gz` exists with the reference genome.

The pipeline generates the following output files.

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

The following files are output when differential expression is enabled.

File Name	Description
Control_vs_Comparison.differential_expression_metrics.csv	Contains differential expression analysis metrics.
Control_vs_Comparison.genes.counts.csv	Describes the number of reads mapped to each gene for each sample in the control and comparison groups.

File Name	Description
Control_vs_ Comparison.genes.heatmap.png	A heat map of the expression of the differentially expressed genes for samples in the control and comparison groups. The heatmap only shows differentially expressed genes with an adjusted P-value <-.05. If there are more than 30 differentially expressed genes, only the top 30 differentially expressed genes are used. If DESeq1 fails to converge or if there are no differentially expressed genes, the file is not generated.
Control_vs_ Comparison.genes.ma.png	Contains the variation of gene expression ratios as a function of average signal intensity. To show the differences between measurements taken in two samples, the plot transforms the data onto M (log ratio) and A (mean average) scales, and then plots the values. The MA plot shows the log2 fold changes attributable to a given variable over the mean of normalized counts for all the samples. If the adjusted P-value is less than 0.1, the points are red. Points that fall out of the window are plotted as open triangles. Upwards pointing triangles represent a positive log fold change. Downwards pointing triangles represent a negative log fold change.
Control_vs_ Comparison.genes.pca.png	Plot displays the first two principal components that explain the most variance.
Control_vs_Comparison.genes.res.csv	Contains DESeq2 results, which describe the mean expression, log2 (fold change), standard error of log2, P-value, adjusted P-value, and the expression status of each gene.
Control_vs_ Comparison.genes.rlog.csv	Contains regularized log-transformed counts calculated by DESeq2.

DRAGEN Single Cell RNA Pipeline

The DRAGEN supports the following features:

- Sample demultiplexing

- Mapping and alignment, including sorting and duplicate marking

- Cell and gene classification

To generate output files, specify a GTF file in the sample sheet or make sure the default `genes.gtf.gz` exists with the reference genome.

The pipeline generates the following output files.

Component	Type	Output File Name
Mapping/aligning	BAM or CRAM	• <sample_name>.bam, or • <sample_name>.cram
Cell/gene classification	TSV, CSV, and MTX	• <sample_name>.scRNA.barcodeSummary.tsv • <sample_name>.scRNA.genes.tsv • <sample_name>.scRNA.matrix.mtx
Analysis reports	HTML	<sample_name>.dragen.scrna-report.*.html

DRAGEN BCL Convert Pipeline

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

The pipeline generates the following reports.

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

Demultiplexing Statistics Report

The demultiplexing statistics report contains information on the number of passing filter reads that are assigned to each sample in the sample sheet. Any reads not clearly associated with a sample are classified as undetermined. The report also includes information about the quality scores of bases in the passing filter (PF) reads assigned to each sample.

The following information is included.

Metric	Description
Lane	The lane on the flow cell the sample was sequenced.
SampleID	The sample ID from the sample sheet. If a read does not correspond with a sample, the field displays `undetermined`.

Metric	Description
Index	The concatenation of Index Read 1 and Index Read 2 from the sample sheet separated by a hyphen. If a read does not correspond to a sample, the field displays `undetermined`.
# Reads	The number of PF reads demultiplexed for the sample in the specified lane.
# Perfect Index Reads	Number of reads with a perfect match to the combined index sequences specified in the sample sheet.
# One Mismatch Index Reads	Number of reads with one error in the combined index sequences specified in the sample sheet.
# of ≥ Q30 Bases (PF)	Number of bases, including adapters, corresponding to reads that pass a Q30 quality threshold.
Mean Quality Score (PF)	The mean quality score for reads corresponding to the sample in the specified lane. The value includes adapter bases.

Adapter Metrics Reports

The adapter metrics file contains the number of adapter and sample bases associated with each read. The following information is included.

Metric	Description
Lane	The lane on the flow cell the sample was sequenced.
Sample_ID	The sample ID from the sample sheet. If a read does not correspond with a sample, the field displays `undetermined`.
index	The index1 sequence from the sample sheet. The field is empty if the index was not specified in the sample sheet or the sample ID value is `undetermined`.
index2	The index2 sequence from the sample sheet. The field is empty if index2 was not specified in the sample sheet or the sample ID value is `undetermined`.
R1_ AdapterBases	Number of bases corresponding to AdapterRead1 in the sample sheet.
R1_ SampleBases	Number of trimmed or masked bases from Read 1 for the corresponding lane and sample.
R2_ AdapterBases	Number of bases corresponding to AdapterRead2 in the sample sheet.

Metric	Description
R2_ SampleBases	Number of trimmed or masked bases from Read 2 for the corresponding lane and sample.
# Reads	Number of reads for the sample in the specified lane.

Index Hopping Counts Report

The index hopping counts report contains the number of reads for each expected and hopped index for dual index runs. The report only includes unique dual indexes per lane where no barcode collision is detected in either index. To generate index-hopping metrics for a lane, every pair of entries within each index must have a hamming distance of at least 2N +1, where N represents the barcode mismatch tolerance specified for the index.

The following information is included.

For nonindex runs, single index runs, or lanes that do not contain unique dual indexes, the file contains only the headers.

Metric	Description
Lane	The lane on the flow cell the sample was sequenced.
# Reads	Number of reads for the sample in the specified lane.
SampleID	The sample ID from the sample sheet. If a read does not correspond with a sample, the field displays `undetermined`.
index	The index1 sequence from the sample sheet. The field is empty if a read is single-ended or the sample ID value is `undetermined`.
index2	The index2 sequence from the sample sheet. The field is empty if a read is single-ended or the sample ID value is `undetermined`.

Top Unknown Barcodes Report

The top unknown barcodes report contains the top 100 index or index pairs per lane that were not identified in the sample sheet according to the number of allowed mismatches. If there are multiple index values placed as the 100th highest index count entry, all index values with the same count are output as the 100th entry.

The following information is included:

Metric	Description
Lane	The lane on the flow cell the sample was sequenced.

Metric	Description
index	The sequence for each unknown index in index Read1. The field is empty if no unknown indexes are found.
index2	The sequence for each unknown index in index Read 2. If the run was single-read or there were no unknown indexes found, the field is empty.
# Reads	Number of reads for the sample in the specified lane.

Illumina DRAGEN QC Reports

For all pipelines, DRAGEN FastQC generates QC plots by default. Aggregated QC results are stored in the `AggregatedFastqcMetrics` folder and per sample results are stored in the `<sample_name>` folder.

QC reports are not generated if the number of samples is greater than 512.

The following QC plots are provided.

QC Plot	Description
adapter_content	The percentage of sequences for each base pair.
positional_mean_quality	Average Phred-scale base quality score for each read position.
gc_content	The GC content percentage for each sequencing read.
positional_quality.read_1	Average Phred-scale quality value of bases with a specific nucleotide and at a given location in Read 1.
gc_quality	
positional_quality.read_2	Average Phred-scale quality value of bases with a specific nucleotide and at a given location in Read 2.
n_content	
read_length	The sequence length for each read.
positional_base_content.read_1	Number of bases of each specific nucleotide at given locations in Read 1.
read_quality	Average Phred-scale quality score for each sequencing read.
positional_base_content.read_2	Number of bases of each specific nucleotide at given locations in Read 2.

DRAGEN Secondary Analysis Output Folder Structure

By default, DRAGEN generates output files in the output folder selected in the Settings tab. For each workflow, DRAGEN produces a summary report in the `report.html` file.

📁**Data**

report.html

report_files

📁 **AggregateFastQCPlots**

    *.png

*stderr_.txt

*stdout_.txt

dragen_prev_48_hrs.log

dlm_prev_48_hrs.log

SampleSheet.csv

Run input files (eg, BED, GTF files)

📁 **sample_name**

    📁 **enrich_caller**, **germline_seq**, **dna_amplicon_seq**, **rna_seq**, or **scrna_seq**

       📁 **sample_name**

          *.png

      dragen_*.log

      sample_name.*.metrics.csv

      [DNA] sample_name.*.vcf.gz

      [DNA] sample_name.*.gvcf.gz—Unavailable for DRAGEN Bio-IT Platform Amplicon (somatic) pipeline.

      sample_name.*.bam or sample_name.*.cram

      Logs

      [RNA] sample_name.fusion_candidates.filter_info

      [RNA] sample_name.fusion_candidates.final

      [RNA] sample_name.quant.genes.sf

      [RNA] sample_name.quant.sf

      sample_name.metrics.json

      [scRNA] sample_dragen-scrna-report.*.html

      [scRNA] sample_name.scRNA.barcodeSummary.tsv

      [Germline] sample_name.roh_metrics.csv

      [Germline] sample_name.roh.bed

      [Germline] sample_name.cyp2d6.tsv

      ▤ `sample_name.fastqc_metrics.csv`

      ▤ `sample_name.trimmer_metrics.csv`

    📁 **[RNA] DifferentialExpression**

      📁 **Comparison1**

        ▤ `Control_vs_Comparison.differential_expression_metrics.csv`

        ▤ `Control_vs_Comparison.genes.counts.csv`

        ▤ `Control_vs_Comparison.genes.disp.pdf`

        ▤ `Control_vs_Comparison.genes.heatmap.pdf`

        ▤ `Control_vs_Comparison.genes.ma.pdf`

        ▤ `Control_vs_Comparison.genes.pca.pdf`

        ▤ `Control_vs_Comparison.genes.res.csv`

        ▤ `Control_vs_Comparison.genes.rlog.csv`

      📁 **ComparisonN**

    📁 **logs**

      ▤ `*.txt`

      ▤ `*.csv`

  📁 **fastq**—Available only if KeepFastq is set to true.

    ▤ `*.fastq.gz`

  📁 **ora_fastq**—Available only if FastqCompressionFormat is set to dragen.

    ▤ `*.fastq.ora`

  📁 **RunInstrumentAnalyticsMetrics**

    📁 **0001**

      ▤ `dataset.json`

      ▤ `fastqc_metrics.csv`

    📁 **0002**

      ▤ `dataset.json`

      ▤ `fastqc_metrics.csv`

    ▤ `Adapter_Metrics.csv`

    ▤ `Demultiplex_Stats.csv`

    ▤ `Index_Hopping_Counts.csv`

  📁 **Reports**

      ▤ `Demultiplex_Stats.csv`

      ▤ `RunInfo.xml`

      ▤ `Trim_Metrics.csv`

      ▤ `fastq_list.csv`

      ▤ `SampleSheet.csv`

      ▤ `Index_Hopping_Counts.csv`

      ▤ `Top_Unknown_Barcodes.csv`

📁 **Read1InstrumentAnalyticsMetrics**—For paired-end reads only.

    📁 **0001**

      ▤ `dataset.json`

    📁 **0002**

      ▤ `dataset.json`

  ▤ `Adapter_Metrics.csv`

  ▤ `Demultiplex_Stats.csv`

  ▤ `Index_Hopping_Counts.csv`

📁 **Read1Metrics**—For paired-end reads only.

  ▤ `Adapter_Metrics.csv`

  ▤ `Index_Hopping_Counts.csv`

Maintenance

This section describes the procedures necessary for maintaining a healthy system. Learn how to install software updates, change the air filter, and perform other periodic maintenance procedures. Keeping the control software up-to-date ensures that your system has the latest bug fixes and features installed for optimum performance.

Clear Hard Drive Space

A sequencing run requires about 200 GB of local hard drive space. A warning notification displays when space is low. Use the following steps to clear space by deleting completed runs and installed reference genomes from a temporary run folder.

⚠️ Only delete runs using the NextSeq 1000/2000 Control Software rather than manually through the operating system. Deleting runs manually can negatively impact the control software.

1. From the control software menu, select **Disk Management**.
   The Disk Management screen appears with a list of runs and reference genomes saved to the local hard drive.
2. For the run you want to delete, select **Delete Run**.
   Deleting a run deletes the local run folder. The output folder, which is a copy of the run folder, is retained.
3. In the dialog box, select **Yes, Delete Run** to confirm deleting the run.
4. Repeat steps 2 and 3 for each run you want to delete.
5. For the genome you want to delete, select **Delete Genome**.
6. In the dialog box, select **Yes, Delete Genome**.
7. Repeat steps 5 and 6 for each genome you want to delete.
8. When you are finished, close Disk Management to return to the Home screen.

Software Updates

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

- NextSeq 1000/2000 Control Software
- NextSeq 1000/2000 recipes
- Universal Copy Service

For Research Use Only. Not for use in diagnostic procedures.

- Real-Time Analysis

ℹ️ DRAGEN modules are not included in the system suite. Install them separately as needed. Access the DRAGEN module software from the support pages.

The system is configured to download software updates automatically or manually:

- **Automatic updates**—Updates are automatically downloaded from BaseSpace Sequence Hub for you to install. This option requires an internet connection, but not a BaseSpace Sequence Hub account.

- **Manual updates**—Updates are manually downloaded from the web, saved locally or to a portable drive, and installed from the saved location. This option does not require an internet connection for the instrument.

Install an Automatic Software Update

1. Make sure that there are no sequencing runs or on-instrument secondary analysis in progress.
2. Log into ilmnadmin.
3. Select **Software Update** from the control software menu.
   Systems configured for automatic updates display an alert when a software update is available.
4. To check for an update, select **Check Online for Software Update**.
5. Select **Update Now** to download the new version of software.
   When the download is complete, the control software is closed and the installation wizard appears. The control software automatically restarts. Any firmware update occurs automatically after the restart.

   ℹ️ Canceling an update is not possible after installation begins. You can only cancel an update during download.

Install a Manual Software Update

1. Log into ilmnadmin.
2. Make sure that there are no sequencing runs or on-instrument secondary analysis in progress.
3. When a software update is available, download the suite installer (*.tar.gz) from the NextSeq 1000 and NextSeq 2000 Sequencing Systems support page. Save the installer to a local or portable drive.
4. If you saved the installer to a portable drive, plug the drive into a USB 3.0 port, located on both the side and back of the instrument.
5. In the control software, select **Software Update** from the control software menu.
6. Select **Choose...** to navigate to the installer.
7. Select **Update Now** to start installation.

The control software displays a busy indicator during installation.

The control software automatically restarts. Any firmware update occurs automatically after the restart.

ℹ️ | Canceling an update is not possible after installation begins. You can only cancel an update during download.

DRAGEN Workflow and License Updates

Only system administrators can install DRAGEN workflows and renew DRAGEN license.

Renew DRAGEN License Online

If the NextSeq 1000/2000 is connected to the internet, update your DRAGEN Bio-IT Platform license as follows.

1. Contact Illumina Technical Support to obtain a new license key.
2. Wait 24 hours for the license to update automatically, or update the license immediately as follows.
   a. Select the control software menu, and then select **DRAGEN**.
   b. Select **Check Online** to check if a new DRAGEN license key is available.
   c. If available, select **Update**.

Renew DRAGEN License Offline

If the NextSeq 1000/2000 is not connected to the internet, update your DRAGEN Bio-IT Platform license as follows.

1. Contact Illumina Technical Support to obtain a new license key. Save the `license.zip` file to a local or portable drive.
2. If you saved the *.zip file to a portable drive, plug the drive into a USB 3.0 port, located on both the side and back of the instrument. Gently move the instrument as needed to access the back.
3. Select the control software menu, and then select **DRAGEN**.
4. Select **Choose** to navigate to the *.zip file, and then select **Open**.

Install DRAGEN Workflows Online

If the NextSeq 1000/2000 is connected to the internet, you can install DRAGEN workflows directly in the NextSeq 1000/2000 Control Software. Installing DRAGEN workflows online is only available in NextSeq 1000/2000 Control Software v1.3.

1. Select the control software menu, and then select **Process Management**.
2. Make sure that there are no sequencing runs or on-instrument secondary analysis in progress.
3. Select the control software menu, and then select **DRAGEN**.

Under Version, the Available Workflows section lists the workflows currently installed on the system.

4. To install DRAGEN workflows in the NextSeq 1000/2000 Control Software, select **Check Online**. Not all DRAGEN versions and workflows are compatible with online installation. Use offline installation for additional workflows.

5. Select the checkbox for the workflows you would like to install. If not installed, make sure to install the latest version of BCL Convert first.
You can view information about the latest version of a workflow in the release notes.

6. Select **Install** to start installation.

7. Enter ilmnadmin for the system password, and then select **Authenticate**.

Install DRAGEN Workflows Offline

1. When a DRAGEN workflow update is available, download the installer (*.tar.gz) from the DRAGEN support page. Save the installer to a local or portable drive.

2. If you saved the installer to a portable drive, plug the drive into a USB 3.0 port, located on both the side and back of the instrument. Gently move the instrument as needed to access the back.

3. Select the control software menu, and then select **Process Management**.

4. Make sure that there are no sequencing runs or on-instrument secondary analysis in progress.

5. Select the control software menu, and then select **DRAGEN**.

6. Under Version, select **Browse for New Version** to navigate to the installer.

7. Select **Install** to start installation.

8. Enter ilmnadmin for the system password, and then select **Authenticate**.
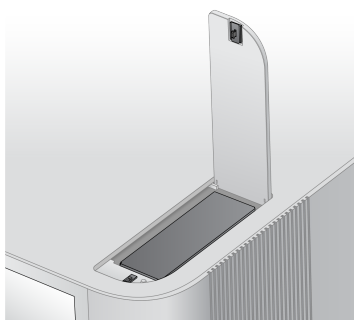
Replace the Air Filter

Use the following instructions to replace an expired air filter every 6 months.

The air filter is a single-use rectangular cartridge that covers the fan on the right side of the instrument. It ensures proper cooling and prevents debris from entering the system. The instrument is shipped with one air filter installed and one spare. Additional spares are included with a valid instrument service contract, or they can be purchased separately from Illumina.
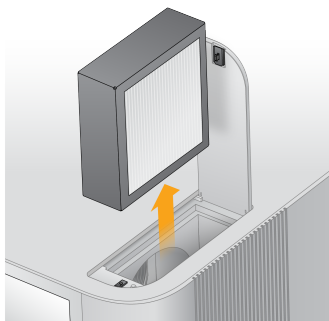
1. On the top of the instrument, press the right side of the top panel to disengage, as shown in the following illustration.
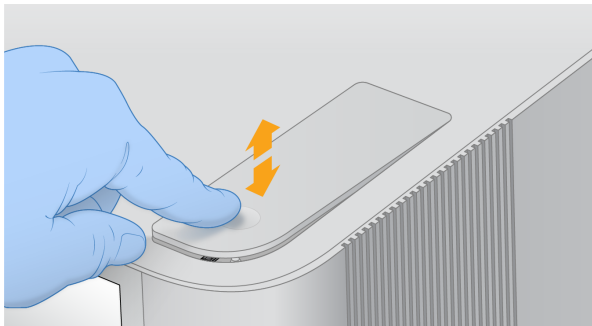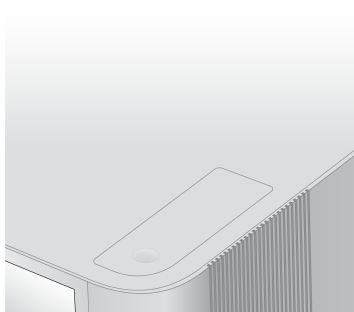

2. Open the panel.

3. Press to release the air filter cartridge, remove from the center of the panel, and discard.

4. Insert a new air filter into the receptacle, and press to secure.
5. Close the top panel and press into place.

6. Return the instrument to the original placement.

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Troubleshooting

This section provides step by step instructions for canceling a run, power cycling the instrument, and other troubleshooting procedures.

Error Message Resolution

This appendix provides detailed instructions for various troubleshooting steps. The following flowchart provides an overview on troubleshooting error messages that appear during initialization, run setup, or sequencing, and are not resolved with a retry.

Many errors can be resolved with a power cycle: turning off the instrument, and then restarting it. See *Power Cycle the Instrument* on page 78 for more information on power cycling.

For Research Use Only. Not for use in diagnostic procedures.
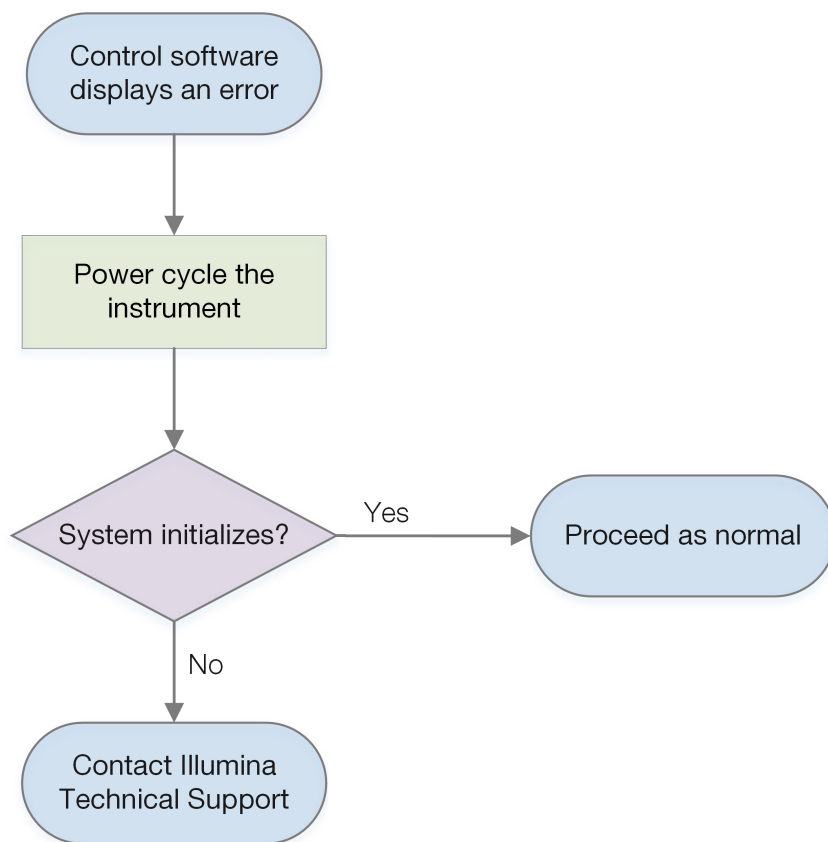
Return Consumables to Storage

Use the following instructions to store a thawed cartridge and flow cell in the event of an instrument error during the instrument pre-run check prior to the fluidics check.

1. Separate the flow cell from the cartridge.
2. Remove and discard diluted library from the reservoir (up to ~18 µl).

> ⚠️ Prepare a fresh dilution of the same library for the next run to avoid sample cross-contamination with residual library in the reservoir.

3. Position the cartridge in 2°C to 8°C storage so that the label faces up and air can circulate on all sides.
   Do not exceed 72 hours. If cartridge was thawed in the refrigerator for 12 hours overnight, do not exceed 60 hours.
4. Return the flow cell to the original silver foil packaging with the desiccant.
5. Tape the foil packaging shut and place in 2°C to 8°C storage.
   Do not exceed 72 hours.

Cancel a Run

1. Select **End Run**.
2. To automatically purge the reagent cartridge, select the **Purge Reagent Cartridge** checkbox.
   The default selection is configured in the NextSeq 1000/2000 Control Software settings.
3. Select **Yes, end the sequencing run**.
   Canceling a run is final. The software cannot resume the run and consumables cannot be reused after the instrument check portion of pre-run checks.

2. Select **Eject Cartridge** to open the visor and eject the tray.

3. Remove the cartridge from the tray.

4. Store or dispose of the cartridge, depending on when the cancellation occurred:

Circumstance	Instance
You canceled before or during the instrument pre-run check and want to reuse consumables.	See *Return Consumables to Storage* on page 77.
All other circumstances.	See *Unload Consumables* on page 48

4. 5. Select **Close Door** to reload the tray and return to the Home screen.
   Sensors confirm cartridge removal.

Requeue a Run

If an error displays for Status of Secondary Analysis in Process Management, you can requeue the run to perform on-instrument DRAGEN analysis again on the generated cBCL files. The original run folder must still be present on the instrument for requeue functionality. Using this requeue functionality does not requeue runs in BaseSpace Sequence Hub. To requeue in BaseSpace Sequence Hub, see Fix Sample Sheet in BaseSpace Sequence Hub Help Center.

1. Update your sample sheet v2, and then save the sample sheet to a portable or mounted network drive.
2. If you saved the sample sheet to a portable drive, plug the drive into a USB 3.0 port, located on both the side and back of the instrument. Gently move the instrument as needed to access the back.
3. Select the control software menu, and then select **Process Management**.
4. Make sure that there are no sequencing runs or on-instrument secondary analyses in progress.
5. Select **Requeue** next to the completed run to requeue.
6. Select **Choose** to navigate to the updated sample sheet, and then select **Open**.
7. Select **Start Requeue**.

Power Cycle the Instrument

Power cycling the instrument safely shuts down and restarts the system to restore a lost connection, align a specification, or resolve an initialization failure. Software messages indicate when to power cycle to resolve an error or warning.

1. From the control software menu, select **Shut Down Instrument**.
2. If the system does not shut down, hold the power button on the right side of the instrument until the lights fade.
3. When the power button pulses, press the power off (**O**) side of the toggle switch on the rear panel. The power button might continue to pulse after the power is turned off.

Figure 8 Toggle Switch Location
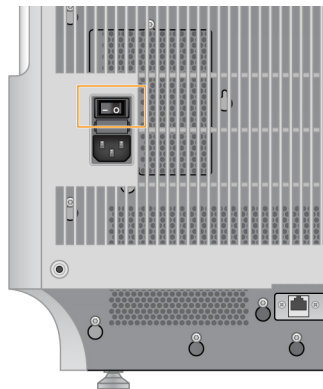
4. Wait 30 seconds.

5. Press the power on (**I**) side of the toggle switch.

6. When the power button pulses, wait 30 seconds, and then press it.

Figure 9 Power Button Location

7. Wait about 5 minutes for the operating system to load. When the operating system is loaded, log on to the system.

    The control software is launched and initializes the system. Wait about 5 minutes for system initialization. The Home screen appears when initialization is complete.

Perform a System Check

A system check is not required for normal operation or instrument maintenance. However an Illumina Technical Support representative might ask you to perform a system check for troubleshooting purposes.

Four subsystem checks take about 58 minutes to troubleshoot pre-run check errors and other issues. The tests confirm whether components are properly aligned and functional.

The test results are output to the `system-check` folder located in `/usr/local/illumina/system-check`.

Make sure to unload the cartridge before running system checks.

Running a System Check

1. From the control software menu, select **System Checks**.

2. Select the checkbox for any of the following system checks you would like to perform.

    • **Network Connectivity**—Checks your network connection status and performance.

    • **Enclosure**—Checks the performance of the thermal system and visor lift mechanism.

    • **Motion**—Checks the travel limits and performance of the Z-stage and XY-stage.

    • **Optics**—Checks the performance of the imaging module.

3. Select **Start**.

Restore to Factory Settings

Restore the system to factory defaults to downgrade the software or recover from an undesirable configuration. This feature should only be used by an Illumina representative.

Capture Installed Image

Capture a system image to backup a successfully functioning software installation. This system image can be restored at a later point in time. It is recommended that you capture the system image immediately after completing the initial installation and changing your password with an Illumina representative.

1. Restart Linux.
2. When prompted to choose an operating system, select **Capture Installed Image**.
   The operating system options appear briefly before automatically proceeding with NextSeq 1000/2000 Control Software.

   > Since only one image is retained in memory, this will overwrite the previously captured image.

3. Wait about 30 minutes for the system to capture the currently installed image.
   The capture can include several reboots. When complete, the system reboots with the currently installed image stored in memory.

Restore Captured Image

Restore the system to the previously captured image to recover from an undesirable configuration.

1. Restart Linux.
2. When prompted to choose an operating system, select **Restore Installed Image**.
   The operating system options appear briefly before automatically proceeding with NextSeq 1000/2000 Control Software.

> Passwords are tied to the system image. After restoration, use the restored image's password to log on to the system.

3. Wait about 30 minutes until restoration is complete.
   The restoration can include several reboots. When complete, the system reboots with the restored image.

Resources & References

Sample Sheet v2 Settings

If following Local mode, you can use the sample sheet v2 file format to configure your run settings. Create the sample sheet in Instrument Run Setup or by editing the *NextSeq 1000 and NextSeq 2000 Sequencing Systems Sample Sheet v2 Template*. When editing the sample sheet make sure that the following sections and fields are included in the order listed and meet the requirements. After editing, use a portable or mounted network drive to transfer the sample sheet to the NextSeq 1000 and NextSeq 2000 Sequencing Systems. When you navigate to the sample sheet in the control software, it is copied to a pre-run folder on the instrument so the portable drive can be removed.

Make sure your sample sheet v2 settings meet the following requirements:

- The index sequences specified in the BCLConvert_Data sample sheet section should match the index kit selected in the NextSeq 1000/2000.

- If using NextSeq 1000/2000 Control Software v1.2, the DRAGEN version specified in the sample sheet must be installed and active on the system. For installation information, see *Software Updates* on page 70.

- If using NextSeq 1000/2000 Control Software v1.3, the DRAGEN version specified in the sample sheet must be installed on the system. The control software automatically detects the DRAGEN version from the sample sheet and prompts you to switch active versions, if needed. For installation information, see *Software Updates* on page 70.

If you are using DRAGEN, you will need to configure additional settings. For more information, see *DRAGEN Sample Sheet Settings* on page 85

Download the sample sheet v2 template from Product Files on NextSeq 1000 and NextSeq 2000 Sequencing Systems support page. If you created a sample sheet using Instrument Run Setup, altering the sample sheet after initial download can result in analysis failure.

File names cannot contain special characters.

[Header] Requirements

The [Header] section includes overall information on your run. The following are the available [Header] fields and descriptions.

Field	Required	Description
FileFormatVersion	Yes	The sample sheet version. Enter 2 for the value.
RunName	No	Unique run name of your preference. The RunName can contain alphanumeric characters, underscores, dashes, and periods. If the RunName contains spaces or special characters, analysis fails.
RunDescription	No	Description of the run.
InstrumentPlatform	No	NextSeq 1000/2000
InstrumentType	No	NextSeq 1000/2000

[Reads] Requirements

The [Reads] section describes the number of sequencing cycles used for genomic and index read 1 and 2. The following are the available [Reads] fields and descriptions.

Field	Required	Description
Read1Cycles	Yes	Number of cycles in the first read. Value must be a whole integer greater than zero.
Read2Cycles	No	Number of cycles in the second read.
Index1Cycles	No	Number of cycles in the first index read. Required when sequencing more than one sample. The maximum is 10 cycles.
Index2Cycles	No	Number of cycles in the second index read. The maximum is 10 cycles.

[Sequencing_Settings] Requirements

Use the [Sequencing_Settings] section to specify the library prep kit you are using.

Field	Required	Description
LibraryPrepKits	No	Your library prep kit. Only one library prep kit is allowed. In the NextSeq 1000/2000 Control Software v1.3, the required custom recipe is automatically selected if the Illumina Stranded Total RNA Prep with Ribo-Zero Plus kit or the Illumina Stranded mRNA Prep kit is specified as the library prep kit. Enter one of the following values. • Illumina Stranded Total RNA Prep with Ribo-Zero Plus kit— `ILMNStrandedTotalRNA` • Illumina Stranded mRNA Prep kit—`ILMNStrandedmRNA`

BCL Convert Requirements

The BCL convert sections provide information on converting your data from BCL to FASTQ. The BCL convert options includes two separate sections: [BCLConvert_Settings] and [BCLConvert_Data]. The BCL convert sections require information about index adapter sequences. To identify the compatible adapter sequence for each read and index, see *Illumina Adapter Sequences (document # 1000000002694)*.

The following are the available [BCLConvert_Settings] fields and descriptions.

Field	Required	Description
SoftwareVersion	Yes	The version of the DRAGEN software currently installed on the system. Use all three integers included in the version name. For example, 3.5.7.
BarcodeMistmatchesIndex1	No	The number of allowed mismatches between the first index read and index sequence. Values can be either 0,1, or 2. The default value is 1.
BarcodeMismatchesIndex2	No	The number of allowed mismatches between the second index read and index sequence. Values can be either 0,1, or 2. The default value is 1.

Field	Required	Description
FastqCompressionFormat	No	To output FASTQ files as a *.gz file, enter `gzip`. To save FASTQ files as *.ora file and use with DRAGEN Decompression, enter `dragen`.
AdapterRead1	No	The sequence to trim or mask from the end of read 1. Read 1 adapter sequence containing A, C, G, or T. AdapterRead1 trims cycles by default.
AdapterRead2	No	The sequence to trim or mask from the end of read 2. Read 2 adapter sequence containing A, C, G, or T. AdapterRead2 trims cycles by default.

OverrideCycles	No	String used to specify UMI cycles and mask out cycles of a read. The following values are allowed:
		• N—Specifies cycles to ignore.
		• Y—Specifies sequencing cycles.
		• I—Specifies index cycles.
		• U—Specifies UMI cycles to be trimmed.
		Each element is separated by semicolons. The following are examples of OverrideCycles input.
		`U8Y143;I8;I8;U8Y143`
		`N10Y66;I6;N10Y66`

The following are the available [BCLConvert_Data] fields and descriptions.

Field	Required	Description
Sample_ID	Yes	The ID of the sample. The sample ID can contain up to 20 alphanumeric characters, hyphens, and underscores. The ID is case sensitive. Separate each identifier with a dash or underscore. For example, Sample1-DQB1-022515.
Index	No	The index sequence associated with the sample. Only A, C, T, G are permitted. Required when sequencing more than one sample.

Field	Required	Description
Index2	No	The second index sequence associated with the sample. Only A, C, T, G are permitted. Make sure the second index (i5) adapter sequences are in forward orientation. DRAGEN automatically reverse complements i5 indexes during secondary analysis.
Lane	No	The lane of the flow cell. Lanes are represented by one integer value.

DRAGEN Sample Sheet Settings

This section describes the sample sheet requirements for each DRAGEN pipeline. Add your DRAGEN pipeline settings as the last section on your sample sheet. You can only use one DRAGEN pipeline.

Each DRAGEN pipeline includes separate sections for settings and data.

DRAGEN Germline Pipeline Requirements

The following are the available [DragenGermline_Settings] fields and descriptions.

Field	Required	Description
SoftwareVersion	Yes	The version of the DRAGEN software currently installed on the system. Use all three integers included in the version name. For example, 3.5.7. The software version must match with the version specified in the BCLConvert_Settings section.
ReferenceGenomeDir	Yes	The reference genome name. For example, hg19_alt_aware. Use the name of the reference genome located in `/usr/local/illumina/genomes`. To use a custom reference genome, see *Reference Builder for Illumina Instruments v1.0.0 App Online Help*.
MapAlignOutFormat	No	The formatting of the output file. Allowed values are bam or cram. If no value is specified, the default is none.
KeepFastq	No	To save FASTQ output files, enter `true`. To remove FASTQ output files, enter `false`.

The following are the available [DragenGermline_Data] fields and descriptions.

Field	Required	Description
Sample_ID	Yes	The ID of the sample. The sample ID can contain up to 20 alphanumeric characters. The ID is case sensitive. Separate each identifier with a dash. For example, Sample1-DQB1-022515. Sample IDs must match with the IDs specified in the BCLConvert_Data section.

DRAGEN RNA Pipeline Requirements

The following are the available [DragenRNA_Settings] fields and descriptions.

Field	Required	Description
SoftwareVersion	Yes	The version of the DRAGEN software currently installed on the system. Use all three integers included in the version name. For example, 3.5.7. The software version must match with the version specified in the BCLConvert_Settings section.
ReferenceGenomeDir	Yes	The reference genome name. For example, hg38_noalt_with_decoy. Use the name of the reference genome located in `/usr/local/illumina/genomes`. To use a custom reference genome, see *Reference Builder for Illumina Instruments v1.0.0 App Online Help*.
RnaGeneAnnotationFile	No	The file containing RNA gene annotations. Only alphanumeric characters are allowed. If not provided, the default annotation file included in the specified reference genome is used.
MapAlignOutFormat	No	The formatting of the output file. Allowed values are bam or cram. If no value is specified, the default is none.
KeepFastq	No	To save FASTQ output files, enter `true`. To remove FASTQ output files, enter `false`.
DifferentialExpressionEnable	No	To enable differential gene expression, enter `true`. Enter `false` to exclude differential gene expression from analysis.

The following are the available [DragenRna_Data] fields and descriptions.

Field	Required	Description
Sample_ID	Yes	The ID of the sample. The sample ID can contain up to 20 alphanumeric characters. The ID is case sensitive. Separate each identifier with a dash. For example, Sample1-DQB1-022515. Sample IDs must match with the IDs specified in the BCLConvert_Data section.
Comparison<N>	No	The value of control or comparison for each sample. If there is no control or comparison value for the sample, the sample is assigned `na`. All samples marked with control are compared with all samples marked with comparison. The `N` value reflects the comparison group of the samples.

DRAGEN Enrichment Pipeline Requirements

The following are the available [DragenEnrichment_Settings] fields and descriptions.

Field	Required	Description
SoftwareVersion	Yes	The version of the DRAGEN software currently installed on the system. Use all three integers included in the version name. For example, 3.5.7. The software version must match with the version specified in the BCLConvert_Settings section.
ReferenceGenomeDir	Yes	The reference genome name. For example, hg38_alt_aware. Reference genomes are located at `/usr/local/illumina/genomes`. To use a custom reference genome, see *Reference Builder for Illumina Instruments v1.0.0 App Online Help*.
BedFile	Yes	The bed file containing the regions to target.
GermlineOrSomatic	Yes	To perform enrichment germline analysis, enter `germline`. To perform enrichment somatic analysis, enter `somatic`.
KeepFastq	No	To save FASTQ output files, enter `true`. To remove FASTQ output files, enter `false`.

Field	Required	Description
MapAlignOutFormat	No	The formatting of the output file. Allowed values are bam or cram. If no value is specified, the default is none.
AuxNoiseBaselineFile	No	The name of the noise baseline file. You can use *.txt or *.gz file format. Noise baseline files are only available when using somatic mode. See *Import Noise Baseline Files* on page 17 for more information.

The following are the available [DragenEnrichment_Data] fields and descriptions.

Field	Required	Description
Sample_ID	Yes	The ID of the sample. The sample ID can contain up to 20 alphanumeric characters. The ID is case sensitive. Separate each identifier with a dash. For example, Sample1-DQB1-022515.Sample IDs must match with the IDs specified in the BCLConvert_Data section.

DRAGEN DNA Amplicon Pipeline Requirements

The following are the available [DragenAmplicon_Settings] fields and descriptions.

Field	Required	Description
SoftwareVersion	Yes	The version of the DRAGEN software currently installed on the system. Use all three integers included in the version name. For example, 3.5.7. The software version must match with the version specified in the BCLConvert_Settings section.
ReferenceGenomeDir	Yes	The reference genome name. For example, hg38_alt_aware. Reference genomes are located at `/usr/local/illumina/genomes`. To use a custom reference genome, see *Reference Builder for Illumina Instruments v1.0.0 App Online Help*.

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Field	Required	Description
DnaBedFile	Yes	The bed file containing the regions to target. The bed file can be input in *.txt or *.gz file format.
DnaGermlineOrSomatic	Yes	To perform DNA Amplicon germline analysis, enter `germline`. To perform DNA Amplicon somatic analysis, enter `somatic`.
KeepFastq	No	To save FASTQ output files, enter `true`. To remove FASTQ output files, enter `false`.
MapAlignOutFormat	No	The formatting of the output file. Allowed values are bam or cram. If no value is specified, the default is none.

The following are the available [DragenAmplicon_Data] fields and descriptions.

Field	Required	Description
Sample_ID	Yes	The ID of the sample. The sample ID can contain up to 20 alphanumeric characters. The ID is case sensitive. Separate each identifier with a dash. For example, Sample1-DQB1-022515.Sample IDs must match with the IDs specified in the BCLConvert_Data section.
DnaOrRna	Yes	The type of Amplicon analysis to perform. Only DNA analysis is supported for DRAGEN v3.8. Enter `dna`.

DRAGEN Single Cell RNA Pipeline Requirements

The following are the available [DragenSingleCellRNA_Settings] fields and descriptions. For information on third-party kit compatibility, see the DRAGEN Bio-IT Platform Product Compatibility support page.

Single Cell Library Kit 1—5

The following sample sheet settings apply to library prep kits with the same genetic structure as DRAGEN Single Cell Library Kits 1—5. Use the DRAGEN Bio-IT Platform Product Compatibility support page to confirm the genetic structure for your kit.

Field	Required	Description
SoftwareVersion	Yes	The version of the DRAGEN software currently installed on the system. Use all three integers included in the version name. For example, 3.5.7. The software version must match with the version specified in the BCLConvert_Settings section.
ReferenceGenomeDir	Yes	The reference genome name. For example, hg38_alt_aware. Reference genomes are located at `/usr/local/illumina/genomes`. To use a custom reference genome, see *Reference Builder for Illumina Instruments v1.0.0 App Online Help* .
RnaLibraryType	No	Enter one of the following values: • `SF`—Stranded forward. `SF` is the default value. • `SR`—Stranded reverse. • `U`—Unstranded.
RnaGeneAnnotationFile	No	The file containing RNA gene annotations. Only alphanumeric characters are allowed. If not provided, the default annotation file included in the specified reference genome is used.
BarcodeRead	No	The location within the sequencing run of the barcode read, which contains both the barcode and the UMI. Values can contain `Read1` or `Read2`. The default value is `Read1`.
BarcodePosition	Yes	The location of the bases corresponding to the barcode within the value entered for BarcodeRead. Base positions are indexed starting at the zero position. Enter the BarcodePosition value in the following format: `0_<barcode end position>` For example, if a barcode contains 16 bases, the value is `0_15`.

Field	Required	Description
UmiPosition	Yes	The location of the bases corresponding to the UMI within the value entered for BarcodeRead. Enter the UmiPosition value in the following format: `<UMI start position>_<UMI end position>` For example, if the UMI contains 10 bases and the barcode contains 16, the value is `16_25`.
BarcodeSequenceWhitelist	No	The name of the file containing the barcode sequences to include. The file name can only contain alphanumeric characters, dashes, underscores, and periods.
KeepFastq	No	To save FASTQ output files, enter `true`. To remove FASTQ output files, enter `false`.
MapAlignOutFormat	No	The formatting of the output file. Allowed values are bam or cram. If no value is specified, the default is none.

The following are the available [DragenSingleCellRNA_Data] fields and descriptions.

Field	Required	Description
Sample_ID	Yes	The ID of the sample. The sample ID can contain up to 20 alphanumeric characters. The ID is case sensitive. Separate each identifier with a dash. For example, Sample1-DQB1-022515.Sample IDs must match with the IDs specified in the BCLConvert_Data section.

Single Cell Library Kit 6

The following sample sheet settings apply to library prep kits with the same genetic structure as DRAGEN Single Cell Library Kits 6. Use the DRAGEN Bio-IT Platform Product Compatibility support page to confirm the genetic structure for your kit.

Field	Required	Description
SoftwareVersion	Yes	The version of the DRAGEN software currently installed on the system. Use all three integers included in the version name. For example, 3.5.7. The software version must match with the version specified in the BCLConvert_Settings section.
ReferenceGenomeDir	Yes	The reference genome name. For example, hg38_alt_aware. Reference genomes are located at `/usr/local/illumina/genomes`. To use a custom reference genome, see *Reference Builder for Illumina Instruments v1.0.0 App Online Help*.
RnaLibraryType	No	Enter one of the following values: • `SF`—Stranded forward. • `SR`—Stranded reverse. • `U`—Unstranded.
RnaGeneAnnotationFile	No	The file containing RNA gene annotations. Only alphanumeric characters are allowed. If not provided, the default annotation file included in the specified reference genome is used.
BarcodeRead	No	The location within the sequencing run of the barcode read, which contains both the barcode and the UMI. Values can contain `Read1` or `Read2`. The default value is `Read1`.

Field	Required	Description
BarcodePosition	Yes	The location of the bases corresponding to the barcodes within the value entered for BarcodeRead. Base positions are indexed starting at the zero position. Enter the BarcodePosition value in the following format: `0_<first barcode end position>+<second barcode start position>_<second barcode end position>+<third barcode start position>_<third barcode end position>` For example, the following structure would result in the value `0_8+21_29+43_51`: • 9 bases in the first barcode (`0_8`). • 12 bases between first and second barcodes. • 9 bases in the second barcode (`21_29`). • 13 bases between second and third barcodes. • 9 bases in the third barcode (`43_51`).
UmiPosition	Yes	The location of the bases corresponding to the UMI within the specified BarCodeRead. Enter the string in the following format: `<UMI start position>_<UMI end position>` For example, if the UMI contains 8 bases and the number of bases before the UMI totals to 51, the value is `52_59`.
BarcodeSequenceWhitelist	No	The name of the file containing the barcode sequence to whitelist. The file name can only contain alphanumeric characters, dashes, underscores, and periods.
KeepFastq	No	To save FASTQ output files, enter `true`. To remove FASTQ output files, enter `false`.
MapAlignOutFormat	No	The formatting of the output file. Allowed values are bam or cram. If no value is specified, the default is none.

The following are the available [DragenSingleCellRNA_Data] fields and descriptions.

Field	Required	Description
Sample_ID	Yes	The ID of the sample. The sample ID can contain up to 20 alphanumeric characters. The ID is case sensitive. Separate each identifier with a dash. For example, Sample1-DQB1-022515.Sample IDs must match with the IDs specified in the BCLConvert_Data section.

Dark Cycle Sequencing

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

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

Use the following steps for dark cycle sequencing.

Edit the Recipe File

1. Download the recipe XML file from the Illumina Support Site.
2. Edit the recipe XML file.
   a. Identify the appropriate protocol section based on your read and index sequencing configuration. There are six different possible protocols per custom recipe that can be edited. For example, the protocol for a single Read 1 with no index sequencing configuration would be `<Protocol Name="1 Read 0 Index" ProtocolType="1Read0Index" >`.
   b. Before `<ReadRef ReadName="Read 1"/>` and `<ReadRef ReadName="Read 2"/>`, enter the following dark cycle step on a new line.
   `<DarkCycle ChemistryName="Dark Cycle Before First Base" />`.
   c. Enter the dark cycle step on a new line for every dark cycle required.
3. Save the recipe XML file.

The following is a sample recipe with dark cycle:

```
<Protocol Name="1 Read 0 Index" ProtocolType="1Read0Index" >
    <ChemistryRef ChemistryName="Start" />
    <ChemistryRef ChemistryName="Prime Cartridge" />
    <ChemistryRef ChemistryName="BIX Mixing" />
    <ChemistryRef ChemistryName="Prime Cartridge" />
    <ChemistryRef ChemistryName="ExAmp Transfer" />
    <ChemistryRef ChemistryName="ExAmp Mixing" />
    <ChemistryRef ChemistryName="Prime Cartridge" />
    <Obdd ChemistryName="Library Denaturation and Dilution" />
    <ChemistryRef ChemistryName="Prime Cartridge" />
```

```
        <Obcg ChemistryName="Cluster Generation" />
        <ChemistryRef ChemistryName="SBS Prime" />
        <ChemistryRef ChemistryName="Read Prep" />
        <DarkCycle ChemistryName="Dark Cycle Before First Base" />
        <ReadRef ReadName="Read 1" />
        <SetThermalZoneTemp Enable="false" Zone="FlowCellHeater" />
    </Protocol>
    <Protocol Name="1 Read 1 Index" ProtocolType="1Read1Index" >
        <ChemistryRef ChemistryName="Start" />
        <ChemistryRef ChemistryName="2min 60C Vacuum Hold" />
        ...
```

Attach the Recipe to the Run

4. In the Run Setup in the control software, select **Choose** under Custom Recipe.

5. Navigate to the updated recipe XML file.

6. Select **Open**.

7. Return to *Initiating a Sequencing Run* on page 43.

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
Email: techsupport@illumina.com

Illumina Technical Support Telephone Numbers

Region	Toll Free	International
Australia	+61 1800 775 688	
Austria	+43 800 006249	+43 1 9286540
Belgium	+32 800 77 160	+32 3 400 29 73
Canada	+1 800 809 4566	
China		+86 400 066 5835
Denmark	+45 80 82 01 83	+45 89 87 11 56
Finland	+358 800 918 363	+358 9 7479 0110
France	+33 8 05 10 21 93	+33 1 70 77 04 46
Germany	+49 800 101 4940	+49 89 3803 5677
Hong Kong, China	+852 800 960 230	
India	+91 8006500375	
Indonesia		0078036510048
Ireland	+353 1800 936608	+353 1 695 0506
Italy	+39 800 985513	+39 236003759
Japan	+81 0800 111 5011	
Malaysia	+60 1800 80 6789	
Netherlands	+31 800 022 2493	+31 20 713 2960
New Zealand	+64 800 451 650	
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Singapore	1 800 5792 745	
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Taiwan, China	+886 8 06651752	
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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