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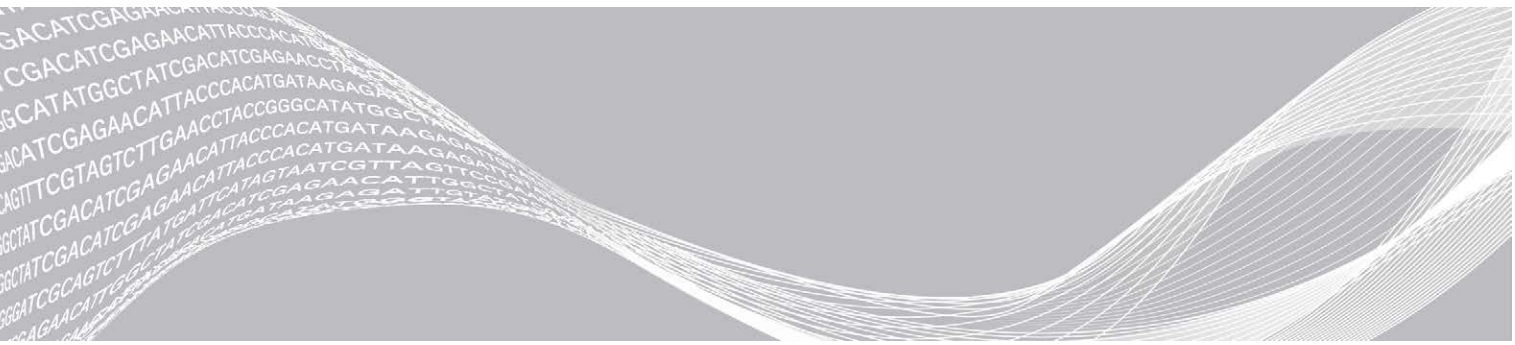
# NextSeq 550

## System Guide



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

# Revision History

| Document | Date | Description of Change |
|---|---|---|
| Material # 20006831<br>Document # 15069765<br>v06 | June<br>2019 | Added workgroup information for BaseSpace Sequence Hub during run set up.<br>Added UNC path information for the output folder.<br>Added troubleshooting for network storage errors.<br>Clarified that air filter directions are for instruments with a filter accessible from the rear panel.<br>Updated location of files that were in the root folder to run specific folders of the output folder. |
| Material # 20006831<br>Document # 15069765<br>v05 | December<br>2018 | Updated software descriptions, screens, and workflow for NextSeq Control Software (NCS) 4.0.<br>Updated the following additional information for NCS 4.0.<br>• Added information on Local Run Manager software.<br>• Updated BaseSpace information to BaseSpace Sequence Hub. BaseSpace Prep tab and BaseSpace Onsite are no longer available.<br>• Added instructions on selecting Local Run Manager or manual run mode. Manual mode replaces standalone mode, with some modifications.<br>• Added option to check for instrument software updates in BaseSpace Sequence Hub.<br>• Added Local Run Manager, Universal Copy Service, and Direct Memory Access driver to the description of the System Suite bundle. Removed BaseSpace Broker and SAV.<br>• Run Copy Service is now Universal Copy Service.<br>• Added option to enable custom recipes when loading the reagent cartridge.<br>• Removed the description of the flow cell image when monitoring run progress.<br>• Removed the select start-up option for kiosk and Windows mode.<br>• Added MethylationEPIC v1.0 as a compatible BeadChip type.<br>• Added maintenance instructions for instruments with an air filter.<br>• Added new icons for attention, information, and minimizing NCS.<br>• Updated instructions for customizing run settings and system settings.<br>• Updated send instrument performance data option.<br>• Updated data transfer icons.<br>• Clarified that for scanning, files queued for transfer have no time limit.<br>• Corrected references of BSM to Buffer Straw Mechanism in motion checks information.<br>• Added six-month Windows password expiration information.<br>Added reagent or spectrophotometric-grade methanol or isopropyl alcohol (99%) for instrument maintenance. |

| Document | Date | Description of Change |
|---|---|---|
| Material # 20006831<br>Document # 15069765<br>v04 | May 2018 | Added support for NextSeq v2.5 reagents.<br>Updated storage/shipping information for NextSeq v2.5 Reagent Kits shipping flow cells at ambient temperatures. NextSeq v2.5 flow cells continue to require previous storage conditions.<br>Added information about NextSeq v2.5 Reagent Kits requiring software updates to version 2.2.<br>Added note regarding mid output kit loading concentration.<br>Added note regarding saving flow cells.<br>Added note recommending that high output flow cells are used for system checks. |
| Material # 20006831<br>Document # 15069765<br>v03 | March 2018 | Removed the default user name and password required to log on to the operating system. Illumina recommends using site-specific credentials.<br>Added information about the Illumina Proactive monitoring service in the Select BaseSpace Configuration section.<br>Updated RTA v2 software references to RTA2. |
| Material # 20006831<br>Document # 15069765<br>v02 | March 2016 | Added section titled Indexing Considerations.<br>Removed steps to inspect the flow cell.<br>Specified loading volume and concentration in the step to Load Libraries onto the Reagent Cartridge. |
| Material # 20001843<br>Document # 15069765<br>v01 | October 2015 | Specified that an equivalent to the recommended supplier of NaOCl is a laboratory-grade equivalent.<br>Added recommendation for annual preventive maintenance service.<br>Reorganized information in the Overview and Getting Started chapters.<br>Added instructions for customizing system settings.<br>Removed Live Help instructions from troubleshooting chapter. This feature was removed from the control software. |
| Part # 15069765 Rev. B | May 2015 | Corrected description of reserved reservoirs on the reagent cartridge. |
| Part # 15069765 Rev. A | May 2015 | Initial release. |

# Table of Contents

# Chapter 1 Overview



## Introduction

The Illumina® NextSeq™ 550 system is a single solution that provides a seamless transition between high-throughput sequencing and array scanning.

### Sequencing Features

- **High-throughput sequencing**—The NextSeq 550 enables sequencing of exomes, whole genomes, and transcriptomes and supports TruSeq™, TruSight™, and Nextera™ libraries.
- **Flow cell types**—Flow cells are available in configurations for high output and mid output. Each flow cell type is kitted with a compatible prefilled reagent cartridge.
- **Real-Time Analysis (RTA)**—Integrated analysis software performs on-instrument data analysis, which includes image analysis and base calling. The NextSeq uses an implementation of RTA called RTA v2, which includes important architecture and feature differences. For more information, see *Real-Time Analysis* on page 55.
- **Cloud-based analysis with BaseSpace™ Sequence Hub**—The sequencing workflow is integrated with BaseSpace Sequence Hub, the Illumina genomics cloud computing environment for run monitoring, data analysis, storage, and collaboration. As the run progresses, output files are streamed in real-time to BaseSpace Sequence Hub for analysis.
- **On-instrument data analysis**— The Local Run Manager software analyzes run data according to the analysis module specified for the run.

### Array Scanning Features

- **Integrated array scanning in control software**—The NextSeq 550 allows you to transition between array scanning and high-throughput sequencing on the same instrument and control software.
- **Extended imaging capability**—The imaging system in the NextSeq 550 includes software and stage modifications that enable imaging of a larger surface area to accommodate BeadChip scanning.
- **BeadChip types**—Compatible BeadChip types include CytoSNP-12, CytoSNP-850K, Karyomap-12, and MethylationEPIC v1.0.
- **BeadChip adapter**—A reusable BeadChip adapter enables easy loading of a BeadChip onto the instrument.
- **Data Analysis**—Use the BlueFuse® Multi software to analyze array data.

## Additional Resources

The following documentation is available for download from the Illumina website.

| Resource | Description |
|---|---|
| *NextSeq System Site Prep Guide (document # 15045113)* | Provides specifications for laboratory space, electrical requirements, and environmental considerations. |
| *NextSeq System Safety and Compliance Guide (document # 15046564)* | Provides information about operational safety considerations, compliance statements, and instrument labeling. |
| *RFID Reader - Model # TR-001-44 User Guide (document # 15041950)* | Provides information about the RFID reader in the instrument, compliance certifications, and safety considerations. |
| *Denaturing and Diluting Libraries for the NextSeq System (document # 15048776)* | Provides instructions for denaturing and diluting prepared libraries for a sequencing run, and preparing an optional PhiX control. This step applies to most library types. |
| *NextSeq Custom Primers Guide (document # 15057456)* | Provides information about using custom sequencing primers in place of Illumina sequencing primers. |
| *BaseSpace help (help.basespace.illumina.com)* | Provides information about using BaseSpace™ Sequence Hub and available analysis options. |
| *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)* | Provides an overview of instrument components and software, instructions for performing sequencing runs, and procedures for instrument maintenance and troubleshooting on the NextSeq 550Dx. |
| *Local Run Manager Software Guide (document # 1000000002702)* | Provides an overview of the Local Run Manager software and instructions for using software features. |

Visit the NextSeq 550 support page on the Illumina website for access to documentation, software downloads, online training, and frequently asked questions.

## NextSeq 550Dx in RUO Mode

The instructions in this guide are also applicable to the NextSeq 550Dx instrument when in research mode with the latest version of RUO instrument software. For a summary of exceptions and other considerations, see *NextSeq 550Dx Research Mode Considerations* on page 65.

## Instrument Components

The NextSeq 550 system includes a touch screen monitor, a status bar, and four compartments.

**Figure 1** Instrument Components


A **Touch screen monitor**—Enables on-instrument configuration and setup using the control software interface.
B **Status bar**—Indicates instrument status as processing (blue), requires attention (orange), ready to sequence (green), or when a wash is due within the next 24 hours (yellow).
C **Buffer compartment**—Holds the buffer cartridge and the spent reagents container.
D **Reagent compartment**—Holds the reagent cartridge.
E **Power button**—Powers the instrument and the instrument computer on or off.
F **Imaging compartment**—Holds the flow cell for sequencing or the BeadChip adapter for scanning.
G **Air filter compartment**—Holds the air filter for instruments with a filter that is accessible from the rear panel.

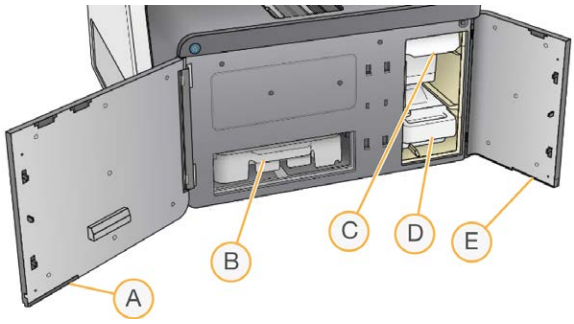
## Imaging Compartment

The imaging compartment houses the stage, which includes three alignment pins for positioning the flow cell for sequencing or the BeadChip adapter for scanning. After loading the flow cell or BeadChip adapter, the imaging compartment door closes automatically and moves components into position.

## Reagent and Buffer Compartments

Setting up a sequencing run on the NextSeq 550 requires access to the reagent compartment and buffer compartment to load run consumables and empty the spent reagents container.

**Figure 2** Reagent and Buffer Compartments




A **Reagent compartment door**—Encloses the reagent compartment with a latch under the lower-right corner of the door. The reagent compartment holds the reagent cartridge. Reagents are pumped through the sippers and fluidics system, and then to the flow cell.
B **Reagent cartridge**—The reagent cartridge is a prefilled single-use consumable.
C **Buffer cartridge**—The buffer cartridge is a prefilled single-use consumable.
D **Spent reagents container**—Spent reagents are collected for disposal after each run.
E **Buffer compartment door**—Encloses the buffer compartment with a latch under the lower-left corner of the door.

## Air Filter Compartment

The air filter compartment holds the air filter for instruments with a filter that is accessible from the rear panel. Replace the air filter every 90 days. For information on replacing the filter, see *Replace Air Filter* on page 37.

## NextSeq Software

The instrument software includes integrated applications that perform sequencing runs or array scanning.

- **NextSeq Control Software (NCS)**—Controls instrument operation and guides you through the steps to set up a sequencing run or array scan.
  - The software is preinstalled on the NextSeq, and runs on-instrument. The NCS performs the run according to the parameters specified in the Local Run Manager software module or in NCS.
  - Before you start the sequencing run, you select a run that you created with Local Run Manager module or in NCS. The NCS software interface guides you through the steps to load the flow cell and reagents.
  - During the run, the software operates the flow cell stage, dispenses reagents, controls fluidics, sets temperatures, captures images of clusters on the flow cell, and provides a visual summary of quality statistics. You can monitor the run in NCS, or in Local Run Manager.
  - During the run, which you can monitor in NCS, or in Local Run Manager, NCS performs the following functions.
    - Operates the flow cell stage
    - Dispenses reagents
    - Controls fluidics
    - Sets temperatures
    - Captures images of clusters on the flow cell
    - Provides a visual summary of quality statistics
- **Local Run Manager software**— Integrated software solution for creating a run and analyzing results (secondary analysis). The software also provides sample tracking and can control user permissions.

- **Real-Time Analysis (RTA) software**—For sequencing runs, RTA performs image analysis and base calling during the run. The NextSeq 550 uses RTA v2, which includes important architecture and feature differences from earlier versions. For more information, see *Real-Time Analysis* on page 55.
- **Universal Copy Service**—Copies sequencing output files from the run folder to the output folder and BaseSpace Sequence Hub (if applicable), where you can access them.

Real-Time Analysis (RTA) and Universal Copy Service run background processes only.

## Status Icons

A status icon in the top-right corner of the control software interface screen signals any change in conditions during run setup or during the run.

| Status Icon | Status Name | Description |
|---|---|---|
|  | Status OK | System is normal. |
|  | Processing | System is processing. |
|  | Warning | A warning has occurred.<br>Warnings do not stop a run or require action before proceeding. |
|  | Error | An error has occurred.<br>Errors require action before proceeding with the run. |
|  | Attention | A notification requiring attention has occurred. Refer to the message for additional information. |
|  | Information | An informational message only. No further action is required. |

When a change in condition occurs, the icon blinks to alert you. Select the icon to view a description of the condition. Select **Acknowledge** to accept the message and **Close** to close the dialog box.

## Navigation Bar Icon

The minimize NCS icon is in the top-right corner of the control software interface.

| Access Icon | Icon Name | Description |
|---|---|---|
|  | Minimize NCS | Select to minimize NCS to access Windows applications and folders. |

# Power Button

The power button on the front of the NextSeq turns on power to the instrument and instrument computer. The power button performs the following actions depending on the state of instrument power.

| Power State | Action |
|---|---|
| Instrument power is off | Briefly press the button to turn on the power. |
| Instrument power is on | Briefly press the button to turn off the power. A dialog box appears on the screen to confirm a normal instrument shutdown. |
| Instrument power is on | Press and hold the power button for 10 seconds to cause a hard shutdown of the instrument and instrument computer.<br>Use this method to turn off the instrument only if the instrument is unresponsive. |

**NOTE**

Turning off the instrument during a sequencing run ends the run immediately. Ending a run is final. Run consumables cannot be reused and sequencing data from the run is not saved.

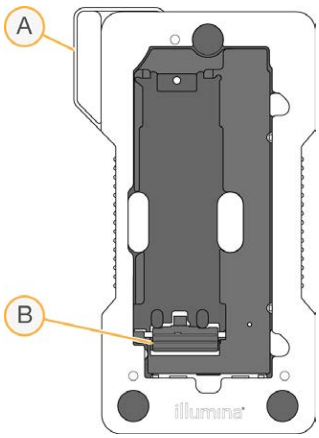
## Windows Password Requirements

The operating system requires a Windows password change every 180 days. When prompted, update your Windows password. If you use Local Run Manager for analysis, also update the password for the Windows account in Local Run Manager. See the section Specify Service Account Settings in *Local Run Manager Software Guide (document # 1000000002702)*.

## Reusable BeadChip Adapter Overview

The reusable BeadChip adapter holds the BeadChip during scanning. The BeadChip is secured in the recessed shelf of the adapter with the retention clip. Then, the BeadChip adapter is loaded onto the stage in the imaging compartment.

**Figure 3** Reusable BeadChip Adapter




A BeadChip adapter
B Retention clip

# Sequencing Consumables Overview

## Contents and Storage

The sequencing consumables required to run the NextSeq are provided separately in a single-use kit. Each kit includes one flow cell, a reagent cartridge, a buffer cartridge, and library dilution buffer. When you receive NextSeq 500/550 Kit:

- Do not open the foil package of the flow cell until instructed to do so.
- Promptly store components at the indicated temperatures to ensure proper performance.
- Store cartridges so that the package labels face up.

| Consumable | Quantity | Storage Temperature | Description |
|---|---|---|---|
| Reagent Cartridge | 1 | -25°C to -15°C | Contains clustering and sequencing reagents |
| Buffer Cartridge | 1 | 15°C to 30°C | Contains buffer and wash solution |
| HT1 | 1 | -25°C to -15°C | Hybridization Buffer |
| Flow Cell | 1 | 2°C to 8°C* | Single-use flow cell |

*Shipped at room temperature for NextSeq v2.5 Reagents kits

Reagents are sensitive to light. Store the reagent cartridge and buffer cartridge in a dark location away from light.

The flow cell, reagent cartridge, and buffer cartridge use radio-frequency identification (RFID) for accurate consumable tracking and compatibility.

All other kits include dual-index sequencing primers and NaOCl in the prefilled cartridge. No additional steps are required.

**CAUTION**

NextSeq v2.5 Reagent kits require NCS v2.2 or later. Make sure that software updates are complete before you prepare samples and consumables.

## Kit Compatibility and Labeling

Kit components are labeled with color-coded indicators to show compatibility between flow cells and reagent cartridges. Always use a compatible reagent cartridge and flow cell. The buffer cartridge is universal.

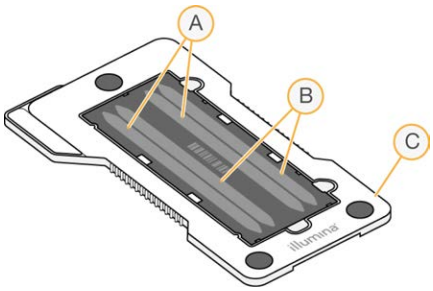
Each flow cell and reagent cartridge is labeled **High** or **Mid**. Always check the label when you prepare consumables for a run.

| Kit Type | Marking on Label |
|---|---|
| High Output Kit Components |   |
| Mid Output Kit Components |   |

There are additional compatibility considerations for NextSeq 550Dx instruments in research mode. See *NextSeq 550Dx Consumables Compatibility* on page 65.

## Flow Cell Overview

**Figure 4** Flow Cell Cartridge




A Lane pair A—Lanes one and three
B Lane pair B—Lanes two and four
C Flow cell cartridge frame

The flow cell is a glass-based substrate on which clusters are generated and the sequencing reaction is performed. The flow cell is encased in a flow cell cartridge.

The flow cell contains four lanes that are imaged in pairs.

- Lanes one and three (lane pair A) are imaged at the same time.
- Lanes two and four (lane pair B) are imaged when imaging of lane pair A is complete.

Although the flow cell has four lanes, only a single library or set of pooled libraries is sequenced on the flow cell. Libraries are loaded onto the reagent cartridge from a single reservoir and transferred automatically to the flow cell to all four lanes.

Each lane is imaged in small imaging areas called tiles. For more information, see *Flow Cell Tiles* on page 60.

## Reagent Cartridge Overview

The reagent cartridge is a single-use consumable with RFID tracking and foil-sealed reservoirs that are prefilled with clustering and sequencing reagents.

**Figure 5** Reagent Cartridge

The reagent cartridge includes a designated reservoir for loading prepared libraries. After the run begins, libraries are transferred automatically from the reservoir to the flow cell.

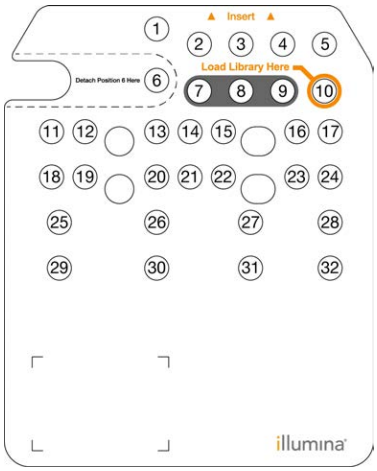
Several reservoirs are reserved for the automatic post-run wash. Wash solution is pumped from the buffer cartridge to the reserved reservoirs, through the system, and then to the spent reagents container.

**WARNING**

**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.

## Reserved Reservoirs

**Figure 6** Numbered Reservoirs




| Position | Description |
|---|---|
| 7, 8, and 9 | Reserved for optional custom primers |
| 10 | Load libraries |

For information about custom primers, see *NextSeq Custom Primers Guide (document # 15057456)*.

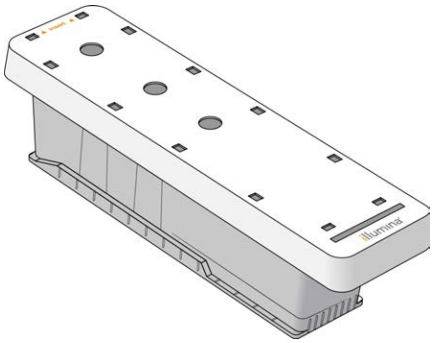
## Removable Reservoir in Position #6

The prefilled reagent cartridge includes a denaturation reagent in position 6 that contains formamide. To facilitate safe disposal of any unused reagent after the sequencing run, the reservoir in position six is removable. For more information, see *Remove Used Reservoir from Position #6* on page 24.

# Buffer Cartridge Overview

The buffer cartridge is a single-use consumable containing three reservoirs that are prefilled with buffers and wash solution. The contents of the buffer cartridge are sufficient for sequencing one flow cell.

**Figure 7** Buffer Cartridge



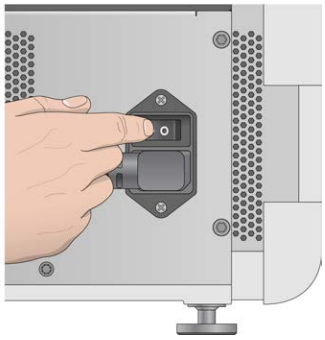
# Chapter 2 Getting Started



## Starting the Instrument

Turn on the power toggle switch to the **I** (on) position.

**Figure 8** Power Switch Located on Back of Instrument



**NOTE**

To start the NextSeq 550Dx instrument in research mode, see *Starting the NextSeq 550Dx Instrument* on page 65.

1 Press the power button above the reagent compartment. The power button turns on the instrument power and starts the integrated instrument computer and software.

**Figure 9** Power Button Located on Front of Instrument


2 Wait until the operating system has finished loading.
The NextSeq Control Software (NCS) launches and initializes the system automatically. After the initialization step is complete, the Home screen opens.

3 If your system has been configured to require login credentials, wait for the system to load, and then log on to the operating system. If necessary, consult your facility administrator for the user name and password.

# Customize System Settings

The control software includes customizable system settings for the following. To change network configuration settings, see *Configure System Settings* on page 52.

- Customize Instrument Identification (Avatar and Nickname)
- Set Keyboard Option and Audio Indicator
- Set Custom Recipes Option
- Set Check for Instrument Software Updates from BaseSpace Sequence Hub
- Set Send Instrument Performance Data Option

## Customize Instrument Avatar and Nickname

1. From the Home screen, select **Manage Instrument**.
2. Select **System Customization**.
3. To assign a preferred avatar image for your instrument, select **Browse** and navigate to the image.
4. In the Nickname field, enter a preferred name for the instrument.
5. Select **Save** to save settings and advance the screen.
   The image and name appear at the upper-left corner of each screen.

## Set Keyboard Option and Audio Indicator

1. From the Home screen, select **Manage Instrument**.
2. Select **System Customization**.
3. Select the **Use on-screen keyboard** checkbox to activate the on-screen keyboard for input to the instrument.
4. Select the **Play audio** checkbox to turn on audio indicators for the following events.
   - Upon instrument initialization
   - When a run is started
   - When certain errors occur
   - When user interaction is required
   - When a run has finished
5. Select **Save** to save settings and advance the screen.

## Set Custom Recipes Option

1. From the Home screen, select **Manage Instrument**.
2. Select **System Customization**.
3. Select the **Enable Custom Recipes** checkbox to enable the selection of a custom recipe when loading a reagent cartridge. For more information, see *Custom Recipes and Recipe Folders* on page 49.
4. Select **Save** to save settings and advance the screen.

## Set Check for Instrument Software Updates from BaseSpace

1. From the Home screen, select **Manage Instrument**.
2. Select **System Customization**.

3 Select **Automatically check for new software updates on BaseSpace** checkbox to activate automatic checks for BaseSpace Sequence Hub updates.
The automatic check for updates is performed every 24 hours. When an update is available, a notification is displayed in the following places.
   - On the Manage Instrument screen on the Software Update icon.
   - On the Manage Instrument button on the Home screen.

4 Select **Save** to save settings and advance the screen.

### Set Send Instrument Performance Data Option

1 From the Home screen, select **Manage Instrument**.

2 Select **System Customization**.

3 Select **Send Instrument Performance Data to Illumina** to enable the Illumina Proactive monitoring service. The name of the setting in the software interface might be different from the name in this guide, depending on the version of NCS in use.
With this setting turned on, instrument performance data are sent to Illumina. This data helps Illumina troubleshoot more easily and detect potential failures, enabling proactive maintenance and maximizing instrument uptime. For more information on the benefits of this service, see *Illumina Proactive Technical Note (document # 1000000052503)*.
This service:
   - Does not send sequencing data.
   - Requires that the instrument be connected to a network with internet access.
   - Is turned on by default. To opt out of this service, disable the **Send Instrument Performance Data to Illumina** setting.

4 Select **Save** to save settings and advance the screen.

## Customize Run Settings

The control software includes customizable settings for run setup preferences and purging of unused reagents.

### Set Run Setup Options

1 From the Home screen, select **Manage Instrument**.

2 Select **System Customization**.

3 Select the **Use Advanced Load Consumables** checkbox to enable the option to load all run consumables from a single screen.

4 Select the **Skip Pre-Run Check Confirmation** checkbox to start sequencing or scanning automatically after a successful automatic check.

5 Select **Save** to save settings and exit the screen.

### Set Automatic Purge Option

1 From the Home screen, select **Manage Instrument**.

2 Select **System Customization**.

3 Select the **Purge Consumables at End of Run** checkbox to purge unused reagents from the reagent cartridge to the spent reagents container automatically after each run.

**NOTE**

Purging consumables automatically adds additional time to the workflow.

4 Select **Save** to save settings and exit the screen.

## User-Supplied Consumables and Equipment

The following consumables and equipment are used on the NextSeq 550.

### User-Supplied Consumables for Sequencing Runs

| Consumable | Supplier | Purpose |
|---|---|---|
| 1 N NaOH<br>(sodium hydroxide) | General lab supplier | Library denaturation, diluted to 0.2 N |
| 200 mM Tris-HCl, pH7 | General lab supplier | Library denaturation |
| Alcohol wipes, 70% Isopropyl<br>or<br>Ethanol, 70% | VWR, catalog # 95041-714<br>(or equivalent)<br>General lab supplier | Flow cell cleaning and general purpose |
| Lab tissue, low-lint | VWR, catalog # 21905-026<br>(or equivalent) | Flow cell cleaning |

### User-Supplied Consumables for Instrument Maintenance

| Consumable | Supplier | Purpose |
|---|---|---|
| NaOCl, 5%<br>(sodium hypochlorite) | Sigma-Aldrich, catalog # 239305<br>(or laboratory-grade equivalent) | Washing the instrument using the manual post-run wash; diluted to 0.12% |
| Tween 20 | Sigma-Aldrich, catalog # P7949 | Washing the instrument using manual wash options; diluted to 0.05% |
| Water, laboratory-grade | General lab supplier | Washing the instrument (manual wash) |
| Reagent or spectrophotometric-grade methanol or isopropyl alcohol (99%), 100 ml bottle | General lab supplier | Cleaning optics components periodically and support the objective cleaning cartridge |
| Air filter | Illumina, catalog # 20022240 | For instruments with an air filter accessible from the rear panel. Cleaning the air the instrument takes in for cooling. |

## Guidelines for Laboratory-Grade Water

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

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

# User-Supplied Equipment

| Item | Source |
|---|---|
| Freezer, -25°C to -15°C, frost-free | General lab supplier |
| Ice bucket | General lab supplier |
| Refrigerator, 2°C to 8°C | General lab supplier |

# Chapter 3 Sequencing



## Introduction

To perform a sequencing run on the NextSeq 550, prepare a reagent cartridge and flow cell. Then follow the software prompts to set up and start the run. Cluster generation and sequencing are performed on-instrument. After the run, an instrument wash begins automatically using components already loaded on the instrument.

### Cluster Generation

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

### Sequencing

Clusters are imaged using two-channel sequencing chemistry and filter combinations specific to each of the fluorescently labeled chain terminators. After imaging of a tile on the flow cell is complete, the next tile is imaged. The process is repeated for each cycle of sequencing. Following image analysis, the software performs base calling, filtering, and quality scoring.

Monitor run progress and statistics in the following locations.

- The NCS interface
- BaseSpace Sequence Hub
- Local Run Manager
- A networked computer using the Sequencing Analysis Viewer (SAV) software. See *Sequencing Analysis Viewer* on page 27.

### Analysis

As the run progresses, the control software automatically transfers base call (BCL) files to BaseSpace Sequence Hub, Local Run Manager, or another specified output location for secondary analysis.

Several analysis methods are available depending on your application. For more information, see the *BaseSpace help (help.basespace.illumina.com)* or *Local Run Manager Software Guide (document # 1000000002702)*.

### Sequencing Run Duration

Sequencing run duration depends on the number of cycles performed. The maximum run length is a paired-end run of 150 cycles each read (2 x 150), plus up to eight cycles each for two index reads.

For expected durations and other system specifications, visit the NextSeq 550 specifications page on the Illumina website.

### Number of Cycles in a Read

In a sequencing run, the number of cycles performed in a read is one more cycle than the number of cycles analyzed. For example, a paired-end 150-cycle run performs reads of 151-cycles (2 × 151) for a total of 302 cycles. At the end of the run, 2 x 150 cycles are analyzed. The extra cycle is required for phasing and prephasing calculations.

## Create Run with Local Run Manager Software

The process to set up run and analysis parameters in Local Run Manager varies depending on the particular analysis workflow module you use. Refer to the Local Run Manager module guide for specific directions on how to create a run.

1 From the home screen, select **Edit Runs**.

2 Select **Create Run** from the Local Run Manager dashboard, and then select an analysis module.

3 Enter a run name, enter samples for the run, and if applicable, import manifests.

4 Save the run and close the Local Run Manager dashboard window.

To create a run in NCS, without Local Run Manager software, use the manual run mode. See *Create Run with NCS* on page 17 and *Run Modes* on page 19.

## Create Run with NCS

If you create a run with NCS (manual run mode), run and analysis parameters are entered immediately before loading the flow cell.

1 Review the run and analysis parameters required in *Enter Run and Analysis Parameters in NCS (Manual Run Mode)* on page 21.

2 Determine the run and analysis parameters now so that there is no delay when starting your sequencing run.

## Prepare the Reagent Cartridge

1 Remove the reagent cartridge from -25°C to -15°C storage.

2 Thaw in a room temperature water bath until thawed (~60 minutes). Do not submerge the cartridge.

3 Gently tap on the bench to dislodge water from the base, and then dry the base.

**NOTE**

**[Alternate method]** Thaw reagents overnight at 2°C to 8°C. Reagents require a minimum of 18 hours to thaw. At this temperature, reagents are stable up to one week.

4 Invert the cartridge five times to mix reagents.

5 Inspect positions 29, 30, 31, and 32 to make sure that reagents are thawed.

6 Gently tap on the bench to reduce air bubbles.

**WARNING**

**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.

## Prepare the Flow Cell

1 Remove a new flow cell package from 2°C to 8°C storage.

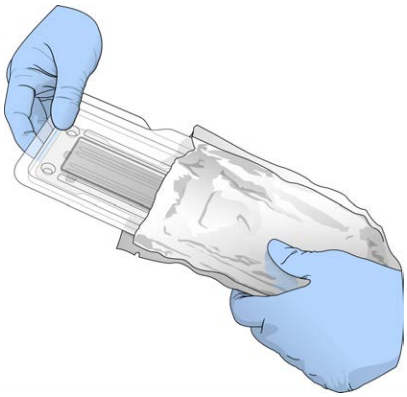
2 Set the unwrapped flow cell package aside at room temperature for 30 minutes.

**NOTE**

If the foil package is intact, the flow cell can remain at room temperature up to 12 hours. Avoid repeated cooling and warming of the flow cell.

3 Remove the flow cell from the foil package.

**Figure 10** Remove from Foil Package



4 Open the clear plastic clamshell package and remove the flow cell.

**Figure 11** Remove from Clamshell Package

5 Clean the glass surface of the flow cell with a lint-free alcohol wipe. Dry the glass with a low-lint lab tissue.

## Prepare Libraries for Sequencing

The library volume and loading concentration differ depending on the version of NCS you are running.

| Control Software Version | Library Volume | Library Concentration |
|---|---|---|
| NCS v1.3, or later | 1.3 ml | 1.8 pM |
| NCS v1.2, or earlier | 3 ml | 3 pM |

## Denature and Dilute Libraries

Denature and dilute your libraries to the following loading volume and concentration.

| Kit Type | Loading Volume | Loading Concentration |
|---|---|---|
| High Output | 1.3 ml | 1.8 pM |
| Mid Output | 1.3 ml | 1.5 pM |

In practice, loading concentration can vary depending on library preparation and quantification methods. For instructions, see the *NextSeq System Denature and Dilute Libraries Guide (document # 15048776)*.

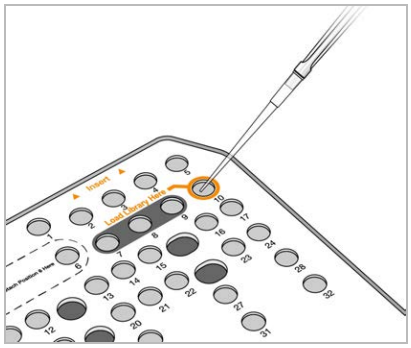
## Load Libraries onto the Reagent Cartridge

1 Clean the foil seal covering reservoir #10 labeled **Load Library Here** using a low-lint tissue.

2 Pierce the seal with a clean 1 ml pipette tip.

3 Load 1.3 ml of prepared 1.8 pM libraries into reservoir #10 labeled **Load Library Here**. Avoid touching the foil seal as you dispense the libraries.

**Figure 12** Load Libraries



## Set Up a Sequencing Run

1 From the Home screen, select **Experiment**.

2 On the Select Assay screen, select **Sequence**.
The Sequence command opens the imaging compartment door, releases consumables from a previous run, and opens the series of run setup screens. A short delay is normal.

## Run Modes

When setting up a sequencing run, you select one of the following run modes to determine where to enter run information and how to analyze data.

| Run Mode | Run Information | Data Analysis* |
|---|---|---|
| Local Run Manager | Enter in Local Run Manager. | The software saves data to the specified output folder for automatic analysis in Local Run Manager. |
| Manual | Enter in the NCS. | The software saves data to a specified output folder for later analysis off the instrument. |

* For analysis purposes, BaseSpace Sequence Hub can pair with either run mode. When the run mode is Local Run Manager and BaseSpace Sequence Hub is configured, both applications analyze the data.

Local Run Manager is the default run mode, and it provides the most streamlined workflow. You create and save runs in Local Run Manager. The information is then sent to the control software, where you select a run and continue run setup. After sequencing, Local Run Manager automatically performs data analysis. Separate sample sheets and analysis applications are not needed.

**NOTE**

Local Run Manager is not a feature of the control software. It is integrated software for recording samples for sequencing, specifying run parameters, and analyzing data.

## BaseSpace Sequence Hub (Optional)

When setting up a sequencing run, you can select one of the following BaseSpace Sequence Hub options.

| Option | Description and Requirements |
|---|---|
| Run Monitoring and Storage | Send InterOp files, log files, and run data to BaseSpace Sequence Hub for remote monitoring and analysis.<br>Requires a BaseSpace Sequence Hub account, an internet connection, and a sample sheet. |
| Run Monitoring Only | Send InterOp and log files to BaseSpace Sequence Hub for remote run monitoring. This option is the default.<br>Requires a BaseSpace Sequence Hub account and an internet connection. |

## Select Run Mode and BaseSpace Sequence Hub

1 On the Run Setup screen, select from one of the following run modes.
- Local Run Manager
- Manual

2 [Optional] Select Use BaseSpace Sequence Hub Setting and select from one of the following.
- Run Monitoring and Storage
- Run Monitoring Only

Enter your BaseSpace Sequence Hub user name and password.

If prompted, select a workgroup to upload run data to. You are prompted only if you belong to multiple workgroups.

3 Select Next.

## Select Run (Local Run Manager Run Mode)

1 Select a run name from the list of available runs.
Use the up and down arrows to scroll through the list or enter a run name in the Search field.

2 Confirm run parameters.
- Run Name—Name of the run as assigned in Local Run Manager.

- **Library ID**—Name of the pooled libraries as assigned in Local Run Manager.
- **Recipe**—Name of the recipe, either **NextSeq High** or **NextSeq Mid** depending on the reagent cartridge used for the run.
- **Read Type**—Single-Read or Paired-End.
- **Read Length**—Number of cycles for each read.
- **[Optional]** Custom Primers, if applicable.

3 **[Optional]** Select the **Edit** icon to change run parameters. When finished, select **Save**.
- **Run parameters**—Change the number of reads or number of cycles per read.
- **Custom primers**—Change the settings for custom primers. For more information, see *NextSeq Custom Primers Guide (document # 15057456)*.
- **Purge consumables for this run**—Change the setting to purge consumables automatically after the current run.

4 Select **Next**.

## Enter Run and Analysis Parameters in NCS (Manual Run Mode)

1 Enter a run name of your preference.

2 **[Optional]** Enter a library ID of your preference.

3 From the Recipe drop-down list, select a recipe. Only compatible recipes are listed.

4 Select a read type, either **Single-Read** or **Paired-End**.

5 Enter the number of cycles for each read in the sequencing run.
- **Read 1**—Enter a value up to 151 cycles.
- **Read 2**—Enter a value up to 151 cycles. This value is typically the same number of cycles as Read 1.
- **Index 1**—Enter the number of cycles required for the Index 1 (i7) primer.
- **Index 2**—Enter the number of cycles required for the Index 2 (i5) primer.

The control software confirms your entries using the following criteria:
- Total cycles do not exceed the maximum cycles allowed
- Cycles for Read 1 are greater than the 5 cycles used for template generation
- Index Read cycles do not exceed Read 1 and Read 2 cycles

6 **[Optional]** If you are using custom primers, select the checkbox for the primers used. For more information, see *NextSeq Custom Primers Guide (document # 15057456)*.
- **Read 1**—Custom primer for Read 1.
- **Read 2**—Custom primer for Read 2.
- **Index 1**—Custom primer for Index 1.
- **Index 2**—Custom primer for Index 2.

7 Set the output folder location for the current run. Select **Browse** to navigate to a network location. For information on output folder requirements, see *Set Output Folder Location* on page 53.

8 Select **Browse** to navigate to a sample sheet.
Systems configured for manual mode with Run Monitoring and Storage in BaseSpace Sequence Hub require a sample sheet.

9 Select **Purge consumables for this run**.
The setting purges consumables automatically after the current run.

10 Select **Next**.

11 [Optional] Select the Edit icon to change run parameters.

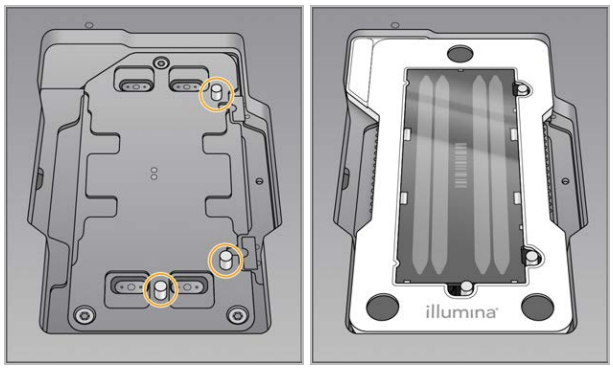
12 Select Next.

## Load the Flow Cell

1 Remove the used flow cell from a previous run.

2 Align the flow cell over the alignment pins and place the flow cell on the stage.

**Figure 13** Load the Flow Cell




3 Select Load.
The door closes automatically, the flow cell ID appears on the screen, and the sensors are checked.

4 Select Next.

## Empty the Spent Reagents Container

1 Remove the spent reagents container and discard the contents in accordance with applicable standards.

**Figure 14** Remove the Spent Reagents Container

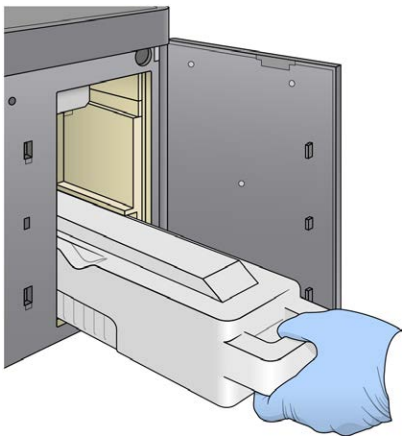
**NOTE**
As you remove the container, place your other hand underneath for support.

**WARNING**

**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.

2 Slide the empty spent reagents container into the buffer compartment until it stops. An audible click indicates that the container is in position.

**Figure 15** Load the Empty Spent Reagents Container

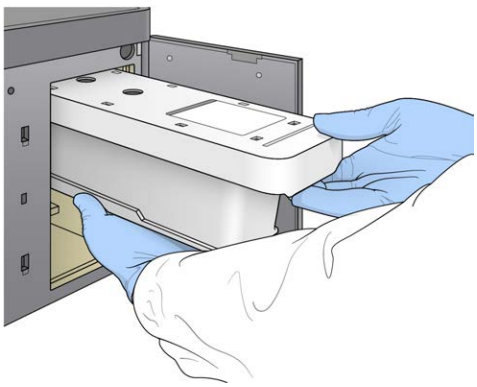


## Load the Buffer Cartridge

1 Remove the used buffer cartridge from the upper compartment.

2 Slide a new buffer cartridge into the buffer compartment until it stops.
An audible click indicates that the cartridge is in position, the buffer cartridge ID appears on the screen, and the sensor is checked.

**Figure 16** Load the Buffer Cartridge



3 Close the buffer compartment door, and select **Next**.

## Load the Reagent Cartridge

1 Remove the used reagent cartridge from the reagent compartment. Dispose of unused contents in accordance with applicable standards.

**WARNING**

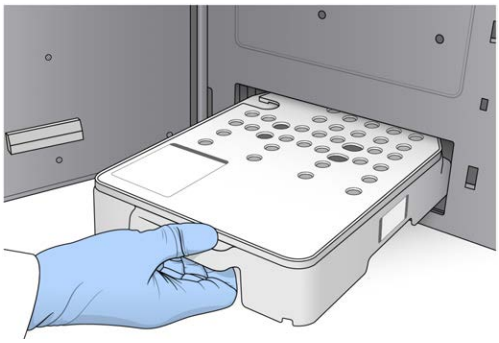
**This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical 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.

**NOTE**

To facilitate safe disposal of unused reagent, the reservoir in position 6 is removable. For more information, see *Remove Used Reservoir from Position #6* on page 24.

2 Slide the reagent cartridge into the reagent compartment until the cartridge stops, and then close the reagent compartment door.

**Figure 17** Load Reagent Cartridge



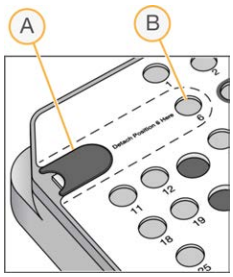
3 Select **Load**.
The software moves the cartridge into position automatically (~30 seconds), the reagent cartridge ID appears on the screen, and the sensors are checked.

4 Select **Next**.

## Remove Used Reservoir from Position #6

1 After you have removed the ***used*** reagent cartridge from the instrument, remove the protective rubber cover over the slot next to position #6.

**Figure 18** Removable Position #6




A Protective rubber cover
B Position #6

2 Press down on the clear plastic tab and push towards the left to eject the reservoir.

3 Dispose of the reservoir in accordance with applicable standards.

## Review Automated Check

The software performs an automated check of the system. During the check, the following indicators appear on the screen:

- Gray checkmark —The check has not been performed yet.
- Progress icon—The check is in progress.
- Green checkmark—The check passed.
- Red —The check did not pass. For any items that do not pass, an action is required before you can proceed. See *Resolve Automatic Check Errors* on page 43.

To stop an automated check that is in progress, select the icon in the lower-right corner. To restart the check, select the icon. The check resumes at the first incomplete or failed check.

To view the results of each individual check within a category, select the icon to expand the category.

**NOTE**

When you perform the first sequencing run with NCS v4.0 or later, it is normal for flow cell registration to take more than 15 minutes during the automated check of the system.

## Start the Run

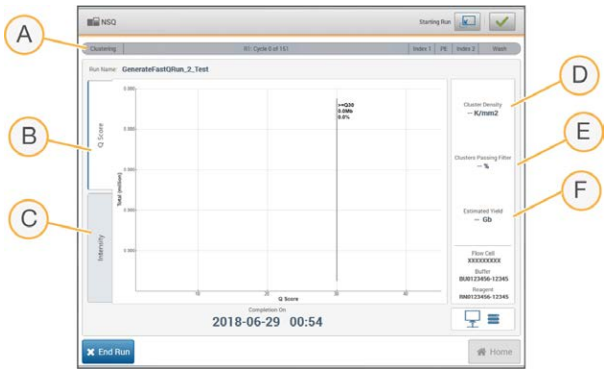
When the automated check is complete, select **Start**. The sequencing run begins.

To configure the system to start the run automatically after a successful check, see *Set Run Setup Options* on page 13.

# Monitor Run Progress

1 Monitor run progress, intensities, and quality scores as metrics appear on the screen.

**Figure 19** Sequencing Run Progress and Metrics




A **Run progress**—Shows the current step and number of cycles completed for each read. The progress bar is not proportional to the run rate of each step. Use the time remaining in the upper-right corner to determine actual duration.
B **Q-score**—Shows the distribution of quality scores (Q-scores). See *Quality Scoring* on page 58.
C **Intensity**—Shows the value of cluster intensities of the 90$^{th}$ percentile for each tile. Plot colors indicate each base: red is A, green is C, blue is G, and black is T. Colors match base indicators used in the Sequencing Analysis Software (SAV).
D **Cluster Density (K/mm²)**—Shows the number of clusters detected for the run.
E **Clusters Passing Filter (%)**—Shows the percentage of clusters passing filter. See *Clusters Passing Filter* on page 58.
F **Estimated Yield (Gb)**—Shows the number of bases projected for the run.

**NOTE**

After you select Home, it is not possible to return to view run metrics. However, run metrics are accessible on BaseSpace Sequence Hub or viewable from a standalone computer using the Sequencing Analysis Viewer (SAV).

## Cycles for Run Metrics

Run metrics appear at different points in a run.

- During the cluster generation steps, no metrics appear.
- The first five cycles are reserved for template generation.
- Run metrics appear after cycle 25, including cluster density, clusters passing filter, yield, and quality scores.

## Data Transfer

Depending on the analysis configuration selected, an icon appears on the screen during the run to indicate the data transfer status.

| Status | Local Run Manager | Output Folder | Illumina BaseSpace Sequence Hub |
|---|---|---|---|
| Connected |  |  |  |
| Connected and transferring data |  |  |  |
| Disconnected |  |  |  |
| Disabled |  |  |  |

If data transfer is interrupted during the run, data are stored temporarily on the instrument computer. When the connection is restored, data transfer resumes automatically. If the connection is not restored before the run ends, manually remove data from the instrument computer before a subsequent run can begin.

### Universal Copy Service

The NextSeq System Software Suite includes a Universal Copy Service. RTA v2 requests the service to copy files from a source location to a destination location and the service processes copy requests in the order received. If an exception occurs, the file is requeued for copy based on the number of files in the copy queue.

## Sequencing Analysis Viewer

The Sequencing Analysis Viewer software shows sequencing metrics generated during the run. Metrics appear in the form of plots, graphs, and tables based on data generated by RTA and written to InterOp files. Metrics are updated as the run progresses. Select **Refresh** at any time during the run to view updated metrics. For more information, see the *Sequencing Analysis Viewer User Guide (part # 15020619)*.

The Sequencing Analysis Viewer is included in the software installed on the instrument computer. You can also install Sequencing Analysis Viewer on another computer linked to the same network as the instrument to monitor run metrics remotely.

## Automatic Post-Run Wash

When the sequencing run is complete, the software initiates an automatic post-run wash. The post-run wash uses wash solution provided in the buffer cartridge and NaOCl provided in the reagent cartridge.

When the sequencing run is complete, the software initiates an automatic post-run wash using the wash solution provided in the buffer cartridge and NaOCl provided in the reagent cartridge.

The automatic post-run wash takes approximately 90 minutes. When the wash is complete, the Home button becomes active. Sequencing results remain visible on the screen during the wash.

## After the Wash

After the wash, the sippers remain in the down position to prevent air from entering the system. Leave the cartridges in place until the next run.

# Scanning



## Introduction

To perform a scan on the NextSeq 550, you need the following run components:

- A hybridized and stained BeadChip
- The reusable BeadChip adapter
- Decode Map (DMAP) files for the BeadChip you are using
- A manifest file for the BeadChip you are using
- A cluster file for the BeadChip you are using

Output files are generated during the scan and then queued for transfer to the specified output folder.

Perform analysis using the BlueFuse Multi software, which requires that scanning data are available in a genotype call (GTC) file format. By default, the NextSeq 550 generates normalized data and associated genotype calls in the format of a GTC file. Optionally, you can configure the instrument to generate additional intensity data (IDAT) files. For more information, see *BeadChip Scan Configuration* on page 53.

### Decode File Client

The DMAP folder contains information that identifies bead locations on the BeadChip and quantifies the signal associated with each bead. A DMAP folder is unique for each BeadChip barcode.

The Decode File Client Utility enables you to download DMAP folders directly from Illumina servers using standard HTTP protocol.

For access to the Decode File Client, go to the Decode File Client support page on the Illumina website (support.illumina.com/array/array_software/decode_file_client/downloads.html). Install the Decode File Client on a computer with access to the network location of the DMAP folder.

For more information, see *Download the DMAP Folder* on page 29.

### Manifest Files and Cluster Files

For each BeadChip, the software requires access to a manifest file and cluster file. Each manifest and cluster file is unique to a BeadChip type. Make sure that you use cluster files that include NS550 in the file name. These files are compatible with the NextSeq system.

- **Manifest file**—Manifest files describe the SNP or probe content on a BeadChip. Manifest files use the *.bpm file format.
- **Cluster files**—Cluster files describe the cluster positions for the Illumina genotyping array and are used when analyzing data to make the genotype call. Cluster files use the *.egt file format.

The location of the files is specified on the BeadChip Scan Configuration screen. From the NCS Home screen, select **Manage Instrument**, **System Configuration**, and then **BeadChip Scan Configuration**.

When the NextSeq 550 instrument is installed, the Illumina representative downloads these files and specifies the path in the control software. There is no need to change these files except in the case of loss or if a new version is available. For more information, see *Replace Manifest Files and Cluster Files* on page 48.

## Download the DMAP Folder

You can access the DMAP folder using the Decode File Client by account or by BeadChip (default view).

### Access DMAP Folder by Account

1 From the main tab of the Decode File Client, select a download option:
- AutoPilot
- All BeadChips not yet downloaded
- All BeadChips
- BeadChips by Purchase Order
- BeadChips by barcode

2 Enter the required information.

3 Locate the DMAP folder that you want to download.

4 Make sure that you have sufficient free space on the download destination.

5 Start the download. View the download status on the Download Status and Log tab.

6 Save the DMAP folder to the specified DMAP folder location.

### Access DMAP Folder by BeadChip

1 Identify BeadChips using two of the following options:
- BeadChip barcode
- BeadChips box ID
- Purchase order number
- Sales order number

2 Locate the DMAP folder that you want to download.

3 Make sure that you have sufficient free space on the download destination.

4 Start the download. View the download status on the Download Status and Log tab.

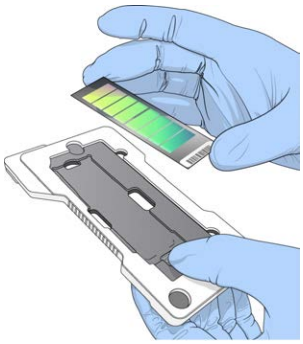
5 Save the DMAP folder to the specified DMAP folder location.

## Load the BeadChip Onto the Adapter

1 Press down on the adapter retention clip. The clip tilts back slightly to open.

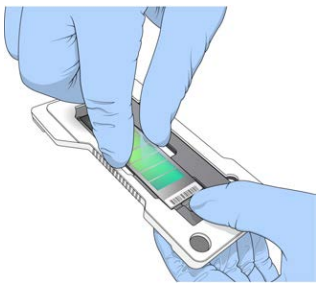
2 Holding the BeadChip by the edges, position the BeadChip with the barcode near the retention clip and place the BeadChip onto the recessed shelf of the adapter.

**Figure 20** Load BeadChip Onto Adapter



3 Using the openings on either side of the BeadChip, make sure that the BeadChip is seated in the recessed shelf of the adapter.

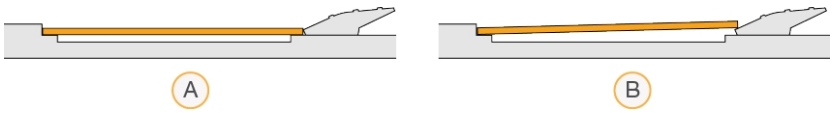
**Figure 21** Seat and Secure BeadChip



4 Gently release the retention clip to secure the BeadChip.

5 Inspect the BeadChip from a side view to make sure that the BeadChip is sitting flat on the adapter. Reposition the BeadChip, if necessary.

**Figure 22** Inspect BeadChip Position




A Correct position—BeadChip is flat on adapter when clip is released.
B Incorrect position—BeadChip is not flat when clip is released.

## Set Up a Scan

1 From the Home screen, select **Experiment**, and then select **Scan**.
The Scan command opens the imaging compartment door, releases consumables from a previous run (if present), and opens the series of scan setup screens. A short delay is normal.

## Unload Sequencing Consumables

If used sequencing consumables are present when you are setting up a scan, the software prompts you to unload the reagent cartridge and buffer cartridge before proceeding to the next step.

1 If prompted, remove used sequencing consumables from a previous sequencing run.

a Remove the reagent cartridge from the reagent compartment. Dispose of unused contents in accordance with applicable standards.
b Remove the used buffer cartridge from the buffer compartment.

**WARNING**

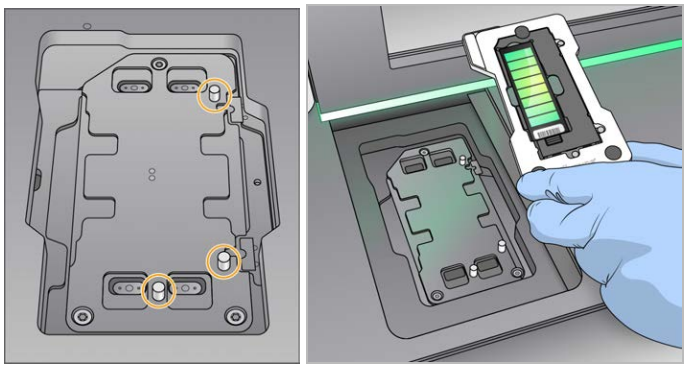
**This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical 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.

2 Close the reagent compartment and buffer compartment doors.

## Load the BeadChip Adapter

1 Use the alignment pins to position the BeadChip adapter on the stage.

**Figure 23** Load the BeadChip Adapter



2 Select **Load**.
The door closes automatically, the BeadChip ID appears on the screen, and the sensors are checked. A short delay is normal. If the BeadChip barcode cannot be read, a dialog box appears that allows you to enter the barcode manually. See *Software Cannot Read the BeadChip Barcode* on page 47.

3 Select **Next**.

## Scan Setup

1 On the Scan Setup screen, confirm the following information:
- **Barcode**—The software reads the BeadChip barcode when the BeadChip is loaded. If the barcode was entered manually, the Edit button appears for further changes.
- **Type**—The BeadChip type field is autopopulated based on the BeadChip barcode.
- **DMAP Location**—The DMAP folder location is specified on the BeadChip Scan Configuration screen. To change the location for the current scan only, select **Browse** and navigate to the correct location.
- **Output Location**—The output location is specified on the BeadChip Scan Configuration screen. To change the location for the current scan only, select **Browse** and navigate to the preferred location.

2 Select **Next**.

## Review Automated Check

The software performs an automated check of the system. During the check, the following indicators appear on the screen:

- Gray checkmark —The check has not been performed yet.
- Progress icon—The check is in progress.
- Green checkmark—The check passed.
- Red —The check did not pass. For any items that do not pass, an action is required before you can proceed. See *Resolve Automatic Check Errors* on page 43.

To stop an automated check that is in progress, select the icon in the lower-right corner. To restart the check, select the icon. The check resumes at the first incomplete or failed check.

To view the results of each individual check within a category, select the icon to expand the category.

**NOTE**

When you perform the first sequencing run with NCS v4.0 or later, it is normal for flow cell registration to take more than 15 minutes during the automated check of the system.

## Start the Scan

When the automated check is complete, select **Start**. The scan begins.

To configure the system to start the scan automatically after a successful check, see *Set Run Setup Options* on page 13.

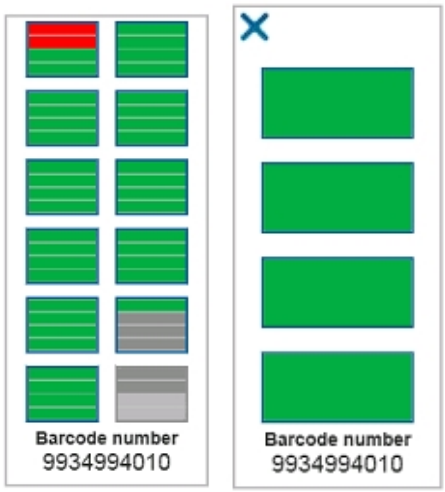
## Monitor Scan Progress

1 Monitor scan progress using the BeadChip image. Each color on the image indicates the scanning status.
   - **Light gray**—Not scanned
   - **Dark gray**—Scanned but not registered.
   - **Green**—Scanned and registered successfully.
   - **Red**—Scan and registration failed.

   If registration fails, you can rescan samples that contain failed sections. See *BeadChip Scan Failure* on page 48.

2 Select the BeadChip image to toggle between a full view and a detail view of a selected sample.
   - The full view shows the samples on the BeadChip and sections within each sample.
   - The detail view shows each section within the selected sample.

**Figure 24** BeadChip Image: Full View and Detail View




**NOTE**

Ending a scan is final. If you end the scan before the scan is complete, scan data are *not* saved.

## Data Transfer

Data are queued for transfer to the scanning output folder when the scan is complete. Data are temporarily written to the instrument computer. The temporary folder is deleted from the instrument computer automatically when a subsequent scan is started.

The time required to transfer data depends on your network connection. Before beginning a subsequent scan, make sure that data have been written to the output folder. To check, make sure that GTC files are present in the barcode folder. For more information, see *Scanning Output Folder Structure* on page 64.

If the connection is interrupted, data transfer resumes automatically when the connection is restored.

# Chapter 5 Maintenance

This section describes the procedures necessary for maintaining a healthy system, including performing a maintenance wash and updating software. Keeping the control software up to date ensures that your system has the latest bug fixes and features installed for optimum performance.

## Introduction

Maintenance procedures include manual instrument washes, replacing the air filter, and system software updates when available.

- Instrument washes—An automatic post-run wash after each sequencing run maintains instrument performance. However, a manual wash is required periodically under certain conditions. See *Perform a Manual Wash* on page 34.
- Software updates—When an updated version of the system software is available, you can install the update automatically using one of the following two methods.
  - Through a connection to BaseSpace Sequence Hub
  - Manually after you download the installer from the Illumina website. See *Software Updates* on page 38.
- Air filter replacement—For instruments with an air filter that is accessible from the rear panel, regular replacement of the air filter ensures proper air flow through the instrument.

## Preventive Maintenance

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

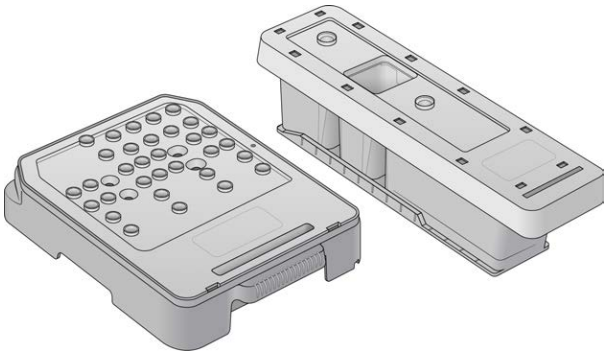
## Perform a Manual Wash

Manual washes are initiated from the Home screen. Wash options include the Quick Wash and the Manual Post-Run Wash.

| Wash Types | Description |
|---|---|
| **Quick Wash**<br>**Duration: 20 minutes** | Flushes the system with a user-supplied wash solution of laboratory-grade water and Tween 20 (buffer wash cartridge).<br>• Required every 14 days that the instrument is idle with reagent cartridge and buffer cartridge in place.<br>• Required every seven days that the instrument is in a dry state (reagent cartridge and buffer cartridge removed).<br>• Required after a shutdown. |
| **Manual Post-Run Wash**<br>**Duration: 90 minutes** | Flushes the system with a user-supplied wash solution of laboratory-grade water and Tween 20 (buffer wash cartridge) and 0.12% sodium hypochlorite (reagent wash cartridge).<br>Required if the automatic post-run wash was not performed. |

A manual wash requires the reagent wash cartridge and buffer wash cartridge provided with the instrument, and a used flow cell. A used flow cell can be used up to 20 times for instrument washes.

**Figure 25** Reagent Wash Cartridge and Buffer Wash Cartridge



## Prepare for a Manual Post-Run Wash

| User-Supplied Consumables | Volume and Description |
|---|---|
| • NaOCl | 1 ml, diluted to 0.12%<br>Loaded onto the reagent wash cartridge (position #28) |
| • 100% Tween 20<br>• Laboratory-grade water | Used to make 125 ml 0.05% Tween 20 wash solution<br>Loaded onto the buffer wash cartridge (center reservoir) |

**NOTE**

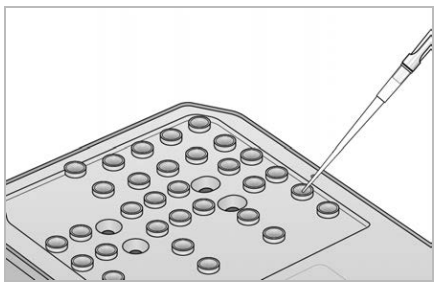
Always use a fresh dilution of NaOCl prepared within the last **24 hours**. If you make a volume larger than 1 ml, store the remaining dilution at 2°C to 8°C for use within the next 24 hours. Otherwise, discard the remaining dilution of NaOCl.

1 Combine the following volumes in a microcentrifuge tube to result in 1 ml 0.12% NaOCl:
   - 5% NaOCl (24 µl)
   - Laboratory-grade water (976 µl)

2 Invert the tube to mix.

3 Add 1 ml 0.12% NaOCl to the reagent wash cartridge. The correct reservoir is equivalent to position #**28** on the prefilled cartridge.

**Figure 26** Load NaOCl



4 Combine the following volumes to result in a 0.05% Tween 20 wash solution:
   - 100% Tween 20 (62 µl)
   - Laboratory-grade water (125 ml)

5 Add 125 ml wash solution to the center reservoir of the buffer wash cartridge.

6 Select **Perform Wash**, and then select **Manual Post-Run Wash**.

## Prepare for a Quick Wash

| User-Supplied Consumables | Volume and Description |
|---|---|
| • 100% Tween 20<br>• Laboratory-grade water | Used to make 40 ml 0.05% Tween 20 wash solution<br>Loaded onto buffer wash cartridge (center reservoir) |

1 Combine the following volumes to result in a 0.05% Tween 20 wash solution:
   - 100% Tween 20 (20 µl)
   - Laboratory-grade water (40 ml)
2 Add 40 ml wash solution to the center reservoir of the buffer wash cartridge.
3 Select **Perform Wash**, and then select **Quick Wash**.

## Load a Used Flow Cell and the Wash Cartridges

1 If a used flow cell is not present, load a used flow cell. Select **Load**, and then select **Next**.
2 Remove the spent reagents container and discard the contents in accordance with applicable standards.

**WARNING**

**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.

3 Slide the empty spent reagents container into the buffer compartment until it stops.
4 Remove the used buffer cartridge from the previous run, if present.
5 Load the buffer wash cartridge containing wash solution.
6 Remove the used reagent cartridge from the previous run, if present.
7 Load the reagent wash cartridge.
8 Select **Next**. The prewash check begins automatically.

## Start the Wash

1 Select **Start**.
2 When the wash is complete, select **Home**.

## After the Wash

After the wash, the sippers remain in the down position to prevent air from entering the system. Leave the cartridges in place until the next run.

## Replace Air Filter

For instruments with an air filter that is accessible from the rear, the air filter ensures air flow through the instrument. The software displays a notification to change the air filter every 90 days. When prompted, select **Remind in 1 day**, or follow the following procedure and select **Filter Changed**. The 90-day countdown resets following **Filter Changed** selection.

1 Remove the new air filter from the package and write the date you install it on the frame of the filter.

2 On the back of the instrument, press down on the top of the filter tray to release the tray.

3 Grasp the top of the filter tray and pull up to lift the tray completely out of the instrument.

4 Remove and discard the old air filter.

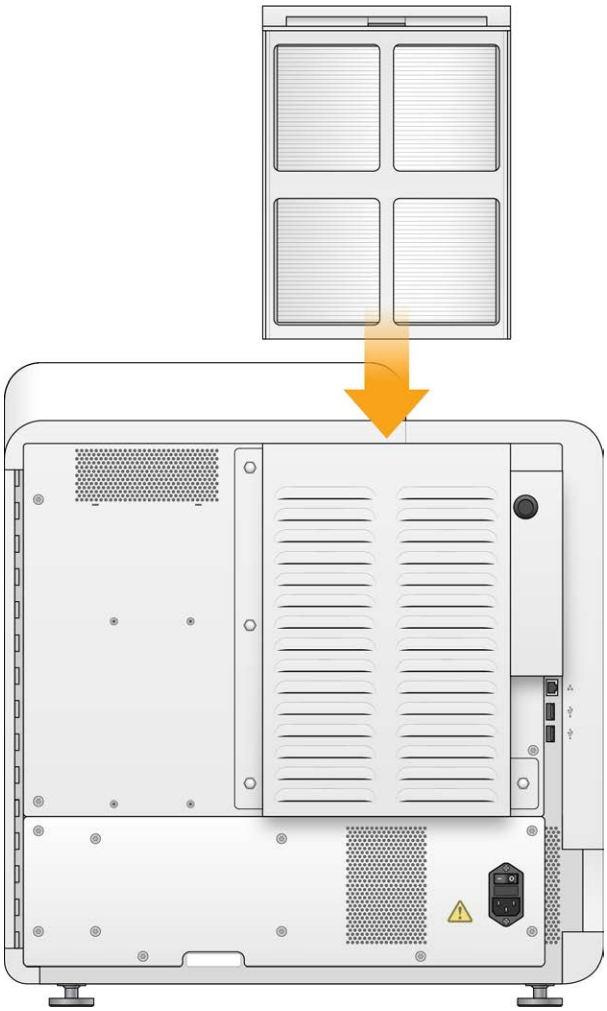
5 Insert the new air filter into the tray.

**NOTE**

The air filter does not work correctly if it is backwards. Make sure to insert the air filter into the tray so that you can see the green Up arrow, and cannot see the warning label. The arrow should point towards the handle of the filter tray.

6 Slide the filter tray into the instrument. Push down on the top of the filter tray until it clicks into place.

**Figure 27** Air filter insertion



## Software Updates

Software updates are packaged in a software bundle called the System Suite, which includes the following software:

- NextSeq Control Software (NCS)
- NextSeq recipes
- Local Run Manager software
- RTA2
- NextSeq Service Software (NSS)
- Universal Copy Service
- Direct Memory Access (DMA) driver

You can install software updates automatically using an internet connection or manually from a network or USB location.

- **Automatic updates**—For instruments connected to a network with internet access, an alert icon appears on the Manage Instrument button on the Home screen when an update is available.
- **Manual updates**—Download the System Suite installer from the NextSeq 550 support page on the Illumina website. If you plan to make a manual update, make sure to complete the update before preparing samples and consumables for a sequencing run.

## Automatic Software Update

1. Select **Manage Instrument**.
2. Select **Software Update**.
3. Select **Install the update already downloaded from BaseSpace**.
4. Select **Update** to begin the update. A dialog box opens to confirm the command.
5. Follow the prompts in the installation wizard:
   a. Accept the licensing agreement.
   b. Review the release notes.
   c. Review the list of software included in the update.

   When the update is complete, the control software restarts automatically.

**NOTE**
If a firmware update is included, an automatic restart of the system is required after the firmware is updated.

## Manual Software Update

1. Download the System Suite installer from the Illumina website and save it to a network location. Alternatively, copy the software installation file to a portable USB drive.
2. Select **Manage Instrument**.
3. Select **Software Update**.
4. Select **Manually install the update from the following location**.
5. Select **Browse** to navigate to the location of the software installation file, and then select **Update**.
6. Follow the prompts in the installation wizard:
   a. Accept the licensing agreement.
   b. Review the release notes.
   c. Review the list of software included in the update.

   When the update is complete, the control software restarts automatically.

**NOTE**
If a firmware update is included, an automatic restart of the system is required after the firmware is updated.

## Shut Down the Instrument

1 Select **Manage Instrument**.

**NOTE**

To shut down the NextSeq 550Dx instrument in research mode, see *NextSeq 550Dx Reboot and Shut Down Options* on page 67.

2 Select **Shutdown Options**.

3 Select **Shutdown**.
The Shut Down command safely shuts down the software and turns off instrument power. Wait at least 60 seconds before turning on the instrument again.

**CAUTION**

*Do not* relocate the instrument. Moving the instrument improperly can affect the optical alignment and compromise data integrity. If you have to relocate the instrument, contact your Illumina representative.

# Appendix A Troubleshooting



## Introduction

For technical questions, visit the NextSeq 550 support pages on the Illumina website. The support pages provide access to documentation, downloads, and frequently asked questions.

Log in to your MyIllumina account for access to support bulletins.

For run quality or performance problems, contact Illumina Technical Support. See *Technical Assistance* on page 73.

Consider sharing a link to the run summary in BaseSpace Sequence Hub with Illumina Technical Support to facilitate troubleshooting. You can also assist troubleshooting when the Illumina Proactive monitoring service is active. For more information on the service, see *Set Send Instrument Performance Data Option* on page 13.

## Troubleshooting Files

An Illumina Technical Support representative might request copies of run-specific or scan-specific files to troubleshoot issues. Typically, the following files are used for troubleshooting.

### Troubleshooting Files for Sequencing Runs

| Key File | Subfolder | Description |
|---|---|---|
| Run information file (RunInfo.xml) | <Run folder name> | Contains the following information:<br>• Run name<br>• Number of cycles in the run<br>• Number of cycles in each read<br>• Whether the read is an indexed read<br>• Number of swaths and tiles on the flow cell |
| Run parameters file (RunParameters.xml) | <Run folder name> | Contains information about run parameters and run components. Information includes the RFID, serial number, part number, and expiration date. |
| RTA configuration file (RTAConfiguration.xml) | Data\Intensities | Contains the RTA configuration settings for the run.<br>The RTAConfiguration.xml file is created at the beginning of the run. |

| Key File | Subfolder | Description |
|---|---|---|
| InterOp files (*.bin) | InterOp | Binary reporting files used for Sequencing Analysis Viewer. InterOp files are updated throughout the run. |
| Log files | Logs | Log files describe each step performed by the instrument for each cycle, and list software and firmware versions used with the run. The file named [InstrumentName]_ CurrentHardware.csv lists the serial numbers of instrument components. |
| Error log files (*ErrorLog*.txt) | RTA Logs | Log of RTA errors. Error log files are updated whenever an error occurs. |
| Global log files (*GlobalLog*.tsv) | RTA Logs | Log of all RTA events. Global log files are updated throughout the run. |
| Lane log files (*LaneLog*.txt) | RTA Logs | Log RTA processing events. Lane log files are updated throughout the run. |

### RTA Errors

To troubleshoot RTA errors, first check the RTA error log, which is stored in the RTALogs folder. This file is not present for successful runs. Files are located in run-specific folders of the output folder. Include the error log when reporting issues to Illumina Technical Support.

## Troubleshooting Files for Array Scans

| Key File | Subfolder | Description |
|---|---|---|
| Scan parameters file (ScanParameters.xml) | <Run folder name> | Contains information about scan parameters. Information includes scan date, BeadChip barcode, cluster file location, and manifest file location. |
| Log files | Logs | Log files describe each step performed on the instrument during the scan. |

| Key File | Subfolder | Description |
|---|---|---|
| Metrics files | [Barcode] | Metrics are provided as sample metrics and as section metrics.<br>**[barcode]_sample_metrics.csv**—For each sample and channel (red and green), lists Percent Off Image, Percent Outliers, P05, P50, P95, Avg FWHM Avg, FWHM Stddev, and Min Registration Score.<br>**[barcode]_section_metrics.csv**—For each section and tile, lists Laser Z-position, Through Focus Z-position, Red FWHM, Green FWHM, Red Avg Pixel Intensity, Green Avg Pixel Intensity, Red Registration Score, and Green Registration Score. |
| Rescan file | [Barcode] | **[barcode]_rescan.flowcell**—Lists the tile locations adjusted for a rescan, which include an increased tile-to-tile overlap. |

## Resolve Automatic Check Errors

If errors occur during the automatic check, use the following recommended actions to resolve the error. Automatic checks differ for sequencing and array scans.

If a pre-run check fails, the reagent cartridge RFID is not locked and can be used for a subsequent run. However, the RFID is locked after the foil seals have been pierced.

| System Checks | Recommended Action |
|---|---|
| Doors Closed | Make sure that the compartment doors are closed. |
| Consumables Loaded | Consumable sensors do not register. Make sure that each consumable is properly loaded.<br>On the run setup screens, select **Back** to return to the loading step, and repeat run setup. |
| Required Software | Critical components of the software are missing.<br>Perform a manual software update to restore all software components. |
| Instrument Disk Space | The instrument hard drive does not have sufficient disk space to perform a run. It is possible that data from a previous run did not transfer.<br>Clear run data from the instrument hard drive. |
| Network Connection | The network connection has been interrupted. Check network status and the physical network connection. |
| Network Disk Space | Either the BaseSpace account is full or the network server is full. |

| Temperature | Recommended Action |
|---|---|
| Temperature | Contact Illumina Technical Support. |
| Temperature Sensors | Contact Illumina Technical Support. |
| Fans | Contact Illumina Technical Support. |

| Imaging System | Recommended Action |
|---|---|
| Imaging Limits | Contact Illumina Technical Support. |
| Z Steps-and-Settle | Contact Illumina Technical Support. |
| Bit Error Rate | Contact Illumina Technical Support. |
| Flow Cell Registration | It is possible that the flow cell is not properly seated.<br>• On the run setup screens, select **Back** to return to the flow cell step. The imaging compartment door opens.<br>• Unload and reload the flow cell to make sure that it is seated properly. |

| Reagent Delivery | Recommended Action |
|---|---|
| Valve Response | Contact Illumina Technical Support. |
| Pump | Contact Illumina Technical Support. |
| Buffer Mechanism | Contact Illumina Technical Support. |
| Spent Reagents Empty | Empty the spent reagents container and reload the empty container. |

## Checks for Sequencing Runs

If a pre-run check fails, the reagent cartridge RFID is not locked and can be used for a subsequent run. However, the RFID is locked after the foil seals have been pierced.

| System Checks | Recommended Action |
|---|---|
| Doors Closed | Make sure that the compartment doors are closed. |
| Consumables Loaded | Consumable sensors do not register. Make sure that each consumable is properly loaded.<br>On the run setup screens, select **Back** to return to the loading step, and repeat run setup. |
| Required Software | Critical components of the software are missing.<br>Perform a manual software update to restore all software components. |
| Instrument Disk Space | The instrument hard drive does not have sufficient disk space to perform a run. It is possible that data from a previous run did not transfer.<br>Clear run data from the instrument hard drive. |
| Network Connection | The network connection has been interrupted. Check network status and the physical network connection. |
| Network Disk Space | Either the BaseSpace account is full or the network server is full. |

| Temperature | Recommended Action |
|---|---|
| Temperature | Contact Illumina Technical Support. |
| Temperature Sensors | Contact Illumina Technical Support. |
| Fans | Contact Illumina Technical Support. |

| Imaging System | Recommended Action |
|---|---|
| Imaging Limits | Contact Illumina Technical Support. |
| Z Steps-and-Settle | Contact Illumina Technical Support. |
| Bit Error Rate | Contact Illumina Technical Support. |
| Flow Cell Registration | It is possible that the flow cell is not properly seated.<br>• On the run setup screens, select **Back** to return to the flow cell step. The imaging compartment door opens.<br>• Unload and reload the flow cell to make sure that it is seated properly. |

| Reagent Delivery | Recommended Action |
|---|---|
| Valve Response | Contact Illumina Technical Support. |
| Pump | Contact Illumina Technical Support. |
| Buffer Mechanism | Contact Illumina Technical Support. |
| Spent Reagents Empty | Empty the spent reagents container and reload the empty container. |

## Checks for Array Scans

| System Checks | Recommended Action |
|---|---|
| Doors Closed | Make sure that the compartment doors are closed. |
| Consumables Loaded | Consumable sensors do not register. Make sure that each consumable is properly loaded.<br>On the run setup screens, select **Back** to return to the loading step, and repeat run setup. |
| Required Software | Critical components of the software are missing.<br>Perform a manual software update to restore all software components. |
| Verify Input Files | Make sure that the path to the cluster file and manifest file is correct and the files are present. |
| Instrument Disk Space | The instrument hard drive does not have sufficient disk space to perform a run. It is possible that data from a previous run did not transfer.<br>Clear run data from the instrument hard drive. |
| Network Connection | The network connection has been interrupted. Check network status and the physical network connection. |
| Network Disk Space | Either the BaseSpace account is full or the network server is full. |

| Imaging System | Recommended Action |
|---|---|
| Imaging Limits | Contact Illumina Technical Support. |
| Z Steps-and-Settle | Contact Illumina Technical Support. |
| Bit Error Rate | Contact Illumina Technical Support. |
| Auto-Center | Unload the BeadChip adapter. Make sure that the BeadChip is seated in the adapter, and then reload the adapter. |

## Spent Reagents Container is Full

Always begin a run with an empty spent reagents container.

If you begin a run without emptying the spent reagents container, system sensors trigger the software to pause the run when the container is full. System sensors cannot pause a run during clustering, paired-end resynthesis, or the automatic post-run wash.

When the run pauses, a dialog box opens with options to raise the sippers and empty the full container.

## Empty Spent Reagents Container

1 Select **Raise Sippers**.

2 Remove the spent reagents container and discard the contents appropriately.

3 Return the empty container to the buffer compartment.

4 Select **Continue**. The run resumes automatically.

## Rehybridization Workflow

A rehybridization run might be necessary if metrics generated during the first few cycles show intensities below 2500. Some low-diversity libraries can show intensities below 1000, which is expected and cannot be resolved with rehybridization.

**NOTE**

The End Run command is final. The run cannot be resumed, run consumables cannot be reused, and sequencing data from the run are not saved.

When you end a run, the software performs the following steps before the run ends:

- Places the flow cell in a safe state.
- Unlocks the flow cell RFID for a later run.
- Assigns a rehybridization expiration date to the flow cell.
- Writes the run logs for completed cycles. A delay is normal.
- Bypasses the automatic post-run wash.

When you start a rehybridization run, the software performs the following steps to perform the run:

- Creates a run folder based on a unique run name.
- Checks that the flow cell rehybridization date has not expired.
- Primes reagents. A delay is normal.
- Skips the clustering step.
- Removes the previous Read 1 primer.
- Hybridizes a fresh Read 1 primer.
- Continues through Read 1 and the remainder of the run based on specified run parameters.

### Points to End a Run for Rehybridization

Later rehybridization is possible only if you end the run at the following points:

- **After cycle 5**—Intensities appear after template registration, which requires the first five cycles of sequencing. Although it is safe to end a run after cycle 1, ending after cycle 5 is recommended. Do not end a run during cluster generation.
- **Read 1 or Index 1 Read**—End the run ***before*** paired-end resynthesis begins. The flow cell cannot be saved for later rehybridization after paired-end resynthesis begins.

### Required Consumables

A rehybridization run requires a new NextSeq reagent cartridge and buffer cartridge regardless of when the run was stopped.

### End the Current Run

1 Select **End Run**. When prompted to confirm the command, select **Yes**.

2 When prompted to save the flow cell, select **Yes**. Saving the flow cell does not ensure that the current run is salvageable. Note the expiration date for rehybridization.

3 Remove the saved flow cell and set aside at 2°C to 8°C until you are ready to set up the rehybridization run.

**NOTE**

You can store the flow cell up to seven days at 2°C to 8°C in the plastic clamshell case ***without*** the desiccant package. For best results, rehybridize the saved flow cell within three days.

## Perform a Manual Wash

1 From the Home screen, select **Perform Wash**.

2 From the Wash Selection screen, select **Manual Post-Run Wash**. See *Perform a Manual Wash* on page 34.

**NOTE**

If you have not removed the reagent cartridge and buffer cartridge from the stopped run, you can use them for the manual wash. Otherwise, perform the manual wash with the reagent wash cartridge and buffer wash cartridge.

## Set Up a Run on the Instrument

1 Prepare a new reagent cartridge.

2 If the saved flow cell was stored, allow it to reach room temperature (15–30 minutes).

3 Clean and load the saved flow cell.

4 Remove the spent reagents container and discard the contents appropriately, and then reload the empty container.

5 On the Run Setup screen, select from one of the following run modes.
- Local Run Manager
- Manual

6 **[Optional]** Select **Use BaseSpace Sequence Hub Setting** and select from one of the following.
- Run Monitoring and Storage
- Run Monitoring Only

Enter your BaseSpace Sequence Hub user name and password.

7 Load the new buffer cartridge and reagent cartridge.

8 Select **Next** to proceed to the pre-run check and start the run.

# BeadChip and Scan Errors

## Software Cannot Read the BeadChip Barcode

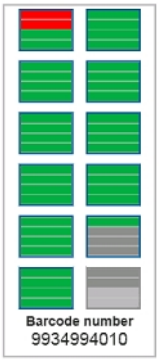
When the barcode error dialog box appears, select from the following options:
- Select **Rescan**. The software attempts to read the barcode again.
- Select text field and enter the numeric barcode as shown in the image. Depending on the BeadChip, barcode numbers have up to 12 digits. Select **Save**. The barcode image is stored in the output folder.
- Select **Cancel**. The imaging compartment door opens to unload the BeadChip adapter.

## BeadChip Scan Failure

Images are registered after they are scanned. Registration identifies beads by correlating locations on the scanned image with information provided in the bead map, or DMAP folder.

Sections that fail registration are indicated in red on the BeadChip image.

**Figure 28** BeadChip Showing Failed Sections




After the scan is complete and scanning data are written to the output folder, the Rescan button becomes active.

When Rescan is selected, the software performs the following steps:

- Rescans samples that contain failed sections using an increased tile-to-tile overlap.
- Generates output files in the original output folder.
- Overwrites previous output files for failed sections.
- Increments the scan counter by one for each rescan, but does so in the background. The software does not rename the output folder.

### Rescan or Start New Scan

1. Select **Rescan** to scan samples that contain failed sections.
2. If the scan continues to fail, end the scan.
3. Remove the BeadChip and adapter and inspect the BeadChip for dust or debris. Use canned air or other compressed dusting method to clear the debris.
4. Reload the BeadChip and start a new scan.
   When a new scan is started, the software performs the following steps:
   - Scans the entire BeadChip.
   - Generates output files in a new output folder.
   - Increments the scan counter by one based on the scan count of the last rescan.

## Replace Manifest Files and Cluster Files

1. Go to the Illumina support page (support.illumina.com) for the BeadChip that you are using, and click the **Downloads** tab.
2. Download the files to be replaced or updated, and copy the files to your preferred network location.

**NOTE**

Make sure that you select manifest and cluster files that are compatible with the NextSeq 550 system. Compatible files include **NS550** in the file name.

3 Only if the location has changed, update the location on the BeadChip Scan Configuration screen, as follows:

   a From the NCS Home screen, select **Manage Instrument**.
   b Select **System Configuration**.
   c Select **BeadChip Scan Configuration**.

4 Select **Browse** and navigate to the location of the replaced or updated files.

## Custom Recipes and Recipe Folders

Do not modify original recipes. Always make a copy of the original recipe with a new name. If an original recipe is modified, the software updater can no longer recognize the recipe for later updates, and newer versions are not installed.

Store custom recipes in the appropriate recipe folder. Recipe folders are organized as follows.

- **Custom**
  - **High**—Customized recipes used with a high-output kit.
  - **Mid**—Customized recipes used with a mid-output kit.
- **High**—Original recipes used with a high-output kit.
- **Mid**—Original recipes used with a mid-output kit.
- **Wash**—Contains the manual wash recipe.

## System Check

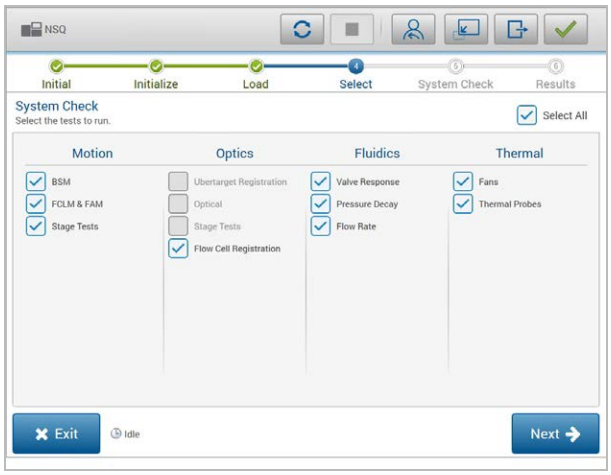
A system check is not required for normal operation or instrument maintenance. However, an Illumina Technical Support representative might ask you to perform a system check for troubleshooting purposes.

**NOTE**

If an instrument wash is due, perform the wash before starting a system check.

Starting a system check automatically closes the control software and launches the NextSeq Service Software (NSS). The service software launches and opens to the Load screen, which is configured to use the advanced loading option.

**Figure 29** Available System Checks




Inactive checkboxes on the Select screen indicate tests that require assistance from an Illumina field representative.

## Perform a System Check

1 From the Manage Instrument screen, select **System Check**. When prompted to close the control software, select **Yes**.

2 Load the consumables as follows:

   a If a used flow cell is not already on the instrument, load a used flow cell.

   **NOTE**
   Illumina recommends using a high output flow cell for system check purposes.

   b Empty the spent reagents container and return it to the instrument.
   c Load the buffer wash cartridge containing 120 ml laboratory-grade water in the center reservoir.
   d Load the reagent wash cartridge. Make sure that the reagent wash cartridge is empty and clean.

3 Select **Load**. The software moves the flow cell and reagent wash cartridge into position. Select **Next**.

4 Select **Next**. The system check begins.

5 **[Optional]** When the system check is complete, select **View** next to the check name to view the values associated with each check.

6 Select **Next**. The system check report opens.

7 Select **Save** to save the report to a zipped file. Navigate to a network location to save the file.

8 When finished, select **Exit**.

9 When prompted to close the service software and restart the control software, select **Yes**. The control software restarts automatically.

## Motion Checks

| System Check | Description |
|---|---|
| BSM | Checks the gain and distance of the Buffer Straw Mechanism (BSM) to confirm that the module is working properly. |
| FCLM & FAM | Checks the gain and distance of the Flow Cell Load Mechanism (FCLM) and Fluid Automation Module (FAM) to confirm that the modules are working properly. |
| Stage Tests | Checks the travel limits and performance of the XY-stage and 6 Z-stages, one for each camera. |

## Optics Check

| System Check | Description |
|---|---|
| Flow Cell Registration | Measures flow cell tilt on an optical plane, tests camera functionality, tests the imaging module, and verifies registration of the flow cell in the correct imaging position. |

## Fluidics Checks

| System Check | Description |
|---|---|
| Valve Response | Checks the accuracy of the valve and pump movements, and tests the pump syringe range of movement. |
| Pressure Decay | Checks the leak rate of a sealed fluidics system, which confirms that the flow cell is properly mounted in the sequencing position. |
| Flow Rate | Checks the functionality of the bubble sensors, which are used to detect the presence of air in the reagent lines. Measures the flow rates to check for occlusions or leaks. |

## Thermal Checks

| System Check | Description |
|---|---|
| Fans | Checks the speed of system fans in pulse per minute (PPM) to confirm that fans are functioning. Fans that are not functioning return a negative value. |
| Thermal Probes | Checks the average temperature of each thermal sensor. Thermal sensors that are not functioning return a negative value. |

# RAID Error Message

The NextSeq computer is equipped with two hard drives. If a hard drive begins to fail, the system generates a RAID error message and suggests that you contact Illumina Technical Support. Usually, a hard drive replacement is required.

You can proceed with the run setup steps and normal operation. The purpose of the message is for scheduling service in advance to avoid interruptions in normal instrument operation. To proceed, select **Acknowledge** and then **Close**.

# Network Storage Error

Network storage errors are the result of one of the following reasons:

- **Insufficient storage space for the output folder**— Increase the amount of space on the storage device or move the output folder to a location with enough storage.

- **Cannot connect to network storage**— Check the path to the output folder. See *Set Output Folder Location* on page 53.
- **The system cannot write to network storage**— Consult your IT administrator to check permissions. The Windows account on the operating system of the instrument requires permission to read and write to the output folder.

The Windows account in Local Run Manager also requires permission to read and write to the output folder. See Specify Service Account Settings in *Local Run Manager Software Guide (document # 1000000002702)*.

## Configure System Settings

The system is configured during installation. However, if a change is required or if the system has to be reconfigured, use the system configuration options.

- **Network Configuration**—Provides options for IP address settings, domain name server (DNS) address, computer name, and domain name.
- **BaseSpace Sequence Hub**—If BaseSpace Sequence Hub is in use, provides location options where data are transferred for storage and analysis.
- **Output Folder Location**—Provides path options to the output folder.
- **BeadChip Scan Configuration**—Provides options to specify the following.
  - Default DMAP folder location
  - Output folder location
  - File format of saved images
  - Output file type

### Set Network Configuration

1 From the Manage Instrument screen, select **System Configuration**.

2 Select **Network Configuration**.

3 Select **Obtain an IP address automatically** to obtain the IP address using DHCP server.

**NOTE**

Dynamic Host Configuration Protocol (DHCP) is a standard network protocol used on IP networks for dynamically distributing network configuration parameters.

Alternatively, select **Use the following IP address** to connect the instrument to another server manually as follows. Contact your network administrator for the addresses specific to your facility.

- Enter IP address. The IP address is a series of four numbers separated by a dot. For example, 168.62.20.37.
- Enter the subnet mask, which is a subdivision of the IP network.
- Enter the default gateway, which is the router on the network that connects to the internet.

4 Select **Obtain a DNS server address automatically** to connect the instrument to the domain name server associated with IP address.

Alternatively, select **Use the following DNS server addresses** to connect the instrument to the domain name server manually as follows.

- Enter the preferred DNS address. The DNS address is the server name used to translate domain names into IP addresses.
- Enter the alternate DNS address. The alternate is used if the preferred DNS cannot translate a particular domain name to an IP address.

5 Select **Save** to advance to the Computer screen.

**NOTE**

The instrument computer name is assigned to the instrument computer at the time of manufacture. Any changes to the computer name can affect connectivity and require a network administrator.

6 Connect the instrument computer to a domain or a workgroup as follows.
- **For instruments connected to the internet**—Select **Member of domain**, and then enter domain name associated with the internet connection at your facility. Domain changes require an administrator user name and password.
- **For instruments not connected to the internet**—Select **Member of work group**, and then enter a work group name. The work group name is unique to your facility.

7 Select **Save**.

## Set BaseSpace Sequence Hub Configuration

1 From the Home screen, select **Manage Instrument**.

2 Select **System Configuration**.

3 Select **BaseSpace Sequence Hub Configuration**.

4 Select from the following options to specify a location where data are transferred for analysis.
- From the Hosting Location list, select the location of the server where data are uploaded to.
- If you have an Enterprise subscription, select the **Private Domain** checkbox and enter the domain name (URL) used for single sign-on to BaseSpace Sequence Hub.

For example: https://**yourlab**.basespace.illumina.com.

5 Select **Save**.

## Set Output Folder Location

The NextSeq requires an output folder for all runs. Use the full Universal Naming Convention (UNC) path to the output folder. The UNC path includes two backslashes, the server name and directory name, but ***not*** a letter for a mapped network drive.

- Paths to the output folder that are one level require a trailing backslash.
  - Example UNC path: \\servername\directory1\
- Paths to the output folder that are two or more levels do not require a trailing backslash.
  - Example UNC path: \\servername\directory1\directory2
- Paths to a mapped network drive cause errors. ***Do not use.***
  - Example of a mapped network drive path: T:\sbsfiles

For Local Run Manager run mode, set the output folder location in the Local Run Manager software. For more information, see the *Local Run Manager Software Guide (document # 1000000002702)*.

## BeadChip Scan Configuration

1 From the Manage Instrument screen, select **System Configuration**.

2 Select **BeadChip Scan Configuration**.

3 To specify a default DMAP folder location, select **Browse** and navigate to the preferred folder location on your facility network.

**NOTE**

Before each scan, download and copy the DMAP content to this location. DMAP content is required for each BeadChip and is unique to each BeadChip barcode.

4 To specify a default output location, select **Browse** and navigate to the preferred location on your facility network.

5 Select an image file format for saved images. The default image type is **JPG**.

6 Select an output file format for scan data. The default output file type is **GTC only**.

7 Select **Save**.

8 From the Scan Map screen, specify the full path to the manifest file and cluster file for each BeadChip type. Select **Browse** for each file type and navigate to the folder location that contains these files.

# Appendix B Real-Time Analysis



## Real-Time Analysis Overview

The NextSeq 550 uses an implementation of Real-Time Analysis (RTA) software called RTA2. RTA2 runs on the instrument computer and extracts intensities from images, performs base calling, and assigns a quality score to the base call. RTA2 and the control software communicate through a web HTTP interface and shared memory files. If RTA2 is terminated, processing does not resume and run data are not saved.

**NOTE**

Demultiplex performance is not calculated. Therefore, the Index tab in Sequencing Analysis Viewer (SAV) is not populated.

### RTA2 Inputs

RTA2 requires the following input for processing:

- Tile images contained in local system memory.
- RunInfo.xml, which is generated automatically at the beginning of the run. The file provides the following information.
  - Run name
  - Number of cycles
  - Whether a read is indexed
  - Number of tiles on the flow cell
- RTA.exe.config, which is a software configuration file in XML format.

RTA2 receives commands from the control software about the location of RunInfo.xml and whether an optional output folder is specified.

### RTA v2 Output Files

Images for each channel are passed in memory as tiles. Tiles are small imaging areas on the flow cell defined as the field of view by the camera. From these images, the software produces output as a set of quality-scored base call files and filter files. All other files are supporting output files.

| File Type | Description |
|---|---|
| Base call files | Each tile that is analyzed is included in an aggregated base call (*.bcl) file for each lane and for each cycle. The aggregated base call file contains the base call and associated quality score for every cluster in that lane. |
| Filter files | Each tile produces filter information that is aggregated into one filter (*.filter) file for each lane. The filter file 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 lane during template generation. |
| Base call index files | A base call index (*.bci) file is produced for each lane to preserve the original tile information. The index file contains a pair of values for each tile, which are tile number and number of clusters for that tile. |

Output files are used for downstream analysis in BaseSpace. Alternatively, use bcl2fastq conversion software for FASTQ conversion and third-party analysis solutions. NextSeq files require bcl2fastq v2.0, or later. For the latest version of bcl2fastq, visit the NextSeq downloads page on the Illumina website.

RTA v2 provides real-time metrics of run quality stored as InterOp files. InterOp files are a binary output containing tile, cycle, and read-level metrics, and are required to view real-time metrics using the Sequencing Analysis Viewer (SAV). For the latest version of SAV, visit the SAV downloads page on the Illumina website.

## Error Handling

RTA2 creates log files and writes them to the RTALogs folder. Errors are recorded in an error file in *.tsv file format.

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

- *GlobalLog*.tsv summarizes important run events.
- *LaneNLog*.tsv lists processing events for each lane.
- *Error*.tsv lists errors that occurred during a run.
- *WarningLog*.tsv lists warnings that occurred during a run.

## Real-Time Analysis Workflow

| | |
|---|---|
| Template generation | Maps cluster locations. |
| ↓ | |
| Registration and intensity extraction | Records the location of each cluster on the flow cell and 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. |

## Template Generation

The first step in the RTA workflow is template generation, which defines the position of each cluster in a tile using X and Y coordinates.

Template generation requires image data from the first five cycles of the run. After the last template cycle for a tile is imaged, the template is generated.

**NOTE**

To detect a cluster during template generation, there must be at least one base other than G in the first **five** cycles. For any index sequences, RTA v2 requires at least one base other than G in the first **two** cycles.

The template is used as a reference for the subsequent step of registration and intensity extraction. Cluster positions for the entire flow cell are written to cluster location (*.locs) files, one file for each lane.

## Registration and Intensity Extraction

Registration and intensity extraction begin after template generation.

- Registration aligns images produced over every subsequent cycle of imaging against the template.
- Intensity extraction determines an intensity value for each cluster in the template for a given image.

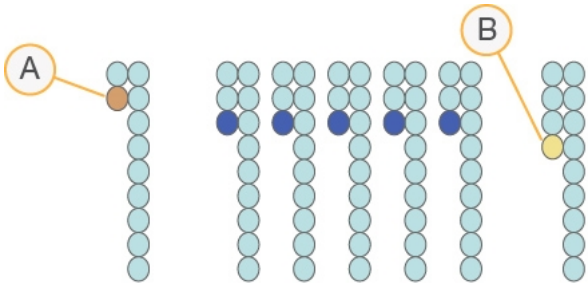
If registration fails for any images in a cycle, no base calls are generated for that tile in that cycle. Use the Sequencing Analysis Viewer (SAV) software to examine thumbnail images and identify images that failed registration.

## 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 30** Phasing and Prephasing




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

RTA2 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 550 uses two-channel sequencing, which requires only two images to encode the data for four DNA bases, one from the red channel and one from the green channel.

Intensities extracted from an image compared to another image result in four distinct populations, each corresponding to a nucleotide. The base calling process determines to which population each cluster belongs.

**Figure 31** Visualization of Cluster Intensities

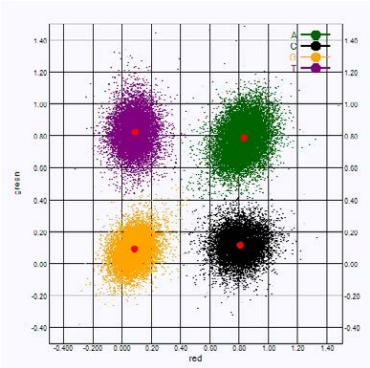


**Table 1 Base Calls in Two-channel Sequencing**

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

## Clusters Passing Filter

During the run, RTA2 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, RTA2 uses a population-based system to determine the chastity of a base call. Clusters pass filter (PF) when no more than one base call in the first 25 cycles has a chastity of < 0.63. Clusters that do not pass filter are not base called.

## Indexing Considerations

The process for base calling index reads differs from base calling during other reads.

Index reads must begin with at least one base other than G in either of the first two cycles. If an Index Read begins with two base calls of G, no signal intensity is generated. Signal must be present in either of the first two cycles to ensure demultiplexing performance.

To increase demultiplexing robustness, select index sequences that provide signal in at least one channel, preferably both channels, for every cycle. Following this guideline avoids index combinations that result in only G bases at any cycle.

- Red channel—A or C
- Green channel—A or T

This base calling process ensures accuracy when analyzing low-plex samples.

# Quality Scoring

A quality score, or 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.

The Q-score is a compact way to communicate small error probabilities. Q(X) represents quality scores, where X is the score. The following table shows the relationship between the quality score and error probability.

| Q-score Q(X) | Error Probability |
|---|---|
| Q40 | 0.0001 (1 in 10,000) |
| Q30 | 0.001 (1 in 1,000) |
| Q20 | 0.01 (1 in 100) |
| Q10 | 0.1 (1 in 10) |

**NOTE**

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

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.

After the Q-score is determined, results are recorded in the base call files.

# Appendix C Output Files and Folders



## Sequencing Output Files

| File Type | File Description, Location, and Name |
|---|---|
| Base call files | Each tile analyzed is included in a base call file, aggregated in one file for each lane, for each cycle. The aggregated file contains the base call and encoded quality score for every cluster for that lane.<br>Data\Intensities\BaseCalls\L00[X]—Files are stored in one folder for each lane.<br>**[Cycle].bcl.bgzf**, where [Cycle] represents the cycle number in four digits. Base call files are compressed using block gzip compression. |
| Base call index file | For each lane, a binary index file lists the original tile information in a pair of values for each tile, which are tile number and number of clusters for the tile.<br>Base call index files are created the first time a base call file is created for that lane.<br>Data\Intensities\BaseCalls\L00[X]—Files are stored in one folder for each lane.<br>**s_[Lane].bci** |
| Cluster location files | For each tile, the XY coordinates for every cluster are aggregated into one cluster location file for each lane. Cluster location files are the result of template generation.<br>Data\Intensities\L00[X]—Files are stored in one folder for each lane.<br>**s_[lane].locs** |
| Filter files | The filter file specifies whether a cluster passed filters. Filter information is aggregated into one filter file for each lane and read.<br>Filter files are generated at cycle 26 using 25 cycles of data.<br>Data\Intensities\BaseCalls\L00[X]—Files are stored in one folder for each lane.<br>**s_[lane].filter** |
| InterOp files | Binary reporting files used for Sequencing Analysis Viewer (SAV). InterOp files are updated throughout the run.<br>InterOp folder |
| RTA configuration file | Created at the beginning of the run, the RTA configuration file lists settings for the run.<br><Run folder Name>, **RTAConfiguration.xml** |
| Run information file | Lists the run name, number of cycles in each read, whether the read is an indexed read, and the number of swaths and tiles on the flow cell. The run info file is created at the beginning of the run.<br><Run folder Name>, **RunInfo.xml** |
| Thumbnail files | A thumbnail image for each color channel (red and green) for tiles 1, 6, and 12 from all cameras, top and bottom surfaces, at every cycle during imaging.<br>Thumbnail_Images\L00[X]\C[X.1]—Files are stored in one folder for each lane and one subfolder for each cycle.<br>**s_[lane]_[tile]_[channel].jpg**—In the file name, the tile is represented with a five-digit number that indicates surface, swath, camera, and tile. For more information, see *Tile Numbering* on page 62 and *Thumbnail Image Naming* on page 63. |

## Flow Cell Tiles

Tiles are small imaging areas on the flow cell defined as the field of view by the camera. The total number of tiles depends on the number of lanes, swaths, and surfaces that are imaged on the flow cell, and how the cameras work together to collect the images.

- High-output flow cells have a total of 864 tiles.
- Mid-output flow cells have a total of 288 tiles.

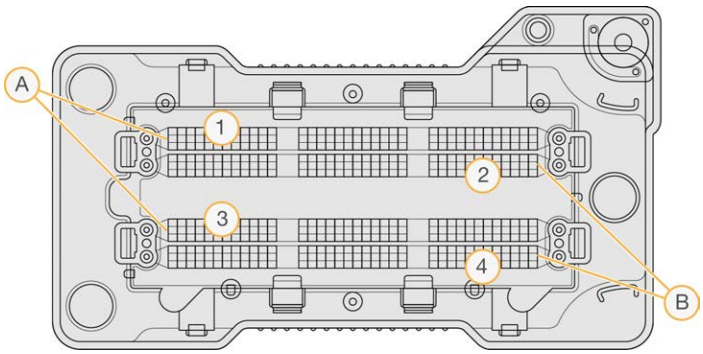
**Table 2 Flow Cell Tiles**

| Flow Cell Component | High Output | Mid Output | Description |
|---|---|---|---|
| Lanes | 4 | 4 | A lane is a physical channel with dedicated input and output ports. |
| Surfaces | 2 | 2 | The flow cell is imaged on two surfaces, the top and bottom. The top surface of 1 tile is imaged, then the bottom surface of the same tile is imaged before moving to the next tile. |
| Swaths per lane | 3 | 1 | A swath is a column of tiles in a lane. |
| Camera segments | 3 | 3 | The instrument uses six cameras to image the flow cell in three segments for each lane. |
| Tiles per swath per camera segment | 12 | 12 | A tile is the area on the flow cell that the camera sees as 1 image. |
| Total tiles imaged | 864 | 288 | The total number of tiles equals lanes × surfaces × swaths × camera segments × tiles per swath per segment. |

## Lane Numbering

Lanes 1 and 3, called lane pair A, are imaged at the same time. Lanes 2 and 4, called lane pair B, are imaged when imaging of lane pair A is complete.

**Figure 32** Lane Numbering

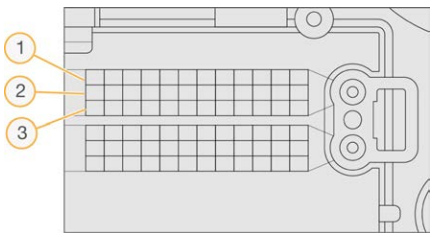



A Lane Pair A—Lanes 1 and 3
B Lane Pair B—Lanes 2 and 4

## Swath Numbering

Each lane is imaged in three swaths. Swaths are numbered 1–3 for high output flow cells.

**Figure 33** Swath Numbering

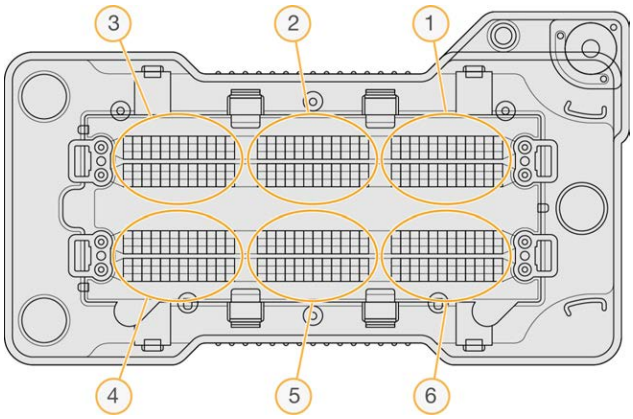


## Camera Numbering

The NextSeq 550 uses six cameras to image the flow cell.

Cameras are numbered 1–6. Cameras 1–3 image lane one. Cameras 4–6 image lane three. After lanes 1 and 3 are imaged, the imaging module moves on the X-axis to image lanes 2 and 4.

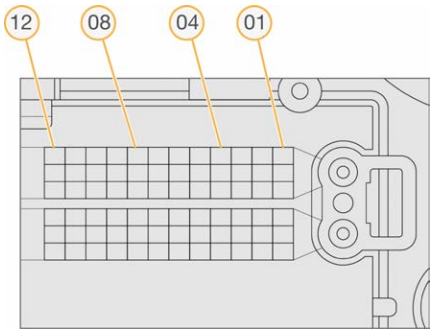
**Figure 34** Camera and Segment Numbering (High output flow cell shown)




## Tile Numbering

There are 12 tiles in each swath of each camera segment. Tiles are numbered 01–12, regardless of swath number or camera segment, and represented in two digits.

**Figure 35** Tile Numbering




The complete tile number includes 5 digits to represent the location, as follows:

- **Surface**—1 represents the top surface; 2 represents the bottom surface
- **Swath**—1, 2, or 3
- **Camera**—1, 2, 3, 4, 5, or 6
- **Tile**—01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, or 12

**Example:** Tile number 12508 indicates top surface, swath 2, camera 5, and tile 8.

The complete five-digit tile number is used in the file name of thumbnail images and empirical phasing files. For more information, see *Sequencing Output Files* on page 60.

### Thumbnail Image Naming

A thumbnail image for each color channel (red and green) for tiles 1, 6, and 12 are generated from all cameras, top and bottom surfaces, at every cycle during imaging. Thumbnail files are generated in the JPG file format.

Each image is named with the tile number as indicated by the following naming convention, which always begins with **s_**:

- **Lane**—1, 2, 3, or 4
- **Tile**—A 5-digit tile number, which indicates surface, swath, camera, and tile
- **Channel**—Red or green

**Example: s_3_12512_green.jpg**, which indicates lane 3, top surface, swath 2, camera 5, tile 12, and the green channel.

## Sequencing Output Folder Structure

The  control software generates the output folder name automatically.

- Data
  - Intensities
    - BaseCalls
      - L001—Base call files for lane 1, aggregated in one file per cycle.
      - L002—Base call files for lane 2, aggregated in one file per cycle.
      - L003—Base call files for lane 3, aggregated in one file per cycle.
      - L004—Base call files for lane 4, aggregated in one file per cycle.
    - L001—An aggregated *.locs file for lane 1.
    - L002—An aggregated *.locs file for lane 2.
    - L003—An aggregated *.locs file for lane 3.
    - L004—An aggregated *.locs file for lane 4.
- Images
  - Focus
    - L001—Focus images for lane 1.
    - L002—Focus images for lane 2.
    - L003—Focus images for lane 3.
    - L004—Focus images for lane 4.
- InterOp—Binary files used by Sequencing Analysis Viewer (SAV).
- Logs—Log files describing operational steps.
- Recipe—Run-specific recipe file named with reagent cartridge ID.
- RTALogs—Log files describing analysis steps.
- Thumbnail_Images—Thumbnail images for tiles 1, 6, and 12 in each swath at every cycle.
- RTAComplete.xml

RTAConfiguration.xml

RunInfo.xml

RunNotes.xml

RunParameters.xml

## Scanning Output Files

| File Type | File Description, Location, and Name |
|---|---|
| GTC files | Genotype call file. A GTC file is generated for each sample scanned on the BeadChip. The file name includes the barcode and sample scanned.<br>**[barcode]_[sample].gtc** |
| Image files | Image files are named according to the area scanned on the BeadChip. The name includes the barcode, sample and section on the BeadChip, swath, and the imaging channel (red or green).<br>**[barcode]_[sample]_[section]_[swath]_[camera]_[tile]_[channel].jpg**<br>• **Barcode**—The file name begins with the BeadChip barcode.<br>• **Sample**—An area of the BeadChip, which is numbered as a row (R0X), top to bottom, and column (C0X) left to right.<br>• **Section**—A numbered row within a sample.<br>• **Swath**—BeadChips are imaged as a collection of overlapping tiles. Therefore, only 1 swath is used to image the section.<br>• **Camera**—The camera used to collect the image.<br>• **Tile**—An imaging area defined as the field of view by the camera.<br>• **Channel**—A channel is either red or green. |

## Scanning Output Folder Structure

- [Date]_[Instrument Name]_[Scan#]_[Barcode]
  - [Barcode]
    - Config
      - Effective.cfg—Records config settings used during the scan.
    - **Focus**—Contains image files used to focus the scan.
    - **Logs**—Contains log files that list each step performed during the scan.
      - PreScanDiagnosticFiles
        - [Date_Time] Barcode Scan
          - ProcessedBarcode.jpg—Image of BeadChip barcode.
        - Scanning Diagnostics (log files)
        - PreScanChecks.csv—Records results of the automatic check.
    - GTC files—Genotype call files (one file per sample).
    - IDAT files—[Optional] Intensity data files (two files per sample; one each per channel).
    - Image files—Scan images for each sample, section, swath, camera, tile, and channel.
    - [Barcode]_sample_metrics.csv
    - [Barcode]_section_metrics.csv
  - ScanParameters.xml

# Appendix D NextSeq 550Dx Research Mode Considerations



## Introduction

Instructions in this guide, with some exceptions, are applicable to the NextSeq 550Dx instrument when in research mode with NCS v4.0 or later. When in research mode with NCS v3.0, refer to the *NextSeq 550Dx Research Mode Instrument Reference Guide (document # 1000000041922)*.

Your source for general Local Run Manager software instructions depends on the mode in use on the NextSeq 550Dx instrument. When in research mode, see the *Local Run Manager Software Guide (document # 1000000002702)*. When in diagnostic mode, see the Local Run Manager software instructions in *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*. Local Run Manager software is not available for NCS v3.0.

The differences between the NextSeq 550Dx in research mode and the NextSeq 550 include the following.

- Consumables compatibility.
- Starting the instrument.
- Instrument reboot and shutdown.

## NextSeq 550Dx Consumables Compatibility

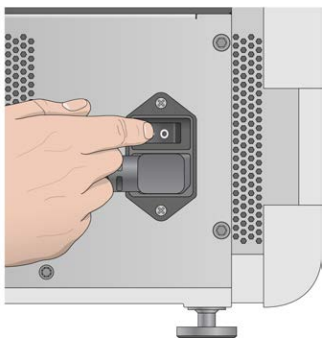
Performing a sequencing run on the NextSeq 550Dx requires a single-use NextSeq 550/550 Kit or NextSeq 550Dx High Output Reagent Kit.

If you use a NextSeq 550Dx High Output Reagent kit for a research mode run, all components must be from the same kit lot. A NextSeq 550/550 Kit cannot be used for a diagnostic mode run.

## Starting the NextSeq 550Dx Instrument

Turn on the power toggle switch to the **I** (on) position.

**Figure 36** Power Switch Located on Back of Instrument



1 Press the power button above the reagent compartment. The power button turns on the instrument power and starts the integrated instrument computer and software.
By default, the instrument boots into diagnostic mode.

**Figure 37** Power Button Located on Front of Instrument

2 Wait until the operating system has finished loading.
The NextSeq 550Dx Operating Software (NOS) launches and initializes the system automatically. After the initialization step is complete, the Home screen opens.

3 Enter your Local Run Manager user name and password.
For information on Local Run Manager passwords, see the *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

4 Select **Login**.
The Home screen opens, with Sequence, Local Run Manager, Manage Instrument, and Perform Wash icons.

5 Use the Reboot to RUO command in NOS to safely shut down the instrument and reboot to research mode.
- Select **Manage Instrument**.
- Select **Reboot / Shutdown**.
- Select **Reboot to RUO**.

6 Wait until the operating system has finished loading.
The NCS launches and initializes the system automatically. After the initialization step is complete, the Home screen opens.

7 If your system has been configured to require login credentials, log in to Windows using the user name and password for your site.

**NOTE**

If you are not sure what mode the instrument is in, see *Instrument Mode Indicators*.

## NextSeq 550Dx Instrument Mode Indicators

The following table lists instrument mode indicators on the NCS or NOS screen. For information on how to switch from research mode to diagnostic mode, see *NextSeq 550Dx Reboot and Shut Down Options* on page 67.

| Mode | Home Screen | Color Bar | Status Icon Orientation |
|---|---|---|---|
| Diagnostic Mode | Welcome to NextSeqDx | Blue | Horizontal |
| Research Mode | Welcome to NextSeq | Orange | Vertical |

## NextSeq 550Dx Reboot and Shut Down Options

Access the following features in the NextSeq 550Dx when in research mode by selecting the Shutdown Options button:

- Reboot to Dx—The instrument opens in diagnostic mode.
- Reboot to RUO—The instrument opens in research mode.
- Shutdown—The instrument opens in diagnostic mode.
- Exit to Windows—Depending on permissions, you can close NCS and view Windows.

**NOTE**

If you use the NextSeq 550Dx in research mode, when you return to diagnostic mode you are prompted to perform a post-run wash.

### Reboot to Diagnostic Mode

Use the Reboot to Dx command to safely shut down the instrument and reboot to diagnostic mode.

1 Select **Manage Instrument**.

2 Select **Shutdown Options**.

3 Select **Reboot to Dx**.

### Reboot to Research Mode

Use the Reboot to RUO command to safely shut down the instrument and reboot to research mode.

1 Select **Manage Instrument**.

2 Select **Shutdown Options**.

3 Select **Reboot to RUO**.

### Shut Down the Instrument

1 Select **Manage Instrument**.

2 Select **Shutdown Options**.

3 Select **Shutdown**.
The Shut Down command safely shuts down the software and turns off instrument power. Wait at least 60 seconds before turning on the instrument again.

**NOTE**

By default, the instrument boots in diagnostic mode when turned on.

**CAUTION**

*Do not* relocate the instrument. Moving the instrument improperly can affect the optical alignment and compromise data integrity. If you have to relocate the instrument, contact your Illumina representative.

## Exit to Windows

The Exit to Windows command provides access to the instrument operating system and any folder on the instrument computer. The command safely shuts down the software and exits to Windows.

1 Select Manage Instrument.

2 Select Shutdown Options.

3 Select Exit to Windows.

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# Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

## Illumina Customer Support Telephone Numbers

| Region | Toll Free | Regional |
|---|---|---|
| North America | +1.800.809.4566 | |
| Australia | +1.800.775.688 | |
| Austria | +43 800006249 | +43 19286540 |
| Belgium | +32 80077160 | +32 34002973 |
| China | 400.066.5835 | |
| Denmark | +45 80820183 | +45 89871156 |
| Finland | +358 800918363 | +358 974790110 |
| France | +33 805102193 | +33 170770446 |
| Germany | +49 8001014940 | +49 8938035677 |
| Hong Kong | 800960230 | |
| Ireland | +353 1800936608 | +353 016950506 |
| Italy | +39 800985513 | +39 236003759 |
| Japan | 0800.111.5011 | |
| Netherlands | +31 8000222493 | +31 207132960 |
| New Zealand | 0800.451.650 | |
| Norway | +47 800 16836 | +47 21939693 |
| Singapore | +1.800.579.2745 | |
| South Korea | +82 80 234 5300 | |
| Spain | +34 911899417 | +34 800300143 |
| Sweden | +46 850619671 | +46 200883979 |
| Switzerland | +41 565800000 | +41 800200442 |
| Taiwan | 00806651752 | |
| United Kingdom | +44 8000126019 | +44 2073057197 |
| Other countries | +44.1799.534000 | |

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

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

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