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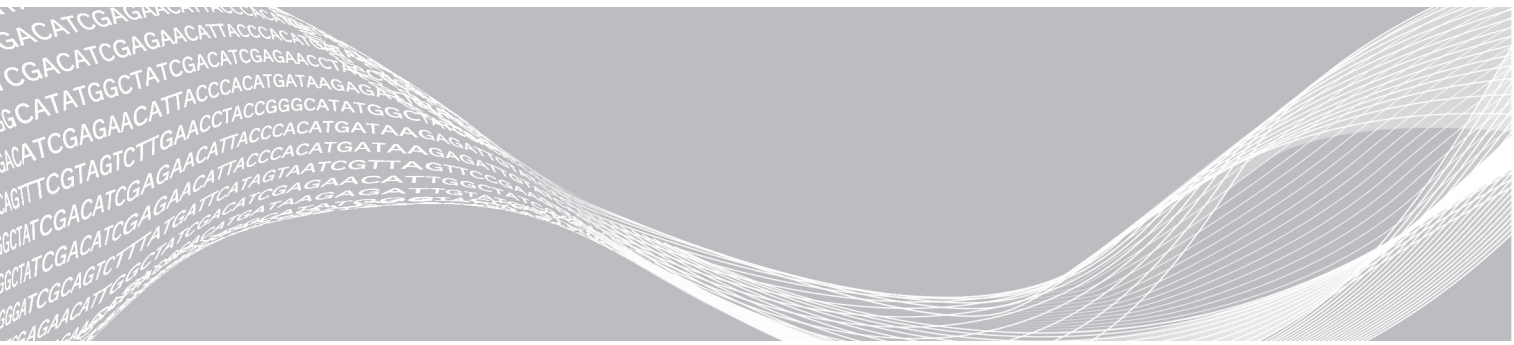
NextSeq 550Dx Research Mode

Instrument Reference Guide



Document # 1000000041922 v03

October 2021

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

Revision History

Document	Date	Description of Change
Document # 1000000041922 v03	October 2021	Added a notice about the 7 day timer in Checks for Sequencing Runs Updated Sequencing workflow by adding section to create run using Local Run Manager Software. Changed stability limit Added Infinium Methylation EPIC to BeachChip types Updated icon images to reflect UI changes.
Document # 1000000041922 v02	November 2020	Updated figure in Perform a Manual Wash to reflect new Reagent Wash and Buffer Wash cartridges. Updated status bar information with additional colors.
Document # 1000000041922 v01	March 2018	Added information about the Illumina Proactive monitoring service in the Configure System Settings section.
Document # 1000000041922 v00	November 2017	Initial release.

Table of Contents

Chapter 1 Overview

About this Guide

This instrument reference guide provides instructions on the use of the NextSeq 550Dx instrument in research (RUO) mode.

Introduction

Sequencing Features

- **High-throughput sequencing**—The NextSeq™ 550Dx instrument enables sequencing of DNA libraries.
- **Real-Time Analysis (RTA)**—Performs image processing and base calling. For more information, see *Real-Time Analysis* on page 51.
- **On-instrument data analysis capability**— Analysis Software software analysis modules specified for the run can analyze run data.
- **Dual Boot**—The NextSeq 550Dx instrument contains separate hard drives that support diagnostic (Dx) and research (RUO) modes.

Array Scanning Features

- **Integrated array scanning in control software**—The NextSeq 550Dx instrument allows you to transition between array scanning and high-throughput sequencing on the same instrument using the same control software.
- **Extended imaging capability**—The imaging system in the NextSeq 550Dx instrument 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, Infinium MethylationEPIC and Karyomap-12.
- **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 550Dx Instrument Site Prep Guide (document # 1000000009869)	Provides specifications for laboratory space, electrical requirements, and environmental considerations.
NextSeq 550Dx Instrument Safety and Compliance Guide (document # 1000000009868)	Provides information about operational safety considerations, compliance statements, and instrument labeling.
RFID Reader Compliance Guide (document # 1000000030332)	Provides information about the RFID reader in the instrument, compliance certifications, and safety considerations.
NextSeq 550Dx Research Mode Instrument Reference Guide (document # 1000000041922)	Provides instructions for operating the instrument and troubleshooting procedures. For use when operating the NextSeq 550Dx instrument in research mode with NextSeq Control Software (NCS) v3.0.
NextSeq 550 System Guide (document # 15069765)	Provides instructions for operating the instrument and troubleshooting procedures. For use when operating the NextSeq 550Dx instrument in research mode with NextSeq Control Software (NCS) v4.0 or later.
NextSeq 550 System Guide	Provides an overview of instrument components, instructions for operating the instrument, and maintenance and troubleshooting procedures.
BaseSpace help	Provides information about using BaseSpace™ Sequence Hub and available analysis options.

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

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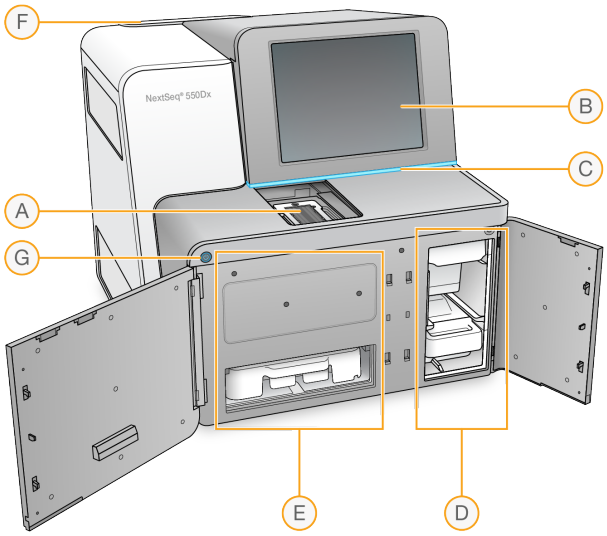
Instrument Components

The NextSeq 550Dx instrument includes a touch screen monitor, a status bar, and 4 compartments.

Figure 1 Instrument Components



A **Imaging compartment**—Holds the flow cell during a sequencing run.
B **Touch screen monitor**—Enables on-instrument configuration and setup using the operating software interface.
C **Status bar**—Indicates instrument status as processing (blue), requiring attention (orange), ready for sequencing (green), initializing (alternating blue and white), not yet initialized (white), or requiring a wash within the next 24 hours (yellow).
D **Buffer compartment**—Holds the buffer cartridge and the spent reagents container.
E **Reagent compartment**—Holds the reagent cartridge.
F **Air filter compartment**—Holds the air filter. Access the filter from the back of the instrument.
G **Power button**—Powers the instrument and the instrument computer on or off.

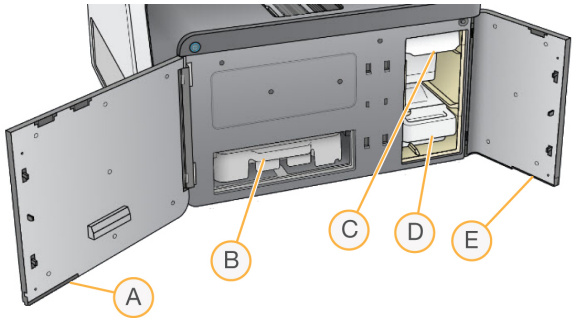
Imaging Compartment

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

Reagent and Buffer Compartments

Setting up a sequencing run on the NextSeq 550Dx instrument 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 area of the door. The reagent compartment holds the reagent cartridge.
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 and is located in the back of the instrument. Replace the air filter every 90 days. For information on replacing the filter, see *Replace Air Filter* on page 36.

NextSeq 550Dx Software

The instrument software includes integrated applications that perform sequencing runs.

- **NextSeq Control Software (NCS)**—The control software guides you through the steps to set up a sequencing run.

- **Real-Time Analysis (RTA) software**—RTA performs image analysis and base calling during the run. The NextSeq 550Dx instrument uses RTA v2, which includes important architecture and feature differences from earlier versions. For more information, see *Real-Time Analysis* on page 51.

Status Icons

A status icon in the top-right corner of the NCS 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. Warnings do not stop a run or require action before proceeding.
	Error	An error has occurred. Errors require action before proceeding with the run.
	Service Needed	A notification requiring attention has occurred. Refer to the message for additional information.

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.

NOTE

Acknowledging a message resets the icon, and the message is grayed out. The message is still visible to the user if they select the icon, but disappears once NCS is restarted.

Power Button

The power button on the front of the NextSeq 550Dx turns on power to the instrument and instrument computer. The power button performs the following actions depending on the state of instrument power. By default, the NextSeq 550Dx boots into diagnostic mode.

For information on initial power up of the instrument, see *Starting the Instrument* on page 9.

For information on shutting down the instrument, see *Shut Down the Instrument* on page 39.

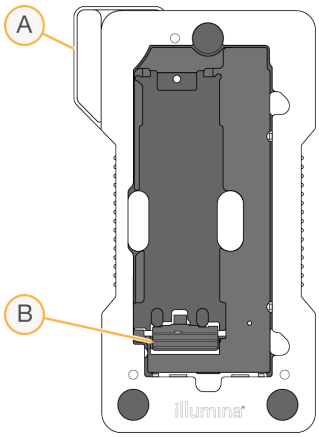
Power State	Action
Instrument power is off	Press the button to turn on the power.
Instrument power is on	Press the button to turn off the power. A dialog box appears on the screen to confirm instrument shutdown.
Instrument power is on	Press and hold the power button for 10 seconds to cause a hard shutdown of the instrument and instrument computer. 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.

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

Reagent Kit Overview

Sequencing Consumables Overview

The sequencing consumables required to run the NextSeq 550Dx are provided separately in a single-use kit. Each kit includes one flow cell, a reagent cartridge, a buffer cartridge, and library dilution buffer. For more information, see the *NextSeq 550Dx High Output Reagent Kit v2 (300 cycles)*, *NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles)*, or *NextSeq 550Dx High Output Reagent Kit v2.5 (75 cycles)* package insert.

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

CAUTION

NextSeq 550Dx High Output Reagent v2.5 kits require NOS 1.3 or later for the instrument to accept the v2.5 Flow Cell Cartridge. Complete software updates before preparing samples and consumables to avoid wasting reagents and or samples.

NOTE

Keep sequencing consumables stored in their boxes until ready for use.

Kit Compatibility Labeling

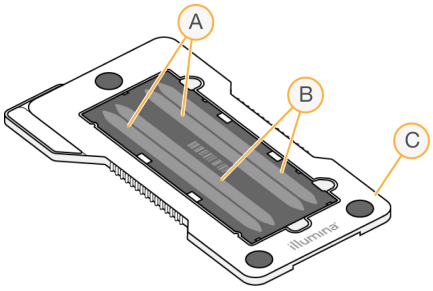
Kit components are labeled with color-coded indicators to show compatibility between flow cells and reagent cartridges. Always use a compatible reagent cartridge and flow cell. The buffer cartridge is universal.

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	

Flow Cell Overview

Figure 4 Flow Cell Cartridge



A Lane pair A—Lanes 1 and 3
B Lane pair B—Lanes 2 and 4
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 4 lanes that are imaged in pairs.

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

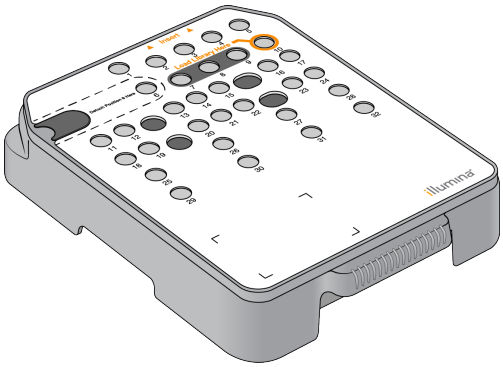
Although the flow cell has 4 lanes, only a single library or set of pooled libraries is sequenced on the flow cell. Libraries are loaded onto the reagent cartridge from a single reservoir and transferred automatically to the flow cell to all 4 lanes.

Each lane is imaged in small imaging areas called tiles. For more information, see *Flow Cell Tiles* on page 55.

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.

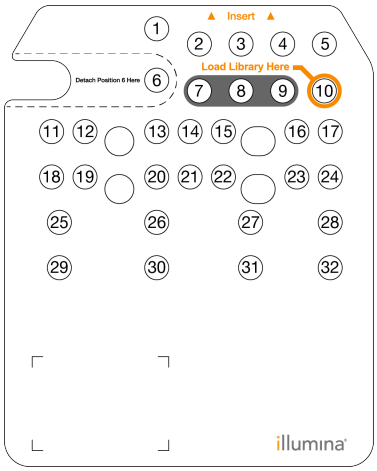
Several reservoirs are reserved for the automatic post-run wash. Wash solution is pumped from the buffer cartridge to the reserved reservoirs, through the system, and then to the spent reagents container.

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**

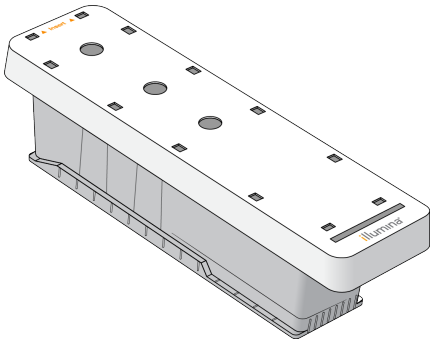
Removable Reservoir in Position #6

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 6 is removable. For more information, see *Remove Used Reservoir from Position #6* on page 20.

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

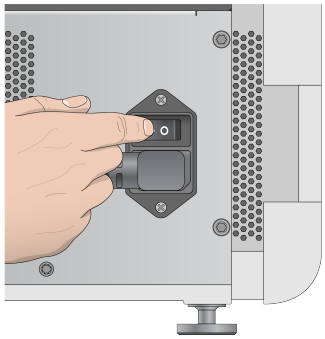


Chapter 2 Getting Started

Starting the Instrument

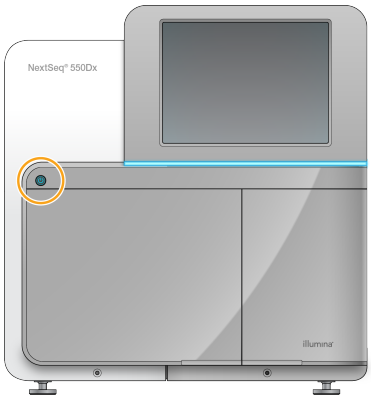
Turn on the power toggle switch to the **I** (on) position.

Figure 8 Power Switch Located on Back of Instrument



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 Enter your Local Run Manager user name and password.
For information on passwords, see *User Passwords* on page 1. For information on setting up an account on Local Run Manager, see *Administrative Settings and Tasks* on page 1.

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

Instrument Mode Indicators

The default mode of the NextSeq 550Dx is diagnostic mode. The following on the NCS screen indicate the mode of the instrument.

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

Customize System Settings

The operating software includes customizable system settings for instrument identification, input preferences, audio settings, and output folder location. To change network configuration settings, see *Configure System Settings* on page 48.

Customization options:

- Customize Instrument Identification (Avatar and Nickname)
- Set Input Option and Audio Indicator
- Set Run Setup Options
- Shut Down Options
- Configuring instrument start after pre-run check
- Choosing to send instrument performance data to Illumina
- Designating a run output folder

Customize Instrument Avatar and Nickname

1. From the Home screen, select **Manage Instrument**.
2. Select **System Customization**.
3. To assign a preferred image for your instrument, select **Browse** and navigate to the image.
4. In the Nick Name 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 Run Setup Options

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

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

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

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

Set Automatic Purge Option

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

2 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.

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

User-Supplied Consumables and Equipment

The following consumables and equipment are used on the NextSeq 550Dx instrument.The following consumables and equipment are used for consumables preparation, sequencing, and instrument maintenance. For more information, see the *NextSeq 550 System Guide*.

Consumables for Sequencing

Consumable	Supplier	Purpose
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	VWR, catalog # 95041-714 (or equivalent) General lab supplier	Flow cell cleaning and general purpose
Lab tissue, low-lint	VWR, catalog # 21905-026 (or equivalent)	Flow cell cleaning and general purpose

Consumables for Maintenance and Troubleshooting

Consumable	Supplier	Purpose
NaOCl, 5% (sodium hypochlorite)	Sigma-Aldrich, catalog # 239305 (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)
Air filter	Illumina, catalog # 20022240	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

Equipment

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

Chapter 3 Sequencing

Introduction

To perform a sequencing run on the NextSeq 550Dx instrument, prepare a reagent cartridge and flow cell, and 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 nucleotides. 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.

Analysis

As the run progresses, the operating software automatically transfers base call (BCL) files to the specified output location for secondary analysis.

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 8 cycles each for 2 index reads.

Number of Cycles in a Read

In a sequencing run, the number of cycles performed in a read is 1 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.

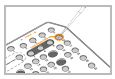
Sequencing Workflow

Create Run

Create Run in the Local Run Manager software module. See the analysis workflow guide for your particular module.

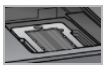
Prepare a new reagent cartridge: thaw and inspect.
Prepare a new flow cell: bring to room temperature, unwrap, and inspect.

Denature and dilute libraries. See the library prep package insert for instructions.

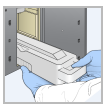


Load the library dilution onto the reagent cartridge in reservoir #10.

From the instrument NCS Home screen, select **Sequence**, your run ID, and begin the run setup steps. Select **Run**.



Load the flow cell.



Empty and reload the spent reagents container.
Load the buffer cartridge and reagent cartridge.

Review Pre-run check results. Select **Start**. (Not required if configured to start automatically).

Monitor the run from the operating software interface or from a networked computer with Local Run Manager.

An instrument wash begins automatically when sequencing is complete.

Prepare the Reagent Cartridge

Make sure to follow reagent cartridge directions carefully for successful sequencing.

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

2 Choose one of the following methods to thaw the reagents. Do not submerge the cartridge. After the cartridge is thawed, dry it before you proceed to the next step.

Temperature	Time to Thaw	Stability Limit
15°C to 30°C water bath	60 minutes	Not to exceed 6 hours
2°C to 8°C	7 hours	Not to exceed 7 days

NOTE If more than one cartridge is thawing in the same water bath, allow for additional thawing time.

3 Invert the cartridge five times to mix reagents.

4 Inspect the bottom of the cartridge to make sure that reagents are thawed and free of precipitates. Confirm that positions 29, 30, 31, and 32 are thawed, as they are the largest and take the longest to thaw.

5 Gently tap on the bench to reduce air bubbles.
For best results, proceed directly to loading the sample and setting up the run.

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 box from 2°C to 8°C storage.

2 Remove the foil package from the box and set 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.

Prepare Libraries for Sequencing

Denature and dilute your libraries to a loading volume of 1.3 ml. In practice, loading concentration can vary depending on library preparation and quantification methods. Dilution of sample libraries depends on the complexity of oligonucleotide pools. For directions on how to prepare sample libraries for sequencing, including library dilution and pooling, see the Instructions for Use section for the applicable library preparation kit. Optimization of cluster density on the NextSeq 550Dx is required.

Denature and Dilute Libraries

Denature and dilute your libraries to a loading volume of 1.3 ml and a loading concentration of 1.8 pM. In practice, loading concentration can vary depending on library preparation and quantification methods. For instructions, see the library prep package insert.

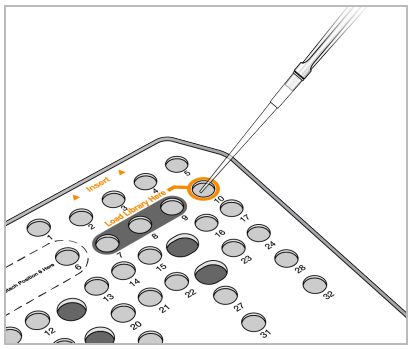
Load Libraries onto the Reagent Cartridge

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 libraries into reservoir #10 labeled **Load Library Here**. Avoid touching the foil seal as you dispense the libraries.

Figure 10 Load Libraries



Set Up a Sequencing Run

Log in to BaseSpace

1 Enter your BaseSpace user name and password.

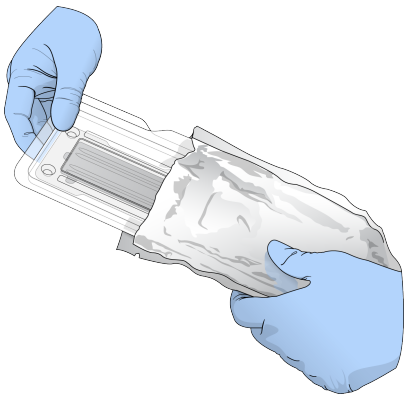
2 Select **Next**.

Load the Flow Cell

1 Remove the used flow cell from a previous run.

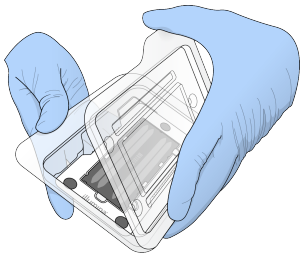
2 Remove the flow cell from the foil package.

Figure 11 Remove from Foil Package



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

Figure 12 Remove from Clamshell Package

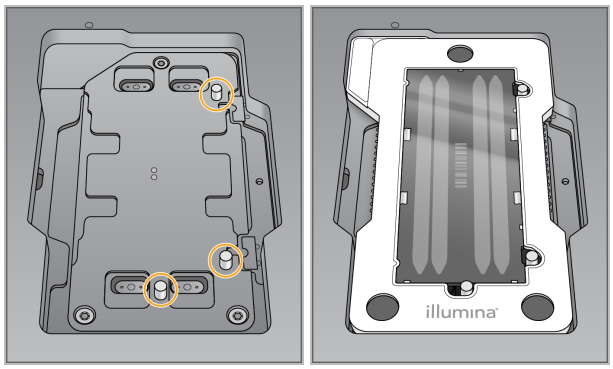


4 Clean the glass surface of the flow cell with a lint-free alcohol wipe. Dry the glass with a low-lint lab tissue.

NOTE Make sure the glass surface of the flow cell is clean. If necessary, repeat the cleaning step.

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

Figure 13 Load the Flow Cell



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

NOTE Keep hands clear of the flow cell door while it is closing to avoid pinching.

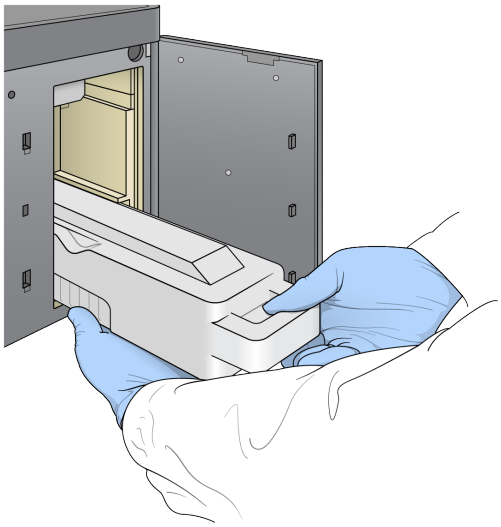
7 Select **Next**.

Empty the Spent Reagents Container

1 Open the buffer compartment door with the latch under the lower-left corner of the door.

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

Figure 14 Remove the Spent Reagents Container



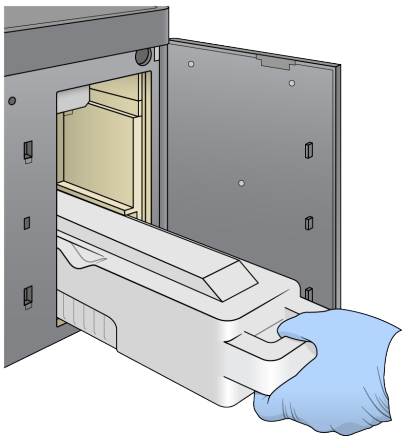
NOTE As you remove the container, place your other hand underneath for support.

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.

3 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

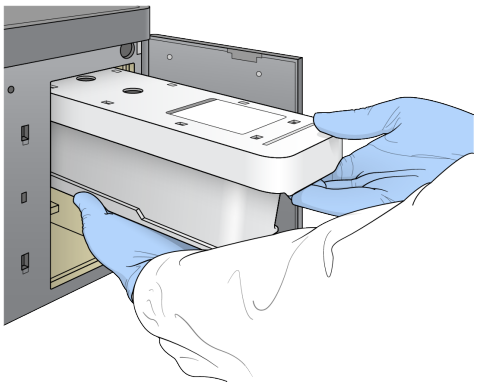


Load the Buffer Cartridge

1 Remove the used buffer cartridge from the upper compartment.
Some force is required to lift and then pull out the buffer cartridge.

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 Open the reagent compartment door using the latch under the lower-right corner of the door.

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

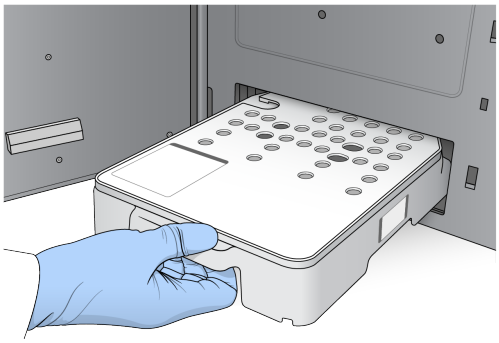
WARNING

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 20.

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

Figure 17 Load Reagent Cartridge



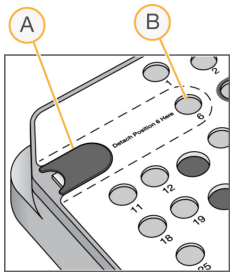
4 Select **Load**.
The software moves the cartridge into position automatically (~30 seconds), the reagent cartridge ID appears on the screen, and the sensors are checked.

5 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.

Specify Run Parameters

The steps on the Run Setup screen differ based on system configuration:

- **BaseSpace or BaseSpace Onsite**—The Run Setup screen lists runs that were set up using the BaseSpace Prep tab. If the intended run does not appear on the Run Setup screen, make sure that the run is marked for sequencing in BaseSpace.
- **Standalone**—The Run Setup screen includes fields for defining run parameters.

Select Available Run (BaseSpace Configuration)

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 Select **Next**.

3 Confirm run parameters.
- **Run Name**—Name of the run as assigned in BaseSpace.
- **Library ID**—Name of the pooled libraries as assigned in BaseSpace.
- **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.
- **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 Parameters (Standalone Configuration)

1 Enter a run name of your preference.

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

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

4 Enter the number of cycles for each read in the sequencing run.
- **Read 1**—Enter a value up to 151 cycles.
- **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.
- **Read 2**—Enter a value up to 151 cycles. This value is typically the same number of cycles as Read 1.

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

5 **[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.
- **Index 1**—Custom primer for Index 1.
- **Index 2**—Custom primer for Index 2.
- **Read 2**—Custom primer for Read 2.

6 **[Optional]** Select the **Advanced Settings** 

button to change run parameters.
- From the Recipe drop-down list, select a recipe. Only compatible recipes are listed.
- **Output folder location**—Change the output folder location for the current run. Select **Browse** to navigate to a network location.
- **Included file**—Select files to include in the Output Folder that can be useful if further analysis is required. For example, manifest files and sample lists.
- **Purge consumables for this run**—Change the setting to purge consumables automatically after the current run.

- **Use run monitoring for this run**—Change the setting to use run monitoring in BaseSpace.

7 Select **Next**.

Review Pre-Run Check

The software performs an automated pre-run 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 42.

To stop an automated pre-run check in progress, select the **Cancel** button. To restart the check, select the **Retry** button. The check resumes at the first incomplete or failed check.

To view the results of each individual check within a category, select the Category tab.

If the instrument is not configured to start the run automatically, start the run after the automated pre-run check is complete.

Start the Run

When the automated pre-run 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 1.

CAUTION

Make sure that you stay logged on to Windows. If you log off the Windows system during a sequencing run, the run stops.

NOTE Reagents cannot sit idle on the instrument for more than 24 hours.

Monitor Run Progress

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

NOTE After you select Home, it is not possible to return to view run metrics. However, run metrics are accessible on BaseSpace 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 5 cycles are reserved for template generation.
- Run metrics appear after cycle 25, including cluster density, clusters passing filter, yield, and quality scores.

Data Transfer

Status	Local Run Manager	Output Folder
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 transfer data from the instrument computer before starting a subsequent run.

Universal Copy Service

The NextSeq 550Dx includes a Universal Copy Service. RTA2 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 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 550Dx instrument, 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 type of BeadChip you are using
- A cluster file for the type of 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 550Dx instrument 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 49.

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 26.

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 550Dx system.

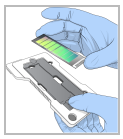
- **Manifest file**—Manifest files describe the SNP or probe content on a BeadChip. Manifest files use the *.bpm file format.
- **Cluster files**—Cluster files describe the cluster positions for the Illumina genotyping array and are used when analyzing data to make the genotype call. Cluster files use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

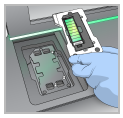
When the NextSeq 550Dx instrument instrument is installed, the Illumina representative downloads these files and specifies the path in the control software. There is no need to change these files except in the case of loss or if a new version is available. For more information, see *Replace Manifest Files and Cluster Files* on page 47.

Scanning Workflow

DMAP — Download the DMAP information and save it to the specified DMAP folder location.



Load the BeadChip onto the BeadChip adapter.



Load the BeadChip adapter onto the instrument.

Specify scan parameters: DMAP folder location and output location.

Review automatic check results.
Select **Start**.

Monitor the scan from the control software interface.

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 2 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.

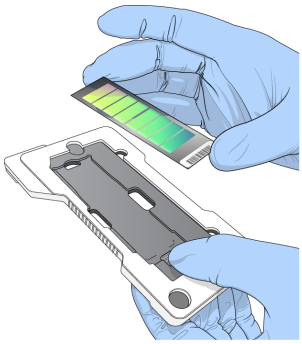
5 Save the DMAP folder to the specified DMAP folder location.

Load the BeadChip Onto the Adapter

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

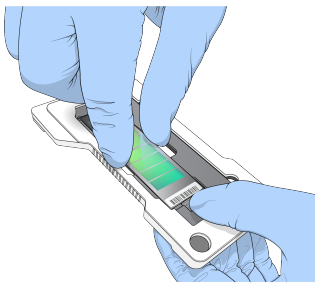
2 Holding the BeadChip by the edges, position the BeadChip with the barcode near the retention clip and place the BeadChip onto the recessed shelf of the adapter.

Figure 19 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.

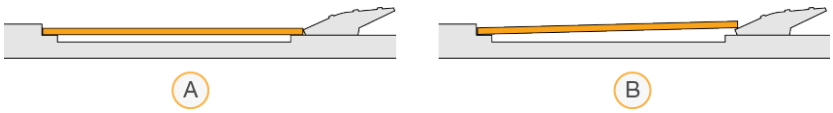
Figure 20 Seat and Secure BeadChip



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 21 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.

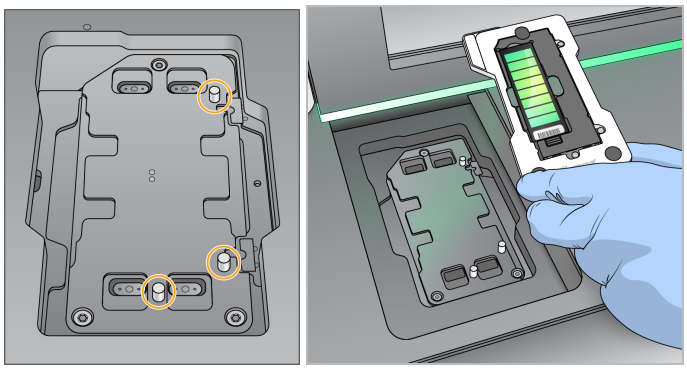
2 Remove the flow cell from the imaging compartment.

3 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 22 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 46.

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 Pre-Run Check

The software performs an automated pre-run 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 42.

To stop an automated pre-run check in progress, select the **Cancel** button. To restart the check, select the **Retry** button. The check resumes at the first incomplete or failed check.

To view the results of each individual check within a category, select the Category tab.

If the instrument is not configured to start the run automatically, start the run after the automated pre-run check is complete.

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 11.

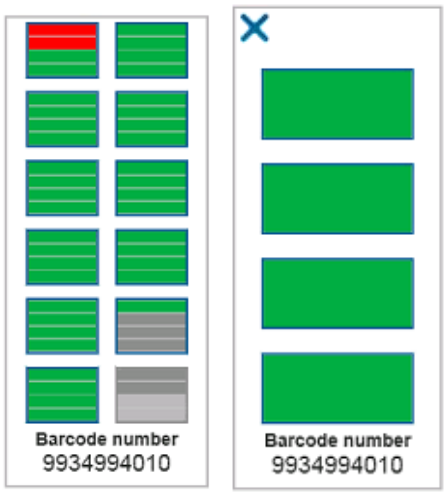
Monitor Scan Progress

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 46.

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 23 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 59.

If the connection is interrupted, data transfer resumes automatically when the connection is restored. Each file has a timer of 1 hour after it has been queued for transfer to the output folder. When the timer expires or if the instrument is rebooted before transfer is complete, data are not written to the output folder.

Chapter 5 Maintenance

Introduction

Maintenance procedures include manual instrument washes and replacing the air filter. Instrument shut down and reboot options are also described.

- **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 33.
- **Air filter replacement**—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 Duration: 20 minutes	Flushes the system with a user-supplied wash solution of laboratory-grade water and Tween 20 (buffer wash cartridge). • Required every 14 days that the instrument is idle with reagent cartridge and buffer cartridge in place. • Required every 7 days that the instrument is in a dry state (reagent cartridge and buffer cartridge removed).
Manual Post-Run Wash 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). 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 24 Original Style Reagent Wash Cartridge and Buffer Wash Cartridge.

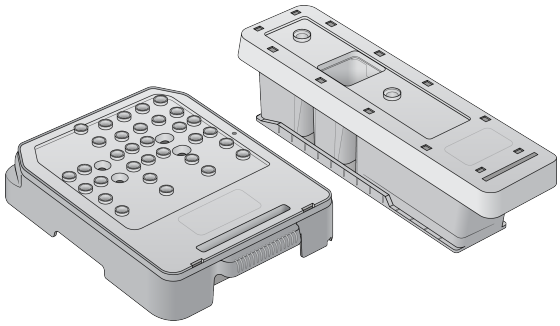
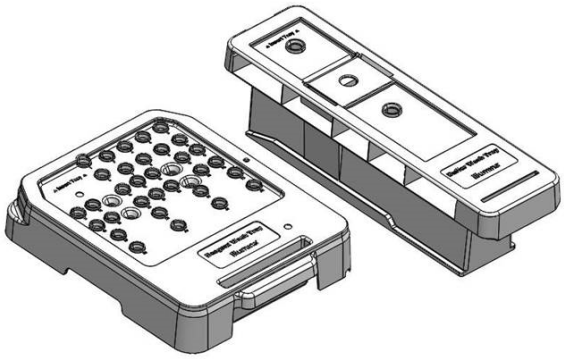


Figure 25 New Style Reagent Wash Cartridge and Buffer Wash Cartridge.



Prepare for a Manual Post-Run Wash

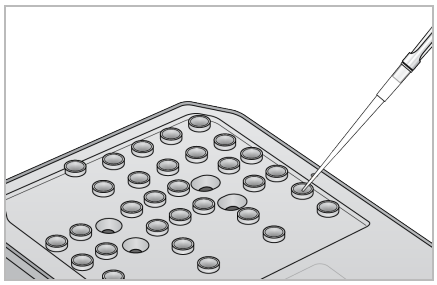
Choose to either prepare for a manual post-run wash as described below, or prepare for a quick wash (next section). If you intend to do a manual post-run wash, skip the quick wash section and continue on to *Load a Used Flow Cell and the Wash Cartridges* on page 35.

User-Supplied Consumables	Volume and Description
NaOCl	1 ml, diluted to 0.12% Loaded onto the reagent wash cartridge (position #28)
100% Tween 20 Laboratory-grade water	Used to make 125 ml 0.05% Tween 20 wash solution 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 of 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:
Original style buffer wash cartridge
- 100% Tween 20 (62 µl)
- Laboratory-grade water (125 ml)
- Add 125 ml wash solution to the center reservoir of the buffer wash cartridge.

New style buffer wash cartridge
- 100% Tween 20 (75 µl)
- Laboratory-grade water (150 ml)
- Add 150 ml wash solution to the center reservoir of the buffer wash cartridge.

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

Prepare for a Quick Wash

You can prepare for a quick wash as described below as an alternative to *Prepare for a Manual Post-Run Wash* on page 34.

User-Supplied Consumables	Volume and Description
100% Tween 20	Used to make 40 ml 0.05% Tween 20 wash solution
Laboratory-grade water	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

New systems come with three spare air filters. These should be stored and used when a prompt is received from the instrument to change the filter.

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.

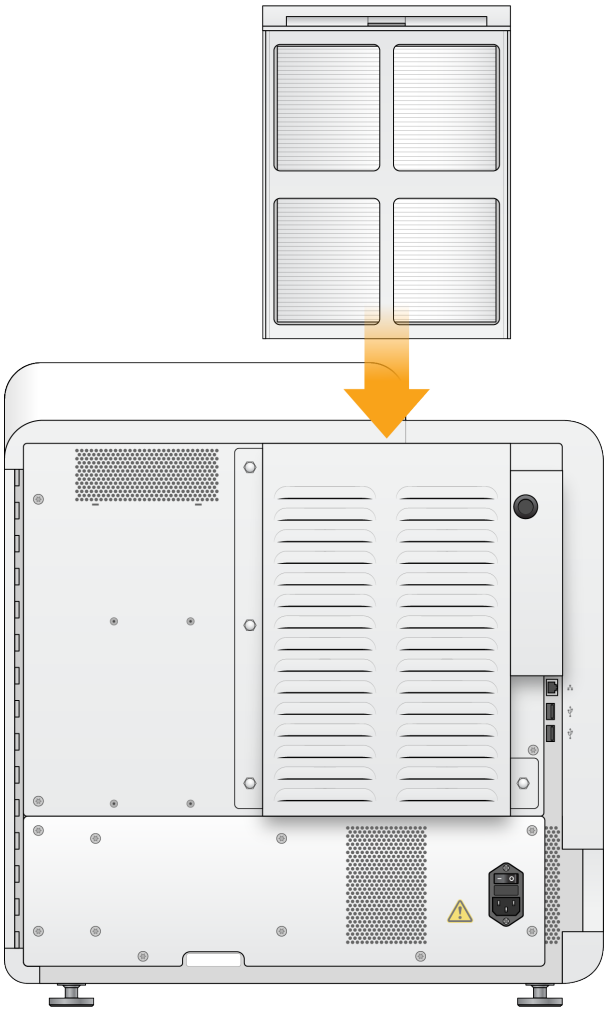
4 Remove and discard the old air filter.

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
- RTA2
- NextSeq Service Software (NSS)
- Sequencing Analysis Viewer (SAV)
- BaseSpace Broker

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 550Dx instrument support page on the Illumina website.

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.

Reboot and Shut Down Options

Access the following features by selecting the Reboot / Shutdown button:

- Reboot to RUO—The instrument opens in research mode.
- Restart —The instrument opens in diagnostic mode.
- Restart to Dx from RUO—The instrument opens in diagnostic mode.
- Shutdown—When powered on again, the instrument opens in diagnostic mode.
- Exit to Windows—Depending on permissions, you may close NCS and view Windows.

Restart to Diagnostic Mode

Use the Restart command to safely shut down the instrument and reboot to diagnostic mode. Diagnostic mode is the default boot mode.

1 Select **Manage Instrument**.

2 Select **Reboot / Shutdown**.

3 Select **Restart**.

Shut Down the Instrument

1 Select **Manage Instrument**.

2 Select **Reboot / Shutdown**.

3 Select **Shutdown**.
The Shutdown 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. Only an Admin user can exit to Windows.

1 Select **Manage Instrument**.

2 Select **Reboot / Shutdown**.

3 Select **Exit to Windows**.

Appendix A Troubleshooting

Introduction

For run quality or performance problems, contact Illumina Technical Support. See *Technical Assistance* on page 65.

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	Folder	Description
Run information file (RunInfo.xml)	Root folder	Contains the following information: • Run name • Number of cycles in the run • Number of cycles in each read • Whether the read is an indexed read • Number of swaths and tiles on the flow cell
Run parameters file (RunParameters.xml)	Root folder	Contains information about run parameters and run components. Information includes the RFID, serial number, part number, and expiration date.
RTA configuration file (RTAConfiguration.xml)	Root folder	Contains the RTA configuration settings for the run. The RTAConfiguration.xml file is created at the beginning of the run.
InterOp files (*.bin)	InterOp	Binary reporting files. 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. Include the error log when reporting issues to Illumina Technical Support.

Troubleshooting Files for Array Scans

Key File	Folder	Description
Scan parameters file (ScanParameters.xml)	Root folder	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.
Metrics files	[Barcode]	Metrics are provided as sample metrics and as section metrics. **[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. **[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.

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 flow cell, reagent cartridge, and buffer cartridge RFIDs will be locked during a control software initialization, which may be required to resolve an error. User must remove the flow cell, reagent cartridge, and buffer cartridge from the instrument prior to a system restart. Additionally, the consumable RFIDs are locked after the foil seals have been pierced. Once a flow cell RFID is read by the software, a 7 hour timer starts before the flow cell is considered locked and unusable.

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. 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. Contact Illumina Technical Support.
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. 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	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. • On the run setup screens, select **Back** to return to the flow cell step. The imaging compartment door opens. • 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. 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. 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. 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 5 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 550Dx 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**. 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 7 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 3 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 33.

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 New Run on the BaseSpace Prep Tab

1 If the instrument is configured for BaseSpace or BaseSpace Onsite, set up a new run on the Prep tab using the same parameters as the original run.

TIP Click the Pools tab, select the appropriate Pool ID to retain the previous run settings, and then assign a unique name for the new run.

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 Load the new buffer cartridge and reagent cartridge.

6 From the Run Setup screen, select from the following options:
- **BaseSpace or BaseSpace Onsite**—Select the run and confirm run parameters.
- **Standalone**—Enter the name of the run and specify the same parameters as the original run.

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

BeadChip and Scan Errors

Software Cannot Read the BeadChip Barcode

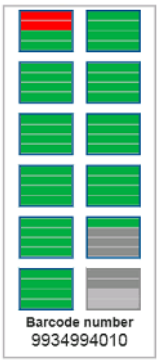
When the barcode error dialog box appears, select from the following options:
- Select **Rescan**. The software attempts to read the barcode again.
- Select text field and enter the numeric barcode as shown in the image. Depending on the BeadChip, barcode numbers have up to 12 digits. Select **Save**. 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 1 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 1 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 550Dx instrument 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.

RAID Error Message

The NextSeq 550Dx computer is equipped with four hard drives, two for diagnostic mode and two for research mode. 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. The RAID warning can only be acknowledged by an administrator. Using your instrument with only one hard drive could lead to the loss of data.

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. Only a Windows administrator account has permission to access system configuration options.

- **Network Configuration**—Provides options for IP address settings, domain name server (DNS) address, computer name, and domain name.

Set Network Configuration

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

2 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 4 numbers separated by a dot, similar to 168.62.20.37, for example.
- 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.

3 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.

4 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.

5 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.

6 Select **Save**.

Set Analysis Configuration

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

2 Select **Analysis Configuration**.

3 Select from the following options to specify a location where data are transferred for subsequent analysis.
- Select **BaseSpace** to send sequencing data to Illumina BaseSpace. **[Optional]** Select the **Output Folder** checkbox, select **Browse**, and navigate to a secondary network location to save BCL files in addition to BaseSpace.
- Select **BaseSpace Onsite**. In the Server Name field, enter the full path to your BaseSpace Onsite server. **[Optional]** Select the **Output Folder** checkbox, select **Browse**, and navigate to a secondary network location to save BCL files in addition to the BaseSpace Onsite server.
- Select **Standalone instrument** to save data to a network location only. Select **Browse** and navigate to a preferred network location. The control software generates the output folder name automatically.
  - **[Optional]** Select **Use Run Monitoring** to monitor the run using visualization tools on BaseSpace. A BaseSpace login and internet connection is required.

4 If you selected BaseSpace or BaseSpace Onsite, set the BaseSpace parameters as follows.
- Enter a BaseSpace **User Name** and **Password** to register the instrument with BaseSpace.
- Select **Use default login and bypass the BaseSpace login screen** to set the registered user name and password as the default login. This setting bypasses the BaseSpace screen during run setup.

5 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 off by default. To opt in to this service, enable the **Send Instrument Performance Data to Illumina** setting.

6 Select **Save**.

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.

9 **[Optional]** Select **Hide Obsolete BeadChips** to remove BeadChips from view that are obsolete.

10 Select **Save**.

Appendix B Real-Time Analysis

Real-Time Analysis Overview

The NextSeq 550Dx instrument 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 operating 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.

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 and provides the run name, number of cycles, whether a read is indexed, and the number of tiles on the flow cell.
- RTA.exe.config, which is a software configuration file in XML format.

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

RTA2 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.bgzf) 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 1 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.

RTA2 provides real-time metrics of run quality stored as InterOp files. InterOp files are a binary output containing tile, cycle, and read-level metrics.

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 5 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 1 base other than G in the first **5** cycles. For any index sequences, RTA2 requires at least 1 base other than G in the first **2** 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, 1 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.

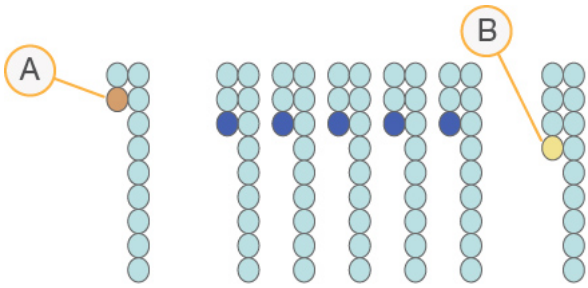
If registration fails for any images in a cycle, no base calls are generated for that tile in that cycle.

Phasing Correction

During the sequencing reaction, each DNA strand in a cluster extends by 1 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 29 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 550Dx instrument uses 2-channel sequencing, which requires only 2 images to encode the data for 4 DNA bases, 1 from the red channel and 1 from the green channel.

Intensities extracted from an image compared to another image result in 4 distinct populations, each corresponding to a nucleotide. The base calling process determines to which population each cluster belongs.

Figure 30 Visualization of Cluster Intensities

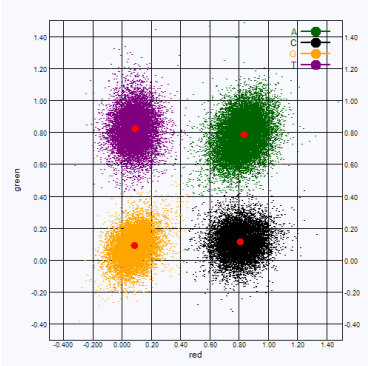


Table 1 Base Calls in 2-Channel Sequencing

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

Clusters Passing Filter

During the run, RTA2 filters raw data to remove reads that do not meet the data quality threshold. Overlapping and low-quality clusters are removed.

For 2-channel analysis, RTA2 uses a population-based system to determine the chastity of a base call. Clusters pass filter (PF) when no more than 1 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 1 base other than G in either of the first 2 cycles. If an Index Read begins with 2 base calls of G, no signal intensity is generated. Signal must be present in either of the first 2 cycles to ensure demultiplexing performance.

To increase demultiplexing robustness, select index sequences that provide signal in at least 1 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. Quality scores are represented as Q(X), 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 base call (*.bcl.bgzf) 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 1 file for each lane, for each cycle. The aggregated file contains the base call and encoded quality score for every cluster for that lane. Data\Intensities\BaseCalls\L00[X]—Files are stored in 1 folder for each lane. [Cycle].bcl.bgzf, where [Cycle] represents the cycle number in 4 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. Base call index files are created the first time a base call file is created for that lane. Data\Intensities\BaseCalls\L00[X]—Files are stored in 1 folder for each lane. s_[Lane].bci
Cluster location files	For each tile, the XY coordinates for every cluster are aggregated into 1 cluster location file for each lane. Cluster location files are the result of template generation. Data\Intensities\L00[X]—Files are stored in 1 folder for each lane. s_[lane].locs
Filter files	The filter file specifies whether a cluster passed filters. Filter information is aggregated into 1 filter file for each lane and read. Filter files are generated at cycle 26 using 25 cycles of data. Data\Intensities\BaseCalls\L00[X]—Files are stored in 1 folder for each lane. s_[lane].filter
InterOp files	Binary reporting files. InterOp files are updated throughout the run. InterOp folder
RTA configuration file	Created at the beginning of the run, the RTA configuration file lists settings for the run. [Root folder], 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. [Root folder], RunInfo.xml

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.

Table 2 Flow Cell Tiles

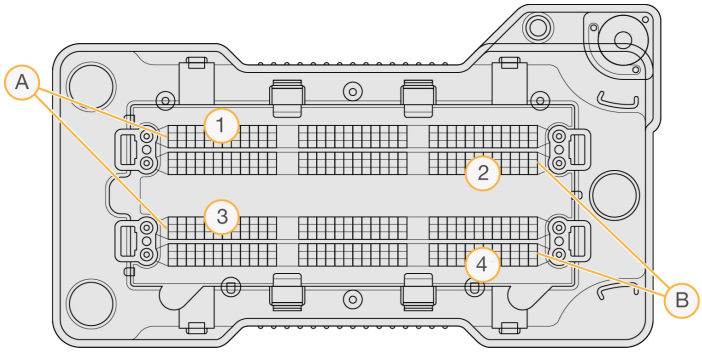
Flow Cell Component	High Output	Description
Lanes	4	A lane is a physical channel with dedicated input and output ports.
Surfaces	2	The flow cell is imaged on 2 surfaces, the top and bottom. The top surface of 1 tile is imaged, then the bottom surface of the same tile is imaged before moving to the next tile.

Flow Cell Component	High Output	Description
Swaths per lane	3	A swath is a column of tiles in a lane.
Camera segments	3	The instrument uses 6 cameras to image the flow cell in 3 segments for each lane.
Tiles per swath per camera segment	12	A tile is the area on the flow cell that the camera sees as 1 image.
Total tiles imaged	864	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 31 Lane Numbering

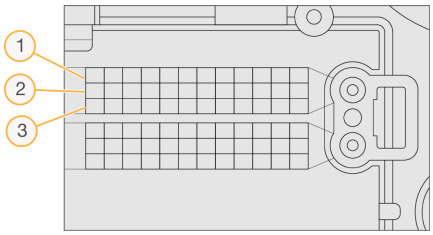


A Lane Pair A—Lanes 1 and 3
B Lane Pair B—Lanes 2 and 4

Swath Numbering

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

Figure 32 Swath Numbering

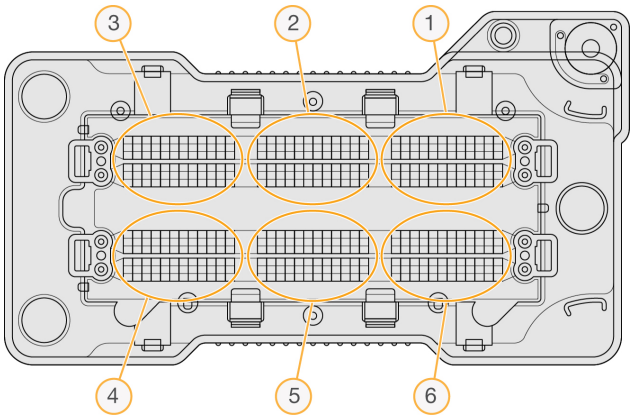


Camera Numbering

The NextSeq 550Dx instrument uses 6 cameras to image the flow cell.

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

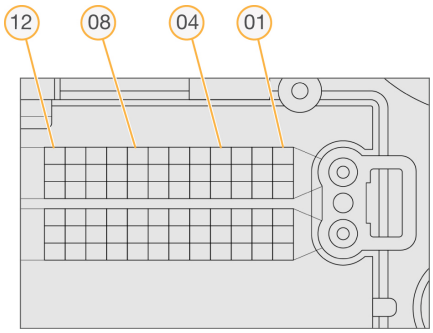
Figure 33 Camera and Segment Numbering (High output flow cell shown)



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 2 digits.

Figure 34 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 5-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 55.

Output Folder Structure

The operating software generates the output folder name automatically.

Data

Intensities

BaseCalls

L001—Base call files for lane 1, aggregated in 1 file per cycle.

- **L002**—Base call files for lane 2, aggregated in 1 file per cycle.
- **L003**—Base call files for lane 3, aggregated in 1 file per cycle.
- **L004**—Base call files for lane 4, aggregated in 1 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.

Logs—Log files describing operational steps.

Recipe—Run-specific recipe file named with reagent cartridge ID.

RTALogs—Log files describing analysis steps.

RTAComplete.txt

RTAConfiguration.xml

RunInfo.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. **[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). **[barcode]_[sample]_[section]_[swath]_[camera]_[tile]_[channel].jpg** • **Barcode**—The file name begins with the BeadChip barcode. • **Sample**—An area of the BeadChip, which is numbered as a row (R0X), top to bottom, and column (C0X) left to right. • **Section**—A numbered row within a sample. • **Swath**—BeadChips are imaged as a collection of overlapping tiles. Therefore, only 1 swath is used to image the section. • **Camera**—The camera used to collect the image. • **Tile**—An imaging area defined as the field of view by the camera. • **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 (1 file per sample).
    - IDAT files—[Optional] Intensity data files (2 files per sample; 1 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

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

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

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

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