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NextSeq 550Dx

Instrument Reference Guide

Revision History

Document	Date	Description of Change
Document # 1000000009513 v08	May 2023	Added reference to optional Illumina DRAGEN Server for NextSeq 550Dx with Illumina Run Manager. Updated Air Filter part number. Updated stability limit for reagent cartridge.
Document # 1000000009513 v07	October 2021	Added Air Filter Compartment 3 spare filters included. Changed reagent cartridge stability limit. Added new buffer wash cartridge to manual wash section. Updated System Check section to include instructions for LRM user credentials. Updated Checks for Sequencing Runs section.
Document # 1000000009513 v06	August 2021	Updated EU Authorized Representative address.
Document # 1000000009513 v05	November 2020	Updated service accounts user name and password requirements. Updated status bar with additional color information. Created a new section called "Set Default Output Folder." Added examples of output folder paths. Added troubleshooting for network storage errors. Added password expiration information.
Document # 1000000009513 v04	April 2020	Updated EU Authorized Representative address. Updated Australian Sponsor address.

Document	Date	Description of Change
Document # 1000000009513 v03	March 2019	Added information about v2.5 (75 cycle) reagent kits.
Document # 1000000009513 v02	January 2019	Added information about v2.5 (300 cycle) reagent kits. Updated the list of additional resources. Noted that the instructions for Local Run Manager in this guide are for use on the diagnostic mode of the instrument. Added instructions on rebooting from research mode to diagnostic mode based on the version of NextSeq Control Software (NCS) that is on the research mode drives. Corrected the Shut Down Options button to Reboot/Shutdown button. Corrected the exit to Windows procedure by adding a step to select Reboot / Shutdown.
Document # 1000000009513 v01	March 2018	Added information about the Illumina Proactive monitoring service in the Customize System Settings section. Updated instructions in the Replace Air Filter section. Changed references to base call files from *.bcl to *.bcl.bgzf to clarify that the files are compressed. Updated instructions on how to resolve a Required Software system check error. Added Australia regulatory marking.
Document # 1000000009513 v00	November 2017	Initial release.

Table of Contents

Overview

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 62.
- **On-instrument data analysis capability**—Local Run Manager software analysis modules specified for the run can analyze run data.
- **Off-instrument data analysis capability**—Illumina Run Manager enables secondary data analysis when the NextSeq 550Dx instrument is paired with an optional Illumina DRAGEN Server for NextSeq 550Dx. The Illumina DRAGEN Server for NextSeq 550Dx is optional and is only available in select countries. Contact an Illumina representative for regional availability.
- **Dual Boot**—The NextSeq 550Dx instrument contains separate hard drives that support diagnostic (Dx) and research (RUO) modes.

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.
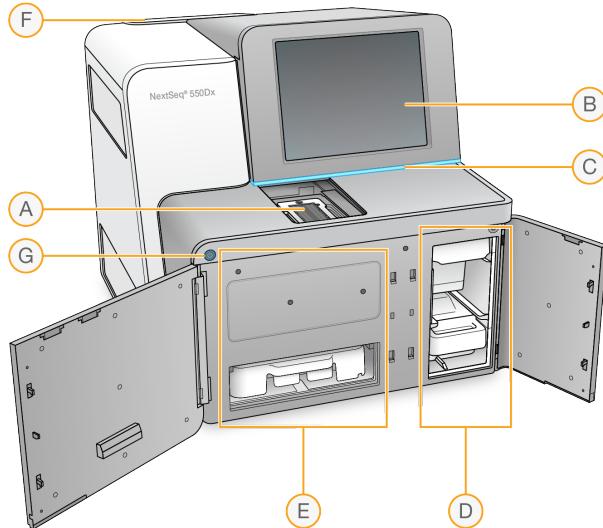
Resource	Description
NextSeq 550Dx Research Mode Instrument Reference Guide (document # 1000000041922)	Provides instructions for operating the instrument and troubleshooting procedures. For use when operating the NextSeq 550Dx instrument in research mode with NextSeq Control Software (NCS) v3.0.
NextSeq 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.
Illumina Run Manager for NextSeq 550Dx Software Guide (document # 200025239)	Provides information about using optional Illumina DRAGEN Server for NextSeq 550Dx with Illumina Run Manager 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.

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.

FOR IN VITRO DIAGNOSTIC USE.

    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.
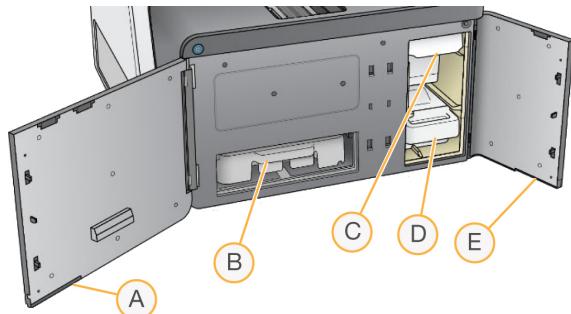
Imaging Compartment

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 45.

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 21.

For information on shutting down the instrument, see *Shut Down the Instrument* on page 25.

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.

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 v2.5 (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.

FOR IN VITRO DIAGNOSTIC USE.
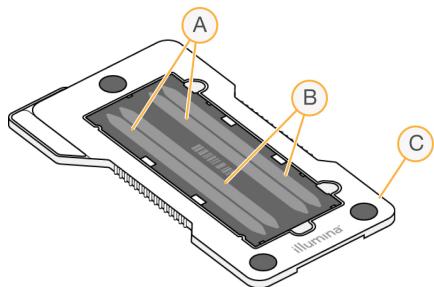
Required Storage for Sequencing Consumables

Item (1 per run)	Storage Requirement
Library dilution buffer	-25°C to -15°C
Reagent cartridge	-25°C to -15°C
Buffer cartridge	15°C to 30°C
Flow cell cartridge	2°C to 8°C*

* The NextSeq 550Dx High Output Flow Cell Cartridge v2.5 is shipped at ambient temperature.

Flow Cell Overview

Figure 3   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.
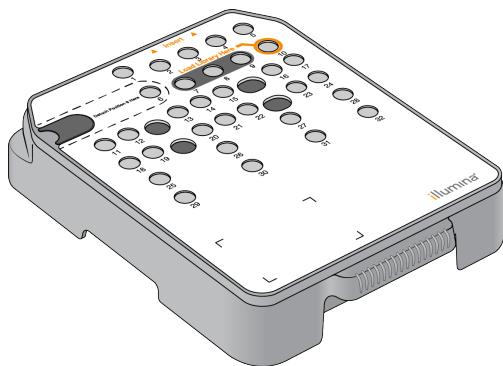
Although the flow cell has 4 lanes, only a single library or set of pooled libraries is sequenced on the flow cell. Libraries are loaded onto the reagent cartridge from a single reservoir and transferred automatically to the flow cell to all 4 lanes.

Each lane is imaged in small imaging areas called tiles. For more information, see *Flow Cell Tiles* on page 69.

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 4   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.
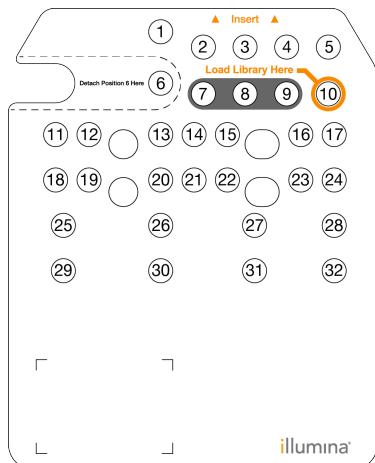
⚠ CAUTION

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

FOR IN VITRO DIAGNOSTIC USE.

Reserved Reservoirs

Figure 5   Numbered Reservoirs

Position	Description
7, 8, and 9	Reserved for optional custom primers
10	**Load libraries**
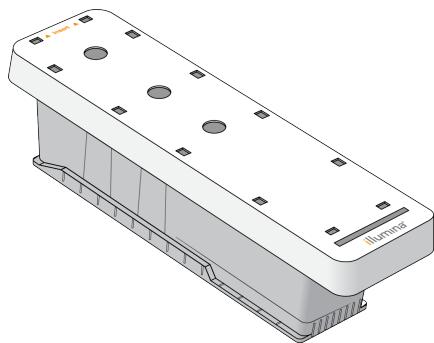
Removable Reservoir in Position #6

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 34.

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 6   Buffer Cartridge

Library Dilution Buffer Overview

The library dilution buffer is in the NextSeq 550Dx Accessory Box. Use the buffer as needed to dilute libraries according to the library preparation instructions to achieve recommended cluster densities.

User-Supplied Consumables and Equipment

The following consumables and equipment are used for consumables preparation, sequencing, and instrument maintenance.

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 # 20063988	Cleaning the air the instrument takes in for cooling

Equipment

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

FOR IN VITRO DIAGNOSTIC USE.

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

NextSeq 550Dx Software

NextSeq 550Dx Software Overview

The software described in this chapter is used to configure, run, and analyze data from the NextSeq 550Dx instrument. The instrument software includes integrated applications that perform sequencing runs. Software updates are performed by Illumina personnel.

- **Local Run Manager software**—Integrated software solution for creating a run and analyzing results (secondary analysis). The software also controls user permissions. For more information, see *Local Run Manager Overview* on page 12.
- **Illumina Run Manager**—Off-instrument software solution for creating a run and enabling performing secondary data analysis. For more information, refer to Illumina Run Manager for NextSeq 550Dx Software Guide (document # 200025239).
- **NextSeq 550Dx Operating Software (NOS)**—Controls instrument operation.
    - The software is preinstalled on the NextSeq 550Dx, and runs on-instrument. The NOS performs the run according to the parameters specified in the Local Run Manager software module.
    - Before you start the sequencing run, you select a run that you created with Local Run Manager. The NOS software interface guides you through the steps to load the flow cell and reagents.
    - During the run, the software operates the flow cell stage, dispenses reagents, controls fluidics, sets temperatures, captures images of clusters on the flow cell, and provides a visual summary of quality statistics. You can monitor the run in NOS, or in Local Run Manager.
- **Real-Time Analysis (RTA) software**—RTA performs image analysis and base calling, also referred to as primary analysis, during the run. For more information, see *Real-Time Analysis* on page 62.

Required Disk Space

The integrated instrument computer has approximately 1.5 TB of storage capacity.

Before starting a run, the software checks available disk space. The amount of disk space required depends on the Local Run Manager analysis module. If there is not enough disk space for the run, a software prompt appears. The message indicates how much disk space is required for the run and how much disk space must be cleared before the run can proceed. If there is insufficient space, delete run folders that do not need to be reanalyzed in Local Run Manager. For more information, see *Delete a Run Folder* on page 18.

Status Icons

A status icon in the top-right corner of the NOS 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 NOS is restarted.

Navigation Bar Icons
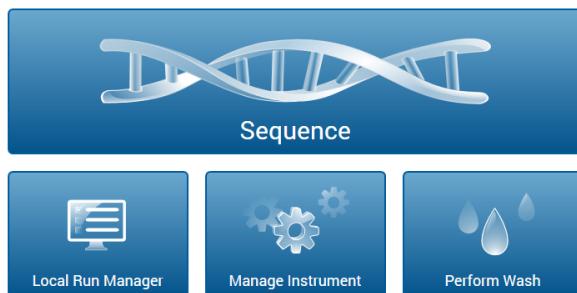
The minimize NOS icon is in the top-right corner of the operating software interface and is only visible to admin users.

Access Icon	Icon Name	Description
	Log Off	Select to log off of the software.
	Minimize NOS	Select to minimize NOS to access Windows applications and folders. This icon appears for admin users only.

NOS Home Screen

The NOS Home Screen features the following four icons.

- **Sequence**—Select to start a sequencing run from a pre-defined run list.
- **Local Run Manager**—Select to launch Local Run Manager for creating a run, monitoring run status, analyzing sequencing data, and viewing results. See *Local Run Manager Overview* on page 12.

NOTE   When using optional Illumina DRAGEN Server for NextSeq 550Dx with Illumina Run Manager, **Illumina Run Manager** displays. For more information about using Illumina Run Manager, refer to Illumina Run Manager for NextSeq 550Dx Software Guide (document # 200025239).

- **Manage Instrument**—Select to control system settings, reboot to research mode, or shut down the instrument software.
- **Perform Wash**—Select to perform a Quick Wash or the Manual Post-Run Wash.

Local Run Manager Overview

The Local Run Manager software is an on-instrument integrated solution for creating a run, monitoring status, analyzing sequencing data, and viewing results.

Local Run Manager instructions in this guide apply when in the diagnostic mode of the instrument. Details listed in this section cover general Local Run Manager functionality. Some features may not apply to all Analysis Modules. For Module specific features, see the respective Local Run Manager guide.

The Local Run Manager has the following features:

- Runs as a service on the instrument computer and integrates with the instrument operating software.
- Records samples to be sequenced.
- Provides a run setup interface specific to the analysis module.
- Performs a series of analysis steps specific to the selected analysis module.

- Shows analysis metrics in the form of tables and graphs upon completion of analysis.

Viewing Local Run Manager

The Local Run Manager interface is viewed within NOS or through a web browser. The supported web browser is Chromium.

NOTE   If you use an unsupported browser, download the supported browser when prompted on the "Confirm Unsupported Browser" message. Select "**here**" to download the supported version of Chromium.

View on a Networked Computer

Open a Chromium web browser on a computer with access to the same network as the instrument and connect using the instrument IP address or instrument name. For example, `http://myinstrument`.

View on the Instrument Monitor

To view the Local Run Manager interface on the instrument monitor, select one of the following options:
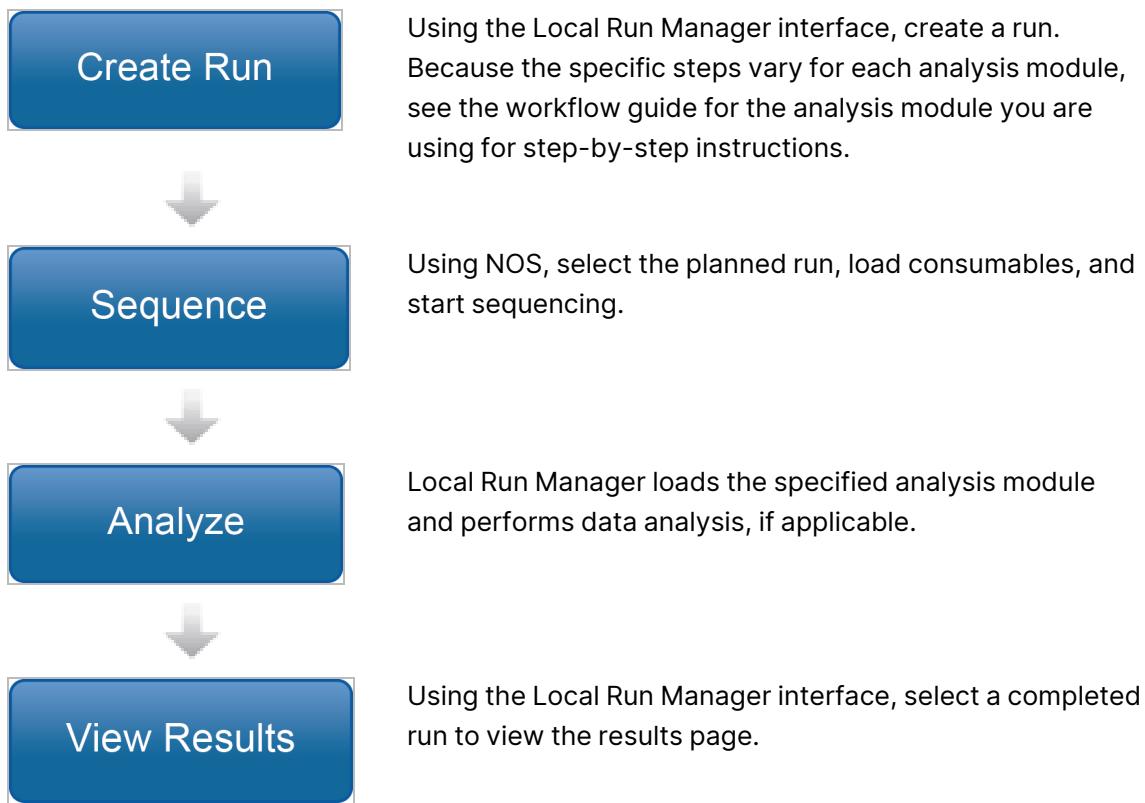
- From the NOS Home Screen, select **Local Run Manager**.
  Select the X in the upper right corner to return to NOS.

- Minimize the NOS, and then open the Chromium web browser on the instrument. Enter **http://localhost** in the address bar.
  Only admins can minimize NOS.

Sequencing During Analysis

The NextSeq 550Dx system computing resources are dedicated to either sequencing or analysis. If a new sequencing run is needed on the NextSeq 550Dx before secondary analysis of an earlier run is complete, the user can cancel secondary analysis from within Local Run Manager and then start a new sequencing run.

To restart the analysis performed by Local Run Manager, use the Requeue feature on the Local Run Manager interface after the new sequencing run is complete. At that point, secondary analysis starts from the beginning. See *Requeue or Stop Analysis* on page 39.

Local Run Manager Workflow

Create Run	Using the Local Run Manager interface, create a run. Because the specific steps vary for each analysis module, see the workflow guide for the analysis module you are using for step-by-step instructions.
↓	
Sequence	Using NOS, select the planned run, load consumables, and start sequencing.
↓	
Analyze	Local Run Manager loads the specified analysis module and performs data analysis, if applicable.
↓	
View Results	Using the Local Run Manager interface, select a completed run to view the results page.

Dashboard Overview

After you log in to the Local Run Manager software, the dashboard opens. From the dashboard, you can perform the following tasks:

- Track sequencing and analysis status
- Sort and filter runs
- Create and edit runs
- Stop or requeue analysis
- View analysis results
- Hide runs
- Pin runs
- Manage Run Folder

The dashboard lists all runs performed on the instrument, 10 runs per page. Use the navigation arrows at the bottom of the list to scroll through pages.

Each run listed includes the run name, analysis module, run status, and the date that the run was last modified. To sort runs, select the column names.

- **Run Name / ID**—Lists the assigned run name and links to the run results pages. After analysis is complete, the analysis folder name is added to the run name column.
- **Module**—Lists the analysis module assigned to the run.
- **Status**—Lists the status of the run and includes a progress status bar. For more information, see *Possible Run States* on page 39.
- **Last Modified**—Lists the date and time that a run or analysis session was last modified. By default, the Active Runs page is sorted by this column.

Local Run Manager Icons

The following icons are used in Local Run Manager to perform various tasks. Note that depending on the size of the screen, some icons are condensed under the ⁞ More Options icon.

Icon	Name	Description
	Copy to clipboard	Copies a field to the computer clipboard.
	Delete run folder	Deletes the data from a run to clear hard drive space.
	Edit	Enables editing of run parameters before sequencing.
	Edit output folder location	Enables editing of run folder path.
	Edit users	Enables editing of passwords and users.
	Export	Command to export items.
	Hide run	Moves a run from the active runs pages to the hidden runs page. View hidden runs using the menu at the lower right corner of the active runs page.
	Locked	Indicates that the run is being sequenced, or another user is editing a run in another browser session.
⁞	More options	Opens available options for an entry on the dashboard. Depending on the size of the screen, the dots can look more round.
	Pin	Pins a run to the active runs page to ensure that the run folder will not be deleted.
	Restore run	Moves a run from the hidden runs page to the active runs page.
	Relink run folder	Enables a run to be relinked after a deleted run folder is restored. The run can then be requeued for analysis.

Icon	Name	Description
⟲	Requeue	Restarts analysis of the selected run.
📌	Run is pinned	Indicates that a run has been pinned to the active runs page.
🗑	Run deleted	Indicates a deleted run.
💾	Save output folder location	Save edits to the path of the output run folder.
🔍	Search	Opens the search field to search run names and sample IDs. Depending on the size of the screen, the icon can be condensed underneath the more options icon.
▼	Show filters	Shows filters.
■	Stop analysis	Stops analysis by the Local Run Manager module.
📌	Unpin	Unpin a run.
👤	User	Opens the user account menu.
⚠	Warnings detected	See the notification text for information about warnings.

Active Runs Page

The dashboard opens to show the Active Runs page. Across the top of the Active Runs page, a summary of active runs provides a count of runs in each of the following run status categories. To filter active runs by status, select the number.

- **Ready**—Indicates that the run is ready for sequencing.
- **In Progress**—Indicates that the run is in the process of sequencing or data analysis.
- **Stopped or Unsuccessful**—Indicates that analysis was stopped manually or that analysis was unsuccessful.
- **Complete**—Indicates that analysis was successfully completed.
- **Total**—The total number of runs on the Active Runs page.

Filter Runs

1. Select a run status category in the summary of active runs to filter the Active Runs page as follows.
   - **Ready**—Filters the list to show only runs that are ready for sequencing.
   - **In Progress**—Filters the list to show only runs that are in progress.
   - **Stopped or Unsuccessful**—Filters the list to show only runs that were stopped or unsuccessful.
   - **Complete**—Filters the list to show only runs that are complete.

- **Total**—Removes filters and shows all active runs.

Hide and Restore Runs

1. From the Active Runs page, select the More Options ⋮ icon, and then select 🗑 **Hide**.

2. When prompted to confirm the move, select **Hide**.
   The run is moved to the Hidden Runs page.

3. To show the Hidden Runs page, select the Active Runs drop-down arrow, and then select **Hidden Runs**.

4. From the Hidden Runs page, select the Restore ↺ icon.

5. When prompted to confirm the move, select **Restore**.
   The run is restored to the Active Runs page.

6. To show the Active Runs page, select the Hidden Runs drop-down arrow, and then select **Active Runs**.

Search for Runs or Samples

1. From the dashboard navigation bar, select the Search 🔍 icon.

2. In the search field, enter a run name or sample ID.
   As you type, a list of possible matches appears to assist your search.

3. Select a match from the list or press **Enter**.

   - If you searched on a run name, the Run Overview tab opens.

   - If you searched on a sample ID, the Samples and Results tab opens.
     For more information, see *View Run and Sample Data* on page 37.

Edit a Run

1. From the Active Runs page, select the More Options ⋮ icon next to the run name that you want to edit.

2. Select ✏ **Edit**.

3. When prompted to confirm the action, select **Continue**.

4. Edit run parameters as needed.

5. When finished, select **Save Run**
   The Last Modified dates for the run is updated on the Active Runs page.

Pin a Run

Pinned runs are prevented from being deleted or hidden. A run cannot be pinned if the run is hidden or its run folder has been deleted.

1. From the Active Runs page, click the more options ⋮ icon next to a run.

2. Select 📌 **Pin**.
   Delete Run Folder and Hide are disabled. Pin is replaced with 📌 **Unpin**.

Delete a Run Folder

You can manually delete run folders to manage storage space. The run cannot be pinned and must be in one of the following states:

- Sequencing Complete
- Primary Analysis Complete
- Primary Analysis Unsuccessful
- Sequencing Errored
- Analysis Errored
- Analysis Complete
- Sequencing Stopped
- Analysis Stopped

1. From the Active Runs page, click the more options ⋮ icon next to the run name that you want to delete.

2. Select 🗑 **Delete Run Folder**.
   The Delete Run Folder option is replaced with 🔗 Relink Run Folder. The Run Deleted 🗑 icon appears on the run.

Relink a Run Folder

Only an admin user can relink deleted run folders.

1. Copy the run folder back to its original location.
   If the run is not saved in its original location, the software displays an error message following an attempt to restore.

2. Hover over the more options ⋮ icon next to the run.

3. Select 🔗 **Relink Run Folder**.

4. Confirm to proceed and restore the run.
   The run is restored to the state it was in before the run folder was deleted.

User Passwords

To access the Local Run Manager interface, you need a valid user name and password to log in to the system. Only an admin user can assign user credentials.

NOTE   User accounts are specific to an instrument. User passwords are not universal across multiple
instruments.

When your password is approaching an expiration date, a message appears at the top of the interface
to remind you to reset your password.

My Account

From My Account, you can view your user name, your assigned role, permissions, and change your
password.

After you have logged in for the first time, you can change your existing password at any time from the
My Account window.

Your current password appears encrypted. Therefore, you have to know your current password to
change it to a new password. Forgotten passwords require assistance from a system administrator or
admin user.

Password States

The following are possible password states:

- **Temporary password**—When an admin user creates a user account, the admin user assigns a
temporary password for the new user.

- **User password**—Upon first access, a new user is prompted to change the temporary password
from the login screen to a user-assigned password.

- **Forgotten password**—If a user forgets a password, an admin user can reassign a temporary
password that can be changed upon next access.

- **Used password**—Any user cannot reuse a password for up to five password cycles.

- **User lockout**—The number of attempts to log in using an invalid password is configured by an
admin user. If a user exceeds the number of allowed attempts, the user account is locked. Only an
admin user can unlock the account, or assign a temporary password.

Change Your Password

1. From the navigation bar at the top of the interface, select the **User** 👤 icon next to your name.

NOTE   Depending on the size of the screen you are viewing the software on, the **User** 👤 icon may be
condensed underneath **More Options** ☰ .

2. From the drop-down list, select **My Account**.

3. Select the **Edit** ✏ icon in the Password section.

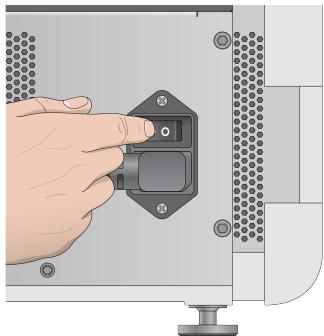4. In the Old Password field, enter your old password.

5. In the New Password field, enter a new password.

6. In the Confirm New Password field, reenter your new password.

7. Select **Save**.

Getting Started

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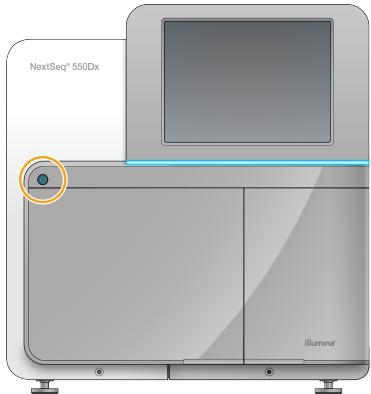
Turn on the power toggle switch to the **I** (on) position.

Figure 7   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 8   Power Button Located on Front of Instrument

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

3. Enter your Local Run Manager user name and password.
    For information on passwords, see *User Passwords* on page 18. For information on setting up an account on Local Run Manager, see *Introduction* on page 47.

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 NOS 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 60.

- 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 avatar 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 Start and Instrument Performance Data

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

2. Select **System Customization**.

3. [Optional] Select the **Automatically start run after pre-run check** checkbox to start sequencing automatically after a successful pre-run check.

4. 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 NOS 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.

5. Select **Save** to save settings and return to the Manage Instrument screen.

Set Default Output Folder

Both the Windows Account in Local Run Manager and the Windows account on the operating system of the instrument require permission to read and write to the output folder. To check permissions, consult your IT administrator. To set up the Windows Account in Local Run Manager, refer to *Specify System Service Accounts Setting* on page 50.

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

2. Select **System Customization**.

3. Select **Browse** to navigate to the folder location.

4. In the output folder, enter the full Universal Naming Convention (UNC) file path.

   - The UNC path includes two backslashes, the server name, and the directory name, but not a letter for a mapped network drive.

   - Paths to the output folder that are one level require a trailing backslash (eg, \\servername\directory1\).

   - Paths to the output folder that are two or more levels do not require a trailing backslash (eg, \\servername\directory1\directory2).

   - Paths to a mapped network drive cause errors. Do not use.

5. Select **Save** to save settings and return to the Manage Instrument screen.

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 NOS and view Windows.

Reboot to RUO Mode

Use the Reboot to RUO command to change the system software to research mode. The admin grants user permission to access this feature.

1. Select **Manage Instrument**.

2. Select **Reboot / Shut Down**.

3. Select **Reboot to RUO**.

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**.

Return to Diagnostic Mode From Research Mode

The command to move from research mode to diagnostic mode differs based on the version of NextSeq Control Software (NCS) on the research mode drives.

1. When in research mode, select **Manage Instrument**.
2. Select from the following options to return to diagnostic mode.
   - NCS v3.0 — Select **Shutdown Options**, and then **Restart**.
   - NCS v4.0 or later — Select **Shutdown Options**, and then **Reboot to Dx**.

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**.

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.
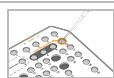
Sequencing Workflow

Create Run	Create Run using the run manager software module. Refer to the run module, application, and analysis workflow guides 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 NOS 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 or Illumina Run Manager.
↓	
	An instrument wash begins automatically when sequencing is complete.

Create Run

Create a sequencing run using Local Run Manager or Illumina Run Manager software. Instructions for using Local Run Manager are included below. For instructions on using Illumina Run Manager, including how to select between Local Run Manager or Illumina Run Manager, refer to the Illumina Run Manager for NextSeq 550Dx Software Guide (document # 200025239). The process to set up a run varies depending on the particular analysis workflow module you use and includes the following steps.

- Select Create Run from the run manager dashboard and then select an analysis module.
- On the Create Run page, enter a run name, and if applicable, enter samples for the run and/or import manifests.

For detailed instructions on specific applications, refer to the module or application guide for the specific assay.

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 5 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.

⚠️ CAUTION

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

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.
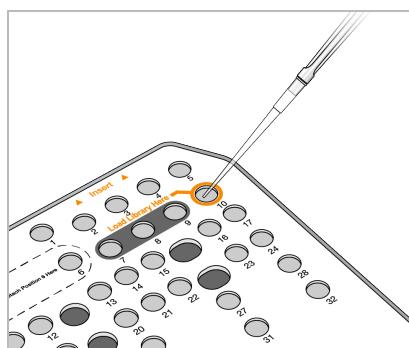
Prepare Libraries for Sequencing

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.

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 9   Load Libraries

Set Up a Sequencing Run

Select Run

1. From the Home screen, select **Sequence**.
2. Select a run from the list.
   For information on creating a sequencing run, see the *Local Run Manager Workflow* on page 14 .
   The imaging compartment door opens, releases consumables from a previous run, and opens the series of run setup screens. A short delay is normal.
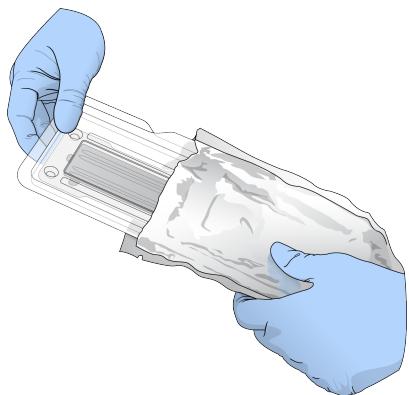3. Select **Next**.

Load the Flow Cell

1. Remove the used flow cell from a previous run.
2. Remove the flow cell from the foil package.

Figure 10   Remove from Foil Package

3. Open the clear plastic clamshell package and remove the flow cell.
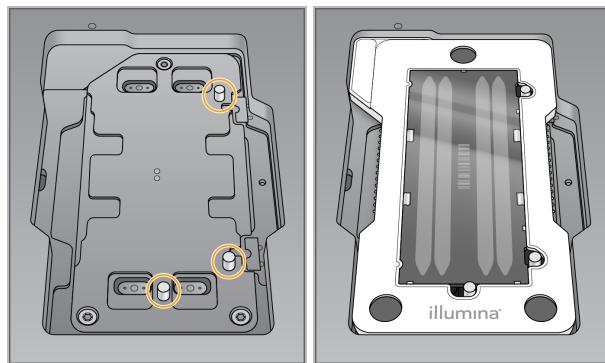
Figure 11 Remove from Clamshell Package

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 12 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 13   Remove the Spent Reagents Container

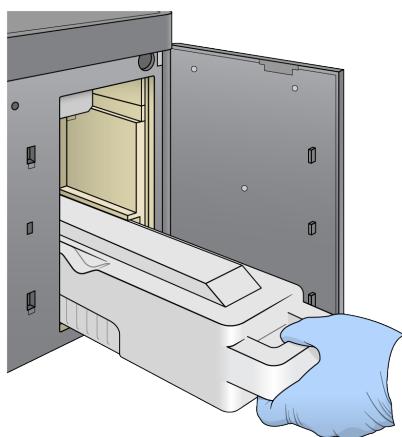NOTE   As you remove the container, place your other hand underneath for support.

⚠ CAUTION

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

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 14   Load the Empty Spent Reagents Container

FOR IN VITRO DIAGNOSTIC USE.

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 15 Load the Buffer Cartridge

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

Load the Reagent Cartridge

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

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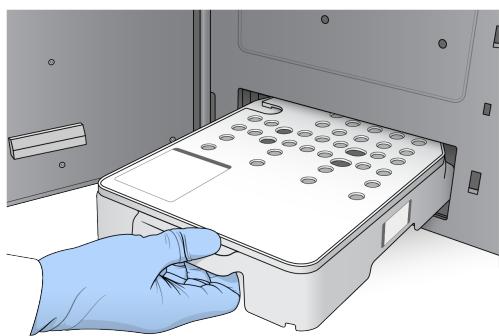
   ⚠ CAUTION

   This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

   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 34.

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

Figure 16   Load Reagent Cartridge
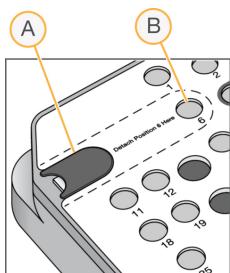

7. Select **Load**.

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

8. 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 17   Removable Position #6

   A. Protective rubber cover
   B. Position #6

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

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

Review 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 57.

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 Start and Instrument Performance Data* on page 23.
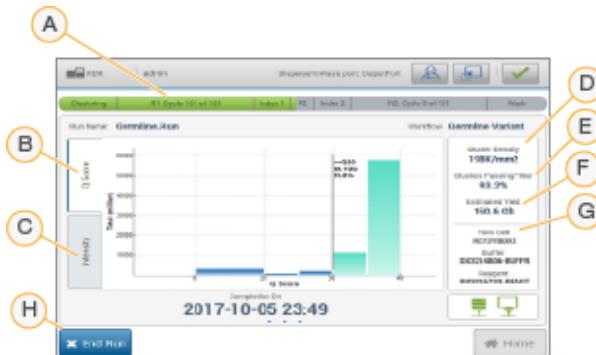
⚠ CAUTION

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.

Figure 18 Sequencing Run Progress and Metrics

A. **Run progress**— Shows the current step and number of cycles completed for each read. The progress bar is not proportional to the run rate of each step. The estimated date and time of run completion is shown at the bottom.
B. **Q-Score**— Shows the distribution of quality scores (Q-scores). See *Quality Scoring* on page 66.

FOR IN VITRO DIAGNOSTIC USE.

C. **Intensity**— Shows the value of cluster intensities of the 90th percentile for each tile. Plot colors indicate each base: red is A, green is C, blue is G, and black is T.

D. **Cluster Density (K/mm²)**—Shows the number of clusters detected for the run.

E. **Clusters Passing Filter (%)**— Shows the percentage of clusters passing filter. See *Clusters Passing Filter* on page 65.

F. **Estimated Yield (Gb)**— Shows the number of bases projected for the run.

G. **Lot Information**— Shows lot numbers of sequencing consumables. For the flow cell it shows the serial number.

H. **End Run**— Stops the sequencing run in progress.

NOTE   After you select Home, it is not possible to return to view run metrics. However, run metrics are accessible using the run managers. Select the Run Manager from the Home screen in NOS, or remotely using a web browser on a networked computer.

⚠   CAUTION

If the sequencing run is stopped prematurely by user, consumables used for run will be rendered unusable.

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		

Status	Local Run Manager	Output Folder
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.

View Run and Sample Data

View run and sample data using the run manager software that was used to create the sequencing run. For viewing run and sample data using Illumina Run Manager, refer to the Illumina Run Manager for NextSeq 550Dx Software Guide (document # 200025239).

To view run and sample data using Local Run Manager, see below.

Select the run name from the Local Run Manager dashboard. A summary of analysis results is presented on the following 3 tabs:

- Run Overview
- Sequencing Information
- Samples and Results

Run Overview Tab

The Run Overview tab lists information about the run, a summary of sequencing metrics, and the location of the run folder.

Section Title	Description
Run Name / Run ID	The run name assigned when the run was created.
Created By	The name of the user who created the run.
Description	The run description, if provided.
Output Run Folder	The path to sequencing run output folder. Select the Copy to Clipboard 📋 icon for quick access to the output folder.
Total Clusters	The number of clusters in a run.
% Clusters PF	The percentage of clusters passing filter.
% ≥ Q30 (Read 1)	The percentage of reads in Read 1 with a quality score of 30 (Q30) or greater.

Section Title	Description
% ≥ Q30 (Read 2)	The percentage of reads in Read 2 with a quality score of 30 (Q30) or greater.
Last Scored Cycle	The last cycle in the run with a quality score. Unless the run ended early, this value represents the last cycle in the run.

Sequencing Information Tab

The Sequencing Information tab provides a summary of sequencing run parameters. The following information is provided on the Sequencing Information tab.

Section Title	Description
Instrument Name	The name of the instrument on which the run was performed.
Sequenced By	The name of the user who started the run.
Sequencing Start	The date and time that the sequencing run began.
RTA Version	RTA software version in use for the run.
Module Version	The version of the analysis module assigned to the run.
Read Lengths	The number of reads and cycles for each read performed during the run.
Flow Cell Information	The barcode, part number, lot number, and expiration date of the flow cell used for the run.
Buffer Information	The barcode, part number, lot number, and expiration date of the buffer cartridge used for the run.
Reagent Cartridge Information	The barcode, part number, lot number, and expiration date of the reagent cartridge used for the run.

Samples and Results Tab

Information provided on the Samples and Results tab is specific to the analysis module used for the run. The Samples and Results tab can contain the following information fields.

Section Title	Description
Select Analysis	A drop-down list of each analysis performed on the data generated from the sequencing run. For each time that analysis is run, a sequential number is assigned. Results are listed separately.

Section Title	Description
Analysis Folder	The path to analysis folder. Select the copy to clipboard 📋 icon for quick access to the analysis folder.
Requeue Analysis	The command to reanalyze the sequencing run data. The command provides the option to edit analysis parameters or proceed without changes.

Possible Run States

The Status column on the dashboard shows the status of each run listed. The following table describes the possible states of a run and the color of the status bar in the Status column.

Status	Status Bar Color	Status Description
Ready for Sequencing	None	Waiting for the sequencing run to begin.
Sequencing Running	Blue	Sequencing in progress.
Sequencing Unsuccessful	Red	Sequencing issue. No activity.
Sequencing Stopped	Red	Sequencing stopped. No activity.
Sequencing Complete	Blue	Sequencing completed. Status bar at 50%.
Primary Analysis Complete	Blue	Sequencing metrics complete. Status bar at 50%.
Primary Analysis Unsuccessful	Red	RTA analysis failed. Status bar at 25%.
Analysis Queued	Blue	Waiting for analysis to begin.
Analysis Running	Blue	Analysis in progress.
Analysis Unsuccessful	Red	Analysis issue. No activity.
Stopping Analysis	Red	Request to stop analysis received.
Analysis Stopped	Red	Analysis stopped. No activity.
Analysis Completed	Green	Activity completed. Status bar at 100%.

Requeue or Stop Analysis

You might requeue analysis after analysis was stopped, if analysis was unsuccessful, or if you want to reanalyze a run with different settings. For requeue or stop analysis using Illumina Run Manager, refer to the Illumina Run Manager for NextSeq 550Dx Software Guide (document # 200025239). To requeue or

stop analysis using Local Run Manager, see below.

You can requeue analysis from the Local Run Manager dashboard or from the Sample and Results Tab. You can cancel Local Run Manager analysis, start a different sequencing run, and then return to requeue analysis on the original run later.

Stop a Local Run Manager Analysis

1. From the Active Runs page, click **More Options** ⋮ next to the run name that you want to stop.
2. Select ■ **Stop Analysis**.

Requeue Analysis from Active Runs

1. From the Active Runs page, select the more options ⫶ icon next to the run name that you want to requeue.
2. Select ⤬ **Requeue**.
3. When prompted, select from the following options:
   - Select **Edit Setup** to change analysis parameters. From the Requeue Analysis page, change preferred settings, and then select **Requeue Analysis**.
   - Select **Requeue**. Analysis begins using current analysis parameters.

Requeue Analysis from Results Page

1. Select the run name on the Active Runs page.
2. [Optional] Change the output folder. See *Change Output Run Folder Location* on page 40.
3. Select the Samples and Results tab.
4. Select ⤬ **Requeue Analysis**.
5. When prompted, select from the following options:
   - Select **Edit Setup** to change analysis parameters. From the Requeue Analysis page, change preferred settings, and then select **Requeue Analysis**.
   - Select **Requeue**. Analysis begins using current analysis parameters.

Change Output Run Folder Location

The output run folder location can be changed before you requeue a run. Changing the output folder is not required, because a requeue of analysis does not overwrite older analyses.

1. From the Active Runs dashboard, select a run from the list.
2. Select **Run Overview**.
3. Select the Edit ✎ icon and enter the new folder path.
   Although the location of the file can change, you cannot change the name of the output run folder. Make sure to follow UNC file path guidelines as described in *Set Default Output Folder* on page 23.

4. Select the Save 🖫 icon.

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.

Unload Consumables

On rare occasions it may be necessary to unload all consumables from the instrument. To do so, use the Quick Wash screen to unload the consumables.

1. Select Perform Wash and then select Quick Wash

2. The flow cell door will open and the reagent cartridge will move to the unload position.

3. Remove the flow cell, buffer cartridge, and reagent cartridge.

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

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

6. Select Exit to return to the NOS home screen.

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 42.

- **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 19   Original Style Reagent Wash Cartridge and Buffer Wash Cartridge.
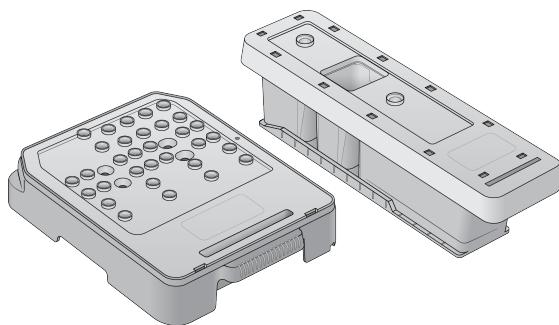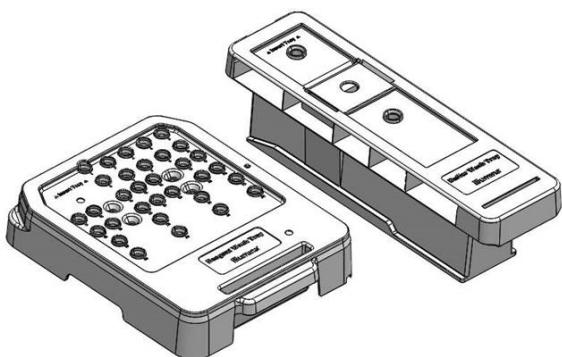

Figure 20   New Style Reagent Wash Cartridge and Buffer Wash Cartridge.

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 45.
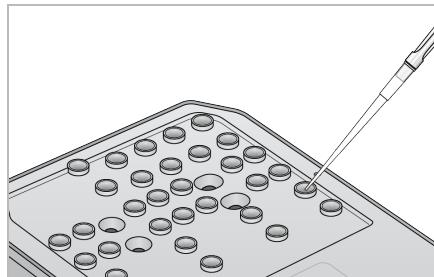
User-Supplied Consumables	Volume and Description
NaOCl	1 ml, diluted to 0.12% Loaded onto the reagent wash cartridge (position #28)
100% Tween 20 Laboratory-grade water	Used to make 125 ml 0.05% Tween 20 wash solution Loaded onto the buffer wash cartridge (center reservoir)

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:

FOR IN VITRO DIAGNOSTIC USE.

- 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 21   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 43.

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

⚠️ CAUTION

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

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.
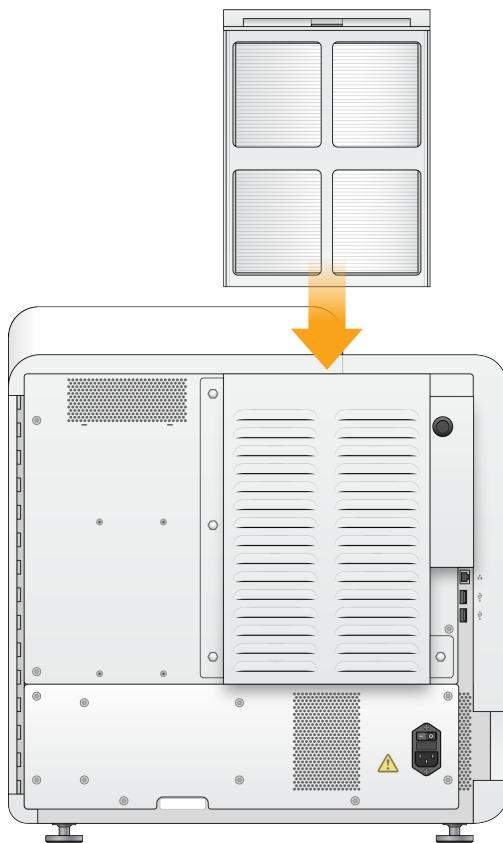
The air filter ensures air flow through the instrument. The software displays a notification to change the air filter every 90 days. When prompted, select **Remind in 1 day**, or follow the following procedure and select **Filter Changed**. The 90 day countdown resets following **Filter Changed** selection.

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 22   Air filter insertion

Local Run Manager Administrative Settings and Tasks

Introduction

The following settings and tasks require administrative user permissions:

- **Manage user accounts**—Adds users, assigns permissions, and sets passwords.
- **Edit NOS system settings**—Allows edits to the NOS through the Manage Instrument icon on the Home screen of the NextSeq 550Dx instrument.
- **Manage Local Run Manager system settings**—Sets system security parameters, maintenance intervals, and service accounts.
- **Relink the Run Folder**—If a run folder is deleted and then restored, allows linking of the run in the restored folder to be requeued for analysis.
- **View audit trails**—Monitors access frequency and user activity.
- Permissions to perform the following task are configurable in user management:
- **Reboot into research use only mode**—Allows reboot to research mode command which changes the system software to research (RUO) mode.
- **Edit module settings**—Allows configuration of module settings, such as the upload of manifest files for use with all runs using a specific analysis module.
- **Requeue analysis**—Allows editing of parameters and then rerunning analysis. See *Requeue or Stop Analysis* on page 39.

User Management

All user accounts are listed on the User Management page, which includes the first name, last name, and user name for each account. Within the details of each account, the role and associated permissions are managed. Two roles are possible, either Admin or User.

- **Admin role**—Admin roles have full permissions, by default.
- **User role**—User roles are configurable based on a subset of possible permissions. All users can create runs regardless of permission settings.

Only an admin user can view the User Management page.

NOTE   Create more than one Admin user account. If the sole admin user on the instrument is locked out, only Illumina Technical Support can unlock the instrument.

NOTE   Depending on the size of the screen you are viewing the software on, the **Tools** menu may be condensed underneath the ☰ menu in the upper left corner.

User Permissions

Permission	Description	Admin Role	User Role
Edit Local Run Manager System Settings	Set security, maintenance, and service accounts settings.	Allowed	Not allowed
Exit to Windows	Exit NOS and access instrument computer.	Allowed	Not allowed
Edit Module Settings	Upload manifest files for analysis modules.	Allowed	Allowed, if selected
Manage User Accounts	Create and edit user accounts.	Allowed	Not allowed
Minimize NOS and Access Computer	Minimize NOS and access instrument computer.	Allowed	Not allowed
Requeue Analysis	Reanalyze runs; edit analysis parameters.	Allowed	Allowed, if selected
Reboot to Research Use Only Mode	Change the instrument software to RUO mode.	Allowed	Allowed, if selected
View Audit Trails	View, filter, and export audit trails.	Allowed	Not allowed

Create a New User

1. From the dashboard navigation bar, select the **Tools** menu, and then select **User Management**.
2. From the User Management page, select **Create User**.
3. In the Create New User dialog box, enter the first and last name of the new user.
4. In the User Name field, enter a user name.
    User names must be unique and cannot be reused or edited later.
5. In the New Password field, enter a temporary password.
    Temporary passwords are not stored in the password history and can be reused.
6. In the Confirm Password field, reenter the temporary password.
7. To select a role, select **Admin** or **User** to toggle between options.
8. Select user permissions based on the specified user role.
9. Select **Create User**.

Reset User Password

1. From the dashboard navigation bar, select the **Tools** menu, and then select **User Management**.
2. Locate the user name you want to edit, and select the **Edit** ✏ icon.
3. In the New Password field, enter a temporary password.
   Temporary passwords are not stored in the password history and can be reused.
4. In the Confirm Password field, reenter the temporary password.
5. Select **Update User**.

Unlock User Password

1. From the dashboard navigation bar, select the **Tools** menu, and then select **User Management**.
2. Locate the user name you want to edit, and select the **Edit** ✏ icon.
3. From the Edit User dialog box, select 🔒 **Unlock User**.
4. In the New Password field, enter a temporary password.
5. In the Confirm Password field, reenter the temporary password.
6. Select **Update User**.

Change User Permissions

1. From the dashboard navigation bar, select the **Tools** menu, and then select **User Management**.
2. Locate the user name you want to edit, and select the **Edit** ✏ icon.
3. To change a role, select **Admin** or **User** to toggle between options.
4. Select user permissions based on the specified user role.
5. Select **Update User**.

Delete a User

1. From the dashboard navigation bar, select the **Tools** menu, and then select **User Management**.
2. Locate the user name you want to edit, and select the **Edit** ✏ icon.
3. In the Create New User dialog box, select **Delete User**.
   After you delete a user, you cannot create an account with that name again.
4. When prompted to delete the user, select **Delete**.

System Settings

System settings are global parameters for user security and automated data maintenance.

- User security settings include duration for password expiration, maximum login attempts, and duration for idle timeout.

- Data maintenance settings include automatic clean up of inactive run folders and database backup frequency, and includes a command for immediate database backup.
- Set analysis service and job service accounts for Windows if your output run folder is on a network path. The default is a local system account.

Only an admin user can view the System Settings page.

NOTE Depending on the size of the screen you are viewing the software on, the **Tools** menu may be condensed underneath the ≡ menu in the upper left corner.

Specify System Security Settings

1. From the dashboard navigation bar, select the **Tools** menu, and then select **System Settings.**
2. Select the Security tab.
3. Specify the number of days before a password expires and has to be reset.
4. Specify the number of days that a user is reminded to reset a password before it expires.
5. Specify the maximum number of attempts that the user can enter invalid login credentials before being locked out of the system.
6. Specify the number of minutes that the system can remain idle before the user is automatically logged out.
7. Select **Save**.

Specify System Maintenance Settings

1. From the dashboard navigation bar, select the **Tools** menu, and then select **System Settings.**
2. Select the Maintenance tab.
3. To enable automatic removal of inactive folders, select the **Enable Automatic Deletion** checkbox. When enabled, the system deletes inactive folders from the default analysis folder after the specified time.
4. If the Enable Automatic Deletion checkbox is selected, specify a number of days of inactivity before the automatic deletion command is activated.
5. To specify a database backup location, enter a preferred path to a backup location. To restore a database that has been backed up, contact Illumina technical support.
6. In the Backup Period field, enter the duration in days between each backup.
7. To create an immediate backup, select **Backup Now**.
8. Select **Save**.

Specify System Service Accounts Setting

1. From the dashboard navigation bar, select the **Tools** menu, and then select **System Settings**.

2. Select the Service Accounts tab.

3. To enable Windows Analysis Service and Job Service accounts, select **Windows Account**.
   Make sure that the service account you enter has permission to read and write to the output run
   folder.

4. In the User Name field, enter a user name.
   For instruments that run Windows 10 with a local account, include **.** in the username (eg,
   .\username). For domain users, include the domain name, then a backslash in the user name (eg,
   domain\username).

5. In the Password field, enter a password.
   The Windows 10 operating system requires a Windows password change every 180 days. Make
   sure to update the Windows Account in Local Run Manager with the same password as the
   Windows operating system.

6. Select **Save**.

Module Settings

The Module Settings page provides a list of installed analysis modules in the left navigation panel. Each
analysis module name opens a page that lists the module version and last modified date.

For modules that require a manifest, add manifest files to the module to make them available for all runs.
Admin user level permissions are required.

1. From the dashboard navigation bar, click the **Tools** menu, and then click **Module Settings**.

NOTE   Depending on the size of the screen you are viewing the software on, the **Tools** menu may be
        condensed underneath the upper left ☰ menu.

2. Click the name of the module in the left navigation panel.

3. Click **Add Manifest(s)**.

4. Navigate to the manifest file, select the manifest that you want to add, and click **Open**.

Audit Trails

Audit trails record information about specific actions, such as user access, user profile changes, and
changes to system, run, or analysis parameters. Each audit trail entry includes the following information:

• When, represented as date YYYY-MM-DD and time in 24-hour time format.

• Who, represented by the user name who initiated the action.

• What, represented by a short predefined description of the action taken.

• Affected Item, represented by 4 affected item categories of User, Run, Analysis, or System.

• To sort the audit trails list, select any column heading to sort in ascending or descending order.

Only an admin user can view the Audit Trails page.

NOTE Depending on the size of the screen you are viewing the software on, the **Tools** menu may be condensed underneath the upper left ☰ menu.

Filter Audit Trails

1. From the dashboard navigation bar, select the **Tools** menu, and then select **Audit Trails**.
2. From the Audit Trails page, select the Filter ▼ icon.

NOTE You can apply filters to previously filtered results to further refine the list. To apply a filter to the entire audit trails database, clear previous filters before proceeding.

Filter by Date

1. Select the Calendar 📅 icon and select a **From** date.
2. Select the Calendar 📅 icon and select a **To** date.
3. Select **Filter**.

Filter by User Name

1. In the Who field, enter a user name.
   You can enter any portion of a user name. Asterisks (*) are not required.
2. Select **Filter**.

Filter by Action

1. In the What field, enter an action description.
   You can enter any portion of an action description. Asterisks (*) are not required.
2. Select **Filter**.

Filter by Affected Item Description

1. In the Affected Item text field, enter any portion of the affected item description.
   The description can be a run name, user name, analysis module name, or report name, for example.
   You can enter any portion of a description. Asterisks (*) are not required.
2. Select **Filter**.

Filter by Affected Item Category

1. To filter by an affected item category, select the Category ❓ icon in the Affected Item field and select from the following options:

    *   👤 **User**—Filters the list to show user actions and user authentication actions.

    *   ⬛ **Run**—Filters the list to show changes to run parameters or run status

    *   🖼️ **Analysis**—Filters the list to show changes in analysis parameters or analysis status.

    *   ⚙️ **System**—Filters the list to show actions at the system level, such as file uploads, system maintenance, or security settings.

2. Select **Filter**.

Export Audit Trails

1. From the dashboard navigation bar, select the **Tools** menu, and then select **Audit Trails**.

2. From the Audit Trails page, select the Filter ▼ icon.

3. Apply preferred filters.

4. Select the Export ⬇️ icon.

    The software generates a report in PDF file format that includes the user name, date of export, and filter parameters.

Audit Trails Icons

The following icons are used on the Audit Trails screen.

Icon	Name	Description
🖼️	Analysis	Indicates a change in analysis parameters or analysis status.
⬛	Run	Indicates a change to run parameters or run status.
⚙️	System	Indicates a change to module settings or system settings.
👤	User	Indicates a user action or user authentication action.

Troubleshooting

Introduction

For run quality or performance problems, contact Illumina Technical Support. See *Technical Assistance on page 76*.

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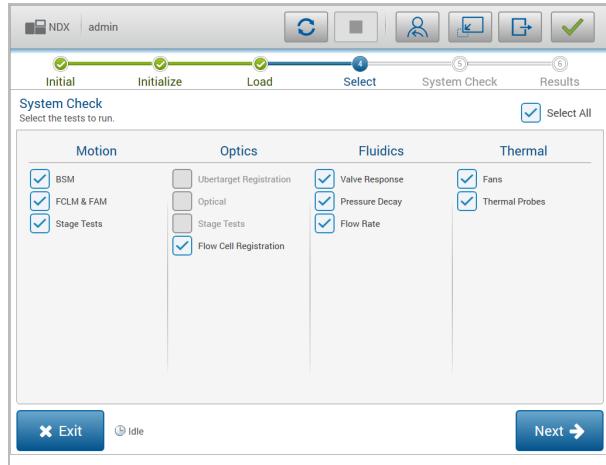
A system check is not required for normal operation or instrument maintenance. However, an Illumina Technical Support representative might ask you to perform a system check for troubleshooting purposes.

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

Starting a system check automatically closes the operating software and launches the NextSeq 550Dx Service Software (NSS). The service software launches and opens to the Load screen, which is configured to use the advanced loading option.

Local Run Manager admin user credentials need to be used to login to Service Software before the load screen appears.

Figure 23   Available System Checks

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

Perform a System Check

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

2. Load the consumables as follows:

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

    b. Empty the spent reagents container and return it to the instrument.

    c. Add 120 ml laboratory-grade water to the center reservoir of the buffer wash cartridge and load it.

    d. Load the reagent wash cartridge. Make sure that the reagent wash cartridge is empty and clean.

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

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

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

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

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

8. When finished, select **Exit**.

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

Motion Checks

System Check	Description
BSM	Checks the gain and distance of the Buffer Straw Mechanism (BSM) to confirm that the module is working properly.
FCLM & FAM	Checks the gain and distance of the Flow Cell Load Mechanism (FCLM) and Fluid Automation Module (FAM) to confirm that the modules are working properly.
Stage Tests	Checks the travel limits and performance of the XY-stage and 6 Z-stages, 1 for each camera.

Optics Check

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

Fluidics Checks

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

Thermal Checks

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

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.

Key File	Folder	Description
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.

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.

System Checks	Recommended Action
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.

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

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.

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.

Network Storage Error

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

- **Insufficient storage space for the output folder**—Increase the amount of space on the storage device or move the output folder to a location with enough storage.
- **Cannot connect to network storage**—Check the path to the output folder. See *Set Default Output Folder* on page 23.

- **The system cannot write to network storage**—Consult your IT administrator to check permissions. The Windows account on the operating system of the instrument requires permission to read and write to the output folder.

The Windows account in Local Run Manager also requires permission to read and write to the output folder. Refer to *Specify System Service Accounts Setting* on page 50.

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**.

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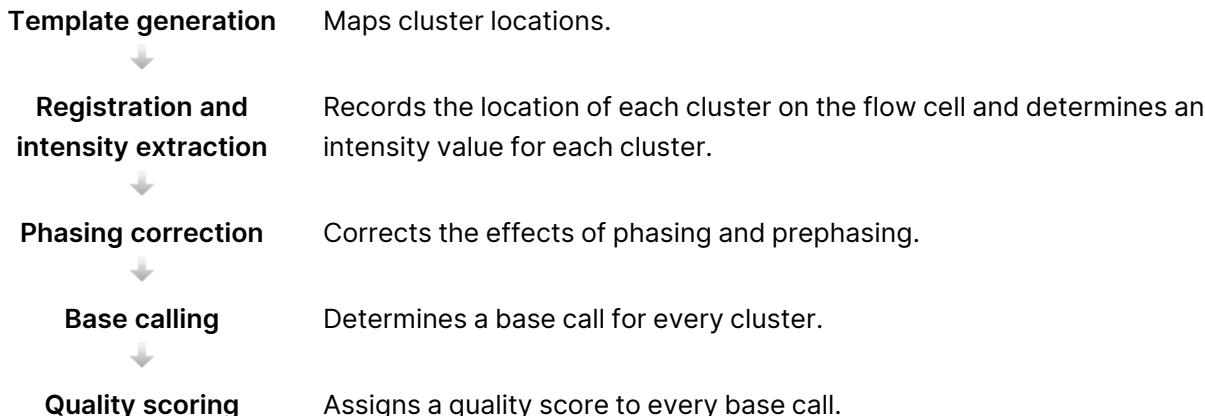
The following log and error files are transferred to the final output destination at the end of processing:

- *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.

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.

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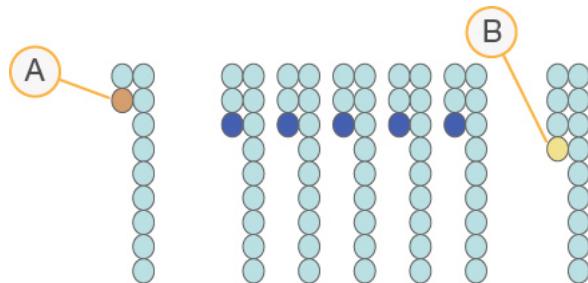
If registration fails for any images in a cycle, no base calls are generated for that tile in that cycle.

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 24   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 25   Visualization of Cluster Intensities
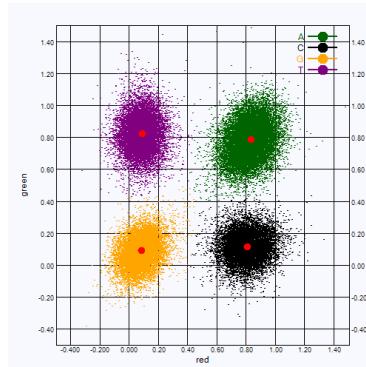
Table 1   Base Calls in 2-Channel Sequencing

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.

Output Files and Folders

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

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.
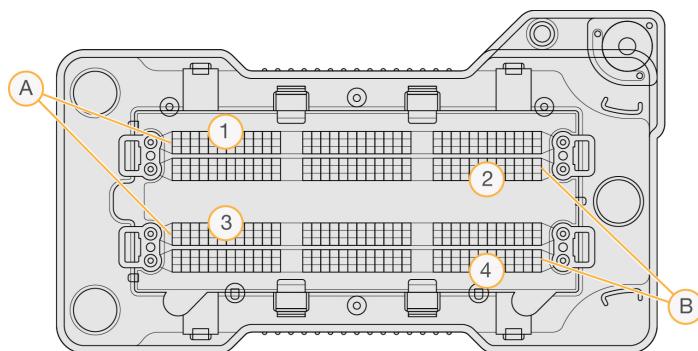
Table 2   Flow Cell Tiles

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.
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 26   Lane Numbering
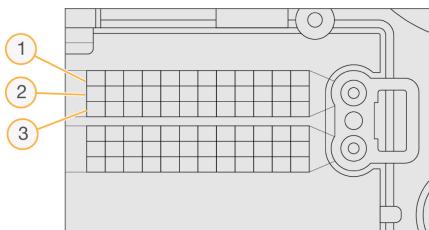
A. Lane Pair A—Lanes 1 and 3
B. Lane Pair B—Lanes 2 and 4

Swath Numbering

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

Figure 27   Swath Numbering
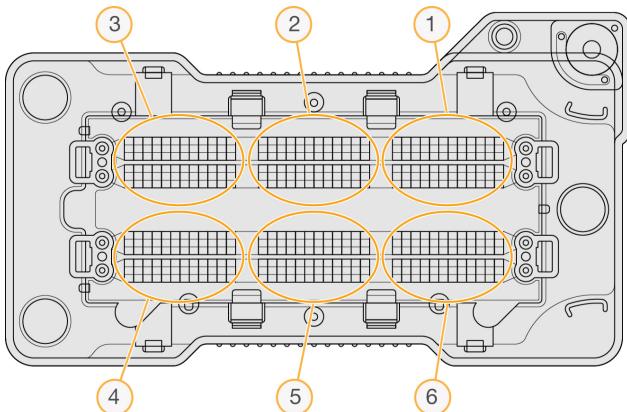
Camera Numbering

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.
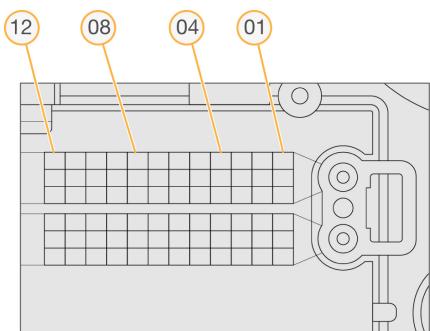
Figure 28   Camera and Segment Numbering (High output flow cell shown)

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 29   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 68.

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website:	www.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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