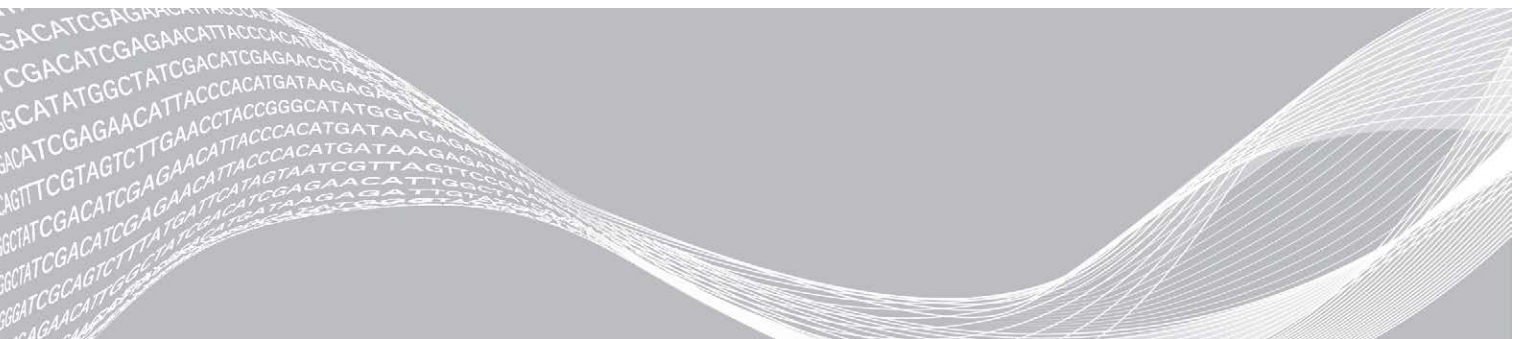


iSeq 100

Sequencing System Guide



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Revision History

Document	Date	Description of Change
Document # 1000000036024 v05	March 2019	<p>Updated software descriptions to iSeq Control Software v1.4:</p> <ul style="list-style-type: none"> • Updated instructions on configuring system settings, including moving and renaming some user interface elements. • Added descriptions of %Clusters PF and %Occupancy metrics, which appear on the Sequencing screen. • Allowed mapped network drive locations for the sample sheets and output folders. • Indicated that the software automatically renames sample sheets to SampleSheet.csv. <p>Added links to the following pages:</p> <ul style="list-style-type: none"> • The iSeq 100 System Sample Sheet Template for Manual Mode. • The bcl2fast Conversion Software support pages. <p>Added volumes of 1 nM 100% PhiX and AmpliSeq Library PLUS for Illumina libraries to prepare.</p> <p>Added instruction to move the reference genomes repository for Local Run Manager to a location other than drive C when restoring to system factory settings.</p> <p>Increased the maximum recommended cycles for Index Read 1 and Index Read 2 to 10 cycles each.</p> <p>Increased the number of cycles the cartridge supports to 322.</p> <p>Referenced the <i>Cluster Density Optimization Guide (document # 1000000071511)</i> for detailed information on optimizing loading concentration.</p> <p>Clarified that a cartridge being thawed in a water bath must be stored at -25°C to -15°C for at least one day before thawing.</p> <p>Corrected AmpliSeq for Illumina Library PLUS to AmpliSeq Library PLUS for Illumina.</p>
Document # 1000000036024 v04	October 2018	<p>Added recommended loading concentrations and dilution instructions for Nextera DNA Flex for Enrichment, TruSeq DNA Nano, and TruSeq DNA PCR-Free libraries.</p> <p>Added information on using a normalization method that does not result in single-stranded libraries.</p> <p>Added descriptions of the two run modes, Local Run Manager and Manual.</p> <p>Added a 5% PhiX spike-in option and defined the purpose of each spike-in percentage.</p> <p>Added the following steps:</p> <ul style="list-style-type: none"> • Switching to the sbsadmin operating system account when installing the control software, analysis modules, and other software. • Power cycling the instrument when restoring to factory settings. <p>Referenced <i>Illumina Adapter Sequences (document # 1000000002694)</i> to determine Index 2 (i5) orientations for a sample sheet.</p> <p>Clarified the following points:</p> <ul style="list-style-type: none"> • Cartridges must be used immediately after thawing. • The loading concentrations listed for Nextera DNA Flex and Nextera Flex for Enrichment libraries are not applicable to other Nextera library types. • SureCell WTA 3' is not a compatible library.

Document	Date	Description of Change
Document # 1000000036024 v03	August 2018	<p>Updated software descriptions to iSeq Control Software v1.3:</p> <ul style="list-style-type: none"> • Added configuration instructions for Universal Copy Service. • Renamed the Network Configuration tab to Network Access. • Added instructions on opening Local Run Manager from the control software. <p>Updated the default output folder location to D:\SequencingRuns. Added instructions on connecting the system to a proxy server. Added requirement to specify a UNC path for output folder and sample sheet locations on the network. Indicated the unique requirements for configuring an output folder location on an internal drive, external drive, or network location. Made instructions on creating a sample sheet for Manual mode the first step of run setup. Corrected instructions on using the system suite installation wizard. Corrected the description of output thumbnail files.</p>
Document # 1000000036024 v02	June 2018	<p>Updated tubes used for diluting libraries to Fisher Scientific catalog # 14-222-158 or equivalent low-bind tubes. Added a section describing the regional availability of Advanced Exchange. Clarified that libraries diluted to the loading concentration must be sequenced that day. Clarified that the reagent cartridge must be removed from the box for thawing.</p>
Document # 1000000036024 v01	May 2018	<p>Updated software descriptions to iSeq Control Software v1.2:</p> <ul style="list-style-type: none"> • Added option to browse to a downloaded software installer from the control software. • Added instructions for saving thumbnails. • Moved network settings to the Network Configuration tab. • Increased maximum uses of reusable test components to 36 and noted that the remaining number of uses appear onscreen. <p>Updated Local Run Manager information:</p> <ul style="list-style-type: none"> • Added steps for opening Local Run Manager and setting up the run. • Added RNA Amplicon as a preinstalled analysis module, and DNA Enrichment and Resequencing as other supported modules. • Updated documentation references to <i>Local Run Manager Software Guide (document #1000000002702)</i>. <p>Updated cartridge thawing instructions:</p> <ul style="list-style-type: none"> • Added a room-temperature thawing option. • Provided more detailed water bath instructions, including storing before thawing. <p>Updated instructions on preparing libraries for sequencing:</p> <ul style="list-style-type: none"> • Updated the Nextera DNA Flex loading concentration to 200 pM. • Added starting loading concentration for unlisted library types. • Added information on the %Occupied metric. • Increased volume of 1 nM PhiX for a spike-in to 50 µl. <p>Updated Illumina catalog numbers for:</p> <ul style="list-style-type: none"> • iSeq 100 Spare Drip Tray Pad to 20023927. • iSeq 100 Spare Air Filter to 20023928. <p>Updated pipette and pipette tip recommendations.</p>

Document	Date	Description of Change
Document # 1000000036024 v01	May 2018	<p>Added the following miscellaneous instructions:</p> <ul style="list-style-type: none"> • Performing validation runs. • Creating a sample sheet when sequencing in Manual mode. • Minimizing the control software to access other applications. <p>Added the following steps to the system check procedure:</p> <ul style="list-style-type: none"> • Unloading and storing reusable test components. • Cleaning visible debris from the reusable test flow cell. <p>Reorganized the following content to improve continuity:</p> <ul style="list-style-type: none"> • Merged instructions for performing a PhiX-only run with the standard sequencing instructions. • Merged flow cell preparation instructions with library dilution instructions. • Consolidated PhiX spike-in instructions. • Moved information on number of cycles in a read. • Moved Real-Time Analysis and renamed to <i>Sequencing Output</i>. <p>Simplified the error messages workflow diagram.</p> <p>Removed information on Tablet and Desktop modes. The operating system runs in Desktop mode by default and Tablet mode is not necessary.</p> <p>Removed requirement to complete and return a decontamination certificate for advanced exchange.</p> <p>Corrected average run size to 2 GB.</p>
Document # 1000000036024 v00	February 2018	Initial release.

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Chapter 1 Overview

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Introduction

The Illumina® iSeq™ 100 Sequencing System provides a targeted approach to next-generation sequencing (NGS). This applications-focused system packages Illumina sequencing technology into a cost-effective desktop instrument.

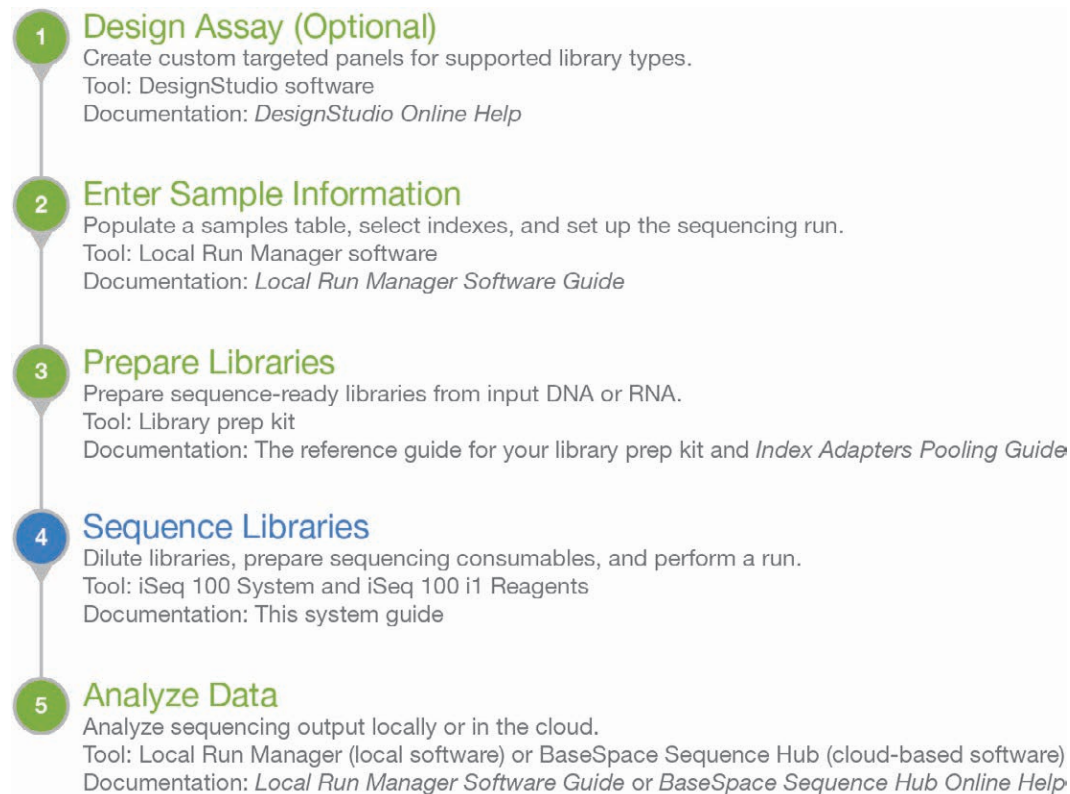
Features

- ▶ **Accessibility and reliability**—The iSeq 100 System has a small footprint and is easy to install and use. Fluidics and imaging components are built into the consumable, simplifying instrument maintenance.
- ▶ **Single-step consumable loading**—A single-use cartridge is prefilled with all reagents required for a run. Library and a sensor-equipped flow cell load directly into the cartridge, which is then loaded onto the instrument. Integrated identification enables accurate tracking.
- ▶ **iSeq 100 System software**—A suite of integrated software controls instrument operations, processes images, and generates base calls. This suite features on-instrument data analysis and data transfer tools for external analysis.
 - ▶ **On-instrument analysis**—Local Run Manager inputs sample information and then analyzes run data using the analysis module specified for the run. The software includes a suite of analysis modules.
 - ▶ **Cloud-based analysis**—The sequencing workflow is integrated with BaseSpace Sequence Hub, the Illumina cloud computing environment for run monitoring, data analysis, storage, and collaboration. Output files are streamed in real time to BaseSpace Sequence Hub for analysis.

Sample to Analysis

The following diagram illustrates the complete sequencing workflow, from experimental design to analyzing data. Tools and documentation are included with each step. This guide covers the sequence libraries step. For other documentation, visit support.illumina.com.

Figure 1 Sample to Analysis Workflow



Additional Resources

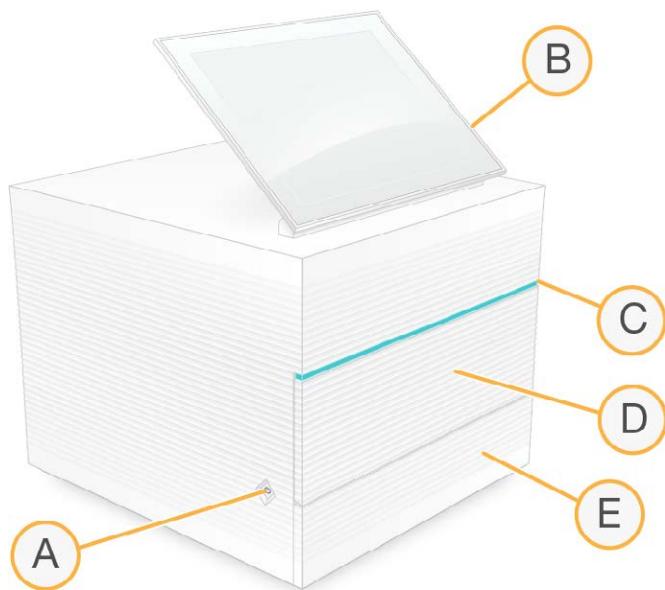
The [iSeq 100 Sequencing System support pages](#) on the Illumina website provide additional system resources. These resources include software, training, compatible products, and the following documentation. Always check support pages for the latest versions.

Resource	Description
Custom Protocol Selector	A tool for generating end-to-end instructions tailored to your library prep method, run parameters, and analysis method, with options to refine the level of detail.
iSeq 100 Sequencing System Setup Poster (document # 1000000035963)	Provides instructions for instrument installation and initiating first time setup.
iSeq 100 Sequencing System Site Prep Guide (document # 1000000035337)	Provides specifications for laboratory space, electrical requirements, and environmental and network considerations.
iSeq 100 Sequencing System Safety and Compliance Guide (document # 1000000035336)	Provides information about operational safety considerations, compliance statements, and instrument labeling.
RFID Reader Compliance Guide (document # 1000000002699)	Provides information about the RFID reader in the instrument, including compliance certifications and safety considerations.

Instrument Components

iSeq 100 Sequencing System comprises a power button, monitor, status bar, consumables compartment, and drip tray.

Figure 2 External System Components



- A **Power button**—Controls instrument power and indicates whether the system is on (glows), off (dark), or off but with AC power (pulses).
- B **Touch-screen monitor**—Enables on-instrument configuration and setup using the control software interface.
- C **Status bar**—Indicates system status as ready to sequence (green), processing (blue), or needs attention (orange).
- D **Consumables compartment**—Contains consumables during a run.
- E **Drip tray door**—Provides access to the drip tray, which catches leaked fluids.

Power and Auxiliary Connections

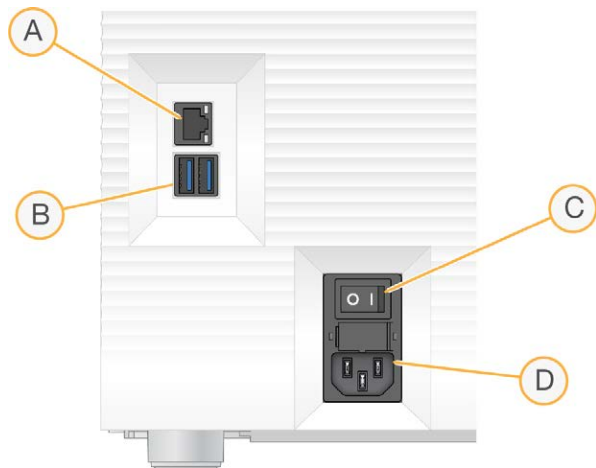
You can move the instrument to access USB ports and other rear panel components.

The back of the instrument has the switch and inlet that controls power to the instrument, and an Ethernet port for an optional Ethernet connection. Two USB ports provide the option to connect a mouse and keyboard, or to upload and download data using a portable device.



NOTE

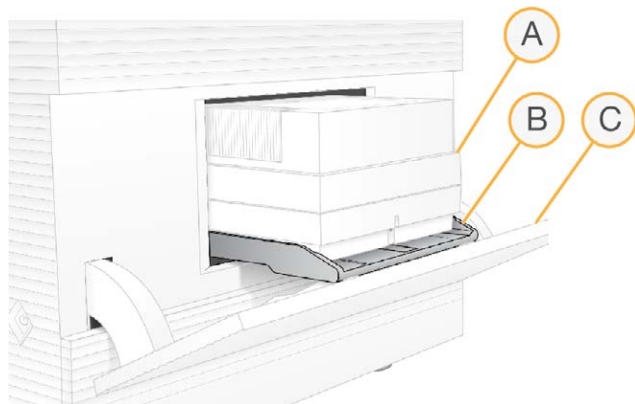
Connecting the system to a keyboard and mouse overrides the onscreen keyboard.

Figure 3 Rear Panel Components

- A **Ethernet port**—Optional Ethernet cable connection.
- B **USB ports**—Two ports for connecting auxiliary components.
- C **Toggle switch**—Turns instrument power on and off.
- D **AC power inlet**—Power cord connection.

Consumables Compartment

The consumables compartment contains the cartridge for a sequencing run.

Figure 4 Loaded Consumables Compartment

- A **Cartridge**—Contains the flow cell, library, and reagents, and collects used reagents during the run.
- B **Tray**—Holds the cartridge during sequencing.
- C **Door**—Opens to a 60 degree angle to provide access to the consumables compartment.

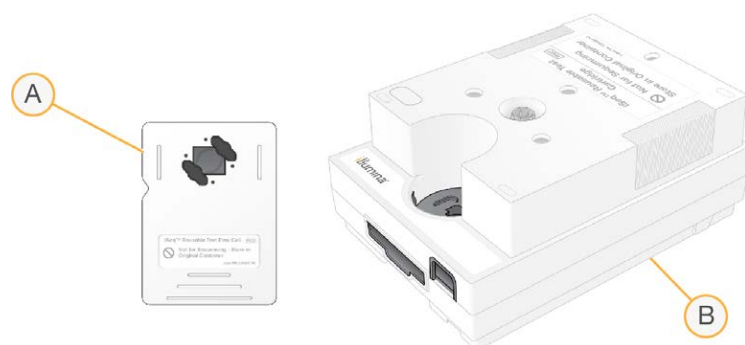
The software opens and closes the compartment door and positions the cartridge for imaging. The door opens down from hinges toward the base of the instrument. Do not place objects on the open door, which is not designed for use as a shelf.

Reusable Test Flow Cell and Cartridge

The instrument ships with the iSeq 100 Reusable Test Flow Cell and iSeq 100 Reusable Test Cartridge to use for system checks.

- ▶ Store in the original packaging at room temperature.
- ▶ Reuse up to 36 times or for 5 years from the date of manufacture, whichever comes first.
 - ▶ During a system check, the software displays the remaining number of uses.
 - ▶ Replace expired reusable test components with the iSeq 100 System Test Kit.

Figure 5 Reusable Test Components



- A Reusable test flow cell
- B Reusable test cartridge

The reusable test components look similar to the sequencing components provided in iSeq 100 i1 Reagent, and the loading orientations are the same. However, the test cartridge does not have a library reservoir and neither test component has the chemistry needed for a run.

System Software

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

- ▶ **iSeq Control Software**—Controls instrument operations and provides an interface for configuring the system, setting up a sequencing run, and monitoring run statistics as sequencing progresses.
- ▶ **Local Run Manager**—Defines run parameters and the analysis method before sequencing. After sequencing, on-instrument data analysis begins automatically.
 - ▶ The system is shipped with the DNA Amplicon, RNA Amplicon, and Generate FASTQ analysis modules installed.
 - ▶ The system also supports the DNA Enrichment and Resequencing analysis modules, which are available on the [Local Run Manager support pages](#).
 - ▶ For more information on Local Run Manager and the analysis modules, see the *Local Run Manager Software Guide (document # 1000000002702)*.
- ▶ **Real-Time Analysis (RTA2)**—Performs image analysis and base calling during the run. For more information, see [Sequencing Output on page 38](#).
- ▶ **Universal Copy Service**—Copies sequencing output files from the run folder to BaseSpace Sequence Hub (if applicable) and the output folder, where you can access them.

Real-Time Analysis and Universal Copy Service run background processes only. Local Run Manager and the control software can require user input.

System Information

The control software menu has an About section where you can view Illumina contact information and the following system information:

- ▶ Serial number
- ▶ Computer name and IP address
- ▶ Recipe fragment version
- ▶ Run count

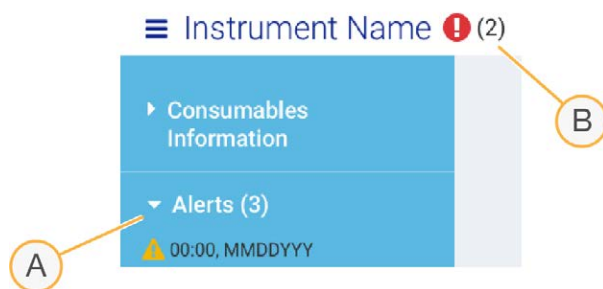
Notifications and Alerts

An icon appears next to the instrument name to indicate notifications. Select the icon to view a list of the notifications, which include warnings and errors.

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

A panel on the left side of run setup screens displays alerts specific to cartridge loading and pre-run checks.

Figure 6 Onscreen Locations



- A Run setup alerts
- B Other notifications

Process Management

The Process Management screen displays hard drive (drive D) space and run status, identifying each run by name, ID, and date. The screen is automatically refreshed every three minutes.

The Status column indicates whether a run is progressing or complete, based on the processing of BCL files. For each run, Process Management also displays the status of background processes Universal Copy Service, BaseSpace Sequence Hub, and Local Run Manager.

Nonapplicable processes do not appear on the screen. For example, if a run is not connected to BaseSpace Sequence Hub, Process Management does not display the BaseSpace status for that run.

- ▶ To troubleshoot status issues, see [Process Management Status on page 44](#).
- ▶ To delete runs and clear space, see [Clear Hard Drive Space on page 31](#).

Status of Universal Copy Service

Universal Copy Service shows the status of files being copied to the output folder:

- ▶ **In Progress**—Universal Copy Service is copying files to the output folder.

- ▶ **Complete**—Universal Copy Services successfully copied all files to the output folder.

Status of BaseSpace Sequence Hub

BaseSpace Sequence Hub shows the upload status:

- ▶ **In Progress**—The control software is uploading files to BaseSpace Sequence Hub.
- ▶ **Complete**—All files are successfully uploaded to BaseSpace Sequence Hub.

Status of Local Run Manager

Local Run Manager shows the analysis status in control software:

- ▶ **Not Started**—The analysis is queued to start or Local Run Manager is waiting for Real-Time Analysis to finish.
- ▶ **In Progress**—Local Run Manager is analyzing files. Check the Local Run Manager software for a more detailed status.
- ▶ **Stopped**—The analysis has stopped, but is incomplete.
- ▶ **Complete**—Local Run Manager has successfully completed the analysis.

For more information on analysis status, check the Local Run Manager software.

iSeq 100 i1 Reagent

Performing a run on the iSeq 100 System requires one single-use iSeq 100 i1 Reagent kit. The kit is available in one size (300-cycle) and two bundles:

- ▶ **Single**—Includes consumables for one run.
- ▶ **Four-pack**—Includes consumables for four runs.

Contents and Storage

The iSeq 100 i1 Reagent kit provides the cartridge and flow cell for sequencing. When you receive the kit:

- ▶ Do not open the white foil packaging until instructed to do so during the cartridge thawing and flow cell preparation procedures. The cartridge is thawed in the bag.
- ▶ Promptly store components at the indicated temperatures to ensure proper performance.
- ▶ Store cartridges so that the package labels face up.
- ▶ Store cartridges for at least one day before thawing in a water bath.

Table 1 Kit Components

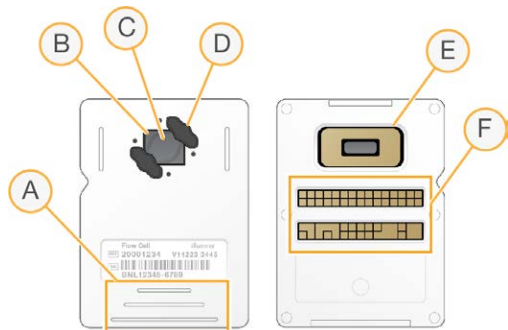
Bundle	Consumable	Quantity	Storage Temperature
Single	Cartridge	1	-25°C to -15°C
	Flow cell	1	2°C to 8°C*
Four-pack	Cartridge	4	-25°C to -15°C
	Flow cell	4	2°C to 8°C*

*Shipped at room temperature.

Both consumables have identifiers for tracking and ensuring compatibility. The cartridge uses radio-frequency identification (RFID). The flow cell uses an electrical interface: electrically erasable programmable read-only memory (EEPROM).

Flow Cell

The iSeq 100 i1 Flow Cell is a patterned, single-lane flow cell built over a complementary metal oxide semiconductor (CMOS) optical sensor. A plastic cartridge encases the glass-based flow cell. Raised grip points on the plastic ensure safe handling.

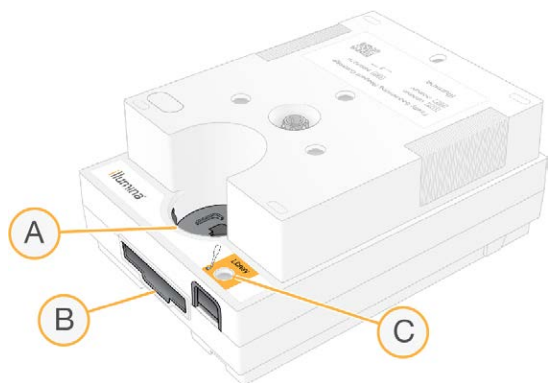


- A Grip points
- B CMOS sensor (top)
- C Imaging area
- D Gasket (one of two)
- E CMOS sensor (bottom)
- F Electrical interface

Millions of nanowells cover the surface of the flow cell. Clusters are generated in the nanowells, from which the sequencing reaction is then performed. The ordered arrangement of the nanowells increases output reads and data. During sequencing, the CMOS sensor captures images for analysis.

Cartridge

The iSeq 100 i1 Cartridge is prefilled with clustering, sequencing, paired-end, and indexing reagents. A foil-sealed reservoir is reserved for libraries, and a slot on the front is reserved for the flow cell. Illuminator light reaches the flow cell through an access window on top of the cartridge.



- A Access window
- B Flow cell slot
- C Library reservoir

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

An underside reservoir collects used reagents. The cartridge also contains pumps, valves, and all other fluidics for the system. Because the cartridge is discarded after a run, instrument washes are not necessary.







Supported Number of Cycles




The 300-cycle label on the cartridge indicates how many cycles are analyzed, not how many cycles are performed. The flow cell is compatible with any number of cycles and any read type.

The cartridge provides sufficient reagent for up to 322 cycles of sequencing. The 322 cycles include 151 cycles each for Read 1 and Read 2, plus up to 10 cycles each for Index 1 and Index 2. For information on how many cycles to sequence, see [Recommended Number of Cycles on page 19](#).

Symbol Descriptions

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

Symbol	Description
	The date the consumable expires. For best results, use the consumable before this date.
	Indicates the manufacturer (Illumina).
	The date when the consumable was manufactured.
	The intended use is Research Use Only (RUO).
	Indicates the part number so that the consumable can be identified.*
	Indicates the batch code to identify the batch or lot that the consumable was manufactured in.*

Symbol	Description
	Indicates that caution is necessary.
	Indicates a health hazard.
	Storage temperature range in degrees Celsius. Store the consumable within the indicated range.

* REF identifies the individual component, while LOT identifies the lot or batch the component belongs to.

Chapter 2 Getting Started

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First Time Setup

The first time the system is turned on, the control software is launched with a series of screens to guide you through first time setup. First time setup includes performing a system check to confirm instrument performance and configuring system settings.

If you want to modify system settings after first time setup, select the System Settings command in the control software. The command opens the Settings, Network Access, and Customization tabs, where you can access all control software settings and Windows network settings.

Operating System Accounts

The Windows operating system has two accounts: administrator (sbsadmin) and user (sbsuser).

The administrator account is intended for IT use, system updates, and installing the control software, Local Run Manager analysis modules, and other software. Perform all other functions, including sequencing, from the user account.

Password Requirements

The operating system requires a password change for both accounts (sbsadmin and sbsuser) at the first logon. Update each password every 180 days, when prompted.

Table 2 Default Password Policies

Policy	Setting
Enforce password history	Five passwords remembered
Lockout threshold	Ten invalid logon attempts
Minimum password length	Ten characters
Password must meet complexity requirements	Disabled
Store passwords using reversible encryption	Disabled

Validation Runs

Optionally perform a validation run before sequencing experimental libraries for the first time. A validation run sequences 100% PhiX, which functions as a control library, to confirm system operation. For instructions, see [Sequencing on page 19](#).

Minimize the Control Software

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

- 1 Swipe up on the touch screen to open the Windows taskbar.

- 2 Select the **iSeq 100 System** icon or another application.
The control software is minimized.
- 3 **[Optional]** Attach a keyboard and mouse to the instrument to facilitate navigation and typing outside of the control software.
- 4 To maximize the control software, swipe up and select **iSeq 100 System**.

Run Settings

Configure options for run setup, run monitoring, and data analysis on the Settings tab in System Settings. This tab displays recommended express settings, which you can apply by selecting the express setup option. Alternatively, select the manual setup option to customize the settings.

Choosing express settings applies the following settings and sends InterOp files, log files, instrument performance data, and run data to BaseSpace Sequence Hub:

- ▶ **Illumina Proactive Support**—Facilitate troubleshooting and detect potential failures, allowing proactive maintenance and maximizing instrument uptime. Turning on Illumina Proactive Support sends instrument performance data (not sequencing data) to BaseSpace Sequence Hub. For more information, see the *Illumina Proactive Technical Note (document # 1000000052503)*.
- ▶ **Local Run Manager**—Use the Local Run Manager software to create runs and analyze run data for a simple, streamlined workflow. Separate sample sheets and analysis applications are not needed.
- ▶ **Remote Run Monitoring**—Use BaseSpace Sequence Hub for remote run monitoring.
- ▶ **Run Analysis, Collaboration, and Storage**—Use BaseSpace Sequence Hub to store and analyze run data and collaborate with colleagues.



NOTE

Local Run Manager automatically starts analysis when the run is complete. However, you can also analyze data in BaseSpace Sequence Hub.

Apply Express Settings

Express setup replaces the current run settings with recommended run settings and localized settings for BaseSpace Sequence Hub. These settings require an internet connection and a BaseSpace Sequence Hub account. For account setup instructions, see the *BaseSpace Sequence Hub Online Help (document # 1000000009008)*.

- 1 On the control software menu, select **System Settings**.
- 2 On the Settings tab, select **Use Express Settings**.
- 3 In the Set Region list, select the geographic location where the system resides or the location closest to where the system resides.
This setting ensures that data are stored in the appropriate location for BaseSpace Sequence Hub.
- 4 If you have an Enterprise subscription, in the Enter Private Domain field, enter the domain name (URL) used for single sign-on to BaseSpace Sequence Hub.
For example: <https://yourlab.basespace.illumina.com>.
- 5 Select **Next**.
- 6 Review the settings. To modify a setting:
 - a Select **Edit** to open the setting.

- b Modify the setting as needed, and then select **Next**.
- c Select **Next** to continue through any subsequent screens.

On the Settings Review screen, a green checkmark indicates enabled settings.

- 7 Select **Save**.
- 8 To close System Settings, select **Exit**.

Manually Configure Settings

Manual setup guides you through each screen on the Settings tab to configure run settings, which have the following requirements:

- ▶ Turning on Illumina Proactive Support and BaseSpace Sequence Hub requires an internet connection. BaseSpace Sequence Hub also requires an account. For account setup instructions, see the *BaseSpace Sequence Hub Online Help (document # 100000009008)*.
- ▶ Using BaseSpace Sequence Hub for data analysis when the system is configured for Manual mode requires a sample sheet. For details, see *Sample Sheet Requirements on page 14*.

1 On the control software menu, select **System Settings**.

2 Select **Set Up Manually**.

3 Choose whether to enable to the Illumina Proactive Support service:

- ▶ To turn it on, select the **Turn on Illumina Proactive Support** checkbox.
- ▶ To turn it off, deselect the **Turn on Illumina Proactive Support** checkbox.

The service sends instrument performance data, such as temperature and run time, to Illumina. These data help Illumina detect potential failures and facilitate troubleshooting. Run data are not sent. For more information, see the *Illumina Proactive Technical Note (document # 1000000052503)*.

4 Select **Next**.

5 Choose whether to connect runs to BaseSpace Sequence Hub:

- ▶ To connect runs, select one of the following checkboxes:
 - ▶ **Turn on run monitoring from anywhere only**—Use BaseSpace Sequence Hub for remote monitoring.
 - ▶ **Turn on run analysis, collaboration, and storage also**—Use BaseSpace Sequence Hub for remote monitoring and analysis.
- ▶ To disconnect runs, deselect the **Turn on run monitoring from anywhere only** and **Turn on run analysis, collaboration, and storage also** checkboxes.

When connected, the control software sends InterOp and log files to BaseSpace Sequence Hub. The run analysis, collaboration, and storage option also sends run data.

6 In the Set Region list, select the geographic location where the system resides or the location closest to where the system resides.

This setting ensures that data are stored in the appropriate location for BaseSpace Sequence Hub.

7 If you have an Enterprise subscription, in the Enter Private Domain field, enter the domain name (URL) used for single sign-on to BaseSpace Sequence Hub.

For example: <https://yourlab.basespace.illumina.com>.

8 Select **Next**.

- 9 Choose whether to integrate the control software with Local Run Manager:
 - ▶ To create runs and analyze data in Local Run Manager, select **Use Local Run Manager**.
 - ▶ To create runs in the control software and analyze data in another application, select **Use Manual Mode**.

Local Run Manager provides the most streamlined workflow, but it is not a feature of the control software. It is integrated software for recording samples for sequencing, creating runs, and analyzing data. Before sequencing, review the *Local Run Manager Software Guide (document # 1000000002702)*.

- 10 Select **Next**.
- 11 Review the settings. To modify a setting:
 - a Select **Edit** to open the setting.
 - b Modify the setting as needed, and then select **Next**.
 - c Select **Next** to continue through any subsequent screens.

On the Settings Review screen, a green checkmark indicates enabled settings.

- 12 Select **Save**.
- 13 To close System Settings, select **Exit**.

Sample Sheet Requirements

When the system is configured for Manual mode and you are analyzing data in BaseSpace Sequence Hub, each run requires a sample sheet. Create a sample sheet by editing the *iSeq 100 System Sample Sheet Template for Manual Mode*, then importing it into the control software during run setup. After import, the software automatically renames the sample sheet to **SampleSheet.csv**.

Download the sample sheet template from the iSeq 100 Sequencing System support pages: [iSeq 100 System Sample Sheet Template for Manual Mode](#).



CAUTION

Enter the Index 2 (i5) adapter sequences in the correct orientation for the iSeq 100 Sequencing System. For index orientation, see *Illumina Adapter Sequences (document # 1000000002694)*.

A sample sheet is also required when the system is configured for Local Run Manager mode. However, Local Run Manager creates the sample sheet for you and saves it to the appropriate location. For all other circumstances, a sample sheet is optional.

Instrument Customization

Name your instrument and configure settings for audio, thumbnail images, and software updates on the Customization tab in System Settings.

Name the Instrument

- 1 On the control software menu, select **System Settings**.
- 2 Select the Customization tab.
- 3 In the Instrument Nickname field, enter a preferred name for the instrument. The name appears along the top of each screen.
- 4 Select **Save**.
- 5 To close System Settings, select **Exit**.

Turn Audio On or Off

- 1 On the control software menu, select **System Settings**.
- 2 Select the Customization tab.
- 3 Choose whether to mute the system:
 - ▶ To turn off the audio, select **Off**.
 - ▶ To turn on the audio, select **On**.
- 4 Select **Save**.
- 5 To close System Settings, select **Exit**.

Save Thumbnails

- 1 On the control software menu, select **System Settings**.
- 2 Select the Customization tab.
- 3 Choose whether to save thumbnail images:
 - ▶ To save all thumbnails, select the **Save all thumbnail images** checkbox.
 - ▶ To save no thumbnails, deselect the **Save all thumbnail images** checkbox.

Saving thumbnail images helps with troubleshooting, but minimally increases run size. By default, all thumbnail images are saved.
- 4 Select **Save**.
- 5 To close System Settings, select **Exit**.

Configure Software Updates

The system can automatically check for and download software updates for you to install, or you can check manually. For more information, see [Software Updates on page 31](#).

- 1 On the control software menu, select **System Settings**.
- 2 Select the Customization tab.
- 3 Choose whether the system automatically checks for software updates:
 - ▶ To check automatically, select the **Autocheck for software updates** checkbox.
 - ▶ To check manually, deselect the **Autocheck for software updates** checkbox.

Autocheck requires an internet connection.
- 4 Select **Save**.
- 5 To close System Settings, select **Exit**.

Network Setup

Operating the system and transferring data requires only a WiFi or Ethernet connection with the default network settings. These settings do not need updating unless your organization has custom network requirements. If it does, consult your IT representative for help changing the default network settings.

The *iSeq 100 Sequencing System Site Prep Guide (document # 1000000035337)* provides guidelines on network settings and control computer security.

Specify the Output Folder Location

Universal Copy Service copies sequencing output files from the run folder to BaseSpace Sequence Hub (when applicable) and the output folder, where you can access them.

An output folder is required unless the system is configured for run monitoring, analysis, collaboration, and storage using BaseSpace Sequence Hub. If an output folder location is not specified, Universal Copy Service copies files to D:\SequencingRuns.

- 1 On the control software menu, select **System Settings**.
- 2 Select the Network Access tab.
- 3 In the Output Folder field, enter a default location for the output folder or select **Browse** to navigate to a location.
 - ▶ **Internal drive**—Enter an existing location on drive D. Drive C lacks sufficient space.
 - ▶ **External drive**—Enter the location of a USB drive connected to the instrument.
 - ▶ **Network location**—Enter a network location.

You can change the default location on a per-run basis.
- 4 Proceed as follows.
 - ▶ If you specified an internal or external drive location, select **Save** and then **Exit** to save the location and close System Settings.
 - ▶ If you specified a network location, continue through steps 5–8 to connect Universal Copy Service to an account that has access to the specified location.
- 5 Under Universal Copy Service, select an account type:
 - ▶ **Local System Account**—The output folder resides in a directory that is accessible with a local account, which has access to most local locations.
 - ▶ **Network Account**—The output folder resides in a directory that requires logon credentials. This setting applies to the default output folder location and any location specified during run setup.
- 6 If you selected Network Account, enter the user name and password for the account.
- 7 Select **Save**.
- 8 To close System Settings, select **Exit**.

Connect to the Internet

Configure a WiFi or Ethernet internet connection in Windows Network & Internet settings, which you can open from the control software. The default Ethernet connection transfers data more reliably.

- 1 On the control software menu, select **System Settings**.
- 2 Select the Network Access tab.
- 3 Select **Network Configuration**, which minimizes the control software and opens Windows Network & Internet settings.
- 4 Configure a WiFi or Ethernet connection.
 - ▶ If configuring WiFi, change the adapter option to **Wi-Fi**.
 - ▶ For detailed configuration instructions, see the Windows 10 help on the Microsoft website.
- 5 When configuration is complete, close Windows settings and maximize the control software.
- 6 On the Network Access tab, select **Save**.
- 7 To close System Settings, select **Exit**.

Connect to a Proxy Server

- 1 Minimize the control software.
- 2 From Windows Start, open the Run dialog box.
- 3 Type **cmd**, and then select **OK**.
- 4 Type the following command:

```
C:\windows\System32\bitsadmin.exe /Util /SetIEProxy LocalSystem Manual_proxy http://<proxyserver>:<proxy port> NULL
```
- 5 Replace `http://<proxyserver>:<proxy port>` with your proxy server address and proxy port, and `NULL` with any bypasses.
- 6 Press Enter to run the command.
- 7 Power cycle the instrument. For instructions, see *Power Cycle the Instrument* on page 45.

User-Supplied Consumables and Equipment

The following user-supplied consumables and equipment are used for sequencing, maintenance, and troubleshooting.

Consumables for Sequencing

Consumable	Supplier	Purpose
Disposable gloves, powder-free	General lab supplier	General purpose.
iSeq 100 i1 Reagent	Illumina, catalog #: <ul style="list-style-type: none"> • 20021533 (single) • 20021534 (four-pack) 	Provides the reagents and flow cell for a run.
Microtubes, 1.5 ml	Fisher Scientific, catalog # 14-222-158, or equivalent low-bind tubes	Diluting libraries to the loading concentration.
Paper towels	General lab supplier	Drying the cartridge after a water bath.
Pipette tips, 20 µl	General lab supplier	Diluting and loading libraries.
Pipette tips, 100 µl	General lab supplier	Diluting and loading libraries.
Resuspension Buffer (RSB)	Illumina, provided with library prep kits	Diluting libraries to the loading concentration.
[Optional] 10 mM Tris-HCl, pH 8.5	General lab supplier	Substitute for RSB to dilute libraries to the loading concentration.
[Optional] PhiX Control v3	Illumina, catalog # FC-110-3001	Performing a PhiX-only run or spiking in a PhiX control.

Consumables for Maintenance and Troubleshooting

Consumable	Supplier	Purpose
Bleach wipes, 10%	WWR, catalog # 16200-218, or equivalent	Decontaminating the instrument and cleaning work surfaces.
Disposable gloves, powder-free	General lab supplier	General purpose.

Consumable	Supplier	Purpose
iSeq 100 i1 Spare Drip Tray Pad ¹	Illumina, catalog # 20023927	Lining the drip tray to absorb any leaked fluids.
iSeq 100 i1 Spare Air Filter ¹	Illumina, catalog # 20023928	Replacing the air filter every six months.
iSeq 100 System Test Kit ²	Illumina, catalog # 20024141	Performing a system check.
Isopropyl alcohol wipes, 70%	WWR, catalog # 95041-714, or equivalent	Cleaning the instrument and reusable test flow cell.
Lab tissue, low-lint	WWR, catalog # 21905-026, or equivalent	Drying the drip tray and reusable test flow cell.
Paper towels	General lab supplier	Drying fluid around the instrument.
[Optional] Bleach solution, 10%	WWR, catalog # 16003-740 (32 oz), 16003-742 (16 oz), or equivalent	Cleaning work surfaces after decontamination.
[Optional] Ethanol wipes, 70%	Fisher Scientific, catalog # 19-037-876, or equivalent	Substitute for isopropyl alcohol wipes to clean the instrument and reusable test flow cell.

¹ The instrument ships with one installed and one spare. When not under warranty, replacements are user-supplied. Keep packaged until use.

² Replaces the reusable test components shipped with the instrument when they expire after 5 years or 36 uses.

Equipment

Item	Source	Purpose
Freezer, -25°C to -15°C	General lab supplier	Storing the cartridge.
Ice bucket	General lab supplier	Setting aside libraries.
Pipette, 10 µl	General lab supplier	Diluting libraries to the loading concentration.
Pipette, 20 µl	General lab supplier	Diluting libraries to the loading concentration.
Pipette, 100 µl	General lab supplier	Diluting libraries to the loading concentration.
Refrigerator, 2°C to 8°C	General lab supplier	Storing the flow cell.
[Optional] Keyboard	General lab supplier	Supplementing the onscreen keyboard.
[Optional] Mouse	General lab supplier	Supplementing the touch screen interface.
[Optional] Water bath	General lab supplier	Thawing the cartridge.

Chapter 3 Sequencing

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Introduction

Cluster generation, sequencing, and analysis comprise sequencing on the iSeq 100 Sequencing System. Each step occurs automatically during a sequencing run. Depending on system configuration, further analysis is performed off-instrument after the run is complete.

- ▶ **Cluster generation**—Library is automatically denatured into single strands and further diluted onboard the instrument. During cluster generation, single DNA molecules are bound to the surface of the flow cell and amplified to form clusters.
- ▶ **Sequencing**—Clusters are imaged using one-dye chemistry, which uses one fluorescent label and two imaging cycles to encode data for the four nucleotides. The first imaging cycle detects adenine (A) and thymine (T). A chemistry cycle then cleaves the dye from A and simultaneously adds a similar dye to cytosine (C). The second imaging cycle detects C and T. After the second imaging cycle, Real-Time Analysis software performs base calling, filtering, and quality scoring. This process is repeated for each cycle of sequencing. For more information on one-dye chemistry, see [Base Calling on page 41](#).
- ▶ **Analysis**—As the run progresses, the control software automatically transfers base call files (*.bcl) to the specified output folder for data analysis. The method of data analysis depends on application and system configuration.

Loading Volume and Concentration

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

Library Type	Loading Concentration (pM)
100% PhiX	60
AmpliSeq Library PLUS for Illumina	50
Nextera DNA Flex	200
Nextera Flex for Enrichment	100
TruSeq DNA Nano	100
TruSeq DNA PCR-Free	100

For other library types, Illumina recommends 50 pM as a starting loading concentration. Optimize this concentration over subsequent runs to identify a loading concentration that consistently yields data that meets specifications.

Loading concentrations that are too high or too low result in suboptimal clustering and run metrics. For more information, see the *Cluster Optimization Overview Guide (document # 1000000071511)*.

Recommended Number of Cycles

For each read, entering a minimum of 26 cycles and a maximum of 151 cycles helps ensure data quality. The exact number of cycles depends on your experiment.

The minimum and maximum cycle numbers include an extra cycle. Always add one cycle to the desired read length to correct the effects of phasing and prephasing. Read length is the number of **sequencing** cycles in Read 1 and Read 2, which excludes extra cycles and index cycles.

Example run setup:

- ▶ For a read length of 36 (single-read), enter **37** in the Read 1 field.
- ▶ For a read length of 150 per read (paired-end), enter **151** in the Read 1 field, and **151** in the Read 2 field.

Best Practices

- ▶ When handling reagents and other chemicals, wear safety glasses, a lab coat, and powder-free gloves. Switch gloves when prompted to prevent cross-contamination.
- ▶ Make sure that you have the required consumables and equipment before starting a protocol. See [User-Supplied Consumables and Equipment on page 17](#).
- ▶ Follow the protocols in the order shown, using the specified volumes, temperatures, and durations.
- ▶ Unless a stopping point is specified, proceed immediately to the next step.

Thaw the Bagged Cartridge

- 1 If you are thawing the cartridge in a water bath, store the cartridge at -25°C to -15°C for at least 1 day before thawing.
A water bath is the fastest of three thaw methods, which are described in step 5.



CAUTION

Thawing a cartridge in a water bath directly from shipment, when it has been stored on dry ice, can adversely affect cartridge performance.

- 2 Put on a new pair of powder-free gloves.
- 3 Remove the cartridge from -25°C to -15°C storage.
- 4 If the cartridge is boxed, remove it from the box but **do not open the white foil bag**.



- 5 Thaw the bagged cartridge using one of the following methods. Use immediately after thawing, without refreezing or otherwise storing.

Method	Thaw Time	Instruction
20°C to 25°C water bath	6 hours, not exceeding 18 hours	<ul style="list-style-type: none"> • Use 6 L (1.5 gal) water per cartridge. • Set a temperature-controlled water bath to 25°C <i>or</i> mix hot and cold water to achieve 20°C to 25°C. • Face the bag label up, submerge the cartridge completely, and apply ~2 kg (4.5 lbs) weight to prevent floating. • Do not stack cartridges in the water bath unless it is temperature-controlled.
2°C to 8°C refrigerator	36 hours, not exceeding 72 hours	Position the cartridge so that the label faces up and air can circulate on all sides, including the bottom.
Room-temperature air (20°C to 25°C)	9 hours, not exceeding 18 hours	Position the cartridge so that the label faces up and air can circulate on all sides, including the bottom.

- 6 If wet from a water bath, dry with paper towels.

Prepare the Flow Cell and Libraries

Before loading the flow cell and libraries into the cartridge, bring the flow cell to room temperature, dilute libraries, and optionally spike in PhiX. Libraries are denatured automatically onboard the instrument.

The following dilution instructions apply to supported Illumina libraries that are double-stranded. Always perform a quality control analysis, optimize the loading concentration for your library, and use a normalization method that generates double-strand libraries. Bead-based normalization that generates single-stranded libraries is not compatible with onboard denaturation.

Dilute Library to 1 nM

- 1 Prepare the flow cell as follows.
 - a Remove a new flow cell from 2°C to 8°C storage.
 - b Set aside the unopened package at room temperature for 10–15 minutes to prevent condensation.

Preparing the flow cell now ensures that it reaches room temperature on time.

- 2 Remove Resuspension Buffer (RSB) from -25°C to -15°C storage. Alternatively, use 10 mM Tris-HCl, pH 8.5 in place of RSB.
- 3 **[Optional]** Remove 10 nM PhiX stock from -25°C to -15°C storage. PhiX is needed only for an optional spike-in or a PhiX-only run.
- 4 Thaw RSB and optional PhiX at room temperature for 10 minutes.
- 5 Using RSB as diluent, prepare the applicable volume of 1 nM library in a *low-bind* microtube:

Library Type	Volume of 1 nM Library to Prepare (µl)
100% PhiX (for a PhiX-only run)	8
AmpliSeq Library PLUS for Illumina	7
Nextera DNA Flex	25
Nextera Flex for Enrichment	12
TruSeq DNA Nano	12
TruSeq DNA PCR-Free	12

Diluting libraries in low-bind microtubes is critical to successful sequencing.

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

- 7 **[Optional]** Store 1 nM library at -25°C to -15°C for up to 1 month.

Dilute 1 nM Library to Loading Concentration

- 1 Combine the following volumes in a low-bind microtube to prepare 100 µl library diluted to the appropriate loading concentration:

Library Type*	Loading Concentration (pM)	1 nM Library Volume (µl)	RSB Volume (µl)
100% PhiX	60	6	94
AmpliSeq Library PLUS for Illumina	50	5	95
Nextera DNA Flex	200	20	80
Nextera Flex for Enrichment	100	10	90
TruSeq DNA Nano	100	10	90
TruSeq DNA PCR-Free	100	10	90

* For unlisted library types, including other Nextera libraries, start with a loading concentration of 50 pM and optimize over subsequent runs.

This table provides example loading concentrations. The iSeq 100 System is compatible with all Illumina library prep kits except SureCell WTA 3', but the optimal loading concentration can vary.

- 2 Vortex briefly, and then centrifuge at 280 × g for 1 minute.
- 3 Set aside diluted library on ice for sequencing.
Sequence libraries diluted to the loading concentration the same day they are diluted.
- 4 If you are *not* adding PhiX or are performing a PhiX-only run, skip the next section and proceed to [Load Consumables Into the Cartridge on page 23](#).

Add a PhiX Control (Optional)

PhiX is a small, ready-to-use Illumina library with balanced nucleotide representation. Adding a 2% PhiX spike-in to your library provides additional metrics. For low-diversity libraries, a 5% spike-in is recommended to increase base diversity.



NOTE

A spike-in as low as 1% is effective for providing additional metrics, but makes pipetting difficult.

- 1 Combine the following volumes in a low-bind microtube to prepare 50 µl 1 nM PhiX:
- ▶ 10 nM PhiX (5 µl)
 - ▶ RSB (45 µl)
- 2 Vortex briefly, and then centrifuge at 280 × g for 1 minute.
- 3 **[Optional]** Store 1 nM PhiX at -25°C to -15°C for up to 1 month.
- 4 Combine the following volumes in a low-bind microtube to dilute PhiX to the same loading concentration as the library, for example:

PhiX Loading Concentration (pM)	1 nM PhiX Volume (µl)	RSB Volume (µl)
50	5	95
100	10	90
200	20	80

- 5 Combine PhiX and library:
 - ▶ For a 2% spike-in, add 2 μl diluted PhiX to 100 μl diluted library.
 - ▶ For a 5% spike-in, add 5 μl diluted PhiX to 100 μl diluted library.
 Actual PhiX percentage varies depending on library quality and quantity.
- 6 Vortex briefly, and then centrifuge at $280 \times g$ for 1 minute.
- 7 Set aside the library with PhiX spike-in on ice.

Load Consumables Into the Cartridge

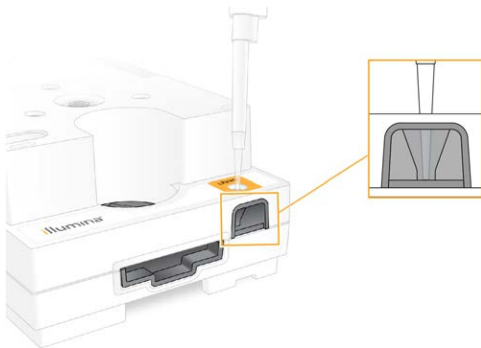
- 1 **[Optional]** To see an instructional video on cartridge preparation and loading, select **Sequence**.
- 2 Open the cartridge bag from the notches.
- 3 Avoiding the access window on top of the cartridge, remove the cartridge from the bag. Discard the bag.
- 4 Invert the cartridge five times to mix reagents.
Internal components can rattle during inversion, which is normal.
- 5 Tap the cartridge (label facing up) on the bench or other hard surface five times to ensure reagent aspiration.

Load Library

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



- 2 Discard the pipette tip to prevent contamination.
- 3 Add 20 μl diluted library to the **bottom** of the reservoir. Avoid touching the foil.



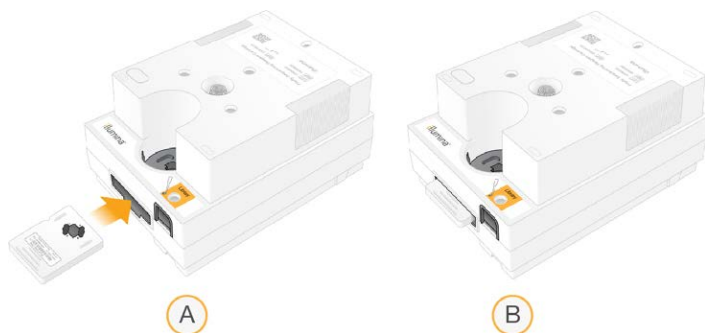
Load the Flow Cell

- 1 Open the white foil package from the notches. Use within 24 hours of opening.

- 2 Pull the flow cell out of the package.
Touch only the plastic when handling the flow cell. Avoid touching the electrical interface, CMOS sensor, glass, and gaskets on either side of the glass.



- 3 Hold the flow cell by the grip points with the label facing up.
- 4 Insert the flow cell into the slot on the front of the cartridge.
An audible click indicates that the flow cell is in place. When properly loaded, the grip protrudes from the cartridge and the glass is visible from the access window.



- A Loading the flow cell
- B Loaded flow cell

- 5 Dispose of the packaging as follows.
 - a Remove the clamshell case from the foil package.
 - b Remove the desiccant from the clamshell case.
 - c Recycle the clamshell case, and discard the foil package and desiccant.
- 6 Proceed depending on whether the system is integrated with Local Run Manager:
 - ▶ If using Local Run Manager, follow *Set Up a Sequencing Run (Local Run Manager)* on page 24.
 - ▶ If not using Local Run Manager, follow *Set Up a Sequencing Run (Manual Mode)* on page 27.

Set Up a Sequencing Run (Local Run Manager)

Setting up a run using Local Run Manager entails creating and saving the run in Local Run Manager, then returning to the control software to load consumables and select the run. Data are saved to the specified output folder for analysis, which Local Run Manager performs automatically when the run is complete.

- 1 Open Local Run Manager locally on the instrument monitor or remotely from another computer:

Access	Open Local Run Manager
Local	From the control software menu, select Local Run Manager , and then select Open Local Run Manager .
Remote	From the control software menu, select About to obtain the system IP address. From a computer on the same network as the instrument, open Local Run Manager in Chromium. Use the system IP address to connect.

- If Chromium appears blank on the instrument monitor, power cycle the instrument and restart run setup. See *Power Cycle the Instrument* on page 45 for instructions.
- In Local Run Manager, create and save the run.
 - ▶ See the *Local Run Manager Software Guide (document # 1000000002702)* for instructions.
 - ▶ Set up a PhiX-only run to be non-indexed.
 Local Run Manager automatically sends saved runs to the control software.
- In the control software, select **Sequence**.
The software opens the door at an angle, ejects the tray, and initiates the series of run setup screens.
- [Optional]** Select **Help** to view an onscreen prompt.
Help prompts appear on each screen to provide supplementary guidance.

Load the Cartridge Onto the Instrument

- Make sure that the cartridge is thawed and contains the flow cell and diluted library.
- Place the cartridge onto the tray so that the access window faces up and the flow cell is inside the instrument. Do not push the cartridge or tray into the instrument.



- Select **Close Door** to retract the cartridge and close the door.
A panel appears on the left side of the screen to show information from the scanned consumables.

Sign In to BaseSpace Sequence Hub

The BaseSpace Sequence Hub screen appears when the system is configured for Run Monitoring or Run Monitoring and Storage.

- To disconnect the current run from BaseSpace Sequence Hub, select **Skip BaseSpace Sequence Hub Sign In**.
Instrument performance data are still sent to Illumina.
- To change connectivity for the current run, select a Configuration option:
 - ▶ **Run Monitoring Only**—Send only InterOp files to BaseSpace Sequence Hub to allow remote monitoring.
 - ▶ **Run Monitoring and Storage**—Send run data to BaseSpace Sequence Hub to allow remote monitoring and analysis.

- 3 Enter your BaseSpace Sequence Hub credentials (email address and password), and then select **Sign In**.
- 4 If the Available Workgroups list appears, select a workgroup to upload run data to. The list appears when you belong to multiple workgroups.
- 5 Select **Run Setup**.

Select a Run

- 1 If the Local Run Manager Log In screen appears, enter your user name and password, and then select **Log In**.
The screen appears when Local Run Manager is configured to require signing in. By default, signing in is not required.
- 2 Select a run from the Run Name list, which lists runs saved in Local Run Manager.
 - ▶ To view an updated list, select **Refresh**.
 - ▶ If the list is empty, select **Open Local Run Manager** to create a run. Selecting Open Local Run Manager minimizes the control software and opens Local Run Manager in Chromium.
- 3 If you left the control software to create a run, return and select the run. Click **Refresh** to view an updated list.
- 4 **[Optional]** Select **Edit** and modify run parameters:
 - a To change Read Type, select **Single Read** or **Paired End**.
 - b To change Read Cycle, enter **26–151** cycles each for Read 1 and Read 2. Add one cycle to the desired number of cycles.
 - c To change the output folder for the current run, enter the path to the location or select **Browse** and navigate to it.
 - d Select **Save**, which updates the run in both the control software and Local Run Manager.
- 5 Select **Start Run** to initiate the pre-run check.

Review Pre-Run Checks

Pre-run checks include an instrument check and a flow check. The flow check pierces the cartridge seals and passes reagent through the flow cell, so consumables cannot be reused after the flow check starts.

- 1 Wait about 15 minutes for pre-run checks to complete.
The run starts automatically after successful completion. Unless the system is muted, a chime sound indicates that the run has started.



CAUTION

Opening the door during the pre-run check or the run can cause run failure.

- 2 If an error occurs during the instrument check, select **Retry** to redo the check. The instrument check precedes the flow check. When a check is in progress, the bar for that check is animated.
- 3 If an error recurs, see [Error Message Resolution on page 44](#) to troubleshoot.

Monitor Run Progress

- 1 Monitor run progress and metrics as they appear on the Sequencing screen after cycle 26.

Metric	Description
%Q30 Read 1	The percentage of Read 1 base calls with a Q-score ≥ 30 .
%Q30 Read 2	The percentage of Read 2 base calls with a Q-score ≥ 30 .
%Clusters PF	The percentage of clusters that passed quality filters.
%Occupancy	The percentage of flow cell wells containing clusters.
Projected Total Yield	The expected number of bases called for the run.

- To monitor file copying and other run processes, select the control software menu, and then select **Process Management**.

Unload Consumables

- When sequencing is complete, select **Eject Cartridge**.
The software ejects the used cartridge from the instrument.
- Remove the cartridge from the tray.
- Remove the flow cell from the cartridge.
- Dispose of the flow cell, which has electronic components, in accordance with applicable standards for your region.
- Dispose of the cartridge, which contains used reagents, in accordance with applicable standards for your region.
A post-run wash is not necessary because fluidics are discarded with the cartridge.



WARNING

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

- Select **Close Door** to reload the tray and return to the Home screen.
The software automatically reloads the tray and sensors confirm cartridge removal.

Set Up a Sequencing Run (Manual Mode)

Setting up a run in Manual mode means specifying run parameters in the control software and performing analysis off-instrument using an application of your choice. The software saves data to the specified output folder for the analysis. Generating FASTQ files requires an additional step.

- If the system is configured for run analysis, collaboration, and storage using BaseSpace Sequence Hub, create a sample sheet for the run:
 - Download the *iSeq 100 System Sample Sheet Template for Manual Mode* from the [iSeq 100 Sequencing System support pages](#).
 - Modify the template as needed and save it in CSV file format. Make sure that:
 - The Index 2 (i5) adapter sequences are in the correct orientation. For orientations, see *Illumina Adapter Sequences (document # 1000000002694)*.

- ▶ Sample sheet values match control software values. For example, enter 151 in the Read 1 field of both the sample sheet and the Run Setup screen.
- 2 In the control software, select **Sequence**.
The software opens the door at an angle, ejects the tray, and initiates the series of run setup screens.
 - 3 **[Optional]** Select **Help** to view an onscreen prompt.
Help prompts appear on each screen to provide supplementary guidance.

Load the Cartridge Onto the Instrument

- 1 Make sure that the cartridge is thawed and contains the flow cell and diluted library.
- 2 Place the cartridge onto the tray so that the access window faces up and the flow cell is inside the instrument. Do not push the cartridge or tray into the instrument.



- 3 Select **Close Door** to retract the cartridge and close the door.
A panel appears on the left side of the screen to show information from the scanned consumables.

Sign In to BaseSpace Sequence Hub

The BaseSpace Sequence Hub screen appears when the system is configured for Run Monitoring or Run Monitoring and Storage.

- 1 To disconnect the current run from BaseSpace Sequence Hub, select **Skip BaseSpace Sequence Hub Sign In**.
Instrument performance data are still sent to Illumina.
- 2 To change connectivity for the current run, select a Configuration option:
 - ▶ **Run Monitoring Only**—Send only InterOp files to BaseSpace Sequence Hub to allow remote monitoring.
 - ▶ **Run Monitoring and Storage**—Send run data to BaseSpace Sequence Hub to allow remote monitoring and analysis (requires a sample sheet).
- 3 Enter your BaseSpace Sequence Hub credentials (email address and password), and then select **Sign In**.
- 4 If the Available Workgroups list appears, select a workgroup to upload run data to.
The list appears when you belong to multiple workgroups.
- 5 Select **Run Setup**.

Enter Run Parameters

- 1 In the Run Name field, enter a unique name of your preference to identify the current run.

The run name can contain alphanumeric characters, hyphens, and underscores.

- For Read Type, select one of the following options:
 - ▶ **Single Read**—Perform one sequencing read, which is the faster, simpler option.
 - ▶ **Paired End**—Perform two sequencing reads, which generates higher-quality data and provides more accurate alignment.
- For Read Cycle, enter the number of cycles to perform in each read.
 - ▶ For Read 1 and Read 2, add one cycle to the desired number of cycles.
 - ▶ For a PhiX-only run, enter **0** in both index fields.

Read	Number of Cycles
Read 1	26–151
Index 1	Up to 10
Index 2	Up to 10
Read 2	26–151

Read 2 is typically the same value as Read 1, including the extra cycle. Index 1 sequences the i7 index adapter, and Index 2 sequences the i5 index adapter.

- To specify an output folder for the current run or upload a sample sheet, select **Advanced**:
 - ▶ In the Output Folder field, enter the path to the output folder location or select **Browse** and navigate to it.
 - ▶ In the Sample Sheet field, enter the path to the sample sheet location or select **Browse** and navigate to it.
- Select **Start Run** to initiate the pre-run check.

Review Pre-Run Checks

Pre-run checks include an instrument check and a flow check. The flow check pierces the cartridge seals and passes reagent through the flow cell, so consumables cannot be reused after the flow check starts.

- Wait about 15 minutes for pre-run checks to complete.
The run starts automatically after successful completion. Unless the system is muted, a chime sound indicates that the run has started.



CAUTION

Opening the door during the pre-run check or the run can cause run failure.

- If an error occurs during the instrument check, select **Retry** to redo the check.
The instrument check precedes the flow check. When a check is in progress, the bar for that check is animated.
- If an error recurs, see [Error Message Resolution on page 44](#) to troubleshoot.

Monitor Run Progress

- Monitor run progress and metrics as they appear on the Sequencing screen after cycle 26.

Metric	Description
%Q30 Read 1	The percentage of Read 1 base calls with a Q-score ≥ 30 .
%Q30 Read 2	The percentage of Read 2 base calls with a Q-score ≥ 30 .
%Clusters PF	The percentage of clusters that passed quality filters.

Metric	Description
%Occupancy	The percentage of flow cell wells containing clusters.
Projected Total Yield	The expected number of bases called for the run.

- To monitor file copying and other run processes, select the control software menu, and then select **Process Management**.

Unload Consumables

- When sequencing is complete, select **Eject Cartridge**.
The software ejects the used cartridge from the instrument.
- Remove the cartridge from the tray.
- Remove the flow cell from the cartridge.
- Dispose of the flow cell, which has electronic components, in accordance with applicable standards for your region.
- Dispose of the cartridge, which contains used reagents, in accordance with applicable standards for your region.
A post-run wash is not necessary because fluidics are discarded with the cartridge.



WARNING

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

- Select **Close Door** to reload the tray and return to the Home screen.
The software automatically reloads the tray and sensors confirm cartridge removal.

Chapter 4 Maintenance

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Clear Hard Drive Space

A sequencing run requires about 2 GB of hard drive space. When space is low, use the following steps to delete completed runs and clear space.

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

Software Updates

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

- ▶ iSeq Control Software
- ▶ iSeq 100 System recipes
- ▶ Universal Copy Service
- ▶ Real-Time Analysis
- ▶ Local Run Manager (framework only)



NOTE

Although Local Run Manager is included in the system suite, the analysis modules are not. Install them separately as needed using the sbsadmin account. Access the analysis module software from the Local Run Manager support pages.

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

- ▶ **Automatic updates**—Updates are automatically downloaded from BaseSpace Sequence Hub for you to install. This option requires an internet connection, but not a BaseSpace Sequence Hub account.
- ▶ **Manual updates**—Updates are manually downloaded from the web, saved locally or to a portable device, and installed from the saved location. This option does not require an internet connection.

Install an Automatic Software Update

- 1 Switch to the sbsadmin operating system account.
- 2 Select the control software menu, and then select **Software Update** to open the Software Update dialog box.
Systems configured for automatic updates display an alert when a software update is available.

- 3 To check for an update, select one of the following options:
 - ▶ **Check for Update**—Check for a software update.
 - ▶ **Autocheck for Updates**—Check for a software update and configure the system to automatically check for future updates.

These options are visible on systems that are connected to the internet but not configured for automatic updates.

- 4 Select **Update** to download the new version of software.
When the download is complete, the control software is closed and the installation wizard appears.
- 5 In the installation wizard, select **Install**.



NOTE

Canceling an update before installation is complete stops the update at the current point. Any changes made up to the cancellation point are reverted to the previous version or not installed.

- 6 When the installation is complete, select **Close**.
The control software automatically restarts. Any firmware update occurs automatically after the restart.

Install a Manual Software Update

- 1 Switch to the sbsadmin operating system account.
- 2 When a software update is available, download the suite installer (*.exe) from the [iSeq 100 Sequencing System support page](#). Save the installer to a local or portable drive.
- 3 If you saved the installer to a portable drive, plug the drive into a USB port on the back of the instrument. Move the instrument as needed to access the back.
- 4 From the control software, select **Software Update** from the control software menu.
- 5 In the Software Update dialog box, expand **Install from local or portable drive**.
- 6 Select **Browse** to navigate to the installer.
- 7 Select **Update** to start installation.
The control software is closed and the installation wizard appears.
- 8 In the installation wizard, select **Install**.



NOTE

Canceling an update before installation is complete stops the update at the current point. Any changes made up to the cancellation point are reverted to the previous version or not installed.

- 9 When the installation is complete, select **Close**.
The control software automatically restarts. Any firmware update occurs automatically after the restart.

Replace the Air Filter

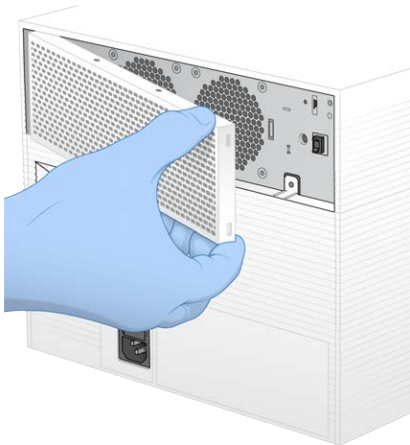
The air filter is a single-use foam piece that covers the two fans on the back of the instrument. It ensures proper cooling and prevents debris from entering the system. The instrument is shipped with one air filter installed and one spare. Additional spares are included with the warranty, or they can be purchased from Illumina.

The software prompts for an air filter change every six months from when first time setup was started. Use the following instructions to replace an expired air filter.

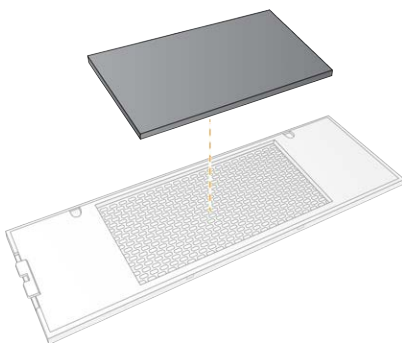
- 1 Position the instrument so you can easily access the back.
- 2 On the back of the instrument, press the right side of the top panel to disengage as shown in the following illustration.



- 3 Remove the panel from the instrument.

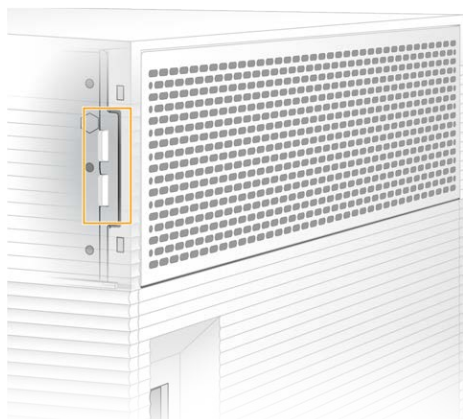


- 4 Remove the foam air filter from the center of the panel and discard.



- 5 Place a new air filter into the panel, and press to secure.

- 6 Insert the two panel hooks into the holes in the instrument and press the panel into place.



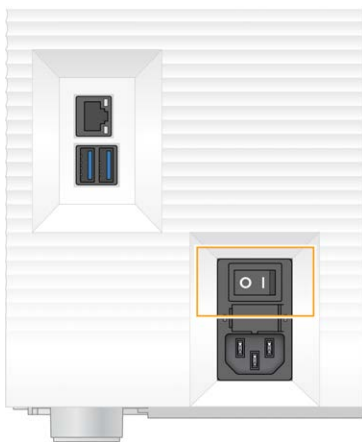
- 7 Return the instrument to the original placement.
- 8 Select **Filter Changed** to proceed.

Relocate the Instrument

Use the following instructions to safely relocate the instrument. Make sure that the new location meets the requirements described in the *iSeq 100 Sequencing System Site Prep Guide (document # 1000000035337)*. If you are returning an instrument, skip this section and see [Advanced Exchange on page 52](#).

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

Figure 7 Toggle Switch Location



- 4 Disconnect the power cord from the wall outlet, and then from the AC power outlet on the rear panel.
- 5 If applicable, disconnect the Ethernet cable from the wall outlet, and then from the Ethernet port on the rear panel.
- 6 Lower the monitor.

- 7 Relocate the instrument to the desired location.
The instrument weighs 15.9 kg (35 lbs) and requires a two-person lift.
- 8 Raise the monitor.
- 9 If the instrument is connected to a network, connect the Ethernet cable to an Ethernet port.
- 10 Connect the power cord to the AC inlet on the rear panel, and then to a wall outlet.
- 11 Press the power on (I) side of the toggle switch.
- 12 When the power button pulses, press it.

Figure 8 Power Button Location



- 13 When the operating system is loaded, log on to Windows.
The control software is launched and initializes the system. The Home screen appears when initialization is complete.

Software Restriction Policies

Windows Software Restriction Policies (SRP) use rules to allow only specified software to run. For the iSeq 100 System, SRP rules are based on certificates, file names and extensions, and directories.

By default, SRP is turned on to prevent unwanted software from running on the control computer. An IT representative or system administrator can add and remove rules to customize the security level. If the system is added to a domain, the local Group Policy Object (GPO) might automatically modify the rules and turn off SRP.

Allowed SRP Rules

On the iSeq 100 Sequencing System, SRP defaults to allowing the following rules.

Certificates

iSeq Application Certificate

Executable Files

Portmon.exe
 Procmon.exe
 Procmon64.exe
 Tcpview.exe

File Extensions

*.cfg
 *.lnk
 *.png
 *.tif
 *.txt
 *.xml

Directories

%HKEY_LOCAL_MACHINE\SOFTWARE\Microsoft\Windows\CurrentVersion\ProgramFilesDir%
 %HKEY_LOCAL_MACHINE\SOFTWARE\Microsoft\Windows NT\CurrentVersion\SystemRoot%
 C:\CrashDumps*
 C:\Illumina Maintenance Logs*
 C:\Illumina Manufacturing Test Results*
 C:\Illumina*
 C:\Program Files (x86)\Chromium\Application*
 C:\Program Files (x86)\Illumina*
 C:\Program Files (x86)\Internet Explorer*
 C:\Program Files\Illumina*
 C:\Program Files\Internet Explorer*
 C:\Program Files\Jenoptik*
 C:\Program Files\Ophir Optronics*
 C:\ProgramData\Illumina*
 C:\ProgramData\Package Cache*
 C:\ProgramData\webex*
 C:\Users*\AppData\Local\GoToAssist Corporate*
 C:\Users*\AppData\Local\Temp\Citrix*
 C:\Users*\AppData\Local\Temp\CitrixLogs*
 C:\Users*\AppData\Local\Temp\LogMeIn*
 C:\Users*\AppData\Local\Temp\LogMeInLogs*
 D:\Recovery*
 GoToAssist Corporate Opener*.exe
 Turnover to customer.bat

Add and Remove SRP Rules

Add and remove SRP rules to customize system security. Modifying the rules requires temporarily turning off SRP.

**CAUTION**

Turning off SRP overrides the default protections.

- 1 Log in to the operating system with the administrator account.
The administrator account has the privileges necessary to modify SRP rules.
- 2 Turn off SRP:
 - a Navigate to the directory C:\Illumina\Security.
 - b Double-click **Disable.reg**.
 - c Select **Yes** to confirm the changes.

When using the touch-screen interface, tapping and holding for about 2 seconds is equivalent to a right-click.

- 3 Select **Start**, and then select **Run**.
- 4 In the Open field, enter **secpol.msc**.
- 5 In the Local Security Policy dialog box, expand **Software Restriction Policies**, and then select **Additional Rules**.
- 6 To add a rule:
 - a On the Action menu, select **New Path Rule**.
 - b In the Path field, enter the certificate, file name, file extension, or directory that you want to allow.
 - c In the Security level list, select **Unrestricted**.
 - d **[Optional]** In the Description field, enter a reason for creating the rule.
 - e Select **OK** to add the rule.
- 7 To delete a rule:
 - a Select the rule you want to delete, and then select **Delete**.
 - b Select **Yes** to confirm the deletion.
- 8 Close the Local Security Policy dialog box.
- 9 **Immediately** reinstate SRP:
 - a Navigate to the directory C:\Illumina\Security.
 - b Double-click **Enable.reg**.
- 10 If SRP rules were modified for the first time, log off and then log on again for the rules to take effect.

Appendix A Sequencing Output

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Real-Time Analysis Overview

The Real-Time Analysis software runs on the instrument control computer. During a sequencing run, it extracts intensities from images to perform base calling, and then assigns a quality score to the base call. iSeq 100 Sequencing System uses the RTA2 implementation of Real-Time Analysis. RTA2 and the control software communicate through a web HTTP interface and shared memory files. If RTA2 is terminated, processing does not resume and run data are not saved.



NOTE

Demultiplex performance is not calculated, so the Index tab in Sequencing Analysis Viewer is not populated.

Input Files

RTA2 requires the following input files for processing:

- ▶ Tile images contained in local system memory.
- ▶ Real-Time Analysis configuration file in XML format.
- ▶ RunInfo.xml, which the control software generates automatically at the beginning of the run.

RTA2 receives commands from the control software that include information about the location of RunInfo.xml and whether an output folder is specified. From RunInfo.xml, RTA2 reads the run name, number of cycles, whether a read is indexed, and the number of tiles on the flow cell.

Output Files

Images are passed in memory to RTA2 as tiles, which are small imaging areas on the flow cell defined by one camera view. The iSeq 100 i1 Flow Cell has 16 tiles.

From these images, RTA2 produces a set of quality-scored base call files and filter files as primary output. Other files support generation of the primary output.

File Type	File Description, Location, and Name
Base call files	Each tile analyzed is included in a base call file, aggregated in one file per cycle. The aggregated file contains the base call and associated quality score for every cluster. Data\Intensities\BaseCalls\L001 [Cycle].bcl.bgzf, where [Cycle] represents the cycle number in four digits. Base call files are compressed using block gzip compression.
Base call index files	A base call index file preserves the original tile information. For each tile, the index file contains the tile number and number of clusters. Data\Intensities\BaseCalls\L001 [Cycle].bcl.bgzf.bci
Cluster locations file	One cluster location (s.locs) file contains the X,Y coordinates for every cluster on the flow cell. Data\Intensities s.locs
Filter files	Filter files specify whether clusters pass filter. For each tile, one filter file is generated. Filter files are generated at cycle 26 using 25 cycles of data. Data\Intensities\BaseCalls\L001 s_[lane].filter

File Type	File Description, Location, and Name
InterOp files	Real-time metrics of run quality that are updated throughout the run. These binary files contain tile, cycle, and read-level metrics, and are needed to view metrics in Sequencing Analysis Viewer. InterOp folder
RTA configuration file	Lists parameters for the run. Created at the beginning of the run, the file combines values from the input configuration file and values that RTA2 defines. [Root folder], RTAConfiguration.xml
Run information file*	Lists the run name, number of cycles per read, whether the read is an Index Read, and the number of swaths and tiles. Created at the beginning of the run. [Root folder], RunInfo.xml
Thumbnail files	Thumbnail images of flow cell tiles. Images\L001\C[X.1]—Files are stored in one folder for each lane and one subfolder for each cycle. s_[lane]_[tile].jpg—The thumbnail image includes the tile number.

*Created by the control software. RTA2 creates all other files listed in the table.

Local Run Manager and BaseSpace Sequence Hub automatically convert base calls files into FASTQ files. When sequencing in Manual mode, use the latest version of the [bcl2fastq2 Conversion Software](#) to convert FASTQ files. Download the software from the [bcl2fastq Conversion Software support pages](#) on the Illumina website.

Output Folder Name and Path



















For each run, the control software automatically generates an output folder and a run folder. Access the run data from the output folder, which is a copy of the run folder. The run folder is for system use.

The path to the output folder is user-defined, but defaults to D:\. The control software names the output folder using the following format.

Format	Example
<YYYYMMDD>_<Instrument ID>_<Run Number>_<Flow Cell ID>	20180331_FFSP247_4_BNS417-05-25-12

The run number increments by one each time the system performs a run. Serial numbers identify the instrument and flow cell.

Output Folder Structure

-  **Recipe**—Run-specific recipe file.
-  **Logs**—Log files describing instrument analytics, operational steps, and other events.
-  **Config**—Configuration settings for the run.
-  RunParameters.xml
-  RunInfo.xml
-  CopyComplete.txt
-  RunCompletionStatus.txt
-  RTAComplete.txt
-  RTAConfiguration.xml
-  **Data**
 -  **Intensities**
 -  **BaseCalls**
 -  **L001**
 -  s.locs
-  **InterOp**
-  **Images**
-  SampleSheet.csv—Sample sheet or the sample manifest.
-  **RTALogs**—Log files describing RTA2 events.

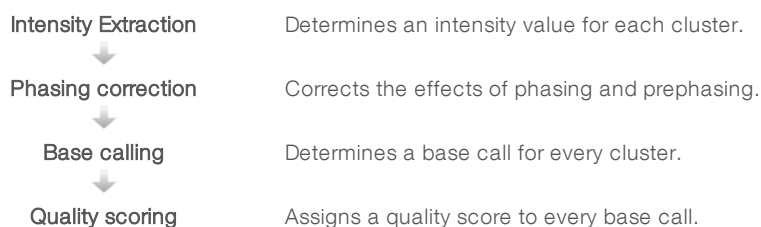
Error Handling

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.
- ▶ *Error*.tsv lists errors that occurred during a run.
- ▶ *WarningLog*.tsv lists warnings that occurred during a run.

Real-Time Analysis Workflow



Intensity Extraction

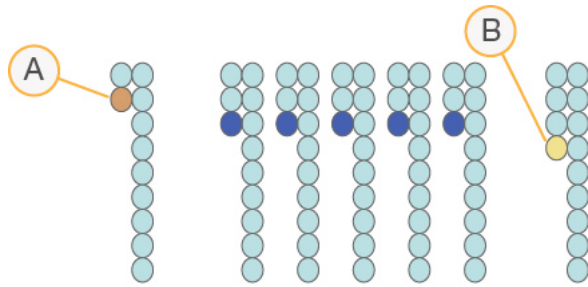
Intensity extraction calculates an intensity value for each nanowell in a given image.

Phasing Correction

During the sequencing reaction, each DNA strand in a cluster extends by one base per cycle. Phasing and prephasing occurs when a strand becomes out of phase with the current incorporation cycle.

- ▶ Phasing occurs when a base falls behind.
- ▶ Prephasing occurs when a base jumps ahead.

Figure 9 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 iSeq 100 System uses one-dye sequencing, which requires one dye and two images to encode data for the four bases.

Intensities extracted from one image and compared to a second image result in four distinct populations, each corresponding to a nucleotide. Base calling determines which population each cluster belongs to.

Figure 10 Visualization of Cluster Intensities

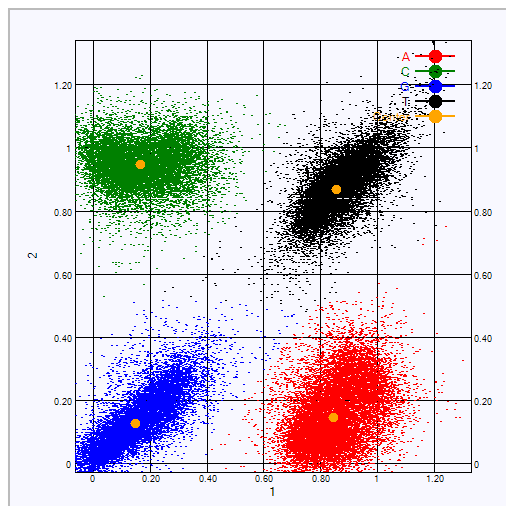


Table 3 Base Calls in One-Dye Sequencing

Base	Dye in First Image	Dye in Second Image	Conclusion From Compared Images
T	On	On	Clusters that show intensity in both images are T bases.
A	On	Off	Clusters that show intensity in the first image only are A bases.
C	Off	On	Clusters that show intensity in the second image only are C bases.
G	Off	Off	Clusters that show intensity in neither image are G bases.

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 one-dye sequencing, RTA2 uses a population-based system to determine the chastity (intensity purity measurement) of a base call. Clusters pass filter (PF) when no more than one base call in the first 25 cycles has a chastity below a fixed threshold.

PhiX alignment is performed at cycle 26 on a subset of tiles for clusters that passed filter. Clusters that do not pass filter are not base called and not aligned.

Index Reads

The process for base calling index reads differs from base calling sequencing reads. The first two cycles of an Index Read cannot start with two G bases, otherwise intensity is not generated. To ensure demultiplexing performance, intensity must be present in either of the first two cycles.

Make sure that **at least** one index adapter sequence in a library pool does not start with two G bases. Select balanced index adapter sequences so that signal is present in at least one image (preferably both images) for every cycle. The plate layout and sequences provided in IDT for Illumina TruSeq UD Indexes are designed to have the proper balance.

For more information on indexing and pooling, see the *Index Adapter Pooling Guide (document # 1000000041074)*.

Quality Scoring

A quality score, or Q-score, is a prediction of the probability of an incorrect base call. A higher Q-score implies that a base call is higher quality and more likely to be correct.

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

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



NOTE

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

Quality scoring calculates a set of predictors for each base call, and then uses the predictor values to look up the Q-score in a quality table. Quality tables are created to provide optimally accurate quality predictions for runs generated by a specific configuration of sequencing platform and version of chemistry.

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

Appendix B Troubleshooting

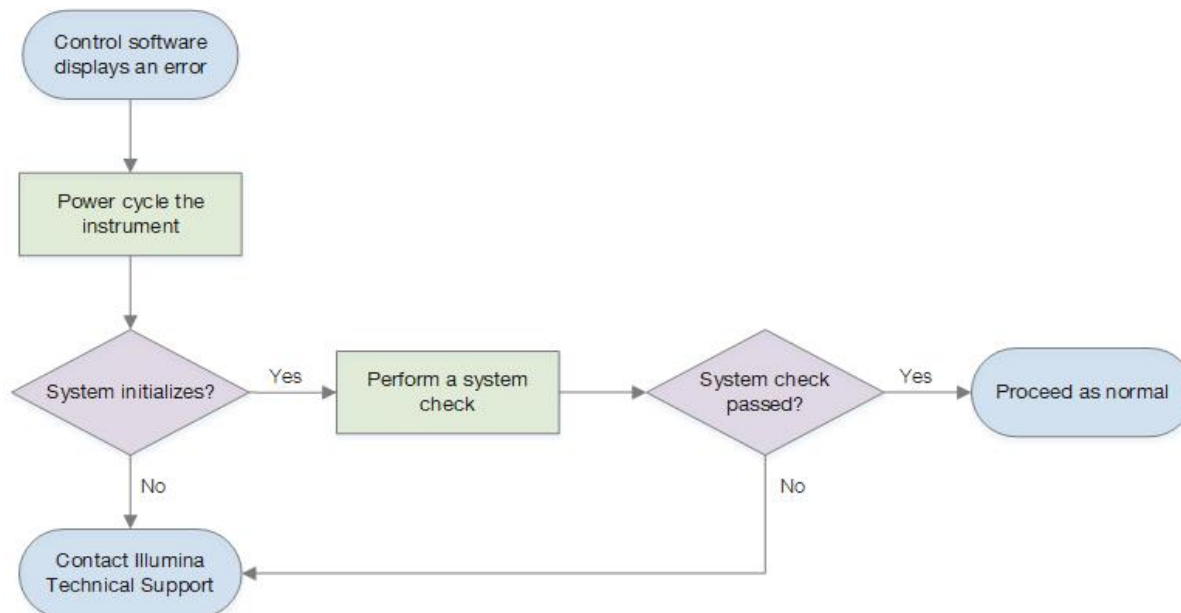
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Error Message Resolution

This appendix provides detailed instructions for various troubleshooting procedures. The following flowchart shows the workflow for troubleshooting error messages that appear during initialization, run setup, pre-run checks, or sequencing that a retry does not resolve.

Many errors can be resolved with a power cycle: turning off the instrument, and then restarting it. Others require a system check to diagnose and resolve.

Figure 11 Error Messages Overview



Process Management Status

To troubleshoot an status issue on the Process Management screen:

- ▶ If the run is in progress, close the Process Management screen, wait about five minutes, and then reopen.
- ▶ If the run is not in progress, power cycle the instrument, and then reopen the Process Management screen. See *Power Cycle the Instrument* on page 45.

Cancel a Started Run

After a run starts, you can cancel it to end the run, eject the cartridge, and return to the Sequence screen.



CAUTION

Canceling a run is *final*. The software cannot resume the run and consumables cannot be reused after the instrument check portion of pre-run checks.

- 1 Select **Stop Run**, and then select **Yes, cancel**.
The Sequencing Canceled screen appears with timestamps for the date and time the run stopped.
- 2 Select **Eject Cartridge** to open the door and eject the tray.
- 3 Remove the cartridge from the tray.
- 4 Store or dispose of the cartridge, depending on when the cancellation occurred:

Circumstance	Instruction
You canceled before or during the instrument check and want to reuse consumables.	Leave the flow cell and library inside the cartridge, and set aside at room temperature for up to 1 hour.
All other circumstances.	Remove the flow cell from the cartridge. Dispose of both components in accordance with applicable regional standards. <ul style="list-style-type: none"> • The flow cell has electronic components. • The cartridge contains used reagents and library.

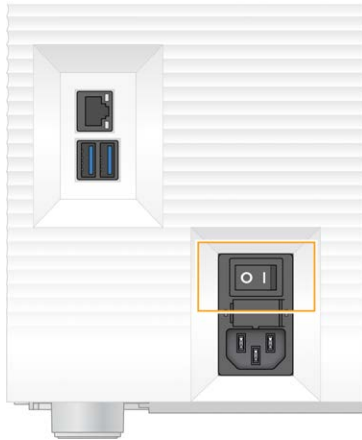
- 5 Select **Close Door** to reload the tray and return to the Sequencing screen.
Sensors confirm cartridge removal.

Power Cycle the Instrument

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

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

Figure 12 Toggle Switch Location



- 4 Wait 30 seconds.
- 5 Press the power on (I) side of the toggle switch.
- 6 When the power button pulses, press it.

Figure 13 Power Button Location



- 7 When the operating system is loaded, log on to Windows.
The control software is launched and initializes the system. The Home screen appears when initialization is complete.

Perform a System Check

A system check takes about 45 minutes and uses the reusable test flow cell and reusable test cartridge to troubleshoot pre-run check errors and other issues. Four subsystem tests confirm whether components are properly aligned and functional.

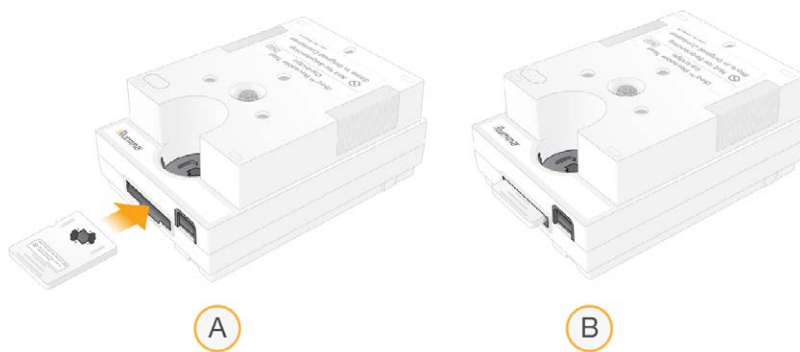
Normal operation and maintenance do not require a system check.

- 1 Remove the reusable test flow cell and reusable test cartridge from room temperature storage.

- 2 From the control software menu, select **System Check**.
The System Check dialog box appears with the mechanical, thermal, optical, and sensor tests selected.
- 3 Select **Unload** to open cartridge compartment door and eject the tray.
- 4 If present, remove the used cartridge from the tray.
- 5 Inspect the glass surface of the reusable flow cell for visible debris. If debris is present, clean as follows.
 - a Clean the glass surface with an alcohol wipe.
 - b Dry with a low-lint lab tissue.
 - c Make sure that the flow cell is free of lint or fibers.

Under normal circumstances, the reusable test flow cell does not require cleaning.

- 6 Hold the reusable test flow cell by the grip points with the label facing up.
- 7 Insert the reusable test flow cell into the slot on the front of the reusable test cartridge.
An audible click indicates that the flow cell is in place. When properly loaded, the grip protrudes from the cartridge and the glass is visible from the access window.



- a Loading the reusable test flow cell
- b Loaded reusable test flow cell

- 8 Place the reusable test cartridge onto the tray so that the access window faces up and the flow cell is inside the instrument.



- 9 Select **Load** to load the reusable test cartridge and close the door.
- 10 Select **Start** to begin the system check.
During the system check, the software ejects and retracts the cartridge one time.



NOTE



The reusable test flow cell and cartridge are valid for 36 uses or 5 years from the date of manufacture, whichever comes first. The remaining number of uses are displayed onscreen.

- When the system check is complete, review whether each test passed or failed.

Result	Indication	Action
All four tests passed	The instrument is functioning properly and the issue is likely consumable- or library-related.	Set up a new run. If consumables from the previous run were stored, use them for the new run.
At least one test failed	The instrument might have a hardware issue.	Contact Illumina Technical Support.

- Select **Unload** to eject the reusable test cartridge.
- Remove the reusable test cartridge from the tray.
- Remove the reusable test flow cell from the cartridge.
- Return the reusable test components to the original packaging and store at room temperature.
- Close the System Check dialog box.

Leak Mitigation

If a poor fluidics connection, cartridge issue, or leak is detected during a pre-run check or sequencing, the software ends the run and notifies you. After assessing the leak and cleaning the instrument, a system check confirms that normal operation can continue.

A drip tray at the base of the instrument catches fluids leaking from the cartridge. However, leaked fluids can reach other areas of the system. Under normal circumstances, the drip tray is dry.

Assess the Leak

- Put on a new pair of powder-free gloves.



WARNING

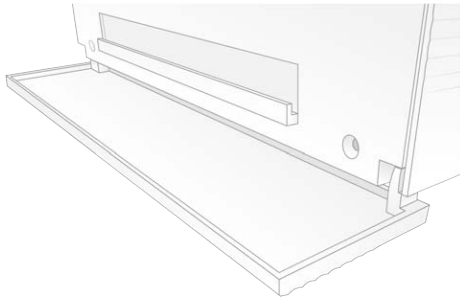
This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste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

- Follow the onscreen prompts to eject the cartridge.
- Inspect the cartridge for visible fluid.
A small amount of fluid (< 500 µl) on the glass surface of the flow cell is acceptable.
- If no fluid (or an acceptable amount of fluid) is visible, proceed to *Clean the Instrument*. After cleaning, a system check confirms normal operation.
- If a significant amount of fluid is visible on the flow cell, cartridge, or instrument, shutdown and disconnect as follows and contact Illumina Technical Support.
 - From the menu, select **Shut Down System**.
 - If the shutdown command is unresponsive, press and hold the power button on the left side of the instrument until the lights fade.

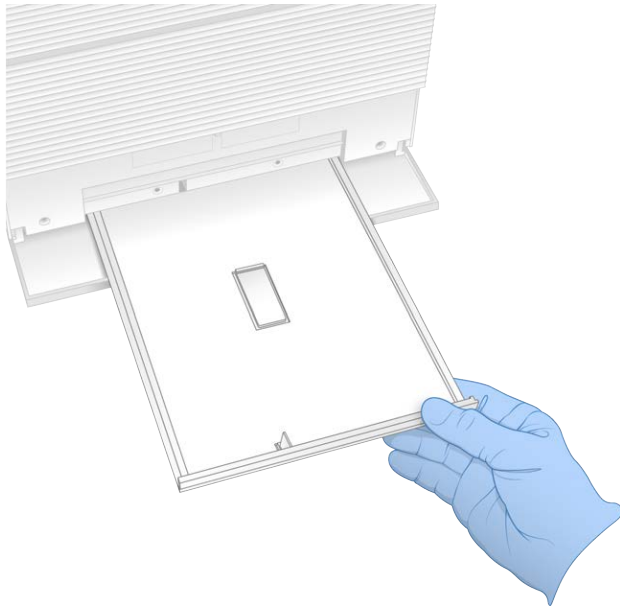
- c When the power button pulses, press the power off (O) side of the toggle switch on the back of the instrument.
- d Wait 30 seconds.
- e Disconnect the power cord from the wall outlet, and then from the AC power outlet on the rear panel.
- f If applicable, disconnect the Ethernet cable from the wall outlet, and then from the Ethernet port on the rear panel.

Clean the Instrument

- 1 For safety, turn off and disconnect the instrument:
 - a From the menu, select **Shut Down System**.
 - b If the shutdown command is unresponsive, press and hold the power button on the left side of the instrument until the lights fade.
 - c When the power button pulses, press the power off (O) side of the toggle switch on the back of the instrument.
 - d Wait 30 seconds.
 - e Disconnect the power cord from the wall outlet, and then from the AC power outlet on the rear panel.
 - f If applicable, disconnect the Ethernet cable from the wall outlet, and then from the Ethernet port on the rear panel.
- 2 Locate the drip tray door below the cartridge compartment on the front of the instrument, and then lower the door.



- 3 Open the drip tray and remove the drip tray pad.



- 4 Wipe any residual fluid from the bottom of the tray using paper towels.
- 5 Dispose of the pad and other consumables in accordance with applicable standards, which vary by region.
For more information, see the Safety Data Sheet (SDS) at support.illumina.com/sds.html.
- 6 Place a new pad onto the drip tray.
- 7 Close the drip tray, and then close the drip tray door.
- 8 Dry any visible fluid on or around the instrument using paper towels.
- 9 Turn on and reconnect the instrument as follows.
 - a If applicable, connect the Ethernet cable to an Ethernet port.
 - b Connect the power cord to the AC inlet on the rear panel, and then to a wall outlet.
 - c Press the power on (I) side of the toggle switch on the rear panel.
 - d When the power button pulses, press it.
 - e When the operating system is loaded, log on to Windows.

The control software is launched and initializes the system. The Home screen appears when initialization is complete.

- 10 Perform a system check to confirm that the system is functioning normally.
Passing the system check indicates that the instrument can resume normal operation. For instructions, see *Perform a System Check* on page 46.

Restore to Factory Settings

Restore the system to factory defaults to downgrade the software, recover from an undesirable configuration, or clear user data before returning an instrument to Illumina. Restoring the system uninstalls control software and clears drive C.

- 1 If the reference genomes repository for Local Run Manager resides on drive C:

- a Move the repository to `D:\Illumina\Genomes` or other local or network folder not on drive C.
 - b In Local Run Manager, reset the repository path to `D:\Illumina\Genomes` or other local or network folder not on drive C. For instructions, see the *Local Run Manager Software Guide (document # 1000000002702)*.
- 2 Restart Windows.
 - 3 When prompted to choose an operating system, select **Restore to Factory Settings**.
The operating system options appear briefly before automatically proceeding with iSeq Control Software.
 - 4 Wait about 30 minutes for the restoration to complete.
The restoration can include several reboots. When complete, the system reboots with the original factory settings, less the control software.
 - 5 Install the control software:
 - c Download the software installer from the iSeq 100 Sequencing System support pages. Save the installer to a network location or portable USB drive.
 - d Copy the installer to `C:\Illumina`.
 - e Open `iSeqSuiteInstaller.exe`, and then follow the prompts to perform the install.
 - f When the update is complete, select **Finish**.
 - g Power cycle the instrument. For instructions, see *Power Cycle the Instrument on page 45*.
 - 6 Follow the onscreen prompts to perform first time setup, including the system check with the reusable test cartridge and reusable test flow cell.
 - 7 Install any Local Run Manager analysis modules:
 - a Switch to the sbsadmin operating system account.
 - b Download the software installers from the Local Run Manager support pages. Save the installers to a network location or portable USB drive.
 - c Copy the installer to `C:\Illumina`.
 - d Open the installer (*.exe), and then follow the prompts to perform the install.
 - e When the update is complete, select **Finish**.

Appendix C Advanced Exchange

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Exchanging the iSeq 100 System

Because the air filter and drip tray pad are the only serviceable parts the iSeq 100 System has, Illumina uses advanced exchange to fix issues that cannot be resolved remotely.

Advanced exchange replaces a damaged or defective system with a refurbished system. To minimize downtime, you receive the replacement system before shipping back the original.

Regional Availability

Advanced Exchange is available in most regions. Other regions can continue to rely on field service engineers. Ask Illumina Technical Support which support models are available in your region.

Receive a Replacement System

- 1 After a system check and other troubleshooting efforts have failed, contact Illumina Technical Support.
 - ▶ If possible, perform another system check with a different reusable test cartridge and reusable test flow cell.
 - ▶ Make system check results available to Technical Support.

If Technical Support cannot resolve the issue remotely, a return is initiated and an order is placed for a replacement system.

- 2 When you receive the replacement system:
 - ▶ Unpack and install it per the *iSeq 100 Sequencing System Setup Poster (document # 1000000035963)*.
 - ▶ **Save all the packaging**, which is used to package the original system and reusable test components for return.
 - ▶ Set aside the return documents, which include a UPS return label and commercial invoice (for international shipments).

Prepare the Original System for Return

Return the original system, reusable test cartridge, and reusable test flow cell to Illumina within 30 days of receiving the replacement.

Shut Down and Disconnect

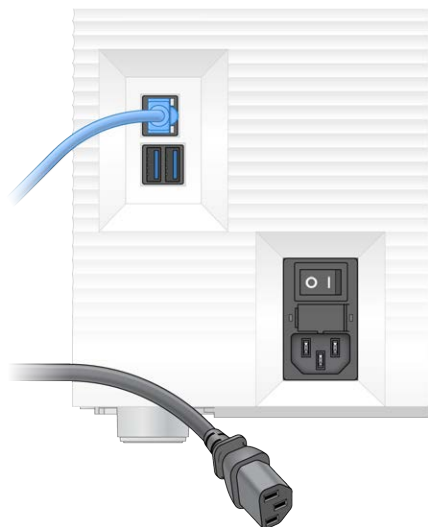
If the system is on, follow steps 1–3 to save and clear data before safely shutting down the system. Follow the remaining steps to remove a cartridge (if necessary) and disconnect cords and cables.

- 1 From File Explorer, copy any files and folders that you want to save to a portable USB drive. The location of sequencing data is user-defined, but **drive D** is the default location.
- 2 Delete any files and folders you do not want to share with Illumina.
- 3 Shut down the system as follows.

- a From the menu, select **Shut Down System**.
 - b If the shutdown command is unresponsive, press and hold the power button on the left side of the instrument until the lights fade.
 - c When the power button pulses, press the power off (O) side of the toggle switch on the back of the instrument.
- 4 If a cartridge is inside the instrument, restart the system and remove the cartridge as follows.
 - a Press the power on (I) side of the toggle switch on the rear panel.
 - b When the power button pulses, press it.
 - c When the operating system is loaded, log on to Windows.
 - d From the control software menu, select **System Check**.
 - e Select **Unload** to eject the cartridge, and then remove the cartridge from the tray.
 - f If ejection fails, contact Illumina Technical Support for further instructions.
 - g Select **Load** to retract the empty tray and close the door.
 - h Close the System Check dialog box, and then shut down the system.

Shutting down and restarting the system is necessary to position the cartridge for removal.

- 5 Disconnect the power cord from the wall outlet, and then from the AC power outlet on the rear panel.



- 6 If applicable, disconnect:
 - ▶ The Ethernet cable from the wall outlet, and then from the Ethernet port on the rear panel.
 - ▶ The keyboard and mouse from the USB ports on the rear panel.

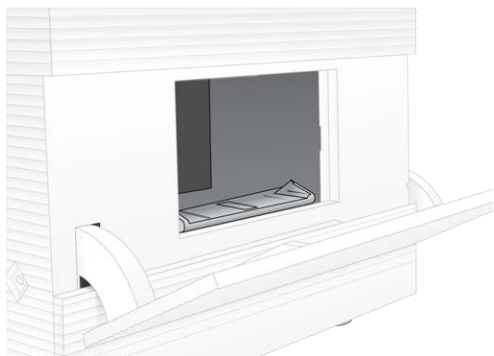
Decontaminate the Instrument

Shipping an instrument requires the following decontamination procedure, which Illumina confirms is complete. Site-specific hazards can require additional decontamination.

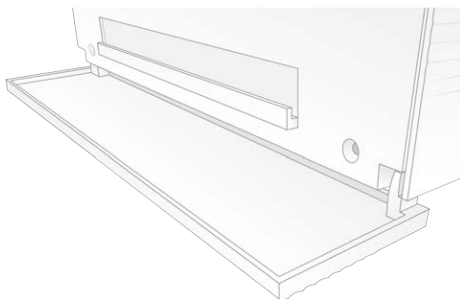
Decontaminate With Bleach

- 1 Put on a new pair of powder-free gloves.
- 2 Lower the instrument monitor.

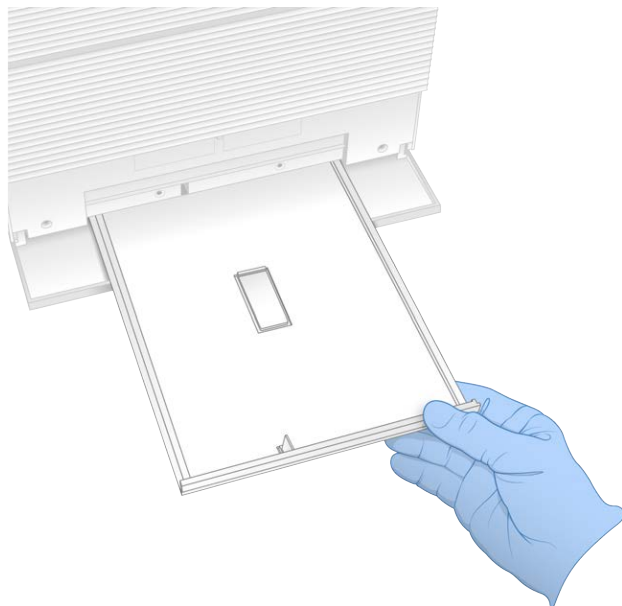
- 3 Gently pull the cartridge compartment door from the side edges to open.



- 4 Clean the entire compartment door with a bleach wipe:
 - ▶ Door interior
 - ▶ Door exterior
 - ▶ Door hinges
- 5 Close the cartridge compartment door.
- 6 Locate the drip tray door below the cartridge compartment on the front of the instrument, and then lower the door.



- 7 Open the drip tray and remove the drip tray pad.



- 8 Wipe any residual fluid from the bottom of the tray using paper towels.
- 9 Dispose of the pad and other consumables in accordance with applicable standards, which vary by region.
For more information, see the Safety Data Sheet (SDS) at support.illumina.com/sds.html.
- 10 Clean the drip tray with a bleach wipe.
- 11 Wait 15 minutes for the bleach to take effect.

Neutralize With Alcohol

- 1 Moisten a cloth or paper towels with water.
Any grade of water is acceptable, including tap water.
- 2 Wipe the following components with the moistened cloth or paper towels:
 - ▶ Drip tray
 - ▶ Cartridge compartment door (interior and exterior, including hinges)The water prevents bleach and alcohol from mixing.
- 3 Reclean the following components with an alcohol wipe:
 - ▶ Drip tray
 - ▶ Cartridge compartment door (interior and exterior, including hinges)The alcohol removes residual bleach, which can cause corrosion.
- 4 Make sure that the drip tray door and cartridge compartment door are closed.
- 5 Clean the lab bench around the instrument with bleach wipes or bleach solution.

Return the Original System

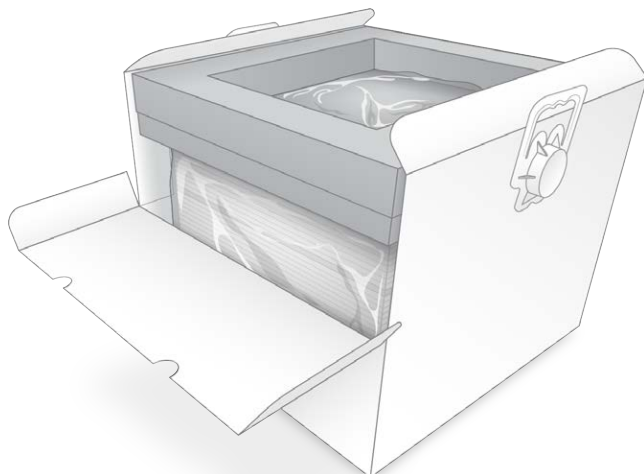
Package the Instrument

- 1 Clear adequate space in the lab for the instrument and packaging.
- 2 Insert the small foam pad between the lowered monitor and the instrument.
- 3 Place the gray plastic bag over the instrument.



- 4 Lower the front flap of the white box.
- 5 Place the instrument into the white box so that the front of the instrument faces you.

- 6 Place the square foam over the instrument so that the thinner sides of the foam rest on the front and back of the instrument. Make sure that the foam is flush with the top of the box.



- 7 Close the front flap, and then close the top of the box.

Package the Reusable Test Components

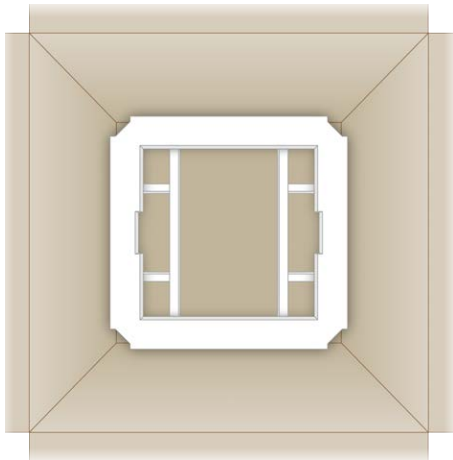
- 1 Put the iSeq 100 i1 Reusable Test Cartridge into the larger resealable bag and seal.
- 2 Put the iSeq 100 i1 Reusable Test Flow Cell into the clamshell case.
- 3 Put the clamshell case into the smaller resealable bag and seal.
- 4 Place both resealable bags into the iSeq 100 Sequencing System Accessories box.



- 5 Close the accessories box.

Ship the System

- 1 If it was removed, place the protective foam base into the bottom of the brown shipping box.



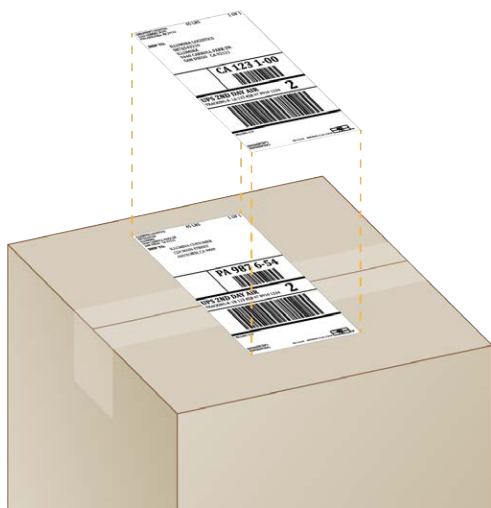
- 2 Lift the white box by the handles (a two-person lift is recommended), and then lower the white box into the brown box. Any orientation is acceptable.



CAUTION

The white box must be shipped inside the brown box. The white box is not designed or labeled for shipping.

- 3 Place the protective foam cover over the top of the white box.
- 4 Place the accessories box into the center of the foam cover.
- 5 Place the black foam pad on top of the accessories box.
- 6 **[Optional]** Place the Ethernet cable and power cord anywhere in the brown box.
- 7 Close the brown box and secure with shipping tape.
- 8 Place the return label over the original shipping label, or remove the original shipping label.



- 9 **[International shipment]** Affix the commercial invoice to the shipping box.
- 10 Ship the instrument to Illumina via UPS.
 - ▶ If your lab has daily deliveries scheduled with UPS, give the labeled shipping box to the driver.
 - ▶ If your lab does not have daily deliveries with UPS, notify Illumina Customer Service so that they can schedule the return shipment for you.

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

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

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

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



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