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NeoPrep[™] Library Prep System Guide



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Customize a short end-to-end workflow guide with the Custom Protocol Selector support.illumina.com/custom-protocol-selector.html

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Revision History

Document	Date	Description of Change
Material # 20000941 Document # 15049720 v01	October 2015	 Updated software descriptions to the NeoPrep Control Software v1.1.0: Added number of test card runs to the About screen Added ability to save license agreement from About screen System Configuration screen reorganized to separate network and BaseSpace configurations Instrument can connect to BaseSpace Onsite Changed 'assay' to 'protocol' Changed 'module' to 'proces' when referring elements of a run Added a filter by protocol type when selecting a BaseSpace run Default protocol version can be specified in Change Version Default on the Confirm screen now provides the capability to select the set of default indexes for each sample row Only consumables required for a protocol are displayed on the Track screen Tracking oil vial and reagent plate serial numbers are required, at a minimum Changed library card loading order to load samples first Renamed button Home at the conclusion of unloading libraries Added Instrument Level screen Access Logs includes system checks Updated System Check instructions and added the ability to retest Changed reference to the main screen as Home Restructured guide contents Removed software screen figures Added Preventive Maintenance section Added test card cleaning instructions
Part # 15049720 Rev. C	June 2015	Added required pipettes to <i>Consumables</i>
Part # 15049720 Rev. B	April 2015	 Added required pipette tip and calibration information to: New <i>Pipette Tip Requirements</i> <i>Tips and Techniques</i> <i>Load the Library Card</i> Changed BaseSpace resource reference to helpcenter
Part # 15049720 Rev. A	March 2015	Initial release.

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Introduction

The Illumina[®] NeoPrep[™] Library Prep System enables library preparation based on the use of digital microfluidics for liquid handling. The system provides a simple user interface and load-and-go operation to limit hands-on time with high-quality and reproducible results.

Features

- Limited upfront sample handling—Samples are prepared before they are loaded on the NeoPrep.
- **BaseSpace**[®] **integration**—The library prep workflow is integrated with BaseSpace, the Illumina genomics computing environment for data analysis, storage, and collaboration. For instruments configured for BaseSpace, library information and run parameters are specified on the BaseSpace Prep tab before setting up the run on the instrument. Runs that were set up in BaseSpace appear on the instrument interface during run setup. As the run progresses, output files are streamed in real time to BaseSpace or BaseSpace Onsite.
- **Simplified library prep**—Delivers highly reproducible, sequencing-ready libraries with minimal hands-on time.
- Assay specific reagents Assay specific reagents are provided in a sealed reagent plate.
- ▶ **Load and go operation**—A graphical user interface guides you through the run set-up to select a protocol, load samples, and reagents onto the library card, and verify before starting a run.
- Digital microfluidics library card—Digital microfluidics precisely manipulate droplets to perform all library prep processes within the tightly controlled environment of the NeoPrep library card.
- **Library prep, quantification, and normalization**—Prepare, quantify, and normalize libraries in a single run without user intervention.
- Ready for pooling, denaturation, dilution—Libraries are ready to be prepared for clustering on any Illumina instrument.

Digital Microfluidics

Digital microfluidics technology is at the core of the NeoPrep operation. An array of electrodes patterned on a printed circuit board (PCB) controls aqueous droplets within an oil-filled chamber. By switching the voltages applied to the electrodes, droplets are dispensed, transported, mixed, and incubated on the library card. The software controls all steps in the complex microfluidics protocols.



Figure 1 Library Card Cutaway - Side View

Library Prep Workflow



Figure 2 Sequencing and NeoPrep Library Prep Workflow

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

Resource	Description
NeoPrep System Site Prep Guide (document # 15050812)	Provides specifications for laboratory space, electrical requirements, and environmental considerations, and system installation instructions.
NeoPrep System Safety and Compliance Guide (document # 15050811)	Provides information about instrument labeling, compliance certifications, and safety considerations.
NeoPrep library prep guides	Provide instructions for preparing libraries using the NeoPrep. See the documentation support page for the library prep kit that you are using.
Illumina Experiment Manager Guide (document # 15031335) and IEM NeoPrep Quick Reference Card (document # 15061111)	Provide information about creating and editing appropriate sample sheets for Illumina sequencing systems and analysis software and record parameters for your sample plate.
BaseSpace help (help.basespace.illumina.com)	Provides information about the BaseSpace sequencing data analysis tool that also enables you to organize samples, libraries, pools, and sequencing runs in a single environment.

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

Instrument Components

The NeoPrep includes a touch screen monitor, a status bar, and a library card compartment.



- A Status bar Indicates instrument status as processing (blue), processing with warnings (blue blinking), needs attention (orange blinking), preparing the run (green blinking), and ready to begin the run or finished with a run (green).
- **B** Touch screen monitor Enables on-instrument configuration and setup using the software interface.
- **C Integrated library prep module**—Contains the enclosed thermal, electrical, magnetic, and optical components.
- **D** Library card compartment—Contains the library card stage that holds the library card throughout the run. Library card stage motors move the stage into the integrated library prep module when the run begins and return the stage to the compartment when the run is complete.

Library Card Compartment

The library card compartment houses the library card for loading and unloading.

Figure 3 Library Card Compartment



- A Library card stage—Houses a library card.
- **B** Library card compartment door—Open to load and unload a library card. Close during operation.
- C Library card compartment light-Illuminates the library card stage when the door is open.
- **D** Library card—Single-use consumable where samples and reagents are loaded and libraries are collected.
- **E** Library card latch release Releases the library card latches for placing and removing the library card from the library card stage.
- F Library card latches—Hold the library card in place on the library card stage.

NeoPrep Software

The NeoPrep Control Software has a touch screen interface with step-by-step instructions for loading oil, samples, reagents, and adapters onto the library card before beginning a run. During the run, the control software operates the library prep, quantification, and normalization processes. The run status appears on the screen as the run progresses.

Status Icons

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

Status Icon	Status Name	Description
\checkmark	OK	System is normal.
\mathbf{V}	Processing	System is processing.
	Warning	A warning has occurred.
		Warnings do not stop a run.
	Error	An error has occurred.
X		Errors require action before proceeding with the run.
0	BaseSpace	The instrument is connected to BaseSpace.

When a change in condition occurs, the icon blinks to alert you. Select the icon to view a description of the condition.

- Select any item listed to see a detailed description of the condition and instructions to resolve the condition, if applicable.
- Select **Acknowledge** to accept the message and **Close** to close the dialog box.

To filter the types of messages that appear in the status window, select the icons along the top margin of the window. Selecting an icon toggles the condition to show or hide.

Power Button

The power button on the front of the NeoPrep turns on power to the instrument. The power button performs the following actions, depending on the state of instrument power.

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



NOTE

Turning off the instrument during a run ends the run immediately. AC power is only removed from the instrument when then the AC cord is unplugged. Ending a run is final. Run consumables cannot be reused and samples are lost.

Library Prep Kit Overview

To perform a run on the NeoPrep Library Prep System, a library prep kit for NeoPrep is required. Illumina offers several compatible options. Each assay-specific kit contains the following consumables for a single run:

- Reagent plate and guide
- Reagent tubes
- Oil vial and funnel
- Library card
- Library card guide
- Library separation tube strips

For information about library prep kit contents, see the library prep guide for the kit that you are using.

Library Card Overview

NeoPrep library prep kits include a single-use, disposable library card designed to facilitate hands-free digital microfluidics library preparation on the NeoPrep. The library card is the vessel for the complete library prep workflow without manual intervention, including optional quantification and normalization.

Design

Each library card consists of a plastic top plate with ports for oil, sample, reagent, and adapter loading. Library cards prepared for use consist of an oil layer between 2 substrates onto which insulated electrodes are patterned. By changing the relative voltages of the patterned electrodes, aqueous droplets containing samples and reagents are manipulated to perform complex protocols. Droplets are dispensed from loading ports and transported to various locations on the library card, where they are mixed and incubated by software control. Reagents are contained in discrete droplets of oil, which isolates the reactions from the lab environment and carryover contamination.

Usage

Library cards are unpacked, placed onto the library card stage, and loaded with oil, samples, reagents, and adapters. Library cards are a single-use consumable. If a run is canceled before completion, the library card cannot be reused.

Handling

To ensure proper protocol performance, the library card loading procedure must be strictly followed. See Chapter 3 Performing A Run and see the assay-specific guide for library card loading and setup procedures. When opening the library card package, do not flex or distort the library card.

Library Card Components

One library card is provided in each NeoPrep library prep kit. Each library card has the following elements.



- A Notched corner—Used as a reference for library card orientation
- B Keyed feature Aligns the library card to the library card stage
- C Sample wells-Input samples are pipetted into the sample wells
- D Adapter wells-Adapters are pipetted into the adapter wells
- **E** Library collection wells—Prepared libraries are extracted from the library collection wells
- F Reagent wells-Reagents are transferred from the reagent plate to the reagent wells
- G Oil well-A layer of oil is added to the library card

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NeoPrep Library Prep System Guide

Starting the Instrument

NOTE

The power switch on the back of the instrument is turned on during installation of the instrument. Unless you plan to unplug the instrument, there is no need to turn off the power switch.

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

Figure 5 Power Switch Located on Back of Instrument



2 Wait a moment, and then briefly press the power button on the front left corner of the instrument. The power button turns on the instrument power and starts the integrated instrument computer and software.

Figure 6 Power Button Located on Front of Instrument



- Wait until the operating system has finished loading.
 The NeoPrep Control Software launches and initializes the system automatically.
 After the initialization step is complete, the Home screen opens.
- 4 If your system has been configured to require login credentials, log in using the default user name and password:
 - User name: sbsuser
 - Password: sbs123

Otherwise, login using the credentials for your site.



Leave the instrument on continuously. If the instrument must be turned off, follow the shutdown procedure described in *Shut Down or Restart the Instrument* on page 41. After shutting down the instrument, always wait a *minimum* of 60 seconds before turning on the instrument.

Customize System Settings

The control software includes customizable system settings for start-up options, input preferences, audio settings, and instrument name.

Select Display, Identification, and Audio Options

- 1 From the Manage Instrument screen, select **System Customization**.
- 2 To assign a preferred image for your instrument, select **Upload Image** and navigate to the image. The image appears at the top of the software interface.
- 3 In the Nick Name field, enter a preferred name for the instrument. The name appear at the top of the software interface.
- 4 Select from the following start-up options:
 - Select **Kiosk Mode** to use the control software interface in full screen.
 - Select Windowed Mode to allow access to Windows on the instrument computer. Interaction with the software interface, such as button location, is likely to be altered in this mode.



Alternatively, use the **Exit to Windows** command on the Shutdown Options screen to access Windows temporarily. The Exit to Windows command closes the control software.

- 5 Select the **Play audio** checkbox to turn on audio indicators for the following events.
 - Upon instrument initialization
 - When a run is started
 - When certain errors occur
 - When user interaction is required
 - When a run has finished
- 6 Slide the **Volume Control** bar to the right to increase the audio volume or to the left to decrease the audio volume.
- 7 Select **Next** to save settings and advance the screen.

Set Input, Tracking, and Loading Guide Requirements

- Select the **Use on-screen keyboard** checkbox to activate the on-screen keyboard for input to the instrument.
 - If the checkbox is selected and a keyboard is connected to an external USB port, then both the on-screen keyboard and the external keyboard can be used.
 - Clear the checkbox to disable the on-screen keyboard and only use a keyboard connected to an external USB port.
- 2 At a minimum, oil vial and reagent plate serial number tracking are required for each run. Select the **Track all consumables** checkbox to require tracking the following Illumina-supplied consumable information for all consumables used in a run.
 - Reference number
 - Serial number
 - Lot number
 - Expiration date

Depending on the protocol, tracking other consumable serial number might be required. See *Track the Consumables* on page 27.

- 3 Select the **Require loading guide** checkbox to require using the control software loading guide instructions when loading the library card. Clear this checkbox to allow the loading guide instructions to be skipped when loading the library card.
- 4 Select the **Require pre-verification** checkbox to require successful verification of the library card before loading oil, samples, reagents, and adapters. If the library card cannot be successfully verified, then the run cannot start.
 - > The library card is always automatically verified when **Start Run** is selected.
 - Clear this checkbox to disable the requirement to Verify Library Card before loading consumables and samples. The verification step can be skipped, but if the library card does not pass verification when **Start Run** is selected, loaded consumables cannot be reused and the samples are lost.
- 5 Select **Next** to save settings and advance the screen.

Set System Time

- 1 Select the **Local Time Zone** from the drop-down list.
- 2 Select the current **Year** from the drop-down list.
- 3 Select the current **Month** from the drop-down list.
- 4 Select the current **Day** from the drop-down list.
- 5 Set current hour, minute, and AM/PM using the up and down arrows.
- 6 Select the **Use 24-hour time** checkbox to display time using a 24-hour clock. Clear this checkbox to display time using a 12-hour clock.
- 7 Select **Update time** to synchronize with the server time.
- 8 Select Finish.

User-Supplied Consumables

The following consumables are required for use with the NeoPrep. For more information, see the *NeoPrep System Site Prep Guide (document # 15050812)*. Use the required pipettes and tips. Using other pipettes and tips can cause improper reagent dispensing and run failure. See the library prep guide, for the kit that you are using for additional user-supplied consumables and equipment requirements.

Pipettes and Tips

Volume	Use	Product Name	Supplier
20 µl	≤20 µl	Pipet-Lite XLS+ 8-channel LTS, 2 μl to 20 μl	Rainin, catalog # L8-20XLS+
		One of the following:	
		• LTS tips 20 µl. Presterilized. Filter	 Rainin, catalog # RT-L10F
		• ART Barrier Pipette Tips 20 μl; 20 μl SoftFit-L	 Fisher Scientific, catalog # 2749RI
200 µl	21–200 µl	Pipet-Lite XLS+ 8-channel LTS, 20 μl to 200 μl	Rainin, catalog # L8-200XLS+
	_	One of the following:	
		• LTS tips 200 µl. Presterilized. Filter	 Rainin, catalog # RT-L200F
		 ART Barrier Pipette Tips 200 μl; 200 μl SoftFit-L 	 Fisher Scientific, catalog # 2769RI

Consumables

Item	Supplier
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	VWR, catalog # 15648-981 or equivalent General lab supplier
Deionized or distilled water	General lab supplier
Disposable gloves, powder-free	General lab supplier
Dust-free tissues	VWR, catalog # 52846-001 or equivalent
Paper towels or lint-free cotton cloths	General lab supplier

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Introduction

Before beginning a run on the NeoPrep, samples must be prepared for loading. To prepare your samples, see the corresponding library prep guide for the kit that you are using.

To perform a run on the NeoPrep, follow the prompts in the control software to set up the run and load the library card. After the run begins, no other user interaction is required until the run is complete. Monitor run progress and statistics from the control software interface.

Tips and Techniques

Sealing a Plate

- Always seal a 96-well plate before centrifuging.
- Apply the adhesive seal to cover the plate and seal with a rubber roller.
- Microseal 'B' adhesive seals are effective at -40°C to 110°C, and suitable for skirted or semiskirted PCR plates.

Handling the Library Card

- To avoid instrument damage, do not place the library card guide on the library card during library card verification or a run.
- Use the library card latch release to load and remove the library card from the library card stage.
 - Do not snap the library card into place.
 - Oil and reagents in a used library card can splash out of the card and onto the instrument.
- Hold the used library card level when removing it from the instrument to avoid spilling its contents.

Library Card Loading Guidelines

- Load the library card while it is on the library card stage to avoid spilling or disturbing the loaded contents.
- This guide provides an overview of the loading process and can be used as a reference. When loading the library card, use the control software loading guide while loading the run for detailed instructions and reminders for each step.
- Do not open the compartment door during library card verification or run.
- Change your gloves after loading the oil.
- Transfer contents from the reagent plate to the corresponding wells on the library card. Reference the corresponding colors and well labels on the reagent plate and library card guides.
- Make sure that pipettes are calibrated before beginning. Uncalibrated pipettes can lead to variations in insert size, reagents not dispensing properly, and result in run failure.
- Use the pipettes and tips specified in *Pipettes and Tips* on page 15 and the library prep guide for the kit that you are using. Other pipettes and tips are not supported and can result in reagents not dispensing properly and run failure.
- Use a multichannel pipette to load samples, reagents, and adapters.
- Not all reagent wells are used for each protocol.
- To avoid instrument damage, make sure that the library card guide is removed from the library card before starting the run.

Library Card Loading Techniques

- Use proper library card loading techniques and specified loading angles.
- Pipette to the first stop to avoid creating bubbles.
- Insert the pipette tips perpendicular to the well.
- Insert the pipette tips to the bottom of the well while dispensing. Do not lift the tips until the reagents are dispensed completely.
- Dispense at an angle by pointing pipette tips under the well label and dotted well outline on the library card guide.
- The pipette loading angle depends on the item being dispensed. The angle is specified in each step of the control software loading guide and is depicted in the library prep guide.
- An icon represents the loading angle and the volume is specified on the control software loading guide. For example, (1/2 5 µl)

 Icon
 Description

 Image: A state of the provided stat

Increase the pipette angle if liquid is not dispensing from the pipette tips.

Handling Samples

- Always track the location of each sample.
- Change tips between each sample to avoid cross-contamination.
- Do not centrifuge samples before loading.
- Some space might be present in the pipette tips during transfer from the sample plate to the library card.
- For protocols with 2 sample insert size options, both insert sizes can be included in a single run.

Collecting Libraries

- Unload the library card while it is on the library card stage.
- > The control software guides you through the steps to unload libraries.
- Use a 200 μl multichannel pipette with filter barrier tips.
- Do not use a 20 µl pipette. It does not fit properly into the library card well.
- Insert pipette tips perpendicularly and touch the tips to the bottom of the collection wells.
- Hold down the library card with one hand while removing the tips from the collection wells to prevent any movement of the card.
- An icon represents the required pipette angle, and the volume is specified on the control software unloading guide. For example, (1 μl)

Inspect each pipette tip to make sure that a blue library droplet is present in the tips indicated by the control software.

Figure 7 Library Droplet in Pipette Tips



- If a blue library droplet is not visible in each expected pipette tip, do the following:
 - > Transfer the extracted liquid to the corresponding plate well containing RSB.
 - Do not dispense the liquid back into the library card, which can introduce air gaps and interfere with library extraction.
 - Use a single-channel pipette to repeat the transfer 1 time for the wells that did not contain the blue droplet. Do not attempt the transfer more than 2 times.
- Vigorously pipette up and down in RSB to dislodge the blue library droplet from the pipette tip.

Handling Library Separation Tube Strips

- Label the tubes to support tracking sample location.
- Use the wells of a plate or another device to hold the library separation tube strips upright.
- Do not centrifuge library separation tube strips.

Library Prep Workflow



Select **Prepare Libraries** from the control software.



Set up your run using the control software or select from a BaseSpace run.



Place the library card onto the instrument.



Load the oil, samples, and reagents onto the library card.



Select Start Run to start the library prep.



Extract the libraries from the library card and separate them from the oil.



Remove the library card from the instrument.

Begin the Run Setup

1 From the Home screen, select **Prepare Libraries**.

The steps to begin the run setup differ depending on how the instrument is configured. For more information, see *Configure System Settings* on page 49.

- BaseSpace or BaseSpace Onsite The run setup screens lists runs that were set up using the BaseSpace Prep tab.
- **Standalone**—The run setup screens include fields for defining run parameters.

Prepare Libraries screens include the following commands:

- **Back**—Returns to the previous screen.
- Exit—Does not save changes and returns to the Prepare Libraries screen.
- Next—Saves changes and progresses to the next screen.

Log in to BaseSpace (BaseSpace Configuration)

📜 NOTE

If the system was configured to use a default login and bypass the BaseSpace login screen, the BaseSpace screen does not appear. For more information, see *Set BaseSpace Configuration* on page 50.

- 1 Enter your BaseSpace user name and password.
- 2 Select Next.

Select a Run (BaseSpace Configuration)

- 1 Select the **I** icon to sort the list alphabetically by run name in ascending or descending order.
- 2 Select the original icon to sort the list in the order that the runs were saved, with the most recently saved run at the top of the list.
- 3 Select **Protocol Type** to sort the list alphabetically by protocol type in ascending order.
- 4 Select **Filter** to view only a specific protocol.
- 5 Select a run from the list runs that were set up in BaseSpace.
- 6 Select Next.

Specify Run Parameters (Standalone Configuration)

- 1 Select the protocol to run using the following methods:
 - Select Select by barcode to identify the protocol automatically using the following methods. For either method, the serial number will appear on the Track screen. See *Track the Consumables* on page 27.
 - Use the barcode scanner to scan the barcode on the reagent plate. An audible beep indicates that the scanner has successfully read the reagent plate barcode. The reagent plate serial number appears on the screen.
 - Enter the reagent plate serial number in the Scan reagent plate barcode field.
 - Select **Select by name** to select the protocol from a list.
 - The protocol options are displayed.
 - Select the desired protocol from the list.
 - If the list is longer than can be displayed on a single screen, navigate through the list using the scroll-bar on the screen.
- 2 Select Next.
- 3 [Optional] To run another different version of the protocol, select Change Version.
 - Select the version to run.
 - Select a different version to run as the default. At initial setup, the default is the latest version
- 4 Select Next.

Set Up the Run

Before library prep can begin, configure and confirm your run on the instrument and track the consumables. The options in the run setup differ depending on the system configuration and customization settings. Review the default settings and modify it as needed.

Configure the Run

NOTE

- The configuration options and settings are dependent upon the protocol. For options and defaults for the selected protocol, see the library prep guide for the kit that you are using.
 Only the PCR Cycles setting is editable when running in BaseSpace mode.
- 1 [Optional] Select **Load Settings** to load a run configuration that was created and saved on the instrument, and then select **Load**.
 - Alphabet—Sorts the list alphabetically in ascending or descending order.
 - Recently Added—Sorts the list in the order that the settings were saved, with the most recently saved settings at the top of the list.
- 2 By default all processes are selected for the run. Deselect the optional processes, if desired.
 - **Prep Library**—Prepares libraries and must be selected.
 - Optional] Quantify—Quantifies samples during the run, after library prep is complete.
 - Optional] Normalize Normalizes final libraries during the run, after quantification is complete. This option can only be selected if Quantify is also selected.
- 3 [Optional] Select Save Settings to save the run configuration.
 - Enter a file name in the File Name field.
 - Select Save to save the configuration. Alternatively, select Back to return to the Configure screen without saving the run configuration.
 - If parameters of a loaded configuration are modified, the configuration must be saved with a new name.
- 4 Review and modify the default parameters, if necessary. Select **Default** next to a parameter to reset it to the default setting.
 - **Sample Count**—The number of samples in the run.
 - ▶ PCR Cycles The number of PCR cycles. Only the default setting is supported.
 - Insert Size The sample insert size. If available, an insert size option must be selected for each sample. If the Mixed option is selected, then the insert size for each sample must be specified when confirming the run settings. For more information, see *Confirm the Run* on page 26.
- 5 Select Next.

Confirm the Run



When running in BaseSpace mode, run confirmation settings cannot be changed.

- 1 Confirm the Run Info:
 - **Run Name**—Name of the run.
 - **Operator**—Person operating the run.
 - Project—Project that the run is being performed for.
 - Secondary Output Folder—Optional location where a copy of the run files is saved, in addition to their standard local saved location on the instrument. This folder is specified in *Set BaseSpace Configuration* on page 50.
 - ▶ **Notes**−Notes about the run.
- 2 [Optional] With the Run Info tab selected, select **Edit** to edit the fields in the run information tab, and then select **Save Changes**. Notes from a run setup in BaseSpace are not editable.
- 3 Confirm the Sample Info:
 - Well—Location of the sample on the library card.
 - **Pool**—Pool that the sample will be in at the end of the run.
 - Sample Name Name of the sample.
 - ▶ Index—Index (adapter name and sequence) to be added to the sample.
 - Insert Size The sample insert size. If the Mixed Insert Size option was selected for the run parameters, then the sample insert size is displayed and step 5 is required. This is not available nor required for some protocols.
- 4 [Optional] Select **Import Sample Sheet** to navigate to a saved sample sheet. Use the Illumina Experiment Manager (IEM) to create an appropriate sample sheet or the BaseSpace Prep tab to organize samples and the library prep run. For more information, see *Additional Resources* on page 4.
- 5 [Optional] With the Sample Info tab selected, select Edit to edit the sample information.
 - Edit the Sample Name field
 - Edit the Index field:
 - Select the index.
 - Select the appropriate index from the list that opens.
 - Select Select.
 - Edit the default set of indexes used for each sample row.
 - Select Default.
 - Select the default index column (A–H, I–P, or Q–X) for samples 1–8 and/or 9–16.
 - Select Select.
 - When running a library prep with 2 different sample insert sizes, specify the insert size for each sample:
 - Select Edit from the Insert Size column. An insert size number is shown.
 - > Select on the insert size number to toggle between the insert size options.
 - Select Save Changes.
- 6 Select Next.

Track the Consumables

Track the library prep kit consumables that are being used for the run.

- For specific library prep kit consumables, see the library prep guide for the kit that you are using.
- Oil vial and reagent plate serial number tracking is required.
- Depending on the protocol, other consumable tracking might be required.

Providing tracking information for all consumables can be disabled and is optional, unless the NeoPrep is configured to require them. See *Track all consumables* in *Customize System Settings* on page 13.

- If all are required, the run setup cannot continue until all information is scanned or manually entered.
- If all are not required, only those that are required appear on the Required tab. It is optional to scan and/or manually enter the information for consumables other than those required.

Perform Consumable Tracking

- 1 To populate the consumable fields on each tab, select the tab. If not all consumables are required, then a Required tab displays only the consumables that require tracking and only the serial number is required for those consumables.
- 2 Do 1 of the following:
 - Use the NeoPrep barcode scanner to scan the consumable serial number barcode. An audible beep indicates when the scanner has successfully read the barcode. The consumable serial number appears on the screen.
 - Manually enter the serial number.

If not all consumables are required, then a Required tab displays only the consumables that require tracking and only the serial number is required for those consumables.

- 3 Manually enter the consumable reference number, lot number, and/or expiration date
- 4 Repeat steps 1–3 for each tab.
- 5 Select Next.

Load the Library Card

The control software loading guide provides step-by-step instructions for loading oil, samples, reagents, and adapters onto the library card for the selected protocol. The instructions are also available in the library prep guide for the selected protocol. Use the arrows to navigate the loading guide:



Procedure

- 1 Put on a new pair of powder-free gloves.
- 2 Follow the on-screen instructions to gather consumables specific for the selected protocol. For more information on assay-specific consumables, see the corresponding library prep guide.



WARNING

Use the pipette tips specified in the library prep guide. Other tips are not supported and can result in reagents not dispensing properly and run failure.

- 3 Vortex the reagent plate for 3 seconds.
- 4 Centrifuge at 600 × g for 5 seconds.If you are not using the reagent plate immediately, set aside on ice.

5 Follow the on-screen instructions to verify the library card.



To avoid instrument damage, make sure that the library card guide is not on the library card.

Figure 8 Library Card on Library Card Stage



- a Open the library card compartment door.
- b Slide the library card latch release to the right and place the library card on the library card stage. Make sure that the keyed features and the notched corners of the library card are on the left.
- c Release the library card latch release, so that the library card latches secure the library card on the library card stage.
- d Close the library card compartment door, and then select **Verify Library Card**. Do not open the compartment door during library card verification.



NOTE

For experienced users, you can skip the loading instructions. Select **Skip Guide** after the library card is verified. If you skip the loading instructions, proceed to *Start the Run* on page 31.

Depending on system setup, the loading guide instructions might be required. See *Require Loading Guide* in *Customize System Settings* on page 13.

6 Place the library card guide on the library card.

Figure 9 Library Card Guide on Library Card



7 Follow the on-screen instructions to load oil, samples, reagents, and adapters onto the library card.



WARNING

The reagent plate contains hazardous materials. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and a laboratory coat. Handle the used reagent plate as chemical waste. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the SDS for the kit that you are using at support.illumina.com/sds.html.

Figure 10 Reagent Plate to Library Card Transfer Layout



- A Reagent plate
- **B** Library card
- C Oil
- **D** Large reagents
- E Small reagents
- F Samples
- G Adapters
- 8 Remove the library card guide. Keep it for later use during the unloading process.



WARNING

To avoid instrument damage, make sure that the library card guide is removed from the library card.

9 Close the library card compartment door.

Start the Run

1 Select **Start Run** to begin the run. Do not open the library card compartment door until the run is complete.

While the instrument is running, the current procedure in process and the estimated run completion time are displayed. Sample and run information are also available:

- Run Data—Provides name, index, quantification, and normalization results for each sample.
- **Run Details**—Provides run information, including the protocol, operator, run ID, library card identifier, processes, and run parameters.



NOTE

Select **Stop** to stop a run. A dialog box opens to confirm the stop. *Stopping a run is final.* A stopped run cannot be resumed. The reagents and samples in the library card are not recoverable.

2 When the run is complete, select **Next** and proceed to *Unload the Libraries*.



Libraries can remain at room temperature on a library card for up to 3 days after a run is complete

Unload the Libraries

The control software unloading guide provides step-by-step instructions to collect libraries from the library card, separate libraries from the oil, and unload the library card from the instrument. The instructions are also available in the library prep guide for the selected protocol.

Use the arrows to navigate the unloading guide:



Make sure that you have all necessary consumables before proceeding.

- RSB (Resuspension Buffer)
- Library separation tube strips (2)
- ▶ 96-well 0.3 ml PCR plates (2)
- Microseal 'B' adhesive seals (2)

WARNING

The used library card contains hazardous materials. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and a laboratory coat. Handle the used reagent plate as chemical waste. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the SDS for the kit that you are using at support.illumina.com/sds.html.

Procedure

- 1 Remove RSB from 2°C to 8°C storage and bring it to room temperature.
- 2 Label wells of 2 new 96-well 0.3 ml PCR plates 1–16.
- 3 Label each tube of a library separation tube strip 1–8 and each tube of another library separation tube strip 9–16.
- 4~ Add 10 μl RSB to each well of a PCR plate.
- 5 Open the library card compartment door and place the library card guide on the library card.
- 6 Use a 200 μ l pipette to transfer 20 μ l from wells 1L–8L, then 9L–16L to corresponding wells 1–16 of the plate containing RSB. Pipette to mix.

Figure 11 Library Card Collection Wells

- A Collection wells 1L–8L
- **B** Collection wells 9L–16L
- 7 Centrifuge briefly.
- 8 Transfer the entire volume from wells 1–8, then 9–16 of the plate to the center indent in the membrane of the corresponding library separation tubes 1–16.
- 9 Let the library separation tubes stand for 10 seconds while the oil is absorbed in the tubes.
- 10 Transfer the entire volume from library separation tubes 1–8, then 9–16 to the corresponding wells 1–16 of a new PCR plate.
- 11 Remove the library card and library card guide from the library card stage.
- 12 Discard the library card in accordance with applicable standards.
- 13 Close the library card compartment door, and then select **Home**.
- 14 See the library prep guide for the protocol you are running for instructions on how to proceed after a library is collected from the library card and separated from the oil.

Maintenance

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Chapter 4

Introduction

Following normal operating procedures fulfills required instrument maintenance.

Preventive Maintenance

Illumina recommends that you schedule a preventive maintenance service and a calibration per year or more frequently if you perform multiple runs per day. If you are not under a service contract, please contact your Territory Account Manager or Illumina Technical Support to arrange for a billable preventive maintenance service.

Cleaning

Clean the instrument and test card as needed.

Cleaning the Instrument

Make sure that the power is turned off and the instrument is unplugged during cleaning. Clean the surface of the library card stage using isopropanol and lab wipes.

Cleaning an Oil Spill



WARNING

On rare occasions, oil can leak or be spilled over the library card stage and leak into the instrument. If this occurs, contact Illumina Technical Support for further instructions. Do not continue to use the instrument.

When a minimal amount of oil is on a visible surface, clean the surface using 70% isopropyl alcohol. Depending on the amount of oil, repeat this procedure as needed. The following materials are required:

- 70% Isopropyl alcohol
- Dust-free tissues

Procedure

- 1 Moisten a dust-free tissue with 70% isopropyl alcohol. Do not soak the tissue.
- 2 Wipe the oil-covered surfaces of the instrument until all oil is absorbed.

Cleaning the NeoPrep Test Card

Clean the NeoPrep Test Card if a test fails and then repeat the test. Also clean the test card it appears dirty or has been left out of it's case.

The following materials are required:

- Alcohol wipes
- Dust-free tissues

Procedure

- 1 Use an alcohol wipe to clean the 2 raised surfaces on the top of the NeoPrep Test Card.
- 2 Rub off any debris, so that the edges of the test card are clean.
- 3 Gently pat dry using a dust-free tissue.
- 4 Use an alcohol wipe to clean the shiny fluorescent tape on the top of the test card.
- 5 Gently pat dry using a dust-free tissue. Make sure that the tape is free of streaks, fingerprints, lint or tissue fibers.
- 6 Use an alcohol wipe to clean the underside of the test card. Make sure to clean the gold squares, as these contact the NeoPrep electrical components.

Decontamination

Decontaminate the instrument under the following conditions:

- If a spill of biologically hazardous material occurs.
- If the instrument is being returned to Illumina.

Make sure that you are familiar with the basic setup and operation of the instrument to perform the decontamination process.

- Use 70% isopropyl alcohol for the decontamination of infectious agents and blood.
- Always wear protective gloves and safety glasses when handling contaminated instruments or performing the decontamination procedure.
- A face shield is recommended whenever there is a possibility of aerosol contamination.

The following materials are required:

- ▶ 70% Isopropyl alcohol
- Deionized or distilled water
- Safety glasses
- Gloves
- Lab coat
- Paper towels or lint-free cotton cloths

Procedure

- 1 Turn off the instrument and unplug it from the power supply. Never immerse the instrument in liquid.
- 2 Moisten a paper towel or cloth with 70% isopropyl alcohol. Do not soak the towel or cloth.
- 3 Wipe all exposed surfaces of the instrument.
- 4 Open the library card compartment door and wipe all interior surfaces of the compartment with 70% isopropyl alcohol.
- 5 Wait 20 minutes. Allow the isopropyl alcohol to dry slowly. Do not wipe dry.
- 6 Moisten a paper towel or cloth with deionized or distilled water and wipe all surfaces that have been cleaned with isopropyl alcohol.
- 7 Use a dry paper towel or cloth and dry all wet surfaces.
- 8 Discard paper towels or cloths and gloves in an approved biohazard container.

Software Updates

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

- NeoPrep Control Software
- Firmware
- Protocols
- BaseSpace Broker

The current version of the System Suite installed on the instrument appears on the control software About screen.

You can install software updates automatically using an internet connection or manually from a network or USB location. Software updates include periodic updated versions of current protocols, and the addition of new protocols.

- Automatic updates For instruments connected to a network with internet access, an alert 1 icon appears on the Manage Instrument button on the Home screen when an update is available.
- Manual updates—If your instrument is not connected to a network with internet access, update manually using the Software Update command from the Manage Instrument screen.

Automatic Software Update

- 1 Select the alert \bigcirc icon to begin the update. A dialog box opens to confirm the command.
- 2 Follow the prompts in the installation wizard:
 - a Accept the licensing agreement.
 - b Review the release notes.
 - c Review the list of software included in the update.

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



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

Manual Software Update

- 1 Download the software installation file and save it to a network location. Alternatively, copy the software installation file to a portable USB drive.
- 2 Select Manage Instrument.
- 3 Select Software Update.
- 4 Select Manually install the update from the following location.
- 5 Select ... to navigate to the location of the software installation file, and then select **Update**.

- 6 Follow the prompts in the installation wizard:
 - a Accept the licensing agreement.
 - b Review the release notes.
 - c Review the list of software included in the update.

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

NOTE

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

Protocol Management

- 1 Select Manage Instrument.
- 2 Select **Protocol Management**.

Installed protocols are listed, including the version number. Protocols that are listed on the Select screen, display a checkmark in the Installed column. Installed protocols also can be configured so that they are not listed on the Select screen. For more information on the Select screen, see *Specify Run Parameters* (*Standalone Configuration*) on page 24.

3 Modify the Installed setting to designate the availability of a protocol on the Select screen.

Protocol available—To make a protocol available for selection on the Select screen, slide the toggle switch in the Installed column to the right. A checkmark is displayed in the Installed column.



Protocols of the same name, but with a different version number can be available at the same time.

- Protocol not available—To make a protocol not visible on the Select screen, slide the toggle switch in the Installed column to the left. An X is displayed in the Installed column. When a protocol is not visible on the Select screen, it cannot be run.
- 4 Select a protocol from the list to see the full protocol name, version, and description on the right side of the screen.
- 5 Select **Back** to save the settings and return to the **Manage Instrument** screen.

Shut Down or Restart the Instrument

Under normal operation, there is no reason to shut down or restart the instrument. However, if the instrument has to be turned off or restarted, use the following instructions.

- 1 Select Manage Instrument.
- 2 Select Shut Down.
- 3 Select from the following options.
 - Select Shut Down to shut down the software and the instrument power.
 - Select **Restart** to shut down and restart Windows, restart control software, and initialize the instrument. This command is typically used as directed by an Illumina Technical Support representative during a troubleshooting session.
 - Select Exit to Windows to close the control software and access the operating system directly. Exit to Windows for administrative tasks. Normal operation is performed from the software interface in kiosk mode.
- 4 If you shut down the instrument, wait at least 60 seconds before turning on the instrument again.

Troubleshooting

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Introduction

For technical questions, visit the NeoPrep support page on the Illumina website. The support pages provide access to documentation, downloads, online training, and frequently asked questions. Log in to your MyIllumina account for access to support bulletins.

For most errors on the NeoPrep, an on-screen message opens with instructions for correcting the error and completing the run. This section also has directions on how to access files and information to facilitate troubleshooting.

For problems with run quality or performance, contact Illumina Technical Support. For contact information, see *Technical Assistance* on page 55.

Access Log Files

Log files provide information on each run and on system checks. An Illumina Technical Support representative might request copies of log files for troubleshooting purposes. A single log file can be sent or copied at a time.

- 1 From the Home screen, select **Access Logs**.
- 2 Select a tab.
 - Runs—Select a run from the list, then select **Details** to view the run details, consumable information, sample information, and standard curves.
 - System Checks—Select a system check from the list, then select Details to view the details.
- 3 To email the selected log, select Email.
 - a Enter the address of the recipient or select **Send to Illumina Technical Support** and enter your site support *#*.
 - b Select Email.
- 4 To save a copy of the selected log to a network location, select **Save**.
 - a Enter the network location or navigate to the network location.
 - b Select Select.
- 5 Select Back.

System Check

A system check tests the optical, magnetic, electrowetting, and thermal elements of the system. This process requires the NeoPrep Test Card provided in the instrument accessories kit. A system check is not required during normal operation or for instrument maintenance. However, an Illumina Technical Support representative might ask you to perform a system self-check during troubleshooting.

Perform a System Check

- 1 From the Manage Instrument screen, select **System Check**.
- 2 Open the library card compartment door.
- 3 Slide the library card latch release to the right and place NeoPrep Test Card on the library card stage. Make sure that the keyed features are on the left and the notched corner is on the right.



NOTE Use the library card latch release and do not snap the NeoPrep Test Card into place on the library card stage.

Figure 12 NeoPrep Test Card On Library Card Stage



- 4 Release the library card latch release, so that the library card latches secure the NeoPrep Test Card on the library card stage.
- 5 Close the library card compartment door.
- 6 Select the tests to run: **Optical**, **Magnetic**, **Electrowetting**, and/or **Thermal**.
- 7 Select **Run**. The system check begins, takes approximately 55 minutes depending on the tests selected, and does not require user intervention.



If an error occurs, follow the instructions provided by the control software. If you are unable to resolve the error, contact Illumina Technical Support.

- 8 When the check is complete, a dialog box appears on the screen with instructions to remove the NeoPrep Test Card. Select **OK**.
- 9 To rerun the check on the currently selected tests, select Retry.
- 10 To reselect tests to run, select **Select Tests** and repeat steps 6–8.
- 11 Open the library card compartment door.
- 12 Slide the library card latch release to the right and remove the NeoPrep Test Card from the library card stage.

- 13 Close the library card compartment door and store the NeoPrep Test Card for future use.
- 14 Select Back.

Level Instrument

Make sure that the NeoPrep is level.

Perform Instrument Level Check

- 1 From the Manage Instrument screen, select **Instrument Level**. The tilt of the instrument appears. Red text indicates that the instrument it not level at the specified side.
- 2 Use the supplied wrench or equivalent tool to adjust the feet of the instrument to level the instrument front to back and side to side. To extend a foot, turn it clockwise as viewed from above. Make sure that the lab bench is level.
- 3 Select Back.

Configure System Settings

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

- Network Configuration—Provides options for IP address settings, domain name server (DNS) address, computer name, and domain name.
- BaseSpace Configuration—Provides options for analysis methods, including BaseSpace, BaseSpace Onsite, standalone mode, and run monitoring in BaseSpace, and settings for a default BaseSpace login and instrument health reporting.

Set Network Configuration

- 1 From the Manage Instrument screen, select **System Configuration**.
- 2 Select Network Configuration.

Setup IP and DNS

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



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

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

- Enter IP address. The IP address is a series of 4 numbers separated by a dot, similar to 168.62.20.37, for example.
- Enter the subnet mask, which is a subdivision of the IP network.
- Enter the default gateway, which is the router on the network that connects to the internet.
- 2 Select **Obtain DNS server address automatically** to connect the instrument to the domain name server associated with IP address.

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

- Enter the preferred DNS address. The DNS address is the server name used to translate domain names into IP addresses.
- Enter the alternate DNS address. The alternate is used if the preferred DNS cannot translate a particular domain name to an IP address.
- 3 Select **Next** to save settings and advance the screen.



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

Setup Computer Name and Domain or Workgroup

1 The **Computer Name** is the name assigned to the instrument computer at the time of manufacture.

Typically, there is no need to change the computer name. Any changes made to the computer name on this screen can affect connectivity and require the user name and password of a network administrator.

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

Setup Message Communication

- 1 In the **SMTP Server** field, enter the name of the email server.
- 2 In the **Port** field, enter the number of the simple mail transfer protocol (SMTP) port. The default is 587.
- 3 Deselect Use SSL to not use standard security technology for encryption.
- 4 In the **User Account** field, enter the email account username.
- 5 In the **Password** field, enter the email account password.
- 6 Select Finish to save settings and return to the System Configuration screen.
 - L NOTE

Changes to the computer name, domain, or workgroup require a restart of the system. The software prompts you to confirm a restart of the system. Select **Continue** to proceed.

Set BaseSpace Configuration

- 1 From the Manage Instrument screen, select System Configuration.
- 2 Select BaseSpace Configuration.
- 3 Select from the following options to interface with BaseSpace, BaseSpace Onsite, or as standalone.
 - Select BaseSpace to send data to IlluminaBaseSpace. [Optional] Select ..., and navigate to a secondary network Output Folder location to save a copy of data in addition to BaseSpace.
 - Select BaseSpace Onsite. In the Api Url field, enter the full path to your BaseSpace Onsite server. [Optional] Select ..., and navigate to a secondary network Output Folder location to save a copy of data in addition to the BaseSpace Onsite server.
 - Select Standalone instrument to save data to a network location only. Select ... and navigate to a preferred network Output Folder location. The control software generates the output folder name automatically.



CAUTION

Do not specify a location on the instrument computer for the output folder. Illumina recommends using a network location only. The capacity of the instrument hard drive is sufficient for multiple runs, however a full drive can prevent subsequent runs.

- 4 Select **Next** to advance to the next screen.
- 5 If you selected BaseSpace or BaseSpace Onsite, set the BaseSpace parameters as follows.
 - Enter a BaseSpaceUser Name and Password to register the instrument with BaseSpace.

- Select Use default login and bypass the BaseSpace login screen to set the registered user name and password as the default login. This setting bypasses the BaseSpace screen during run setup.
- 6 If you selected BaseSpace, select **Send instrument health information to Illumina Technical Support** to send log files to Illumina. This option is not available with BaseSpace Onsite.
- 7 Select Finish.

Resolve Errors

If pre-run check errors occur, use the following recommended actions to resolve the error or follow the instructions provided by the control software. If you are unable to resolve the error, contact Illumina Technical Support.

Pre-run Check	Recommended Action
Door Closed	Make sure that the library card compartment door is closed
Library Card Verification	Library card sensors do not register. Make sure that the library card is properly loaded on the library card stage.
Required Software	Critical components of the software are missing. Perform a manual software update to restore all software components.
Instrument Disk Space	The instrument hard drive does not have sufficient disk space to perform a run. It is possible that data from a previous run did not transfer. Clear the instrument hard drive from any run data.
Network Connection	The network connection has been interrupted. Confirm network status and confirm the physical network connection.
Network Disk Space	The network server is full. Backup and remove unneeded files.
Tilt Fault	Use the supplied wrench or equivalent tool to adjust the feet of the instrument to level the instrument front to back and side to side. To extend a foot, turn it clockwise as viewed from above. Also make sure that the lab bench is level. See <i>Level Instrument</i> on page 48.

Stop a Run

The NeoPrep is designed to complete a run from beginning to end without user intervention. However, it is possible to stop a run from the Prepare Libraries screen. You might stop a run if the run was set up incorrectly or if you experience a hardware error.

You can stop a run before the run has completed using the **Stop** button on the Prepare Libraries screen. A dialog box opens to confirm the stop. *Stopping a run is final.* A stopped run cannot be resumed. The NeoPrep moves the library card from the integrated library prep module to the library card compartment. The reagents and samples in the library card are not recoverable.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

 Table 1
 Illumina General Contact Information

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

 Table 2
 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number	
North America	1.800.809.4566	Italy	800.874909	
Australia	1.800.775.688	Netherlands	0800.0223859	
Austria	0800.296575	New Zealand	0800.451.650	
Belgium	0800.81102	Norway	800.16836	
Denmark	80882346	Spain	900.812168	
Finland	0800.918363	Sweden	020790181	
France	0800.911850	Switzerland	0800.563118	
Germany	0800.180.8994	United Kingdom	0800.917.0041	
Ireland	1.800.812949	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**.





Illumina 5200 Illumina Way San Diego, California 92122 U.S.A. +1.800.809.ILMN (4566) +1.858.202.4566 (outside North America) techsupport@illumina.com www.illumina.com