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cBot™ User Guide

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Part # 15006165 Rev. K
October 2012

Revision History

Part #	Revision	Date	Description of Change
15006165	K	October 2012	Added support for template hybridization on a Rapid flow cell.
15006165	J	July 2012	Added sequencing primers requirements for dual-indexed TruSeq HT libraries.
15006165	H	April 2012	Updated information for sequencing dual-indexed libraries. Added the following procedures: • Reagent preparation instructions, including instructions for preparing HP10 • Primer rehybridization procedure
15006165	G	October 2011	Added new section titled *Dual-Indexed Sequencing Modifications*.
15006165	F	June 2011	Updated procedure for preparing template DNA to include instructions for higher concentrations, and added note about high NaOH concentration.
15006165	E	April 2011	Updated software descriptions to cBot software v1.4. Added the following information: • TruSeq Cluster Kit v3 and catalog number • Description of keyed corner as visual orientation when loading HiSeq Flow Cell v3 • New section titled *Version Compatibility of Run Components* that lists compatible software and recipe versions for various flow cell types Updated recommended DNA template storage to a concentration of 2 nM, and adjusted protocol for preparing DNA using a 2 nM template.
15006165	D	October 2010	Updated software descriptions to cBot software v1.3. Added the following information:

Part #	Revision	Date	Description of Change
			• Recommended cluster densities based on version of analysis software • Instructions for upgrading the software • Instructions for recovering a run
15006165	C	May 2010	Updated software descriptions to cBot software v1.1. Added flow cell storage recommendation. Increased water wash volume to 12 ml and DECON to 10 ml.
15006165	B	March 2010	Corrected centrifuge instructions for thawing the reagent plate. Added the following information: • Instructions for loading the HiSeq flow cell, and associated templates and primers • Instructions for installing the adapter plate • HiSeq Cluster Generation Kit catalog numbers and kit descriptions • Instructions for setting local date and time using the Time tab • Monthly maintenance wash procedure
15006165	A	October 2009	Initial release

Table of Contents

Overview

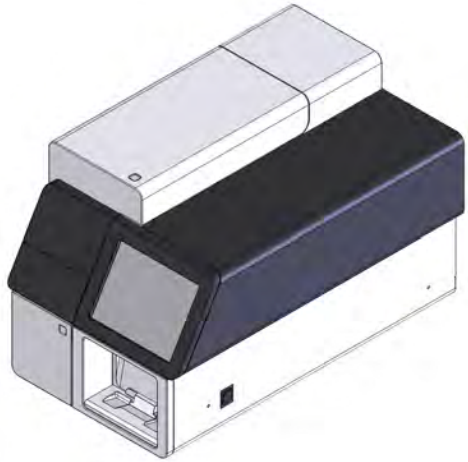
Chapter 1

Introduction

The cBot System supports Genome Analyzer flow cells as well as HiSeq High Output and Rapid flow cells. It provides complete automation of a complex process. With very little hands-on time and no reagent preparation, the cBot uses bridge amplification to create hundreds of millions of single-molecule DNA templates simultaneously in less than five hours.

The cBot dispenses reagents from a pre-aliquoted well plate and controls reaction times, flow rates, and temperatures. A run is set up using the cBot software interface, which simplifies operation and provides a visual report of run status. An on-instrument barcode reader records the reagents and flow cell used for each experiment.

Figure 1 Illumina cBot



Audience and Purpose

The *cBot User Guide* provides information about installing, operating, and maintaining the cBot. This guide includes descriptions of site requirements, instrument components, and software features.

This guide is for laboratory personnel and other individuals responsible for the following:

- Operating the cBot

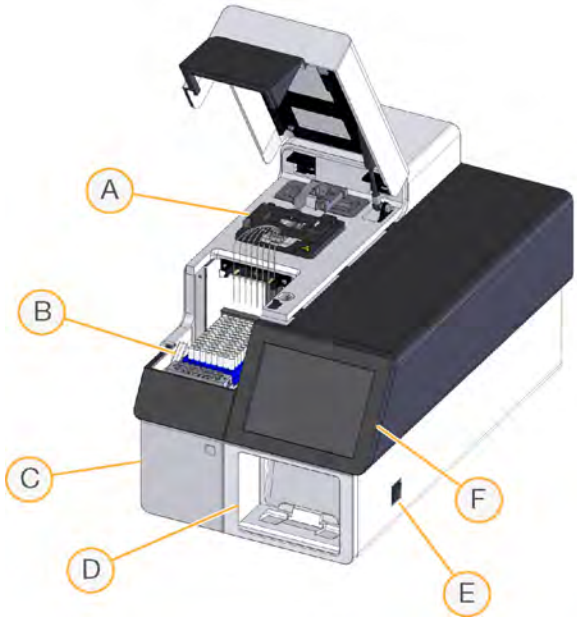
- Performing instrument maintenance
- Training personnel on the cBot

cBot Components

The cBot components include an on-instrument touch screen monitor and barcode scanner. The thermal stage and reagent stage are located under the cBot lid. The waste bottle is conveniently stored in the waste bottle compartment.

The cBot uses sensors to detect the presence of run components, and provides prompts if a component is missing or installed incorrectly. The instrument lid must remain closed for safe operation during the run. The instrument software prompts you to close the lid before proceeding with the run.

Figure 2 cBotComponents



A **Thermal Stage**—The thermal stage holds the flow cell and interchangeable flow cell adapter plate, and controls the flow cell temperature throughout the run.

B **Reagent Stage**—The reagent stage holds the 96-well reagent plate, the templates, and any custom or specialty primers.

C **Waste Bottle Compartment**—The waste bottle compartment holds a sensor-controlled 250 ml waste bottle that collects reagents after they have passed through the flow cell and manifold.

D **Barcode Scanner**—The on-instrument barcode scanner records the unique ID of the reagent plate and flow cell used with each run. The cBot uses this information to verify the compatibility of a selected protocol and reagents.

E **Power Switch**—The power switch turns on the instrument. The start switch, located to the left of the waste bottle compartment, starts the instrument software.

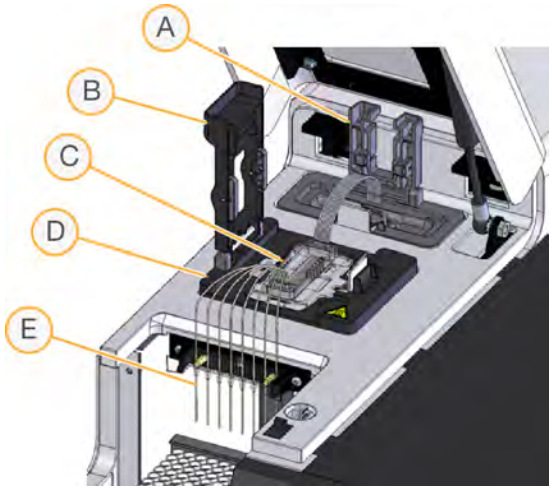
F **Touch Screen Monitor**—The touch screen monitor displays the software interface, which guides you through each step of the cluster generation process.

Thermal Stage

The thermal stage holds the flow cell, and the manifold is seated over the flow cell. The flow cell clamp locks the flow cell and manifold in place.

CAUTION
Never touch the aluminum thermal block on the thermal stage. The heater poses a serious burn hazard if touched during operation. For more safety information, see the *cBot Safety and Compliance Guide*, Part # 15027616.

Figure 3 Thermal Stage With Sipper Comb for High Output Flow Cell



A Output Clamp
B Flow Cell Clamp
C Flow Cell and Manifold
D Thermal Stage
E Sipper Comb

The manifold is a single-use component that delivers reagents from the reagent plate to the inlet end of the manifold, through the flow cell, and then to the waste bottle from the outlet end of the manifold:

- **Inlet**—The inlet end of the manifold, positioned toward the front of the cBot, contains a series of sippers called the sipper comb. The sipper comb pierces the foil-sealed reagent tubes seated in the reagent plate.
- **Outlet**—The outlet end of the manifold, positioned toward the rear of the cBot, receives the waste flowing out of the flow cell, and then transfers it to the waste container. The outlet clamp locks the outlet end in place.

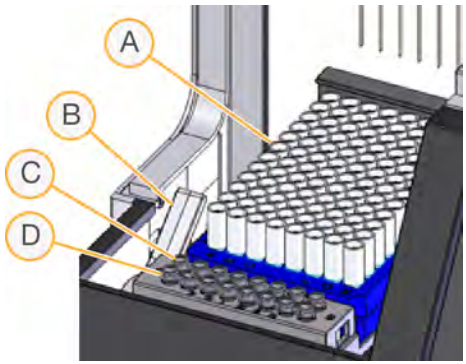
Flow Cell Adapter Plates

The HiSeq flow cell and Genome Analyzer flow cell require specific adapter plates. For more information, see *Changing the Adapter Plate* on page 112.

Reagent Stage

The reagent stage holds a 96-well cBot reagent plate. The plate is locked into position by the reagent plate lever. Toward the front of the reagent stage are two eight-tube strip holders, one for an eight-tube strip containing your prepared library templates, and another for an eight-tube strip containing optional custom primers.

Figure 4 cBot Reagent Stage With 96-Well Reagent Plate



A 96-Well Reagent Plate
B Reagent Plate Lever
C Templates
D Custom Primers

Illumina-Supplied Consumables

The following kits are available for use on the cBot.

TruSeq Cluster Kits

TruSeq Cluster Kits are available for the Genome Analyzer and the HiSeq.

Each kit contains an instrument-specific flow cell, a flow cell-specific manifold, and the required reagents for performing one cluster generation run on the cBot.

Cluster Kits for the HiSeq

Kit Name	Catalog #
TruSeq SR Cluster Kit v3 - HS (cBot)	Catalog # GD-401-3001
TruSeq PE Cluster Kit v3 - HS (cBot)	Catalog # PE-401-3001
TruSeq Rapid Duo Sample Loading Kit Note: This kit enables a different sample to be loaded into each lane of a Rapid flow cell.	Catalog # CT-402-4001

Cluster Kits for the Genome Analyzer

Kit Name	Catalog #
TruSeq SR Cluster Kit v2 - GA (cBot)	Catalog # GD-300-2001
TruSeq PE Cluster Kit v2 - GA (cBot)	Catalog # PE-300-2001

Sequencing Primers for Dual-Indexed Libraries

This section applies to only High Output flow cells. Sequencing dual-indexed Nextera libraries requires the Read 1 sequencing primer provided in the TruSeq Dual Index Sequencing Box, in addition to cluster generation reagents provided in the TruSeq Cluster Kit. For more information, see *Sequencing Dual-Indexed Libraries* on page 9.

Kit Name	Catalog #
TruSeq Dual Index Sequencing Primer Box, Single Read	Catalog # FC-121-1003
TruSeq Dual Index Sequencing Primer Box, Paired End	Catalog # PE-121-1003

Rehybridization Kit

Use the Rehybridization Kit after extended flow cell storage or to facilitate run recovery. For more information, see *Performing a Primer Rehybridization Run* on page 74.

Kit Name	Catalog #
TruSeq cBot Multi-Primer Re-hybridization Kit v2	Catalog # GD-304-2001

Sequencing Dual-Indexed Libraries

This section applies to only High Output runs. Dual-indexed Nextera libraries and dual-indexed TruSeq HT libraries have different sequencing primer requirements:

- **Nextera libraries**—Nextera libraries require additional sequencing primers provided in the TruSeq Dual Index Sequencing Primer Box. These primers are used throughout the sequencing process, including Read 1 (applied during cluster generation), any Index Reads, and Read 2.
  - Sequencing runs on single-read flow cells require the sequencing primers provided in the single-read kit.
  - Sequencing runs on paired-end flow cells require the sequencing primers provided paired-end kit, even if you plan to perform a single-read run on a paired-end flow cell.
- **TruSeq HT libraries**—When sequencing on a paired-end flow cell, TruSeq HT libraries can use the sequencing primers provided in the TruSeq Cluster Kit throughout the sequencing process. However, when performing a dual-indexed run on a single-read flow cell, TruSeq HT libraries require the Index 2 Read sequencing primer (HP9) from the Dual Index Sequencing Primer Box (Single-Read).

NOTE
Sequencing primers provided in the TruSeq Dual Index Sequencing Primer Box are compatible with all TruSeq and Nextera libraries. If your flow cell contains a combination of TruSeq and Nextera libraries in different lanes, use the sequencing primers in the TruSeq Dual Index Sequencing Primer Box for the entire run.

Read 1 Sequencing Primer Requirements

The following table lists required Read 1 sequencing primers by library type.

Run Type	Read 1 Primer (cBot)
Nextera libraries	HP10
TruSeq HT libraries	HP6 or HP10
TruSeq LT/v2 libraries	HP6 or HP10
TruSeq Small RNA libraries	HP6 or HP10

NOTE
TruSeq HT libraries are dual-indexed libraries. TruSeq LT libraries are single-indexed libraries and include libraries prepared with TruSeq v2 kits.

cBot Recipes for Dual-Indexed Libraries

To make sure that the appropriate sequencing primer is used during the run, you must select the correct recipe for dual-indexed runs based on the type of flow cell you are using.

Flow Cell	Recipe Name
HiSeq Flow Cell v3	**Single-Read:** SR_Amp_Lin_Block_TubeStripHyb_v8.0.xml **Paired-End:** PE_Amp_Lin_Block_TubeStripHyb_v8.0.xml
GA Flow Cell	**Single-Read:** SR_Amp_Lin_Block_TubeStripHyb_v7.0.xml **Paired-End:** PE_Amp_Lin_Block_TubeStripHyb_v7.0.xml

You can download cBot recipes and software from the Illumina website on the cBot support page.

Component	Component Version
Software Version	cBot Software v1.5 or later
Recipe Version	cBot Recipe Installer v1.0.13 or later

Version Compatibility of Run Components

The following table lists compatible versions of cBot-compatible reagent kits and instrument software. For best performance and run results, always use compatible versions.

Kit Version	Recipe Version	Software Version
TruSeq Rapid Duo Sample Loading Kit	Version 8 recipes	cBot v1.5 or later
TruSeq Dual Index Sequencing Primer Box*	Version 8 recipes (HiSeq) Version 7 recipes (GA)	cBot v1.4.36 or later
TruSeq Cluster Kit v3 - HS	Version 8 recipes	cBot v1.4 or later
TruSeq Cluster Kit v2 - GA	Version 7 recipes	cBot v1.3 or later

* Required for sequencing dual-indexed Nextera libraries.

Starting the cBot

To start the cBot, first use the power switch and then the start button.

Figure 5 Power Switch and Start Button

A Start Button
B Power Switch

1 Locate the power switch on the right side of the instrument and toggle the switch to the **ON** position.

2 Locate the start button to the left of the waste bottle compartment door and press to start the cBot software.
The software initiates a start-up routine. When the start-up routine is complete, the Start screen opens.

Chapter 2

Using the Software Interface

Introduction

The cBot software interface provides ease of use in setting up your run, confirming installation of run components, and monitoring the run as it progresses.

The following software screens are used during a cluster generation run:

- **Start screen**—See *Start Screen* on page 15.
- **Run Setup screens**—See *Run Setup Screens* on page 16.
- **Run Status screen**—See *Run Status Screen* on page 20.

Using the cBot software interface, you can configure input requirements, wash preferences, email notifications, and remote monitoring. For more information, see *Configuring the cBot* on page 99.

What's New in v1.5

The cBot software v1.5 enables the use of TruSeq Rapid Kits. If the reagent kit bar code is GA#######-RR (Rapid Run where # is 1-9), cBot Control Software does the following:

- Verifies that the recipe selected has a <SupportedKits> section with a <ReagentKit> element with Version set to "R"
- Analyzes the flow check for only lanes 1 and 2 (labeled as 7 and 8 on the strip tube holder)
- Shows alternate images in the right-hand help box whenever the 2-line Rapid manifold is in view
- Shows alternate images in the right-hand help box whenever the 3-rows-loaded Rapid reagent kit is in view
- Displays an additional flow check button on the manual commands page for the Rapid Run manifold

Start Screen

The cBot Start screen opens when the system power is on and the software is running. You can leave your cBot sitting idle on the Start screen between runs.

Figure 6 cBot Start Screen

A **Menu Button**—The Start screen menu contains options for accessing the configuration screen, manual command settings, protocol editor, and viewing the About screen.

B **Sensor Status**—Provides status of cBot components. Sensors read the status of the cBot lid, waste bottle, coolant system, and sipper comb.

C **User Name**—Select **User Name** to enter the name of the person performing the run. You can configure the user name to be optional input or required input.

D **Start Button**—Select **Start** to proceed to the Run Setup screen. Make sure that you have made any necessary changes to run configurations before proceeding.

E **Error Messages**—Error messages appear if required input is missing or if the system is not responding. For example, if you select Start without entering a user name, and user name is required input, a message appears.

Run Setup Screens

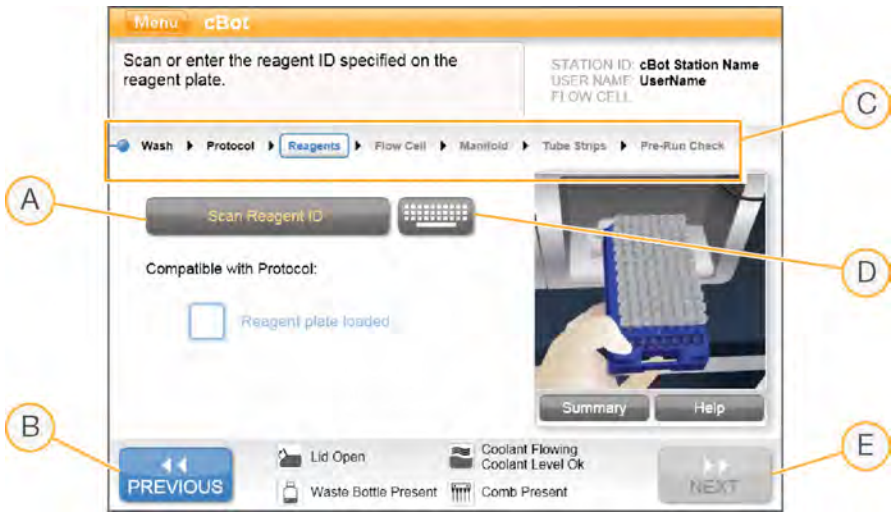
The Run Setup screens are seven sequential screens that guide you through the run setup steps, as follows:

- **Wash**—Perform a pre-run wash.
- **Protocol**—Name your experiment and select a protocol.
- **Reagents**—Record the reagent kit ID and load reagents.
- **Flow Cell**—Record the flow cell ID and load the flow cell.
- **Manifold**—Load the manifold, secure the clamps, and secure the sipper comb.
- **Tube Strips**—Enter a templates name and load the eight-tube strip containing templates. If you use a protocol that requires custom primers, enter a primers name and load the eight-tube strip containing primers.
- **Pre-Run Check**—The software automatically checks the run readiness of each component and performs a flow check using eight integrated bubble sensors to test the fluidics before each run.

Run Setup Screen Features

Each Run Setup screen contains instructional messages, sensor status, and input fields.

Figure 7 Run Setup Screen Buttons



A Required Input
B Previous Button
C Run Setup Steps
D Optional Keyboard Input
E Next Button (shown inactive)

Required input, such as reagent ID and flow cell ID can be scanned using the barcode scanner, or you can select the keyboard icon to activate the on-screen keyboard and manually enter the component ID.

Other input is recorded by the system using instrument sensors.

- **Manual Input**—Input you enter manually is indicated by a full checkbox. On the Wash screen, for example, you must manually select the checkbox to indicate that the reservoir is filled with water.
- **System Input**—Input entered by the system is indicated by a half checkbox. On the Wash screen, for example, sensors detect if the manifold is installed or removed.

Figure 8 Run Setup Screen Input Types

A **Instructional Messages**—Provides reminders and warnings if run components are missing or installed incorrectly.
B **System Input**—System input is indicated by a half checkbox. On the Wash screen, for example, sensors detect if the manifold is installed or removed.

C **Manual Input**—Manual input is indicated by a full checkbox. On the Wash screen, for example, you must manually select the checkbox to indicate that the reservoir is filled with water

D **Sensor Icons**—Indicates when run components are properly loaded, when the waste bottle is full, or if there is a problem in the cooling system. For more information, see *Sensor Status Icons* on page 18.

E **Summary**—Provides a list of component IDs and completed run setup steps.

F **Help**—Opens the cBot online help containing detailed instructions and videos.

Sensor Status Icons

The sensor status icons indicate if a component is properly installed and ready for the run. The following tables describe the various states of the sensor status icons.

Flow Cell

Icon	Indication/Meaning
	Genome Analyzer flow cell adapter plate installed.
	HiSeq flow cell adapter plate installed.
	Flow cell adapter plate type unknown.

cBot Lid

Icon	Indication/Meaning
	cBot lid is open. You must close the lid during operation.
	cBot lid is closed.

Waste Bottle

Icon	Indication/Meaning
	Waste bottle is present and ready for use.
	Waste bottle is full.
	Waste bottle is missing.

Coolant

Icon	Indication/Meaning
	Coolant is flowing and coolant level is good.
	Warning: Coolant is flowing, but coolant level is low.
	Error: Coolant is not flowing, but coolant level is good.
	Error: Coolant is not flowing and coolant level is low.

Manifold and Sipper Comb

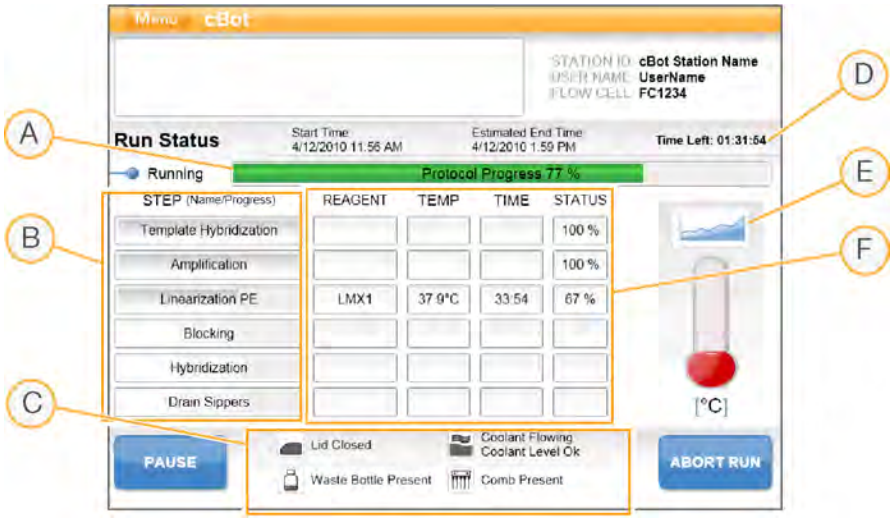
Icon	Indication/Meaning
	Manifold is loaded and sipper comb is secure.
	Manifold is missing or sipper comb is not secure.

Run Status Screen

The Run Status screen provides the current status of a cluster generation run and includes the following run details:

- Status bar showing run progress
- Start date and time, end date and time, and time remaining
- Cluster generation protocol steps with status bar for each step
- Reagent currently in use
- Current temperature (°C)
- Status of the command in the current step

Figure 9 Run Status Screen

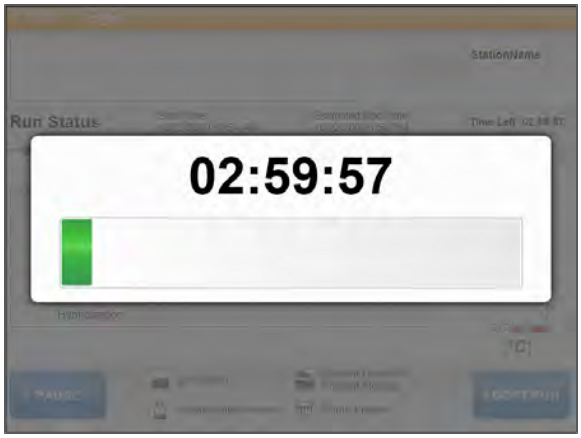


A Visual Status Bar
B Protocol Steps
C Sensor Icons
D Run Time Remaining
E Toggle Between Run Steps and Temp Graph
F Protocol Step Details

Run Status Screensaver

After a period of inactivity, the Run Status screen changes to a screensaver that shows the run countdown time and status bar. Touch the countdown on the screen to toggle through view options: status bar only, countdown only, or both countdown and status bar. Touch the lower portion of the screen to return to the Run Status screen.

Figure 10 Run Status Screensaver

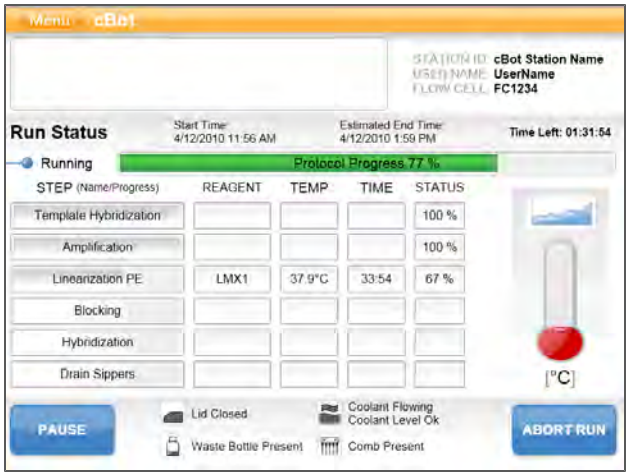


Pausing or Aborting a Run

You can pause or abort the run using commands on the Run Status screen.

- **Pause**—Completes the current command in the protocol, and then pauses the run. Allow a few minutes before the run pauses. When the run is paused, the sippers are lifted from the reagent tubes, the reagent stage returns the home position, and the Pause button changes to the Resume button.
  - When the run is active, select **Pause** to pause the run.
  - When the run is paused, select **Resume** to resume the run.

Figure 11 Run Status Screen in Pause Mode



- **Abort Run**—Aborts the run without the option of resuming. At this point, you are prompted to unload the run components.

NOTE
Aborting a run is final. You cannot resume the run from this point. Select **Unload** and unload run components.

Remote Monitoring Overview

Remote monitoring is a feature that allows you to monitor the status of your cBot from another computer. To take advantage of the remote monitoring feature, make sure that the following requirements are met:

- Your cBot must be connected to a network.
- **Allow Remote Access** is selected on the Remote tab. For more information, see *Enable Remote Monitoring* on page 104.

Add Your cBot

1. Use your web browser to navigate to the remote monitoring IP address listed on the Remote tab of the cBot configuration screen.
2. In the text box on the remote monitoring screen, enter the IP address of your cBot. This is the same IP address listed on the Remote tab of your cBot. For more information, see *Enable Remote Monitoring* on page 104.
3. Click **Add Instrument**. An image that represents your instrument appears on the screen.

Figure 12 Remote Monitoring Screen

The remote monitoring screen shows each of the instruments you have added by instrument name. At a glance, this screen allows you to monitor the progression of the run and see the current state of the instrument. There are four possible states: running, paused, stopped, or error.

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Chapter 3

Performing a Cluster Generation Run

Introduction

A cluster generation run on the cBot requires very little preparation. All steps are performed on the cBot with the exception of preparing the DNA template and reagent plate before starting the run, and confirming reagent delivery after the run. See *Cluster Generation Workflow* on page 27.

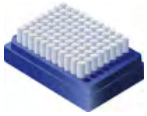
To set up a run using the cBot software interface, you first need to select a protocol, and then load the flow cell, the single-use manifold, the reagent plate, and your templates. If you wish to customize your experiment, additional primers can be applied to your run.

NOTE

When running in Rapid mode, the HiSeq draws all sequencing primers from a single tube; consequently, only a single tube of sequencing primer can be used in each primer hybridization step on the HiSeq in Rapid Run mode. Therefore the same sequencing primer must be used for both lanes of a Rapid flow cell.

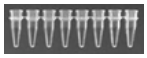
Following run setup, a cluster generation run takes about four hours to complete.

Cluster Generation Workflow



Prepare the reagent plate for use by thawing, vortexing, and piercing the foil over each tube in row 10.

For High Output dual-indexed Nextera libraries, prepare HP10.



Dilute and denature your libraries, and then load your libraries into an eight-tube strip.

Perform a pre-run wash.

Using the cBot software interface, select a protocol and load the reagent plate, flow cell, manifold, and tube strips.

Select **Pre-Run Check** to initiate the automated pre-run check.

Select **Start** to begin the run. Monitor your run from the Run Status screen.

Unload run components and confirm reagent delivery.

Perform a post-run wash.

About Template DNA

To achieve the highest quality data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of every flow cell. This requires accurate quantification of template DNA libraries. For recommended quantification methods, see the sample prep documentation for the kit that you used to create your libraries.

For Rapid Run flow cells, use the same loading concentration as you would for on-board cluster generation on the HiSeq and Genome Analyzer.

Recommended Cluster Densities

HiSeq Flow Cell

Cluster Kit Version	Cluster Density	When Measured With
TruSeq Cluster Kit v3	750–850 K/mm²	RTA v1.12 or later
TruSeqDuo cBot Sample Loading Kit	850–1000 K/mm²	RTA v1.12 or later

Genome Analyzer Flow Cell

Cluster Kit Version	Cluster Density	When Measured With
TruSeq Cluster Kit v2	700–800 K/mm²	RTA v1.8 or later

Template DNA Concentration

The first time you process template DNA, it is useful to try a concentration titration range to optimize the number of clusters formed. If the concentration is too low, fewer clusters are generated and result in a low sequencing yield. If the concentration is too high, clusters are too dense and can complicate data analysis, and cluster positions may not be accurately identified.

Template DNA Storage

Illumina recommends storing prepared template DNA at a concentration of 2 nM.

Adjust the concentration of prepared DNA template to 2 nM using Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20. The addition of 0.1% Tween 20 helps to prevent adsorption of the template to plastic tubes upon repeated freeze-thaw cycles, which would decrease the cluster numbers over time.

Preparing Template DNA

This section explains how to prepare your template DNA for cluster generation on HiSeq v3 and Genome Analyzer flow cells.

Illumina-Supplied Consumables

- HT1 (Hybridization Buffer), pre-chilled

User-Supplied Consumables

- 0.1 N NaOH
- Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20
- 0.2 ml eight-tube strip

CAUTION

Always prepare freshly diluted NaOH. Using freshly diluted NaOH is essential in order to completely denature samples for cluster generation.

Denature Template DNA

Perform the following steps to denature the template DNA with 0.1 N NaOH to a final DNA concentration of 20 pM. This is suitable for performing the hybridization step on the cBot at a final DNA concentration up to 20 pM.

NOTE

If your application requires higher than a 20 pM final concentration of your library, make sure that your concentration of NaOH is not higher than 0.05 N in the denaturation solution and not more than 0.001 N (1 mM) in the final solution after diluting with HT1.

Higher concentrations of NaOH in the loaded library will inhibit library hybridization and decrease cluster density.

1. Combine the following volumes of template DNA and 0.1 N NaOH in a microcentrifuge tube:
   - 2 nM template DNA (10 µl)
   - 0.1 N NaOH (10 µl)
2. Vortex briefly to mix the template solution.
3. Centrifuge the template solution to 280 xg for one minute.

4 Incubate for five minutes at room temperature to denature the template into single strands.

5 Transfer 20 µl of denatured template to a tube containing 980 µl of pre-chilled HT1 (Hybridization Buffer).

6 Place the denatured template DNA on ice until you are ready to proceed to final dilution.

Dilute Denatured DNA

Perform the following steps to dilute the denatured DNA with pre-chilled HT1 to a total volume of 1,000 µl.

1 Dilute the denatured DNA to the desired concentration using the following example:

Final Concentration	10 pM	12 pM	15 pM	18 pM	20 pM
20 pM denatured DNA	500 µl	600 µl	750 µl	900 µl	1,000 µl
Pre-chilled HT1	500 µl	400 µl	250 µl	100 µl	0 µl

2 Invert several times to mix, and then pulse centrifuge the template solution.

3 Place the denatured and diluted template DNA on ice until you are ready to prepare the eight-tube strip.

Denature and Dilute PhiX Control

If you require a control lane, use the following instructions to denature and dilute the 10 nM PhiX library.

NOTE
Generally, you do not need to use a control lane if you are sequencing a balanced genome. In the case of an unbalanced or skewed base composition (e.g., bisulfite-treated samples) a control lane is recommended for phasing and matrix estimation.

1 Combine the following volumes to dilute the PhiX library to 2 nM:
- 10 nM PhiX library (2 µl)

- 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20 (8 µl)

2 Combine the following volumes of 2 nM PhiX library and 0.1 N NaOH in a microcentrifuge tube to result in a 1 nM PhiX library:
   - 2 nM PhiX library (10 µl)
   - 0.1 N NaOH (10 µl)

3 Vortex briefly to mix the 1 nM PhiX library solution.

4 Centrifuge the template solution to 280 xg for one minute.

5 Incubate for five minutes at room temperature to denature the PhiX library into single strands.

6 Transfer 980 µl of pre-chilled HT1 (Hybridization Buffer) to the tube containing 20 µl of denatured PhiX library to result in a 20 pM PhiX library.

NOTE
The denatured 20 pM PhiX library can be stored up to three weeks at -15° to -25°C. After three weeks, cluster numbers tend to decrease.

7 Dilute the denatured 20 pM PhiX library to 12 pM as follows:
   - 20 pM denatured PhiX library (600 µl)
   - Pre-chilled HT1 (400 µl)

8 Place the denatured PhiX library on ice until you are ready to prepare the eight-tube strip.

Load Templates Into an Eight-Tube Strip

This section pertains to High Output and Rapid run modes.

High Output

1 Label the tubes of an eight-tube strip 1–8.

2 If you are using a PhiX control lane, dispense 120 µl of the denatured PhiX library into tube 4 of the eight-tube strip. This places the control sample in lane 4 of the flow cell.

3 Dispense 120 µl of template DNA into each of the remaining tubes. For Rapid Run flow cells, you will load the sample into lanes 1 and 2.

4 Set aside on ice until you are ready to load it onto the cBot.

Rapid Run

1 Label the tubes of an eight-tube strip 1–8.

2 Dispense 135 µl of template DNA into tubes 1 and 2.

3 Set aside on ice until you are ready to load it onto the cBot.

Preparing the Reagent Plate

Reagents take a minimum of one hour to thaw using a room temperature water bath. Alternatively, you can thaw reagents at 2° to 8°C overnight or a maximum of 16 hours. After thawing, carefully follow the steps to prepare reagents for use on the cBot.

Best Practices

- Wear a fresh pair of gloves when setting up a run.
- Hold the 96-well reagent plate by the plate base to avoid dislodging any reagent tubes.
- Always check that the tubes are securely seated in the reagent plate before and after vortexing or inverting reagents. Loose reagent tubes can damage the cBot manifold and stop the run.

  NOTE
  The clear plastic lid on the reagent plate protects the foil seals from being damaged or punctured during thawing. Remove the protective lid only when necessary, such as when you are checking that tubes are securely seated.

- After completing the reagent preparation steps, ***promptly*** load them onto the cBot and begin your run.

Thaw Reagents

1. Remove the reagent plate from -15° to -25°C storage.
2. Press down on the outside edge of each row of tubes in the reagent plate to make sure that they are securely seated in the plate. Take care not puncture the foil seals.
3. Place the reagent plate in a water bath containing only enough room temperature deionized water to submerge the reagent plate base. To prevent contamination, do not allow the water to select the foil seals. Allow the reagents to thaw in the water bath for at least 60 minutes.

   NOTE
   Reagent tubes with greater volumes may take longer to thaw. Inspect the reagents to make sure that they have completely thawed.

Prepare Reagents

NOTE
To ensure proper run performance, it is ***important*** that you thoroughly mix reagents and collect reagent droplets as described in the following steps.

1. Hold the reagent plate by the base and place your other hand on top of the tubes, then invert the reagent plate five times to mix the thawed reagents.
2. While holding your hand on top of the tubes, vortex the plate for approximately 10 seconds to dislodge any trapped air bubbles.
3. Tap the reagent plate on a hard surface 5–10 times to collect any reagent droplets to the bottom of the tubes. Alternatively, pulse centrifuge the reagent plate to collect reagent droplets.
4. Visually inspect the reagent plate to make sure that no air bubbles exist at the bottom of the tubes.
5. Visually inspect the numbering on the tube labels to make sure that they are in the correct order.
6. ***Promptly*** proceed to setting up the run. Do not return reagents to 2° to 8°C storage or allow reagents to sit at room temperature.

Important Step Before You Load Reagents

This section pertains to only High Output flow cells and rehybridization plates.

The tube strip in row 10 is sealed with a non-pierceable red foil. You *must* completely remove the foil before placing reagents on the instrument when instructed to do so by the cBot software prompts.

1. With one hand, gently hold each end of the tube strip in row 10 to secure the tubes in the plate.
2. Using your other hand, carefully peel the red foil from the eight-tube strip, taking care not to allow NaOH to spill on your skin or garments, or in your eyes. Discard the foil appropriately.
3. After removing the foil, press down on the tubes to make sure that they are securely seated in the plate and did not become dislodged when you removed the foil.

Preparing HP10 for Dual-Indexed Nextera Libraries

This section pertains to only High Output flow cells and rehybridization plates.

Perform the following steps to prepare HP10, the Read 1 Sequencing Primer for dual-indexed Nextera libraries.

NOTE
HP10 is used in an eight-tube strip seated in the tube strip holder labeled **Primers**, not on the reagent plate.

Illumina-Supplied Consumables

- HP10 (Read 1 Sequencing Primer) provided in the TruSeq Dual Index Sequencing Primer Box

User-Supplied Consumables

- Eight-tube strip, 0.2 ml

Procedure

1. Prepare the cBot reagent plate as described in *Preparing the Reagent Plate* on page 34.
2. Remove HP10 from -15° to -25°C storage.
3. Thaw in a beaker filled with room temperature deionized water for about 20 minutes, or until fully thawed.
4. Aliquot 150 µl HP10 into each tube of a fresh 0.2 ml eight-tube strip.
5. Set aside on ice until you are ready to load reagents and set up your run.
6. When you are ready to load reagents, place the eight-tube strip in the cBot tube strip holder in the **Primers** position. For more information, see *Load Primers* on page 49.

Performing a Pre-Run Wash

A wash is recommended before every cluster generation run on the cBot.

1 Select **User Name**. The keyboard opens.

2 Using the on-screen keyboard, type your name and then select **Enter**.

3 Select **Start** to proceed to the pre-run wash.

NOTE
The cBot software checks the system to make sure that the flow cell and manifold have been removed before beginning a wash.

4 Confirm that the system-controlled checkbox next to **Manifold Removed** is selected. If it is not selected, you must remove the manifold before proceeding. For more information, see *Unload Run Components* on page 53.
If the pre-run wash has been configured as optional, you can select **Skip** to bypass the pre-run wash.

5 Raise the cBot lid by gently lifting from the top-right corner.

6 Fill the wash reservoir, located behind the thermal stage, with approximately 12 ml deionized water.

Figure 13 Fill the Wash Reservoir

7 Close the cBot lid.

8 Select the checkbox on the screen to indicate that water is present. The Wash button becomes active.

9 Select **Wash**. After the wash is finished, the Wash Reservoir Dry button becomes active.

10 Blot out any excess water remaining in the wash reservoir with a low-lint wipe, taking care not to rub the outlet ports as this can cause fibers to clog the holes.

Figure 14 Dry the Wash Reservoir

11 Select the checkbox on the screen to indicate that the wash reservoir is dry. The Next button becomes active.

12 Select **Next** to proceed to selecting the protocol.

Setting Up the Run

This section describes how to set up a cluster generation run and load run components on the cBot. cBot cluster generation protocols perform either of the following:

- A complete cluster generation run, including steps for amplification, linearization, blocking, and primer hybridization
- Template hybridization only for Rapid Run Flow Cells.

NOTE

Steps for loading the flow cell, templates, and primers differ depending on the flow cell you are using. All flow cells are loaded onto the cBot with ports facing up, which reverses the lane orientation on the HiSeq flow cell. You must load templates and primers in the same orientation as the flow cell.

Select a Protocol

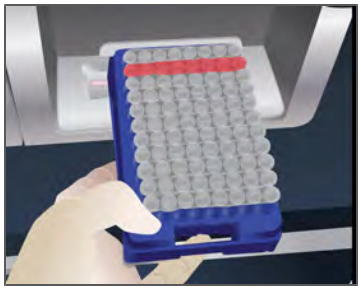
If you are planning to alter the protocol in any way, you must do so before setting up the run. For more information, see *Protocol Editor* on page 83.

1. Select **Experiment Name**. The keyboard opens.
2. Using the on-screen keyboard, type your experiment name and then select **Enter**.
3. Select the appropriate protocol for your experiment from the list of protocols. Select the scroll bar to scroll through available protocols.
4. Select **Next** to proceed to the reagents step.

Load Reagents

1. Remove the clear plastic lid from the cBot reagent plate.
2. Gently press down on the tubes in the reagent plate to make sure they are securely seated in the plate.
3. Select **Scan Reagent ID** to activate the barcode scanner.
   Alternatively, you can enter the reagent ID using the keyboard. Select the keyboard icon to activate the keyboard option.
4. With the barcode label facing the instrument, hold the reagent plate level and move the reagent plate into the light path of the barcode scanner.

Figure 15 Scan Reagent ID (showing a reagent plate for a High Output flow cell)



You will hear a beep when the scanner has successfully read the barcode. The reagent ID appears on the screen.

5 Raise the cBot lid.

6 **Remove the red foil seal from HP5 in row 10 (if you are not preparing a flow cell for a Rapid Run)**:

- a With one hand, gently hold each end of the tube strip in row 10 to secure the tubes in the plate.
- b Using your other hand, carefully peel the red foil from the eight-tube strip, taking care not to allow NaOH to spill on your skin or garments or in your eyes. Discard the foil appropriately.
- c After removing the foil, press down on the tubes to make sure they are still securely seated in the plate.
- d Select the checkbox on the screen to indicate that the HP5 row 10 seal is removed.

7 With the reagent plate in one hand, use the other hand to pull the spring-loaded reagent plate lever towards you to release the clamp.

8 Place the reagent plate onto the reagent stage, positioned with row 1 facing towards the front of the instrument directly behind the eight-tube strip holder. Make sure that the beveled corner of the plate is positioned in the front-right corner.

NOTE
Rapid Duo kits only have 3 loaded rows.

Figure 16 Position the Reagent Plate (showing a reagent plate for a High Output flow cell)

9 Release the reagent plate lever to the secure the reagent plate.

10 Select the checkbox on the screen to indicate that the reagent plate is loaded.

11 Select **Next** to proceed to loading the flow cell.

Load the Flow Cell

1 Lift the flow cell clamp.

2 Wash the adapter plate on the thermal stage with a small amount of deionized water, and wipe dry with a lint-free cleaning tissue. Do not allow fluids to drip inside the instrument.

3 Remove the flow cell from the storage tube using plastic forceps or metal forceps with tips wrapped tightly in parafilm.

4 Gently dry the flow cell with a lens cleaning tissue using a sweeping motion. Repeat until the flow cell is completely dry.

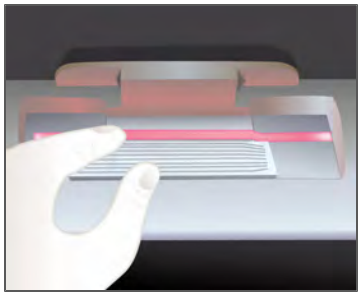
NOTE
If you plan to store the flow cell after completing cluster generation, make sure you retain the storage tube and storage buffer.

5 Select **Scan Flow Cell ID** to activate the barcode scanner.
Alternatively, you can enter the reagent ID using the keyboard. Select the keyboard icon to activate the keyboard option.

6 Hold the flow cell close to the scanner tray with the barcode positioned toward the instrument.
The white background of the barcode scanner tray is necessary for successful scanning.

7 Slowly slide the flow cell into the light path of the barcode scanner so the entire barcode crosses the scan light at the same time.

Figure 17 Scan Flow Cell ID (showing a High Output flow cell)



You will hear a beep when the scanner has successfully read the barcode. The flow cell ID appears on the screen.

8 Holding the flow cell by the edges, rinse the flow cell with deionized water.

9 Gently dry the flow cell with a lens cleaning tissue using a sweeping motion. Repeat until the flow cell is completely clean and dry.

CAUTION
Lane orientation for the HiSeq flow cell is opposite of the Genome Analyzer flow cell. The Genome Analyzer flow cell lane orientation is lane 1–8, left to right, while the HiSeq flow cell lane orientation is lane 8–1, left to right.

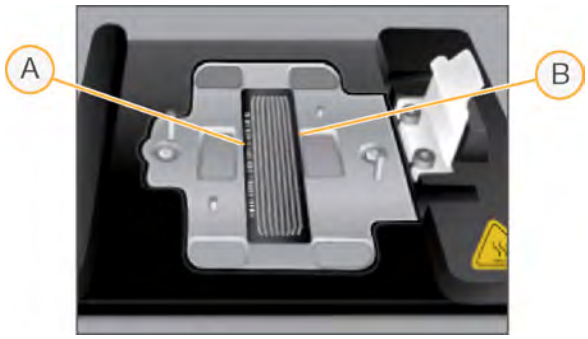
Position the HiSeq Flow Cell

1 **High Output:** Place the flow cell on the thermal stage with holes facing upward, lane 1 on the right side, and lane 8 and the barcode on the left side.
Rapid Run: Place the flow cell on the thermal stage with holes facing upward, lane 1 on the right side, and lane 2 and the barcode on the left side.

NOTE

HiSeq Flow Cell v3 (High Output) and HiSeq Rapid Flow Cell have a mechanically keyed corner, which provides a visual orientation for loading the flow cell. Install HiSeq Flow Cell so that the keyed corner is on the output end facing towards the back of the instrument and on the right side of the flow cell by lane 1.

Figure 18 Position HiSeq High Output Flow Cell



A Lane 8 and Barcode (lane 2 will be on this side for a rapid flow cell)
B Lane 1

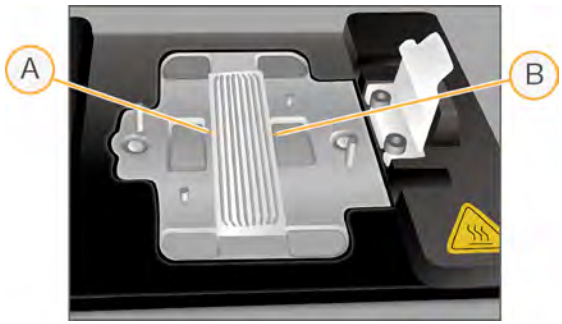
2 Select the checkbox to indicate that you have loaded the flow cell.

3 Select **Next** to proceed to loading the manifold.

Position the GA Flow Cell

1 Place the flow cell on the thermal stage with the holes facing upward, lane 1 on the left side, and lane 8 and the barcode on the right side.

Figure 19 Position Genome Analyzer Flow Cell



A Lane 1
B Lane 8 and Barcode

2 Select the checkbox to indicate that you have loaded the flow cell.

3 Select **Next** to proceed to loading the manifold.

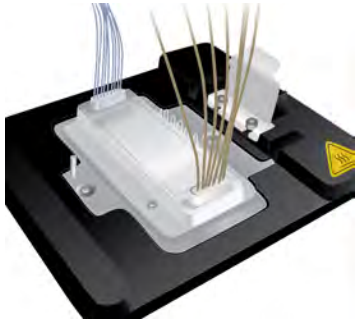
Load the Manifold

Make sure that you use the manifold provided in the same cluster kit as the flow cell. Manifolds are specific to the version and type of flow cell.

1 Remove the manifold from the packaging and inspect the sippers on the sipper comb.
   - Make sure that the sippers are straight and have not been bent or damaged.
   - Make sure that the black rubber gaskets are evenly seated.

2 Position the manifold over the flow cell with the sipper comb pointing toward the front of the cBot.

Figure 20 Position the Manifold (showing a manifold for a High Output flow cell)



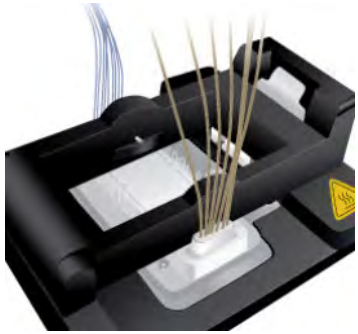
3 Align the manifold with the guide pins on the thermal stage, and set the manifold into place on top of the flow cell.

4 Wiggle the manifold to make sure it is evenly seated over the flow cell. The manifold must be evenly seated to form a tight seal.

5 Select the checkbox to indicate that the manifold is seated.

6 Close the flow cell clamp to lock the manifold in position, making sure that the bracket snaps securely under the white clip.

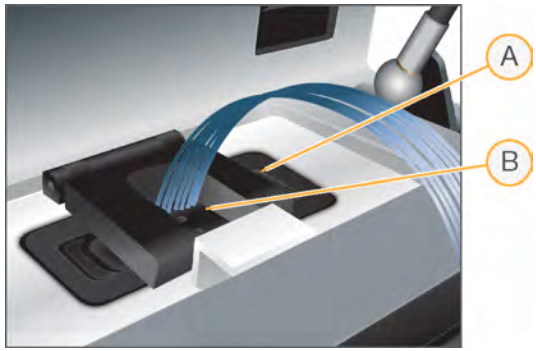
Figure 21 Close the Flow Cell Clamp



7 Select the checkbox to indicate that the flow cell clamp is closed.

8 Connect the outlet end of the manifold to the outlet port in the wash reservoir, and make sure that it is evenly seated.

Figure 22 Secure Outlet End



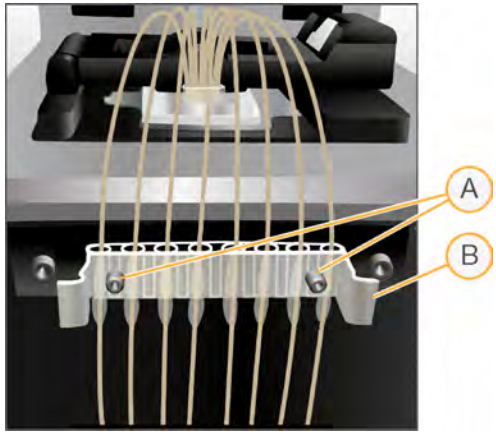
A Output Clamp
B Outlet Port

9 Snap the outlet clamp closed to secure the outlet end of the manifold.

10 Select the checkbox to indicate that you have connected the manifold to the outlet port and the rear clamp is secured.

11 Align the sipper comb with the two metal guide pins on the front edge of the thermal stage.

Figure 23 Secure the Sipper Comb



A Metal Guide Pins
B Plastic Tabs on Sipper Comb For High Output Flow Cell

12 Snap the sipper comb into place using the plastic tabs on either side of the sipper comb.
When the sipper comb is secure, the system checks the final checkbox and the Next button is activated.

13 Make sure that the sipper comb sippers are straight and perpendicular to the reagent plate.

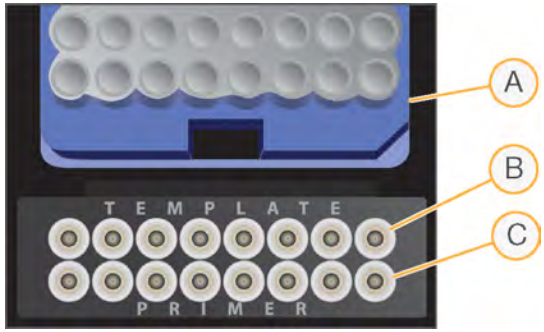
14 Select **Next** to proceed to loading the tube strips.

Load Templates

1 Select **Enter Template Name**. The keyboard opens.

2 Using the on-screen keyboard, type the template ID and then select **Enter**.

Figure 24 Load Templates and Primers (showing a template for a High Output flow cell)



A Reagent Plate
B Templates
C Primers

CAUTION
Lane orientation for the HiSeq flow cell is opposite of the Genome Analyzer flow cell. the HiSeq flow cell lane orientation is lane 8–1 (for a High Output Flow Cell) and 2–1 (for a Rapid Flow Cell), left to right, when loaded on the cBot. (The Genome Analyzer flow cell lane orientation is lane 1–8, left to right.)

Load Templates for the HiSeq Flow Cell

1 **High Output Flow Cell:** Load the eight-tube strip containing the template into the TEMPLATE row of the tube strip holder such that the tube labeled #8 is on the left side and the tube labeled #1 is on the right side, as shown in Figure 25.
Rapid Flow Cell: Load the two-tube strip containing the template into the TEMPLATE row of the tube strip holder such that the tube labeled #2 is on the left side and the tube labeled #1 is on the right side, as shown in Figure 26.
This orientation aligns the tube strip with the lanes on the HiSeq flow cell when correctly loaded onto the cBot.

Figure 25 Tube Strip Orientation for HiSeq High Output Flow Cell

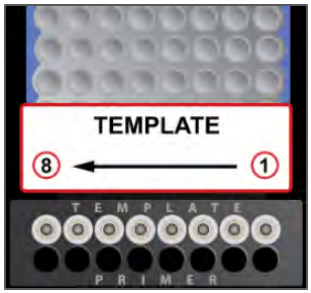
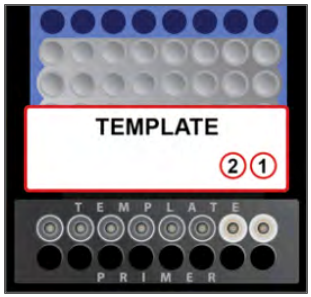


Figure 26 Tube Strip Orientation for HiSeq Rapid Flow Cell



2 Select the checkbox to indicate that you have loaded templates.

3 If you are using additional primers, proceed to *Load Primers* on page 49. Otherwise, close the cBot lid. The Next button becomes active.

Load Templates for GA the Flow Cell

1 Load the eight-tube strip containing the template into the TEMPLATE row of the tube strip holder such that the tube labeled #1 is on the left side and the tube labeled #8 is on the right side.

Figure 27 Tube Strip Orientation for Genome Analyzer Flow Cell

2 Select the checkbox to indicate that you have loaded templates.

3 If you are using additional primers, proceed to *Load Primers* on page 49. Otherwise, close the cBot lid. The Next button becomes active.

Load Primers

The option to load the strip tube containing primers appears if the protocol you selected uses custom or specialty primers. Perform the following steps to load primers. Otherwise, close the cBot lid and select **Next** to proceed to the pre-run check.

1 Select **Enter Primer Name**. The keyboard opens.

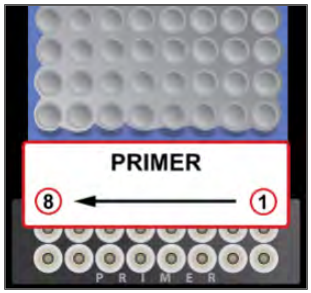
2 Using the on-screen keyboard, type the primer ID, and then select **Enter**.

Load Primers for the HiSeq Flow Cell

This section pertains to only High Output mode.

1 Load the eight-tube strip containing primers into the PRIMER row of the tube strip holder such that the tube labeled #8 is on the left side and the tube labeled #1 is on the right side, as shown in the figure below. This orientation aligns the tube strip with the lanes on the HiSeq flow cell when correctly loaded onto the cBot.

Figure 28 Tube Strip Orientation for HiSeq High Output Flow Cell.



2 Select the checkbox to indicate that you have loaded additional primers.

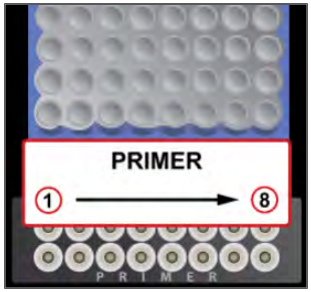
3 Close the cBot lid. The Next button becomes active.

4 Select **Next** to proceed. The system automatically performs a pre-run check.

Load Primers for the GA Flow Cell

1 Load the eight-tube strip containing primers into the PRIMER row of the tube strip holder such that the tube labeled #1 is on the left side and the tube labeled #8 is on the right side, as shown in the figure below.

Figure 29 Tube Strip Orientation for Genome Analyzer Flow Cell



2 Select the checkbox to indicate that you have loaded additional primers.

3 Close the cBot lid. The Next button becomes active.

4 Select **Next** to proceed. The system automatically performs a pre-run check.

Perform a Pre-Run Check

The pre-run check reads the instrument sensors to detect the correct installation of run components, and then performs a flow check using bubble sensors to detect air in the lines. The pre-run check takes approximately three minutes.

After successful completion of the pre-run check, select **Start**. The Run Status screen opens and the run begins.

Run Component Errors

If the pre-run check fails due to errors related to run components, perform the following steps:

1. Check any run component with an indicated error to make sure that it is present and loaded correctly.
2. Select **Rerun Check** to repeat the sensor check.
3. If the check continues to fail and your system is configured to allow sensor bypass, do the following:
   a. Visually inspect run components to confirm correct orientation.
   b. Select **Bypass Sensor Check** and **Rerun Check**. The system proceeds to the flow check.

   If the Bypass Sensor Check option is not visible, your system is not configured to allow sensor bypass.

Flow Check Failure

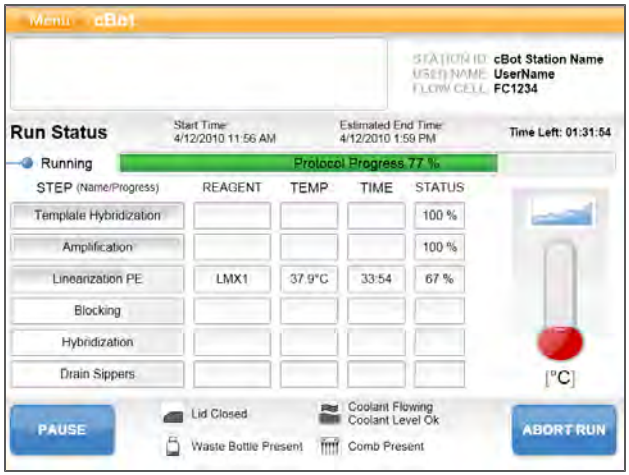
Flow check failure might be caused by an improperly loaded flow cell, a faulty manifold, or a hardware clog. Before using the option to bypass the flow check, perform the troubleshooting steps described in *Troubleshooting Flow Check Failure* on page 66.

NOTE
The software allows you to perform the flow check up to three times.

Monitoring the Run

The Run Status screen allows you to monitor the run in progress.

Figure 30 Run Status Screen



Allow approximately four hours for the run to complete. After the run is complete, the cBot holds the flow cell at 20°C. The flow cell may remain on the instrument at this temperature overnight.

(High Output flow cell) If you need to store a High Output flow cell, store it in storage buffer in the flow cell tube at 2° to 8°C. The flow cell is stable after primer hybridization for up to ten days when properly stored at 2° to 8°C in storage buffer in the tube.

(Rapid flow cell) If you need to store a Rapid flow cell, store it in storage buffer in the flow cell tube at 4°C. The flow cell is stable after annealing the DNA for about one hour when properly stored at 4°C in storage buffer in the tube.

Run Data Report

The run data report provides a summary of the run in progress. You can view the report at any time during the run or at the end of the run. Select **Menu** and then select **Run Data**.

Performing Post-Run Procedures

Post-run procedures confirm that the run successfully completed. Post-run procedures include viewing the run data report, unloading run components, performing an instrument wash, and checking reagent delivery.

View the Run Data Report

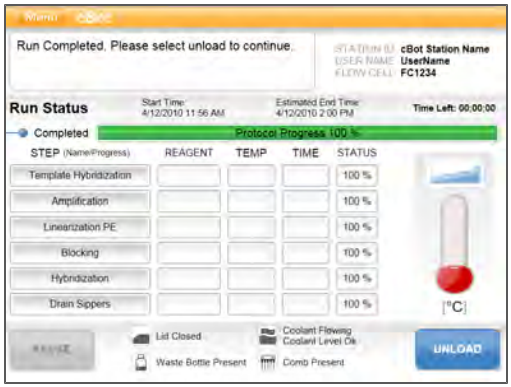
At the end of the run, the run data report automatically opens to alert you that the run is complete. The run data report lists the following information:

- Protocol name
- Flow cell ID
- Reagent ID
- Template name
- Start time and finish time

Unload Run Components

1 When the run is complete, select **Unload** to proceed.

Figure 31 Run Complete, Unload Components



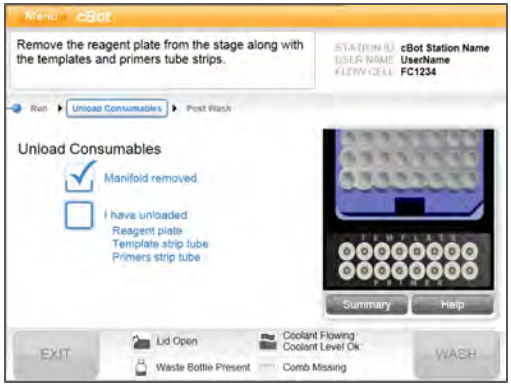
2 Raise the cBot lid by gently lifting from the top-right corner.

3 Release the outlet clamp securing the outlet end of the manifold.

4 Disconnect the outlet end of the manifold from the outlet port in the wash reservoir.

5 Remove the sipper comb from the metal guide pins using the plastic tabs on either side of the sipper comb.
When the sipper comb is removed, the system selects the checkbox indicating that the manifold is removed.

Figure 32 Manifold Removed



6 Release the flow cell clamp.

7 Remove the manifold from the cBot, making sure that the flow cell is still on the thermal stage. It is possible for the flow cell to stick to the bottom of the manifold.

8 Carefully lift the flow cell from the thermal stage.

9 Pull the reagent plate lever toward you to release the reagent plate.

10 Remove the reagent plate from the reagent stage. Set aside until you are ready to check reagent delivery.

11 Remove the eight-tube strip containing the templates. Set aside until you are ready to confirm reagent delivery.

12 If applicable, remove the eight-tube strip containing additional primers. Set aside until you are ready to confirm reagent delivery.

13 Select the checkbox to indicate that you have unloaded the reagents, templates, and primers. The Wash button becomes active when all components have been removed.

a If a component is not removed and an error appears, remove the component.
b If the component has been removed and the error persists, select **Bypass Sensor Check** to proceed to the post-run wash.

If the Bypass Sensor Check option is not visible, your system is not configured to allow sensor bypass.

14 Select **Wash** to proceed to the post-run wash.
If you have configured the post-run wash as optional, you can select **Exit** to bypass the wash.

Flow Cell Storage

High Output: If you need to store the flow cell, store it in storage buffer in the flow cell tube at 4°C. The flow cell is stable after primer hybridization for up to ten days when properly stored at 4°C in storage buffer in the flow cell tube.

Rapid Run: If you are performing a Rapid Run, you must perform your run on the same day as sample loading. If you need to store the flow cell, store it in storage buffer in the flow cell tube at 4°C.

Perform a Post-Run Wash

1 Wash the plate on the thermal stage with deionized water to remove any salt residue, and dry with a lint-free cleaning tissue.

2 Fill the wash reservoir with approximately 12 ml deionized water. You must have a sufficient volume of water to prevent air from entering the lines.

3 Close the cBot lid.

4 Select the checkbox on the screen to indicate that water is present. The Wash button becomes active.

5 Select **Wash**.

6 When the wash is complete, blot out any excess water remaining in the wash reservoir, taking care not to rub the outlet ports as this can cause fibers to clog the holes.

7 Select the checkbox on the screen to indicated that the wash reservoir is dry. The Exit button becomes active.

8 Select **Exit**. The Start screen opens. Your cBot is ready for another run.

Confirm Reagent Delivery

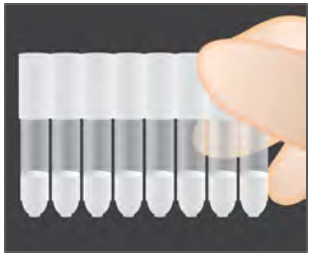
1 Visually inspect the foil-sealed tops of each tube strip and confirm that each reagent tube seal was pierced by the sipper comb.

2 Remove each tube strip from the reagent plate base:
   a Hold the reagent plate base firmly with two hands.
   b With your finger tips under the reagent plate base, gently press upward on the center tubes of the tube strip, releasing the tube strip from the base.
   c Lift the tube strip out of the base.

3 Visually inspect each tube to confirm that reagent delivery was successful from all tubes. Successful delivery is indicated by an approximately equal remaining volume in each tube.

NOTE
Very small differences in delivery per lane are normal and do not affect performance.

Figure 33 Successful Reagent Delivery for a High Output Flow Cell



NOTE
For Rapid Run, all 8 wells of the strip tube will be filled, but only lanes 1 and 2 will be used. Consequently, tubes 1 and 2 will have a different level of fluid than tubes 3 through 8.

Figure 34 Successful Reagent Delivery for a Rapid Flow Cell

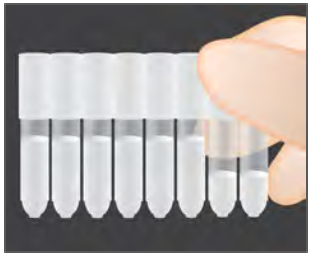
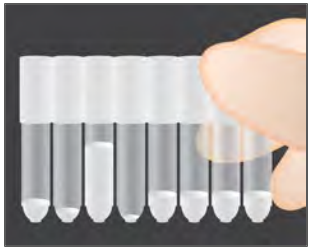


Figure 35 Unsuccessful Reagent Delivery



4 If reagent delivery was not successful from all tubes and the foil-sealed tops of each tube is pierced, take a picture of the tube strip and email Illumina Technical Support.

5 Inspect the eight-tube strips containing templates.

6 If you used custom primers with your run, inspect the eight-tube strips containing primers.

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Maintenance Procedures

Chapter 4

Performing Periodic Maintenance

Perform the basic maintenance steps described in this section to ensure optimal performance.

Table 1 cBot Periodic Maintenance

Maintenance	Frequency	Description
Instrument wash	At least one water wash between every run and if the instrument is idle for more than a day.	Always perform an instrument wash after each run to clear salts and enzymes from the instrument hardware and to prevent clogs. If the instrument has been idle for more than 24 hours, a pre-run wash is recommended. For more information, see *Performing a Pre-Run Wash* on page 37.
Empty waste bottle	Between every run.	To make sure that your run is not interrupted, empty the waste bottle between runs.
Clean surfaces	Once a week.	Use deionized water and a lint-free cleaning tissue to clean the surface of the thermal stage and the reagent stage. Clean the surface of the template and primer tube strip holders.
Clean barcode scanner window	Once a week.	Use deionized water and a lint-free cleaning tissue to clean the barcode scanner window.
Maintenance Wash	Once a month.	Use 5% DECON (or 100 mM NaOH) to remove traces of reagents from internal cBot components and inhibit the growth of microorganisms. For more information, see *Performing a Monthly Maintenance Wash* on page 61.
Check coolant level	Every three months.	Make sure that the green coolant is visible through the coolant window on the rear panel of the instrument. If necessary, use a mirror to view the coolant window. If the coolant is low, use a wide coin or standard screwdriver to remove the coolant reservoir cap, and fill the reservoir to just below the reservoir cap. Use only Illumina-supplied coolant (part # 1003709). If you need additional coolant, contact your Illumina FAS or FSE.

Performing a Monthly Maintenance Wash

Perform a monthly maintenance using 5% DECON to remove traces of reagents from internal cBot components and inhibit microbial growth. In regions where DECON is not available, 100 mM NaOH may be substituted.

The maintenance wash requires approximately ten minutes of hands-on time and consists of four wash steps:

- Water Wash
- DECON or NaOH Wash
- Water Wash (First Rinse)
- Water Wash (Final Rinse)

User-Supplied Consumables

- Deionized water
- 5% DECON or 100 mM NaOH
- Low-lint lab tissues

Perform a Water Wash

1. Confirm that all run components are removed before proceeding. For more information, see *Unload Run Components* on page 53.
2. From the Start screen, select **Menu** and select **Manual Commands**. The Manual Commands screen opens.
3. Select **Commands** to open the Commands tab.
4. Fill the wash reservoir with approximately 12 ml deionized water.
5. Select **Wash** on the Commands tab.
6. After the wash is finished, blot out any excess water remaining in the wash reservoir with a low-lint wipe, taking care not to rub the outlet ports as this can cause fibers to clog the holes.
7. Proceed to the DECON or NaOH wash.

Perform a DECON (or NaOH) Wash

This wash cleanses the system with DECON or NaOH.

1 Fill the wash reservoir with 10 ml of 5% DECON or 100 mM NaOH.

2 Select **Wash**.

CAUTION

DECON is a highly alkaline wash solution. Wear gloves when blotting 5% DECON to protect your skin.

Do not allow DECON to sit in the wash reservoir for long periods of time as the high pH may corrode the metal pins over repeated, extended contact.

3 After the wash is finished, blot out any excess 5% DECON remaining in the wash reservoir with a low-lint wipe, taking care not to rub the outlet ports as this can cause fibers to clog the holes.

4 ***Immediately*** proceed to the water wash to prevent DECON from drying and clogging the wash reservoir holes.

Perform a Water Wash (First Rinse)

The first water wash rinses NaOH from the system.

1 Fill the wash reservoir with approximately 12 ml deionized water.

2 Select **Wash**.

3 After the wash is finished, blot out any excess water remaining in the wash reservoir with a low-lint wipe, taking care not to rub the outlet ports as this can cause fibers to clog the holes.

4 Proceed to the final water wash to remove all remaining traces of the 5% DECON from the wash reservoir and internal cBot components.

Perform a Water Wash (Final Rinse)

A second water wash thoroughly rinses the system.

1 Fill the wash reservoir with approximately 12 ml of clean, deionized water.

2 Select **Wash**.

3 After the wash is finished, blot out any excess water remaining in the wash reservoir with a low-lint wipe, taking care not to rub the outlet ports as this can cause fibers to clog the holes.

4 Close the cBot lid and empty the waste bottle. The cBot is ready for the next cluster generation run.

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Troubleshooting

Chapter 5

Troubleshooting Flow Check Failure

Perform the following procedure to troubleshoot flow check failure. Do not select the option to bypass flow check until you have completed this procedure and safely determined that the flow cell is properly positioned on the instrument, and the manifold and hardware are working properly.

Since High Output flow cells and Rapid flow cells utilize different flow checks, you will want to ensure that you are using the correct recipe, manifold, and flow cell combination if the flow check fails.

Make sure that you have enough HT1 in row 1 of the reagent plate. If it gets low due to repeated flow checks, add HT1 provided in the TruSeq Cluster Kit.

NOTE
Bypassing the flow check can result in some lanes not clustering successfully.

1 Note which lanes failed the flow check. This information is provided on the top-left corner of the interface screen. Do one of the following:
   - If all eight lanes failed, the flow cell is probably loaded improperly. Remove the manifold and confirm that the flow cell holes are facing upward, and the flow cell orientation is correct for the flow cell you are using.
   - If only some lanes failed, the flow cell might not be properly seated. Remove the manifold, reseat the flow cell, and then reinstall the manifold.

2 Select **Rerun Check** to repeat the flow check. This is the second attempt.

3 If the flow check failed a second time, note which lanes failed the flow check, and do one of the following:
   - If all eight lanes failed again, you probably have a faulty manifold. Remove the manifold and replace it with a new manifold. For more information, see *Load the Manifold* on page 44.
   - If different lanes failed, you probably do not have a faulty manifold. Visually inspect the volumes of HT1 in row 1 to make sure the tubes contain equal volumes.

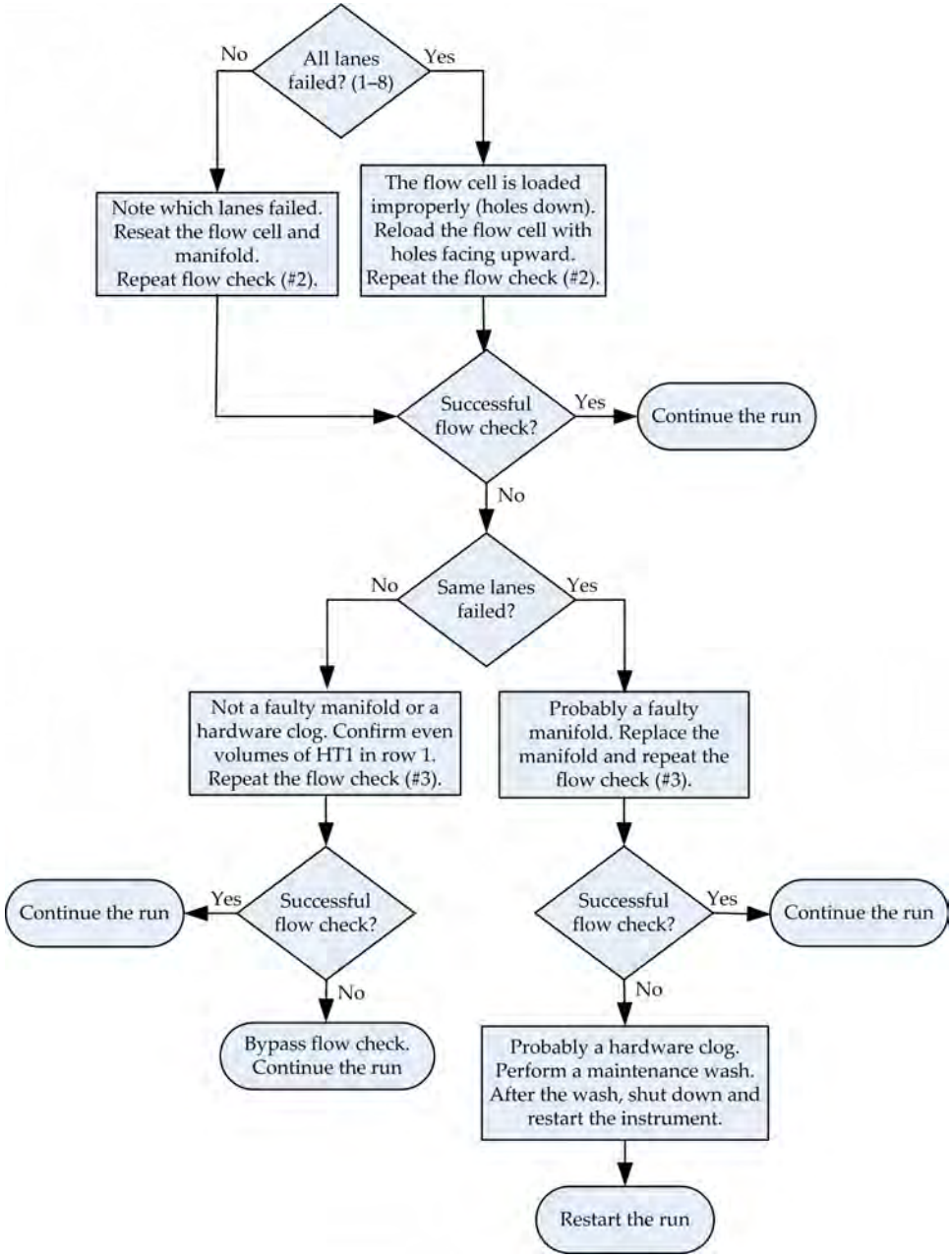
4 Select **Rerun Check** to repeat the flow check for a third time.
   - If this flow check fails after replacing the manifold, go to step 5.
   - If this check fails and you did not need to replace the manifold, go to step 6.

5 If the flow check failed a third time after replacing the manifold, you might have a clog in the hardware. Do the following to correct the problem:
   a Visually inspect the volumes of HT1 in row 1 to make sure the tubes contain equal volumes. A hardware clog is indicated by higher volumes in the tubes that correspond to the lanes that repeatedly failed the flow check. This further confirms a hardware clog.
   b Unload the run components and perform a maintenance wash. For more information, see *Performing a Monthly Maintenance Wash* on page 61.
   c After the wash, turn off the instrument using the power switch. After a few seconds, turn the power switch on, and then press the start button to restart the software. You have to power cycle the instrument in this way to reset the allowable number of pre-run check attempts.
   d Follow the software prompts to reload run components and set up your run.
6 If the flow check failed a third time and you have confirmed that you do not have a faulty manifold, you can now safely use the option to bypass the flow check:
   a Select **Bypass Flow Check** to proceed with the run.
   b After the run, check reagent delivery from all tubes. For more information, see *Confirm Reagent Delivery* on page 56.

Troubleshooting Flowchart

The following flowchart illustrates the troubleshooting procedure. Steps to repeat the flow check include a number to indicate how many of the three allowed flow checks have been performed at that point in the procedure.

Figure 36 Troubleshooting Flow Chart


All lanes failed? (1–8)
No
Yes
Note which lanes failed. Reseat the flow cell and manifold. Repeat flow check (#2).
The flow cell is loaded improperly (holes down). Reload the flow cell with holes facing upward. Repeat the flow check (#2).
Successful flow check?
Yes
Continue the run
No
Same lanes failed?
No
Yes
Not a faulty manifold or a hardware clog. Confirm even volumes of HT1 in row 1. Repeat the flow check (#3).
Probably a faulty manifold. Replace the manifold and repeat the flow check (#3).
Successful flow check?
Yes
Continue the run
No
Bypass flow check. Continue the run
Successful flow check?
Yes
Continue the run
No
Probably a hardware clog. Perform a maintenance wash. After the wash, shut down and restart the instrument.
Restart the run

Troubleshooting Run Problems

Use the following table to troubleshoot possible problems encountered during a cluster generation run.

Problem	Possible Cause	Action
Temperature out of range	Often indicates just that the cBot did not reach the set temperature in the expected time. This is usually not a problem. Can also indicate a potential Peltier or Peltier control board failure.	Email Illumina Technical Support.
Coolant is flowing and coolant level is low	Coolant has slowly evaporated to a low level.	Add Illumina-supplied coolant (part # 1003709) to the coolant reservoir.
Coolant is not flowing and coolant level is low	Coolant level may be too low to generate flow.	Add Illumina-supplied coolant (part # 1003709) to the coolant reservoir.
Coolant is not flowing and coolant level is not low	Potential coolant pump failure.	Email Illumina Technical Support.
System is in a locked state	Potential software error.	Email Illumina Technical Support.

Resetting the Barcode Scanner

The cBot barcode scanner is ready for use when you receive your instrument. However, in the event that the barcode scanner is reset to an incorrect configuration, use the following instructions to restore it to the default configuration.

1 Print out the 11 barcodes on the following pages.

2 Cut each barcode into an individual strip.

3 From the start screen, select **Menu** and select **Manual Commands**.

4 From the manual commands screen, select **General** to access the barcode reader manual control inputs.

Figure 37 Manual Commands, General Tab

5 First, select **Turn Off** and then select **Turn On**. This activates the barcode scanner. The laser line should be visible on the scanner plate under the LCD screen.

6 Scan the first 11 barcodes, one at a time in sequential order (#1–#11) as follows:

a Place barcode #1 underneath the barcode scanner, then select **Turn Off**, and then **Turn On**. A beep indicates that the barcode has been successfully scanned.

b Repeat the previous step for barcodes #2–#11. Make sure you scan them in sequential order.

Table 2 Barcodes for Resetting the Barcode Scanner

Scanning Order	Barcode
1	Set All Defaults
2	*Continuous (04h)
3	Erase All Rules
4	Begin New Rule
5	Send All Data That Remains
6	Send Enter Key

Scanning Order	Barcode
7	Save Rule
8	Laser On Time
9	9
10	9
11	Level (00h)

Performing a Primer Rehybridization Run

A primer rehybridization run repeats the rehybridization step of a cluster generation run to ensure proper hybridization of the Read 1 sequencing primer. If you experience the following conditions during a sequencing run, you can perform a primer rehybridization run on the cBot:

- The first base report from the sequencing run indicates low cluster numbers or low cluster intensities.
- Fluidics issues occurred during Read 1 or the Index Read of a sequencing run.

If you have any concerns before sequencing the flow cell, perform a primer rehybridization run. Primer rehybridization runs will not cause a problem or damage the clusters on the flow cell, even if you need to repeat the process multiple times.

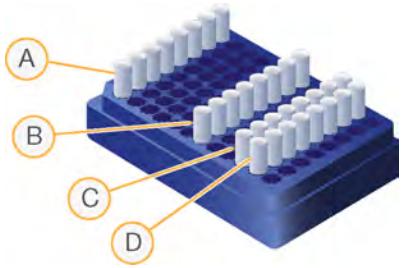
Rehybridization Kit Overview

Store the rehybridization kit at -15° to -25°C until you are ready to use it.

The cBot Rehybridization Kit contains four rows of foil-sealed eight-tube strips filled with primer hybridization reagents, one of which is the Read 1 sequencing primer.

NOTE
The tube strip in row 10 (HP5) is sealed with a non-pierceable red foil. Make sure that you remove the foil seal prior to loading reagents onto the instrument.

Figure 38 Rehybridization Kit



A Row 1—HT1 (Hybridization Buffer)
B Row 7—HT2 (Wash Buffer)
C Row 10—HP5 (0.1 N NaOH)
D Row 11—HP6 (Read 1 Sequencing Primer)

NOTE

When rehybridizing dual-indexed libraries, use HP10 from the TruSeq Dual Index Sequencing Primer Box instead of HP6. See *Preparing HP10 for Dual-Indexed Nextera Libraries* on page 36.

You must use cBot software v1.4.36 or later and compatible recipes from cBot Recipe Installer v1.0.13 or later.

Additional Consumables

- **Flow Cell**—The flow cell must have previously completed the cluster generation process on the cBot.
- **Manifold**—One of the following manifolds (sold separately):
  - **GA Flow Cell**—cBot Manifold, Catalog # SY-301-2014
  - **HiSeq Flow Cell**—cBot Manifold, Catalog # SY-401-2015
- **Optional custom primers**—User-supplied custom sequencing primers, if required for your experiment.
- **Dual-index sequencing primers**—To rehybridize a flow cell containing dual-indexed Nextera libraries, you need HP10 from the TruSeq Dual Index Sequencing Primer Box. See *Preparing HP10 for Dual-Indexed Nextera Libraries* on page 36.

Prepare Reagents

Make sure that reagents are completely thawed before loading them onto the instrument. Reagents take approximately 60 minutes to thaw using a water bath. For more information, see *Preparing the Reagent Plate* on page 34.

1. Remove the thawed reagent plate from the water bath and gently pat dry with paper towels.
2. Hold the reagent plate by the base and place your other hand on top of the tubes, then invert the reagent plate five times to mix the thawed reagents.
3. While holding your hand on top of the tubes, vortex the plate for approximately 10 seconds to dislodge any trapped air bubbles.
4. Tap the reagent plate on a hard surface 5–10 times to collect any reagent droplets to the bottom of the tubes. Alternatively, pulse centrifuge the reagent plate to collect reagent droplets.

5 Visually inspect the reagent plate to make sure that there are no air bubbles at the bottom of the tubes.

6 Visually inspect the numbering on the tube labels to make sure that they are in the correct positions: row 1, row 7, row 10, and row 11.

7 Immediately proceed to setting up the run. Do not allow reagents to sit at room temperature for an extended period of time.

Select a Protocol

Select one of the following rehybridization protocols:

- Without custom primers—**Repeat_Hyb_v#**
- With custom primers or dual-index primer HP10—**Repeat_TubeStripHyb_v#**

Flow Cell	Recipe Version
HiSeq Flow Cell v3	v8 recipes
GA Flow Cell	v7 recipes

Continue Run Setup Steps

1 Remove the foil seal from the eight-tube strip containing HP5 in row 10. See *Important Step Before You Load Reagents* on page 35 for directions.

2 Load the rehybridization reagent plate onto the instrument. See *Load Reagents* on page 39.

3 Load the flow cell. See *Load the Flow Cell* on page 41.

4 Load the manifold. See *Load the Manifold* on page 44.

5 If you are using custom primers, load the tube strip containing your primers in the tube strip holder labeled **Primers**. See *Load Primers* on page 49.

6 After passing the pre-run check, select **Start**.

Start a New Sequencing Run

1 When rehybridization is complete, remove the flow cell from the cBot.

2 Load the rehybridized flow cell on the sequencing instrument using the run setup procedure for your sequencer.

3 Begin a *new* sequencing run. ***Do not attempt to resume the original run.***

Recovering a Run

If a run crashes, you need to restart the cBot. When the instrument is restarted, you can recover the run or choose to cancel the partial run completely.

1 Restart the instrument. Upon restart, the following message appears on the start screen:
"A recoverable run exists. Select Start to recover the run."

2 Select **Start**. The cBot Recover Run dialog box opens.

3 Select **OK**. The software opens to the pre-run check screen. Upon successful completion of the pre-run check, the run restarts from the point where it stopped. Alternatively, select **Cancel** to completely cancel the partial run.

Upgrading the Software

Using software v1.3 or later, you can upgrade instrument software from the configuration screen using a USB flash drive.

1 Insert the USB flash drive containing the new software version installer (for example, cBotSetupX86_1.3.1.0.exe) into one of the two USB ports on the front of the instrument. The installer must reside in the root directory of the USB flash drive.

2 Select **Menu** in the upper-left corner of the screen, and select **Configure**. The keyboard opens.

3 Log in using the default password and select **Enter** to close the keyboard. The configure screen opens.
   a Select the **Shift** key to display lower case letters.
   b Type the default password, **admin**. The default password must be entered using lower case letters.

4 Select **Menu** in the upper-left corner of the screen, and select **Upgrade**.

Figure 39 Upgrade Software Menu

5 A dialog box appears with one of the following messages:

Message	Action
The software installer version is greater than the version currently installed on the cBot	Select **OK** to proceed with the installation of the newer version.
cBot cannot find a valid software installer	You can either insert a valid cBot upgrade and select **OK** to try again, or **Cancel** to abort the upgrade.
The software installer version is equal or lower than the version currently installed on the cBot	Select **Cancel** to abort the upgrade, or **OK** to proceed with installation of a previous version.

WARNING

The USB flash drive must remain in the USB port for the duration of the upgrade process until the instrument reboots automatically. Do not interact with the system during the upgrade.

6 When the cBot reboot is complete and the login screen opens, you can remove the USB flash drive.

Upgrading Recipes

Using software v1.4, you can upgrade recipe versions independent of software upgrades using a USB flash drive containing the recipe installer.

1 Insert the USB flash drive containing new recipe installer into one of the two USB ports on the front of the instrument.

NOTE
The installer must reside in the root directory of the USB flash drive, not in a folder.

2 Select **Menu** in the upper-left corner of the screen, and select **Configure**.

3 Log in using the default password and select enter to close the keyboard. The configure screen opens.
   a Select the **Shift** key to display lower case letters.
   b Type the default password, **admin**. The default password must be entered using lower case letters.

4 Select **Menu** in the upper-left corner of the screen, and select **Upgrade Recipes**.

Figure 40 Upgrade Recipes Menu

WARNING
The USB flash drive must remain in the USB slot for the duration of the upgrade process until the instrument reboots automatically. Do not interact with the system during the upgrade.

Upon completion of the upgrade, the cBot instrument reboots automatically. The reboot process takes about ten minutes to complete.

5 When the cBot reboot is complete and the login screen opens, you can remove the USB flash drive.

Protocol Editor

You can edit protocols according to your needs using the Protocol Editor. For example, you may want to repeat steps in a protocol, or change the number of amplification cycles in the chemistry section.

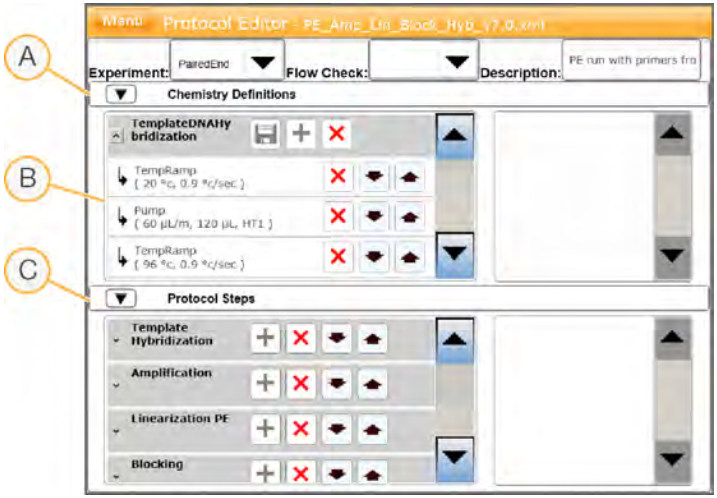
Each protocol consists of two main sections:

- **Chemistry section**—Contains instructions for pumping reagents, temperature changes, and wait durations. The chemistry section of the protocol appears in upper portion of the Protocol Editor screen.
- **Protocol section**—Contains a series of steps made up of chemistry definitions. The protocol section appears in the lower portion of the Protocol Editor screen.

If you edit an existing protocol, remember to rename your protocol.

1. From the Start screen, select **Menu**, and then select **Protocol Editor**. The Protocol Editor opens.
2. From the Protocol Editor, select **Menu**, and then select one of the following commands from the menu:
   - Select **Open** to open an existing protocol.
   - Select **Load from Library** to load an existing chemistry definition or protocol step stored in the cBot library.
   - Select **New Chemistry Definition** or **New Protocol Step** to create a new definition or step and store it in the cBot library.

Figure 41 Protocol Editor, Expanded Steps



A Chemistry Section
B Expanded Chemistry Section
C Protocol Section

3 Use the down arrow to the left of the step to expand the commands in the step or the up arrow to collapse the commands.

4 To edit a step in a chemistry definition, highlight the step. Selections appear in the right-hand panel to change the pump, temperature ramp, or wait commands.

5 To edit a step in the protocol, highlight the step. Selections appear in the right-hand panel to change to the number of cycles for the selected chemistry definition.

6 Use the Protocol Editor icons to the right of the step name to rearrange, delete, or copy steps and commands. For more information, see *Protocol Editor Icons* on page 85.

Protocol Editor Icons

Icon	Description
	Moves the highlighted step below the following step in the protocol.
	Moves the highlighted step above the preceding step in the protocol.
	Deletes the highlighted step.
	Repeats the highlighted step.
	Saves your changes to the protocol library.

Shutting Down the cBot

It is not necessary to shut down your cBot between runs. It is designed to continue running in an idle state on the Start screen. In the case you need to shut down your cBot, you can access the command from the configuration screen.

1 Select **Menu** and select **Configure**. The keyboard opens.

2 Enter the default password using the keyboard:
   a Select the **Shift** key to display lower case letters and type the default password, **admin**. The default password must be entered using lower case letters.
   b Select **Enter**. The configuration screen appears.

3 From the configuration screen, select **Menu** and select **Shut Down Station**. The cBot software shuts down.

Figure 42 Shut Down Station

4 To turn off your cBot, toggle the power switch on the right side panel to the OFF position.

Reboot in FSE Mode

The option to reboot in FSE Mode is for use by a trained Illumina Field Application Scientist (FAS) or Field Service Engineer (FSE) to update software or service the instrument.

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Cluster Generation Chemistry

Appendix A

Introduction

During cluster generation, template molecules are attached to a flow cell and then amplified locally to form clonal clusters. The flow cells containing the clusters can then be sequenced on an Illumina sequencing instrument.

This section provides an overview of the chemistry of cluster generation.

Adapter Ligation and Selection

Before cluster generation can occur, the template fragments need to contain the right 5′ and 3′ adapters, and have the proper length. This is done during sample preparation using the following steps:

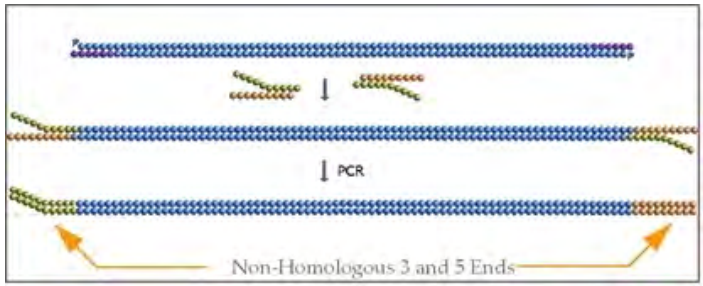
1 In most protocols, samples are fragmented to get proper sized fragments.

2 Samples that consist of RNA are reverse transcribed to get a DNA template.

3 Two different Y-shaped adapters are ligated to the DNA fragments.

4 The fragments are PCR-amplified with primers annealing to the Y-shaped adapters. The Y-shaped adapters have been devised to generate fragments containing two non-homologous 5′ and 3′ ends.

Figure 43 Ligation of Y-shaped Adapters and PCR



5 Fragments are size-fractionated on a gel.

The sample is now ready for cluster generation.

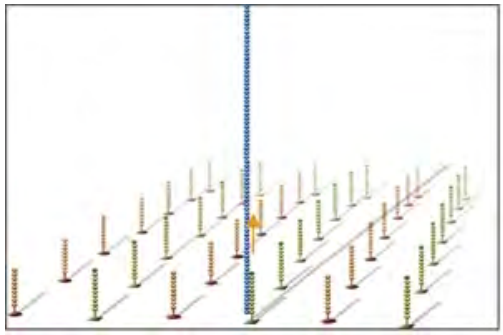
Cluster Generation

Cluster generation is performed on the cBot using the following steps:

1 The DNA fragment library is diluted, denatured and introduced into the lanes of the flow cell. The flow cell is a silica slide with eight lengthwise lanes containing a dense lawn of oligonucleotides.

2 The templates are captured by the oligonucleotides attached to the surface of the flow cell.

Figure 44 Capture and Extension of Template

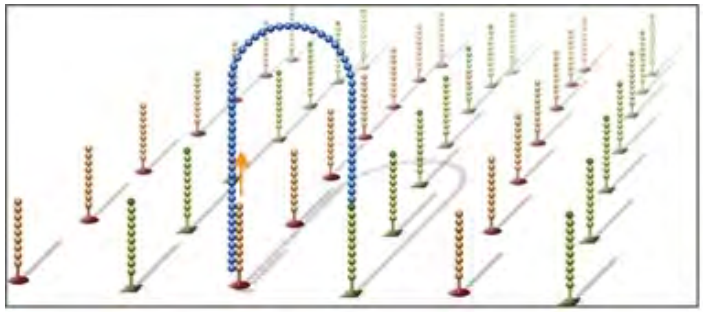


3 Templates bound to the primers are 3′-extended producing covalently-attached discrete single molecules.

4 The double-stranded molecule is denatured, and the original template is washed away.

5 Free ends of the bound templates hybridize to adjacent lawn primers to form U-shaped bridges.

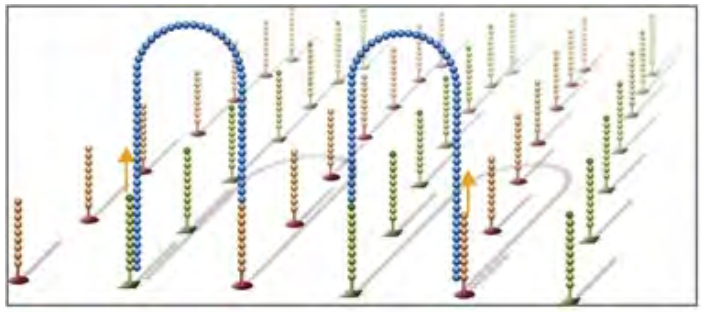
Figure 45 Bridge Formation



6 The DNA bridge is copied from the primer to create a double-stranded DNA bridge.

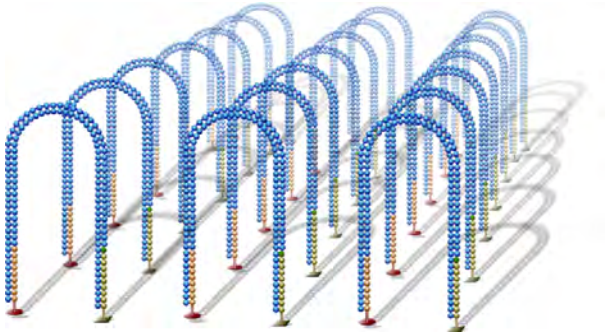
7 The resulting dsDNA is denatured, hybridized to lawn-primers to form new bridges and extended again.

Figure 46 Further Amplification of Template



8 This process of iso-thermal bridge amplification is repeated 35 times to create a dense cluster of over 2000 molecules.

Figure 47 Clonal Cluster



9 The reverse strands in the cluster are removed by cleavage at the reverse strand-specific lawn primers, leaving a cluster with forward strands only.

10 The free 3′-OH ends are blocked to prevent non-specific priming.

11 Sequencing primers are hybridized to the free ends of the DNA templates.

The flow cell with the synthesized clonal clusters is now ready to be sequenced on the Illumina sequencing instrument.

Specifications and Installation

Appendix B

Preparing Your Site

This section describes the cBot physical dimensions, electrical requirements, and environmental constraints.

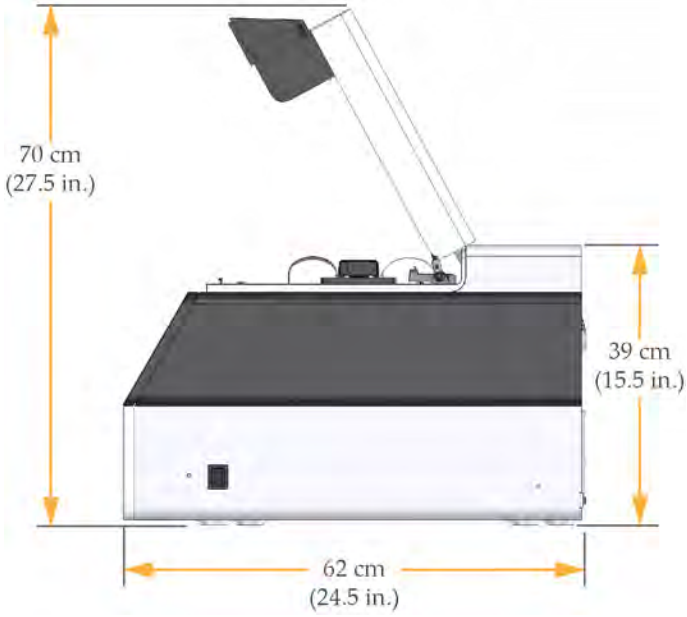
Lab Space and Dimensions

Reserve sufficient laboratory space and a lab bench that is capable of safely holding the weight of the cBot. Place the instrument at least six inches away from the wall so that you can easily reach the power connections on the back of the cBot.

Table 3 cBotDimensions

Width	Height (Lid Closed)	Height (Lid Open)	Depth	Weight
38 cm (15 in.)	39 cm (15.5 in.)	70 cm (27.5 in.)	62 cm (24.5 in.)	31 kg (68 lbs.)

Figure 48 cBot Dimensions, Lid Open



Reserve sufficient laboratory space to unpack your cBot. The cBot shipping container has the following dimensions:

Table 4 Shipping Container Dimensions

Width	Height	Depth	Weight
52 cm (20.5 in.)	51 cm (20.25 in.)	75 cm (29.5 in.)	34 kg (75 lbs.)

Electrical Requirements

The cBot is certified to the following electrical standards:

- Conforms to UL STD 61010-1
- Certified to CSA STD C22.2 No 61010-1
- Low Voltage Directive (Product Safety) IEC 61010-1
- EMC Directive IEC 61326-1

Power Consumption

- The line voltage of the cBot is 100–240 volts AC, running at 50/60 Hz.
- The cBot typically consumes approximately 300 watts and has a maximum of 500 watts.
- Illumina recommends that you connect the cBot to an uninterruptible power supply (UPS) to protect the instrument in the event of a power surge or loss.
- The cBot requires a 6–10 amp grounded, dedicated line with proper voltage (100–240 volts AC) and an electrical ground.

Power Cords

- The cBot comes with an international standard IEC 60320 receptacle and is shipped with a region-specific power cord.
- Allow a maximum of 2 m (6 ft.) between the instrument AC power inlet and facility power.
- Never use an extension cord to connect your cBot.

Fuses

- Do not replace any fuses. Email Illumina Technical Support for preventative maintenance.

- The cBot uses only manufacturer recommended fuses:
  - Littelfuse: part # 218008.HXP
  - Time Lag (SLO BLO) Type
  - 250 VAC, 5A
  - 5×20 mm

Coin Cell Battery

The coin cell battery on the instrument computer motherboard is ***not*** a user-replaceable part.

The coin cell battery is ***not*** rechargeable. Under no circumstances attempt to recharge the battery.

Environmental Constraints

Consult your facilities department regarding environmental constraints before installing your cBot.

Table 5 Environmental Constraints

Condition	Acceptable Range
Temperature	22°C ±3°C
Humidity	Relative humidity between 20–80%, non-condensing
Ventilation	Maximum thermal output is approximately ~2550 Btu/h (~750 W)

Configuring the cBot

You can configure the cBot using the touch screen monitor. Configuration steps include naming your instrument, determining run requirements, and selecting input types required for each run during run setup. Using a network connection, you can enable remote monitoring, email alerts, and LIMS support.

Start Screen Menu

1 Select **Menu** in the upper-left corner of the screen, and select **Configure**. The keyboard opens.

Figure 49 Start Screen Menu

2 Enter the default password using the keyboard:

a Select the **Shift** key to display lower case letters.

b Type the default password, **admin**. The default password must be entered using lower case letters.

Figure 50 Keyboard

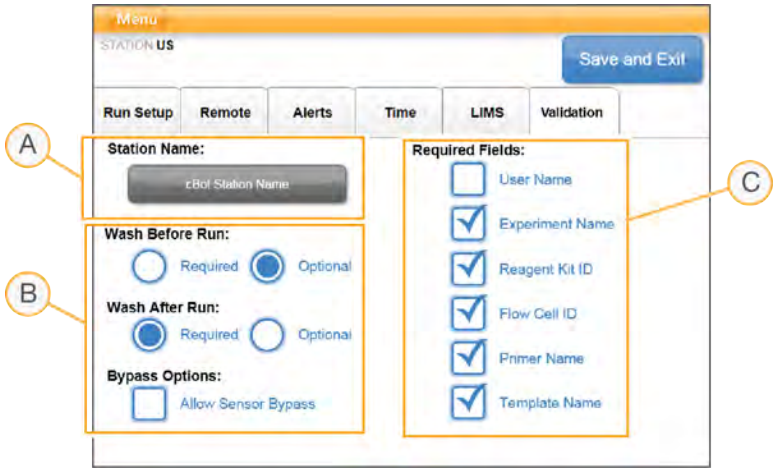
c Select **Enter**. The keyboard closes.

Configure Run Requirements

The configuration screen contains four tabs: the Run Setup tab, the Remote tab, the Alerts tab, and the Time tab.

From the Run Setup tab, you can name your instrument, configure wash and sensor bypass options, and indicate which fields are required input before each run can begin. These settings can be modified as needed before the start of each run.

Figure 51 Run Setup Tab



A Station Name
B Wash Options and Bypass Options
C Required Fields

1 Select **StationName** to name your cBot. The keyboard opens.

NOTE
Select the **Shift** key to activate lower case letters.
Select the **Alt** key to activate symbol keys.

2 Type the name of your instrument using the on-screen keyboard, and then select **Enter**.

3 Select the radial buttons to select pre-run and post-run wash options.

NOTE
For optimal performance,Illumina recommends an instrument water wash before and after each run.

4 To provide the option to allow a run to proceed even if an invalid sensor reading occurs during the pre-run check, select the checkbox **Allow Sensor Bypass**. This option is disabled by default.
If this feature is enabled, the option appears on the pre-run check screen if the pre-run check fails due to sensor failure. You must visually confirm that the run

components are loaded correctly before bypassing sensors and proceeding to the fluidics check. For more information, see *Perform a Pre-Run Check* on page 51.

5 Select the checkbox to select which fields are required input at the start of each run. Fields include user name, experiment name, reagent kit ID, flow cell ID, primer name, and template name.

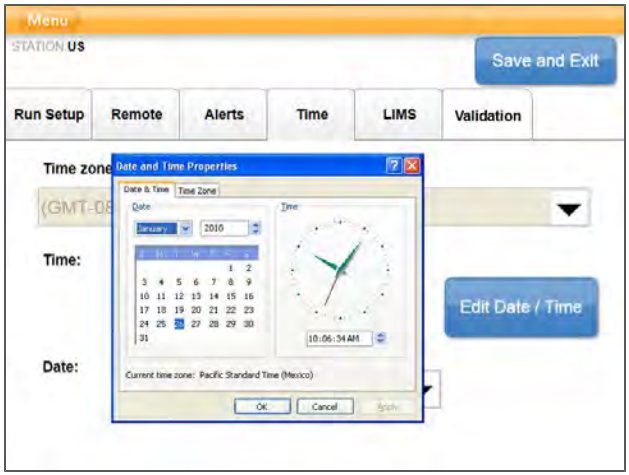
Set Date and Time

From the Time tab, you can set your instrument to the current date and your local time.

1 Select the Time tab.

2 Select **Edit Date/Time**. The Windows Date and Time Properties dialog box appears.

Figure 52 Time Tab



3 Select the appropriate time zone for your area, the current date, and the current time.

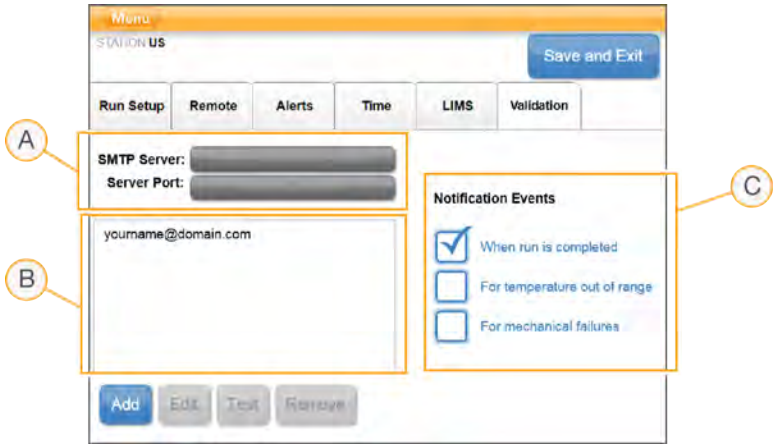
4 Select **OK** to close the Windows Date and Time Properties dialog box. The changes appear on the Time Tab.

Set Up Email Alerts

From the Alerts tab, you can configure the system to send an email alert if a system issue occurs or when a run is complete. A network connection is required.

1 Select the Alerts tab.

Figure 53 Alerts Tab



A Email Server
B Email Addresses
C Notification Events

2 Select **SMTP Server**. The keyboard opens.

NOTE
Consult your facility administrator for the name of your SMTP server and service port.

3 Using the on-screen keyboard, enter the smtp server name, and then select **Enter**.

4 Select **Server Path**. The keyboard opens.

5 Using the on-screen keyboard, enter the email smtp server port, and then select **Enter**.

6 Enter the email address for each intended alert recipient:

a Select **Add** to add the email address of alert recipients. The keyboard opens.

b Using the on-screen keyboard, enter an email address, and then select **Enter**.
c Select **Add** again to enter an additional email address.
d To test an email address, highlight the address and select **Test**.

7 In the Notification Events area, select the checkbox to identify which events trigger an email alert.

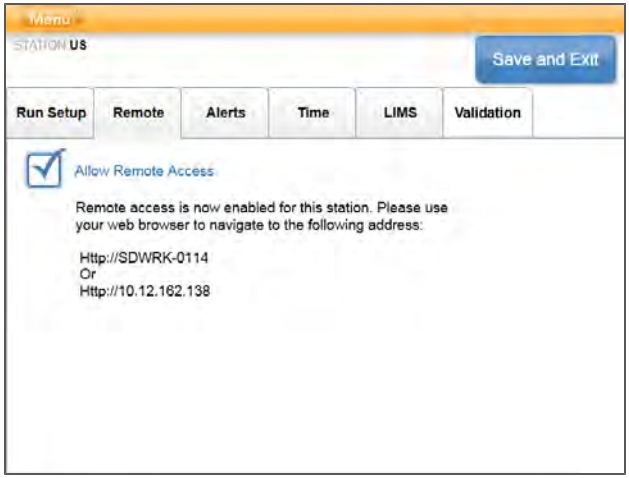
Enable Remote Monitoring

From the Remote tab, you can configure the system to monitor your run remotely using the remote monitoring feature. A network connection is required.

1 Select the Remote tab.

2 Select the checkbox next to **Allow Remote Access**.
The IP address of the instrument appears on the screen.

Figure 54 Remote Tab



3 Use your web browser from another computer to access the remote monitoring feature. For more information, see *Remote Monitoring Overview* on page 23.

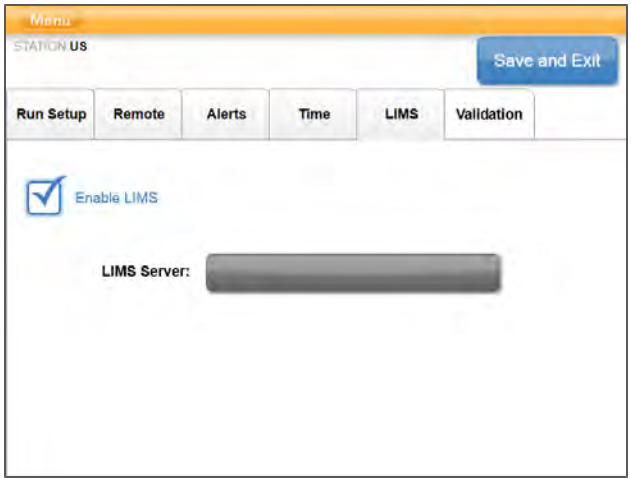
Enable LIMS Support

From the LIMS tab, you can set your instrument to the current date and your local time.

1 Select the LIMS tab.

2 Select the checkbox next to **Enable LIMS**.

Figure 55 LIMS Tab



3 Select the field next to LIMS server. The keyboard opens.

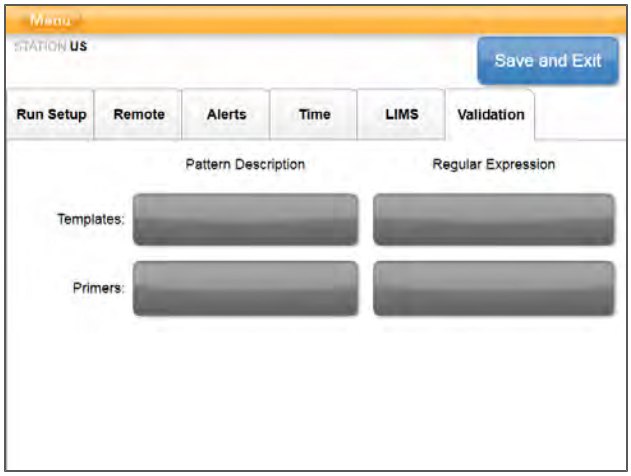
4 Using the on-screen keyboard, enter the LIMS server name, and then select **Enter**.

Set Validation Expressions

From the Validation tab, you can set regular expressions for your template and primer IDs. This allows you to standardize how template and primer IDs are entered during run setup on the cBot.

1 Select the Validation tab.

Figure 56 Validation Tab



2 To set expressions for template IDs, select the fields next to **Templates**. The keyboard opens.

a Enter a pattern description for the template ID.

b Enter a regular expression for template ID.

The following table shows examples of validation expressions:

Pattern Description	Regular Expression	Valid Value
Template - (###)	Template - \(\d{3}\)	Template - (123)
ILMN - <A to F>###	ILMN - [a - fA - F]\d{3}	ILMN - D341
PR - <Number from 30 - 59>	PR - [3 - 5] [0 - 9]	PR - 41

3 Repeat these steps to set expressions for custom primer IDs.

Close Configuration Screen

When you have finished configuring the setup options, select **Save and Exit**. The cBot Start screen opens. You are ready to start a cluster generation run.

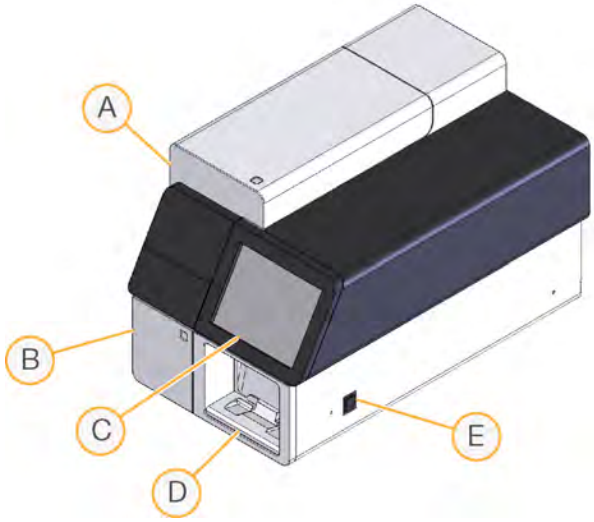
Installing the cBot

Installing the cBot takes approximately 15 minutes. It is shipped in one box with the following contents:

- cBot instrument
- HiSeq Flow cell adapter plate (The GA adapter plate is installed on the instrument.)
- Power cord
- Waste bottle
- Quick setup poster
- User guide

Exterior Components

Figure 57 cBotExterior Components



A **Lid**—Covers the thermal stage, reagent stage, and wash reservoir.
B **Waste Bottle Compartment**—Holds the sensor-controlled waste bottle.
C **Monitor**—Displays the cBot user interface.
D **Barcode Scanner**—Scans barcode ID of reagent plate and flow cell.
E **Power Switch**—Turns on your instrument.

Unpack and Inspect

NOTE

If your cBot was stored in a cold location, allow it to reach room temperature before proceeding.

Save the packing material and shipping box for use in case you ever need to ship your cBot to Illumina for servicing.

1 Snip the shipping straps wrapped around the shipping box.

NOTE

Do not cut the shipping tape on top of the box. The top of the shipping box is designed to lift off of the instrument.

2 Lift the top of the shipping box straight up using the cutout handles on each end.

3 Locate the power cord and waste bottle, and set them aside.

CAUTION

Never lift the cBot from the front or rear panels, barcode scanner, or any location other than the long side panels. These panels are not designed to support the weight of the instrument.

4 With one person standing in front of the instrument and one person standing behind it, lift the instrument from the packaging by placing your hands under the long side panels. Cutouts in the foam packing mark the best positions for lifting.

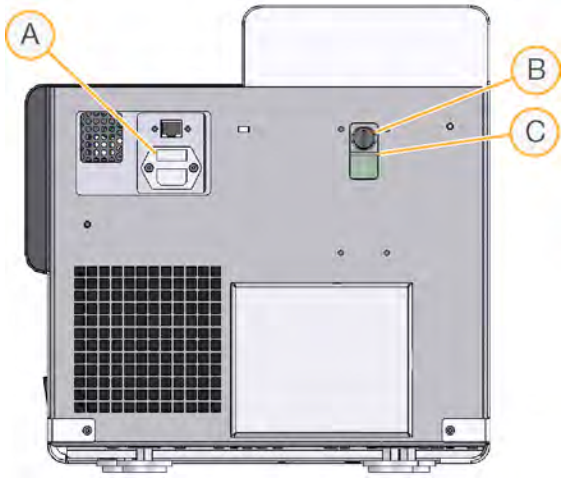
5 Place the cBot on a lab bench or suitable location, positioning it so you can inspect all sides of the instrument.

6 Inspect the condition of the following components:

- Make sure that the instrument panels are intact.
- Inspect the touch screen for cracks or scratches.
- Make sure that coolant did not leak during shipment. Check for moisture inside the box.

If any of the above items are unsatisfactory, immediately contact Illumina Technical Support for further instructions.

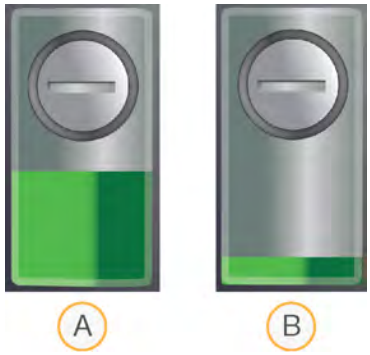
Figure 58 cBot Rear Panel Components



A Power Connection
B Coolant Reservoir
C Coolant Level

7 Check the coolant level through the view port on the rear panel of the instrument. The green liquid coolant level should be just below the coolant reservoir cap.

Figure 59 Coolant Level



A Coolant Level Good
B Coolant Level Low

If the coolant level is low, top up the coolant. Use the same coolant provided with the Illumina sequencing instrument (Part # 1003709).

a Use a wide coin or flat screwdriver to remove the reservoir cap.
b Fill the reservoir with Illumina-supplied coolant to just below the fill port.
c Replace the reservoir cap.

Position and Connect

1 Remove the protective foam block from the barcode scanner.

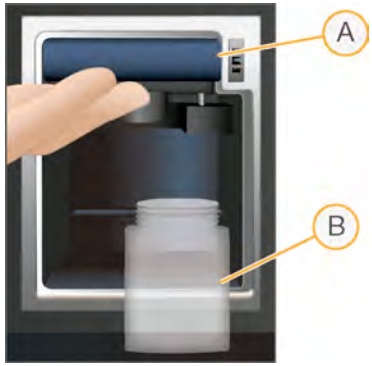
2 Lift the cBot lid and remove the protective foam block from the reagent area.

3 Press the top-right corner of the waste compartment door, then quickly release to open the door.

4 Remove the protective foam block from the waste bottle compartment.

5 Lift the waste bottle lever and position the waste bottle under the lever, slightly tipping the mouth of the bottle to clear the spout.

Figure 60 Install the Waste Bottle



A Waste Bottle Lever
B Waste Bottle

6 Lower the lever to secure the waste bottle into position. The spout should sit inside the mouth of the wash bottle.

7 Remove the protective sheet from the cBot monitor.

8 Connect the power cord to the AC power inlet on the rear panel, and then connect to facility power.

9 If you are planning to configure your cBot for a network connection, connect a user-supplied RJ45 Ethernet cable to the Ethernet port.

10 Position your cBot on the lab bench at least 15.25 cm (6 in.) away from the wall or other obstruction. Make sure that it is sitting level on the bench.

11 Locate the power switch on the right side of the instrument and toggle the switch to the **ON** position.

12 Locate the start button to the left of the waste bottle compartment door and press to start the cBot software.

Figure 61 cBotStart Button

A Start Switch
B Power Switch

The software initiates a start-up routine. When the start-up routine is complete, the Start screen opens.

Changing the Adapter Plate

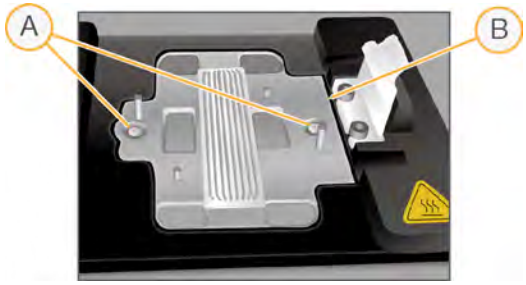
You can use a Genome Analyzer flow cell or a HiSeq flow cell on the cBot. Each flow cell type requires that a specific adapter plate is installed on the cBot prior to starting the run. Icons on the start page indicate which adapter plate is currently installed on the cBot.

1 Open the cBot lid by gently lifting from the top-right corner.

2 Lift the flow cell clamp.

3 Loosen the two captive Phillips head screws securing the adapter plate.

Figure 62 Flow Cell Adapter Plate for High Output Flow Cell



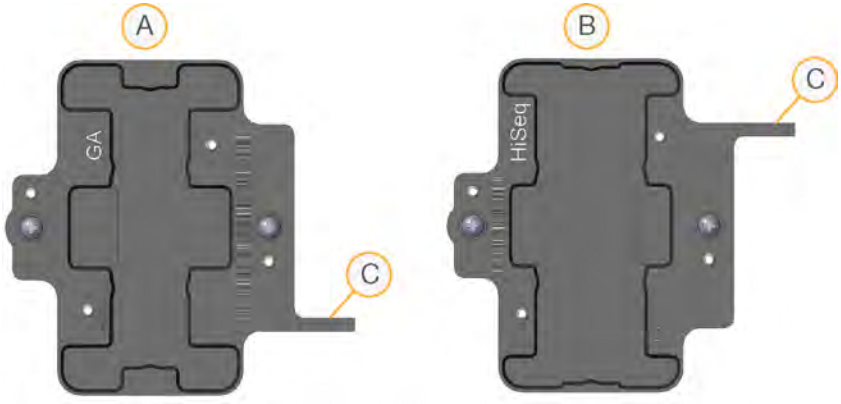
A Captive Screws
B Adapter Plate

4 Lift the existing adapter plate from the thermal stage and set aside.

5 Make sure that the thermal stage is clean. If any salt build up is present, wipe the stage with a lint-free cleaning tissue slightly moistened with water.

6 Position the new adapter plate on the thermal stage, aligning the sensor arm with the corresponding slot on the right side of the thermal stage.

Figure 63 Sensor Arm Location



A Genome Analyzer Adapter Plate
B HiSeq Adapter Plate
C Adapter Plate Sensor Arm

7 Tighten the two Phillips head screws to secure the adapter plate.

8 For optimal heat transfer, make sure that the adapter plate is sitting flat and the screws are tightened evenly.

9 Wipe the installed adapter plate with a lint-free cleaning tissue moistened with water, and then wipe dry with a clean tissue. Do not allow fluids to drip inside the instrument.

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 6 Illumina General Contact Information

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

Table 7 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
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

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at www.illumina.com/msds.

Product Documentation

You can obtain PDFs of additional product documentation from the Illumina website. Go to www.illumina.com/support and select a product. To download documentation, you will be asked to log in to MyIllumina. After you log in, you can view or save the PDF. To register for a MyIllumina account, please visit my.illumina.com/Account/Register.