MiniSeq

System Guide

Document # 1000000002695 v04 Material # 20014309

ii

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Revision History

Document	Date	Description of Change
Material # 20014309 Document # 1000000002695 v04	September 2020	Updated loading concentration and software information to be inclusive of rapid kits.
Material # 20014309 Document # 1000000002695 v03	February 2020	Updated workflow information for Manual and Local Run Manager run options. Updated preinstalled genomes: Removed Bacillus_cereus_ATCC_ 10987 and added HumanRNAFusion. Removed information about BaseSpace Onsite as it is no longer supported. Minor text edits.
Material # 20014309 Document # 1000000002695 v02	March 2018	Added information about the Illumina Proactive monitoring service in the Configure Analysis Settings section. Removed the default user name and password required to log on to the operating system. Illumina recommends using site-specific credentials. Minor text edits.
Material # 20014309 Document # 1000000002695 v01	September 2016	Updated software descriptions to MiniSeq Control Software v1.1.8, which includes Demo Mode. Updated the automatic post-run wash duration to 60 minutes. Added a server configuration step to the instructions for selecting BaseSpace for analysis. Noted that mapped drives are not supported by the Local Run Manager software.
Material # 20002370 Document # 1000000002695 v00	January 2016	Initial release.

Table of Contents

Chapter 1 Overview

Introduction

The Illumina® MiniSeq™ System provides high-quality, industry-standard Illumina sequencing technology with the convenience of an easy-to-use cost-effective desktop system.

Features

▶ **High-quality sequencing**—The MiniSeq System enables small genome, amplicon, targeted enrichment, and RNA sequencing using low library volumes.

▶ **MiniSeq system software**—The MiniSeq System includes a suite of integrated software that controls instrument operations, processes images, and generates base calls. The suite includes on-instrument data analysis software and data transfer tools for analysis using other options, such as BaseSpace Sequence Hub.

  ▶ **On-instrument data analysis**—The Local Run Manager software analyzes run data according to the analysis module specified for the run. A suite of analysis modules are included in the software.

  ▶ **BaseSpace® Sequence Hub integration**—The sequencing workflow is integrated with BaseSpace Sequence Hub, the Illumina genomics computing environment for run monitoring, data analysis, storage, and collaboration. Output files are streamed in real time to BaseSpace Sequence Hub for analysis.

▶ **Convenient consumable loading**—A clamping mechanism positions the flow cell as it is loaded onto the instrument. A single-use prefilled reagent cartridge provides reagents required for a run and subsequent instrument wash. The flow cell and reagent cartridge include integrated identification to enable accurate tracking.

Additional Resources

The MiniSeq System support pages on the Illumina website provide additional resources. These resources include software, training, compatible products, and the following documentation. Always check support pages for the latest versions.

Resource	Description
Custom Protocol Selector	A wizard for generating customized end-to-end documentation that is tailored to the library prep method, run parameters, and analysis method used for the sequencing run.
MiniSeq System Site Prep Guide (document # 1000000002696)	Provides specifications for laboratory space, electrical requirements, and environmental considerations.
MiniSeq System Safety and Compliance Guide (document # 1000000002698)	Provides information about operational safety considerations, compliance statements, and instrument labeling.
RFID Reader Compliance Guide (document # 1000000002699)	Provides information about the RFID reader in the instrument, compliance certifications, and safety considerations.

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Resource	Description
MiniSeq System Denature and Dilute Libraries Guide (document # 1000000002697)	Provides instructions for denaturing and diluting prepared libraries for a sequencing run, and preparing an optional PhiX control.
Local Run Manager Software Guide (document # 1000000002702)	Provides information about using the Local Run Manager software and available analysis options.

Instrument Components

The MiniSeq System includes a touch screen monitor, a status bar, a flow cell compartment, and a reagent compartment.

Figure 1   Instrument Components
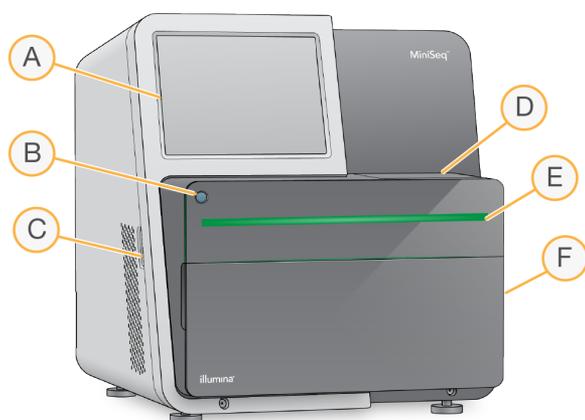
A   **Touch screen monitor**–Enables on-instrument configuration and setup using the control software interface.
B   **Power button**-Turns on the integrated instrument computer and operating system.
C   **USB ports**–Convenient connections for peripheral components.
D   **Flow cell compartment**–Holds the flow cell during a sequencing run.
E   **Status bar**–Indicates instrument status as processing (blue), requires attention (orange), ready to sequence (green), or when a wash is due within the next 24 hours (yellow).
F   **Reagent compartment**–Holds the reagent cartridge and spent reagents bottle.

Flow Cell Compartment

The flow cell stage includes the flow cell latch, which secures the flow cell when it is closed. As the latch closes, pins near the base of the latch align the flow cell ports with fluidics connections.

Document # 1000000002695 v04 Material # 20014309

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2

Figure 2 Flow Cell Compartment
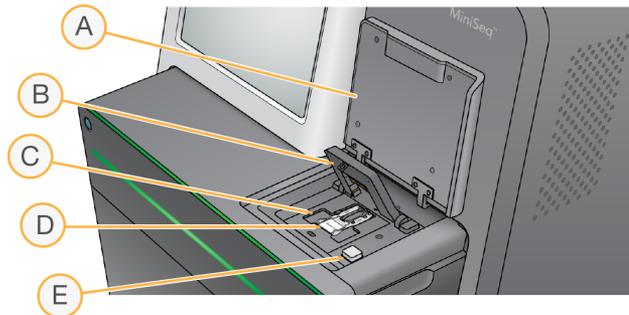
A Flow cell compartment door
B Flow cell latch
C Flow cell stage
D Flow cell
E Flow cell latch release button

The thermal station, located beneath the flow cell stage, controls changes in temperature required for cluster generation and sequencing.

> **NOTE**
> Do not place objects on the instrument near the flow cell compartment.

Reagent Compartment

Setting up a sequencing run on the MiniSeq System requires access to the reagent compartment to load run consumables and empty the spent reagents bottle.

Figure 3 Reagent Compartment
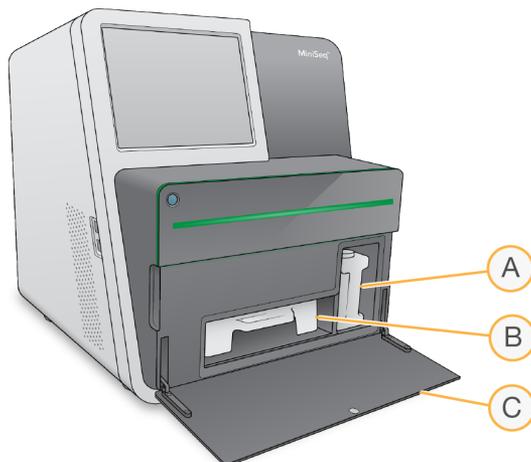
A **Spent reagents bottle**—Includes a threaded cap to prevent spills when carrying.
B **Reagent cartridge**—Provides reagents in a single-use prefilled consumable.
C **Reagent compartment door**—Provides access to the reagent compartment.

The reagent compartment door opens outward from hinges at the bottom edge of the instrument. To open the door, gently pull forward from the side edges of the door.

> **NOTE**
>
> Do not place objects on the reagent compartment door. The compartment door is not designed for use as a shelf.

Power Button

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

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

> **NOTE**
>
> Under normal conditions, do not turn off the instrument power.
>
> Turning off the instrument during a sequencing run ends the run immediately. Ending a run is final. Run consumables cannot be reused and sequencing data are not saved.

System Software

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

▶ **MiniSeq Control Software**—The control software guides you through the steps to set up a sequencing run, controls instrument operations, and shows an overview of run statistics as the run progresses.

▶ **Real-Time Analysis (RTA) software**—RTA performs image analysis and base calling during the run. See *Real-Time Analysis Overview* on page 42.

▶ **Local Run Manager**—Before sequencing, use Local Run Manager to specify run parameters and the analysis method. After sequencing, on-instrument data analysis begins automatically. For more information, see the *Local Run Manager Software Guide (document # 1000000002702)*.

Status Icons

A status icon in the top-right corner of the control software interface screen indicates a change in condition during run setup or during the run.

Status Icon	Status Name	Description
	Status OK	System is normal.
	Processing	System is processing.

Status Icon	Status Name	Description
	Attention	Attention is required.
	Warning	A warning has occurred. Warnings do not stop a run or require action before proceeding.
	Error	An error has occurred. Errors require action before proceeding with the run.

When a change in condition occurs, the icon blinks to alert you. Select the icon to view a description of the condition. Select **Acknowledge** to accept the message and **Close** to close the dialog box.

Sequencing Consumables Overview

Performing a sequencing run on the MiniSeq System requires a single-use MiniSeq Kit. Each kit includes a flow cell and the reagents required for a sequencing run.

The flow cell and reagent cartridge use radio-frequency identification (RFID) for accurate consumable tracking and to ensure compatibility with specified run parameters.

Flow Cell

The flow cell is a glass-based substrate on which clusters are generated and the sequencing reaction is performed. The flow cell is encased in a flow cell cartridge.

Figure 4   Flow Cell Components
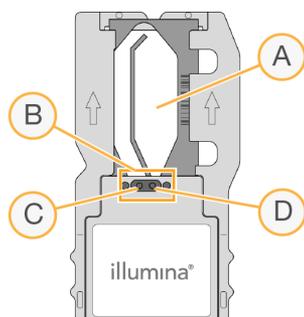
A   Imaging area
B   Flow cell gasket
C   Outlet port
D   Inlet port

Reagents enter the flow cell through the inlet port, pass through the single-lane imaging area, and then exit the flow cell through the outlet port.

The flow cell is shipped dry in a flow cell tube that is wrapped in a foil package. Store the flow cell in the sealed foil package at 2°C to 8°C until use. For more information, see *Prepare the Flow Cell* on page 13.

Document # 1000000002695 v04 Material # 20014309

5

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Reagent Cartridge Overview

The reagent cartridge is a single-use consumable with foil-sealed reservoirs that are prefilled with clustering, sequencing, and wash reagents.

Figure 5 Reagent Cartridge

The reagent cartridge includes a designated reservoir for loading prepared libraries. After the run begins, libraries are transferred automatically from the reagent cartridge to the flow cell.

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

Store the reagent cartridge at -25°C to -15°C until use. For more information, see *Prepare the Reagent Cartridge* on page 13.

Reserved Reservoirs

Figure 6 Numbered Reservoirs

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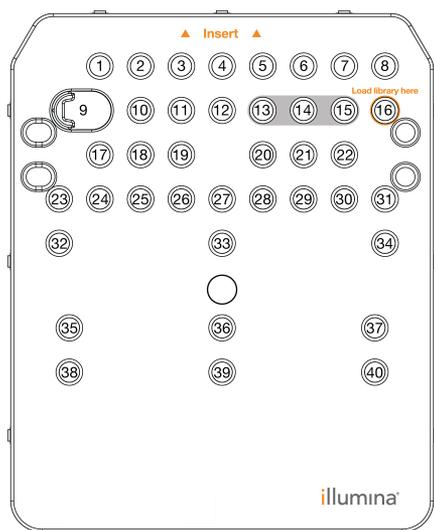
Position	Description
13, 14, and 15	Reserved for optional custom primers
16	**Load libraries**

Removable Reservoir in Position #9

The prefilled reagent cartridge includes a denaturation reagent in position #9 that contains formamide. To facilitate safe disposal of any unused reagent after the sequencing run, this reservoir is removable. For more information, see *Remove Used Reservoir from Position #9* on page 24.

Preinstalled Databases and Genomes

For most analysis methods, a reference is required to perform alignment. Several reference databases and genomes are preinstalled on the instrument computer.

Preinstalled	Description	
Databases	• miRbase for human • dbSNP for human • RefGene for human	
Genomes	• *Arabidopsis thaliana* • *Bacillus_cereus_ATCC_10987* • cow (*Bos taurus*) • *E. coli* strain DH10B • *E. coli* strain MG1655 • fruit fly (*Drosophila melanogaster*) • human (*Homo sapiens*) build hg19	• *HumanRNAFusion* • mouse (*Mus musculus*) • PhiX • rat (*Rattus norvegicus*) • *Rhodobacter sphaeroides* 2.4.1 • *Staphylococcus aureus* NCTC 8325 • yeast (*Saccharomyces cerevisiae* S288C)

Chapter 2 Getting Started

Starting the Instrument

Make sure that the instrument has been properly installed and initialized, and that instrument setup is complete. Starting the instrument before it is ready can damage the system.

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

Figure 7   Power Switch Located on Back of Instrument
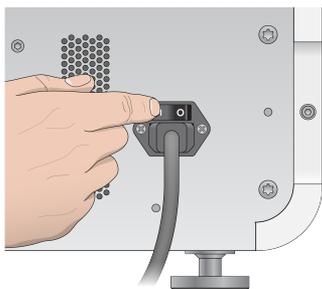
2   Press the power button above the reagent compartment.
    The power button turns on the integrated instrument computer and operating system.

Figure 8   Power Button Located on Front of Instrument
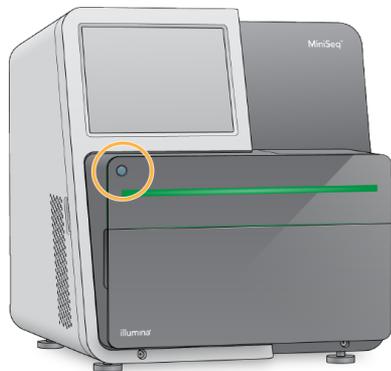
3   Wait until the operating system has finished loading.
    The Windows home screen opens after initialization. Press any key to open the Windows login window.

4   Log in to the desired Windows account. If necessary, consult your facility administrator for the user name and password.

5   If a general user account is selected, then the MiniSeq Control Software launches and initializes the system automatically. If an administrator account is selected, then the MiniSeq Control Software must be launched by double clicking the MiniSeq System icon on the desktop.

Document # 1000000002695 v04 Material # 20014309

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8

Customize System Settings

The control software includes customizable settings for instrument identification and the following workflow preferences:

▶ Using the on-screen keyboard for run setup steps.

▶ Purging consumables at end of run.

▶ Enabling audio indicators.

▶ Sending instrument performance data to Illumina.

▶ Skipping pre-run check confirmation to start the run automatically.

▶ Automatically checking for software updates (in BaseSpace Sequence Hub).

▶ Enabling customer recipes.

Customize Instrument Identification

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

2 To assign an avatar image for the instrument, select **Browse** and navigate to a preferred image.

3 In the Nick Name field, enter a preferred name for the instrument.

4 Select **Save** to save settings and advance the screen.
The image and name appear in the upper-left corner of each screen.

Set Automatic Purge Option

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

2 Select the **Purge consumables at end of run** checkbox.
This setting purges unused reagents from the reagent cartridge to the spent reagents bottle automatically after each run. If this setting is disabled, unused reagents remain in the reagent cartridge.

> **NOTE**
> Purging consumables automatically adds additional time to the workflow. For example, purging reagents after a 300-cycle run (2 × 151), takes approximately 50 minutes.

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

Set Automatic Start Option

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

2 Select the **Skip pre-run check confirmation** checkbox.
This setting starts the sequencing run automatically after a successful automatic check. If this setting is disabled, start the run manually after the pre-run check.

3 Select **Save**.

Set Automatic Check for Software Updates

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

2 Select the **Automatically check for software updates** checkbox.

Document # 1000000002695 v04 Material # 20014309

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9

An internet connection is required.

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

Set On-Screen Keyboard Option

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

2   Select the **Use on-screen keyboard** checkbox.
    This setting enables the on-screen keyboard for input during run setup steps.

3   Select **Save**.

Enable Audio Indicators

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

2   Select the **Play audio** checkbox to turn on audio indicators for the following events.
    ▶   Upon instrument initialization
    ▶   When a run is started
    ▶   When errors occur
    ▶   When user interaction is required
    ▶   When a run has finished

3   Select **Save**.

Send Instrument Performance Data to Illumina

The Send Instrument Performance Data to Illumina option is enabled by default. This sends non-run instrument performance data to a BaseSpace Sequence Hub server.

User-Supplied Consumables and Equipment

The following consumables and equipment are used for sequencing and system maintenance.

Consumables for Sequencing

Consumable	Supplier	Purpose
1 N NaOH (sodium hydroxide)	General lab supplier	Library denaturation, diluted to 0.1 N
Isopropyl alcohol wipes, 70% or Ethanol, 70%	VWR, catalog # 95041-714 (or equivalent) General lab supplier	Flow cell cleaning and general purpose
Disposable gloves, powder-free	General lab supplier	General purpose
Lab tissue, low-lint	VWR, catalog # 21905-026 (or equivalent)	Flow cell cleaning

Document # 1000000002695 v04 Material # 20014309

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10

Consumables for Maintenance and Troubleshooting

Consumable	Supplier	Purpose
NaOCl, 5% (sodium hypochlorite)	Sigma-Aldrich, catalog # 239305 (or laboratory-grade equivalent)	Performing a manual post-run wash; diluted to 0.12%
Tween 20	Sigma-Aldrich, catalog # P7949	Performing a manual instrument wash; diluted to 0.05%
Water, laboratory-grade	General lab supplier	Performing a manual instrument wash

Guidelines for Laboratory-Grade Water

Always use laboratory-grade water or deionized water to perform instrument procedures. Never use tap water. Use only the following grades of water or equivalents:

▶ Deionized water

▶ Illumina PW1

▶ 18 Megohms (MΩ) water

▶ Milli-Q water

▶ Super-Q water

▶ Molecular biology grade water

Equipment

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

Chapter 3 Sequencing

Introduction

To perform a sequencing run on the MiniSeq System, prepare run consumables and then follow the software prompts to set up the sequencing run.

Workflow Overview

Cluster Generation

During cluster generation, single DNA molecules are bound to the surface of the flow cell, and then amplified to form clusters.

Sequencing

Clusters are imaged using 2-channel sequencing chemistry and filter combinations specific to each of the fluorescently labeled chain terminators. After imaging of a tile on the flow cell is complete, the next tile is imaged. The process is repeated for each cycle of sequencing. Following image analysis, the software performs base calling, filtering, and quality scoring.

Analysis

As the run progresses, the control software automatically transfers base call (BCL) files to the specified output location for data analysis. Several analysis methods are available depending on your application and the selected analysis configuration for your system.

Sequencing Run Duration

Sequencing run duration depends on the number of cycles performed. The maximum run length is a 150-cycle paired-end run, plus up to two index reads of eight cycles each.

For expected durations and other system specifications, visit the MiniSeq System specifications page on the Illumina website.

Number of Cycles in a Read

In a sequencing run, the number of cycles performed in a read is 1 more cycle than the number of cycles analyzed. For example, to perform a 150-cycle paired-end run, set up the run to perform 151 cycles per read (2 × 151) for a total of 302 cycles. At the end of the run, 2 × 150 cycles are analyzed. The extra cycle in each read is used for phasing and prephasing calculations.

Document # 1000000002695 v04 Material # 20014309

12

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Prepare Consumables

Prepare the Reagent Cartridge

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

2 Thaw reagents using the following water bath options. Do not submerge the cartridge. When thawed, dry the base before proceeding.

Method	Time to Thaw	Stability Limit
37°C water bath	35 minutes	Up to 2 hours
Room temperature water bath (19°C to 25°C)	90 minutes	Up to 24 hours

If multiple cartridges are thawing in the same water bath, allow additional thawing time. Alternatively, thaw reagents using the following options.

Method	Time to Thaw	Stability Limit
Room temperature air (19°C to 25°C)	5 hours	Up to 24 hours
Chilled at 2°C to 8°C	18 hours	Up to 72 hours

3 Invert the cartridge 5 times to mix reagents.

4 Inspect the large reservoirs from the bottom of the cartridge to make sure that reagents have thawed and the reservoirs are free of ice crystals.

5 Gently tap on the bench to reduce air bubbles.

Prepare the Flow Cell

1 Remove a new flow cell package from 2°C to 8°C storage.

2 Set the unopened flow cell package aside at room temperature for 30 minutes.

> NOTE
> Avoid repeated cooling and warming of the flow cell.

3 Remove the flow cell container from the foil package.

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

5 Grip the flow cell by the plastic cartridge and remove it from the container.

Figure 9   Remove Flow Cell

6 Clean the glass surface of the flow cell with a lint-free alcohol wipe.

7   Dry with a lint-free lens cleaning tissue. Use care around the black flow cell gasket.

8   Inspect the flow cell ports for obstructions. Make sure that the gasket is well-seated.

## Prepare Libraries for Sequencing

Denature and Dilute Libraries

Before loading libraries onto the reagent cartridge, denature and dilute libraries and add an optional PhiX control. For more information, see *MiniSeq System Denature and Dilute Libraries Guide (document # 1000000002697)*.

The loading volume for MiniSeq System is 500 µl. The loading concentration is 1.4 pM for standard kits and 1.6 pM for rapid kits. In practice, loading concentration can vary depending on library preparation and quantification methods.

Load Libraries onto the Reagent Cartridge

1   Clean the foil seal covering reservoir **#16** labeled **Load library here** using a low-lint tissue.

2   Pierce the seal with a clean 1 ml pipette tip.

3   Add 500 µl prepared 1.4 pM or 1.6 pM libraries into reservoir **#16**. Avoid touching the foil seal as you dispense the libraries.

Figure 10   Load Libraries
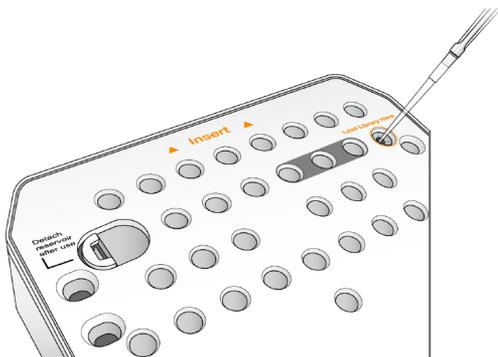
Set Up a Sequencing Run

Run setup steps differ based on system configuration:

▶   **Standalone configuration**—The system prompts users to define run parameters on the control software Run Setup screen.

▶   **Local Run Manager configuration**—Select from a list of runs predefined in Local Run Manager. If User Management is enabled under System Settings, login information is required. User Management is disabled by default.

Set Up a Run (Manual Configuration)

1   From the Home screen, select **Sequence**.
    The Sequence command releases consumables from a previous run and opens the series of run setup screens.

Document # 1000000002695 v04 Material # 20014309

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14

2　From the Run Mode screen, select **Manual**.

3　**Optional**—Select **Use BaseSpace Sequence Hub**. Select run monitoring and storage or run monitoring only. When enabled, a BaseSpace Sequence Hub login and internet connection are required.

Enter Run Parameters

1　Enter a run name of your preference.

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

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

4　Enter the number of cycles for each read in the sequencing run.
   ▷ **Read 1**—Enter a value up to 151 cycles.
   ▷ **Index 1**—Enter up to 8 cycles for the Index 1 (i7) primer.
   ▷ **Index 2**—Enter up to 8 cycles for the Index 2 (i5) primer.
   ▷ **Read 2**—Enter a value up to 151 cycles. This value is typically the same number of cycles as Read 1.
   The control software confirms the number of specified cycles using the following criteria:
   ▷ Total cycles do not exceed the maximum cycles allowed based on the reagent cartridge loaded for the run.
   ▷ Cycles for Read 1 are greater than the 6 cycles required for template generation.
   ▷ Index Read cycles do not exceed Read 1 and Read 2 cycles.

   > **NOTE**
   > Make sure that you specify the appropriate number of Index Read cycles for the libraries that you are sequencing. For more information, see the library prep documentation.

5　**[Optional]** If you are using custom primers, select the checkbox for the primers used.
   ▷ **Read 1**—Custom primer for Read 1.
   ▷ **Index 1**—Custom primer for Index 1.
   ▷ **Index 2**—Custom primer for Index 2.
   ▷ **Read 2**—Custom primer for Read 2.

6　**[Optional]** Change settings for the current run.
   ▷ **Purge consumables for this run**—Change the setting to purge consumables automatically after the current run.
   ▷ **Output folder**—Change the output folder location for the current run. Select **Browse** to navigate to a folder location.
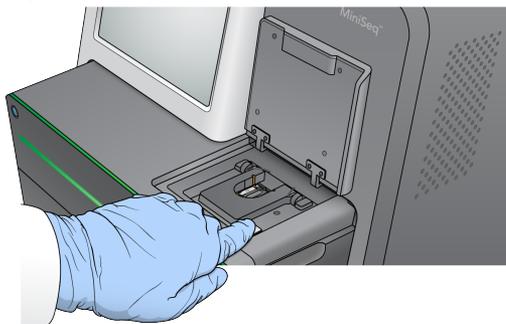
7　Select **Next**.

   > **NOTE**
   > Do not open the reagent compartment door or the flow cell compartment door during the automated check or during the sequencing run.

Load the Flow Cell

1　Open the flow cell compartment door.

2　Press the release button to the right of the flow cell latch.

Figure 11 Open Flow Cell Latch

3   If present, remove the used flow cell from a previous run.

4   Make sure that the flow cell stage is clean. If debris is present, clean the flow cell stage with an alcohol wipe.

5   Place the flow cell on the flow cell stage over the alignment pins.

Figure 12 Place Flow Cell on Stage

6   Close the flow cell latch to secure the flow cell.

Figure 13 Close Flow Cell Latch

7   Close the flow cell compartment door.

Load the Reagent Cartridge

1   Open the reagent compartment door.

2   If present, remove the used reagent cartridge.

> NOTE
> To facilitate safe disposal of unused reagent containing formamide, the reservoir in position #9 is removable. See *Remove Used Reservoir from Position #9* on page 24.

Document # 1000000002695 v04 Material # 20014309

16

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3   Slide the reagent cartridge into the reagent compartment until the cartridge stops.

Figure 14   Load Reagent Cartridge
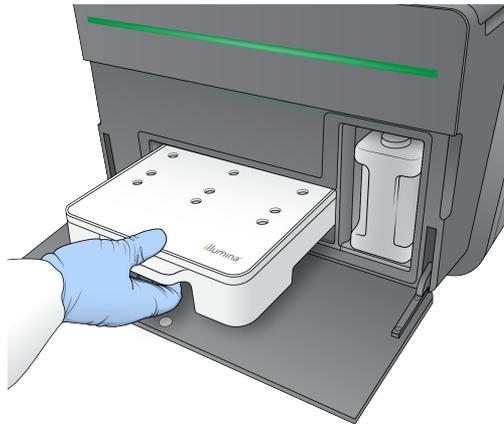
Empty the Spent Reagents Bottle
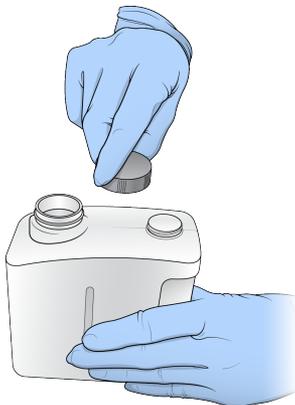
1   Remove the spent reagents bottle from the compartment.

Figure 15   Remove the Spent Reagents Bottle
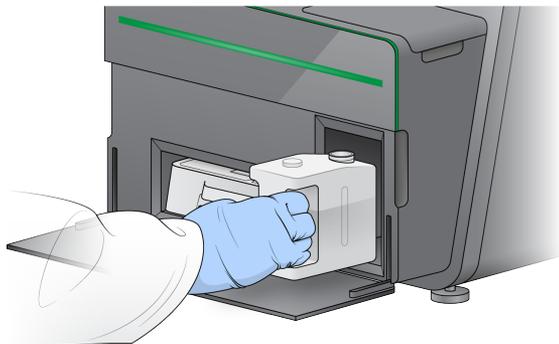
2   To prevent spills when carrying the spent reagents bottle, seal the bottle opening with the threaded cap.

Figure 16   Seal the Spent Reagents Bottle

Document # 1000000002695 v04 Material # 20014309

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17

3   Discard the contents in accordance with applicable standards.

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

4   With the threaded cap removed, slide the empty spent reagents bottle into the compartment until it stops.

5   Close the compartment door and select **Next**.

Confirm Run Parameters

1   Confirm run parameters.
    The control software confirms the number of specified cycles using the following criteria:
    ▶ Total cycles do not exceed the maximum cycles allowed based on the reagent cartridge loaded for the run.
    ▶ Cycles for Read 1 are greater than the 6 cycles required for template generation.
    ▶ Index Read cycles do not exceed Read 1 and Read 2 cycles.

> **NOTE**
>
> Make sure that you specify the appropriate number of Index Read cycles for the libraries that you are sequencing. For more information, see the library prep documentation.

2   **[Optional]** Select **Edit** to change run parameters. When finished, select **Save**.
    ▶ **Run parameters**—Change the read type or number of cycles per read.
    ▶ **Custom primers**—Change the settings for custom primers.

3   Select **Next**.

> **NOTE**
>
> Do not open the reagent compartment door or the flow cell compartment door during the automated check or during the sequencing run.

Review Pre-Run Check

1   Review the automated check results.
    ▶ To stop a check in progress, select **Cancel**.
    ▶ For any items that do not pass, an action is required before you can proceed. See *Automatic Check Errors* on page 33.
    ▶ To restart the check, select **Retry**. The check resumes at the first incomplete or failed check.

2   To start the run, select from the following options.
    ▶ If the system is not configured to start automatically after a successful check, select **Start**.
    ▶ If the system is configured to start automatically after a successful check, the sequencing run begins automatically. You do not have to be present. However, if any errors occur during the check, the run does not begin automatically.

Document # 1000000002695 v04 Material # 20014309

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

18

Set Up a Run (Local Run Manager Configuration)

1   From the Home screen, select **Sequence**.
    The Sequence command releases consumables from a previous run and opens the series of run setup screens.

2   From the Run Setup screen, select **Local Run Manager**.

3   **Optional**–Select **Use BaseSpace Sequence Hub**. Select run monitoring and storage or run monitoring only. When enabled, a BaseSpace login and internet connection are required.

Log in to Local Run Manager

1   Enter your user name and password.
    Login credentials are not required when Local Run Manager User Management is tuned off.
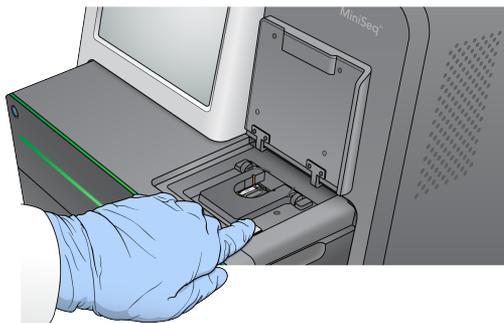
2   Select **Next**.

Select Available Run

1   Select a run name from the list of available runs.
    Use the up and down arrows to scroll through the list or enter a run name in the Search field.

2   Select **Next**.

Load the Flow Cell

1   Open the flow cell compartment door.

2   Press the release button to the right of the flow cell latch.

Figure 17 Open Flow Cell Latch

3   If present, remove the used flow cell from a previous run.

4   Make sure that the flow cell stage is clean. If debris is present, clean the flow cell stage with an alcohol wipe.

5   Place the flow cell on the flow cell stage over the alignment pins.

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19

Figure 18   Place Flow Cell on Stage
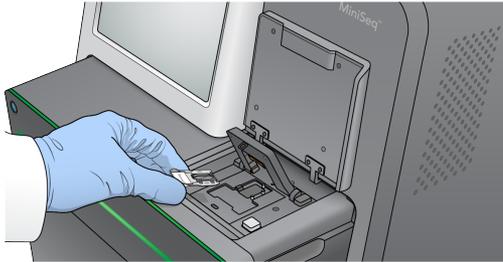
6   Close the flow cell latch to secure the flow cell.

Figure 19   Close Flow Cell Latch

7   Close the flow cell compartment door.

Load the Reagent Cartridge

1   Open the reagent compartment door.

2   If present, remove the used reagent cartridge.

> **NOTE**
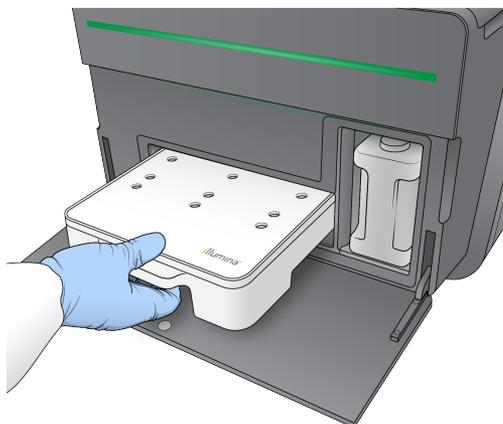> To facilitate safe disposal of unused reagent containing formamide, the reservoir in position #9 is removable. See *Remove Used Reservoir from Position #9* on page 24.

3   Slide the reagent cartridge into the reagent compartment until the cartridge stops.

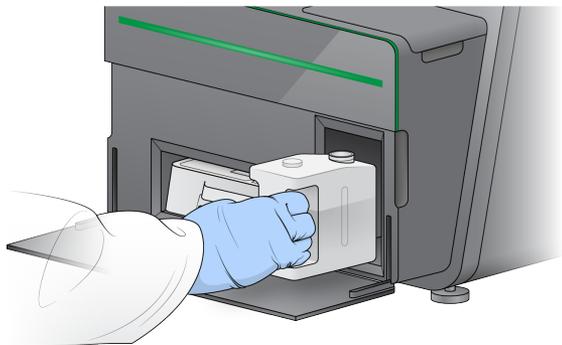Figure 20   Load Reagent Cartridge

4   From the Recipe drop-down list, select a recipe. Only compatible recipes are listed.
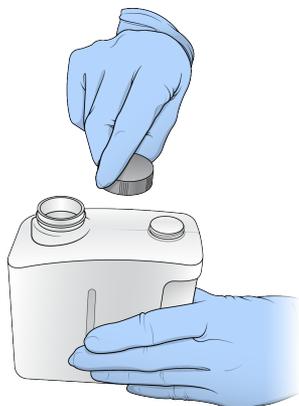
Empty the Spent Reagents Bottle

1 Remove the spent reagents bottle from the compartment.

Figure 21 Remove the Spent Reagents Bottle

2 To prevent spills when carrying the spent reagents bottle, seal the bottle opening with the threaded cap.

Figure 22 Seal the Spent Reagents Bottle

3 Discard the contents in accordance with applicable standards.

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

4 With the threaded cap removed, slide the empty spent reagents bottle into the compartment until it stops.

5 Close the compartment door and select **Next**.

Confirm Run Parameters

1 Confirm run parameters.

Document # 1000000002695 v04 Material # 20014309

21

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The control software confirms the number of specified cycles using the following criteria:

▶ Total cycles do not exceed the maximum cycles allowed based on the reagent cartridge loaded for the run.

▶ Cycles for Read 1 are greater than the 6 cycles required for template generation.

▶ Index Read cycles do not exceed Read 1 and Read 2 cycles.

> **NOTE**
> Make sure that you specify the appropriate number of Index Read cycles for the libraries that you are sequencing. For more information, see the library prep documentation.

2 **[Optional]** Select **Edit** to change run parameters. When finished, select **Save**.

▶ **Run parameters**—Change the read type or number of cycles per read.

▶ **Custom primers**—Change the settings for custom primers.

3 Select **Next**.

> **NOTE**
> Do not open the reagent compartment door or the flow cell compartment door during the automated check or during the sequencing run.

Review Pre-Run Check

1 Review the automated check results.

▶ To stop a check in progress, select **Cancel**.

▶ For any items that do not pass, an action is required before you can proceed. See *Automatic Check Errors* on page 33.

▶ To restart the check, select **Retry**. The check resumes at the first incomplete or failed check.

2 To start the run, select from the following options.

▶ If the system is not configured to start automatically after a successful check, select **Start**.

▶ If the system is configured to start automatically after a successful check, the sequencing run begins automatically. You do not have to be present. However, if any errors occur during the check, the run does not begin automatically.

Monitor Run Progress

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

Figure 23 Sequencing Run Progress and Metrics
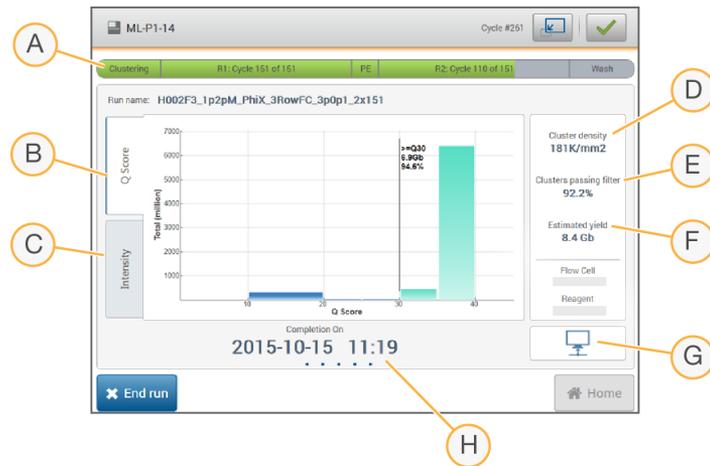
A **Run progress**—Shows the current step and number of cycles completed for each read. The progress bar is not proportional to the run rate of each step.

B **Q-Score**—Shows the distribution of quality scores (Q-scores). See *Quality Scoring* on page 45.

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

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

E **Clusters passing filter (%)**—Shows the percentage of clusters passing filter. See *Clusters Passing Filter* on page 44.

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

G **Data transfer status**—Shows the status of data transfer based on analysis configuration.

H **Time to completion**—Shows the run completion date and time (yyyy-mm-dd hh:mm).

> **NOTE**
>
> After you select Home, it is not possible to return to view run metrics. However, run metrics are accessible on BaseSpace, from a networked computer using Sequencing Analysis Viewer, or from a networked computer using Local Run Manager.

Cycles for Run Metrics

Run metrics appear at different points in a run.

▶ During the cluster generation steps, no metrics appear.

▶ The first 5 cycles are reserved for template generation.

▶ At cycle 6, raw cluster density and cycle 1 intensities are available.

▶ After cycle 25, clusters passing filter, yield, and quality scores are available.

Data Transfer Status

Depending on the analysis configuration selected, an icon appears on the screen during the run to indicate connection status.

Document # 1000000002695 v04 Material # 20014309

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23

Status	BaseSpace	Local Run Manager	Standalone
Connected			
Transferring data			
Disconnected			
Disabled			

It is possible that multiple icons appear on the screen. For example, if run data are being transferred to BaseSpace and an additional output folder location, a BaseSpace icon and a standalone icon appear.

Universal Copy Service

The MiniSeq System Software Suite includes a Universal Copy Service. As RTA generates files, the service copies files to the specified output folder location.

If data transfer is interrupted during the run, data are stored temporarily on the instrument computer. When the connection is restored, data transfer resumes automatically during the run. If the connection is not restored before the run ends, manually move data to the preferred location.

Transfer to BaseSpace

The Universal Copy Service transfers run data to BaseSpace. Data is transferred continuously from the Universal Copy Service to BaseSpace.

If you specify an additional location for run data, data are transferred to that location regardless of Universal Copy Service status.

Automatic Post-Run Wash

When the sequencing run is complete, the software initiates an automatic post-run wash using the wash solution and NaOCl provided in the reagent cartridge.

The automatic post-run wash takes approximately 60 minutes. When the wash is complete, the Home button becomes active. Sequencing results remain visible on the screen during the wash.
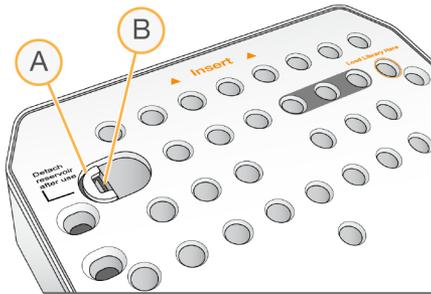
After the Wash

After the wash, the sippers remain in the down position to prevent air from entering the system. Leave the cartridge in place until the next run.

Remove Used Reservoir from Position #9

The reservoir in position #9 of the reagent cartridge contains formamide. Before discarding the used reagent cartridge, you can remove the reservoir from position #9 to discard it separately.

1   Wearing gloves, press downwards on the white breakaway tab at position #9 to break the 3 connection points.

Document # 1000000002695 v04 Material # 20014309

24

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Figure 24   Breakaway Tab at Position #9

A   Breakaway tab shown intact
B   Reservoir clip

2   Slide the breakaway tab sideways towards the left edge of the cartridge, so that the breakaway tab slides under the cartridge cover.

Figure 25   Breakaway Tab Removed, Reservoir Clip Exposed
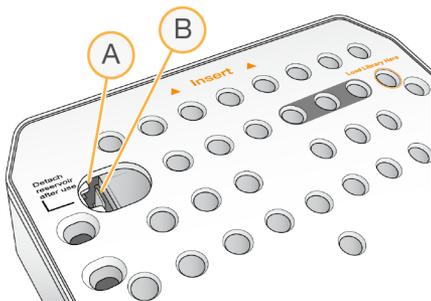
A   Breakaway tab shown under cartridge cover
B   Reservoir clip

3   Press the clear plastic reservoir clip down and towards the right.
The reservoir releases from position under the reagent cartridge.

4   Dispose of the reservoir in accordance with applicable standards.

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

Document # 1000000002695 v04 Material # 20014309

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25

Chapter 4 Maintenance

Introduction

Maintenance procedures include manual instrument washes and system software updates when available. No other periodic maintenance is required.

▶ **Instrument washes**—An automatic post-run wash after each sequencing run maintains instrument performance. However, a manual instrument wash is required under certain conditions. See *Perform a Manual Instrument Wash* on page 26.

▶ **Software updates**—When a new version of the system software suite is available, you can update the software automatically through a connection to BaseSpace or manually after downloading the installer from the Illumina website. See *Software Updates* on page 29.

Preventive Maintenance

Illumina recommends that you schedule a preventive maintenance service each year. If you are not under a service contract, contact your Territory Account Manager or Illumina Technical Support to arrange for a billable preventive maintenance service.

Perform a Manual Instrument Wash

Manual instrument wash options include the Quick Wash and the Manual Post-Run Wash.

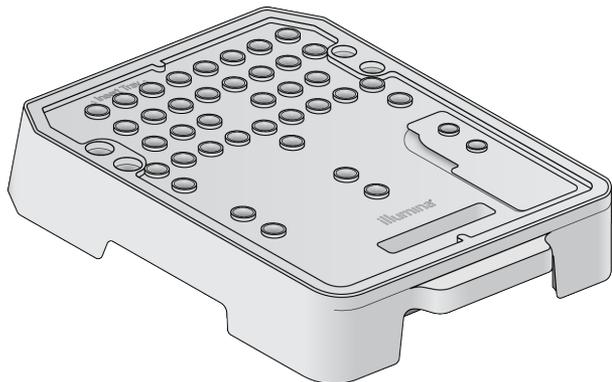| Wash Types	Description
Quick Wash Duration: 20 minutes	A quick wash is required every 7 days that the instrument is idle or after an instrument shutdown. The wash flushes the system with a user-supplied wash solution of laboratory-grade water and Tween 20.
Manual Post-Run Wash Duration: 90 minutes	A manual post-run wash is required when the automatic post-run wash is not performed, such as when a run is ended early and the flow cell is saved for later rehybridization. The wash flushes the system with user-supplied 0.12% sodium hypochlorite and a wash solution of laboratory-grade water and Tween 20.

> **NOTE**
>
> Always use a fresh dilution of NaOCl prepared within the last **24 hours**. If you make a volume larger than 1 ml, store the remaining dilution at 2°C to 8°C for use within the next 24 hours. Otherwise, discard the remaining dilution of NaOCl.

A manual instrument wash requires the wash cartridge and a wash flow cell provided with the instrument. Alternatively, you can use a used flow cell for the wash.

Document # 1000000002695 v04 Material # 20014309

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26

Figure 26   Wash Cartridge

Prepare for a Manual Post-Run Wash

1   Combine the following volumes to result in 0.12% NaOCl.
   ▶   5% NaOCl (31 µl)
   ▶   Laboratory-grade water (1269 µl)

2   Add 1.3 ml of 0.12% NaOCl to the wash cartridge.
   The correct reservoir is equivalent to position **#31** on the prefilled reagent cartridge.

Figure 27   Positions for NaOCl and Wash Solution
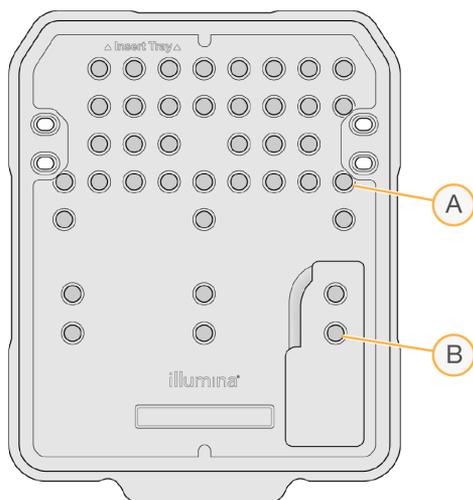
   A   0.12% NaOCl
   B   Wash solution

3   Combine the following volumes to result in a 0.05% Tween 20 wash solution.
   ▶   100% Tween 20 (40 µl)
   ▶   Laboratory-grade water (80 ml)

4   Add 80 ml wash solution to the wash cartridge.
   The correct reservoir is equivalent to position **#40** on the prefilled reagent cartridge.

5   From the Home screen, select **Perform Wash**, and then select **Manual post-run wash**.

Document # 1000000002695 v04 Material # 20014309

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27

Prepare for a Quick Wash

1   Combine the following volumes to result in a 0.05% Tween 20 wash solution.
    ▶ 100% Tween 20 (20 µl)
    ▶ Laboratory-grade water (40 ml)

2   Add 40 ml wash solution to the wash cartridge.
    The correct reservoir is equivalent to position **#40** on the prefilled reagent cartridge.

Figure 28   Position for Wash Solution
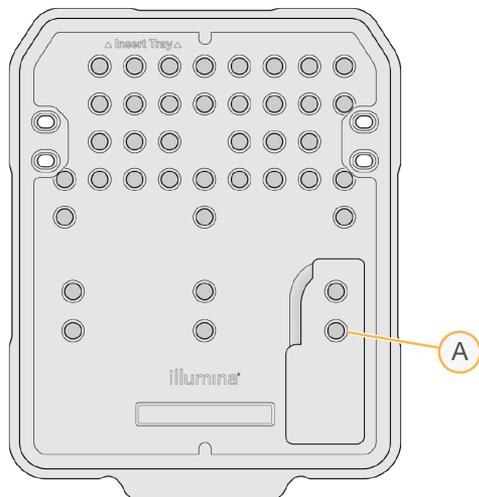
A   Wash solution

3   From the Home screen, select **Perform wash**, and then select **Quick Wash**.

Load the Wash Flow Cell and Wash Cartridge

1   Load the wash flow cell. Close the flow cell clamp and flow cell door.

> **NOTE**
> Alternatively, you can load a used flow cell.

2   Remove the used reagent cartridge from the previous run, if present.

3   Load the prepared wash cartridge.

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

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

5   Slide the empty spent reagents bottle into the compartment until it stops.

6   Close the reagent compartment door.

7 Select **Next**.

Start the Wash

1 After the check is complete, select **Start**.

2 When the wash is complete, select **Home**.

After the Wash

After the wash, the sippers remain in the down position to prevent air from entering the system. Leave the cartridge in place until the next run.

Software Updates

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

▶ MiniSeq Control Software

▶ MiniSeq recipes

▶ RTA2

▶ Local Run Manager

▶ MiniSeq Service Software

▶ Universal Copy Service

Software release notes are available on the MiniSeq System support page on the Illumina website.

You can install software updates automatically using an internet connection or manually from a network or USB location.

▶ **Automatic updates**—For instruments connected to a network with internet access, an alert 🛑 icon appears on the Manage Instrument button on the Home screen when an update is available.

▶ **Manual updates**—Download the System Suite installer from the MiniSeq System support page on the Illumina website.

> NOTE
> Cancelling an update before installation is complete stops the update at the current point in the installation. Any changes made up to the point of cancellation are not uninstalled or reverted to the previous version.

Automatic Software Update

1 Select **Manage Instrument**.

2 Select **Software Update**.

3 Select **Install the update already downloaded from BaseSpace**.

4 Select **Update** to begin the update. A dialog box opens to confirm the command.

5 Follow the prompts in the installation wizard:

   a Accept the licensing agreement.
   b Review the list of software included in the update.

Document # 1000000002695 v04 Material # 20014309

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29

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

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

Manual Software Update

1   Download the System Suite installer from the Illumina website and save it to a whitelisted network location. Illumina recommends D:\Illumina or C:\Illumina. Alternatively, copy the software installation file to a portable USB drive.

2   Select **Manage Instrument**.

3   Select **Software Update**.

4   Select **Manually install the update from the following location**.

5   Select **Browse** to navigate to the location of the software installation file, and then select **Update**.

6   Follow the prompts in the installation wizard:

a   Accept the licensing agreement.
b   Review the list of software included in the update.

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

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

Rapid Kit Software Requirements

The use of rapid kits with the MiniSeq System requires MiniSeq Control Software v2.1 or higher. An Administrator is required to perform the update from v2.0 to v2.1. Windows 10 is required for installation of MiniSeq Control Software v2.0 or higher.

Document # 1000000002695 v04 Material # 20014309

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30

31

Appendix A Troubleshooting

Troubleshooting Files

Key File	Folder	Description
Run information file (RunInfo.xml)	Root folder	Contains the following information: • Run name • Number of cycles in the run • Number of cycles in each read • Whether the read is an indexed read • Number of swaths and tiles on the flow cell
Run parameters file (RunParameters.xml)	Root folder	Contains information about run parameters and run components. Information includes the RFID, serial number, lot number, and expiration date.
RTA configuration file (RTAConfiguration.xml)	Data\Intensities	Contains the RTA configuration settings for the run. The RTAConfiguration.xml file is created at the beginning of the run.
InterOp files (*.bin)	InterOp	Binary reporting files used for Sequencing Analysis Viewer. InterOp files are updated throughout the run.
Log files	Logs	Log files describe each step performed by the instrument for each cycle, and list software and firmware versions used with the run. The file named **[InstrumentName]_CurrentHardware.csv** lists the serial numbers of instrument components.
Error log files (*ErrorLog*.txt)	RTA Logs	Log of RTA errors. Error log files are updated whenever an error occurs.
Global log files (*GlobalLog*.tsv)	RTA Logs	Log of all RTA events. Global log files are updated throughout the run.
Lane log files (*LaneLog*.txt)	RTA Logs	Log RTA processing events. Lane log files are updated throughout the run.

Troubleshooting Resources

For technical questions, visit the MiniSeq System support pages on the Illumina website. The support pages provide access to documentation, downloads, and frequently asked questions.

Log in to your MyIllumina account for access to support bulletins.

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

Consider sharing a link to the run summary in BaseSpace with Illumina Technical Support to facilitate troubleshooting.

Document # 1000000002695 v04 Material # 20014309

32

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Process Status

The MiniSeq Control Software lists the status of at least 3 runs in the system Temp folder. From the Manage Instrument screen, select **Process status**.

For each run name, the system lists the status of the following components:

▶ **Real-Time Analysis (RTA)**—Based on the processing of BCL files

▶ **Local Run Manager**—If Local Run Manager was used for the run

▶ **File Copy**—Based on file transfer using the Run Copy Service

▶ **BaseSpace**—If BaseSpace was used for the run

Sequencing Archive Folder

The MiniSeq Control Software saves run summary files on the system computer at **D:\IIIumina\MiniSeq** Sequencing Archive for each run performed on the instrument.

In this folder, a subfolder exists for each run performed on the instrument contains the following files:

▶ **RunCompletionStatus.xml**—Contains the completion status, run folder name, number of cycles planned and performed, cluster density, clusters passing filter, and estimated yield for the run.

▶ **RunParameters.xml**—Contains information about run parameters and run components. Information includes the RFID, serial number, lot number, and expiration date.

Automatic Check Errors

If errors occur during the automatic pre-run check, use the following recommended actions to resolve the error.

If a pre-run check fails, the reagent cartridge RFID is not locked and can be used for a subsequent run. However, the RFID is locked after the foil seals have been pierced.

System Checks	Recommended Action
Doors closed	Make sure that the compartment doors are closed.
Consumables loaded	Consumable sensors do not register. Make sure that each consumable is properly loaded. On the run setup screens, select **Back** to return to the loading step, and repeat run setup.
Required software	Critical components of the software are missing. Perform a manual software update to restore all software components.
Instrument disk space	The instrument hard drive does not have sufficient disk space to perform a run. Clear run data from the instrument hard drive.
Network connection	The connection to the specified location of the output folder has been interrupted. Although the check is labeled network connection, the system checks the connection to any specified location of the output folder on a server, an external hard drive, or the local drive. Check the status of the connection to the specified output folder location.
Network disk space	The specified location of the output folder is full. Although the check is labeled network disk space, the system checks any specified location of the output folder on a server, an external hard drive, or the local hard drive. Clear disk space from the specified output folder location.

Temperature	Recommended Action
Temperature ramp	Contact Illumina Technical Support.
Temperature sensors	Contact Illumina Technical Support.
Fans	Contact Illumina Technical Support.

Imaging System	Recommended Action
Imaging limits	Contact Illumina Technical Support.
Z step-and-settle	Contact Illumina Technical Support.
Bit error rate	Contact Illumina Technical Support.
Flow cell registration	It is possible that the flow cell is not properly seated. • On the run setup screens, select **Back** to return to the flow cell step. • Unload and reload the flow cell to make sure that it is seated properly.

Reagent Delivery	Recommended Action
Valve response	Contact Illumina Technical Support.
Pump	Contact Illumina Technical Support.

Hard Drive Space

The instrument computer hard drive can hold approximately 45 runs based on data generated from a run using the following run parameters:

▶ Approximately 5–6 GB of space is required for 150-cycle paired-end run.

▶ An additional 10 GB of space is required for analysis files when using the Local Run Manager Resequencing analysis module.

For each run performed, a temporary run folder is created as part of the software operation. As files are written to the temporary run folder, the files are copied to the output folder. Therefore, if you specify an output folder location on the instrument hard drive, 2 copies of that run are written to the hard drive. The software saves the most recent 3 temporary run folders.

When using the Local Run Manager software for analysis, temporary files are not deleted, by default. The retention policy is set manually from the Local Run Manager System Settings screen.

Eventually, temporary files can fill the hard drive space. Consider using a network location for run data and setting a reasonable Local Run Manager retention policy based on the number of runs that you perform.

RTA Errors

To troubleshoot RTA errors, first check the RTA error log, which is stored in the RTALogs folder. This file is not present for successful runs. Include the error log when reporting issues to Illumina Technical Support.

Error Handling

RTA2 creates log files and writes them to the RTALogs folder. Errors are recorded in an error file in *.tsv file format.

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

▶ *GlobalLog*.tsv summarizes important run events.

▶ *LaneNLog*.tsv lists processing events. N is always 1 on a MiniSeq flow cell.

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

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

Rehybridization Workflow

A rehybridization run might be necessary if metrics generated during the first few cycles show intensities below 2500. Some low-diversity libraries can show intensities below 1000, which is expected and cannot be resolved with rehybridization.

> **NOTE**
> The End Run command is final. The run cannot be resumed, run consumables cannot be reused, and sequencing data from the run are not saved.

When you end a run and save the flow cell, the software performs the following steps before the run ends:

▶ Places the flow cell in a safe state.

▶ Unlocks the flow cell RFID for a later run.

▶ Assigns a rehybridization expiration date to the flow cell.

▶ Writes the run logs for completed cycles. A delay is normal.

▶ Bypasses the automatic post-run wash.

When you start a rehybridization run, the software performs the following steps to perform the run:

▶ Creates a run folder based on a unique run name.

▶ Checks that the flow cell rehybridization date has not expired.

▶ Primes reagents. A delay is normal.

▶ Skips the clustering step.

▶ Removes the previous Read 1 primer.

▶ Hybridizes a fresh Read 1 primer.

▶ Continues through Read 1 and the remainder of the run based on specified run parameters.

Points to End a Run for Rehybridization

Later rehybridization is possible only if you end the run at the following points:

▶ **After cycle 5**—Intensities appear after template registration, which requires the first 5 cycles of sequencing. Although it is safe to end a run after cycle 1, ending after cycle 5 is recommended. Do not end a run during cluster generation.

▶ **Read 1 or Index 1 Read**—End the run *before* paired-end resynthesis begins. The flow cell cannot be saved for later rehybridization after paired-end resynthesis begins.

Required Consumables

A rehybridization run requires a new MiniSeq reagent cartridge regardless of when the run was stopped.

End the Current Run

1   Select **End Run**. When prompted to confirm the command, select **Yes**.

Document # 1000000002695 v04 Material # 20014309

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35

2   When prompted to save the flow cell, select **Yes**. Note the expiration date for rehybridization.

3   Remove the saved flow cell and set aside at 2°C to 8°C until you are ready to set up the rehybridization run.

> **NOTE**
> You can store the flow cell up to 7 days at 2°C to 8°C in the flow cell container with the cap closed. For best results, rehybridize the saved flow cell within 3 days.

Perform a Manual Wash

1   From the Home screen, select **Perform Wash**.

2   From the Wash Selection screen, select **Manual Post-Run Wash**. See *Perform a Manual Instrument Wash* on page 26.

> **NOTE**
> If you have not yet removed the reagent cartridge from the stopped run, you can use them for the manual wash. Otherwise, perform the manual wash with the wash cartridge.

Set Up a Run on the Instrument

1   Prepare a new reagent cartridge.

2   If the saved flow cell was stored, allow it to reach room temperature (15-30 minutes).

3   Clean and load the saved flow cell.
    The system reads the flow cell RFID as a saved flow cell and confirms a valid rehybridization date.

4   Remove the spent reagents bottle and discard the contents appropriately, and then reload the empty container.

5   Load the new reagent cartridge.

6   From the Run Setup screen, select from the following options:
    ▶ **Local Run Manager configuration**—Select the run and confirm run parameters.
    ▶ **Manual configuration**—Enter the name of the run and specify the same parameters as the original run.

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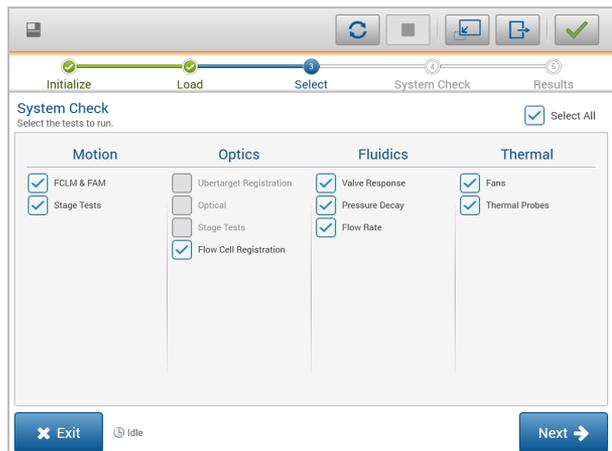
System Check

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

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

Starting a system check automatically closes the control software and launches the MiniSeq Service Software. The service software launches and opens to the Load screen, which is configured to use the advanced loading option.

Document # 1000000002695 v04 Material # 20014309

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36

Figure 29   Available System Checks

After loading consumables, the Select screen opens, which lists available system checks. Inactive checkboxes on the Select screen indicate tests that require assistance from an Illumina field representative.

Perform a System Check

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

2   Add 40 ml deionized water to the wash cartridge.
The correct reservoir is equivalent to position **#40** on the prefilled reagent cartridge.

Figure 30   Position for Wash Solution
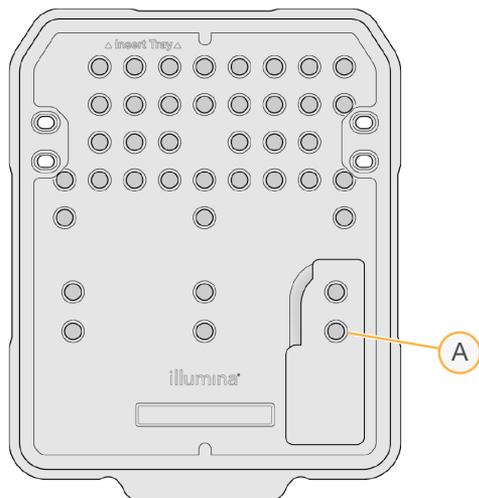
A   Wash solution

3   Load the consumables as follows:

a   If a used flow cell is not already on the instrument, load a used flow cell.
b   Empty the spent reagents bottle and return it to the instrument.

    c   Load the wash cartridge.

4   Select **Load**.
The software moves the flow cell and wash cartridge into position.

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

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

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

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

9   When finished, select **Exit**.

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

Motion Checks

System Check	Description
FCLM & FAM	Checks the gain and distance of the Flow Cell Load Mechanism (FCLM) and Fluidics Automation Module (FAM) to confirm that the modules are working properly.
Stage Tests	Checks the travel limits and performance of the XY-stage and Z-stage.

Optics Check

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

Fluidics Checks

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

Thermal Checks

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

Network Configuration Settings

Network settings are configured during installation. If the system has to be reconfigured, you can change settings or reset settings on the Network Configuration screen. Configuration settings include the IP address, domain name server (DNS) address, and domain name.

> **NOTE**
> You must be logged in as a Windows OS Administrator user to change these settings.

Set Network Configuration

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

2 Select **Network configuration**.

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

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

Alternatively, select **Use the following IP address** to connect the instrument to another server manually as follows. Contact your network administrator for the addresses specific to your facility.
  ▶ Enter IP address. The IP address is a series of 4 numbers separated by a dot, similar to 168.62.20.37, for example.
  ▶ Enter the subnet mask, which is a subdivision of the IP network.
  ▶ Enter the default gateway, which is the router on the network that connects to the internet.

4 Select **Obtain a DNS server address automatically** to connect the instrument to the domain name server associated with IP address.
Alternatively, select **Use the following DNS server addresses** to connect the instrument to the domain name server manually as follows.
  ▶ Enter the preferred DNS address. The DNS address is the server name used to translate domain names into IP addresses.
  ▶ Enter the alternate DNS address. The alternate is used if the preferred DNS cannot translate a particular domain name to an IP address.

5 Select **Save**.

Configure Computer Domain

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

1 Connect the instrument computer to a domain or a workgroup as follows.
  ▶ **For instruments connected to the internet**—Select **Member of domain**, and then enter domain name associated with the internet connection at your facility.

> **NOTE**
> Domain changes require an administrator user name and password.

Document # 1000000002695 v04 Material # 20014309

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39

 ▶ **For instruments not connected to the internet**–Select **Member of work group**, and then enter a work group name. The work group name is unique to your facility.

2 Select **Save**.

Custom Genomes

You can upload your own reference in FASTA format to the instrument computer. You can upload several single FASTA files *or* a single multi-FASTA file (recommended), but not a combination of both.

To troubleshoot issues with a custom genome file, confirm the following requirements.

1 Make sure that the file uses a *.fa or *.fasta extension and that it is stored in a dedicated folder for the reference.

2 Make sure that the chromosome name does not contain any of following characters:

```
# – ? ( ) [ ] / \ = + < > : ; " ' , * ^ | &
```

For best results, use only alpha-numeric characters as chromosome names.

Shut Down the Instrument

Under normal conditions, there is no reason to shut down the instrument.

1 Select **Manage Instrument**.

2 Select **Shutdown options**.

3 Select **Shut down**.
The command safely shuts down the software and turns off instrument power. Wait at least 60 seconds before turning on the instrument again. A wash is required before the next sequencing run.

CAUTION

Do not relocate the instrument. Moving the instrument improperly can affect the optical alignment and compromise data integrity. If you have to relocate the instrument, contact your Illumina representative.

Document # 1000000002695 v04 Material # 20014309

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40

Appendix B Real-Time Analysis

Real-Time Analysis Overview 42
Input and Output Files 42
Real-Time Analysis Workflow 43

Real-Time Analysis Overview

Real-Time Analysis is a software that runs on the instrument computer, extracts intensities from images to perform base calling, and then assigns a quality score to the base call.

The MiniSeq System uses an implementation of Real-Time Analysis called RTA2. The system control software and RTA2 communicate through a web HTTP interface and shared memory files. If RTA2 is terminated, processing does not resume and run data are not saved.

Input and Output Files

Input Files

Real-Time Analysis software requires the following input for processing:

▶ Tile images contained in local system memory.

▶ **RunInfo.xml**, which is generated automatically at the beginning of the run and provides the run name, number of cycles, whether a read is indexed, and the number of tiles on the flow cell.

Real-Time Analysis software receives commands from the control software about the location of **RunInfo.xml** and whether an optional output folder is specified.

Output Files

Images for each channel are passed in memory as tiles. Tiles are small imaging areas on the flow cell defined as the field of view by the camera. From these images, the software produces output as a set of quality-scored base call files and filter files. Output files are used for downstream analysis on BaseSpace or by Local Run Manager analysis modules.

File Type	Description
Base call files	Each tile that is analyzed is included in an aggregated base call (*.bcl) file for each lane and for each cycle. The aggregated base call file contains the base call and associated quality score for every cluster in that lane.
Filter files	Each tile produces filter information that is aggregated into 1 filter (*.filter) file for each lane. The filter file specifies whether a cluster passes filters.
Cluster location files	Cluster location (*.locs) files contain the X,Y coordinates for every cluster in a tile. A cluster location file is generated for each lane during template generation.
Base call index files	A base call index (*.bci) file is produced for each lane to preserve the original tile information. The index file contains a pair of values for each tile, which are tile number and number of clusters for that tile.

RTA2 provides real-time metrics of run quality stored as InterOp files. InterOp files are a binary output containing tile, cycle, and read-level metrics, and are used to view real-time metrics using the Sequencing Analysis Viewer software.

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42

Real-Time Analysis Workflow

Template generation ↓	Maps cluster locations.
Registration and intensity extraction ↓	Records the location of each cluster on the flow cell and determines an intensity value for each cluster.
Phasing correction ↓	Corrects the effects of phasing and prephasing.
Base calling ↓	Determines a base call for every cluster.
Quality scoring	Assigns a quality score to every base call.

Template Generation

The first step in the RTA workflow is template generation, which defines the position of each cluster in a tile using X and Y coordinates.

Template generation requires image data from the first 5 cycles of the run. After the last template cycle for a tile is imaged, the template is generated.

> **NOTE**
> To detect a cluster during template generation, there must be at least 1 base other than G in the first **5** cycles.

The template is used as a reference for the subsequent step of registration and intensity extraction. Cluster positions for the entire flow cell are written to cluster location (*.locs) files, 1 file for each lane.

Registration and Intensity Extraction

Registration and intensity extraction begin after template generation.

▶ Registration aligns images produced over every subsequent cycle of imaging against the template.

▶ Intensity extraction determines an intensity value for each cluster in the template for a given image.

If registration fails for any images in a cycle, no intensities are extracted and all bases are called as N for that tile in that cycle. Use the Sequencing Analysis Viewer software to identify tiles and cycles that have failed registration. Registration failures are easily identified as tiles and cycles that have 0 in the P90 column on the Imaging tab.

Phasing Correction

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

▶ Phasing occurs when a base falls behind.

▶ Prephasing occurs when a base jumps ahead.

Document # 1000000002695 v04 Material # 20014309

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43

Figure 31 Phasing and Prephasing
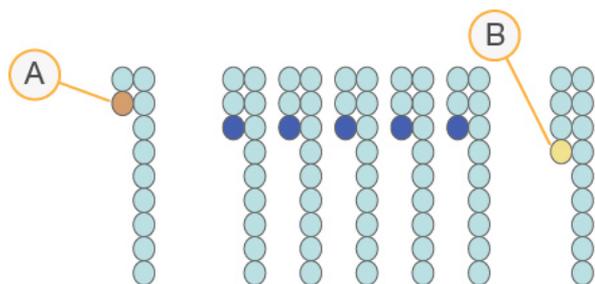
A   Read with a base that is phasing
B   Read with a base that is prephasing

RTA2 corrects the effects of phasing and prephasing, which maximizes the data quality at every cycle throughout the run.

Base Calling

Base calling determines a base (A, C, G, or T) for every cluster of a given tile at a specific cycle. The MiniSeq System uses 2-channel sequencing, which requires only 2 images to encode the data for 4 DNA bases, 1 from the red channel and 1 from the green channel.

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

Figure 32 Visualization of Cluster Intensities
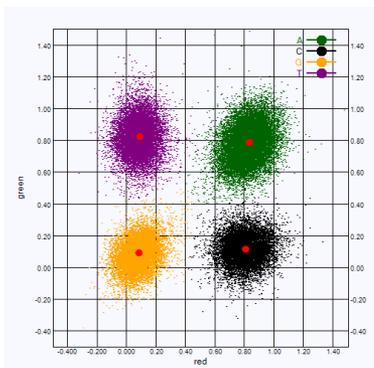
Table 1 Base Calls in 2-Channel Sequencing

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

Clusters Passing Filter

During the run, RTA2 filters raw data to remove clusters that do not meet the data quality threshold.

For 2-channel analysis, RTA2 uses a population-based system to determine the chastity of a base call. Clusters pass filter (PF) when no more than 1 base call in the first 25 cycles has an unacceptable chastity value. Clusters that do not pass filter are not base called in future cycles.

Indexing Considerations

The process for base calling index reads differs from base calling during other reads.

Index reads must begin with at least one base other than G in either of the first two cycles. If an Index Read begins with two base calls of G, no signal intensity is generated. Signal must be present in either of the first two cycles to ensure demultiplexing performance.

To increase demultiplexing robustness, select index sequences that provide signal in at least one channel, preferably both channels, for every cycle. Following this guideline avoids index combinations that result in only G bases at any cycle.

▶ Red channel—A or C

▶ Green channel—A or T

This base calling process ensures accuracy when analyzing low-plex samples.

Quality Scoring

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

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

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

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

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

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

Document # 1000000002695 v04 Material # 20014309

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45

Appendix C Output Files

Output Files

Sequencing Output Files

File Type	File Description, Location, and Name
Base call files	Each tile analyzed is included in a base call file, aggregated in a file for each cycle. The aggregated file contains the base call and encoded quality score for every cluster. Data\Intensities\BaseCalls\L001 [Cycle].bcl.bgzf, where [Cycle] represents the cycle number in 4 digits. Base call files are compressed using block gzip compression.
Base call index file	A binary index file lists the original tile information in a pair of values for each tile, which are tile number and number of clusters for the tile. Base call index files are created the first time a base call file is created. Data\Intensities\BaseCalls\L001 s_[Lane].bci
Cluster location files	For each tile, the XY coordinates for every cluster are aggregated into a cluster location file. Cluster location files are the result of template generation. Data\Intensities\L001 s_[lane].locs
Filter files	The filter file specifies whether a cluster passed filters. Filter information is aggregated into a filter file for each read. Filter files are generated at cycle 26 using 25 cycles of data. Data\Intensities\BaseCalls\L001 s_[lane].filter
InterOp files	Binary reporting files used for Sequencing Analysis Viewer. InterOp files are updated throughout the run. InterOp folder
RTA configuration file	Created at the beginning of the run, the RTA configuration file lists settings for the run. [Root folder], RTAConfiguration.xml
Run information file	Lists the run name, number of cycles in each read, whether the read is an Index Read, and the number of swaths and tiles on the flow cell. The run info file is created at the beginning of the run. [Root folder], RunInfo.xml

Sequencing Output Folder Structure

The control software generates the output folder name automatically.

📁 Configs

📁 Data

    📁 Intensities

       📁 BaseCalls

          📁 L001—Base call files, aggregated per cycle

       📁 L001—An aggregated *.locs file

Document # 1000000002695 v04 Material # 20014309

46

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📁 **Images**
    📁 **Focus**
        📁**L001**–Focus images
📁 **InstrumentAnalyticsLogs**–Log files describing instrument analytics steps
📁 **InterOp**–Binary files used by Sequencing Analysis Viewer
📁 **Logs**–Log files describing operational steps
📁 **Recipe**–Run-specific recipe file named with reagent cartridge ID
📁 **RTALogs**–Log files describing analysis steps
📄 RTAComplete.xml
📄 RTAConfiguration.xml
📄 RunInfo.xml
📄 RunNotes.xml
📄 RunParameters.xml

Analysis Input File Requirements

Local Run Manager requires the following files generated during the sequencing run to perform analysis or to requeue analysis. Some analysis modules require additional input files to perform analysis. For more information, see the workflow guide for the analysis module you are using.

File Name / Type	Description
RTAComplete.txt	A marker file that indicates RTA processing is complete. The presence of this file triggers Local Run Manager to queue analysis.
RunInfo.xml	Contains high-level run information, such as the number of reads and cycles in the sequencing run, and whether a read is indexed.
Base call files (*.bcl)	Contains the base call and encoded quality score for every cluster for each tile, aggregated in a file for each cycle.
Filter files (*.filter)	Specifies whether a cluster passed filters. Filter information is aggregated into a filter file for each read.
Cluster location files (*.locs)	Contains the XY coordinates for every cluster for each tile, aggregated into a cluster location file

Index

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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Email: techsupport@illumina.com

Illumina Customer Support Telephone Numbers

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China	400.066.5835	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
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Germany	+49 8001014940	+49 8938035677
Hong Kong, China	800960230	
Ireland	+353 1800936608	+353 016950506
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New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
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Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
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Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

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

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