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Genome Analyzer *IIx* User Guide
(SCS v2.10)

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Revision History

NOTE
For information about performing runs on the Genome Analyzer using SCS v2.9, see the *Genome Analyzer IIx User Guide (Using SCS v2.9)*, Part # 15018814.

Part #	Revision	Date	Description of Change
15030966	C	July 2012	Added the following information: • List of supported library types for dual-indexed sequencing • Sequencing primers requirements for TruSeq HT libraries Updated the following information: • Listed primer requirements for TruSeq HT libraries and Nextera libraries • Replaced run duration specifications with a link to the Illumina website, GAIIx specifications page
15030966	B	April 2012	Added the following information: • Description of dual-indexed libraries • Overviews of single-indexed and dual-indexed sequencing workflows Updated the following information: • Corrected PE pre-run wash volume to 17 ml • Increased number of required SBS kits for a 36-cycle single-indexed run from two to three kits • Added the paired-end priming volume to expected delivery for Read 2 resynthesis. • Noted that a dual-indexed single-read run on paired-end flow cell must use the dual-indexed paired-end reagents and recipe. • Changed references to Nextera libraries to dual-indexed libraries.
15030966	A	December 2011	Initial release. Supports SCS v2.10, and v8.5 and v10 recipes. • Includes workflows for non-indexed, single-indexed, and dual-indexed sequencing. • Describes the dual-indexed sequencing of Illumina Nextera™ DNA libraries using sequencing primers provided in the TruSeq™ Dual Index Sequencing Primer Box.

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Overview

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Introduction

NOTE
This guide describes how to perform runs on the Genome Analyzer using Sequencing Control Software (SCS) v2.10. For information about performing runs on the Genome Analyzer using SCS v2.9, see the *Genome Analyzer IIx User Guide (Using SCS v2.9)*, Part # 15018814.

The Genome Analyzer System is a groundbreaking platform for sequence analysis and functional genomics. Dramatically improving speed and reducing costs, it is suitable for a range of applications including whole-genome and candidate-region sequencing, gene expression discovery and profiling, DNA-protein interaction profiling, and small RNA identification and quantitation.

The Genome Analyzer System uses a straightforward and flexible process, and consists of four main steps:

1. **Sample preparation**—Performed using the Illumina sample preparation kit for your specific application.
2. **Cluster generation**—Performed on the cBot.
3. **Sequencing**—Performed on the Genome Analyzer.
4. **Data analysis**—Data analysis starts on-instrument with Real Time Analysis (RTA) software, which is integrated with the Genome Analyzer Sequencing Control Software (SCS). Further data analysis is performed off-instrument with Illumina Consensus Assessment of Sequence and Variation (CASAVA) software.

Audience and Purpose

This guide contains information about the Genome Analyzer. It provides an overview of instrument components and software features, and full instructions for performing a sequencing run.

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

- Operating the instrument
- Performing sequencing runs
- Performing instrument and component maintenance
- Training personnel on the instrument

Genome Analyzer Overview

The Genome Analyzer sequences clustered template DNA using a robust four-color sequencing by synthesis (SBS) technology that employs reversible terminators with removable fluorescence. This approach provides a high degree of sequencing accuracy even through homopolymeric regions.

High sensitivity fluorescence detection is achieved using laser excitation and internal reflection optics. Sequence reads are aligned against a reference genome and genetic differences are called using specially developed data analysis software.

The instrument software performs real time data analysis, which saves downstream analysis time and allows you to monitor important quality metrics during the sequencing run.

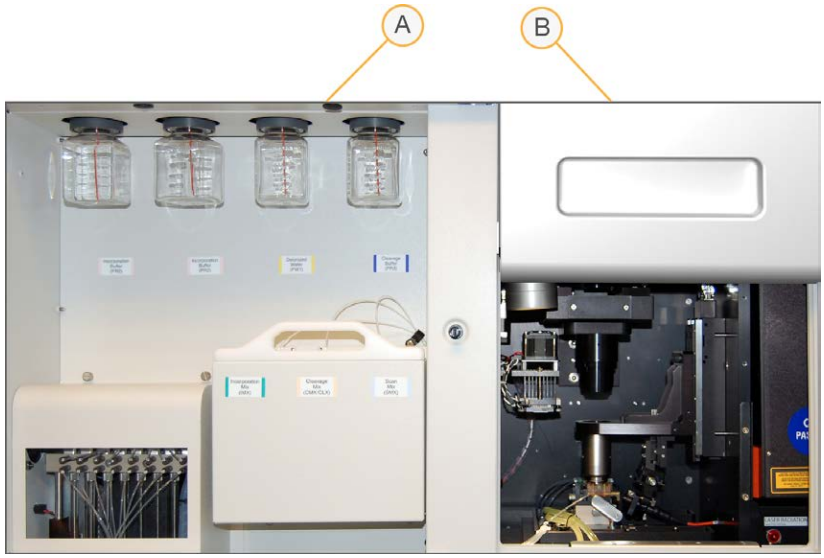
Figure 1 Genome Analyzer IIx

Genome Analyzer Components

All operator activity on the instrument occurs in two main compartments:

- Reagent Compartment
- Imaging Compartment

Figure 2 Main Compartments



A Reagent Compartment
B Imaging Compartment

Reagent Compartment

The reagent compartment holds active reagents, buffers, and wash solutions.

Figure 3 Reagent Compartment

A Fluidics Pump
B Room Temperature Reagents (Positions 4, 5, 2, and 7)
C Chilled Reagents (Positions 1, 6, and 3)

The fluidics pump directs waste to the Genome Analyzer waste bottle.

Reagent bottles in positions 4, 5, 2, and 7 are connected to threaded bottle receptacles on the instrument. Each reagent position is numbered according to the position it connects to on the fluidics pump.

Reagent bottles in positions 1, 6, and 3 are connected to fluidics lines in the reagent chiller. The reagent chiller maintains reagent temperature at 4°C. It is not intended to cool down reagents.

Imaging Compartment

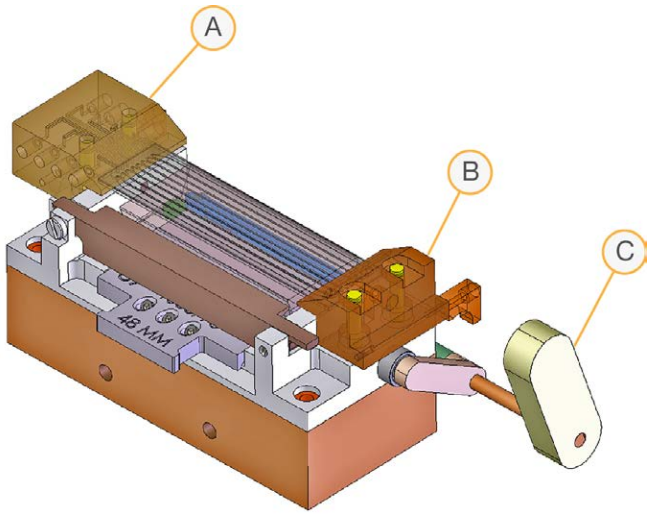
The imaging compartment houses the imaging station, thermal station, and flow cell stage.

The imaging station, or Z stage, includes optical components for imaging the flow cell. The imaging station moves along the Z axis (up an down) to focus the imaging area.

The thermal stage controls the temperature of the flow cell, and includes the Peltier heater, fan, and heat sink. Similar to the imaging stage, the thermal stage also moves along the Z axis.

The flow cell stage holds the flow cell mount and moves along the X (left-right) and Y (front-back) axes. The flow cell is clamped under front and rear plumbing manifolds that allow reagents to pass through the eight lanes of the flow cell.

Figure 4 Plumbing Manifolds



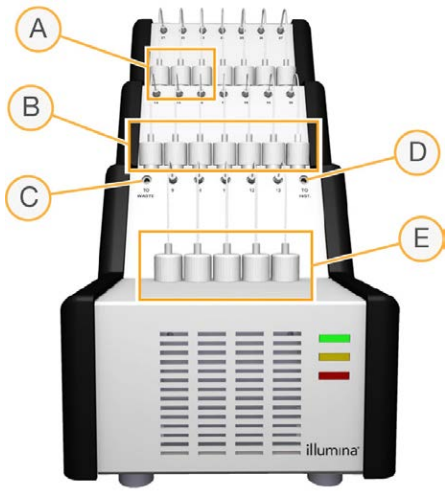
A Rear Manifold
B Front Manifold
C Manifold Handle

One imaging area on the flow cell is called a tile. The design of the manifolds on the Genome Analyzer IIx allow imaging of 120 tiles in each lane of the flow cell.

Paired-End Module Overview

The Paired-End Module is an auxiliary instrument connected to the Genome Analyzer via an external VICI valve that provides reagents used during Read 2 resynthesis, a process that prepares the templates for the second round of sequencing (Read 2). The Paired-End Module is also used for preparation of Index Reads in single-indexed or dual-indexed sequencing protocols.

Figure 5 Paired-End Module



A Positions 21–23
B Positions 14–20
C Connection to Waste
D Connection to Instrument
E Positions 9–13

The Paired-End Module is fitted and installed to port position 8 of the Genome Analyzer internal VICI valve prior to starting the first read. To enable the Paired-End Module for your run, select **Enable Paired-End Module** in the Run Parameters window. For more information, see *Run Parameters Window* on page 26.

Paired-End Sequencing Overview

The paired-end sequencing process sequences the same population of clusters on the same flow cell twice, reading from both ends of the template. Paired-end sequencing consists of Read 1, then Read 2 resynthesis using the Paired-End Module, and then Read 2.

Read 1

Read 1 is performed using paired-end sequencing recipes and reagents provided in the TruSeq SBS Kit.

Paired-end sequencing recipes are similar to single-read recipes with the important exception that they end with a deblock cycle. The flow cell is then equilibrated in high salt buffer, which removes any fluorescent background prior to sequencing Read 2.

Failure to use a paired-end recipe will result in a very high fluorescent background for Read 2 and can compromise the intensity of the second read, both of which have a significant impact on data quality.

Read 2 Resynthesis

Following the completion of Read 1, the flow cell is prepared for Read 2 while still mounted on the Genome Analyzer with the Paired-End Module attached, allowing for the *in situ* treatment of the flow cell. During Read 2 resynthesis, the newly sequenced strands are removed and the complementary strands are bridge amplified to form clusters.

The Paired-End Module supplies paired-end reagents to the flow cell for Read 2 resynthesis. Paired-end reagents are provided in the TruSeq PE Cluster Kit.

The following steps are performed during Read 2 resynthesis:

- **Primer Dehybridization**—Removes the extended sequencing primer used in Read 1.
- **Deprotection**—Prepares the flow cell for the next step.
- **Resynthesis**—Regenerates the previously linearized strand.
- **Linearization 2**—Selectively removes the strand that was sequenced in Read 1 to allow hybridization of the second sequencing primer to the synthesized DNA strand.
- **Blocking**—Blocks one end of the linearized strand to prepare for hybridization of the second sequencing primer.
- **Denaturation and hybridization**—Denatures the linearized strand and hybridizes the second sequencing primer.

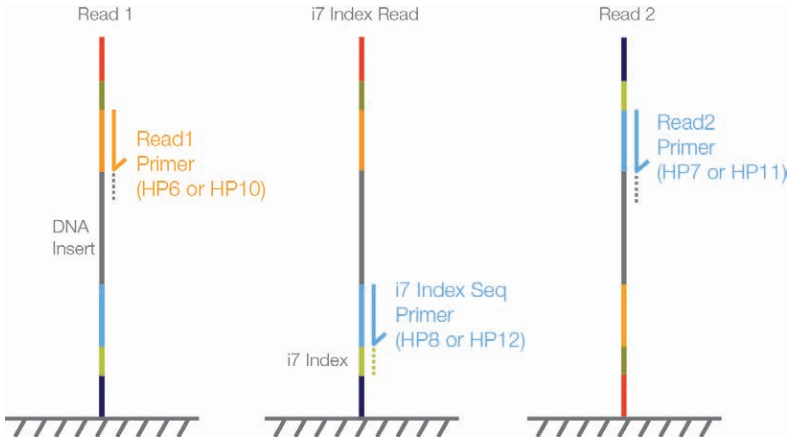
Read 2

After the original templates are removed, the reverse strands undergo another round of sequencing using paired-end sequencing recipes and reagents provided in the TruSeq SBS Kit.

Single-Indexed Sequencing Overview

Single-indexed sequencing includes one Index Read following Read 1.

Figure 6 Single-Indexed Sequencing



Read 1

Read 1 follows the standard Read 1 sequencing protocol using reagents provided in the TruSeq SBS Kit. The Read 1 sequencing primer is annealed to the template strand during the cluster generation process on the cBot.

- When sequencing dual-indexed Nextera libraries, you must use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP10**, which is used on the cBot in place of HP6. For more information, see the *cBot User Guide*, Part # 15006165.
- When sequencing single-indexed TruSeq LT/v2 libraries or dual-indexed TruSeq HT libraries, you can use either HP6 or HP10.

Index Read Preparation

The Read 1 product is removed and the Index 1 (i7) sequencing primer is annealed to the same template strand, producing the Index 1 (i7) Read.

- When sequencing dual-indexed Nextera libraries, you must use the appropriate sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP12**, which is used in place of HP8.
- When sequencing single-indexed TruSeq LT/v2 libraries or dual-indexed TruSeq HT libraries, you can use either HP8 or HP12.

Index 1 (i7) Read

Following Index Read preparation, the Index 1 (i7) Read is performed.

- When sequencing single-indexed libraries, the Index Read consists of seven cycles of sequencing.
- When sequencing dual-indexed libraries (Nextera or TruSeq HT), the Index Read consists of eight cycles of sequencing.

Read 2 Resynthesis

The Index Read product is removed and the original template strand is used to regenerate the complementary strand. Then, the original template strand is removed to allow hybridization of the Read 2 sequencing primer.

- When sequencing dual-indexed Nextera libraries, you must use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP11**, which is used in place of HP7.
- When sequencing single-indexed TruSeq LT/v2 libraries or dual-indexed TruSeq HT libraries, you can use either HP7 or HP11.

Read 2

Read 2 follows the standard paired-end sequencing protocol using reagents provided in the TruSeq SBS Kit.

Dual-Indexed Sequencing Overview

NOTE
Performing dual-indexed sequencing runs requires Sequencing Control Software (SCS) v2.10 and v10 recipes.

Dual-indexed sequencing includes two Index Reads following Read 1.

Figure 7 Dual-Indexed Single-Read Sequencing

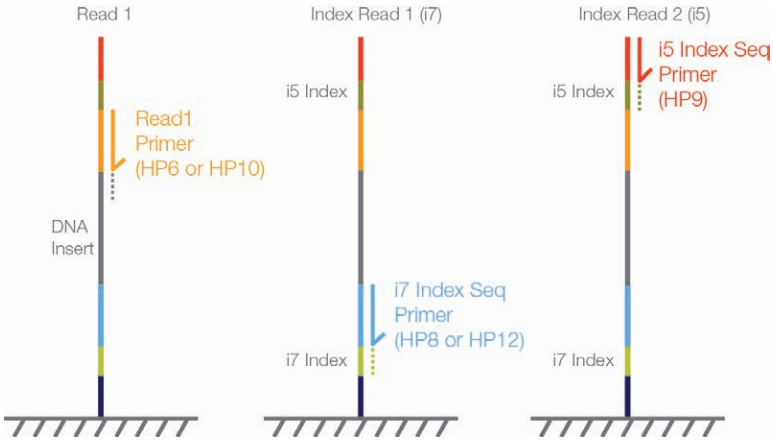
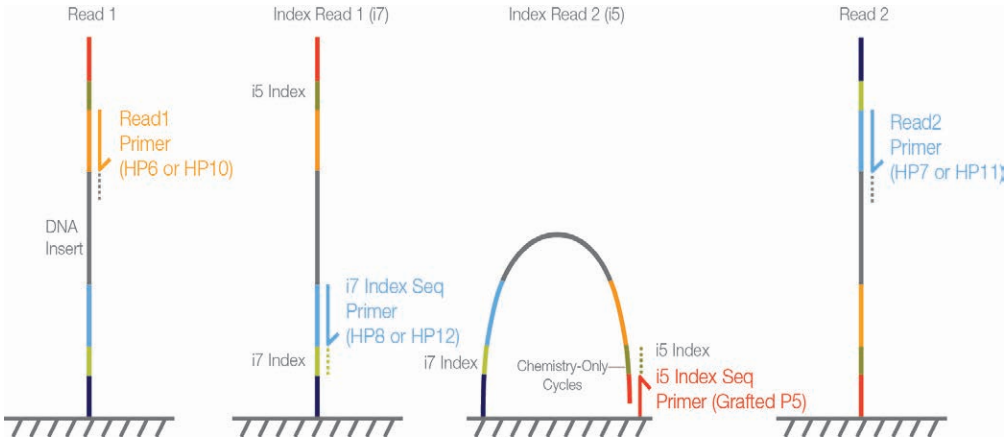


Figure 8 Dual-Indexed Paired-End Sequencing



Read 1

Read 1 follows the standard Read 1 sequencing protocol using reagents provided in the TruSeq SBS Kit. The Read 1 sequencing primer is annealed to the template strand during the cluster generation process on the cBot.

- When sequencing dual-indexed Nextera libraries, you must use the appropriate sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP10**, which is used on the cBot in place of HP6. For more information, see the *cBot User Guide*, Part # 15006165.
- When sequencing dual-indexed TruSeq HT libraries, you can use either HP6 or HP10.

Index Read Preparation

The Read 1 product is removed and the Index 1 (i7) sequencing primer is annealed to the same template strand, producing the Index 1 (i7) Read.

- When sequencing dual-indexed Nextera libraries, you must use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP12**, which is used in place of HP8.
- When sequencing dual-indexed TruSeq HT libraries, you can use either HP8 or HP12.

Index 1 (i7) Read

Following Index Read preparation, the Index 1 (i7) Read is performed. When sequencing dual-indexed libraries (Nextera or TruSeq HT), the Index Read consists of eight cycles of sequencing.

Index 2 (i5) Read

The process for Index 2 (i5) Read is different for runs performed on single-read flow cells than for runs performed on paired-end flow cells:

- **Single-Read Flow Cells**—The Index 1 (i7) Read product is removed and the Index 2 (i5) Sequencing Primer Mix, **HP9**, is annealed to the same template strand. HP9 is provided in the TruSeq Dual Index Sequencing Primer Box and required for dual-indexed runs on single-read flow cells regardless of the library type (Nextera or TruSeq HT). The run proceeds through eight cycles of sequencing.
- **Paired-End Flow Cells**—The Index 1 (i7) Read product is removed. The Resynthesis Mix, **RMX**, deprotects the grafted P5 primer on the surface of the flow cell and the template anneals to the grafted P5 primer. The run proceeds through an additional seven chemistry-only cycles (no imaging occurs), followed by eight cycles of sequencing.

Read 2 Resynthesis

The Index Read product is removed and the original template strand is used to regenerate the complementary strand. Then, the original template strand is removed to allow hybridization of the Read 2 sequencing primer.

- When sequencing dual-indexed Nextera libraries, you must use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP11**, which is used in place of HP7.
- When sequencing dual-indexed TruSeq HT libraries, you can use either HP7 or HP11.

Read 2

Read 2 follows the standard paired-end sequencing protocol using reagents provided in the TruSeq SBS Kit.

Dual-Indexed Libraries

Dual-indexed libraries are libraries prepared with adapters containing two indices:

- Index 1 (i7), an eight-base index adjacent to the P7 strand
- Index 2 (i5), an eight-base index adjacent to the P5 strand

During sample preparation, up to 12 unique Index 1 sequences are arrayed across the columns of a 96-well plate and up to eight unique Index 2 sequences are arrayed down the rows, which create up to 96 uniquely dual-indexed adapters.

Supported Dual-Indexed Libraries

Dual-indexed sequencing on the Genome Analyzer is supported for libraries prepared with the following Illumina sample prep kits:

- TruSeq HT Sample Prep Kits
- Nextera DNA Sample Prep Kits
- Nextera XT Sample Prep Kits

Sequencing Consumables

Illumina-supplied consumables are required for sequencing on the Genome Analyzer. The kits that you need depend on the type of run you perform and the type of libraries you are sequencing.

The following table lists the type of reagents required for a run:

Run Type	Library Type	Required Consumables
Any sequencing run	Any library type	TruSeq SBS Kit
Any indexed sequencing run	Any library type	TruSeq Cluster Kit, SR or PE
Any paired-end sequencing run	Any library type	TruSeq PE Cluster Kit
All run types	Nextera libraries	TruSeq Dual Index Sequencing Primer Box
Dual-indexed single-read run Single-read flow cell	TruSeq HT libraries	TruSeq Dual Index Sequencing Primer Box

For more information, see *Sequencing Primer Requirements* on page 16.

SBS Reagents

One SBS kit provides sequencing reagents for 36 cycles of sequencing. For longer runs, you need multiple kits. For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

Kit Name	Catalog #
TruSeq SBS Kit v5 - GA (36 Cycle)	FC-104-4002

TruSeq SBS Kit v5 Contents

All reagents provided in the TruSeq SBS Kit are labeled with the prefix GA plus a barcode number that uniquely identifies the container.

SBS Box 1: Store at 2° to 8°C

Reagent Name	Reagent Description
PW1	Wash Solution
PR1	High Salt Buffer
PR2	Incorporation Buffer
PR3	Cleavage Buffer

SBS Box 2: Store at -15° to -25°C

Reagent Name	Reagent Description
IMR	Incorporation Mix Reagent
LFN	Long-Read FFN
HDP	High Density Polymerase
CLM	Cleavage Mix
SMX	Scan Mix

The SMX instrument tube is not required for use on the GAIIx.

Indexing Reagents

Indexing reagents are provided in the TruSeq Cluster Kit (SR or PE). One kit provides indexing reagents for sequencing one flow cell.

Kit Name	Catalog #
TruSeq SR Cluster Kit v2 - GA (cBot)	GD-300-2001
TruSeq PE Cluster Kit v2 - GA (cBot)	PE-300-2001
TruSeq SR Cluster Kit v5 - GA (Cluster Station)	GD-203-5001
TruSeq PE Cluster Kit v5 - GA (Cluster Station)	PE-203-5001

NOTE
For all sequencing runs using Nextera libraries and for dual-indexed runs using TruSeq HT libraries on a single-read flow cell, additional sequencing primers are required for the Index Reads. For more information, see *Sequencing Primers for Dual-Indexed Libraries* on page 15.

Indexing Reagent Box Contents

TruSeq Cluster Kit (PE or SR), Indexing Box: Store at -15° to -25°C

Reagent Name	Description
HP3	Denaturation Solution
HT2	Wash Buffer
HP8	Index Sequencing Primer

Paired-End Reagents

Paired-end reagents for paired-end sequencing runs are provided in the TruSeq PE Cluster Kit. One kit provides paired-end reagents for sequencing one flow cell.

Kit Name	Catalog #
TruSeq PE Cluster Kit v2 - GA (cBot)	PE-300-2001
TruSeq PE Cluster Kit v5 - GA (Cluster Station)	PE-203-5001

NOTE
Dual-indexed Nextera libraries require the Read 2 sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box. For more information, see *Sequencing Primers for Dual-Indexed Libraries* on page 15.

Paired-End Reagent Box Contents

TruSeq PE Cluster Kit, Paired-End Box: Store at -15° to -25°C

Reagent Name	Description
HT2	Wash Buffer
RMX	Resynthesis Mix

Reagent Name	Description
AT2	100% Formamide
APM2	Amplification Premix
AMX2	Amplification Mix 2
LMX2	Linearization Mix 2
BMX	Blocking Mix
HP7	Read 2 Sequencing Primer Mix
HP3	Denaturation Solution

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact.

Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

For more information, see the MSDS for this kit on the Illumina website.

Sequencing Primers for Dual-Indexed Libraries

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.

One TruSeq Dual Index Sequencing Primer Box provides sufficient reagents for sequencing one flow cell.

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

Dual Index Sequencing Primer Box Contents, Single Read

Reagent Name	Storage	Description
HP10	-15° to -25°C	Read 1 Sequencing Primer Mix
HP12	-15° to -25°C	Index 1 (i7) Sequencing Primer Mix
HP9	-15° to -25°C	Index 2 (i5) Sequencing Primer Mix

Dual Index Sequencing Primer Box Contents, Paired End

Reagent Name	Storage	Description
HP10	-15° to -25°C	Read 1 Sequencing Primer Mix
HP12	-15° to -25°C	Index 1 (i7) Sequencing Primer Mix
HP11	-15° to -25°C	Read 2 Sequencing Primer Mix

Sequencing Primer Requirements

The following table lists required sequencing primers by library type and flow cell type.

Run Type	Read 1 (cBot)	Index 1 (i7)	Index 2 (i5)	Read 2
Nextera libraries Single-Read Flow Cell	HP10	HP12	HP9	--
Nextera libraries Paired-End Flow Cell	HP10	HP12	Grafted P5 on flow cell	HP11
TruSeq HT libraries Single-Read Flow Cell	HP6 or HP10	HP8 or HP12	HP9	--
TruSeq HT libraries Paired-End Flow Cell	HP6 or HP10	HP8 or HP12	Grafted P5 on flow cell	HP7 or HP11
TruSeq LT/v2 libraries Single-Read Flow Cell or Paired-End Flow Cell	HP6 or HP10	HP8 or HP12	--	HP7 or HP11
TruSeq Small RNA libraries Single-Read Flow Cell or Paired-End Flow Cell	HP6 or HP10	HP8 or HP12	--	HP7 or HP11

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

User-Supplied Consumables

Consumable	Supplier	Use
Bottles, Falcon conical, 175 ml	BD, catalog # 352076	Used for runs and washes in positions 1, 3, and 6 (GAIIx only).
Bottles, Nalgene, 125 ml	ThermoFisher Scientific, catalog # 342040-0125	Used for instrument washes in positions 2, 4, 5, and 7, and combining reagents for long runs.
Bottle, Storage, 150 ml	Corning, catalog # 431175	Used in reagent positions 4, 5, and 7 when combining reagents for long runs.
Ethanol, 100% or Spectrophotometer-grade methanol	General lab supplier	Used to clean the prism and flow cell.
Gloves, disposable, powder-free	General lab supplier	General use.
Lens paper, 4 x 6 in.	VWR, catalog # 52846-001	Cleaning the prism and flow cell.
Oil, Immersion, refractive index 1.473	Cargille, catalog # 19570	Used to load the flow cell.
Tubes, conical, 50 ml	BD, catalog # 352070	Used for runs and washes in positions 1, 3, and 6 (GAII only).
Tubes, conical, 15 ml	General lab supplier	Used for collecting waste volumes.
Tubes, Sarstedt conical, 15 ml	General lab supplier	Used for washes on the Paired-End Module.
Water, MilliQ water or laboratory grade	Millipore	Used for washing the Paired-End Module.
1 N NaOH	General lab supplier	Used for monthly maintenance and storage washes.

Version Compatibility

Every recipe name ends in a version number. Be sure to select a recipe version that is compatible with the version of SBS reagents you are using and the type of library you are sequencing.

Software Version	Recipe Version	SBS Kit Version
SCS v2.10	v8.5 recipes or v10 recipes	TruSeq SBS Kit v5

Recipe Versions by Library Type

Run Type	Library Type	Recipe Version	Cycles in Index Read
Non-Indexed Run	Non-Indexed Libraries	v10	No Index Read included
Single-Indexed Run	Single-Indexed Libraries	v8.5	Performs a seven-cycle Index Read
	Dual-Indexed Libraries	v10 (Eight-cycle Index Read)	Performs an eight-cycle Index Read
Dual-Indexed Run	Single-Indexed Libraries	--	*Not applicable*
	Dual-Indexed Libraries	v10 (Eight-cycle Index Reads)	Performs eight-cycle Index Reads

For recipe descriptions, see *Sequencing Recipes* on page 19.

Sequencing Recipes

Recipes are XML files containing a series of commands required for a sequencing run. Sequencing recipes are specific to a type of run: single-read, paired-end, single-indexed, or dual-indexed.

Recipe names contain indicators that specify which type of run they perform. The following indicators are used in sequencing recipe names:

- **SR**—Single-read sequencing recipe.
- **PE**—Paired-end sequencing recipe.
- **MP**—Indexed sequencing recipe (single-indexed or dual-indexed).
  - **+7**—Seven-cycle single-indexed sequencing recipe, which is compatible with single-indexed libraries.
  - **+8**—Eight-cycle single-indexed sequencing recipe, which is compatible with dual-indexed libraries.
  - **+8+8**—Eight-cycle dual-indexed sequencing recipe, which is compatible with dual-indexed libraries.

Paired-end and indexing recipes are specific to one of two types of Paired-End Module, either the Paired-End Module "classic" or the Paired-End Module IIx:

- **PEM**—Paired-End Module "classic" recipe.
- **PEM2X**—Paired-End Module IIx recipe. These recipes also include px in the recipe version. You must use a version px recipe with the Paired-End Module IIx.

Non-Indexed Single-Read Recipes

Step	Non-Indexed Single-Read Run
Pre-Run Wash	GA2_PreWash_v10
Prime	GA2_Prime_v10
Sequencing (151 Cycles)	GA2_151Cycle_SR_v10
	The sequencing recipe performs the following steps: **Read 1:** • First Base Incorporation • Single-Read Run
Post-Run Wash	GA2_PostWash_v10

Single-Indexed Single-Read Recipes

Step	Single-Indexed Single-Read Run
Pre-Run Wash	GA2-PEM_MP_PreWash_SR_v10
Prime	GA2_Prime_v10
Sequencing (151 Cycles)	GA2-PEM_MP_151+7Cycle_SR_v8.5 (for single-indexed libraries) GA2-PEM2X_MP_151+7Cycle_SR_v8.5px (for single-indexed libraries) GA2-PEM_MP_151+8Cycle_SR_v10 (for dual-indexed libraries) GA2-PEM2X_MP_151+8Cycle_SR_v10px (for dual-indexed libraries)
	The sequencing recipe performs the following steps: **Read 1:** • PEM Water Prime • First Base Incorporation

Step	Single-Indexed Single-Read Run
	• Single-Read Run **Index 1 (i7) Read:** • Prime Paired-End Module • Index 1 (i7) Read Preparation • Index 1 (i7) Read First Base Incorporation • Index 1 (i7) Read
Post-Run Wash	GA2-PEM_MP_PostWash_SR_v10

Dual-Indexed Single-Read Recipes

Step	Dual-Indexed Single-Read Run
Pre-Run Wash	GA2-PEM_MP_PreWash_SR_v10
Prime	GA2_Prime_v10
Sequencing (151 Cycles)	GA2-PEM_MP_151+8+8Cycle_SR_v10 (for dual-indexed libraries) GA2-PEM2X_MP_151+8+8Cycle_SR_v10px (for dual-indexed libraries)
	The sequencing recipe performs the following steps: **Read 1:** • PEM Water Prime • First Base Incorporation • Single-Read Run **Index 1 (i7) Read:** • Prime Paired-End Module • Index 1 (i7) Read Preparation • Index 1 (i7) Read First Base Incorporation • Index 1 (i7) Read **Index 2 (i5) Read:** • Prime Paired-End Module • Index 2 (i5) Read Preparation • Index 2 (i5) Read First Base Incorporation • Index 2 (i5) Read
Post-Run Wash	GA2-PEM_MP_PostWash_SR_v10

Non-Indexed Paired-End Recipes

Step	Non-Indexed Paired-End Run
Pre-Run Wash	GA2-PEM_PreWash_PE_v10
Prime	GA2_Prime_v10
Sequencing (2x151 Cycles)	GA2-PEM_2x151Cycle_v10 GA2-PEM2X_2x151Cycle_v10px
	The sequencing recipe performs the following steps: **Read 1:** • Read 1 First Base Incorporation • Read 1 **Read 2 Preparation (Resynthesis):** • PEM Water Prime • Prime Paired-End Module • Read 2 Resynthesis

Step	Non-Indexed Paired-End Run
	Read 2: • Read 2 First Base Incorporation • PEM Water Wash • Read 2
Post-Run Wash	GA2-PEM_PostWash_PE_v10

Single-Indexed Paired-End Recipes

Step	Single-Indexed Paired-End Run
Pre-Run Wash	GA2-PEM_PreWash_PE_v10
Prime	GA2_Prime_v10
Sequencing (2x151 Cycles)	GA2-PEM_MP_151+7+151Cycle_v8.5 (for single-indexed libraries) GA2-PEM2X_MP_151+7+151Cycle_v8.5px (for single-indexed libraries) GA2-PEM_MP_151+8+151Cycle_v10 (for dual-indexed libraries) GA2-PEM2X_MP_151+8+151Cycle_v10px (for dual-indexed libraries)
	The sequencing recipe performs the following steps: **Read 1:** • PEM Water Prime • Read 1 First Base Incorporation • Read 1 **Index 1 (i7) Read:** • Prime Paired-End Module • Index 1 (i7) Read Preparation • Index 1 (i7) Read First Base Incorporation • Index 1 (i7) Read Sequencing **Read 2 Preparation (Resynthesis):** • Prime Paired-End Module • Read 2 Resynthesis **Read 2:** • Read 2 First Base Incorporation • PEM Water Wash • Read 2
Post-Run Wash	GA2-PEM_PostWash_PE_v10

Dual-Indexed Paired-End Recipes

Step	Dual-Indexed Paired-End Run
Pre-Run Wash	GA2-PEM_PreWash_PE_v10
Prime	GA2_Prime_v10
Sequencing (2x151 Cycles)	GA2-PEM_MP_151+8+8+151Cycle_PE_v10 (for dual-indexed libraries) GA2-PEM2X_MP_151+8+8+151Cycle_PE_v10px (for dual-indexed libraries)
	The sequencing recipe performs the following steps: **Read 1:** • PEM Water Prime • Read 1 First Base Incorporation • Read 1

Step	Dual-Indexed Paired-End Run
	Index 1 (i7) Read: • Prime Paired-End Module • Index 1 (i7) Read Preparation • Index 1 (i7) Read First Base Incorporation • Index 1 (i7) Read Sequencing **Index 2 (i5) Read:** • Prime Paired-End Module • Index 2 (i5) Read Preparation • Index 2 (i5) Read First Base Incorporation • Index 2 (i5) Read Sequencing **Read 2 Preparation (Resynthesis):** • Prime Paired-End Module • Read 2 Resynthesis **Read 2:** • Read 2 First Base Incorporation • PEM Water Wash • Read 2
Post-Run Wash	GA2-PEM_PostWash_PE_v10

Starting and Stopping Recipes

To start a recipe, click **File** | **Open Recipe** from the Data Collection Software window. The Recipe Viewer opens. Select a recipe from the list and click **OK**. The recipe opens in the recipe panel in the Data Collection Software window. For more information, see *Recipe Viewer* on page 38.

Click **Stop** at the bottom of the recipe panel to pause or stop the recipe. Click **Resume** to resume the recipe. The protocol resumes from the selected step in the recipe shown on the Recipe tab. For more information, see *Stopping and Restarting a Recipe* on page 40.

Approximate Run Duration

Sequencing run duration depends on the number of cycles you perform. With SCS v2.10, you can perform a paired-end sequencing run up to 2 x 151 cycles plus any Index Reads. For expected run durations, visit the Genome Analyzer IIx specifications page on the Illumina website (http://www.illumina.com/systems/genome_analyzer_iix/performance_specifications.ilmn).

Genome Analyzer Software

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Introduction

NOTE
This guide describes how to perform run on the Genome Analyzer using Sequencing Control Software (SCS) v2.10. For information about performing runs using SCS v2.9, see the *Genome Analyzer IIx User Guide (Using SCS v2.9)*, Part # 15018814.

Three software applications are installed on the instrument computer:

- **Sequencing Control Software**—The Sequencing Control Software (SCS) performs the run based on the recipe selected and parameters specified in the Run Parameters window.
- **Real Time Analysis**—The integrated primary analysis software, Real Time Analysis (RTA), performs base calling and assigns a quality score to each base for each cycle. For more information, see *Real Time Analysis Overview* on page 52.
- **Sequencing Analysis Viewer**—The Sequencing Analysis Viewer (SAV) provides detailed quality statistics and updated metrics as the run progresses. For more information, see *Sequencing Analysis Viewer* on page 52.

What's New in SCS v2.10

- New version 10 recipes enable dual-indexed sequencing of dual-indexed libraries. For more information, see *Dual-Indexed Sequencing Overview* on page 10.
- Version 8.5 and version 10 recipes provided with SCS v2.10 include new user waits to improve workflow for reagent replenishment.
- User interface enhancements include additional options for file saving in the Run Parameters window.

Starting the Genome Analyzer

1. Turn the main switch on the instrument to the ON position.
   After a short delay the instrument emits a regular buzzing sound from the top-right-rear region of the chassis. This sound is a normal indicator of stable instrument initialization.
2. Restart the computer and log in to the operating system using the default values:
   User name: sbsuser
   Password: sbs123
   If the default values do not work, consult your facility administrator for the correct user name and password for your site.
3. Delete the data from all previous runs to ensure adequate disk space.

   NOTE
   Illumina recommends reformatting the data drive to prevent fragmentation of the drive.

4. Perform a quick reformat of the data drive as follows:
   a. Right-click **My Computer** and select **Manage**.
   b. In the tree on the left side of the screen, select **Storage** | **Disk Management**.
   c. Right-click **D Partition** and select **Format**.
   d. Select the **Perform a Quick Format** checkbox. Leave all other parameters at the default values.
   e. Click **OK**.
5. If you are using a Paired-End Module, turn the power switch to the ON position.
6. Double-click the Illumina Genome Analyzer Data Collection icon on the computer desktop.
   The Run Parameters window opens. For more information, see *Run Parameters Window* on page 26.

Best Practices

- Illumina recommends that you leave the Genome Analyzer on at all times. Turn it off only if it will remain idle for more than three days.
- Before each run, restart SCS to ensure that the software is properly initialized.

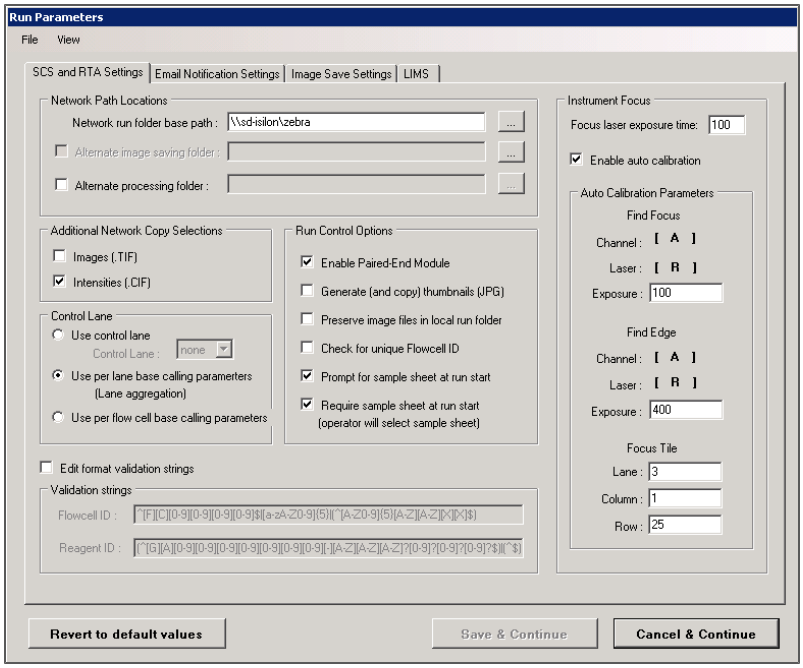
Run Parameters Window

The Run Parameters window is the first window that opens when you launch SCS. Options in the Run Parameters window determine parameters for the run, such as network paths, image file settings, control lane settings, focus settings, and email notifications.

The Run Parameters window contains four tabs:

- SCS and RTA Settings
- Email Notification Settings
- Image Save Settings
- LIMS Settings (for potential future LIMS integration; currently not functional)

Figure 9 Run Parameters Window



By default, the Run Parameters window opens when you launch the software.

- To change this setting, click **View** in the Run Parameters window and clear the **Show On StartUp** checkbox. The next time you launch the software, the Data Collection Software screen opens. You can access the Run Parameters window from the Data Collection toolbar, unless a recipe is running on the instrument.
- To restore this setting, select **Edit | Run Parameters** in the Data Collection Software window. The Run Parameters window opens. From the Run Parameters window, select **View | Show On StartUp**.

SCS and RTA Settings Tab

The SCS and RTA Settings tab contains settings for the network location of run results, preferences for Real Time Analysis (RTA), and settings for image files, control lane use, and focus calibration.

Network Location

- **Network Run Folder Path**—Enter or browse to the location of your network folder. Illumina recommends that you specify the full network path. Do not include a back-slash (\) at the end of the network path.

  If you are using your local drive, enter the path on the D: drive.
- **Alternate Image Saving Folder**—This option allows you to store images in an alternate location. Select the checkbox and enter or browse to an alternate storage location. This does not apply to thumbnails, only images.

  NOTE
  If you are using a Dell 690 instrument computer, image saving and copying is no longer supported. You can select image saving and an alternate image folder; however, these settings are reset automatically when you click **Save and Continue**.
- **Alternate Processing Folder**—This option allows you to copy the RTA run data to an alternate location. The alternate folder is not a replacement for the network run folder. It is only a duplicate of the main network run folder in a different location.

Network Copy Selections

- **Images (*.tif)**—Select this option to copy a subset of images to the specified network folder. This option must be selected to enable the Image Save Settings tab options. For more information, see *Image Save Settings Tab* on page 30.
- **Intensities (*.cif)**—Select this option to copy cluster intensity files (*.cif) to the network folder that you specified in the **Network run folder path** field.

Control Lane

- **Use Control Lane**—You can select **None**, or a value from 1 to 8 to indicate which lane on the flow cell is the control lane.

  NOTE
  A control lane is essential for sample libraries with biased nucleotide content, such as methylation bisulfite sequencing or miRNA samples. If a lane is specified, that lane is used for estimating base calling parameters for all lanes.
- **Use Per Lane Base Calling Parameters (Lane Aggregation)**—Select this option if you are not using a control lane. This setting calculates parameters for each lane individually. This is the default setting.
- **Use Per Flow Cell Base Calling Parameters**—Select this option if you are not using a control lane, and all lanes contain similar samples. This setting calculates parameters based on an aggregate of the entire flow cell for all lanes.

Run Control Options

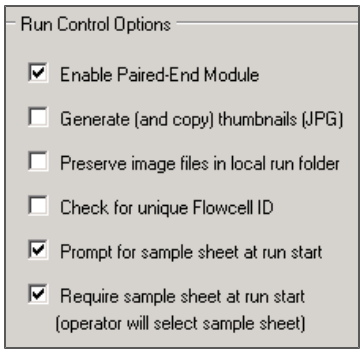
- **Enable Paired-End Module**—Select this option if you are performing a sequencing run that uses the Paired-End Module. The Paired-End Module must be turned on to use this option. This option is enabled by default.
- **Enable Base Calling and Quality Scoring**—This option is enabled by default.
- **Generate (and copy) Thumbnails (*.jpg)**—Select this option to generate thumbnails for all images and copy them to your network location. If selected, thumbnails are always copied to the network. This option is not recommended if you are saving a subset of images.

- **Preserve Image Files in Local Run Folder**—This option preserves images from the instrument computer after analysis.

  NOTE
  Illumina recommends that you disable this feature for read lengths greater than 30 cycles.

- **Check for Unique Flow Cell ID**—If selected, the software verifies that the flow cell ID is unique, and will not allow the same flow cell ID to be used for a different run on the same instrument.
- **Prompt for Sample Sheet at Run Start**—Select this option to enable the prompt for sample sheet at the start of the run. This setting allows you to browse to any location accessible from the instrument computer to locate your sample sheet. You must also select **Require sample sheet at run start** to activate this option.

Figure 10 Prompt for Sample Sheet Settings

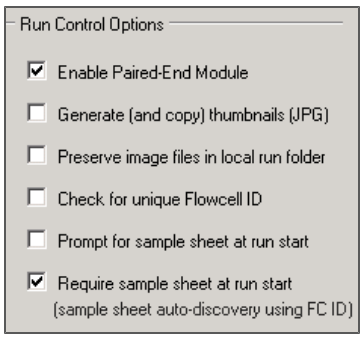


NOTE
To view indexing results in Sequencing Analysis Viewer (SAV), it is essential that you load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to properly report indexing information on the Indexing tab. For more information, see the *Sequencing Analysis Viewer User Guide*, Part # 15020619.

- **Require Sample Sheet at Run Start**—Select this option if you are using a sample sheet. This option must be selected to activate the option **Prompt for sample sheet at run start**.

  If you select only **Require sample sheet at run start**, you are not prompted for a sample sheet at the start of the run. Instead, the software automatically looks for a *.csv file named with the flow cell ID for the run. If the software cannot find a sample sheet by that name, SCS opens the Sample Information dialog box. Browse to select the appropriate sample sheet for this run.

Figure 11 Auto-Discovery Sample Sheet Settings



Instrument Focus Options

- **Focus Laser Exposure Time**—This field contains the exposure used when taking focus images. This value is set by the Illumina Field Service Engineer.
- **Enable Auto Calibration**—Select this option to enable auto calibration. If this option is cleared, focus calibration must be performed manually and only after the first fluorescently-tagged base has been incorporated. If prompted to perform Autofocus Calibration prior to first base incorporation, click **No**.
- **Auto Calibration Parameters**—Specify channel, laser, and exposure values for **Find Focus** and **Find Edge**. Enter the **Focus Tile** values for lane, column, and row.

Validation Strings

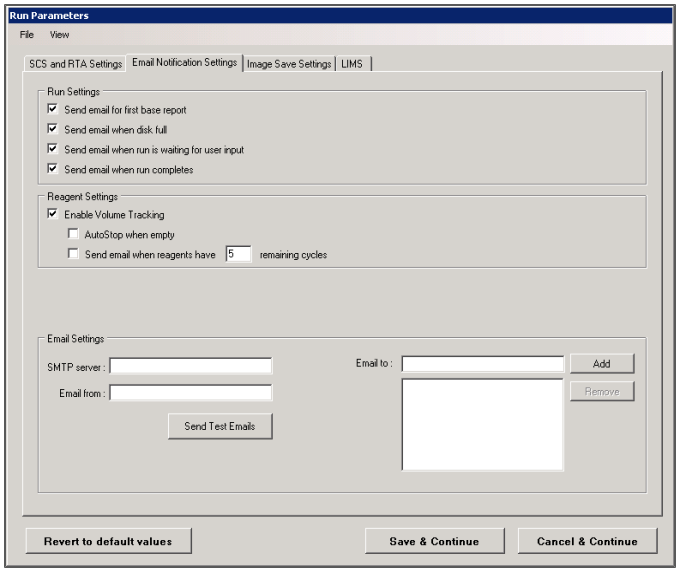
- **Edit Format Validation Strings**—Enables **Validation strings** settings. This option is disabled by default.
- **Validation Strings**—These fields show the validation expressions for the flow cell ID and reagent ID. You can edit the expression if you are using a customized identification system.

Email Notification Settings Tab

In the Email Notification Settings tab, you can specify which events trigger email notifications to the specified email recipients.

To enable this feature, you must have an Internet connection. Check with your facility administrator to make sure that your email is properly configured.

Figure 12 Email Report Settings Tab



Run Settings

- **Send email for first base report**—Alerts you when the first base report is available for review.
- **Send email when disk is full**—Alerts you when you must clear disk space before the run can continue.

- **Send email when run is waiting for user input**—Alerts you when reagents need to be loaded during a run, such as indexing reagents or paired-end reagents, or when water must be loaded for a PEM water prime or PEM wash.
- **Send email when run is complete**—Alerts you at run completion so that you can weigh reagents and set up a post-run wash.

Reagent Settings

- **Enable Volume Tracking**—Enables reagent volume tracking and alerts you when reagent volumes need to be replenished. If left unchecked, reagent tracking is disabled.
  - **AutoStop when empty**—Stops the current run when it reaches the number of cycles entered in the Reset Reagents Volume dialog box.
    The system is placed in a safe state and the flow cell is flushed with PR1 (High Salt Buffer) to protect the clusters from degradation. This occurs after imaging of the current cycle is complete.
  - **Send Email**—Use this option to email warning messages when the specified number of cycles remain before reagents need replacing. Specific the number of remaining cycles that when reached trigger notification.

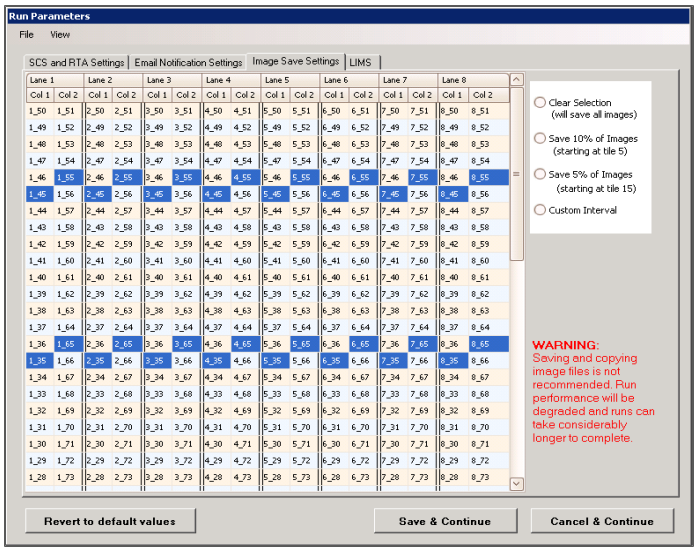
Email Settings

- **SMTP Server**—Enter the SMTP server address that is accessible from the instrument.
- **Email from**—Enter the originating email address. You can use any email that has a valid account on the POP3 server.
- **Email to**—Enter the email address of each notification recipient in the **To** field, and then click **Add**.

Image Save Settings Tab

You can save a subset of images from your run using the settings on the Image Save Settings tab. To enable this feature, select **Images (.TIF)** in the Network Copy Selections group on the SCS and RTA Settings tab.

Figure 13 Image Save Settings Tab: Clear Selection View



NOTE
Illumina does not recommend saving images if you are saving thumbnails. Image save settings do not apply to thumbnail images.

Select any of the following options to specify preferences for saving images:

- **Clear Selection**—Saves all images. This is not recommended.
- **Save 10% of Images (starting at tile 5)**—Saves images every 10th tile, starting with tile number 5. For example, tile 5, 15, 25, etc.
- **Save 5% of Images (starting at tile 15)**—Saves images every 20th tile, starting with tile number 15. For example, tile 15, 35, 55, etc.
- **Custom Interval**—You can customize the tiles by visually selecting tiles on the grid, or you can use the Custom Interval feature. To do this, select **Custom Interval**, and specify a start tile and a new interval other than those already listed on the screen. The software automatically populates this setting for each lane. If you select this option, you must specify **Save 1 Image out of every** X, where X is a number you supply, as well as the beginning tile number.
- For additional information, see *Recommended File Saving Options* on page 51.

Continue to Data Collection

After you have finished selecting the settings in the Run Parameters window click one of the following buttons:

- **Cancel & Continue**—Cancels any recent changes you made in the Run Parameters window and launches the Data Collection Software.
- **Save & Continue**—Saves the parameters you specified and launches the Data Collection Software.
- **Revert to Default Values**—Resets the run parameters to the installed configuration. If you use this option, the **Save & Continue** option disappears and the original settings are applied. Click **Cancel & Continue** to launch the Data Collection Software.

You can return to the Run Parameters window from the Data Collection Software by clicking **Edit | Run Parameters** in the Data Collection toolbar, as long as the instrument is not currently running a recipe.

Data Collection Software Interface

The Data Collection Software interface has two main windows:

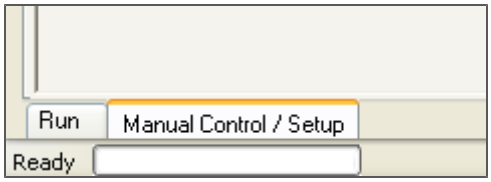
- **Run Window**—From the Run window, you can view recipe details and run progression, and monitor scanning and temperatures.
- **Manual Control/Setup Window**—From the Manual Control/Setup window, you can start the command to load a flow cell, set laser controls and camera position, and initialize the software.

Initializing the Software

Before you can use the Data Collection Software, you must initialize the software.

1 Click **Manual Control/Setup** in the lower left corner of the Data Collection Software window.

Figure 14 Manual Control/Setup Tab

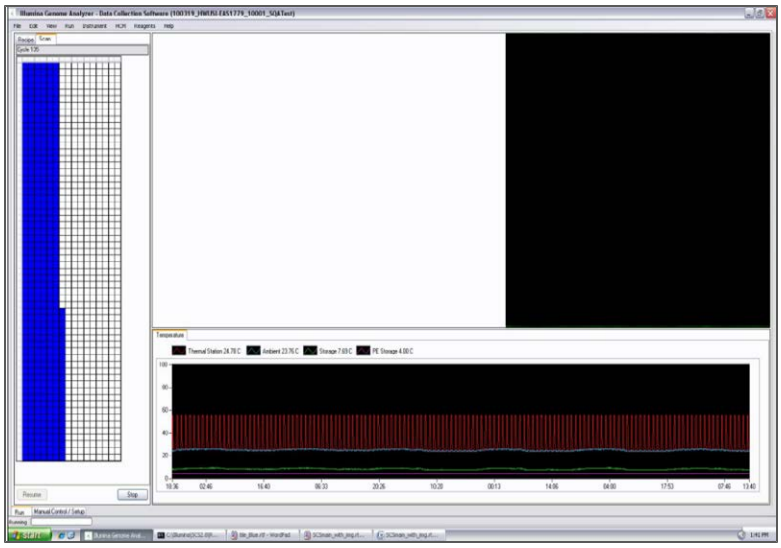


2 In the Manual Control/Setup tab, perform any operation, such as **Take a Picture**, to trigger the initialization. A status bar at the lower left portion of the screen shows the progress of the initialization routine.
The software ignores input until the initialization routine is complete.

Run Window

Click **Run** in the lower-left corner to open the Run window. The Run window contains the Recipe tab, the Scan tab, and the Temperature tab.

Figure 15 Run Window

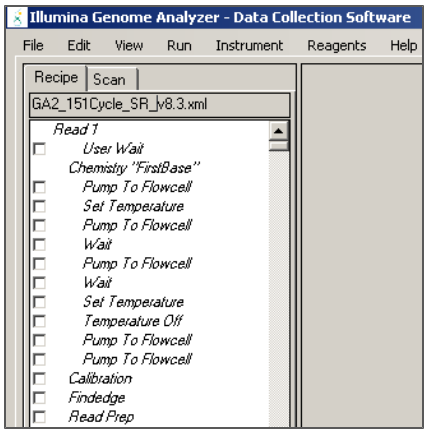


Recipe Tab

The Recipe tab shows the progression of each step in a recipe. To show the parameters specific to any step in the recipe, such as temperature, duration, dispense rates, and volumes, hover your mouse over that step until the tooltip appears. The recipe steps are automatically checked in the interface as they are completed.

You must open a recipe file to show the steps in the Recipe tab. For more information, see *Recipe Viewer* on page 38.

Figure 16 Recipe Tab

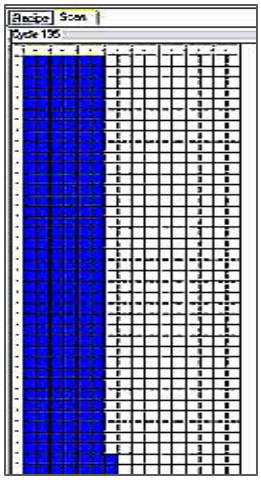


Scan Tab

The Scan tab shows the progression of imaging steps. The column on the left side shows the entire flow cell, using color codes for each tile. Colors and their significance are described below.

- White tiles are queued for imaging.
- Blue tiles have been imaged.
- Gray tiles are not defined in the current run.

Figure 17 Scan Tab

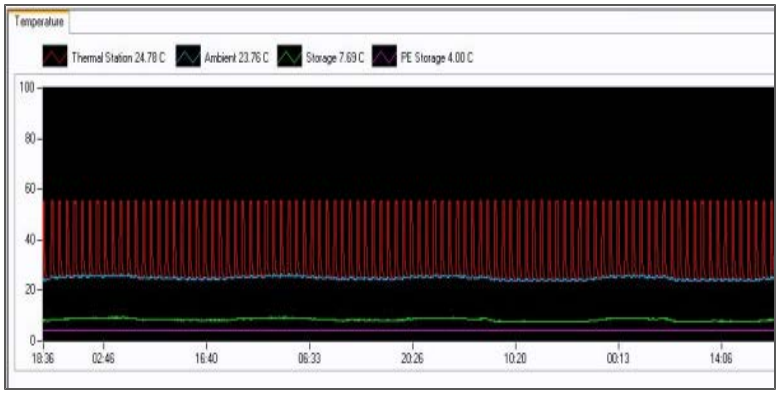


NOTE
The Genome Analyzer Data Collection software window must be maximized to show all of the tiles correctly.

Temperature Tab

The Temperature tab is located in the lower-right portion of the Run window. This tab allows you monitor various run temperatures.

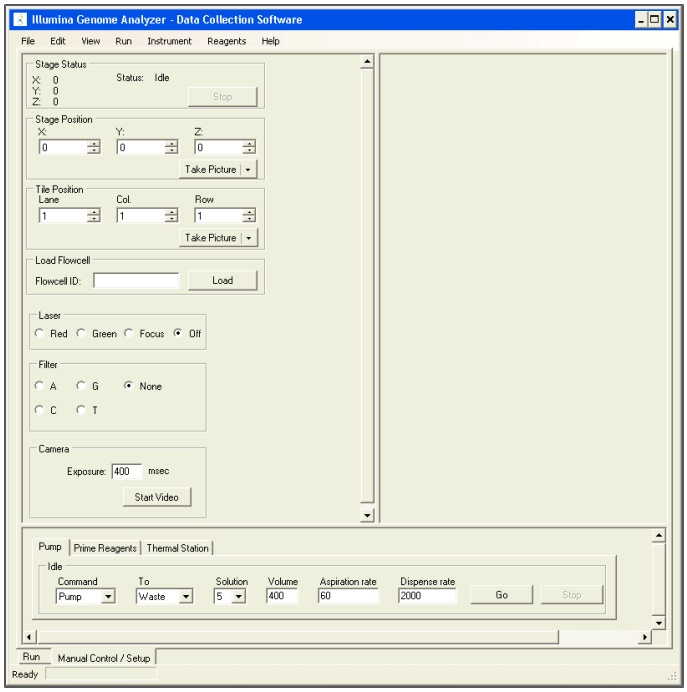
Figure 18 Temperature Tab



Manual Control/Setup Window

The Manual Control/Setup window includes commands for loading the flow cell, specifying laser color, turning the laser on, moving the camera to a specific tile on the flow cell, taking a picture, and starting a video. It also contains manual settings for pump controls, priming reagents, and adjusting thermal station temperature. To access the Manual Control/Setup window, click **Manual Control/Setup** in the lower-left corner.

Figure 19 Manual Control/Setup Tab



Laser Icon

If your instrument is equipped with an Illumina laser engine, the laser status icon appears in the lower right corner of the main window. Instruments equipped with non-Illumina laser engines, do not include the laser status icon. The type of laser engine does not impact instrument performance.

When you see the icon, go to the Manual Control/Setup tab and select **Instrument | Power Lasers On and Wait** from the SCS toolbar.

Icon	Description
	The laser status icon appears red when the laser is off. After a period of four hours without activity, the laser icon reverts to red indicating the laser is off.
	The laser status icon appears yellow while the laser is warming up. This usually takes about ten minutes.
	When the laser is powered on, the status icon appears green.

Stage and Tile Position

The Manual Control/Setup tab contains several settings used for manual focus calibration and troubleshooting. If you selected **Use auto calibration** in the Run Parameters window, you do not need to focus manually. For more information about manual focus and manual priming, see *Using Manual Controls* on page 154.

Use the drop-down menu selection for stage position or tile position to move to the specified location or take a picture.

- To move to the specified position without taking a picture, select the drop-down arrow to the right of Take Picture. Click **Move**.
- To take a picture at the specified position, click **Take Picture**.

Figure 20 Move Stage Position

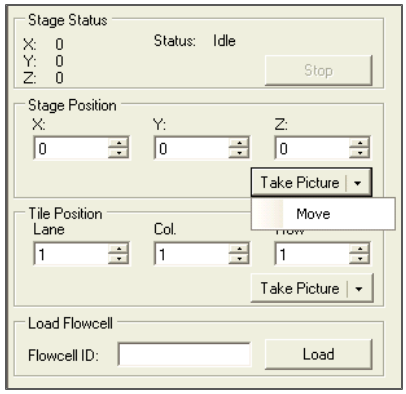
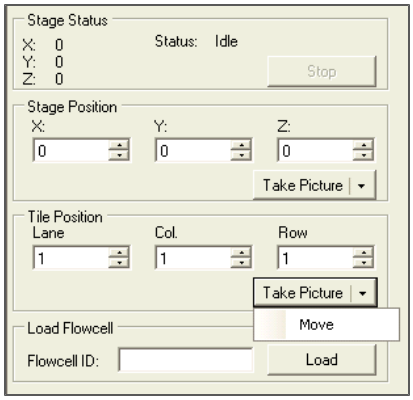


Figure 21 Move to Tile Position



Load Flow Cell

Use this function to move the stage into position for loading or unloading a flow cell. The flow cell ID must be entered before you click the Load button. An error message appears if the field is left blank.

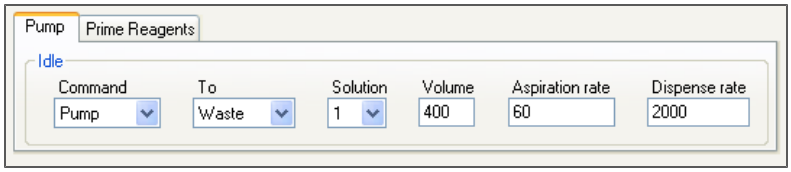
If you are unloading a flow cell, the flow cell ID from the previous run is stored.

Pump Tab

Some setup and maintenance protocols require that you set pump control parameters. These parameters are defined in the Pump tab in the Manual Control/Setup window.

After entering pump control parameters, you can press **Enter** on the keyboard or click **Go**.

Figure 22 Pump Tab



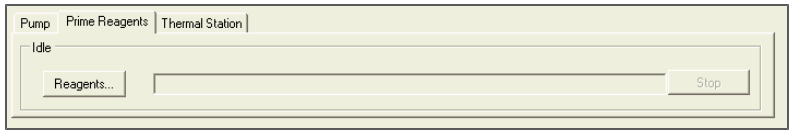
- **Command**—Transfers liquid from the port indicated in the **Solution** field to the location indicated in the To field. This command is used in many procedures, such as washes.
- **To**—Directs the liquid through the flow cell or to waste.
- **Solution**—The port from which the instrument draws liquid.
  When configured for the Paired-End Module, the solution range is 1–28; otherwise, it is 1–8.
  Solution 8 (in single-read recipes) and 28 (in paired-end recipes) is currently air, rather than a liquid. Select this position when you remove flow cells from the system, so that fluid does not siphon through the lines.
- **Volume**—The volume transferred in a single pump event, in microliters.
- **Aspiration rate**—The rate at which liquid is removed from the source, in microliters/minute.
- **Dispense rate**—The rate at which liquid is placed into the target location, in microliters/minute.

Prime Reagents Tab

If your system is configured with the Paired-End Module, the Prime Reagents tab appears in the user interface. You can use the settings on this tab to manually prime reagent positions for troubleshooting purposes.

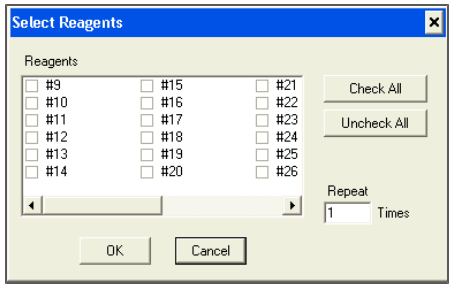
1 Click **Reagents**. The Select Reagents window opens.

Figure 23 Prime Reagents Tab



2 Select the check box next to the reagent positions to prime. Alternatively, you can use Check All to select all reagent positions. Make sure each position is filled to prevent air from entering the lines.

Figure 24 Select Reagents Window

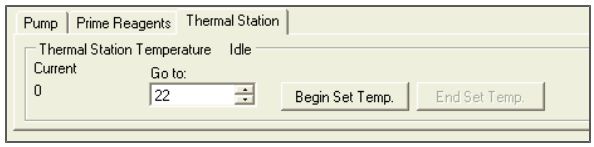


3 Click **OK** to begin the priming process.

Thermal Station Tab

The thermal station settings manually adjust temperature for troubleshooting purposes.

Figure 25 Thermal Station Tab



Using Sequencing Recipes

Recipes are XML files containing a series of commands. To perform a sequencing run, you open and execute the appropriate recipe.

Sequencing recipes contain the following sections:

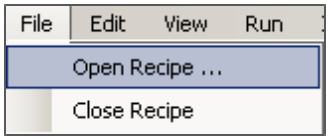
- **Tile Selection**—Determines which rows and lanes are imaged during the incorporation cycles. You can shorten the run by limiting the number of tiles imaged, although this generates concomitantly less data.
- **Chemistry Definitions**—Contains a series of operations and chemistry commands which are later called upon in the protocol section of the recipe.
- **Protocol**—This command invokes chemistry definitions in a particular sequence to perform the run.
  - **Incorporation**—Performs imaging of the current cycle, and then calls the chemistry step for removal and washing of the fluorescent bases and the subsequent addition of the next base in the sequence.
  - **Calibrate**—Invokes the automated process for focus calibration following first base incorporation.
  - **Findedge**—Invokes the automated process for aligning the X axis and finding the flow cell edge.

Recipe Viewer

The Recipe Viewer lists all recipes found in the default location of <install directory>\Illumina\<SCS version>\DataCollection\bin\Recipes.

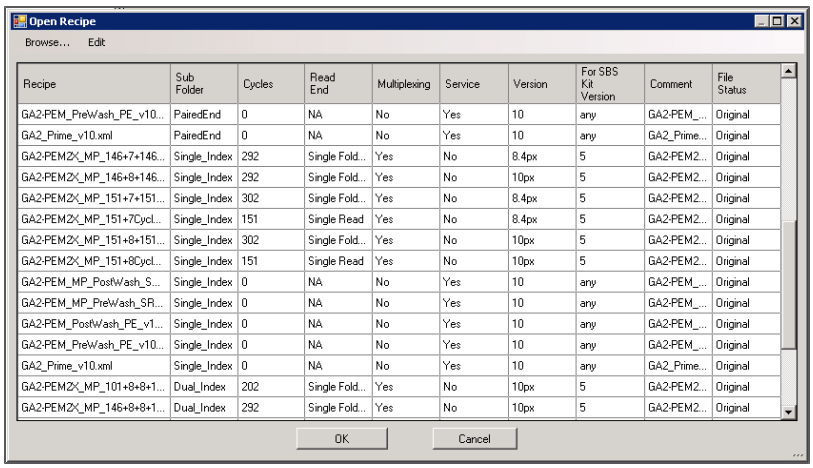
To access the Recipe Viewer, click **File** | **Open Recipe** in the toolbar at the top of the Data Collection Software window.

Figure 26 Recipe Menu



Select a recipe from the list and click **OK**, or double-click a recipe to open it from the Recipe Viewer. The recipe opens in the Recipe tab of the Run window.

Figure 27 Recipe Viewer



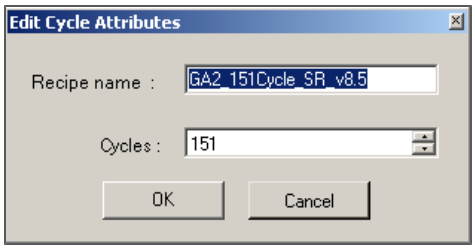
The Recipe Viewer shows the following recipe attributes:

- **Recipe**—Shows the recipe name.
- **Subfolder**—Shows the subfolder where the recipe resides in the default recipe location.
- **Cycles**—Shows the number of cycles that the recipe performs.
- **Read End**—Indicates the type of sequencing read the recipe performs: Single-Read, Paired-End, Single Folder, Service and Maintenance, and Multiplexing.
- **Multiplexing**—Indicates whether this recipe includes an Index Read. If so, this recipe is used for indexed sequencing runs.
- **Service**—Indicates whether this recipe is used for service and maintenance. Service and maintenance recipes cannot be edited with the Recipe Viewer.
- **Version**—Shows the version number of the recipe. Recipe versions help you identify whether a recipe is compatible with a given version of the software or version of sequencing reagents.
- **For SBS Kit Version**—Lists the SBS kit version compatible with the given recipe.
- **Comment**—Shows comments about the recipe. Hover your mouse over the comment field to view the details.
- **File Status**—Shows the status of each recipe. The following are possible status values:
  - **Original**—Indicates that the recipe is an unchanged default version.
  - **User Edited**—Indicates that the recipe was edited but not renamed.
  - **User Created**—Indicates that the recipe was edited and renamed.
  - **Invalid**—Indicates that the recipe does not conform to the recipe format or that it is corrupt. A recipe with Invalid status will not run on the instrument.

Editing the Cycle Number

To increase or decrease the number of cycles in an installed recipe or to change the name of a recipe that you plan to edit, select **Edit | Attributes** from the Recipe Viewer toolbar. The Edit Cycle Attributes dialog box opens.

Figure 28 Edit Cycle Attributes



Use the following table as a guideline for determining the total number of cycles in your run. These examples are based on 151-cycle runs. If your run is shorter, replace 151 for Read 1 and Read 2 with the number of cycles for your run.

Run Type	Read 1	Index Read 1	Index Read 2	Read 2	Total # of Cycles
Non-Indexed Single-Read	151 cycles	--	--	--	151 cycles
Single-Indexed Single-Read (single-indexed libraries)	151 cycles	7 cycles	--	--	158 cycles

Run Type	Read 1	Index Read 1	Index Read 2	Read 2	Total # of Cycles
Single-Indexed Single-Read (dual-indexed libraries)	151 cycles	8 cycles	--	--	159 cycles
Dual-Indexed Single-Read	151 cycles	8 cycles	8 cycles	--	167 cycles
Non-Indexed Paired-End	151 cycles	--	--	151 cycles	302 cycles
Single-Indexed Paired-End (single-indexed libraries)	151 cycles	7 cycles	--	151 cycles	309 cycles
Single-Indexed Paired-End (dual-indexed libraries)	151 cycles	8 cycles	--	151 cycles	310 cycles
Dual-Indexed Paired-End	151 cycles	8 cycles	8 cycles	151 cycles	318 cycles

Illumina strongly recommends that you rename an edited recipe before saving it. If you change the number of cycles in a recipe, the software prompts you to rename the recipe.

NOTE
For paired-end dual-indexed runs, the seven chemistry-only cycles performed during the Index 2 (i5) Read are calculated for reagent use; however, these cycles are not counted in the total number of cycles performed in the run.

Stopping and Restarting a Recipe

Stopping a Recipe

Click **Stop** at the bottom of the recipe panel to pause or stop the recipe. Some commands stop immediately, while others need to finish before stopping. Completed steps have a check mark beside them.

When a run is stopped, the system is placed into a partial safe state. The partial safe state function executes a pump initialization.

Restarting a Recipe

At the beginning of a run, the active recipe file is automatically copied to the run folder. If the run stops before completion, follow these steps to restart the run from the point where it left off.

1 If you closed the application, loaded another recipe, or otherwise navigated away from the run, select **File | Open Recipe**. Navigate to the run folder and open the recipe from there.

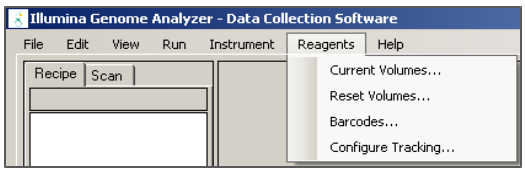
2 Do one of the following to select the restart point:
- Highlight the first protocol step that does not have a check mark.
- Highlight any *italicized* command, whether it is before or after the point where you left off. Italicized commands denote valid restart points.

The **Resume** button becomes active when you select a valid restart point. If you stopped during an Incorporation imaging step, then the imaging cycle restarts from the first tile. If an error occurs, then the run stops automatically and the instrument is placed in a safe state.

Reagent Tracking

To access the reagent tracking commands, click **Reagents** from the toolbar at the top of the Data Collection Software window. You can access these features at any time during a sequencing run.

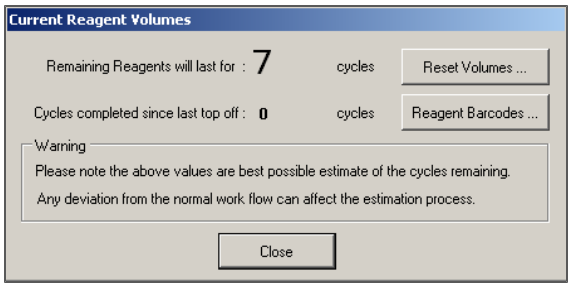
Figure 29 Reagent Commands



Current Reagent Volumes

The Current Reagent Volumes dialog box shows the number of cycles remaining before the reagent volumes become depleted, along with the number of cycles completed since the last time the reagents were replenished.

Figure 30 Current Reagent Volumes



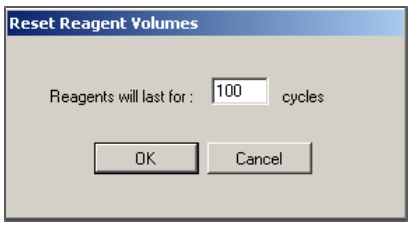
The Current Reagent Volumes dialog box opens automatically when either of the following conditions occur:

- **When a new run is started**—Click **Reset Reagent Volumes** and set the number of cycles that freshly loaded reagents are expected to last. Click **Reagent Barcodes** and enter reagent barcode information for new reagents.
- **When the number of cycles remaining runs down**—If the number of cycles that reagents are expected to last was entered as 12 cycles, for example, this dialog box opens after ten cycles have passed.

Reset Reagent Volumes

When you load a fresh set of reagents or top up reagents during a run, use the Reset Reagent Volumes dialog box to enter the number of cycles the reagents are expected to last. This value is based on the number of cycles you plan to run.

Figure 31 Reset Reagent Volumes



Reagent Barcodes

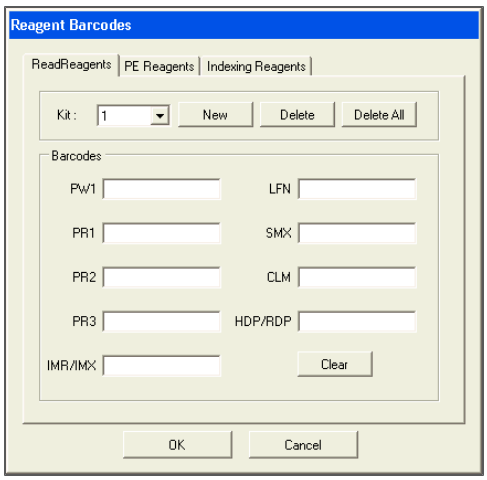
When you load reagents, enter the barcodes in the Reagent Barcode dialog box. The reagent barcodes are stored in the ReagentsId.xml file in the run folder.

The barcode dialog box for sequencing (SBS) reagents opens regardless of the recipe you have open. However, the tabs for paired-end reagents and indexing reagents only appear if a related recipe is open.

Read Reagents Tab

For long runs requiring multiple sequencing kits, click **New** and enter barcodes for additional kits.

Figure 32 Read Reagents Tab



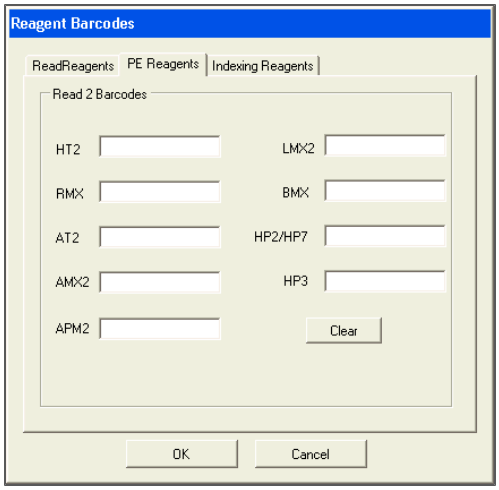
If the reagents are expected to last only through the end of the run (according to the number of cycles entered in Reset Reagent Volumes), clear the reagent barcodes at the end of a run or at the start of the next run.

If any reagents are expected to last for more cycles than the current run, do not clear the barcodes. This eliminates the need to re-enter barcodes for remaining reagents used in the next run. If the remaining reagents will not be used, click **Clear**. This clears the barcodes for the kit number shown in the Kit field.

PE Reagents Tab

The PE Reagents tab is enabled when a paired-end sequencing recipe is open. For paired-end runs, click the PE Reagents tab and enter the reagents used for Read 2.

Figure 33 PE Reagents Tab

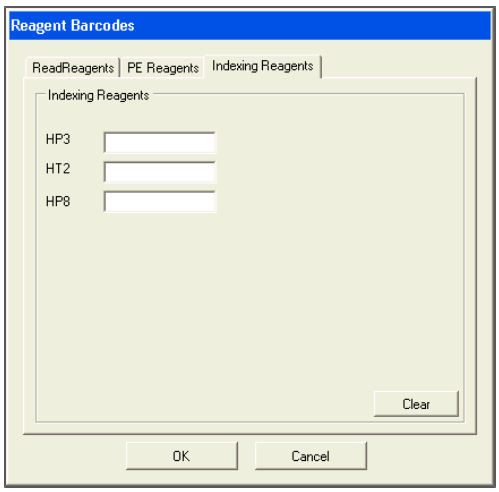


For information about which sequencing primers you should use for your run, see *Sequencing Primer Requirements* on page 16.

Indexing Reagents Tab

The Indexing Reagents tab is enabled when an indexed sequencing recipe is open. For indexed runs, click the Indexing Reagents tab and enter the reagents used for Index Read preparation (Index 1 (i7) Read, and for dual-indexed runs, Index 2 (i5) Read).

Figure 34 Indexing Reagents Tab



For information about which sequencing primers you should use for your run, see *Sequencing Primer Requirements* on page 16.

Image Controls

The Genome Analyzer software provides various tools for adjusting the image. The image display uses 8 bits of data to display color, while TIFF are saved as 16-bit color images. The Show False Color and Auto Scale options provide methods for simulating a larger dynamic range on the computer monitor.

Table 1 Image Controls

Adjustment	Action
Zoom in	Click and drag a rectangle over the area you want to see.
Move the zoomed image	Right-click and select **Center Here**. The display adjusts so that the area you clicked on is at the center.
Zoom out	Right-click over the image and select **Zoom Out** to move one level. Right-click over the image and select **Zoom Out All** to zoom out all the way.
Modify the color display	Right-click over the image and select **Show** | **Color** or **Show** | **False Color**. **Show Color** displays the laser light colors used to acquire the image: blue for the focus tracking images, and red (A/C) or green (G/T) for the intensity values. **Show False Color** lets you customize the color contrast so that it is easier to see peak intensities. Select from: • None • Blue-Green • Green-Blue • Blue-Red • Red-Blue • Red-Green • Green-Red When you select a two-color option, SCS uses the upper 8 bits of the Tiff 16-color range for one color (e.g., blue), and the lower 8 bits for the other color (e.g., green).
View or hide the center mark	Right-click over the image and select **Show** | **Center Mark** to toggle the display of the center mark, which indicates current X and Y values.
See intensity values	Roll the mouse over a point of raised intensity to display the pixel position and intensity value.
Scale the intensity values	Right-click over the image and select **Auto Scale** | **On** or **Auto Scale** | **Off**. When auto scaling is on, the minimum intensity value is mapped to 0 and the maximum intensity value is mapped to 255. When auto scaling is off, the system maps the 12-bit range to an intensity range that you define in RCMConfig.xml. All data intensity values at or below the minimum are mapped to 0 and all values at or above the maximum are mapped to 255. Generally, this mapping is a linear function.
See focus quality and uniformity	Hover the mouse over the image to show focal quality and uniformity.
Show region of interest	Right-click over the image and select **Show** | **ROI** to show the region of interest (ROI), which is the area that is saved during the sequencing runs. The ROI is indicated by the dotted lines on the display. This feature allows you to check for good alignment of the ROI edge and lane edge.
Save the image	Right-click on the image and select **Save As** to save the image as a TIF file.

Auto Calibration

The auto calibration feature determines the best focus by stepping through a series of images, adjusting the Z position with each step. The software performs a coarse focus, followed by a fine focus, and then determines the best Z position.

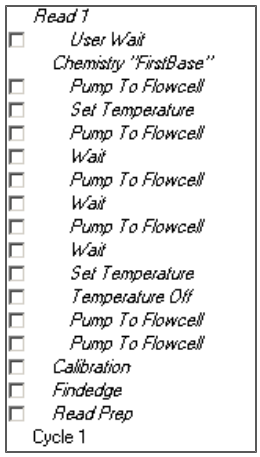
During auto calibration, the software uses a region of 128 x 128 pixels in the middle of the image to determine the best focus. If no clusters are found within this region, auto calibration fails and an error message appears. For more information, see *Troubleshooting Calibration Failure* on page 152.

To enable auto calibration, select the **Enable auto calibration** checkbox in the Run Parameters window. For more information, see *Run Parameters Window* on page 26.

To initiate auto calibration, you can either use the recipe command or you can initiate calibration manually:

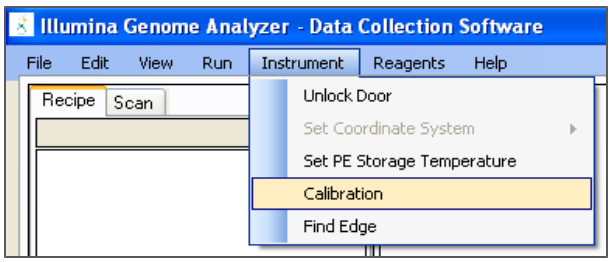
- **Recipe Commands**—The command to run auto calibration is integrated in the first-base incorporation section of the recipe, followed immediately by the command to find the flow cell edge directly.

  Figure 35 Auto Calibration Command in Recipe



- **Manual Command**—You can manually initiate auto calibration after the completion of first base incorporation by selecting **Instrument** | **Calibration** from the Data Collection toolbar.

  Figure 36 Auto Calibration Command in Toolbar

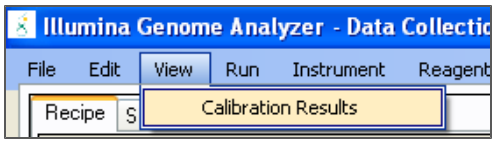


When auto calibration is complete, select **Instrument** | **Find Edge** to find the flow cell edge.

Calibration Results Window

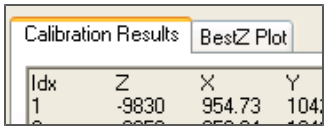
You can view calibration results at any time during the run by selecting **View** | **Calibrations Results** from the Data Collection toolbar.

Figure 37 View Calibration Results



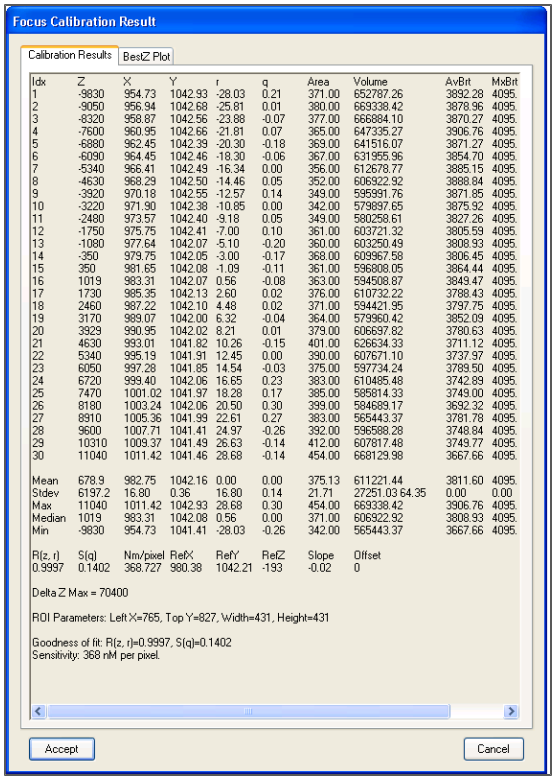
This window contains two tabs: Calibration Results and BestZ Plot.

Figure 38 Calibration Results Window Tabs



- **Calibration Results Tab**—The Calibration Results tab shows the different Z positions that were used to find focus, along with the calibration values.

Figure 39 Focus Calibration Results Window



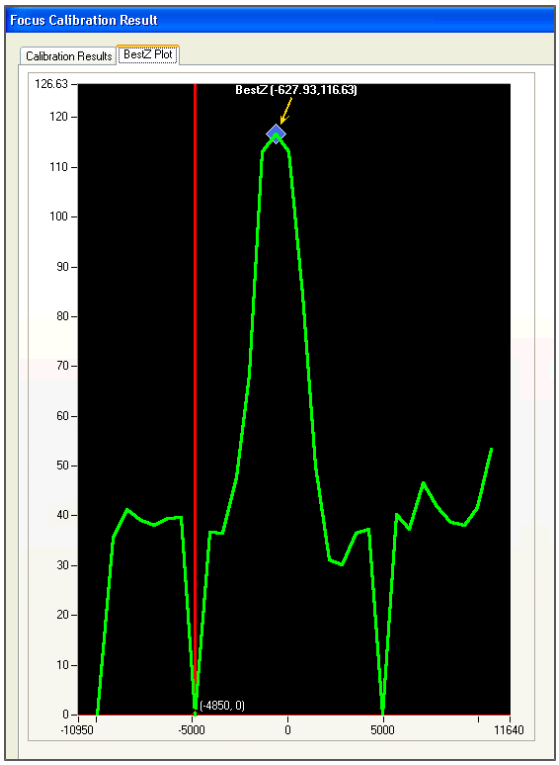
During the calibration process, 30 images are collected; however, outliers might be omitted. As a result, you might see fewer than 30 data points in the Focus Calibration Results window.

This window shows the following settings:

- **Goodness of fit:** ≥ 0.9900
- **Sensitivity:** 350–400

- **BestZ Plot Tab**—The BestZ Plot tab graphs each of the points used in auto calibration and highlights the optimal Z position. You can move the crosshairs to each point on the graph to read the Z position.

Figure 40 Focus Calibration Results, BestZ Plot Tab



If auto calibration fails, see *Troubleshooting Calibration Failure* on page 152.

Clearing Calibration Results

The Genome Analyzer software stores the most recent calibration results, even after you close the software.

The calibration results are cleared if any of the following occurs:

- Auto calibration is restarted.
- Auto calibration fails or is interrupted. No new results are stored.
- You click **Load Flowcell** on the Manual Control/Setup tab.

Run Folders

Each sequencing run generates a run folder that contains data files and log files for that run. When you start a run, the software prompts you to enter the folder name for the run folder. By default, the folder is named in the following format:

YYMMDD_<Workstation Name>_<Run Number>

Example: 070320_WORKSTATION-487_00002

The run number increments by one each time you perform a run on a given workstation. Typically, users add the flow cell ID to the run folder name. The run folder name must not contain any spaces.

All run folders are stored in a single folder. The name and location of this folder are set in <install location>\bin\Config\RCMConfig, in this line:

<Run Path="D:\Runs" />

To change the run folder path, either edit this line in RCMConfig or select **Run | Select Run Folder Root** in the Data Collection Software. Enter the full path and folder name.

Contents of Run Folders

Subfolder	Key Files/Folders	Description
[Root level]	Configuration files	All active configuration files for this run are copied to the root of the run folder path.
	run.completed file	Appears in the folder when a run is successfully completed. You can tell whether a run is still in progress by checking for this file.
	Sample Sheet	If you create a sample sheet, it is copied to this location.
	Recipe_*.xml	When you start a run, a copy of the recipe is moved to the run folder. The name will be prefixed by "Recipe". If you stop the run, open this recipe to start up again where you left off.
	RunInfo.xml	The file RunInfo.xml identifies the boundaries of the reads (including indexing reads), and contains run-specific information such as instrument used and flow cell ID.
	Log file RunLog_<date>_<time>.xml	Whenever you stop a run, a log file detailing the duration of every command appears in the root of the run folder. If you stop and resume the run multiple times, there will be a separate log file for each session. Other log files for the run can be found in <install location>\bin\Config\LogFiles.
Images	Contains one folder for each lane; each lane folder contains one subfolder for each cycle	Each cycle folder contains TIFF files for each lane, named in the format <sample>_<lane>_<pos>_<base>.tif
	Focus folders (optional)	Contains the TIFF focus images. This folder is created if you selected save focus images on the Run Parameters window. For more information, see *Run Parameters Window* on page 26.
Data	Status.htm	Contains run and analysis-specific metrics generated during the run.
	Intensities folder	Contains RTA results.

Subfolder	Key Files/Folders	Description
		This folder is saved on the network folder under <Run Folder>\Data, and is given the same name as the run folder on the instrument computer.
Data\ Intensities	BaseCalls folder	Contains RTA base calling results.
	config.xml file	Contains run and image analysis-specific information.
AnalysisLogs	Contains one QC file per cycle	Each XML file is named as follows: <QC_tab_ name>CycleNumber.xml. For example, FocusQuality15.xml contains focus quality information for all tiles of cycle 15.
Config		Contains configuration parameters for the run, which are stored in XML files.
Calibration_ <DateTime>	Calibration results and image files	
ReadPrep1	Contains Images and Data folders for Read 1	For PE runs only—Contains quality results from Read 1 <ReadPrep> cycle processed by RTA.
ReadPrep2	Contains Images and Data folders for Read 2	For PE runs only—Contains quality results from Read 2 <ReadPrep> cycle processed by RTA.

Available Disk Space Checking

At the end of each cycle, the software checks available disk space. If there is insufficient disk space to accommodate one cycle, the software pauses the run and opens a dialog box that shows how much disk space remains. If this message appears, you must make disk space available to continue the run.

When sufficient disk space is available, the dialog box closes and the run automatically resumes. If you close the dialog box before making space available, you must manually resume the run after sufficient disk space is available.

Recommended File Saving Options

The table below lists the recommended options when copying files to the network. Illumina recommends that you use one of the following configurations to ensure system performance.

	*.cif [1]	*.tif [2]	Thumbnails [3]
Option 1	X	—	—
Option 2	X	X	—

1 The *.cif files are automatically deleted from the local computer after the run completes. Network transfer of *.cif files is necessary to perform off-line base calling at a later time.

2 You might see slower performance (this option is not available on the Dell 690)

3 If you are saving a subset of images, it is not recommended that you also save thumbnails.

Estimated File Size

The table below lists the estimated file size (in Gigabytes) at the recommended densities. Double these file sizes for paired-end runs.

Read Length	*.bcl [1]	*.cif [1]	Images 10% subset [2]	Thumbnails	.bro
51 cycles	21.0 Gb	153.0 Gb	144.5 Gb	14.0 Gb	0.106 Gb
76 cycles	30.0 Gb	228.0 Gb	215.3 Gb	20.8 Gb	0.157 Gb
101 cycles	40.0 Gb	303.0 Gb	286.1 Gb	27.7 Gb	0.209 Gb
125 cycles	50.0 Gb	375.0 Gb	354.1 Gb	34.2 Gb	0.258 Gb
151 cycles	60.0 Gb	453.0 Gb	427.7 Gb	41.3 Gb	0.312 Gb

1 Note that bcl and cif file sizes are dependent on cluster density. Sizes listed above are estimated for 750 K/mm^2.

2 Images and thumbnails are fixed file sizes.

Real Time Analysis Overview

Real Time Analysis (RTA) runs automatically on the instrument computer, and is configured through the SCS interface at the beginning of a run.

NOTE
If for any reason RTA stops functioning, the software automatically resumes analysis during the next cycle at the appropriate point on the flow cell. Do not restart the application manually.

Monitoring Runtime Statistics

Real Time Analysis automatically generates quality metrics when image analysis begins. Not all metrics are available at the early cycles because some processes need multiple cycles to generate data. The following summary describes points in the run when metrics are available:

- **Image Analysis**—Begins after cycle 5. The software first needs to generate a template with cluster locations. At cycle 5, the software catches up with the image analysis.

  NOTE
  Because the software catches up with the image analysis during cycle 5, cycle 5 takes significantly longer to finish. The software waits for the analysis to end before proceeding with the next cycle. This behavior is normal.

- **Phasing Estimates and Base Calls**—The software generates phasing/prephasing estimates and base calls after cycle 12.
- **Quality Scores**—The software generates quality scoring after cycle 25.
- **Error Rates**—If a PhiX control was included, RTA generates error rates after cycle 25.
- **In-Line Controls**—If you prepared your samples with the TruSeq sample prep kits, RTA will perform in-line control alignment at cycle 52 of each read or the end of the read if the read length is less than 52 cycles.
- **Index Count**—After the last index read is completed, RTA will report the index count per lane if a sample sheet was provided.

Sequencing Analysis Viewer

The Sequencing Analysis Viewer (SAV) is an application that allows you to view important sequencing metrics generated by Real Time Analysis (RTA) in plots, graphs, and tables. SAV automatically opens to the analysis page after image analysis begins.

Statistics are updated as soon as new analysis information is available. Select **Refresh** at any time during the run to view updated metrics as the run progresses.

SAV can be viewed from a remote location by installing SAV on another computer with access to the network location of the output run folder. You can download a copy of SAV from the Illumina website. A MyIllumina login is required.

For a detailed description of available metrics and installation instructions, see the *Sequencing Analysis Viewer User Guide*, Part # 15020619.

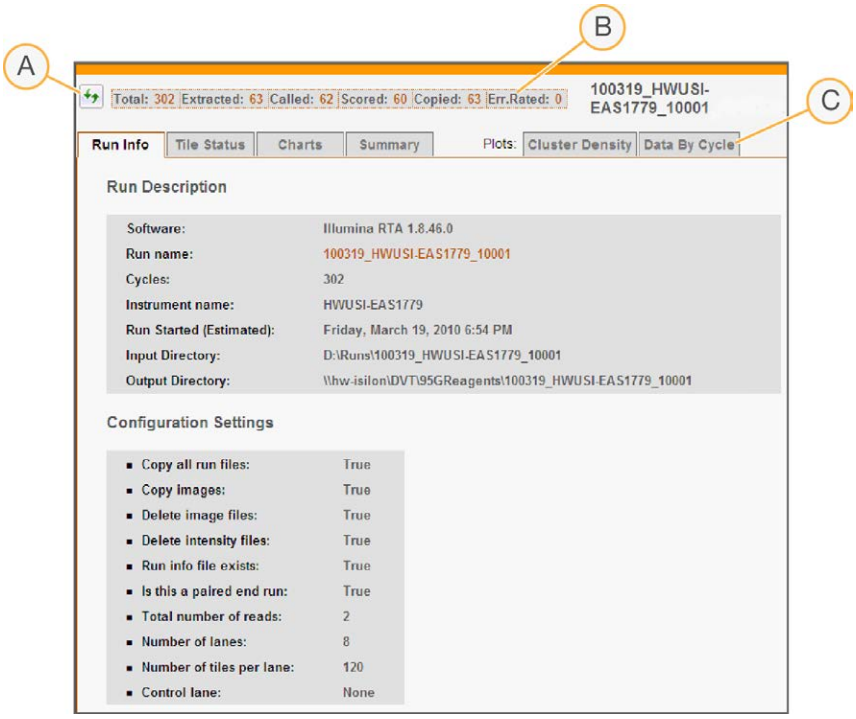
Analysis Page

The analysis page reports the progress of the analysis, and provides access to the most important runtime statistics.

Statistics are updated as soon as new analysis information is available. The runtime statistics can be viewed from any computer with access to the experiment run folder. Navigate to the <RunFolder>\Data folder and open the Status file. The Status file is transferred to the Data subfolder in the analysis folder at the end of each cycle that is processed.

NOTE
Your browser might warn you about active content. If so, click the warning in the information bar and allow blocked content.

Figure 41 Status Page



A Refresh Button
B Progress by Cycle
C Links to Runtime Statistics

Data Transfer

The output from a sequencing run is a set of quality-scored base call (*.bcl) files, which are generated from the raw image files and contain the base calls per cycle. By default, images are deleted from the instrument computer after image analysis. This is necessary for longer runs, since there is not enough space on the instrument computer to store all images. Images are deleted only after image analysis is complete.

If for any reason image analysis falls too far behind, the software stops sequencing and places the flow cell in a safe state. Sequencing resumes when image analysis issues are resolved.

Basecalling_Netcopy_complete

Data transfer is complete when the Basecalling_Netcopy_complete files are present in the root level of the run folder. There should be one generic file named Basecalling_Netcopy_complete.txt, plus one file for each read.

Analysis of RTA Data

CASAVA v1.8 has the capability to use *.bcl files directly without conversion into *_qseq.txt files. For more information, see the *CASAVA v1.8 User Guide*, Part # 15011196.

NOTE
CASAVA v1.8.2 or later is required to analyze RTA data output from dual-indexed sequencing runs.

If you need to perform base calling offline, use the Off-line Basecaller (OLB). For more information, see the *Off-line Basecaller Software User Guide*, Part # 15009920.

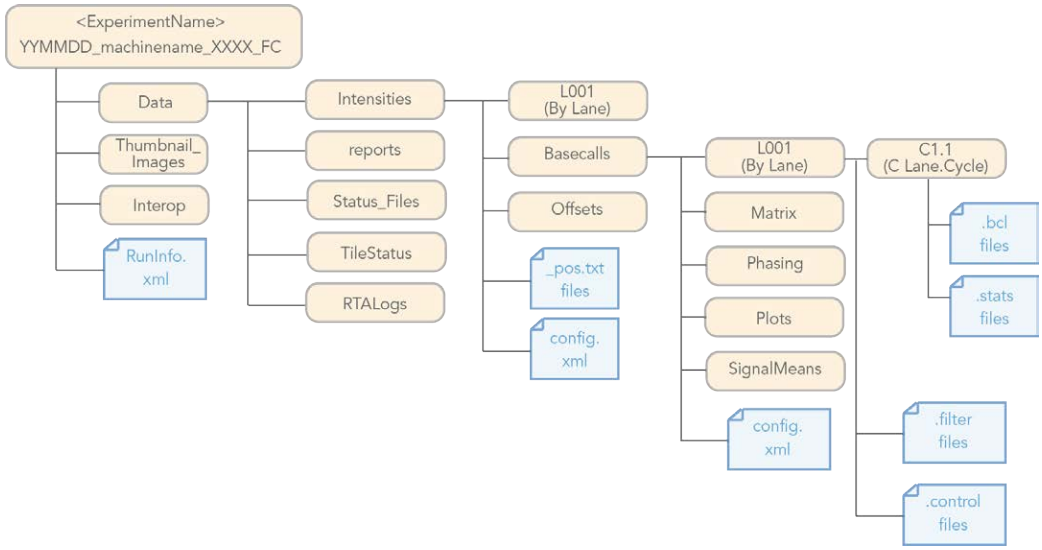
If you are using CASAVA software v1.7 or earlier, base calling data is generated in a binary format and needs to be converted to the text-based sequence files (*_qseq.txt) using the BCL Converter. For more information, see the *BCL Converter User Guide*, Part # 15011195.

Real Time Analysis Output Data

RTA output results are saved to the network location that you specified during the run setup steps.

All analysis results are saved in an analysis output folder that is given the same name as the run folder on the instrument computer. The following figure illustrates the organization of the network analysis folder, including the location of the most important output files (blue boxes).

Figure 42 Real Time Analysis Folder



Main Sequence Output Files

The following output file types contain primary analysis data generated by Real Time Analysis:

Base Call Files

A base call (*.bcl) file contains base calls and quality scores for one cycle and one tile. They can be used for later analysis off-instrument.

NOTE
If you are using CASAVA v1.7 or earlier, the *.bcl files need to be converted to the _qseq.txt format in OLB or the BCL Converter to enable downstream analysis. For more information, see the *BCL Converter User Guide* or the *Off-line Basecaller Software User Guide*.

This step is not necessary with CASAVA v1.8 or later.

Stats Files

The Stats file is a binary file containing base calling statistics for clusters passing filter. These files are used by the BCL Converter and CASAVA v1.8.

Filter Files

The filter (*.filter) files are binary files containing filter results. These files are used by the BCL Converter and CASAVA v1.8.

Control Files

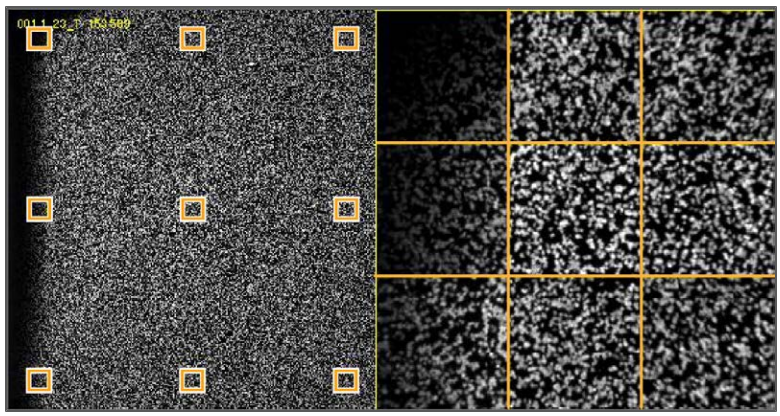
The control (*.control) files are binary files containing control results. These files are used by the BCL Converter and CASAVA v1.8.

Thumbnail Images

You can configure SCS to generate thumbnail images in *.jpg format. Thumbnail images are generated for each cycle and base.

SCS collects images from nine sections of a tile. These nine images are combined in one thumbnail image and can be used to troubleshoot a run. Thumbnail images are not suitable for image analysis.

Figure 43 Thumbnail Image



Analysis Results

Table 2 Contents of Network Analysis Folder

Key File	Subfolder	Description
*.bcl files	AnalysisFolder\Data\Intensities\Basecalls\L00X\CX.X	Each *.bcl file contains RTA base calling and base quality scoring results for one cycle, one tile. These files are the input into CASAVA v1.8.2 or the BCL Converter. For more information, see *Main Sequence Output Files* on page 55.
*.stats files	AnalysisFolder\Data\Intensities\Basecalls\L00X\CX.X	*.stats files contain RTA base calling statistics for a given cycle/tile. These files are used by CASAVA v1.8.2 or later.
*.filter files	AnalysisFolder\Data\Intensities\Basecalls	*.filter files contain filter results per tile. These files are used by CASAVA v1.8.2 or later.
SampleSheet.csv	AnalysisFolder\ AnalysisFolder\Data\Intensities\Basecalls\	Contains sample information, including indexing information. RTA copies the Sample Sheet file in the root folder of the run to the Basecalls folder.
config.xml	AnalysisFolder\Data\Intensities\ AnalysisFolder\Data\Intensities\Basecalls\	Contains meta-information about the run, image analysis, and base calling.
RunInfo.xml	AnalysisFolder\	Identifies the boundaries of the reads (including indexing reads), the Flowcell ID (if available), and the quality table selected for run.

Key File	Subfolder	Description
ErrorLog.txt	AnalysisFolder\Data	Any error during analysis is captured in the ErrorLog file. If there are no errors, no ErrorLog.txt file is generated.
CopyLog.txt	AnalysisFolder\Data	All copy events during analysis are captured in the CopyLog file.
*.cif files	AnalysisFolder\Data\Intensities\L00X\CX.X	Each binary *.cif file contains RTA image analysis results for one cycle, one tile. These files should be used in the OLB analysis software if you want to perform BUSTARD base calling; for a description, see the *Off-line Basecaller Software User Guide*.
*_pos.txt files	AnalysisFolder\Data\Intensities\	Reports the cluster coordinates. There is one *_pos.txt file for each tile. These files should be used in the OLB analysis software if you want to perform BUSTARD base calling.
*.jpg files	AnalysisFolder\Thumbnail_Images\L00X\CX.X	SCS can generate thumbnail images of nine locations from each tile. The thumbnails are generated for each cycle and base, and can be used to troubleshoot a run. For more information, see *Thumbnail Images* on page 56.

Setting Up a Sequencing Run

Chapter 3

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Introduction

The steps to set up a run are the same for any type of run with the exception of which pre-run wash recipe to use and the number of SBS Kits required for the length of your run.

- To ensure that the correct reagent positions are washed prior to your run, select the appropriate wash recipe for your run type. For more information, see *Sequencing Recipes* on page 19.
- Make sure that you prepare and combine reagents according to the number of cycles you plan to run. For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

Use a lab tracking form to record wash delivery, reagent lot numbers, and reagent weights. You can download a lab tracking form from the Illumina website.

Performing a Pre-Run Wash

Perform a pre-run wash before loading reagents on the Genome Analyzer. Run time is approximately 15 minutes for the single-read recipe and approximately 40 minutes for the paired-end recipe.

The wash recipe flushes 1 ml of PW1 (wash solution) through each reagent port on the instrument. The wash recipes used with paired-end, single-indexed, and dual-indexed sequencing runs also flush 1 ml water through reagent ports on the Paired-End Module.

After the wash, check the total volume in the waste container closely to confirm the stability of the reagent delivery system. Expected volumes from the wash cycle are a primary indicator of a stable fluid delivery system. Dry syringes and excessive bubbles indicate a problem.

Illumina-Supplied Consumables

- PW1 (provided in the SBS Kit)

User-Supplied Consumables

- Lens cleaning tissue
- 125 ml Nalgene bottles (ThermoFisher Scientific, catalog # 342040-0125)
- 175 ml Falcon bottles (BD, catalog # 352076)
- MilliQ water or laboratory grade water (for washing the Paired-End Module)

Procedure

1 Load a used flow cell.

2 In the Pump area, make sure the following values are set to prevent siphoning of reagents:
Command: Pump
To: Flow cell
Solution: 8 (PEM not enabled); 28 (PEM enabled)
Volume: 0
Aspiration Rate: 250
Dispense Rate: 2,500
With the cursor in the **Dispense Rate** field, press **Enter**.

3 If a used flow cell is not already installed on the instrument, load a used flow cell. For more information, see *Load the Flow Cell* on page 84.

4 Load PW1 solution as follows:
- 10 ml PW1 onto positions 1, 3, and 6
- 40 ml PW1 onto positions 2, 4, 5, and 7

NOTE
Rotate the tubes while holding the caps stationary to prevent crimps and twisting in the liquid delivery lines.

5 [Paired-End Module] Place at least 5 ml of laboratory grade water into 15 ml conical tubes in the following positions on the Paired-End Module:
- **Any paired-end run**—Positions 9–22
- **Single-indexed single-read run**—Positions 19, 21, 22, and 23
- **Dual-indexed single-read run**—Positions 19, 21, 22, and 23

6 Bundle the Genome Analyzer waste tubing, keeping the ends even. Wrap the tubes with parafilm and place the tube ends into a 50 ml bottle.

7 From the Run tab, select **File** | **Open Recipe** and open one of the following recipes:

Type of Run	Recipe Name
Non-Indexed Single-Read Run	GA2_PreWash_v10.xml
Single-Indexed Single-Read Run, or Dual-Indexed Single-Read Run	GA2-PEM_MP_PreWash_SR_v10.xml
Non-Indexed Paired-End Run, or Single-Indexed Paired-End Run, or Dual-Indexed Paired-End Run	GA2-PEM_PreWash_PE_v10.xml

8 Click **Start**.

9 When the wash is complete, check the delivery volume in the 50 ml tube, and record the volume on the lab tracking form.

Recipe Name	Recipe Version	Expected Volume
GA2_PreWash_v<#>.xml	v10	7 ml
GA2-PEM_MP_PreWash_SR_v<#>.xml	v10	12 ml
GA2-PEM_PreWash_PE_v<#>.xml	v10	17 ml

10 If the measured volume is less than 90% of the expected value, do the following:
   a Check for leaks.
   b Place each waste tubing in a separate 50 ml tube.
   c Repeat the wash cycle.
   d Measure each 1 ml delivery.

NOTE

Keep one 125 ml Nalgene bottle containing PW1 on position 2 during the sequencing run. Save the other bottles containing PW1 for the post-run wash.

Preparing SBS Reagents

One kit provides reagents for 36 cycles of sequencing. For runs longer than 36 cycles, you must prepare reagents from multiple kits. For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

If you are using a Genome Analyzer II, the preparation of reagents and schedule for replenishing reagents differs for positions 1, 3, and 6. See *Preparing and Replenishing Reagents on GAII* on page 173.

Reagent Containers

Bottle	Positions and Reagents	Catalog #
	175 ml Bottle Positions 1, 3, and 6 (IMR, SMX, and CLM) 175 ml bottles hold sufficient reagents for up to 151 cycles of sequencing.	BD Catalog # 352076
	125 ml Bottle Positions 4, 5, and 7 (PR1, PR2, and PR3) 125 ml Nalgene bottles connect to threaded bottle receptacles on the instrument. SBS reagents are kitted in 125 ml bottles.	ThermoFisher Scientific Catalog # 342040-0125
	150 ml Bottles Positions 4, 5, and 7 (PR1, PR2, and PR3) [Optional, User-Supplied] 150 ml bottles minimize the need to replenish PR1, PR2, and PR3 during the run. Remove the threaded bottle adapter before loading 150 ml bottles.	Corning Catalog # 431175

Thaw Reagents

For paired-end runs, thaw reagents for Read 1 and any indexing cycles. Thaw reagents for Read 2 only when you are ready to prepare reagents prior to the start of Read 2.

1 Remove IMR, LFN, and SMX from -15° to -25°C storage, and thaw at room temperature or in a beaker containing deionized water for about one hour.

If you use the beaker method, make sure that the water line does not reach the cap of the tube to prevent contamination.

Leave the HDP in -15° to -25°C storage until you are ready to use it to prepare the IMR.

CAUTION
It is important to keep the CLM away from the other components to avoid cross-contamination.
After handling the CLM container, be sure to discard your gloves and replace them with a new pair each time.

2 Remove the CLM from -15° to -25°C storage and thaw it at room temperature or in a *separate* beaker containing deionized water for about one hour.
If you use the beaker method, make sure that the water line does not reach the cap of the tube to prevent contamination.

3 Record the lot numbers of each reagent on the lab tracking form.

4 Immediately after the reagents have thawed, place them on ice. Be sure to keep the CLM in a *separate* ice bucket during reagent preparation.

Prepare PR1, PR2, and PR3

1 Invert the bottle of PR1 several times to mix the reagent.

2 Invert the bottle of PR2 several times to mix the reagent.

3 Invert the bottle of PR3 several times to mix the reagent.

4 When preparing multiple kits, combine reagents in either a 125 ml bottle or an optional user-supplied 150 ml bottle.

NOTE
The 125 ml bottle holds up to 170 ml of reagent.
The 150 ml bottle holds up to 200 ml of reagent.
Take care to avoid spillage when preparing and loading reagents.

5 Set aside excess PR1, PR2, and PR3 at room temperature to replenish during the run.

Prepare IMR

1 Pour the contents of the IMR container into one 175 ml Falcon bottle.

2 Transfer 3.52 ml of LFN to the IMR reagent.

3 Remove the HDP tube from -15° to -25°C storage and briefly pulse centrifuge.

4 Transfer 330 µl of HDP into the IMR/LFN mix.

5 Cap the bottle of IMR mix tightly and invert five times to mix.

6 Centrifuge at 1,000 xg for one minute at 4°C.

7 When preparing multiple kits, combine up to 185 ml of IMR into one 175 ml bottle.

NOTE
The 175 ml bottle holds up to 185 ml of reagent when it is filled to the top.
Take care to avoid spillage when preparing and loading reagents.

8 Place the IMR on ice until you are ready to load reagents.

Prepare SMX

1 Invert the bottle of SMX several times to mix well.

2 Pour the SMX into one 175 ml Falcon bottle.

3 If your run requires more than three kits, use SMX from a fourth kit to fill the bottle with 185 ml of SMX.

NOTE
The 175 ml bottle holds up to 185 ml of reagent when it is filled to the top. Take care to avoid spillage when preparing and loading reagents.

4 Cap the container and place the SMX on ice until you are ready to load reagents.

5 Set aside excess SMX at 2° to 8°C to replenish reagent during the run.

Prepare CLM

1 Invert the CLM tube several times to mix well.

2 Centrifuge at 1,000 xg for one minute at 4°C.

3 Pour the contents of the CLM container into one 175 ml Falcon bottle.

4 If your run requires more than four kits, use CLM from a fifth kit to fill the bottle with 185 ml of CLM.

NOTE
The 175 ml bottle holds up to 185 ml of reagent when it is filled to the top. Take care to avoid spillage when preparing and loading reagents.

5 Cap the container and place the CLM in a *separate* ice bucket until you are ready to load reagents.

6 Discard your gloves and replace them with a new pair.

Reagent Kits and Replenishing Cycles

This section provides the number of TruSeq SBS Kits required for each reagent used in a sequencing run of various lengths and run types, and the required cycles to replenish reagents.

If you are using a Genome Analyzer II, the schedule for replenishing reagents differs for positions 1, 3, and 6. See *Preparing and Replenishing Reagents on GAII* on page 173.

Using the Reagent Kits and Replenishing Cycles Tables

- The number of required kits are listed for the first read and any indexing cycles. The number of required kits for Read 2 are listed separately. Always prepare fresh SBS reagents for Read 2.
- The "X" indicates which reagents you need to replenish at the indicated cycles.
- The "X" at the start of Read 1 and start of Read 2 indicates that a full set of fresh reagents are required before starting the read.
- For single-read runs, disregard the Read 2 portion of the table.
- For paired-end dual-indexed runs, the seven chemistry-only cycles performed during the Index 2 (i5) Read are calculated for reagent use; however, these cycles are not counted in the total number of cycles performed in the run.

For reagent preparation instructions, see *Preparing SBS Reagents* on page 63.

Best Practices for Replenishing Reagents

If you need to replenish reagents during the run, use the following best practices to ensure the best results:

- Replace PR1, PR2, PR3, IMR and CLM during an imaging step.
- Replace CLM last when replacing reagents during an imaging step. Always discard your gloves and replace them with a new pair after handling CLM.
- Replace SMX early in a chemistry step.
- Use the Reagent Volumes feature in the Data Collection Software to track the number of cycles remaining before reagents need to be replenished.
  For more information, see *Reagent Tracking* on page 41.

151-Cycle Non-Indexed Run

	Container Size:	175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
	Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
	Number of SBS Kits:	4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 105–108				X	X	X			
	Cycle 151									
	Number of SBS Kits:	4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 152	X	X	X	X	X	X	X	X	X
	Cycle 256–259				X	X	X			
	Cycle 302									

- SMX—Combine 185 ml of prepared SMX. Discard the remaining reagent.

- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Set aside remaining reagent to replenish during the run.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard the remaining reagent.

126-Cycle Non-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 105–108				X	X	X			
	Cycle 126									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 127	X	X	X	X	X	X	X	X	X
	Cycle 231–234				X	X	X			
	Cycle 252									

- SMX—Combine 185 ml of prepared SMX. Discard the remaining reagent.
- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Set aside remaining reagent to replenish during the run.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard the remaining reagent.

101-Cycle Non-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 101									
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 102	X	X	X	X	X	X	X	X	X
	Cycle 202									

- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Discard the remaining reagent.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard the remaining reagent.

51-Cycle Non-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	51									
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 2	Start of Read 2 Cycle 52	X	X	X	X	X	X	X	X	X
	102									

- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Discard the remaining reagent.

36-Cycle Non-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		1	1	1	1	1	1	1	1	1
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 36									
Number of SBS Kits:		1	1	1	1	1	1	1	1	1
Read 2	Start of Read 2 Cycle 37	X	X	X	X	X	X	X	X	X
	Cycle 72									

151-Cycle Single-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	4	5	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 105–108				X	X	X			
	Cycle 151									
	User Wait after Read 1		X					X	X	X
Index Read	Cycle 152									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 159/160	X	X	X	X	X	X	X	X	X
	Cycle 264–267				X	X	X			
	Cycle 309/310									

- SMX—Combine 185 ml of prepared SMX. Set aside remaining reagent to replenish after Read 1.
- CLM—Combine 185 ml of prepared CLM. Discard remaining reagent.
- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Set aside remaining reagent to replenish during the run.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Set aside remaining reagent to replenish after Read 1.

146-Cycle Single-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 105–108				X	X	X			
	Cycle 146									
	User Wait after Read 1		X							
Index Read	Cycle 147									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 154/155	X	X	X	X	X	X	X	X	X
	Cycle 259–262				X	X	X			
	Cycle 299/300									

- SMX—Combine 185 ml of prepared SMX. Set aside remaining reagent to replenish after Read 1.
- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Set aside remaining reagent to replenish during the run.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard remaining reagent.

126-Cycle Single-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 105–108				X	X	X			
	Cycle 126									
	User Wait after Read 1									
Index Read	Cycle 127									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 134/135	X	X	X	X	X	X	X	X	X
	Cycle 238–241				X	X	X			
	Cycle 259/260									

- SMX—Combine 185 ml of prepared SMX. Discard remaining reagent.
- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Set aside remaining reagent to replenish during the run.

101-Cycle Single-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 101									
	User Wait after Read 1									
Index Read	Cycle 102									
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 109/110	X	X	X	X	X	X	X	X	X
	Cycle 209/210									

- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Discard remaining reagent.

51-Cycle Single-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 51									
	User Wait after Read 1									
Index Read	Cycle 52									
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 2	Start of Read 2 Cycle 59/60	X	X	X	X	X	X	X	X	X
	Cycle 109/110									

- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Discard remaining reagent.

36-Cycle Single-Indexed Run

Container Size:	175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:	2	2	2	2	1	2	2	1	2

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 36									
	User Wait after Read 1									
Index Read	Cycle 37									
Number of SBS Kits:		1	1	1	1	1	1	1	1	1
Read 2	Start of Read 2 Cycle 43/44	X	X	X	X	X	X	X	X	X
	Cycle 79/80									

151-Cycle Dual-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	5	5	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 105–108				X	X	X			
	Cycle 151									
	User Wait after Read 1		X					X	X	X
Index Reads	Cycle 152									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 168	X	X	X	X	X	X	X	X	X
	Cycle 272–275				X	X	X			
	Cycle 318									

- SMX—Combine 185 ml of prepared SMX. Set aside remaining reagent to replenish after Read 1.
- CLM—Combine 185 ml of prepared CLM. Discard remaining reagent.
- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Set aside remaining reagent to replenish during the run.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Set aside remaining reagent to replenish after Read 1.

146-Cycle Dual-Indexed Run

Container Size:	175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:	4	5	5	3	2	3	3	2	3

	Container Size:	175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
	Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 105–108				X	X	X			
	Cycle 146									
	User Wait after Read 1		X					X	X	X
Index Reads	Cycle 147									
	Number of SBS Kits:	4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 163	X	X	X	X	X	X	X	X	X
	Cycle 267–272				X	X	X			
	Cycle 308									

- SMX—Combine 185 ml of prepared SMX. Set aside remaining reagent to replenish after Read 1.
- CLM—Combine 185 ml of prepared CLM. Discard remaining reagent.
- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Set aside remaining reagent to replenish during the run.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Set aside remaining reagent to replenish after Read 1.

133-Cycle Dual-Indexed Run

	Container Size:	175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
	Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
	Number of SBS Kits:	4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 105–108				X	X	X			
	Cycle 133									
	User Wait after Read 1		X							
Index Reads	Cycle 134									
	Number of SBS Kits:	4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 150	X	X	X	X	X	X	X	X	X
	Cycle 254–257				X	X	X			
	Cycle 282									

- SMX—Combine 185 ml of prepared SMX. Set aside remaining reagent to replenish after Read 1.
- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Set aside remaining reagent to replenish during the run.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard remaining reagent.

126-Cycle Dual-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 105–108				X	X	X			
	Cycle 126									
	User Wait after Read 1		X							
Index Reads	Cycle 127									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 143	X	X	X	X	X	X	X	X	X
	Cycle 238–241				X	X	X			
	Cycle 268									

- SMX—Combine 185 ml of prepared SMX. Set aside remaining reagent to replenish after Read 1.
- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Set aside remaining reagent to replenish during the run.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard remaining reagent.

101-Cycle Dual-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		3	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 101									
	User Wait after Read 1									
Index Reads	Cycle 102									
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 118	X	X	X	X	X	X	X	X	X
	Cycle 218									

- SMX—Combine 185 ml of prepared SMX. Discard remaining reagent.
- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Discard remaining reagent.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard remaining reagent.

93-Cycle Dual-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 93									
	User Wait after Read 1									
Index Reads	Cycle 94									
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 110	X	X	X	X	X	X	X	X	X
	Cycle 202									

- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Discard remaining reagent.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard remaining reagent.

51-Cycle Dual-Indexed Run

Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 51									
	User Wait after Read 1									
Index Reads	Cycle 52									
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 2	Start of Read 2 Cycle 68	X	X	X	X	X	X	X	X	X
	Cycle 118									

- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Discard remaining reagent.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard remaining reagent.

36-Cycle Dual-Indexed Run

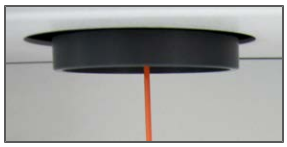
Container Size:		175 ml Bottle			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 36									
	User Wait after Read 1									
Index Reads	Cycle 37									
Number of SBS Kits:		1	1	1	1	1	1	1	1	1
Read 2	Start of Read 2 Cycle 53	X	X	X	X	X	X	X	X	X
	Cycle 88									

- PR1, PR2, and PR3 in 125 ml bottles—Combine 150–170 ml of each prepared reagent. Discard remaining reagent.
- PR1, PR2, and PR3 in 150 ml bottles—Combine 200 ml of each prepared reagent. Discard remaining reagent.

Loading SBS Reagents

1 Weigh each reagent and record the weight on the lab tracking form.

2 If you are using optional 150 ml bottles in positions 4, 5, and 7, remove the bottle adapters before loading reagents.



3 Invert each container several times to mix the reagent.

4 Centrifuge the SMX, IMR, and CLM at 4°C to 1000 xg for 1 minute.

5 Load reagents in the following positions. Load CLM last to avoid cross-contamination.

Figure 44 Reagent Positions

Table 3 Reagent Positions

Position	Contents	Description	Container Size
1	IMR	Incorporation Mix	175 ml Bottle
2	PW1	Wash Solution	125 ml Bottle
3	SMX	Scan Mix	175 ml Bottle
4	PR1	High Salt Buffer	125 ml Bottle or 150 ml bottle
5	PR2	Incorporation Buffer	125 ml Bottle or 150 ml bottle
6	CLM	Cleavage Mix	175 ml Bottle
7	PR3	Cleavage Buffer	125 ml Bottle or 150 ml bottle

6 Insert the appropriate reagent tube into the cap of each container in positions 1, 3, and 6.

Tracking Reagents

The reagent tracking feature helps you track how many cycles the current volume of reagents will last. For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

1. Select **Reagents** | **Current Volumes** from the toolbar at the top of the Data Collection software screen. The Current Reagent Volumes dialog box opens.
2. Click **Reset Volumes**. The Reset Reagent Volumes dialog box opens.
3. Enter the estimated number of cycles that you expect the reagents to last and click **OK**. The number of cycles remaining for the next set of reagents is updated.
4. Click **Close**. Alternatively, you can leave the dialog box open while the run executes.
   The Current Reagent Volumes dialog box opens when the number of cycles completed equals the value set in Reset Volumes, minus the number of cycles before email notification set in the Reagent Configuration.
5. To record the barcode ID of the reagents, click **Reagent Barcodes**. The Reagent Barcode dialog box opens.
6. Enter the barcode IDs for each reagent and click **OK**. Barcode IDs for each run are stored in ReagentIds.xml.
7. If you are performing a multiplexed run, click the Indexing Reagents tab. The Indexing Reagents tab is only visible if a multiplexing recipe is open.
8. If you are performing a paired-end run, click the PE Reagents tab. The PE Reagents tab is only visible if a paired-end recipe is open.
9. Click **OK**.

Priming Reagents

To confirm the stability of the reagent delivery system, collect priming deliveries through all valve ports out to a waste volume, and then check total volume.

NOTE
Priming volumes are a key indicator of a stable fluid delivery system. The measured volumes must be within 10% of normal for optimal sequencing performance.

1. Loosen and remove the waste tubing from the waste bottle, bundle all waste tubes so that the ends are even with each other, and then wrap them with parafilm.
2. Place the bundled tube ends into a 50 ml conical tube.
3. Select **File** | **Open Recipe** and open GA2_Prime_v10.xml.
4. Click **Start**.
5. Collect the waste and ensure that the volume is 6.4 ml.
6. Record the delivery volume on the lab tracking form.
   If the measured volume differs from the expected value by more than 10%, repeat the priming procedure.
   If the delivered volume still differs from the expected volume by more than 10%, see *Troubleshooting Priming Volume* on page 150.
7. Return the waste tubing to the waste bottle.

Unloading the Flow Cell and Prism

Perform the following steps to safely remove the used flow cell. You also need to remove the prism for cleaning.

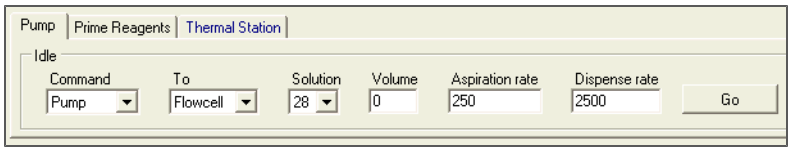
1 From the Manual Control/Setup tab, click **Load** in the Load Flow Cell area to bring the stage to the front of the instrument and raise the lens.

2 In the Pump area, enter the following settings to prevent siphoning of reagents:

Command: Pump
To: Flow cell
Solution: 8 (PEM not enabled); 28 (PEM enabled)
Volume: 0
Aspiration rate: 250
Dispense rate: 2500

Click **Go** or press **Enter** .

Figure 45 Pump Settings to Prevent Siphoning

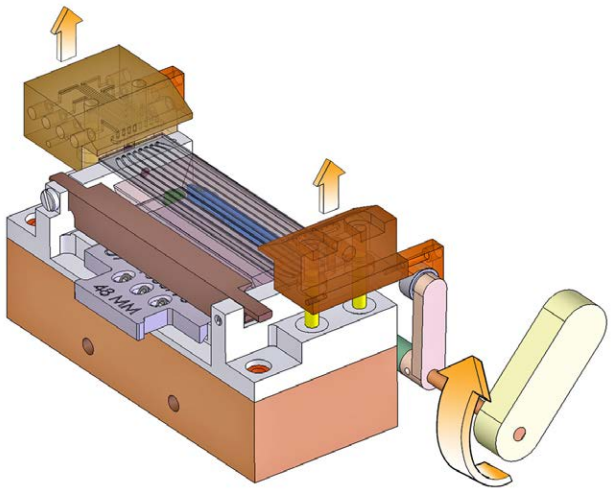


3 Select **Instrument** | **Unlock Door** to release the door to the imaging compartment, and then raise the door.

4 With the cursor in the **Dispense Rate** field, press **Enter**.

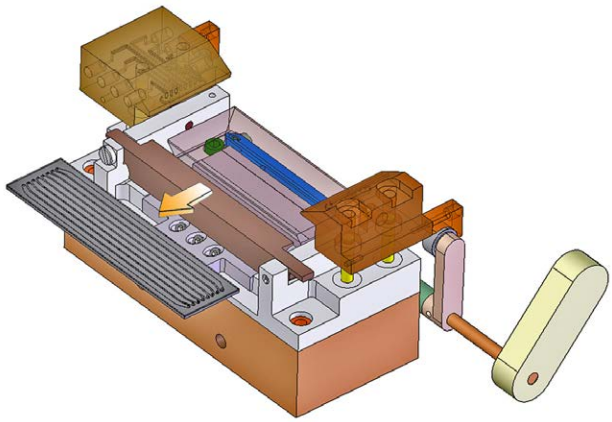
5 Turn the manifold handle clockwise to lift the manifolds.

Figure 46 Lift Front and Rear Manifolds



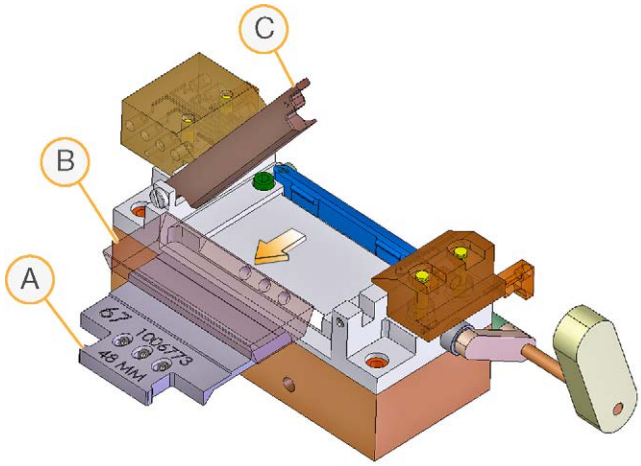
6 Slide the flow cell to the left to clear the manifolds, and then lift it out of the instrument.

Figure 47 Remove the Flow Cell



7 Raise the beam dump.

Figure 48 Remove the Prism



A Prism Base Handle
B Prism
C Beam Dump in Up Position

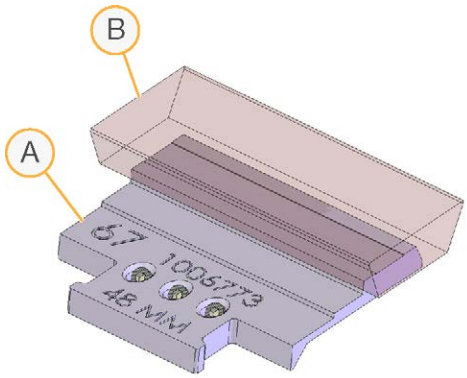
CAUTION
Always wear powder-free latex gloves when handling the prism. Hold the prism by the metal prism base only. Avoid chipping the prism, as this may degrade optical function.

8 Hold the metal prism base by the prism base handle and slide the metal prism base to the left to remove the prism.

Installing the Prism

The prism serves a critical optical function and sits under the flow cell on top of the metal prism base.

Figure 49 Prism



A Prism Base
B Prism

CAUTION
Exercise extreme care when handling the prism to prevent chipping, as this may degrade optical function. Hold the prism by the metal prism base only. The right side is the surface of laser entry.

User-Supplied Consumables

- Lint-free lens cleaning tissue
- 100% ethanol or Spectrophotometer-grade methanol

Clean the Prism

1. Wearing new powder-free latex gloves, wipe down any spilled oil from the mounting rails, manifolds, Peltier heater, and beam dump.
2. Gently wash the prism with a stream of ethanol or methanol, and wipe the metal prism base with a lens cleaning tissue.
3. Using a lens cleaning tissue wet with ethanol or methanol, wipe off the surface of the prism in a single sweeping motion until it is clean.
4. To tell if the prism is clean, observe it under direct light from a variety of angles. Pay particular attention to the surface that will face the laser entry (right-hand side when sitting in the holder) and the large top surface.
5. Protect the prism from dust until you load it onto the instrument.

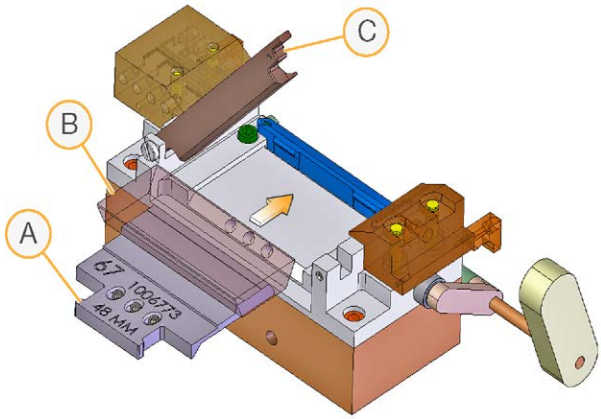
Install the Prism

1. With a lens cleaning tissue wet with ethanol or methanol, wipe the recessed surface of the prism holder to remove oil that may have been spilled during the previous run.
2. Lift the beam dump.

CAUTION
Be very careful not to touch the laser mount when you install the prism. A service call is required for calibration.

3 Slide the metal prism base to the right until it stops.

Figure 50 Install the Prism



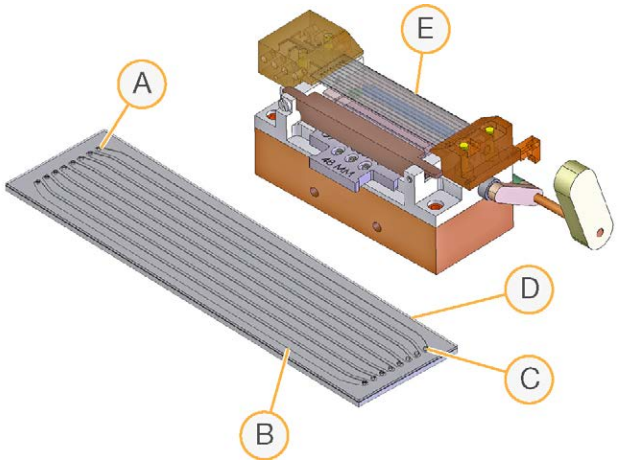
A Prism Base
B Prism
C Beam Dump in Up Position

4 With prism in place, lower the beam dump until it locks into position.

Loading the Flow Cell

The flow cell is located above the prism and rests on the manifold mounting rails. It is important that the flow is clean before loading, that the lanes are free of bubbles, and that oil is properly applied between the prism and flow cell.

Figure 51 Loading the Flow Cell



A Outlet Ports
B Lane 1 on Left Side Edge
C Inlet Ports
D Barcode on Right Side Edge
E Flow Cell Installed

User-Supplied Consumables

- Lint-free lens cleaning tissue
- 100% ethanol or Spectrophotometer-grade methanol

Clean the Flow Cell

1 Wearing new gloves, hold the edges of the flow cell with two fingers. Ensure the inlet and outlet ports are facing *up*.

NOTE
When cleaning the flow cell, use gentle pressure.

2 Wet a lens cleaning tissue with ethanol or methanol, and wipe off each side of the flow cell with a single sweeping motion. Repeat, refolding the tissue with each wipe, until the flow cell is completely clean.

3 Protect the flow cell from dust until you load it onto the instrument.

Enter the Flow Cell ID

TIP
Placing the flow cell on clean white background makes it easier to read the barcode.

1 In the Load Flowcell area on the Manual Control/Setup screen, enter the flow cell ID.

- If you leave the ID field blank, a warning appears, and you must enter an ID to continue.
- You can enter any characters from the keyboard. The barcode IDs are validated using the validation expression in the RCMConfig.xml file (FlowCellID= "[F][C][0-9][0-9][0-9][0-9]").

NOTE
After running a new recipe on a previously abandoned run, tracking of the flow cell ID may prevent you from using the same flow cell. To continue, set the <IgnoreFlowCellIDCheck> field in the RCMConfig.xml file to **true**.

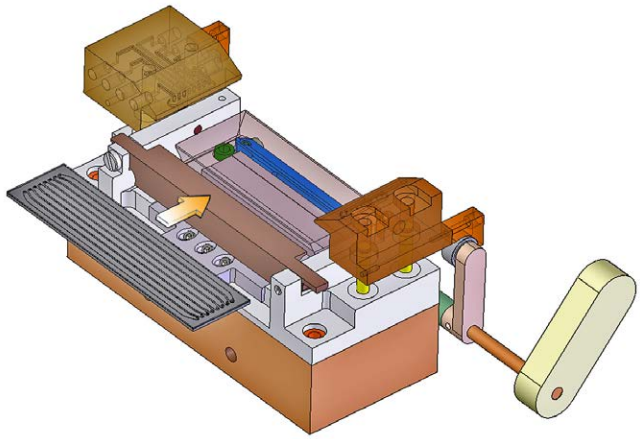
2 Click **Load**. The stage moves to the loading position.

Load the Flow Cell

1 Using a lens cleaning tissue, gently apply pressure on the underside of the front manifold to absorb excess liquid.

2 With the inlet and outlet ports facing *up*, slide the flow cell on top of the front and rear mounting rails. Press it gently against the stops on the right side.

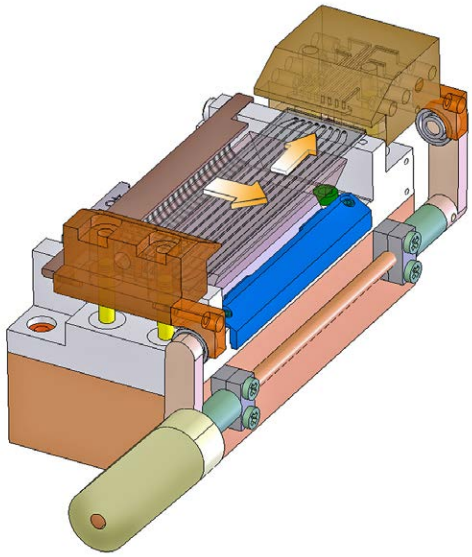
Figure 52 Load the Flow Cell



3 Slide the flow cell toward the rear until you encounter the rear stop.

4 Test proper placement by applying gentle pressure toward the rear, then toward the right to ensure the flow cell is pressed against both stops.

Figure 53 Position the Flow Cell

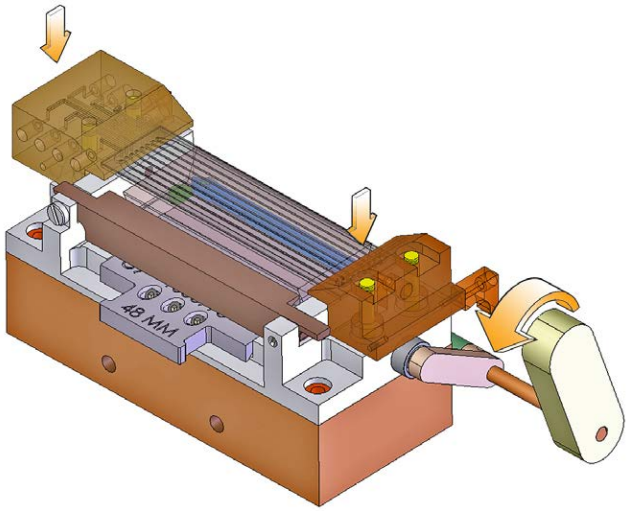


CAUTION
The manifolds are spring-loaded. Carefully control the spring action when lowering the manifolds onto the flow cell.

5 While holding the flow cell against the stops with one hand, carefully rotate the manifold handle counterclockwise with the other hand to lower the manifolds into place.

6 Gently press downward on both manifolds to ensure they have seated properly.

Figure 54 Lower the Manifold



Check Reagent Delivery

This procedure pumps PR2 (Incorporation Buffer) through the flow cell to check for leaks. Excessive air bubbles passing through the lanes indicate leaks at the manifold.

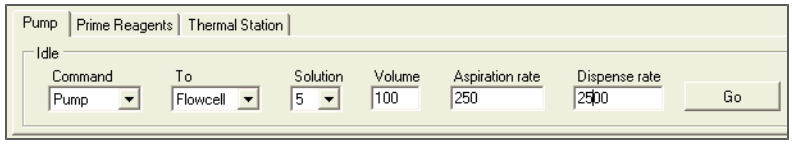
1 Bundle all of the lines together with parafilm, making sure to keep the ends even, and then place the bundle into a 1.5 ml tube.

2 In the Pump area on the Manual Control/Setup tab, enter the following settings to prevent siphoning of reagents:

Command: Pump to Flow Cell
To: Flow cell
Solution: 5
Volume: 100
Aspiration Rate: 250
Dispense Rate: 2500

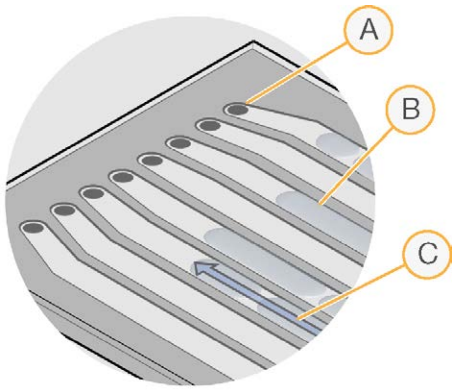
Click **Go** or press **Enter**.

Figure 55 Pump Settings to Prevent Siphoning



3 Visually confirm that liquid is flowing through the flow cell and look closely for any air bubbles moving toward the rear flow cell port of each lane.

Figure 56 Check for Bubbles

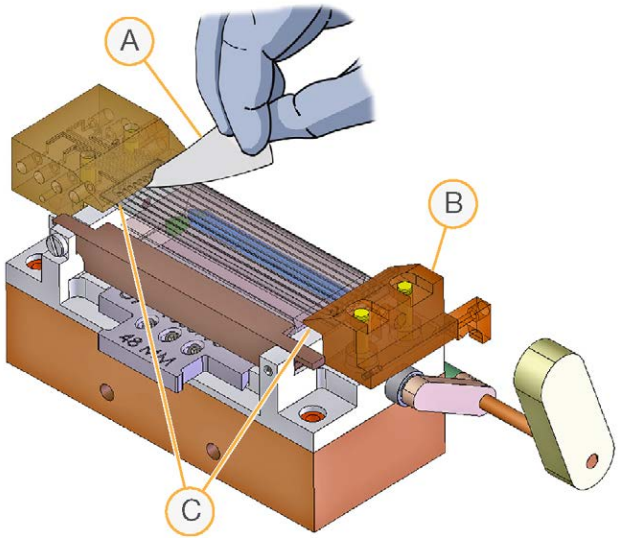


A Outlet Port
B Example of Bubbles
C Flow Direction

If bubbles persist, it might indicate that the flow cell is not properly seated on the flow cell stage. Check that the front manifold is properly seated against the flow cell, and try the leak test again.

4 When the liquid has successfully displaced the air in all eight lanes, check for leaks where the flow cell touches the manifold using a lens cleaning tissue.

Figure 57 Test for Leaks



A Lens Cleaning Tissue
B Manifolds in Down Position
C Test Locations for Leaks

If a leak is present, reseat the flow cell and repeat the leak test. See *Troubleshooting Leaks* on page 151.

5 Measure the flow for each of the eight lanes three times and record the measured volumes on the lab tracking form. The expected volume is 800 µl.
The average measured volume must be within 10% of expected for optimal sequencing performance. If the average measurement differs from the expected volume by more than 10%, have the instrument checked by an Illumina Field Service Engineer.

Apply Oil

Immersion oil between the prism and flow cell is a critical optical element. The layer of oil must be uniform and continuous to create total internal reflection.

- Too little oil can cause a loss of illumination in lane 8 and nearest the inlet and outlet manifolds.
- Too much oil can cause the oil to wick over the right side of the prism or on top of the flow cell resulting in images that are out of focus. Wicking may happen immediately or later during a run when the oil heats up.

User-Supplied Consumables

- Immersion oil, refractive index 1.473 (Cargille, catalog # 19570)

Best Practices

- Always work from the left side of the flow cell. Excess oil on the right surface of the prism can wick over the edge causing optical aberrations.
- Let all of the dispensed oil wick between the flow cell and the prism as far as it will go before dispensing more.

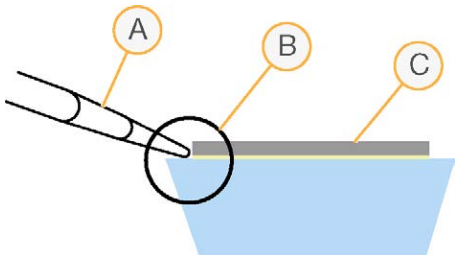
- The pipette tip must not move faster than the leading edge of the oil wicking under the flow cell.
- Avoid touching the edge of the flow cell with the pipette tip. The oil can wick to the top surface of the flow cell.
- Do not apply oil too close to the ends of the prism. Maintain a distance of 1 cm from each end of the prism.

Procedure

1 Aspirate 150 µl of oil into the pipette, ensuring that there are no air bubbles in the oil in the pipette tip. You may not need to dispense all of the immersion oil.
If you are using a Genome Analyzer II, aspirate 100 µl of oil.

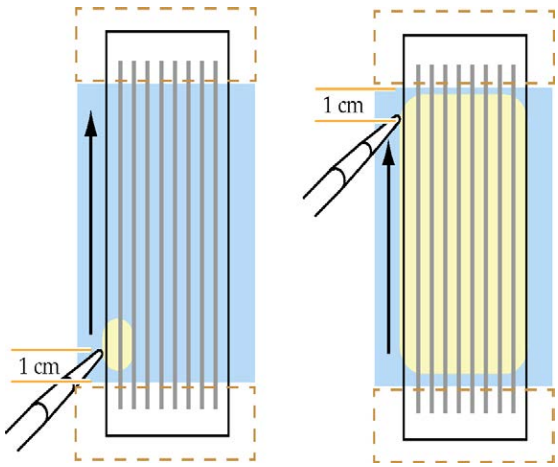
2 Place the pipette tip on the prism at the gap between the top surface of the prism and the front-left side of the flow cell, about 1 cm beyond the inlet manifold. Hold the pipette with two hands, using one hand to support and guide the tip.

Figure 58 Pipette Tip Location



A Pipette
B Left Side of Flow Cell
C Flow Cell

Figure 59 Pipette Tip Direction



3 Dispense the oil *slowly*. Before the oil wicks to the right side of the flow cell, slide the pipette tip in small steps towards the rear, steadily dispensing more oil along the way.
Observe the movement of the oil. Ensure there are no bubbles forming between the flow cell and the prism.

4 Stop moving the pipette when the tip is about 1 cm short of the rear manifold.

You have dispensed enough oil when it has wicked to the right edge of the flow cell.

5 Inspect the gap underneath the flow cell and ensure the following statements are true:
- There is a uniform layer of oil.
- No bubbles exist between the flow cell and prism.
- There is no oil on the top of the flow cell.
- The right surface of the prism is clean.

If anything is unsatisfactory, remove the flow cell and prism, clean them both thoroughly with alcohol wipes, reload, and repeat the application.

6 Use an ethanol wipe to clean the bottom surface of the Peltier heater. This ensures that no splashed immersion oil will be stamped on the flow cell by the Peltier.

7 Close the instrument door.

Performing Single-Read Runs

Chapter 4

Introduction 92
Non-Indexed Single-Read Workflow 93
Performing a Non-Indexed Single-Read Run 94
Single-Indexed Single-Read Workflow 95
Performing a Single-Indexed Single-Read Run 97
Dual-Indexed Single-Read Workflow 101
Performing a Dual-Indexed Single-Read Run 103

Introduction

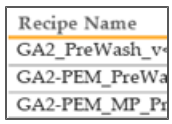
This section describes three types of single-read sequencing runs:

- *Performing a Non-Indexed Single-Read Run* on page 94
- *Performing a Single-Indexed Single-Read Run* on page 97
- *Performing a Dual-Indexed Single-Read Run* on page 103

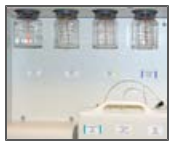
Each run type differs in workflow and requires specific reagents and recipes.

For more information, see *Sequencing Consumables* on page 13 and *Sequencing Recipes* on page 19.

Non-Indexed Single-Read Workflow



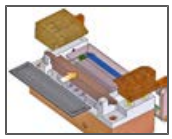
Load water onto the GA and PEM, and perform a pre-run wash using a single-read pre-run wash recipe.



Prepare SBS reagents for the number of cycles in your run.

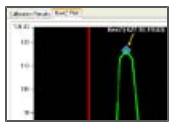
Load SBS reagents.

Start the priming recipe to prime reagents.



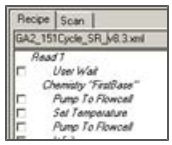
Install the prism and flow cell, and check for reagent delivery.

If no leaks are present, apply oil to the flow cell.



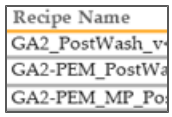
Start the sequencing recipe.

When prompted after first base incorporation, check quality metrics in the calibration results window and first base report.



Resume the recipe to perform Read 1.

Replenish SBS reagents as needed during the run.



When the run is complete, replace reagents with containers of water.

Perform a post-run wash using a single-read post-run wash recipe.

Performing a Non-Indexed Single-Read Run

After completing the run preparation steps, you are ready to start the single-read sequencing recipe.

Start the Single-Read Recipe

1. If necessary, modify the recipe to perform the number of cycles required for your run using the recipe editor.
2. Select **File** | **Open** and open GA2_151Cycle_SR_v<#>.xml.
3. Click **Start**. The software makes a copy of the recipe file and stores it in the current run folder. If you need to stop work at any point, you can reopen the recipe from that location and continue from where you left off.
4. If prompted, browse to the sample sheet for this flow cell, and then click **OK**.

Check Quality Metrics

The recipe proceeds to first base incorporation, which incorporates the first nucleotide. First base incorporation takes approximately 20 minutes.

Directly following first base incorporation, the recipe performs auto calibration to adjust the Z position and find the best focus.

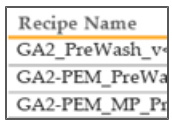
1. Click **View** | **Calibration Results** from the Data Collection toolbar to open the Focus Calibration Result window.
2. Click the Calibration Results tab and check the following specifications:
   Goodness of fit: ≥ 0.9900
   Sensitivity: 350–400
3. If both values are within the specified range, click **Accept**. If either or both of the values do not meet the specification, move to another tile and repeat auto calibration.
4. View the First Base Report after the completion of first base incorporation. The First Base Report summarizes cluster counts, intensity values, focus metrics, focus position, and flow cell tilt.

Continue the Sequencing Run

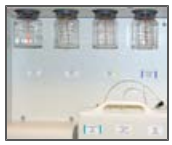
If you are satisfied with the results of first base incorporation, follow these instructions to continue the sequencing run.

1. Click **OK** to resume the recipe.
2. As needed, replenish reagents at the recommended cycles for your run.
   For more information, see *Reagent Kits and Replenishing Cycles* on page 66.
3. When the run is complete, weigh each of the SBS reagents on the Genome Analyzer. Record the weights on the lab tracking form.
4. Proceed to *Performing a Post-Run Wash* on page 143.

Single-Indexed Single-Read Workflow



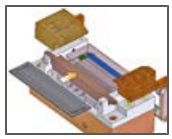
Load water onto the GA and PEM, and perform a pre-run wash using a indexed single-read pre-run wash recipe. The indexed single-read pre-run wash recipe washes the GA and PEM.



Prepare SBS reagents for the number of cycles in your run.

Load SBS reagents.

Start the priming recipe to prime reagents.



Install the prism and flow cell, and check for reagent delivery.

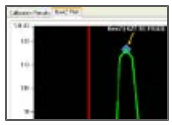
If no leaks are present, apply oil to the flow cell.

Start the sequencing recipe.

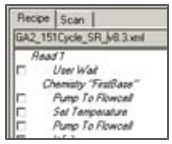
Load water onto the Paired-End Module in preparation of the PEM water prime, and then resume the recipe.

When prompted after the PEM water prime is complete, check the volume in each tube to confirm proper delivery.

Resume the sequencing recipe to proceed to first base incorporation.



When prompted after first base incorporation, check quality metrics in the first base report and calibration results window.



Resume the recipe to begin Read 1.

Replenish SBS reagents during Read 1 as needed.

Prepare indexing reagents.

When prompted after completion of Read 1, load indexing reagents onto the Paired-End Module, and replenish SBS reagents as needed.

Resume the recipe to perform Index 1 (i7) Read.

When prompted after completion of the Index Read, replace reagents on the Paired-End Module with containers of water in preparation of the PEM water wash.

Resume the recipe to perform the PEM water wash.

Recipe Name
GA2_PostWash_v
GA2-PEM_PostWa
GA2-PEM_MP_Po

After the completion of the PEM water wash, replace all reagents with containers of water.

Perform a post-run wash using an indexed single-read post-run wash recipe.

Performing a Single-Indexed Single-Read Run

After completing the run preparation steps, you are ready to start the single-index single-read sequencing recipe.

You must use the appropriate recipe version and sequencing primers for the libraries you are sequencing. For more information, see *Sequencing Primer Requirements* on page 16.

Start a Single-Indexed Single-Read Recipe

1. If necessary, modify the recipe to perform the number of cycles required for your run using the recipe editor.
2. Select **File** | **Open** and open one of the following recipes depending on the Paired-End Module you are using and the type of libraries you are sequencing:

Use With	Recipe Name
Single-indexed libraries	GA2-PEM_MP_151+7Cycle_SR_v8.5 GA2-PEM2X_MP_151+7Cycle_SR_v8.5px
Dual-indexed libraries	GA2-PEM_MP_151+8Cycle_SR_v10 GA2-PEM2X_MP_151+8Cycle_SR_v10px

3. Click **Start**.
4. If prompted, browse to the sample sheet for this flow cell, and then click **OK**.

NOTE
To view indexing results in Sequencing Analysis Viewer (SAV), it is essential that you load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to properly report indexing information. For more information, see the *Sequencing Analysis Viewer User Guide*, Part # 15020619.

Perform the PEM Water Prime

The PEM water prime verifies correct operation of the Paired-End Module.

1. When prompted, load 2 ml water in positions 19, 21, and 22.
2. Click **OK**. The recipe primes water through the lines of the Paired-End Module.
3. Upon completion, measure the volume in each tube to confirm proper delivery and record the volume on the lab tracking form.
4. Click **OK** to proceed to first base incorporation.

Check Quality Metrics

The recipe proceeds to first base incorporation, which incorporates the first nucleotide. First base incorporation takes approximately 20 minutes.

Directly following first base incorporation, the recipe performs auto calibration to adjust the Z position and find the best focus.

1. Click **View** | **Calibration Results** from the Data Collection toolbar to open the Focus Calibration Result window.
2. Click the Calibration Results tab and check the following specifications:

Goodness of fit: ≥ 0.9900
Sensitivity: 350–400

3 If both values are within the specified range, click **Accept**. If either or both of the values do not meet the specification, move to another tile and repeat auto calibration.

4 View the First Base Report after the completion of first base incorporation. The First Base Report summarizes cluster counts, intensity values, focus metrics, focus position, and flow cell tilt.

Perform Read 1

If you are satisfied with the results of first base incorporation, follow these instructions to perform Read 1.

1 Click **OK** to resume the recipe and continue the run.

2 As needed, replenish reagents at the recommended cycles for your run.
For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

3 When Read 1 is complete, weigh each of the reagents used for Read 1. Record the weights on the lab tracking form.

Preparing Indexing Reagents

Indexed sequencing requires indexing reagents provided in the TruSeq Cluster Kit. Make sure that you use the appropriate sequencing primer for the library type that you are sequencing.

Library Type	Index 1 (i7)
Nextera libraries	HP12
TruSeq HT libraries	HP8 or HP12
TruSeq LT/v2 libraries	HP8 or HP12
TruSeq Small RNA libraries	HP8 or HP12

Indexing reagents take less than 30 minutes to prepare.

Table 4 Indexing Reagents

Reagents	Kit Name
HP3	TruSeq PE Cluster Kit (Indexing Reagents Box)
HT2	TruSeq PE Cluster Kit (Indexing Reagents Box)
HP8 or HP12	TruSeq PE Cluster Kit (Indexing Reagents Box) TruSeq Dual Index Sequencing Primer Box

NOTE
HP8, provided in the TruSeq Cluster Kit, is not compatible with dual-indexed Nextera libraries. You must use **HP12** from the TruSeq Dual Index Sequencing Primer Box.

Thaw Reagents

1 Remove the reagents from -15° to -25°C storage. Confirm that you are using the appropriate sequencing primers for your library type and run type.

2 Thaw reagents in a beaker filled with room temperature deionized water for about 20 minutes, or until reagents are fully thawed.

Prepare HP3

1 Invert the tube of HP3 five times to mix the reagent, and then pulse centrifuge.

2 Transfer 1,900 µl of PW1 into a 15 ml Sarstedt conical tube and add 100 µl of HP3.

3 Invert the tube five times to mix the reagent.

4 Label the tube of diluted HP3 **Reagent #19**.

5 Centrifuge at 1,000 rpm for one minute.

6 Set aside at room temperature.

Prepare HT2

1 Invert the tube of HT2 five times to mix the reagent.

2 Label the tube of HT2 **Reagent #21**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside at room temperature.

Prepare HP8

1 Invert the tube of HP8 five times to mix the reagent.

2 Briefly pulse centrifuge to collect droplets.

3 Label the tube of HP8 **Reagent #22**.

4 Set aside at room temperature.

Prepare HP12

You must use HP12 for sequencing dual-indexed Nextera libraries.

1 Invert the tube of HP12 five times to mix the reagent.

2 Briefly pulse centrifuge to collect droplets.

3 Label the tube of HP12 **Reagent #22**.

4 Set aside at room temperature.

Load Indexing Reagents

When prompted after the completion of Read 1, load indexing reagents.

1 Weigh each reagent before loading it onto the Paired-End Module and record the weight on the lab tracking form.

2 Load reagents onto the Paired-End Module in the following positions:

Position	Reagent	Description
19	HP3	Denaturation Solution
21	HT2	Wash Buffer
22	HP8 or HP12	Index 1 (i7) Sequencing Primer Mix

3 Connect the reagent tubes to the corresponding port position on the Paired-End Module.

4 Place the waste tube into a waste container or a 15 ml conical tube.

5 When you have finished loading reagents, click **OK** to resume the sequencing recipe.

Perform the Index Read

The recipe primes the Paired-End Module for Index Read preparation and proceeds directly to the Index Read.

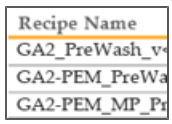
Wash the Paired-End Module

1 When prompted, replace reagent tubes on the Paired-End Module with 15 ml conical tubes containing at least 10 ml of MilliQ water or laboratory-grade water.

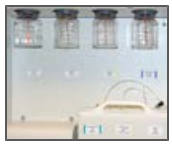
2 Click **OK** to proceed to the Paired-End Module water wash.

3 Proceed to *Performing a Post-Run Wash* on page 143.

Dual-Indexed Single-Read Workflow



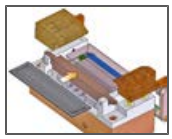
Load water onto the GA and PEM, and perform a pre-run wash using a indexed single-read pre-run wash recipe. The indexed single-read pre-run wash recipe washes the GA and PEM.



Prepare SBS reagents for the number of cycles in your run.

Load SBS reagents for Read 1.

Start the priming recipe to prime reagents.



Install the prism and flow cell, and check for reagent delivery.

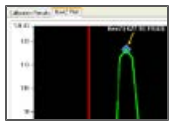
If no leaks are present, apply oil to the flow cell.

Start the sequencing recipe.

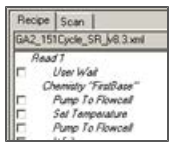
Load water onto the Paired-End Module in preparation of the PEM water prime, and then resume the recipe.

When prompted after the PEM water prime is complete, check the volume in each tube to confirm proper delivery.

Resume the sequencing recipe to proceed to first base incorporation.



When prompted after first base incorporation, check quality metrics in the first base report and calibration results window.



Resume the recipe to begin Read 1.

Replenish SBS reagents during Read 1 as needed.

Prepare indexing reagents.

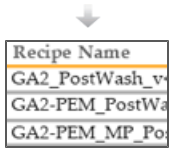
When prompted after completion of Read 1, load indexing reagents onto the Paired-End Module.

Replenish SBS reagents as needed.

Resume the recipe to perform Index 1 (i7) Read and Index 2 (i5) Read.

When prompted after completion of the Index Reads, replace reagents on the Paired-End Module with containers of water in preparation of the PEM water wash.

Resume the recipe to perform the PEM water wash.



After the completion of the PEM water wash, replace all reagents with containers of water.

Perform a post-run wash using an indexed single-read post-run wash recipe.

Performing a Dual-Indexed Single-Read Run

After completing the run preparation steps, you are ready to start the dual-index single-read sequencing recipe.

You must use the appropriate recipe version and sequencing primers for the libraries you are sequencing. For more information, see *Sequencing Primer Requirements* on page 16.

NOTE

It is possible to perform a dual-indexed single-read run using a paired-end flow cell containing paired-end libraries. To do so, set up a ***dual-indexed paired-end run*** and edit the recipe to stop the run after the Index 2 (i5) Read and before the start of Read 2 resynthesis.

It is essential to the success of the Index 2 Read that you use the dual-indexed paired-end recipe and associated reagents with a paired-end flow cell.

Start a Dual-Indexed Single-Read Recipe

1. If necessary, modify the recipe to perform the number of cycles required for your run using the recipe editor.
2. Select **File** | **Open** and open one of the following recipes depending on the Paired-End Module you are using:

Use With	Recipe Name
Dual-indexed libraries only	GA2-PEM_MP_151+8+8Cycle_SR_v10 GA2-PEM2X_MP_151+8+8Cycle_SR_v10px

3. Click **Start**.
4. If prompted, browse to the sample sheet for this flow cell, and then click **OK**.

NOTE

To view indexing results in Sequencing Analysis Viewer (SAV), it is essential that you load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to properly report indexing information. For more information, see the *Sequencing Analysis Viewer User Guide*, Part # 15020619.

Perform the PEM Water Prime

The PEM water prime verifies correct operation of the Paired-End Module.

1. When prompted, load 2 ml water in positions 19, 21, 22, and 23.
2. Click **OK**. The recipe primes water through the lines of the Paired-End Module.
3. Upon completion, measure the volume in each tube to confirm proper delivery and record the volume on the lab tracking form.
4. Click **OK** to proceed to first base incorporation.

Check Quality Metrics

The recipe proceeds to first base incorporation, which incorporates the first nucleotide. First base incorporation takes approximately 20 minutes.

Directly following first base incorporation, the recipe performs auto calibration to adjust the Z position and find the best focus.

1 Click **View** | **Calibration Results** from the Data Collection toolbar to open the Focus Calibration Result window.

2 Click the Calibration Results tab and check the following specifications:
Goodness of fit: ≥ 0.9900
Sensitivity: 350–400

3 If both values are within the specified range, click **Accept**. If either or both of the values do not meet the specification, move to another tile and repeat auto calibration.

4 View the First Base Report after the completion of first base incorporation. The First Base Report summarizes cluster counts, intensity values, focus metrics, focus position, and flow cell tilt.

Perform Read 1

If you are satisfied with the results of first base incorporation, follow these instructions to perform Read 1.

1 Click **OK** to resume the recipe and continue the run.

2 As needed, replenish reagents at the recommended cycles for your run.
For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

3 When Read 1 is complete, weigh each of the reagents used for Read 1. Record the weights on the lab tracking form.

Preparing Indexing Reagents

Indexed sequencing requires indexing reagents provided in the TruSeq Cluster Kit. Make sure that you use the appropriate sequencing primer for the library type that you are sequencing.

Indexing reagents take less than 30 minutes to prepare.

Library Type and Flow Cell Type	Index 1 (i7)	Index 2 (i5)
Nextera libraries Single-Read Flow Cell	HP12	HP9
TruSeq HT libraries Single-Read Flow Cell	HP8 or HP12	HP9

Indexing reagents take less than 30 minutes to prepare.

Table 5 Indexing Reagents

Reagents	Kit Name
HP3	TruSeq PE Cluster Kit (Indexing Reagents Box)
HT2	TruSeq PE Cluster Kit (Indexing Reagents Box)
HP8 or HP12	TruSeq PE Cluster Kit (Indexing Reagents Box) TruSeq Dual Index Sequencing Primer Box
HP9	TruSeq Dual Index Sequencing Primer Box

NOTE
HP8, provided in the TruSeq PE Cluster Kit, is not compatible with dual-indexed Nextera libraries. You must use **HP12** from the TruSeq Dual Index Sequencing Primer Box.

Thaw Reagents

1 Remove the reagents from -15° to -25°C storage. Confirm that you are using the appropriate sequencing primers for your library type and run type.

2 Thaw reagents in a beaker filled with room temperature deionized water for about 20 minutes, or until reagents are fully thawed.

Prepare HP3

NOTE
In order to provide sufficient HP3 for both Index Reads of a dual-indexed run, the preparation instructions for HP3 differ from instructions for single-indexed runs.

1 Invert the tube of HP3 five times to mix the reagent, and then pulse centrifuge.

2 Transfer 3,420 µl of PW1 into a 15 ml Sarstedt conical tube and add 180 µl of HP3.

3 Invert the tube five times to mix the reagent.

4 Label the tube of diluted HP3 **Reagent #19**.

5 Centrifuge at 1,000 rpm for one minute.

6 Set aside at room temperature.

Prepare HT2

1 Invert the tube of HT2 five times to mix the reagent.

2 Label the tube of HT2 **Reagent #21**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside at room temperature.

Prepare HP8

1 Invert the tube of HP8 five times to mix the reagent.

2 Briefly pulse centrifuge to collect droplets.

3 Label the tube of HP8 **Reagent #22**.

4 Set aside at room temperature.

Prepare HP12

You must use HP12 for sequencing dual-indexed Nextera libraries.

1 Invert the tube of HP12 five times to mix the reagent.

2 Briefly pulse centrifuge to collect droplets.

3 Label the tube of HP12 **Reagent #22**.

4 Set aside at room temperature.

Prepare HP9

You must use HP9 for dual-indexed runs on a single-read flow cell.

1 Invert the tube of HP9 five times to mix the reagent.

2 Briefly pulse centrifuge to collect droplets.

3 Label the tube of HP9 **Reagent #23**.

4 Set aside at room temperature.

Load Indexing Reagents

When prompted after the completion of Read 1, load indexing reagents.

1 Weigh each reagent before loading it onto the Paired-End Module and record the weight on the lab tracking form.

2 Load reagents onto the Paired-End Module in the following positions:

Position	Reagent	Description
19	HP3	Denaturation Solution
21	HT2	Wash Buffer
22	HP8 or HP12	Index 1 (i7) Sequencing Primer Mix
23	HP9	Index 2 (i5) Sequencing Primer Mix

3 Connect the reagent tubes to the corresponding port position on the Paired-End Module.

4 Place the waste tube into a waste container or a 15 ml conical tube.

5 When you have finished loading reagents, click **OK** to resume the sequencing recipe.

Perform the Index Reads

The recipe primes the Paired-End Module for Index 1 (i7) Read preparation and proceeds directly to the Index 1 (i7) Read, followed by Index 2 (i5) Read preparation and the Index 2 (i5) Read.

Wash the Paired-End Module

1 When prompted, replace reagent tubes on the Paired-End Module with 15 ml conical tubes containing at least 10 ml of MilliQ water or laboratory-grade water.

2 Click **OK** to proceed to the Paired-End Module water wash.

3 Proceed to *Performing a Post-Run Wash* on page 143.

Performing Paired-End Runs

Chapter 5

Introduction 108
Non-Indexed Paired-End Workflow 109
Performing a Non-Indexed Paired-End Run 111
Single-Indexed Paired-End Workflow 118
Performing a Single-Indexed Paired-End Run 120
Dual-Indexed Paired-End Workflow 129
Performing a Dual-Indexed Paired-End Run 131

Introduction

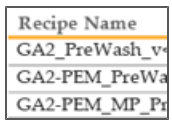
This section describes three types of paired-end sequencing runs:

- *Performing a Non-Indexed Paired-End Run* on page 111
- *Performing a Single-Indexed Paired-End Run* on page 120
- *Performing a Dual-Indexed Paired-End Run* on page 131

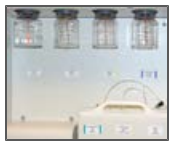
Each run type differs in workflow and requires specific reagents and recipes.

For more information, see *Sequencing Consumables* on page 13 and *Sequencing Recipes* on page 19.

Non-Indexed Paired-End Workflow



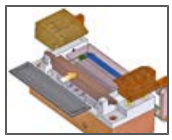
Load water onto the GA and PEM, and perform a pre-run wash using a paired-end pre-run wash recipe. The paired-end pre-run wash recipe washes the GA and PEM.



Prepare SBS reagents for the number of cycles in Read 1.

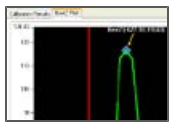
Load SBS reagents for Read 1.

Start the priming recipe to prime reagents.



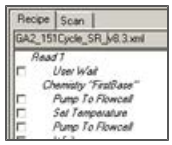
Install the prism and flow cell, and check for reagent delivery.

If no leaks are present, apply oil to the flow cell.



Start the sequencing recipe.

When prompted after first base incorporation, check quality metrics in the calibration results window.



Resume the recipe to perform Read 1.

Replenish SBS reagents during Read 1 as needed.

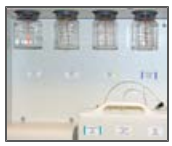
When prompted after the completion of Read 1, load water onto the Paired-End Module in preparation of the PEM water prime, and then resume the recipe.

Prepare paired-end reagents for Read 2 Resynthesis.

When prompted after the PEM water prime is complete, check the volume in each tube to confirm proper delivery. Resume the recipe.

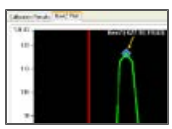
When prompted, load paired-end reagents onto the Paired-End Module.

Resume the recipe to begin Read 2 resynthesis.



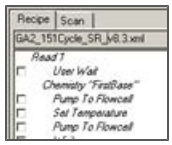
Prepare SBS reagents for Read 2.

When prompted after the completion of Read 2 resynthesis, load SBS reagents for Read 2 first base incorporation.



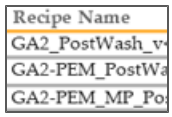
When prompted after Read 2 first base incorporation, check quality metrics in the first base report.

Exchange reagents on the Paired-End Module with containers of water in preparation of the PEM water wash.



Resume the recipe to perform the PEM water wash and proceed Read 2.

Replenish SBS reagents during Read 2 as needed.



When prompted after the completion of Read 2, replace all reagents with containers of water.

Perform a post-run wash using a paired-end post-run wash recipe.

Performing a Non-Indexed Paired-End Run

After completing the run preparation steps, you are ready to start the paired-end sequencing recipe.

You must use the appropriate recipe version and sequencing primers for the libraries you are sequencing. For more information, see *Sequencing Primer Requirements* on page 16.

Start a Paired-End Recipe

1. If necessary, modify the recipe to perform the number of cycles required for your run using the recipe editor.
2. Select **File** | **Open Recipe** and open one of the following recipes depending on the Paired-End Module you are using:
   - GA2-PEM_2x151_PE_v10.xml
   - GA2-PEM2X_2x151_PE_v10px.xml
3. Click **Start**. The software makes a copy of the recipe file and stores it in the current run folder. If you need to stop work at any point, you can reopen the recipe from that location and continue from where you left off.
4. If prompted, browse to the sample sheet for this flow cell, and then click **OK**.

Check Quality Metrics

The recipe proceeds to first base incorporation, which incorporates the first nucleotide. First base incorporation takes approximately 20 minutes.

Directly following first base incorporation, the recipe performs auto calibration to adjust the Z position and find the best focus.

1. Click **View** | **Calibration Results** from the Data Collection toolbar to open the Focus Calibration Result window.
2. Click the Calibration Results tab and check the following specifications:
   Goodness of fit: ≥ 0.9900
   Sensitivity: 350–400
3. If both values are within the specified range, click **Accept**. If either or both of the values do not meet the specification, move to another tile and repeat auto calibration.
4. View the First Base Report after the completion of first base incorporation. The First Base Report summarizes cluster counts, intensity values, focus metrics, focus position, and flow cell tilt.

Perform Read 1

If you are satisfied with the results of first base incorporation, follow these instructions to perform Read 1.

1. Click **OK** to resume the recipe and continue the run.
2. As needed, replenish reagents at the recommended cycles for your run.
   For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

3 When Read 1 is complete, weigh each of the reagents used for Read 1. Record the weights on the lab tracking form.

Perform the PEM Water Prime

The PEM water prime verifies correct operation of the Paired-End Module.

1 Click **OK** to resume the recipe.

2 Load 2 ml water in positions 10, 11, 12, 13, 14, 15, 16, 19, and 21.

3 Click **OK**. The recipe primes water through the lines of the Paired-End Module.

4 Upon completion, measure the volume in each tube to confirm proper delivery and record the volume on the lab tracking form.

5 Click **OK** to proceed.

Prepare Paired-End Reagents

Paired-end sequencing requires paired-end reagents provided in the TruSeq Cluster Kit.

Dual-indexed Nextera libraries require HP11 (Read 2 sequencing primer) provided in the TruSeq Dual Index Sequencing Primer Box.

Library Type	Read 2
Nextera libraries	HP11
TruSeq HT libraries	HP7 or HP11
TruSeq LT/v2 libraries	HP7 or HP11
TruSeq Small RNA libraries	HP7 or HP11

Paired-end reagents take less than 30 minutes to prepare.

NOTE

HP7, provided in the TruSeq PE Cluster Kit, is not compatible with dual-indexed Nextera libraries. You must use **HP11** from the TruSeq Dual Index Sequencing Primer Box (PE).

Table 6 Paired-End Reagents

Reagents	Kit Name
RMX	TruSeq PE Cluster Kit (PE Reagents Box)
LMX2	TruSeq PE Cluster Kit (PE Reagents Box)
BMX	TruSeq PE Cluster Kit (PE Reagents Box)
AMX2	TruSeq PE Cluster Kit (PE Reagents Box)
APM2	TruSeq PE Cluster Kit (PE Reagents Box)
AT2	TruSeq PE Cluster Kit (PE Reagents Box)
HP7 or	TruSeq PE Cluster Kit (PE Reagents Box)
HP11	TruSeq Dual Index Sequencing Primer Box
HP3	TruSeq PE Cluster Kit (PE Reagents Box)
HT2	TruSeq PE Cluster Kit (PE Reagents Box)

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact.

Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

For more information, see the MSDS for this kit on www.illumina.com.

Thaw Reagents

1 Remove the reagents from -15° to -25°C storage. Confirm that you are using the appropriate sequencing primer for your library type and run type.

2 Thaw reagents in a beaker filled with room temperature deionized water for about 20 minutes, or until reagents are fully thawed.

3 After reagents have completely thawed, place LMX2, BMX, AMX2, and RMX on ice.

Prepare RMX

1 Invert the tube of RMX five times to mix the reagent.

2 Label the tube of RMX **Reagent #10**.

3 Briefly centrifuge the reagent at 1,000 rpm for one minute or less. Alternatively, gently tap or flick the tube to make sure that reagent droplets collect at the bottom of the tube.

NOTE
Do not vortex RMX.

4 Set aside on ice.

Prepare LMX2

1 Invert the tube of LMX2 five times to mix the reagent.

2 Label the tube of LMX2 **Reagent #11**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside on ice.

Prepare BMX

1 Invert the tube of BMX five times to mix the reagent.

2 Label the tube of BMX **Reagent #12**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside on ice.

Prepare AMX2

1 Invert the tube of AMX2 five times to mix the reagent.

2 Label the tube of AMX2 **Reagent #13**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside on ice.

Prepare APM2

1 Invert the tube of APM2 five times to mix the reagent.

2 Label the tube of APM2 **Reagent #14**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside at room temperature.

Prepare AT2

1 Invert the tube of AT2 five times to mix the reagent.
2 Label the tube of AT2 **Reagent #15**.
3 Centrifuge the reagent at 1,000 rpm for one minute.
4 Set aside at room temperature.

Prepare HP7

1 Invert the tube of HP7 five times to mix the reagent.
2 Label the tube of HP7 **Reagent #16**.
3 Centrifuge the reagent at 1,000 rpm for one minute.
4 Set aside at room temperature.

Prepare HP11

You must use HP11 for sequencing dual-indexed Nextera libraries.

1 Invert the tube of HP11 five times to mix the reagent.
2 Label the tube of HP11 **Reagent #16**.
3 Centrifuge the reagent at 1,000 rpm for one minute.
4 Set aside at room temperature.

Prepare HP3

1 Invert the tube of HP3 five times to mix the reagent, and then pulse centrifuge.
2 Transfer 2.85 ml of PW1 into a 15 ml Sarstedt conical tube and add 150 µl of HP3.
3 Invert the tube five times to mix the reagent.
4 Label the conical tube of diluted HP3 **Reagent #19**.
5 Centrifuge at 1,000 rpm for one minute.
6 Set aside at room temperature.

Prepare HT2

1 Invert the tube of HT2 five times to mix the reagent.
2 Label the tube of HT2 **Reagent #21**.
3 Centrifuge the reagent at 1,000 rpm for one minute.
4 Set aside at room temperature.

Load Paired-End Reagents

When prompted after the PEM water prime, load paired-end reagents.

1 Weigh each reagent and record the weight on the lab tracking form.
2 Briefly pulse centrifuge each reagent prior to loading it onto the Paired-End Module.

3 Load reagents onto the Paired-End Module in the following positions:

Position	Reagent	Description
10	RMX	Resynthesis Mix
11	LMX2	Linearization Mix 2
12	BMX	Blocking Mix
13	AMX2	Amplification Mix 2
14	APM2	AMX2 Premix
15	AT2	100% Formamide
16	HP7 or HP11	Read 2 Sequencing Primer
19	HP3 (Diluted)	Denaturation Solution
21	HT2	Wash Buffer

4 Connect the reagent tubes to the corresponding port position on the Paired-End Module.

5 Place the waste tube into a waste container or a 15 ml conical tube.

6 When you have finished loading reagents, click **OK** to resume the sequencing recipe.
The recipe primes reagents on the Paired-End Module and proceeds to Read 2 resynthesis.

Perform Read 2 Resynthesis

The sequencing recipe automatically primes paired-end reagent positions using the internal priming pump on the Paired-End Module, and dispenses waste to the waste bottle, bypassing the flow cell.

Priming the Paired-End Module takes approximately 15 minutes. Read 2 resynthesis takes approximately four hours after priming is complete. There is no need to remount the flow cell or perform a leak test.

1 After Read 2 resynthesis, weigh each of the reagents used on the Paired-End Module.

2 Record the weights on the lab tracking form.
The following table lists reagent delivery for Read 2 resynthesis, and includes the priming volume. Volumes can vary ±15%.

Table 7 Reagent Delivery During Read 2 Resynthesis

Position	Reagent	Delivery (PEM) (µl or mg)	Delivery (PEM IIx) (µl or mg)
10	RMX	1200	1350
11	LMX2	1200	1350
12	BMX	1720	1870
13	AMX2	4920	5070
14	APM2	5310	5310
15	AT2	4110	4110
16	HP7 or HP11	1350	1350
19	HP3 (Diluted)	1950	1950
21	HT2	7110	7160

Prepare SBS Reagents for Read 2

Prepare and combine fresh sequencing reagents for Read 2 according to the length of your read. For more information, see *Preparing SBS Reagents* on page 63 and *Reagent Kits and Replenishing Cycles* on page 66.

Load SBS Reagents for Read 2

When prompted after the completion of Read 2 resynthesis, load SBS reagents for Read 2.

1. Weigh each of the reagents before loading them onto the Genome Analyzer and record the weight on the lab tracking form.
2. Invert each container several times to mix the reagent.
3. Centrifuge SMX, IMR, and CLM at 4°C to 1000 xg for 1 minute.
4. Load reagents in the appropriate positions. Load CLM last to avoid cross-contamination.

Table 8 Reagent Positions

Position	Contents	Description	Container Size
1	IMR	Incorporation Mix	175 ml Bottle
2	PW1	Wash Solution	125 ml Bottle
3	SMX	Scan Mix	175 ml Bottle
4	PR1	High Salt Buffer	125 ml Bottle or 150 ml bottle
5	PR2	Incorporation Buffer	125 ml Bottle or 150 ml bottle
6	CLM	Cleavage Mix	175 ml Bottle
7	PR3	Cleavage Buffer	125 ml Bottle or 150 ml bottle

5. Insert the appropriate reagent tube into the cap of each container in positions 1, 3, and 6.
6. Click **OK** to resume the sequencing recipe and proceed to first base incorporation.

Check Quality Metrics for Read 2

1. When prompted after the completion of Read 2 first base incorporation, open the Focus Calibration Result window.
2. Make sure that the instrument is still in focus. Do one of the following before proceeding:
   - Click **OK** to accept the current calibrated focus and resume the recipe. The flow cell is automatically flushed with SMX (Scan Mix).
   - Refocus using the auto calibration feature. Select **Instrument | Calibrate** from the Data Collection toolbar. Upon completion, click **OK** to resume the recipe.

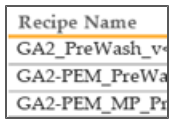
Wash the Paired-End Module

1. When prompted, replace all reagent tubes on the Paired-End Module with 15 ml conical tubes containing at least 10 ml of MilliQ water or laboratory grade water.
2. Click **OK** to proceed to the Paired-End Module water wash.
   Directly following the Paired-End Module water wash, the recipe begins Read 2.

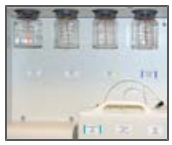
Perform Read 2

1. Replenish reagents at the recommended cycles for your run, as needed.
   For more information, see *Reagent Kits and Replenishing Cycles* on page 66.
2. When Read 2 is complete, weigh each of the reagents on the Genome Analyzer and record the weights on the lab tracking form.
3. Proceed to *Performing a Post-Run Wash* on page 143.

Single-Indexed Paired-End Workflow



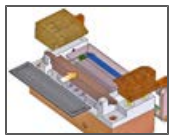
Load water onto the GA and PEM, and perform a pre-run wash using an indexed paired-end pre-run wash recipe. The indexed paired-end pre-run wash recipe washes the GA and PEM.



Prepare SBS reagents for the number of cycles in Read 1.

Load SBS reagents for Read 1.

Start the priming recipe to prime reagents.



Install the prism and flow cell, and check for reagent delivery.

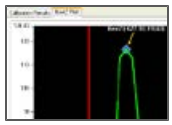
If no leaks are present, apply oil to the flow cell.

Start the sequencing recipe.

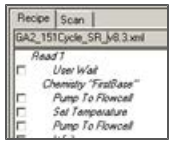
Load water onto the Paired-End Module in preparation of the PEM water prime, and then resume the recipe.

When prompted after the PEM water prime is complete, check the volume in each tube to confirm proper delivery.

Resume the sequencing recipe to proceed to first base incorporation.



When prompted after first base incorporation, check quality metrics in the first base report and calibration results window.



Resume the recipe to begin Read 1.

Replenish SBS reagents during Read 1 as needed.

Prepare indexing reagents.

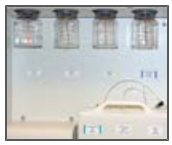
When prompted after completion of Read 1, load indexing reagents onto the Paired-End Module, and replenish SBS reagents as needed.

Resume the recipe to perform Index 1 (i7) Read.

Prepare paired-end reagents for Read 2 Resynthesis.

When prompted after completion of the Index Read, load paired-end reagents onto the Paired-End Module.

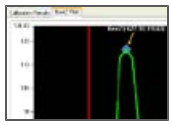
Resume the recipe to proceed to priming and Read 2 resynthesis.



Prepare fresh SBS reagents for Read 2.

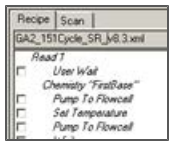
When prompted after Read 2 resynthesis, load SBS reagents for Read 2.

Resume the sequencing recipe to proceed to first base incorporation.



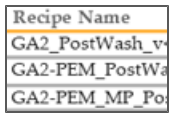
When prompted after Read 2 first base incorporation, check quality metrics in the first base report.

Exchange reagents on the Paired-End Module with containers of water in preparation of the PEM water wash.



Resume the recipe to wash the Paired-End Module and then begin Read 2.

Replenish SBS reagents during Read 2 as needed.



When prompted after the completion of Read 2, replace all reagents with containers of water.

Perform a post-run wash using an indexed paired-end post-run wash recipe.

Performing a Single-Indexed Paired-End Run

After completing the run preparation steps, you are ready to start the single-index paired-end sequencing recipe.

You must use the appropriate recipe version and sequencing primers for the libraries you are sequencing. For more information, see *Sequencing Primer Requirements* on page 16.

Start a Single-Indexed Paired-End Run

1. If necessary, modify the recipe to perform the number of cycles required for your run using the recipe editor.
2. Select **File** | **Open** and open one of the following recipes depending on the Paired-End Module you are using and the type of libraries you are sequencing:

Use With	Recipe Name
Single-indexed libraries	GA2-PEM_MP_151+7+151Cycle_v8.5 GA2-PEM2X_MP_151+7+151Cycle_v8.5px
Dual-indexed libraries	GA2-PEM_MP_151+8+151Cycle_v10 GA2-PEM2X_MP_151+8+151Cycle_v10px

3. Click **Start**.
4. If prompted, browse to the sample sheet for this flow cell, and then click **OK**.

NOTE
To view indexing results in Sequencing Analysis Viewer (SAV), it is essential that you load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to properly report indexing information. For more information, see the *Sequencing Analysis Viewer User Guide*, Part # 15020619.

Perform the PEM Water Prime

The PEM water prime verifies correct operation of the Paired-End Module.

1. When prompted, load 2 ml water onto the Paired-End Module in positions 10, 11, 12, 13, 14, 15, 16, 19, 21, and 22.
2. Click **OK**. The recipe primes water through the lines of the Paired-End Module.
3. Upon completion, measure the volume in each tube to confirm proper delivery and record the volume on the lab tracking form.
4. Click **OK** to proceed to first base incorporation.

Check Quality Metrics

The recipe proceeds to first base incorporation, which incorporates the first nucleotide. First base incorporation takes approximately 20 minutes.

Directly following first base incorporation, the recipe performs auto calibration to adjust the Z position and find the best focus.

1. Click **View** | **Calibration Results** from the Data Collection toolbar to open the Focus Calibration Result window.

2 Click the Calibration Results tab and check the following specifications:
Goodness of fit: ≥ 0.9900
Sensitivity: 350–400

3 If both values are within the specified range, click **Accept**. If either or both of the values do not meet the specification, move to another tile and repeat auto calibration.

4 View the First Base Report after the completion of first base incorporation. The First Base Report summarizes cluster counts, intensity values, focus metrics, focus position, and flow cell tilt.

Perform Read 1

If you are satisfied with the results of first base incorporation, follow these instructions to perform Read 1.

1 Click **OK** to resume the recipe and continue the run.

2 As needed, replenish reagents at the recommended cycles for your run.
For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

3 When Read 1 is complete, weigh each of the reagents used for Read 1. Record the weights on the lab tracking form.

Preparing Indexing Reagents

Indexed sequencing requires indexing reagents provided in the TruSeq Cluster Kit. Make sure that you use the appropriate sequencing primer for the library type that you are sequencing.

Library Type	Index 1 (i7)
Nextera libraries	HP12
TruSeq HT libraries	HP8 or HP12
TruSeq LT/v2 libraries	HP8 or HP12
TruSeq Small RNA libraries	HP8 or HP12

Indexing reagents take less than 30 minutes to prepare.

Table 9 Indexing Reagents

Reagents	Kit Name
HP3	TruSeq PE Cluster Kit (Indexing Reagents Box)
HT2	TruSeq PE Cluster Kit (Indexing Reagents Box)
HP8 or HP12	TruSeq PE Cluster Kit (Indexing Reagents Box) TruSeq Dual Index Sequencing Primer Box

NOTE
HP8, provided in the TruSeq Cluster Kit, is not compatible with dual-indexed Nextera libraries. You must use **HP12** from the TruSeq Dual Index Sequencing Primer Box.

Thaw Reagents

1. Remove the reagents from -15° to -25°C storage. Confirm that you are using the appropriate sequencing primers for your library type and run type.
2. Thaw reagents in a beaker filled with room temperature deionized water for about 20 minutes, or until reagents are fully thawed.

Prepare HP3

1. Invert the tube of HP3 five times to mix the reagent, and then pulse centrifuge.
2. Transfer 1,900 µl of PW1 into a 15 ml Sarstedt conical tube and add 100 µl of HP3.
3. Invert the tube five times to mix the reagent.
4. Label the tube of diluted HP3 **Reagent #19**.
5. Centrifuge at 1,000 rpm for one minute.
6. Set aside at room temperature.

Prepare HT2

1. Invert the tube of HT2 five times to mix the reagent.
2. Label the tube of HT2 **Reagent #21**.
3. Centrifuge the reagent at 1,000 rpm for one minute.
4. Set aside at room temperature.

Prepare HP8

1. Invert the tube of HP8 five times to mix the reagent.
2. Briefly pulse centrifuge to collect droplets.
3. Label the tube of HP8 **Reagent #22**.
4. Set aside at room temperature.

Prepare HP12

You must use HP12 for sequencing dual-indexed Nextera libraries.

1. Invert the tube of HP12 five times to mix the reagent.
2. Briefly pulse centrifuge to collect droplets.
3. Label the tube of HP12 **Reagent #22**.
4. Set aside at room temperature.

Load Indexing Reagents

When prompted after the completion of Read 1, load indexing reagents.

1. Weigh each reagent before loading it onto the Paired-End Module and record the weight on the lab tracking form.
2. Load reagents onto the Paired-End Module in the following positions:

Position	Reagent	Description
19	HP3	Denaturation Solution
21	HT2	Wash Buffer
22	HP8 or HP12	Index 1 (i7) Sequencing Primer Mix

3 Connect the reagent tubes to the corresponding port position on the Paired-End Module.

4 Place the waste tube into a waste container or a 15 ml conical tube.

5 When you have finished loading reagents, click **OK** to resume the sequencing recipe.

Perform the Index Read

The recipe primes the Paired-End Module for Index Read preparation and proceeds directly to the Index Read.

Prepare Paired-End Reagents

Paired-end sequencing requires paired-end reagents provided in the TruSeq Cluster Kit.

Dual-indexed Nextera libraries require HP11 (Read 2 sequencing primer) provided in the TruSeq Dual Index Sequencing Primer Box.

Library Type	Read 2
Nextera libraries	HP11
TruSeq HT libraries	HP7 or HP11
TruSeq LT/v2 libraries	HP7 or HP11
TruSeq Small RNA libraries	HP7 or HP11

Paired-end reagents take less than 30 minutes to prepare.

NOTE

HP7, provided in the TruSeq PE Cluster Kit, is not compatible with dual-indexed Nextera libraries. You must use **HP11** from the TruSeq Dual Index Sequencing Primer Box (PE).

Table 10 Paired-End Reagents

Reagents	Kit Name
RMX	TruSeq PE Cluster Kit (PE Reagents Box)
LMX2	TruSeq PE Cluster Kit (PE Reagents Box)
BMX	TruSeq PE Cluster Kit (PE Reagents Box)
AMX2	TruSeq PE Cluster Kit (PE Reagents Box)
APM2	TruSeq PE Cluster Kit (PE Reagents Box)
AT2	TruSeq PE Cluster Kit (PE Reagents Box)
HP7 or	TruSeq PE Cluster Kit (PE Reagents Box)
HP11	TruSeq Dual Index Sequencing Primer Box
HP3	TruSeq PE Cluster Kit (PE Reagents Box)
HT2	TruSeq PE Cluster Kit (PE Reagents Box)

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin

contact, and eye contact.

Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

For more information, see the MSDS for this kit on www.illumina.com.

Thaw Reagents

1. Remove the reagents from -15° to -25°C storage. Confirm that you are using the appropriate sequencing primer for your library type and run type.
2. Thaw reagents in a beaker filled with room temperature deionized water for about 20 minutes, or until reagents are fully thawed.
3. After reagents have completely thawed, place LMX2, BMX, AMX2, and RMX on ice.

Prepare RMX

1. Invert the tube of RMX five times to mix the reagent.
2. Label the tube of RMX **Reagent #10**.
3. Briefly centrifuge the reagent at 1,000 rpm for one minute or less. Alternatively, gently tap or flick the tube to make sure that reagent droplets collect at the bottom of the tube.

   NOTE
   Do not vortex RMX.

4. Set aside on ice.

Prepare LMX2

1. Invert the tube of LMX2 five times to mix the reagent.
2. Label the tube of LMX2 **Reagent #11**.
3. Centrifuge the reagent at 1,000 rpm for one minute.
4. Set aside on ice.

Prepare BMX

1. Invert the tube of BMX five times to mix the reagent.
2. Label the tube of BMX **Reagent #12**.
3. Centrifuge the reagent at 1,000 rpm for one minute.
4. Set aside on ice.

Prepare AMX2

1. Invert the tube of AMX2 five times to mix the reagent.
2. Label the tube of AMX2 **Reagent #13**.
3. Centrifuge the reagent at 1,000 rpm for one minute.
4. Set aside on ice.

Prepare APM2

1 Invert the tube of APM2 five times to mix the reagent.
2 Label the tube of APM2 **Reagent #14**.
3 Centrifuge the reagent at 1,000 rpm for one minute.
4 Set aside at room temperature.

Prepare AT2

1 Invert the tube of AT2 five times to mix the reagent.
2 Label the tube of AT2 **Reagent #15**.
3 Centrifuge the reagent at 1,000 rpm for one minute.
4 Set aside at room temperature.

Prepare HP7

1 Invert the tube of HP7 five times to mix the reagent.
2 Label the tube of HP7 **Reagent #16**.
3 Centrifuge the reagent at 1,000 rpm for one minute.
4 Set aside at room temperature.

Prepare HP11

You must use HP11 for sequencing dual-indexed Nextera libraries.

1 Invert the tube of HP11 five times to mix the reagent.
2 Label the tube of HP11 **Reagent #16**.
3 Centrifuge the reagent at 1,000 rpm for one minute.
4 Set aside at room temperature.

Prepare HP3

1 Invert the tube of HP3 five times to mix the reagent, and then pulse centrifuge.
2 Transfer 2.85 ml of PW1 into a 15 ml Sarstedt conical tube and add 150 µl of HP3.
3 Invert the tube five times to mix the reagent.
4 Label the conical tube of diluted HP3 **Reagent #19**.
5 Centrifuge at 1,000 rpm for one minute.
6 Set aside at room temperature.

Prepare HT2

1 Invert the tube of HT2 five times to mix the reagent.
2 Label the tube of HT2 **Reagent #21**.
3 Centrifuge the reagent at 1,000 rpm for one minute.
4 Set aside at room temperature.

Load Paired-End Reagents

When prompted after completion of the Index Read, load paired-end reagents.

1 Weigh each reagent and record the weight on the lab tracking form.

2 Briefly pulse centrifuge each reagent prior to loading it onto the Paired-End Module.

NOTE
Replace diluted HP3 and HT2 used during the Index Read with fresh reagent for Read 2 resynthesis.

3 Load reagents onto the Paired-End Module in the following positions:

Position	Reagent	Description
10	RMX	Resynthesis Mix
11	LMX2	Linearization Mix 2
12	BMX	Blocking Mix
13	AMX2	Amplification Mix 2
14	APM2	AMX2 Premix
15	AT2	100% Formamide
16	HP7 or HP11	Read 2 Sequencing Primer
19	HP3 (Diluted)	Denaturation Solution
21	HT2	Wash Buffer

4 Connect the reagent tubes to the corresponding port position on the Paired-End Module.

5 Place the waste tube into a waste container or a 15 ml conical tube.

6 When you have finished loading reagents, click **OK** to resume the sequencing recipe and proceed to Read 2 resynthesis.

Perform Read 2 Resynthesis

The sequencing recipe automatically primes paired-end reagent positions using the internal priming pump on the Paired-End Module, and dispenses waste to the waste bottle, bypassing the flow cell.

Priming the Paired-End Module takes approximately 15 minutes. Read 2 resynthesis takes approximately four hours after priming is complete. There is no need to remount the flow cell or perform a leak test.

1 After Read 2 resynthesis, weigh each of the reagents used on the Paired-End Module.

2 Record the weights on the lab tracking form.
The following table lists reagent delivery for Read 2 resynthesis, and includes the priming volume. Volumes can vary ±15%.

Table 11 Reagent Delivery During Read 2 Resynthesis

Position	Reagent	Delivery (PEM) (µl or mg)	Delivery (PEM IIx) (µl or mg)
10	RMX	1200	1350
11	LMX2	1200	1350

Position	Reagent	Delivery (PEM) (µl or mg)	Delivery (PEM IIx) (µl or mg)
12	BMX	1720	1870
13	AMX2	4920	5070
14	APM2	5310	5310
15	AT2	4110	4110
16	HP7 or HP11	1350	1350
19	HP3 (Diluted)	1950	1950
21	HT2	7110	7160

Prepare SBS Reagents for Read 2

Prepare and combine fresh sequencing reagents for Read 2 according to the length of your read. For more information, see *Preparing SBS Reagents* on page 63 and *Reagent Kits and Replenishing Cycles* on page 66.

Load SBS Reagents for Read 2

When prompted after the completion of Read 2 resynthesis, load SBS reagents for Read 2.

1. Weigh each of the reagents before loading them onto the Genome Analyzer and record the weight on the lab tracking form.
2. Invert each container several times to mix the reagent.
3. Centrifuge SMX, IMR, and CLM at 4°C to 1000 xg for 1 minute.
4. Load reagents in the appropriate positions. Load CLM last to avoid cross-contamination.

Table 12 Reagent Positions

Position	Contents	Description	Container Size
1	IMR	Incorporation Mix	175 ml Bottle
2	PW1	Wash Solution	125 ml Bottle
3	SMX	Scan Mix	175 ml Bottle
4	PR1	High Salt Buffer	125 ml Bottle or 150 ml bottle
5	PR2	Incorporation Buffer	125 ml Bottle or 150 ml bottle
6	CLM	Cleavage Mix	175 ml Bottle
7	PR3	Cleavage Buffer	125 ml Bottle or 150 ml bottle

5. Insert the appropriate reagent tube into the cap of each container in positions 1, 3, and 6.
6. Click **OK** to resume the sequencing recipe and proceed to first base incorporation.

Check Quality Metrics for Read 2

1. When prompted after the completion of Read 2 first base incorporation, open the Focus Calibration Result window.

2 Make sure that the instrument is still in focus. Do one of the following before proceeding:
   - Click **OK** to accept the current calibrated focus and resume the recipe. The flow cell is automatically flushed with SMX (Scan Mix).
   - Refocus using the auto calibration feature. Select **Instrument | Calibrate** from the Data Collection toolbar. Upon completion, click **OK** to resume the recipe.

Wash the Paired-End Module

1 When prompted, replace all reagent tubes on the Paired-End Module with 15 ml conical tubes containing at least 10 ml of MilliQ water or laboratory grade water.

2 Click **OK** to proceed to the Paired-End Module water wash.
Directly following the Paired-End Module water wash, the recipe begins Read 2.

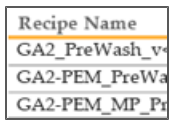
Perform Read 2

1 Replenish reagents at the recommended cycles for your run, as needed.
For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

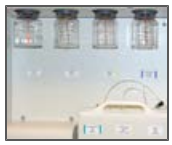
2 When Read 2 is complete, weigh each of the reagents on the Genome Analyzer and record the weights on the lab tracking form.

3 Proceed to *Performing a Post-Run Wash* on page 143.

Dual-Indexed Paired-End Workflow



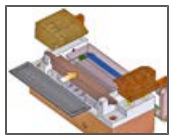
Load water onto the GA and PEM, and perform a pre-run wash using an indexed paired-end pre-run wash recipe. The indexed paired-end pre-run wash recipe washes the GA and PEM.



Prepare SBS reagents for the number of cycles in Read 1.

Load SBS reagents for Read 1.

Start the priming recipe to prime reagents.



Install the prism and flow cell, and check for reagent delivery.

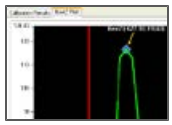
If no leaks are present, apply oil to the flow cell.

Start the sequencing recipe.

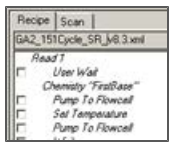
Load water onto the Paired-End Module in preparation of the PEM water prime, and then resume the recipe.

When prompted after the PEM water prime is complete, check the volume in each tube to confirm proper delivery.

Resume the sequencing recipe to proceed to first base incorporation.



When prompted after first base incorporation, check quality metrics in the first base report and calibration results window.



Resume the recipe to begin Read 1.

Replenish SBS reagents during Read 1 as needed.

Prepare indexing reagents.

When prompted after completion of Read 1, load indexing reagents onto the Paired-End Module.

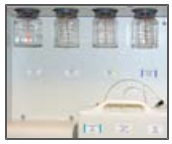
Replenish SBS reagents as needed.

Resume the recipe to perform Index 1 (i7) Read and Index 2 (i5) Read.

Prepare paired-end reagents for Read 2 Resynthesis.

When prompted after completion of the Index Reads, load paired-end reagents onto the Paired-End Module.

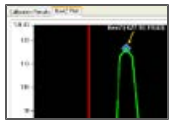
Resume the recipe to proceed to priming and Read 2 resynthesis.



Prepare fresh SBS reagents for Read 2.

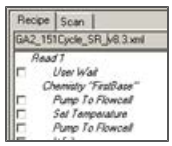
When prompted after Read 2 resynthesis, load SBS reagents for Read 2.

Resume the sequencing recipe to proceed to first base incorporation.



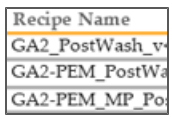
When prompted after Read 2 first base incorporation, check quality metrics in the first base report.

Exchange reagents on the Paired-End Module with containers of water in preparation of the PEM water wash.



Resume the recipe to wash the Paired-End Module and then begin Read 2.

Replenish SBS reagents during Read 2 as needed.



When prompted after the completion of Read 2, replace all reagents with containers of water.

Perform a post-run wash using an indexed paired-end post-run wash recipe.

Performing a Dual-Indexed Paired-End Run

After completing the run preparation steps, you are ready to start the dual-index paired-end sequencing recipe.

You must use the appropriate recipe version and sequencing primers for the libraries you are sequencing. For more information, see *Sequencing Primer Requirements* on page 16.

NOTE
It is possible to perform a dual-indexed single-read run using a paired-end flow cell containing paired-end libraries. To do so, set up a dual-indexed paired-end run and edit the recipe to stop the run after the Index 2 (i5) Read and before the start of Read 2 resynthesis.

Start a Dual-Indexed Paired-End Recipe

1. If necessary, modify the recipe to perform the number of cycles required for your run using the recipe editor.
2. Select **File** | **Open** and open one of the following recipes depending on the Paired-End Module you are using:

Use With	Recipe Name
Dual-indexed libraries only	GA2-PEM_MP_151+8+8+151Cycle_PE_v10 GA2-PEM2X_MP_151+8+8+151Cycle_PE_v10px

3. Click **Start**.
4. If prompted, browse to the sample sheet for this flow cell, and then click **OK**.

NOTE
To view indexing results in Sequencing Analysis Viewer (SAV), it is essential that you load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to properly report indexing information. For more information, see the *Sequencing Analysis Viewer User Guide*, Part # 15020619.

Perform the PEM Water Prime

The PEM water prime verifies correct operation of the Paired-End Module.

1. When prompted, load 2 ml water onto the Paired-End Module in positions 10, 11, 12, 13, 14, 15, 16, 19, 21, and 22.
2. Click **OK**. The recipe primes water through the lines of the Paired-End Module.
3. Upon completion, measure the volume in each tube to confirm proper delivery and record the volume on the lab tracking form.
4. Click **OK** to proceed to first base incorporation.

Check Quality Metrics

The recipe proceeds to first base incorporation, which incorporates the first nucleotide. First base incorporation takes approximately 20 minutes.

Directly following first base incorporation, the recipe performs auto calibration to adjust the Z position and find the best focus.

1 Click **View** | **Calibration Results** from the Data Collection toolbar to open the Focus Calibration Result window.

2 Click the Calibration Results tab and check the following specifications:
Goodness of fit: ≥ 0.9900
Sensitivity: 350–400

3 If both values are within the specified range, click **Accept**. If either or both of the values do not meet the specification, move to another tile and repeat auto calibration.

4 View the First Base Report after the completion of first base incorporation. The First Base Report summarizes cluster counts, intensity values, focus metrics, focus position, and flow cell tilt.

Perform Read 1

If you are satisfied with the results of first base incorporation, follow these instructions to perform Read 1.

1 Click **OK** to resume the recipe and continue the run.

2 As needed, replenish reagents at the recommended cycles for your run.
For more information, see *Reagent Kits and Replenishing Cycles* on page 66.

3 When Read 1 is complete, weigh each of the reagents used for Read 1. Record the weights on the lab tracking form.

Preparing Indexing Reagents

Indexed sequencing requires indexing reagents provided in the TruSeq Cluster Kit. Make sure that you use the appropriate sequencing primer for the library type that you are sequencing.

Indexing reagents take less than 30 minutes to prepare.

Library Type and Flow Cell Type	Index 1 (i7)	Index 2 (i5)
Nextera libraries Paired-End Flow Cell	HP12	RMX
TruSeq HT libraries Paired-End Flow Cell	HP8 or HP12	RMX

Indexing reagents take less than 30 minutes to prepare.

Table 13 Indexing Reagents

Reagents	Kit Name
HP3	TruSeq PE Cluster Kit (Indexing Reagents Box)
HT2	TruSeq PE Cluster Kit (Indexing Reagents Box)
HP8 or HP12	TruSeq PE Cluster Kit (Indexing Reagents Box) TruSeq Dual Index Sequencing Primer Box
RMX	TruSeq PE Cluster Kit (PE Reagents Box)

NOTE
HP8, provided in the TruSeq Cluster Kit, is not compatible with dual-indexed Nextera libraries. You must use **HP12** from the TruSeq Dual Index Sequencing Primer Box.

Thaw Reagents

1 Remove the reagents from -15° to -25°C storage. Confirm that you are using the appropriate sequencing primers for your library type and run type.

2 Thaw reagents in a beaker filled with room temperature deionized water for about 20 minutes, or until reagents are fully thawed.

Prepare HP3

NOTE
In order to provide sufficient HP3 for both Index Reads of a dual-indexed run, the preparation instructions for HP3 differ from instructions for single-indexed runs.

1 Invert the tube of HP3 five times to mix the reagent, and then pulse centrifuge.

2 Transfer 3,420 µl of PW1 into a 15 ml Sarstedt conical tube and add 180 µl of HP3.

3 Invert the tube five times to mix the reagent.

4 Label the tube of diluted HP3 **Reagent #19**.

5 Centrifuge at 1,000 rpm for one minute.

6 Set aside at room temperature.

Prepare HT2

1 Invert the tube of HT2 five times to mix the reagent.

2 Label the tube of HT2 **Reagent #21**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside at room temperature.

Prepare HP8

1 Invert the tube of HP8 five times to mix the reagent.

2 Briefly pulse centrifuge to collect droplets.

3 Label the tube of HP8 **Reagent #22**.

4 Set aside at room temperature.

Prepare HP12

You must use HP12 for sequencing dual-indexed Nextera libraries.

1 Invert the tube of HP12 five times to mix the reagent.

2 Briefly pulse centrifuge to collect droplets.

3 Label the tube of HP12 **Reagent #22**.

4 Set aside at room temperature.

Prepare RMX

Prepare RMX for dual-indexed runs on a paired-end flow cell.

1 Invert the tube of RMX five times to mix the reagent.

2 Label the tube of RMX **Reagent #10**.

3 Briefly centrifuge the reagent at 1,000 rpm for up to one minute. Alternatively, gently tap or flick the tube to make sure that reagent droplets collect at the bottom of the tube.

NOTE
Do not vortex RMX.

4 Set aside on ice.

Load Indexing Reagents

When prompted after the completion of Read 1, load indexing reagents.

1 Weigh each reagent before loading it onto the Paired-End Module and record the weight on the lab tracking form.

2 Load reagents onto the Paired-End Module in the following positions:

Position	Reagent	Description
10	RMX	Resynthesis Mix
19	HP3	Denaturation Solution
21	HT2	Wash Buffer
22	HP8 or HP12	Index 1 (i7) Sequencing Primer Mix

3 Connect the reagent tubes to the corresponding port position on the Paired-End Module.

4 Place the waste tube into a waste container or a 15 ml conical tube.

5 When you have finished loading reagents, click **OK** to resume the sequencing recipe.

Perform the Index Reads

The recipe primes the Paired-End Module for Index 1 (i7) Read preparation and proceeds directly to the Index 1 (i7) Read, followed by Index 2 (i5) Read preparation and the Index 2 (i5) Read.

Prepare Paired-End Reagents

Paired-end sequencing requires paired-end reagents provided in the TruSeq Cluster Kit.

Dual-indexed Nextera libraries require HP11 (Read 2 sequencing primer) provided in the TruSeq Dual Index Sequencing Primer Box.

Library Type	Read 2
Nextera libraries	HP11
TruSeq HT libraries	HP7 or HP11

Paired-end reagents take less than 30 minutes to prepare.

NOTE
HP7, provided in the TruSeq PE Cluster Kit, is not compatible with dual-indexed Nextera libraries. You must use **HP11** from the TruSeq Dual Index Sequencing Primer Box (PE).

Table 14 Paired-End Reagents

Reagents	Kit Name
LMX2	TruSeq PE Cluster Kit (PE Reagents Box)
BMX	TruSeq PE Cluster Kit (PE Reagents Box)
AMX2	TruSeq PE Cluster Kit (PE Reagents Box)
APM2	TruSeq PE Cluster Kit (PE Reagents Box)
AT2	TruSeq PE Cluster Kit (PE Reagents Box)
HP7 or HP11	TruSeq PE Cluster Kit (PE Reagents Box) TruSeq Dual Index Sequencing Primer Box
HP3	TruSeq PE Cluster Kit (PE Reagents Box)
HT2	TruSeq PE Cluster Kit (PE Reagents Box)

You do not need to prepare RMX for dual-indexed paired-end runs. RMX is used during the Index 2 (i5) Read of a dual-indexed paired-end run and is not used again during Read 2 resynthesis.

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact.

Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

For more information, see the MSDS for this kit on www.illumina.com.

Thaw Reagents

1. Remove the reagents from -15° to -25°C storage. Confirm that you are using the appropriate sequencing primer for your library type and run type.
2. Thaw reagents in a beaker filled with room temperature deionized water for about 20 minutes, or until reagents are fully thawed.
3. After reagents have completely thawed, place LMX2, BMX, and AMX2 on ice.

Prepare LMX2

1. Invert the tube of LMX2 five times to mix the reagent.
2. Label the tube of LMX2 **Reagent #11**.
3. Centrifuge the reagent at 1,000 rpm for one minute.
4. Set aside on ice.

Prepare BMX

1. Invert the tube of BMX five times to mix the reagent.
2. Label the tube of BMX **Reagent #12**.
3. Centrifuge the reagent at 1,000 rpm for one minute.
4. Set aside on ice.

Prepare AMX2

1. Invert the tube of AMX2 five times to mix the reagent.
2. Label the tube of AMX2 **Reagent #13**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside on ice.

Prepare APM2

1 Invert the tube of APM2 five times to mix the reagent.

2 Label the tube of APM2 **Reagent #14**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside at room temperature.

Prepare AT2

1 Invert the tube of AT2 five times to mix the reagent.

2 Label the tube of AT2 **Reagent #15**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside at room temperature.

Prepare HP7

1 Invert the tube of HP7 five times to mix the reagent.

2 Label the tube of HP7 **Reagent #16**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside at room temperature.

Prepare HP11

You must use HP11 for sequencing dual-indexed Nextera libraries.

1 Invert the tube of HP11 five times to mix the reagent.

2 Label the tube of HP11 **Reagent #16**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside at room temperature.

Prepare HP3

1 Invert the tube of HP3 five times to mix the reagent, and then pulse centrifuge.

2 Transfer 2.85 ml of PW1 into a 15 ml Sarstedt conical tube and add 150 µl of HP3.

3 Invert the tube five times to mix the reagent.

4 Label the conical tube of diluted HP3 **Reagent #19**.

5 Centrifuge at 1,000 rpm for one minute.

6 Set aside at room temperature.

Prepare HT2

1 Invert the tube of HT2 five times to mix the reagent.

2 Label the tube of HT2 **Reagent #21**.

3 Centrifuge the reagent at 1,000 rpm for one minute.

4 Set aside at room temperature.

Load Paired-End Reagents

When prompted after completion of the Index Reads, load paired-end reagents.

1 Weigh each reagent and record the weight on the lab tracking form.

2 Briefly pulse centrifuge each reagent prior to loading it onto the Paired-End Module.

NOTE
Replace diluted HP3 and HT2 used during the Index Read with fresh reagent for Read 2 resynthesis.

3 Load reagents onto the Paired-End Module in the following positions:

Position	Reagent	Description
11	LMX2	Linearization Mix 2
12	BMX	Blocking Mix
13	AMX2	Amplification Mix 2
14	APM2	AMX2 Premix
15	AT2	100% Formamide
16	HP7 or HP11	Read 2 Sequencing Primer
19	HP3, diluted	Denaturation Solution
21	HT2	Wash Buffer

NOTE
RMX is already loaded in position 10. RMX and HP12 can remain on the Paired-End Module.

4 Connect the reagent tubes to the corresponding port position on the Paired-End Module.

5 Place the waste tube into a waste container or a 15 ml conical tube.

6 When you have finished loading reagents, click **OK** to resume the sequencing recipe and proceed to Read 2 resynthesis.

Perform Read 2 Resynthesis

The sequencing recipe automatically primes paired-end reagent positions using the internal priming pump on the Paired-End Module, and dispenses waste to the waste bottle, bypassing the flow cell.

Priming the Paired-End Module takes approximately 15 minutes. Read 2 resynthesis takes approximately four hours after priming is complete. There is no need to remount the flow cell or perform a leak test.

1 After Read 2 resynthesis, weigh each of the reagents used on the Paired-End Module.

2 Record the weights on the lab tracking form.
The following table lists reagent delivery for Read 2 resynthesis, and includes the priming volume. Volumes can vary ±15%.

Table 15 Reagent Delivery During Read 2 Resynthesis

Position	Reagent	Delivery (PEM) (µl or mg)	Delivery (PEM IIx) (µl or mg)
11	LMX2	1200	1350
12	BMX	1720	1870
13	AMX2	4920	5070
14	APM2	5310	5310
15	AT2	4110	4110
16	HP7 or HP11	1350	1350
19	HP3 (Diluted)	1950	1950
21	HT2	7110	7160

NOTE
For dual-indexed paired-end runs, RMX (position 10) is used during Index 2 (i5) preparation. It is not used during Read 2 resynthesis.

Prepare SBS Reagents for Read 2

Prepare and combine fresh sequencing reagents for Read 2 according to the length of your read. For more information, see *Preparing SBS Reagents* on page 63 and *Reagent Kits and Replenishing Cycles* on page 66.

Load SBS Reagents for Read 2

When prompted after the completion of Read 2 resynthesis, load SBS reagents for Read 2.

1 Weigh each of the reagents before loading them onto the Genome Analyzer and record the weight on the lab tracking form.

2 Invert each container several times to mix the reagent.

3 Centrifuge SMX, IMR, and CLM at 4°C to 1000 xg for 1 minute.

4 Load reagents in the appropriate positions. Load CLM last to avoid cross-contamination.

Table 16 Reagent Positions

Position	Contents	Description	Container Size
1	IMR	Incorporation Mix	175 ml Bottle
2	PW1	Wash Solution	125 ml Bottle
3	SMX	Scan Mix	175 ml Bottle
4	PR1	High Salt Buffer	125 ml Bottle or 150 ml bottle
5	PR2	Incorporation Buffer	125 ml Bottle or 150 ml bottle
6	CLM	Cleavage Mix	175 ml Bottle
7	PR3	Cleavage Buffer	125 ml Bottle or 150 ml bottle

5 Insert the appropriate reagent tube into the cap of each container in positions 1, 3, and 6.

6 Click **OK** to resume the sequencing recipe and proceed to first base incorporation.

Check Quality Metrics for Read 2

1. When prompted after the completion of Read 2 first base incorporation, open the Focus Calibration Result window.
2. Make sure that the instrument is still in focus. Do one of the following before proceeding:
   - Click **OK** to accept the current calibrated focus and resume the recipe. The flow cell is automatically flushed with SMX (Scan Mix).
   - Refocus using the auto calibration feature. Select **Instrument | Calibrate** from the Data Collection toolbar. Upon completion, click **OK** to resume the recipe.

Wash the Paired-End Module

1. When prompted, replace all reagent tubes on the Paired-End Module with 15 ml conical tubes containing at least 10 ml of MilliQ water or laboratory grade water.
2. Click **OK** to proceed to the Paired-End Module water wash.
   Directly following the Paired-End Module water wash, the recipe begins Read 2.

Perform Read 2

1. Replenish reagents at the recommended cycles for your run, as needed.
   For more information, see *Reagent Kits and Replenishing Cycles* on page 66.
2. When Read 2 is complete, weigh each of the reagents on the Genome Analyzer and record the weights on the lab tracking form.
3. Proceed to *Performing a Post-Run Wash* on page 143.

Performing Post-Run Procedures

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Performing Post-Run Procedures

Perform the following three procedures after each run:

- **Weighing reagents**—Post-run reagent weight is a good indicator of fluidics system performance and can be used for troubleshooting purposes.
- **Checking error logs**—Error logs report any network connection issues that might have occurred during the run.
- **Performing a post-run wash**—Regular instrument washes ensure continued performance in the following ways:
  - Flushes any remaining reagents from the fluidics lines
  - Prevents salt accumulation and crystallization in the fluidics lines
  - Prevents cross-contamination from the previous run

Weighing Reagents

1. When the sequencing run is complete, weigh each of the reagents on the Genome Analyzer.
2. Record the weights on the lab tracking form.

Checking the Error Log

After the run is complete, check the error log.

1. Open the ErrorLog.txt located in <Run Folder>\Data.
2. Check the entries. Consider the following:
   - During a typical run, you might run experience copy issues that log the error “Unable to copy Queued file (retry later)". When copying resumes successfully, RTA proceeds normally. A few entries of this type in the error log is acceptable.
   - If you see numerous “Unable to copy Queued file (retry later)" entries (dozens or hundreds), you might have a problem with your network.

Performing a Post-Run Wash

After every single-read run or after the second read of a paired-end run, you must perform a thorough instrument wash.

The wash recipe flushes 4 ml of wash PW1 (wash solution) through each reagent port on the instrument. The wash recipes used with paired-end, single-indexed, and dual-indexed sequencing runs also flush 1 ml of wash solution through reagent ports on the Paired-End Module.

Run time varies depending on the recipe you are using:

- Run time for the single-read recipe is approximately 45 minutes.
- Run time for the paired-end recipe is approximately 60 minutes.

Illumina-Supplied Consumables

- PW1 (provided in the SBS Kit)

User-Supplied Consumables

- Lens cleaning tissue
- 125 ml Nalgene bottles (ThermoFisher Scientific, catalog # 342040-0125)
- 175 ml Falcon bottles (BD, catalog # 352076)
- MilliQ water or laboratory grade water (for washing the Paired-End Module)

Procedure

3 Load PW1 solution as follows:
   - 10 ml PW1 onto positions 1, 3, and 6
   - 40 ml PW1 onto positions 2, 4, 5, and 7

   You can use the same wash containers set aside after the pre-run wash.

   NOTE
   Rotate the tubes while holding the caps stationary to prevent crimps and twisting in the liquid delivery lines.

4 [Paired-End Module] Place at least 5 ml of laboratory grade water into 15 ml conical tubes in the following positions on the Paired-End Module:
   - **Any paired-end run**—Positions 9–22
   - **Single-indexed single-read run**—Positions 19, 21, 22, and 23
   - **Dual-indexed single-read run**—Positions 19, 21, 22, and 23

5 Bundle the Genome Analyzer waste tubing, keeping the ends even. Wrap the tubes with parafilm and place the tube ends into a 50 ml bottle.

6 From the Run tab, select **File** | **Open Recipe** and open one of the following recipes:

Type of Run	Recipe Name
Non-Indexed Single-Read Run	GA2_PostWash_v10.xml
Single-Indexed Single-Read Run, or Dual-Indexed Single-Read Run	GA2-PEM_MP_PostWash_SR_v10.xml
Non-Indexed Paired-End Run, or Single-Indexed Paired-End Run, or Dual-Indexed Paired-End Run	GA2-PEM_PostWash_PE_v10.xml

7 Click **Start** to begin the wash.

Performing a Monthly Maintenance Wash

The Maintenance Wash has two parts: the 1 ml water wash and the 4 ml NaOH wash. Perform the maintenance wash once monthly for maintenance, and any time you plan to leave the Genome Analyzer sitting idle for more than three days. The monthly maintenance wash uses the same instrument cycle as the pre-run wash, but uses a stronger base (1 N NaOH) and filtered cleaning liquids.

Run time varies depending on the recipe you are using. The run time for the single-read recipe is approximately 45 minutes. The run time for the paired-end recipe is approximately 1 hour and 24 minutes.

Illumina-Supplied Consumables

- PW1 (Wash Solution)

User-Supplied Consumables

- Lens cleaning tissue
- 1 N NaOH (0.5 L, filtered with a 0.2 µm nylon filter)
- Four 125 ml Nalgene bottles (ThermoFisher Scientific, catalog # 2019-0125)
- Three 175 ml Falcon bottles (BD, catalog # 352076)
- MilliQ water or laboratory grade water (for washing the Paired-End Module)

Procedure

1 Load a used flow cell.

NOTE
When the manifolds are raised for the first time, place the fluidics on standby. For more information, see *Unloading the Flow Cell and Prism* on page 79.

2 Select **File** | **Open Recipe**.

3 Open one of the following recipes:
- For single-read sequencing, open GA2_MaintenanceWash_v<#>.xml.
- For paired-end sequencing, open GA2-PEM_MaintenanceWash_v<#>.xml.

4 Load PW1 wash solution as follows:
- 10 ml onto positions 1, 6, and 3
- 40 ml onto positions 4, 5, 2, and 7

CAUTION
Rotate the tubes while holding the caps stationary to prevent crimps and twisting in the liquid delivery lines.

5 [Paired-End Module] Place 10 ml of laboratory grade water into 15 ml conical tubes in positions 9–23.

6 Click **OK** to start the prewash.

7 After the pre-run wash is complete, load filtered 1 N NaOH as follows:
- 25 ml onto positions 1, 6, and 3
- 50 ml onto positions 4, 5, 2, and 7

8 [Paired-End Module] Place 10 ml of filtered 1 N NaOH into 15 ml conical tubes in positions 9–23.

9 Click **OK** to start the NaOH wash.

10 After the NaOH wash is complete, load PW1 wash solution as follows:
- 15 ml onto positions 1, 6, and 3
- 50 ml onto positions 4, 5, 2, and 7

11 [Paired-End Module] Place 15 ml of laboratory grade water into 15 ml conical tubes in positions 9–23.

12 Click **OK** to start the wash.

Performing a Storage Wash

If you plan to leave the Genome Analyzer idle for more than three days, perform the storage wash following the maintenance wash.

Illumina-Supplied Consumables

- PW1 (Wash Solution)

User-Supplied Consumables

- Lens cleaning tissue
- 1 N NaOH (0.5 L, filtered with a 0.2 µm nylon filter)
- Four 125 ml Nalgene bottles
  (ThermoFisher Scientific, catalog # 342040-0125)
- 175 ml Falcon bottles (BD, catalog # 352076) (Genome Analyzer IIx)
- 50 ml conical tubes (Genome Analyzer II)
- MilliQ water or laboratory grade water
  (for washing the Paired-End Module)

Procedure

1. Load PW1 solution as follows:
   - 10 ml PW1 onto positions 1, 6, and 3
   - 40 ml PW1 onto positions 4, 5, 2, and 7
2. [Paired-End Module] Place at least 5 ml of laboratory grade water into 15 ml conical tubes onto positions 9–23.
3. Click the Run tab.
4. Select **File** | **Open Recipe**.
5. Open one of the following recipes:

Recipe Name	Positions Washed
GA2_PostWash_v<#>.xml	Positions 1–7 on the Genome Analyzer used for single-read runs
GA2-PEM_PostWash_v<#>.xml	Positions 1–7 on the Genome Analyzer and positions 9–22 on the Paired-End Module

6. Click **Start**.
7. When the run finishes, click the Manual Control tab.
8. In the Pump area, set the parameters as follows:
   Solution: 8 (PEM not enabled); 28 (PEM enabled)
   Volume: 0
9. With the cursor in the **Volume** field, press **Enter**.
10. Leave the used flow cell in the instrument to prevent siphoning.
11. Close the instrument software and shut down the computer.
12. Turn the instrument power switch to the OFF position.

Resuming Use After Storage

Perform the following steps to resume instrument use after short-term or long-term storage:

1 Turn on the instrument.

2 Start the computer and log on to the operating system.

3 Open the instrument software.

4 Load PW1 solution as follows:
- 10 ml PW1 onto positions 1, 6, and 3
- 40 ml PW1 onto positions 4, 5, 2, and 7

5 [Paired-End Module] Place at least 5 ml of laboratory grade water into 15 ml conical tubes onto positions 9–23.

6 Click the Run tab, and select **File** | **Open Recipe**.

7 Open one of the following recipes:

Recipe Name	Positions Washed
GA2_PostWash_v<#>.xml	Positions 1–7 on Genome Analyzer
GA2-PEM_PostWash_v<#>.xml	Positions 1–7 on Genome Analyzer and positions 19–23 on the Paired-End Module

8 Click **Start**.

Troubleshooting

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Troubleshooting Priming Volume

If the delivered priming volume differs from the expected volume by more than 10%, do the following:

1. Click the Manual Control/Setup tab. In the Pump area, set the following values to prevent siphoning of reagents:
   Command: Pump
   To: Flow cell
   Solution: 8 (PEM not enabled); 28 (PEM enabled)
   Volume: 0
   Aspiration Rate: 250
   Dispense Rate: 2,500
2. Click **Load Flow Cell** to bring the stage to the front of the instrument and raise the lens.
3. In the **Instrument** drop-down list, select **Unlock Door**. Raise the door.
4. Briefly lift the manifolds and gently reposition the flow cell.
5. Repeat the priming procedure.

Troubleshooting Leaks

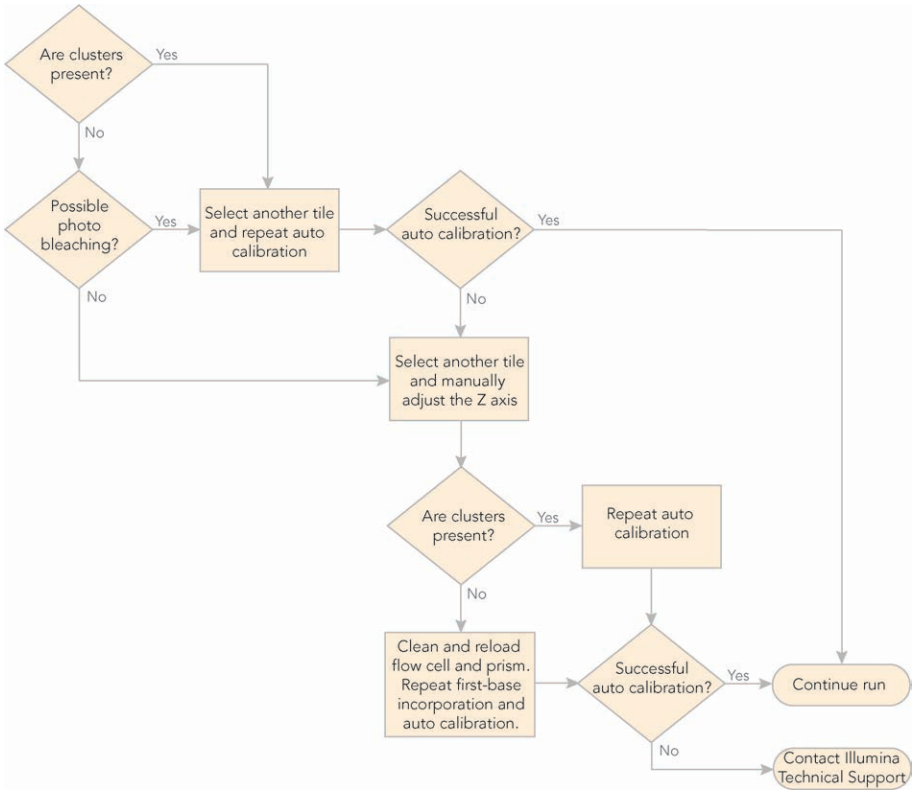
Always perform a leak test after loading the flow cell. If the flow cell is not seated properly, leaks might appear between the flow cell and the manifolds. If a leak is present, perform the following steps:

1. Click the Manual Control/Setup tab. In the Pump area, set the values as follows:
   - **Command**: Pump to Flow Cell
   - **To**: Flow cell
   - **Solution**: 8 (PEM not enabled); 28 (PEM enabled)
   - **Volume**: 0
   - **Aspiration Rate**: 250
   - **Dispense Rate**: 2,500
2. Click **Load Flow Cell** to bring the stage to the front of the instrument and raise the lens.
3. Lift the manifolds and clean the interface between the manifold and flow cell with a moist lens cleaning tissue.
4. Dry and re-seat the flow cell.
5. In the Pump area, set the values as follows:
   - **Command**: Pump to Flow Cell
   - **To**: Flow cell
   - **Solution**: 5
   - **Volume**: 100
   - **Aspiration Rate**: 250
   - **Dispense Rate**: 2,500
6. Pump another 100 µl of the Incorporation Buffer (solution 5) through the system.

Troubleshooting Calibration Failure

Auto calibration can fail if no clusters are present in the tile image or if there is surface contamination on the flow cell or prism. If auto calibration fails, an error message appears. The instrument is placed in a safe state and PR1 (High Salt Buffer) is flushed through the flow cell.

Figure 60 Troubleshooting Calibration Failure



1 Click **OK** and visually inspect an image to make sure clusters are present. Clusters must be present for successful calibration.

2 Move to another tile using the auto calibration settings in the Run Parameters window, or using the settings on the Manual Control/Setup tab in the Data Collection Software window.

NOTE
Illumina recommends that you move to another tile to avoid bleaching of the currently selected tile. Photo bleaching starts to diminish the intensity of the cluster signals after a few seconds of total exposure.

3 Repeat auto calibration manually:
   a Pump SMX (Scan Mix) to the flow cell before initiating auto calibration.
   b Move to another tile to avoid photo bleaching of the previously imaged tile.
   c Select **Instrument** | **Calibration** from the Data Collection Software toolbar.

4 If calibration failed again, manually adjust the Z axis until clusters are visible:
   a Click **Take Picture** on the Manual Control/Setup tab.
   b Move the Z position in increments of 300 nm, decreasing to 100 nm as the focus improves. Repeat steps a–b each time you move the Z position.

5 If you are able to identify clusters in the image, even if they are slightly out of focus, do the following:
   a Select **Instrument | Set Coordinate System | Set Current Z as Origin**.
   b Repeat auto calibration.

NOTE
Surface contamination is the most common cause for poor focus. For example, a little oil might have contaminated the surface when you applied oil to the flow cell/prism interface.

6 If clusters are not visible, do the following:
   a Clean and reload the flow cell and prism.
   b Repeat first base incorporation and auto calibration. Make sure you pump SMX (Scan Mix) through the flow cell before the imaging step.

Auto Calibration Warnings

When you select **View | Calibration** in the Data Collection toolbar, the Focus Calibration Results window shows the following settings:

- **Goodness of fit:** ≥ 0.9900
- **Sensitivity:** 350–400

A warning appears at the bottom of the window if either parameter is out of range. You might see any of the following warnings:

- Warning: CurveFit received _____ images, expecting 21
- Warning: Sigma(q) = _____ exceeds allowed threshold 0.5
- Warning: Correlation coefficient R(z, r) = _____ is less than allowed threshold 0.95
- Warning: Focus Calibration sensitivity is too low. Nm/pixel _____ exceeds allowed threshold 500.00
- Warning: Mean spot picture quality = _____ is less than allowed threshold 0.90. Increase exposure.

Using Manual Controls

The following table describes the areas of the Manual Control/Setup window used during manual focus.

Table 17 Manual Controls

Control	Description
Tile	Enter lane, column, and row coordinates to move the objective to a certain tile. The XY center point for flow cells is typically Lane 5, Column 1, Row 25. This is in the middle of the flow cell.
X/Y (µm)	Shows the current position of the flow cell under the objective lens. Because of minute variations between flow cells, you need to fine-tune the X value so that the edge of lane 1 is at the center of the image after you load a new flow cell. You can adjust the position by entering new values into the **Stage Position** fields and pressing **Enter**. The laser remains stationary, and the flow cell moves underneath it. Increase X to move the right edge of the flow cell under the laser (the stage moves toward the left). Increase Y to move the back of the flow cell under the laser, near the output ports (the stage moves toward the front). You do not need to reset Y=0 unless there is a significant hardware change, in which case an engineer will reset it. After you set the X value correctly, re-zero the X coordinate using the **Instrument** | **Set Coordinate System** menu.
Z (nm)	Shows the current position of the objective lens relative to the flow cell. To adjust the position, enter new values into the **Stage Position** boxes and press **Enter**. The flow cell remains stationary, and the lens moves up and down. Increase Z to move the lens away from the flow cell vertically. Decrease Z to move the lens closer to the flow cell. Ideally, the value of Z at the focal position should be zero (0). The farthest you can safely move below the focal point is 40,000 nm (Z = -40000). The position of the flow cell surface varies from one flow cell to another. You might need to move down 20, 000 nm to find the focal plane of a new flow cell. When you find the focus, re-zero the Z axis and perform autofocus calibration before completing the run. The focal position on the Z axis must be higher than Z= -20,000 (as noted above, it should ideally be zero), or the objective will not have enough room to move down without hitting the flow cell while tracking focus position during the run.
Laser	Controls which laser is used during the exposure. **Red laser**—For use with A and C filters. **Green laser**—For use with G and T filters. **Focus**—Used by Illumina Technical Support and during autofocus calibration to illuminate the focus spot. **Off** (default)—Closes laser shutter for the photo, using only ambient reflected light for the photo. Support scientists sometimes use this to check the optical path.
Filter	Moves the filter wheel to view only the light from a particular base (A, C, G, T). When you select one of the laser settings, the software automatically defaults to the correct filter setting. If you selected the Focus laser, the software defaults the filter setting to None.
Camera	**Exposure**—Lets you set the exposure time in milliseconds. **Start Video**—Click to show a series of images in a sequential loop (like a movie), using the current configuration. Click the button again to stop the video.

Performing Manual Focus

To focus manually, you take photographs from different positions and adjust the focus along the Z axis as necessary. Focus is calibrated manually only if auto calibration is not enabled in the Run Parameters window. For more information, see *Run Parameters Window* on page 26. For a description of auto calibration, see *Auto Calibration* on page 45.

Default XYZ Coordinates

When you initialize the Genome Analyzer, the lens orients itself using limit switches inside the instrument. The default XYZ coordinates are identified in the HCMConfig.xml file. These coordinates are specific to each machine and are configured during installation. Generally, the coordinates are set so that X=0 should put the left edge of lane 1 in the center of the screen. Y=0 puts the objective at the forward-most point of the flow cell. Z=0 should put the clusters in focus.

When you refocus and save the new origin points, the coordinates in the HCMConfig.xml file are updated and become the new defaults for that instrument.

WARNING

Never use the HCMConfig.xml file from any other Genome Analyzer. The coordinates will not be correct for that instrument, and the lens, flow cell, or other equipment could be damaged or broken when the lens returns to the home position.

Adjust the X Axis

Set the position of the flow cell along the X axis so that the zero point is at the left edge of Lane 1, near the bottom of the flow cell. This is done in the following five steps:

- *Move the Stage* on page 155
- *Set Initial Focus* on page 156
- *Find the Left Edge* on page 157
- *Confirm the Left Edge* on page 158
- *Set Current X as Origin* on page 159

Move the Stage

Use the following sequence of stage adjustments to safely move the stage to the proper starting position without running the risk of hardware collision.

1. Click the Manual Control/Setup tab.
2. Set the **X** and **Y** values as follows:
   X (µm): 5,000
   Y (µm): 15,000
3. Click the down arrow next to the Take Picture button and click **Move**. This moves the stage to a position near the middle of the flow cell.
4. Set **Z (nm)**: 0 (zero) and click **Move**.
   This moves the objective stage close to the plane of focus prior to finding the edge of the flow cell. This assumes that you reset the Z position to zero when you focused it during the last run. For more information, see *Adjust the Z Axis* on page 161.
5. Set the **X** and **Y** values as follows:

X (µm): 0 (zero)
Y (µm): 0 (zero)

6 Click **Move**. This moves the objective stage to a position near the bottom left of the flow cell. The left edge of Lane 1 is underneath the objective.

NOTE
The left part of the image might be dark and without any clusters. This area is outside of lane 1 where no clusters have formed.

Set Initial Focus

NOTE
Problems with focusing can be caused by too much oil. Inspect the flow cell surface for dirt or oil. If the surface is not clean, remove and clean the prism and flow cell. Then, skip the first base incorporation step after reloading the prism and flow cell.
Remember to add fresh Scan Mix prior to taking pictures.

Set the initial focus in channel T so the left edge of Lane 1 appears as a sharp line. Fine-tuning the focus for all channels is performed in *Adjust the Z Axis* on page 161.

1 Flush 75 µl of SMX (Scan Mix) prior to imaging.

2 Set the following values:
Laser: Green
Filter: T
Exposure (msec): 100

3 Click **Take Picture**.

4 Right-click over the image and select **Auto Scale** | **On.**

NOTE
If the clusters in the image are too dim, increase the exposure time to 200 msec. If the clusters are saturated (mouse over cluster and see if intensity reads 4095), reduce the exposure time.

5 Evaluate the clusters in the image.
- If the clusters are too dim, increase the exposure time to 200 msec.
- If the clusters are saturated, hover your mouse over the cluster image to check the cluster intensity. If it reads 4095, reduce exposure time.

6 Adjust the Z position as needed to achieve focus. Each time you move the Z position, click **Take Picture**.
- Initial moves should use the full tile image until the clusters become distinct points on the image. Subsequent moves can be smaller as you approach the optimal focus position. Zoom in on the image for final adjustment.
- Generally, start with a move no larger than 1,000 nanometers. If you see you are far out of focus, you can use 2,500 nm moves. Make sure not to step over the focus.
- A distinct halo effect, especially around the smaller (less intense) clusters, is characteristic of images acquired with the lens just above optimal focus position. Lower the Z value.
- A uniform loss of sharpness and intensity is characteristic of images acquired with the lens just below the optimal focus position. Increase the Z value.

Figure 61 Lens Too High

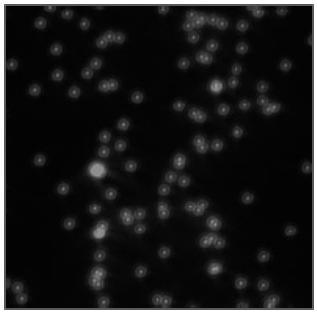


Figure 62 Lens Too Low

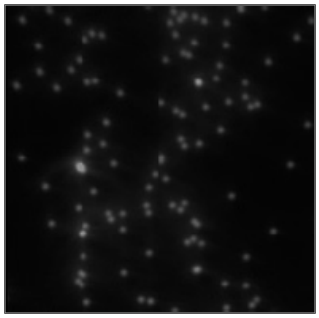
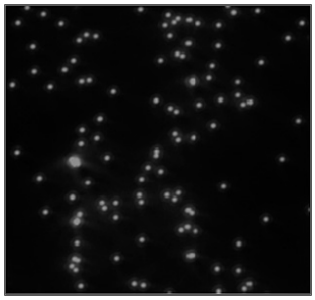


Figure 63 Lens Properly Positioned



7 After the clusters come into focal range, adjust the Z position in steps of 100–500 nm for each change.

8 Evaluate the image to determine the success of first base incorporation. You should see many clusters.

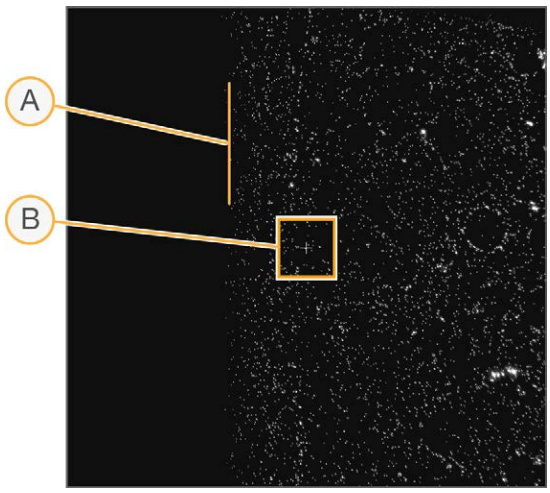
9 Do one of the following:
- If first base incorporation failed, discontinue the run and perform the post-run instrument wash.
- If first base incorporation was successful, proceed to *Find the Left Edge* on page 157.

Find the Left Edge

Now you are ready to set the position of the flow cell along the X axis.

1 Click **Take Picture**. To see the left edge, zoom out to the whole tile image. The edge of Lane 1 appears on the screen. The screen is 2,048 x 2,048 pixels in size, with each pixel representing approximately 371 nm. The crosshair indicates the center of the image (1,024 x 1,024 pixels).

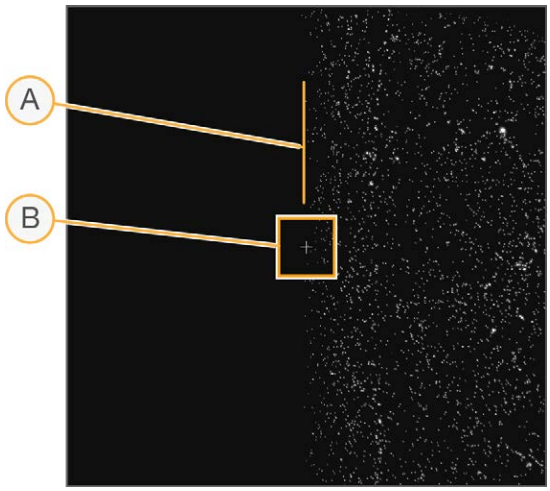
Figure 64 Crosshair at Center of Image



A Edge of Lane
B Crosshair

2 Confirm that the left edge of Lane 1 is close to the center of the image. To identify the distance between the edge and the crosshair, use your mouse to position the arrow over the edge of Lane 1. The pop-up window reveals the position of the arrow in pixels.

Figure 65 Left Edge of Lane 1 on the Screen



A Edge of Lane
B Crosshair

3 If the edge is more than 3–5 pixels from the vertical crosshair, move the stage in the X axis to bring the edge closer to the crosshair.

- Increase the X value to move the edge of Lane 1 to the left.
- Decrease the X value to move the edge of Lane 1 to the right.

NOTE
You cannot change the X origin by more than 1,000 µm at a time.

Confirm the Left Edge

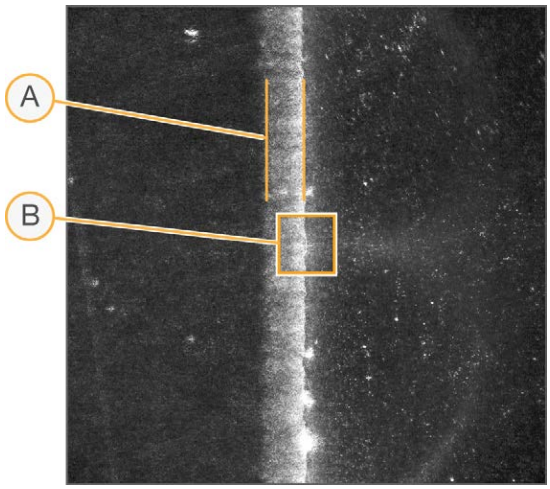
Next, you need to confirm that you have found the left edge of Lane 1.

1 Set the following values:
Laser: Green
Filter: None
Exposure (msec): 3

2 Click **Take Picture**.

3 Confirm that edge of Lane 1 appears on the screen as a blurred, wide line, and the vertical crosshair is within 3–5 pixels of the blurred edge.

Figure 66 Blurred Edge of Lane 1



A Blurred Edge of Lane
B Crosshair

NOTE
If you do not see a blurred edge, or if the blurred edge is not in close proximity to the crosshair, you might be imaging the laser footprint edge. This indicates a misalignment in the instrument, and needs to be corrected by Illumina Technical Support.

Set Current X as Origin

1 Select **Instrument** | **Set Coordinate System** | **Set Current X as Origin**.

2 Click **OK** to confirm that you want to reset the coordinates and XY drift.
This automatically adjusts the values in the HCMConfig.xml file. You will adjust the XY drift (or rotation of the part about the Z axis) in *Set XY Drift* on page 159.

WARNING
Do not adjust the Y axis.
Only an Illumina Field Service Engineer should adjust the Y axis, and only in the case of manifold, XY stage, stage, or optical column replacement.

Set XY Drift

If the flow cell is rotated on the Z axis, the lanes will not be aligned with X at both ends, and imaging may drift too far outside the lanes. To prevent this, set a drift correction factor to adjust the X coordinate for each tile location.

Move the stage up in the Y direction to find the left edge of lane 1 at the outlet end of the lane, and set the drift using the following sequence to avoid hardware collision.

1 Set the **X** and **Y** values as follows:

X (µm): 0 (zero)
Y (µm): 37,000

2 Click **Move**. This moves the stage to a position near the upper left of the flow cell. The left edge of Lane 1 is underneath the objective.

3 Set the following values:
Laser: Green
Filter: T
Exposure (msec): 100 (increase to 200 msec if the image is too dim)

4 Click **Take Picture**. The edge of Lane 1 appears on the screen. The screen is 2,048 x 2,048 pixels in size, with each pixel representing approximately 371 nm. The crosshair indicates the center of the image (1,024 x 1,024 pixels).
You may need to adjust focus for the new tile location, since focus between tiles at opposite ends of the flow cells may differ. For more information, see *Set Initial Focus* on page 156.

5 Confirm that the left edge of Lane 1 appears close to the center of the image. To identify the distance between the edge and the crosshair, use your mouse to position the arrow over the edge of Lane 1. The pop-up window reveals the position of the arrow in pixels.

6 If the edge is more than 3–5 pixels from the vertical crosshair, move the stage in the X axis to bring the edge closer to the crosshair.
- Increase the X value to move the edge of Lane 1 to the left.
- Decrease the X value to move the edge of Lane 1 to the right.

7 Select **Instrument | Set Coordinate System | Set Current X as top-left edge to determine XY drift**.
The current Y is validated and the XY drift is computed. A message appears that reads "The drift was set to 0.nnnnn," for example.

8 Confirm that the current Y and the computed XY drift are within range. If one of the values is out of range, do one of the following:
- If the Y coordinate is not large enough an error message appears that reads: "Current Y=nnnnn is not far enough (min = mmmmm) from the coordinate system origin."
  Go back to step 1 and make sure you enter 35,000 (thirty-five thousand) as the Y value.
- If the drift is too large, the error message reads: "Flow cell XY Drift, resulting from TopLeftX = 333 and TopLeftY = 33333, exceeds allowed limit = 0.zzzz."
  The flow cell has not been registered correctly. Confirm that the flow cell is seated correctly against the pins. If necessary, clean and reload the prism and flow cell.

Confirm the Footprint

1 Set the following values:
Laser: Green
Filter: T
Exposure (msec): 100 (increase to 200 msec if the image is too dim, decrease if the image is saturated)

2 Go to lane 5, column 1, row 25. Take a picture with the T filter. Adjust focus if necessary.
The left side of the image should have a black band with no clusters; this is the edge of the wall of the lane and will be cropped by the region of interest (ROI). However, if there is a black band on the top, bottom, or right of the column 1 image, the footprint is not aligned properly.

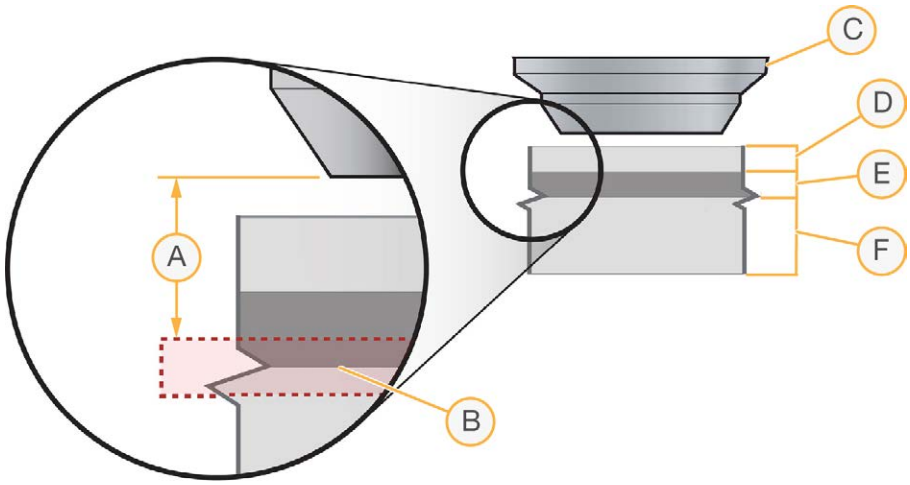
3 Next, move to column 2 of the same lane and row, and click **Take Picture**.

4 Confirm that the black band without clusters is on the right, and no bands are on the top, bottom, or left. If bands are present, the footprint is out of alignment.
If the footprint is misaligned, contact Illumina Technical Support.

Adjust the Z Axis

To adjust the Z axis, you take photos over Lane 5 to determine the optimal position of focus for the flow cell using the Focus Quality (FQ) metric. You should adjust the X axis and the XY drift before performing this procedure.

Figure 67 Focusing Z Axis



A Focal Distance
B Cluster Surface
C Objective at Height Z=0
D 300 µm
E Wet Layer 100 µm
F Glass Layer 700 µm

CAUTION
Minimize the number and duration of exposures during manual focus on a given tile. Photo bleaching starts to diminish the intensity of cluster signals after a few seconds of total exposure.

1 In the Tile area, set the coordinates to Lane 5, Column 1, Row 25. This is the approximate center of the flow cell.

NOTE
Typically, the center tile is used for setting the focus. Alternatively, you can move closer to the start of imaging at Lane 1, Column 1, Row 5.

Make sure that the calibration curve is of good quality and the flow cell drift is less than 5,000 nm, as calculated in the First-Cycle report.

2 Find the best focus for channel A by recording the FQ values for a range of Z positions:

a Set the following values:
Laser: Red
Filter: A
Exposure (msec): 100 (increase to 200 msec if the image is too dim, decrease if the image is saturated)

b Click **Take Picture**.

c Mouse over the image to see the FQ value, and record the FQ value and Z position.

NOTE
Focus Quality depends on cluster size and has a "focused" optimum value from 80–85.

d Move the Z position in increments of 300 nm, decreasing to 100 nm as the focus improves (higher FQ value). Repeat steps b–c each time you move the Z position.

e When you are able to identify the peak FQ value, move the Z stage to the associated Z position. Select **Instrument** | **Set Coordinate System** | **Set Current Z as Origin**.

3 Find the optimal focus for channels A, C, G, and T by recording the FQ values for a range of Z positions, and determining the Z position with the highest combined FQ value.

a Record the FQ values for channel A, C, G, and T by repeating steps 2b–c.
- Channel A: Laser Red, Filter A
- Channel C: Laser Red, Filter C
- Channel G: Laser Green, Filter G
- Channel T: Laser Green, Filter T

b Move the Z stage 300 nm up and down in 100 nm steps. Take pictures at every step, and record the FQ values and associated Z positions for all channels.

c Add all four FQ values for every Z position in the last column.

d Determine the Z position with the highest sum of FQ values. This is the optimal Z position.

NOTE
At the correct depth of focus, the FQ values will remain stable with little change over a range of 500 nm. You should calibrate auto focus and set Z=0 in the center of this 500 nm range.

e Move the Z stage to the optimal Z position, and select **Instrument** | **Set Coordinate System** | **Set Current Z as Origin**.

Stopping the Current Read

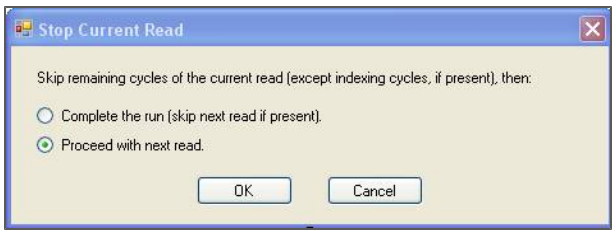
If the quality of the current read is deteriorating, SCS enables you to skip the rest of the cycles and continue to the next read. This does not apply to index reads. Continuing with the next read may still yield meaningful data, while saving time, storage, and reagents.

When you stop the current read, the software completes analysis of the data collected at that point, and enables all subsequent steps of the off-line analysis, such as alignment. Meta-data integrity is preserved. The resulting run, including analysis, should appear as if the skipped cycles were initially excluded from the recipe.

To stop the current read, perform the following:

1 Select **Run** | **Stop Current Read** in the toolbar. The Stop Current Read dialog box opens.

Figure 68 Stop Current Read Dialog Box



2 In the Stop Current Read dialog box, choose one of these options:

- **Complete the run**—Skips all remaining cycles of the current read. For multiplexing recipes, executes all indexing cycles and finishes the run.
- **Proceed with next read**—Skips the remaining cycles from the current read and jumps to the next read.

The option may be disabled if it does not apply to a particular situation, for example, if it would not result in skipping any imaging cycles.

3 Click **OK**. After SCS completes the current recipe command, it will skip the cycles as soon as it arrives at an appropriate stage to do so.

NOTE
"Stop Current Read" requests disable the **Stop Current Read** menu item and enable **Cancel Stopping Read**. Selecting **Cancel Stopping Read** erases any remaining stop requests.

After completion of the current recipe step, the following events occur:

- The run jumps to the appropriate cycle, then continues as usual. If a command is executed to complete the run, the run executes End chemistry before completion.
- SCS modifies RunInfo.xml to reflect the actual number of cycles that were collected and analyzed.
- RTA restarts using the updated RunInfo.xml file to reflect configuration file changes.
- All skipped cycles are marked with a strikethrough font in the SCS interface and cannot be executed.
- Cycle numbers that appear in the Image folder as part of the folder names are normalized to eliminate gaps. These cycle numbers are passed to event scripts; however, the user interface shows the original cycle numbers.

Sample Sheets and Recipe Commands

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Sample Sheets

The sample sheet is a comma-separated values file that describes the location of each sample on the flow cell, and the indices used, if any.

NOTE
Using a sample sheet is essential for indexed sequencing runs in order for Sequencing Analysis Viewer (SAV) to properly report indexing information for CASAVA to demultiplex the samples.

Sample sheets contains the following fields:

Table 18 Sample Sheet Columns

Field	Description
FCID	Flow cell ID
Lane	Positive integer, indicating the lane number (1-8)
SampleID	ID of the sample
SampleRef	The reference sequence for the sample
Index	Index sequence, or 0 for no indexing
Description	Description of the sample
Control	Y indicates this lane is a control lane, N means sample
Recipe	Recipe used during sequencing
Operator	Name or ID of the operator
SampleProject	The project name associated with the sample

Illumina Experiment Manager

You can use the Illumina Experiment Manager, which is a wizard-based application that guides you through the steps to create your sample sheet based on the parameters for your run.

You can download a copy of the Illumina Experiment Manager from the Illumina website at http://www.illumina.com. Go to the Genome Analyzer support page and click **Downloads**. A MyIllumina login is required.

Sample Sheet Selection

You can configure sample sheet selection in the Run Parameters window using one of the following two options:

- **Browse to a sample sheet**—Select **Prompt for Sample Sheet at Run Start** in the Run Parameters window to configure the software to prompt you to browse to a sample in any location accessible from the instrument computer. You must also select **Require sample sheet at run start** to activate this option.
- **Auto-Discovery Using Flow Cell ID**—Select only **Require sample sheet at run start** to configure the software to automatically look for a sample sheet named with the flow cell ID specified in the Load Flow Cell dialog box in the network location specified on the Run Parameters window. If it cannot find one, SCS opens the Sample Information dialog box. Use the browse function to select the proper sample sheet for this run.

Creating or Editing a Sample Sheet

If you need to create or edit a sample sheet, use an application that can process comma-separated values files, such as Excel.

Figure 69 Example of Sample Sheet Opened in Excel

	A	B	C	D	E	F	G	H	I	J
1	FCID	Lane	SampleID	SampleRef	Index	Desc	Control	Recipe	Operator	SampleProject
2	FC612PV	1	Sample1	E_coli	ATCACGA		N		YourName	YourProject
3	FC612PV	1	Sample2	E-Coli	ATAGCTT		N		YourName	YourProject
4	FC612PV	2	Sample3	Human	ATCACGT		N		YourName	YourProject
5	FC612PV	2	Sample4	E_coli	CGATGTA		N		YourName	YourProject
6	FC612PV	3	Sample5	E_coli	CTTGTAC		N		YourName	YourProject
7	FC612PV	3	Sample6	E_coli	ATCACGA		N		YourName	YourProject
8	FC612PV	4	Sample7	Human	CGATGTC		N		YourName	YourProject
9	FC612PV	4	Sample8	Human	ATACAGT		N		YourName	YourProject
10	FC612PV	5	Sample9	E_coli	AGCACTA		N		YourName	YourProject
11	FC612PV	5	Sample10	E_coli	TTCGATA		N		YourName	YourProject
12	FC612PV	6	Sample11	E_coli	TGCACTA		N		YourName	YourProject
13	FC612PV	7	Sample12	E_coli	AGCACTA		N		YourName	YourProject
14	FC612PV	8	Sample13	Human	CATGTTC		N		YourName	YourProject

1 Create a new sample sheet using a text editor such as Excel or Notepad, or the Illumina Experiment Manager.

2 Enter the information for each sample in the appropriate columns.

NOTE
Make sure that the sampleID and SampleRef correspond to the correct DNA samples and references used to avoid misidentifying samples and references.

3 Save the Excel file in the .csv format in a location accessible to the GA instrument computer. Start the name with the flow cell ID, followed by SampleSheet, so the final name will be in this format:

`FCxxxxx_SampleSheet.csv`

NOTE
If you used the Illumina Experiment Manager to create your sample sheet, you can also edit the sample sheet in the Experiment Manager.

Sample Sheet for Dual Indexing

When creating a sample sheet for dual indexing, enter the sequence for Index 1 and the sequence for Index 2, separated by a dash.

For example, enter TCCTGAGC-TAGATCGC.

Figure 70 Example of Sample Sheet for Dual Indexing

	A	B	C	D	E	F	G	H	I	J
1	FCID	Lane	SampleID	SampleRef	Index	Desc	Control	Recipe	Operator	SampleProject
2	FC363RWAAXX	1	Sample1	E_coli	TCCTGAGC-TAGATCGC		N		YourName	YourProject
3	FC363RWAAXX	1	Sample2	E-Coli	GGACTCCT-CTCTCTAT		N		YourName	YourProject
4	FC363RWAAXX	2	Sample3	Human	TAGGCATG-TAGATCGC		N		YourName	YourProject
5	FC363RWAAXX	3	Sample4	E_coli	TCCTGAGC-AGAGTAGA		N		YourName	YourProject
6	FC363RWAAXX	3	Sample5	E_coli	GGACTCCT-CTCTCTAT		N		YourName	YourProject
7	FC363RWAAXX	3	Sample6	E_coli	TAGGCATG-CTCTCTAT		N		YourName	YourProject
8	FC363RWAAXX	4	Sample7	Human	TCCTGAGC-TATCCTCT		N		YourName	YourProject
9	FC363RWAAXX	4	Sample8	Human	GGACTCCT-AGAGTAGA		N		YourName	YourProject
10	FC363RWAAXX	5	Sample9	E_coli	TAGGCATG-TATCCTCT		N		YourName	YourProject
11	FC363RWAAXX	5	Sample10	E_coli	TCCTGAGC-AGAGTAGA		N		YourName	YourProject
12	FC363RWAAXX	6	Sample11	E_coli	GGACTCCT-AGAGTAGA		N		YourName	YourProject
13	FC363RWAAXX	7	Sample12	E_coli	TAGGCATG-AGAGTAGA		N		YourName	YourProject
14	FC363RWAAXX	8	Sample13	Human	CGAGGCTG-TAGATCGC		N		YourName	YourProject

Recipe Commands

The following commands may appear in any Genome Analyzer recipe.

Table 19 Recipe Commands

Command	Mandatory Attributes	Action
TempSet	**Temperature**—Flow cell temperature in degrees Celsius. **Duration**—Length of time that the temperature will be maintained, in milliseconds.	Sets the flow cell temperature.
TempRamp	**Temperature**—Target flow cell temperature in degrees Celsius. **Rate**—The rate of change, in degrees per second.	Ramps the temperature of the flow cell.
Wait	**Duration**—The wait time, in milliseconds.	Sets the time for the chemistry step to complete.
UserWait	[Optional] **Message**—An explanatory or instructional message that appears in a dialog box when the run pauses.	Normally, this is used when the run requires user input to continue. Click **OK** to continue the run or **Cancel** to stop the run.
PumpToFlowcell	**Solution**—The number of the solution to be pumped. **AspirationRate**—Pump rate in µl/minute. **DispenseRate**—Pump rate in µl/minute. **Volume**—Pump volume in µl.	Pumps reagent from the named bottle/tube through the flow cell.
Calibrate		Initiates auto calibration, performs a coarse focus, followed by a fine focus, and then determines the best Z position.
Findedge		Aligns the X axis and finds the left edge of lane 1 on the flow cell.
Incorporation	[Optional] **ChemistryName**—Invokes a particular chemistry as part of the incorporation cycle, such as FirstBase. **ExposureA**, **ExposureG**, **ExposureC**, and **ExposureT**—The image exposure time for each image channel during the incorporation cycle, in milliseconds.	Sets the exposure time for each image channel during incorporation. May also invoke incorporation chemistry.
RowRange	**Min**—The first row in the range. **Max**—The last row in the range.	Identifies a range of adjacent rows for incorporation.
LaneRange	**Min**—The first lane in the range. **Max**—The last lane in the range.	Identifies a range of adjacent lanes for incorporation. LaneRange only applies to the first four lanes.
Lane	**Index**—The number of the lane for incorporation.	Identifies one or more lanes for incorporation. Write separate <Lane Index= ...> commands for each lane.

Command	Mandatory Attributes	Action
		You can include <Row> or <RowRange> commands inside the <Lane> command to limit the number of rows within each lane.
Row	**Index**—The number of the row for incorporation.	Identifies one or more rows for incorporation. Write separate <Row Index= ...> commands for each lane.
ReadPrep (Appears only in paired-end recipes)	**ChemistryName**—Invokes a particular chemistry as part of the incorporation cycle, such as FirstBase. **ExposureA**, **ExposureG**, **ExposureC**, and **ExposureT**—The image exposure time for each image channel during the incorporation cycle, in milliseconds.	Applies chemistry and performs test scan with separate online analysis before each paired-end read. The second occurrence of this element marks the "turn around" point of the paired-end run and determines the length of the first read.

ReadPrep Cycles

In addition to Incorporation, ReadPrep is another cycle type in the protocol section of the sequencing recipes. The ReadPrep cycle performs scans and collects data before each paired-end read. The second occurrence of this element marks the "turnaround point" of the paired-end run and determines the length of the first read. ReadPrep does not affect cycle numbers assigned by the software to any Incorporation elements.

Each ReadPrep cycle is processed by RTA as the first cycle of a new run. Corresponding results are written to separate sub-folders of the run folder, "Read 1" and "Read 2." After the second ReadPrep element, the main run resumes as if no ReadPrep cycles existed in the recipe.

Service Recipes

Service recipes are protocols for maintaining the flow cell and instrument. The RecipeFile element in a service recipe reads <RecipeFile Type="Service">. A non-service recipe has a type of "Normal."

A service recipe causes the software to skip the sample sheet dialog box and to suppress the run folder dialog box. All information relating to the run, such as the recipe and config files, is saved in the following folder:

```
<install directory>\DataCollection_v<#>\bin
```

The folder containing the information from this run is named for the date and time that the recipe was executed.

Configuring Tile Selection

You can edit sequencing recipes to select only certain tiles for imaging. By default, all lanes and rows are imaged, which maximizes the amount of data captured for each experiment. However, in some cases you might want to shorten the run by reducing the area to be imaged. To narrow the number of lanes or rows for an experiment, use the Row, Lane, RowRange, and LaneRange tags.

CAUTION
Exercise care when editing a recipe. Always make a backup copy of the recipe before altering it.

The Tile Selection portion of the recipe indicates the specific tiles to be imaged. Only a subset of the tiles are imaged during first base incorporation, while all tiles are imaged during the complete sequencing run.

```
<TileSelection>
```

The Incorporation section of the recipe refers to images taken after nucleotide incorporation occurs.

```
<Incorporation>
```

By specifying the Lane Index within the Incorporation section you can modify the number of tiles to be imaged per lane.

```
<Lane Index="1">
```

For example, in Lane 4, only five rows will be imaged.

```
<Lane Index="4">
<RowRange Min="33" Max="37" />
</Lane>
```

RowRange indicates which tiles to image in the specified lane. RowRange Min="23" Max="27" indicates five rows will be imaged. This command is inclusive; that is, 23, 24, 25, 26, and 27 will be imaged.

```
<RowRange Min="23" Max="27" />
<RowRange Min="33" Max="37" />
<RowRange Min="43" Max="47" />
```

The following example is an easy way to image the same tiles in each lane:

```
<Incorporation>
<Row Range Min="1" Max "35">
</Incorporation>
```

Reduce the Number of Rows

The following example chooses rows 6–10, 21–25, 36–40 for Incorporation. All lanes within those rows are selected by default, unless the LaneRange command is used to reduce the number.

```
<TileSelection>
<Incorporation>
<RowRange Min="6" Max="10" />
<RowRange Min="21" Max="25" />
<RowRange Min="36" Max="40" />
</Incorporation>
</TileSelection>
```

Reduce the Number of Lanes

The following example chooses only the middle four lanes for incorporation imaging.
<TileSelection>

```
<Incorporation>
<Lane Index="3"/>
<Lane Index="4"/>
<Lane Index="5"/>
<Lane Index="6"/>
</Incorporation>
</TileSelection>
```

ReadPrep

Used only in paired-end recipes, the ReadPrep section refers to a sampling of tiles, much like the sampling during first base incorporation, and determines cluster intensity, cluster number, and focus quality for each lane on the flow cell prior to starting Read 2.

```
<ReadPrep>
```

By specifying the Row Index you can modify which tiles are used in the ReadPrep cycle. Row 26 is approximately in the middle of the flow cell, and rows 5 and 45 are toward each end.

```
<Row Index="5" />
<Row Index="26" />
<Row Index="45" />
```

End of ReadPrep section.

```
</ReadPrep>
```

Preparing and Replenishing Reagents on GAII

Appendix B

Preparing SBS Reagents 174
Reagent Kits and Replenishing Cycles for GAII 177

Preparing SBS Reagents

One kit provides reagents for 36 cycles of sequencing. For runs longer than 36 cycles, you must prepare reagents from multiple kits. For more information, see *Reagent Kits and Replenishing Cycles for GAII* on page 177.

When preparing reagents for longer runs, you have two options for reagent containers in positions 4, 5, and 7.

NOTE
Prepare and load IMR and CLM in the original tubes. Transfer prepared SMR to the instrument tube provided in the kit.

Reagent Containers

- **Positions 1, 3, and 6 (IMR, SMX, and CLM)**—Three 50 ml tubes (BD, catalog # 352070) connect to fluidics lines in the reagent chiller.
- **Positions 4, 5, and 7 (PR1, PR2, and PR3)**—125 ml Nalgene bottles connect to threaded bottle receptacles on the instrument. SBS reagents are kitted in 125 ml bottles. However, you need additional 125 ml bottles to prepare reagents for runs longer than 36 cycles.
- [Optional/User-Supplied] 150 ml bottles minimize the need to replenish PR1, PR2, and PR3 during the run. Remove the threaded bottle adapters before loading 150 ml bottles.

Position	Reagent Name	Container Size
1	IMR	50 ml tube
2	PW1	125 ml bottle
3	SMX	50 ml tube
4	PR1	125 ml bottle or optional 150 ml bottle
5	PR2	125 ml bottle or optional 150 ml bottle
6	CLM	50 ml tube
7	PR3	125 ml bottle or optional 150 ml bottle

Thaw Reagents

For paired-end runs, thaw reagents for Read 1 and any indexing cycles. Thaw reagents for Read 2 only when you are ready to prepare reagents prior to the start of Read 2.

1 Remove IMR, LFN, and SMX from -15° to -25°C storage, and thaw at room temperature or in a beaker containing deionized water for about one hour.
If you use the beaker method, make sure that the water line does not reach the cap of the tube to prevent contamination.
Leave the HDP in -15° to -25°C storage until you are ready to use it to prepare the IMR.

CAUTION
It is important to keep the CLM away from the other components to avoid cross-contamination.
After handling the CLM container, be sure to discard your gloves and replace them with a new pair each time.

2 Remove the CLM from -15° to -25°C storage and thaw it at room temperature or in a *separate* beaker containing deionized water for about one hour.

If you use the beaker method, make sure that the water line does not reach the cap of the tube to prevent contamination.

3 Record the lot numbers of each reagent on the lab tracking form.

4 Immediately after the reagents have thawed, place them on ice. Be sure to keep the CLM in a *separate* ice bucket during reagent preparation.

Prepare PR1, PR2, and PR3

1 Invert the bottle of PR1 several times to mix the reagent.

2 Invert the bottle of PR2 several times to mix the reagent.

3 Invert the bottle of PR3 several times to mix the reagent.

4 When preparing multiple kits, combine reagents in either a 125 ml bottle or an optional user-supplied 150 ml bottle.

NOTE
The 125 ml bottle holds up to 170 ml of reagent.
The 150 ml bottle holds up to 200 ml of reagent.
Take care to avoid spillage when preparing and loading reagents.

5 Set aside excess PR1, PR2, and PR3 at room temperature to replenish during the run.

Prepare IMR

1 Transfer 3.52 ml of LFN to the IMR reagent.

2 Remove the HDP tube from -15° to -25°C storage and briefly pulse centrifuge.

3 Transfer 330 µl of HDP into the IMR/LFN mix.

4 Cap the bottle of IMR mix tightly and invert five times to mix.

5 Centrifuge at 1,000 xg for one minute at 4°C.

6 When preparing multiple kits, combine reagent into a 150 ml bottle or user-supplied foil-wrapped 125 ml bottle, depending on the number of kits you need to prepare for your run.

NOTE
The 125 ml bottle holds up to 170 ml of reagent.
The 150 ml bottle holds up to 200 ml of reagent.
Take care to avoid spillage when preparing and loading reagents.

7 Set aside excess IMR to replenish the reagent during the run.

8 Place the IMR on ice until you are ready to load reagents.

Prepare SMX

1 Invert the bottle of SMX several times to mix well.

2 Transfer 50 ml of SMX into the SMX instrument tube provided in the kit. Make sure the tube is not filled beyond the 50 ml mark. Reagent can spill into the threads of the cap and cause later problems in the fluidics lines.

3 When preparing reagents from multiple kits, combine reagent into a 150 ml bottle or user-supplied foil-wrapped 125 ml bottle, depending on the number of kits you need to prepare for your run.

NOTE
The 125 ml bottle holds up to 170 ml of reagent.
The 150 ml bottle holds up to 200 ml of reagent.
Take care to avoid spillage when preparing and loading reagents.

4 Set aside excess SMX to replenish the reagent during the run.

5 Cap the container and place the SMX on ice until you are ready to load reagents.

Prepare CLM

1 Invert the CLM tube several times to mix well.

2 Centrifuge at 1,000 xg for one minute at 4°C.

3 When preparing multiple kits, combine reagent into a 150 ml bottle or user-supplied foil-wrapped 125 ml bottle, depending on the number of kits you need to prepare for your run.

NOTE
The 125 ml bottle holds up to 170 ml of reagent.
The 150 ml bottle holds up to 200 ml of reagent.
Take care to avoid spillage when preparing and loading reagents.

4 Cap the container and place the CLM in a *separate* ice bucket until you are ready to load reagents.

5 Set aside excess CLM to replenish the reagent during the run.

6 Discard your gloves and replace them with a new pair.

Reagent Kits and Replenishing Cycles for GAII

This section provides the number of TruSeq SBS Kits required for each reagent used in a sequencing run of various lengths and run types, and the required cycles to replenish reagents on the Genome Analyzer II.

Using the Reagent Kits and Replenishing Cycles Tables

- The number of required kits are listed for the first read and any indexing cycles. The number of required kits for Read 2 are listed separately. Always prepare fresh SBS reagents for Read 2.
- The "X" indicates which reagents you need to replenish at the indicated cycles.
- The "X" at the start of Read 1 and start of Read 2 indicate that a full set of fresh reagents are required before starting the read.
- For single-read runs, disregard the Read 2 portion of the table.
- For paired-end dual-indexed runs, the seven chemistry-only cycles performed during the Index 2 (i5) Read are calculated for reagent use; however, these cycles are not counted in the total number of cycles performed in the run.

151-Cycle Non-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 105–108	X	X	X	X	X	X			
	Cycle 140–143	X	X	X						
	Cycle 151									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 152	X	X	X	X	X	X	X	X	X
	Cycle 186–189	X	X	X						
	Cycle 221–224	X	X	X						
	Cycle 256–259	X	X	X	X	X	X			
	Cycle 291–294	X	X	X						
	Cycle 302									

126-Cycle Non-Indexed Run

Container Size:	50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:	4	4	4	3	2	3	3	2	3

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 105–108	X	X	X	X	X	X			
	Cycle 126									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 127	X	X	X	X	X	X	X	X	X
	Cycle 161–164	X	X	X						
	Cycle 196–199	X	X	X						
	Cycle 231–234	X	X	X	X	X	X			
	Cycle 252									

101-Cycle Non-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 101									
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 102	X	X	X	X	X	X	X	X	X
	Cycle 136–139	X	X	X						
	Cycle 171–174	X	X	X						
	Cycle 202									

51-Cycle Non-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37		X	X						
	51									
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 2	Start of Read 2 Cycle 52	X	X	X	X	X	X	X	X	X
	Cycle 87–90		X	X						
	102									

36-Cycle Non-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		1	1	1	1	1	1	1	1	1
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 36									
Number of SBS Kits:		1	1	1	1	1	1	1	1	1
Read 2	Start of Read 2 Cycle 37	X	X	X	X	X	X	X	X	X
	Cycle 72									

151-Cycle Single-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	4	5	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 105–108	X	X	X	X	X	X			
	Cycle 140–143	X	X	X						
	Cycle 151									
	User Wait after Read 1									
Index Read	Cycle 152									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 159/160	X	X	X	X	X	X	X	X	X
	Cycle 193–196	X	X	X						
	Cycle 228–231	X	X	X						
	Cycle 263–266	X	X	X	X	X	X			
	Cycle 298–301	X	X	X						
	Cycle 309/310									

146-Cycle Single-Indexed Run

Container Size:	50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:	4	4	4	3	2	3	3	2	3

	Container Size:	50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
	Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 105–108	X	X	X	X	X	X			
	Cycle 140–143	X	X	X						
	Cycle 146									
	User Wait after Read 1									
Index Read	Cycle 147									
	Number of SBS Kits:	4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 154/155	X	X	X	X	X	X	X	X	X
	Cycle 188–191	X	X	X						
	Cycle 223–226	X	X	X						
	Cycle 258–261	X	X	X	X	X	X			
	Cycle 293–295	X	X	X						
	Cycle 299/300									

126-Cycle Single-Indexed Run

	Container Size:	50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
	Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
	Number of SBS Kits:	4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 105–108	X	X	X	X	X	X			
	Cycle 126									
	User Wait after Read 1									
Index Read	Cycle 127									
	Number of SBS Kits:	4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 134/135	X	X	X	X	X	X	X	X	X
	Cycle 168–171	X	X	X						
	Cycle 203–206	X	X	X						
	Cycle 238–241	X	X	X	X	X	X			
	Cycle 259/260									

101-Cycle Single-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 101									
	User Wait after Read 1									
Index Read	Cycle 102									
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 109/110	X	X	X	X	X	X	X	X	X
	Cycle 143–146	X	X	X						
	Cycle 178–181	X	X	X						
	Cycle 209/210									

51-Cycle Single-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37		X	X						
	Cycle 51									
	User Wait after Read 1									
Index Read	Cycle 52									
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 2	Start of Read 2 Cycle 59/60	X	X	X	X	X	X	X	X	X
	Cycle 94–97		X	X						
	Cycle 109/110									

36-Cycle Single-Indexed Run

Container Size:	50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:	2	2	2	2	1	2	2	1	2

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 36									
	User Wait after Read 1									
Index Read	Cycle 37									
Number of SBS Kits:		1	1	1	1	1	1	1	1	1
Read 2	Start of Read 2 Cycle 43/44	X	X	X	X	X	X	X	X	X
	Cycle 79/80									

151-Cycle Dual-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	5	5	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 105–108	X	X	X	X	X	X			
	Cycle 140–143	X	X	X						
	Cycle 151									
	User Wait after Read 1							X	X	X
Index Reads	Cycle 152									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 168	X	X	X	X	X	X	X	X	X
	Cycle 202–205	X	X	X						
	Cycle 237–239	X	X	X						
	Cycle 272–275	X	X	X	X	X	X			
	Cycle 314–317	X	X	X						
	Cycle 318									

146-Cycle Dual-Indexed Run

Container Size:	50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:	4	5	5	3	2	3	3	2	3

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 105–108	X	X	X	X	X	X			
	Cycle 140–143	X	X	X						
	Cycle 146									
	User Wait after Read 1							X	X	X
Index Reads	Cycle 147									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 163	X	X	X	X	X	X	X	X	X
	Cycle 198–201	X	X	X						
	Cycle 233–236	X	X	X						
	Cycle 268–271	X	X	X	X	X	X			
	Cycle 303–305	X	X	X						
	Cycle 308									

133-Cycle Dual-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 105–108	X	X	X	X	X	X			
	Cycle 133									
	User Wait after Read 1	X	X	X						
Index Reads	Cycle 134									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 150	X	X	X	X	X	X	X	X	X
	Cycle 185–188	X	X	X						
	Cycle 220–222	X	X	X						
	Cycle 254–257	X	X	X	X	X	X			
	Cycle 282									

126-Cycle Dual-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 105–108	X	X	X	X	X	X			
	Cycle 126									
	User Wait after Read 1	X	X	X						
Index Reads	Cycle 127									
Number of SBS Kits:		4	4	4	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 143	X	X	X	X	X	X	X	X	X
	Cycle 178–181	X	X	X						
	Cycle 213–216	X	X	X						
	Cycle 248–251	X	X	X	X	X	X			
	Cycle 268									

101-Cycle Dual-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		3	4	4	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 101									
	User Wait after Read 1	X	X	X						
Index Reads	Cycle 102									
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 118	X	X	X	X	X	X	X	X	X
	Cycle 153–156	X	X	X						
	Cycle 188–191	X	X	X						
	Cycle 218									

93-Cycle Dual-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37	X	X	X						
	Cycle 70–75	X	X	X						
	Cycle 93									
	User Wait after Read 1	X	X	X						
Index Reads	Cycle 94									
Number of SBS Kits:		3	3	3	3	2	3	3	2	3
Read 2	Start of Read 2 Cycle 110	X	X	X	X	X	X	X	X	X
	Cycle 146–149	X	X	X						
	Cycle 181–183	X	X	X						
	Cycle 202									

51-Cycle Dual-Indexed Run

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 35–37		X	X						
	Cycle 51									
	User Wait after Read 1		X	X						
Index Reads	Cycle 52									
Number of SBS Kits:		2	2	2	2	1	2	2	1	2
Read 2	Start of Read 2 Cycle 68	X	X	X	X	X	X	X	X	X
	Cycle 103–105		X	X						
	Cycle 118									

36-Cycle Dual-Indexed Run

Container Size:	50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:	IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Number of SBS Kits:	2	2	2	1	1	1	1	1	1

Container Size:		50 ml Tubes			125 ml Bottle			150 ml Bottle (Optional)		
Reagent:		IMR	SMX	CLM	PR1	PR2	PR3	PR1	PR2	PR3
Read 1	Start of Read 1	X	X	X	X	X	X	X	X	X
	Cycle 36									
	User Wait after Read 1		X	X						
Index Reads	Cycle 37									
Number of SBS Kits:		1	1	1	1	1	1	1	1	1
Read 2	Start of Read 2 Cycle 53	X	X	X	X	X	X	X	X	X
	Cycle 88									

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Technical Assistance

For technical assistance, contact Illumina Customer Support.

Table 20 Illumina General Contact Information

Illumina Website	http://www.illumina.com
Email	techsupport@illumina.com

Table 21 Illumina Customer Support Telephone Numbers

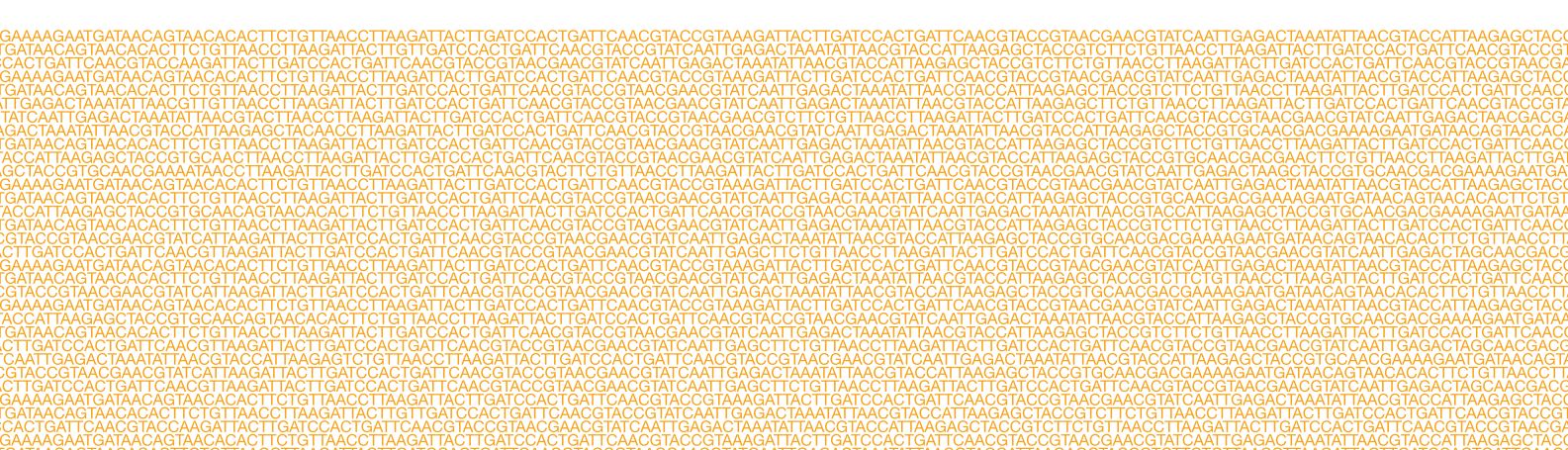
Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	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 http://www.illumina.com/msds.

Product Documentation

You can obtain PDFs of additional product documentation from the Illumina website. Go to http://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 https://my.illumina.com/Account/Register.



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