Applied Biosystems

Applied Biosystems SOLiD™ 3 System

Instrument Operation Guide

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

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Contents

Preface

Safety information

Note: For general safety information, see this Preface and Appendix J, "Safety" on page 157. For important safety information related to the use of the Covaris™ S2 system, please refer to the user documentation of the product.When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, results in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Applied Biosystems instruments* (see "Safety symbols" on page 158).

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "MSDSs" on page 167.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

Hazard symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**CAUTION!** Potential slipping hazard.	**ATTENTION!** Risque potentiel d'avoir un sol glissant.
	**WARNING!** Hot lamp.	**AVERTISSEMENT!** Lampe brûlante.
	WARNING! Hot. Do not remove lamp until **15** min after disconnecting supply.	**AVERTISSEMENT!** Lampe brûlante, après avoir déconnecté le câble d'alimentation de l'appareil, attendre environ **15** minutes avant d'effectuer un remplacement de la lampe.
	WARNING! Hot. Replace lamp with an Applied Biosystems lamp.	**AVERTISSEMENT!** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
	CAUTION! Hot surface.	**ATTENTION!** Surface brûlante.
	CAUTION! Replace only with Applied Biosystems recommended light source (PN 4388441).	**ATTENTION!** La Lampe devra être remplacée par un model recommandé par Applied Biosystems, Réf de la lampe: 4388441.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT!** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Applied Biosystems.
	**CAUTION!** Moving parts. Crush/pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

How to use this guide

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

How to obtain support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

1 Introduction

SOLiD™ 3 System run types

On the Applied Biosystems SOLiD™ 3 System, you can perform three types of runs: *workflow analysis (WFA), sequencing (standard)*, and *multiplex sequencing* (see Table 1).

Table 1 Run types on the SOLiD™3 system

	WFA	Sequencing (standard)	Multiplex sequencing
Purpose	• Assess various preparations of templated beads to determine potential quality of sequence data • Evaluate fraction of P2-positive beads • Use as a tool to determine deposition density for sequencing slides	Generate sequencing data for fragment or mate-paired libraries	Generate multiplex sequencing data for fragment libraries
Run summary	• P1 and P2 bead counting • Single ligation cycle • Report generation	Up to 10 ligation cycles for each of 5 primers resulting in 50 bases per tag‡	Up to 10 ligation cycles for each of 5 primers resulting in 50 bases per tag *and* 1 ligation cycle for each of 5 primers resulting in 5 bases per barcode tag
Estimated run time§	~4 to 5 hours	• ~3 to 4 days for 25 bp • ~6 to 7 days for 50 bp	• ~6 to 7 days for 50 bp • ~1 day for barcode
Deposition chamber	4-well	• 1-well • 4-well • 8-well	• 1-well • 4-well • 8-well
Number of beads	15 million beads per well	• 310 million beads per well (1-well) • 60 million beads per well (4-well) • 30 million beads per well (8-well)	• 310 million beads per well (1-well) • 60 million beads per well (4-well) • 30 million beads per well (8-well)

‡ One tag for fragment libraries and 2 tags for mate-paired libraries
§ Total run time for dual slide run. Times may deviate depending on imaging time.

Use Figure 1 to choose the run type that most closely meets your sequencing needs:

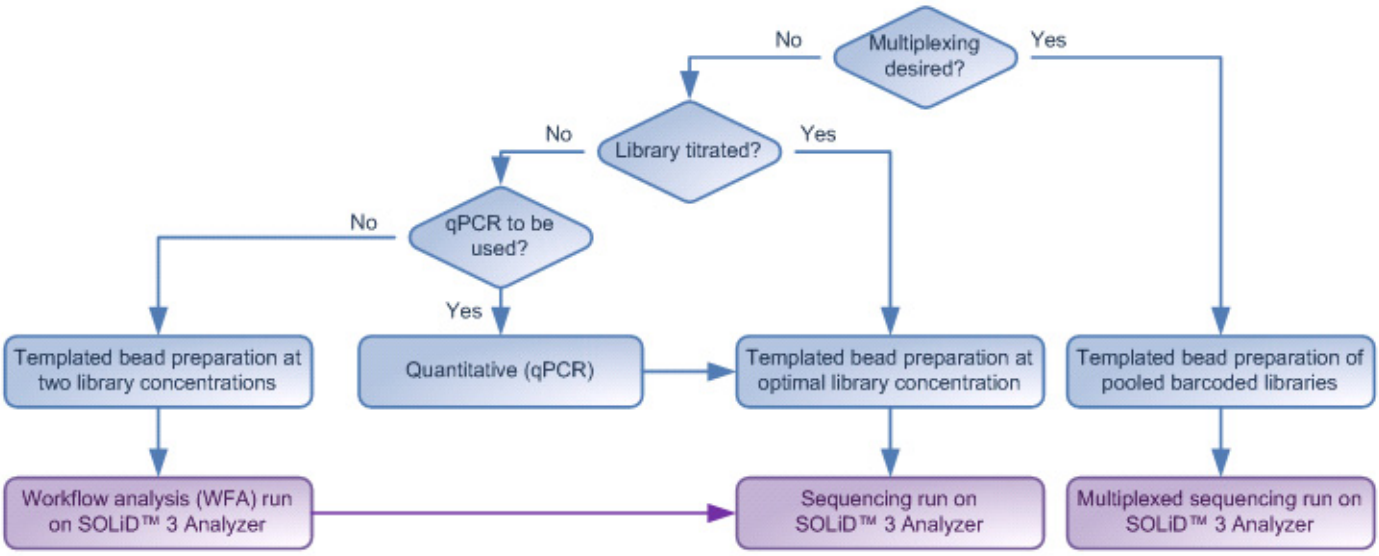


Figure 1 Relationship between the three types of SOLiD™ runs.

Workflow analysis (WFA) run

You can optimize sequencing results by performing workflow analysis (WFA) runs. A WFA run analyzes a quadrant of a slide that undergoes a single ligation cycle. The quadrant contains beads deposited at a lower density than the density of beads deposited for a sequencing run.

A WFA run determines the:

- *Optimal library concentration*: the library concentration for optimal preparation of templated beads using the library. You use this library concentration for any preparation of templated beads for that library as long as the scale of templated bead preparation is the same.
- *Bead enrichment efficiency*: the proportion of beads that have been successfully amplified using emulsion PCR (ePCR) as a fraction of the total number of beads prepared. You use this value to more accurately deposit successfully amplified beads for a sequencing run.

WFA runs require the same materials as those materials needed for sequencing runs. If you perform multiple WFA runs routinely, you should order additional SOLiD™ Instrument Buffer Kits.

To perform a WFA run, prepare slide and install reagents according to the procedure in Chapter 2, “Prepare and install slides and reagents” on page 11, then set up and monitor the run according to the procedure in Chapter 3, “Set Up, Control, and Monitor the Run” on page 41.

Sequencing run

During a SOLiD™ sequencing run, two probe sets are used to maximize the fraction of "*mappable*" beads, read length, and sequencing throughput. (Mappable beads are beads amplified with template that map to the reference genome.) This protocol must be used for sequencing 50-bp reads of both mate-paired and fragment libraries. Compared to terminator-based sequencing chemistry, with SOLiD sequencing, base information is not collected; instead, five rounds of primers (Primers A, B, C, D, and E) are used to sequence template by ligation of di-base labeled probes (see Figure 2 and Figure 4 on page 4). For sequencing of fragment libraries, the set of primers used are specific to the P1 Adaptor.

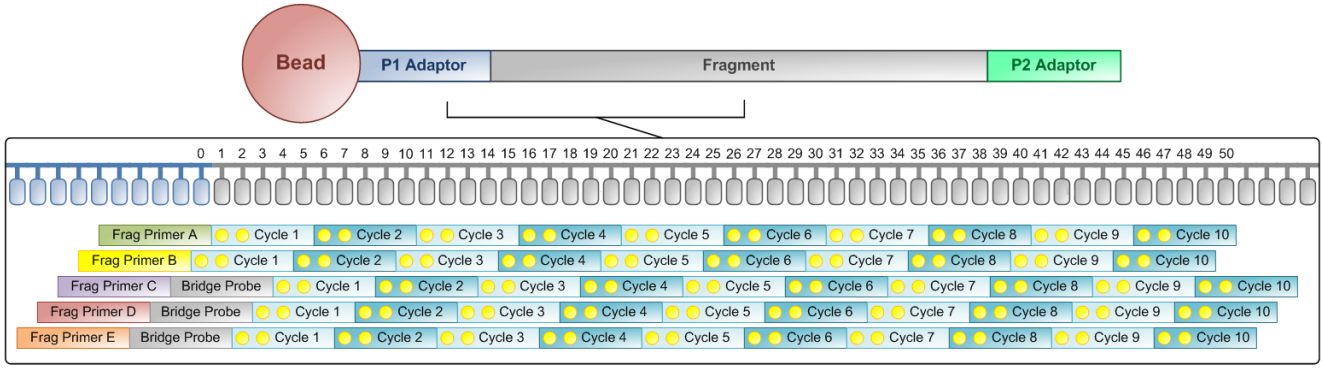


Figure 2 SOLiD™ 3 System interrogation of nucleotide positions for a 50-bp fragment sequencing run.

The typical workflow for a fragment sequencing run is shown below (see Figure 3).

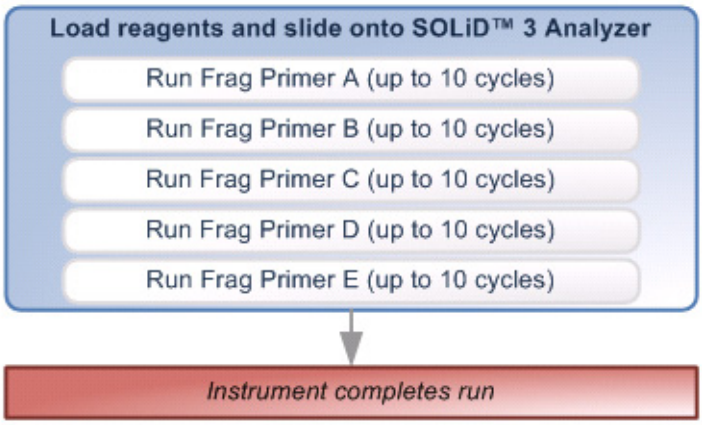


Figure 3 Typical workflow for a fragment sequencing run.

For sequencing of mate-paired libraries, the set of primers used to sequence one of the tags is specific to the P1 Adaptor, while the set of primers used to sequence the other tag is specific to the Internal Adaptor (see Figure 4 on page 4).

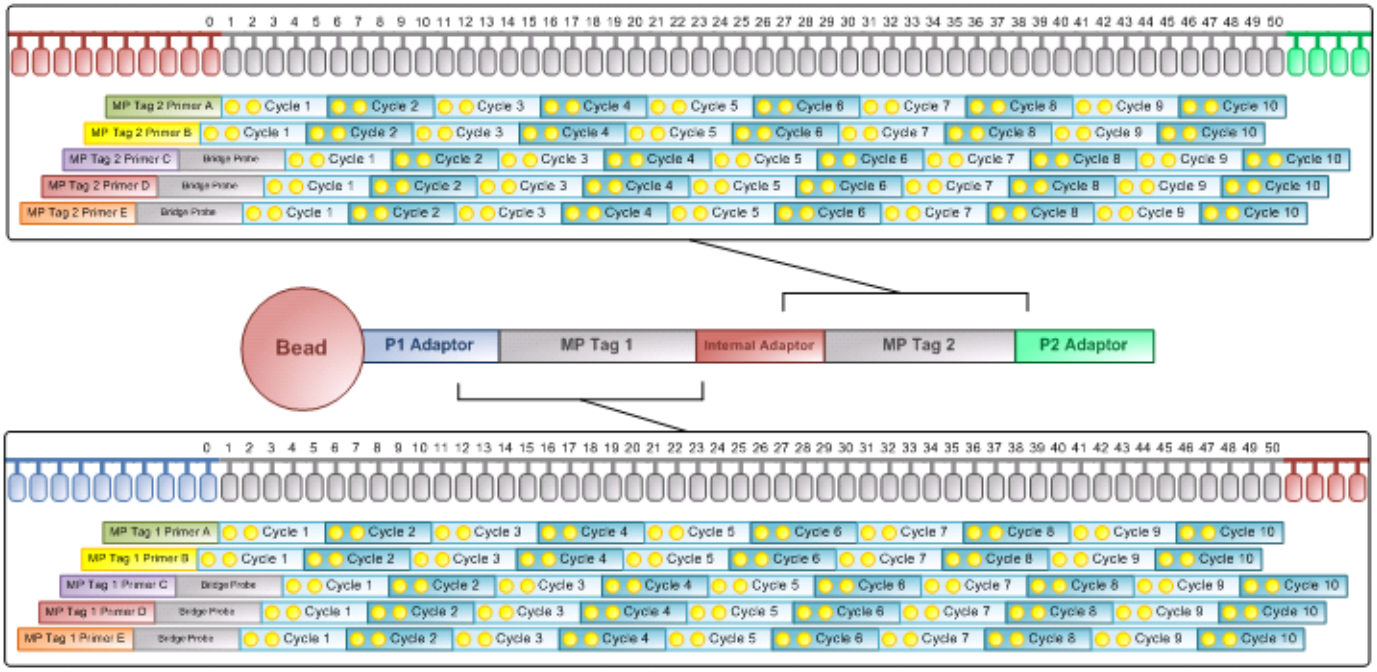


Figure 4 SOLiD™ 3 System interrogation of nucleotide positions for a 50-bp mate-paired sequencing run.

The typical workflow of a mate-paired sequencing run is shown below (see Figure 5 on page 5).

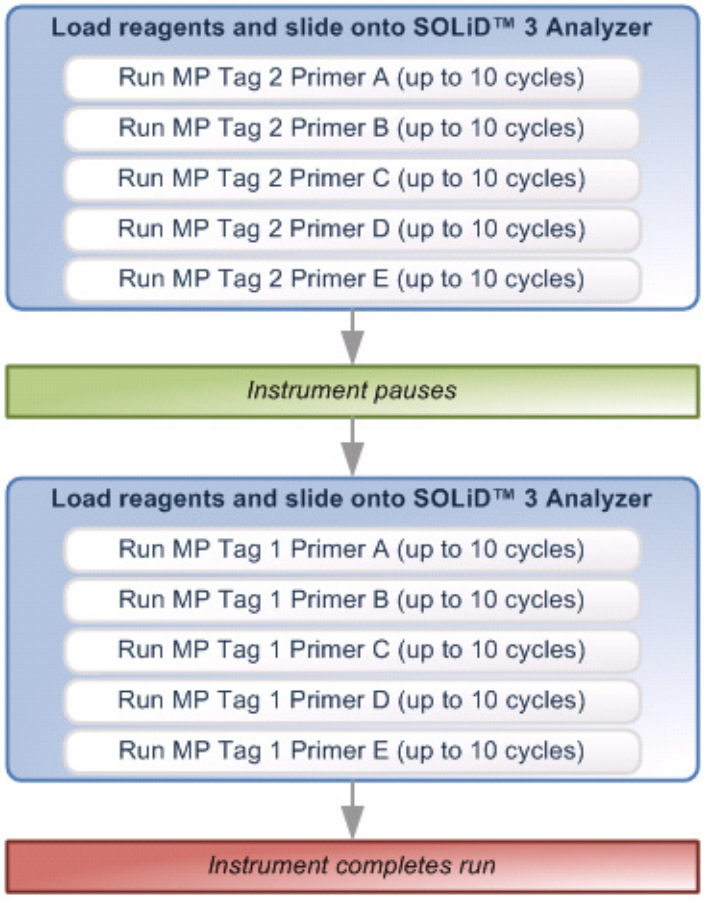


Figure 5 Typical workflow of a mate-paired sequencing run.

Multiplexed sequencing

For sequencing of barcoded fragment libraries, the set of primers used to sequence the fragment is specific to the P1 Adaptor, while the set of primers used to sequence the barcode sequence is specific to the Internal Adaptor (see Figure 6 on page 6).

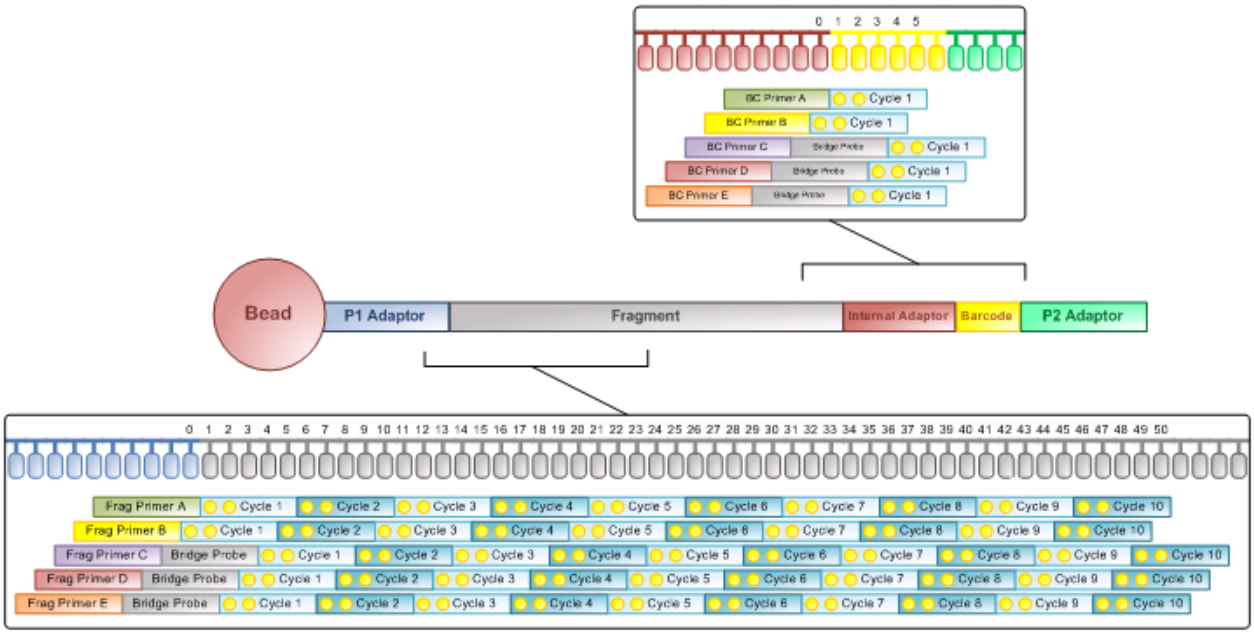


Figure 6 SOLiD™ 3 System interrogation of nucleotide positions for a 50-bp fragment and 5-bp barcode sequencing run.

The typical workflow of a multiplex sequencing run is shown in Figure 7 on page 7.

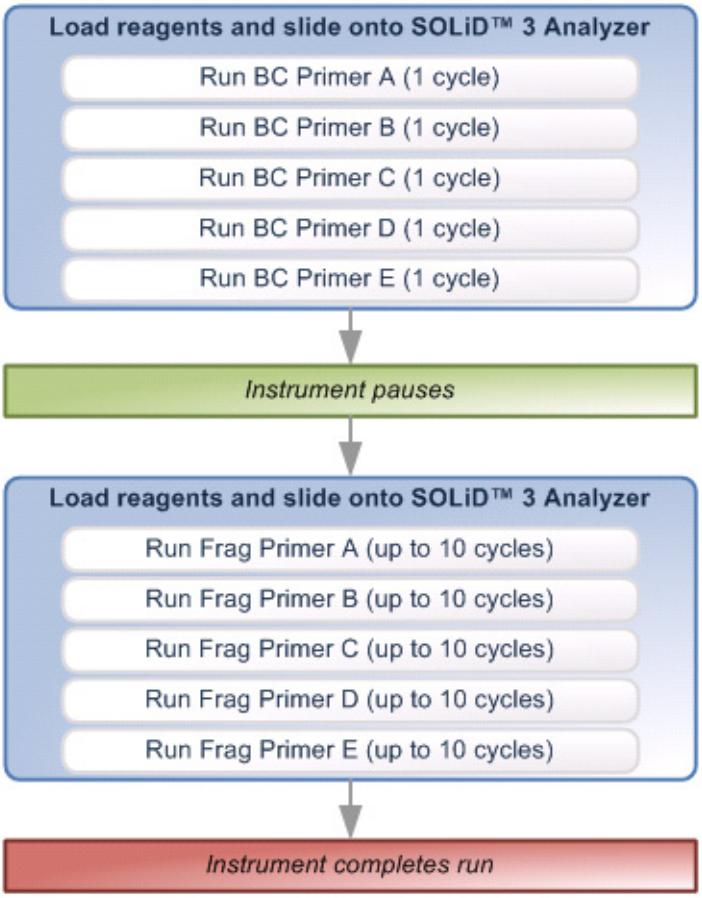


Figure 7 Typical workflow of multiplex fragment sequencing run.

To perform a sequencing or multiplex sequencing run, prepare slides and install slides and reagents according to the procedure in Chapter 2, “Prepare and install slides and reagents” on page 11, then set up and monitor the run according to the procedure in Chapter 3, “Set Up, Control, and Monitor the Run” on page 41.

Data analysis

Data analysis may be organized into primary, secondary, and tertiary analysis (see Table 2).

Table 2 Types of data analysis

Type of Analysis	Description
Primary	Includes universal processes for data generation, collection, and raw processing
Secondary	Analyzes application-specific data at the sequence level
Tertiary	Generates biological interpretation specific to an application

The software tools used to set and control data analysis include SOLiD™ Instrument Control Software (ICS), SOLiD™ Experiment Tracking System (SETS), and SOLiD™ Analysis Tools (SAT) (see Table 3). The relationship between these software tools in the SOLiD™ sequencing run workflow is shown in Figure 8 on page 9.

Table 3 SOLiD™ software tools

Software Application	Type	Function
ICS	Windows application	Instrument operation
SETS	Browser-based application	Reports and reanalysis
SAT	Linux command line tool	Command line tool for analysis

SAT automatically processes the image during the instrument run, performs data filtering, calculates quality values, aligns to a reference genome, and generates base calls. The result can be exported automatically to offline compute clusters for further analysis. SETS allows you to reanalyze data and review/export results. For offline analysis, Global SETS encompasses SETS and SAT for offline analysis and can carry on a compute job from the auto-exported data. For detailed data analysis using SETS and SAT, refer to the *Applied Biosystems SOLiD™ SETS Software Getting Started Guide* (PN 4389302) and the *Applied Biosystems SOLiD™ Analysis Tools (SAT) User Guide* (PN 4392959), respectively.

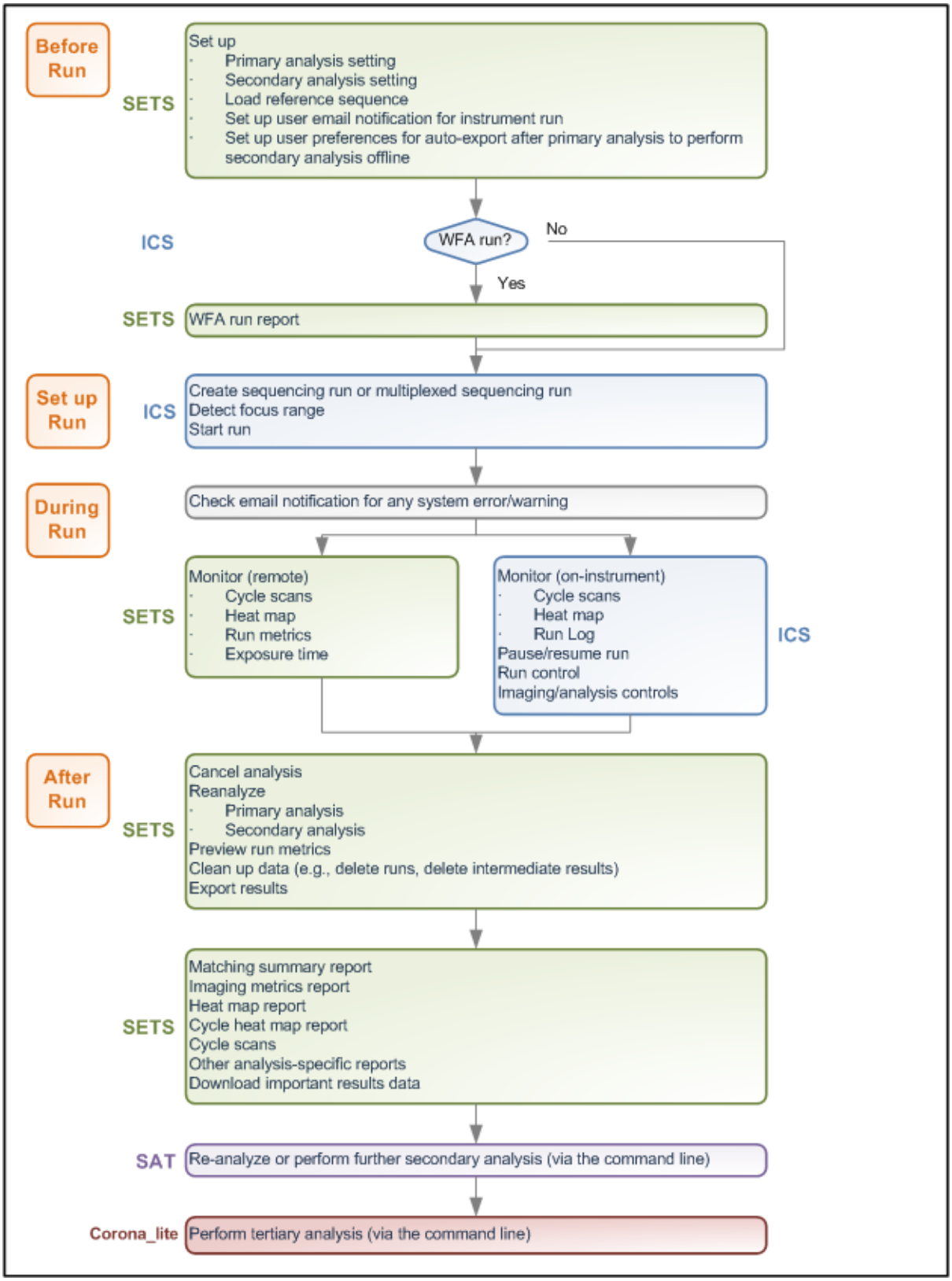


Figure 8 Relationship between software tools in SOLiD™ sequencing run workflow.

For additional secondary and tertiary analysis tools, visit the SOLiD™ Software Development Community website (**http://solidsoftwaretools.com**). One can integrate standalone tools from the SOLiD™ Software Development Community with the SAT pipeline to perform more automated analysis. For details, see the *SOLiD™ Analysis Tools (SAT) User Guide* (PN 4392959).

2 Prepare and install slides and reagents

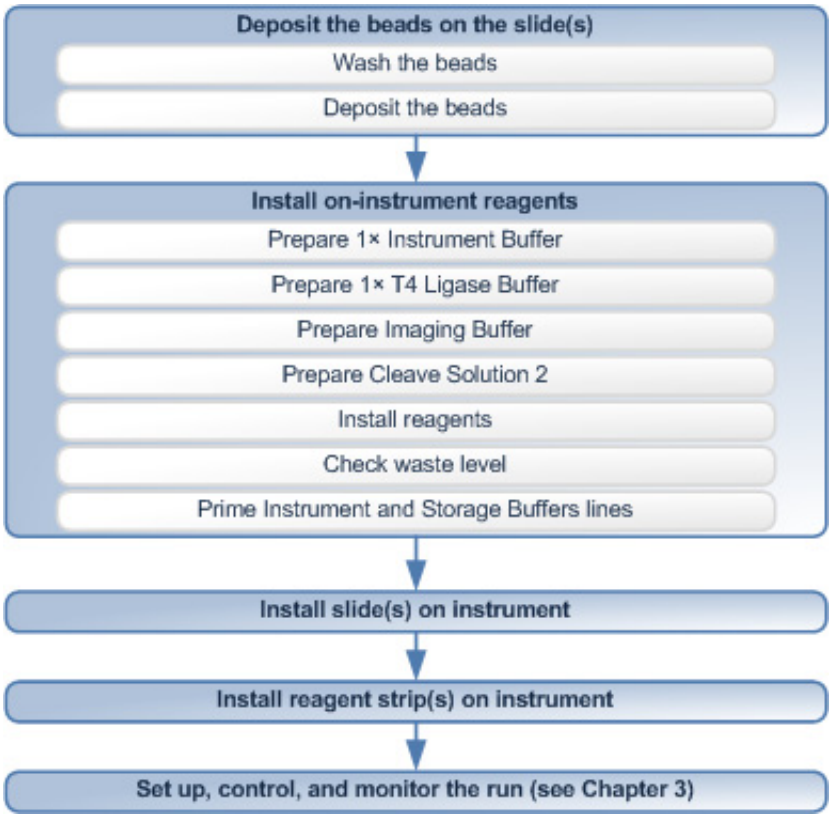
This chapter covers:

Materials and equipment required

See Appendix A, “Set up and perform a workflow analysis (WFA) run” on page 86 for a list of equipment, kits, and consumables necessary to set up a workflow analysis (WFA) run.

See Appendix A, “Set up and perform a sequencing run” on page 90 for a list of equipment, kits, and consumables necessary to set up a sequencing run.

Workflow



Deposit the beads on the slide(s)

For a WFA run, the beads are quantitated using the SOLiD™ Bead Concentration Chart (PN 4415131), and 15 million beads are deposited in one well of a four-well SOLiD™ Deposition Chamber.

For a sequencing run, the choice of SOLiD™ Deposition Chamber depends on factors such as the requirements of the experiment, number of libraries being assessed, the size of the genome, and the sequencing coverage required. Three SOLiD™ Deposition Chamber designs are available for use (see Table 4).

Table 4 Three deposition chamber designs

Deposition Chamber	Number of image panels
1-Well	2357
4-Well	432 per well
8-Well	186 per well

Install on-instrument reagents

1X Instrument Buffer is prepared from glycerol and 10X Instrument Buffer provided in the SOLiD™ Instrument Buffer Kit. 1X Instrument Buffer may be formulated in 8-L batches as needed or may be prepared in larger volumes and stored at 4 °C until ready for use. T4 Ligase Buffer, Imaging Buffer, and Cleave Solution 2 are each prepared by

combining the two parts provided in the SOLiD™ Instrument Buffer Kit. The chiller block should be cooled prior to installation of buffers. The tubing from the Instrument and Storage Buffer bottles to the flowcell is long, and the lines must be primed before the slides are installed in the flowcell.

Install slide(s) on the instrument

The slide is removed from the Deposition Chamber and prepared for installation on the instrument. Each flowcell can be loaded with the slide *independently* of each other.

Install reagent strip(s) on the instrument

The workflow analysis or sequencing reagent strips are installed on the reagent strip chiller block. The chiller block should be cooled prior to installation of reagent strips.

Tips

General

- Prior to deposition, slides must be stored appropriately and kept dry in a desiccator to ensure optimal bead deposition and to minimize loss of P2-enriched beads. Remove the slides from the desiccator only when you are ready to deposit the beads onto the slide.
- Perform all steps requiring 0.5-mL, 1.5-mL, and 2.0-mL tubes with Eppendorf LoBind tubes.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols.

Covaris™ S2 System

- The procedures are optimized for the Covaris™ S2 System. The Covaris S2 System must be specially adapted to prepare beads for the Applied Biosystems SOLiD™ 3 System. Do not use the Covaris S1 sonicator or an unadapted Covaris S2 System for bead preparation. For more information, contact an Applied Biosystems SOLiD™ System applications specialist.
- Ensure that the Covaris S2 System is degassed, that no bubbles are present in the system, and that the instrument and tube are properly aligned for appropriate sonication of beads.
- To ensure optimal sonication by the Covaris™ S2 System, use the appropriate adaptor with the Covaris S2 System. For sample volumes ≤ 200 µL, use a 0.5-mL LoBind tube and 0.65-mL tube adaptor. For sample volumes between 200 µL and 600 µL, use a 1.5-mL LoBind tube and 1.5-mL tube adaptor. For sample volumes between 600 µL and 1.2 mL, use a 2.0-mL LoBind tube and the same adaptor as used for the 1.5-mL tubes. Place the tube collar at the indicator line of the adaptor.

Deposit the beads

For the following hazards, see the complete safety alert descriptions in Appendix J, "Safety" on page 157:

WARNING! CHEMICAL HAZARD. Deposition Buffer.

Wash the beads

Note: The bead wash procedure is for one WFA or sequencing run sample.

1. Sonicate P2-enriched beads using the Covalent Declump 1 program on the Covaris™ S2 System (for program conditions, see "Covalent Declump 1" on page 140). Pulse-spin, but do not pellet the beads.
2. If a WFA run has already been performed, use the results from the WFA report to estimate the bead concentration and proceed to step 5; otherwise, use the SOLiD™ Bead Concentration Chart (PN 4415131) to estimate the bead concentration (see Figure 9).

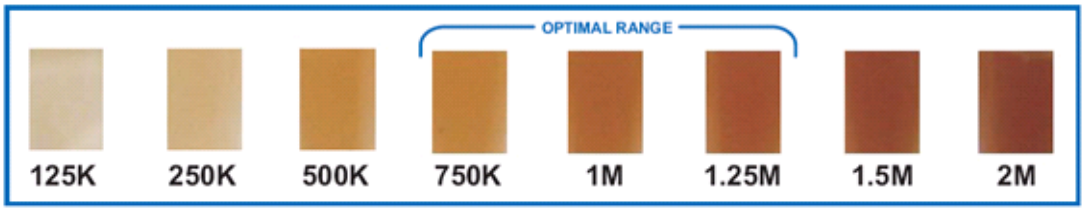


Figure 9 The SOLiD™ Bead Concentration Chart. For best results, use the *Applied Biosystems SOLiD™ Bead Concentration Chart* (PN 4415131), supplied separately.

3. Adjust the volume of beads so that the color of the bead solution matches a color in the optimal range (750,000 beads/µL to 1.25 million beads/µL; see Figure 9 and Figure 10 on page 16).

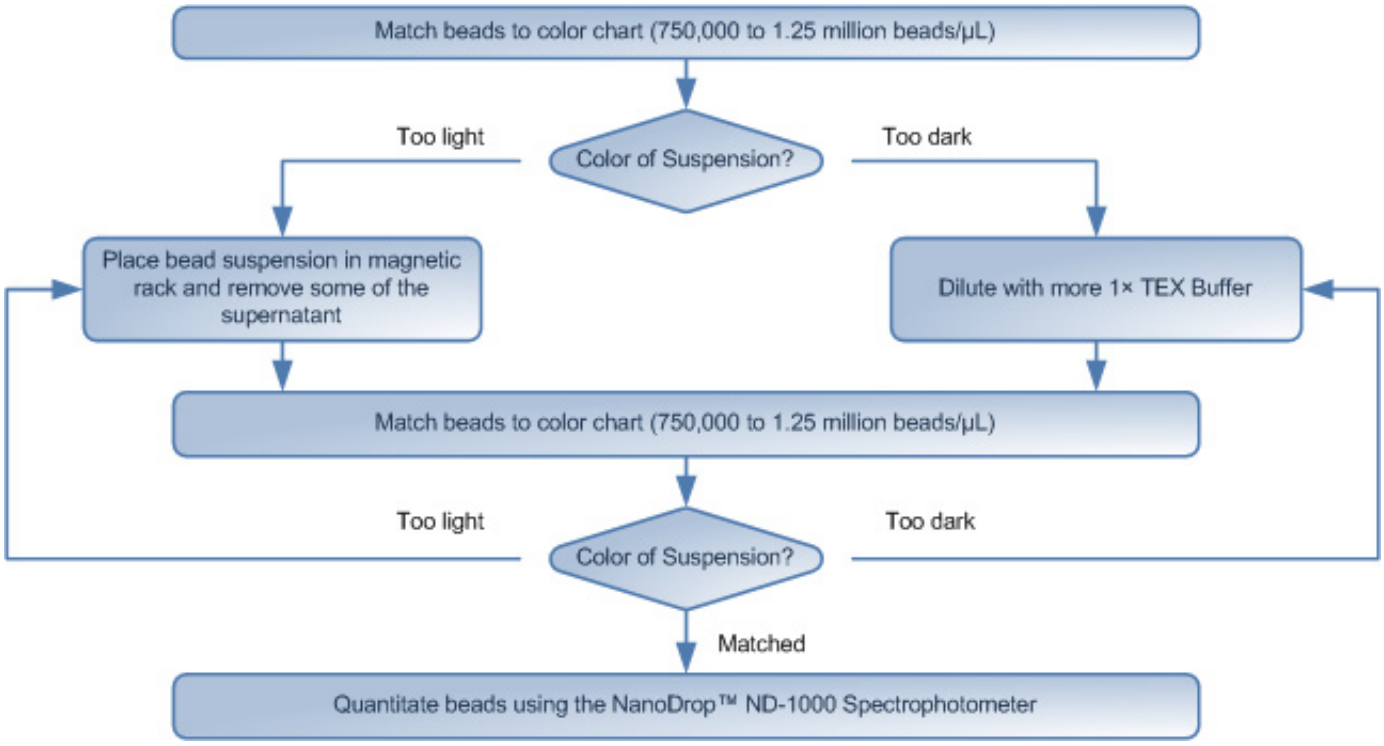


Figure 10 SOLiD™ Bead Concentration Chart workflow.

4. When the bead concentration is within the accurate range, quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer [refer to "Quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer," in the *Applied Biosystems SOLiD™ 3 System Templated Bead Preparation Guide* (PN 4407421)].

5. Transfer the appropriate number of beads (see Table 5) to a 1.5-mL LoBind tube and store the remaining beads at 4 °C.

Table 5 Beads to use according to the type of run and deposition chamber

Type of run	Deposition chamber	Number of beads per well‡
WFA	4-well	15 million
Sequencing	1-Well	310 million
Sequencing	4-Well	60 million
Sequencing	8-Well	30 million

‡ Note: The calculated bead concentration based on the WFA report is the most accurate (see "Determine the bead deposition density for a sequencing run" on page 54). If WFA run results are not available, it is recommended to target an additional 50% over the bead concentration determined by the SOLiD™ Bead Concentration Chart followed by NanoDrop® measurement.

6. Place the tube of aliquoted beads in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

7. Resuspend the beads in 400 µL of Deposition Buffer, vortex thoroughly, then pulse-spin.

8. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

9. Repeat steps 7 and 8 *twice*.

10. Resuspend the beads in the volume of Deposition Buffer indicated on the sticker attached to the lid of the SOLiD™ Deposition Chamber (see Table 6).

Table 6 Approximate Deposition Buffer volumes

Deposition chamber	Volume per well (µL)
1-Well	550
4-Well	400
8-Well	300

Deposit the beads

1. Thoroughly clean, rinse, and dry the SOLiD™ Deposition Chamber overnight before deposition:

   a. Clean the SOLiD Deposition Chamber using a sonicator or Extran 300 detergent (see "Clean the SOLiD™ Deposition Chamber using a sonicator" on page 98 or "Clean the SOLiD™ Deposition Chamber using Extran 300" on page 100).

   Note: Do not wash the SOLiD Deposition Chamber with ethanol because ethanol damages the adhesive on the O-ring.

   b. Blot the SOLiD Deposition Chamber dry on a lab wipe. Be particularly careful to dry around the O-ring and to remove fluid from the fill ports.

   c. Place the SOLiD Deposition Chamber in an incubator at 37 °C for at least one hour to complete drying.

2. Insert a new slide into the SOLiD™ Slide Carrier (see Figure 11 on page 19):

   IMPORTANT! Do not touch the slide surface.

   a. Move the retainers so that the slide can fit into the SOLiD Slide Carrier. Push down on the two spring knobs in the SOLiD Slide Carrier and slide the knobs towards the outside edges of the carrier (see Figure 11 on page 19, Figure 11a).

   b. Place the slide against the alignment nubs in the SOLiD Slide Carrier.

   c. Ensure that the slide is precisely positioned in the SOLiD Slide Carrier and then slide the retainers inward until they hold the slide in position (see Figure 11 on page 19, Figure 11b).

   Note: To move the retainers over the slide, do not push down the knobs.

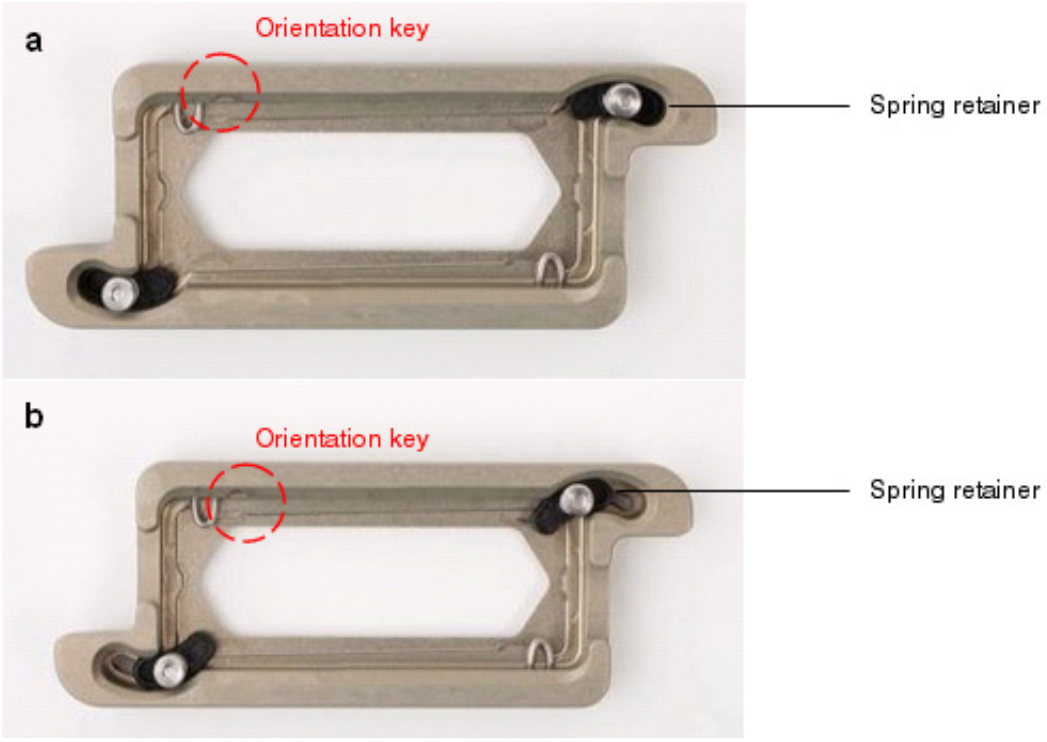


Figure 11 Slide carrier (a) with spring knobs pushed out and (b) with spring knobs pushed in.

3. Place the SOLiD Slide Carrier assembly into SOLiD Deposition Chamber base, then place the appropriate SOLiD Deposition Chamber lid on top (see Figure 12).

> **Note:** The SOLiD Deposition Chamber top must engage with the orientation key to fit properly. The orientation key is in the lower-left position when the SOLiD Slide Carrier assembly is placed on the instrument. This orientation must be maintained in order to preserve the correct sample order when the 4-well or 8-well SOLiD Deposition Chamber is used.

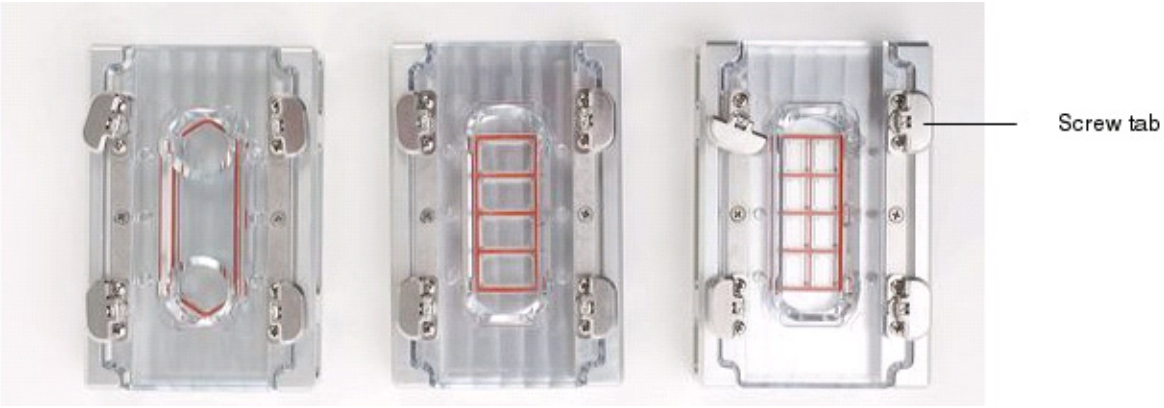


Figure 12 SOLiD™ Deposition Chambers: 1-well, 4-Well, and 8-well.

4. Tighten the four screw tabs on the SOLiD Deposition Chamber in a crisscross pattern until the lid is securely attached.

5. Twist the tabs flat.

6. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System (for program conditions, see "Covalent Declump 3" on page 141). Afterwards, pulse-spin, but do not pellet the beads.

7. Repeat step 6.

8. Use a pipettor with an appropriate tip to pipette the bead solution up and down a few times, then withdraw the sample of templated beads from the microcentrifuge tube.

   IMPORTANT! Samples must be deposited onto the slide immediately after sonication to minimize clumping and maximize monolayering.

9. Perform one of the following steps:

If the SOLiD™ Deposition Chamber has…	Then perform steps…
1 well	10 to 12
4 or 8 wells	13 to 16

10. Elevate and tilt the SOLiD Deposition Chamber with the entry porthole of the well at the lowest point.

11. Carefully pipette a sample of templated beads into the well through the porthole. As the area of the well fills, lower the top of the SOLiD Deposition Chamber so that it becomes level.

12. Proceed to step 15.

13. With the SOLiD Deposition Chamber *flat*, carefully pipette a sample of templated beads into one of the 4 or 8 wells through the porthole.

14. Repeat steps 8 to 13 to fill each of the remaining wells with each sample of templated beads. Note each sample's well position relative to the slide orientation.

15. Place 3-mm adhesive disks over all the portholes in the SOLiD Deposition Chamber.

16. Incubate the SOLiD Deposition Chamber at 37 °C for 1.5 hours.

Install on-instrument reagents

For the following hazards, see the complete safety alert descriptions in Appendix J, "Safety" on page 157:

WARNING! CHEMICAL HAZARDS. 10× Instrument Buffer, Glycerol.

CAUTION! CHEMICAL HAZARDS. Glycerol.

Note: For information about recommended fill volumes for on-instrument reagents, see "Recommended fill volumes for on-instrument reagents" on page 122.

Prepare 1× Instrument Buffer

Note: Prepare buffers just prior to use on the SOLiD™ 3 Analyzer.

1. Add 800 mL of 10× Instrument Buffer to an empty 8-L reagent bottle.

   IMPORTANT! Rinse the 8-L Instrument Buffer bottle with deionized water between runs to prevent microbial buildup and contamination.

2. Add 1600 mL of glycerol to the reagent bottle. Use a graduated cylinder to measure the glycerol.
3. Add 5600 mL of double-distilled water, rinsing residual glycerol from the graduated cylinder.
4. Using a clean magnetic stir bar, mix the solution for 10 minutes to ensure homogeneity.
5. Remove the stir bar and install the prepared buffer on the SOLiD™ 3 Analyzer or store at 4 °C until ready for use.

Prepare 1× T4 Ligase Buffer

1. Transfer the contents of the 1× T4 Ligase Buffer Part 1 tube to the 1× T4 Ligase Buffer Part 2 bottle.
2. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.

Prepare Imaging Buffer

1. Transfer the contents of the Imaging Buffer Part 1 bottle to the contents of the Imaging Buffer Part 2 bottle.
2. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.

Prepare Cleave Solution 2

1. Transfer the contents of the Cleave Solution 2 Part 1 bottle to the contents of the Cleave Solution 2 Part 2 bottle.
2. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.

Install reagents

1. Double-click the **SOLiD™ Instrument Control Software** icon to launch the SOLiD Instrument Control Software, if it is not already open.

2. Under the System Status menu, select **Cooling** from the Chiller drop-down menu (Figure 13).

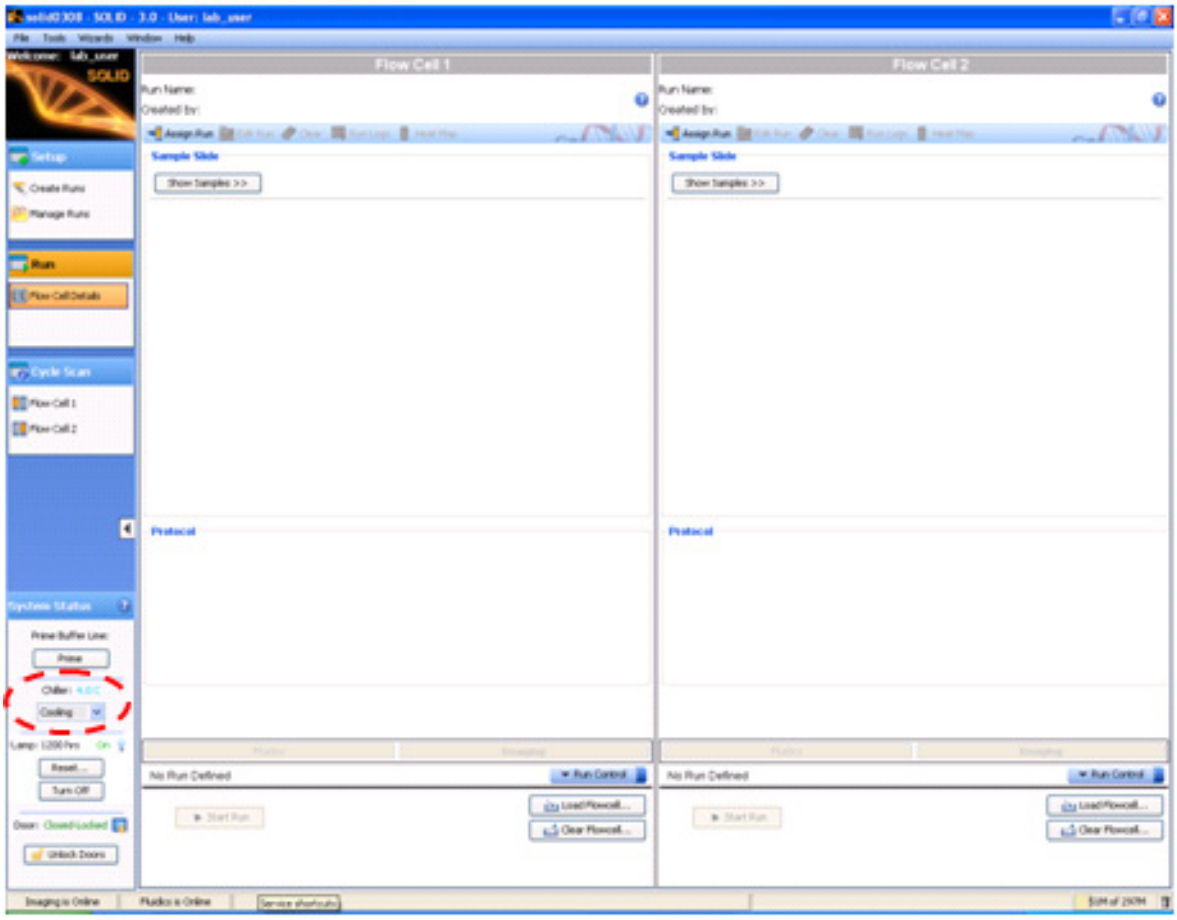


Figure 13 Select Cooling to cool the flowcell.

3. After the chiller temperature is < 10 °C, install the buffers on the SOLiD™ 3 Analyzer according to the following steps.

4. Install the prepared 1X Instrument Buffer and Storage Buffer into the appropriate positions in the cabinet (see Figure 14 on page 24).

Figure 14 Positions of buffers and waste in the cabinet.

5. Install Cleave Solution 1, prepared Cleave Solution 2, and Reset Buffer in the appropriate positions on the side of the instrument (see Figure 15).

Figure 15 Positions of reagent bottles on the side of the instrument.

6. Install the prepared Imaging Buffer, prepared 1X T4 Ligase Buffer, and Phosphatase Buffer into the appropriate positions in the chiller block (see Figure 16).

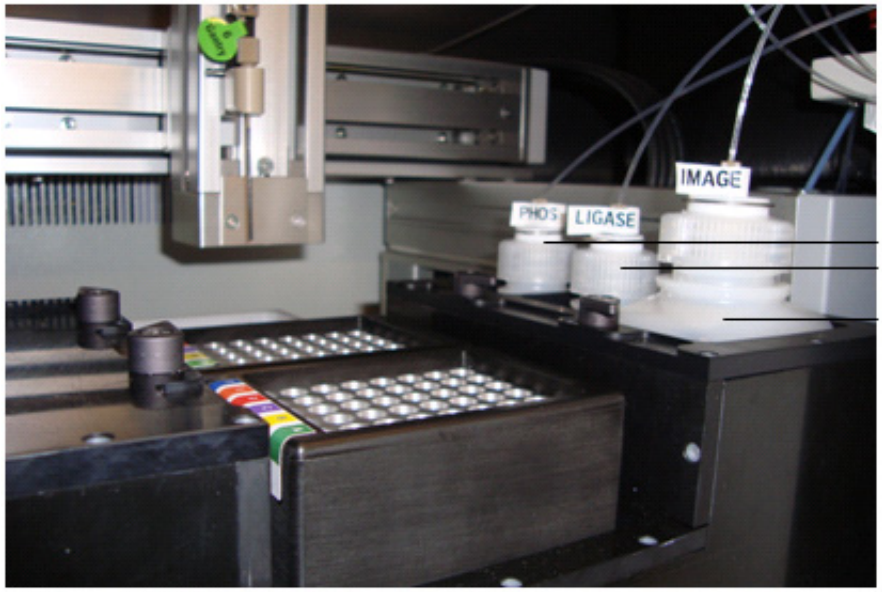


Figure 16 Positions of buffer bottles in the chiller block.

Check the waste level

CAUTION! POTENTIAL SLIPPING HAZARD. Opening the door to the computer rack requires moving the side cart. To move the side cart, the tubing must be detached from the waste container. This may cause a spill. The spill can create a potential slipping hazard. If the waste container contents are spilled, clean up immediately.

1. Check the level of waste in the 10-L carboy.
2. If the carboy is more than ¼ full, properly dispose of the waste according to your institution's environmental health and safety guidelines.

Prime Instrument and Storage Buffer lines

IMPORTANT! The priming of the lines must be performed prior to each run whether the Instrument and/or Storage Buffers were changed or not.

1. Under the System Status menu, click **Prime** (see Figure 17 on page 26).

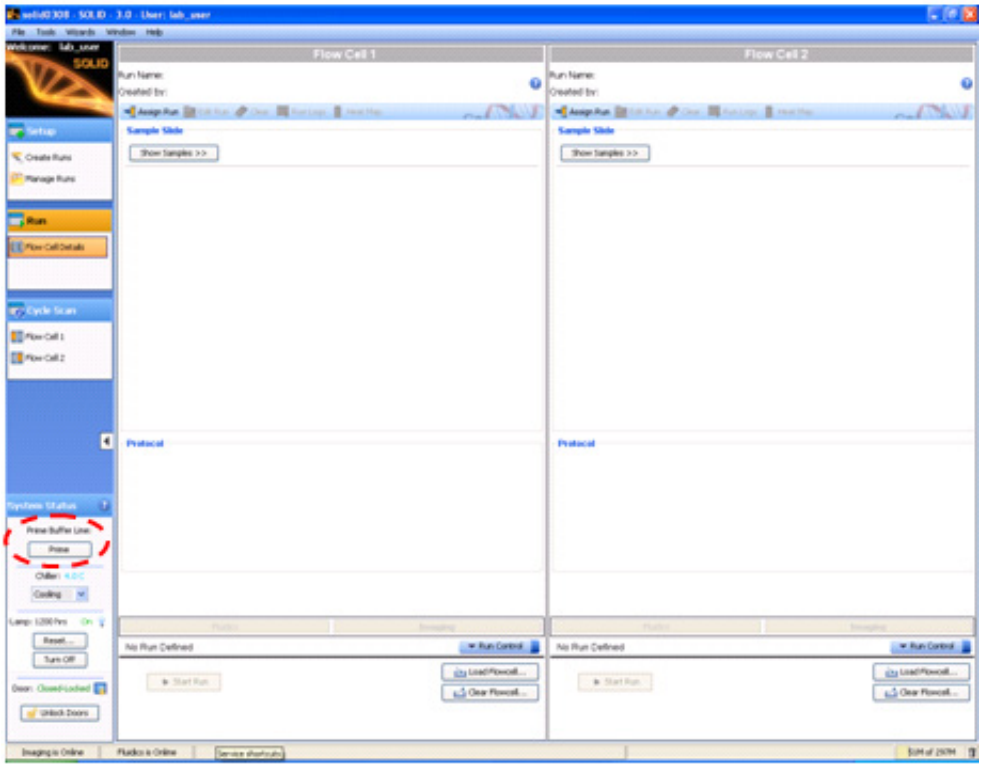


Figure 17 Click Prime to prime the instrument and storage buffer lines.

2. During priming, open the middle front door of the SOLiD™ 3 Analyzer to check that the syringe is filled with buffer when the plunger is at the aspiration stage (see Figure 18 on page 27). Confirm that the buffer lines are filled with buffer. If the syringe is not filled with buffer even at the last stroke, click **Prime** again.

Figure 18 Syringe on the SOLiD™ 3 Analyzer.

Install the slide(s) on the instrument

For the following hazards, see the complete safety alert descriptions in Appendix J, "Safety" on page 157:

WARNING! CHEMICAL HAZARD. 2-Butanol, 1× Bead Wash Buffer.

Prepare the slide

IMPORTANT! Before removing the slide from the SOLiD™ Deposition Chamber, ensure that either the instrument flowcell is ready or a SOLiD™ Slide Storage Chamber is available.

1. Remove the 3-mm adhesive disks.
2. Pour enough Deposition Buffer to cover the top of the SOLiD Deposition Chamber (1.5 mL in each port for the 1-well SOLiD Deposition Chamber and 6 mL for the 4-well and 8-well SOLiD Deposition Chambers).
3. Using an appropriate pipettor and pipette tip, press down the plunger button, then place the tip into one of the portholes of the well. Slowly release the pipettor plunger button, then aspirate the Deposition Buffer. Fresh Deposition Buffer is drawn into the well to replace the old Deposition Buffer, and aspiration removes unattached beads. Repeat this procedure for the other wells.
4. Gently loosen the SOLiD Deposition Chamber screws. As the screws are loosened, more fresh Deposition Buffer is drawn into the Deposition Chamber.
5. Open the SOLiD Deposition Chamber lid, then carefully remove the SOLiD Slide Carrier assembly from the Deposition Chamber.
6. Immediately pour the minimum amount of Overlay Buffer needed to completely cover the bead spots on the slide. Pouring Overlay Buffer onto the bead spots prevents the slide from drying out during subsequent handling.
7. Immediately place the SOLiD Slide Carrier assembly onto the instrument or into the SOLiD Slide Storage Chamber.

STOPPING POINT. If you are storing the slide, place the SOLiD™ Slide Carrier assembly into the SOLiD™ Slide Storage Chamber, then fill with 5 mL Slide Storage Buffer. Store the slide at 4 °C until the slide is ready for use (see Figure 19 on page 29).

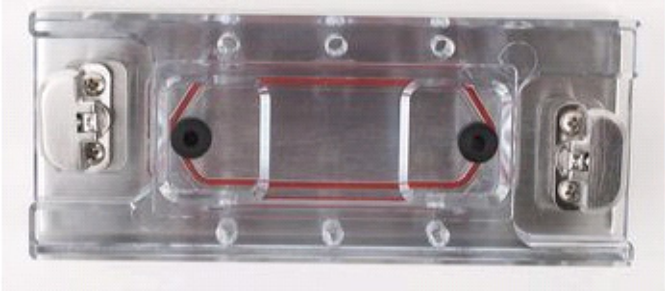


Figure 19 SOLiD™ Slide Storage Chamber.

Install the slide

For the following hazards, see the complete safety alert descriptions in Appendix J, "Safety" on page 157:

WARNING! CHEMICAL HAZARD. 2-Butanol, 1× Bead Wash Buffer.

IMPORTANT! Before removing a slide from a previous run, ensure that the run, images, and data collected from the previous run are satisfactory. For more information, refer to the *Applied Biosystems SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302).

1. Check to see if a SOLiD Slide Carrier assembly from a previous run is present in the flowcell chamber. If a SOLiD Slide Carrier assembly *is* present in the flowcell chamber, proceed with steps 2 to 4. If a SOLiD Slide Carrier assembly is *not* present in the flowcell chamber, skip to step 5.

2. For each flowcell to be used, click the **Clear Flowcell** button at the bottom of the flowcell panel (see Figure 20 on page 30).

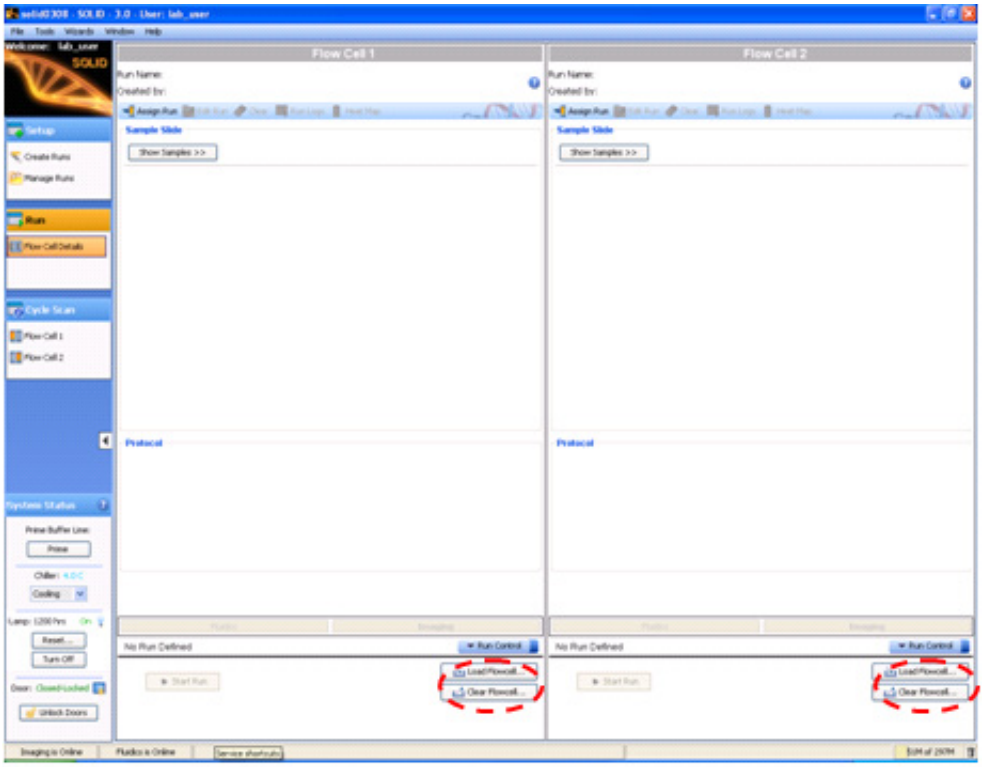


Figure 20 Click **Clear Flowcell** to flush the contents from the flowcell.

3. After clearing each flowcell, open the appropriate flowcell chamber.

4. Remove the SOLiD Slide Carrier assembly from the previous run. If the slide will be reused, place the SOLiD Slide Carrier assembly into a SOLiD Slide Storage Chamber, then fill the chamber with Slide Storage Buffer. Store the SOLiD Slide Storage Chamber at 4 °C.

WARNING! PHYSICAL INJURY HAZARD. Hot Surface. Surface of flowcell may be hot. Use care when working around the flowcell to avoid being burned.

5. Clean the flowcell block with 70% ethanol and Kimwipes® to remove residue.

6. Inspect the O-ring and reseat it if necessary (see Figure 21 on page 31; for details, see "Install the SOLiD™ Flowcell O-Ring" on page 102). Check the O-ring for cuts and abrasions. If any abnormalities are observed, replace it. Inspect the O-ring grooves for debris or contamination and clean with water as needed.

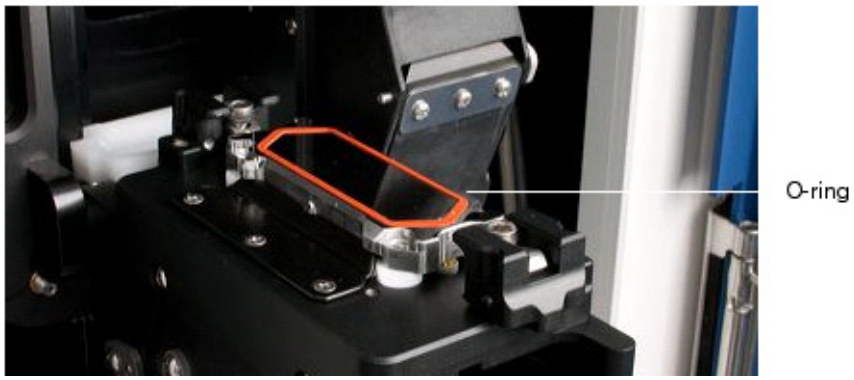


Figure 21 O-ring installed on flowcell.

7. Ensure that the instrument and storage buffer lines are primed before loading a slide, or the slide will dry out.

8. Insert the SOLiD Slide Carrier onto the instrument. Work quickly to prevent the slide from drying out.

   a. Remove the SOLiD Slide Carrier assembly from the SOLiD Deposition Chamber or from the SOLiD Slide Storage Chamber.

   WARNING! PHYSICAL INJURY HAZARD. Hot Surface. Surface of flowcell may be hot. Use care when working around the flowcell to avoid being burned.

   b. Place the SOLiD Slide Carrier assembly into the open flowcell, engaging the alignment key on the carrier with the corresponding part on the flowcell.

   c. Slide the two SOLiD Slide Carrier lock-down tabs on the flowcell inward until they are positioned over and flush with the carrier (see Figure 22 on page 32).

      IMPORTANT! Ensure that the tabs are flush with the carrier. If necessary, loosen the Allen screws further, then slide the tabs over the SOLiD Slide Carrier.

   d. To properly seat the carrier on the flowcell, gradually tighten the 2 Allen screws on both lock-down tabs in an alternating fashion to 20 inch-pounds.

   e. Rotate the flowcell up and lock it into the scan position.

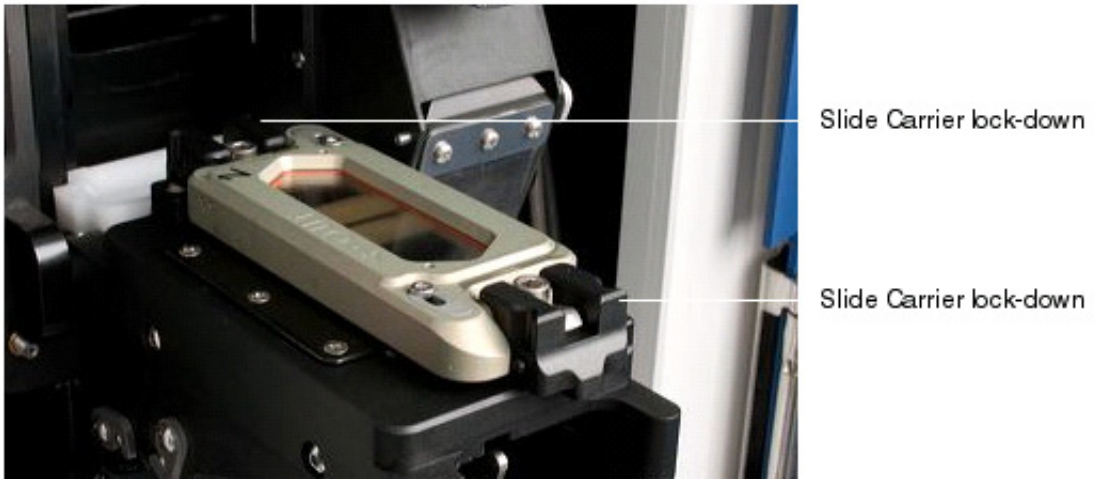


Figure 22 SOLiD™ Slide Carrier lockdown tabs.

9. Close the instrument doors.

10. Click the **Load Flowcells** button located at the bottom of the flowcell panel. Each flowcell has its own Load Flowcells button (see Figure 23).

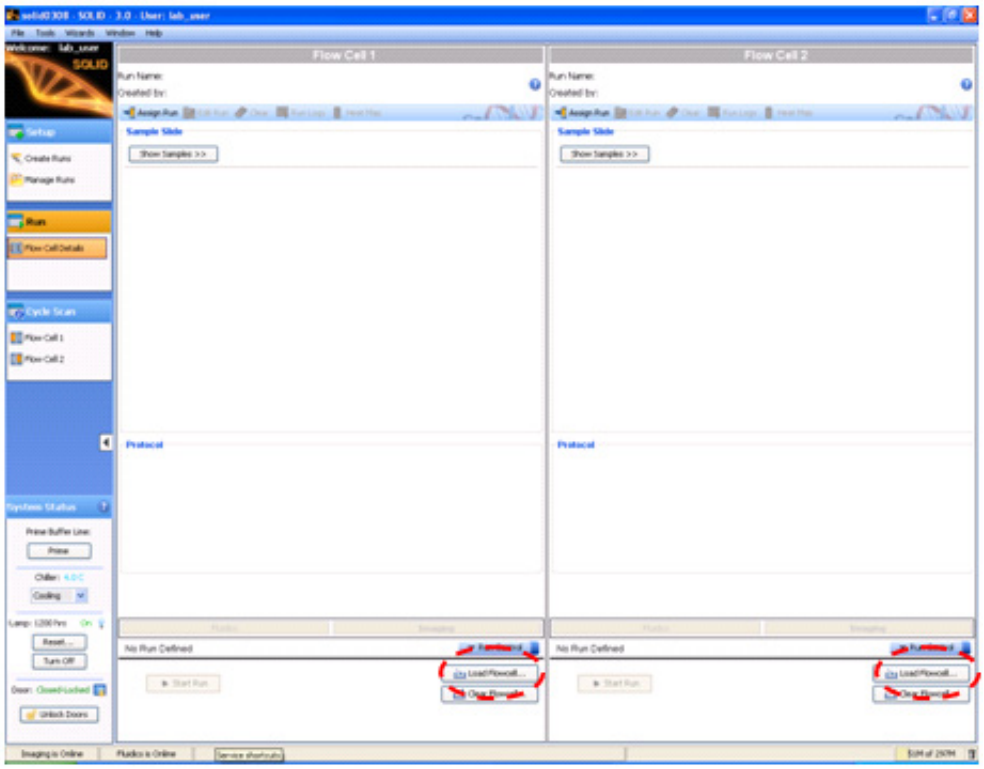


Figure 23 Click Load Flowcells to load the flowcell with buffer.

11. Click **Yes** to start loading the flowcell.

    IMPORTANT! The flowcell should be loaded within 1 to 2 minutes of slide installation. Slides should be installed one at a time, with flowcells loaded before installing a second slide.

12. Check for leaks to ensure that the slide does not dry out.

13. If only one flowcell will be used, ensure that unused flowcell SOLiD Slide Carrier lock-down tabs are pushed all the way in and tightened before closing the unused flowcell.

Install reagent strip(s)

For the following hazards, see the complete safety alert descriptions in Appendix J, "Safety" on page 157:

WARNING! CHEMICAL HAZARDS. Sodium Acetate Solution, Sodium Acetate Solution, 20× SSPE Buffer.

1. Clean the needle and bottom of the needle holder with a Kimwipe® (see Figure 24). If needed, first moisten the Kimwipe with deionized water or use a pre-moistened alcohol pad.

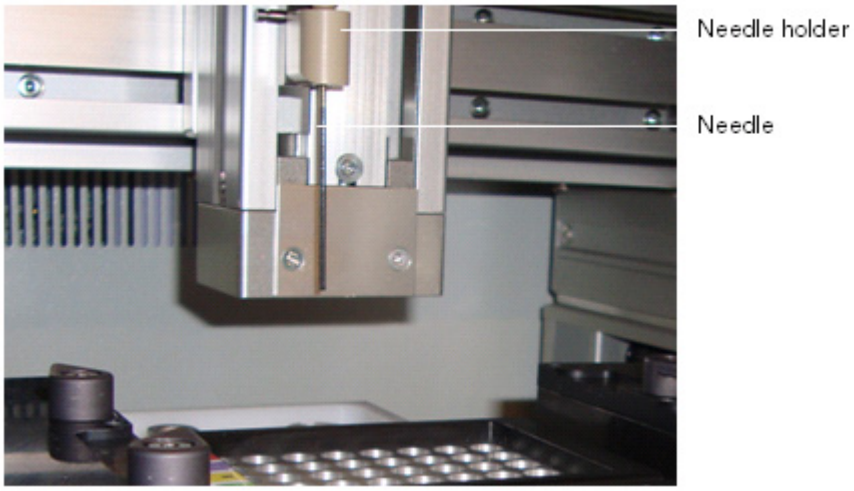


Figure 24 Needle and needle holder.

2. Thaw the appropriate reagent strip(s) on ice.

3. Place the reagent strip(s) in an ABgene® 96-well square-well storage plate and centrifuge at 160 × g for 2 minutes. For reagents needed for a WFA or sequencing run, see Figure 25 on page 35 to Figure 29 on page 37. For information about contents of reagent tubes, see "Reagent strip layouts" on page 124.

   IMPORTANT! For barcoded fragment libraries of short insert sizes such as small RNA samples or SAGE tags, consult your field applications specialist on the recommended reagents for sequencing the target read on barcoded samples, which is different from the SOLiD™ Fragment Library Sequencing - Master Mix 50.

Figure 25 Reagent strip block layout for WFA.

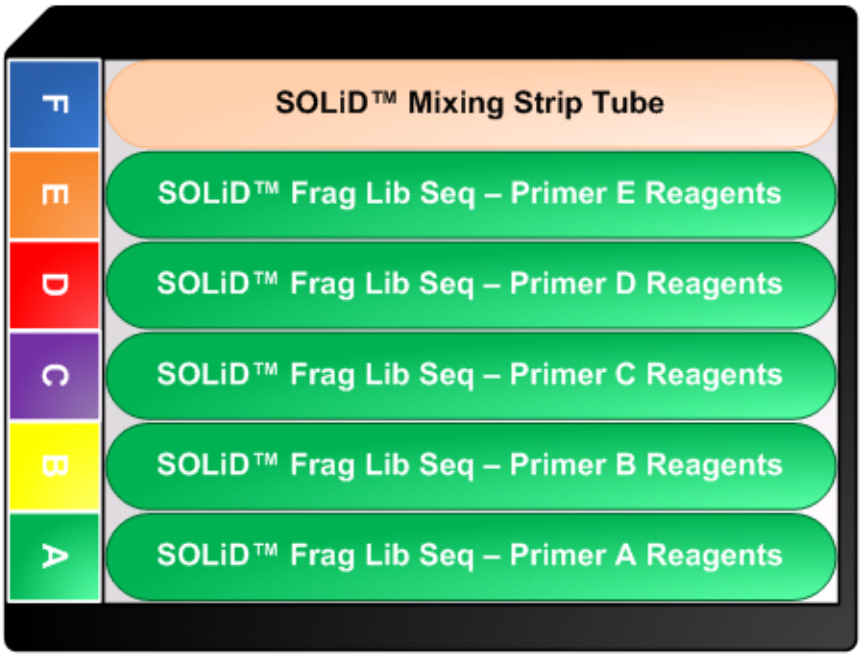


Figure 26 Reagent strip block layout to sequence fragment (50 bp).

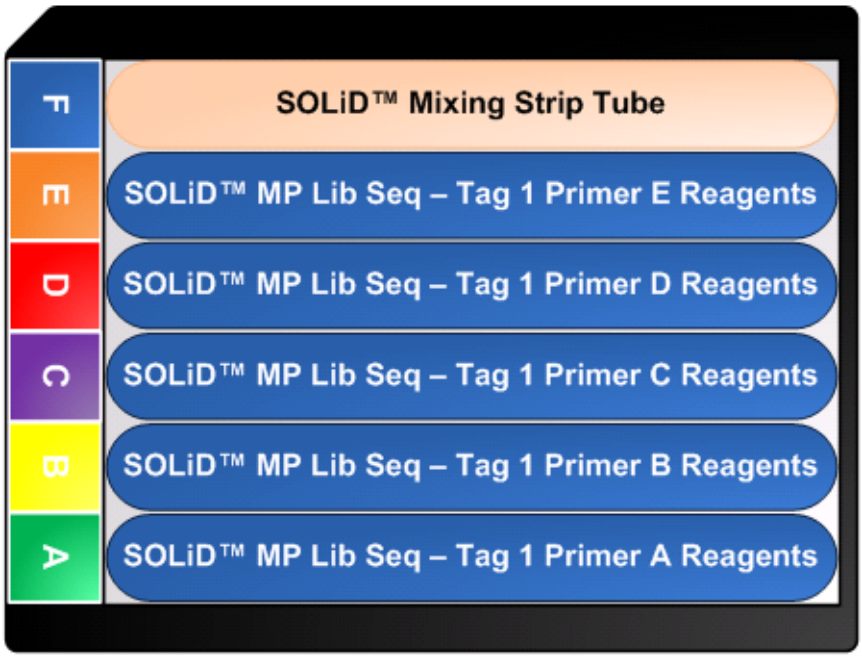


Figure 27 Reagent strip block layout to sequence mate-paired tag 1 (50 bp).

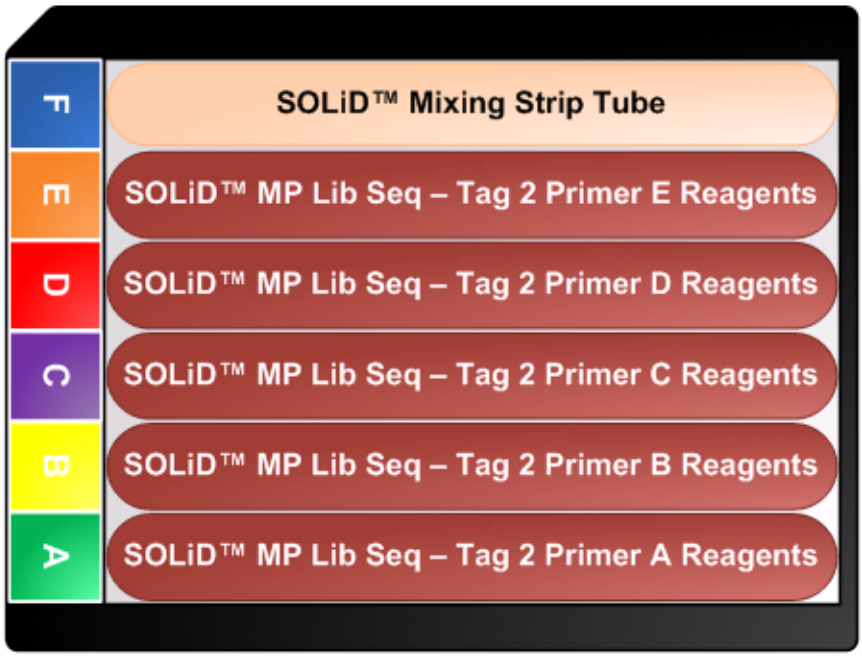


Figure 28 Reagent strip block layout to sequence mate-paired tag 2 (50 bp).

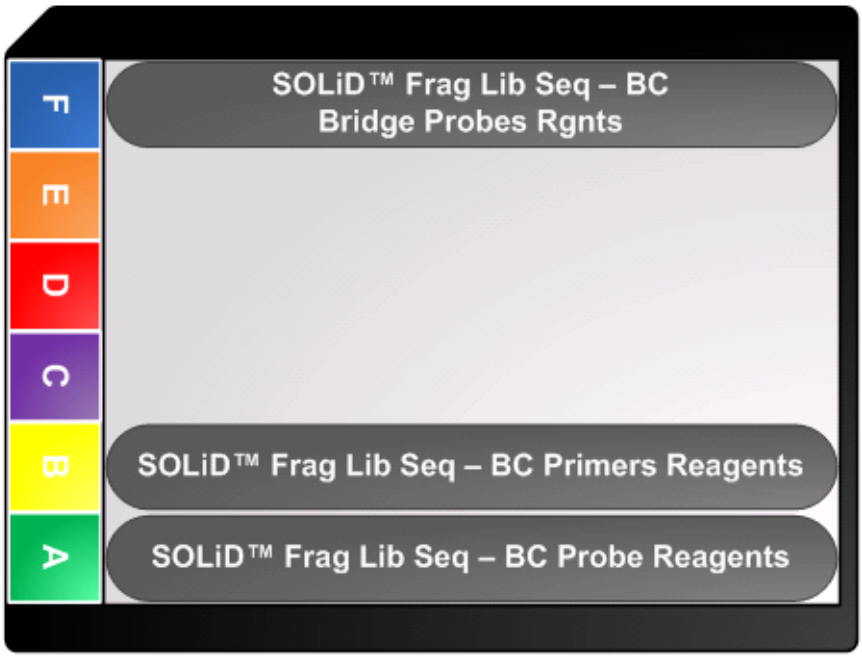


Figure 29 Reagent strip block layout to sequence barcode (5 bp).

4. Verify that the reagent strip blocks are oriented and seated properly in the chiller block. The block must engage the orientation key to fit properly. The orientation key (cut corner) is in the upper-left position when the block is placed on the instrument (see Figure 30 on page 38).

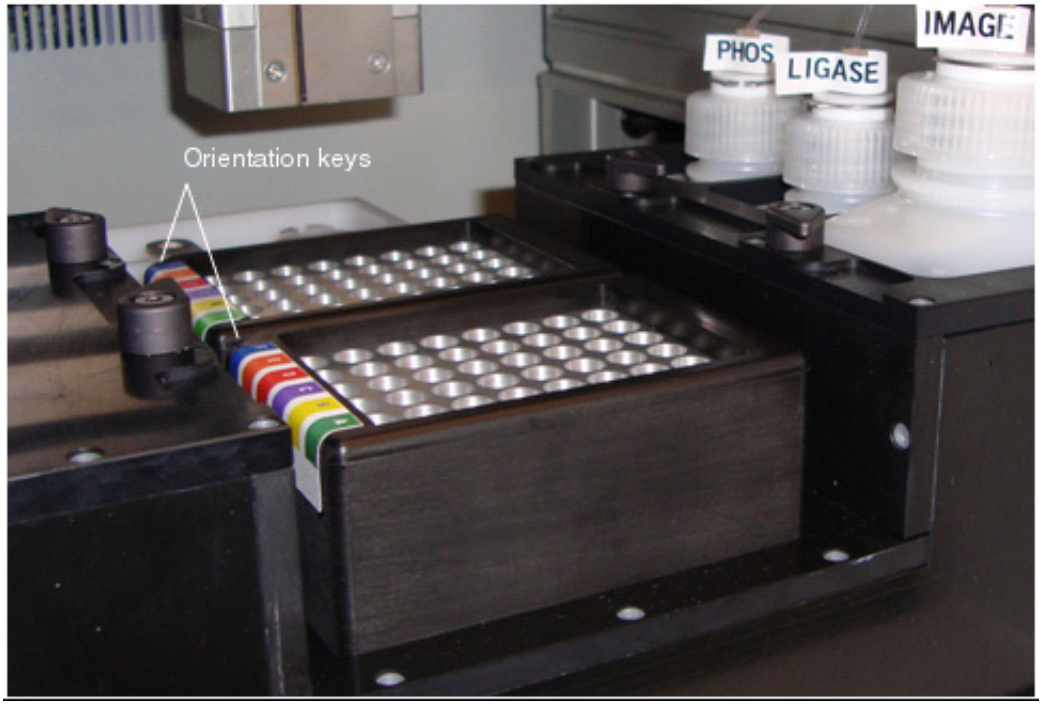


Figure 30 The Reagent Strip Block.

IMPORTANT! Ensure that the chiller block temperature is below 10 °C before proceeding with reagent strip installation. Under the System Status menu, select **Cooling** from the Chiller drop-down menu (Figure 13 on page 23).

5. When the temperature is less than 10 °C, place the reagents in the appropriate location in the chiller block (see Table 7 and Figure 31 on page 39).

Table 7 Where to place the strip tubes

If using flowcell…	Then place the strip tube(s) in the…
1	Front block
2	Rear block

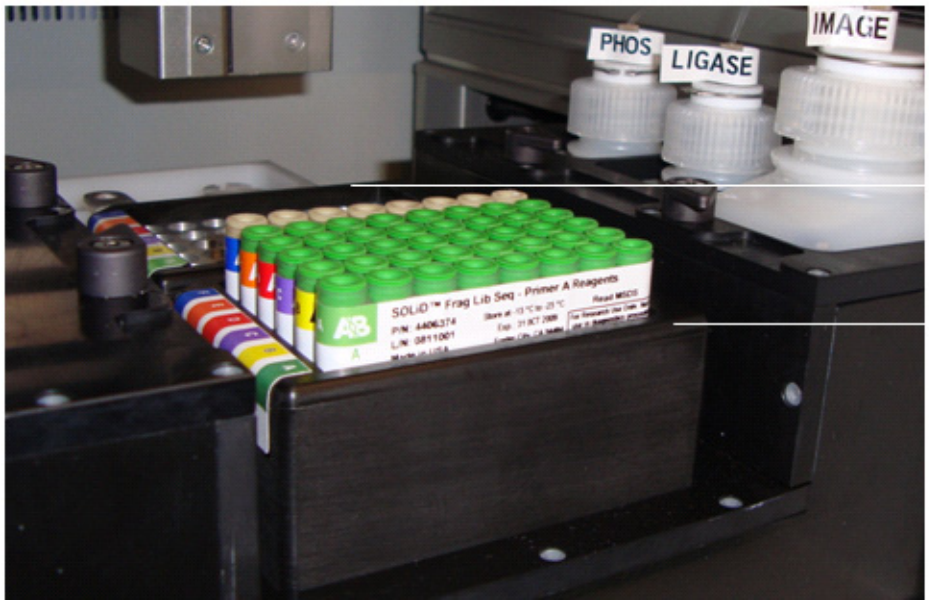


Figure 31 Position of the sequencing reagent blocks for flowcells 1 and 2.

6. Place the cover over the reagent strips and secure them using the cover fasteners (see Figure 32).

Note: Ensure that the top and bottom of the cover is free of splattered wet or dry reagents (see "Clean the reagent strip cover" on page 103).

Figure 32 Securely fastened reagent strip covers.

3 Set Up, Control, and Monitor the Run

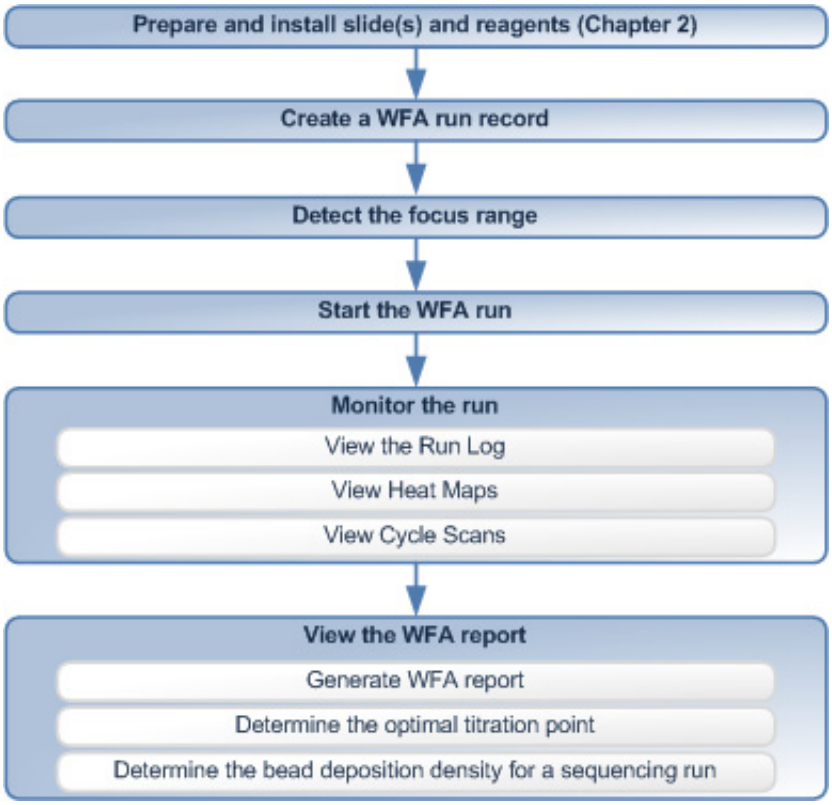
This chapter covers:

Section 3.1 Set up and perform a workflow analysis (WFA) run

Materials and equipment required

See Appendix A on page 85 for a list of equipment, kits, and consumables necessary to set up a workflow analysis (WFA) run.

Workflow



Create a WFA run record

A WFA run record is created using the SOLiD™ Instrument Control Software (ICS).

Detect the focus range

Two methods exist for determining the focus range: automatic and manual. Users should first attempt automatic range detection. If automatic detection fails, use the manual mode.

Start the WFA run

Start the run by using the SOLiD™ Instrument Control Software. Before starting a run, you may need to remove previous data may need to ensure maximum disk space for new results.

Monitor the run

While executing a WFA run, the instrument performs a Prescan. In a Prescan, all of the beads are labeled and their positions on the slide are recorded to derive a focal map followed by a single ligation cycle. The run can be monitored through the ICS or the SETS browser by using the Run Log, Heat Map, and Cycle Scans.

Each flowcell generates a separate Run Log that records high-level events, such as fluidic modules and slide scanning. The start and stop times of these events, as well as any pauses or errors during the run, are also recorded. The Run Log is particularly useful in helping you anticipate and schedule reagent refills or troubleshoot instrument errors.

Heat maps are generated from the analysis of the focal map images and analysis of each ligation cycle. A heat map is a colorized display of a particular metric (*bead count*, *bead signal*, or *image signal*) across all the panels for a run (for definitions of the metrics, see the "Glossary" on page 173).

For each flowcell, the corresponding Cycle Scans window provides nearly real-time feedback on initial data quality on a per-cycle basis.

View the WFA report

You can view a WFA report in the SOLiD™ Experiment Tracking System (SETS) after the run is complete. Three important metrics are generated in the WFA report: *P2#/P1# ratio*, *On-Axis beads*, and *Titration Metric*. These metrics guide the selection of the best-performing bead population based on different titration points used in ePCR (for definitions of the metrics, see the "Glossary" on page 173). In general, the closer the image data points are on-axis, the higher the quality of data obtained due to good bead deposition and chemistry.

From the WFA data, you can also estimate the bead deposition density to be used when preparing a slide for sequencing. Differences between bead concentration measured on the NanoDrop® ND-1000 Spectrophotometer and the concentration actually detected by the bead counting algorithm on the instrument may occur. In order to maximize throughput in a SOLiD™ sequencing run, a bead density of 130,000 P2-positive beads per panel is recommended.

Create a WFA run record

There are two ways to create a WFA run. The first method is to use the Run wizard (see below). The second method is to import a .txt file that contains the run definition (see "Set up a run by importing a Run Definition file" on page 106). Importing a .txt file saves time re-entering information of a repeated run. The .txt file can be generated on an off-instrument computer.

1. Click **Create Runs** in the Setup task pane on the left menu pane of the ICS (see Figure 33 on page 46).

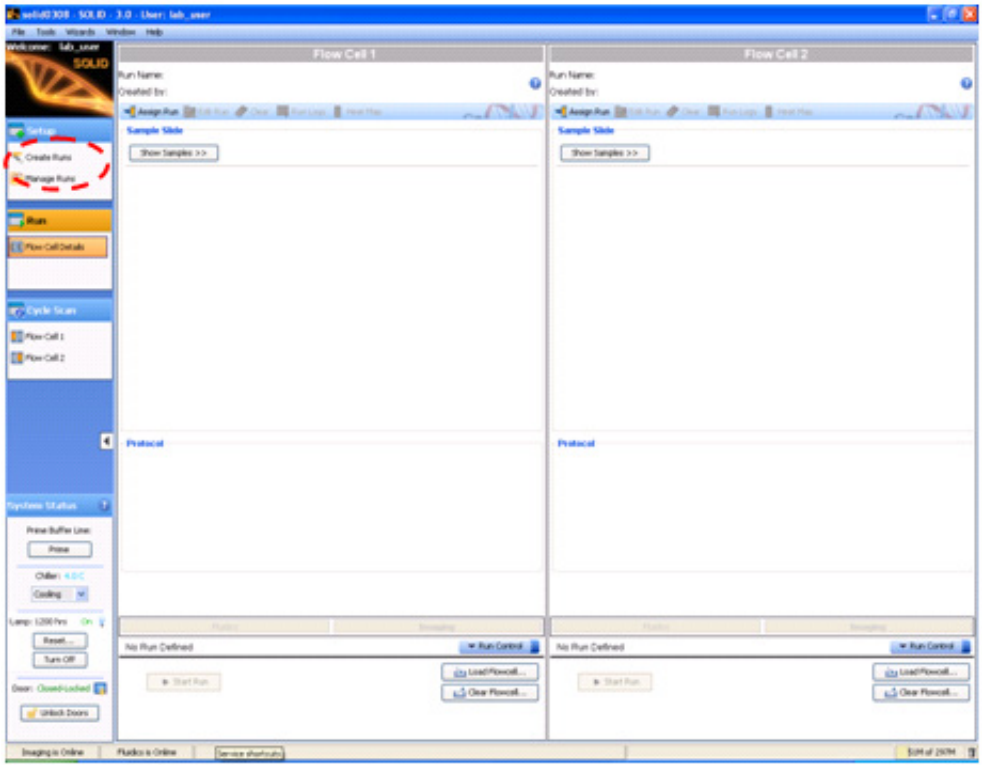


Figure 33 Use the run wizard to create a WFA run.

2. Complete the information in the Select Run Type and Mask pane (see Figure 34 on page 47):
   a. Select the **WFA** option.
   b. (Optional) Type a new run name.
   c. (Optional) Enter a description.
   d. Ensure that the mask **4_spot_WFA_mask_sf** is selected.
   e. Click **Next**.

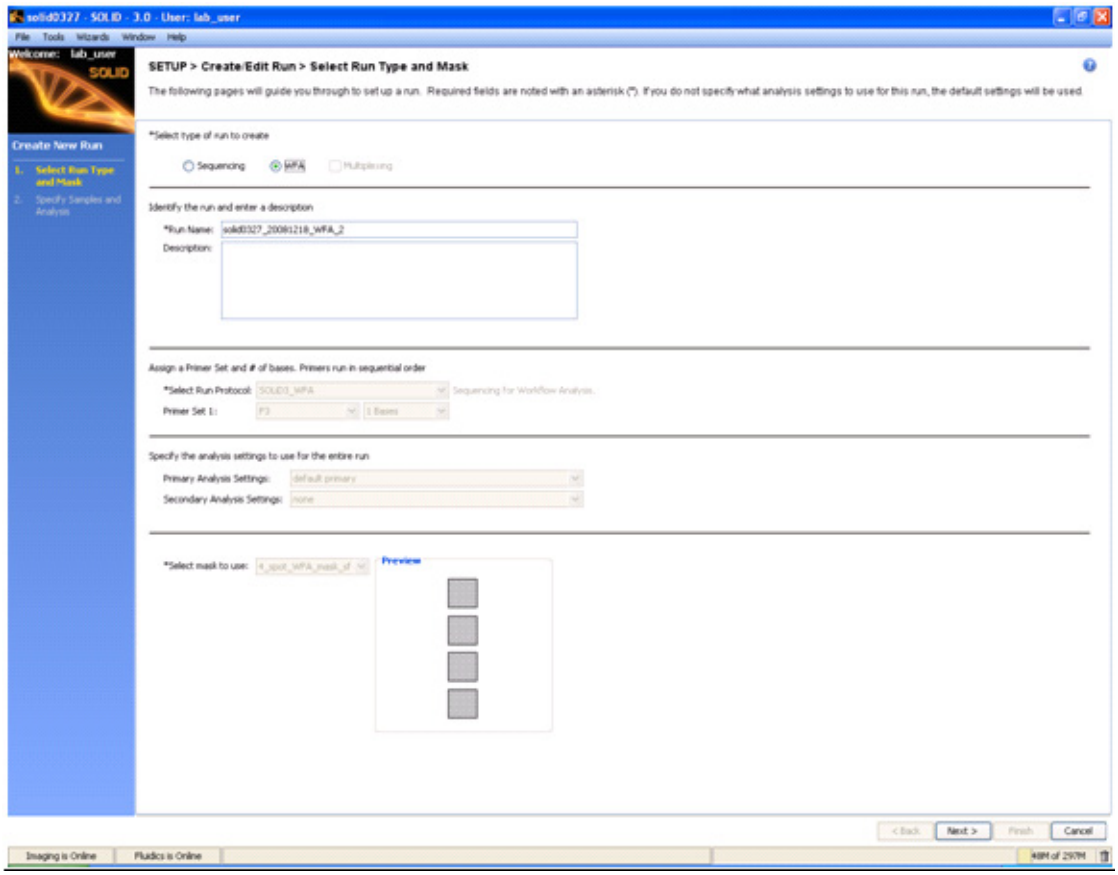


Figure 34 Complete the information in the Select Run Type and Mask pane.

3. Complete the information in the Specify Samples and Analysis pane (see Figure 35 on page 48):
   a. Assign samples to spots.
   b. Click **Finish**.

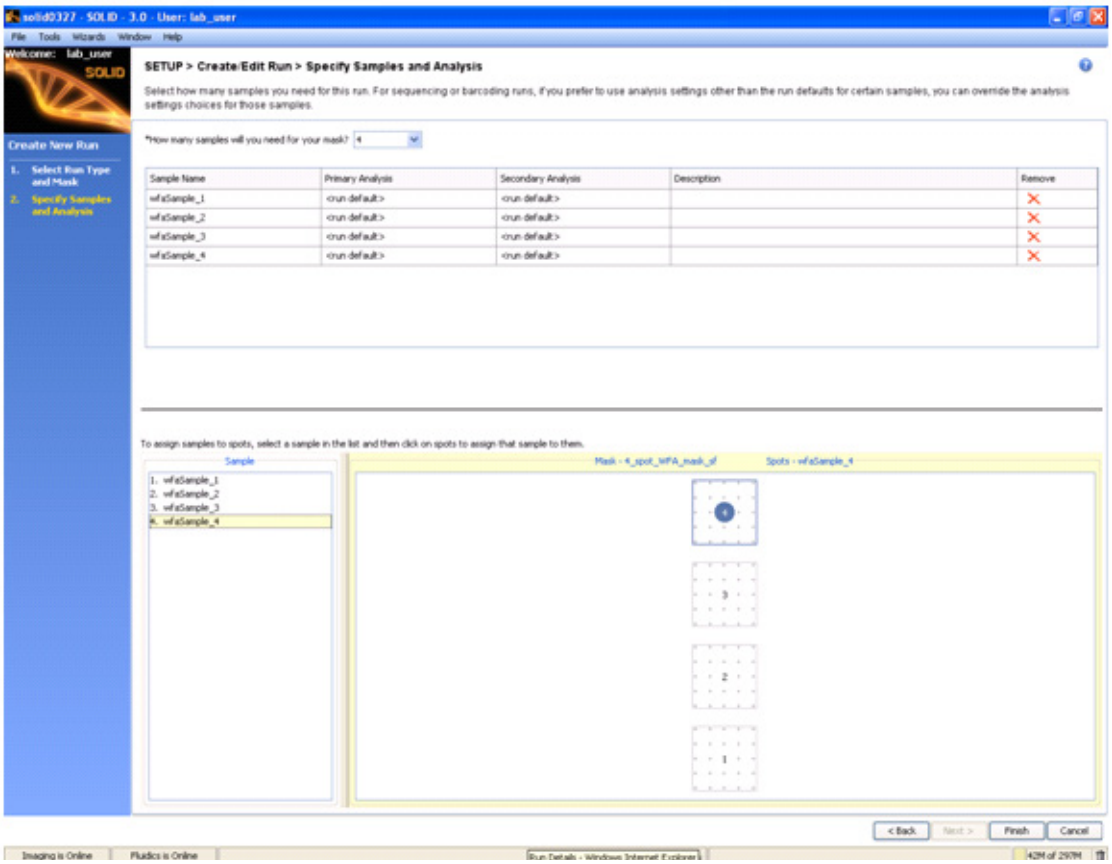


Figure 35 Complete the information in the Specify Samples and Analysis pane.

4. Choose either to assign a run to a flowcell for immediate use or to store the run in the instrument database for later use, then click **OK**.

5. To assign a run previously saved to the database:

   a. Click on **Manage Runs** in the task pane.

   b. Click the run, then select **Assign to Flowcell**.

   c. Choose a flowcell, then click **OK**.

6. (Optional) Repeat the above steps for the other flowcell if performing a WFA run on a second slide.

Detect the focus range

Note: If automatic focus range detection fails, determine the focus range manually (see "Manually find the focus range" on page 107).

1. Close the front doors of the SOLiD™ 3 Analyzer. Open the Imager window by choosing **Windows** ▸ **Imaging System**.
2. Select **Tools** ▸ **Detect Focusing Range**.
3. In the focusing range dialog, specify the stage template file by entering the name directly or by clicking the **Browse** button to navigate to a suitable one (see Figure 36). The stage template must match the slide in the target flowcell(s). If you created a run from the ICS, then select the file **imagingMap.STG** from the subdirectory in C:\Runs whose name matches the name of the run (for example, select C:\Runs\Solid0327_20081209_2_Oct_Test\imagingMap.STG).

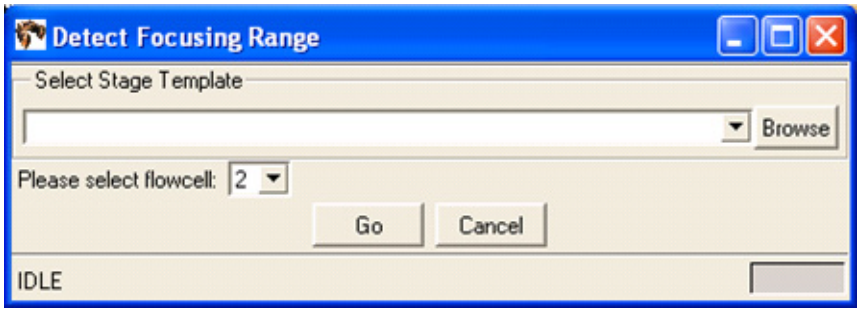


Figure 36 Detect Focusing Range dialog.

4. Select the flowcell using the drop-down menu, then press **Go**. The Imager works for several minutes while it determines the range. The blue progress bar indicates how close it is to completion (see Figure 37). You can also click **Cancel** so that the Imager aborts the ranging operation.

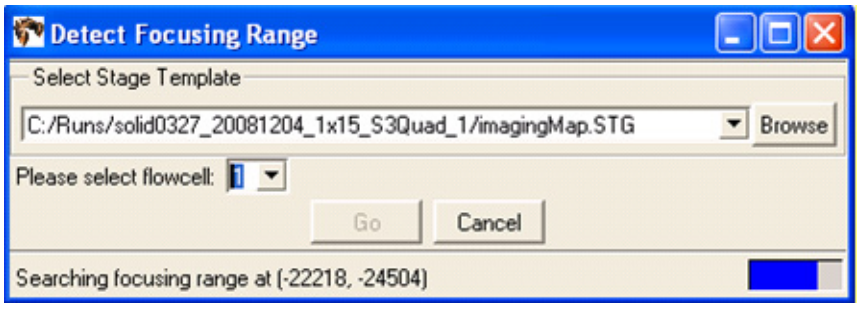


Figure 37 Detect Focusing Range Dialog while detection in progress.

5. When the Imager is done, a dialog appears (see Figure 38 on page 50). Click **Yes** if you want to replace the values in the local settings file. Click **No** if you want the Imager to discard the newly calculated focus range.

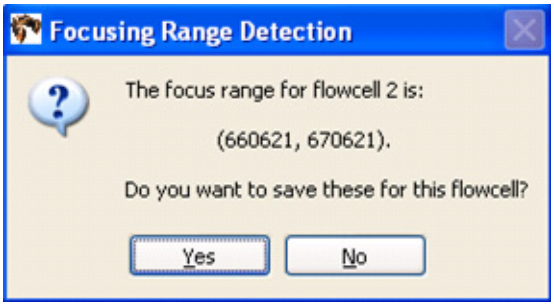


Figure 38 Confirm that you want to replace the local settings file.

6. Verify the validity of the newly calculated focus range by taking images at random locations.

IMPORTANT! You should see images of beads, ensuring that the algorithm was able to focus on the beads and not on other artifacts (see Figure 39). If you do not see bead images or if you see out-of-focus bead images, set the focus range manually (see "Manually find the focus range" on page 107).

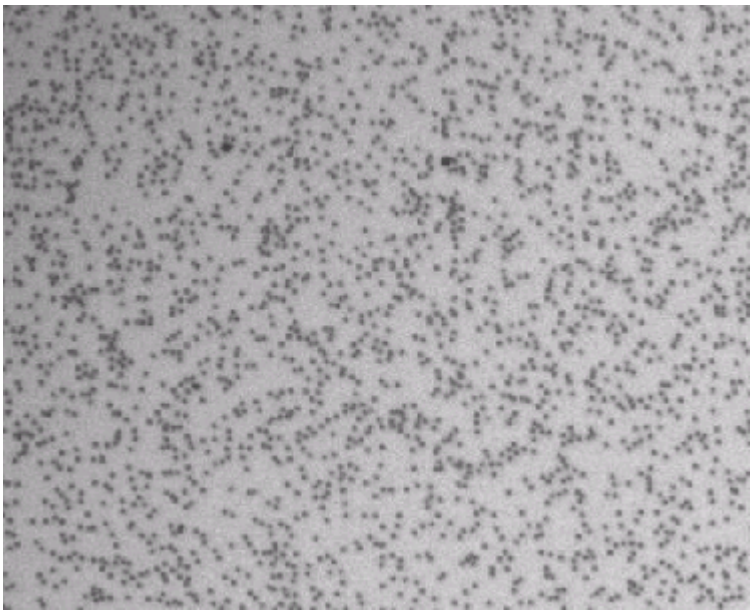


Figure 39 Beads in focus.

Start the WFA run

1. Click **Start Run**.

2. If there is not enough room to store the data, then the Start Run dialog displays (see Figure 40). Choose the appropriate option (see Table 8).

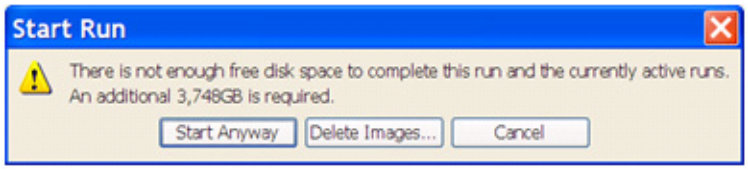


Figure 40 This Start Run dialog appears if there is not enough room to store data for the run.

Table 8 Choose one of the three options to manage disk space.

Option	Description
Start Anyway	Initiates the run. The instrument pauses itself when it runs out of free disk space.
Delete Images‡	Launches Historical Runs page in SETS. Images and/or results can be deleted through SETS
Cancel	Aborts the run.

‡ For more information on creating more available disk space, see the *Applied Biosystems SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302).

IMPORTANT! Before deleting any images, ensure that data analysis from the previous run is satisfactory and complete. For more information, refer to the *Applied Biosystems SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302).

3. After the run has been initiated, you can click the **Run Log**, **Cycle Scans**, and **Heat Map** buttons located at the top of the appropriate flowcell panel to learn more information about the current run (see Figure 41 on page 52).

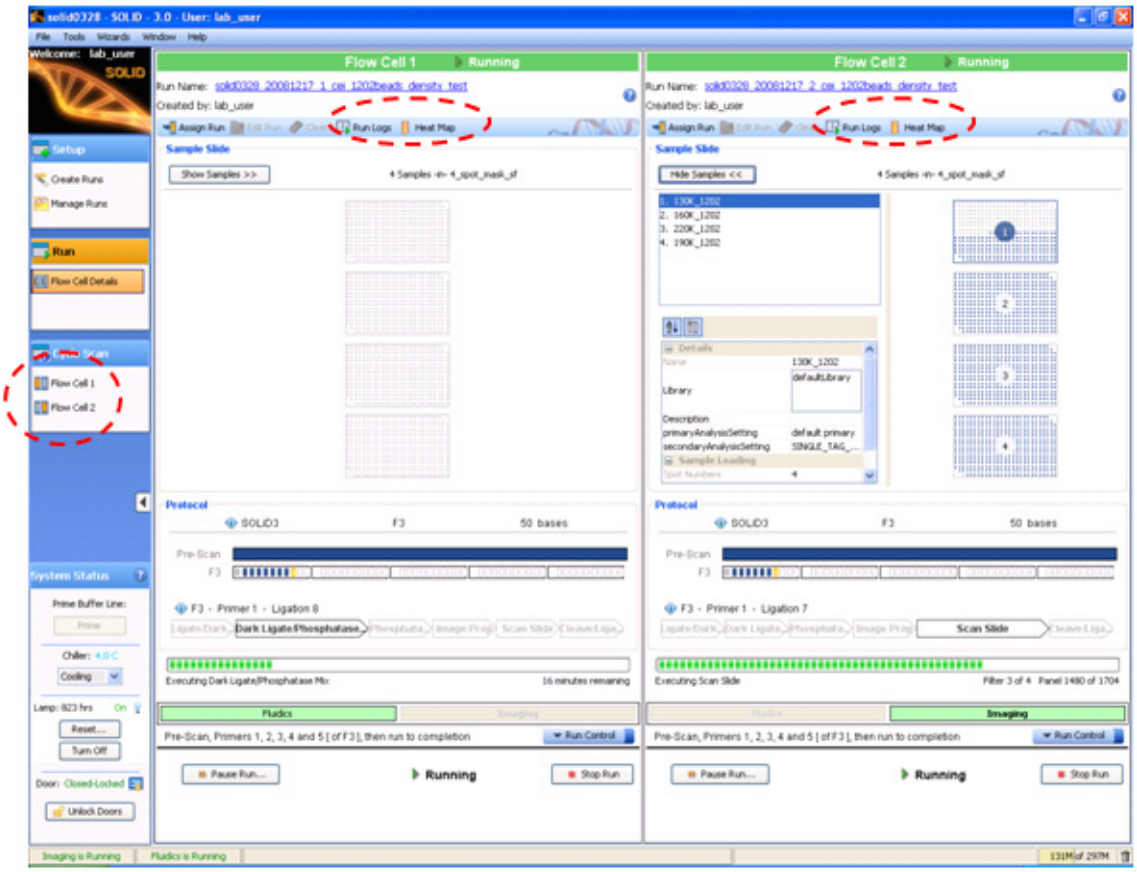


Figure 41 How to learn more about the current run.

IMPORTANT! Do not disturb the SOLiD™ 3 Analyzer while in operation and do not open the flowcell during a pause in the run. Significant perturbations (for example, opening system parts, vibrations) during the run is detrimental to the results.

Monitor the WFA run

Note: To monitor the run remotely, use SETS from any computer to connect to the networked instrument [refer to the *Applied Biosystems SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302)]. If desired, set up e-mail notification regarding the instrument run and system information using SETS (refer to the *SOLiD™ 3 System SETS Software Getting Started Guide*).

View the run log

1. Click **Run Log** located at the top right corner of the flowcell panel. A dialog box opens, describing a series of instrument events.
2. After you finish viewing the Run Log, click **Close**, located at the bottom of the Run Log window.

View heat maps

1. To view the heat map showing bead densities found in the focal map images, click **Heat Map** located at the top right-hand corner of the flowcell panel (see Figure 41 on page 52).
2. Look for:
   - Uniform deposition of beads on the slide.
   - The actual average bead deposition density/panel value being similar in value to the targeted average bead deposition density/panel of 25,000 beads/panel for WFA run. A large number of missing panels could indicate a deposition problem.

   Note: The heat map may not immediately be available after the completion of the Prescan. The software must process all the images collected during the Prescan before the heat map is available. This process may take up to 30 minutes, depending on the number of panels imaged.
3. After you finish viewing the Heat Map, click **Close** located at the bottom of the Heat Map window.

View cycle scans

1. Select the appropriate flowcell (**Flow Cell 1** or **Flow Cell 2**) in the Cycle Scan menu on the task bar on the left.

   Note: Details regarding which parameters to monitor are described in the sequencing run section (see "Monitor the sequencing run" on page 78).
2. Double-click any cycle to view the heat map for that cycle (see Figure 65 on page 82).
3. Left-click any square panel on the heat map to open the panel browser window. The panel browser allows you to view the focal map and the image for each fluorescent dye signal (see Figure 66 on page 83).

4. After you finish viewing the Cycle Scans, close the Cycle Scans window.

Note: If the WFA run appears problematic, you can: (1) Allow the run to continue and troubleshoot after the run or (2) pause the run and troubleshoot. Consult an Applied Biosystems SOLiD™ Field Applications Specialist.

View the WFA report

Generate the WFA report

The WFA report is automatically generated and available in SETS when the WFA run finishes (see Figure 42). Refer to the *Applied Biosystems SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302).

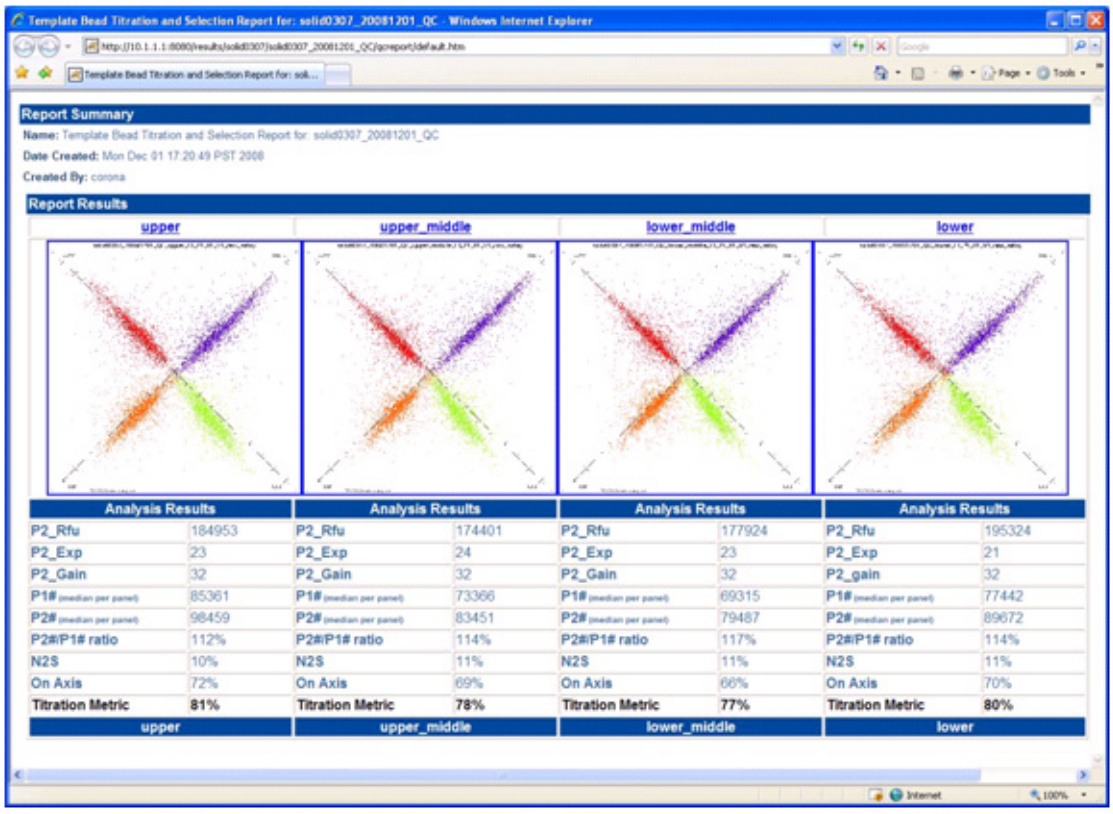


Figure 42 One WFA report for a single 4-well slide displayed in SETS.

Determine the optimal titration point

Determine which titration has the highest titration metric. This titration is the optimal titration point.

Determine the bead deposition density for a sequencing run

1. Calculate the concentration of P2-positive beads using the formula below, where *X* is the volume of templated beads used for the WFA sample (equivalent to 15 million beads) and *P2#* is given by the WFA report:

$$X\ \mu L = \frac{15 \times 10^6 \text{ beads}}{\#\ \text{beads}/\mu L\ \text{(according to NanoDrop™)}}$$

$$Y\ \text{P2-positive beads}/\mu L = \frac{\text{P2\# beads/panel} \times 432\ \text{panels}}{X\ \mu L}$$

Example:

For a sample with a concentration of 500,000 beads/µL measured by NanoDrop™ ND-1000, where the WFA report indicates a P2# value of 20,000 beads/panel.

$$X\ \mu L = \frac{15 \times 10^6 \text{ beads}}{500{,}000\ \text{beads}/\mu L} = 30\ \mu L$$

$$Y\ \text{P2-positive beads}/\mu L = \frac{20{,}000\ \text{beads/panel} \times 432\ \text{panels}}{30\ \mu L}$$

$$= 288{,}000\ \text{P2-positive beads}/\mu L$$

2. Use the calculated concentration in place of the value determined by the NanoDrop® ND-1000 Spectrophotometer for more accurate deposition densities when preparing slides for sequencing. It is recommended to use this resulting calculated bead concentration to determine the volume of beads for deposition. Then, multiply that volume by 120% to calculate the volume of beads for deposition:

$$Z\ \mu L\ \text{bead solution} = \frac{\text{Desired \# P2-positive beads to be deposited}}{Y\ \text{P2-positive beads}/\mu L} \times 120\%$$

Example:

For a sample with a concentration of 288,000 P2-positive beads/µL to be deposited in one well of an 8-well SOLiD™ Deposition Chamber at a density of 130,000 beads/panel.

$$Z\ \mu L\ \text{bead solution} = \frac{30\ \text{million P2-positive beads}}{288{,}000\ \text{P2-positive beads}/\mu L} \times 120\%$$

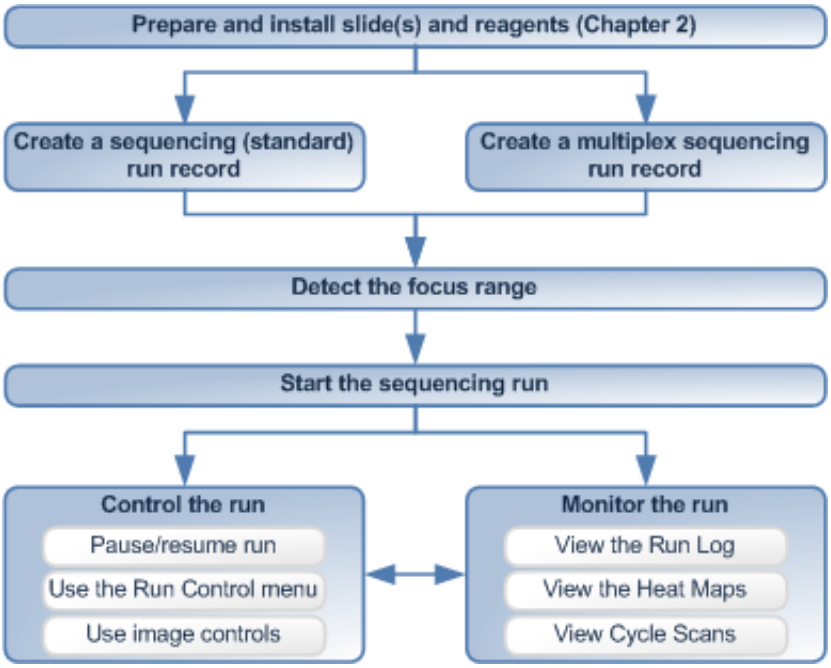
$$= 125\ \mu L\ \text{bead solution}$$

Section 3.2 Set up and perform a sequencing run

Materials and equipment required

See Appendix A on page 85 for a list of equipment, kits, and consumables necessary to set up a sequencing run.

Workflow



Create a sequencing (standard) run record

A sequencing (standard) run record is created using the SOLiD™ Instrument Control Software (ICS). Primary Analysis Settings and Secondary Analysis Settings should be created prior to creating a sequencing run record using SETS [refer to the *Applied Biosystems SOLiD™ 3 SETS Software Guide* (PN 4389302)].

Create a multiplex sequencing run record

A multiplex sequencing run record is created using the SOLiD™ Instrument Control Software (ICS). At this step, barcodes are matched to libraries. Primary Analysis Settings and Secondary Analysis Settings should be created prior to creating a sequencing run record using SETS [refer to the *Applied Biosystems SOLiD™ 3 SETS Software Guide* (PN 4389302)].

Detect the focus range

Two methods exist for determining the focus range: automatic and manual. Users should first attempt automatic range detection. If automatic detection fails, use the manual mode.

Start the sequencing run

The run is started using the SOLiD™ Instrument Control Software. Before starting a run, previous data should be removed to ensure maximum disk space for new results. The instrument is limited to run 5 primers per flowcell. If you are sequencing mate pairs or barcoded fragments, the instrument automatically pauses and stays paused until the reagents are replaced and the run is resumed.

Control the run

You can control how the SOLiD™ 3 System collects sequencing data with the SOLiD™ Instrument Control Software (ICS). With the ICS, you can repeat a primer to improve the real-time primary analysis results or set an early pause point to change reagents on your schedule. You can choose to turn off imaging of specific samples to collect only the best sequencing data.

To control the ICS, use the Run Control drop-down menu. Use the Run Control menu only if you understand clearly the series of fluidic and imaging steps in a run. Skipping or repeating certain steps could lead to errors in the resulting data. For example, with the ICS menus, you can repeat a ligation; however, you must first cleave the fluorescent label from the ligation product.

Monitor the run

While executing a sequencing run, the instrument performs a Prescan. In a Prescan, all of the beads are labeled and their positions on the slide are recorded to derive a focal map followed by a single ligation cycle. The run can be monitored using the Run Log, Heat Map, and Cycle Scans through the Instrument Control Software (ICS) or from the SETS browser.

Each flowcell generates a separate Run Log that records high-level events, such as fluidic modules and slide scanning. The start and stop times of these events and any pauses or errors that occur during the run, are also recorded. The Run Log is particularly useful in helping you anticipate and schedule reagent refills or troubleshoot instrument errors.

Heat maps are generated from the analysis of the focal map images and analysis of each ligation cycle. A heat map is a colorized display of a particular metric (*bead count*, *bead signal*, or *image signal*) across all the panels for a run (for definitions of the metrics, see the "Glossary" on page 173).

For each flowcell, the corresponding Cycle Scans window provides nearly real-time feedback on initial data quality on a per-cycle basis.

Create a sequencing (standard) run record

Note: For instructions to set up a multiplex sequencing run record, go to the next section "Create a multiplex sequencing run record" on page 63.

There are two ways to create a sequencing run. The first method is to use the Run wizard (see below). The second method is to import a .txt file that contains the run definition (see "Set up a run by importing a Run Definition file" on page 106). It saves time re-entering information of a repeated run. This file can be generated on an off-instrument computer.

1. Click **Create Runs** in the Setup task pane on the left menu pane of the ICS (see Figure 43 on page 60).

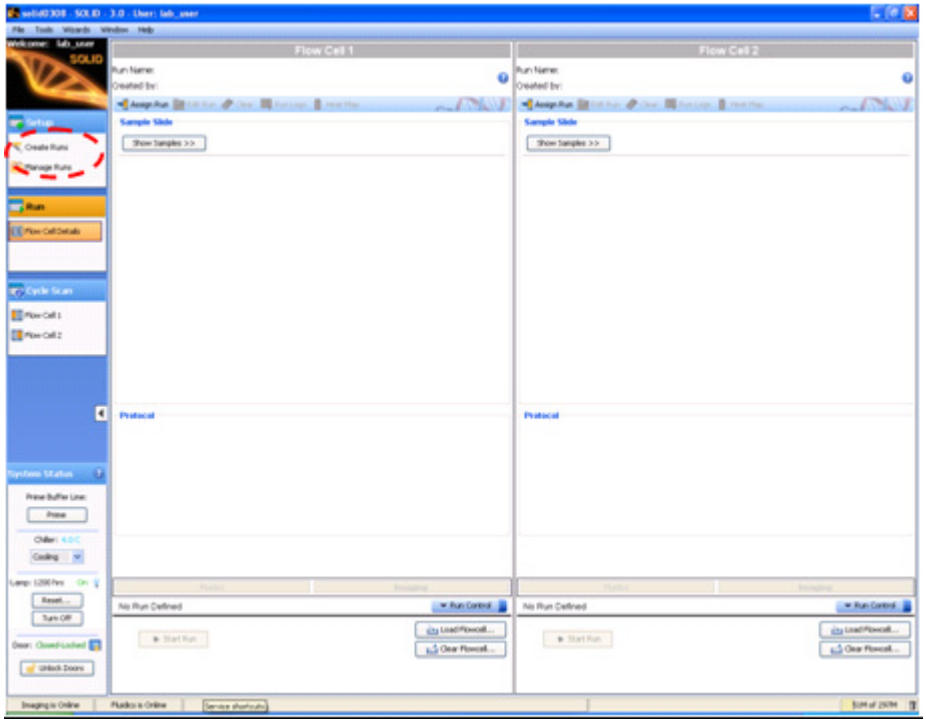


Figure 43 Use the run wizard to create a sequencing run.

2. Complete the information in the Select Run Type and Mask pane (see Figure 44 on page 61):

   a. Select the **Sequencing** option.

   b. (Optional) Type a new run name.

   c. (Optional) Enter a description.

   d. Ensure that the Run Protocol is set to **SOLiD3**.

   e. Select Primer Set 1 and Primer Set 2.

   - If sequencing a fragment library, leave **F3** as Primer Set 1.
   - If sequencing a mate-paired library, select **R3** as Primer Set 1 and **F3** for Primer Set 2.

   > **IMPORTANT!** R3 tag must be run *first* in a mate-paired sequencing run. The reagent strips for the F3 tag do not contain Focal Map reagents.

   f. Enter read lengths for Primer Set 1, and if used, 2. A typical read length is 50 bases.

   g. Select Primary Analysis Settings and Secondary Analysis Settings that are the defaults for the run.

   h. Select the appropriate mask to use.

i. Click **Next**.

Note: If a message window appears during these steps, then continue setting up run as indicated. If a message remains after all selections have been made, correct the entries before clicking **Next**.

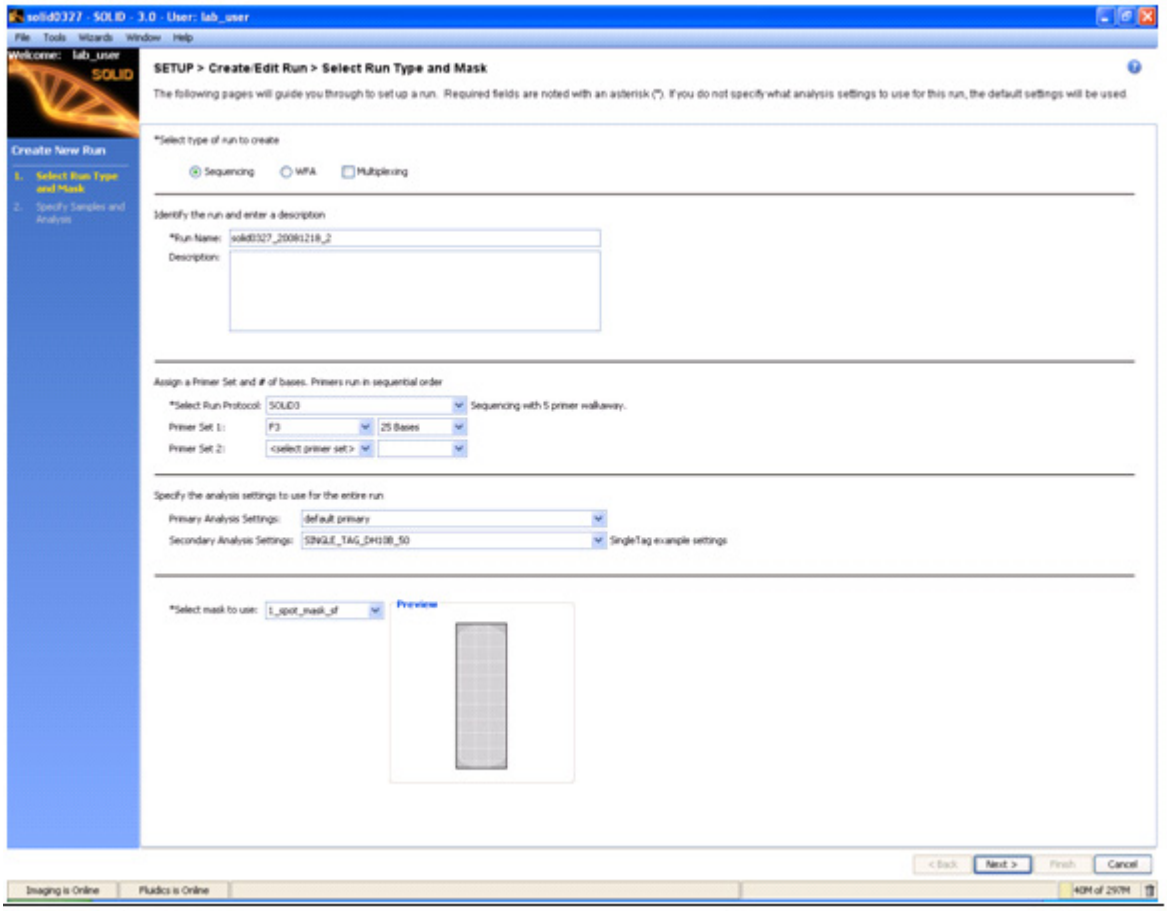


Figure 44 Complete the information in the Select Run Type and Mask pane.

3. Complete the information in the Specify Samples and Analysis pane (see Figure 45 on page 62):

   a. Select the number of samples that will be used in the run. If a single sample will be run in multiple spots of the same slide, it counts as only one sample. Otherwise, the number of samples typically matches the number of spots on the mask.

   b. (Optional) Edit the Sample Name, Library, Analysis settings, and Description.

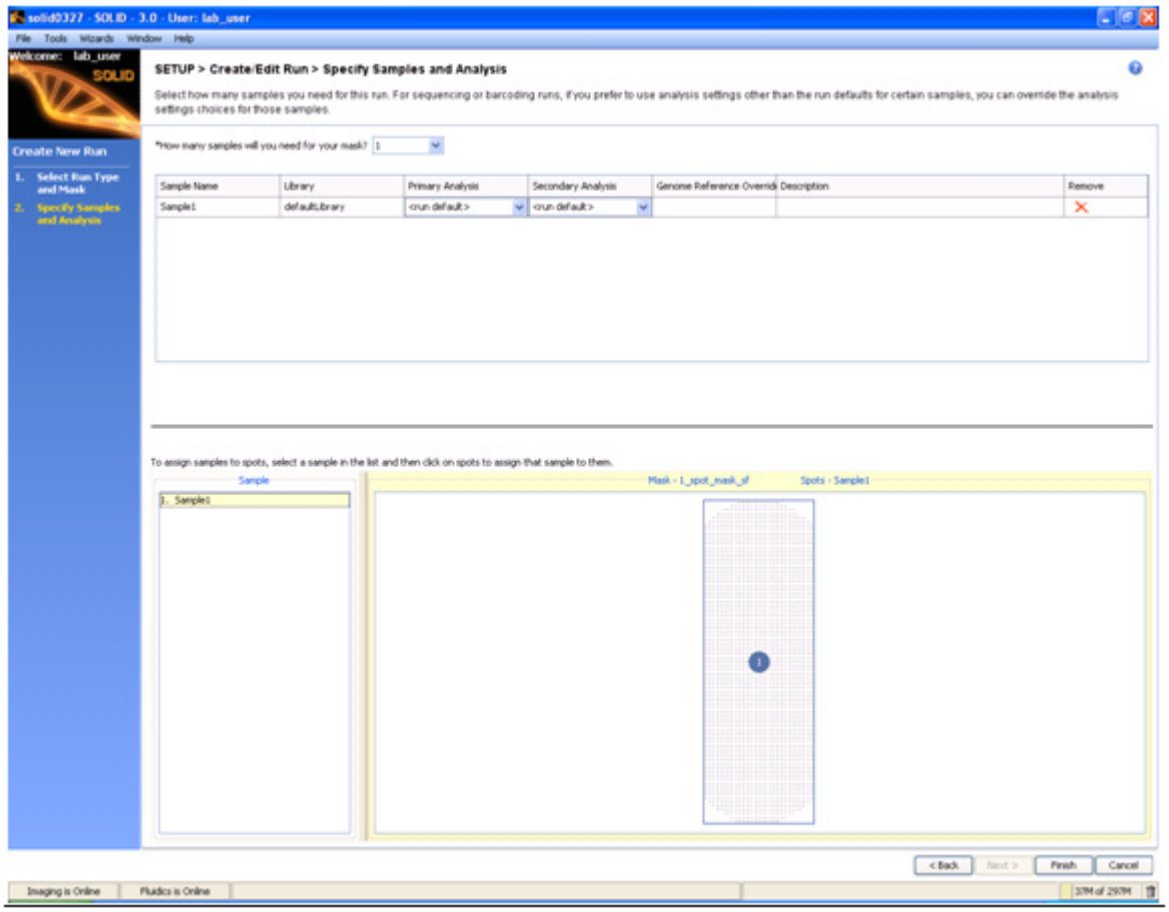


Figure 45 Complete the information in the Specify Samples and Analysis pane.

4. Assign Samples to spots in the mask. A blue or white circle on the mask indicates that the numbered sample has been assigned to a spot. Clicking on a white circle selects that spot, and clicking on a blue circle un-assigns that spot. To assign a sample, select it from the Sample list, then click on a spot with no sample assigned to it.

5. Choose either to assign run to a flowcell for immediate use or to an instrument database to store for later use, then click **OK**.

6. To assign a run previously saved to the database:

   a. Click on **Manage Runs** in the task pane.

   b. Click the run, then select **Assign to Flowcell**.

   c. Choose a flowcell, then click **OK**.

Create a multiplex sequencing run record

Note: For instructions to set up a sequencing (standard) run record, go to the previous section "Create a sequencing (standard) run record" on page 59.

There are two ways to create a multiplex sequencing run. The first method is to use the Run wizard (see below). The second method is to import a Run Definition file created offline (see "Set up a run by importing a Run Definition file" on page 106).

1. Click **Create Runs** in the Setup task pane on the left menu pane of the ICS (see Figure 46).

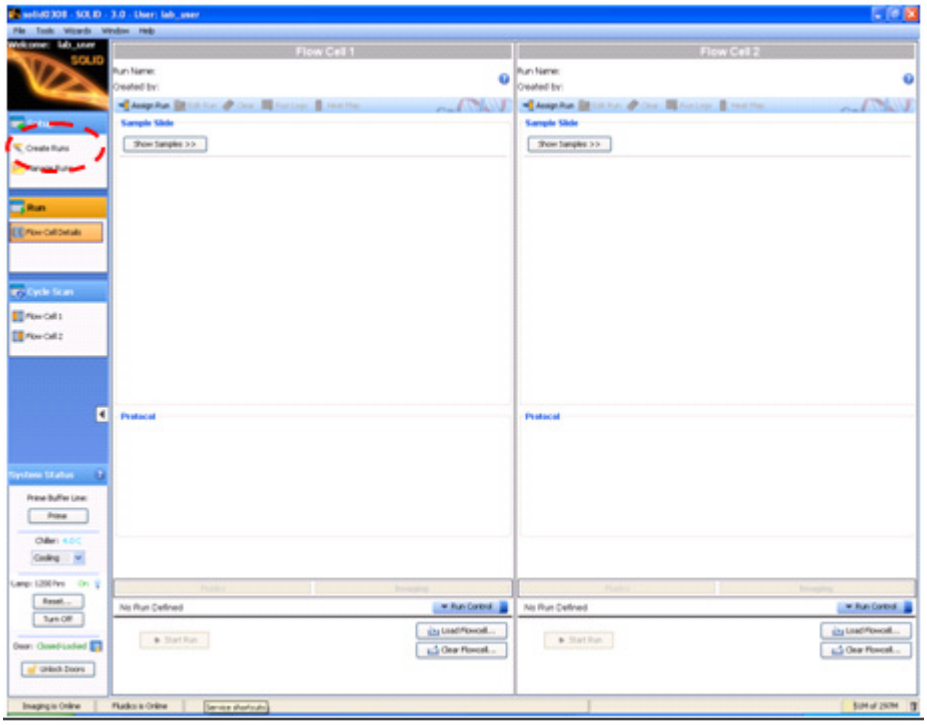


Figure 46 Use the run wizard to create a multiplex sequencing run.

2. Complete the information in the Select Run Type and Mask pane (see Figure 47 on page 64):
   a. Select the **Sequencing** option.
   b. Ensure that the **Multiplexing** option is checked.
   c. (Optional) Type a new run name.
   d. (Optional) Enter a description.
   e. Ensure that the Run Protocol is set to **SOLiD3_Multiplex**.
   f. Select **BC** for Primer Set 1. Enter the read length for the BC Primer set, which is usually 5 bases.

g. Select **F3** for Primer Set 2. Enter the read length for the F3 Primer sets, which is usually 50 bases.

Note: For barcoded fragment libraries of short inserts such as small RNA samples, consult your field applications specialist for the recommended sequencing reagents and read length.

h. Select Primary Analysis Settings and Secondary Analysis Settings that are the defaults for the run.

i. Select the appropriate mask to use.

j. Click **Next**.

Note: If a message window appears during these steps, then continue setting up run as indicated. If a message remains after all selections have been made, correct the entries before clicking **Next**.

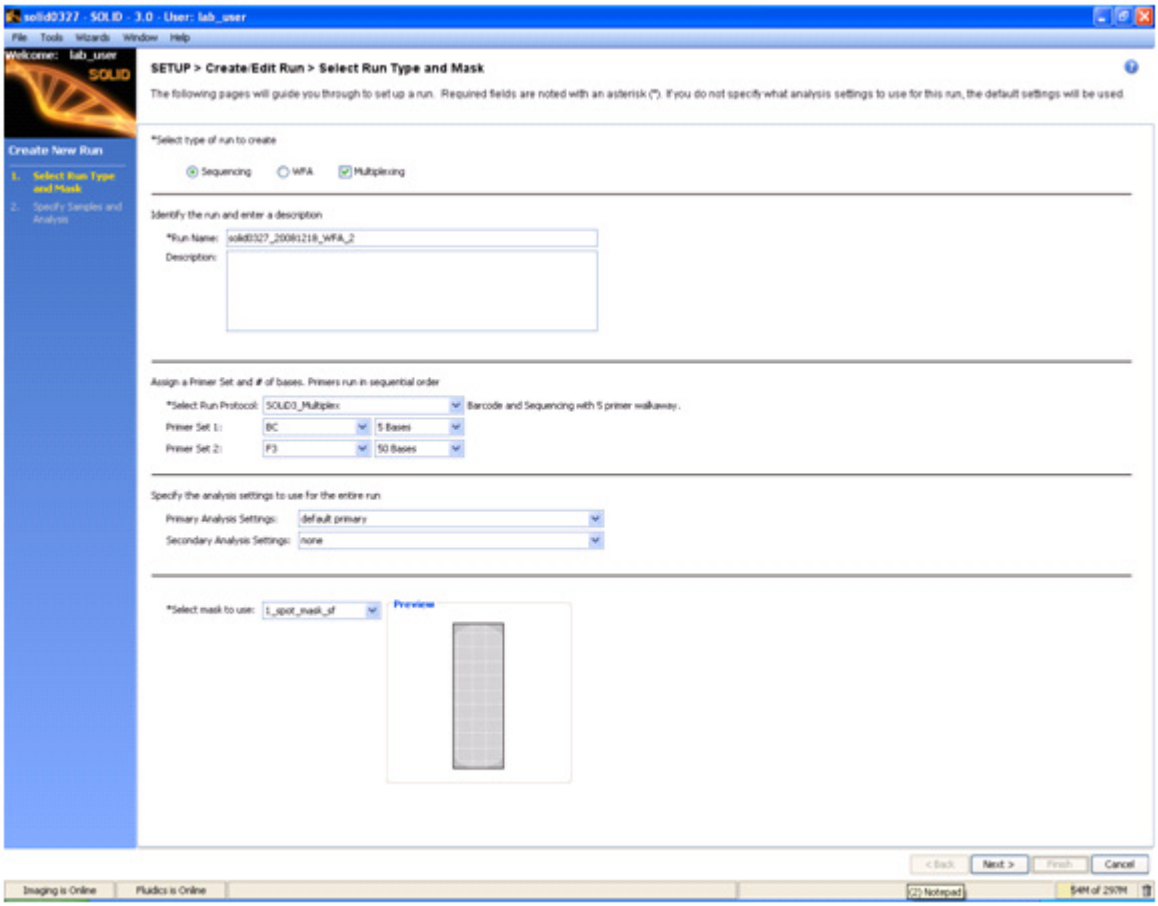


Figure 47 Complete the information in the Select Run Type and Mask pane.

3. Complete the information in the Specify Samples and Analysis pane (see Figure 48 on page 65):

   a. Select the number of samples that will be used in the run. A sample consists of a bead sample that may contain many barcoded libraries. A single sample will run in multiple spots on the same slide counts as only one sample; otherwise, the number of samples typically matches the number of spots on the mask.

   b. Click the first sample name to select it. Change the sample name if desired. After you select the sample, you are able to enter the names and other information about the libraries that are in the sample.

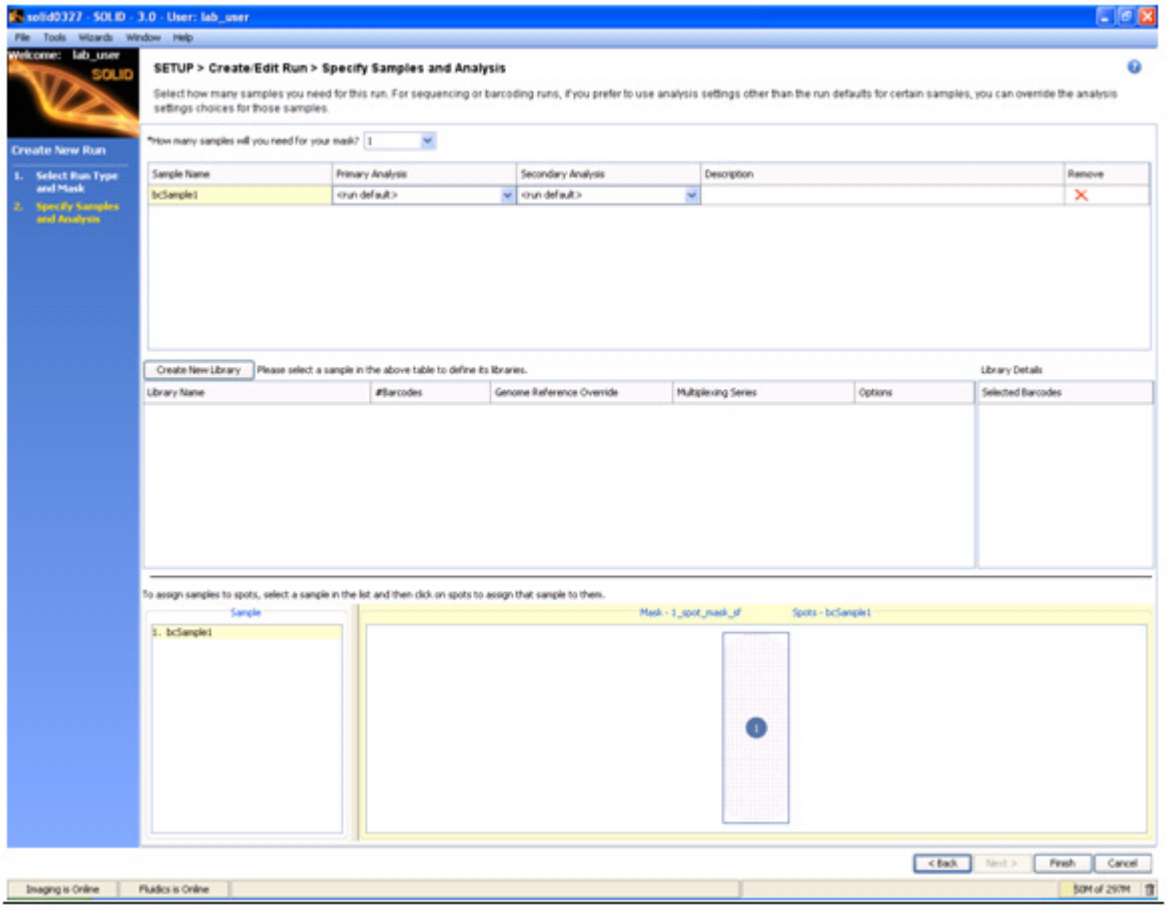


Figure 48 Complete the information in the Specify Samples and Analysis pane.

4. Click **Create New Library** to begin entering library information.

5. Enter information for the first library present in the sample (see Figure 49 on page 66):

   a. Enter the library name.

   b. Select a Multiplexing Series.

   c. Click the selection box for the barcode or barcodes assigned to the library.

d. Click **Save** when information for the first library has been completed.

Note: When entering information on the library, follow these guidelines:

- Only barcodes from a single Multiplexing Series may be used in any individual sample.
- Each barcode may only be assigned to a single library per sample, but a single library may have multiple barcodes.
- The read length of the barcode tag is a property of the Multiplexing Series. Thus Multiplexing Series defined with 5-base barcodes may only be sequenced with 5 base reads of the BC tag (see Table 9 on page 67).

The Libraries panel of the Specify Samples and Analysis screen now shows information on the first library.

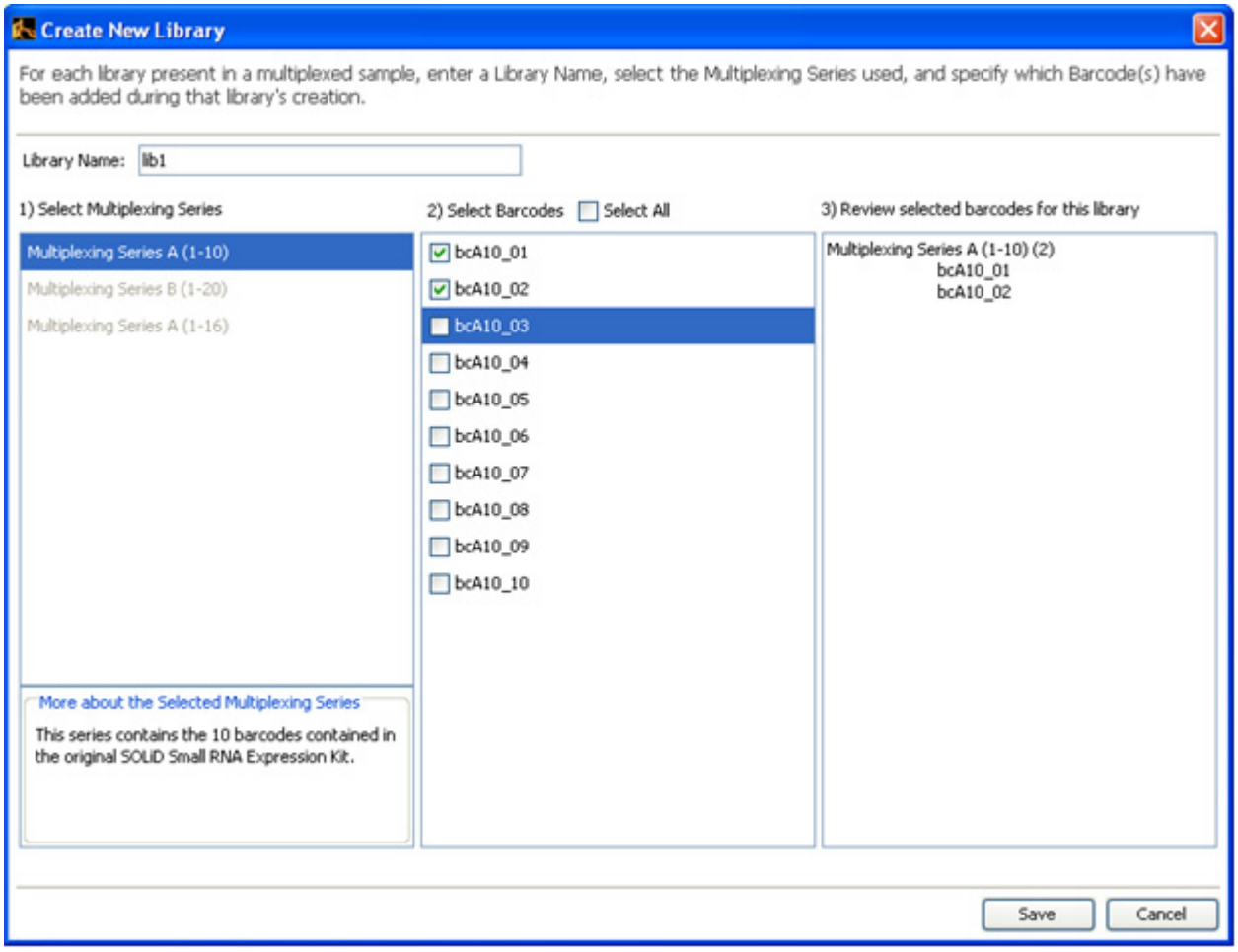


Figure 49 Enter the information for the libraries in the sample.

Table 9 Contents of the Multiplexing Series

Multiplexing Series	Contents
A (1–10)	10 barcodes that are in the SOLiD™ Small RNA Expression Kit
A (1–16)	16 barcodes that are in the SOLiD™ Multiplexing Early Access set
B (1–20)	20 barcodes that are supplied with the SOLiD™ 3 System

6. Repeat steps 4 to 5 for all remaining libraries.The Libraries panel of the Specify Samples and Analysis screen now shows information for all libraries (see Figure 50).

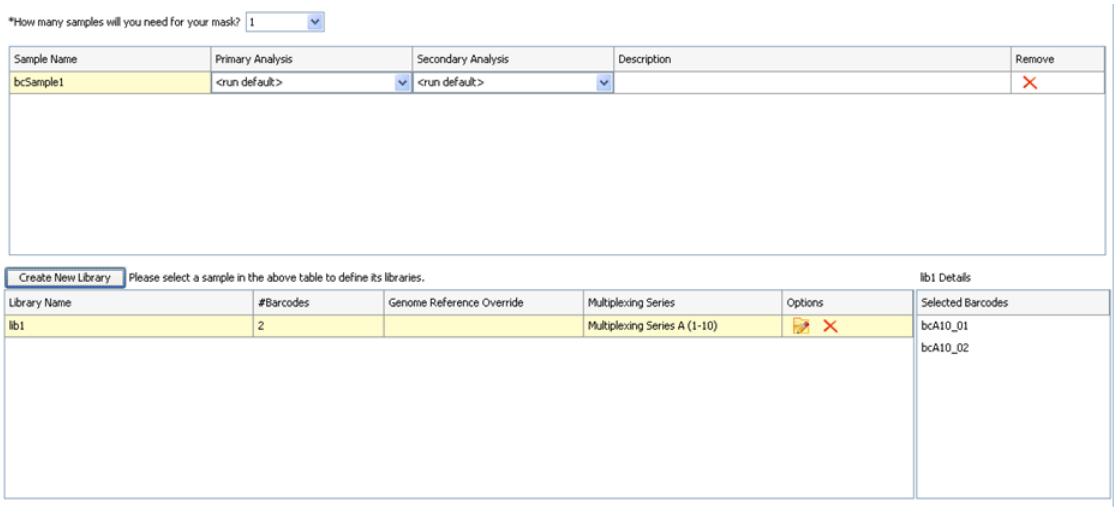


Figure 50 The Libraries panel shows information on all libraries.

7. If any library uses a Genome Reference other than the default, enter the exact name of the Genome Reference fasta file, including the .fasta extension.

8. Assign samples to spots in the mask. A blue or white circle on the mask indicates that the numbered sample has been assigned to a spot. Clicking on a white circle selects that spot, and clicking on a blue circle un-assigns that spot. To assign a sample, select it from the Sample list, then click on a spot with no sample assigned to it.

9. Click **Finish**.

10. Choose to assign the run to a flowcell for immediate use or to an instrument database to store for later use, then click **OK**.

11. To assign a run previously saved to the database:

    a. Click on **Manage Runs** in the task pane.

    b. Click the run, then select **Assign to Flowcell**.

    c. Choose a flowcell, then click **OK**.

Detect the focus range

Note: If automatic focus range detection fails, determine the focus range manually (see "Manually find the focus range" on page 107).

1. Close the front doors of the SOLiD™ 3 Analyzer. Open the Imager window by choosing **Windows** ▸ **Imaging System**.
2. Select **Tools** ▸ **Detect Focusing Range**.
3. In the focusing range dialog, specify the stage template file by entering the name directly or by clicking the **Browse** button to navigate to a suitable one (see Figure 51). The stage template must match the slide in the target flowcell(s). If you created a run from the ICS, then select the file **imagingMap.STG** from the subdirectory in C:\Runs whose name matches the name of the run (for example, select C:\Runs\Solid0327_20081209_2_Oct_Test\imagingMap.STG).

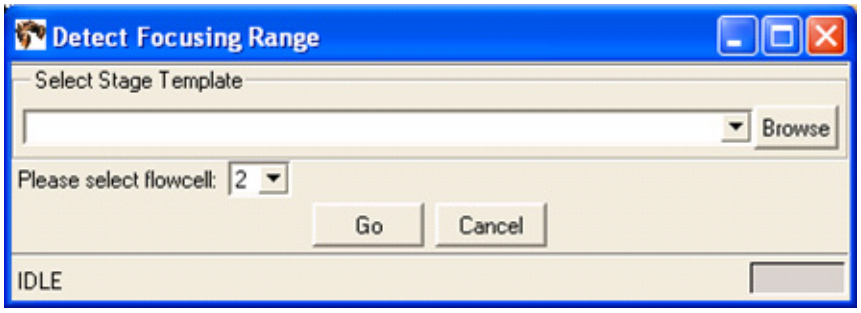


Figure 51 Detect Focusing Range dialog.

4. Select the flowcell using the drop-down menu, then press **Go**. The Imager works for several minutes while it determines the range. The blue progress bar indicates how close it is to completion (see Figure 52). You can also click **Cancel** so that the Imager aborts the ranging operation.

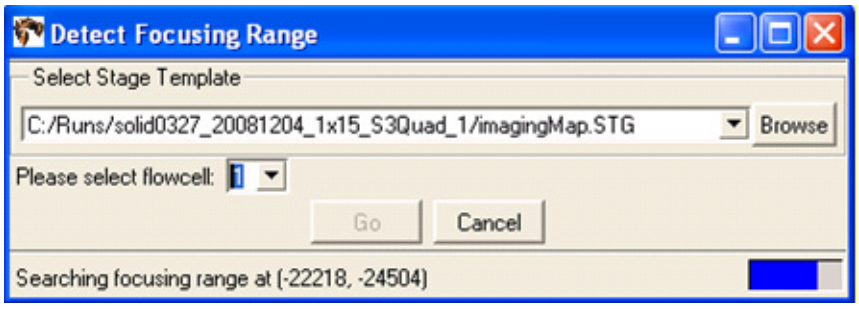


Figure 52 Detect Focusing Range Dialog while detection in progress.

5. When the Imager is done, a dialog appears (see Figure 53 on page 69). Click **Yes** if you want to replace the values in the local settings file. Click **No** if you want the Imager to discard the newly calculated focus range.

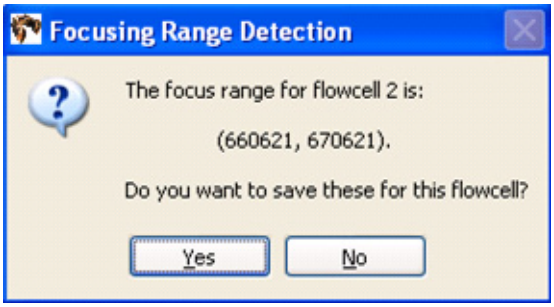


Figure 53 Confirm that you want to replace the local settings file.

6. Verify the validity of the newly calculated focus range by taking images at random locations.

   IMPORTANT! You should see images of beads, ensuring that the algorithm was able to focus on the beads and not on other artifacts (see Figure 54). If you do not see bead images or if you see out-of-focus bead images, set the focus range manually (see "Manually find the focus range" on page 107).

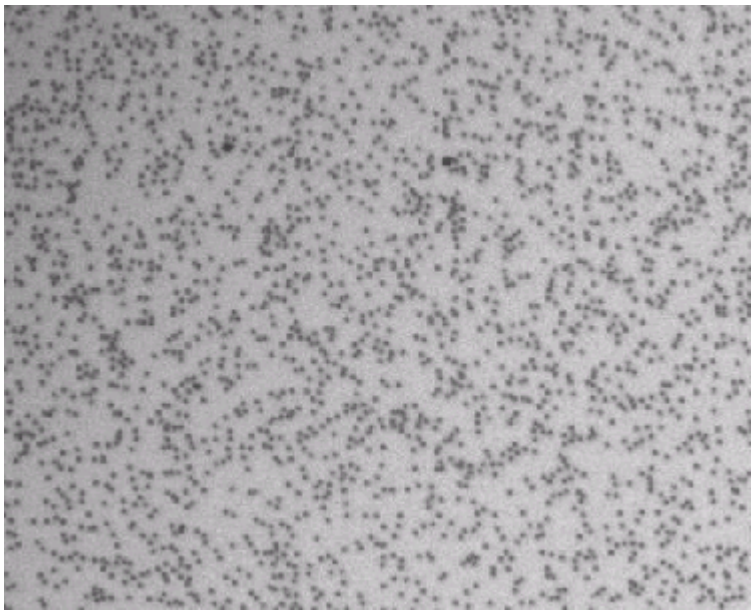


Figure 54 Beads in focus.

Start the sequencing run

1. Ensure that there is adequate disk space for images and results of the sequence run (for the minimum needed disk space, see Table 10). To know the amount of disk space that is available, click **Manage Runs** on the task pane.

Table 10 Minimum required disk space to store images and results

Run Type	Minimum space needed for images	Minimum space needed for results
Fragment (50 bp)	1.9 TB	700 GB
Mate-paired (2 × 25 bp)	1.9 TB	700 GB
Mate-paired (2 × 50 bp)	3.8 TB	1.4 TB
Barcoded fragment (50, 5	2.1 TB	770 GB

2. Click **Start Run**.

3. If there is not enough room to store the data for the run then the Start Run dialog box displays (see Figure 55). Choose the appropriate option (see Table 11).

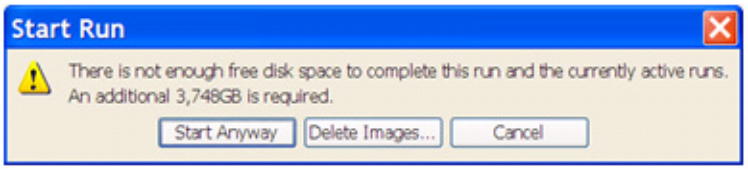


Figure 55 This Start Run dialog appears if there is not enough room to store data for the run.

Table 11 Choose one of the three options to manage disk space.

Option	Description
Start Anyway	Initiates the run. The instrument pauses itself when it runs out of free disk space.
Delete Images‡	Launches Historical Runs page in SETS. Images and/or results can be deleted through SETS
Cancel	Aborts the run.

‡ For more information on creating more available disk space, see the *Applied Biosystems SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302).

IMPORTANT! Before deleting any images, ensure that data analysis from the previous run is satisfactory and complete. For more information, refer to the *Applied Biosystems SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302).

4. After the run has been initiated, you can click the **Run Log** and **Heat Map** buttons located at the top of the appropriate flowcell panel to learn more information about the current run Figure 56. You can also use select the appropriate flowcell in the Cycle Scans menu on the task bar on the left

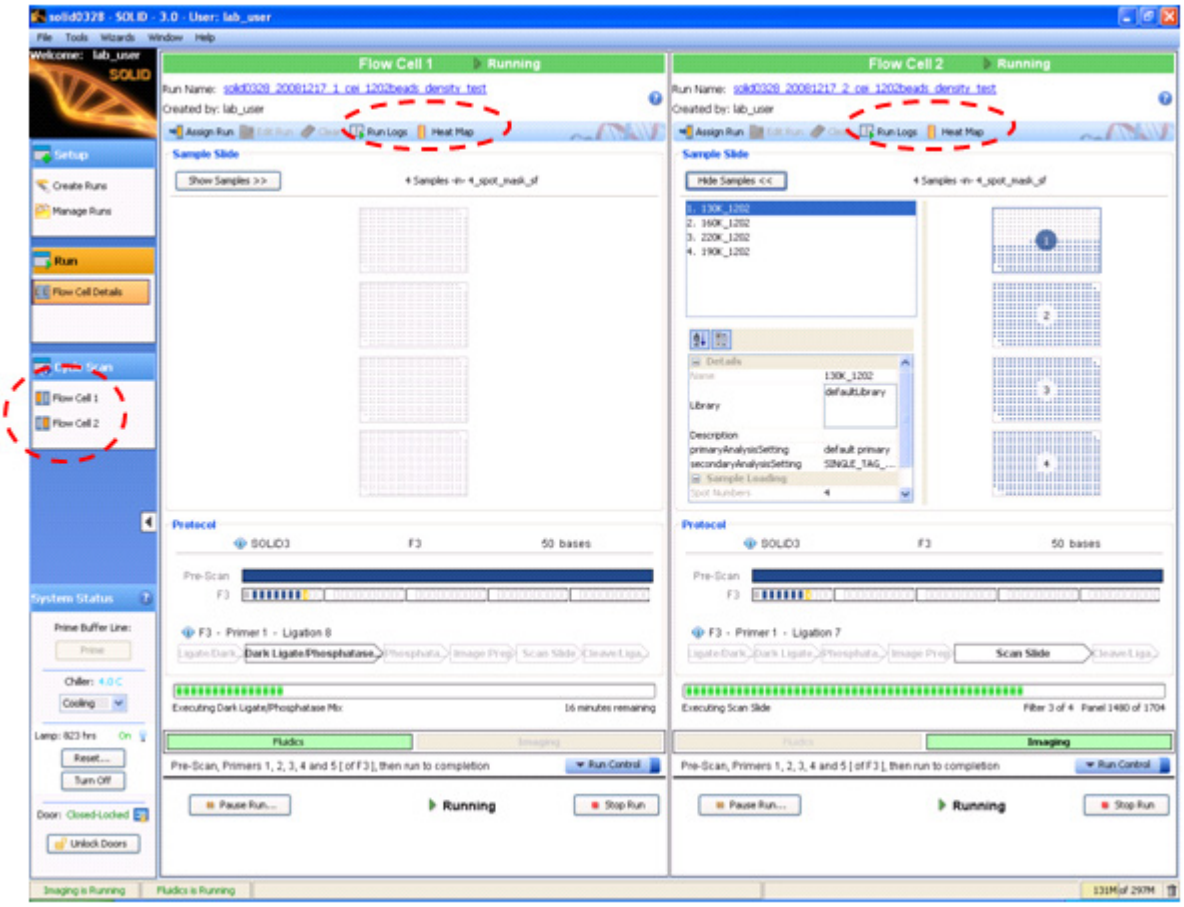


Figure 56 How to learn more about the current run.

IMPORTANT! Do not disturb the SOLiD™ 3 Analyzer while in operation and do not open the flowcell during a pause in the run. Significant perturbations (for example, opening system parts, vibrations) during the run is detrimental to the results.

Control the run

Pause/Resume Run

Five modules contain a pre-mixing step for the next module:

- Ligate/Dark Ligate Mix
- Dark Ligate/Phosphatase Mix
- Cleave/Ligate Mix
- Prime/Bridge Ligate Mix
- Bridge Ligate/Phosphatase Mix

When you try to pause a run in the middle of the module, a dialog displays (see Figure 57).

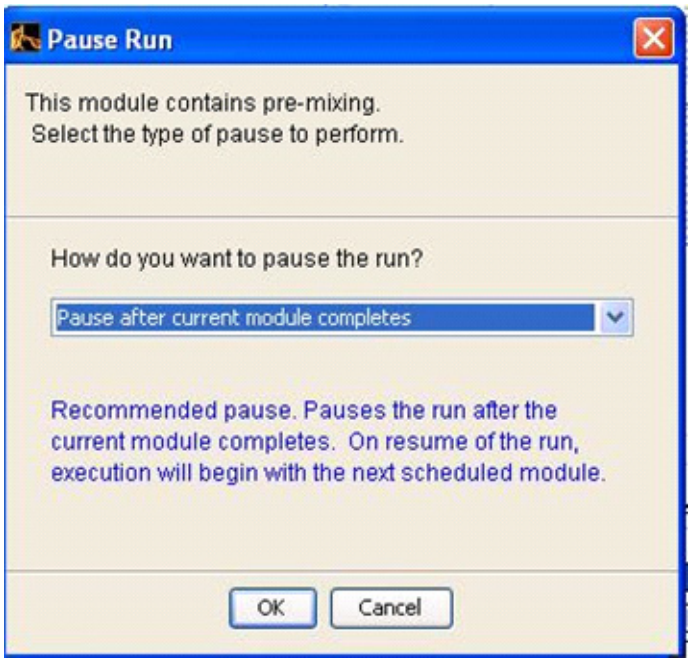


Figure 57 Pause Run dialog for pre-mixing module.

The default setting is to pause after the current module completes so that pre-mixing is not interrupted. If you want to pause the run within a pre-mixing module, select **Pause anyway** (see Figure 58 on page 73).

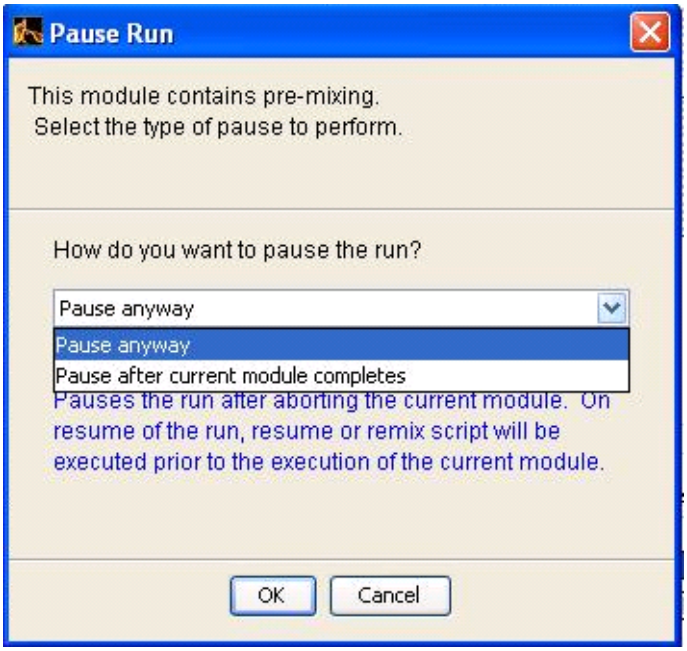


Figure 58 Pause Run dialog options for pre-mixing module.

When you resume a run to use the pre-mixed reagents in the strip tube, a dialog box displays (see Figure 59). While it is recommended to simply resume the run, the system provides an option to remix the reagents by clicking **Remix/Resume**. In this case, the previous pre-mixed reagent is delivered to the waste and a new reagent is mixed.

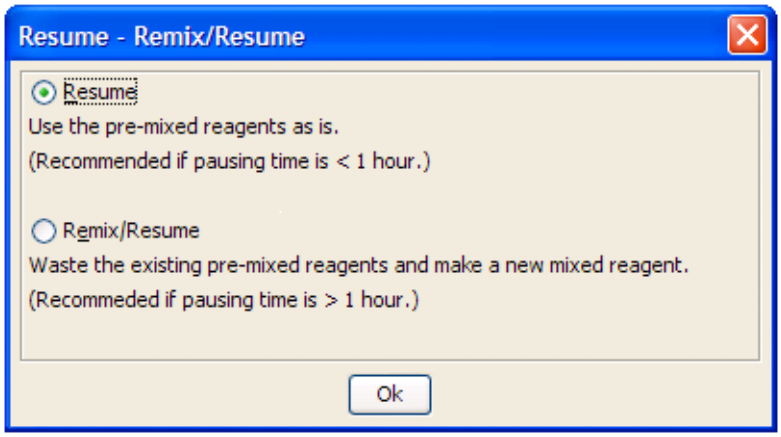


Figure 59 The options to resume or remix/resume the run are available.

Note: One reagent strip contains reagents enough for 10 cycles per primer. Therefore, if the remix option is used, the cycle number which can be used with the reagent strip is reduced.

Use the Run Control menu

1. Click **Run Control** to display the Run Control menu (see Figure 60).

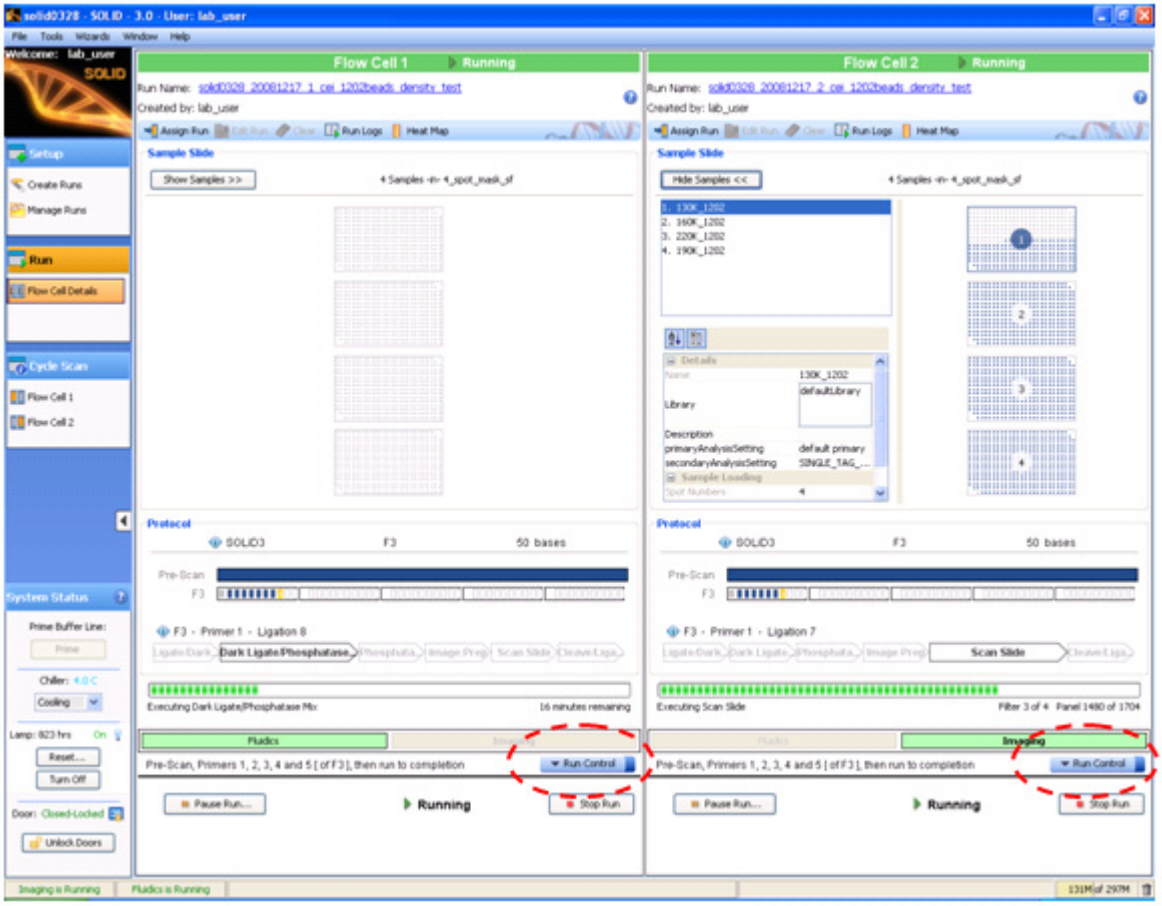


Figure 60 Click **Run Control** to display the Run Control menu for that flowcell.

Run Control commands (see Table 12)

IMPORTANT! Do not disturb the SOLiD™ 3 Analyzer while in operation and do not open the flowcell during a pause in the run. Significant perturbations (for example, opening system parts, vibrations) during the run is detrimental to the results.

Table 12 Run Control commands

Command‡	Command is available while the instrument is…
Stop Run	Running or paused
Reset Current Primer	Paused within Primer Cycle
Change Primer Schedule	Paused
Set Early Pause Point	Running or paused
Change Run Progress Point	Paused

‡ Certain commands have sub-menus that allow you to control every step in a run protocol.

Stop Run

The Stop Run command launches a Stop Run dialog box. Choose the appropriate option in the dialog according to Table 13.

Table 13 Options available in the Stop Run dialog

Option	Description
Abort Run	Run aborts and analysis jobs are cancelled.
Set run as completed	Run is set as completed. Secondary analysis jobs starts.

Change Primer Schedule

The Change Primer Schedule command allows you to choose a first and second primer, in any order, from primers A through E of any of the three primer sets (F3/R3/BC). Depending on how you set up the run, only primers from a selected primer set are available in the sub-menu.

Set Early Pause Point

The Set Early Pause Point command allows you to define when the instrument pauses. This command can be used to replenish reagents at a more convenient time than that the time defined by the software. Note that if the Change Run Progress Point command (see below) is selected, any changes to the Early Pause Point resets to the default.

Depending on the progress of the run, various sub-menu commands are available under the Set Early Pause Point (see Figure 61 on page 76).

- Prescan
  - Reset
  - P2 Label
  - Image Prep
  - Focus Map
- Sequence Primer A and B
  - Priming
    - Reset
    - Prime/Ligate Mix
  - Ligation
    - Ligate/Dark Ligate Mix
    - Dark Ligate/Phosphatase Mix
    - Phosphatase
    - Image Prep
    - Scan Slide
    - Cleave/Ligate Mix
- Sequence Primer C, D, and E
  - Priming
    - Reset
    - Prime/Bridge Ligate Mix
    - Bridge Ligate/Phosphatase Mix
    - Phosphatase
    - Cleave/Ligate Mix
  - Ligation
    - Ligate/Dark Ligate Mix
    - Dark Ligate/Phosphatase Mix
    - Phosphatase
    - Image Prep
    - Scan Slide
    - Cleave/Ligate Mix
- Slide Store
  - Store

Figure 61 Set Early Pause Point sub-menu commands.

Change Run Progress Point

The Change Run Progress Point command allows you to back up or skip to any specific point in the run. Ensure that you select points that are consistent with the progress of the run. The choices available under the Change Run Progress Point command include those described above for the Set Early Pause Point command.

Repeating primers can be performed by using the Change Run Progress Point command, selecting a primer to repeat, resuming the run, and using the Set Early Pause Point command to pause the run after the repeated primer has completed.

Use imaging and analysis controls for specific samples

The Instrument Control Software (ICS) allows you to control imaging for individual samples on a slide. With the controls, you can deposit fragment and mate-paired library samples onto the same slide and turn off imaging of a sample. To access the imaging and analysis controls, in the Sample Slide display of the ICS, right-mouse click a sample name to display options menu. These options are not available until the focal map (prescan) is complete.

The options menu includes:

- Enabled

- Imaging Turned Off
- Spot discarded

Enabled

Enabled is the default setting and allows for both imaging and analysis of the spot.

Imaging Turned Off

Imaging Turned Off turns off imaging but allows analysis to complete.

Spot Discarded

Spot Discarded turns off both imaging and analysis. Spot Discarded can be used to remove problematic samples from the software workflow. Use of Spot Discarded updates the Sample Slide display according to the selection in Spot Discarded. Use of Spot Discarded also affects subsequent cycles (see Figure 62).

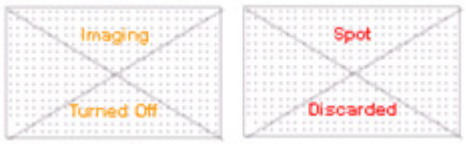


Figure 62 The Sample Slide display updates after using Imaging Turned Off or Spot Discarded.

Monitor the sequencing run

Note: To monitor the run remotely, use SETS from any computer to connect to the networked instrument [refer to the *Applied Biosystems SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302)]. If desired, set up e-mail notification regarding instrument run and system information using SETS (refer to the *SOLiD™ 3 System SETS Software Getting Started Guide*).

View the run log

1. Click **Run Log** located at the top right corner of the flowcell panel to open a dialog box describing a series of instrument events.
2. After you finish viewing the Run Log, click **Close** located at the bottom of the Run Log window.

View heat maps

1. To view the heat map showing bead densities found in the focal map images, click **Heat Map** located at the top right corner of the flowcell panel (see Figure 63 on page 79).
2. Look for:
   - Uniform deposition of beads on the slide.
   - The actual average bead deposition density/panel value being similar in value to the targeted average bead deposition density (for example, 130,000 beads/panel). A large number of missing panels could indicate a deposition problem.

   Note: The heat map may not immediately be available after the completion of the Prescan. The software must process all the images collected during the Prescan before the heat map is available. This process may take up to 30 minutes depending on the number of panels imaged.
3. After you finish viewing the Heat Map, click **Close** located at the bottom of the Heat Map window.

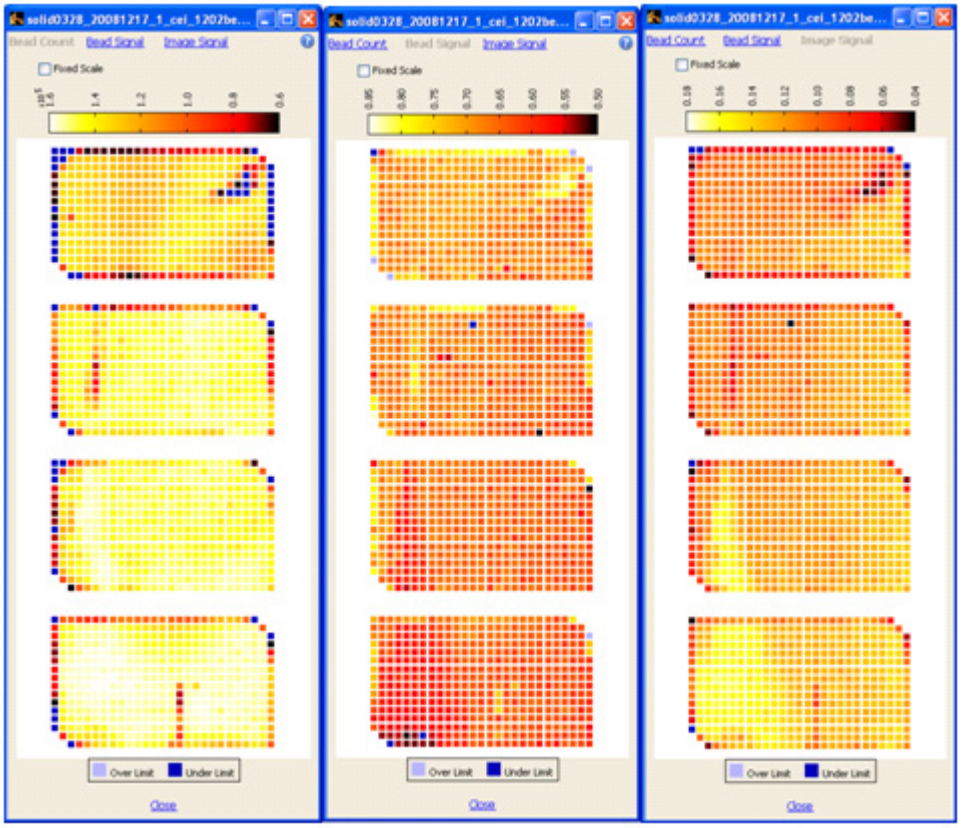


Figure 63 Bead Count (left), Bead Signal (center), and Image Signal (right) heat maps.

View cycle scans

1. Select the appropriate flowcell (**Flow Cell 1** or **Flow Cell 2**) in the Cycle Scan menu on the task bar on the left.

   The top section of the Cycle Scans window lists all the scans per ligation cycle for the slide, and the bottom section shows scan information sorted by sample for the scan selected in the top section. Use the parameters shown in Figure 64 on page 80 to assess the progress of the sequencing run (see Table 14 on page 81).

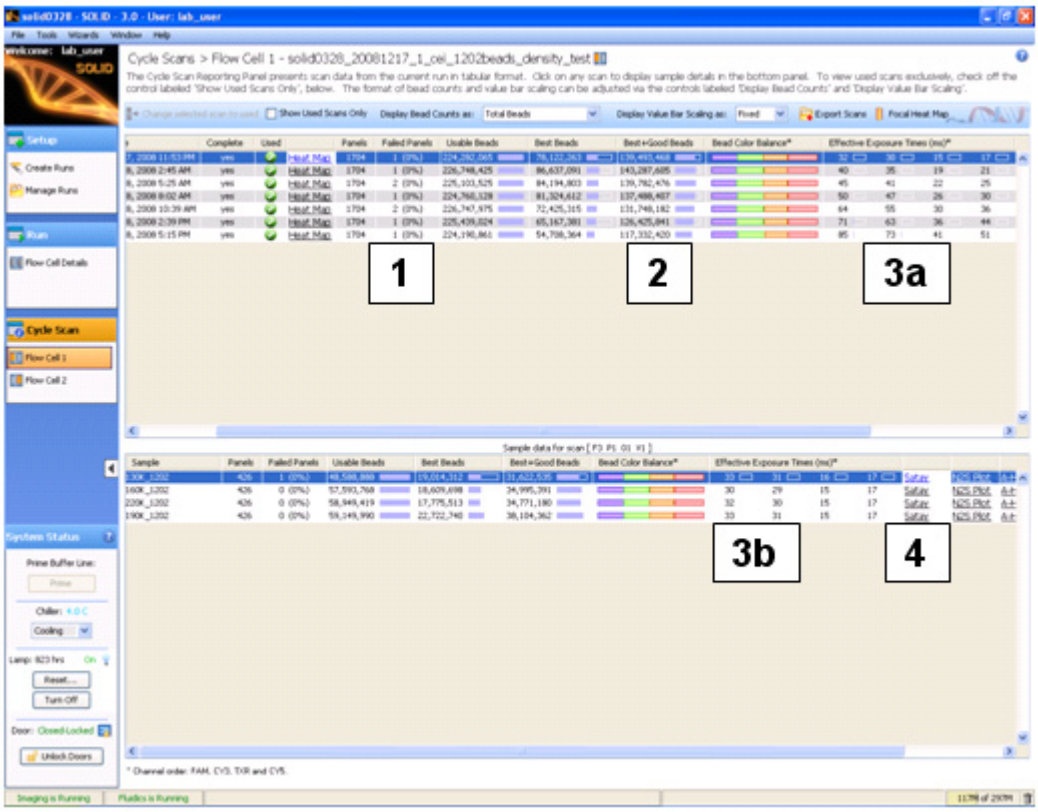


Figure 64 Parameters available in the Cycle Scans window to assess the progress of the sequencing run.

Table 14 Use the Cycle Scans window to distinguish normal runs from problematic runs.

Parameter	Normal run	Problematic run
(1) Failed panels (number of panels that failed image alignment during color-calling).	Gradual decline from ligation cycle 1 to ligation cycle 5 or higher for each sequencing primer. In general, the number of failed panels should be relatively small and consistent.	Run begins with extremely high number of failed panels or dramatic increase in any subsequent ligation cycle for each sequencing primer.
(2) The fraction of (Best + good beads)/usable beads	The fraction can vary depending on the quality of the library, the efficiency of the PCR and the enrichment process. As a guideline, the fraction is around 0.5 to 0.6 in the first ligation cycle of each primer and drops to 0.2 to 0.3 in the last cycle.	A significant drop in the fraction of good beads in the initial ligation cycles would indicate a reason to pause the run and to troubleshoot the performance.
(3a/3b) Effective exposure times‡	Gradual increase from ligation cycles 1 to 5 or higher for each sequence primer. Performance varies from slide to slide and as a function of the age of the SOLiD™ Light Source.§ As a guideline, the effective exposure time is typically 50 to 200 ms in the first ligation cycle and increases to 500 to 1000 ms in the fifth cycle.	One second or greater in ligation cycle 1 or when instrument times out when the effective exposure time exceeds 2 s. Long exposure times may indicate replacement of the SOLiD™ Light Source.§
(4) Satay plots	The first cycle of any primer should show a relatively “clean” Satay plot, with most points clustered on the four color axes and with minimal fraction of the points clustered around the origin. The quality of the Satay plot typically degrades gradually with each ligation cycle for a single primer cycle, becoming more “fuzzy” in the last cycle.	An abnormal “fuzzy” Satay plot in the first cycle is a reason to pause the run and troubleshoot the performance.

‡ Exposure time is indicative of the signal intensity of the beads. The instrument uses an auto-exposure routine, on a per sample basis, to maximize bead signal with minimal image saturation. Shorter exposure times are associated with efficient ligation of the fluorescent probes.

§ You should replace the SOLiD™ Light Source in the Applied Biosystems SOLiD™ 3 Analyzer every 1500 hours of use. Refer to Appendix B for instructions.

2. Double-click any cycle to view the heat map for that cycle (see Figure 65 on page 82).

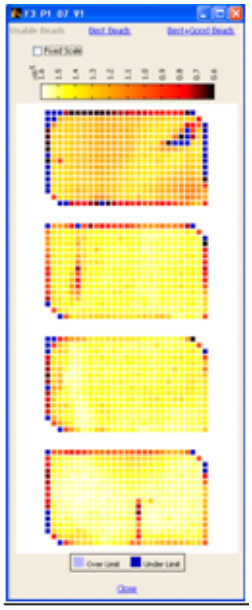


Figure 65 Double-click any cycle to view the heat map for that cycle.

3. Left-click any square panel on the heat map to open the panel browser window. The panel browser allows you to view the focal map and the image for each fluorescent dye signal (see Figure 66 on page 83).

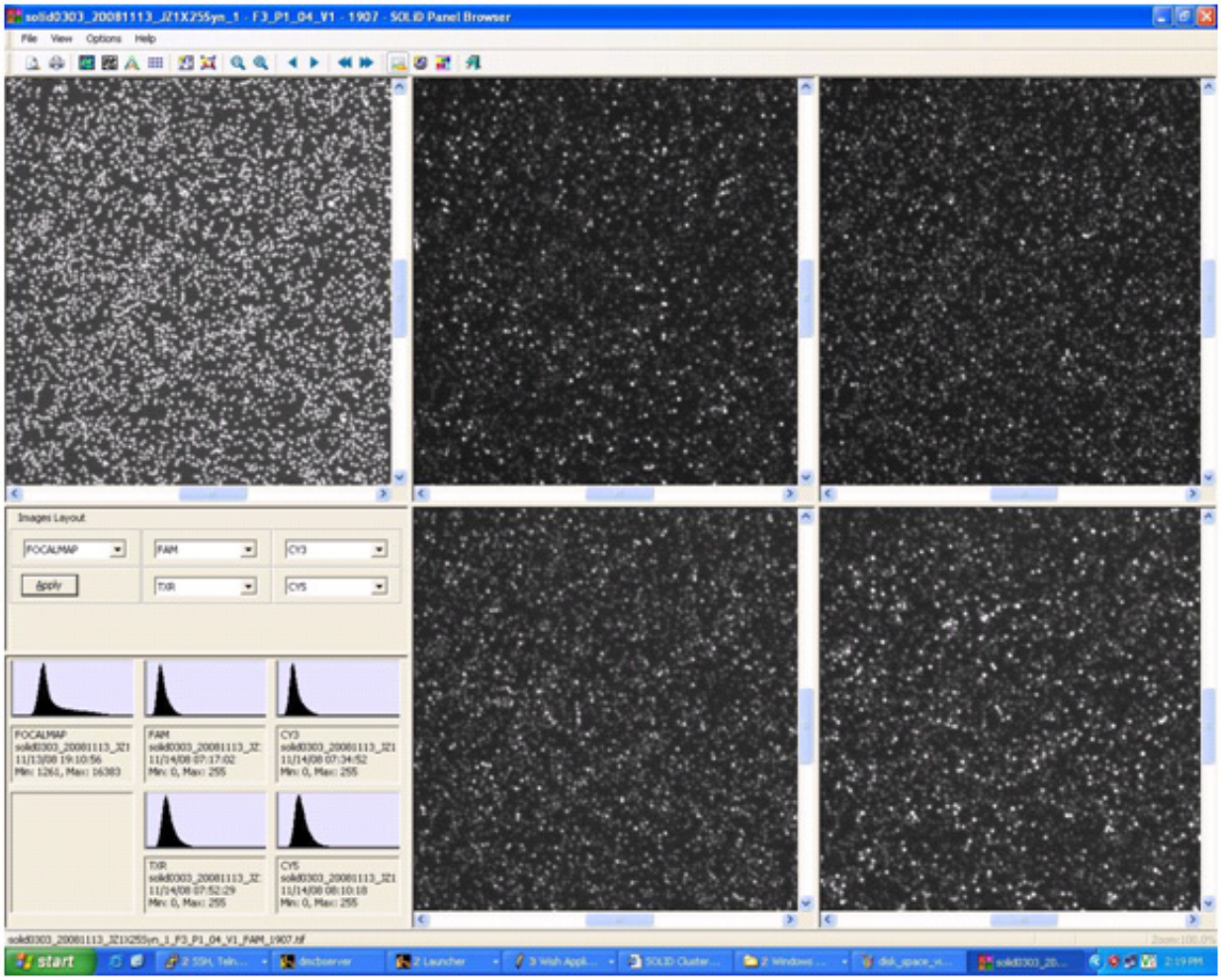


Figure 66 View the focal map and the image for each fluorescent dye signal.

4. After you finish viewing the Cycle Scans, close the Cycle Scans window.

Note: If the sequencing run appear problematic, you can: (1) Allow the run to continue and troubleshoot after the run or (2) pause the run and troubleshoot. Consult an Applied Biosystems SOLiD™ Field Applications Specialist.

A Required Materials

This appendix covers:

Set up and perform a workflow analysis (WFA) run

Required Applied Biosystems reagent kits

Item (part number)	Components	Kit component(s) used in…
SOLiD™ Workflow Analysis Reagents (4406463)	SOLiD™ Workflow Analysis Reagents	WFA
SOLiD™ 3 Instrument Buffer Kit (4406479)	Reset Buffer Glycerol 10× Instrument Buffer Cleave Solution 1 Cleave 2 Kit (Cleave 2 Parts 1 and 2) Storage Buffer Imaging Buffer Kit (Imaging Buffer Parts 1 and 2) 1× T4 Ligase Buffer Kit (1× Ligase Buffer Parts 1 and 2) 1× Phosphatase Buffer	Sequencing and/or WFA
SOLiD™ Slide Pack Kit (4412172)	Slide Storage Buffer Sequencing Slides	Bead deposition
SOLiD™ Bead Deposition Kit (4387895)	Deposition Buffer Overlay Buffer	Bead deposition
	10× Terminal Transferase Buffer 10× Cobalt chloride Terminal Transferase Bead Linker	Templated bead preparation

Required equipment

Item‡	Source
SOLiD™ 3 System	• Applied Biosystems 4406205 (110 V) • Applied Biosystems 4406208 (220 V)
Upgrade Kit from SOLiD™ 2.0 to SOLiD™ 3	Applied Biosystems 4409522
SOLiD™ 3 Analyzer	Applied Biosystems 4406207
SOLiD™ Light Source	Applied Biosystems 4388441
SOLiD™ Slide Storage Chamber	Applied Biosystems 4406354

Item‡	Source
SOLiD™ Deposition Chambers 1-Well§	Applied Biosystems 4406352
SOLiD™ Deposition Chambers 4-Wells§	Applied Biosystems 4406358
SOLiD™ Deposition Chambers 8-Wells§	Applied Biosystems 4406359
SOLiD™ Slide Carriers§	Applied Biosystems 4406360
SOLiD™ Uninterruptible Power Supply (UPS)	• Applied Biosystems 4397781 (SOLiD™ UPS North America) • Applied Biosystems 4393695 (220 V; SOLiD™ UPS International)
SOLiD™ Offline Cluster	• Applied Biosystems 4413002 (North America) • Applied Biosystems 4425588 (International)
SOLiD™ Accessory Disk Drive	Applied Biosystems 4426101
SOLiD™ Bead Concentration Chart	Applied Biosystems 4415131
Covaris™ S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris™ S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube For system materials summary, see "Covaris™ S2 System Materials Summary," *SOLiD™ 3 System Site Preparation Guide*.	• Applied Biosystems 4387833 (110 V) • Applied Biosystems 4392718 (220 V) or Covaris™ Inc.
6-Tube Magnetic Stand	Applied Biosystems AM10055
Microcentrifuge 5417R, refrigerated, without rotor	• Eppendorf# 022621807 (120 V/60 Hz) • Eppendorf# 022621840 (230 V/50 Hz)

Item‡	Source
FA-45-24-11, fixed-angle rotor, 24 × 1.5/2 mL, including aluminum lid, aerosol-tight	Eppendorf# 022636006
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Scienceware Dry-Keeper AutoDesiccator Cabinet, Tall#	VWR 24983-455
Tabletop Centrifuge (for 96-well plate)	MLS‡‡
Vortexer	MLS
Picofuge	MLS
Magnetic stirrer	MLS
Incubator (37 °C)	MLS
Refrigerator (4 °C)	MLS
Freezer (– 20 °C)	MLS
Pipettors, 20 µL	MLS
Pipettors, 200 µL	MLS
Pipettors, 1000 µL	MLS

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.
§ The SOLiD™ 3 Analyzer is shipped with 2 of each size Deposition Chambers and Slide Carriers. Two Slide Storage Chambers are provided for use with all chambers.
# Or equivalent but validation of the equipment for library preparation is required.
‡‡ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Required consumables

Item‡	Source
SOLiD™ Flowcell O-rings, 10-pack	Applied Biosystems 4398217
Nuclease-free water	Applied Biosystems AM9932
ABgene® 96 1.2-mL square-well storage plates	ABgene AB-1127
3-mm adhesive disks	Grace Bio-Labs ST200
Ethylene glycol	American Bioanalytical AB00455-01000
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit§	Thermo Scientific PR-1

Item‡	Source
1.5-mL LoBind Tubes	Eppendorf 022431021
Kimwipes®	MLS#
Filtered pipettor tips	MLS

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.

§ The NanoDrop® Conditioning Kit is useful for "reconditioning" the sample measurement pedestals to a hydrophobic state if they become "unconditioned" (see NanoDrop user's manual for more information). The PR-1 kit consists of a container of specially formulated polishing compound and a supply of convenient applicators.

For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Set up and perform a sequencing run

Required Applied Biosystems reagent kits

Item (part number)	Components	Kit component(s) used in…
SOLiD™ Fragment Library Sequencing Kit – Master Mix 50 (4406370)	SOLiD™ Frag Lib Seq – Primer A Reagents SOLiD™ Frag Lib Seq – Primer B Reagents SOLiD™ Frag Lib Seq – Primer C Reagents SOLiD™ Frag Lib Seq – Primer D Reagents SOLiD™ Frag Lib Seq – Primer E Reagents SOLiD™ Mixing Strip Tube	Fragment sequencing
SOLiD™ Mate-Paired Library Sequencing Kit (4406398)	SOLiD™ MP Lib Seq – Tag 1 Primer A Reagents SOLiD™ MP Lib Seq – Tag 1 Primer B Reagents SOLiD™ MP Lib Seq – Tag 1 Primer C Reagents SOLiD™ MP Lib Seq – Tag 1 Primer D Reagents SOLiD™ MP Lib Seq – Tag 1 Primer E Reagents SOLiD™ MP Lib Seq – Tag 2 Primer A Reagents SOLiD™ MP Lib Seq – Tag 2 Primer B Reagents SOLiD™ MP Lib Seq – Tag 2 Primer C Reagents SOLiD™ MP Lib Seq – Tag 2 Primer D Reagents SOLiD™ MP Lib Seq – Tag 2 Primer E Reagents SOLiD™ Mixing Strip Tubes	Mate-pair sequencing
SOLiD™ Fragment Library Sequencing Kit – Barcode Set (4406447)	SOLiD™ Frag Lib Seq – BC Probe Reagents SOLiD™ Frag Lib Seq – BC Primers Reagents SOLiD™ Frag Lib Seq – BC Bridge Probes Reagents	Barcode sequencing

Item (part number)	Components	Kit component(s) used in…
SOLiD™ 3 Instrument Buffer Kit (4406479)	– Reset Buffer – Glycerol – 10× Instrument Buffer – Cleave Solution 1 – Cleave 2 Kit (Cleave 2 Parts 1 and 2) – Storage Buffer – Imaging Buffer Kit (Imaging Buffer Parts 1 and 2) – 1× T4 Ligase Buffer Kit (Ligase Buffer Parts 1 and 2) – 1× Phosphatase Buffer	Sequencing and/or WFA
SOLiD™ Slide Pack Kit (4412172)	Slide Storage Buffer Sequencing Slides	Bead deposition
SOLiD™ Bead Deposition Kit (4387895)	Deposition Buffer Overlay Buffer	Bead deposition
	10 × Terminal Transferase Buffer 10 × Cobalt chloride Terminal Transferase Bead Linker	Templated bead preparation

Optional Applied Biosystems kits

Item (part number)	Components	Kit component(s) used in…
SOLiD™ Fragment Library Sequencing – Primer A Master Mix 50 (4406550)	SOLiD™ Frag Lib Seq – Primer A Reagents	Fragment sequencing
SOLiD™ Fragment Library Sequencing – Primer B Master Mix 50 (4406601)	SOLiD™ Frag Lib Seq – Primer B Reagents	Fragment sequencing
SOLiD™ Fragment Library Sequencing – Primer C Master Mix 50 (4406556)	SOLiD™ Frag Lib Seq – Primer C Reagents	Fragment sequencing
SOLiD™ Fragment Library Sequencing – Primer D Master Mix 50 (4406559)	SOLiD™ Frag Lib Seq – Primer D Reagents	Fragment sequencing
SOLiD™ Fragment Library Sequencing – Primer E Master Mix 50 (4406562)	SOLiD™ Frag Lib Seq – Primer E Reagents	Fragment sequencing
SOLiD™ Mate-Paired Library Sequencing – Tag 1 Primer A Master Mix 50 (4406565)	SOLiD™ MP Lib Seq – Tag 1 Primer A Reagents	Mate-pair sequencing

Item (part number)	Components	Kit component(s) used in…
SOLiD™ Mate-Paired Library Sequencing – Tag 1 Primer B Master Mix 50 (4406568)	SOLiD™ MP Lib Seq – Tag 1 Primer B Reagents	Mate-pair sequencing
SOLiD™ Mate-Paired Library Sequencing – Tag 1 Primer C Master Mix 50 (4406571)	SOLiD™ MP Lib Seq – Tag 1 Primer C Reagents	Mate-pair sequencing
SOLiD™ Mate-Paired Library Sequencing – Tag 1 Primer D Master Mix 50 (4406574)	SOLiD™ MP Lib Seq – Tag 1 Primer D Reagents	Mate-pair sequencing
SOLiD™ Mate-Paired Library Sequencing – Tag 1 Primer E Master Mix 50 (4406577)	SOLiD™ MP Lib Seq – Tag 1 Primer E Reagents	Mate-pair sequencing
SOLiD™ Mate-Paired Library Sequencing – Tag 2 Primer A Master Mix 50 (4406580)	SOLiD™ MP Lib Seq – Tag 2 Primer A Reagents	Mate-pair sequencing
SOLiD™ Mate-Paired Library Sequencing – Tag 2 Primer B Master Mix 50 (4406583)	SOLiD™ MP Lib Seq – Tag 2 Primer B Reagents	Mate-pair sequencing
SOLiD™ Mate-Paired Library Sequencing – Tag 2 Primer C Master Mix 50 (4406586)	SOLiD™ MP Lib Seq – Tag 2 Primer C Reagents	Mate-pair sequencing
SOLiD™ Mate-Paired Library Sequencing – Tag 2 Primer D Master Mix 50 (4406589)	SOLiD™ MP Lib Seq – Tag 2 Primer D Reagents	Mate-pair sequencing
SOLiD™ Mate-Paired Library Sequencing – Tag 2 Primer E Master Mix 50 (4406592)	SOLiD™ MP Lib Seq – Tag 2 Primer E Reagents	Mate-pair sequencing
SOLiD™ Mixing Strip Tube (4406595)	SOLiD™ Mixing Strip Tubes	Fragment or mate-pair sequencing

Required equipment

Item‡	Source
SOLiD™ 3 System	• Applied Biosystems 4406205 (110 V) • Applied Biosystems 4406208 (220 V)
SOLiD™ 3 Analyzer	Applied Biosystems 4406207
Upgrade Kit from SOLiD™ 2.0 to SOLiD™ 3	Applied Biosystems 4409522

Item‡	Source
SOLiD™ Light Source	Applied Biosystems 4388441
SOLiD™ Slide Storage Chamber	Applied Biosystems 4406354
SOLiD™ Deposition Chambers 1-Well§	Applied Biosystems 4406352
SOLiD™ Deposition Chambers 4-Wells§	Applied Biosystems 4406358
SOLiD™ Deposition Chambers 8-Wells§	Applied Biosystems 4406359
SOLiD™ Slide Carriers§	Applied Biosystems 4406360
SOLiD™ Uninterruptible Power Supply (UPS)	• Applied Biosystems 4397781 (SOLiD™ UPS North America) • Applied Biosystems 4393695 (220 V; SOLiD™ UPS International)
SOLiD™ Offline Cluster	• Applied Biosystems 4413002 (North America) • Applied Biosystems 4425588 (International)
SOLiD™ Accessory Disk Drive	Applied Biosystems 4426101
SOLiD™ Bead Concentration Chart	Applied Biosystems 4415131
Covaris™ S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris™ S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube For system materials summary, see "Covaris™ S2 System Materials Summary," *SOLiD™ 3 System Site Preparation Guide*.	• Applied Biosystems 4387833 (110 V) • Applied Biosystems 4392718 (220 V) or Covaris™ Inc.

Item‡	Source
6-Tube Magnetic Stand	Applied Biosystems AM10055
Microcentrifuge 5417R, refrigerated, without rotor	• Eppendorf# 022621807 (120 V/60 Hz) • Eppendorf# 022621840 (230 V/50 Hz)
FA-45-24-11, fixed-angle rotor, 24 × 1.5/2 mL, including aluminum lid, aerosol-tight	Eppendorf# 022636006
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Scienceware Dry-Keeper AutoDesiccator Cabinet, Tall#	VWR 24983-455
Tabletop Centrifuge (for 96-well plate)	MLS‡‡
Vortexer	MLS
Picofuge	MLS
Magnetic stirrer	MLS
Incubator (37 °C)	MLS
Refrigerator (4 °C)	MLS
Freezer (– 20 °C)	MLS
Pipettors, 20 µL	MLS
Pipettors, 200 µL	MLS
Pipettors, 1000 µL	MLS

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.
§ The SOLiD™ 3 Analyzer is shipped with 2 of each size Deposition Chambers and Slide Carriers. Two Slide Storage Chambers are provided for use with all chambers.
# Or equivalent but validation of the equipment for library preparation is required.
‡‡ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Required consumables

Item‡	Source
SOLiD™ Flowcell O-ring. 10-pack	Applied Biosystems 4398217
Nuclease-free water	Applied Biosystems AM9932
ABgene® 96 1.2-mL square-well storage plates	ABgene AB-1127
3-mm adhesive disks	Grace Bio-Labs ST200
Ethylene glycol	American Bioanalytical AB00455-01000

Item‡	Source
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit§	Thermo Scientific PR-1
1.5-mL LoBind Tubes	Eppendorf 022431021
Kimwipes®	MLS#
Filtered pipettor tips	MLS

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.

§ The NanoDrop® Conditioning Kit is useful for "reconditioning" the sample measurement pedestals to a hydrophobic state if they become "unconditioned" (see NanoDrop user's manual for more information). The PR-1 kit consists of a container of specially formulated polishing compound and a supply of convenient applicators.

For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

B Supplemental Procedures

This appendix covers:

Clean the SOLiD™ Deposition Chamber using a sonicator

Clean the SOLiD™ Deposition Chamber a day before slide deposition with a sonicator or with Extran 300 (see "Clean the SOLiD™ Deposition Chamber using Extran 300" on page 100).

Required equipment

Item	Source
Sonicator‡	Branson Ultrasonics, Inc. 8510R-DTH
2-L beaker	MLS§

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance

§ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Required consumables

Item	Source
Bleach	MLS
Deionized water	MLS
Hot water	MLS

1. Thoroughly wipe the beaker and the inside of the sonicator with 10% bleach, then fill the sonicator to the fill line with deionized water. Clean the sonicator and replace the deionized water every week. Clean the beaker before use.
2. Set the sonicator temperature to 25 °C, then allow it to equilibrate before use.
3. Immediately after the SOLiD Deposition Chamber assembly is disassembled, thoroughly rinse the SOLiD Deposition Chamber with hot water to remove residual buffers from previous deposition. Rinse the base of the chamber with deionized water and allow it to dry overnight.
4. Degas the sonicator for 5 minutes before use.
5. Place the SOLiD Deposition Chamber and SOLiD™ Slide Carrier into the beaker. Place the beaker in the sonicator, then sonicate the SOLiD Deposition Chamber and the SOLiD Slide Carrier for 10 minutes.

6. After sonication, remove the SOLiD Deposition Chamber and SOLiD Slide Carrier from the sonicator, then rinse the SOLiD Deposition Chamber and SOLiD Slide Carrier thoroughly with deionized water (3 to 5 minutes for each chamber). When rinsing the chamber, be careful with the face and O-ring that come into contact with the slide.

   IMPORTANT! Do not use abrasive chemicals or wipes, because they can damage the chamber and gasket.

7. Dry the SOLiD Deposition Chamber and SOLiD Slide Carrier overnight or at 37 °C for 1 hour before use.

Clean the SOLiD™ Deposition Chamber using Extran 300

Clean the SOLiD™ Deposition Chamber a day before slide deposition with Extran 300 or with a sonicator (see "Clean the SOLiD™ Deposition Chamber using a sonicator" on page 98).

Required equipment

Item	Source
Tub	MLS‡
Pipette or graduated cylinder	MLS

‡ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Required consumables

Item	Source
Extran 300	VWR EM-EX0996-2
Deionized water	MLS
Hot water	MLS

1. Prepare enough 1:1000 dilution of Extran 300 and deionized water to fill a large tub suitable for washing SOLiD™ Deposition Chambers, leaving enough empty volume in the tub for the addition of the SOLiD Deposition Chambers (for example, 5 mL of Extran 300 in 4995 mL deionized water for a 7-L tub).
2. Immediately after the SOLiD Deposition Chamber assembly is disassembled, thoroughly rinse the SOLiD Deposition Chamber assembly with hot water, removing residual buffers from the previous deposition. Rinse the base of the chamber with deionized water and allow it to dry overnight.
3. Place the SOLiD Deposition Chamber and SOLiD™ Slide Carrier into the tub to soak for 5 minutes.
4. Gently agitate the tub for 2 minutes. Be careful not to let the face of the SOLiD Deposition Chamber rub against the tub or other SOLiD Deposition Chambers.
5. Allow the SOLiD Deposition Chamber and SOLiD Slide Carrier to soak for another 5 minutes.

6. After the second soak, remove the SOLiD Deposition Chamber and SOLiD Slide Carrier from the tub and rinse thoroughly with deionized water (3 to 5 minutes for each chamber). When rinsing the chamber, be careful with the face and O-ring that come into contact with the slide.

   IMPORTANT! Do not use abrasive chemicals or wipes, because they can damage the chamber and gasket.

7. Dry the SOLiD Deposition Chamber and SOLiD Slide Carrier overnight before use.

Install the SOLiD™ Flowcell O-Ring

After each run, check the SOLiD™ Flowcell O-ring for cuts and abrasions. If you see any abnormalities, replace the O-ring. Inspect the O-ring groove for debris or contamination. Clean the O-ring groove with water as needed.

Required equipment

Item	Source
SOLiD™ Flowcell O-ring, 10-pack	Applied Biosystems 4398217

Procedure

1. Insert the O-ring into the groove on the flowcell so that the smooth side is on top (see Figure 67).
2. Run your finger around the O-ring to make sure that there are no high spots.

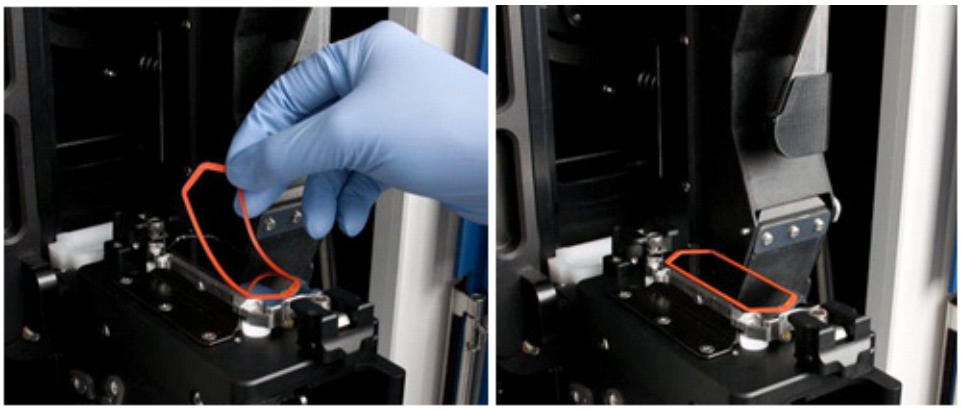


Figure 67 Install the SOLiD™ O-Ring on a flowcell.

Clean the reagent strip cover

Inspect the top and bottom of the reagent strip covers after each run for splattered wet or dry reagents. If you see reagent on a cover, clean the cover (see Table 15).

Required equipment

Item	Source
Pipette or graduated cylinder	MLS‡
Scrub brush	MLS

‡ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Required consumables

Item	Source
Extran 300	VWR EM-EX0996-2
Kimwipes®	MLS
Deionized water	MLS
Cotton swabs	MLS

Table 15 Clean the reagent strip cover according to location or amount of reagent splatter

If you see...	Then
Minor splashes	Wipe the reagent strip cover with a Kimwipe®. If the reagents are dried, first wet the Kimwipe moistened with deionized water or a dilution of Extran 300 wash detergent in deionized water
Reagents in the holes of the of the reagent strip cover	Wipe the inside surfaces of the holes with a cotton swab. If the reagents are dried, first wet the cotton swab as necessary with deionized water or a dilution of Extran 300 wash detergent in deionized water.
Large areas of dried reagents	Wash the covers in a dilution of Extran wash detergent (10 mL Extran 300 in 1 L of deionized water). If needed, use a scrub brush.

Replace the SOLiD™ Light Source

WARNING! PHYSICAL INJURY HAZARD. Hot Surface. Surface of the SOLiD™ Light Source may be hot. Use care when working around the SOLiD Light Source to avoid being burned.

Replace the SOLiD™ Light Source in the SOLiD™ 3 Analyzer every 1500 hours.

Required equipment

Item	Source
SOLiD™ Light Source	Applied Biosystems 4388441

1. Under the System Status menu on the ICS, click the **Turn Off** button for the lamp (see Figure 68).

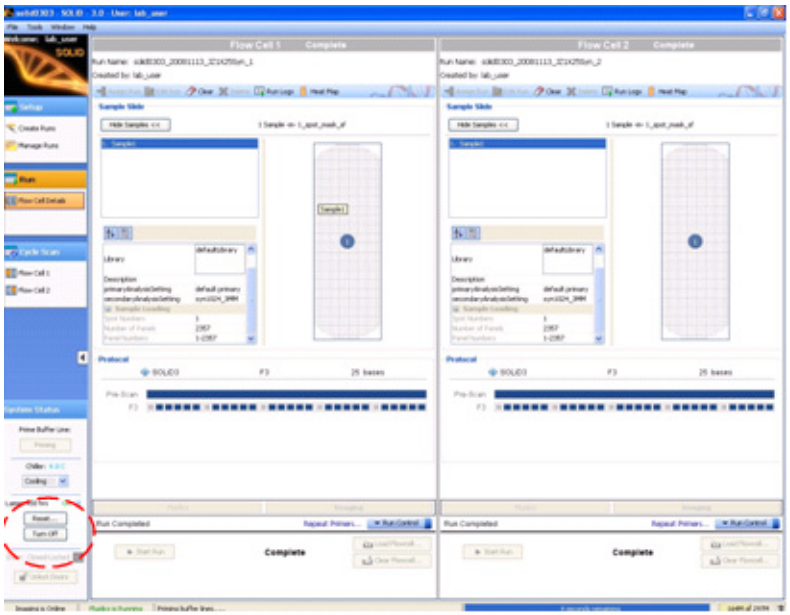


Figure 68 Lamp controls in the ICS.

2. Let the lamp cool for at least 15 minutes.
3. Unscrew the 4 screws retaining the light box access cover on the top of the instrument.
4. Remove the cover to the light source from the housing.
5. Pull the light source straight up and out of unit.
6. Slide a new SOLiD™ Light Source into place. Ensure that the light source is oriented in the correct direction (see Figure 69 on page 105).

7. Refit and screw down the access cover to the housing.
8. Restart power to the SOLiD Light Source.
9. In the System Status menu on the ICS, click **Reset** to reset the lamp timer.

Figure 69 Orientation of the SOLiD™ Light Source.

Set up a run by importing a Run Definition file

A sequencing run can be set up by importing a Run Definition file that has been created offline. Setting up a run by importing a run definition file saves time re-entering information of a repeated run.

1. Acquire a template Run Definition file. The most reliable way of acquiring the file is to create a run using the Run Wizard, assign it to the database, then select **Export Run** from the Manage Runs window.
2. Create a Run Definition with the information appropriate to your run. Save the file as a .txt file.
3. Click **Manage Runs**, select **Import Run**, then select the Run Definition file.
4. To assign a run previously saved to the database:
   a. Click on **Manage Runs** in the task pane.
   b. Click the run, then select **Assign to Flowcel**l.
   c. Choose a flowcell, then click **OK**.

Manually find the focus range

You should first attempt automatic range detection (see "Detect the focus range" on page 44). If automatic detection fails, then use the manual mode.

Select the flowcell, then find the beads on the slide

1. Close the front doors of the SOLiD™ 3 Analyzer. Open the Imager window by choosing **Window** ▸ **Imaging System**.

2. Select the **Show Flowcell** box in the bottom left hand corner of the Imager window, then choose the number of the flowcell to set (Flowcell **1** or Flowcell **2**; see Figure 70).

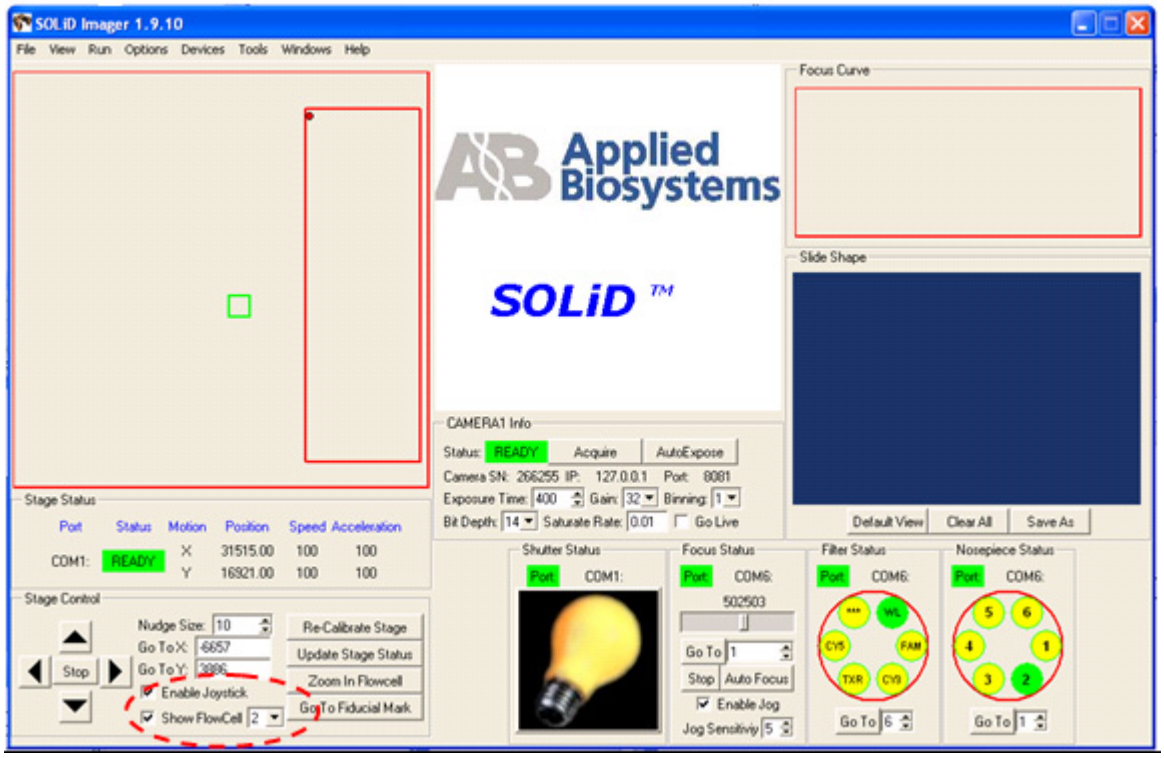


Figure 70 Click Show Flowcell to find the beads on the slide.

3. From the View menu, choose **Stage Template**, then navigate to the C:\Runs directory. Find the folder in the Runs directory that corresponds to the run just set up, then choose the .STG file for the run. Choosing Stage Template and the .STG file superimposes the slide layout on the Imager screen.

4. Use the mouse to drag the green-box cursor to a position on the upper-left side of the slide. Dragging the green-box cursor moves the flowcell stage to that corner of the slide (see Figure 71 on page 108).

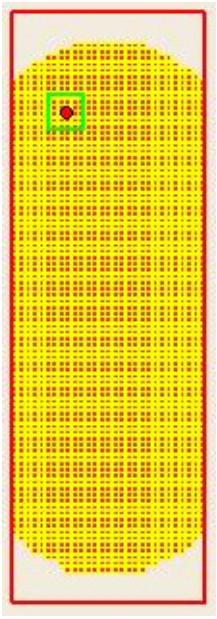


Figure 71 Use the mouse to drag the green-box cursor and to move the flowcell stage the same way.

Find the focal range

1. Set the Filter Status to **WL** (white light; see Figure 72).

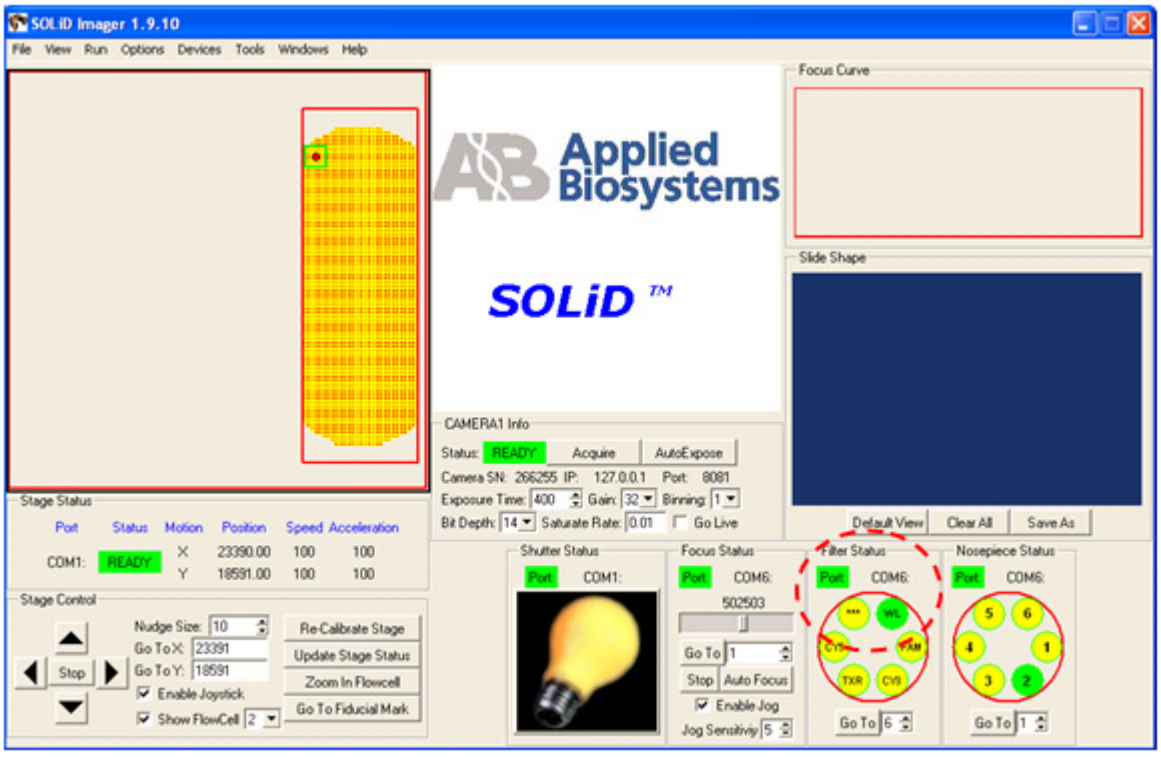


Figure 72 Click WL (white light) to set the Filter Status.

2. In the Imager window, click the **AutoFocus** button, then wait about 20 seconds for the Imager to focus on the slide (see Figure 73). The Camera 1 indicator displays BUSY while autofocus is running. When the Camera 1 indicator displays READY, click **AutoExpose**, then wait for the Camera 1 indicator to show READY. Click **AutoFocus** so that the Imager can focus again (this time with correct exposure settings). Note where the focus peak falls in the focus range by looking at the value that displays where the peak is located.

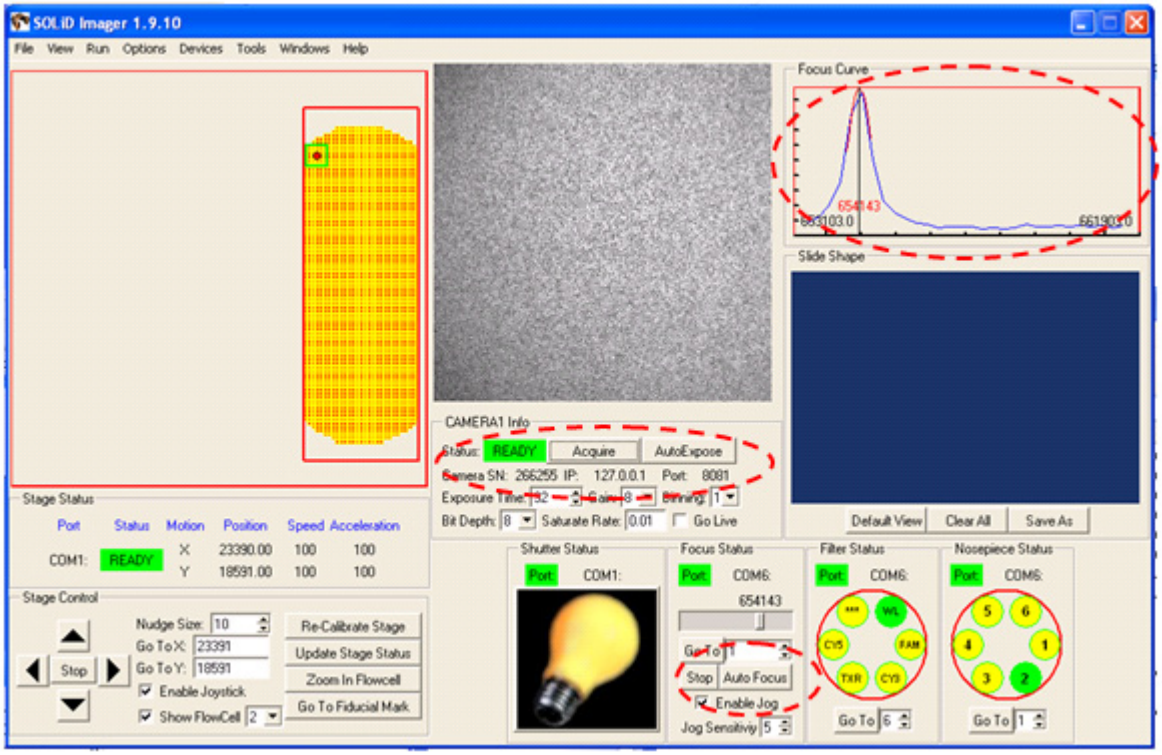


Figure 73 Click AutoFocus and AutoExpose so that the focus peak falls in the focus range.

3. When the Imager displays READY, click the **Acquire** button, then examine the image on the screen (see Figure 74 on page 110).

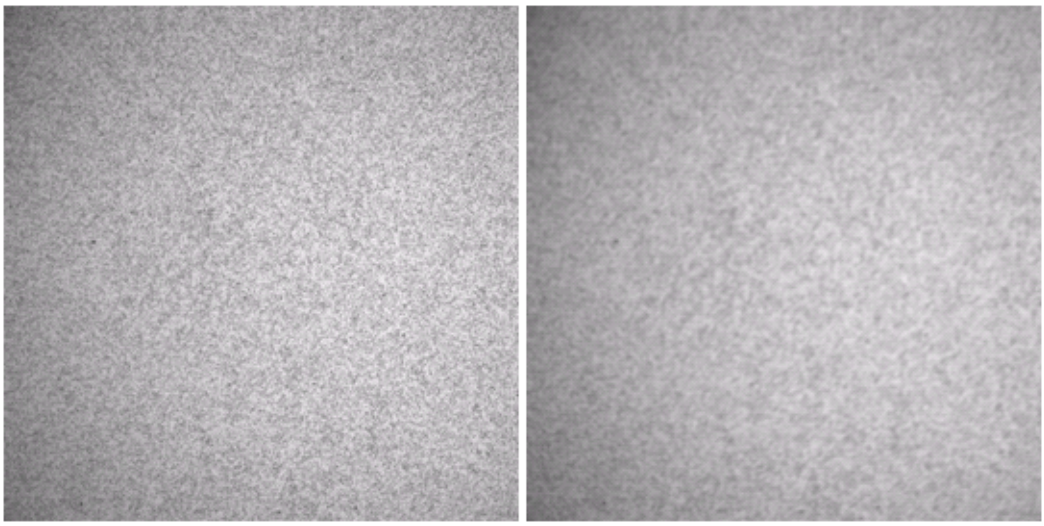


Figure 74 Examine the image on the screen.

Note: To zoom on the image, double-left-click the mouse. To un-zoom, double-right-click. To drag the image, hold down the left mouse button.

4. If the image is *in* focus, proceed to "Calculate, then set the focal range" on page 114. If the image is *out* of focus, proceed to step 5.

5. Check the **Go Live** check box in the Imager window. By checking the **Go Live** check box, a live image of the flowcell displays. You can use this image to manually find the focal range (see Figure 75 on page 111).

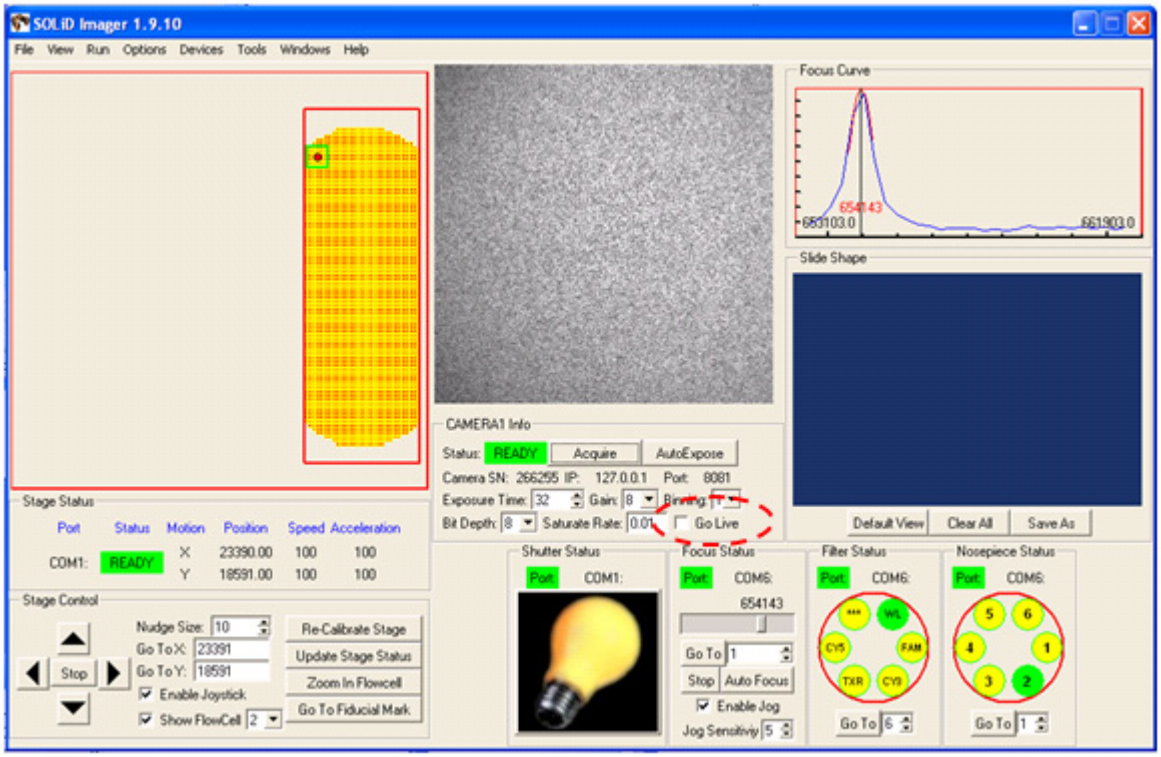


Figure 75 Check Go Live to manually find the focal range.

6. Click the focus slider bar in the Imager window. A black box appears (see Figure 76 on page 112).

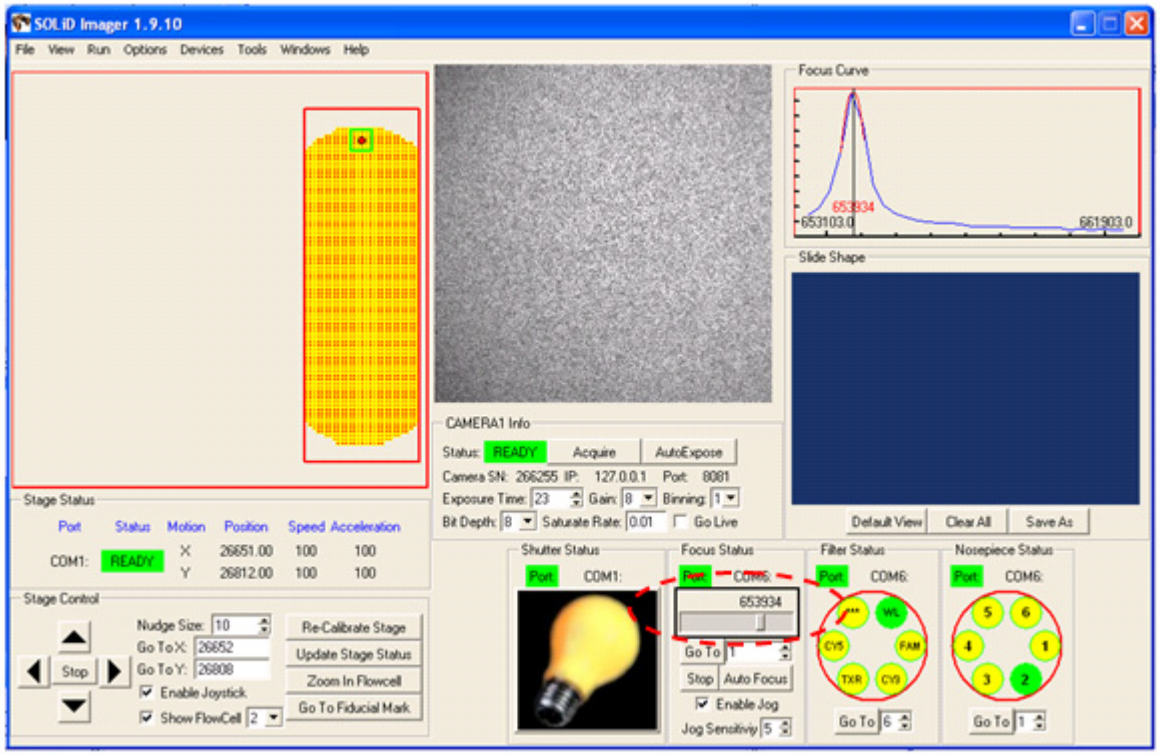


Figure 76 Use the black box to change the focal distance.

7. When the black box is visible, hold down the **Ctrl** key and hold down either the **right** or **left** arrow key on the keyboard. Holding down **Ctrl** and the arrow key (**right** or **left** arrow) simultaneously changes the focal distance in 300-count intervals.

8. While watching the live image on the screen, scan the focal distance:
   - Use the **Ctrl**+***right*** arrow keys to scan the focal distance upward. When the live image is in focus, release the keys, then record the value shown in the black box of the Imager window.
   - If an in-focus image *cannot* be found, then scan downward using the **Ctrl**+***left*** arrow keys, past the starting point, while watching the live image on the screen. When the live image is in focus, release the keys, then record the value shown in the black box of the Imager window.

   The value in the black box of the imager window when the image is in focus is the *nominal Z distance*.

   For examples of in-focus images, see Figure 77 on page 113.

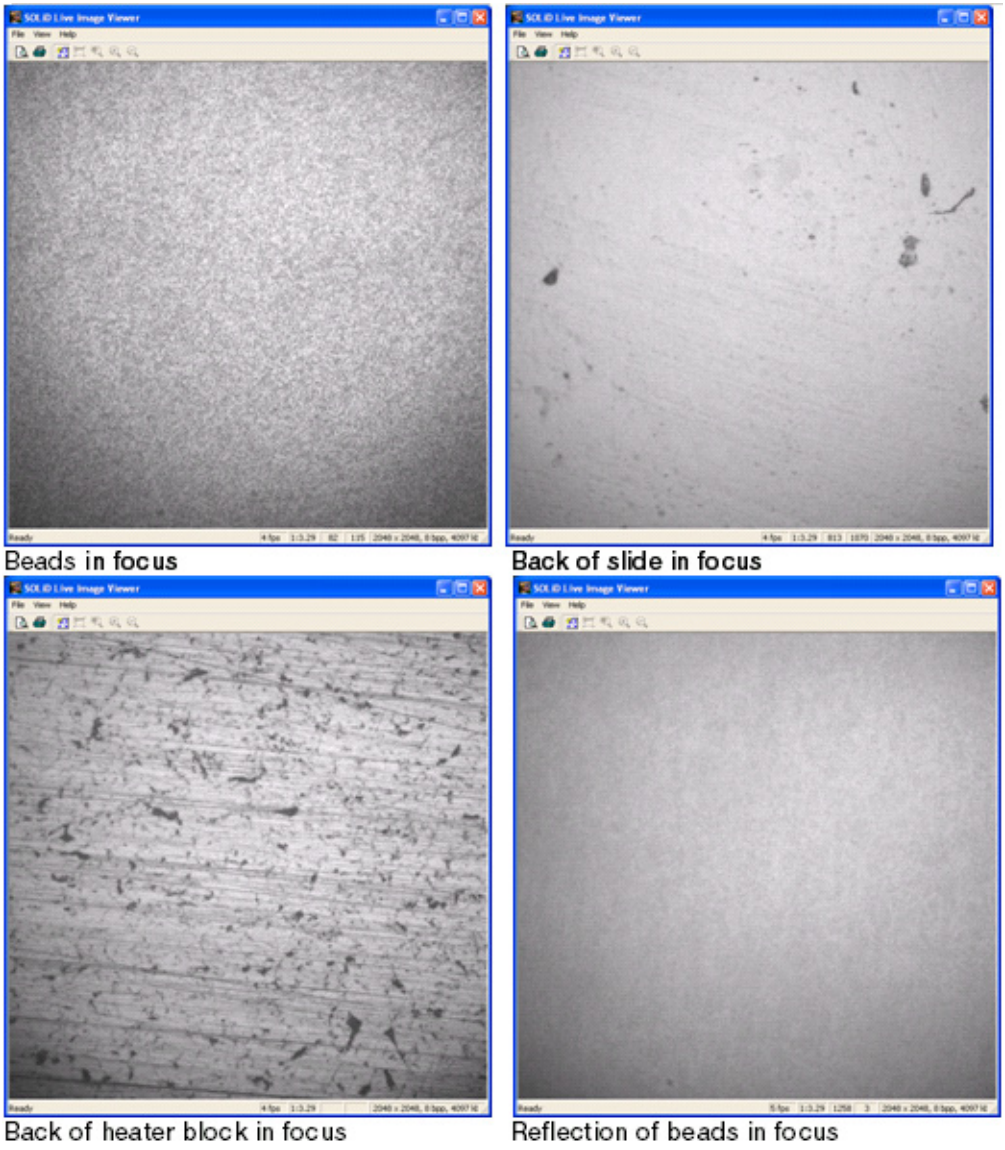


Figure 77 Examples of in-focus images.

9. Uncheck the **Go Live** check box in the Imager window to close the live-image window.

Calculate, then set the focal range

1. When the image is in focus, record the value of the *Z* distance in the Focus Status pane. This is the *nominal Z distance* for this flowcell. Be sure to record the correct *Z* distance value, not the value in the GoTo window (see Figure 78).

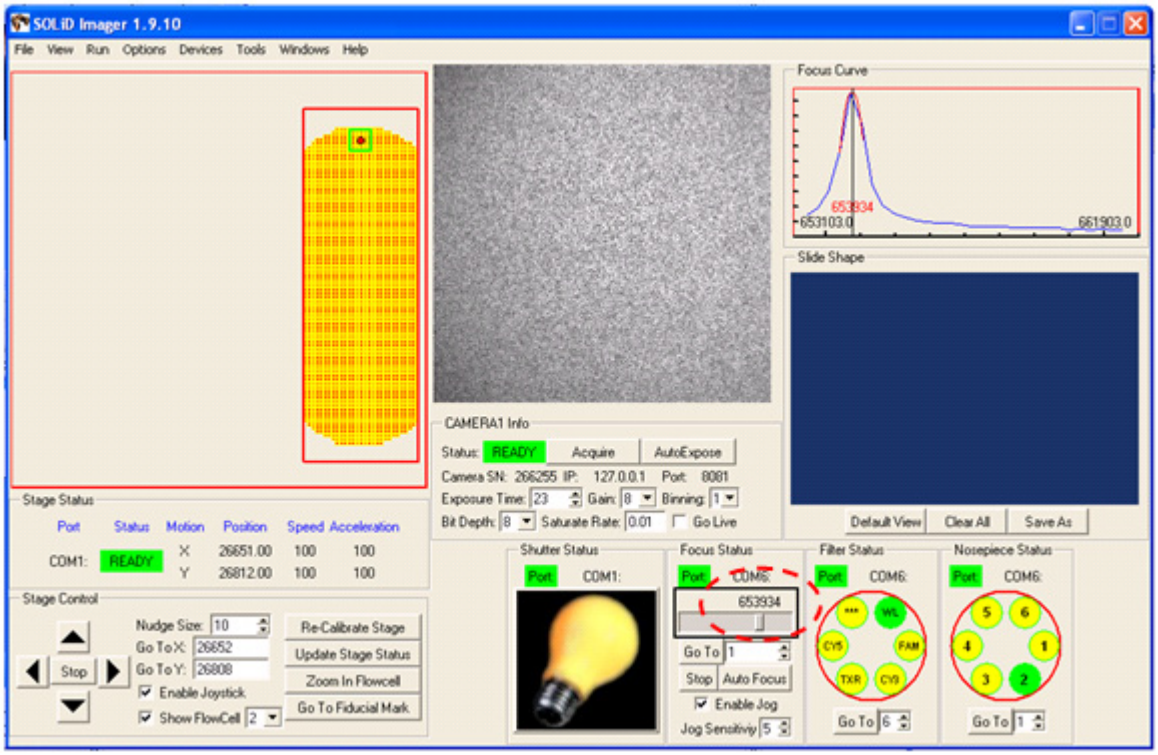


Figure 78 Record the nominal Z distance.

2. Choose **Options** ▸ **Focusing**.

3. Ensure that **Do auto-exposure with auto-focus** is checked. Enter a value 5000 *less than* the nominal *Z* distance for *Z* Min. Enter a value 5000 *greater than* the nominal *Z* distance for *Z* Max. Enter **30** for the Number of Steps and choose **Histogram Range** from the drop-down menu in the Focusing Method Selection pane (see Figure 79 on page 115).

> **IMPORTANT!** Leave the Auto-Focus Options window *open* on the Desktop. Do *not* click the **Save to File** button. Values entered in the Focusing box are used by the Imager software even if the values are not saved.

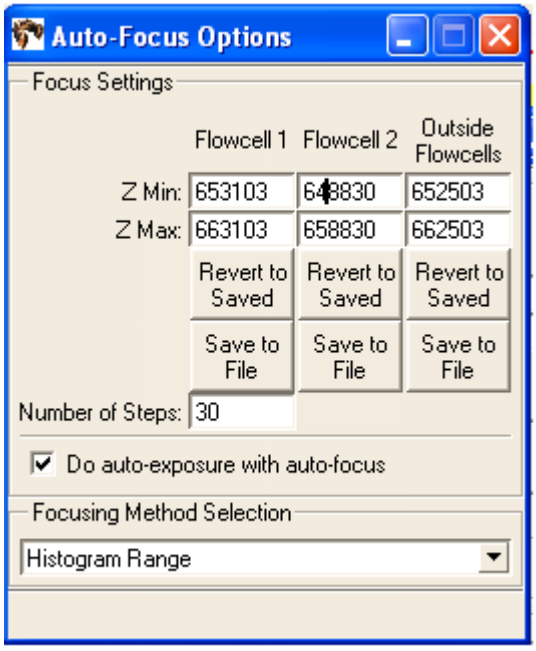


Figure 79 Enter auto-focus options.

4. Use the mouse to move the green box to the upper-left corner of the slide.

5. In the Imager window, click the **Autofocus** button, then wait about 20 seconds for the Imager to focus on the slide. The Camera 1 indicator displays BUSY while the autofocus is running. When the Camera 1 indicator displays READY, click **AutoExpose** and wait for the Camera 1 indicator to display READY. Click AutoFocus so that the Imager can focus again (this time with the correct exposure settings).

6. When the Imager displays READY, click the **Acquire** button, then confirm that the image is in focus.

7. Record the *Z* distance value from the Focus Status pane (see Figure 80 on page 116).

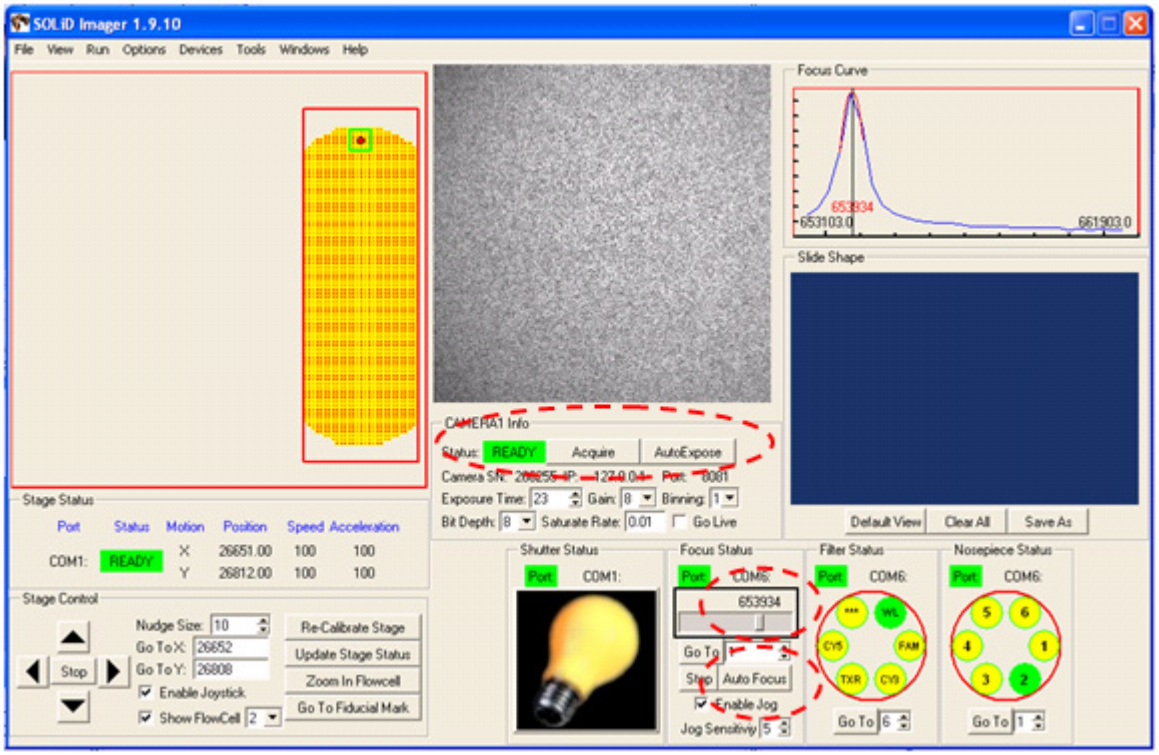


Figure 80 Record the *Z* distance value.

8. Open Notepad, then record the measured focal value for the spot on the slide that has been focussed.

9. Repeat steps 4 to 8 for the top right, bottom left, bottom right, and center of the slide. The range of these values should not exceed 5000 units. Calculate the average value of the highest and lowest spots and record this value in Notepad (see Figure 81 on page 117).

> **IMPORTANT!** If the range of these values exceeds 5000 units, the flowcell is out of alignment. Call your Applied Biosystems Field Service Engineer to align the flowcell.

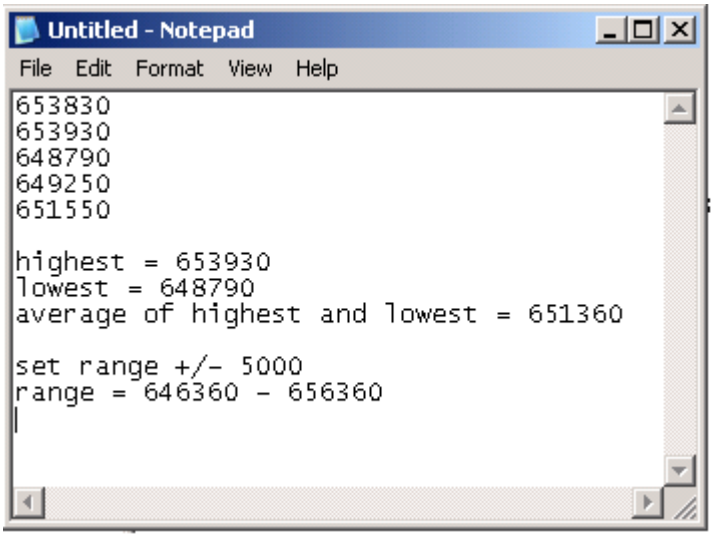


Figure 81 Record the average focal value in Notepad.

10. Subtract 5000 counts from the calculated average, then enter that value in the *Z* Min box. Add 5000 counts to the average, then enter that value into the *Z* Max box. Confirm that the Number of Steps is **30**. Click **Save to File** (see Figure 82).

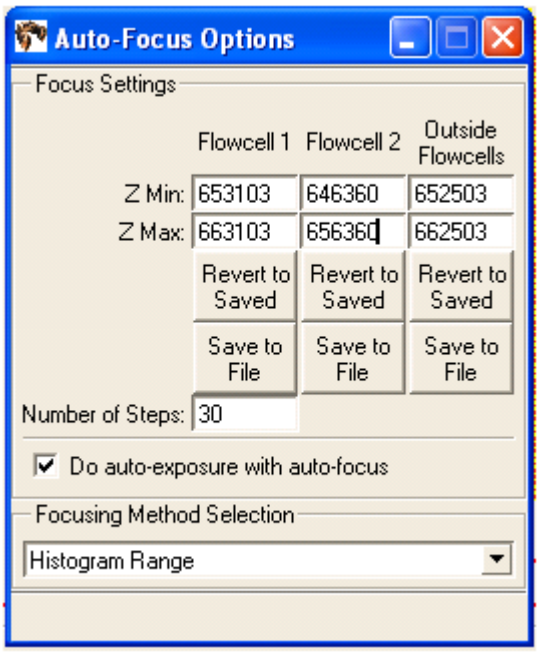


Figure 82 Enter the focus settings.

11. Click **Yes** when the warning displays (see Figure 83 on page 118).

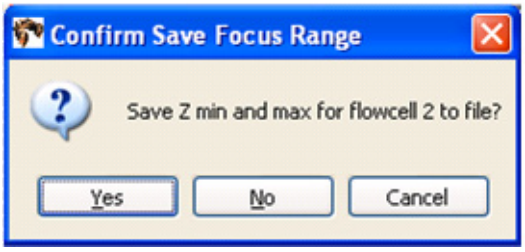


Figure 83 Click Yes when the Save Focus Range Warning displays.

12. When the Flowcell Selection window displays, choose the number of the flowcell (**1** or **2**) that has just been measured and click **OK**. The focal range is saved.

13. (Optional) Repeat all previous steps starting from "Select the flowcell, then find the beads on the slide" on page 107.

Shut down the SOLiD™ 3 Analyzer

The instrument can be shut down using the Instrument Shutdown wizard in the ICS. For instruments that have UPS, in the event of power failure, an uninterrupted power supply (UPS) is activated, UPS systematically shuts down the instrument: Analysis jobs are stopped and Linux is shut down. Slides are preserved in Storage Buffer.

Required equipment

Item	Source
UPS	Applied Biosystems 4397781 (North America) 4393695 (International)

1. Open the Instrument Shutdown wizard by choosing **Wizards** ▸ **Instrument Shutdown**.
2. Follow the instructions in the wizard.

Reset the robot position

The Robot standby script sends the robot back to home position.

1. Open the Utility Scripts menu by choosing **Tools** ▸ **Utility Scripts**.
2. Select **Robot standby**.
3. Select **Run Script**.

Store the slide in a flowcell

The Store flowcell script fills the flowcell with Storage Buffer.

1. Open the Utility Scripts menu by choosing **Tools** ▸ **Utility Scripts**.
2. Select **Store flowcell**.
3. Select **Run Script**.

C On-Instrument Reagent Volumes and Reagent Strip Layouts

This appendix covers:

Recommended fill volumes for on-instrument reagents

Fragment sequencing (50 bp)

Table 16 Fill volumes for fragment sequencing (50 bp)

	Volume (mL)	
	1 Flowcell	**2 Flowcells**
Instrument Buffer	2949	5710
Storage Buffer	422	746
Cleave 1 Solution	167	311
Cleave 2 Solution	167	311
Reset Buffer	91	163
Ligase Buffer	60	110
Phosphatase Buffer	36	62
Imaging Buffer	110	196

Mate-pair sequencing (25 or 50 bp)

Table 17 Fill volumes for mate-pair sequencing (25 or 50 bp)

	Volume per tag (mL)			
	25 bp		**50 bp**	
	1 Flowcell	**2 Flowcells**	**1 Flowcell**	**2 Flowcells**
Instrument Buffer	1629	3069	2949	5710
Storage Buffer	289	479	422	746
Cleave 1 Solution	92	161	167	311
Cleave 2 Solution	92	161	167	311
Reset Buffer	91	163	91	163
Ligase Buffer	35	59	60	110
Phosphatase Buffer	23	35	36	62
Imaging Buffer	67	111	110	196

Barcode sequencing (5 bp)

Table 18 Fill volumes for barcode sequencing (5 bp)

	Volume (mL)	
	1 Flowcell	**2 Flowcells**
Instrument Buffer	638	1088
Storage Buffer	189	279
Cleave 1 Solution	32	41
Cleave 2 Solution	32	41
Reset Buffer	91	163
Ligase Buffer	16	21
Phosphatase Buffer	13	15
Imaging Buffer	33	43

Workflow analysis (WFA)

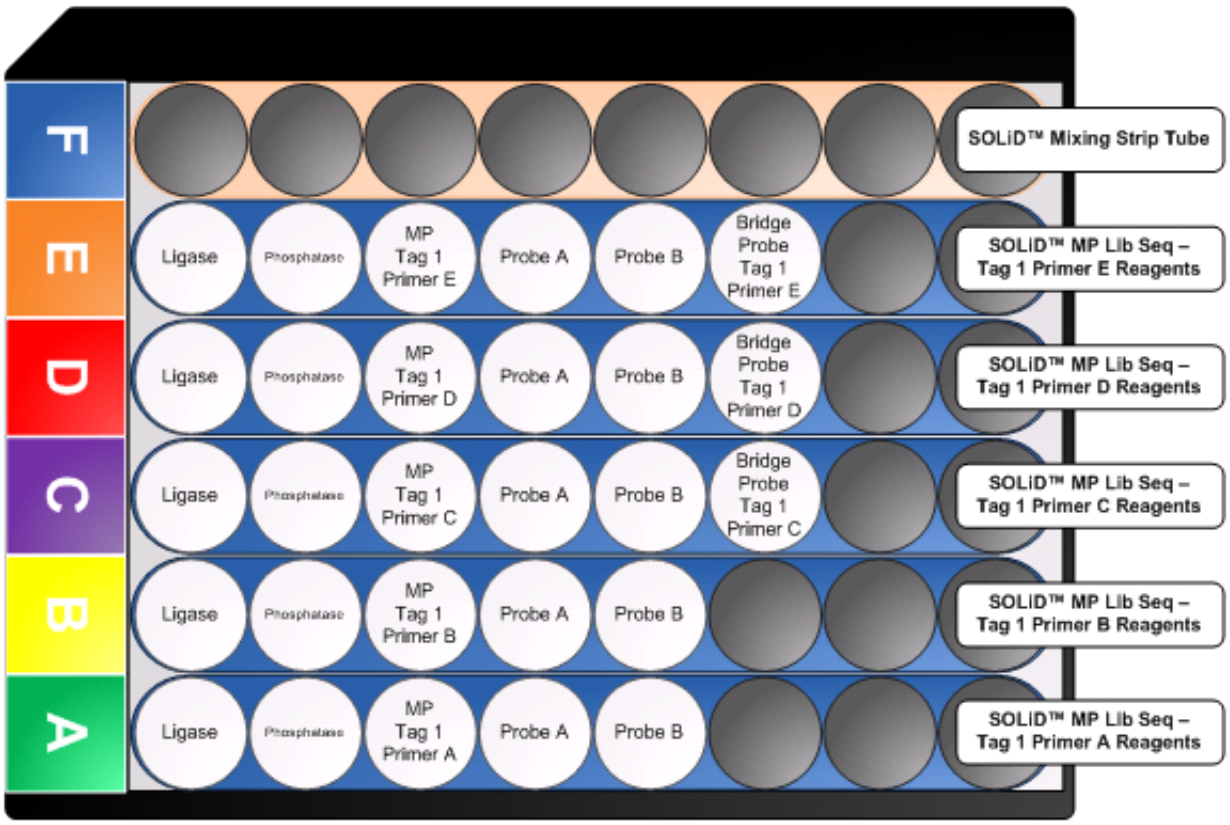
Table 19 Fill volumes for workflow analysis (WFA)

	Volume (mL)	
	1 Flowcell	**2 Flowcells**
Instrument Buffer	294	400
Storage Buffer	116	134
Cleave 1 Solution	0	0
Cleave 2 Solution	0	0
Reset Buffer	43	67
Ligase Buffer	12	12
Phosphatase Buffer	0	0
Imaging Buffer	26	30

Reagent strip layouts

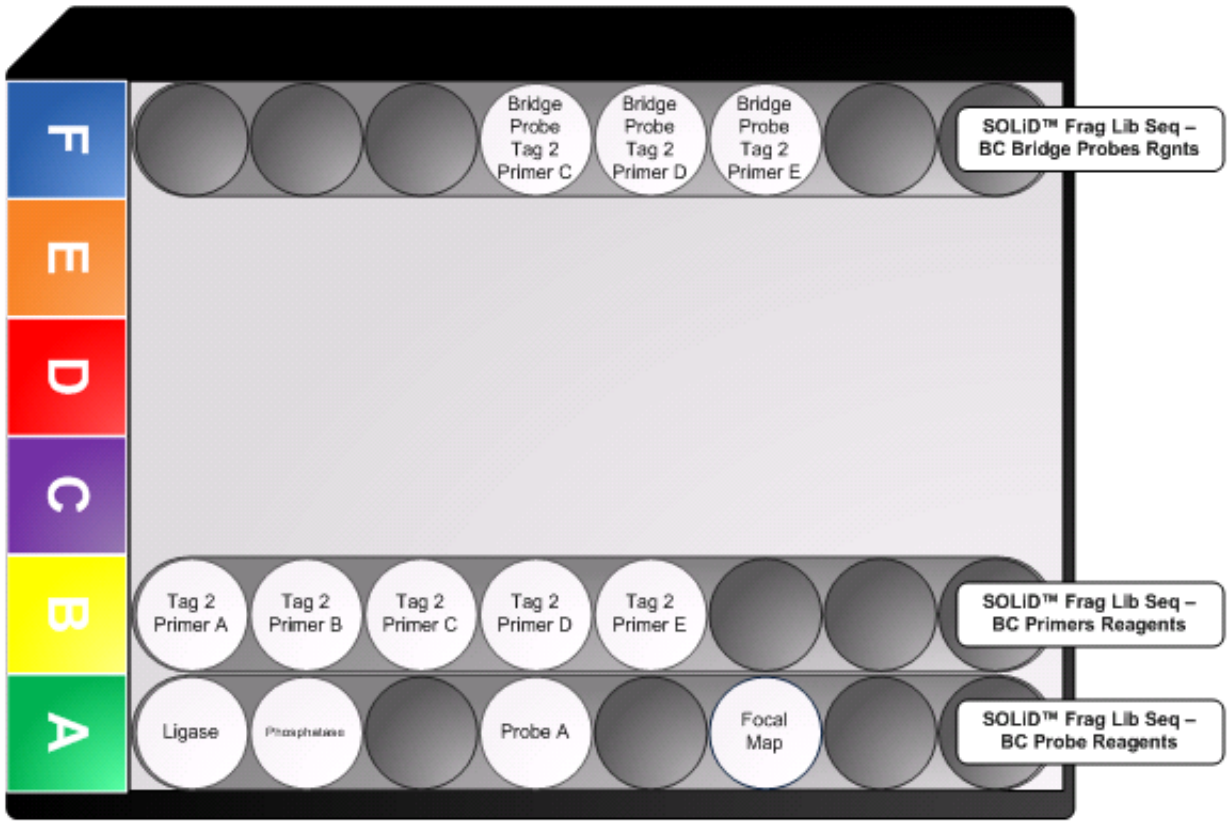
Fragment sequencing

Mate Pair Tag 1 sequencing



Mate Pair Tag 2 sequencing

Barcode sequencing



Workflow analysis (WFA)

D Instrument Process Times

This appendix covers:

Times for individual processes

Table 20 Times for individual processes

Process	Estimated Time (h)	Steps	Estimated time per primer (h)
Focal Map	~2 to 3	Reset P2 Label Image	
Sequencing Primers 1 & 2 and Ligation	~3 to 4	Reset Prime Ligate Dark Ligate Phosphatase Image Cleave	~3 to 4 (1 cycle or 5 bp) ~15 to 17 (5 cycles or 25 bp) ~28 to 33 (10 cycles or 50 bp)
Sequencing Primers 3, 4, & 5 and Ligation	~4 to 5	Reset Prime Bridge Probe Ligate Dark Ligate Phosphatase Image Cleave	~4 to 5 (1 cycle or 5 bp) ~16 to 18 (5 cycles or 25 bp) ~29 to 34 (10 cycles or 50 bp)
Ligation cycle (without priming)	~2.5 to 3	Ligate Dark Ligate Phosphatase Image Cleave	

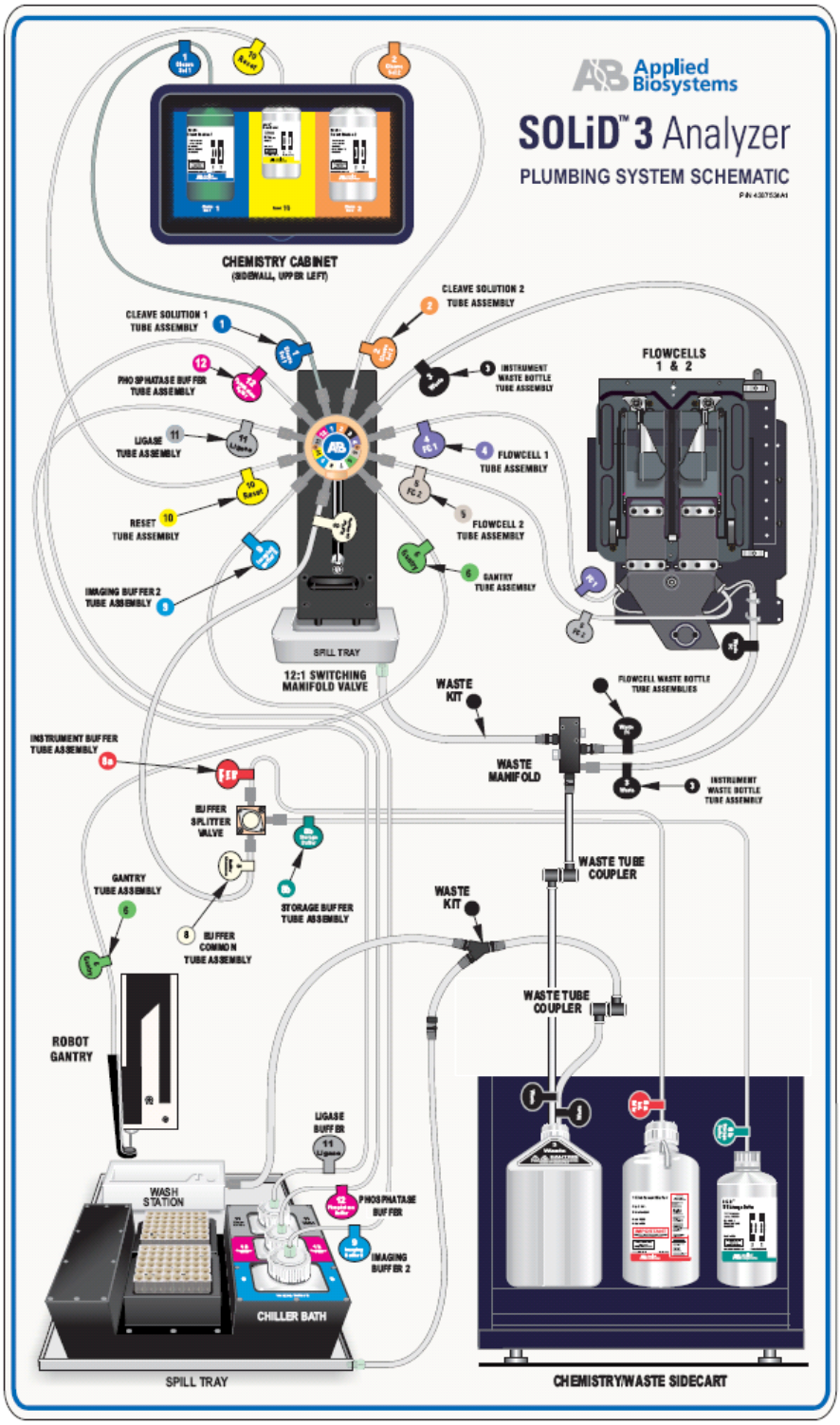
Times for entire processes

Table 21 Times for entire processes

Process	Total Runtime‡
Workflow Analysis (WFA)	~4 to 5 hours
Fragment (50 bp)	~6 to 7 days
Mate Pair (2 × 25 bp)	~7 to 8 days
Mate Pair (2 × 50 bp)	~13 to 14 days
Barcode (5 bp)	~1 day

‡ The estimated time is for a dual slide run. Estimated times may vary due to differences in imaging time.

E SOLID™ 3 Analyzer Plumbing System Schematic



F Checklists and workflow tracking forms

This appendix covers:

Workflow checklists: set up a workflow analysis or sequencing run

	Equipment	Reagents	Preparation Steps
Deposit the beads	☐ Covaris™ S2 System ☐ SOLiD™ Bead Concentration Chart ☐ SOLiD™ Deposition Chamber ☐ SOLiD™ Slide Carrier ☐ NanoDrop™ ND-1000 ☐ 6 Tube magnetic rack ☐ Incubator (37 °C) ☐ Pipettors	☐ 1X TEX Buffer ☐ Deposition Buffer ☐ Double distilled filtered water ☐ 1.5-mL LoBind tubes ☐ 3-mm adhesive disks ☐ Filtered pipettor tips	☐ Clean and dry the SOLiD™ Deposition Chamber overnight
Install on-instrument reagents	☐ SOLiD™ 3 Analyzer ☐ 8-L reagent bottle ☐ Graduated cylinder ☐ Magnetic stir bar ☐ Pipettors	☐ 10× Instrument Buffer ☐ Storage Buffer ☐ 1× T4 DNA Ligase Buffer (Parts 1 and 2) ☐ Phosphatase Buffer ☐ Imaging Buffer (Parts 1 and 2) ☐ Cleave Solution 1 ☐ Cleave Solution 2 (Parts 1 and 2) ☐ Reset Buffer ☐ Glycerol ☐ Double-distilled water	
Install slide(s)	☐ SOLiD™ 3 Analyzer ☐ Pipettors ☐ Allen wrench ☐ SOLiD™ Slide Storage Chamber (optional)	☐ Deposition Buffer ☐ Overlay Buffer ☐ Filtered pipettor tips ☐ 70% ethanol ☐ Kimwipes® ☐ Slide Storage Buffer (optional)	
Install reagent strip(s)	☐ Tabletop centrifuge	☐ 96-well square-well storage plate	☐ Thaw appropriate reagent strip(s)

Workflow tracking: set up and perform a workflow analysis (WFA) run

Slide:				**Date:**
Run:				**Date:**
Sample Name				
Sample information (pre-WFA)				
A600				
Concentration (beads/µL)				
Deposition Volume (µL)				
WFA report				
P2_rfu				
P2_Exp				
P2_Gain				
P1#				
P2#				
P2#/P1# ratio				
N2S				
On Axis				
Titration Metric				
Sample information (post-WFA)				
P2 Concentration (beads/µL)				
Volume Left				
Beads Left				

Lot numbers	
Slide	
Deposition Buffer	
Overlay Buffer	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer Part 1	
T4 Ligase Buffer Part 2	
Phosphatase Buffer	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2 Part 1	
Cleave Solution 2 Part 2	
Reset Buffer	
Slide Storage Buffer	
Workflow Analysis Reagents	

Workflow tracking: set up a sequencing run (1-well)

Slide:	**Date:**
Run:	**Date:**

Sample Name	
Sample information	
A600	
Concentration (beads/µL)	
Deposition Volume (µL)	
Volume Left	
Beads Left	

Lot numbers	
Slide	
Deposition Buffer	
Overlay Buffer	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer Part 1	
T4 Ligase Buffer Part 2	
Phosphatase Buffer	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2 Part 1	
Cleave Solution 2 Part 2	
Reset Buffer	
Slide Storage Buffer	
Fragment Library Seq Kit	
Frag Lib Seq – Primer A	
Frag Lib Seq – Primer B	
Frag Lib Seq – Primer C	
Frag Lib Seq – Primer D	
Frag Lib Seq – Primer E	
Mate-Paired Library Seq Kit	
MP Lib Seq – Tag 1 Primer A	
MP Lib Seq – Tag 1 Primer B	
MP Lib Seq – Tag 1 Primer C	
MP Lib Seq – Tag 1 Primer D	
MP Lib Seq – Tag 1 Primer E	
MP Lib Seq – Tag 2 Primer A	
MP Lib Seq – Tag 2 Primer B	
MP Lib Seq – Tag 2 Primer C	
MP Lib Seq – Tag 2 Primer D	
MP Lib Seq – Tag 2 Primer E	
Frag Barcode Library Seq Kit	
BC Lib Seq – BC Probe	
BC Lib Seq – BC Primers	
BC Lib Seq – BC Bridge Probes	

Workflow tracking: set up a sequencing run (4-well)

Slide:				**Date:**
Run:				**Date:**
Samples 1 to 4				
Sample Name				
Sample information				
A600				
Concentration (beads/µL)				
Deposition Volume (µL)				
Volume Left				
Beads Left				

Lot numbers	
Slide	
Deposition Buffer	
Overlay Buffer	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer Part 1	
T4 Ligase Buffer Part 2	
Phosphatase Buffer	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2 Part 1	
Cleave Solution 2 Part 2	
Reset Buffer	
Slide Storage Buffer	
Fragment Library Seq Kit	
Frag Lib Seq – Primer A	
Frag Lib Seq – Primer B	
Frag Lib Seq – Primer C	
Frag Lib Seq – Primer D	
Frag Lib Seq – Primer E	
Mate-Paired Library Seq Kit	
MP Lib Seq – Tag 1 Primer A	
MP Lib Seq – Tag 1 Primer B	
MP Lib Seq – Tag 1 Primer C	
MP Lib Seq – Tag 1 Primer D	
MP Lib Seq – Tag 1 Primer E	
MP Lib Seq – Tag 2 Primer A	
MP Lib Seq – Tag 2 Primer B	
MP Lib Seq – Tag 2 Primer C	
MP Lib Seq – Tag 2 Primer D	
MP Lib Seq – Tag 2 Primer E	
Frag Barcode Library Seq Kit	
BC Lib Seq – BC Probe	
BC Lib Seq – BC Primers	
BC Lib Seq – BC Bridge Probes	

Workflow tracking: set up a sequencing run (8-well)

Slide:				**Date:**
Run:				**Date:**
Samples 1 to 4				
Sample Name				
Sample Information				
A600				
Concentration (beads/µL)				
Deposition Volume (µL)				
Volume Left				
Beads Left				
Samples 5 to 8				
Sample Name				
Sample information				
A600				
Concentration (beads/µL)				
Deposition Volume (µL)				
Volume Left				
Beads Left				

Lot numbers	
Slide	
Deposition Buffer	
Overlay Buffer	
Instrument Buffer	
Storage Buffer	
Ligase Buffer	
Phosphatase Buffer	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2 Part 1	
Cleave Solution 2 Part 2	
Reset Buffer	
Slide Storage Buffer	
Fragment Library Seq Kit	
Frag Lib Seq – Primer A	
Frag Lib Seq – Primer B	
Frag Lib Seq – Primer C	
Frag Lib Seq – Primer D	
Frag Lib Seq – Primer E	
Mate-Paired Library Seq Kit	
MP Lib Seq – Tag 1 Primer A	
MP Lib Seq – Tag 1 Primer B	
MP Lib Seq – Tag 1 Primer C	
MP Lib Seq – Tag 1 Primer D	
MP Lib Seq – Tag 1 Primer E	
MP Lib Seq – Tag 2 Primer A	
MP Lib Seq – Tag 2 Primer B	
MP Lib Seq – Tag 2 Primer C	
MP Lib Seq – Tag 2 Primer D	
MP Lib Seq – Tag 2 Primer E	
Frag Barcode Library Seq Kit	
BC Lib Seq – BC Probe	
BC Lib Seq – BC Primers	
BC Lib Seq – BC Bridge Probes	

G The Covaris™ S2 System

This appendix covers:

Operation notes

Fill the tank

Fill the tank with fresh deionized water to the proper fill line. The water should cover the visible part of the tube.

Degas the water

Degas the water for 30 minutes. To maintain degassed water, keep the pump continuously on during operation and sample processing.

Set the chiller

Set the chiller temperature to between 2 to 5 °C to ensure that the temperature reading in the water bath displays 5 °C. The circulated water chiller should be supplemented with 20% ethylene glycol.

Perform required maintenance of the Covaris™ S2 System

The Covaris S2 System requires regular maintenance to work properly. Perform the tasks in the table below (see Table 22):

Table 22 Required maintenance of the Covaris™ S2 System

Required maintenance task	Frequency to perform task
Degas water for 30 minutes prior to use	Before every use
Change water	Daily
Clean with bleach	Every two weeks

Covaris™ S2 Programs

Covalent Declump 1

Table 23 Covalent Declump 1: 1 cycle Treatment 1 followed by 1 cycle Treatment 2

	Treatment 1	Treatment 2
Duty Cycle	2%	5%
Intensity	6	9
Cycles/Burst	100	100
Time	5 sec	30 sec
Target wattage power performance estimate (W)‡	4	15

‡ Not programmed.

Covalent Declump 3

Table 24 Covalent Declump 3: 3 cycles Treatment 1 followed by 1 cycle Treatment 2

	Treatment 1	Treatment 2
Duty Cycle	2%	5%
Intensity	6	9
Cycles/Burst	100	100
Time	5 sec	30 sec
Target wattage power performance estimate (W)‡	4	15

‡ Not programmed.

H Instrument Warranty Information

This appendix covers:

Computer configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited product warranty

Applied Biosystems warrants that all standard components of the SOLiD™ 3 Analyzer, IKA® ULTRA-TURRAX® Tube Drive, the Covaris™ S2 System, APC UPS, and the recirculating chiller will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. Applied Biosystems warrants the Genomic Solutions Hydroshear will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will replace a defective Hydroshear during the warranty period. The following parts of the Hydroshear are use- replaceable and not covered by the warranty on the Hydroshear: shearing assembly, syringes, syringe adapters, syringe shields, and output tubing. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its SOLiD 3 Analyzer, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days from the date the warranty begins. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on or in the instrument are themselves warranted to be free of defects in materials and workmanship for a period of ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the operating software of the product, if any, will be free of defects in materials and workmanship under normal use. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media.

Unless indicated herein, Applied Biosystems makes no warranty whatsoever in regard to products or parts furnished by third parties, including but not limited to the non-APC- branded UPS or APC UPS, Covaris S2, Genomic Solutions Hydroshear, Recirculating Chiller, and IKA ULTRA-TURRAX purchased or obtained from a third party. Such products or parts will be subject to the warranties, if any, of their respective manufacturers to the extent they are 'transferable or otherwise available to Applied Biosystems' buyer.

Applied Biosystems at its sole discretion may refuse to provide buyer with support or service for buyer's use of Covaris S2 in a method not described in a SOLiD System protocol.

Applied Biosystems does not warrant that the operation of the instrument or its operating software will be uninterrupted or be error-free.

Warranty period effective date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for hardware and software installed by Applied Biosystems personnel. For all hardware and software installed by the buyer or anyone other than Applied Biosystems, and for all other products, the applicable warranty period begins the date the product is delivered to the buyer.

Warranty claims

Warranty claims must be made within the applicable warranty period, or, for chemicals or other consumable products, within thirty (30) days after receipt by the buyer unless otherwise specified in the documentation accompanying the product.

Warranty exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specifications or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; modification or repair of the product not authorized by Applied

Biosystems; relocation or movement of the instrument by buyer or by any third party not acting on behalf of Applied Biosystems; or intrusive activity, including without limitation, computer viruses, hackers or other unauthorized interactions with instrument or software that detrimentally affects normal operations.

Parts in contact with any liquid are considered wetted and may be deemed user-replaceable and not be covered by the above warranties, including, but not limited to, seals, filters, gaskets, shearing assemblies, valves, syringes, syringe adapters, syringe shields, and output tubing.

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Damages If shipping damage to the product is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims After a damage inspection report is received by Applied Biosystems, Applied Biosystems will process the claim unless other instructions are provided.

Returns Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the address designated by the Applied Biosystems representative.

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J Safety

This appendix covers:

Instrumentation safety

Symbols on instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or with text that explains the relevant hazard (see "Safety labels on instruments" on page 160). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.

Symbol	Description
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Indicates the presence of a radiological hazard and to proceed with appropriate caution.
	Indicates the presence of a slipping hazard and to proceed with appropriate caution.
	Indicates the presence of an ultraviolet light (in the instrument?) and to proceed with appropriate caution.

Environmental symbols on instruments

The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See **www.appliedbiosystems.com** for a list of customer service offices in the European Union.

Safety labels on instruments

The SOLiD™ 3 System contains warnings at the locations listed in Table 25.

Table 25 Where to find safety labels on the SOLiD™ 3 System

Safety label	Located by...
CAUTION! Hot Surfaces.	Flow cell cover and inside arc lamp box
CAUTION! Replace only with CERMAX lx300f, 300 W Lamp	Arc lamp box cover
WARNING! HOT! Do not remove lamp until 15 minutes after disconnecting supply!Surfaces.	Arc lamp box cover
CAUTION! Crush/Pinch hazard	Syringe pump

General instrument safety

WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and lifting the instrument

CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and lifting stand-alone computers and monitors

WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs). See "About MSDSs" on page 167.

Cleaning or decontaminating the instrument

CAUTION! Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Physical hazard safety

Ultraviolet light

WARNING! ULTRAVIOLET LIGHT HAZARD. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and always prevent others from UV exposure. Follow the manufacturer's recommendations for appropriate protective eyewear and clothing.

Moving parts

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Solvents and pressurized fluids

WARNING! PHYSICAL INJURY HAZARD. Always wear eye protection when working with solvents or any pressurized fluids.

- Be aware that polymeric tubing is a polymeric material. Use caution when working with any polymer tubing that is under pressure.

  Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40 mL/min) may cause a static charge to build up on the surface of the tubing. Electrical sparks may result.

Electrical safety

WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the SOLiD™ 3 System without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

WARNING! ELECTRICAL HAZARD. Grounding circuit continuity is required for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

WARNING! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

WARNING! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The SOLiD™ 3 System has an installation (overvoltage) category of II, and is classified as portable equipment.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- Canadian EMC standard
- European safety and EMC standards
- Australian EMC Standards

U.S. and Canadian safety standards

The SOLiD™ 3 System has been tested to and complies with standard:

UL 61010-1/CSA C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

Canadian EMC standard

This instrument has been tested to and complies with ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."

European safety and EMC standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

EN 61010-2-081, "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 167.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.

- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Safety alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see "Safety alert words" on page vii.

Chemical alerts

General alerts for all chemicals

Causes eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Specific chemical alerts

DANGER! CHEMICAL HAZARD. 2-Butanol is a flammable liquid and vapor. Causes eye, skin, and respiratory tract irritation. Harmful if inhaled. Keep away from heat, sparks and flame. Avoid breathing vapor. Do not get in eyes or on skin. Use only with adequate ventilation. Avoid contact with eyes, skin and clothing.

WARNING! CHEMICAL HAZARD. Cleave Solution 1. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death. Instrument waste when spilled should be cleaned up immediately to prevent slipping injuries. Instrument waste can be classified as Hazard Class 9 (silver and mercaptans) with an US EPA Code:

D011 (silver). However, at the relatively low mg/L concentrations found in this waste stream, shipments of lab-quantity volumes would be well below the RQ – meaning that "RQ" would not need to be included in the DOT description on the hazardous waste shipping manifest.

WARNING! CHEMICAL HAZARD. Cleave Solution 2.

WARNING! CHEMICAL HAZARD. Deposition Buffer.

CAUTION! CHEMICAL HAZARD. Glycerol may cause eye, skin, and respiratory tract irritation. May be harmful if swallowed.

WARNING! CHEMICAL HAZARD. 10× Instrument Buffer.

WARNING! CHEMICAL HAZARD. Reset Buffer is harmful if inhaled, swallowed, or absorbed through skin. It causes eye, skin, and respiratory tract irritation.

WARNING! CHEMICAL HAZARD. Sodium Acetate Solution.

WARNING! CHEMICAL HAZARD. 20× SSPE Solution.

WARNING! CHEMICAL HAZARD. Storage Buffer.

Glossary

barcode	Unique sequence identifier added to the sample during library construction
barcoded fragment library	Fragment library with a barcode sequence appended to the 3′ end of the sheared DNA fragments
BC tag	Barcode tag
bead count	The number of beads detected in a panel
bead signal	The average signal intensity of every pixel associated with a bead
best beads	Number of beads that meet a stringent set of criteria based on spectral purity and intensity
F3 tag	Tag to be sequenced using primers specific to the P1 Adaptor sequence
fragment library	Library consisting of a sheared DNA fragment with P1 and P2 Adaptors ligated to the 5′ end and 3′ end respectively
good beads	The number of beads that meet criteria (less stringent than best beads criteria) based on spectral purity and intensity
image signal	The average signal intensity of every pixel whether or not it is associated with a bead or not
internal adaptor	Double-stranded oligonucleotide located between two tags to be sequenced
library	Set of DNA tags prepared from the same biological sample to be sequenced on the SOLiD™ System
mappable beads	Beads with template that map back to the reference genome
mate-paired library	Library consisting of two DNA tags a known distance apart linked by an internal adaptor with P1 and P2 Adaptors ligated to the 5′ end and 3′ end, respectively
multiplexing	Method to analyze multiple biological samples in a single spot using barcodes
N2S plot	Plot indicating noise-to-signal for each dye
on-axis beads	The frequency of template-positive beads that meet a defined threshold of spectral purity and signal intensity after a single ligation step
optimal titration point	Library template concentration that gives the best sequencing results

P1 Adaptor	Double-stranded oligonucleotide ligated at the 5′ end of the library
P2 Adaptor	Double-stranded oligonucleotide ligated at the 3′ end of the library
P2#/P1# ratio	The frequency of template-positive beads (P2#) relative to total beads (P1#) deposited on the slide; this metric is also referred to as "% P2 Positive" value
P2-positive beads	SOLiD™ P1 DNA beads with fully extended and amplified template
pulse-spin	Place the tube in a picofuge and spin for a few seconds to bring down any beads or liquid stuck on the walls of the tube
R3 tag	Tag to be sequenced using primers specific to the Internal Adaptor sequence
remove the supernatant	Use a pipette to carefully remove the liquid from the tube without disturbing any beads
resuspend the beads	The beads can be resuspended in one of two ways: • Gently pipette the solution up and down until the beads are suspended. Using a slower speed to aspirate and expel the solution minimizes the amount of beads that stick to the inside of the pipette tip. • Vortex the solution until all of the beads are suspended. Place the beads in a picofuge and pulse-spin for a few seconds to bring down any beads stuck on the walls of the tube. Do not over-spin the beads or the beads aggregate into a pellet.
Satay plot	Indicator of spectral purity and signal intensity of the beads
sonicate the beads	Place the tube containing the beads in the appropriate tube holder, then place in the Covaris™ S2 System; afterwards, run the appropriate program
tag	A length of DNA to be sequenced
templated bead preparation	Process of adding library template to beads by emulsion PCR, enriching the beads to remove beads without template, then modifying the 3′ end of the template on the beads to prepare for bead deposition and sequencing
templated beads	SOLiD™ P1 DNA Beads with amplified library template attached
titration	Library template concentration used to prepare an emulsion
titration metric	Product of % P2 positive beads and the On-Axis beads; the titration that generates the highest titration metric value is the optimal titration point for a given library
usable beads	Number of beads that are called during color-calling
workflow analysis (WFA) run	Type of run on the SOLiD™ system in which a small portion of templated beads are deposited and analyzed to test for templated bead quality

Related documentation

Document	Part number	Description
Applied Biosystems SOLiD™ 3 System Library Preparation Guide	4407413	Describes how to prepare fragment and mate-paired libraries for templated bead preparation and sequencing on the SOLiD™ 3 System.
Applied Biosystems SOLiD™ 3 System Library Preparation Quick Reference Card	4407414	Provides brief, step-by-step procedures for preparing libraries.
Applied Biosystems SOLiD™ 3 System Templated Bead Preparation Guide	4407421	Describes how to prepare templated beads by emulsion PCR (ePCR), required before sequencing on the SOLiD™ 3 System.
Applied Biosystems SOLiD™ 3 System Templated Bead Preparation Quick Reference Card	4407429	Provides brief, step-by-step procedures for preparing templated beads by emulsion PCR (ePCR), required before sequencing on the SOLiD™ 3 System.
Applied Biosystems SOLiD™ 3 System Instrument Operation Quick Reference Card	4407431	Provides brief, step-by-step procedures for loading and running the SOLiD™ 3 System.
SOLiD™ 3 System Site Preparation Guide	4386998	Provides all the information that you need to set up the SOLiD™ 3 System.
Applied Biosystems SOLiD™ 3 System SETS Software Getting Started Guide	4389302	Describes how to monitor the run, modify run settings, and/or perform data analysis for the SOLiD™ 3 System.
Applied Biosystems SOLiD™ 3 System Instrument Control Software (ICS) Help	—	Provides convenient information for setting up a run on the SOLiD™ 3 System (see the Instrument Control Software).
SOLiD™ Analysis Tools (SAT) User Guide	4392959	Provides in-depth information on sequencing analysis with the SOLiD™ 3 System.

Note: For additional documentation, see "How to obtain support" on page ix.

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating *only* to documentation. To order documents, download PDF files, or for help with a technical question, see "How to obtain support" on page ix.

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