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by life technologies

Applied Biosystems SOLiD™ 4 System Instrument Operation Guide

April 2010

Library Preparation | Templated Bead Preparation | **Instrument Operation**

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

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Contents

Safety information

Note: For general safety information, see this section and Appendix J, "Safety" on page 185. 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 186).

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 195.

IMPORTANT! For the SDSs 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 Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(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.
	**CAUTION!** Potential overhead hazard.	**ATTENTION!** Présence d'objet pouvant heurter la tête.

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, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

1 Introduction

SOLiD™ 4 System run types

On the Applied Biosystems SOLiD™ 4 System, you can perform a workflow analysis (WFA) run and a sequencing run (see Table 1).

Table 1 Run types on the SOLiD™ 4 System

	WFA	Sequencing
Purpose	• Assess various preparations of templated beads to determine the potential quality of sequence data • Evaluate the fraction of P2-positive beads • Use as a tool to determine the deposition density for sequencing slides	Generate sequencing data for fragment or mate-paired libraries
Run summary	• P1 and P2 bead counting • Single ligation cycle • Report generation	Multiple ligation cycles for each of 5 primers, resulting in up to 50 bases of sequence information per tag‡
Estimated run time§	~4 to 5 hours	• ~4 to 14 days
Deposition chamber	4-well	• 1-well • 4-well • 8-well
Number of beads	15 million beads per well	• 708 million beads per well (1-well) • 128 million beads per well (4-well) • 56 million beads per well (8-well)

‡ One tag for fragment sequencing and two tags for mate-pair sequencing and paired-end sequencing. The reverse read tag in paired-end sequencing is currently limited to 25 bases. Only 5 or 10 bases of the barcode tag need to be sequenced.

§ Total run time for dual slide run. Run time varies with read length and type of sequencing run. Times may deviate depending on imaging time.

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

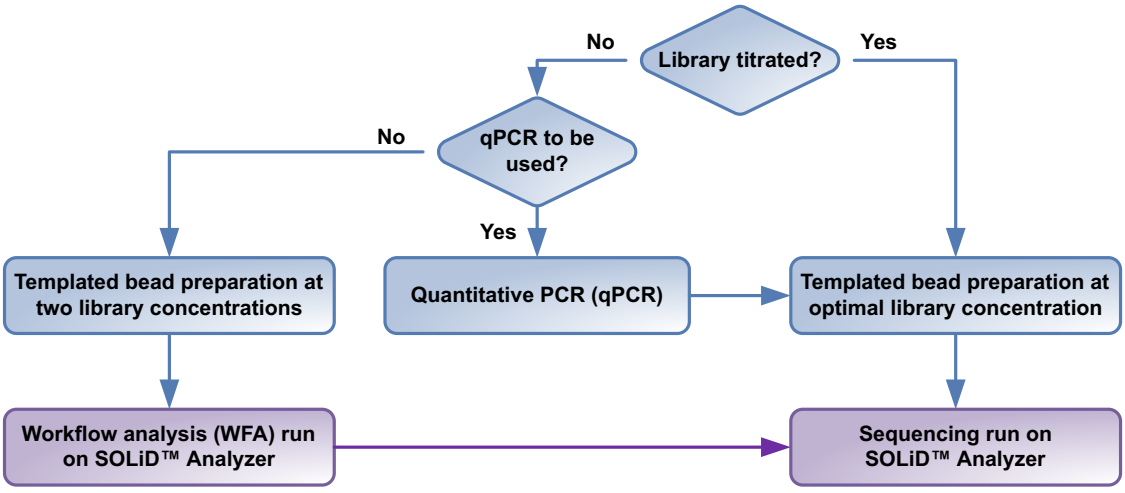


Figure 1 Relationship between the workflow analysis (WFA) and sequencing runs on the SOLiD™ System.

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 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 19, then set up and monitor the run according to the procedure in Chapter 3, “Set Up, Control, and Monitor the Run” on page 53.

Sequencing run

There are five types of sequencing runs that can be performed on the SOLiD™ 4 System: fragment sequencing, paired-end sequencing, mate-pair sequencing, multiplex fragment sequencing, and multiplex paired-end sequencing (see Figure 2).

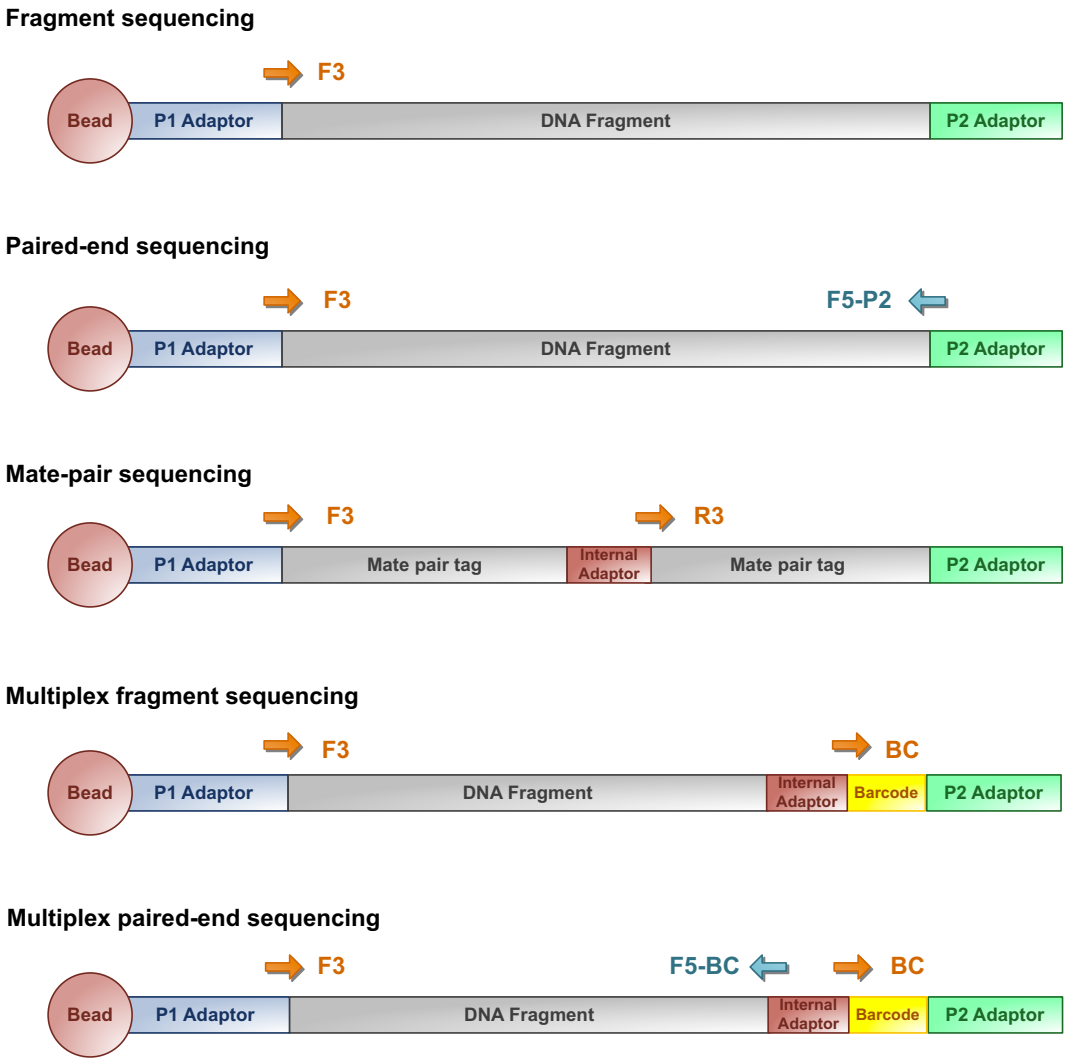


Figure 2 Types of sequencing runs.

The type of sequencing run depends on the type of library to be sequenced (see Table 2).

Table 2 Types of sequencing runs possible for each library type

Library type	Possible sequencing run type(s)
Fragment library	• Fragment sequencing • Paired-end sequencing
Mate-paired library	• Mate-pair sequencing
Barcoded fragment library	• Multiplex fragment sequencing • Multiplex paired-end sequencing

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.)

Compared to terminator-based sequencing chemistry, with SOLiD™ System 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 for each tag (for examples, see Figure 3).

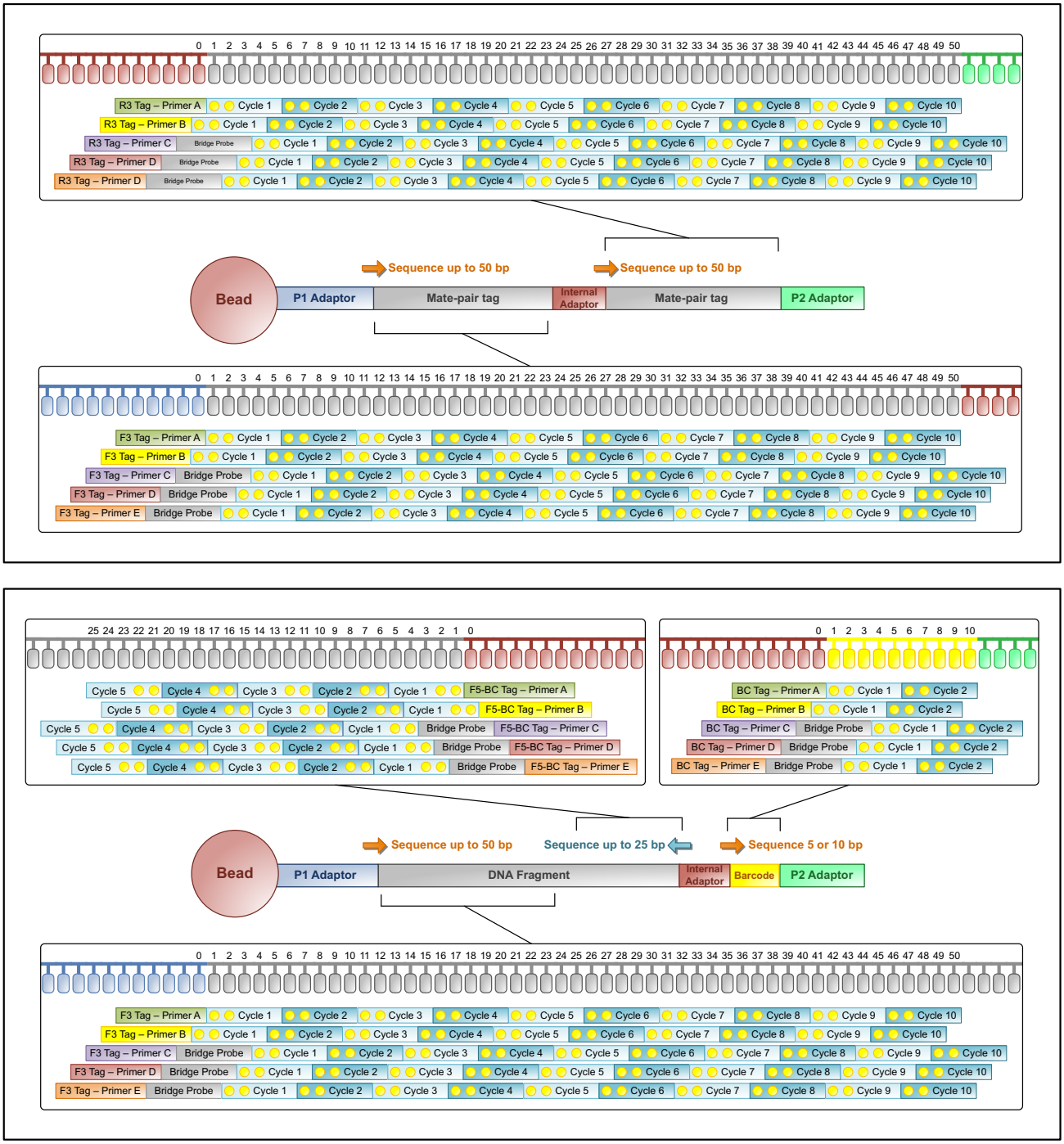


Figure 3 Di-base sequencing in a mate-pair sequencing run (top) and in a multiplex paired-end sequencing run (bottom).

The workflow for each type of sequencing run is shown in Figure 4.

Figure 4 Sequencing run workflows.

Software operation and data analysis

The SOLiD™ 4 System comprises multiple complementary analysis software components that complete primary analysis (image acquisition, signal processing, color calling, and quality control) and secondary analysis (alignment to a reference genome, SNP identification, and base calling) of fragment, mate-pair, and paired-end sequencing experiments.

For information describing the relationship between ICS (SOLiD™ Instrument Control Software), SETS (SOLiD™ Experimental Tracking System), and BioScope Software, refer to the *Applied Biosystems SOLiD™ 4 Software Operation and Data Analysis Quick Reference Guide* (PN 4448432).

For additional secondary and tertiary analysis tools, visit the SOLiD™ Software Development Community website (**http://solidsoftwaretools.com**). You can integrate standalone tools from the SOLiD™ Software Development Community with BioScope to perform more automated analysis. For details, refer to the *BioScope™ Software for Scientists Guide* (PN 4448431).

2 Prepare and Install Slides and Reagents

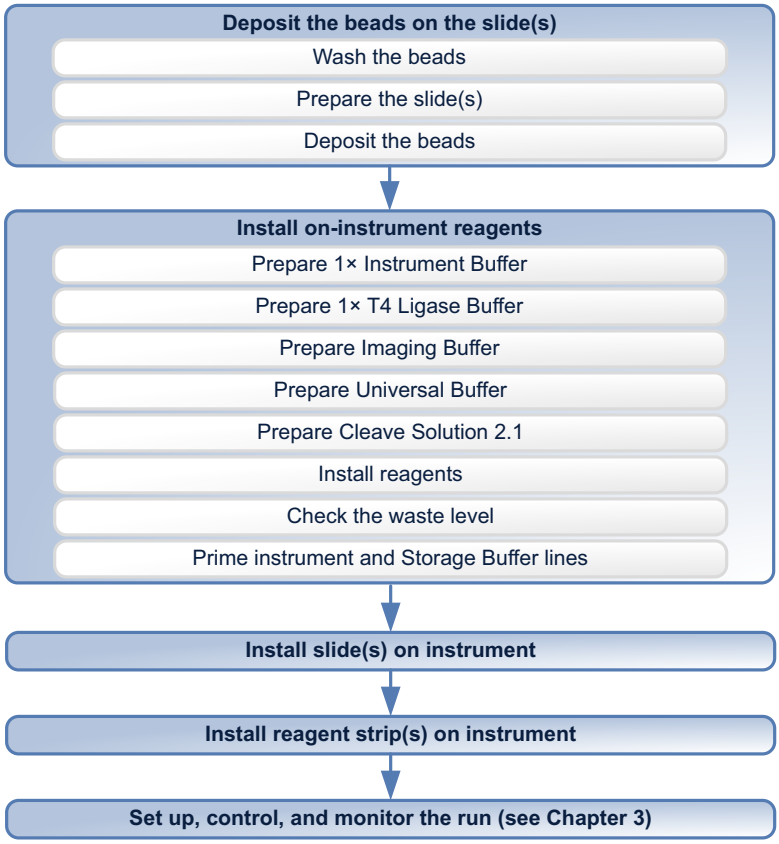
This chapter covers:

Materials and equipment required

See Appendix A, "Set up and perform a workflow analysis (WFA) run" on page 102 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 105 for a list of equipment, kits, and consumables necessary to set up a sequencing run.

Workflow



Workflow overview

Deposit the beads on the slide(s)

For a WFA run, the beads are quantitated using the *Applied Biosystems SOLiD™ Bead Concentration Chart* (PN 4415131), and 15 million beads are deposited in one well of a 4-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 3).

Table 3 Three deposition chamber designs

Deposition chamber	Number of image panels
1-Well	2357
4-Well	426 per well
8-Well	186 per well

Install on-instrument reagents

Prepare 1X Instrument Buffer using glycerol and 10X Instrument Buffer provided in the SOLiD™ Instrument Buffer Kit. You may formulate 1X Instrument Buffer in 8-L batches as needed or prepare it in larger volumes and store at 4 °C until ready for use.

Prepare T4 Ligase Buffer, Imaging Buffer, Universal Buffer, and Cleave Solution 2.1 by combining the two parts provided in the SOLiD™ ToP Instrument Buffer Kit.

Cool the chiller block prior to installation of buffers.

Because the tubing from the Instrument and Storage Buffer bottles to the flowcell is long, prime the lines before installing the slides in the flowcell.

Install slide(s) on the instrument

Remove the slide from the Deposition Chamber and prepare it for installation on the instrument. Each flowcell can be loaded with the slide *independently* of each other.

Install reagent strip(s) on the instrument

Install the workflow analysis or sequencing reagent strips on the reagent strip chiller block. Cool the chiller block prior to installation of reagent strips.

Tips

General

- Prior to deposition, store the slides at –20 °C to ensure optimal bead deposition and to minimize loss of P2-enriched beads.
- Remove the slides from storage at –20 °C 5 minutes prior to use.

  If only one slide from the two-pack is used, place the remaining slide back into the box and place the box back into the resealable pouch. The pouch should be resealed and stored at–20 °C. The stored slide should be used within 1 month.
- Use Eppendorf LoBind Tubes to perform all steps requiring 0.5-mL, 1.5-mL, and 2.0-mL tubes. LoBind Tubes from other vendors may have a chemical coating that can have adverse effects on bead deposition.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols (160 × g, 167 × g).

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™ 4 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 on the slides

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 168). 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 *Applied Biosystems SOLiD™ Bead Concentration Chart* (PN 4415131) to estimate the bead concentration (see Figure 5).

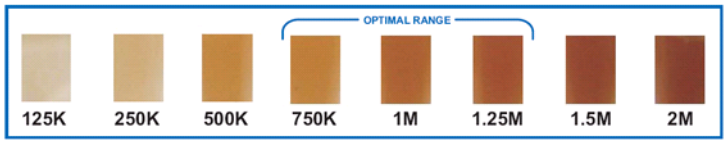


Figure 5 The SOLiD™ Bead Concentration Chart. For best results, use the *Applied Biosystems SOLiD™ Bead Concentration Chart* (PN 4415131), supplied separately.

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 5 and Figure 6).

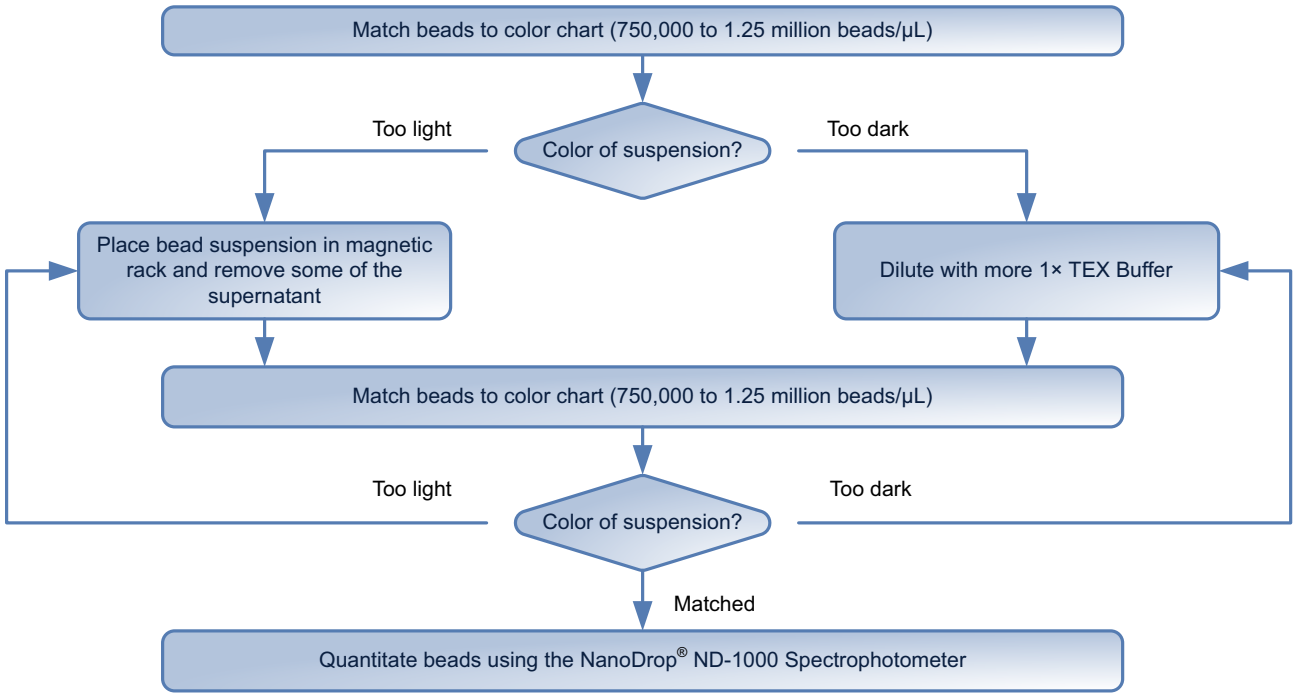


Figure 6 SOLiD™ Bead Concentration Chart workflow.

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™ 4 System Templated Bead Preparation Guide* (PN 4448378)].

5. Transfer the appropriate number of beads to a 1.5-mL LoBind Tube and store the remaining beads at 4 °C.

Table 4 Number of beads to use according to the type of run and deposition chamber

Type of run	SOLiD™ Deposition Chamber	Target number of P2-positive beads per well‡	Maximum threshold number of beads per well‡
WFA	4-Well	15 million	30 million
Sequencing	1-Well	708 million	778 million
Sequencing	4-Well	128 million	141 million
Sequencing	8-Well	56 million	61 million

‡ Note: The targeted bead deposition density is 300,000 P2-positive beads per panel, and the maximum threshold bead deposition density for all beads (P2-positive or not) is 330,000 beads per panel. Exceeding the maximum threshold number of beads per well may result in decreased sequence quality. Bead deposition densities may deviate from the targeted bead deposition density of 300,000 P2-positive beads per panel due to variability in the bead quantitation process. The calculated bead concentration based on the WFA report is the most accurate because it specifically measures the concentration of P2-positive beads (see "Determine the bead deposition density for a sequencing run" on page 66). If WFA run results are not available, it is possible to estimate the bead concentration using the SOLiD™ Bead Concentration Chart and NanoDrop® spectrometer measurement. It is recommended you target an additional overage volume to account for measurement variability, especially for off-instrument bead quantitation. Typical overages can range as high as 20% to 50% and can vary with the library, operator, sample type, and other factors.

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. Remove the SOLiD™ XD slide(s) from –20 °C storage to equilibrate at room temperature.

8. Resuspend the beads in 400 µL of SOLiD™ XD Slide Deposition Buffer v2. Vortex thoroughly, then pulse-spin.

   IMPORTANT! Do not pellet the beads.

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

   Note: It may take longer than 1 minute for the solution to clear.

10. Repeat steps step 8 and step 9 *twice*.

11. Resuspend the beads in the volume of SOLiD™ XD Slide Deposition Buffer v2 listed in Table 5 (calibration of the SOLiD™ Deposition Chamber with the SOLiD™ 4 Slide Carrier or SOLiD™ Opti Slide Carrier).

IMPORTANT! For upgraded systems with new slide carriers, the SOLiD™ Deposition Chamber should be recalibrated using the SOLiD™ 4 Slide Carrier to determine the exact deposition volume.

Table 5 Approximate Deposition Buffer volumes

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

Note: Deposition Buffer volume is dependent on the slide carrier as well as the Deposition Chamber, and volumes vary from slide carrier to slide carrier. In addition, residual SOLiD™ XD Slide Deposition Buffer v2 that remains on the surface of the slide after washing may alter the volume slightly.

Prepare the slide(s)

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

   a. Clean the SOLiD™ Deposition Chamber by rinsing it with MilliQ water.

   IMPORTANT! For upgrade customers, the SOLiD™ Deposition Chambers and SOLiD™ Opti Slide Carriers must be cleaned thoroughly using a sonicator or Extran 300 detergent before use with SOLiD™ XD Slides to remove all traces of glycerol (see "Clean the SOLiD™ Deposition Chamber using a sonicator" on page 114 or "Clean the SOLiD™ Deposition Chamber using Extran 300" on page 116). Once all traces of glycerol have been removed from the SOLiD™ Deposition Chamber, it should not be exposed to glycerol-based solutions such as Overlay Buffer (from previous versions of SOLiD™) or Slide Storage Buffer.

   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.

2. Insert a new slide into the SOLiD™ 4 or Opti Slide Carrier (see Figure 7).

   IMPORTANT! Do not touch the slide surface.

   IMPORTANT! The SOLiD™ 4 and Opti Slide Carriers are painted blue across the side (see Figure 7). If you are using the SOLiD™ ToP reagent strips for sequencing, you must use either the SOLiD™ 4 Slide Carrier or the SOLiD™ Opti Slide Carrier. Using a different slide carrier may lead to run failure.

   a. Move the retainers out so that the slide can fit into the SOLiD™ 4 or Opti Slide Carrier. To do this, push down on the two spring knobs in the SOLiD™ 4 or Opti Slide Carrier and slide the knobs towards the outside edges of the carrier (see Figure 7 A).

   b. Place the slide against the alignment nubs in the SOLiD™ 4 or Opti Slide Carrier.

   c. Ensure that the slide is precisely positioned in the SOLiD™ 4 or Opti Slide Carrier and then slide the retainers inward until they hold the slide in position (see Figure 7 B).

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

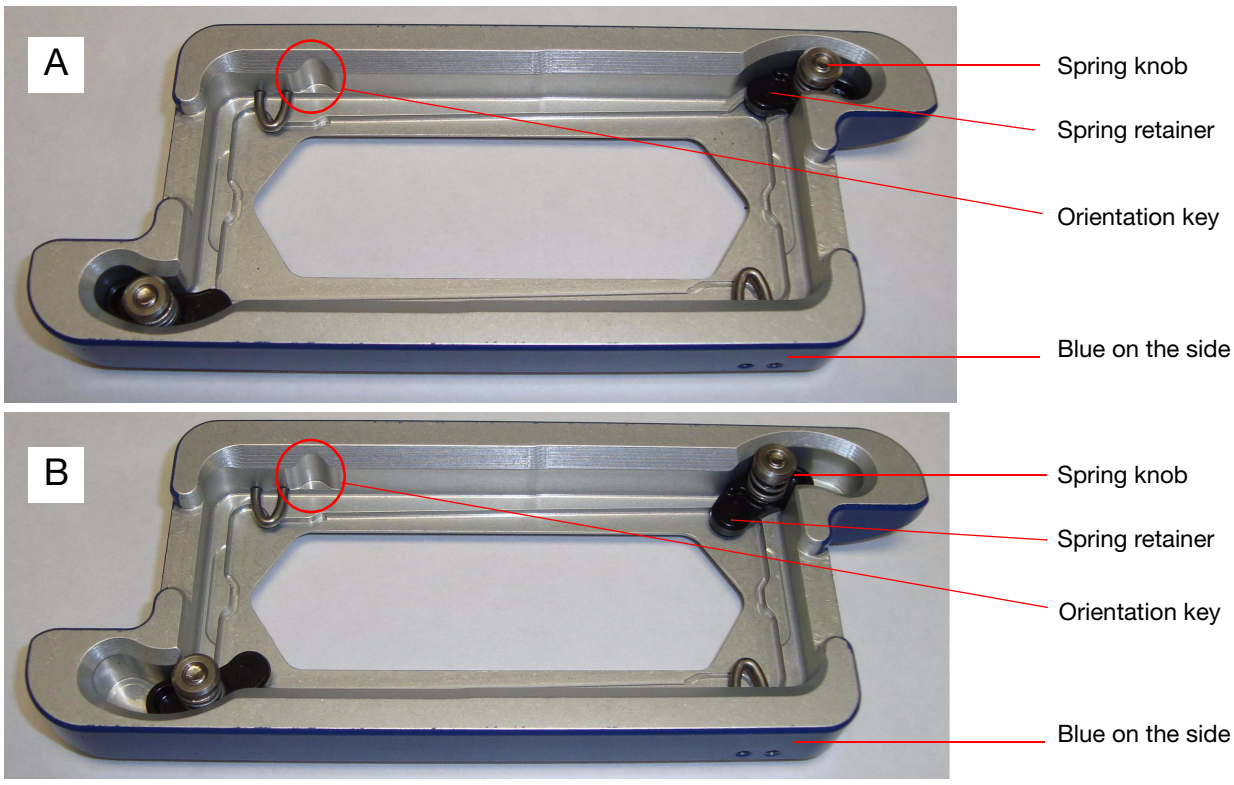


Figure 7 (A) The SOLiD™ 4 Slide Carrier with spring knobs and retainers pushed out and (B) with spring knobs and retainers pushed in (the slide is held under the retainers).

3. Pipet 3 mL SOLiD™ XD Slide Prep Reagent over the surface of the slide. Make sure the entire slide is covered with SOLiD™ XD Slide Prep Reagent (see Figure 8).

4. Let the reagent sit for 2 minutes.

   IMPORTANT! Do not let the slide dry out.

Figure 8 Cover the slide completely with SOLiD™ XD Slide Prep Reagent and let sit for 2 minutes—do not let it dry out.

5. Lay a Kimwipes® wiper (or other lab paper towel) on the lab bench. Hold the slide in the carrier perpendicular to the lab bench, and decant the SOLiD™ XD Slide Prep Reagent off of the surface of the slide onto the Kimwipes® wiper or paper towel. If needed, carefully put the towel in the corner of the slide to soak up excess reagent (see Figure 9).

   IMPORTANT! Do not let the Kimwipes® wiper touch the surface of the slide.

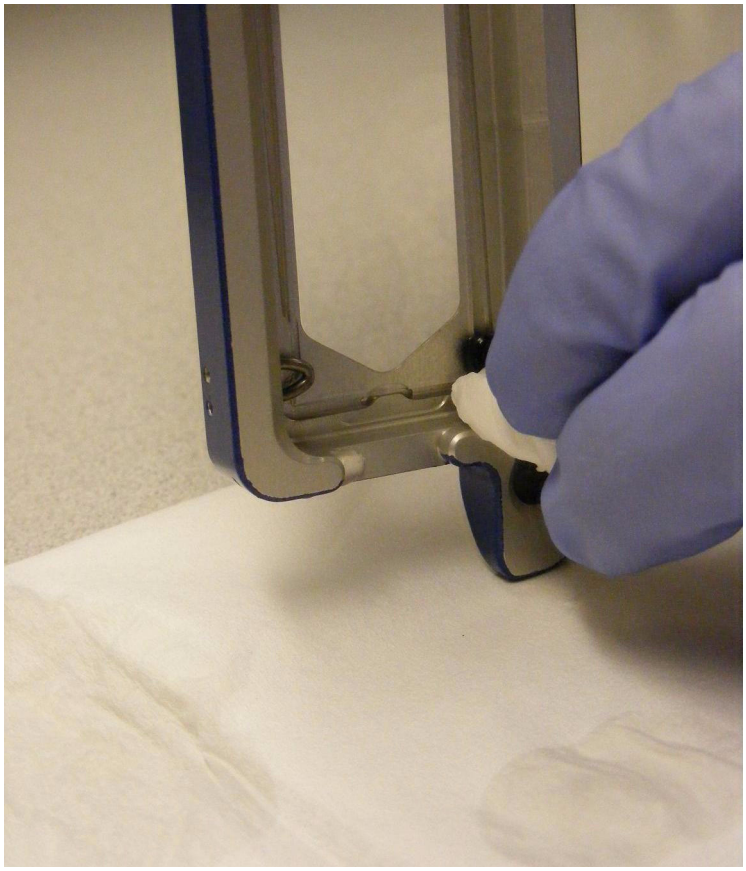


Figure 9 Carefully use a lab towel to soak up excess SOLiD™ XD Slide Prep Reagent, but do not touch the slide surface.

6. Pipet 3 mL SOLiD™ XD Slide Deposition Buffer v2 over the surface of the slide. Make sure the entire slide is covered with SOLiD™ XD Slide Deposition Buffer v2.

7. Decant the SOLiD™ XD Slide Deposition Buffer v2 off of the surface of the slide onto a Kimwipes® wiper or paper towel (as described in step 5).

   Note: A minimal amount of SOLiD™ XD Slide Deposition Buffer v2 on the slide surface after washing will not have a detrimental effect on deposition.

8. Repeat step 6 and step 7 twice.

9. Immediately place the SOLiD™ 4 or Opti Slide Carrier assembly into SOLiD™ Deposition Chamber base, then place the appropriate SOLiD™ Deposition Chamber lid on top (see Figure 10).

   IMPORTANT! After washing, do not let the slide sit for more than 10 minutes before depositing beads.

   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 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.

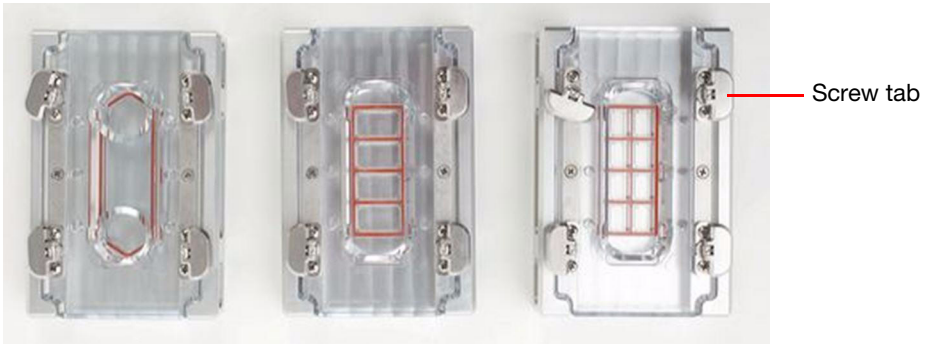


Figure 10 SOLiD™ Deposition Chambers: 1-Well, 4-Well, and 8-Well.

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

11. Twist the tabs flat.

Deposit the beads

1. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System (for program conditions, see "Covalent Declump 3" on page 169). Afterwards, pulse-spin, but do not pellet the beads.

2. Repeat step 1.

3. Use a pipettor with an appropriate tip to pipet 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.

4. Perform one of the following sequences:

If the SOLiD™ Deposition Chamber has…	Then perform steps…
1 well	5, 6, and 9 to 11
4 or 8 wells	7 to 11

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

6. Carefully pipet 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. Proceed to step 9.

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

8. Repeat step 7 to fill each of the remaining wells with each sample of templated beads. Note each sample's well position relative to the slide orientation.

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

10. Centrifuge the slide in the SOLiD™ Deposition Chamber at 167 × g for 10 minutes.

11. Incubate the SOLiD™ Deposition Chamber at room temperature for 1 hour.

Install on-instrument reagents

Note: For information about recommended fill volumes for on-instrument reagents, see "Recommended fill volumes for on-instrument reagents" on page 148.

Prepare 1× Instrument Buffer

Note: Prepare buffers just prior to use on the SOLiD™ 4 Analyzer.

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

   IMPORTANT! Regular cleaning of the 8-L Instrument Buffer bottle is required for every run (see "Clean the Instrument Buffer bottle" on page 118). Failure to clean the Instrument Buffer bottle regularly may allow microbial contaminants to proliferate in the system. Never top off the Instrument Buffer bottle.

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™ 4 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 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 Universal Buffer

1. Transfer the contents of the Universal Buffer Part 1 bottle to the Universal 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.

3. After mixing, apply the Universal Buffer label supplied with the bottle.

Prepare Cleave Solution 2.1

1. Transfer the contents of the Cleave Solution 2.1 Part 1 bottle to the contents of the Cleave Solution 2.1 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. If needed, flush the tubing of the SOLiD™ 4 Analyzer fluidics system (see "Flush the fluidic lines" on page 120).

   Note: If the SOLiD™ 4 Analyzer is in continuous use, the fluidics system should be flushed every three months. If the SOLiD™ 4 Analyzer will sit idle for more than two weeks, the fluidics system should be flushed and the instrument powered down with the fluidics lines empty.

2. Double-click the SOLiD™ **Instrument Control Software** icon to launch the SOLiD™ Instrument Control Software, if it is not already open.
3. Under the System Status menu, select **Cooling** from the Chiller drop-down menu (see Figure 11).

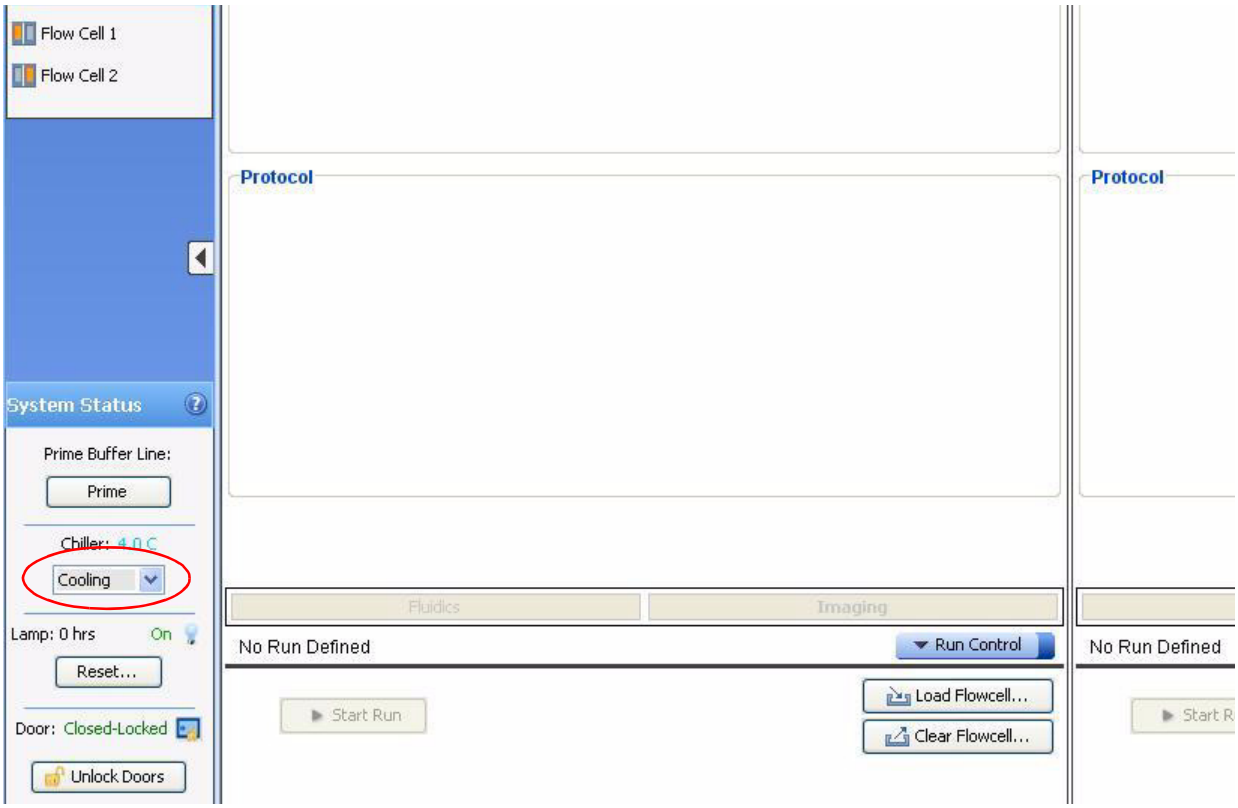


Figure 11 Select Cooling to cool the flowcell.

4. After the chiller temperature is <10 °C, install the prepared 1✕ Instrument Buffer and Storage Buffer into the appropriate positions in the cabinet (see Figure 12).

> **CAUTION! POTENTIAL OVERHEAD HAZARD**. Use caution when working inside the cabinet.

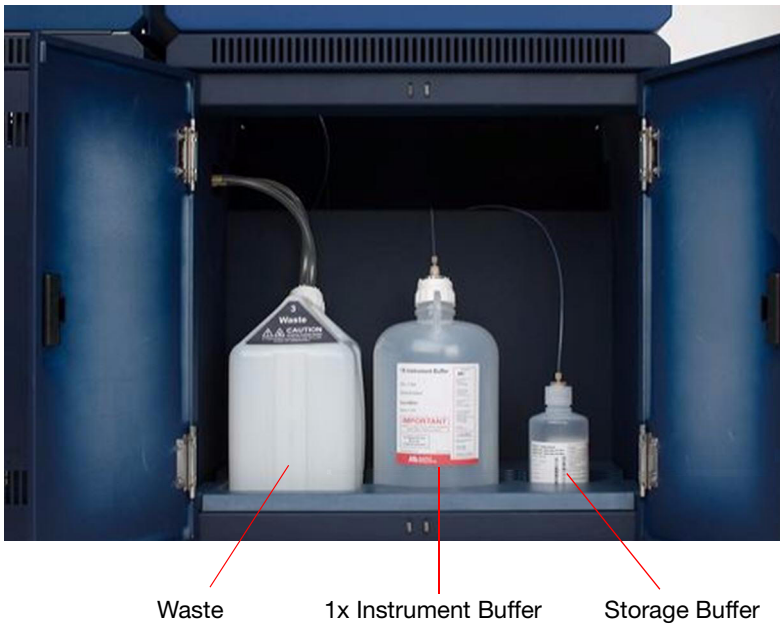


Figure 12 Positions of buffers and waste in the cabinet.

5. Install Cleave Solution 1, prepared Cleave Solution 2.1, and Reset Buffer in the appropriate positions on the side of the instrument (see Figure 13). Make sure that the tubes on the caps of the reagent bottles are fully screwed on.

> **CAUTION! POTENTIAL OVERHEAD HAZARD**. Use caution when working inside the cabinet. Keep the instrument side door closed over the Cleave Solution and Reset Buffer bottles.

Figure 13 Positions of reagent bottles on the side of the instrument.

6. Install the prepared Imaging Buffer, prepared 1X T4 Ligase Buffer, and prepared Universal Buffer into the appropriate positions in the chiller block (see Figure 14). Make sure that the tubes on the caps of the reagent bottles are fully screwed on.

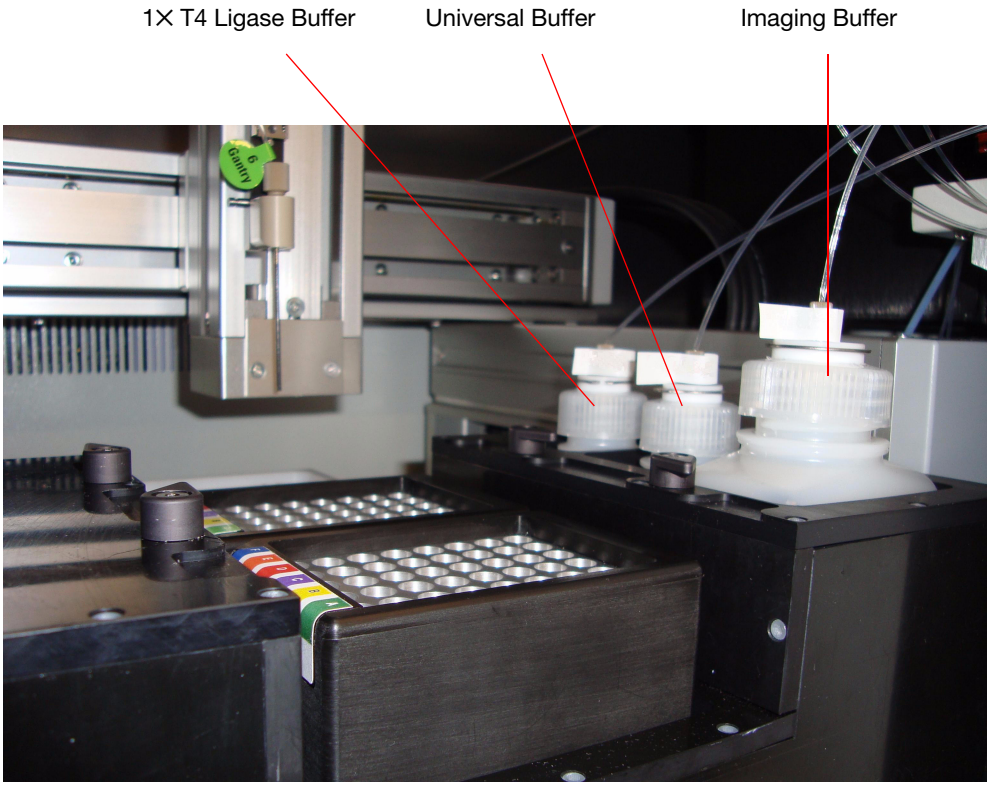


Figure 14 Positions of buffer bottles in the chiller block.

Check the waste level

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 15).

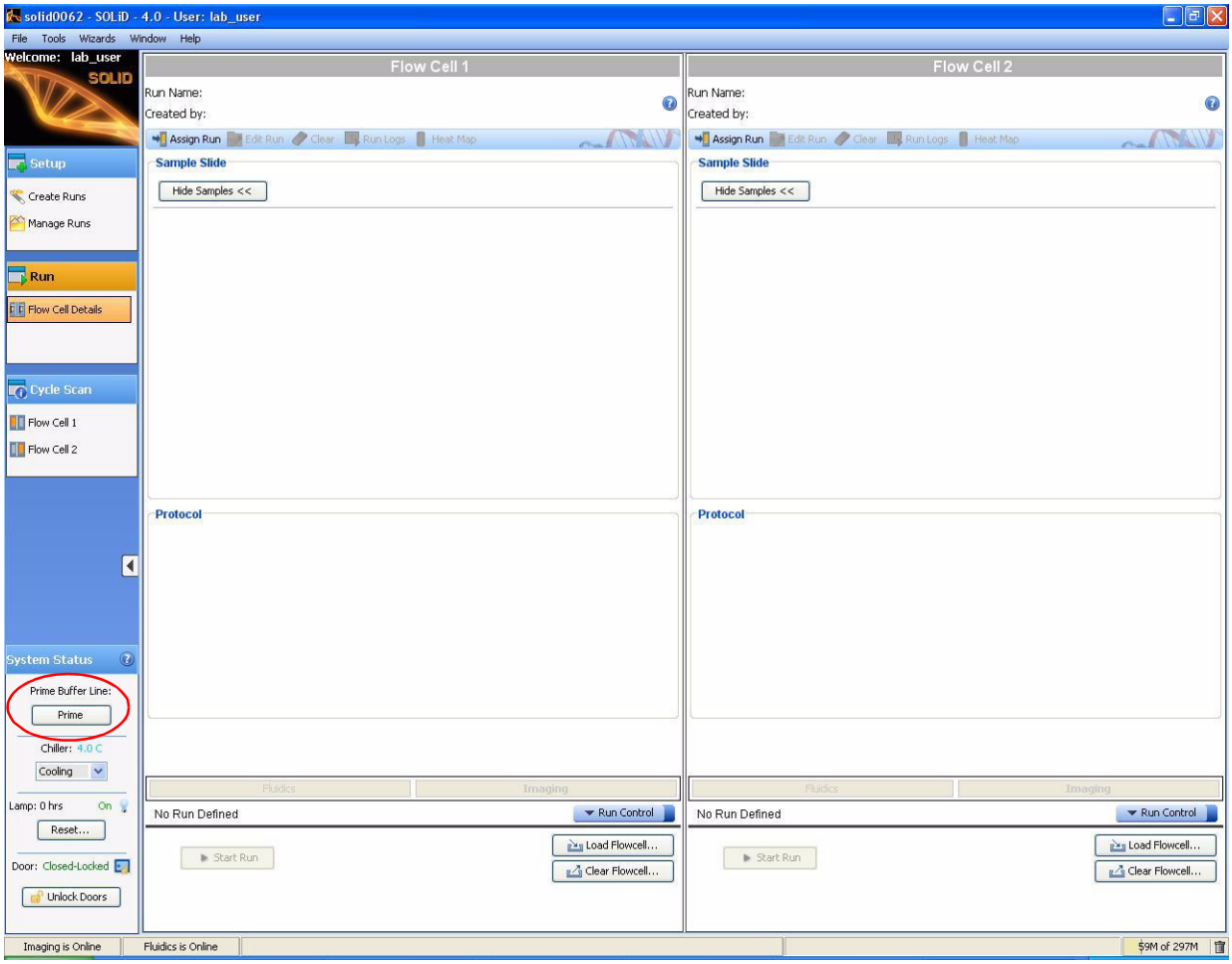


Figure 15 Click Prime to prime the instrument and storage buffer lines.

2. During priming, open the middle front door of the SOLiD™ 4 Analyzer to check that the syringe is filled with buffer when the plunger is at the aspiration stage (see Figure 16). 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 16 Syringe on the SOLiD™ 4 Analyzer.

Install slide(s) on the instrument

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 SOLiD™ XD Slide Deposition Buffer v2 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 SOLiD™ XD Slide Deposition Buffer v2. Fresh SOLiD™ XD Slide Deposition Buffer v2 is drawn into the well to replace the old Deposition Buffer. Repeat this procedure for the other wells.
4. Gently loosen the SOLiD™ Deposition Chamber screws. As the screws are loosened, more fresh SOLiD™ XD Slide Deposition Buffer v2 is drawn into the Deposition Chamber.
5. Open the SOLiD™ Deposition Chamber lid, then carefully remove the SOLiD™ 4 or Opti Slide Carrier assembly from the Deposition Chamber.
6. Immediately pour Slide Storage Buffer over the slide to completely cover the beads. Allow the Slide Storage Buffer to flow off onto a paper towel or into a waste container.
7. Repeat step 6.
8. Immediately place the SOLiD™ 4 or Opti Slide Carrier assembly onto the instrument or into the SOLiD™ Slide Storage Chamber.

   IMPORTANT! The Deposition Chamber should not have any contact with Slide Storage Buffer or Overlay Buffer (from previous versions of SOLiD™ instruments).

STOPPING POINT. If you are storing the slide, place the SOLiD™ 4 or Opti 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 17).

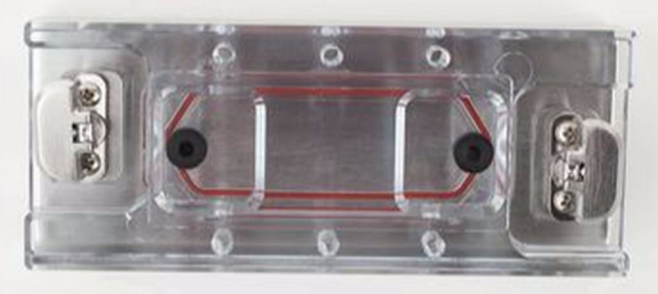


Figure 17 SOLiD™ Slide Storage Chamber.

Install the slide

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™ 4 System SETS Software User Guide* (PN 4448411).

1. Check to see if a SOLiD™ 4 or Opti Slide Carrier assembly from a previous run is present in the flowcell chamber. If a SOLiD™ 4 or Opti Slide Carrier assembly *is* present in the flowcell chamber, proceed with steps 2 to 4. If a SOLiD™ 4 or Opti 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 18).

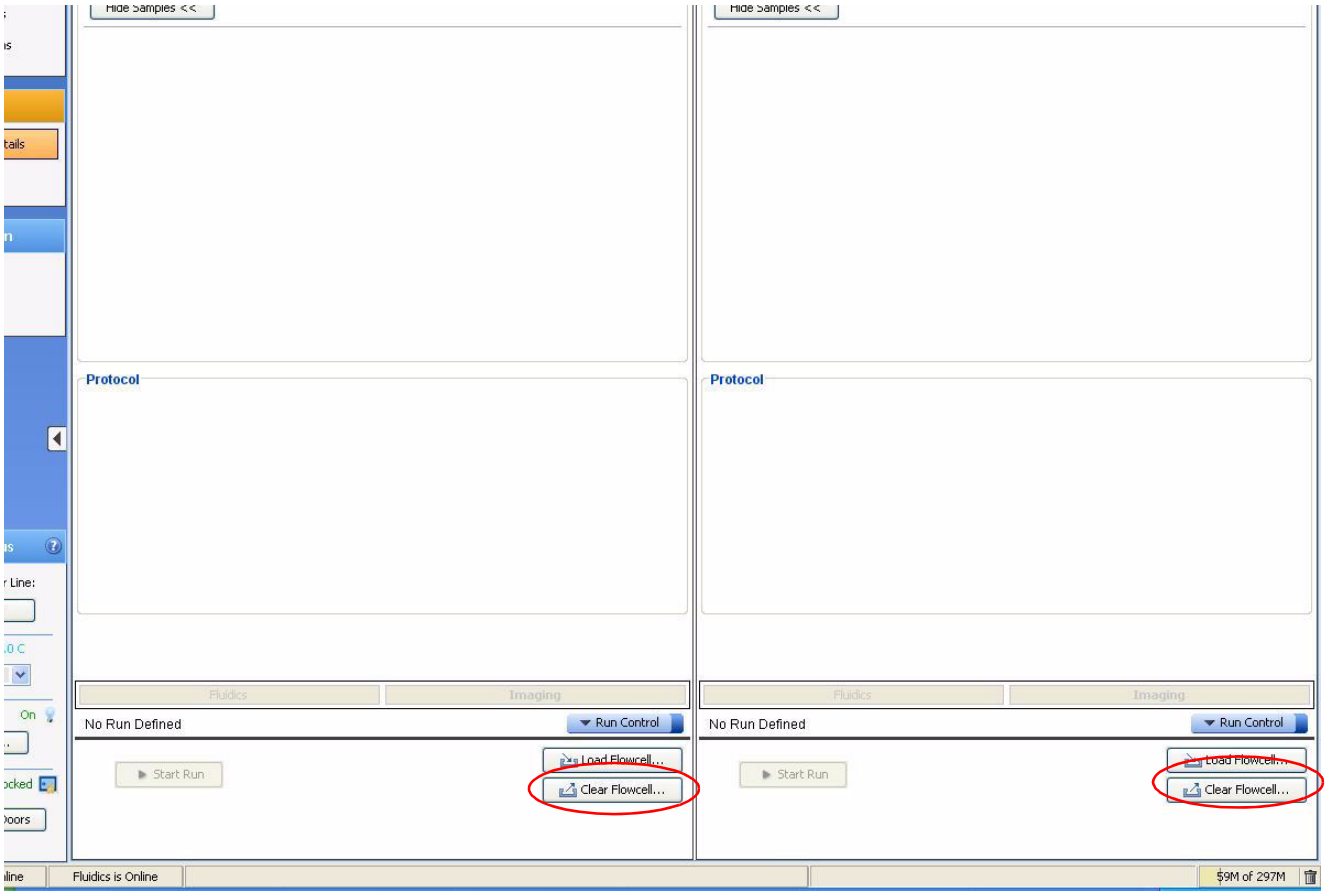


Figure 18 Click Clear Flowcell to flush the contents from the flowcell.

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

4. Remove the SOLiD™ 4 or Opti Slide Carrier assembly from the previous run. If the slide will be reused, place the SOLiD™ 4 or Opti 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.

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

6. Inspect the O-ring and reseat it if necessary (see Figure 19; for details, see "Install the SOLiD™ System Flowcell O-ring" on page 124). 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.

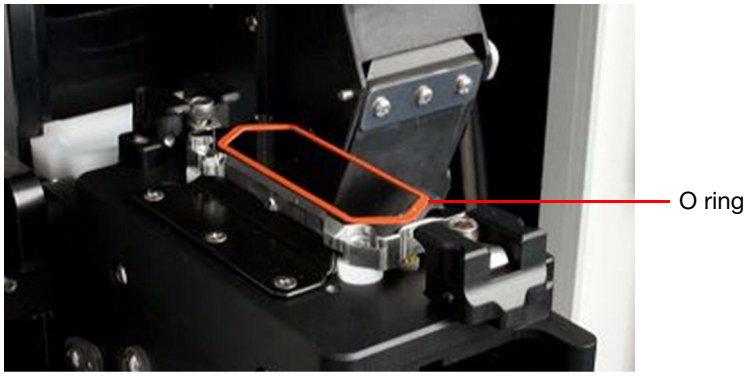


Figure 19 O-ring installed on flowcell.

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™ 4 or Opti Slide Carrier onto the instrument. Work quickly to prevent the slide from drying out.

   a. Remove the SOLiD™ 4 or Opti Slide Carrier assembly from the SOLiD™ Deposition Chamber or from the SOLiD™ Slide Storage Chamber.

   b. Place the SOLiD™ 4 or Opti 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 slide carrier lock-down tabs on the flowcell inward until they are positioned over and flush with the carrier (see Figure 20).

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

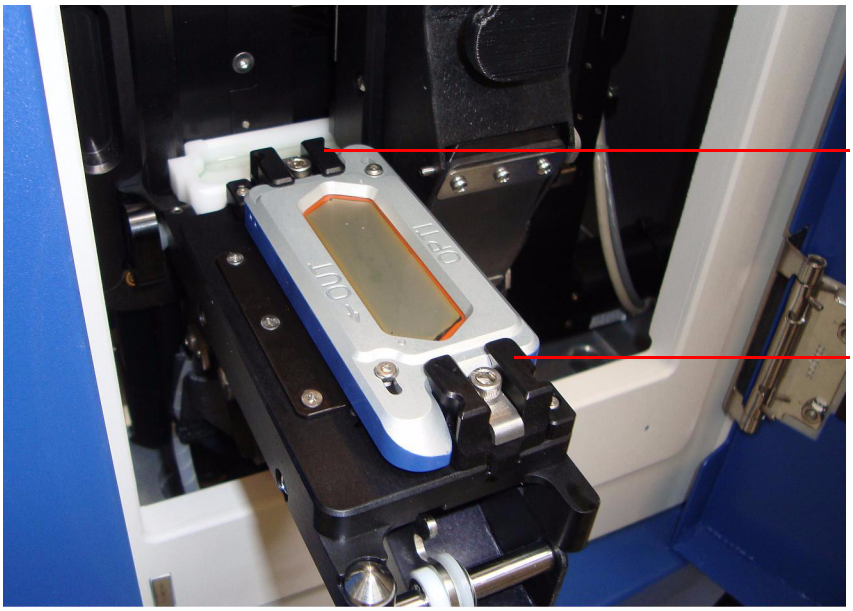


Figure 20 Slide carrier lockdown tabs.

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 21).

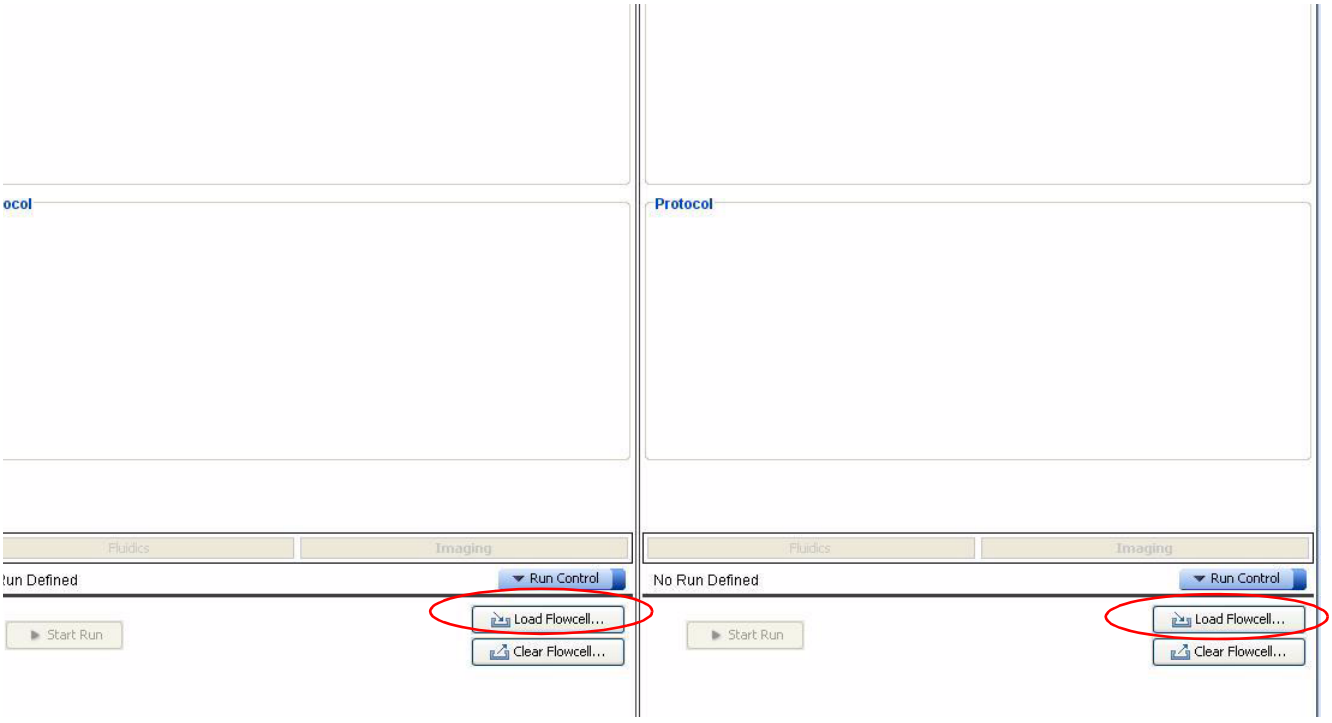


Figure 21 Click Load Flowcells to load the flowcell with buffer.

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 slide carrier lock-down tabs are pushed all the way in and tightened before closing the unused flowcell.

Install reagent strip(s) on the instrument

1. Clean the needle and bottom of the needle holder with a Kimwipes® wiper (see Figure 22). If needed, first moisten the Kimwipes® wiper with deionized water or use a pre-moistened alcohol pad.

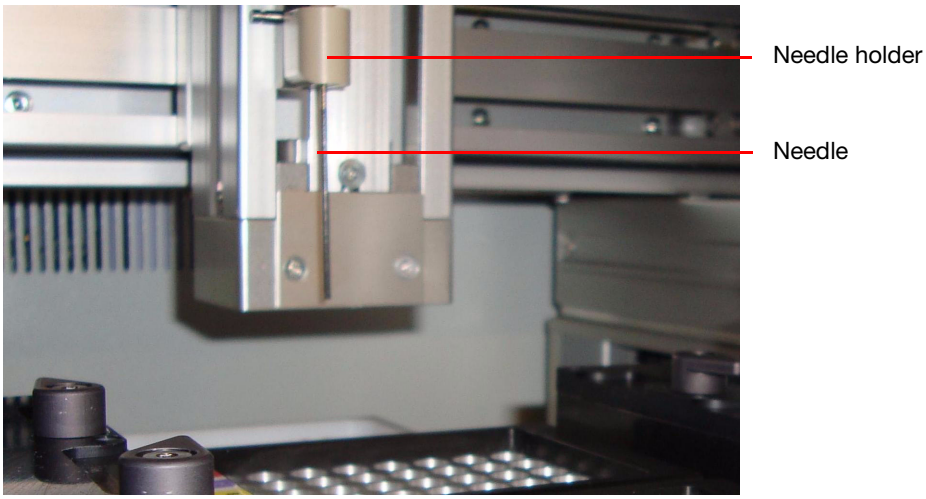


Figure 22 Needle and needle holder.

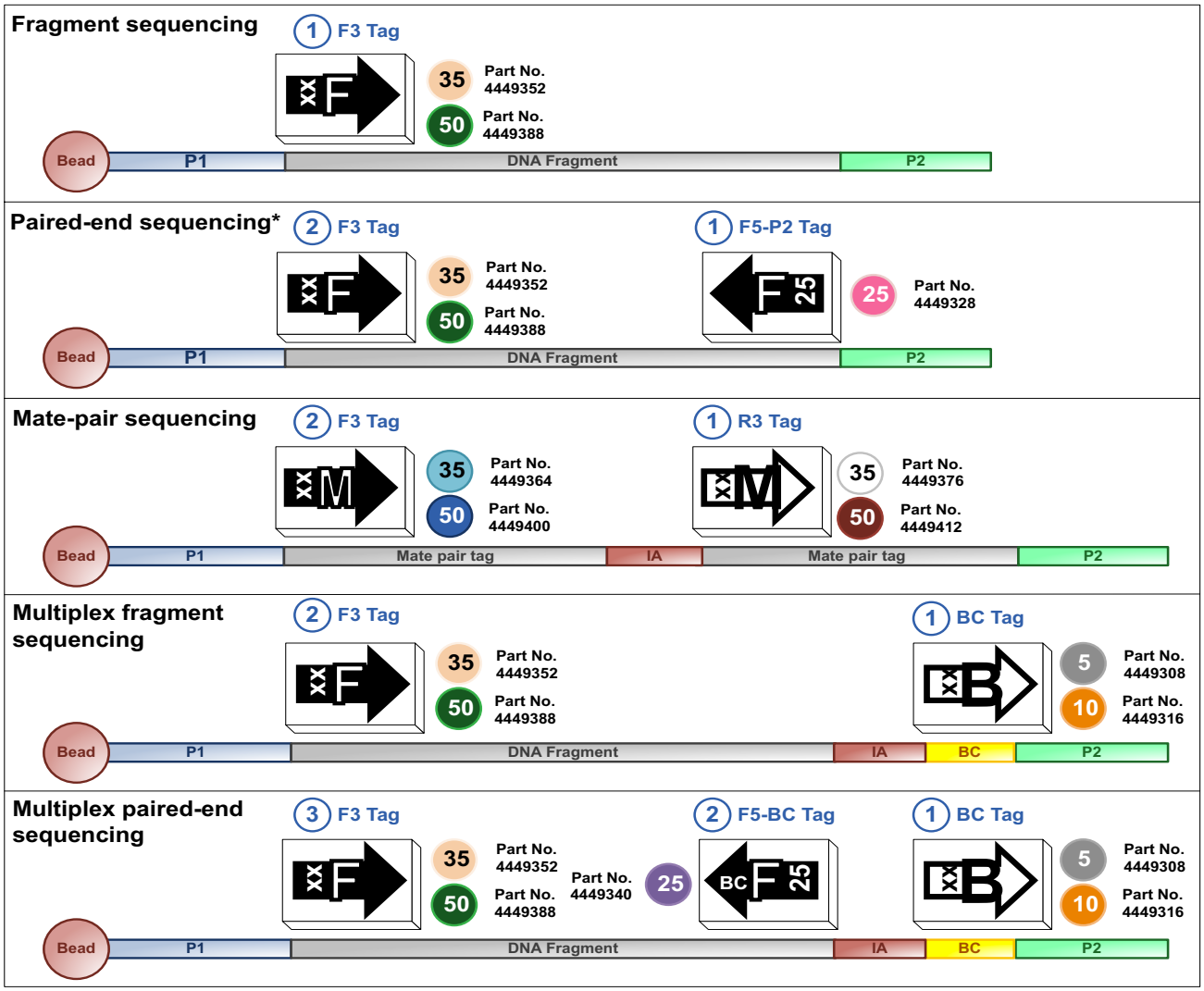
2. Thaw the appropriate reagent strip(s) on ice.

   See Figure 23 to determine which reagent strips should be used for a sequencing run. Use reagent strips from the boxes that match the arrow symbol for the appropriate library and tag.

   IMPORTANT! Verify that there is sufficient volume of Reset Buffer for each tag sequenced. The SOLiD™ ToP Instrument Buffer contains sufficient Reset Buffer to reset two tags (see "On-Instrument Reagent Volumes and Reagent Strip Layouts" on page 147). For multiplex sequencing, a bottle of Reset Buffer is included with multiplex sequencing kits to account for sequencing the BC tag.

   The blue circled numbers indicate the order in which the tags should be sequenced for each library type. The XX represents read length options in bases (35 and 50 for F3 and R3 tags; 5 and 10 for BC tag).

   Note: The 5-bp barcode sequencing reagents can be used if only barcodes 1 to 16 are being used. The 10-bp barcode sequencing reagents are needed for any set that includes barcodes 17 to 96. For example, if you are sequencing 32 barcoded fragment libraries using barcodes 1 to 32, you should use the 10-bp barcode sequencing reagents. Only 5-bp or 10-bp barcode reads (not both) may be sequenced on a single slide.



* For non-multiplex paired-end sequencing of libraries prepared using the SOLiD™ Total RNA-Seq Kit, use the SOLiD™ ToP Sequencing Kit – BC Frag. Lib., F5-BC Tag, MM25 (PN 4449340) to sequence the F5-BC tag instead of the F5-P2 tag.

Figure 23 Reagent strip box symbols corresponding to fragment sequencing, paired-end sequencing, mate-pair sequencing, multiplex fragment sequencing, and multiplex paired-end sequencing.

3. Place the reagent strip(s) in an ABgene® 96-well square-well storage plate and centrifuge at 160 × g for 2 minutes.

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 24).

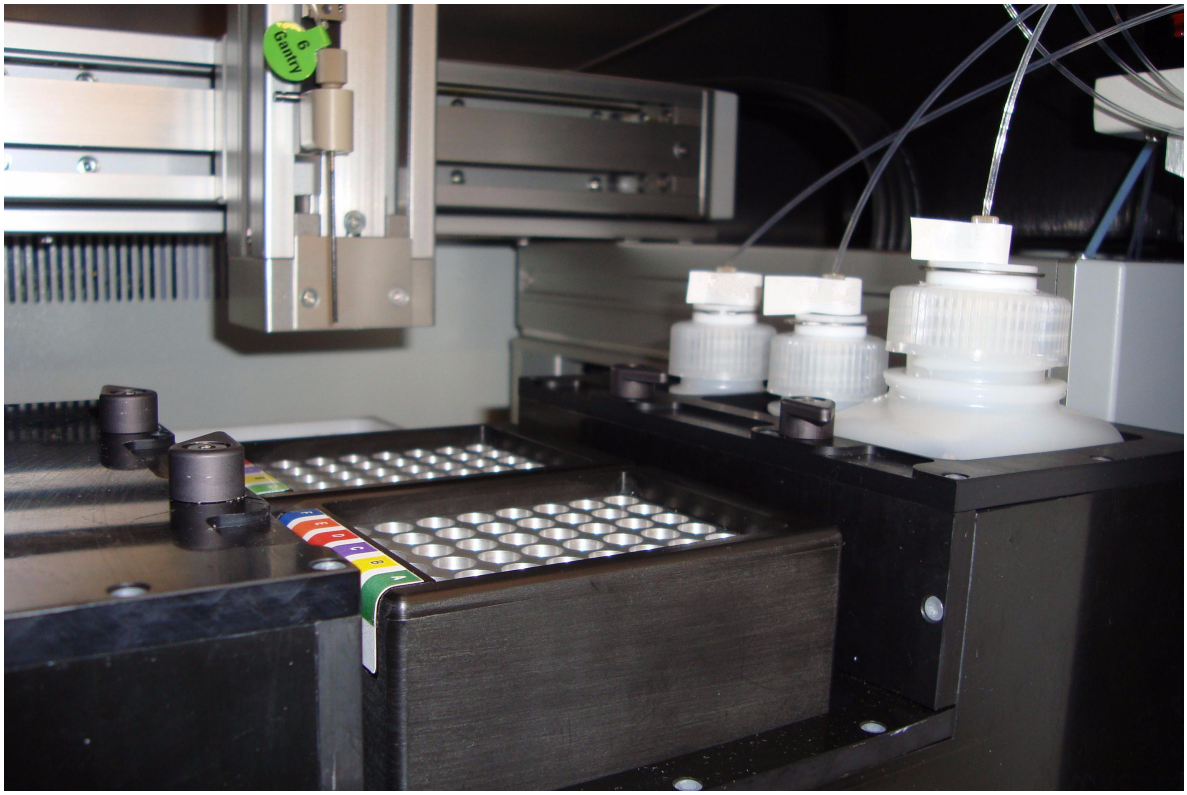


Figure 24 The Reagent Strip Block.

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 (see Figure 11 on page 33).

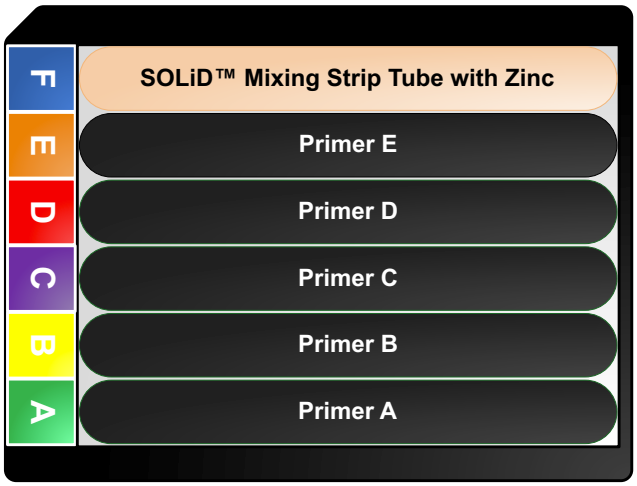
5. When the temperature is less than 10 °C, place the reagents in the appropriate location in the chiller block (see Table 6 and Figure 28 on page 50).

   See Figures 25 to 27 for the appropriate location of reagent strips on the chiller block. For information about the contents of the reagent strip tubes, see "Reagent strip layouts" on page 151.

Table 6 Where to place the strip tubes

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

Figure 25 Reagent strip block layout for WFA.



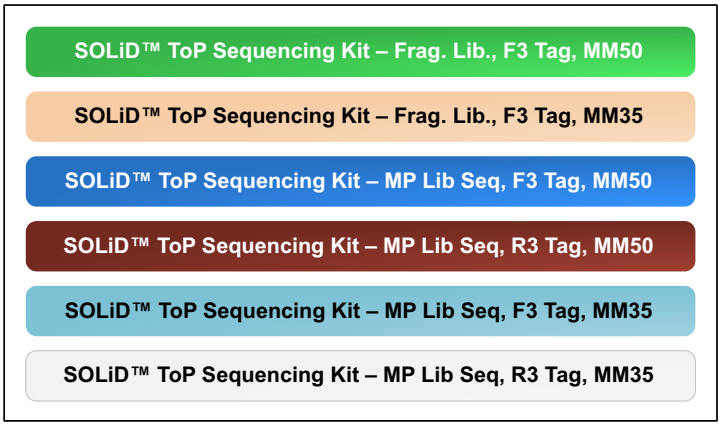


Figure 26 Reagent strip block layouts for sequencing F3 and R3 tags.

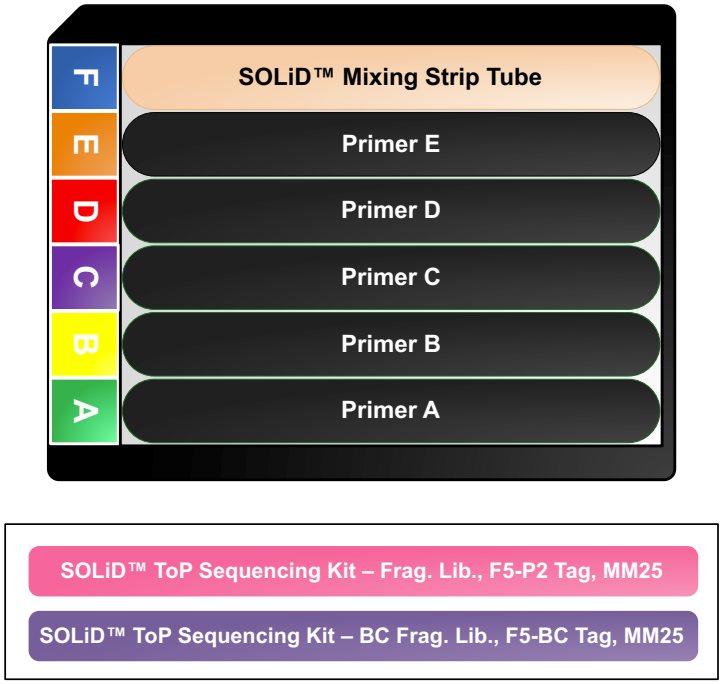


Figure 27 Reagent strip block layouts for sequencing F5-P2 and F5-BC tags.

Figure 28 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 29).

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 125).

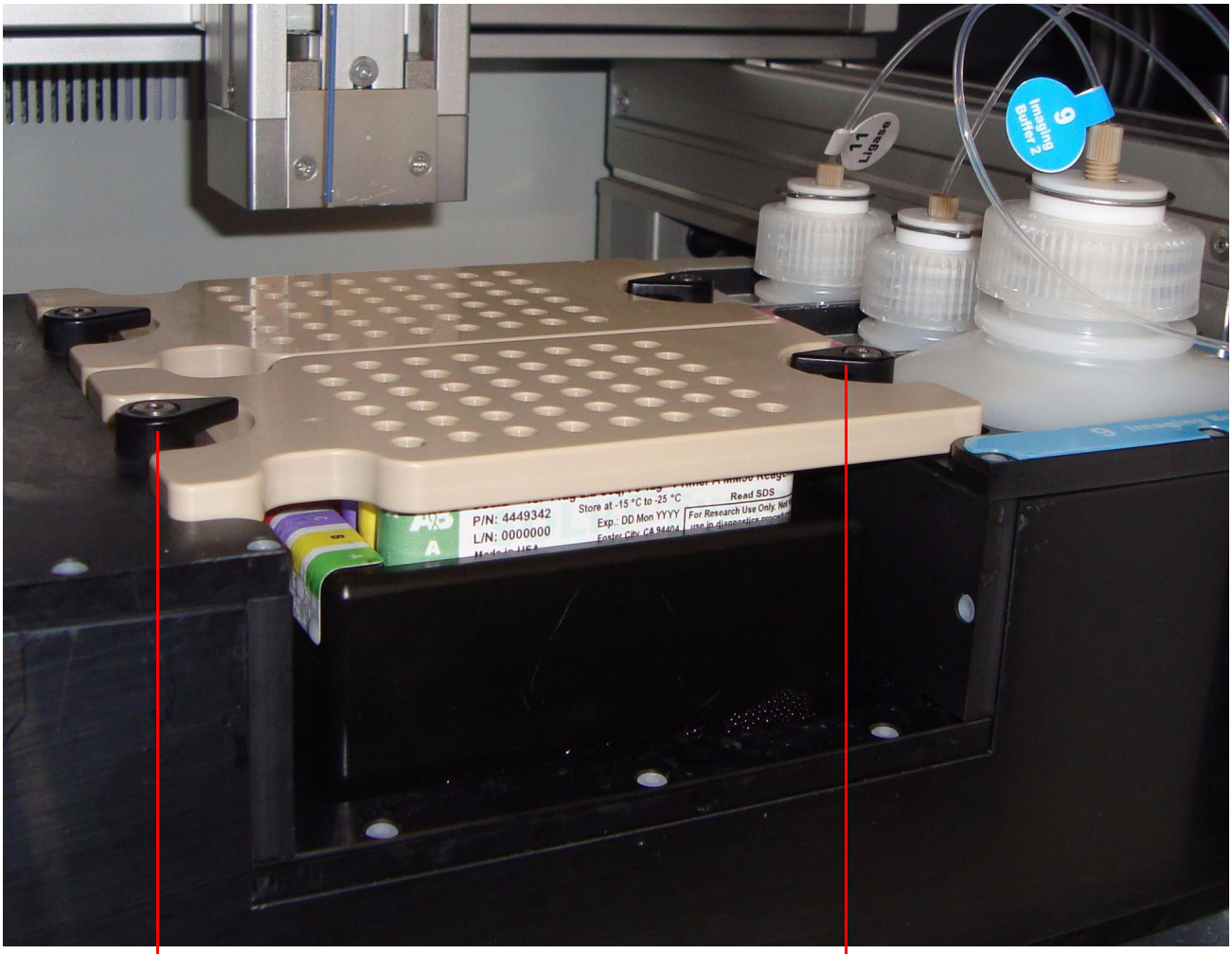


Figure 29 Securely fastened reagent strip covers.

3 Set Up, Control, and Monitor the Run

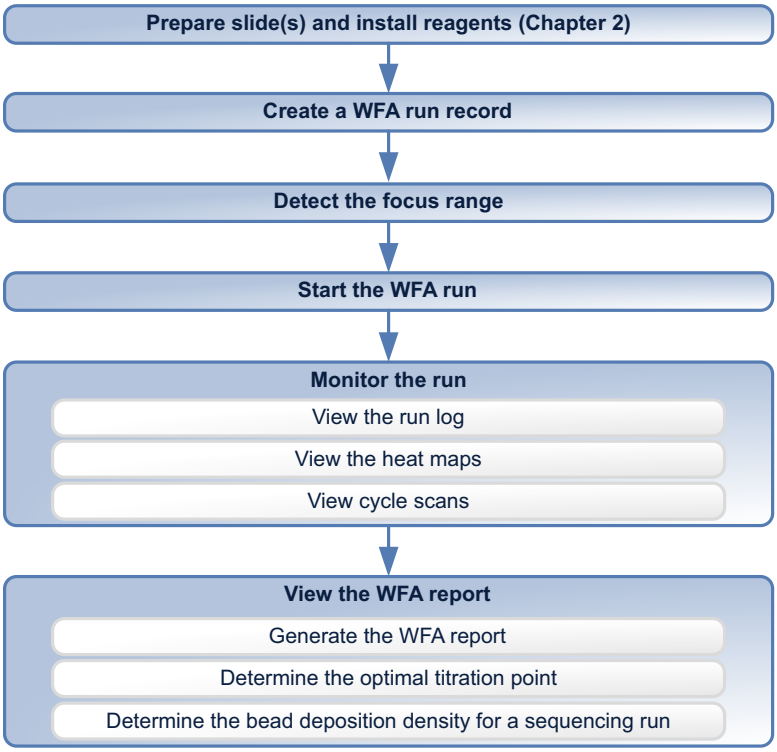
This chapter covers:

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

Materials and equipment required

See Appendix A on page 101 for a list of equipment, kits, and consumables necessary to set up a workflow analysis (WFA) run.

Workflow



Workflow overview

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. You 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 and then they undergo 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 199).

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 199). 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™ System sequencing run, a bead density of 300,000 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 127). 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 30 on page 57).

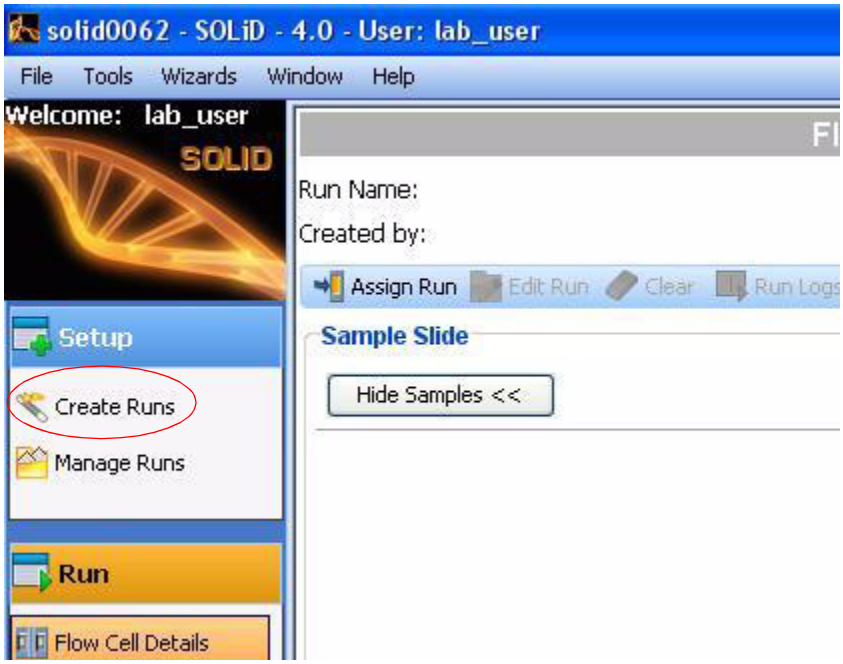


Figure 30 Use the run wizard to create a WFA run.

2. Complete the information in the Select Run Type and Mask pane (see Figure 31 on page 58):
   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**.

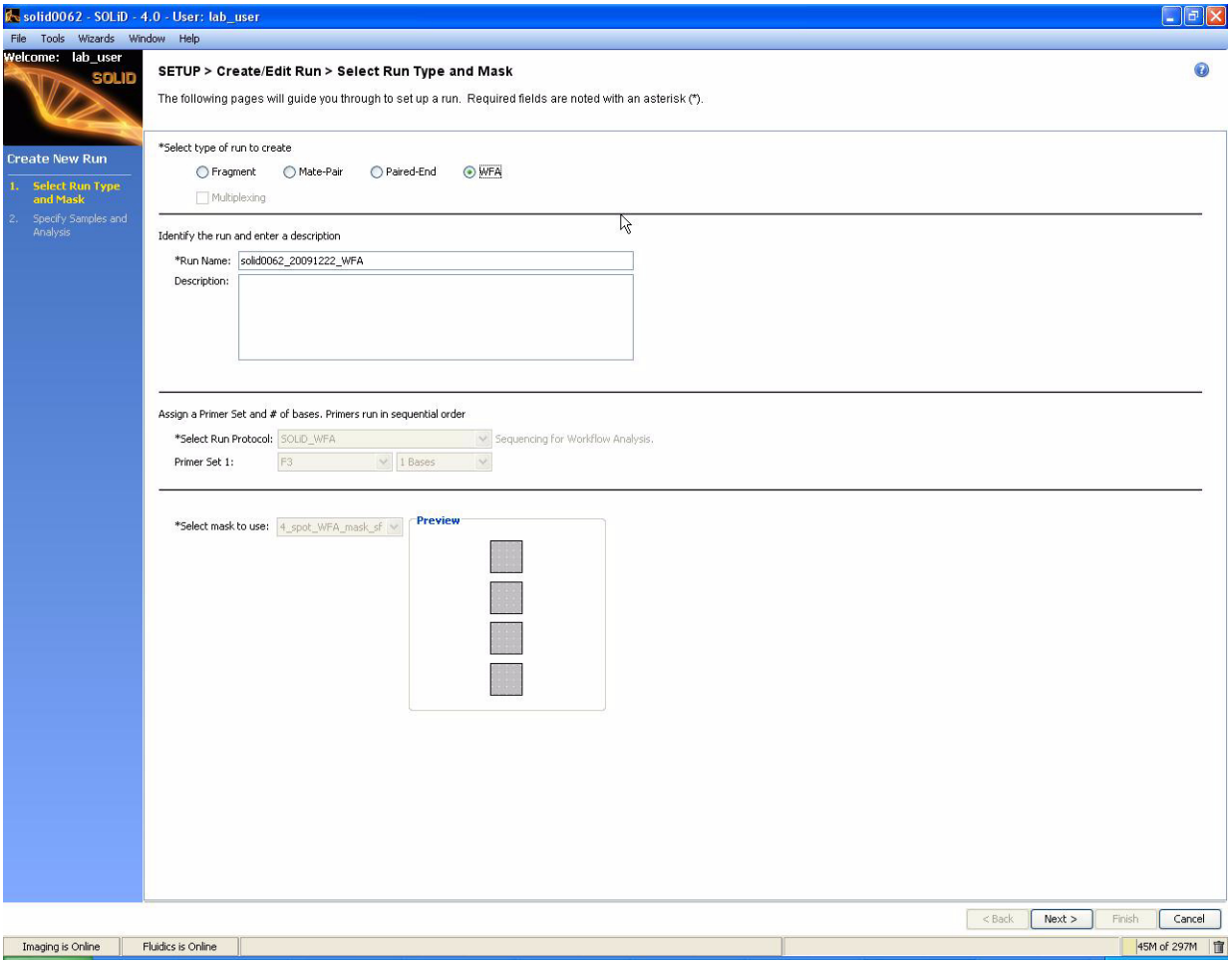


Figure 31 Complete the information in the Select Run Type and Mask pane.

3. Complete the information in the Specify Samples and Analysis pane (see Figure 32):

   a. Assign samples to spots.

   b. Click **Finish**.

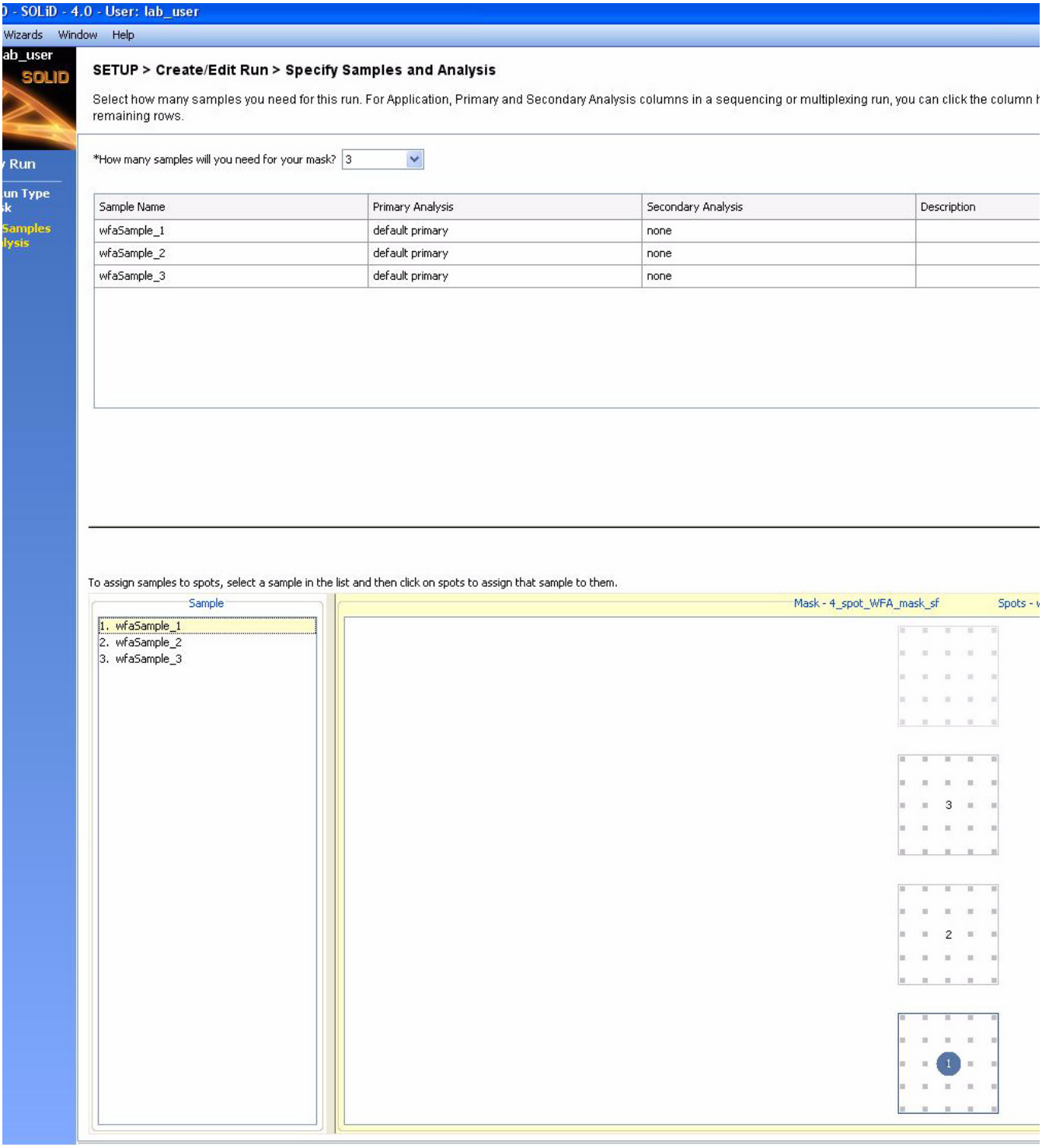


Figure 32 Complete the information in the Specify Samples and Analysis pane.

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 Flowcel**l.

   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 132).

1. Close the front doors of the SOLiD™ 4 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 of the stage template file directly or by clicking the **Browse** button to navigate to a suitable stage template file (see Figure 33).

   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**).

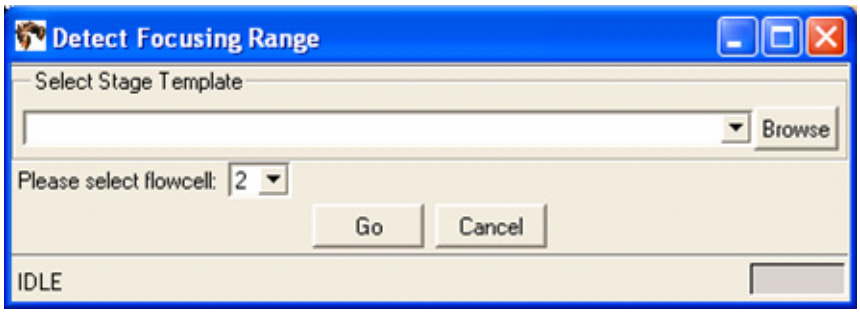


Figure 33 Detect Focusing Range dialog.

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 34). You can also click **Cancel** so that the Imager aborts the ranging operation.

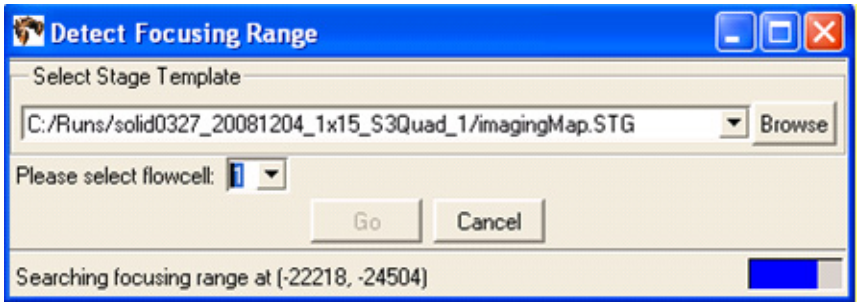


Figure 34 Detect Focusing Range Dialog while detection in progress.

5. When the Imager is done, a dialog appears (see Figure 35). 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.

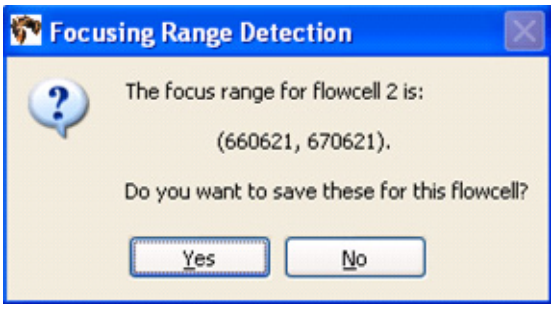


Figure 35 Confirm that you want to replace the local settings file.

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 36). 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 132).

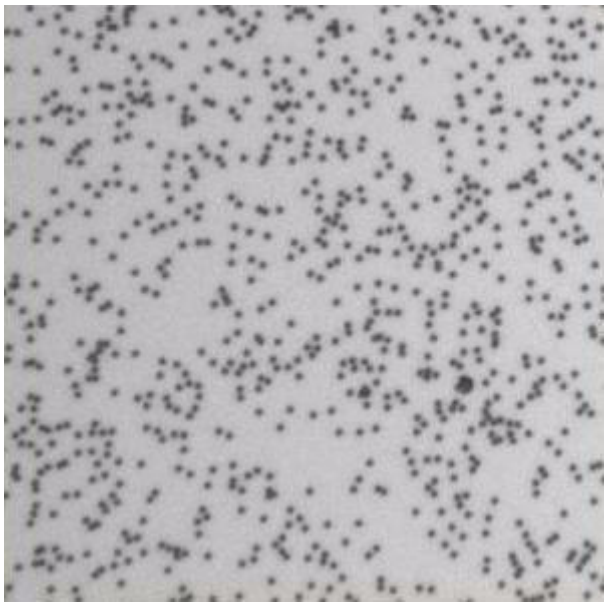


Figure 36 Beads in focus.

Start the WFA run

Note: Before starting the run, make sure the air filter at the back of the instrument is not clogged with dust (see "Clean the air filter" on page 119).

1. Click **Start Run**.
2. If there is not enough room to store the data, the Start Run dialog appears (see Figure 37). Choose the appropriate option (see Table 7).

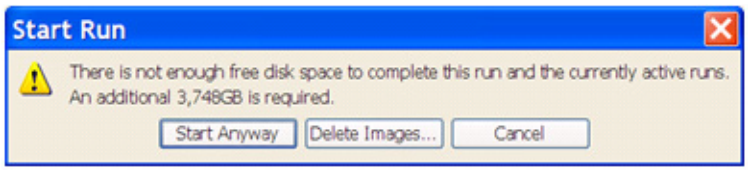


Figure 37 This Start Run dialog appears if there is not enough room to store data for the run.

Table 7 Options for managing 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, refer to the *Applied Biosystems SOLiD™ 4 System SETS Software User Guide* (PN 4448411).

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™ 4 System SETS Software User Guide* (PN 4448411).

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 38).

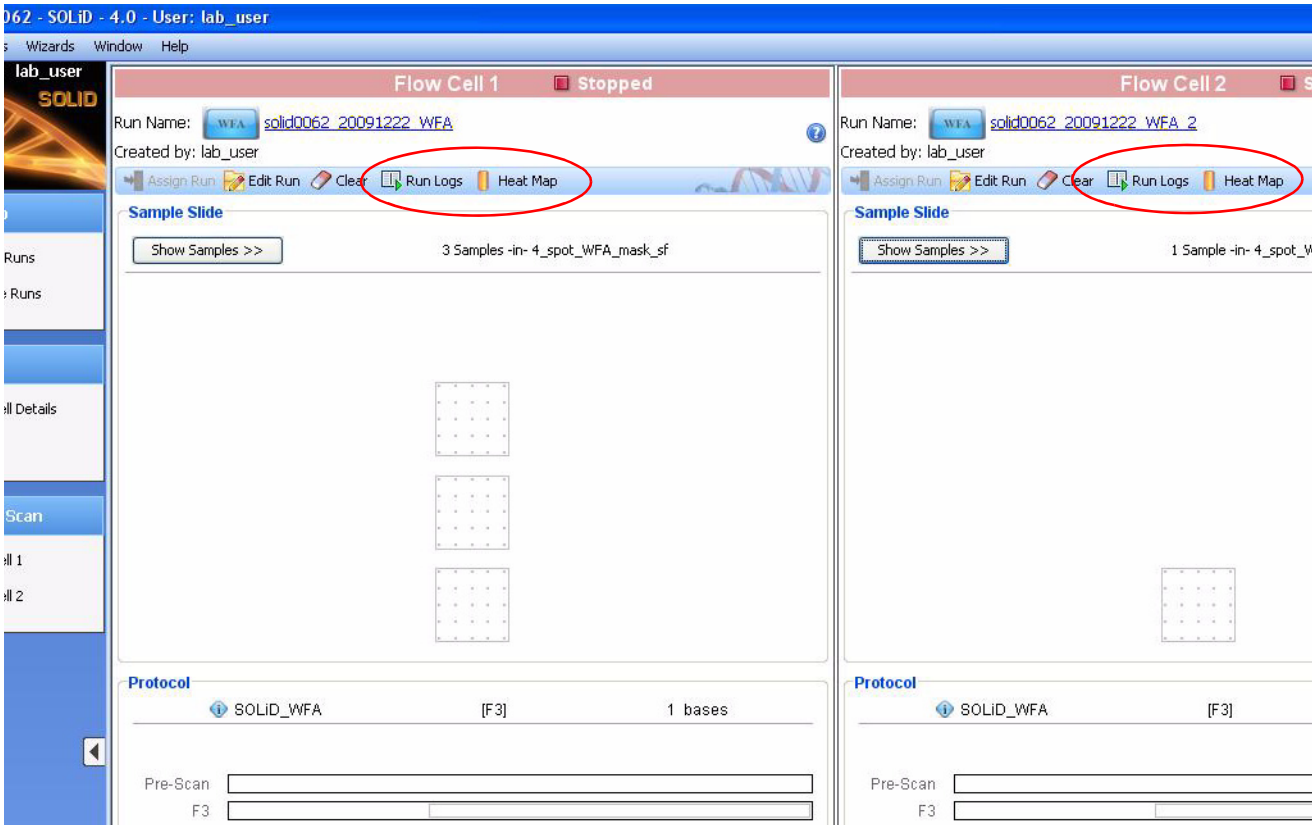


Figure 38 How to learn more about the current run.

IMPORTANT! Do not disturb the SOLiD™ 4 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™ 4 System SETS Software User Guide* (PN 4448411)]. If desired, set up e-mail notification regarding the instrument run and system information using SETS (refer to the *Applied Biosystems SOLiD™ 4 System SETS Software User 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 the 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 38 on page 64).
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 run" on page 95).
2. Click the heat map link to view the heat map for that cycle (see Figure 62 on page 99).
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 63 on page 100).

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 39). Refer to the *Applied Biosystems SOLiD™ 4 System SETS Software User Guide* (PN 4448411).

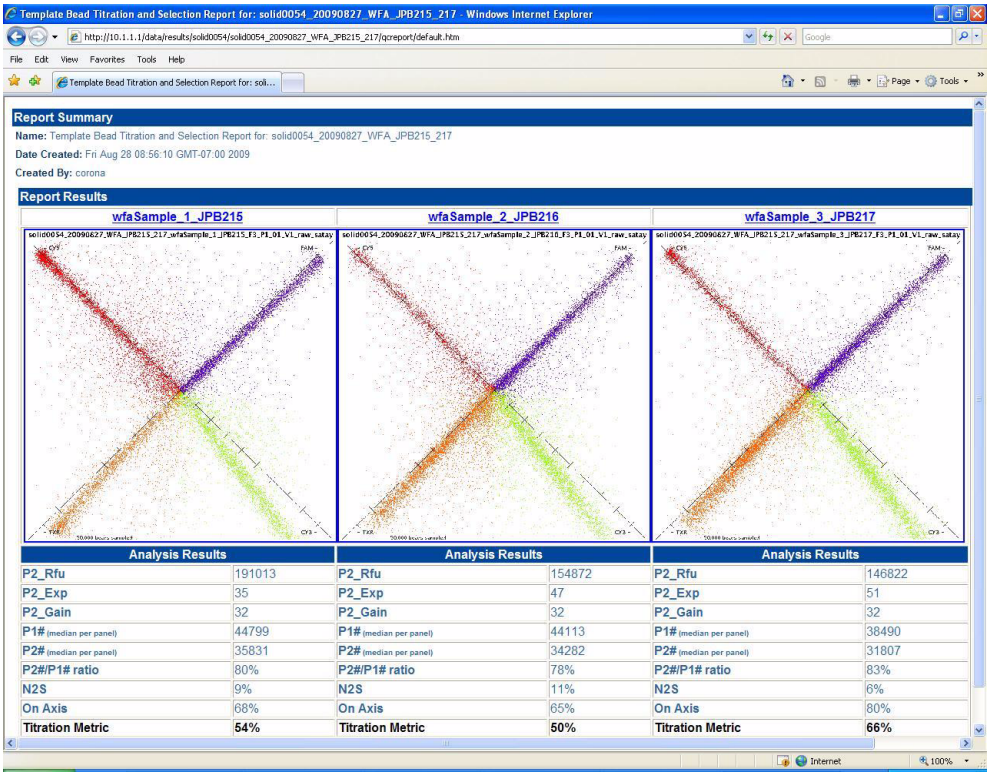


Figure 39 One WFA report for a single 4-well slide displayed in SETS.

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:

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

$$Y\ \text{P2-positive beads}/\mu L = \frac{\text{P2\# beads/panel} \times 426\ \text{panels}}{X\ \mu L}$$

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.

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

$$Y\ \text{P2-positive beads}/\mu L = \frac{20{,}000\ \text{beads/panel} \times 426\ \text{panels}}{30\ \mu L}$$

$$= 284{,}000\ \text{P2-positive beads}/\mu L$$

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 that you use this resulting calculated bead concentration to determine the volume of beads for deposition.

3. Multiply that volume by 120% to calculate the volume of beads for deposition.

Note: The 20% overage has been estimated empirically and is attributable to bead loss during washing and bead deposition steps. The overage factor may be adjusted by individual operators based on their experiences.

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

Example:

For a sample with a concentration of 284,000 P2-positive beads/µL to be deposited in one well of an 8-well SOLiD™ Deposition Chamber at a density of 300,000 beads/panel.

$$Z\ \mu L\ \text{bead solution} = \frac{56\ \text{million P2-positive beads}}{284{,}000\ \text{P2-positive beads}/\mu L} \times 120\%$$

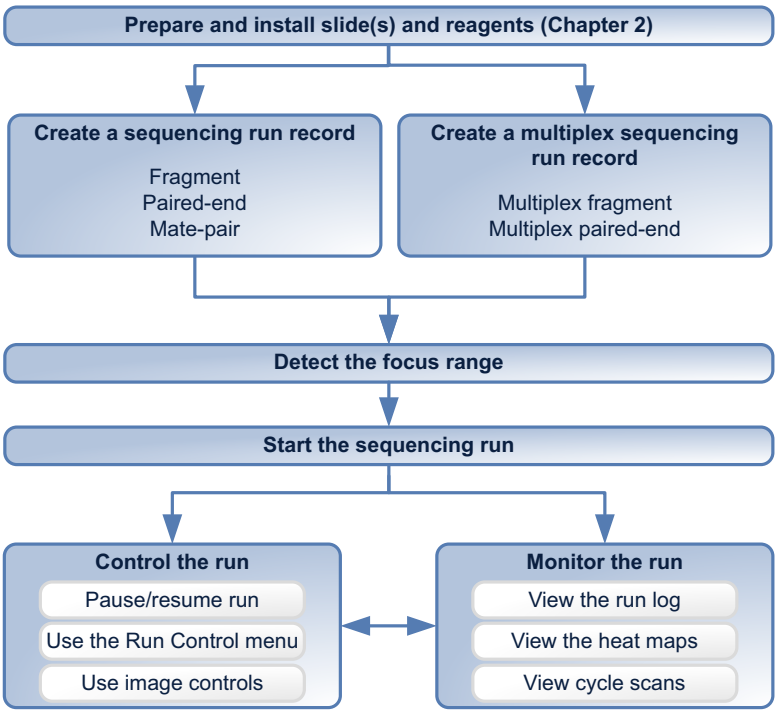
$$= 237\ \mu L\ \text{bead solution}$$

Section 3.2 Set up and perform a sequencing run

Materials and equipment required

See Appendix A on page 101 for a list of equipment, kits, and consumables necessary to set up a sequencing run.

Workflow



Workflow overview

Create a sequencing (non-multiplex) run record

A sequencing (non-multiplex) 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™ 4 System SETS Software User Guide* (PN 4448411)].

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. The run record file can be created using a spreadsheet program either on or off the instrument.

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™ 4 System SETS Software User Guide* (PN 4448411)].

Detect the focus range

Two methods exist for determining the focus range: automatic and manual. You 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 performing a sequencing run with multiple tags, 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™ 4 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 199).

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 (non-multiplex) 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 75.

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 127). 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 40).

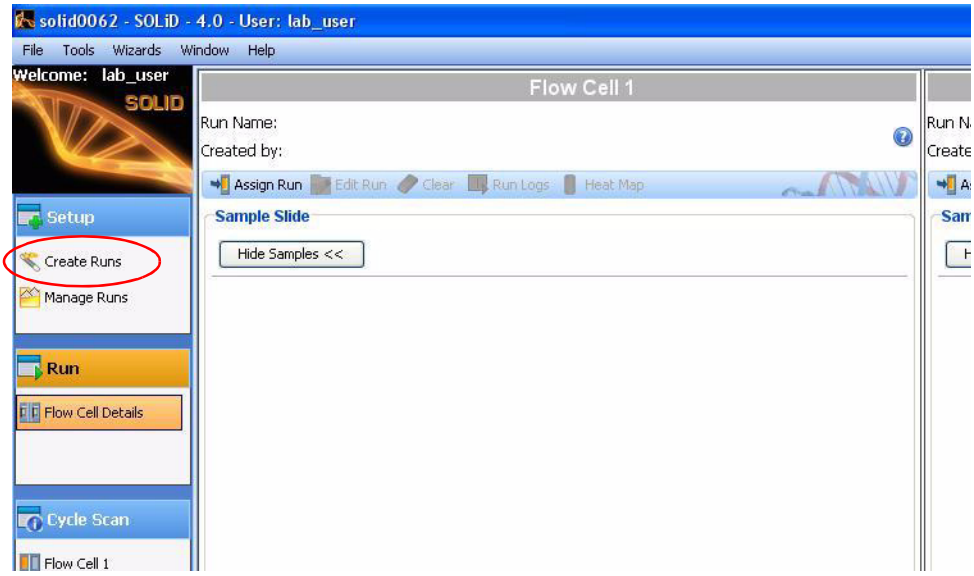


Figure 40 Use the run wizard to create a sequencing run.

2. Complete the information in the Select Run Type and Mask pane (see Figure 41):
   a. Select the type of run:
      - If performing a fragment sequencing run, select **Fragment**
      - If performing a mate-pair sequencing run, select **Mate-Pair**
      - If performing a paired-end sequencing run, select **Paired-End**
   b. (Optional) Type a new run name.
   c. (Optional) Enter a description.
   d. Ensure that the Run Protocol is set to **SOLiD4**.
   e. Select **Primer Set 1** and **Primer Set 2**.
      - If performing a fragment sequencing run, leave **F3** as Primer Set 1.

- If performing a mate-pair sequencing run, select **R3** as Primer Set 1 and **F3** for Primer Set 2.

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

- If performing a paired-end sequencing run, select **F5-P2** as Primer Set 1 and **F3** for Primer Set 2.
- If performing a paired-end sequencing run on a library prepared using the SOLiD™ Total RNA-Seq Kit, select **F5-BC** as Primer Set 1 and **F3** for Primer Set 2.

f. Enter read lengths for Primer Set 1, and if used, 2. A typical read length for the F3 and R3 tags is 35 to 50 bases. A typical read length for the F5-P2 tag or F5-BC tag is 25 bases.

g. Select the appropriate mask to use.

h. 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**.

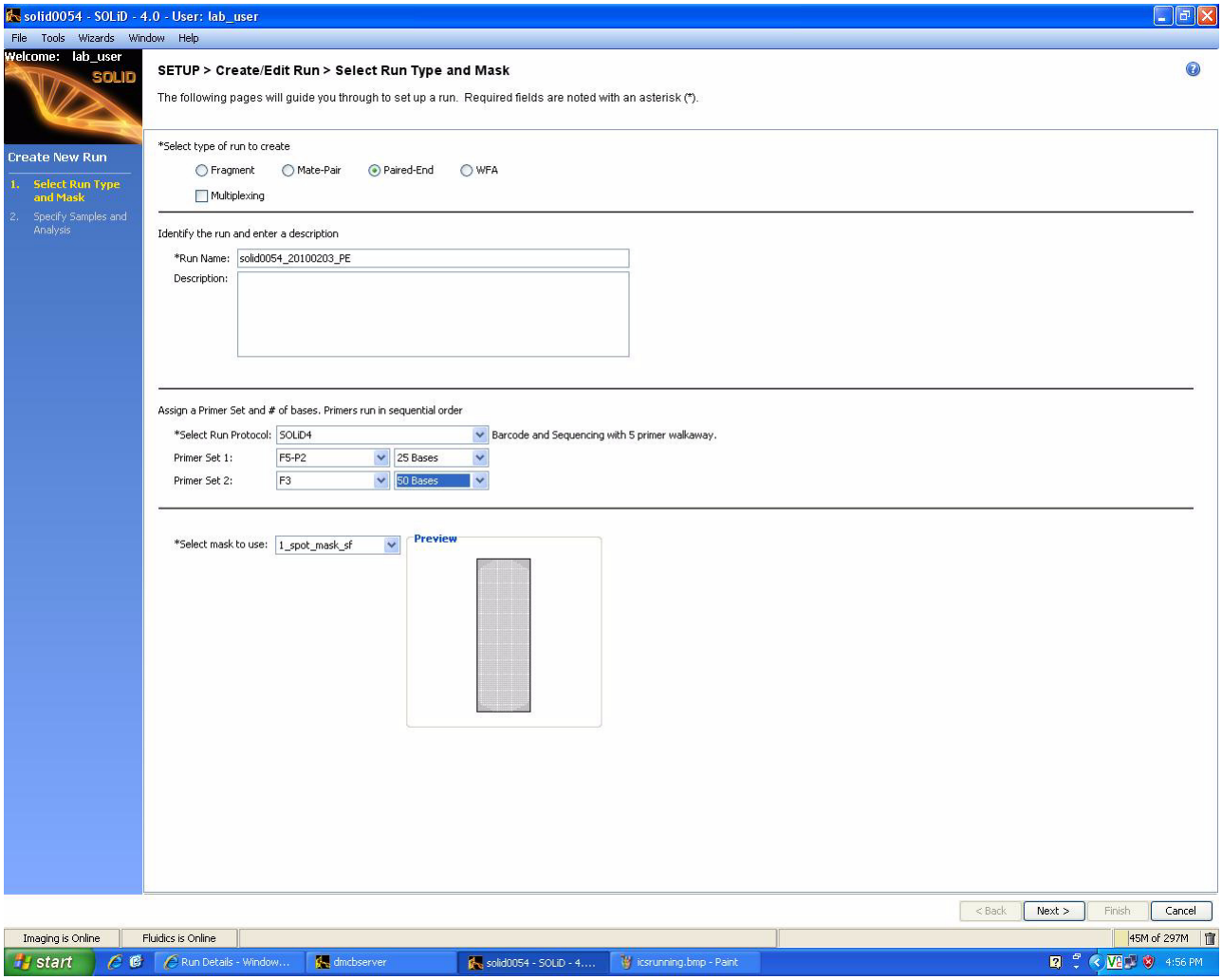


Figure 41 Complete the information in the Select Run Type and Mask pane.

3. Complete the information in the Specify Samples and Analysis pane (see Figure 42):

   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.

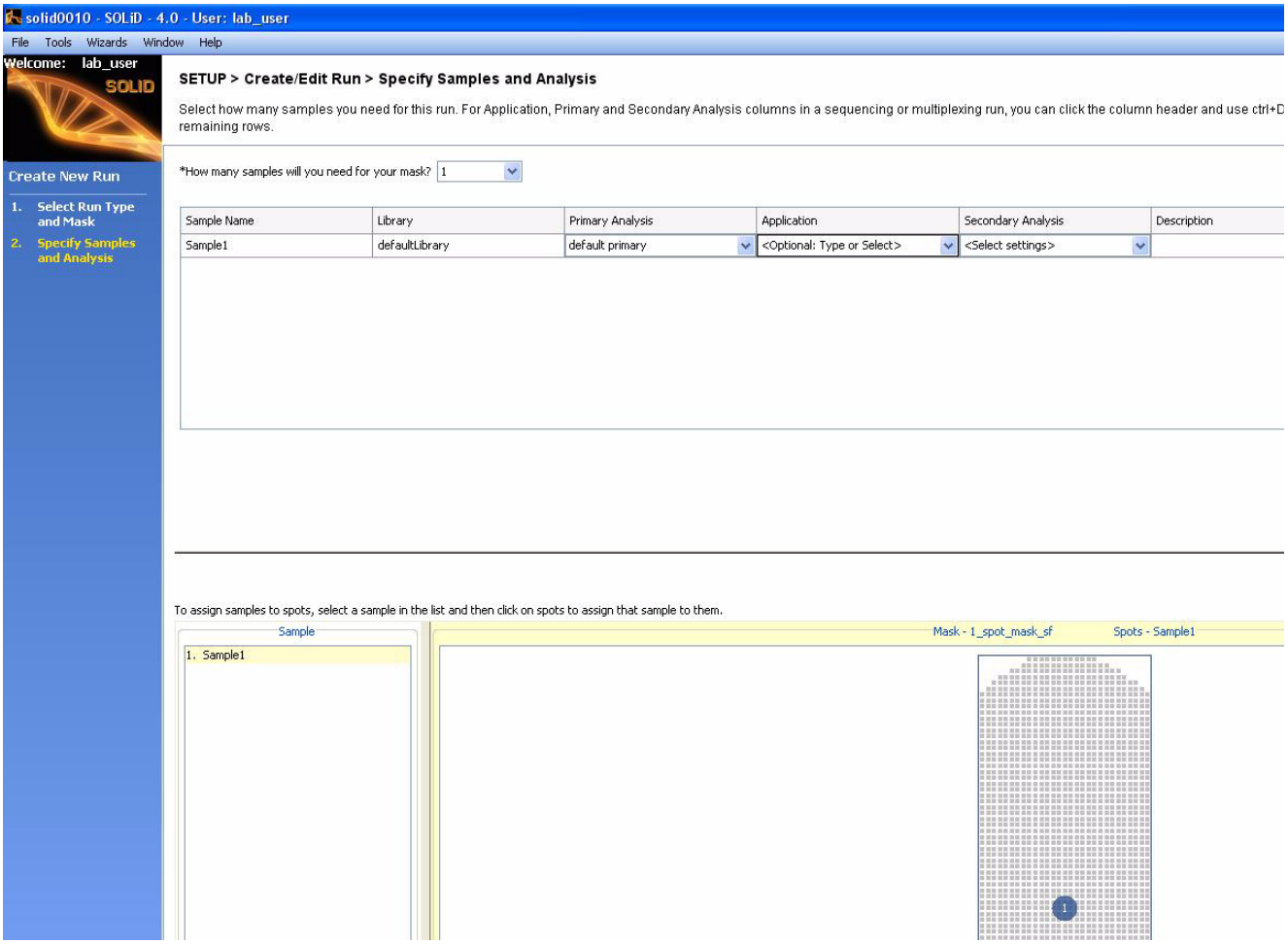


Figure 42 Complete the information in the Specify Samples and Analysis pane.

4. Assign Samples to spots in the mask on the Specify Samples and Analysis pane. 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. Click **Finish** to return to the Setup Task pane.

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

7. 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 (non-multiplex) run record, go to the previous section "Create a sequencing (non-multiplex) run record" on page 71.

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 127).

IMPORTANT! For all barcodes ≥ Barcode 16, ten bases of barcode tag must be read. While creating a multiplex sequencing run file on the System, the barcode definition file for the 1-96 series already specifies ten bases of sequence. If at least 1 library on the slide uses barcode 17 or higher, ten bases of barcode tag will be read for all other libraries on the slide, even if they are on separate spots.

1. (Optional) If desired, modify the Barcode Error Correction Level. A Barcode Error Correction Level is set at 0 mismatches by default. A Barcode Error Correction Level of 1 mismatch increases the number of reads by about 10%, but lower-quality sequencing reads are included in the data.

   For information about how to modify the Barcode Error Correction Level, see "Modify the Barcode Error Correction Level" on page 145.

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

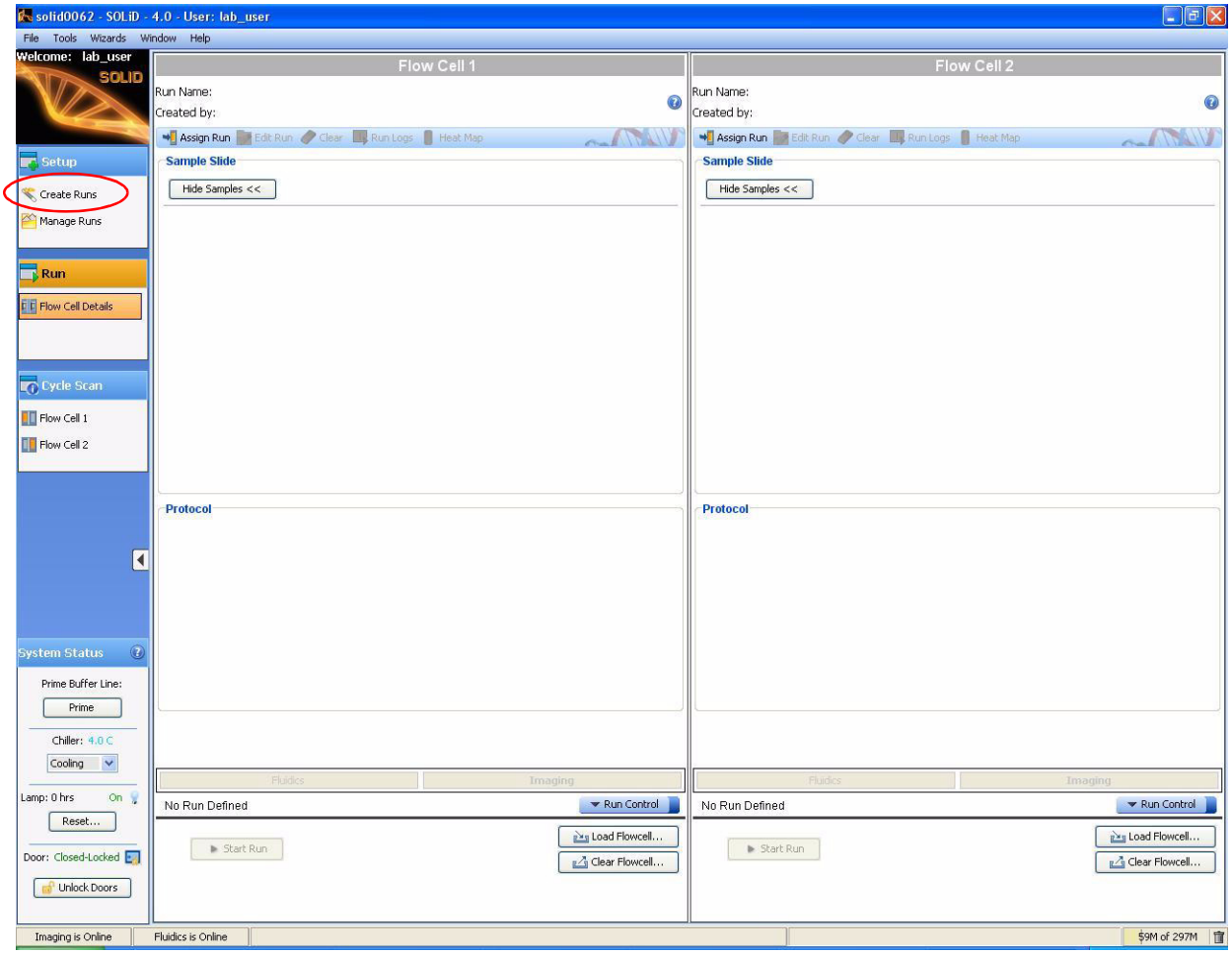


Figure 43 Use the run wizard to create a multiplex sequencing run.

3. Complete the information in the Select Run Type and Mask pane (see Figure 44):

   a. Select the type of run:
      - If sequencing only one end of the barcoded fragment library, select **Fragment**.
      - If sequencing both ends of the barcoded fragment library, select **Paired-End**.

   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 **SOLiD4 Multiplex**.

   f. Select **BC** for Primer Set 1. Enter the read length for the BC Primer according to the barcodes used in the libraries:

Barcodes used on the slide	Set the read length to...(bases)
Barcodes 1 to 16 only	5
Barcodes 17 to 96	10

Note: If you do not select the correct read length for the number of barcodes specified in your run definition file, the SOLiD™ System software will display an error. If at least 1 library on the slide uses Barcode 17 or higher, ten bases of barcode tag should be read.

g. Select Primer Set 2 (and Primer Set 3):
   - If sequencing only one end of the barcoded fragment library, select **F3** for Primer Set 2.
   - If sequencing both ends of the barcoded fragment library, select **F5-BC** for Primer Set 2 and **F3** for Primer Set 3.

h. Enter read lengths for Primer Set 2, and if used, Primer Set 3. A typical read length for the F3 tag is 35 to 50 bases. A typical read length for the F5-BC tag is 25 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.

i. Select the appropriate mask to use.

j. Click **Next**.

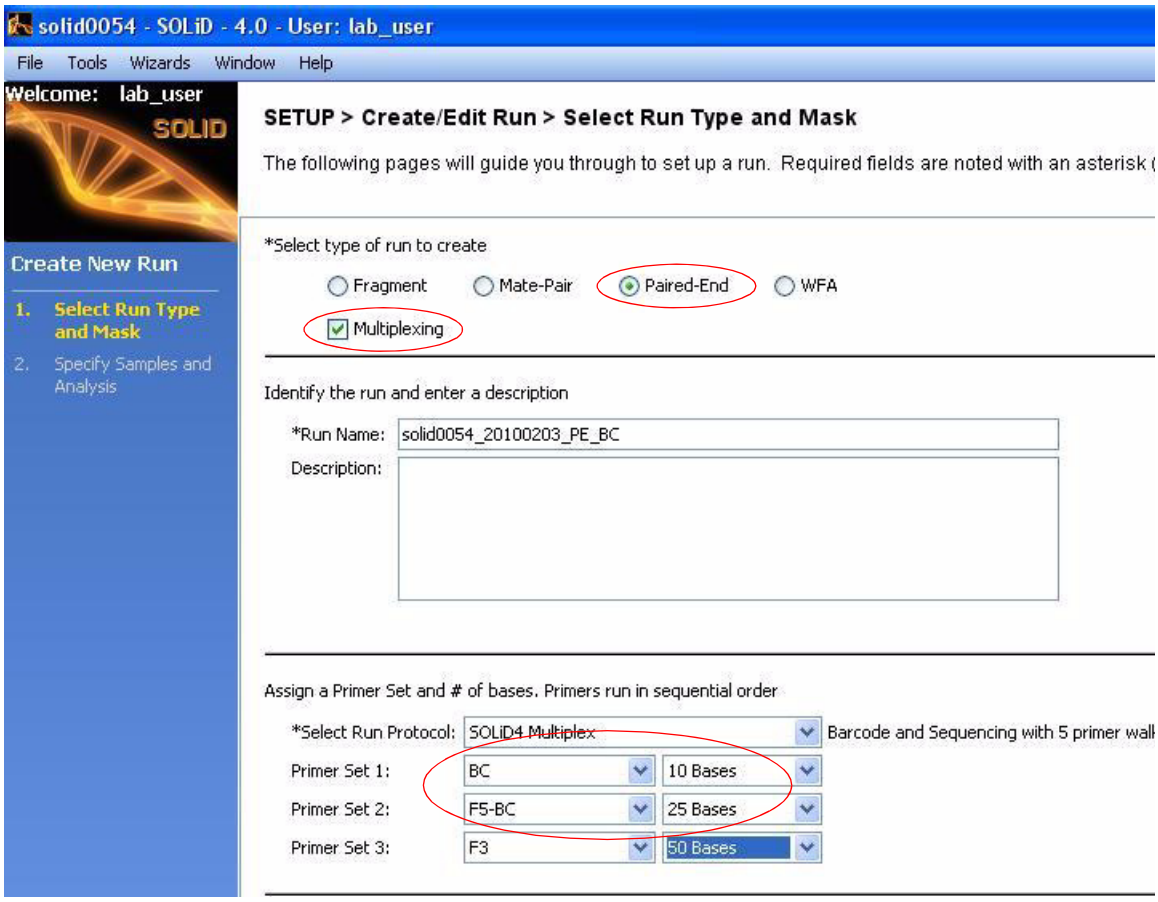


Figure 44 Complete the information in the Select Run Type and Mask pane.

4. Complete the information in the Specify Samples and Analysis pane (see Figure 45 on page 79):

   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 that 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, primary analysis, and description of libraries that are in the sample.

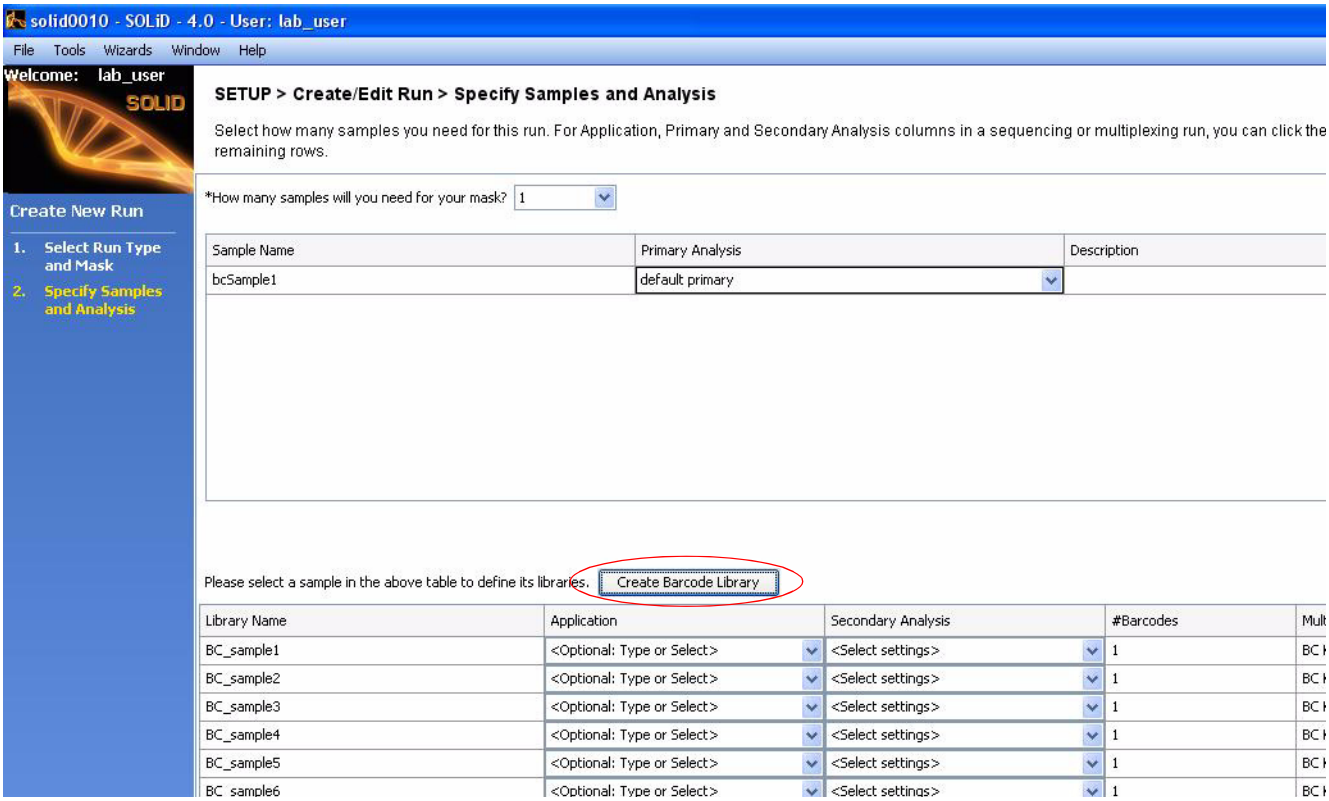


Figure 45 Complete the information in the Specify Samples and Analysis pane.

5. If performing a paired-end sequencing run, click **Create New Library** to begin entering library information for that sample (see Figure 45).

   For a barcode sample in a fragment sequencing run, select **Barcoded Sample** and click **Create New Library** to begin entering library information for that sample (see Figure 45).

   IMPORTANT! Each barcoded sample requires at least one library.

   If there are any non-barcoded fragment library samples to be sequenced on the same slide as barcoded samples, select **Non-barcoded Sample** for that sample.

   Note: Non-barcoded samples cannot be sequenced on the same slide as barcoded samples for paired-end sequencing runs.

6. Enter information for the first library present in the sample (see Figure 46):
   a. Enter the library name.
   b. Select a Multiplexing Series.

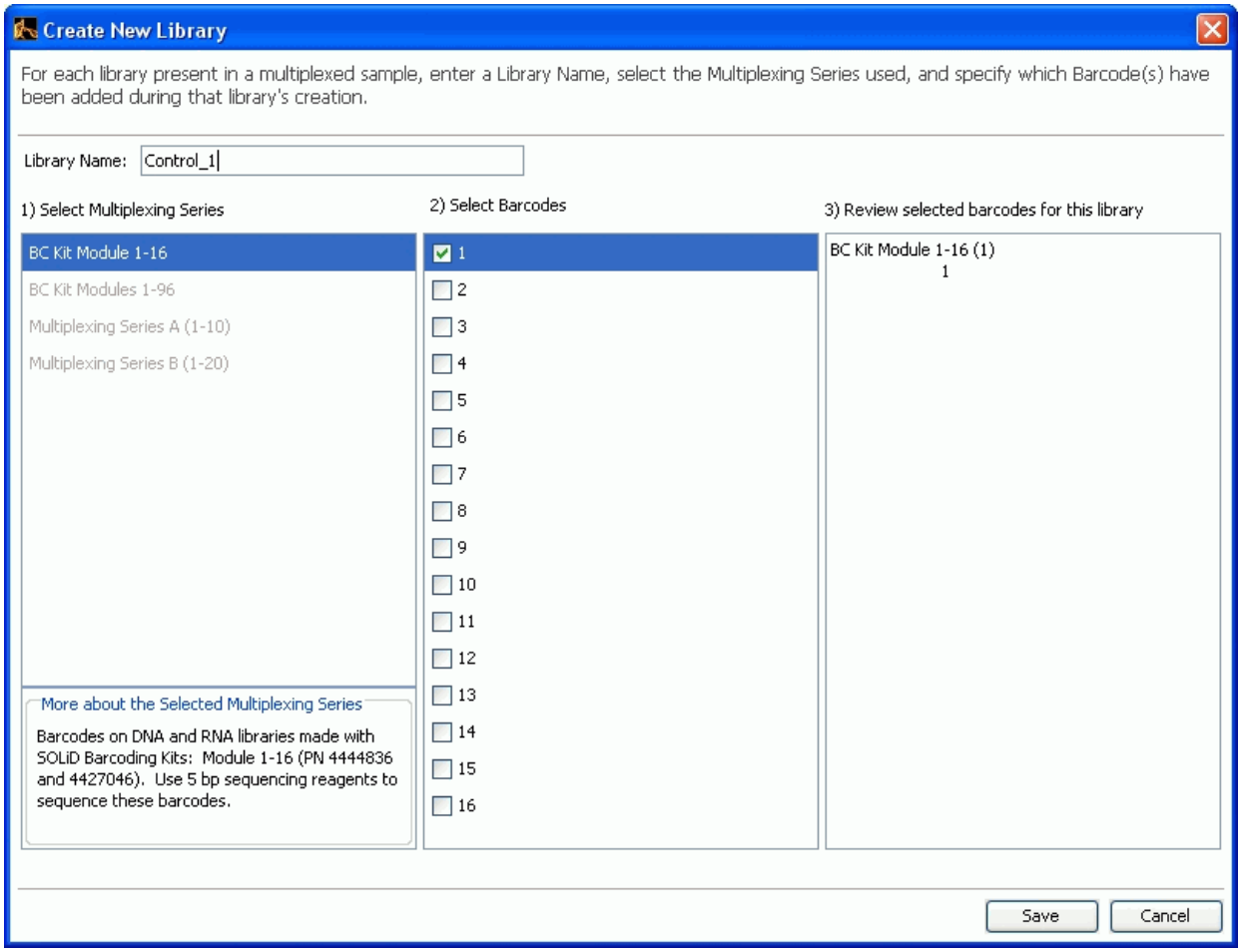


Figure 46 Enter the information for the libraries in the sample.

IMPORTANT! Be sure to select the correct Multiplexing Series for your run. You can obtain additional information by selecting the Multiplexing Series and looking at the lower left panel: **More about the Selected Multiplexing Series**. If at least 1 library uses Barcode 17 or higher on the slide, you should select the **BC Kit Modules 1–96** option for each library on the slide.

Table 8 Contents of the Multiplexing Series‡

Multiplexing Series	Contents
BC Kit Module 1-16	16 barcodes in the SOLiD™ Fragment Library Barcoding Kit Module 1-16 (PN 4444636) and SOLiD™ Transcriptome Multiplexing Kit (PN 4427046)
BC Kit Modules 1-96	96 barcodes in the SOLiD™ Fragment Library Barcoding Kit 1-96 (PN 4449637), or 48 barcodes in the SOLiD™ RNA Barcode Modules
Multiplexing Series A (1–10)	10 barcodes that are in the SOLiD™ Small RNA Expression Kit
Multiplexing Series B (1–20)	20 barcodes that are supplied with the SOLiD™ 3 System or 16 barcodes supplied with the SOLiD™ 3 Plus System Use the “Multiplexing Series B (1-20)” option with the SOLiD™ Transcriptome Multiplex Kit, which contains up to 16 barcodes

‡ For the latest information regarding Multiplexing Series and barcode kits, contact your Field Applications Specialist.

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

8. Click **Save** when you have completed the information for the first library.

9. (Optional) Repeat step 5 to step 7 for all remaining libraries. The Libraries panel of the Specify Samples and Analysis screen now shows information for all libraries for a particular sample (see Figure 47).

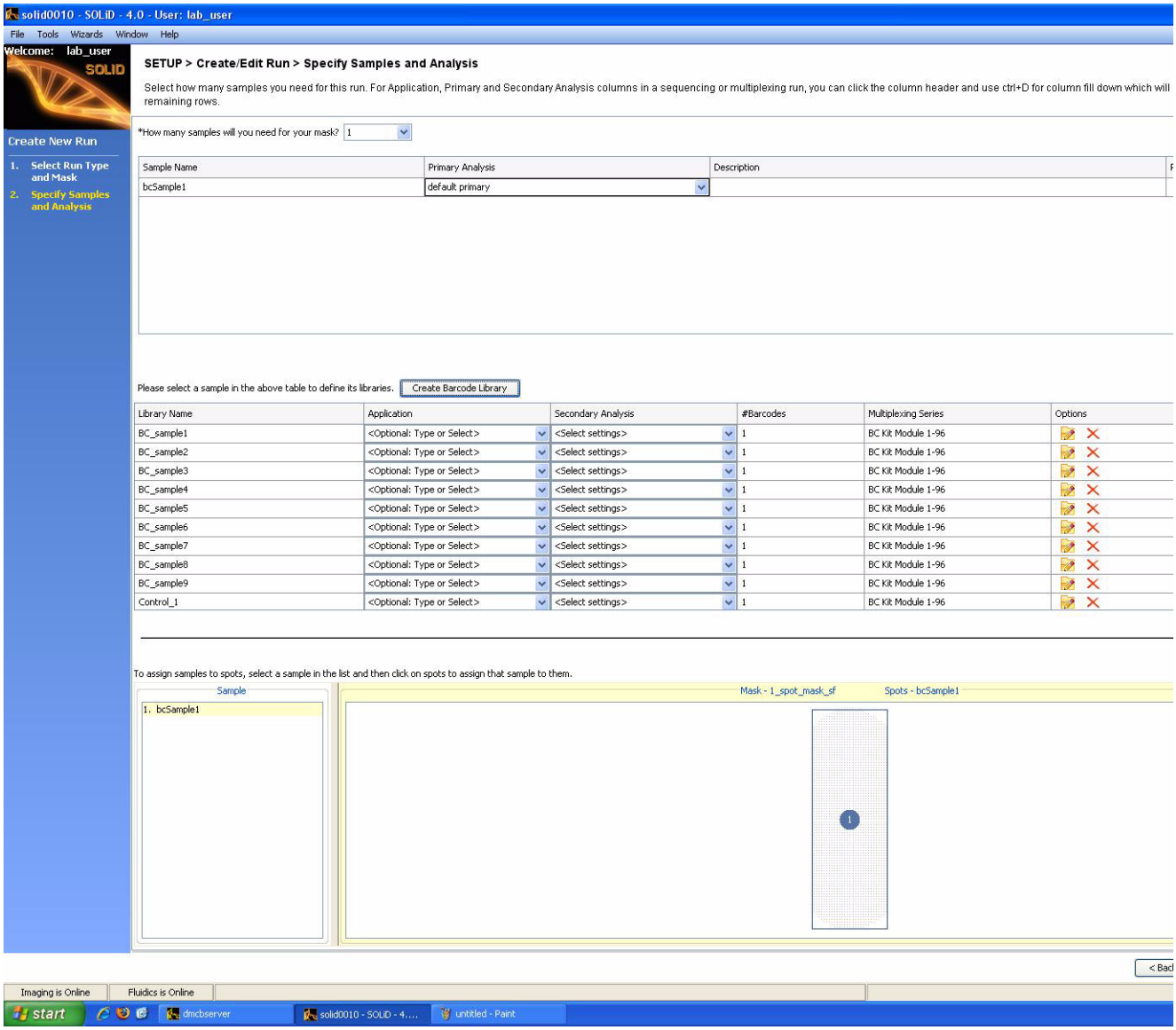


Figure 47 The Libraries panel shows information for all libraries.

IMPORTANT! Review the Selected Barcodes for each library to ensure that they are correct, and be sure that the libraries are correctly assigned to the sample. After a run is started, you cannot change the barcode or library assignment.

10. Repeat step 5 to step 9 for all remaining samples.

11. Assign samples to unassigned spots in the mask. White circles are unassigned, and blue circles are assigned. To assign a sample:

    a. Select it from the Sample list.

    b. Click a white (unassigned) spot.

    To remove a sample from an assigned (blue) spot, click the spot. The sample is removed, and the spot turns white.

12. Once all of the samples have at least 1 library, click **Finish** and select **Save to Database**.

13. Click **Manage Runs** in the task pane on the left menu pane of the ICS (see Figure 48).

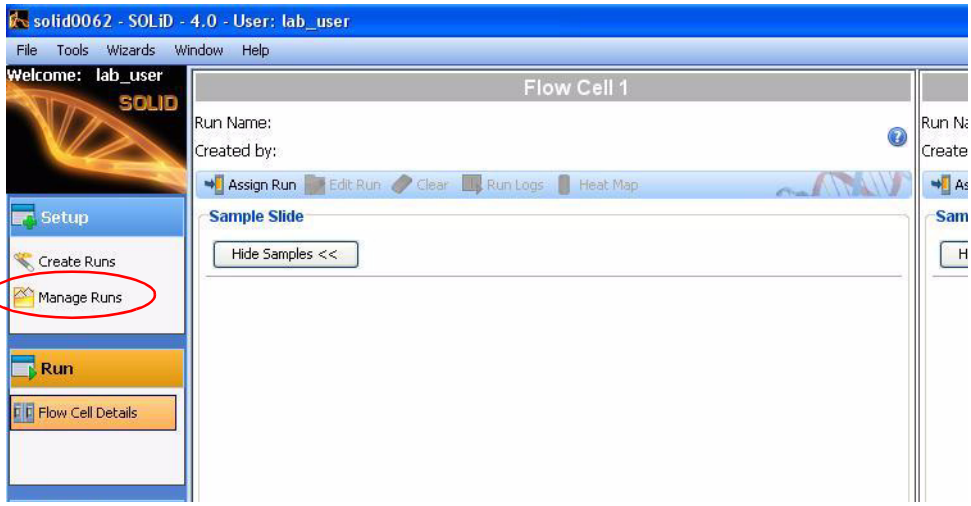


Figure 48 Manage Runs.

14. (Optional) To complete the information in the run record using a separate spreadsheet program, see "Set up a run by importing a Run Definition file" on page 127.

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

16. 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 132).

1. Close the front doors of the SOLiD™ 4 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 49). 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, in Figure 50, C:\Runs\Solid0327_20081209_2_Oct_Test\imagingMap.STG is selected).

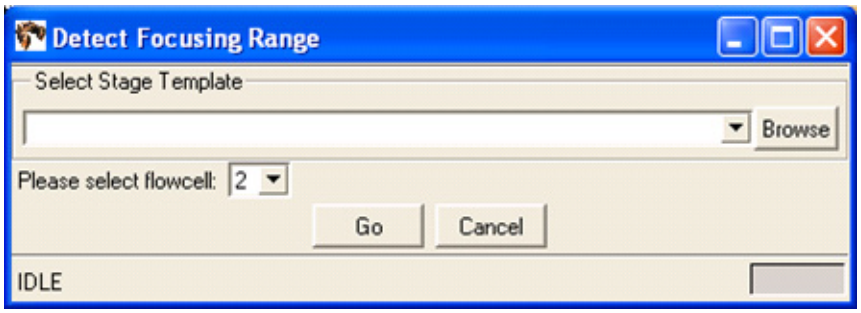


Figure 49 Detect Focusing Range dialog.

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 50). You can also click **Cancel** so that the Imager aborts the ranging operation.

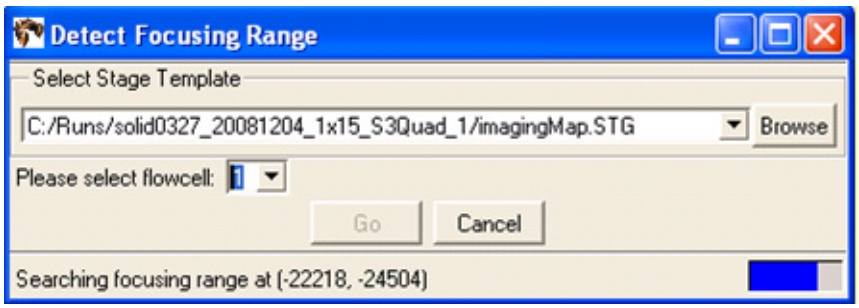


Figure 50 Detect Focusing Range Dialog while detection is in progress.

5. When the Imager is done, a dialog appears (see Figure 51). 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.

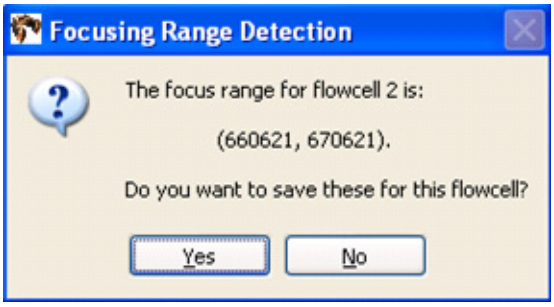


Figure 51 Confirm that you want to replace the local settings file.

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 52). 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 132).

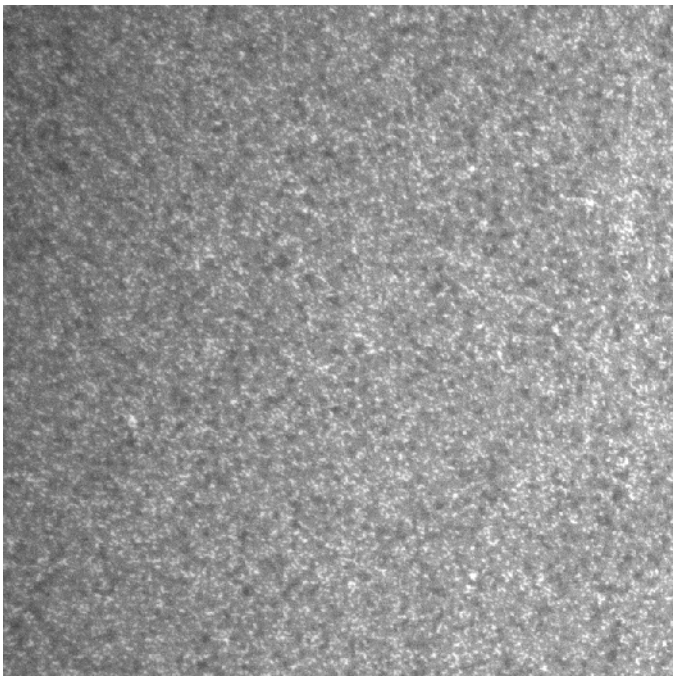


Figure 52 Beads in focus.

Start the sequencing run

Note: Before starting the run, make sure that the air filter at the back of the instrument is not clogged with dust (see "Clean the air filter" on page 119).

1. Ensure that there is adequate disk space for images and results of the sequence run (for the minimum needed disk space, see Table 9). To know the amount of disk space that is available, click **Manage Runs** on the task pane.

Table 9 Minimum required disk space to store images and results

Run Type	Minimum space needed for images	Minimum space needed for Primary Analysis results (spch, csfasta, QV.qual)	Minimum space needed for Secondary Analysis results (mapping/pairing)
Fragment (35 bp)	2.7 TB	1.3 TB	<200 GB
Fragment (50 bp)	3.8 TB	1.8 TB	<200 GB
Mate-paired (25/25 bp)	3.8 TB	1.8 TB	<200 GB
Mate-paired (35/35 bp)	5.3 TB	2.4 TB	<200 GB
Mate-paired (50/50 bp)	7.6 TB	3.3 TB	<200 GB
Paired-end (35/25 bp)	4.6 TB	2.2 TB	<200 GB
Paired-end (50/25 bp)	5.7 TB	2.7 TB	<200 GB
Multiplex fragment (35/5 bp)	3.1 TB	1.5 TB	<200 GB
Multiplex fragment (35/10 bp)	3.4 TB	1.7 TB	<200 GB
Multiplex fragment (50/5 bp)	4.2 TB	2.1 TB	<200 GB
Multiplex fragment (50/10 bp)	4.6 TB	2.3 TB	<200 GB
Multiplex paired-end (35/25/5 bp)	4.6 TB	2.4 TB	<200 GB
Multiplex paired-end (35/25/10 bp)	5.3 TB	2.6 TB	<200 GB
Multiplex paired-end (50/25/5 bp)	6.1 TB	2.9 TB	<200 GB
Multiplex paired-end (50/25/10 bp)	6.5 TB	3.1 TB	<200 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 appears (see Figure 53). Choose the appropriate option (see Table 10).

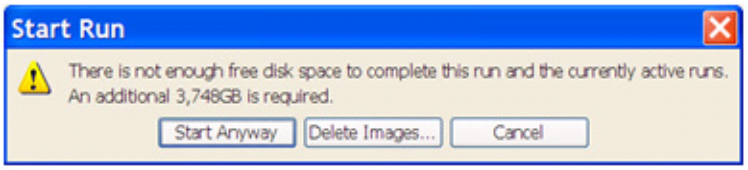


Figure 53 This Start Run dialog appears if there is not enough room to store data for the run.

Table 10 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™ 4 System SETS Software User Guide* (PN 4448411).

IMPORTANT! Before deleting a image, ensure that data analysis from the previous run is satisfactory and complete. For more information, refer to the *Applied Biosystems SOLiD™ 4 System SETS Software User Guide* (PN 4448411).

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 (see Figure 54). You can also select the appropriate flowcell in the Cycle Scans menu on the task bar on the left.

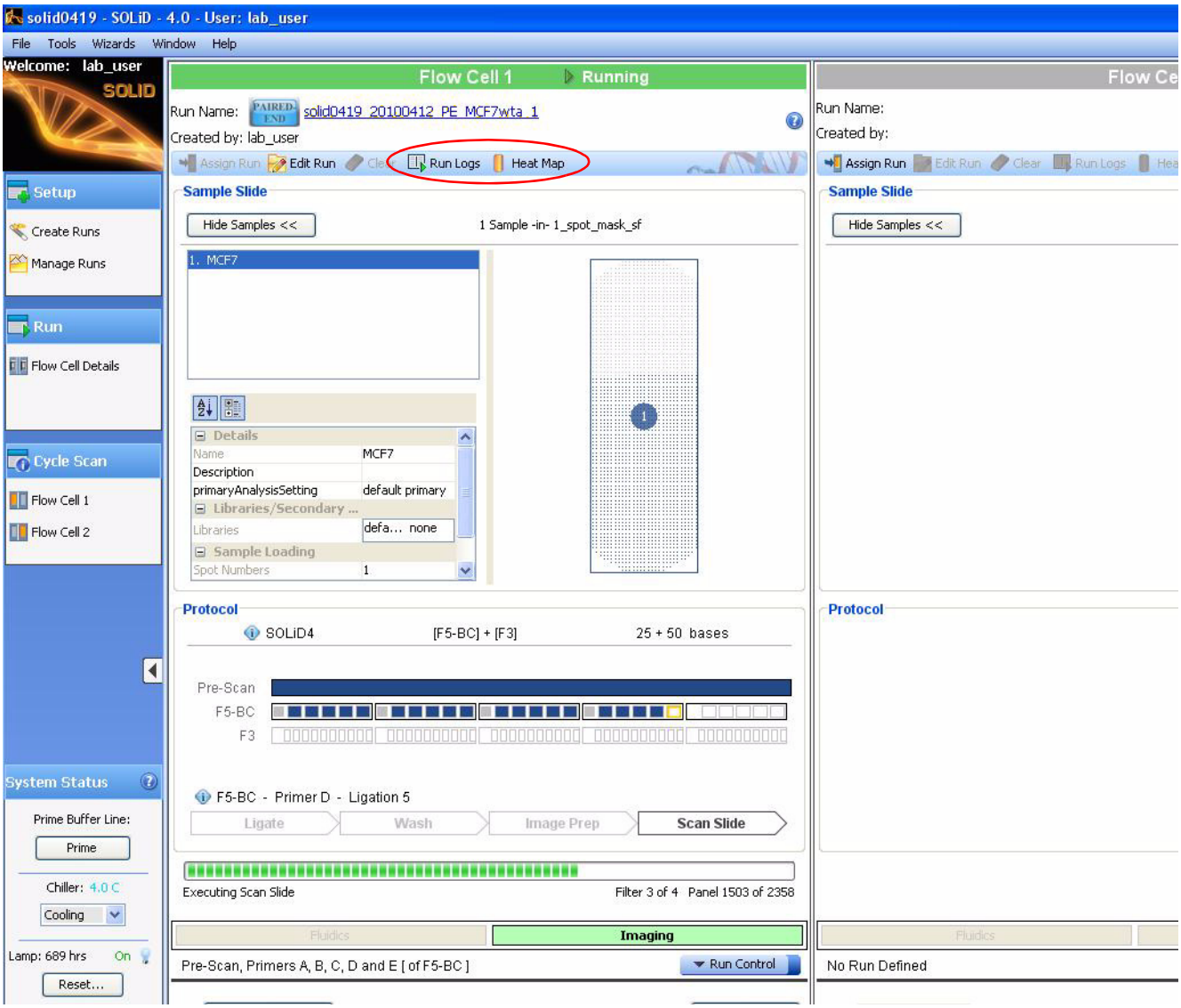


Figure 54 How to learn more about the current run.

IMPORTANT! Do not disturb the SOLiD™ 4 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

Note: Special considerations need to be made when using the Pause Run/Resume Run function in certain modules:

When the run has been paused in the middle of or at the end of the Scan Slide module, and if the Scan Slide module of the same cycle needs to be repeated, it is recommended that you resume the run starting from the Image Prep module.

Pausing the run is generally followed by automatic filling of the flowcell with Storage Buffer. Resuming from the Image Prep module fills the flowcell with Imaging Buffer again, which is required to perform the functions in the Scan Slide module. You can resume from the Image Prep module using the Change Run Progress Point command in the Run Control menu (see "Use the Run Control menu" on page 92).

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

- Prime/Ligate Mix
- Ligate/Dark Ligate Mix
- Dark Ligate/Phosphatase Mix
- Cleave/Ligate Mix
- Prime/Bridge Ligate Mix
- Bridge Ligate/Phosphatase Mix
- Dark Ligate/Capper Enzyme Mix
- Cleave/RevPhosphatase Mix
- RevPhosphatase/Ligate Mix
- RevPhosphatase/Bridge Ligate Mix
- Bridge Ligate/Capper Enzyme Mix

When you try to pause a run in the middle of the module, a dialog displays (see Figure 55).

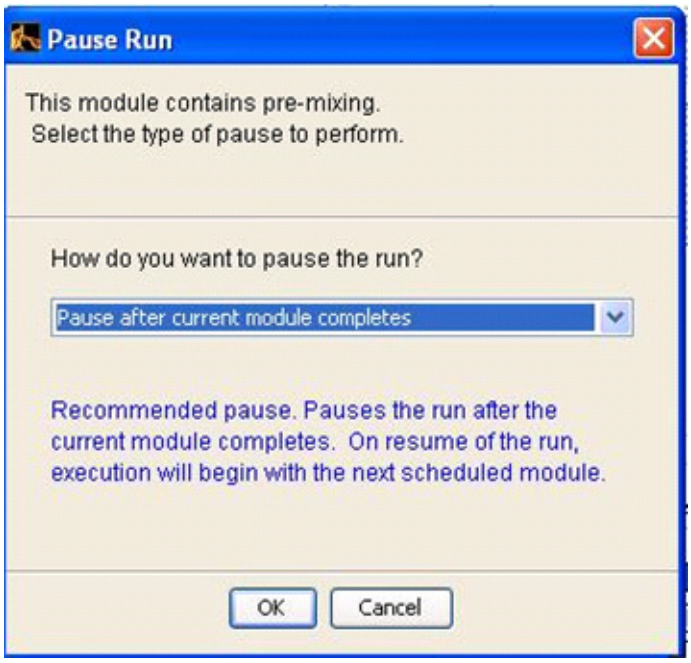


Figure 55 Pause Run dialog for pre-mixing module.

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 56).

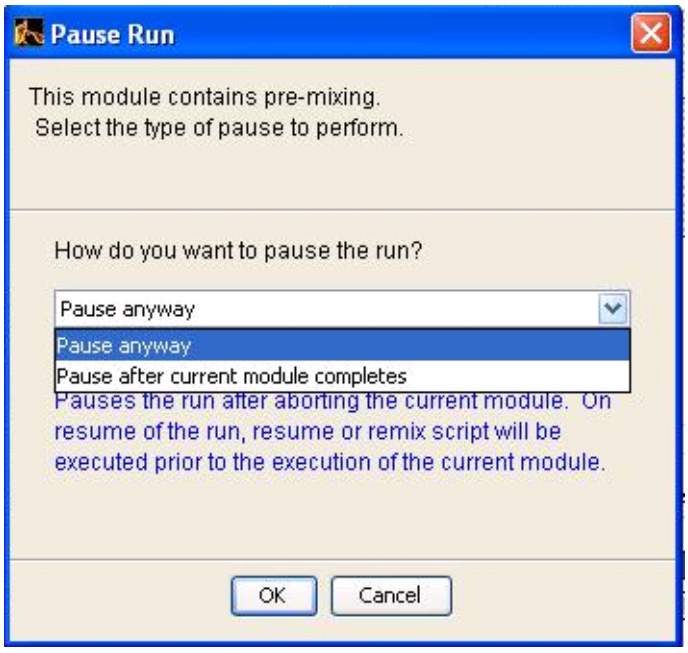


Figure 56 Pause Run dialog options for pre-mixing module.

When you resume a run to use the pre-mixed reagents in the strip tube, a dialog box displays (see Figure 57). 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.

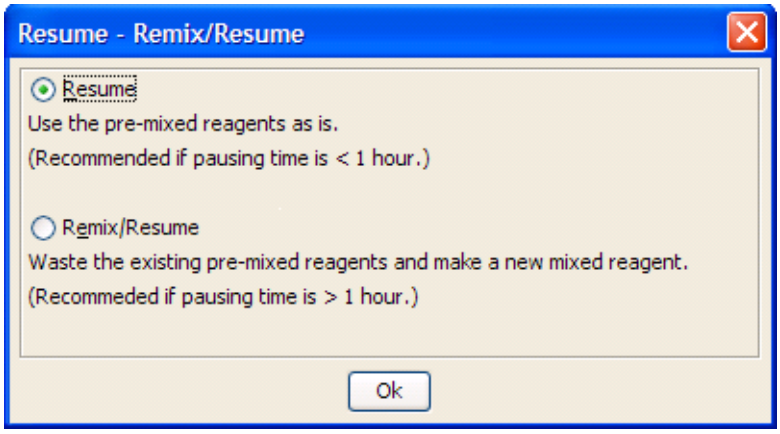


Figure 57 The options to resume or remix/resume the run are available.

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 58).

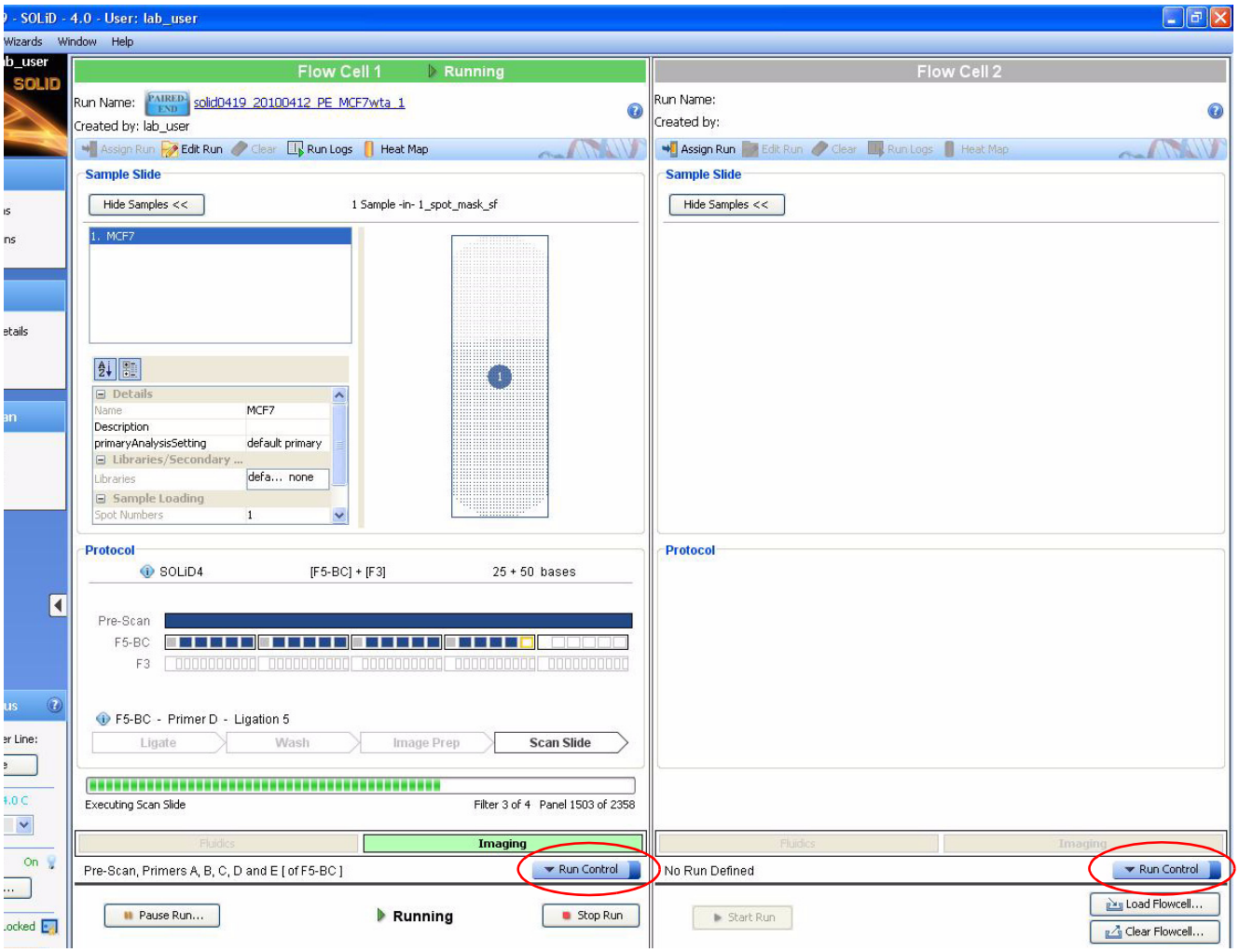


Figure 58 Click **Run Control** to display the Run Control menu for that flowcell.

Run Control commands

IMPORTANT! Do not disturb the SOLiD™ 4 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 11 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 12.

Table 12 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 start.

Change Primer Schedule

The Change Primer Schedule command allows you to choose a first and second primer, in any order. 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 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.

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.

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 spots

The Instrument Control Software (ICS) allows you to control imaging for individual spots 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 spot. To access the imaging and analysis controls, in the Sample Slide display of the ICS, right-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 59).

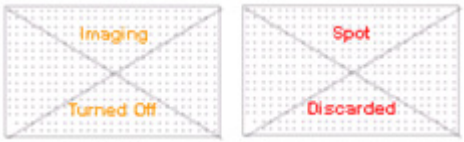


Figure 59 The Sample Slide display updates after using Imaging Turned Off or Spot Discarded.

Monitor the run

Note: To monitor the run remotely, use SETS from any computer to connect to the networked instrument (refer to the *Applied Biosystems SOLiD™ 4 System SETS Software User Guide* [PN 4448411]). If desired, set up e-mail notification regarding instrument run and system information using SETS (refer to the *Applied Biosystems SOLiD™ 4 System SETS Software User 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 the 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 60).
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, 300,000 beads/panel). A large number of missing panels could indicate a deposition problem.

     Note: The heat map is not available until after the completion of the first sequencing cycle. The software refines the focal map using images collected during the first sequencing cycle and does not display the heat map until after all of the images collected during both the Prescan and cycle 1 have been processed. This may take up to 30 minutes depending on the number of panels that were imaged.
3. After you finish viewing the Heat Map, click **Close** located at the bottom of the Heat Map window.

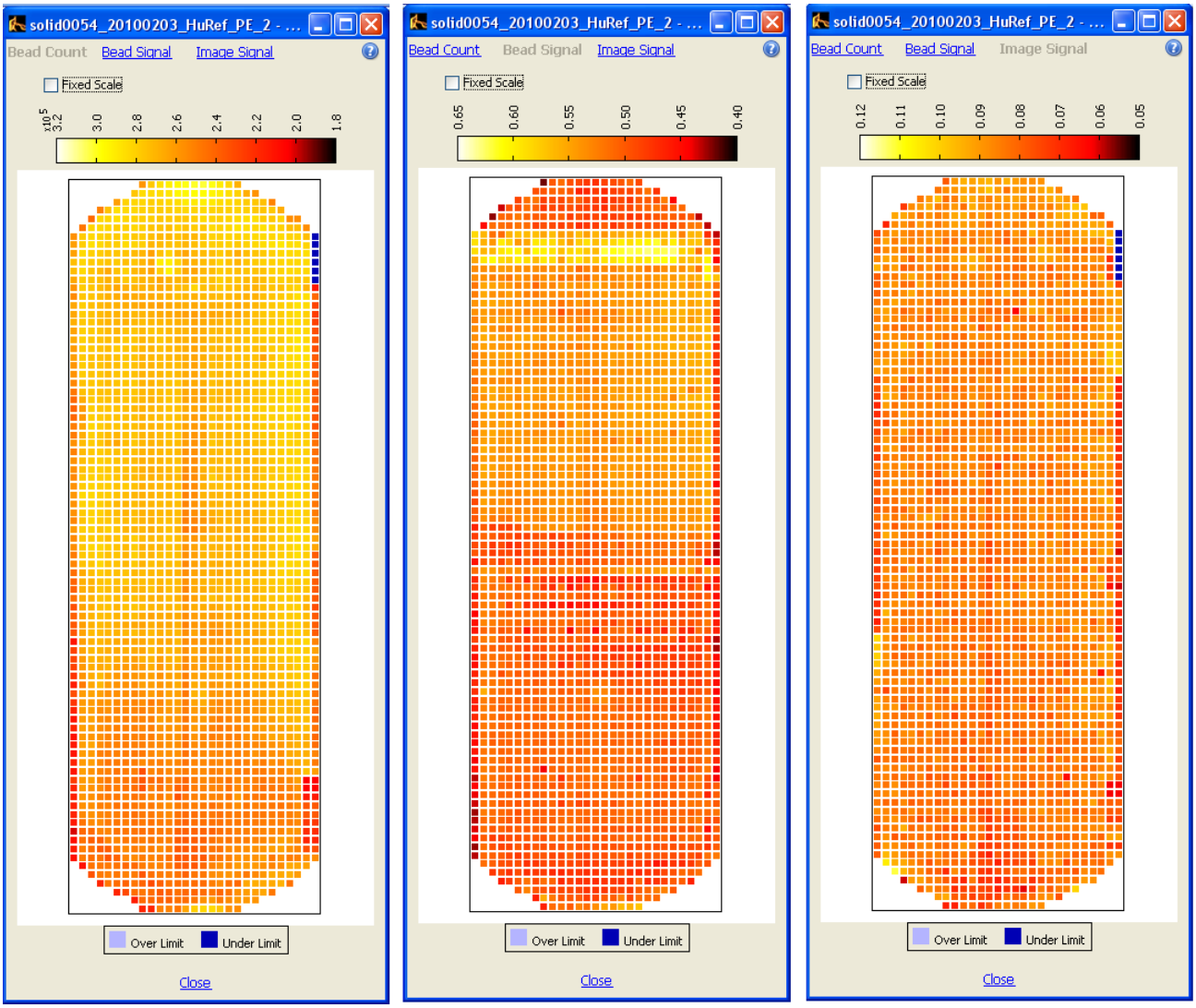


Figure 60 Bead Count (left), Bead Signal (center), and Image Signal (right) heat maps.

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 61 to assess the progress of the sequencing run (see Table 13).

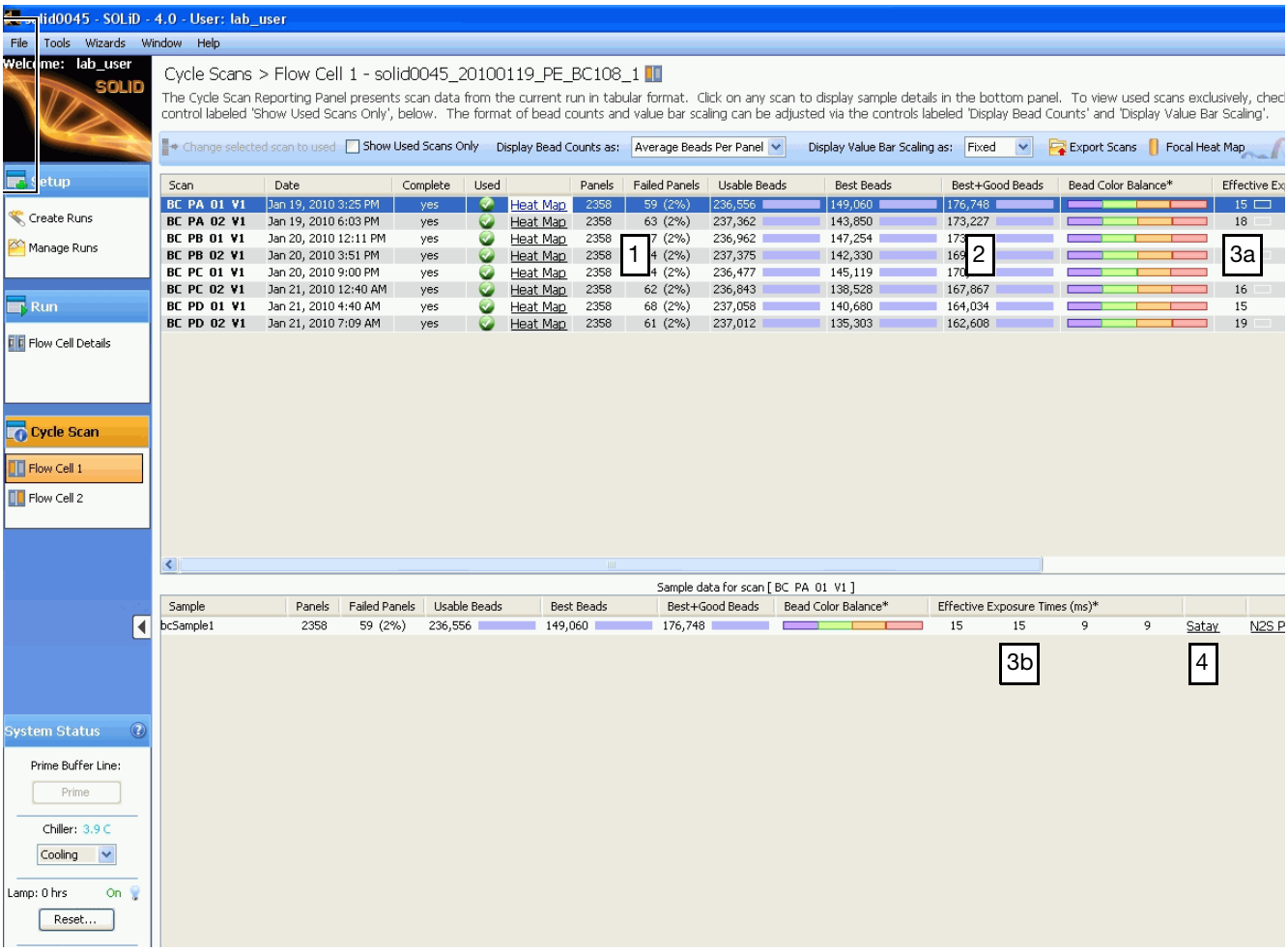


Figure 61 Parameters available in the Cycle Scans window to assess the progress of the sequencing run: 1 Failed panels; 2 Best + Good Beads / usable beads; 3a/3b Effective exposure times; 4 Satay plots.

Table 13 Cycle Scans window: Distinguish normal runs from problematic runs

Parameter	Normal run	Problematic run
(1) Failed panels (number of panels that failed image alignment during color-calling).	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 20 to 40 ms in the first ligation cycle and increases to 100 to 300 ms in the tenth cycle.	250 ms or greater in ligation cycle 1 or when instrument times out when the effective exposure time exceeds 500 ms. 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™ 4 Analyzer every 1500 hours of use. See Appendix B for instructions.

2. Click the heat map link to view the heat map for that cycle (see Figure 62).

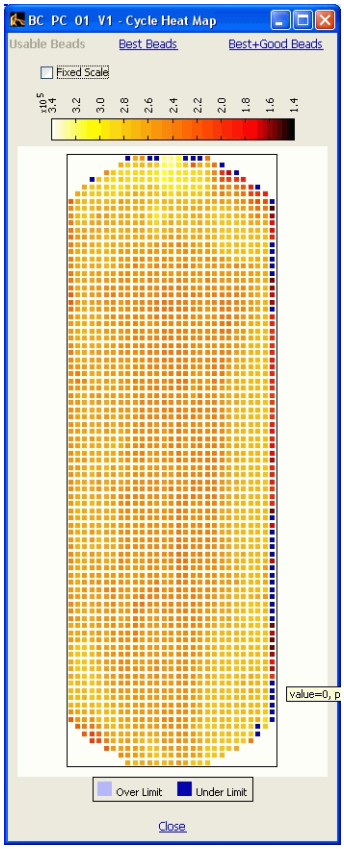


Figure 62 Click the heat map link to view the heat map for that cycle.

3. Left-double-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 63).

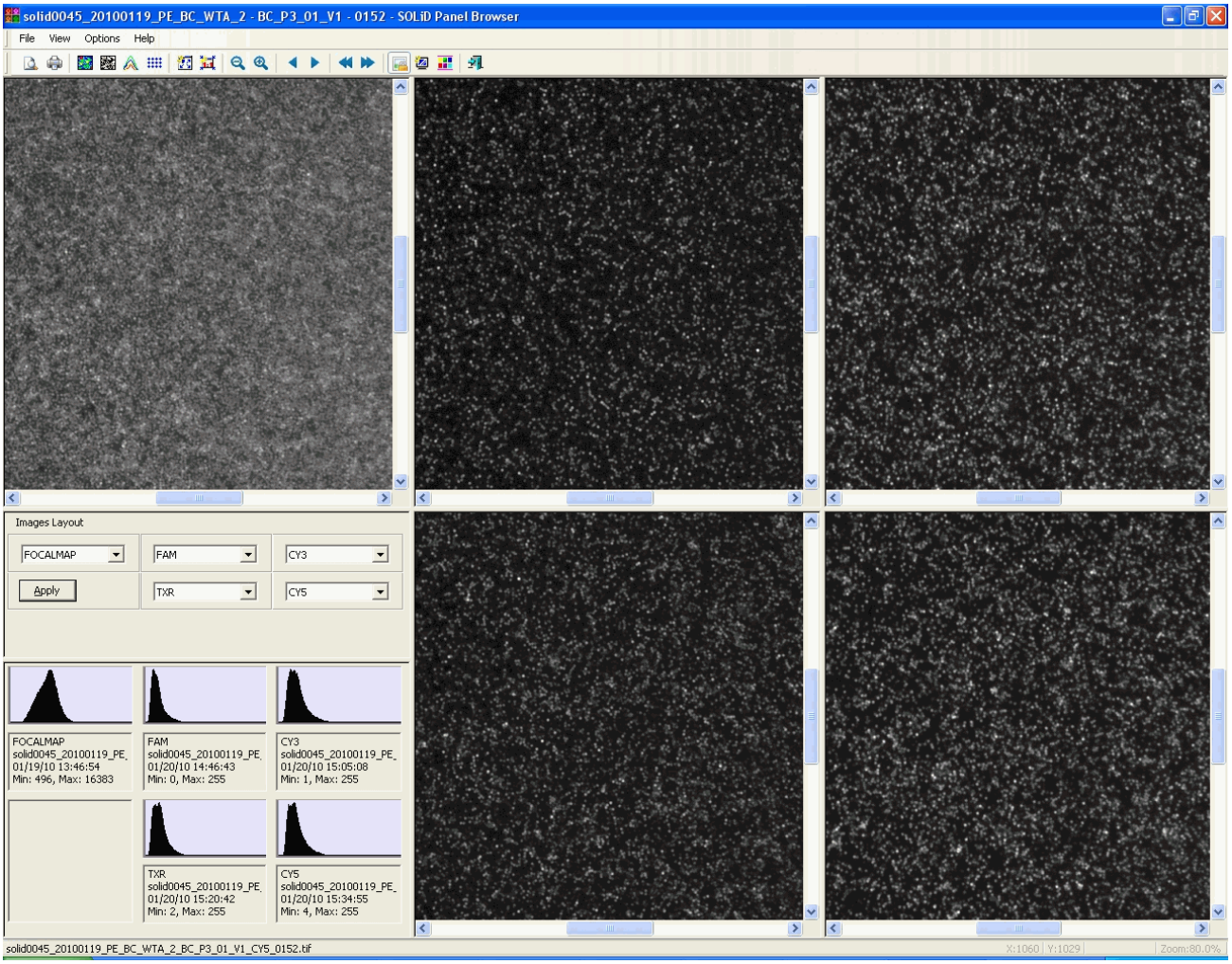


Figure 63 View the focal map and the image for each fluorescent dye signal.

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

Note: If the sequencing run 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™ System Field Applications Specialist.

A

Required Materials

This appendix covers:

Set up and perform a workflow analysis (WFA) run

Required Applied Biosystems reagent kits

Table 14 Required Applied Biosystems reagent kits: WFA run

Item (part number)	Components	Kit component(s) used in...
SOLiD™ ToP Instrument Buffer Kit (4452688)	Reset Buffer Glycerol 10× Instrument Buffer Cleave Solution 1 Cleave 2.1 Kit (Cleave 2.1 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) Universal Buffer Kit (Universal Buffer Parts 1 and 2)	Sequencing and/or WFA
SOLiD™ XD Slide & Deposition Kit v2 (4456997)‡	Slide Prep Reagent 4 × 2 Sequencing Slides Slide Deposition Buffer v2 (4456998)§ Slide Storage Buffer	Bead deposition
SOLiD™ ToP Workflow Analysis Reagents (4453237)	SOLiD™ ToP Workflow Analysis Reagents	WFA
SOLiD™ Flowcell O-rings (4398217)	10-pack of flowcell O-rings	Sequencing and/or WFA

‡ If this kit is not available, you can use the SOLiD™ XD Slide & Deposition Kit (4448393).
§ If the SOLiD™ XD Slide Deposition Buffer v2 (4456998) is not available, you can use SOLiD™ XD Slide Deposition Buffer (4448388).

Required equipment

Table 15 Required equipment: WFA run

Item‡	Source
SOLiD™ 4 System	Applied Biosystems PN 4452773 (110 V) Applied Biosystems PN 4452774 (220 V)
SOLiD™ 4 Analyzer	Applied Biosystems PN 4452775
SOLiD™ 3 Plus to SOLiD™4 Upgrade Kit SOLiD™ 3 to SOLiD™4 Upgrade Kit	Applied Biosystems PN 4452784 Applied Biosystems PN 4452785
SOLiD™ Light Source	Applied Biosystems PN 4383441
SOLiD™ Slide Storage Chamber	Applied Biosystems PN 4406354
SOLiD™ Deposition Chambers, 1-Well§	Applied Biosystems PN 4406352
SOLiD™ Deposition Chambers, 4-Well§	Applied Biosystems PN 4406358
SOLiD™ Deposition Chambers, 8-Well§	Applied Biosystems PN 4406359
SOLiD™ Uninterruptible Power Supply (UPS)	Applied Biosystems PN 4397781 (SOLiD™ UPS North America) Applied Biosystems PN 4393695 (220 V; SOLiD™ UPS International)
SOLiD™ Accessory Disk Drive	Applied Biosystems PN 4426101
SOLiD™ Bead Concentration Chart	Applied Biosystems PN 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 • 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 • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTubes (1 pack of 25) For system materials summary, refer to "Covaris™ S2 System Materials Summary," *Applied Biosystems SOLiD™ 4 System Site Preparation Guide*.	Applied Biosystems PN 4387833 (110 V) Applied Biosystems PN 4392718 (220 V) or Covaris
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
Tabletop Centrifuge (for 96-well plate)	Major Laboratory Supplier (MLS)
Vortexer	MLS
Picofuge	MLS
Magnetic stirrer	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™ 4 Analyzer is shipped with 2 of each size SOLiD™ Deposition Chambers and SOLiD™ 4 Slide Carriers. Two SOLiD™ Slide Storage Chambers are provided for use with all chambers.
# Or equivalent but validation of the equipment for library preparation is required.

Required consumables

Table 16 Required consumables: WFA run

Item‡	Source
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
1.5-mL LoBind Tubes	Eppendorf 022431021
Kimwipes®	Major Laboratory Supplier (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” (refer to the 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.

Set up and perform a sequencing run

Required Applied Biosystems reagent kits

Table 17 Required Applied Biosystems reagent kits: Sequencing run (for part numbers of individual boxes, see Figure 23 on page 46).

Item (part number)	Components	Kit component(s) used in…
SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35 (4449352)	SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD™ Mixing Strip Tube with Zinc	Fragment sequencing up to 35 bases
SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 (4449388)	SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD™ Mixing Strip Tube with Zinc	Fragment sequencing up to 50 bases
SOLiD™ ToP Paired End Sequencing Kit – Frag. Lib., MM35/25 (4452690)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – Frag. Lib., F5-P2 Tag, MM25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer A Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer B Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer C Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer D Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer E Master Mix 25 SOLiD™ Mixing Strip Tube	Paired-end sequencing up to 35 bases for the forward read and 25 bases for the reverse read

Item (part number)	Components	Kit component(s) used in…
SOLiD™ ToP Paired End Sequencing Kit – Frag. Lib., MM50/25 (4452693)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – Frag. Lib., F5-P2 Tag, MM25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer A Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer B Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer C Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer D Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-P2 Tag – Primer E Master Mix 25 SOLiD™ Mixing Strip Tube	Paired-end sequencing up to 50 bases for the forward read and 25 bases for the reverse read
SOLiD™ ToP Mate-Paired Sequencing Kit – M-P. Lib., MM35/35 (4452684)	SOLiD™ ToP Sequencing Kit – M-P. Lib., F3 Tag, MM35 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – M-P. Lib., R3 Tag, MM35 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer A Master Mix 35 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer B Master Mix 35 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer C Master Mix 35 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer D Master Mix 35 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer E Master Mix 35 SOLiD™ Mixing Strip Tube with Zinc	Mate-pair sequencing up to 35 bases for each tag

Item (part number)	Components	Kit component(s) used in…
SOLiD™ ToP Mate-Paired Sequencing Kit – M-P. Lib., MM50/50 (4452685)	SOLiD™ ToP Sequencing Kit – M-P. Lib., F3 Tag, MM50 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD™ ToP MP Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – M-P. Lib., R3 Tag, MM50 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer A Master Mix 50 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer B Master Mix 50 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer C Master Mix 50 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer D Master Mix 50 SOLiD™ ToP MP Lib Seq, R3 Tag – Primer E Master Mix 50 SOLiD™ Mixing Strip Tube with Zinc	Mate-pair sequencing up to 50 bases for each tag
SOLiD™ ToP Fragment BC Sequencing Kit – BC Frag Lib., MM35/5 (4452696)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM5 Reset Buffer	Multiplex sequencing up to 35 bases for the F3 tag of barcoded fragment libraries (Barcodes 1 to 16)
SOLiD™ ToP Fragment BC Sequencing Kit – BC Frag Lib., MM35/10 (4452698)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM10 Reset Buffer	Multiplex sequencing up to 35 bases for the F3 tag of barcoded fragment libraries (Barcodes 1 to 96)

Item (part number)	Components	Kit component(s) used in…
SOLiD™ ToP Fragment BC Sequencing Kit – BC Frag Lib., MM50/5 (4452697)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM5 Reset Buffer	Multiplex sequencing up to 50 bases for the F3 tag of barcoded fragment libraries (Barcodes 1 to 16)
SOLiD™ ToP Fragment BC Sequencing Kit – BC Frag Lib., MM50/10 (4452699)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM10 Reset Buffer	Multiplex sequencing up to 50 bases for the F3 tag of barcoded fragment libraries (Barcodes 1 to 96)
SOLiD™ ToP Paired End Sequencing Kit – BC Frag. Lib., MM35/25/5 (4452691)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – BC Frag. Lib., F5-BC Tag, MM25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer A Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer B Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer C Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer D Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer E Master Mix 25 SOLiD™ Mixing Strip Tube SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM5 Reset Buffer	Multiplex paired-end sequencing up to 35 bases for the F3 tag of barcoded fragment libraries (Barcodes 1 to 16)

Item (part number)	Components	Kit component(s) used in…
SOLiD™ ToP Paired End Sequencing Kit – BC Frag. Lib., MM35/25/10 (4452692)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – BC Frag. Lib., F5-BC Tag, MM25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer A Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer B Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer C Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer D Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer E Master Mix 25 SOLiD™ Mixing Strip Tube SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM10 Reset Buffer	Multiplex paired-end sequencing up to 35 bases for the F3 tag of barcoded fragment libraries (Barcodes 1 to 96)
SOLiD™ ToP Paired End Sequencing Kit – BC Frag. Lib., MM50/25/5 (4452694)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – BC Frag. Lib., F5-BC Tag, MM25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer A Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer B Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer C Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer D Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer E Master Mix 25 SOLiD™ Mixing Strip Tube SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM5 Reset Buffer	Multiplex paired-end sequencing up to 50 bases for the F3 tag of barcoded fragment libraries (Barcodes 1 to 16)

Item (part number)	Components	Kit component(s) used in…
SOLiD™ ToP Paired End Sequencing Kit – BC Frag. Lib., MM50/25/10 (4452695)	SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD™ ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD™ Mixing Strip Tube with Zinc SOLiD™ ToP Sequencing Kit – BC Frag. Lib., F5-BC Tag, MM25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer A Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer B Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer C Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer D Master Mix 25 SOLiD™ ToP Frag Lib Seq, F5-BC Tag – Primer E Master Mix 25 SOLiD™ Mixing Strip Tube SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM10 Reset Buffer	Multiplex paired-end sequencing up to 50 bases for the F3 tag of barcoded fragment libraries (Barcodes 1 to 96)
SOLiD™ ToP Instrument Buffer Kit (4452688)	Reset Buffer Glycerol 10× Instrument Buffer Cleave Solution 1 Cleave 2.1 Kit (Cleave 2.1 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) Universal Buffer Kit (Universal Buffer Parts 1 and 2)	Sequencing and/or WFA
SOLiD™ Flowcell O-rings (4398217)	10-pack of flowcell O-rings	Sequencing and/or WFA
SOLiD™ XD Slide & Deposition Kit v2 (4456997)‡	Slide Prep Reagent 4 × 2 Sequencing Slides Slide Deposition Buffer v2 (4456998)§ Slide Storage Buffer	Bead deposition
Other components	SOLiD™ Mixing Strip Tube with Zinc (4449506) SOLiD™ Mixing Strip Tube (4406595)	

‡ If this kit is not available, you can use the SOLiD™ XD Slide & Deposition Kit (4448393).
§ If the SOLiD™ XD Slide Deposition Buffer v2 (4456998) is not available, you can use SOLiD™ XD Slide Deposition Buffer (4448388).

Required equipment

Table 18 Required equipment: Sequencing run

Item‡	Source
SOLiD™ 4 System	Applied Biosystems PN 4452773 (110 V) Applied Biosystems PN 4452774 (220 V)
SOLiD™ 4 Analyzer	Applied Biosystems PN 4444317
SOLiD™ 3 Plus to SOLiD™4 Upgrade Kit SOLiD™ 3 to SOLiD™4 Upgrade Kit	Applied Biosystems PN 4452784 Applied Biosystems PN 4452785
SOLiD™ Light Source	Applied Biosystems PN 4388441
SOLiD™ Slide Storage Chamber	Applied Biosystems PN 4406354
SOLiD™ Deposition Chambers, 1-Well§	Applied Biosystems PN 4406352
SOLiD™ Deposition Chambers, 4-Well§	Applied Biosystems PN 4406358
SOLiD™ Deposition Chambers, 8-Well	Applied Biosystems PN 4406359
SOLiD™ 4 Slide Carriers§	Applied Biosystems PN 4453027
SOLiD™ Uninterruptible Power Supply (UPS)	Applied Biosystems PN 4397781 (SOLiD™ UPS North America) Applied Biosystems PN 4393695 (220 V; SOLiD™ UPS International)
SOLiD™ Accessory Disk Drive	Applied Biosystems PN 4426101
SOLiD™ Bead Concentration Chart	Applied Biosystems PN 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 • 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 • Covaris-2 Series Machine Holder for (one) microTube • Covaris microTube Prep Station • Covaris Water Tank Label Kit • Covaris microTubes (1 pack of 25) For system materials summary, refer to "Covaris™ S2 System Materials Summary," *Applied Biosystems SOLiD™ 4 System Site Preparation Guide*.	Applied Biosystems PN 4387833 (110 V) Applied Biosystems PN 4392718 (220 V) or Covaris
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

Item‡	Source
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Tabletop Centrifuge (for 96-well plate)	Major Laboratory Supplier (MLS)
Vortexer	MLS
Picofuge	MLS
Magnetic stirrer	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™ 4 Analyzer is shipped with 2 of each size SOLiD™ Deposition Chambers and SOLiD™ 4 Slide Carriers. Two SOLiD™ Slide Storage Chambers are provided for use with all chambers.
# Or equivalent but validation of the equipment for library preparation is required.

Required consumables

Table 19 Required consumables: Sequencing run

Item‡	Source
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
1.5-mL LoBind Tubes	Eppendorf 022431021
Kimwipes®	Major Laboratory Supplier (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” (refer to the 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.

B Supplemental Procedures

This appendix covers:

Clean the SOLiD™ Deposition Chamber using a sonicator

Only users upgrading from a SOLiD™ 3 or SOLiD™ 3 Plus System need to 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 116). The cleaning procedure needs to be performed only once after upgrading to the SOLiD™ 4 System.

Required equipment

Table 20 Required equipment: Clean the Deposition Chamber with a sonicator

Item	Source
Sonicator‡	Branson Ultrasonics, Inc. 8510R-DTH
2-L beaker	Major Laboratory Supplier (MLS)

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

Required consumables

Table 21 Required consumables: Clean the Deposition Chamber with a sonicator

Item	Source
Bleach	Major Laboratory Supplier (MLS)‡
Deionized water	MLS
Hot water	MLS

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

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™ 4 or SOLiD™ Opti Slide Carrier into the beaker. Place the beaker in the sonicator, then sonicate the SOLiD™ Deposition Chamber and the SOLiD™ 4 or SOLiD™Opti Slide Carrier for 10 minutes.

6. After sonication, remove the SOLiD™ Deposition Chamber and SOLiD™ 4 or SOLiD™ Opti Slide Carrier from the sonicator, then rinse the SOLiD™ Deposition Chamber and SOLiD™ 4 or SOLiD™ Opti 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 the SOLiD™ 4 or SOLiD™ Opti Slide Carrier overnight or at 37 °C for 1 hour before use.

Clean the SOLiD™ Deposition Chamber using Extran 300

Only users upgrading from a SOLiD™ 3 or SOLiD™ 3 Plus System need to 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 114). The cleaning procedure needs to be performed only once after upgrading to the SOLiD™ 4 System.

Required equipment

Table 22 Required equipment: Clean the Deposition Chamber with Extran 300

Item	Source
Tub	Major Laboratory Supplier (MLS)
Pipette or graduated cylinder	MLS

Required consumables

Table 23 Required consumables: Clean the Deposition Chamber with Extran 300

Item	Source
Extran 300	VWR EM-EX0996-2
Deionized water	Major Laboratory Supplier (MLS)‡
Hot water	MLS

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

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™ 4 or SOLiD™ Opti 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™ 4 or SOLiD™ Opti Slide Carrier to soak for another 5 minutes.

6. After the second soak, remove the SOLiD™ Deposition Chamber and SOLiD™ 4 or SOLiD™ Opti 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™ 4 or SOLiD™ Opti Slide Carrier overnight before use.

Clean the Instrument Buffer bottle

Regular cleaning of the Instrument Buffer bottle is required for every run. Failure to clean the Instrument Buffer bottle regularly may allow microbial contaminants to proliferate in the system.

If the level of Instrument Buffer in the Instrument Buffer bottle falls below the recommended fill volume, do not add new Instrument Buffer to "top off" the Buffer that has been standing in the Instrument Buffer bottle. Topping off can lead to contamination.

Required equipment

Table 24 Required equipment: Clean the Instrument Buffer bottle

Item	Source
Beaker or graduated cylinder	Major Laboratory Supplier (MLS)
Bottle brush	MLS

Required consumables

Table 25 Required consumables: Clean the Instrument Buffer bottle

Item	Source
Deionized water	Major Laboratory Supplier (MLS)‡
Bleach	MLS
Water	MLS

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

1. Rinse the Instrument Buffer bottle three times with approximately 1 L of deionized water.
2. Inspect the Instrument Buffer bottle for visible signs of microbial contaminants.
3. If any contaminants are present, clean the Instrument Buffer bottle with bleach:
   a. Pour approximately 500 mL of bleach into the Instrument Buffer bottle.
   b. Add approximately 500 mL of water to the bleach in the bottle.
   c. Scrub the bottle with a bottle brush.
   d. Rinse the bottle at least two times with water.
   e. Rinse the bottle at least three more times with deionized water.

Clean the air filter

Regular cleaning of the air filter is required once a month. Failure to clean the air filter may cause the filter to clog with dust, which may lead to poor air flow and temperature control for the flowcells. You should clean the filter in between runs (but not during runs).

Required consumables

Table 26 Required consumables: Clean the air filter

Item	Source
Kimwipes® wipers	Major Laboratory Supplier (MLS)

1. At the back of the instrument, remove the plastic filter cover with the filter (see Figure 64).

Figure 64 Remove the plastic filter cover.

2. Separate the plastic cover from the air filter (see Figure 65).

Figure 65 Separate the plastic cover (left) from the air filter (right).

3. Use a Kimwipes® wiper to wipe the filter clean. If the filter is heavily clogged, rinse the filter under running water and dry thoroughly overnight.

4. Reinstall the filter and plastic filter cover on the instrument.

Flush the fluidic lines

If the SOLiD™ 4 Analyzer is in continuous use, the fluidics system in the instrument generally does not need much routine care beyond regular cleaning of the Instrument Buffer bottle and flushing of the lines every three months. However, if the SOLiD™ 4 Analyzer will be sitting idle for more than two weeks, flush the fluidic system and power off the instrument with the fluidic lines empty.

Note: The instrument flush procedure comprises two scripts. Each script takes approximately 13 minutes to complete.

Required consumables

Table 27 Required consumables: Flush the fluidic lines

Item	Source
Deionized water	Major Laboratory Supplier (MLS)
Slides	MLS

1. Open the Instrument Shutdown wizard by choosing **Wizards ▸ Instrument Shutdown** (Figure 66).

Figure 66 Open Instrument Shutdown Wizard.

2. In the Wizard's introduction screen, click **Next**.

3. In the System Wash screen, click **Unlock Doors** (Figure 67).

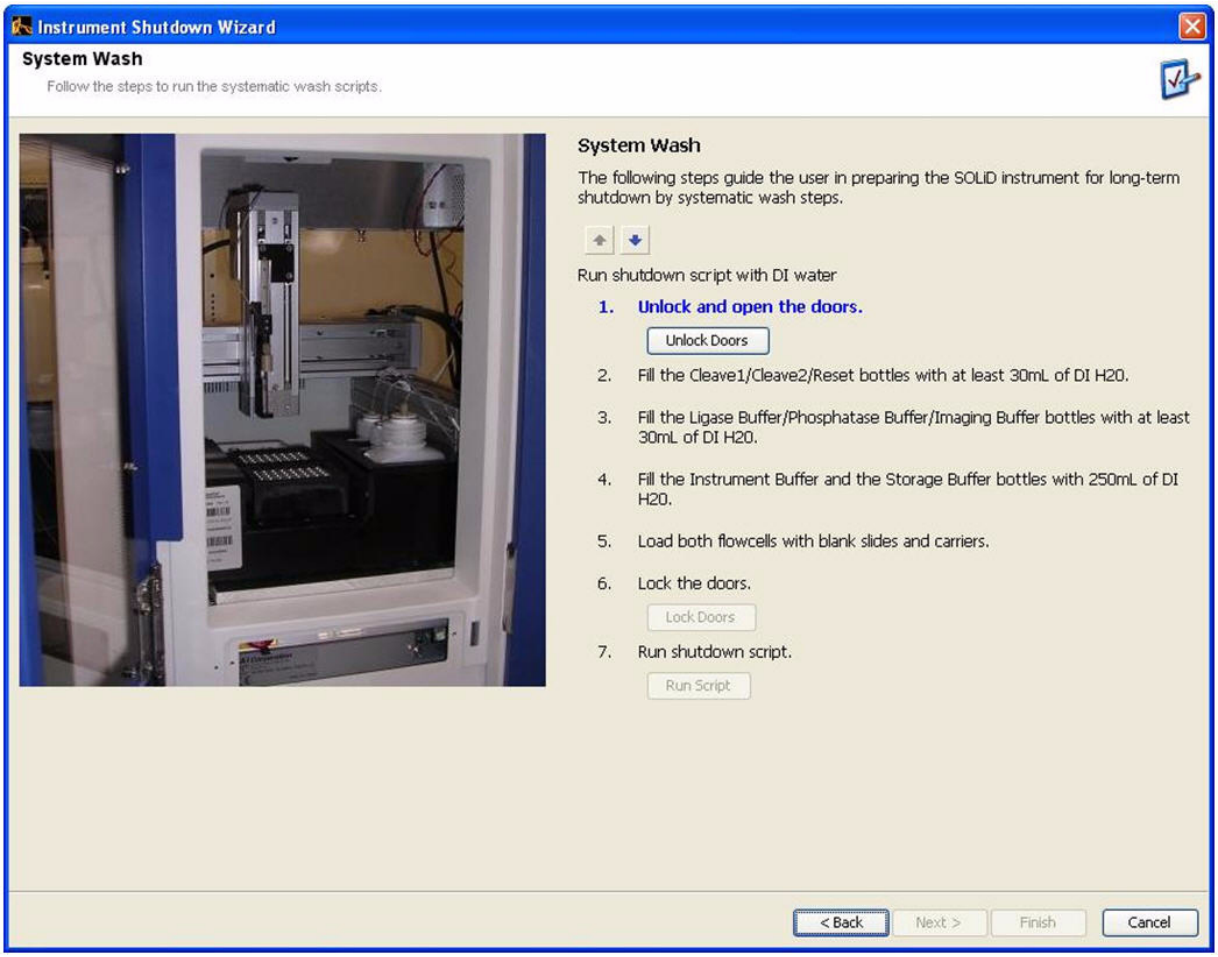


Figure 67 Complete the actions in the first System Wash screen.

4. Replace all of the bottles from the side of the instrument, the chiller block, and the cabinet with rinsed-out bottles.

   All bottles *except* the Instrument Buffer and Storage Buffer bottles should contain at least 30 mL of deionized water. The Instrument Buffer and Storage Buffer bottles. These two bottles require 250 mL.

5. Load a slide onto each of the flowcells. You can load used slides or 1-inch × 3-inch standard microscope slides for this step.

6. Click **Lock Doors**.

7. Click **Run Script** to begin instrument flush.

   Note: The script takes approximately 13 minutes to complete.

8. After the script has completed, click **Next**.

9. In the System Wash screen, click **Unlock Doors** and remove all of the bottles from the side of the instrument, the chiller block, and the cabinet.

   You will now perform a dry flush of the system (Figure 68).

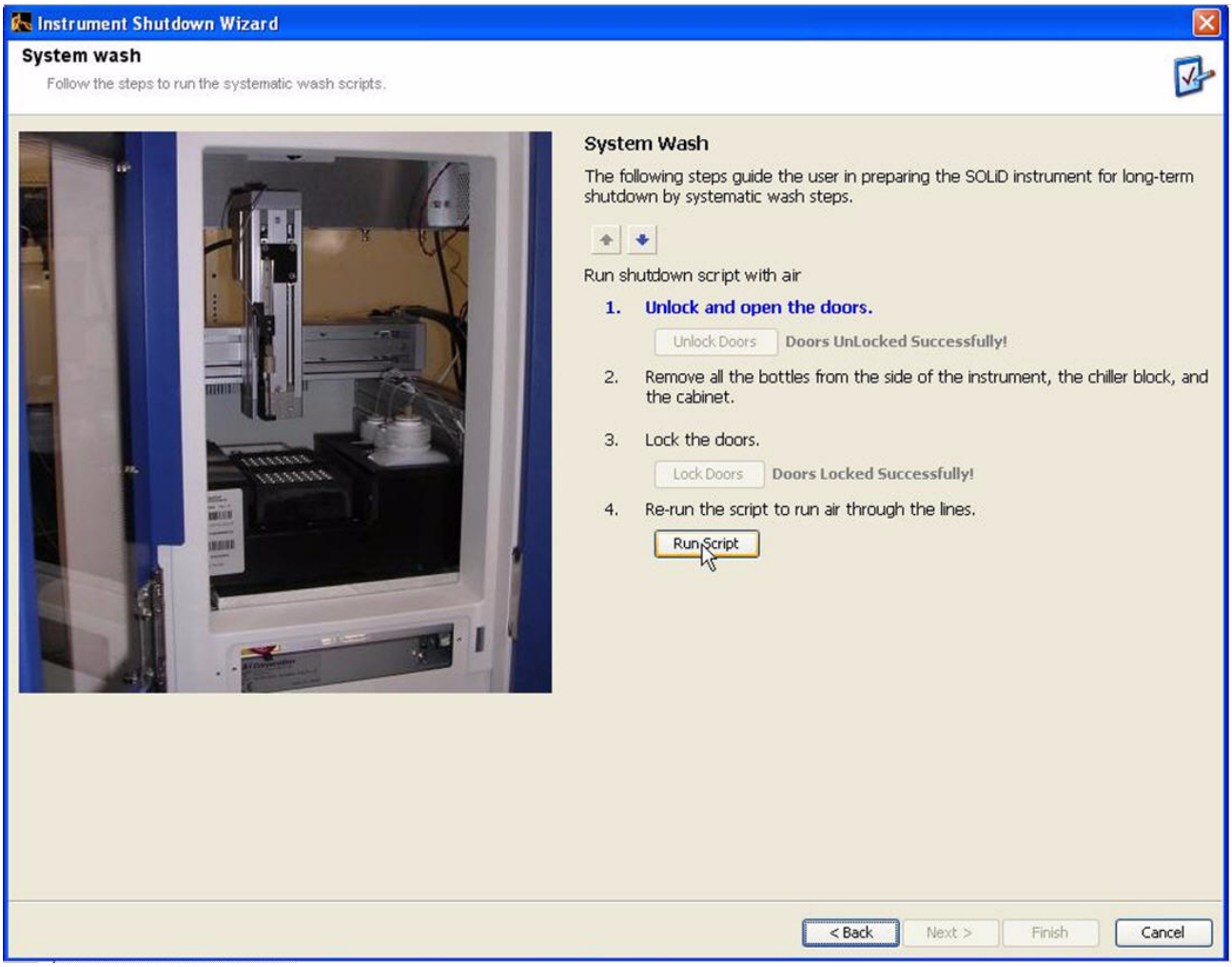


Figure 68 Complete the actions in the second System Wash screen.

10. To begin the dry instrument flush, click **Run Script**.

    Note: This script takes approximately 13 minutes to complete.

11. Perform one of the following:
    - SOLiD™ 4 Analyzer **in continuous use**: If the SOLiD™ 4 Analyzer is *in continuous use* and you are performing this flush every three months as required for preventive maintenance, click **Cancel** (Figure 69). Do not shut down the power to the Linux head node and compute nodes.
    - SOLiD™ 4 Analyzer **idle for more than two weeks**: If the SOLiD™ 4 Analyzer *will not be used for more than two weeks*, click **ShutDown** (Figure 69). Close down the SOLiD™ Instrument Control Software and power off the instrument.

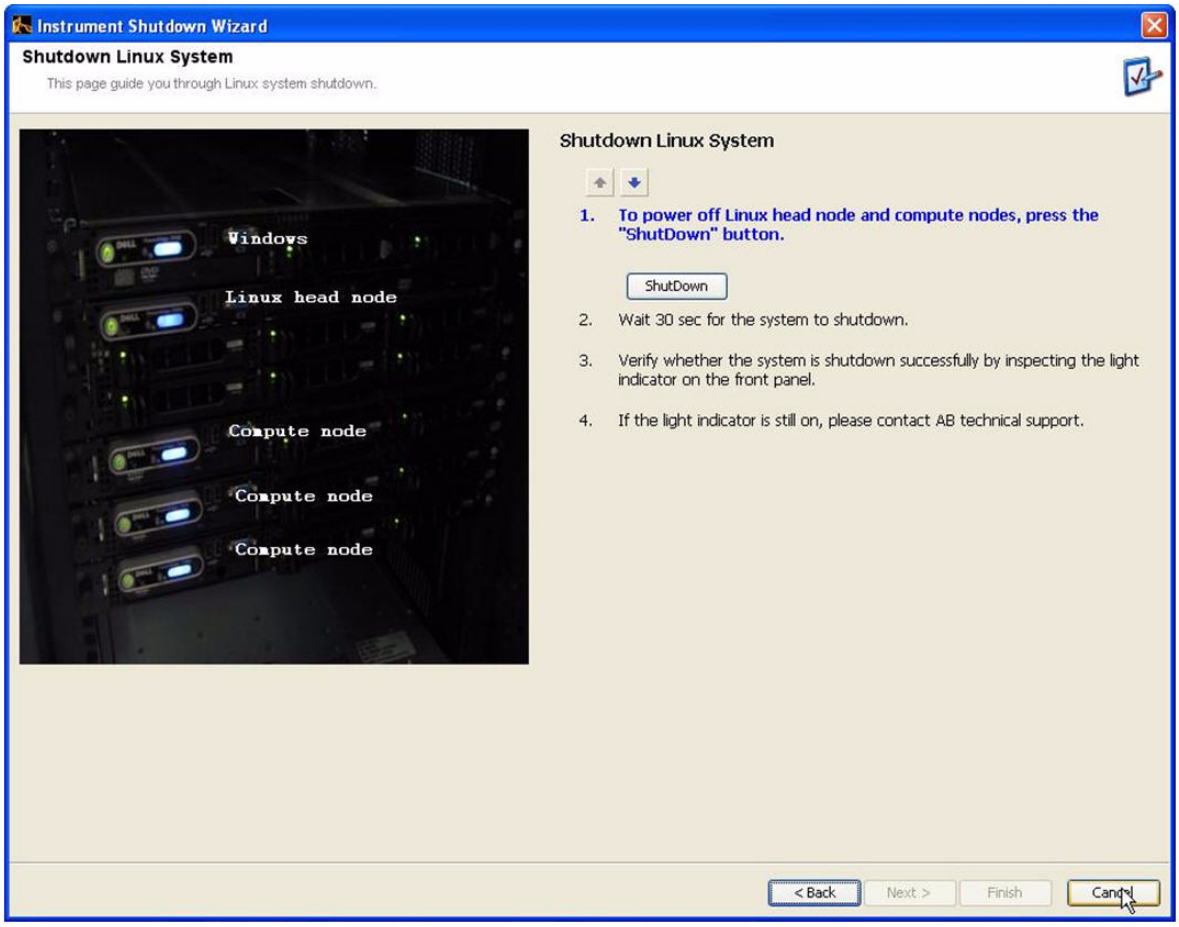


Figure 69 Click Cancel if performing regular maintenance or ShutDown to power off the instrument.

Install the SOLiD™ System Flowcell O-ring

After each run, check the SOLiD™ System 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

Table 28 Required equipment: Install the SOLiD™ Flowcell O-ring

Item	Source
SOLiD™ System 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 70).
2. Run your finger around the O-ring to make sure that there are no high spots.

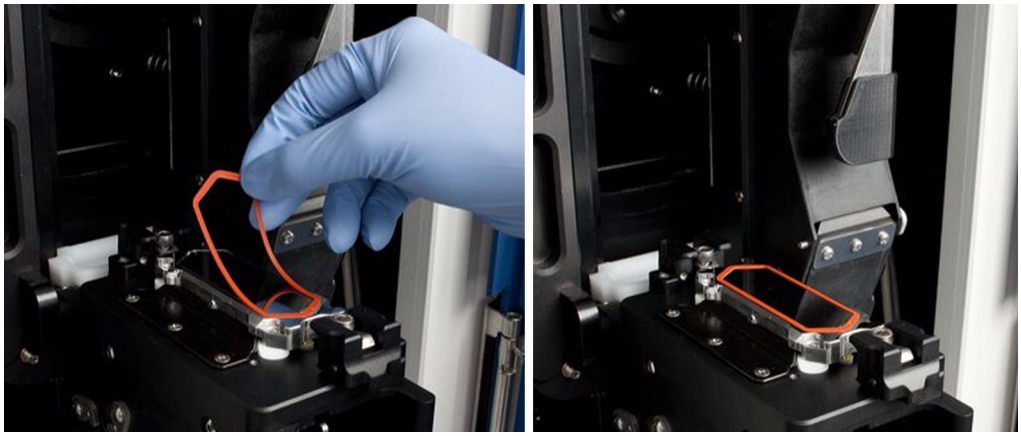


Figure 70 Install the SOLiD™ O-Ring on a flowcell.

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 31).

Required equipment

Table 29 Required equipment: Clean the reagent strip cover

Item	Source
Pipette or graduated cylinder	Major Laboratory Supplier (MLS)‡
Scrub brush	MLS

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

Required consumables

Table 30 Required consumables: Clean the reagent strip cover

Item	Source
Extran 300	VWR EM-EX0996-2
Kimwipes® wipers	Major Laboratory Supplier (MLS)
Deionized water	MLS
Cotton swabs	MLS

Table 31 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 Kimwipes® wiper. If the reagents are dried, first wet the Kimwipes® wiper 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™ 4 Analyzer every 1500 hours.

Required equipment

Table 32 Replace the SOLiD™ Light Source

Item	Source
SOLiD™ Light Source	Applied Biosystems 4388441

1. Unscrew the 4 screws retaining the light box access cover on the top of the instrument.
2. Remove the cover to the light source from the housing.
3. Pull the light source straight up and out of the unit.
4. Slide a new SOLiD™ Light Source into place. Ensure that the light source is oriented in the correct direction (see Figure 71).
5. Refit and screw down the access cover on the housing.
6. In the System Status menu on the ICS, click **Reset** to reset the lamp timer.

Figure 71 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. Click **Manage Runs** in the task pane on the left menu pane of the ICS (see Figure 72).

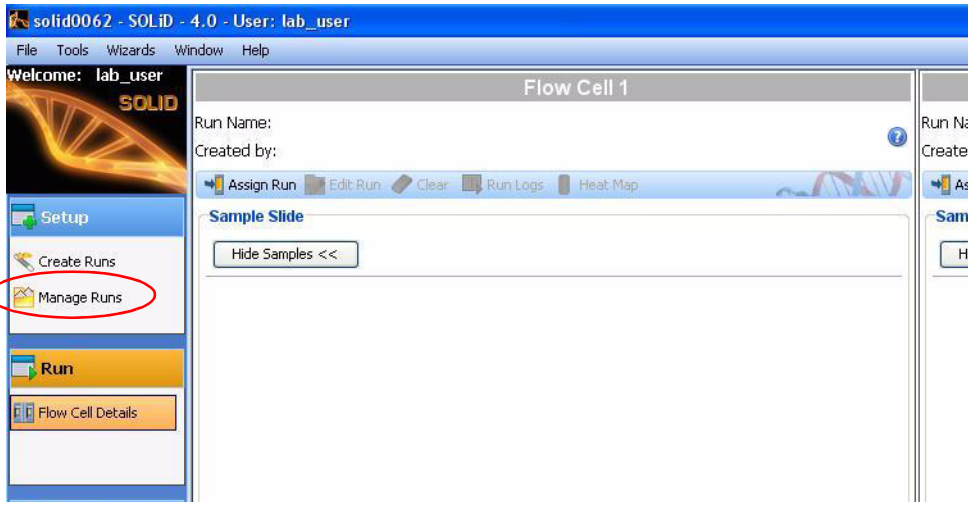


Figure 72 Manage Runs.

2. Click **Export Run** (see Figure 73).

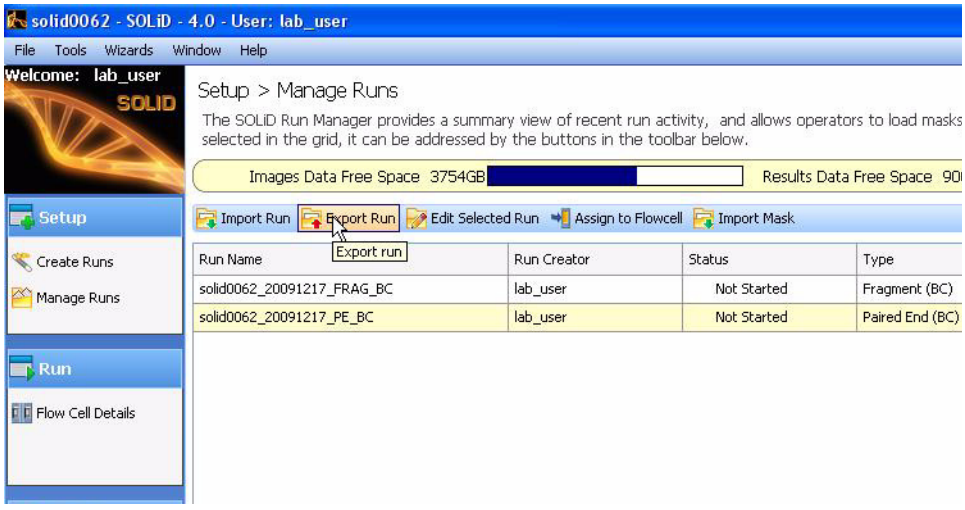


Figure 73 Export Run.

3. Open the Run Definition file that you exported using scalc (see Figure 74). If desired, you can use another spreadsheet program.

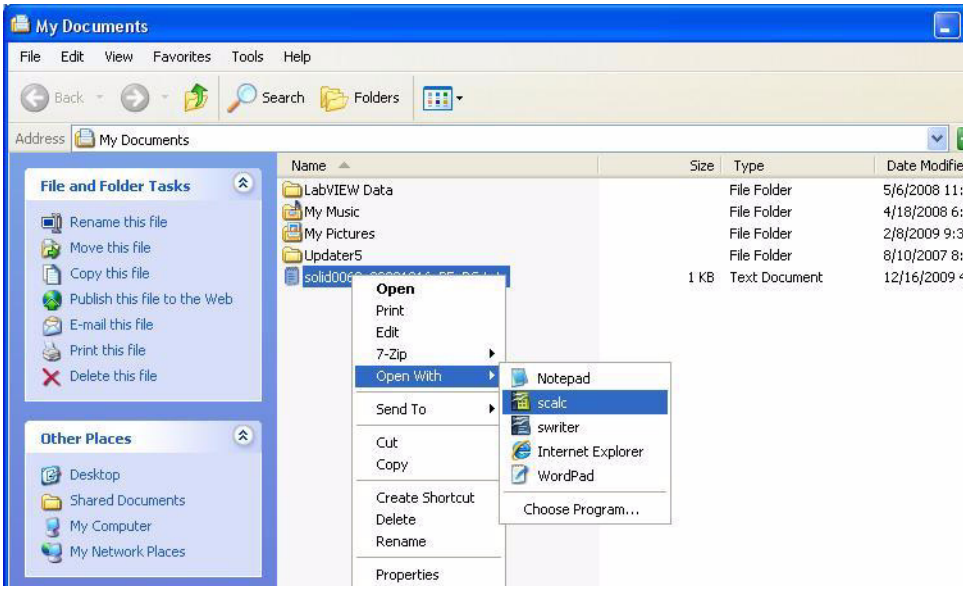


Figure 74 Open the Run Definition file.

4. In the Text Importer window, empty the Text Delimiter box by clicking in the **Text Delimiter** box and pressing the **Backspace** key to delete the quotation marks (see Figure 75). Then click **OK**.

5.

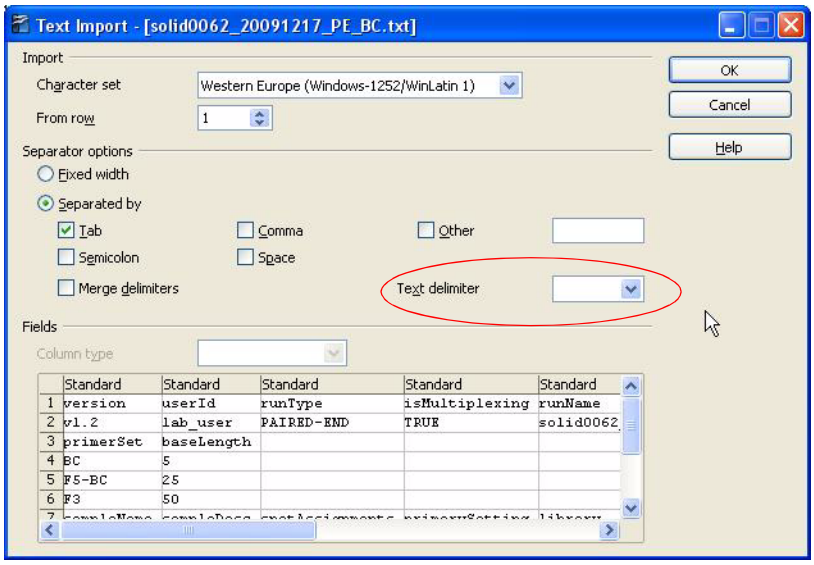


Figure 75 Empty the Text Delimiter box.

6. Select **Tools** ▸ **Autocorrect Options**. In the Custom Quotes tab, uncheck the **Replace** box in the Double Quotes section (see Figure 76). If this box is checked, the file will not be imported correctly into ICS because directional quotation marks will be used instead of non-directional (straight) quotation marks.

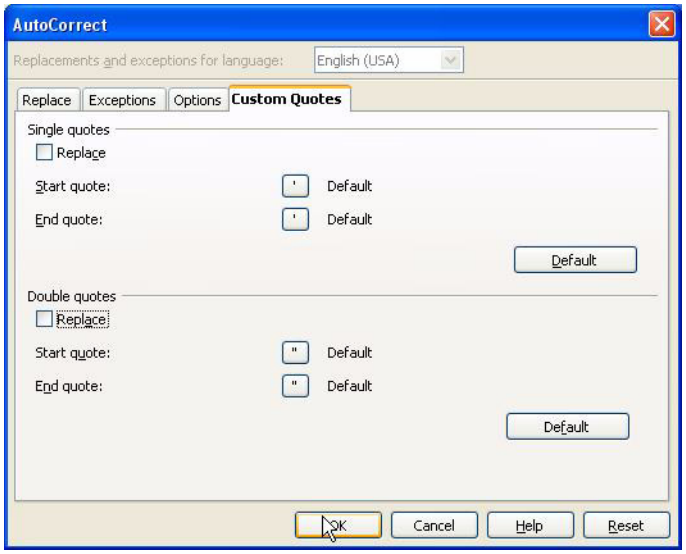
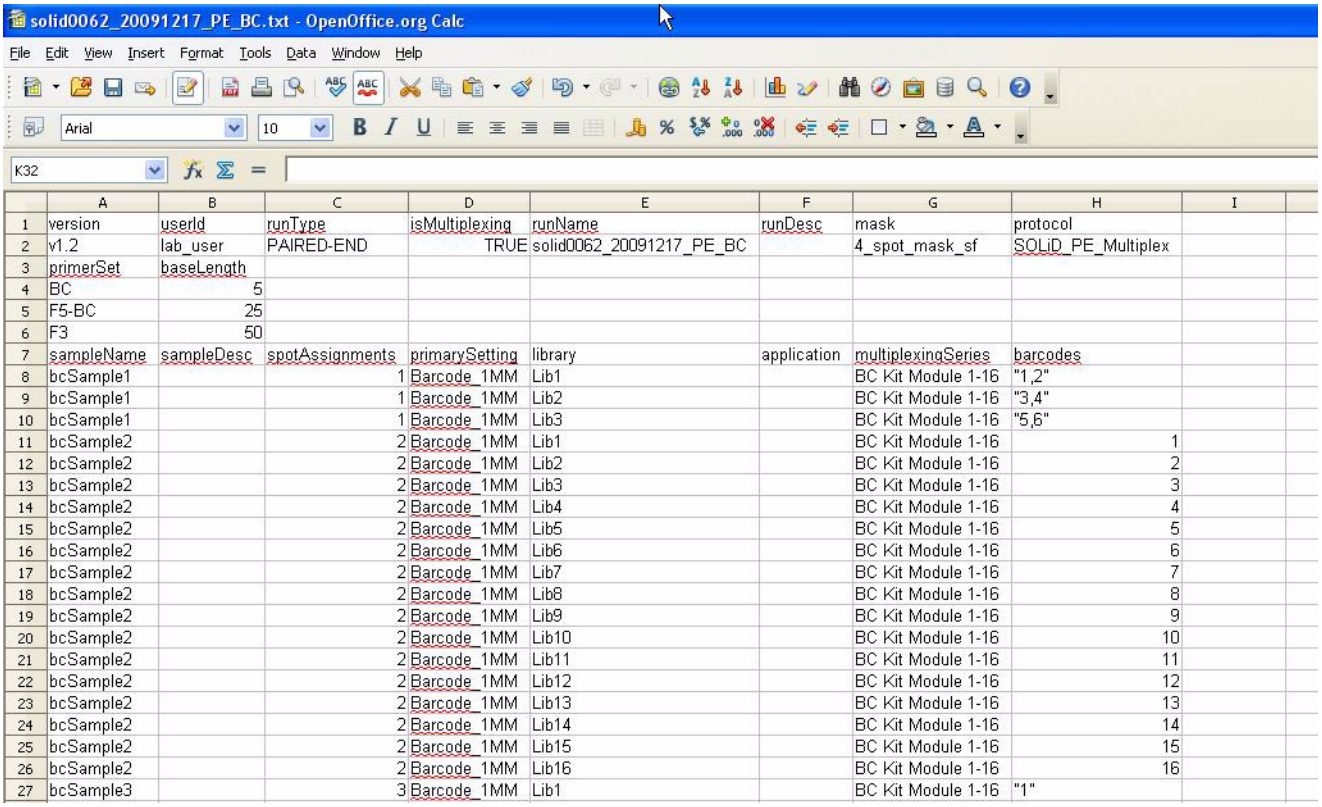


Figure 76 Uncheck **Replace** in the **Double quotes** section.

7. Finish entering the sample, library, and if applicable, barcode information using the fill down function. You can access this function by selecting **Edit** ▸ **Fill** ▸ **Down**. If you assign multiple barcodes to a single library, enter the barcodes in quotes, separated by commas (see Figure 77).



solid0062_20091217_PE_BC.txt - OpenOffice.org Calc

File Edit View Insert Format Tools Data Window Help

Arial 10

K32

	A	B	C	D	E	F	G	H	I
1	version	userId	runType	isMultiplexing	runName	runDesc	mask	protocol	
2	v1.2	lab_user	PAIRED-END	TRUE	solid0062_20091217_PE_BC		4_spot_mask_sf	SOLiD_PE_Multiplex	
3	primerSet	baseLength							
4	BC	5							
5	F5-BC	25							
6	F3	50							
7	sampleName	sampleDesc	spotAssignments	primarySetting	library	application	multiplexingSeries	barcodes	
8	bcSample1		1	Barcode_1MM	Lib1		BC Kit Module 1-16	"1,2"	
9	bcSample1		1	Barcode_1MM	Lib2		BC Kit Module 1-16	"3,4"	
10	bcSample1		1	Barcode_1MM	Lib3		BC Kit Module 1-16	"5,6"	
11	bcSample2		2	Barcode_1MM	Lib1		BC Kit Module 1-16	1	
12	bcSample2		2	Barcode_1MM	Lib2		BC Kit Module 1-16	2	
13	bcSample2		2	Barcode_1MM	Lib3		BC Kit Module 1-16	3	
14	bcSample2		2	Barcode_1MM	Lib4		BC Kit Module 1-16	4	
15	bcSample2		2	Barcode_1MM	Lib5		BC Kit Module 1-16	5	
16	bcSample2		2	Barcode_1MM	Lib6		BC Kit Module 1-16	6	
17	bcSample2		2	Barcode_1MM	Lib7		BC Kit Module 1-16	7	
18	bcSample2		2	Barcode_1MM	Lib8		BC Kit Module 1-16	8	
19	bcSample2		2	Barcode_1MM	Lib9		BC Kit Module 1-16	9	
20	bcSample2		2	Barcode_1MM	Lib10		BC Kit Module 1-16	10	
21	bcSample2		2	Barcode_1MM	Lib11		BC Kit Module 1-16	11	
22	bcSample2		2	Barcode_1MM	Lib12		BC Kit Module 1-16	12	
23	bcSample2		2	Barcode_1MM	Lib13		BC Kit Module 1-16	13	
24	bcSample2		2	Barcode_1MM	Lib14		BC Kit Module 1-16	14	
25	bcSample2		2	Barcode_1MM	Lib15		BC Kit Module 1-16	15	
26	bcSample2		2	Barcode_1MM	Lib16		BC Kit Module 1-16	16	
27	bcSample3		3	Barcode_1MM	Lib1		BC Kit Module 1-16	"1"	

Figure 77 Complete the sample, library, and barcode information.

IMPORTANT! Be sure to review each sample's library and barcode assignment before starting a run. After a run starts, you cannot change the library or barcode assignment.

8. Save the file by selecting **File ▸ Save**. Do not select "Save As," because scalc does not allow files to be saved as .txt files.

9. Click **Keep Current Format**.

10. In ICS, click **Manage Runs**.

11. Click **Import Run** and select the run definition file you modified with scalc (see Figure 78).

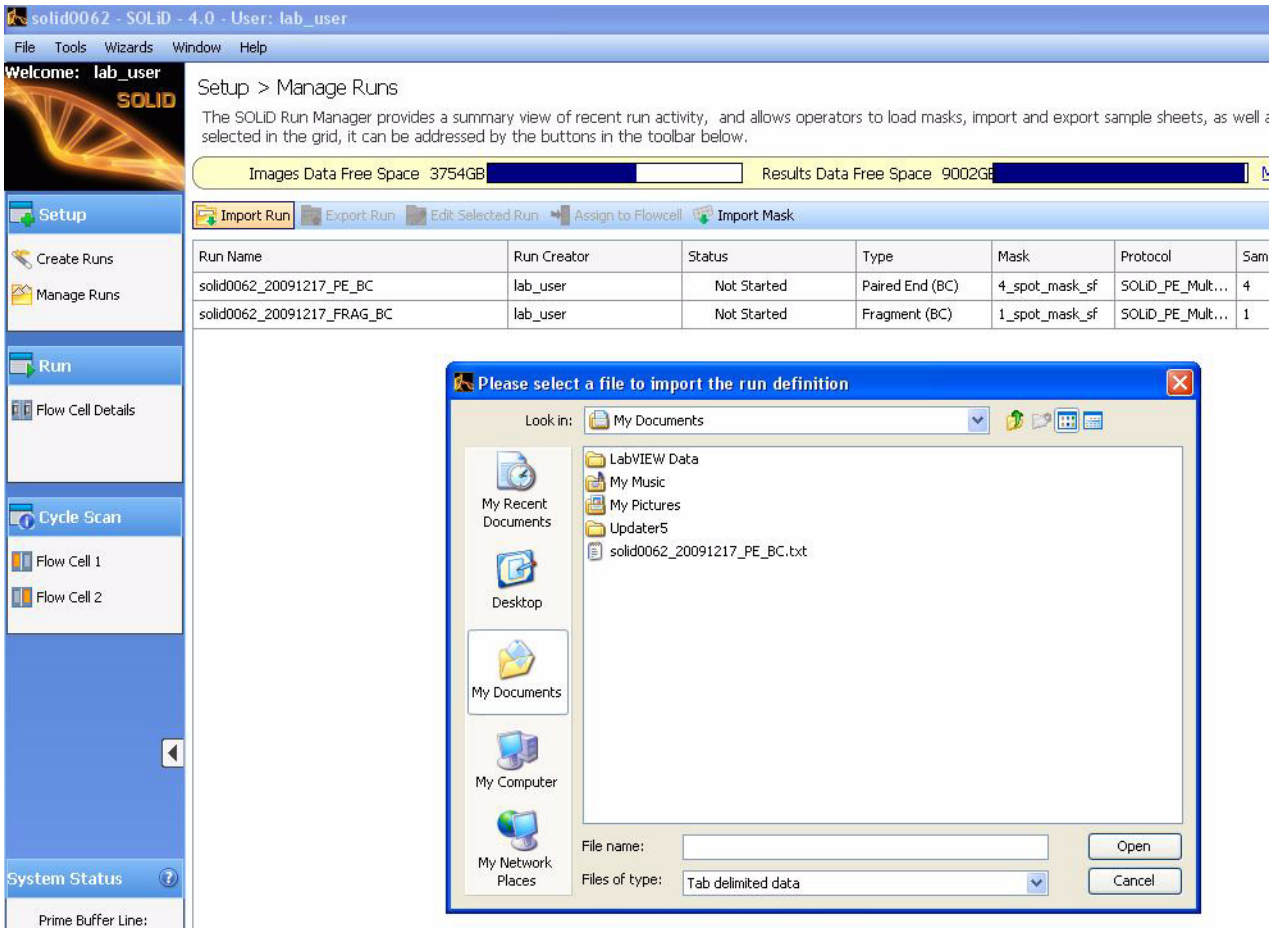


Figure 78 Import the run definition file.

12. 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**.

Manually find the focus range

You should first attempt automatic range detection (see "Detect the focus range" on page 55). 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™ 4 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 79).

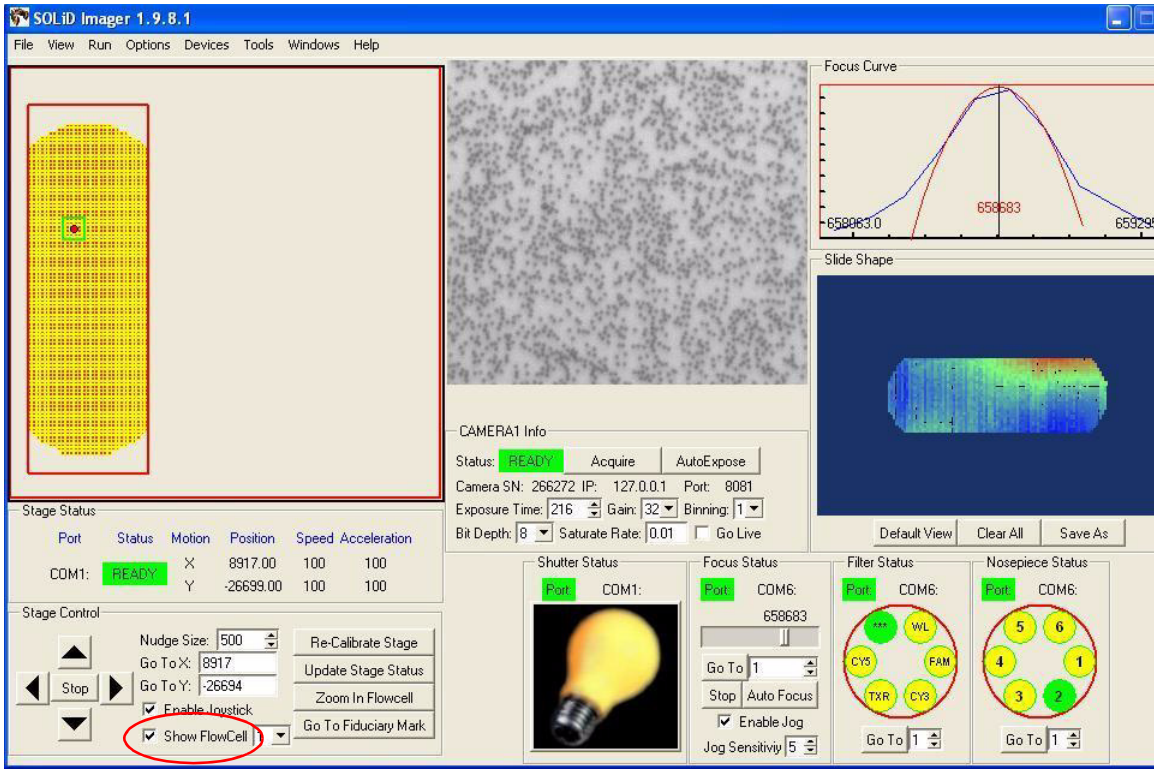


Figure 79 Click Show Flowcell to find the beads on the slide.

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 80 on page 133).

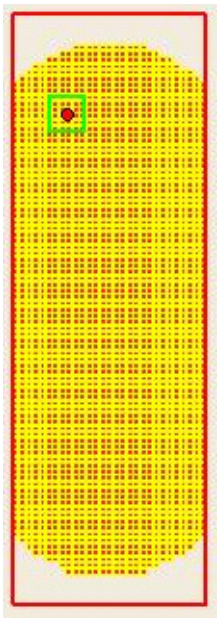


Figure 80 Use the mouse to drag the green-box cursor and to move the flowcell stage the same way.

Find the focal range

1. Set the Filter Status to **WL** (white light; see Figure 81).

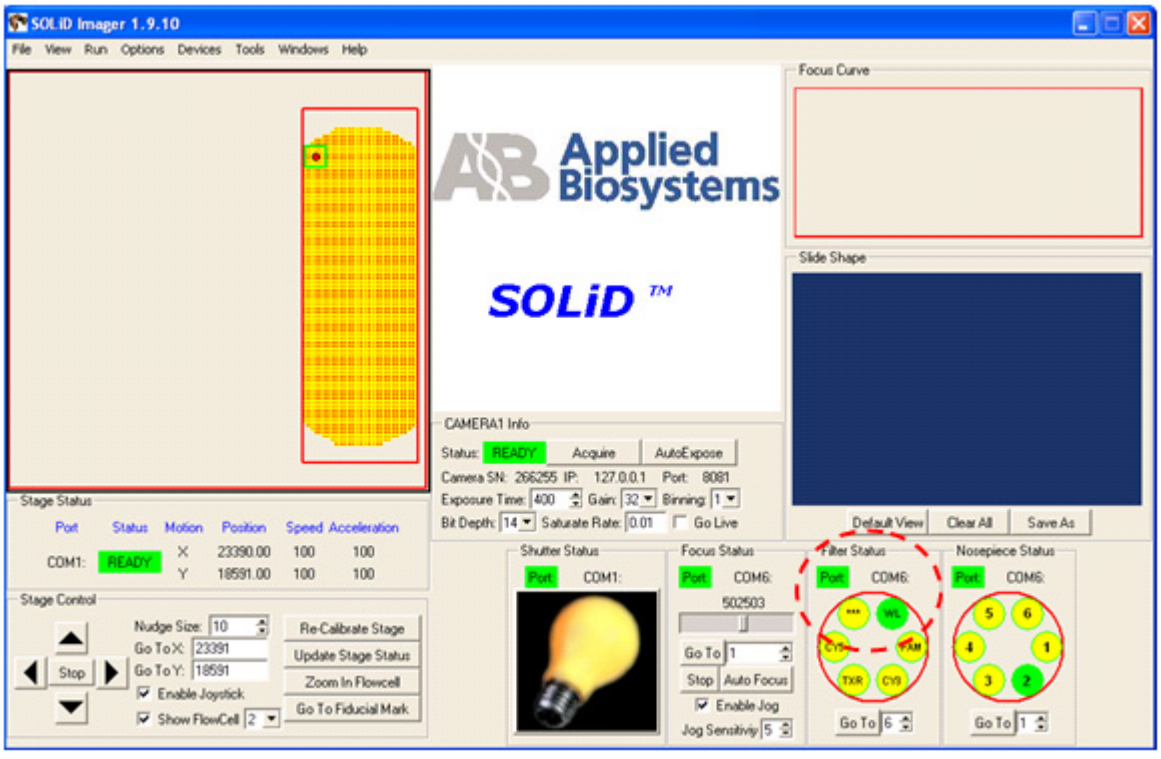


Figure 81 Click WL (white light) to set the Filter Status.

2. In the Imager window, click the **AutoFocus** button, then wait about 20 seconds for the Imager to focus on the slide (see Figure 82). 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.

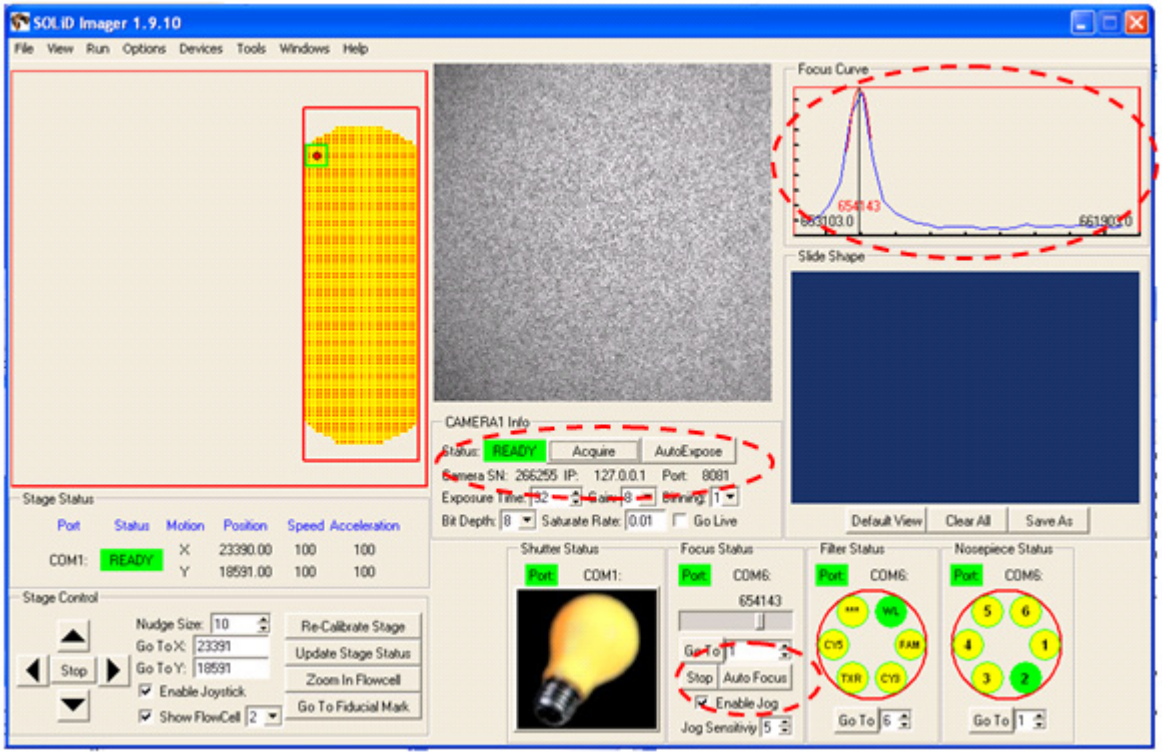


Figure 82 Click AutoFocus and AutoExpose so that the focus peak falls in the focus range.

3. When the Imager displays READY, click the **Acquire** button, then examine the image on the screen (see Figure 83).

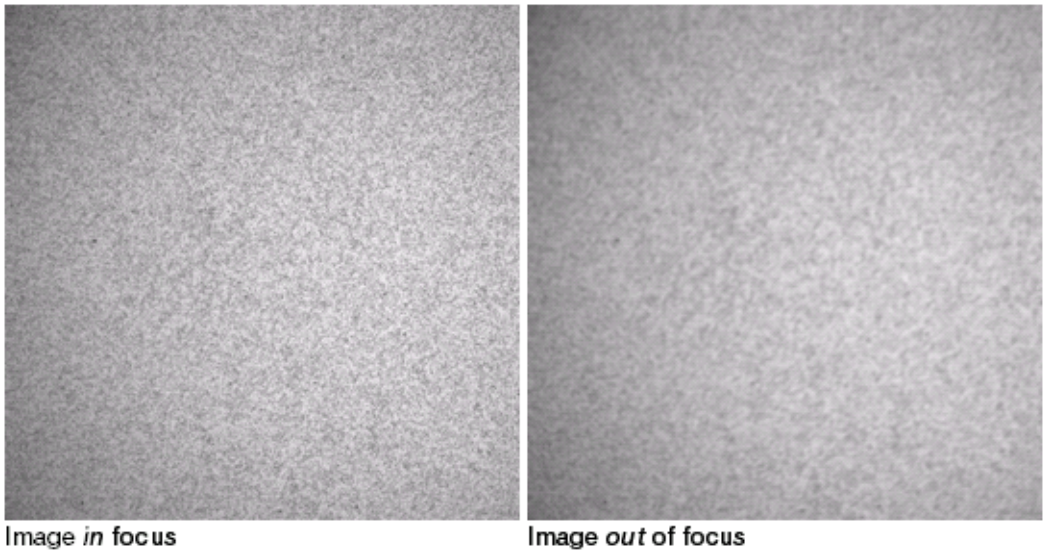


Figure 83 Examine the image on the screen.

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 139. 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 84).

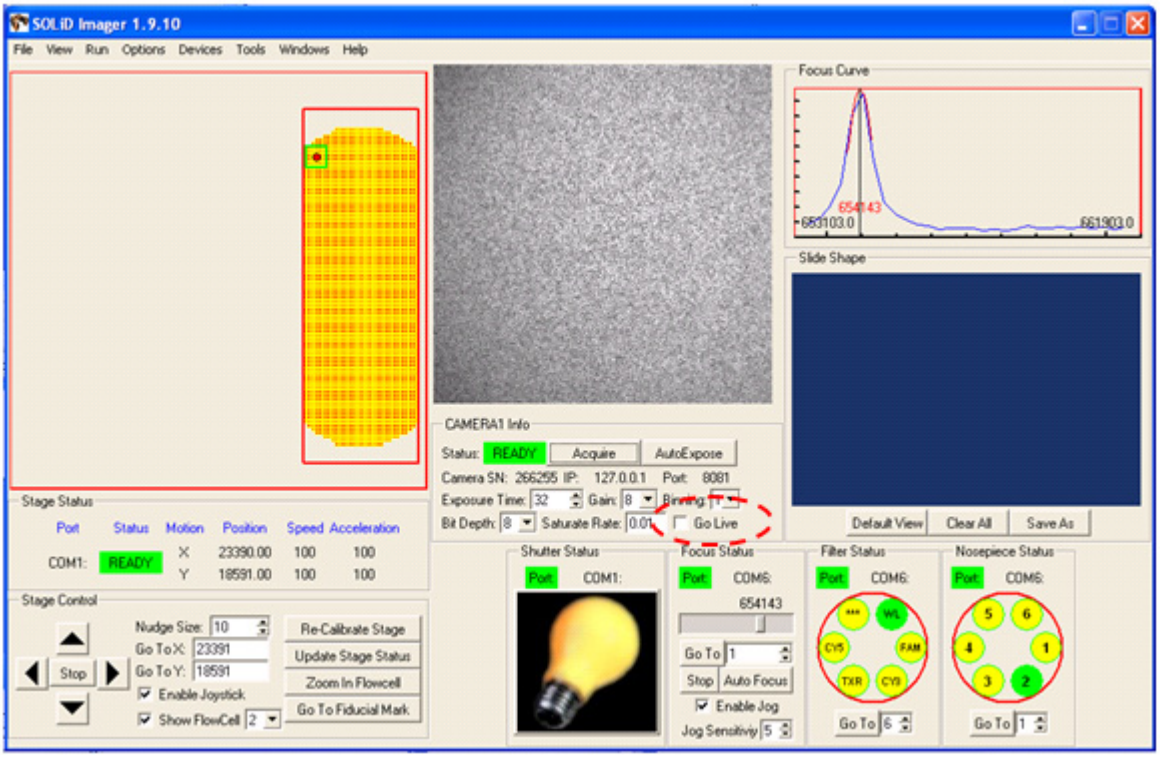


Figure 84 Check Go Live to manually find the focal range.

6. Click the focus slider bar in the Imager window. A black box appears (see Figure 85).

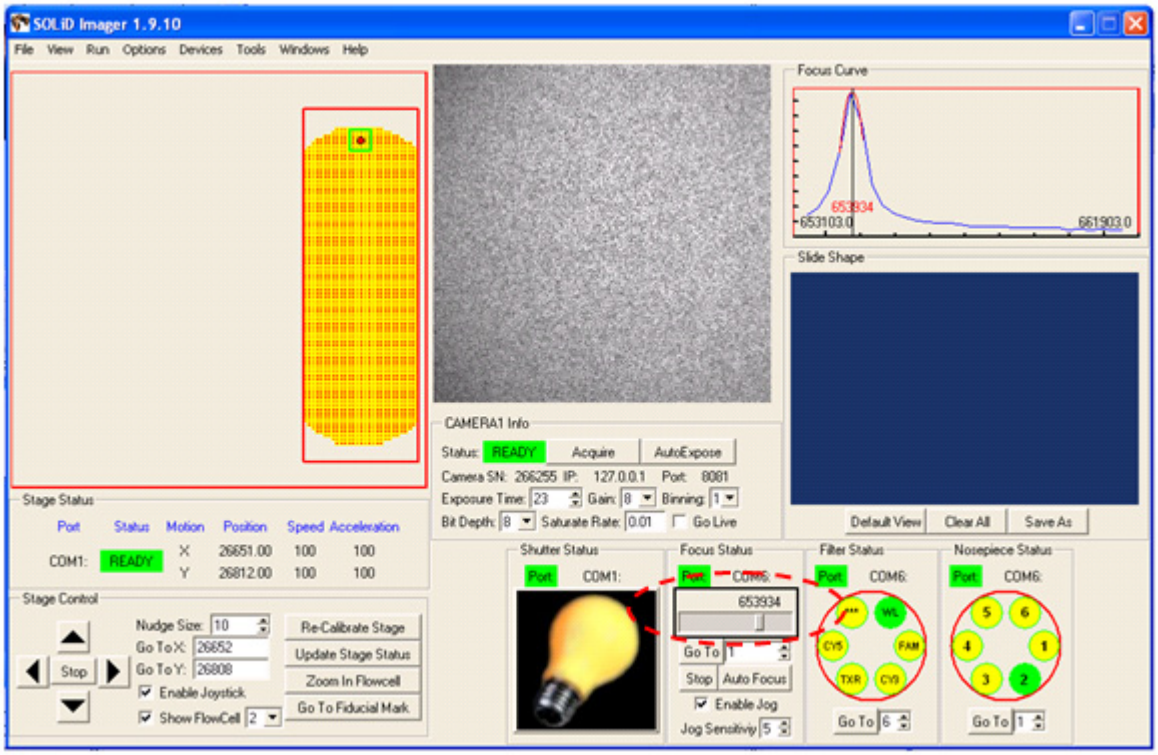


Figure 85 Use the black box to change the focal distance.

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 86.

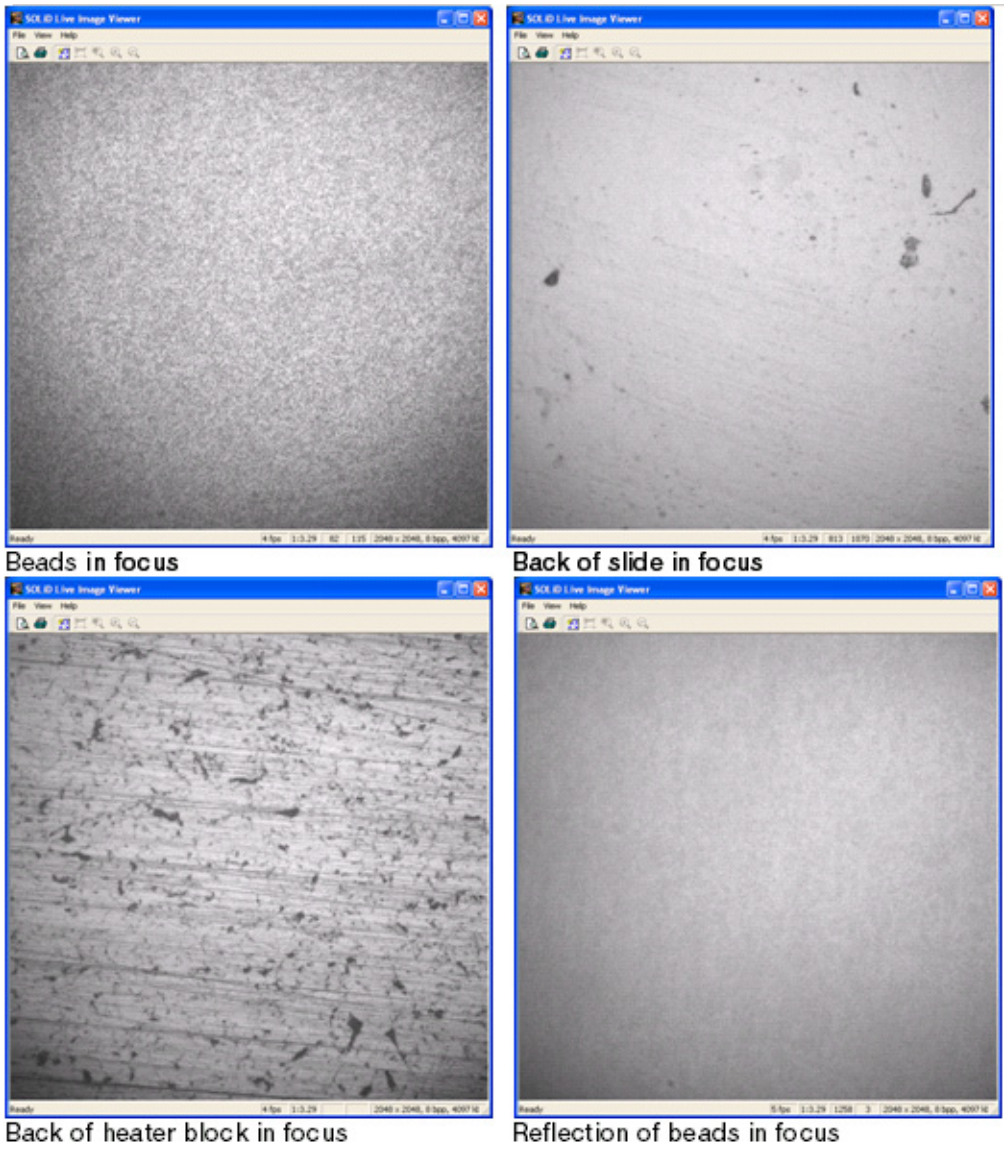


Figure 86 Examples of in-focus images.

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 87).

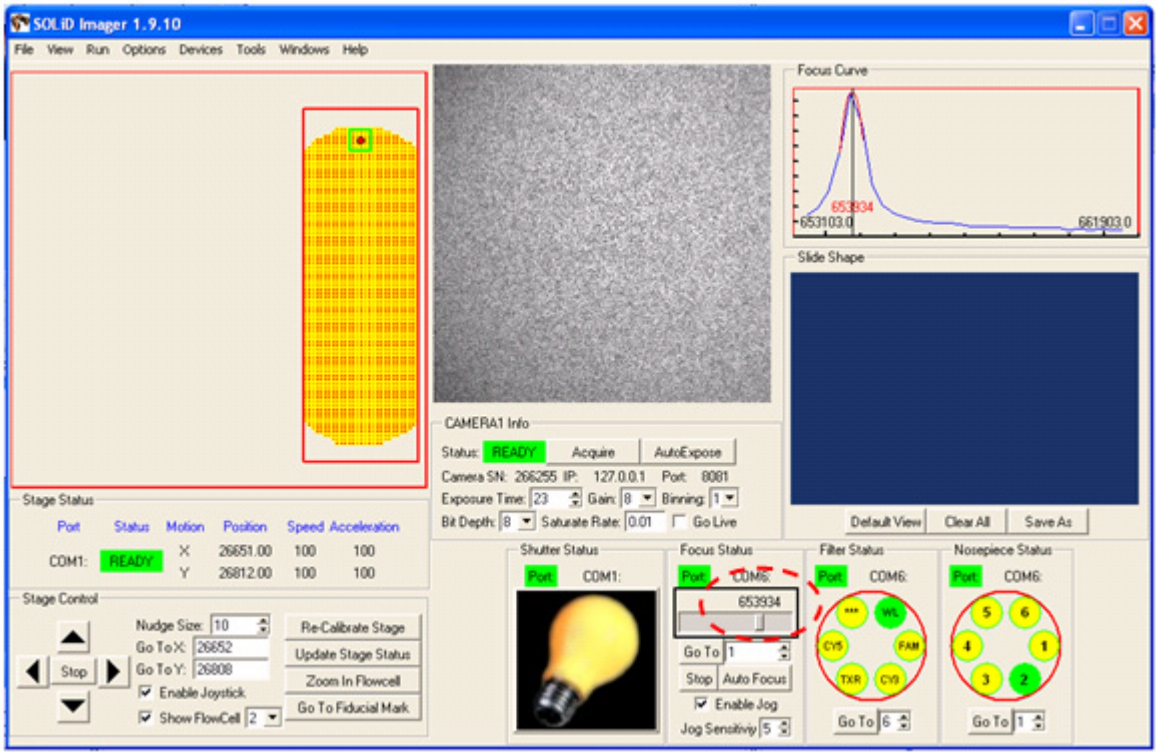


Figure 87 Record the nominal Z distance.

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 88).

> **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.

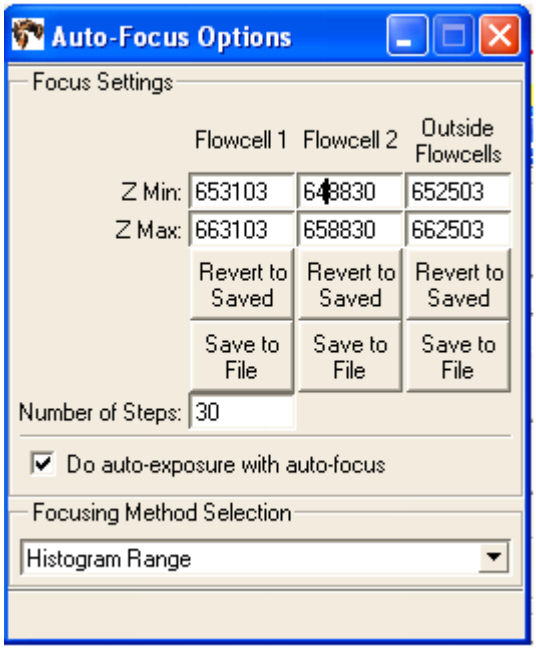


Figure 88 Enter auto-focus options.

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 89).

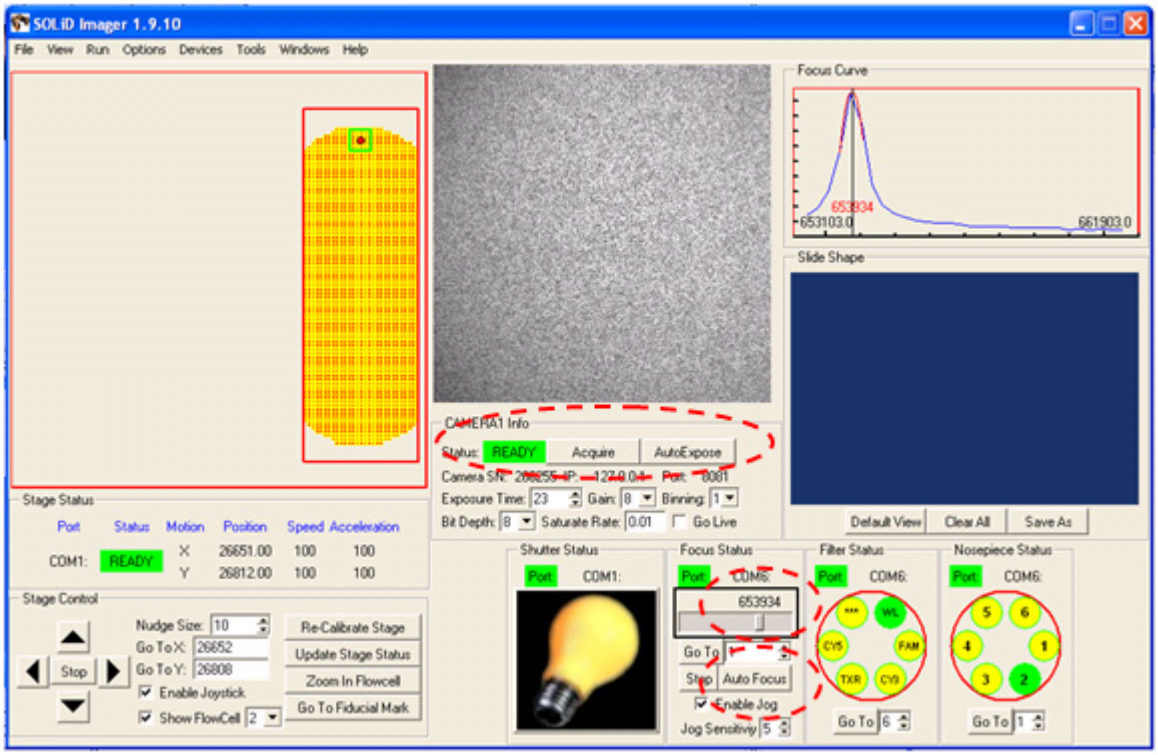


Figure 89 Record the *Z* distance value.

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 90).

> **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.

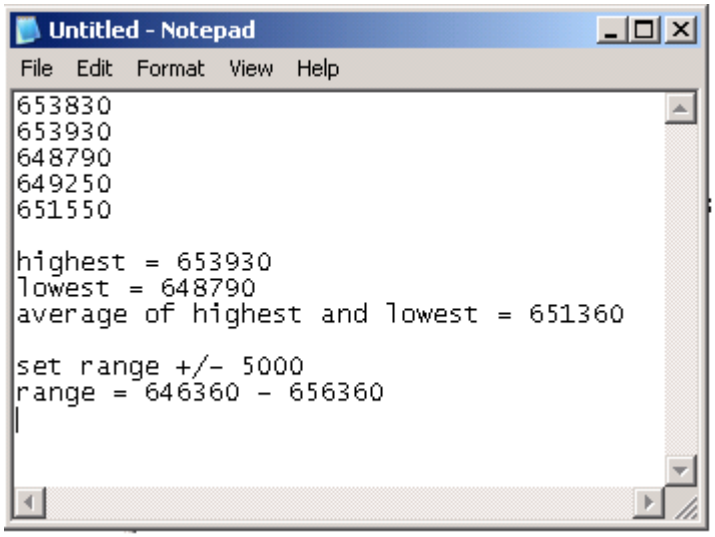


Figure 90 Record the average focal value in Notepad.

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 91).

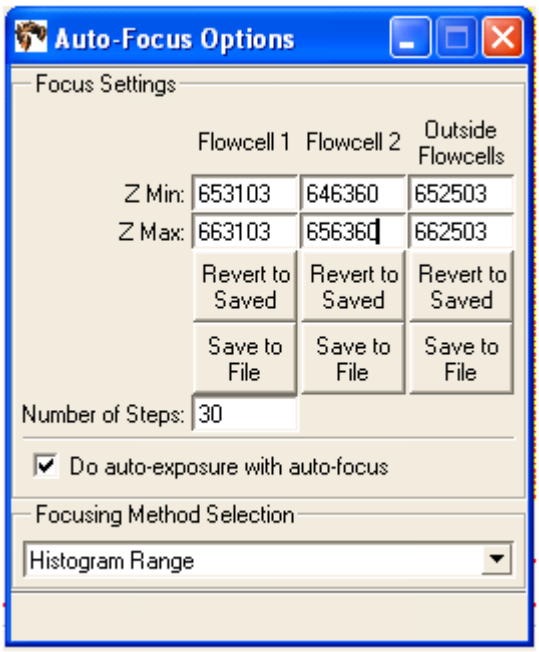


Figure 91 Enter the focus settings.

11. Click **Yes** when the warning displays (see Figure 92).

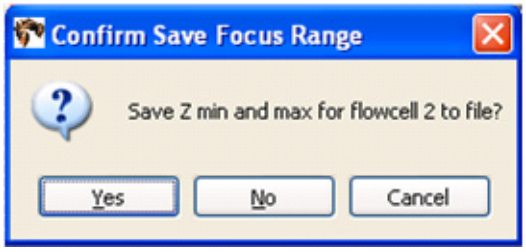


Figure 92 Click Yes when the Save Focus Range Warning displays.

12. When the Flowcell Selection window appears, 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 132.

Shut down the SOLiD™ 4 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

Table 33 Required equipment: Shut down the instrument

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**.

Modify the Barcode Error Correction Level

A Barcode Error Correction Level is set at 0 mismatches by default. A Barcode Error Correction Level of 1 mismatch increases the number of reads by about 10%, but lower-quality sequencing reads are included in the data.

To modify the Barcode Error Correction Level:

1. In SETS, log in as the Administrator.
2. Select **Analysis** ▸ **Primary Analysis Settings** ▸ **New**.
3. In the Barcode Error Correction Level drop-down list, select 0 or 1 (see Figure 93).
4. Save the primary analysis setting, then apply the setting to the multiplex sequencing run when the run is created.

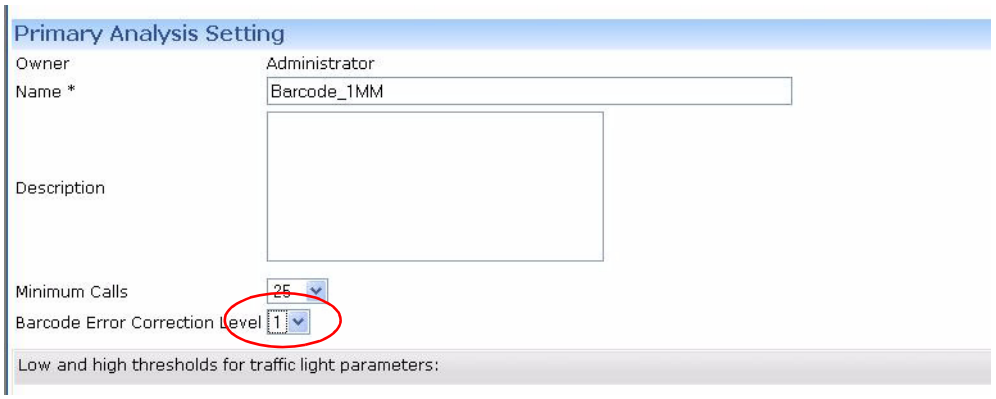


Figure 93 In Barcode Error Correction Level, select 0 or 1.

C

On-Instrument Reagent Volumes and Reagent Strip Layouts

This appendix covers:

Recommended fill volumes for on-instrument reagents

Volumes of on-instrument reagents

IMPORTANT! Verify that there is sufficient volume of Reset Buffer for each tag sequenced. The SOLiD™ ToP Instrument Buffer contains sufficient Reset Buffer to reset two tags (see "On-Instrument Reagent Volumes and Reagent Strip Layouts" on page 147). For multiplex sequencing, a bottle of Reset Buffer is included with multiplex sequencing kits to account for sequencing the BC tag.

Table 34 Volumes of on-instrument reagents provided in the SOLiD™ ToP Instrument Buffer Kit

	Volume (mL)
1 × Instrument Buffer‡	8000
Cleave 1 Solution	350
Cleave 2.1 Solution‡	350
Reset Buffer	170
Imaging Buffer‡	250
Ligase Buffer‡	120
Universal Buffer‡	75
Storage Buffer	825

‡ Volumes shown are after preparation according to the instructions in "Install on-instrument reagents" on page 32.

Fragment sequencing (F3 Tag)

Table 35 Fill volumes for fragment sequencing (35 or 50 bp)

	Volume (mL)			
	35 bp		50 bp	
	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells
Instrument Buffer	2157	4125	2949	5710
Storage Buffer	343	587	423	748
Cleave 1 Solution	124	224	169	316
Cleave 2.1 Solution	124	224	169	316
Reset Buffer	91	164	91	164
Ligase Buffer	42	74	56	101
Universal Buffer	26	41	33	55
Imaging Buffer	82	142	107	191

Paired-end sequencing (F5-BC/F5-P2 Tag)

Table 36 Fill volumes for paired-end sequencing (25 bp)

	Volume (mL)			
	F5-P2 Tag		F5-BC Tag	
	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells
Instrument Buffer	1783	3378	1783	3378
Storage Buffer	398	698	398	698
Cleave 1 Solution	70	117	70	117
Cleave 2.1 Solution	70	117	70	117
Reset Buffer	91	164	91	164
Ligase Buffer	34	57	34	57
Universal Buffer	33	55	33	55
Imaging Buffer	66	109	66	109

Mate-pair sequencing (F3/R3 Tags)

Table 37 Fill volumes for mate-pair sequencing (25, 35, or 50 bp)

	Volume per tag (mL)					
	25 bp		35 bp		50 bp	
	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells
Instrument Buffer	1629	3069	2157	4125	2949	5710
Storage Buffer	289	480	343	587	423	748
Cleave 1 Solution	93	163	124	224	169	316
Cleave 2.1 Solution	93	163	124	224	169	316
Reset Buffer	91	164	91	164	91	164
Ligase Buffer	33	55	42	74	56	101
Universal Buffer	21	32	26	41	33	55
Imaging Buffer	66	109	82	142	107	191

Barcode sequencing (BC Tag)

Table 38 Fill volumes for barcode sequencing (5 or 10 bp)

	Volume (mL)			
	5 bp		10 bp	
	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells
Instrument Buffer	638	1088	883	1577
Storage Buffer	189	279	227	356
Cleave 1 Solution	32	41	47	72
Cleave 2.1 Solution	32	41	47	72
Reset Buffer	91	164	91	164

	Volume (mL)			
	5 bp		10 bp	
	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells
Ligase Buffer	15	19	18	25
Universal Buffer	13	14	15	19
Imaging Buffer	33	43	41	59

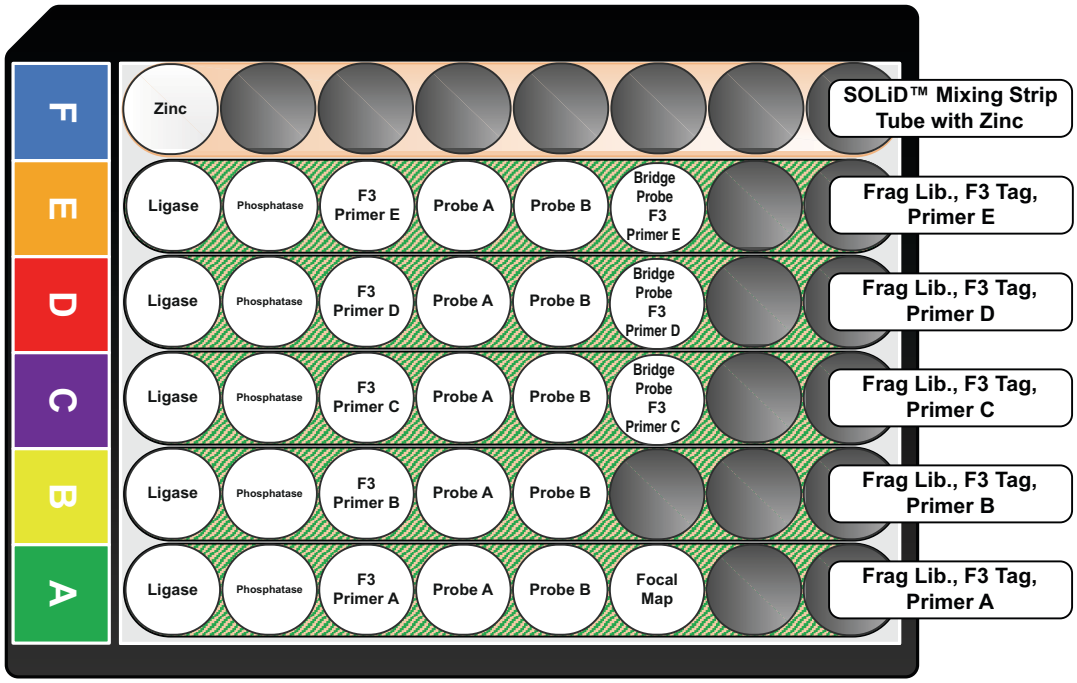
Workflow analysis (WFA)

Table 39 Fill volumes for workflow analysis (WFA)

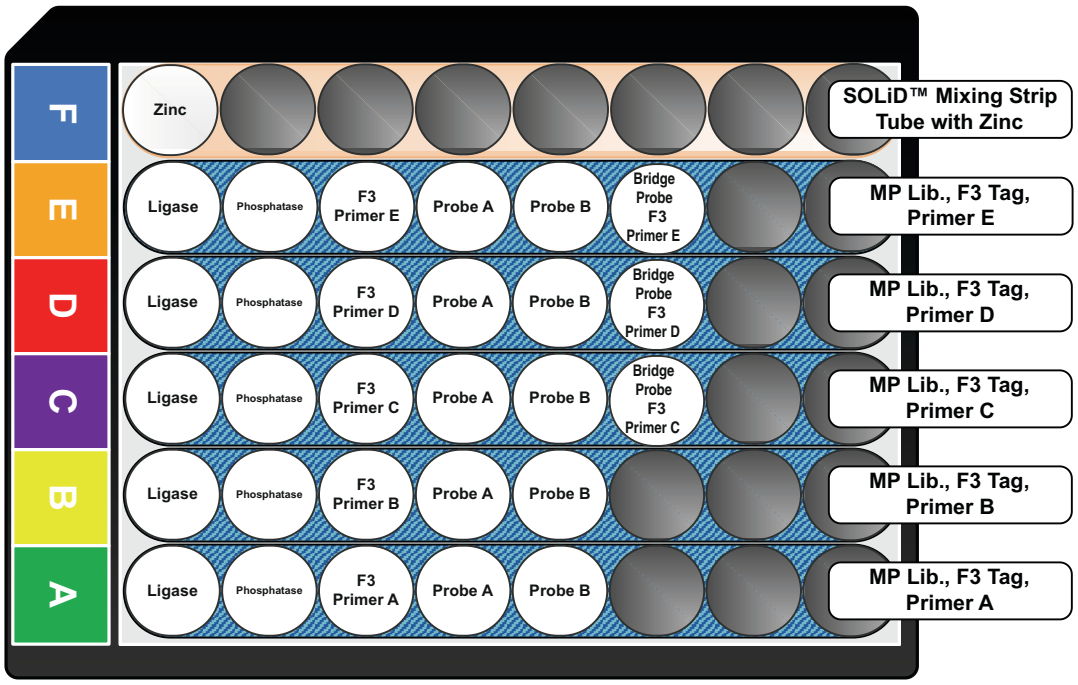
	Volume (mL)	
	1 Flowcell	2 Flowcells
Instrument Buffer	294	400
Storage Buffer	116	134
Cleave 1 Solution	0	0
Cleave 2.1 Solution	0	0
Reset Buffer	43	67
Ligase Buffer	12	12
Universal Buffer	0	0
Imaging Buffer	26	30

Reagent strip layouts

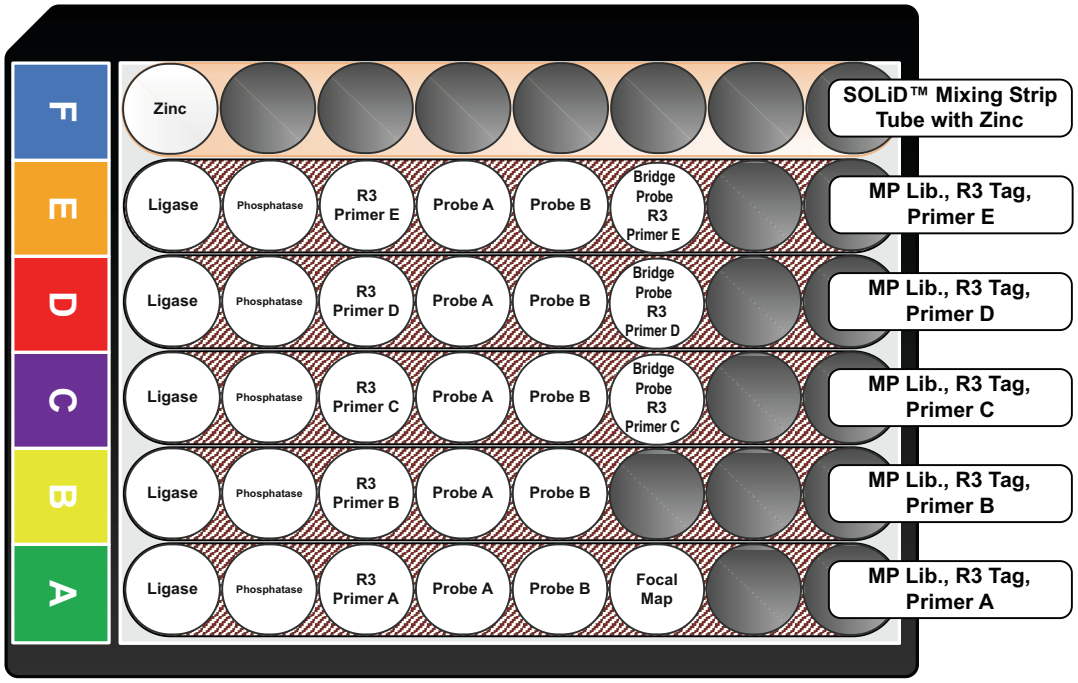
Fragment/paired-end sequencing, F3 Tag



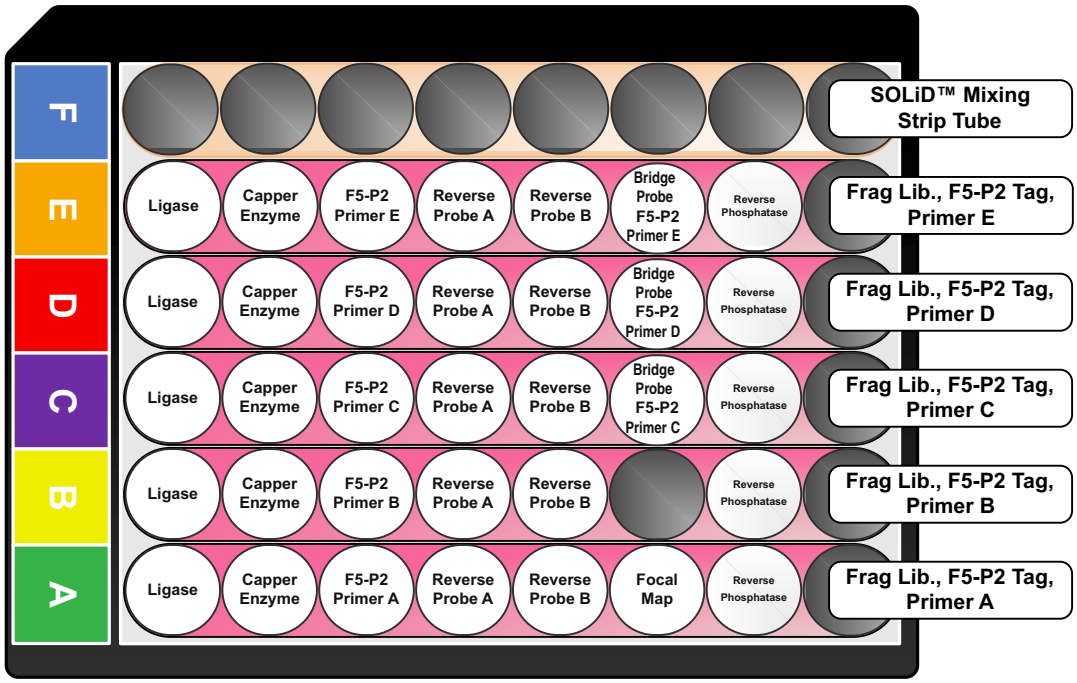
Mate-Pair sequencing, F3 Tag



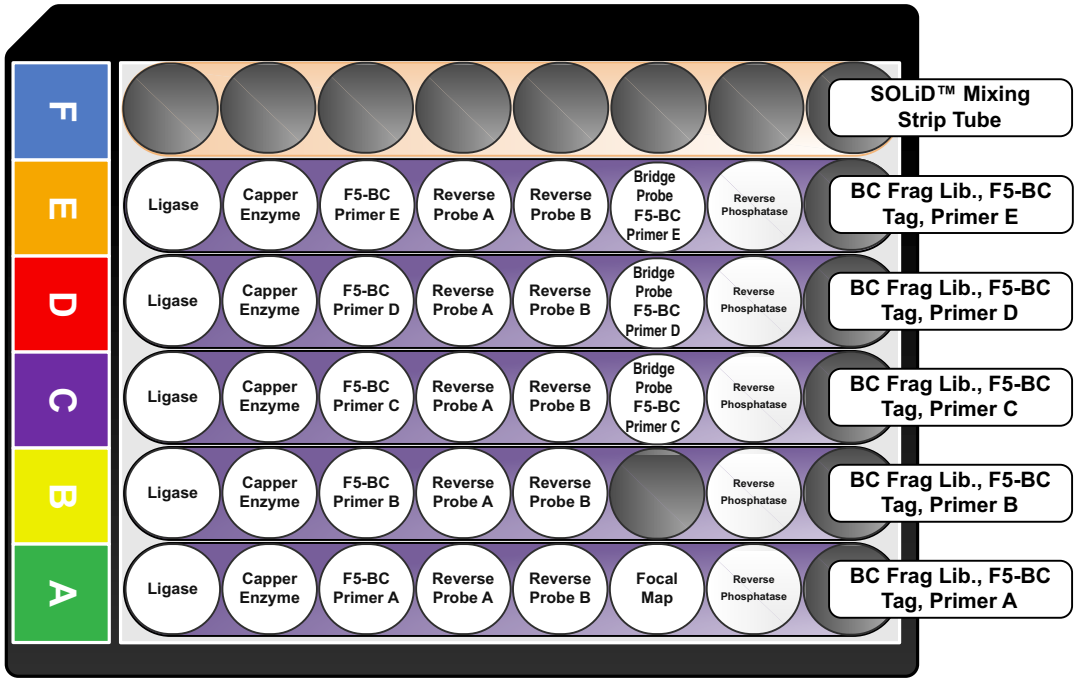
Mate-Pair sequencing, R3 Tag



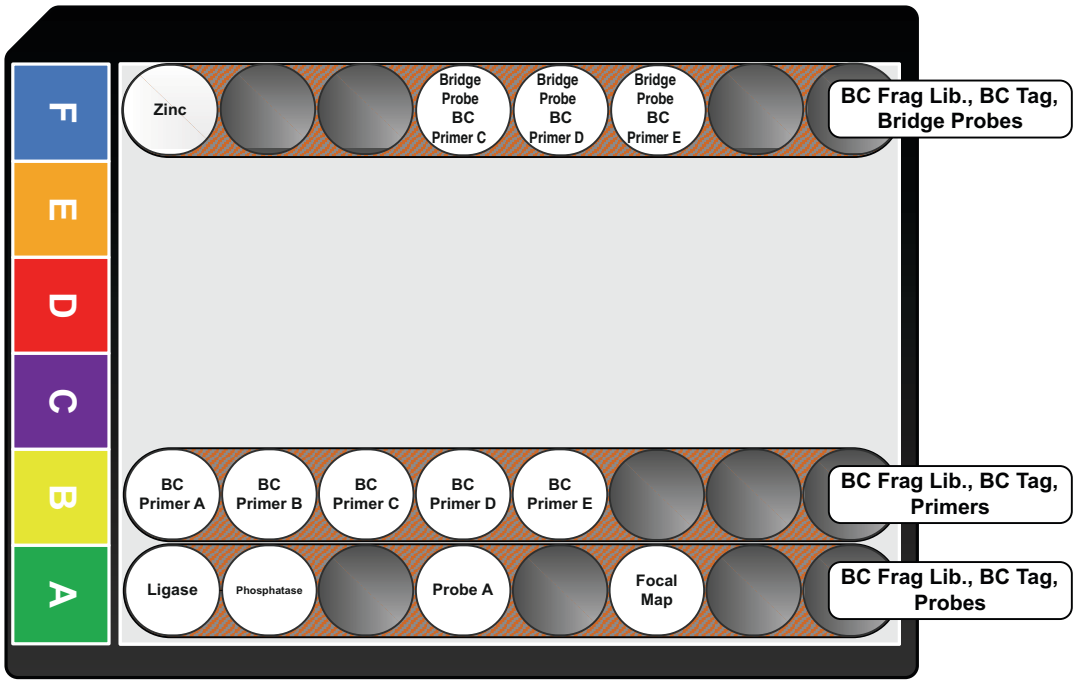
Paired-end sequencing, F5-P2 Tag



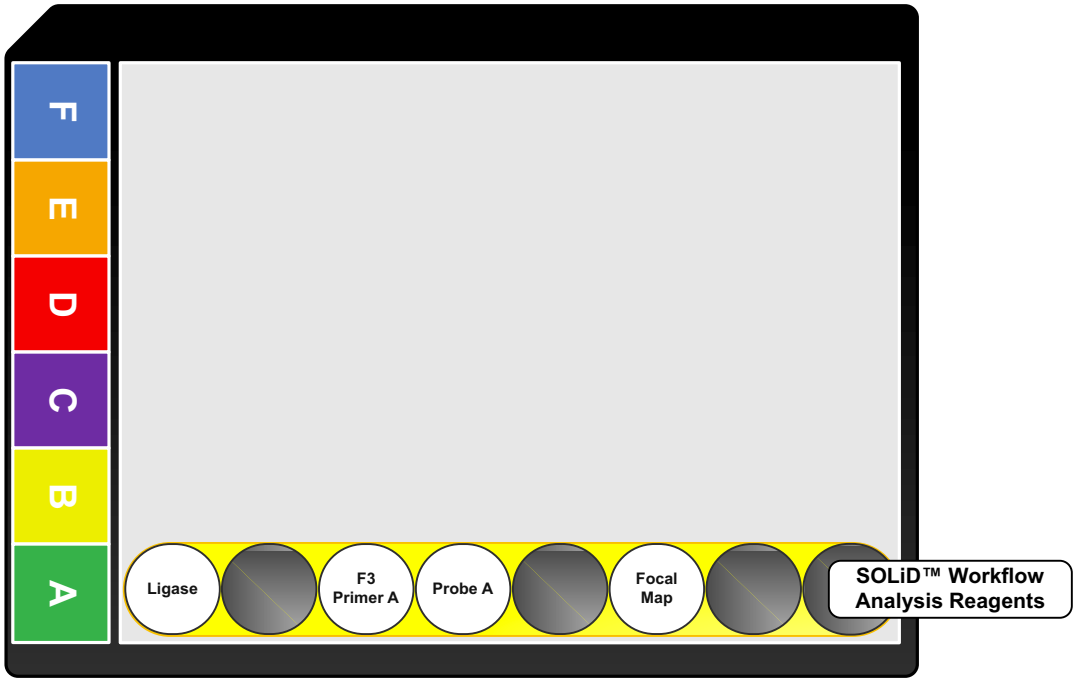
Multiplex paired-end sequencing, F5-BC Tag



Multiplex fragment/paired-end sequencing, BC Tag



Workflow analysis (WFA)



D Instrument Process Times

This appendix covers:

Times for individual processes

Table 40 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 (F3/R3)	~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) ~18 to 22 (7 cycles or 35 bp) ~28 to 33 (10 cycles or 50 bp)
Sequencing Primers 3, 4, & 5 and Ligation (F3/R3)	~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) ~19 to 23 (7 cycles or 35 bp) ~29 to 34 (10 cycles or 50 bp)
Ligation cycle without priming (F3/R3)	~2.5 to 3	Ligate Dark Ligate Phosphatase Image Cleave	—
Sequencing Primers 1 & 2 and Ligation (F5-P2/F5-BC)	~3.5	Reset Prime Ligate Dark Ligate Capper Enzyme Image Cleave RevPhosphatase	~15.5 to 21.5 (5 cycles or 25 bp)

Process	Estimated Time (h)	Steps	Estimated time per primer (h)
Sequencing Primers 3, 4, & 5 and Ligation (F5-P2/F5-BC)	~4.5 to 5.5	Reset Prime Bridge Probe Ligate Dark Ligate Capper Enzyme Image Cleave RevPhosphatase	~16.5 to 21.5 (5 cycles or 25 bp)
Ligation cycle without priming (F5-P2/F5-BC)	~3 to 4	Ligate Dark Ligate Capper Enzyme Image Cleave RevPhosphatase	—

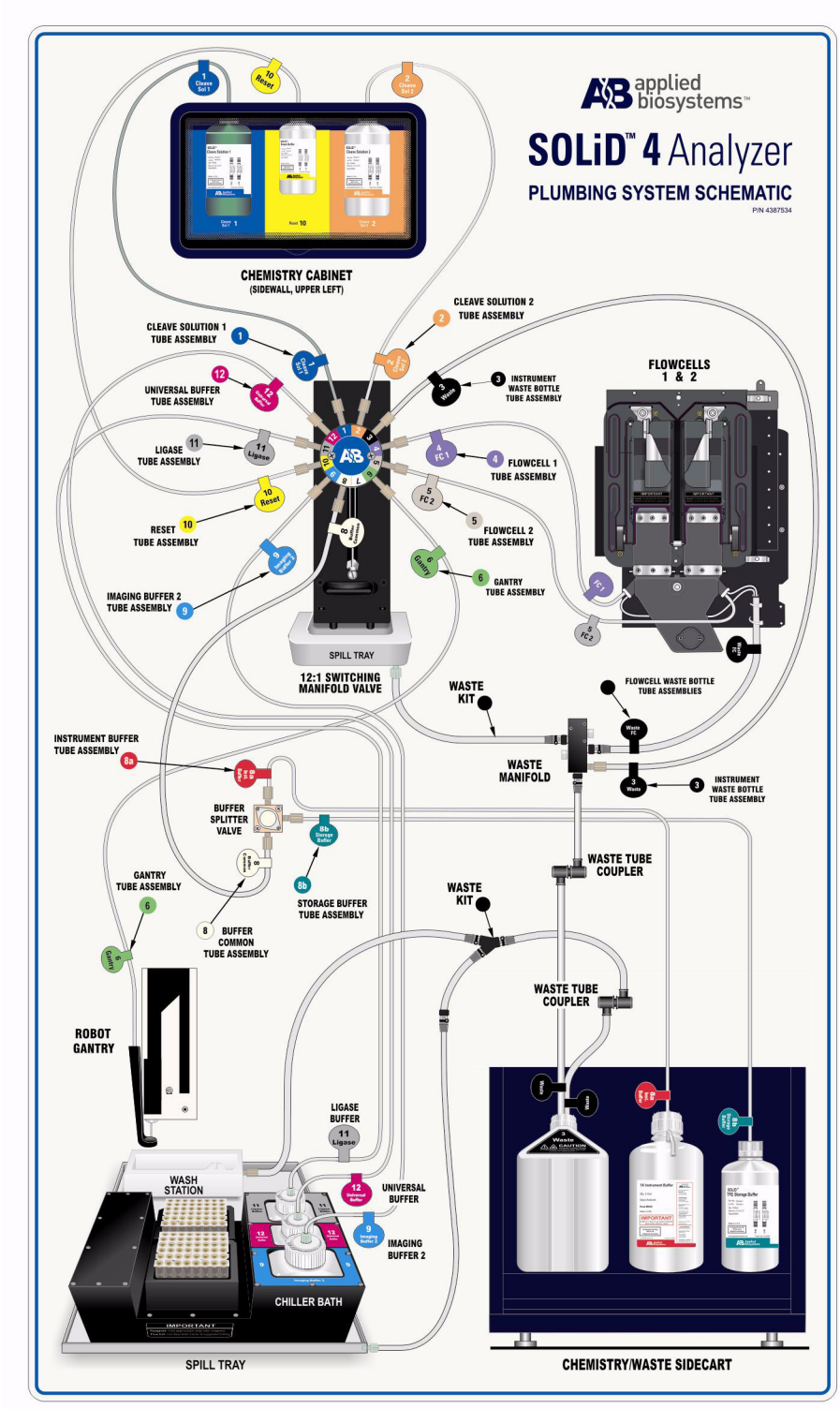
Times for entire processes

Table 41 Times for entire processes

Process	Total Runtime‡
Workflow Analysis (WFA)	~4 to 5 hours
Fragment (35 bp)	~4 to 4.5 days
Fragment (50 bp)	~6 to 7 days
Mate-paired (25/25 bp)	~7 to 8 days
Mate-paired (35/35 bp)	~8 to 9 days
Mate-paired (50/50 bp)	~13 to 14 days
Paired-end (35/25 bp)	~8 to 9 days
Paired-end (50/25 bp)	~10 to 11.5 days
Multiplex fragment (35/5 bp)	~5 to 5.5 days
Multiplex fragment (35/10 bp)	~6 to 6.5 days
Multiplex fragment (50/5 bp)	~7 to 8 days
Multiplex fragment (50/10 bp)	~8 to 9 days
Multiplex paired-end (35/25/5 bp)	~9 to 10 days
Multiplex paired-end (35/25/10 bp)	~10 to 11 days
Multiplex paired-end (50/25/5 bp)	~11 to 12.5 days
Multiplex paired-end (50/25/10 bp)	~12 to 13.5 days

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

E SOLiD™ 4 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 ☐ Vortexer ☐ Picofuge ☐ Pipettors	☐ 1X TEX Buffer ☐ Deposition Buffer v2 ☐ Double distilled filtered water ☐ 1.5-mL LoBind Tubes ☐ 3-mm adhesive disks ☐ SOLiD™ System Sequencing Slide ☐ Slide Prep Reagent ☐ Filtered pipettor tips	—
Install on-instrument reagents	☐ SOLiD™ 4 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) ☐ Universal Buffer (Parts 1 and 2) ☐ Imaging Buffer (Parts 1 and 2) ☐ Cleave Solution 1 ☐ Cleave Solution 2.1 (Parts 1 and 2) ☐ Reset Buffer ☐ Glycerol ☐ Double-distilled water	—
Install slide(s)	☐ SOLiD™ 4 Analyzer ☐ Pipettors ☐ Allen wrench ☐ SOLiD™ Slide Storage Chamber (optional)	☐ Deposition Buffer v2 ☐ Filtered pipettor tips ☐ 70% ethanol ☐ Kimwipes® Slide Storage Buffer	—
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 v2	
Slide Prep Reagent	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer Part 1	
T4 Ligase Buffer Part 2	
Universal Buffer Part 1	
Universal Buffer Part 2	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2.1 Part 1	
Cleave Solution 2.1 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 v2	
Slide Prep Reagent	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer Part 1	
T4 Ligase Buffer Part 2	
Universal Buffer Part 1	
Universal Buffer Part 2	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2.1 Part 1	
Cleave Solution 2.1 Part 2	
Reset Buffer	
Slide Storage Buffer	
F3 Tag Sequencing Kit	
F3 – Primer A	
F3 – Primer B	
F3 – Primer C	
F3 – Primer D	
F3 – Primer E	
F3 – Mixing Strip Tube	
R3/F5-P2/F5-BC Tag Sequencing Kit	
R3/F5-P2/F5-BC – Primer A	
R3/F5-P2/F5-BC – Primer B	
R3/F5-P2/F5-BC – Primer C	
R3/F5-P2/F5-BC – Primer D	
R3/F5-P2/F5-BC – Primer E	
R3/F5-P2/F5-BC – Mixing Strip Tube	
BC Tag Sequencing Kit	
BC – Bridge Probes	
BC – Primers	
BC – 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 v2	
Slide Prep Reagent	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer Part 1	
T4 Ligase Buffer Part 2	
Universal Buffer Part 1	
Universal Buffer Part 2	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2.1 Part 1	
Cleave Solution 2.1 Part 2	
Reset Buffer	
Slide Storage Buffer	
F3 Tag Sequencing Kit	
F3 – Primer A	
F3 – Primer B	
F3 – Primer C	
F3 – Primer D	
F3 – Primer E	
F3 – Mixing Strip Tube	
R3/F5-P2/F5-BC Tag Sequencing Kit	
R3/F5-P2/F5-BC – Primer A	
R3/F5-P2/F5-BC – Primer B	
R3/F5-P2/F5-BC – Primer C	
R3/F5-P2/F5-BC – Primer D	
R3/F5-P2/F5-BC – Primer E	
R3/F5-P2/F5-BC – Mixing Strip Tube	
BC Tag Sequencing Kit	
BC – Bridge Probes	
BC – Primers	
BC – 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 v2	
Slide Prep Reagent	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer	
Universal Buffer Part 1	
Universal Buffer Part 2	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2.1 Part 1	
Cleave Solution 2.1 Part 2	
Reset Buffer	
Slide Storage Buffer	
F3 Tag Sequencing Kit	
F3 – Primer A	
F3 – Primer B	
F3 – Primer C	
F3 – Primer D	
F3 – Primer E	
F3 – Mixing Strip Tube	
R3/F5-P2/F5-BC Tag Sequencing Kit	
R3/F5-P2/F5-BC – Primer A	
R3/F5-P2/F5-BC – Primer B	
R3/F5-P2/F5-BC – Primer C	
R3/F5-P2/F5-BC – Primer D	
R3/F5-P2/F5-BC – Primer E	
R3/F5-P2/F5-BC – Mixing Strip Tube	
BC Tag Sequencing Kit	
BC – Bridge Probes	
BC – Primers	
BC – 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 42):

Table 42 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 43 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 44 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:

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Applied Biosystems warrants that all standard components of the SOLiD™ 4 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® DNA Shearing Device 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® DNA Shearing Device during the warranty period. The following parts of the HydroShear® DNA Shearing Device are use- replaceable and not covered by the warranty on the HydroShear® DNA Shearing Device: 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™ 4 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.

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Warranty period effective date

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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 188). 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.
	**CAUTION!** Potential overhead hazard.

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™ 4 System contains warnings at the locations listed in Table 45.

Table 45 Where to find safety labels on the SOLiD™ 4 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 Safety Data Sheets (SDSs). See "About SDSs" on page 195.

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™ 4 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™ 4 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™ 4 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 Safety Data Sheet (SDS) 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 Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 195.)
- 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 SDS.
- 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 SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

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

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

Obtaining SDSs

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

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS 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 SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to 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 Safety Data Sheets (SDSs) 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 SDS.
- 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 SDS.
- 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* (**http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**).
- 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 7.

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
F5-P2 tag	Tag to be sequenced using reverse ligation chemistry and using primers specific to the P2 Adaptor sequence (for paired-end sequencing of non-barcoded fragment libraries)
F5-BC tag	Tag to be sequenced using reverse ligation chemistry and using primers specific to the Internal Adaptor sequence (for paired-end sequencing of barcoded fragment libraries)
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 pipet 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™ 4 System Library Preparation Guide	4445673	Provides procedures for preparing libraries.
Applied Biosystems SOLiD™ 4 System Library Preparation Quick Reference Card	4445674	Provides brief, step-by-step procedures for preparing libraries.
Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide	4448378	Describes how to prepare templated beads by emulsion PCR (ePCR), required before sequencing on the SOLiD™ 4 System.
Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Quick Reference Card	4448329	Provides brief, step-by-step procedures for preparing templated beads by emulsion PCR (ePCR), required before sequencing on the SOLiD™ 4 System.
Applied Biosystems SOLiD™ 4 System Instrument Operation Quick Reference Card	4448380	Provides brief, step-by-step procedures for loading and running the SOLiD™ 4 System.
Applied Biosystems SOLiD™ 4 System Site Preparation Guide	4448639	Provides all the information that you need to set up the SOLiD™ 4 System.
Applied Biosystems SOLiD™ 4 System SETS Software User Guide	4448411	Provides an alternate platform to monitor runs, modify settings, and reanalyze previous runs that are performed on the SOLiD™ 4 System.
Applied Biosystems SOLiD™ 4 System ICS Software Help	—	Describes the software and provides procedures for common tasks (see the Instrument Control Software).
BioScope™ Software for Scientists Guide	4448431	Provides a bioinformatics analysis framework for flexible application analysis (data-generated mapping, SNPs, count reads) from sequencing runs.
Working with SOLIDBioScope.com™ Quick Reference Card	4452359	Provides an online suite of software tools for Next Generation Sequencing (NGS) analysis. SOLiDBioScope.com™ leverages the scalable resources of cloud computing to perform computation-intensive NGS data processing.
Applied Biosystems SOLiD™ 4 System Software Integrated Workflow Quick Reference Guide	4448432	Describes the relationship between the software comprising the SOLiD 4 platform and provides quick step procedures on operating each software to perform data analysis.

Document	Part number	Description
Applied Biosystems SOLiD™4 System Product Selection Guide	4452360	Provides a quick guide to the sequencing kits you need to perform fragment, paired-end, mate-pair, multiplex fragment, and multiplex paired-end sequencing.
Applied Biosystems SOLiD™ System SOLiD™ 3 Plus to SOLiD™ 4 System User Documentation Changes	4451929	Provides a brief summary of changes made between the SOLiD™ 3 Plus System documentation and the SOLiD™ 4 System documentation.
Applied Biosystems SOLiD™ 4 Upgrade Checklist	4449773	Provides a checklist to ensure that all necessary preparations are made before upgrading to the SOLiD™ 4 System and provides a list of consumables you can order.

Note: For additional documentation, see "How to obtain support" on page 9.

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 9.

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