

Set up and perform a workflow analysis (WFA) run

Note: For safety and biohazard guidelines, refer to the “Safety” section in the *Applied Biosystems SOLiD™ 4 System Library Preparation Guide* (PN 4445674) and the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* (PN 4448379). For every chemical, read the SDS and follow the handling instructions. Wear appropriate protective eye wear, clothing, and gloves.

Deposit the beads

1. Wash the beads:
 - a. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System. Pulse-spin the beads.
 - b. Use the *Applied Biosystems SOLiD™ Bead Concentration Chart* (PN 4415131) to adjust the volume of beads in 1X TEX Buffer so that the color of the bead solution matches a color in the optimal range (750,000 to 1.25 million).
 - c. Quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer.
 - d. Transfer 15 million beads to a 1.5-mL LoBind Tube, then store the remaining beads at 4 °C.
 - e. Place the tube of beads in a magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.
 - f. Resuspend the beads in 400 µL of SOLiD™ XD Slide Deposition Buffer v2. Vortex, then pulse-spin the beads.
 - g. Place the tube of beads in a magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.
 - h. Repeat [step 1f](#) and [step 1g](#) twice.
 - i. Resuspend the beads in the volume of SOLiD™ XD Slide Deposition Buffer v2 indicated on the 4-Well SOLiD™ Deposition Chamber.
2. Prepare the slide(s):
 - a. Insert a new slide into the SOLiD™ 4 or Opti Slide Carrier.
 - b. Pipet 3 mL SOLiD™ XD Slide Prep Reagent over the entire surface of the slide and let sit for 2 minutes. **IMPORTANT!** Do not let the slide dry out.
 - c. Decant the SOLiD™ XD Slide Prep Reagent off of the surface of the slide onto a Kimwipes® wiper by laying the Kimwipes® wiper on the lab bench and holding the slide in the carrier perpendicular to the lab bench. If needed, carefully put the Kimwipes® wiper in the corner of the slide to soak up excess reagent *without touching* the slide surface.

- d. Pipet 3 mL SOLiD™ XD Slide Deposition Buffer v2 over the entire surface of the slide.
 - e. Decant the SOLiD™ XD Slide Deposition Buffer v2 off of the surface of the slide in the same manner as described in [step 2c](#).
 - f. Repeat [steps 2d](#) and [step 2e](#) twice.
3. Deposit the beads:
 - a. Place the slide carrier assembly into the 4-Well SOLiD™ Deposition Chamber.
 - b. Sonicate the beads using the Covalent Declump 3 program on the Covaris S2 System. Pulse-spin the beads.
 - c. Repeat [step 3b](#).
 - d. *Immediately* transfer the templated beads into one of the 4 wells in the SOLiD™ Deposition Chamber.
 - e. Repeat [step 3b](#) to [step 3d](#) for each WFA sample. Note the relative location of each sample well.
 - f. Place 3-mm adhesive disks over all the portholes in the SOLiD™ Deposition Chamber.
 - g. Centrifuge the SOLiD™ Deposition Chamber at 167 × g for 10 minutes.
 - h. Incubate the SOLiD™ Deposition Chamber at room temperature for 1 hour.

Install on-instrument reagents

1. Open the SOLiD™ Instrument Control Software, if it is not already open.
2. In the System Status menu, set the Chiller to **Cooling**. Install reagents only after the Chiller temperature reads <10 °C.
3. Prepare the 1X Instrument Buffer:
 - a. Add 800 mL of 10X Instrument Buffer to an empty 8-L reagent bottle.
 - b. Add 1600 mL of glycerol to the reagent bottle using a graduated cylinder.
 - c. Add 5600 mL of double-distilled water, rinsing residual glycerol from the graduated cylinder.
 - d. Use a magnetic stir bar to mix the solution for 10 minutes.
 - e. Remove the stir bar, then install the buffer or store at 4 °C.
4. Prepare the 1X T4 Ligase Buffer by transferring the contents of the 1X T4 Ligase Buffer Part 1 tube to the 1X T4 Ligase Buffer Part 2 bottle. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.

5. Prepare the Universal Buffer by transferring the contents of the Universal Buffer Part 1 bottle to the Universal Buffer Part 2 bottle. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.
6. After mixing, apply the Universal Buffer label supplied with the bottle.
7. Prepare the Imaging Buffer by transferring the contents of the Imaging Buffer Part 1 bottle to the Imaging Buffer Part 2 bottle. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.
8. Prepare the Cleave 2.1 solution by transferring the contents of the Cleave 2.1 Part 1 bottle to the Cleave 2.1 Part 2 bottle. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.
9. Install the prepared 1X Instrument Buffer and Storage Buffer into the appropriate positions in the cabinet.
10. Install the Cleave Solution 1, prepared Cleave Solution 2.1, and Reset Buffer into the appropriate positions on the side of the instrument. Verify that there is sufficient volume of Reset Buffer for each tag sequenced.
11. Install the prepared Imaging Buffer, prepared 1X T4 Ligase Buffer, and prepared Universal Buffer into the appropriate positions in the chiller block.
12. Check the level of waste in the 10-L carboy. Properly dispose of waste, if necessary.
13. Under the System Status menu, click **Prime**. While priming, check the syringe to ensure that it is filled with buffer during aspiration. If it is not full, click **Prime** again.

Install slides on the instrument

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.
3. Aspirate the Deposition Buffer from the SOLiD™ Deposition Chamber using a different pipettor tip for each of the wells.
4. Open the SOLiD™ Deposition Chamber, then carefully remove the slide carrier assembly.
5. Place the slide carrier assembly onto the instrument or into a SOLiD™ Slide Storage Chamber.
6. For each flowcell to be used, click **Clear Flowcell**.
7. When prompted, open the flowcell chamber. Remove the slide carrier assembly from the previous run, if necessary.
8. Clean the flowcell block with 70% ethanol and a Kimwipes® wiper.
9. Inspect the O-ring and reseal or replace it, if necessary.

10. Insert the SOLiD™ 4 or Opti Slide Carrier assembly onto the instrument, then tighten the lock-down tabs.
11. Close the flowcell chamber, then click **OK** to prime the flowcell with 1X Instrument Buffer.
12. Check for and eliminate leaks.
13. If only one flowcell is used, ensure that the lock-down tabs are tightened before closing the flowcell.
14. Click **Load Flowcells** for the appropriate flowcell.

Install reagent strip(s)

1. Clean the needle and needle holder.
2. Thaw the appropriate reagent strip(s), then centrifuge the strips at 160 × g for 3 minutes (see “Reagent strip layouts” on page 7).
3. Verify that the reagent strip blocks are oriented and seated properly in the chiller block, then place the reagent strips in the appropriate location in the chiller block (flowcell 1 = front block, flowcell 2 = rear block).
4. Clean the cover, if needed.
5. Place the cover over the reagent strips, then fasten it in place.

Create a WFA run record

Create a WFA run by using the Run wizard or by importing a Run Definition file created offline (for detailed information, refer to “Create a WFA run record,” in Section 3.1 of the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* [PN 4448379]).

Detect the focus range

1. Close the front doors of the SOLiD™ 4 Analyzer.
2. Choose **Window ▶ Imaging System**.
3. Select **Tools ▶ Detect Focusing Range**. Navigate to C:\Runs, then select the **imagingMap.STG** file located in the directory of the appropriate run.
4. Select the flowcell, then click **Go**.
5. When the Imager dialog appears, 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.
6. Check the images at random locations to ensure that the beads are in focus.

Start the WFA run

1. Click **Start Run**.
2. If needed, use the SOLiD™ Experimental Tracking System (SETS) to create more available disk space.

Monitor the run

Tools	Tasks
Run log	Check for any errors.
Heat maps	<ul style="list-style-type: none"> • Look for uniform deposition of beads on the slide. • Check if the bead density is ~25,000 beads/panel.
Cycle scans [‡]	<ul style="list-style-type: none"> • Check: <ul style="list-style-type: none"> – Failed panels = number of panels that failed image alignment during color-calling. – Fraction of (best + good beads)/ usable beads. – Effective exposure times. – Satay plots. • View the image signals for a specific panel by using the panel browser. Open the panel browser by double-clicking any cycle to view the heat map, then left-click any square panel on the heat map.

[‡]For guidelines, refer to “Monitor the run,” in Section 3.1 of the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* (PN 4448379).

View the WFA report

1. View the WFA report using the SOLiD™ Experimental Tracking System (SETS).
2. Determine the optimal titration point based on the highest titration metric.
3. Calculate the concentration of P2-positive beads:

$$\begin{aligned}
 X \mu\text{L} &= \frac{15 \times 10^6 \text{ beads}}{\# \text{ beads}/\mu\text{L} \text{ (according to NanoDrop®)}} \\
 &= \frac{\text{P2\# beads/panel} \times 426 \text{ panels}}{X \mu\text{L}}
 \end{aligned}$$

4. Use the calculated concentration in place of the value determined by the NanoDrop® ND-1000 Spectrophotometer for more accurate deposition densities.

For more details about bead density, refer to “Determine the bead deposition density for a sequencing run” in Section 3.1 of the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* (PN 4448379).

Set up and perform a sequencing run

Deposit the beads

1. Wash the beads:

- Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System. Pulse-spin the beads.
- If a WFA run was performed, use the results from the WFA report to estimate the bead concentration. If results are unavailable:
 - Use the *Applied Biosystems SOLiD™ Bead Concentration Chart* (PN 4415131) to adjust the volume of beads in 1X TEX Buffer so that the color of the bead solution matches a color in the optimal range (750,000 to 1.25 million).
 - Quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer.
- Transfer the appropriate number of beads (see table below) to a 1.5-mL LoBind Tube, then store the remaining beads at 4 °C.

Number of beads to use according to deposition chamber

SOLiD™ Deposition Chamber	Target number of P2-positive beads per well (million) [‡]
1-Well	708
4-Well	128
8-Well	56

[‡]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. For details, refer to "Wash the beads" in Chapter 2 of the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* (PN 4448379).

- Place the tube of beads in a magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.
 - Resuspend the beads in 400 µL of SOLiD™ XD Slide Deposition Buffer v2. Vortex, then pulse-spin the beads.
 - Place the tube of beads in a magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.
 - Repeat [step 1f](#) and [step 1g](#) twice.
 - Resuspend the beads in the volume of Deposition Buffer v2 indicated on the SOLiD™ Deposition Chamber.
- ### 2. Prepare the slide(s):
- Insert a new slide into the SOLiD™ 4 or Opti Slide Carrier.

- Pipet 3 mL SOLiD™ XD Slide Prep Reagent over the entire surface of the slide and let sit for 2 minutes. **IMPORTANT!** Do not let the slide dry out.
- Decant the SOLiD™ XD Slide Prep Reagent off of the surface of the slide onto a Kimwipes® wiper by laying the Kimwipes® wiper on the lab bench and holding the slide in the carrier perpendicular to the lab bench. If needed, carefully put the Kimwipes® wiper in the corner of the slide to soak up excess reagent *without touching* the slide surface.
- Pipet 3 mL SOLiD™ XD Slide Deposition Buffer v2 over the entire surface of the slide.
- Decant the SOLiD™ XD Slide Deposition Buffer v2 off of the surface of the slide in the same manner as described in [step 2c](#).
- Repeat steps [step 2d](#) and [step 2e](#) twice.

3. Deposit the beads:

- Place the slide carrier assembly into the SOLiD™ Deposition Chamber.
- Sonicate the beads using the Covalent Declump 3 program on the Covaris S2 System. Pulse-spin the beads.
- Repeat [step 3b](#).
- Immediately* transfer the templated beads into one of the wells in the SOLiD™ Deposition Chamber.
- Repeat steps b to d for each sequencing sample. Note the relative location of each sample well.
- Place 3-mm adhesive disks over all the portholes in the SOLiD™ Deposition Chamber.
- Centrifuge the SOLiD™ Deposition Chamber at 167 X g for 10 minutes.
- Incubate the SOLiD™ Deposition Chamber at room temperature for 1 hour.

Install on-instrument reagents

- Open SOLiD™ Instrument Control Software, if it is not already open.
- In the System Status menu, set the Chiller to **Cooling**. Install reagents only after the Chiller temperature reads <10 °C.
- Prepare the 1X Instrument Buffer:
 - Add 800 mL of 10X Instrument Buffer to an empty 8-L reagent bottle.
 - Add 1600 mL of glycerol to the reagent bottle using a graduated cylinder.
 - Add 5600 mL of double-distilled water, rinsing residual glycerol from the graduated cylinder.

- d. Use a magnetic stir bar to mix the solution for 10 minutes.
 - e. Remove the stir bar, then install the buffer or store at 4 °C.
4. Prepare the 1X T4 Ligase Buffer by transferring the contents of the 1X T4 Ligase Buffer Part 1 tube to the 1X T4 Ligase Buffer Part 2 bottle. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.
 5. Prepare the Universal Buffer by transferring the contents of Universal Buffer Part 1 bottle to the Universal Buffer Part 2 bottle. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.
 6. After mixing, apply the Universal Buffer label supplied with the bottle.
 7. Prepare the Imaging Buffer by transferring the contents of the Imaging Buffer Part 1 bottle to the Imaging Buffer Part 2 bottle. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.
 8. Prepare the Cleave 2.1 solution by transferring the contents of the Cleave 2.1 Part 1 bottle to the Cleave 2.1 Part 2 bottle. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.
 9. Install the prepared 1X Instrument Buffer and Storage Buffer into the appropriate positions in the cabinet.
 10. Install Cleave Solution 1, prepared Cleave Solution 2.1, and Reset Buffer into the appropriate positions on the side of the instrument. Verify that there is sufficient volume of Reset Buffer for each tag sequenced.
 11. Install the prepared Imaging Buffer, prepared 1X T4 Ligase Buffer, and prepared Universal Buffer into the appropriate positions in the chiller block.
 12. Check the level of waste in the 10-L carboy. Properly dispose of waste, if necessary.
 13. Under the System Status menu, click **Prime**. While priming, check the syringe to ensure that it is filled with buffer during aspiration. If it is not full, click **Prime** again.
5. Place the slide carrier assembly onto the instrument or into a SOLiD™ Slide Storage Chamber.
 6. For each flowcell to be used, click **Clear Flowcell**.
 7. When prompted, open the flowcell chamber. Remove the slide carrier assembly from the previous run, if necessary.
 8. Clean the flowcell block with 70% ethanol and a Kimwipes® wiper.
 9. Inspect the O-ring and reseal or replace it, if necessary.
 10. Insert the SOLiD™ 4 or Opti Slide Carrier assembly onto the instrument, then tighten the lock-down tabs.
 11. Close the flowcell chamber, then click **OK** to prime the flowcell with 1X Instrument Buffer.
 12. Check for and eliminate leaks.
 13. If only one flowcell is used, ensure that the lock-down tabs are tightened before closing the flowcell.
 14. Click **Load Flowcells** for the appropriate flowcell.

Install reagent strip(s)

1. Clean the needle and needle holder.
2. Thaw the appropriate reagent strip(s), then centrifuge the strips at 160 × g for 3 minutes (see [“Reagent strip layouts” on page 7](#)).
3. Verify that the reagent strip blocks are oriented and seated properly in the chiller block, then place the reagent strips in the appropriate location in the chiller block (flowcell 1 = *front* block, flowcell 2 = *rear* block).
4. Clean the cover, if needed.
5. Place the cover over the reagent strips, then fasten it in place.

Create a sequencing run record

Create a sequencing run by using the Run wizard or by importing a Run Definition file created offline (for details, refer to “Create a sequencing (standard) run record” or “Create a multiplex sequencing run record” in Chapter 3, Section 3.2 of the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* [PN 4448379]).

Detect the focus range

1. Close the front doors of the SOLiD™ 4 Analyzer.
2. Choose **Window ▶ Imaging System**.
3. Select **Tools ▶ Detect Focusing Range**. Navigate to C:\Runs, then select the **imagingMap.STG** file located in the directory of the appropriate run.
4. Select the flowcell, then click **Go**.

Install the slides on the instrument

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.
3. Aspirate the Deposition Buffer from the SOLiD™ Deposition Chamber using a different pipettor tip for each of the wells.
4. Open the SOLiD™ Deposition Chamber, then carefully remove the slide carrier assembly.

- When the Imager dialog appears, 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.
- Check the images at random locations to ensure that the beads are in focus.

Start the sequencing run

- Click **Start Run**.
- If needed, use the SOLiD™ Experimental Tracking System (SETS) to create more available disk space.

Control the run

Use:

Tools	Tasks
Run control	<ul style="list-style-type: none"> Stop the run. Reset current primer. Change primer schedule. Set early pause point. Change run progress point.
Imaging and analysis controls for specific spots	<ul style="list-style-type: none"> Enabled (default): allow imaging and analysis of the spot. Image turned off: turn off imaging but allows analysis to complete. Spot discarded: turn off both imaging and analysis.

Monitor the run

Use:

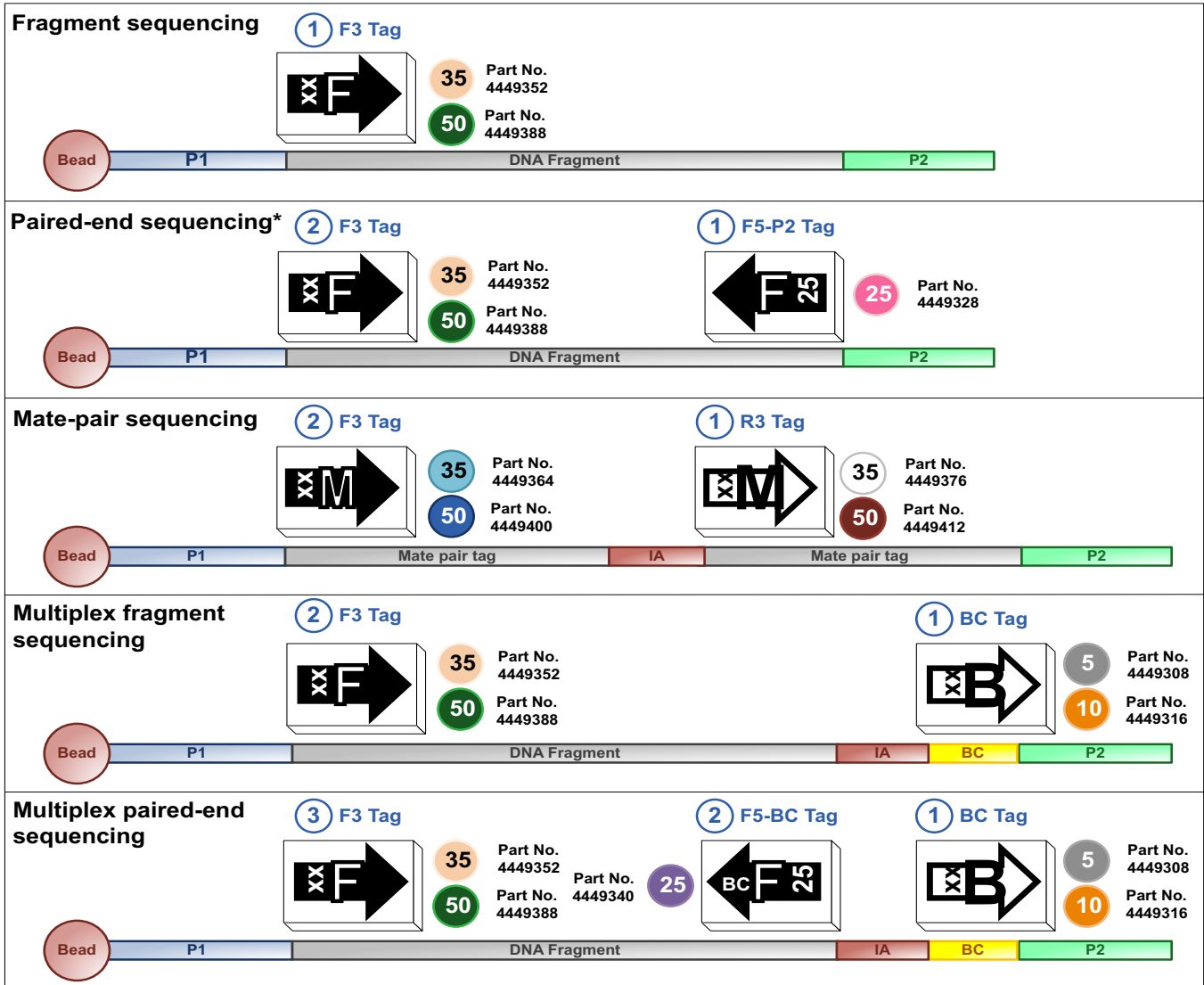
Tools	Tasks
Run log	Check for any errors.
Heat maps	<ul style="list-style-type: none"> Look for uniform deposition of beads on the slide. Check if the bead deposition density is close to the targeted bead deposition density.
Cycle scans [‡]	<ul style="list-style-type: none"> Check: <ul style="list-style-type: none"> Failed panels = number of panels that failed image alignment during color-calling. Fraction of (best + good beads)/usable beads. Effective exposure times. Satay plots. View the image signals for a specific panel using the panel browser. Open the panel browser by double-clicking any cycle to view the heat map, then left-click any square panel on the heat map.

[‡] For guidelines, refer to “Monitor the run,” in Chapter 3, Section 3.2 of the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* (PN 4448379).

Reagent strip layouts

Reagent strip box symbols corresponding with sequencing of fragment, mate-paired, and barcoded fragment libraries. For more information, refer to the *Applied Biosystems SOLiD™ 4 System Product Selection Guide (4452360)*.

Note: Verify that there is sufficient volume of Reset Buffer for each tag sequenced.

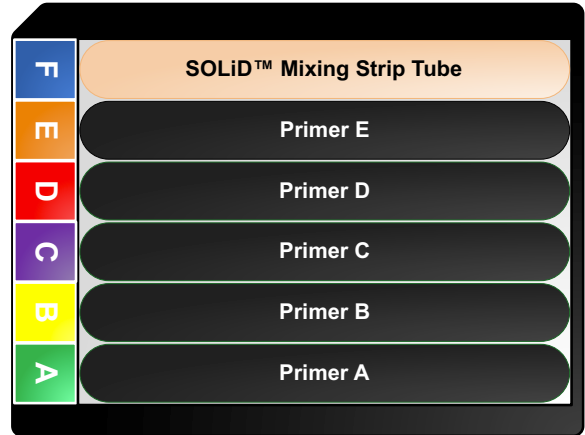


* For non-multiplex paired-end sequencing of libraries prepared using the SOLiD™ Total RNA-Seq Kit, use the SOLiD™ ToP Sequencing Kit – BC Frag. Lib., F5-BC Tag, MM25 (PN 4449340) to sequence the F5-BC tag instead of the F5-P2 tag.

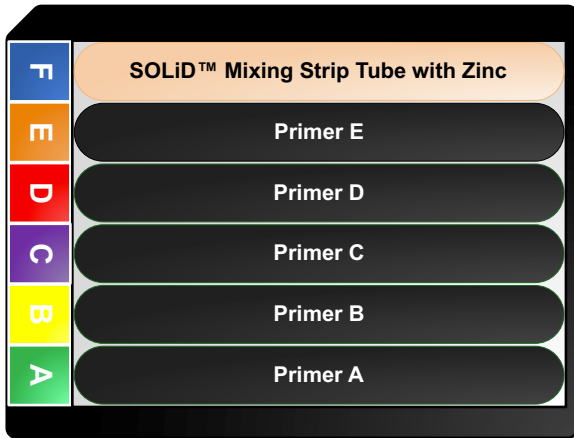
WFA



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

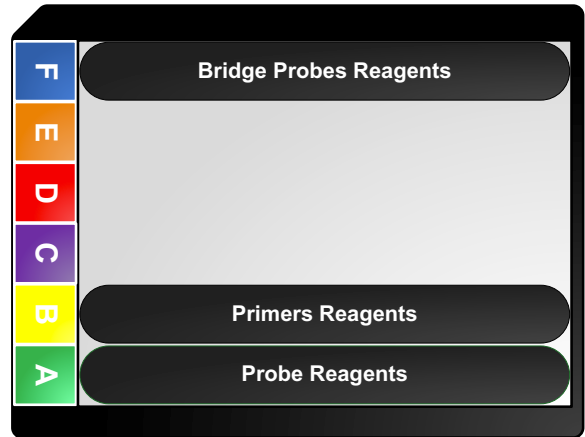


Fragment/Paired-end/Mate-pair sequencing (F3/R3 tags)



- SOLiD™ ToP Sequencing Kit – Frag. Lib., F5-P2 Tag, MM25
- SOLiD™ ToP Sequencing Kit – BC Frag. Lib., F5-BC Tag, MM25

Barcode sequencing (BC tag)



- SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM5
- SOLiD™ ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM10

- SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50
- SOLiD™ ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35
- SOLiD™ ToP Sequencing Kit – MP Lib Seq, F3 Tag, MM 50
- SOLiD™ ToP Sequencing Kit – MP Lib Seq, R3 Tag, MM 50
- SOLiD™ ToP Sequencing Kit – MP Lib Seq, F3 Tag, MM 35
- SOLiD™ ToP Sequencing Kit – MP Lib Seq, R3 Tag, MM 35

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