USER GUIDE

applied biosystems® by life technologies™

Fragment Library Preparation Using the AB Library Builder™ System

SOLiD™ 4 System

Publication Part Number 4462553 Rev. A
Revision Date March 2011

design experiment

▶ **prepare libraries**

prepare beads

run sequencer

analyze data

life technologies™

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

4462553 Rev. A

03/2011

Contents

About This Guide

Safety information

Note: For important instrument safety information, refer to the *AB Library Builder™ System User Guide* (Part no. 4463421). For general safety information, see this section and Appendix F, "Safety" on page 83. 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, will result 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*.

SDSs

The 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 84.

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

About the Products

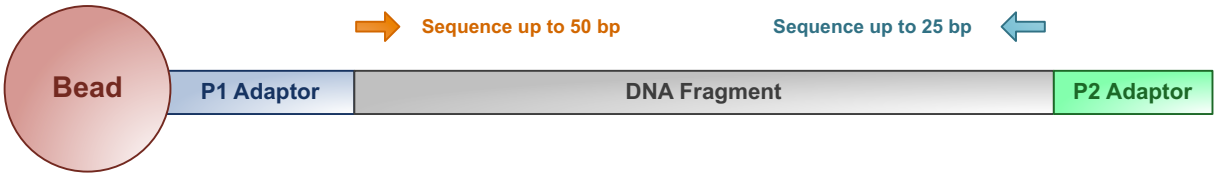
Note: For complete site preparation and operating instructions of the AB Library Builder™ System, refer to the *AB Library Builder™ System Site Preparation Guide* (Part no. 4465106) and the *AB Library Builder™ System User Guide* (Part no. 4463421): **http://www.appliedbiosystems.com/librarybuilderguides**

For a more detailed overview of library types and the library preparation workflows, see "Supplemental Background Information" on page 61.

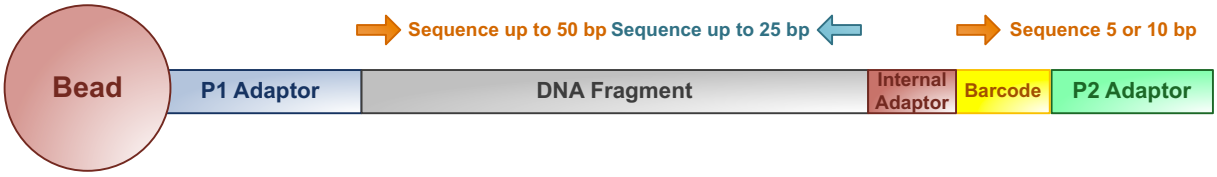
Library preparation

Library preparation is the first step in which samples are adapted for sequencing on the SOLiD™ 4 System. During library preparation, forward and reverse adaptors are added to the ends of DNA inserts (The bead is for illustration purposes only and is not added until the bead preparation step):

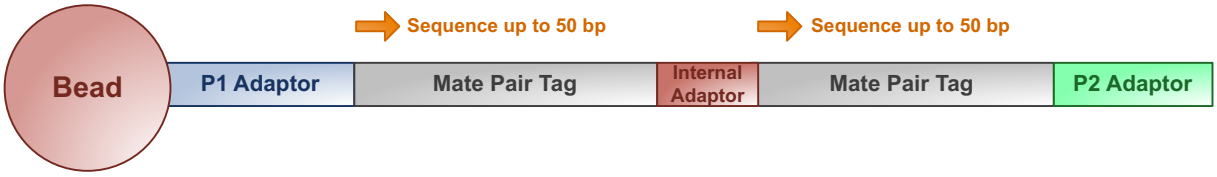
Fragment Library



Barcoded Fragment Library



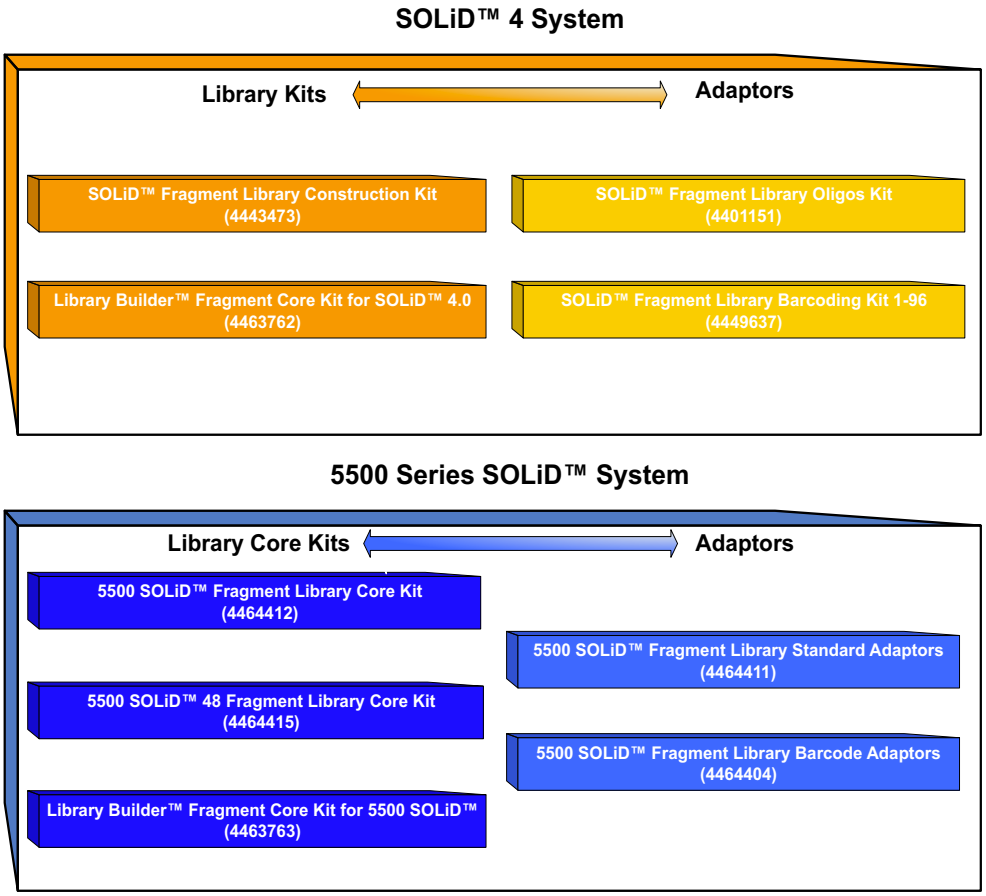
Mate-Paired Library



Product information

Purpose of the product

To prepare fragment and barcoded fragment libraries for sequencing on the SOLiD™ 4 System, Life Technologies offers a system of kits and adaptors to customize preparation of single to multiplexed, barcoded libraries (Life Technologies part numbers are in parentheses. For comparison, the 5500 Series SOLiD™ Sequencers kits and adaptors are shown):



How to use the library kits and adaptors

This user guide, the *Fragment Library Preparation Using the AB Library Builder™ System: SOLiD™ 4 System User Guide*, describes how to use the Library Builder™ Fragment Core Kit for SOLiD™ 4.0 with the SOLiD™ Fragment Library Oligos Kit or the SOLiD™ Fragment Library Barcode Kit.

Used with a wide range of adaptors, the Library Builder™ Fragment Core Kit for SOLiD™ 4.0 contains reagents and a protocol card to prepare standard libraries (100–250 bp, before adaptor ligation) or express libraries (100–550 bp, before adaptor ligation). The protocol card directs the instrument to end-repair, ligate, and size-select libraries.

After automated library preparation, nick-translate and quantitate the library for templated bead preparation on the Applied Biosystems SOLiD™ EZ Bead™ System [refer to *SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (Part no. 4441486)].

Kit contents and storage conditions

Kit contents

The Library Builder™ Fragment Core Kit for SOLiD™ 4.0 (Part no. 4463762) contains materials sufficient to prepare up to 13 standard or express fragment libraries:

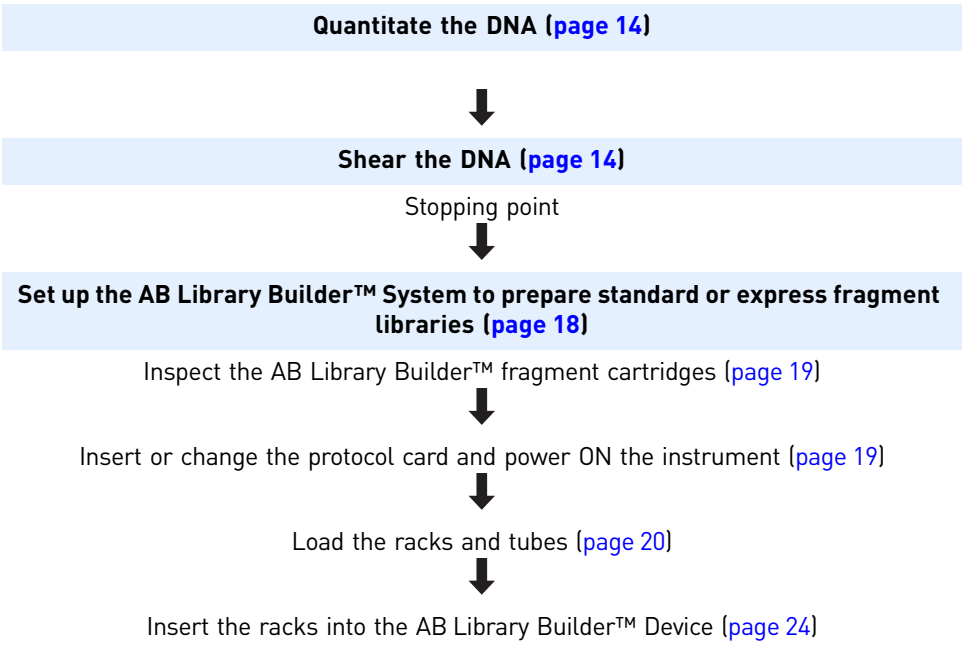
Part	Description	Storage temperature
AB Library Builder™ Fragment Reagents Module for SOLiD™ 4.0	• 13 cartridges. Each cartridge contains ready-to-use reagents. • 13 tubes of 5× Reaction Buffer tubes. • 1 tube of Shear Buffer. • 5 tubes of Platinum® PCR Amplification mix.	–20°C
AB Library Builder™ Plastics Module	Sample and elution tubes, tips, and tip holders.	Room temperature

The adaptors that are required to prepare fragment libraries with the AB Library Builder™ System are sold separately:

Part	Description	Storage temperature
SOLiD™ Fragment Library Oligos Kit (4401151)	One each	–20°C
SOLiD™ Fragment Library Barcoding Kit 1–96 (4449637)	One each	–20°C

Prepare to Build the Library

Workflow



Procedural guidelines

- The protocol is designed for 10 ng–5 µg of genomic DNA or PCR product.
- Use good laboratory practices to minimize cross-contamination of products.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols. Applied Biosystems recommends the Eppendorf 5417R tabletop microcentrifuge.
- Perform all steps requiring 0.5-mL and 1.5-mL tubes with 0.5-mL Eppendorf LoBind Tubes (Eppendorf Part no. 022431005) and 1.5-mL Eppendorf LoBind Tubes (Eppendorf Part no. 022431021).
- Thaw reagents on ice or at room temperature before use, but thaw Shear Buffer at room temperature.

Quantitate the DNA

For accuracy, determine sample DNA concentration using a double-stranded DNA-specific fluorescence assay. Use the HS Assay Kit to measure dsDNA concentrations from 10 pg/µL to 100 ng/µL. For samples outside this range, use the dsDNA BR for higher concentrations of DNA or PicoGreen® dsDNA Assay Kit for lower concentrations:

- Invitrogen Qubit™ dsDNA HS Assay Kit (Invitrogen Part no. Q32851 or Q32854)

  or
- Invitrogen Qubit™ dsDNA BR Assay Kit (Invitrogen Part no. Q32850 or Q32853).

  or
- Invitrogen Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen Part no. P7589)

Shear the DNA

This step involves fragmenting the input DNA into small fragments with a mean fragment size of 165 bp by using a Covaris® System. The conditions have been tested for shearing 10 ng–5 µg DNA in a total volume of 120 µL. For certain DNA samples, optimizing the shearing protocol may be necessary.

Shear the DNA

You can shear the DNA with two supported shearing systems:

- The Covaris® S2 System (see "Shear the DNA with the Covaris® S2 System".

  or
- The Covaris® S220 System (see "Shear the DNA with the Covaris® S220 System" on page 16).

Shear the DNA with the Covaris® S2 System

1. Prepare the Covaris® S2 Tank:
   a. Ensure that the water in the Covaris® S2 tank is filled with fresh deionized water to fill-line level 12 on the graduated fill-line label.

      The water should cover the visible glass part of the tube.
   b. Set the chiller temperature to 2–5°C to ensure that the temperature reading in the water bath displays 5°C.
   c. Supplement the circulated water chiller — ***not*** the actual water bath —with 20% ethylene glycol.
2. Dilute the desired amount of DNA to 120 µL in 1× Low TE Buffer in a LoBind tube. Shear Buffer reduces DNA damage from fragmentation:

Component	Amount
DNA	10 ng to 5 µg
1× Low TE Buffer	Variable µL
Shear Buffer	1.2 µL
Total	**120 µL**

3. Load the DNA into the Covaris® S2 System:
   a. Place a Covaris® microTUBE into the loading station.
   b. Keeping the snap-cap on the tube, use a tapered pipette tip to slowly transfer the 120 µL of DNA sample through the pre-split septa.

      Be careful not to introduce a bubble into the bottom of the tube.

      Note: To load and unload the Covaris® microTUBE correctly from the microTUBE holder, see "Load and unload Covaris® microTUBE vials from the Covaris® microTUBE holder" on page 49.

4. Shear the DNA using the following Covaris® S2 System conditions:

IMPORTANT! Ensure that the bath temperature limit is set at 15°C, and keep the bath temperature to ≤10°C.

Condition	Setting
Number of cycles	**6**
Bath temperature	**5°**C
Bath temperature limit	**15°**C
Mode	**Frequency sweeping**
Water quality testing function	**Off**
Duty cycle	**10%**
Intensity	**5**
Cycles/burst	**100**
Time	**60 seconds**

5. Remove the sheared DNA:
   a. Place the Covaris® microTUBE into the loading station.
   b. While keeping the snap-cap on, insert a pipette tip through the pre-split septa, then slowly remove the sheared DNA.
   c. Transfer 110 µL of the sheared DNA into a new 1.5-mL sample tube provided in the Library Builder™ Fragment Core Kit for SOLiD™ 4.0.

Shear the DNA with the Covaris® S220 System

IMPORTANT! Ensure that the bath temperature during shearing is 5–10°C. Higher shearing temperatures can be harmful to DNA.

1. For each library dilute the components below in a 1.5-mL LoBind Tube. Shear Buffer reduces DNA damage from fragmentation:

   Note: Applied Biosystems has validated this protocol for fragment library preparation using Shear Buffer. To see if it is appropriate in your protocol, test the Shear Buffer first.

Component	Amount
DNA	10 ng–5 µg
1X Low TE Buffer	Variable µL
(*Optional*) Shear Buffer	1.2 µL
Total	**120 µL**

2. Prepare the Covaris® S220 Tank:
   a. Ensure that the water in the Covaris® S220 tank is filled with fresh deionized water to fill-line level 12 on the graduated fill-line label.

      The water should cover the visible glass part of the tube.
   b. Set the chiller temperature to 2–5 °C to ensure that the temperature reading in the water bath displays 5°C.
   c. Supplement the circulated water chiller with 20% ethylene glycol.
3. Load the DNA:
   a. Place a Covaris® microTUBE into the loading station.
   b. Keeping the snap-cap on the tube, use a tapered pipette tip to slowly transfer the 120 µL of DNA sample through the pre-split septa.

      Be careful not to introduce a bubble into the bottom of the tube.

      Note: To load and unload the Covaris® microTUBE correctly from the microTUBE holder, see "Load and unload Covaris® microTUBE vials from the Covaris® microTUBE holder" on page 49.

4. Shear the DNA using the following Covaris® S220 System conditions:

IMPORTANT! Ensure that the bath temperature limit is set at 15°C, and keep the bath temperature to ≤10°C.

Condition	Setting
Number of cycles	**6**
Bath temperature	**5°**C
Bath temperature limit	**15°**C
Mode	**Frequency sweeping**
Water quality testing function	**Off**
Duty Factor	**10%**
Peak Incident Power	**175 Watts**
Cycles/burst	**100**
Time	**60 seconds**

5. Remove the sheared DNA:
   a. Place the Covaris® microTUBE into the loading station.
   b. While keeping the snap-cap on, insert a pipette tip through the pre-split septa, then slowly remove the sheared DNA.
   c. Transfer 110 µL of the sheared DNA into a new 1.5-mL sample tube provided in the Library Builder™ Fragment Core Kit for SOLiD™ 4.0.

STOPPING POINT Store the DNA in a supplied Sample Tube at 4°C for short-term storage or at –20°C for long-term storage, or proceed directly to "Set up the AB Library Builder™ System to prepare standard or express fragment libraries" on page 18.

Set up the AB Library Builder™ System to prepare standard or express fragment libraries

About standard and express fragment library preparation

AB Library Builder™ System prepares standard (size-selected) or express (not size-selected) DNA fragment libraries. Use the AB Library Builder™ System with the Library Builder™ Fragment Core Kit for SOLiD™ 4.0 and Agencourt AMPure® XP Reagent to end-repair, size-select (optional), ligate, and purify fragment libraries.

To install and set up the AB Library Builder™ System, refer to the *AB Library Builder™ System Site Preparation Guide* (Part no. 4465106) and the *AB Library Builder™ System User Guide* (Part no. 4463421).

IMPORTANT! To avoid data loss or run cancellation, always follow these practices:

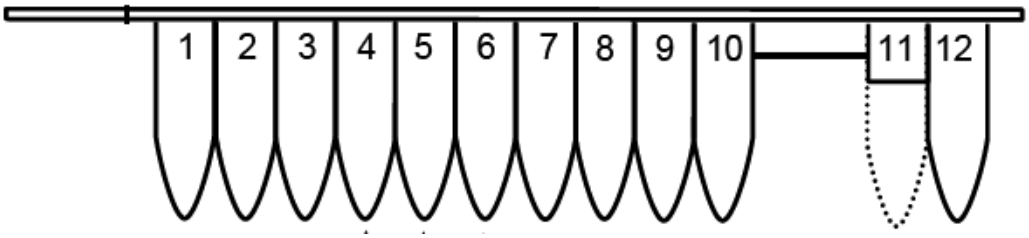
- Before you insert or remove a protocol card, power the instrument OFF
- Before you power the instrument ON, insert the protocol card, then close the instrument door.
- To pause the instrument during a run, press **Stop** before you open the instrument door
- When you are *not* performing an extraction run or instrument test, you can open the instrument door with the power OFF or ON
- Do not move instrument components such as the platform, magnets, and syringes while the instrument is powered ON.

Before a run, follow these procedures to set up the instrument:

1. "Inspect the AB Library Builder™ fragment cartridges" on page 19.
2. "Insert or change the protocol card and power ON the instrument" on page 19.
3. "Load the racks and tubes" on page 20.
4. "Insert the racks into the AB Library Builder™ Device" on page 24.

Inspect the AB Library Builder™ fragment cartridges

Each cartridge contains 12 compartments for reagents:



Cartridge compartment numbers	Volumes
1	1300 µL
2–3	1200 µL
4	20 µL
5–7	Empty
8	90 µL
9–10	20 µL
11: Tube to be added after 5X Reaction Buffer tube prepared	Adaptor volumes to be calculated
12: Unsealed compartment for beads	—

Insert or change the protocol card and power ON the instrument

IMPORTANT! Do not remove the protocol card while the instrument is on. Removing the card stops the run, and it may cause instrument data file loss. To remove the card, see page 20.
If you accidentally remove the protocol card during a run, power off the instrument immediately to minimize potential for instrument data loss.

For guidelines on handling protocol cards, see the*AB Library Builder™ System User Guide* (Part no. 4463421).

1. Confirm that the power switch is in the OFF position.

   Note: If you insert the card while the instrument is on, the instrument does not recognize the card.

2. Open the card slot.

3. To remove a card that is already installed in the slot, push the button located below the card slot (see the photo below), then pull the card out of the slot. Place the card in the plastic cover in the box.

   IMPORTANT! Do not remove the protocol card while the instrument is on.

4. Insert the appropriate protocol card in the slot with the arrow on the protocol card pointing toward the instrument and the label facing left.
5. Push the card completely into the card slot, then close the card slot.
6. Close the door to the AB Library Builder™ Device.
7. Power ON the instrument.

   When the card is fully inserted in the correct orientation, the display briefly shows information including the instrument version, then displays the Main menu.
8. Press **START.**

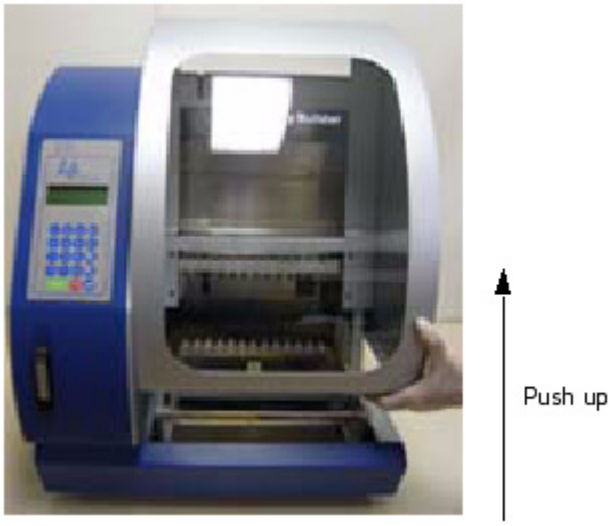
Load the racks and tubes

Note: To ensure the best pipetting performance, use the cartridge rack and tip and tube rack shipped with the instrument; these racks are calibrated with the instrument at the factory. Before using other racks on a specific instrument, run the installation test to qualify the racks for use on that instrument. Refer to the *AB Library Builder™ System User Guide* for details

Wear gloves when you handle samples or load the cartridges, tips, and tubes in the rack.

Remove the racks from the instrument

Open the instrument door (push up the door), then remove the tip and tube rack and the cartridge rack:



Load the cartridge rack

1. Remove up to 13 cartridges from the kit box.

   IMPORTANT!
   One cartridge is required per sample. Use only AB Library Builder™ Cartridges.

   IMPORTANT!
   Do not switch the supplied pre-filled reagents with any other buffers, because the protocols are specifically optimized with the reagents supplied with the kit.

2. Thaw the cartridges at room temperature or on ice for ≤2 hours or until completely thawed.

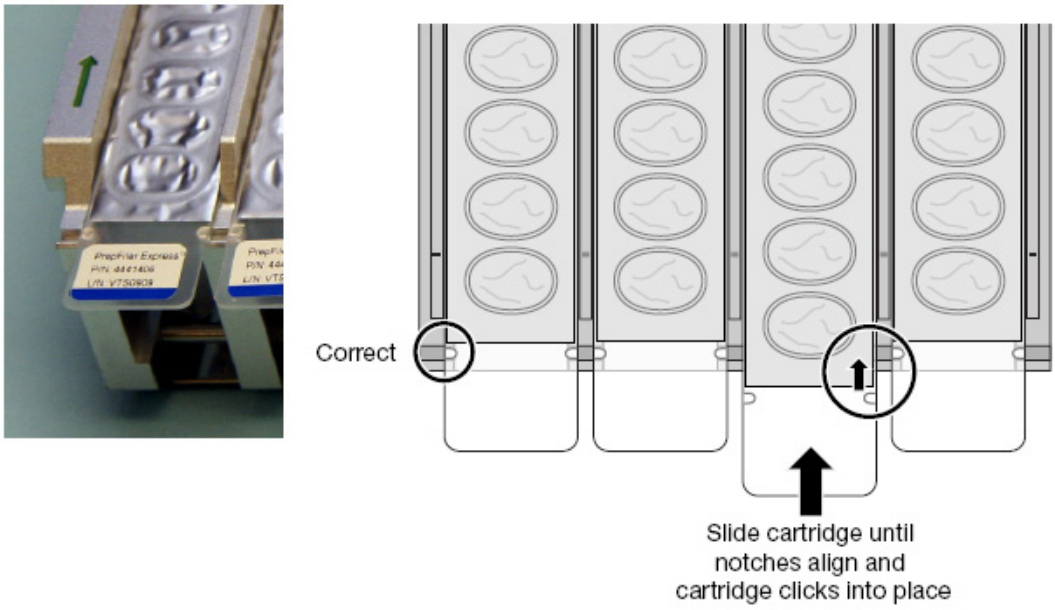
   IMPORTANT! Avoid leaving the cartridges at room temperature for longer than necessary to completely thaw them. Avoid repeated freeze-thawings of unused cartridges.

   IMPORTANT! Before loading the cartridges into the cartridge rack, ensure that the cartridges are *completely* thawed, particularly reagents in cartridge wells 2 and 3.

3. Gently tap each cartridge on the laboratory bench until any liquid droplets that might be underneath the foil seal are deposited into the bottom of the well.

4. Load the reagent cartridges into the cartridge rack by sliding each reagent cartridge along the groove in the direction of the arrow until the reagent cartridge clicks into place. Make sure that the notches in the cartridge align with the notches in the cartridge rack.

   Note: An incorrectly loaded cartridge rack may cause the instrument to stop during a run.



Load adaptors in the cartridges

IMPORTANT! If you are preparing barcoded libraries for multiplexed sequencing, for each sequencing run, use at least one of the following full sets of four barcodes: Barcodes-T-001–004, 005–008, 009–012, 013–016, 017–020, 021–024, 025–028, 029–032, 033–036, 037–040, 041–044, 045–048, 049–052, 053–056, 057–060, 061–064, 065–068, 069–072, 073–076, 077–080, 081–084, 085–088, 089–092, or 093–096. Use only one of the barcoded-T-0XX adaptors for each ligation reaction, unless < 4 libraries are being barcoded.

Use the barcodes according to these conditions:

- If <4 libraries are prepared for sequencing, then use multiple barcodes per library in equal ratios. For example, for 2 libraries, use 2 barcodes for each library. For 3 libraries, use 4 barcode adaptors for each library for a total of 12 barcodes.
- If ≥4 libraries are prepared for sequencing and libraries are split into sets of 4 to use full sets of barcodes, then use one set of barcodes for the remaining libraries (1,2,or 3 libraries). There is no need to use multiple barcodes per library in equal ratios.

1. Prepare the 5✕ Reaction Buffer tubes:
   a. Spin the supplied 5✕ Reaction Buffer tubes for each sample.
   b. Calculate the amount of P1 Adaptor (*Y*) needed:

$$\textbf{\textit{Y}}\ \mu\text{L P1 Adaptor needed} = \#\ \mu\text{g DNA} \times A \times \frac{9.2\ \text{pmol}}{1\ \mu\text{g DNA}} \times B \times \frac{1\ \mu\text{L P1 Adaptor needed}}{50\ \text{pmol}}$$

where:

A = (value below), if...	Library type
0.3	Standard fragment
0.66	Express fragment
B = (value below), if...	**Amount DNA**
35 - (5 × n µg DNA)	n = 1–5 µg
30	300 ng–1 µg
***Y* µL P1 Adaptor = (value below) if...**	
0.5 µL	<300 ng (standard fragment library)
1.1 µL	<300 ng (express fragment library
4.25 µL	>1.2 µg (barcoded express fragment library)

c. Calculate the amount of P2 Adaptor required:

P2 = if...	Adaptor type
P1	Standard
9 × P1	Barcoded

d. Calculate the amount of 1X Low TE Buffer required based on the volumes of P1 and P2:

µL 1X Low TE Buffer needed = 42.5 – P1 – P2

e. Add 1X Low TE Buffer, P1 and P2 Adaptors to the appropriate 5X Reaction Buffer tube. Vortex, then pulse-spin the tube and place it in open position 11 of the cartridge.

Load Agencourt AMPure® XP Reagent in the cartridges

Thoroughly resuspend Agencourt AMPure® XP Reagent, then carefully transfer 500 µL to unsealed position 12.

Load the tip and tube rack

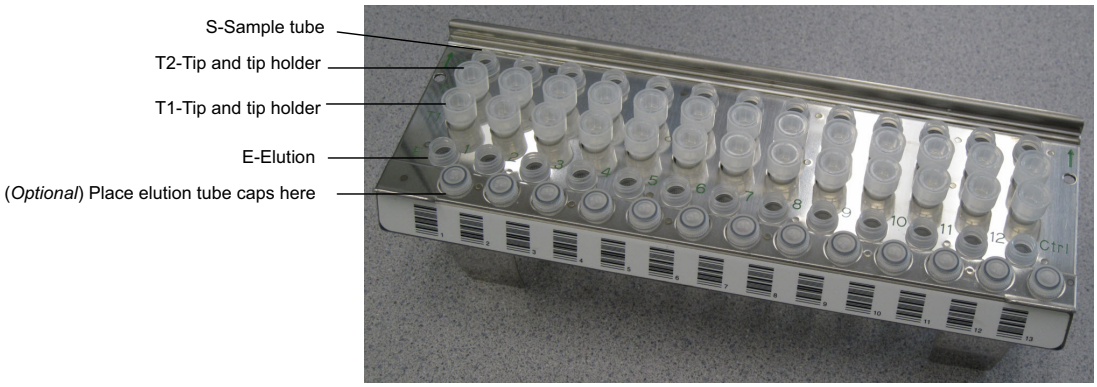
IMPORTANT! If you are processing fewer than 13 samples, make sure to load the tips and tubes in the same positions as the reagent cartridges that are loaded in the cartridge rack.

Load the tip and tube rack in the following order:

1. **Row S** (fourth row): Load with sample/elution tubes containing 110 µL of sheared DNA. If the sample volume is <110 µL, then add 1× TE to the sample for a total volume of 110 µL. Ensure that the cap is off.
2. **Row T1 and T2** (second and third rows): Load with AB Library Builder™ Tips inserted into tip holders.

   Note: Two tip and tip holder sets are required per sample.

3. **Row E** (first row): Load with *labeled* sample/elution tubes, with the caps removed and secured:



Insert the racks into the AB Library Builder™ Device

IMPORTANT!

- Insert the cartridge rack before the tip and tube rack. Changing the order of loading the racks may cause the instrument to stop during a run.
- Use only AB Library Builder™ Sample Tubes (sample/elution tubes). Other tubes may be picked up by the nozzle tips due to differences in tube height and shape, stopping the run.

Insert the cartridge rack

IMPORTANT! Before inserting the cartridge rack into AB Library Builder™ Device, ensure that the cartridges are *completely* thawed, particularly reagents in cartridge wells 2 and 3.

Insert the loaded cartridge rack into the instrument:

WARNING! Do not touch the surface of the heat block. The temperature of the heat block can reach 95°C. Touching the block can cause burns.

Insert the tip and tube rack

Insert the loaded tip and tube rack into the instrument with row E in the front:

Build the Library

Workflow

Start the run (page 27) (run time: ~2.5 h)

⬇

Set up for a new run (page 28)

Note: For additional instructions on instrument operation, see "AB Library Builder™ System operation" on page 51.

Start the run

1. Press **START** to select the AB Library Builder™ System Kit option.
2. Confirm that you have loaded and inserted the cartridge rack and tip and tube rack correctly.
3. Select the script for the kit you are using, then follow the on-screen prompts.
4. (*Optional*) Scan the sample, elution tube, and sample lane barcodes [refer to the *AB Library Builder™ System User Guide* (Part no. 4463421)].
5. Close the door to the AB Library Builder™ Device.
6. Press **START**.

   The screen shows the current step and the approximate incubation time remaining.

   IMPORTANT! Do not open the door during a protocol run. To pause or cancel the run, see "AB Library Builder™ System operation" on page 51.

   Note: If you lose power or the power cord is unplugged, the run stops. When the power resumes, the digital display shows the Main menu. You cannot resume the run. If the tips are still on the syringe unit when the power resumes, return the tips to the original positions as described in "AB Library Builder™ System operation" on page 51.

7. At the end of the run (the instrument beeps briefly and the digital display shows "Finished Protocol"). To unload the instrument:
   a. Press  to return to the Main menu, then open the instrument door.
   b. Remove the elution tubes, confirm that they are properly labeled, then cap the elution tubes containing the library in 100 µL.

c. If the library has a brown tint, place each tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle; then transfer the *supernatant* to a new tube.

d. Remove the tip and tube rack and cartridge rack.

e. Properly dispose of the used reagent cartridges, tips, and tubes.

f. Close the instrument door.

g. Clean the tip and tube rack as needed.

Note: No cooling period is required between runs.

STOPPING POINT Store the DNA in a supplied Sample Tube at 4°C for short-term storage or at –20°C for long-term storage, or proceed directly to "Nick-Translate and Amplify the Library" on page 29.

Set up for a new run

WARNING! Do not clean the instrument with acids, or bases (such as bleach). Acids and bases can react with the guanidine thiocyanate in the lysis buffer and generate toxic gas.

1. Follow the set-up procedures for a new run (see "Set up the AB Library Builder™ System to prepare standard or express fragment libraries" on page 18).

   Note: To set up for a new run using the *same* protocol card, leave the instrument on. To set up for a new run with a *different* protocol card, power off the instrument, then change the protocol card (see "Insert or change the protocol card and power ON the instrument" on page 19.).

2. Start the run (see "Start the run" on page 27).

Nick-Translate and Amplify the Library

Workflow

Nick-translate the libraries: *ONLY* for NON-BARCODED (standard fragment) libraries	**Nick-translate *and* amplify the libraries: *REQUIRED* for BARCODED fragment libraries OPTIONAL for *non*-barcoded libraries**
Prepare the reaction, then nick-translate the non-barcoded (standard fragment) library (page 29)	Prepare the reaction, then nick-translate and amplify the library (page 31)
↓	↓
Purify the nick-translated, non-barcoded library (page 30)	Purify the nick-translated, amplified library (page 32)
↓	↓
Quantitate the DNA (page 34)	**Quantitate the DNA (page 34)**
Stopping point	Stopping point
↓	↓
Check the size distribution of the library (page 34)	**Check the size distribution of the library (page 34)**
Stopping point	Stopping point
↓	↓
(Optional) Pool equal molar barcoded libraries of similar size (page 34)	**(Optional) Pool equal molar barcoded libraries of similar size (page 34)**
Stopping point	Stopping point

Nick-translate the non-barcoded library

Prepare the reaction, then nick-translate the *non-barcoded* (standard fragment) library

IMPORTANT! For *barcoded* libraries, proceed to "Nick-translate and amplify the library" on page 31.

1. In a new 1.5-mL LoBind Tube, combine for each library:

Component	Volume
Platinum® PCR Amplification Mix	400 µL
Non-barcoded library	100 µL
Total	**500 µL**

2. Vortex the reaction for 5 seconds, then pulse-spin.

3. Distribute 125-µL aliquots of combined library and PCR master mix between four, 0.2-mL PCR tubes.

4. Incubate the library at 72°C for 20 minutes.

Purify the nick-translated, *non-barcoded* library

1. Resuspend the Agencourt AMPure® XP Reagent and allow the mixture to come to room temperature (~30 minutes).

2. Prepare 70% ethanol for *N* number of libraries:

Component	Volume
Nuclease-Free Water	600 µL × *N*
Ethanol, Absolute	1400 µL × *N*
Total	**2000 µL × *N***

3. For every nick-translated library, label a new 1.5-ml LoBind Tube.

4. Combine each set of the identical 4 PCR reactions (125 µL) to the appropriately labeled 1.5-mL LoBind Tube. The total combined volume of the amplified library is 500 µL.

5. Bind the DNA to the resuspended, ambient Agencourt AMPure® XP Reagent:

   a. For each library, prepare the bead suspension:

Component	Volume
Nick-translated library	500 µL
Agencourt AMPure® XP Reagent	750 µL†

† Equal to 1.5 volumes of sample reaction.

   b. Vortex the beads for 10 seconds, then pulse-spin.

   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.

   d. Place each tube in a DynaMag™-2 magnetic rack for at least 3 minutes until the solution is clear of brown tint when viewed at an angle; then, remove and discard the supernatant.

6. Wash the DNA **2** times. For each wash:

   a. Without removing the tube from the magnet, add 750 µL of *freshly prepared* 70% ethanol and incubate for 30 seconds. Do not disturb the pellet.

   b. Aspirate and discard ethanol.

7. Remove the tube from the DynaMag™-2 magnetic rack, pulse-spin the tube, return the tube to the magnetic rack; then remove and discard the supernatant with a 20-µL pipettor.

8. Open each tube, then dry the beads at room temperature (20–25°C) for ≤5 minutes.

9. Elute the DNA:
   a. Remove each tube from the DynaMag™-2 magnetic rack, then add 50–100 µL Low TE Buffer directly to the pellet to disperse the beads.
   b. Vortex the beads for 10 seconds, then pulse-spin.
   c. Incubate the beads for 2 minutes at room temperature.
   d. Place the tube in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears.
   e. Transfer the *supernatant* containing the amplified library to a new 1.5-mL LoBind Tube.
10. Proceed to "Quantitate the DNA" on page 34.

Nick-translate and amplify the library

IMPORTANT! For *barcoded* fragment libraries for ePCR and sequencing on the SOLiD™ 4 System, nick-translation *and* amplification are *required.*

Library amplification is useful to increase the amount of rare or low-input samples and to enrich targeted sequences. Library amplification can, however, bias the library and introduce base incorporation errors.

Prepare the reaction, then nick-translate and amplify the library

1. In a new 1.5-mL LoBind Tube, combine for a PCR master mix, depending on the preparation of *non-barcoded* fragment libraries or *barcoded* fragment libraries:

***Barcoded* fragment libraries**

Component	Volume per amplification	Master mix for *N* libraries
Platinum® PCR Amplification Mix	380 µL	380 µL × (1.1 × *N*)
Multiplex Library PCR-1, 50 µM	10 µL	10 µL × (1.1 × *N*)
Multiplex Library PCR-2, 50 µM	10 µL	10 µL × (1.1 × *N*)
Total	**400 µL**	**400 µL × (1.1 × *N*)**

***Non-barcoded* standard fragment libraries**

Component	Volume per amplification	Master mix for *N* libraries
Platinum® PCR Amplification Mix	380 µL	380 µL × (1.1 × *N*)
Library PCR Primer 1, 50 µM	10 µL	10 µL × (1.1 × *N*)
Library PCR Primer 2, 50 µM	10 µL	10 µL × (1.1 × *N*)
Total	**400 µL**	400 µL × (1.1 × *N*)

2. Transfer 400 µL of the PCR master mix to *each* library. Each library is 100 µL in an elution tube so that the total volume of the mix is 500 µL.
3. Vortex the reaction for 5 seconds, then pulse-spin.

4. Distribute 125-µL aliquots of combined library and PCR master mix between four, 0.2-mL PCR tubes.

IMPORTANT! The current protocol is optimized for maximum yield from input DNA. In many cases, library amplification is not needed. Quantitate the library to assess the need to amplify it. If library amplification is needed, minimize the number of cycles, based on the amount of starting input DNA. Use minimal cycling to avoid over-amplification and production of redundant molecules.

5. Determine the number of PCR cycles:

Starting amount of DNA	Number of cycles
10–100 ng	10 cycles
100 ng–1 µg	6–8 cycles
1–2 µg	4–6 cycles
2–5 µg, *non-barcoded* standard fragment libraries	2–3 cycles
2–5 µg, *barcoded* fragment libraries	3–6 cycles

IMPORTANT! Minimize the number of PCR cycles to avoid over-amplification and redundant molecules. Base the number of cycles on the amount of starting input DNA.

6. Run the PCR for each 125-µL aliquot:

Stage	Step	Temp	Time
Holding	Nick translation	72°C	20 min
Holding	Denature	95°C	5 min
Cycling	Denature	95°C	15 sec
	Anneal	62°C	15 sec
	Extend	70°C	1 min
Holding	Extend	70°C	5 min
Holding	—	4°C	∞

Purify the nick-translated, amplified library

1. Resuspend the Agencourt AMPure® XP Reagent and allow the mixture to come to room temperature (~30 minutes).
2. Prepare 70% ethanol for *N* number of libraries:

Component	Volume
Nuclease-Free Water	600 µL × *N*
Ethanol, Absolute	1400 µL × *N*
Total	**2000 µL × *N***

3. For every amplified library, label a new 1.5-ml LoBind Tube.
4. Combine each set of the identical 4 PCR reactions (125 µL) to the appropriately labeled 1.5-mL LoBind Tube. The total combined volume of the amplified library is 500 µL.
5. Bind the DNA to the resuspended, ambient Agencourt AMPure® XP Reagent:
   a. For each library, prepare the bead suspension:

Component	Volume
Nick-translated and amplified library	500 µL
Agencourt AMPure® XP Reagent	750 µL†

† Equal to 1.5 volumes of sample reaction.

   b. Vortex the beads for 10 seconds, then pulse-spin.
   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.
   d. Place each tube in a DynaMag™-2 magnetic rack for at least 3 minutes until the solution is clear of brown tint when viewed at an angle; then, remove and discard the supernatant.
6. Wash the DNA **2** times. For each wash:
   a. Without removing the tube from the magnet, add 750 µL of *freshly prepared* 70% ethanol and incubate for 30 seconds. Do not disturb the pellet.
   b. Aspirate and discard ethanol.
7. Remove the tube from the DynaMag™-2 magnetic rack, pulse-spin the tube, return the tube to the magnetic rack; then remove and discard the supernatant with a 20-µL pipettor.
8. Open each tube, then dry the beads at room temperature (20–25°C) for ≤5 minutes.
9. Elute the DNA:
   a. Remove each tube from the DynaMag™-2 magnetic rack, then add 50–100 µL Low TE Buffer directly to the pellet to disperse the beads.
   b. Vortex the beads for 10 seconds, then pulse-spin.
   c. Incubate the beads for 2 minutes at room temperature.
   d. Place the tube in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears.
   e. Transfer the *supernatant* containing the amplified library to a new 1.5-mL LoBind Tube.

Quantitate the DNA

Measure the DNA concentration by using:

- 2 µL of sample with the Qubit™ dsDNA HS Assay Kit (Invitrogen Part no. Q32851) and the Qubit® 2.0 Fluorometer (Invitrogen Part no. Q32866). Use the Qubit™ dsDNA HS Assay Kit to measure dsDNA concentrations from 10 pg/µL to 100 ng/µL. For samples outside this range, use the Qubit™ dsDNA BR Assay Kit for higher concentrations of DNA or the Invitrogen Quant-iT™ PicoGreen® dsDNA Assay Kit for lower concentrations

  or
- 2 µL of sample in the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 57)

  or
- 1 µL of sample in the Agilent Technologies 2100 Bioanalyzer™. If you used the bioanalyzer, see "Check the size distribution of the library".

  and/or
- The appropriate volume in qPCR [refer to the *Applied Biosystems SOLiD™ Library TaqMan® Quantitation Kit* protocol (Invitrogen Part no. A12120)]

STOPPING POINT Store the DNA in Elution Buffer (E1) at 4°C for short-term storage or at –20°C for long-term storage. Proceed directly to emulsion PCR [refer to the *SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (Part no. 4441486)] or "Check the size distribution of the library".

Check the size distribution of the library

Use 1 µL of sample in the Agilent Technologies 2100 Bioanalyzer™. If you see the expected size distribution, proceed directly to emulsion PCR [refer to the *SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (Part no. 4441486)]. If you do *not* see the expected size distribution, troubleshoot or contact your Life Technologies Applications Specialist.

STOPPING POINT Store the DNA in Low TE Buffer at 4°C for short-term storage or at –20°C for long-term storage; or proceed to "(Optional) Pool equal molar barcoded libraries of similar size".

(*Optional*) Pool equal molar barcoded libraries of similar size

IMPORTANT! To avoid library bias, do *not* pool the libraries until after gel purification if:

- the libraries are of dissimilar sizes
- it is unacceptable to pool libraries of unequal library representation
- you prefer not to pool libraries of similar sizes

1. Quantitate the libraries to be pooled by qPCR (see "Quantitate the DNA" on page 34.
2. Mix together equal molar amounts of each barcoded library of *similar* size in an appropriately sized 1.5-mL LoBind Tube. Vortex the tube.
3. (*Optional*) size-select the pooled libraries [see "(Optional) Size-select and pool libraries" on page 54].

STOPPING POINT Store the library DNA in Elution Buffer (E1) at 4°C, or proceed directly to templated bead preparation [refer to *SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (Part no. 4441486)].

Troubleshooting

For symptoms other than those listed in this section, contact Technical Support ("Obtaining support" on page 88).

Observation	Possible Cause	Recommended action
Before loading the cartridges in the cartridge rack		
Precipitate in AB Library Builder™ 5X Reaction Buffer tubes	5X Reaction Buffer tubes were exposed to low temperatures during shipping or storage.	To dissolve precipitate that may have formed during shipping or storage, incubate the 5X Reaction Buffer tubes at 37°C for 5 minutes or until precipitate is no longer visible.
***During* the automated run**		
No power (the digital display is blank and the fan does not turn on when you power on)	AC power cord is not connected	Check AC power cord connections at both ends. Use the correct cords.
	Fuse has blown	Check the integrity of the fuse and replace it if necessary (refer to the *AB Library Builder™ System User Guide*). If the problem persists after connecting the correct power cord and replacing the fuse, contact Technical Support ("Obtaining support" on page 88).
The digital display is blank, but the fan turns on when you power on.	Protocol card is not inserted correctly	Power off the instrument and re-insert the protocol card in the proper orientation into the card slot (see "Insert or change the protocol card and power ON the instrument" on page 19."). Insert it completely into the slot by manually pushing the card.
	Protocol card was inserted when the instrument was powered on	Power off the instrument, then power on the instrument.
Error code displayed	—	See "Instrument error codes" on page 40.
Reagent cartridges, tips, or tubes are not inserted in the correct positions.	—	Press **STOP** to pause the run. Open the door, add the missing items, then press **START** to resume the run. Do not open the door without pausing the run.

Observation	Possible Cause	Recommended action
Run stops after an initial start (you may also see an error code).	• Instrument door opened during the run • Reagent cartridges, tips, or tubes incorrectly loaded in the rack • Racks incorrectly loaded on the instrument	IMPORTANT! If you open the instrument door while the instrument is running, the run stops, and it cannot be restarted. If you need to open the instrument door during a run, first press **Stop** to pause the run, then open the door. 1. Follow the procedure in "Instrument error codes" on page 40. 2. Before starting a new run, make sure that the reagent cartridges, tips, and tubes are correctly loaded: • Slide the reagent cartridges into the cartridge rack as described in ""Load the racks and tubes" on page 20." on page 18. • Load the cartridge rack before the tip and tube rack for proper positioning. • Do not cap the tubes. 3. If the instrument continues to stop during the run, contact Applied Biosystems Technical Support.
	Reagent cartridges not completely thawed	1. Stop the run. 2. Remove the tip and tube rack, then remove the cartridge rack. 3. Inspect cartridge wells 2 and 3 for ice. 4. If any well is frozen, close the door to the AB Library Builder™ Device, then thaw the cartridges completely. 5. Replace the tips in position T2. 6. Insert the cartridge rack then the tip and tube rack onto the AB Library Builder™ Device. 7. Restart the run.
No DNA yield	No sample added to tube causes?	Add samples to tubes, load new reagent cartridges, then perform the run again.
No liquid in tip, or liquid in tip not moving	No sample added to tube, leading to wet filter barrier on the tip and blockage of nozzles	Add samples to tubes, load new reagent cartridges, then perform the run again.
Buffer in the bottom tray	Motor movements are not smooth	Schedule preventive maintenance annually to ensure proper motor movements.
	Reagent cartridges, tips, or tubes incorrectly loaded in the rack	If you are processing fewer than 13 samples, make sure to load the tips and tubes in the same positions as the reagent cartridges that are loaded in the cartridge rack. See below for leakage from tips.
Leakage from tips or uneven liquid handling between nozzles	D-Rings are not greased regularly or they need replacement	You can continue the run, but maintain the D-rings as scheduled. To prevent leakage, maintain or replace the D-rings (refer to the (*AB Library Builder™ System User Guide*).
Blockage of tips	Too much starting material causing clumps or aggregates	Contact Technical Support ("Obtaining support" on page 88). In future runs, use the sample volume recommended in the user guide for the kit you are using.

Observation	Possible Cause	Recommended action
***After* the automated run**		
No elution volume	Sample volume is lower than the recommended volume, leading to wet filter barrier on the tip and blockage of nozzles.	In future runs, use the recommended sample volume for the protocol you are using. Long-term operation with lower-than-recommended sample volumes can lead to issues with liquid handling performance.
No amplifiable library	Insufficient or no adaptors added to the 5X Reaction Buffer tube	Add sufficient adaptor according to the adaptor calculations, and insert the tube in position 11 of the cartridge (see "Load adaptors in the cartridges" on page 22).
	Enzymes or buffer not at bottom of wells	Tap the wells down against a hard surface to move enzymes and buffer to bottom of wells, then inspect the wells.
Observed DNA peak size is significantly different from the expected DNA peak size	Incorrect volume in sheared DNA or prepared 5X Reaction Buffer tube	Add the correct volumes to the sheared DNA and 5X Reaction Buffer tubes.
	Enzymes or buffer not at bottom of wells	Tap the wells down against a hard surface to move enzymes and buffer to bottom of wells, then inspect the wells.
Final library is brownish	Beads in final library	1. Place the tube with the final library in a DynaMag™-2 magnetic rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle. 2. Without disturbing the pellet, carefully transfer the ***supernatant***, which contains the final library, to a new 1.5-mL LoBind Tube.

Instrument error codes

If an extraction run is interrupted by an error, you cannot resume the interrupted run. Follow the procedure below to resolve the error before you start a new run.

If you observe an error code:

1. Make a note of the error code, including the line number. Common error codes are listed in the following table:

Code	Problem	Code	Problem
10	Failed return to origins, protocol cannot run	22	M axis time out, protocol in run
11	Limit error, protocol can not run	23	Y axis time out, protocol in run
12	Failed to return to Z Axis, protocol in run	24	Open door in motion
13	Failed to return to P axis, protocol in run	25	Abnormal input from bottom sensor in motion
14	Failed to return to M axis, protocol in run	26	Failed to initialize heating block
15	Failed to return to Y axis, protocol in run	27	Failed to initialize motion control board
16	Z axis limit error, protocol in run	110	System error; (Assigned greater than 10)
19	Y axis end limit, protocol in run		
20	Z axis time out, protocol in run		
21	P axis time out, protocol in run		

2. Press **ESC** to return to the Main menu.
3. If there are tips attached to the nozzles, press **1** to select the Manual screen, then press **2** to return the tips to the original position.
4. Power OFF the instrument, remove the protocol card, wait 5 minutes, insert the protocol card, then power on the instrument.
5. Run the axis test (refer to the *AB Library Builder™ System User Guide*).
6. If the axis test:
   - Is successful, start a new extraction run. Use new samples and plastics where required.
   - Is *not* successful, contact Technical Support ("Obtaining support" on page 88).

A Ordering Information

This appendix covers materials for *barcoded* fragment library preparation:

Sufficient reagents are supplied in the AB Library Builder™ System Kit to prepare 13 libraries for high-throughput sequencing with the SOLiD™ System.

Upon receipt of the AB Library Builder™ System Kit, immediately store each components at the temperature specified on the label.

Required Applied Biosystems reagent kits

Item (part number)†	Components	Kit components used in...
Library Builder™ Fragment Core Kit for SOLiD™ 4.0 (Part no. 4463762)	• AB Library Builder™ Fragment Reagents Module for SOLiD™ 4.0 • AB Library Builder™ Plastics Module	AB Library Builder™ System
SOLiD™ Fragment Library Oligos Kit (4401151)	SOLiD™ Library Oligos Kit 1 – P1 Adaptor (ds)	Ligation of adaptors
	SOLiD™ Library Oligos Kit 1 – P2 Adaptor (ds)	
	SOLiD™ Library Oligos Kit 1 – Library PCR Primer 1	Library amplification
	SOLiD™ Library Oligos Kit 1 – Library PCR Primer 2	
SOLiD™ Fragment Library Barcoding Kit 1–96 (4449637)	• Multiplex Library P1 Adaptor, 50 µM • Multiplex Library PCR-1, 50 µM • Multiplex Library PCR-2, 50 µM • Barcodes 001–096, 50 µM	Preparation of 96 barcoded fragment libraries

Item (part number)†	Components	Kit components used in...
SOLiD™ Fragment Library Barcoding Kit Module 1–16 (4444837)	• Multiplex Library P1 Adaptor, 50 µM • Multiplex Library PCR-1, 50 µM • Multiplex Library PCR-2, 50 µM • Barcodes 0XX, 50 µM	Preparation of up to 96 barcoded fragment libraries in increments of 16 libraries
SOLiD™ Fragment Library Barcoding Module 17–32 (4449636)		
SOLiD™ Fragment Library Barcoding Module 33–48 (4449635)		
SOLiD™ Fragment Library Barcoding Module 49–64 (4449641)		
SOLiD™ Fragment Library Barcoding Module 65–80 (4449642)		
SOLiD™ Fragment Library Barcoding Module 81–96 (4449643)		

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

Required equipment

Item†	Source
AB Library Builder™ System The system includes: • Library Builder Fragment Core Kits for SOLiD™ 4 and 5500 series Protocol Card • AB Library Builder™ Device • Tip and Tube Tray • Reagent Cartridge Rack • Barcode Reader • RS232C Cable • CommViewer Barcode Software CD-ROM • 13 empty reagent cartridges • 52 sample/elution tubes	Applied Biosystems Part no. 4463592

Item†	Source
AB Library Builder™ System with Service Installation The system includes: • Library Builder Fragment Core Kits for SOLiD™ 4 and 5500 series Protocol Card • AB Library Builder™ Device • Tip and Tube Tray • Reagent Cartridge Rack • Barcode Reader • RS232C Cable • CommViewer Barcode Software CD-ROM • 13 empty reagent cartridges • 52 sample/elution tubes	Applied Biosystems Part no. 4463794
Covaris® S220 System‡ (110 V for U.S. customers) (220 V for international customers) The Covaris® S220 System includes: • Covaris® S220 sonicator • Universal Voltage Kit • Latitude® laptop from Dell Inc. • MultiTemp III Thermostatic Circulator • Covaris®-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris®-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris®-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris®-2 Series Machine Holder for (one) microTUBE • Covaris® microTUBE Prep Station • Covaris® Water Tank Label Kit • Covaris® microTUBEs (1 pack of 25)	Applied Biosystems 4465653
Covaris® S2 System§ (110 V for U.S. customers) (220 V for international customers)	**Note:** Fragment libraries can be prepared with the Covaris® S2 System. New users should purchase the Covaris® S220 System.

Item†	Source
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
96-well GeneAmp® PCR System 9700 (thermal cycler)	• Applied Biosystems N8050200 (Base) • Applied Biosystems 4314443 (Block)‡
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
DynaMag™- 2 Magnet (magnetic rack)	Invitrogen 123-21D
Vortexer	Major Laboratory Supplier (MLS)‡‡
PicoFuge® Microcentrifuge	MLS
Pipettors, 2 µL	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.

‡ Or the Covaris® S2 System.

§ Or the Covaris® S220 System.

†† Or equivalent but validation of the equipment for library preparation is required.

‡‡ 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.

Optional equipment

Item†	Source
E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit	Invitrogen G6465
Qubit™ Quantitation Starter Kit	Invitrogen Q32860
Qubit® 2.0 Fluorometer	Invitrogen Q32866

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

Replacement parts

Product name†	Vendor
AB Library Builder™ Tips and Tip Holders	4463781
AB Library Builder™ and Tube Rack	4463776
AB Library Builder™ Cartridge Rack	4463782
AB Library Builder™ D-Ring Tool	4465603
AB Library Builder™ Barcode Reader	4465657
AB Library Builder™ Sample Tubes	4463779
AB Library Builder™ D-Rings	4465602
AB Library Builder™ Plastics Module	4465605
Agilent DNA 1000 Kit	Agilent Technologies 5067-1504

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

Required consumables

Item†	Source
1× Low TE Buffer	Applied Biosystems 4389764
Nuclease-free Water, 1 L	Applied Biosystems AM9932

Item†	Source
MicroAmp® Optical 8-Tube Strip, 0.2 mL	Applied Biosystems 4316567
Invitrogen Qubit™ dsDNA HS Assay Kit *or* Invitrogen Qubit™ dsDNA BR Assay Kit *or* Invitrogen Quant-iT™ PicoGreen® dsDNA Assay Kit	Invitrogen Q32851 or Q32854 Invitrogen Q32850 or Q32853 Invitrogen P7589
Agencourt AMPure® XP: 5 mL Kit *or* 60 mL Kit *or* 450 mL Kit	Beckman Coulter Genomics A63880 *or* A63881 *or* A63882
Covaris® microTUBEs	Covaris 520045
Ethanol, absolute	Sigma-Aldrich E7023
Ethylene glycol	American Bioanalytical AB00455-01000
50-bp ladder	Invitrogen 10416-014
0.5-mL LoBind Tubes	Eppendorf 022431005
1.5-mL LoBind Tubes	Eppendorf 022431021
Filtered pipettor tips	Major Laboratory Supplier (MLS)‡

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

‡ 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.

Optional consumables

Product name†	Vendor
50-bp ladder	Invitrogen 10416-04
SOLiD™ Library Size Selection Gel	Applied Biosystems 4443733
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit‡	Thermo Scientific PR-1
Agilent DNA 1000 Kit	Agilent Technologies 5067-1504

† 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® Conditioning Kit 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.

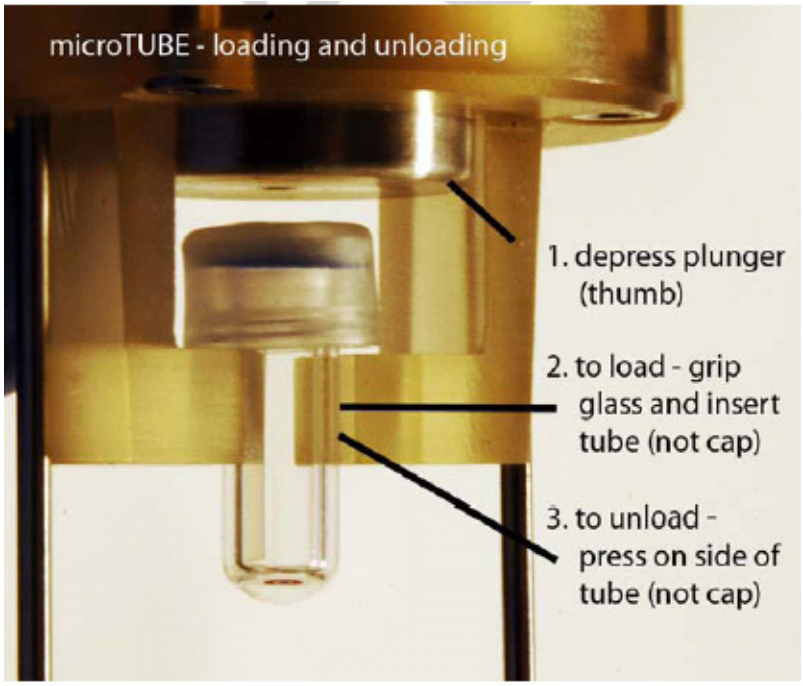
B Supplemental Procedures

This appendix covers:

Load and unload Covaris® microTUBE vials from the Covaris® microTUBE holder

Load Covaris® microTUBE vials

1. Use a thumb to push the stainless steel plunger up into the body of the microTUBE holder.
2. Place the body of the microTUBE against the two amber plastic prongs with the cap of the microTUBE positioned above the prongs.
3. Use a finger to press against the middle of the glass tube (*not* against the cap). With a single motion, push the tube between the prongs to position the tube:



IMPORTANT! Do not press against the cap to load or unload microTUBE vials, because pressing against the cap may dislodge or damage the cap.

4. Release the plunger. The plunger pushes the tube until the base of the cap rests against the prongs. The tube and holder are now ready to be inserted into the S Series instrument.

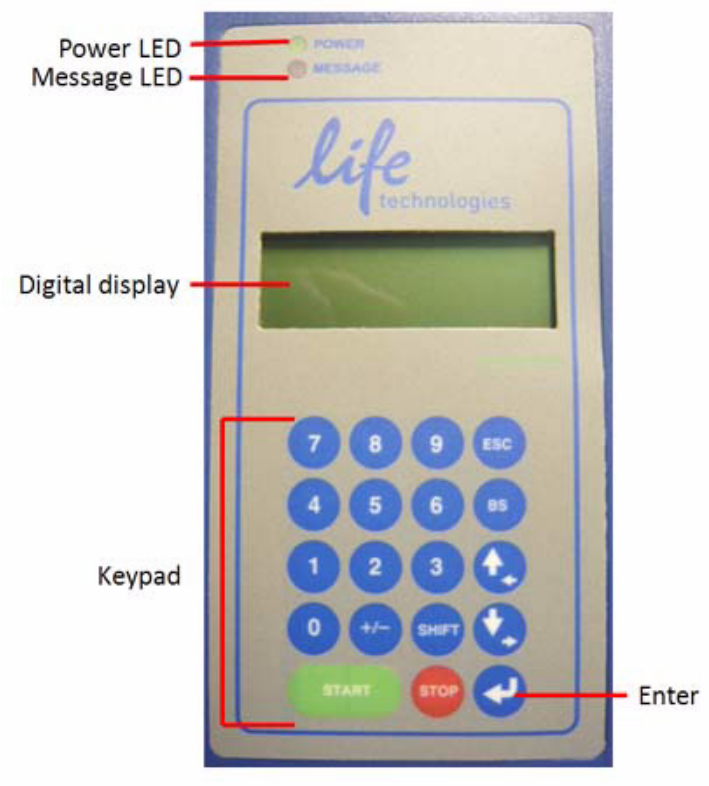
Unload Covaris® microTUBE vials

1. Use a thumb to push the stainless steel plunger up into the body of the microTUBE holder to relieve pressure on the cap.
2. Press against the side of the glass tube (*not* against the cap) to free the microTUBE from the grip of the holder.

AB Library Builder™ System operation

Parts of the front panel

The front panel provides tools for operating the instrument and tools for the service engineer to maintain the instrument:



The front panel contains:

- A digital display that shows the steps of the protocol that is in use.

  The digital display consists of 4 lines of information and menu choices.

  For the Main menu, Tests menu, and Manual menu:

  – The first line shows the current menu name
  – The second and third line show the executable commands for the current menu
  – The fourth line describes the keys to use for executing the commands

  For the protocols screen, the display provides current information on the protocol step and allows you to choose options.
- Two LEDs: Green indicates the power is ON, and blinking red indicates an error code

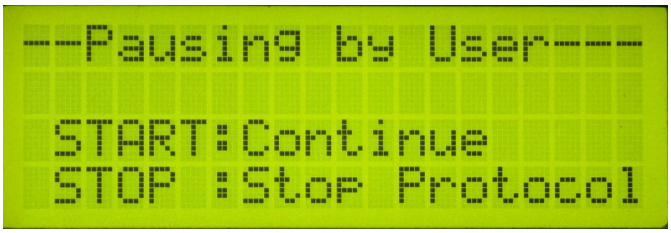
- The Keypad to enter parameters and operate the instrument:

Key	Description
0–9	To choose menu
ESC	To previous menu
START	To run or resume protocol
STOP	To stop or pause protocol
	Enter (to confirm or enter the next menu)
BS	Backspace key to delete the last digit/character
SHIFT	Shift + Up/Down arrow keys to move the cursor right or left during time/date setup

Manage the run

Pause a run

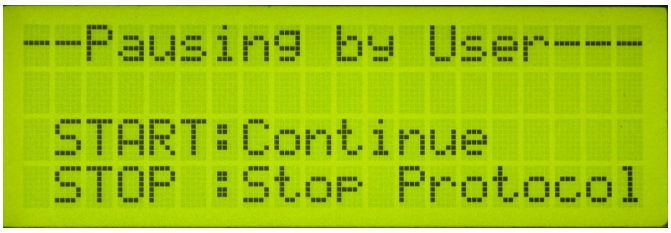
1. Press **Stop** to pause the run.
   The display shows the following:



2. To resume the run after a pause, press **Start**.
   The run continues from the last step before the pause.

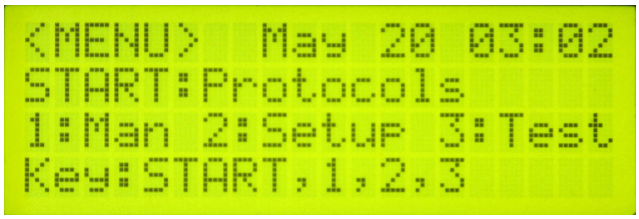
Cancel a run

1. Press **Stop** to pause the run:
   The display shows the following:

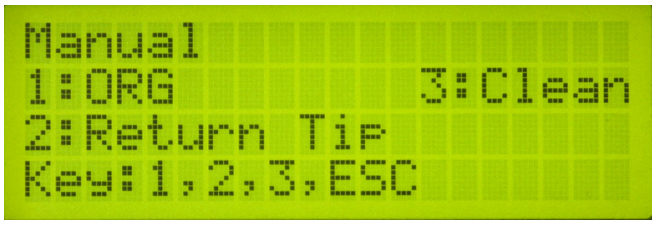


2. Press **Stop** again.

The instrument stops after the current step is completed. The screen returns to the Main menu:



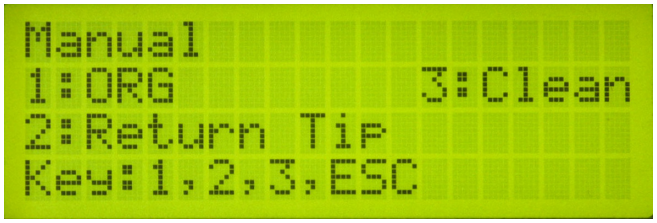
3. Press **1** to go to the Manual screen:



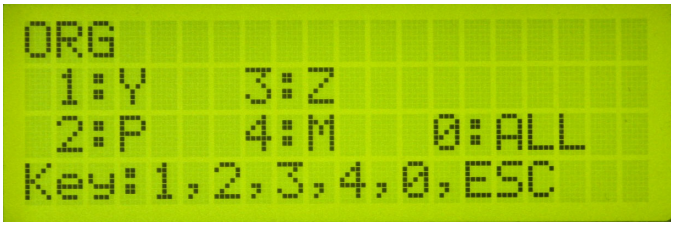
4. Move the axes to the original positions and/or return the tip to the origin as follows:

   Note: When the run is interrupted, the axes and tip do not automatically return to the original positions.

   - **If the tips need to be returned to the holders** – Press **2** (Return Tip) to return the tips to the tip holders and move all axes to the original position:

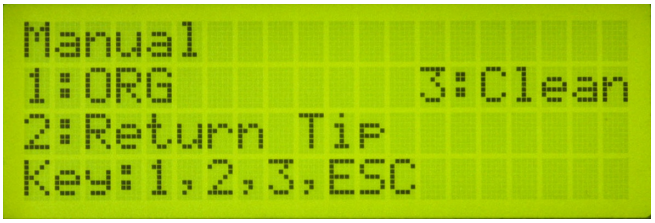


   - **If the tips do *not* need to be returned to the holders**:
     – Press **1** (ORG) to go to the ORG screen:



     – Move each individual axis to the origin by pressing **1**, **2**, **3**, **4**, respectively, or press **0** to return all axes to the origin.

5. Press **ESC** to return to Main menu:



You are now ready to set up for a new run.

(*Optional*) Size-select and pool libraries

Size-select barcoded libraries

Use a size selection gel to narrow the size distribution of barcoded libraries. The DNA is run on a SOLiD™ Library Size Selection gel. The correctly sized ligation products (~240–270 bp) are electrophoresed to the collection wells of the SOLiD™ Size Selection Gel. The eluates in each collection well are pooled.

Prepare the SOLiD Library Size Selection gel

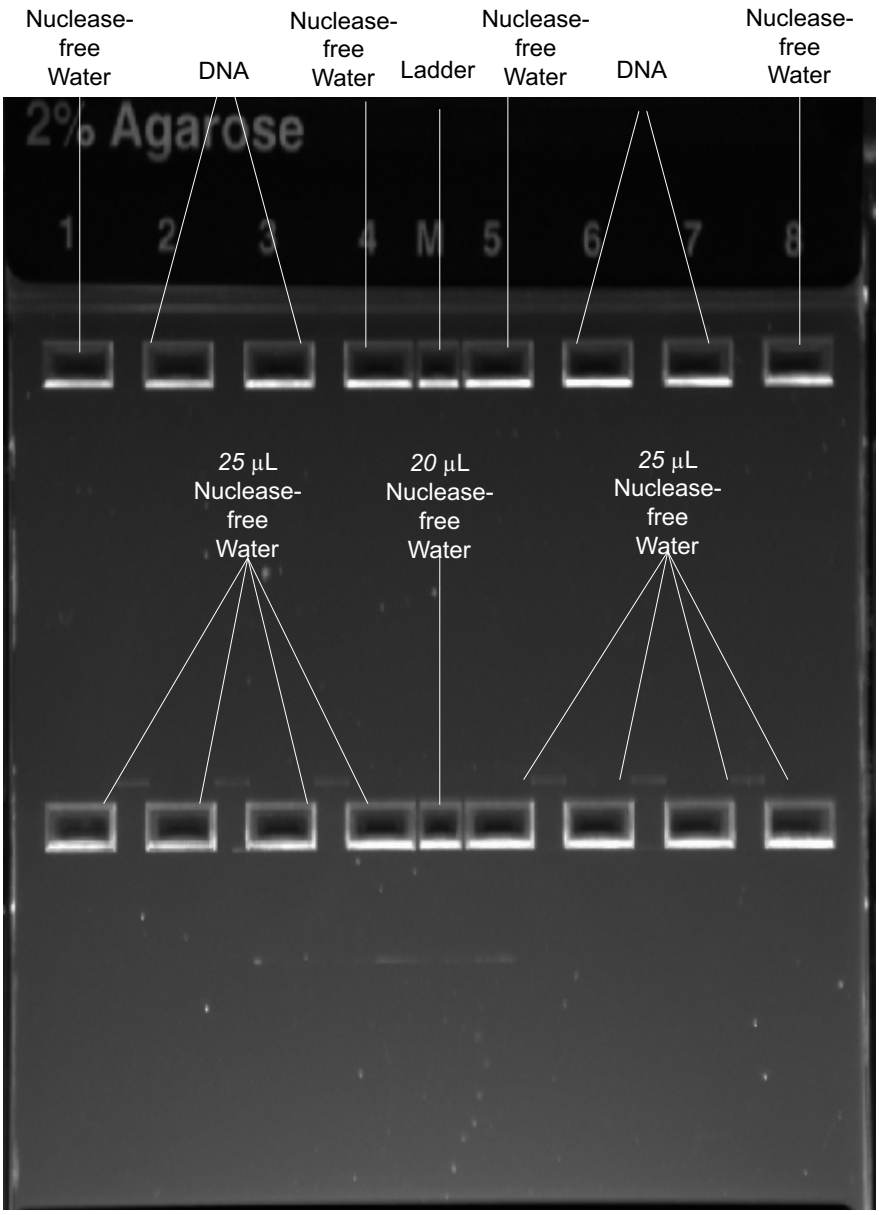
1. Remove a SOLiD™ Library Size Selection gel from its package. Remove the combs from *top* sample-loading wells and *middle* collection wells.
2. Set the SOLiD Library Size Selection Gel on the E-Gel® iBase™ system linked with the E-Gel® Safe Imager™ Real-Time Transilluminator.

Load the gel

For exact fill volumes of the wells, refer to the *Invitrogen E-Gel® SizeSelect™ Agarose Gels Quick Reference Card*.

1. Load 16 µL (≤1 µg/lane) of the (pooled) library DNA into wells 2, 3, 6 or 7 of the top *row* of wells. If the sample volume is < 20 µL, add Nuclease-free Water to the well for a total volume of 20 µL. Skip the center well (smaller well in the top center of the gel for the ladder); and skip a single well to the right and left of the center top well. Skip the two outermost wells (to avoid edge effects). Do not load more than 1 µg of DNA per lane.
2. Load 10 µL of 50-bp ladder at 0.1 µg/µL to the center top well. Add 7 µL of water to fill the well.
3. Fill the empty wells in the top row with 20 µL of Nuclease-free Water.
4. Fill each of the collection wells in the *middle* of the gel with 25 µL of Nuclease-free Water. Add 20 µL of Nuclease-free Water to the middle center well.

   The following figure shows you where to load DNA, ladder, and Nuclease-free Water on a SOLiD™ Library Size Selection gel to size-select the DNA ("*M*" is the middle well for the ladder):

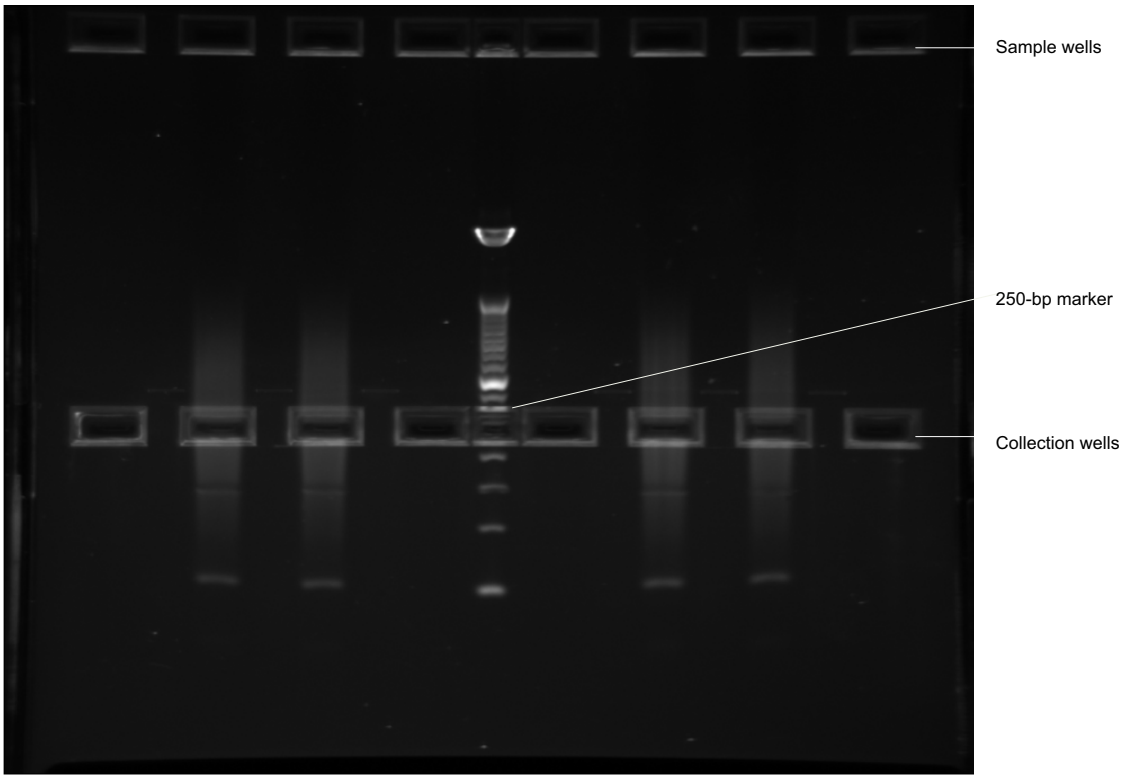


Run the SOLiD™ Library Size Selection Gel

1. Run the gel:
   - iBase™ system program: **SizeSelect 2%**
   - Run time: **14:30** (14 minutes and 30 seconds)

   Monitor the SOLiD™ Library Size Selection gel in real-time with the E-Gel® Safe Imager Real-Time Transilluminator.
2. During the run, fill the middle collection wells with additional Nuclease-free Water to ensure optimal migration of the DNA through the wells.

3. When the 250-bp band (~240–270-bp region) from the marker (ladder) lane is at the top of the collection well, stop the run if the run has not already stopped:



Note: After amplification, the total size of the product is ~240–270 bp, and the estimated insert size after size selection is ~150–180 bp.

Collect the sample from the SOLiD™ Library Size Selection Gel

1. Collect the solution from the wells and pool according to samples.
2. Wash each collection well with 25 µL with Nuclease-free Water, then retrieve the wash solution with the solution collected in Step 6.
3. (*Optional*) Concentrate the DNA with a SOLiD™ Library purification column.

Pool remaining libraries that will be combined into a single emulsion

1. Quantitate the libraries to be pooled by qPCR (see "Quantitate the DNA" on page 34).
2. Mix together *equal molar* amounts of each barcoded library in a single 1.5-mL LoBind Tube. Vortex the tube.

STOPPING POINT Store the purified DNA in Elution Buffer (E1) at – 20°C, or proceed directly to emulsion PCR, as describe in the *SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (Part no. 4441486).

Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer

The Thermo Scientific NanoDrop® 1000 Spectrophotometer measures nucleic acid samples from 2 ng/µL–3700 ng/µL without dilution.

Materials and equipment required

Required equipment	
Item†	**Source**
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Pipettors (20 µL)	Major Laboratory Supplier (MLS)‡

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

‡ 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	
Item†	**Source**
Nuclease-free Water (1 L)	Applied Biosystems AM9932
CF-1 Calibration Fluid Kit‡	Thermo Scientific CF-1
PR Conditioning Kit	Thermo Scientific PR-1
Filtered pipettor tips	Major Laboratory Supplier (MLS)

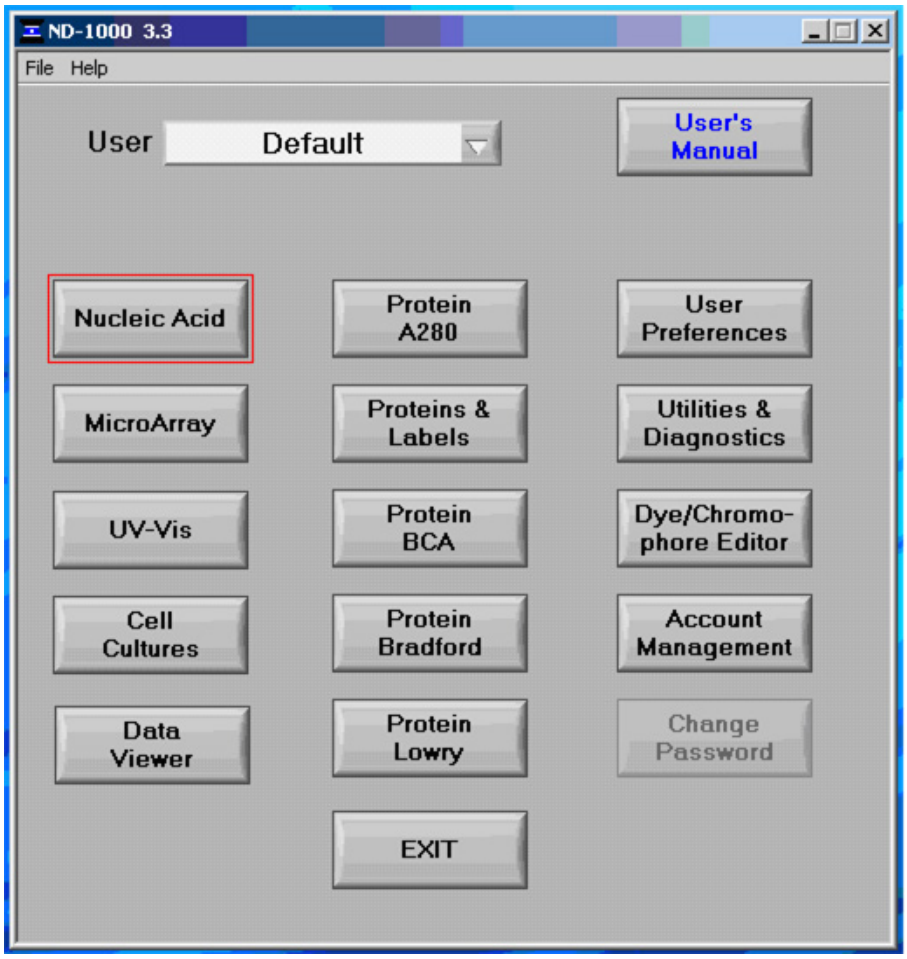
† Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.

‡ The NanoDrop® Conditioning Kit is useful for "reconditioning" the sample measurement pedestals to a hydrophobic state if they become "unconditioned." (Refer to the NanoDrop® Conditioning Kit 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.

Procedure

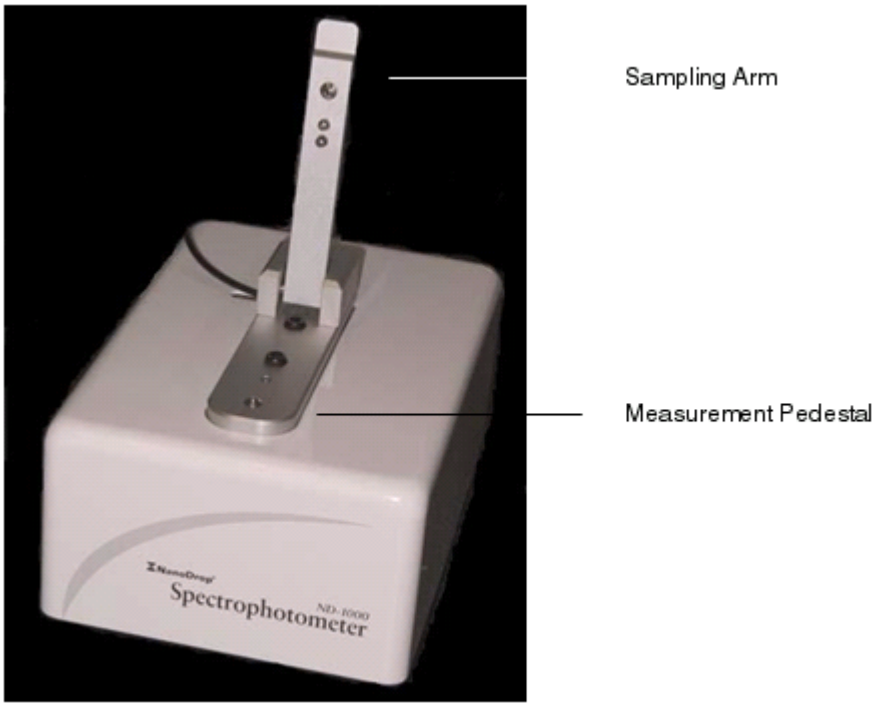
1. Ensure that the NanoDrop® ND-1000 Spectrophotometer is properly calibrated. Use the CF-1 Calibration Fluid Kit if necessary.

2. Open the NanoDrop® ND-1000 Spectrophotometer software to display a dialog box:

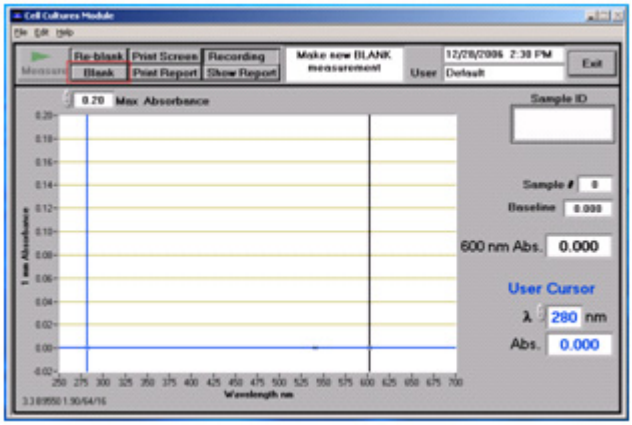


3. Select the **Nucleic Acid** button.

4. Lift the sampling arm and load 2 µL of Nuclease-free Water onto the lower measurement pedestal and lower the sampling arm:



5. In the dialog box, click **OK** and allow the instrument to initialize.
6. Lift the sampling arm and use Kimwipes® to remove water from the measurement pedestal and the sampling arm.
7. Load 2 µL of the same buffer that was used to resuspend or elute the DNA onto the measurement pedestal and lower the sampling arm.
8. Click **Blank** and allow the instrument to take a measurement:



9. Lift the sampling arm and wipe away the buffer from both the upper and lower measurement pedestals with Kimwipes®. The instrument is now ready to take readings.
10. Load 2 µL of DNA sample onto the lower measurement pedestal and lower the sampling arm.

C Supplemental Background Information

This appendix covers:

Why prepare fragment libraries?

Features

- Appropriate for sequence lengths ≤300 bp.
- Adaptors on each end of sheared DNA insert.
- Multiplexed sequencing, if the libraries are barcoded.
- The protocol is designed for 10 ng–5 µg of genomic DNA.
- Compared to mate-paired libraries, fragment libraries yield a higher recovery of unique molecules, when normalized to the same input amount.

Applications

- Targeted resequencing, primary library
- Genomic resequencing
- Methylation analysis

Complexity

The amount of library used depends on the application and information needed. For deeper coverage of large and complex genomes (for example, human genomes), more DNA is required to prepare libraries. For smaller and less complex genomes (for example, microbial genomes), less DNA can be used. For information about specific applications, go to the 5500 Series SOLiD™ Sequencers website:

www.appliedbiosystems.com/solid5500

Or, contact your field applications specialist.

Preparing standard fragment libraries

Preparing standard fragment libraries involves shearing DNA into small fragments and ligating P1 and P2 Adaptors (see Figure 1 and Figure 2).

Figure 1 Basic fragment library preparation workflow overview.

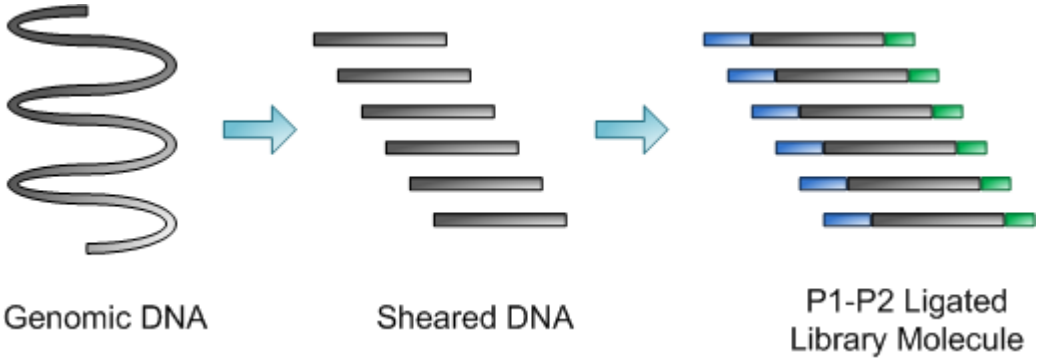
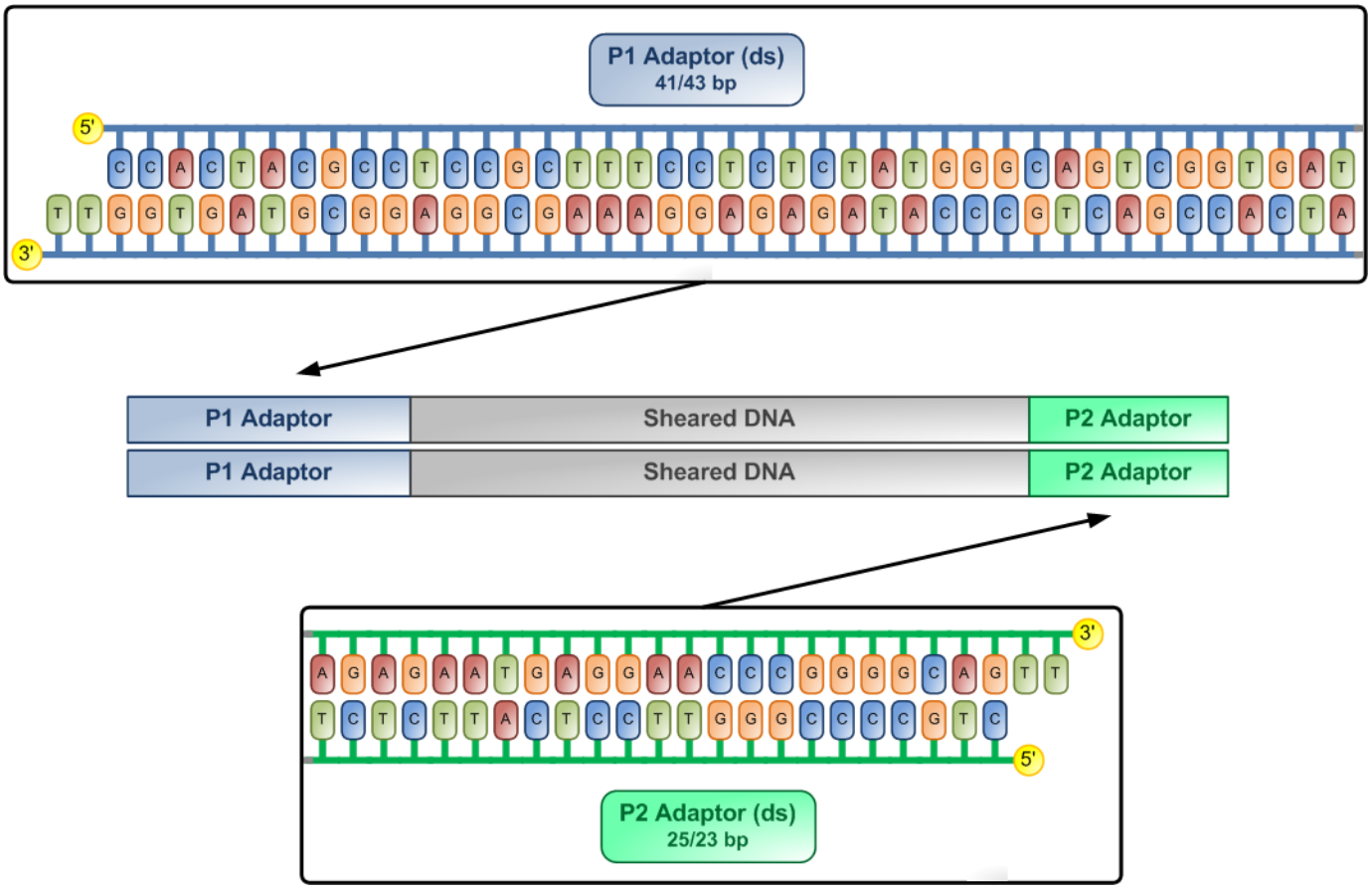
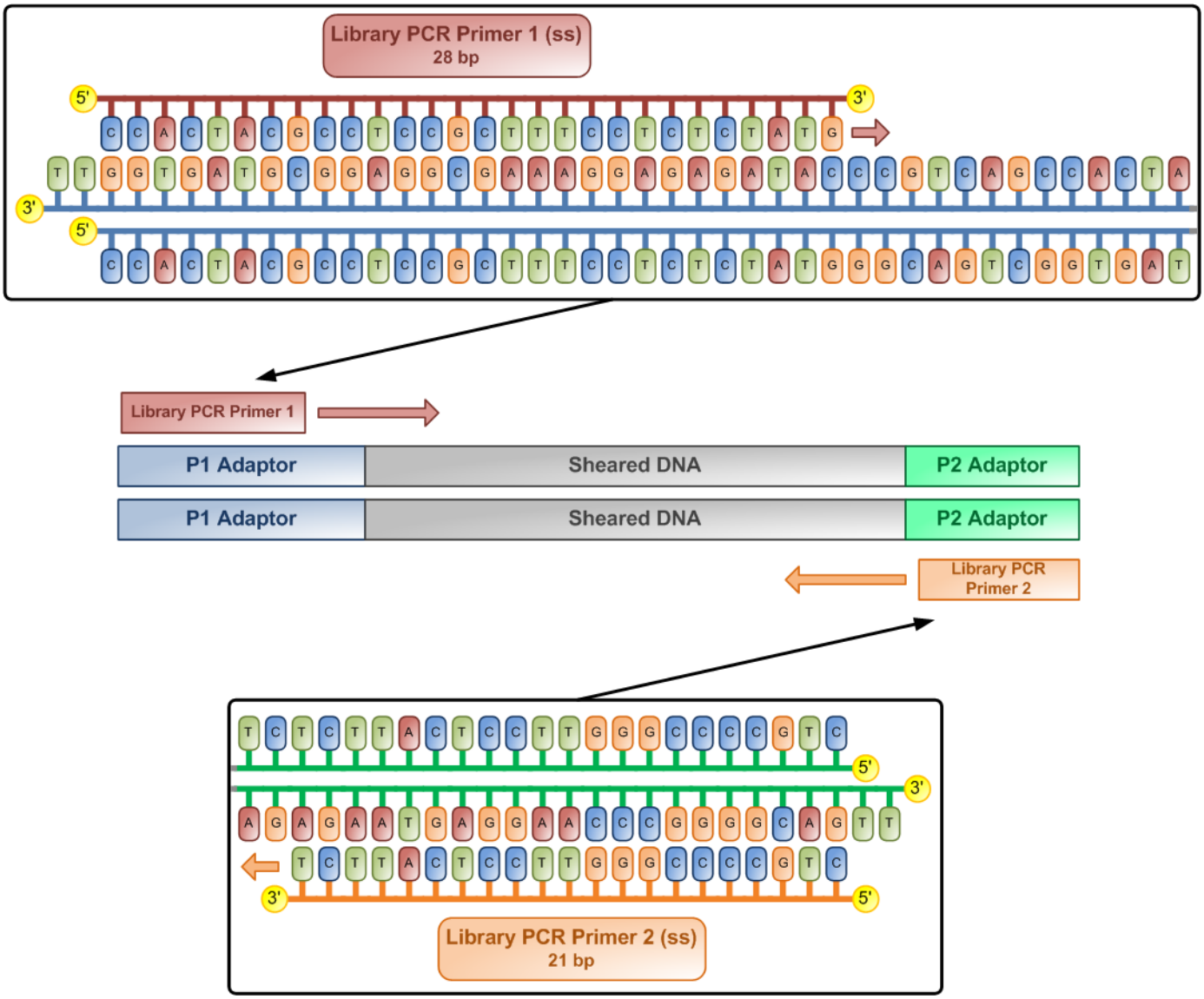


Figure 2 Fragment library design.



After P1 and P2 Adaptors are ligated to the sheared DNA, the library is amplified using primers specific to the P1 and P2 Adaptors (see Figure 3). Library PCR Primer 1 is a 3′ -truncated version of the 5′ -strand sequence of P1, while Library PCR Primer 2 is a 3′ -truncated version of the 5′ -strand sequence of P2. These primers can be used only for library amplification and not for alternative or modified library construction adaptor design, as they do not have 3′ sequences compatible with the sequencing primers.

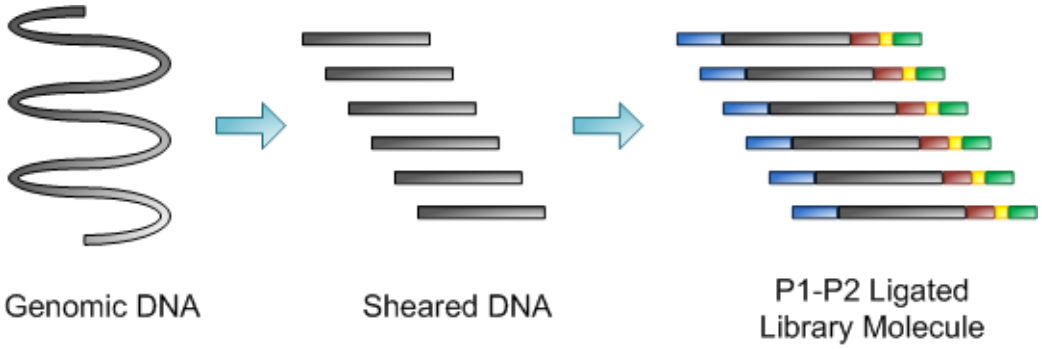
Figure 3 Fragment library amplification design.



Preparing barcoded libraries

Preparing barcoded libraries generates a fragment library (150 to 180 bp, before adaptor ligation), tagged with a unique sequence identifier, or barcode, to enable multiplexed sequencing analysis. This method involves shearing DNA into small fragments and ligating Multiplex P1 and Multiplex P2 Adaptors specific for barcoded library preparation (see Figure 4).

Figure 4 Basic barcoded fragment library preparation workflow overview.

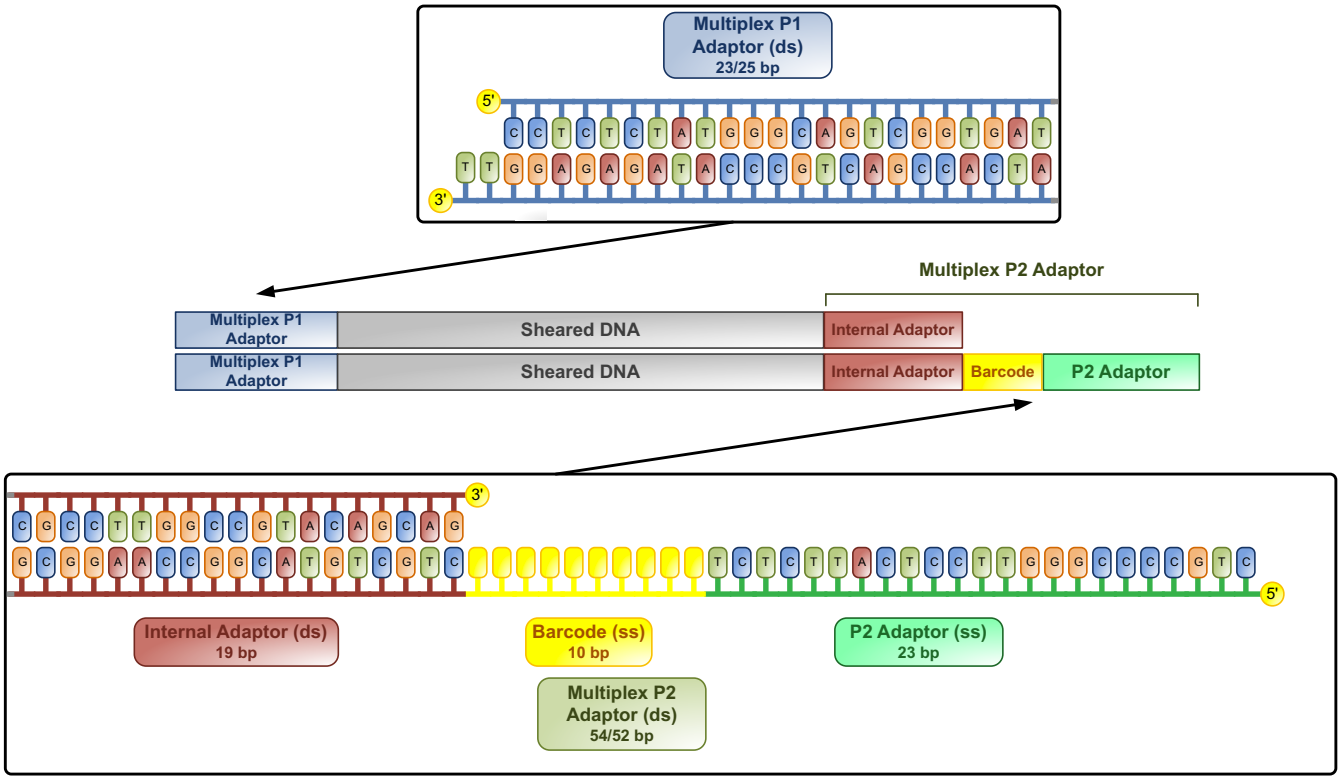


The Multiplex P2 Adaptor consists of 3 segments of sequence:

- Internal adaptor sequence, which is necessary for sequencing the barcode
- Barcode sequence
- P2 adaptor sequence, which is used for library amplification and emulsion PCR

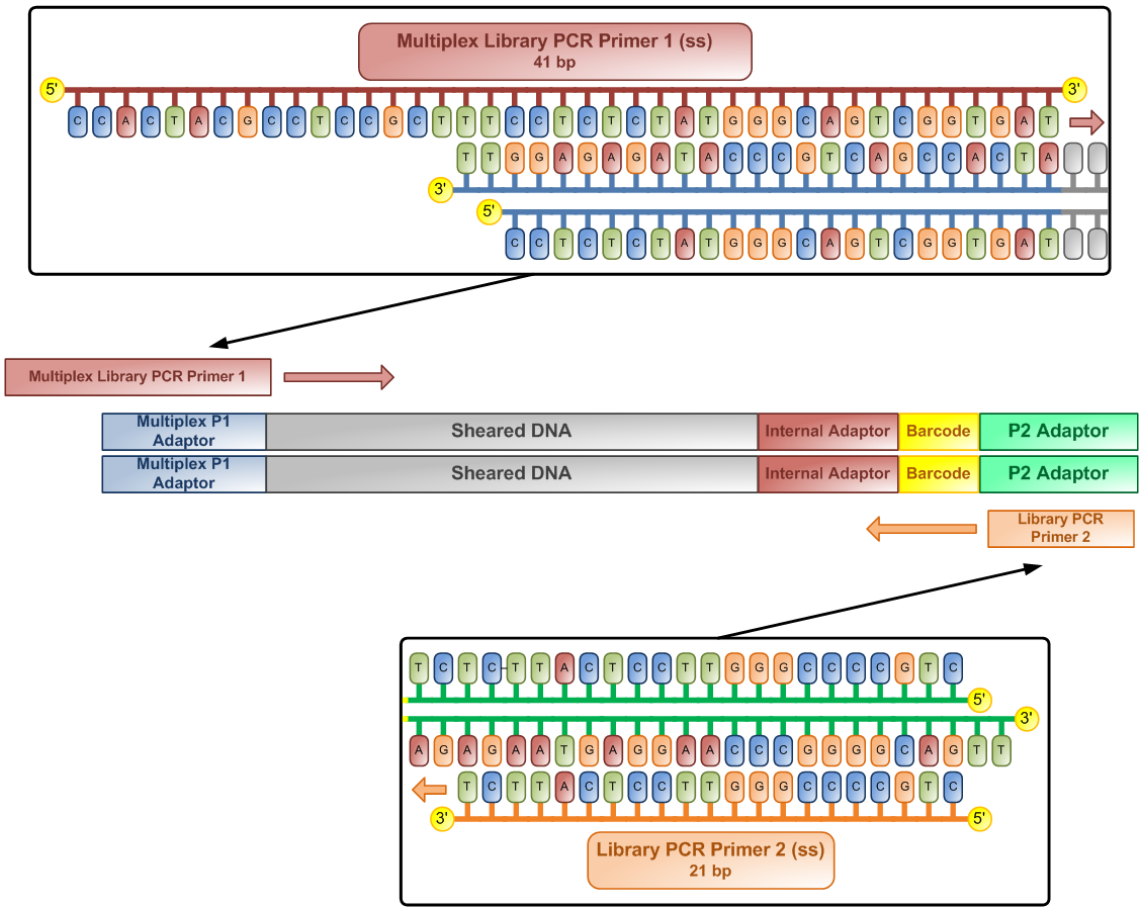
The Multiplex P1 Adaptor is a truncated version of the standard P1 Adaptor. The Multiplex P1 Adaptor is shorter to make up for the increased length of the Multiplex P2 Adaptor. Different libraries to be multiplexed in the same sequencing run are ligated to Multiplex P2 Adaptors with different barcode sequences. Ninety-six barcode sequences are available to tag different libraries (see Figure 5 on page 65).

Figure 5 Barcoded fragment library design.



After Multiplex P1 and Multiplex P2 Adaptors are ligated to the sheared DNA, the library is amplified using primers specific to the Multiplex P1 and Multiplex P2 Adaptors (see Figure 6 on page 66). Multiplex Library PCR Primer 1 is a 3′ -extended version of the 5′ -strand sequence of Multiplex P1 that adds back the truncated part of the standard P1 sequence, while Library PCR Primer 2 is a 3′ -truncated version of the 5′ -strand sequence of standard P2. Amplification with Multiplex Library PCR Primer 1 adds back the P1 sequence that was truncated in the Multiplex P1 Adaptor. These primers can be used only for library amplification and not for alternative or modified library construction adaptor design, because they do not have 3′ sequences compatible with the sequencing primers.

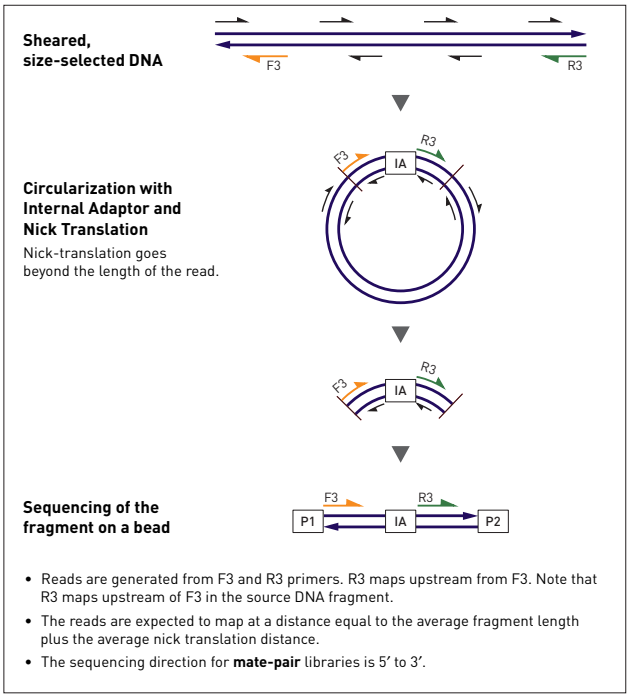
Figure 6 Barcoded fragment library amplification design.



For RNA applications, an alternative method to generate barcoded libraries is described in the protocols for the SOLiD™ RNA Barcode Module 1-16 (PN 4427046), SOLiD™ RNA Barcode Module 17-32 (PN 4453189), and SOLiD™ RNA Barcode Module 33-48 (PN 4453191).

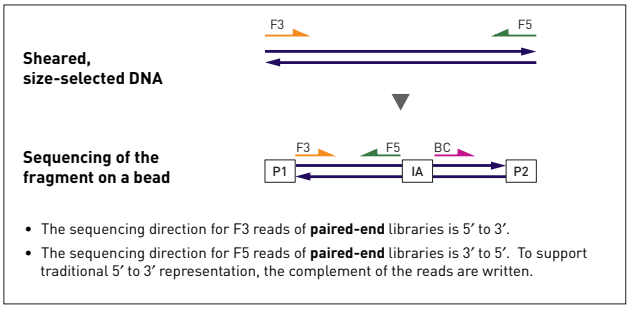
Sequence orientation from source DNA to sequence map

Mate-pair



- Reads are generated from F3 and R3 primers. R3 maps upstream from F3. Note that R3 maps upstream of F3 in the source DNA fragment.
- The reads are expected to map at a distance equal to the average fragment length plus the average nick translation distance.
- The sequencing direction for **mate-pair** libraries is 5′ to 3′.

Paired-end



- The sequencing direction for F3 reads of **paired-end** libraries is 5′ to 3′.
- The sequencing direction for F5 reads of **paired-end** libraries is 3′ to 5′. To support traditional 5′ to 3′ representation, the complement of the reads are written.

For more information on sequencing tags, refer to *5500 Series SOLiD™ Sequencers User Guide* (Part no. 4456991).

D Library Construction Oligonucleotide Sequences

PCR Primer and adaptor sequences

Note: The internal adaptor used for DNA fragment libraries is different from the internal adaptor used for RNA libraries.

Note: The "~" is a phosphorothioate bond, which protects a sequence from nucleases.

Adaptor sequence	Length (nt)
P1 Adaptor, 50 µM	
5′ -CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT-3′	41
5′ -ATCACCGACTGCCCATAGAGAGGAAAGCGGAGGCGTAGTGGTT-3′	43
P2 Adaptor, 50 µM	
5′ -AGAGAATGAGGAACCCGGGGCAGTT-3′	25
5′ -CTGCCCCGGGTTCCTCATTCTCT-3′	23
Library PCR Primer 1, 50 µM	
5′ -CCACTACGCCTCCGCTTTCCTCTCTATG-3′	28
Library PCR Primer 2, 50 µM	
5′ -CTGCCCCGGGTTCCTCATTCT-3′	21

Multiplex adaptor and barcoded sequences

Barcoded adaptor sequence	Length (nt)
Multiplex Library P1 Adaptor, 50 µM	
5′ -ATCACCGACTGCCCATAGAGAGGTT-3′	25
5′ -CCTCTCTATGGGCAGTCGGTGAT-3′	23
Multiplex Library PCR-1, 50 µM	
5′ -CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT-3′	41
Multiplex Library PCR-2, 50 µM	
5′ -CTGCCCCGGGTTCCTCATTCT-3′	21
Barcode-001, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGTAAGAGG**CTGCTGTACGGCCAAGGCG-3′	52

Barcoded adaptor sequence	Length (nt)
Barcode-002, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGGAGTGGT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-003, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATAGGTTATA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-004, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGATGCGGTC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-005, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGGTGTAAG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-006, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGAGGGACA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-007, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGTTATGCC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-008, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGCGAGGAT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-009, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGTTGCGAC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-010, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGTAAGCT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-011, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCGACACG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-012, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGAGGAAAA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-013, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGTAAGGC**CTGCTGTACGGCCAAGGCG-3′	52

Barcoded adaptor sequence	Length (nt)
Barcode-014, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCGGCAGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-015, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGTTGAATG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-016, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGAGACGTT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-017, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGCTCACCGC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-018, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGCGGATGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-019, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATGGTAACTG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-020, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTCAAGCTTT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-021, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCGGTTCC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-022, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGAAGATGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-023, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGTGCTTG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-024, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGTCGGTAT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-025, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AACATGATGA**CTGCTGTACGGCCAAGGCG-3′	52

Barcoded adaptor sequence	Length (nt)
Barcode-026, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CGGGAGCCCG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-027, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CAGCAAACTT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-028, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGCTTACTAC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-029, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAATCTAGGG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-030, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTAGCGAAGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-031, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCTGGTGCGT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-032, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGTTGGGTG**CCTGCTGTACGGCCAAGGCG-3′	52
Barcode-033, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CGTTGGATAC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-034, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**TCGTTAAAGG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-035, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGCGTAGGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-036, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTTCTCACAT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-037, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CTGTTATACC**CTGCTGTACGGCCAAGGCG-3′	52

Barcoded adaptor sequence	Length (nt)
Barcode-038, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTCGTCTTAG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-039, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**TATCGTGAGT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-040, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAAAGGGTTA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-041, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**TGTGGGATTG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-042, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAATGTACTA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-043, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CGCTAGGGTT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-044, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGGATGATC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-045, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTACTTGGCTC**TGCTGTACGGCCAAGGCG-3′	52
Barcode-046, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGTCGTCGAA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-047, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGGGATGG**CCTGCTGTACGGCCAAGGCG-3′	52
Barcode-048, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCCGTAAGTG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-049, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATGTCATAAG**CTGCTGTACGGCCAAGGCG-3′	52

Barcoded adaptor sequence	Length (nt)
Barcode-050, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAAGGCTTGC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-051, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGCAGGAGT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-052, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTAATTGTAA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-053, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTCATCAAGT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-054, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAAAGGCGGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-055, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGCTTAAGCG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-056, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCATGTCACC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-057, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CTAGTAAGAA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-058, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**TAAAGTGGCG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-059, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGTAATGTC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-060, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCCTCGGT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-061, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGATTATCG**CTGCTGTACGGCCAAGGCG-3′	52

Barcoded adaptor sequence	Length (nt)
Barcode-062, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGTGAGGGT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-063, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGGTTCGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-064, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCTACACC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-065, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGATCAAGC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-066, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GATGTAATGT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-067, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTCCTTAGGG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-068, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCATTGACGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-069, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GATATGCTTT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-070, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCCCTACAGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-071, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ACAGGGAACG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-072, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGTGAATA**CCTGCTGTACGGCCAAGGCG-3′	52
Barcode-073, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCAATGACGT**CTGCTGTACGGCCAAGGCG-3′	52

Barcoded adaptor sequence	Length (nt)
Barcode-074, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGACGCTGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-075, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTATCTGGGC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-076, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGTTTTAGG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-077, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATCTGGTCTT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-078, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGCAATCATC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-079, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGTAGAATTA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-080, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTTTACGGTG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-081, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAACGTCATT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-082, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGAAGGGAG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-083, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGATGGCGTA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-084, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGATGAAC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-085, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGAAAGCGTT**CTGCTGTACGGCCAAGGCG-3′	52

Barcoded adaptor sequence	Length (nt)
Barcode-086, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGTACCAGGA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-087, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATAGCAAAGC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-088, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTTGATCATG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-089, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGCTGTCTA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-090, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGACCTACT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-091, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGTATTGGG**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-092, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGGGATTAC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-093, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTTACGATGC**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-094, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATGGGTGTTT**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-095, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGTCCGGCA**CTGCTGTACGGCCAAGGCG-3′	52
Barcode-096, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AATCGAAGAG**CTGCTGTACGGCCAAGGCG-3′	52

E Checklist and workflow tracking form

This appendix covers:

Workflow checklists: prepare a standard or express fragment library with the AB Library Builder™ System Kit

Note: The checklist includes only equipment and reagents needed to prepare libraries and excludes the usual and necessary standard laboratory equipment, such as pipettes, filtered pipette tips, tubes, vortexers, microcentrifuges, and nuclease-free water.

	Equipment	Reagents	Preparation steps
Shear the DNA	☐ Covaris® S2 or S220 System ☐ Covaris® microTube adaptor ☐ Covaris® microTube loading station ☐ Covaris® microTube	☐ 1× Low TE Buffer ☐ Shearing Buffer ☐ Ethylene glycol	☐ Degas the water in the Covaris® S2 or S220 System for 30 minutes prior to use. ☐ Supplement the circulated water chiller with 20% ethylene glycol. ☐ Thaw Shear Buffer at room temperature.
Set up the AB Library Builder System™	☐ Protocol Card ☐ Cartridge Rack ☐ Tip and Tube Rack ☐ AB Library Builder™ System	☐ Library Builder™ Fragment Core Kit for SOLiD™ 4.0 ☐ SOLiD™ Fragment Library Oligos Kit *or* ☐ SOLiD™ Fragment Library Barcoding Kit 1–96 ☐ Agencourt AMPure® Reagent Kit ☐ 1x Low TE	☐ Thaw cartridges completely. ☐ Add Agencourt AMPure® XP Reagent to cartridges. ☐ Add library adaptors to Ligation Buffer Tubes. ☐ Load all tubes, tips, and cartridges into the AB Library Builder™ Device.
Nick-translate, then amplify the library	☐ Thermal cycler ☐ PCR strip tubes	☐ AB Library Builder™ Amplification Reagents ☐ Agencourt AMPure™ XP Reagent	☐ Thaw Library PCR Primers 1 and 2 on ice. ☐ Thaw Platinum® PCR Amplification Mix on ice.
Quantitate	☐ Real-time PCR system	☐ SOLiD™ Library TaqMan® Quantitation Kit ☐ qPCR plate and optical covers	—
(*Optional*) Pool equimolar libraries of similar size	—	—	—
(*Optional*) Gel-purify the libraries	☐ iBase™ System ☐ E-gel® Safe Imager™ instrument	☐ E-Gel® 2% SizeSelect™ gel ☐ 50 bp DNA Ladder ☐ Nuclease-free Water	☐ Thaw 50-bp DNA Ladder on ice.
(*Optional*) Pool remaining libraries to be combined into a single	—	—	—

Workflow tracking: prepare a standard or express fragment library with the AB Library Builder™ System Kit

Sample:	**Barcode:**

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Quantitative PCR	

Lot number	
Step	**Lot number**
Adaptors and primers used:	

Sample:	**Barcode:**

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Quantitative PCR	

Lot number	
Step	**Lot number**
Adaptors and primers used:	

Sample:	**Barcode:**

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Quantitative PCR	

Lot number	
Step	**Lot number**
Adaptors and primers used:	

Sample:	**Barcode:**

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Quantitative PCR	

Lot number	
Step	**Lot number**
Adaptors and primers used:	

F Safety

This appendix covers:

Note: For instrument safety and biohazard guidelines, refer to the "Safety" section in the *AB Library Builder™ System User Guide* (Part no. 4463421).

General chemical safety

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 84.)
- 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

WARNING! Do not add acids, or bases (such as bleach), to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.

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/biosafety/publications/index.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

Documentation and Support

Related documentation

Document	Part number	Description
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 Guide	4448379	Describes how to load and run the SOLiD™ 4 System for sequencing.
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 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.

Document	Part number	Description
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 compute-intensive NGS data processing.
Applied Biosystems SOLiD™ 4 System Software Integrated Workflow Quick Reference Guide	4448432	Describes the relationship between the softwares comprising the SOLiD 4 platform and provides quick step procedures on operating each software to perform data analysis.
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 orderable consumables.

Note: For additional documentation, see "Obtaining support".

Obtaining support

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

www.appliedbiosystems.com

At the Applied Biosystems website, 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.

Glossary

barcode	A short, unique sequence that is incorporated into a library that enables identification of the library during multiplex sequencing.
barcoded library	A library that has a unique barcode sequence incorporated that enables identification of the library during multiplex sequencing.
fragment library	A library that has a single insert prepared from genomic DNA for sequencing on the SOLiD™ System. Fragment libraries compatible with the 5500 Series SOLiD™ Sequencers can be sequenced with a forward-only run or with a paired-end run.
internal adaptor (IA)	The internal adaptor sequence is incorporated into the template during library construction and provides a common hybridization target for SOLiD™ sequencing primers. The IA sequence is different in DNA-source libraries and RNA-source libraries, therefore sequencing primers specific for RNA and DNA libraries must be used for reverse reads (F5 tag). The IA-containing adaptors used during mate-paired library preparation are different from the adaptors used for fragment library preparation, but the sequencing primers used for forward reads originating in the IA sequence (R3 and BC tags) are the same. See the *5500 Series SOLiD™ Systems Sequencing Products Ordering Guide* for a schematic of sequencing primers compatible with each type of SOLiD™ library.
library	A set of DNA or cDNA molecules prepared from the same biological specimen and prepared for sequencing on the SOLiD™ System.
Library PCR Primer 1	Single-stranded oligonucleotide used in library amplification and corresponding to the P1 Adaptor sequence
Library PCR Primer 2	Single-stranded oligonucleotide used in library amplification and corresponding to the P2 Adaptor sequence
mate-paired library	Library consisting of two DNA segments that reside a known distance apart in the genome, linked by an internal adaptor, and with P1 and P2 Adaptors ligated to the 5' and 3' ends of the template strand, respectively.
Multiplex Library PCR Primer 1	Single-stranded oligonucleotide used in barcoded fragment library amplification and corresponding to the Multiplex P1 Adaptor sequence
Multiplex P1 Adaptor	Double-stranded oligonucleotide ligated at the 5′ end of the barcoded fragment library
Multiplex P2 Adaptor	Double-stranded oligonucleotide ligated at the 3′ end of the barcoded fragment library; contains the barcode sequence

multiplex sequencing	Sequencing runs in which multiple barcoded libraries are simultaneously sequenced in a single flowchip lane. Each bead is assigned to the correct library after the sequencing run according to the sequence of its barcode.
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
tag	There are two uses for this term. • A length of DNA or cDNA to be sequenced; especially, a relatively short stretch of DNA or cDNA that is used to infer information about the longer native molecule from which it is derived, such as in SAGE™ analysis and mate-pair library sequencing. • Sequencing data from a single bead with a single primer set; sometimes used interchangeably with *read*.
templated bead preparation	Process of covalently attaching and clonally amplifying template strands 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

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