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# SOLiD™ Fragment Library Barcoding Kit Module 1–16

## Protocol

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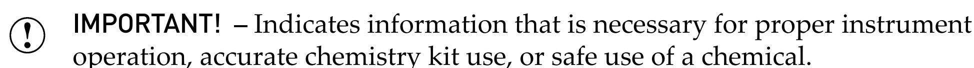
# About This Guide

## Safety information

**Note:** For general safety information, see this section and Appendix B, "Safety" on page 31. 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

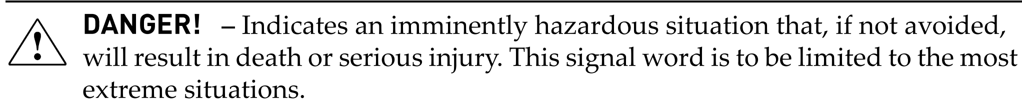
Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:



**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.

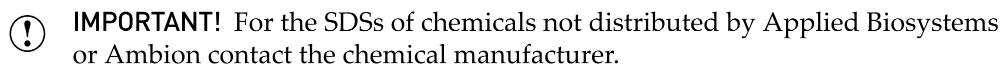
**WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.



**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.

### 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 33.



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

# Prepare a barcoded fragment library with the SOLiD™ Fragment Library Barcoding Kit Module 1–16

## Product information

### Purpose of the product

Use the Applied Biosystems SOLiD™ Fragment Library Barcoding Kit Module 1–16 (PN 4444836) to generate a fragment library (150 to 180 bp, before adaptor ligation) that is tagged with a unique sequence identifier, or *barcode*. The barcode tag enables multiplexed sequencing analysis using the barcode sequences derived from the Barcoding Kit Module 1–16.

The fragmented double-stranded DNA is ligated with a truncated Multiplex P1 Adaptor and a Multiplex P2 Adaptor with a barcode. The Multiplex P2 Adaptor consists of three segments: (1) an internal adaptor sequence (derived from the sequence used for mate-paired libraries); (2) a barcode decamer sequence; and (3) a standard P2 adaptor sequence. Because the Multiplex P2 Adaptor is longer than the standard P2 adaptor, the Multiplex P1 Adaptor is truncated to keep the total length of the adaptors approximately the same as for a non-barcoded library.

This protocol is designed for 500 ng to 5 µg of genomic DNA or ligated PCR product. If the starting amount of genomic DNA is outside the range of 500 ng to 5 µg, you should modify the protocol. For technical assistance, contact your local field application specialist.

If you are trying to construct a targeted, resequencing library with small-sized PCR products (≤500 bp), then you must first perform a PCR-product ligation step. For a concatenation protocol, contact your field application specialist.

### Module contents

Sufficient reagents are supplied in the SOLiD™ Fragment Library Barcoding Kit Module 1–16 to prepare a minimum 16 barcoded fragment libraries for high-throughput sequencing with the SOLiD™ System.

**Table 1** SOLiD™ Fragment Library Barcoding Kit Module 1–16 components

| Component | Amount (µL) |
|---|---|
| Multiplex Library P1 Adaptor, 50 µM | 1120 |
| Multiplex Library PCR-1, 50 µM | 1280 |
| Multiplex Library PCR-2, 50 µM | 1280 |
| Barcodes 001–016, 50 µM | 70 each |

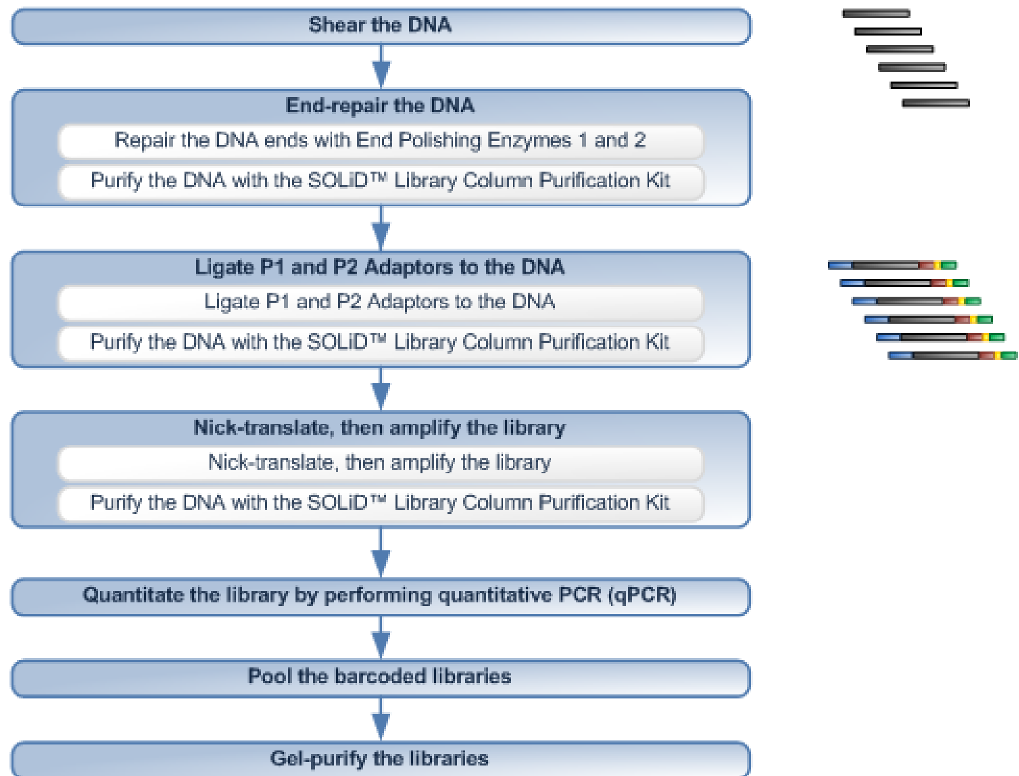
## Storage

Upon receipt of the SOLiD™ Fragment Library Barcoding Kit Module 1–16, immediately store the components at –20 °C.

## Materials and equipment required

The SOLiD™ Fragment Library Barcoding Kit Module 1-16 should be used together with a SOLiD™ Fragment Library Construction Kit. See Appendix A, “Ordering Information” on page 23 for a list of equipment, kits, and consumables necessary for this procedure.

# Workflow




### Shear the DNA

This step involves sonicating the input DNA into small fragments with a mean fragment size of 165 bp and a fragment size range of 150 to 180 bp (before adaptor ligation) using the Covaris™ S2 System. The conditions have been tested for shearing 500 ng to 5 µg DNA in a total volume of 100 µL. For certain DNA samples, optimizing the shearing protocol may be necessary.

### End-repair the DNA

End Polishing Enzyme 1 and End Polishing Enzyme 2 are used to convert DNA that has damaged or incompatible 5′ -protruding and/or 3′ -protruding ends to 5′ -phosphorylated, blunt-ended DNA. The conversion to blunt-ended DNA results from 5′ -to-3′ polymerase and the 3′ -to-5′ exonuclease activities of End Polishing Enzyme 2. End Polishing Enzyme 1 and optimized buffer are also included for phosphorylation of the 5′ -ends of the blunt-ended DNA to allow for subsequent ligation.

### Purify the DNA with the SOLiD™ Library Column Purification Kit

Sample purification is recommended with the PureLink™ columns supplied in the SOLiD™ Library Column Purification Kit. PureLink™ columns have a 40-µg capacity, but it may be necessary to use multiple columns during a purification step for higher yields.

### Ligate P1 and P2 Adaptors to the DNA

Multiplex P1 and P2 Adaptors are ligated to the ends of the end-repaired DNA. You can design experiments to use as few as 4 barcodes for color balance, as long as at least one of the following full sets of four barcodes are used: Barcodes 1–4, 5–8, 9–12, or 13–16.

### Nick-translate, then amplify the library

The adaptor-ligated, purified DNA undergoes nick translation, then amplification using Multiplex Library PCR-1, Multiplex Library PCR-2, and Platinum® PCR Amplification Mix. After amplification, the PCR samples are purified with the SOLiD Library Column Purification Kit. Before column purification of the nick-translated libraries, you can pool equivalent amounts of barcoded libraries of similar size, or you can purify each barcoded library separately.

### Quantitate the library by performing quantitative PCR (qPCR)

Quantitate the library by using the SOLiD™ TaqMan® Quantitation Kit. For more information on quantitation by TaqMan® or SYBR® assays, refer to the *Applied Biosystems SOLiD™ 4 System Library Preparation Guide*, "SOLiD™ 4 System Library Quantitation" (PN 4445673) [or to the *Applied Biosystems SOLiD™ 3 Plus System Library Preparation Guide*, "SOLiD™ 3 Plus System Library Quantitation" (PN 4442697)].

### Pool the barcoded libraries

**Note:** If libraries are *not* of similar size but will be gel-purified, then gel-purify the libraries first before pooling the libraries (see "Gel-purify the libraries").

Equal molar amounts of each barcoded library are mixed together. Combined barcoded libraries of similar size can be processed together through templated bead preparation. Refer to the *Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378) [or to the *Applied Biosystems SOLiD™ 3 Plus System Templated Bead Preparation Guide* (PN 4442695)]. If you want to perform fewer steps and accurate quantitation of each barcoded library is not critical, then you can pool the barcoded libraries in binding buffer at an earlier step, just prior to nick translation and amplification.

### Gel-purify the libraries

The library is run on a SOLiD™ Library Size Selection gel. The correctly sized ligation products (240 to 270 bp) are electrophoresed to the collection wells of the SOLiD™ Library Size Selection gel. If needed, the eluate can be concentrated using the SOLiD Library Column Purification Kit. Pool any remaining libraries that will be combined into a single emulsion.

# Tips

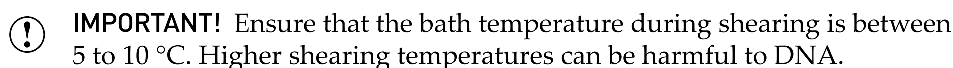
- Use good laboratory practices (change gloves frequently) to minimize cross-contamination of products.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols. Applied Biosystems recommends the Eppendorf 5417R tabletop microcentrifuge.
- Perform all steps requiring 0.5-mL and 1.5-mL tubes with Eppendorf LoBind tubes.
- Thaw reagents on ice before use.

# Procedure

Experiments should be designed so that for each multiplexed sequencing run, at least one of the following full sets of four barcodes should be used: Barcodes 1–4, 5–8, 9–12, or 13–16.

## Shear the DNA

### Shear the DNA using the Covaris™ S2 System



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

1. Dilute the desired amount of DNA in 100 µL in 1✕ Low TE Buffer in a LoBind tube (see Table 2).

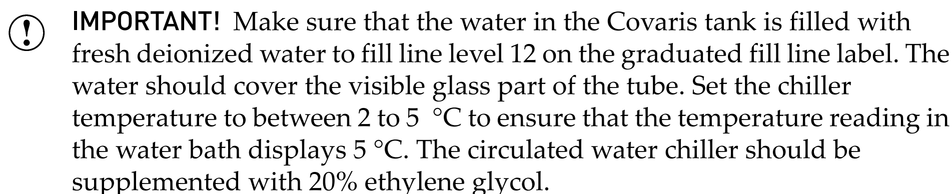
**Table 2** Dilute the DNA for shearing

| Component | Amount |
|---|---|
| DNA | 500 ng to 5 µg |
| 1✕ Low TE Buffer | Variable |
| Total | 100 µL |

1. Place a Covaris™ microTUBE into the loading station. Keep the cap on the tube and use a tapered pipette tip to slowly transfer the 100 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, refer to "Load and unload Covaris™ microTUBE vials from the Covaris™ microTUBE holder" in the *Applied Biosystems SOLiD™ 4 System Library Preparation Guide* (PN 4445673) [or in the *Applied Biosystems SOLiD™ 3 Plus System Library Preparation Guide* (PN 4442697)].

2. Shear the DNA using the following Covaris S2 System conditions:
   - Number of Cycles: **6**
   - Bath Temperature: **5** °C
   - Bath Temperature Limit: **30** °C
   - Mode: **Frequency sweeping**
   - Water Quality Testing Function: **Off**
   - Duty cycle: **10%**
   - Intensity: **5**
   - Cycles/burst: **100**
   - Time: **60 seconds**

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

3. Place the Covaris microTUBE into the loading station. While keeping the snap-cap on, insert a pipette tip through the pre-split septa, then slowly remove the sheared DNA. Transfer the sheared DNA into a new 1.5-mL LoBind tube.

## End-repair the DNA

### Repair the DNA ends with End Polishing Enzyme 1 and End Polishing Enzyme 2

1. Combine and mix the following components in a 1.5-mL LoBind tube (see Table 3):

**Table 3** Mix for end-repair of DNA

| Component | Volume (µL) |
|---|---|
| Sheared DNA | 100 |
| 5X End-Polishing Buffer | 40 |
| dNTP Mix, 10 mM | 8 |
| End Polishing Enzyme 1, 10 U/µL | 4 |
| End Polishing Enzyme 2, 5 U/µL | 16 |
| Nuclease-free water | 32 |
| Total | 200 |

2. Incubate the mixture at room temperature for 30 minutes.

### Purify the DNA with SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-S) with 55% isopropanol to the end-repaired DNA.
2. Apply about 700 µL of end-repaired DNA in the Binding Buffer (B2-S) to the column(s). The maximum yield of DNA can be achieved if the amount of DNA loaded to a SOLiD™ Library column is ≤5 µg. Use more columns if necessary.
3. Let the column(s) stand for 2 minutes at room temperature.
4. Centrifuge the column(s) at ≥10,000 × g (13,000 rpm) for 1 minute and discard the flow-through.
5. Repeat steps 2 and 4 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
6. Add 650 µL of Wash Buffer (W1) to wash the column(s).
7. Centrifuge the column(s) at ≥10,000 × g (13,000 rpm) for 2 minutes, then discard the flow-through. Repeat to remove residual wash buffer.
8. Air-dry the column(s) for 2 minutes to evaporate any residual alcohol. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
9. Add 50 µL of Elution Buffer (E1) to the column(s) to elute the DNA, then let the column(s) stand for 2 minutes.
10. Centrifuge the column(s) at ≥10,000 × g (13,000 rpm) for 1 minute.

11. Add the eluate from step 10 back to the column(s), then let the column(s) stand for 2 minutes. Repeat step 10.

12. If necessary, pool the eluted DNA.

13. If the starting DNA input amount is ≥500 ng, quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer. If the starting DNA input amount is < 500 ng, assume 70% recovery of input material after shearing.

STOPPING POINT Store the purified DNA in Elution Buffer (E1) at 4 °C, or proceed directly to "Ligate Multiplex P1 and Multiplex P2 Adaptors to the DNA".

## Ligate Multiplex P1 and Multiplex P2 Adaptors to the DNA

### Ligate Multiplex P1 and Multiplex P2 Adaptors to the DNA

1. Calculate the amount of adaptor needed, *Y*, for the reaction based on the amount of DNA from the last purification step [for calculation details, refer to "Formulas and Calculations" appendix, Fragment Library section, in the *Applied Biosystems SOLiD™ 4 System Library Preparation Guide* (or in the *Applied Biosystems SOLiD™ 3 Plus System Library Preparation Guide*)]. If DNA fragments were sheared using the standard protocol for fragment library preparation, the average insert size should be approximately 165 bp (before adaptor ligation).

$$\boldsymbol{X}\ \text{pmol/µg DNA} = 1\ \text{µg DNA} \times \frac{10^6\ \text{pg}}{1\ \text{µg}} \times \frac{1\ \text{pmol}}{660\ \text{pg}} \times \frac{1}{\text{Average insert size}}$$

$$\boldsymbol{Y}\ \text{µL adaptor needed} = \#\ \text{µg DNA} \times \frac{\boldsymbol{X}\ \text{pmol}}{1\ \text{µg DNA}} \times 30 \times \frac{1\ \text{µL adaptor needed}}{50\ \text{pmol}}$$

**Example:**

For 1 µg of purified end-repaired DNA with an average insert size of 165 bp

$$\boldsymbol{X}\ \text{pmol/µg DNA} = 1\ \text{µg DNA} \times \frac{10^6\ \text{pg}}{1\ \text{µg}} \times \frac{1\ \text{pmol}}{660\ \text{pg}} \times \frac{1}{165} = 9.2\ \text{pmol/µg DNA}$$

$$\boldsymbol{Y}\ \text{µL adaptor needed} = 1\ \text{µg DNA} \times \frac{9.2\ \text{pmol}}{1\ \text{µg DNA}} \times 30 \times \frac{1\ \text{µL adaptor needed}}{50\ \text{pmol}}$$

$$= 5.5\ \text{µL adaptor needed}$$

**IMPORTANT!** For each multiplexed sequencing run, use at least one of the following full sets of four barcodes: Barcodes 1–4, 5–8, 9–12, or 13–16. Use only one of the barcoded Multiplex P2 Adaptors for each ligation reaction, unless fewer than four libraries are being barcoded. If fewer than four samples are to be prepared for sequencing, use multiple barcodes per sample in equal ratios (see next step).

2. For each library, combine (see Table 4):

**Table 4** Ligation mix

| Component | Volume (µL) |
|---|---|
| Multiplex Library P1 Adaptor, 50 µM | *Y* |
| Barcode-0XX, 50 µM | *Y* |
| 5X T4 Buffer | 40 |
| DNA | 48 to 50 |
| T4 Ligase, 5 U/µL | 10 |
| Nuclease-free water | Variable |
| Total | 200 |

3. Incubate at room temperature for 15 minutes.

## Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes (800 µL) of Binding Buffer (B2-L) with 40% isopropanol to the sample.

**Note:** You can proceed in one of two ways:

- *Purify each barcoded library separately* if the libraries are of significantly different sizes (go to step 2). Individually purified barcoded libraries are then pooled after qPCR quantification (see "Pool the barcoded libraries" on page 19). You can normalize the amount of each barcoded library.

*or*

- *Pool barcoded libraries* before proceeding to step 2 to reduce the number of tubes during preparation; however, each library may be unequally represented after sequencing. Pool equivalent amounts of barcoded libraries before column purification if the libraries are of similar size and unequal library representation is acceptable. Ensure that each library is in binding buffer before pooling.

2. Apply about 700 µL of the (individual or pooled) barcoded library DNA in the binding buffer to the column(s). The maximum yield of DNA can be achieved when the amount of DNA loaded to a PureLink™ column is ≤5 µg. Use more columns if necessary.
3. Let the column stand for 2 minutes at room temperature.
4. Centrifuge the column at ≥10,000 × g (13,000 rpm) for 1 minute, then discard the flow-through.
5. Repeat steps 2 and 4 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
6. Add 650 µL of Wash Buffer (W1) to wash the column(s).
7. Centrifuge the column(s) at ≥10,000 × g (13,000 rpm) for 2 minutes, then discard the flow-through. Repeat to remove residual wash buffer.
8. Air-dry the column(s) for 2 minutes to evaporate any residual alcohol. Transfer the column(s) to clean 1.5-mL LoBind tube(s).

9. Add 50 µL of Elution Buffer (E1) to the column(s) to elute the DNA, then let the column(s) stand for 2 minutes.

10. Centrifuge the column(s) at ≥10,000 × g (13,000 rpm) for 1 minute.

11. Add the eluate from step 10 back to the column(s), then let the column(s) stand for 2 minutes. Repeat step 10.

12. If necessary, pool the eluted DNA.

STOPPING POINT Store the purified DNA in Elution Buffer (E1) at 4 °C, or proceed directly to "Nick-translate, then amplify the library".

## Nick-translate, then amplify the library

### Nick-translate, then amplify the library

1. Prepare a PCR reaction mix (see Table 5). Ensure that you use the *Multiplex* Library PCR-1, 50 µM.

**Table 5** PCR reaction mix: a mix for nick translation and amplification of the library

| Component | Volume (µL) |
|---|---|
| Platinum® PCR Amplification Mix | 400 |
| Multiplex Library PCR-1, 50 µM | 10 |
| Multiplex Library PCR-2, 50 µM | 10 |
| Adaptor-ligated, purified DNA | 48 to 50 |
| Nuclease-free water | Variable |
| Total | 500 |

2. Pipette 125 µL of the PCR reaction mix into each of four PCR tubes. Depending on the pooling conditions, the number of PCR reactions can be scaled up.

3. Run the PCR (Table 6).

   **IMPORTANT!** The number of cycles should be minimized and determined based on the amount of starting input DNA. Minimal cycling is desirable to avoid over-amplification and production of redundant molecules.

**Table 6** PCR conditions to nick-translate and amplify the library

| Stage | Step | Temp | Time |
|---|---|---|---|
| Holding | Nick translation | 72 °C | 20 min |
| Holding | Denature | 95 °C | 5 min |
| Cycling (2 to 8 cycles)‡ | Denature | 95 °C | 15 sec |
| | Anneal | 62 °C | 15 sec |
| | Extend | 70 °C | 1 min |
| Holding | Extend | 70 °C | 5 min |
| Holding | — | 4 °C | ∞ |

‡ Starting amount of DNA: number of cycles:
500 ng to 1 µg: 6 to 8 cycles
1 µg to 2 µg: 4 to 6 cycles
2 µg to 5 µg: 3 to 6 cycles

4. Pool all four of the PCR tubes into a new 1.5-mL LoBind tube.

## Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-L) with 40% isopropanol to the sample.
2. Apply about 700 µL of PCR product in the Binding Buffer (B2-L) to the column(s). The maximum yield of DNA can be achieved if the amount of DNA loaded to a SOLiD™ Library column is ≤5 µg. Use more columns if necessary.
3. Let the column(s) stand for 2 minutes at room temperature.
4. Centrifuge the column(s) at ≥10,000 × g (13,000 rpm) for 1 minute and discard the flow-through.
5. Repeat steps 2 and 4 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
6. Add 650 µL of Wash Buffer (W1) to wash the column(s).
7. Centrifuge the column(s) at ≥10,000 × g (13,000 rpm) for 2 minutes, then discard the flow-through. Repeat to remove residual wash buffer.
8. Air-dry the column(s) for 2 minutes to evaporate any residual alcohol. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
9. Add 50 µL of Elution Buffer (E1) to the column(s) to elute the DNA, then let the column(s) stand for 2 minutes.
10. Centrifuge the column(s) at ≥10,000 × g (13,000 rpm) for 1 minute.
11. Add the eluate from step 10 back to the column(s), then let the column(s) stand for 2 minutes. Repeat step 10.

12. If necessary, pool the eluted DNA.

STOPPING POINT Store the purified DNA in Elution Buffer (E1) at 4 °C for short-term storage or at –20 °C for long-term storage, or proceed directly to “Quantitate the library by performing quantitative PCR (qPCR)”.

## Quantitate the library by performing quantitative PCR (qPCR)

Quantitate your library by quantitative PCR. For the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), refer to the *Applied Biosystems SOLiD™ 4 System Library Preparation Guide*, "SOLiD™ 4 System Library Quantitation " (PN 4445673).

STOPPING POINT Store the DNA in Elution Buffer (E1) at 4 °C for short-term storage or at –20 °C for long-term storage, or proceed directly to “Pool the barcoded libraries”.

## Pool the barcoded libraries

**IMPORTANT!** If you:

- *Pooled the libraries after ligation of the P1 and P2 adaptors*, then skip this step and proceed to “Gel-purify the libraries”.
- *Are working with libraries of dissimilar sizes*, then do *not* pool the libraries until after gel purification. Proceed to “Gel-purify the libraries”.

Pool libraries of similar size. To pool for multiplexed libraries, simply mix equal molar amounts of each barcoded library together in a single tube. Vortex the tube.

STOPPING POINT Store the library DNA in Elution Buffer (E1) at 4 °C, or proceed directly to “Gel-purify the libraries”.

## Gel-purify the libraries

### Size-select the DNA fragments with a 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. Set the SOLiD Library Size Selection gel on the E-Gel® iBase™ system linked with the E-Gel Safe Imager™ Real-Time Transilluminator.

2. Load the SOLiD Library Size Selection gel as follows:

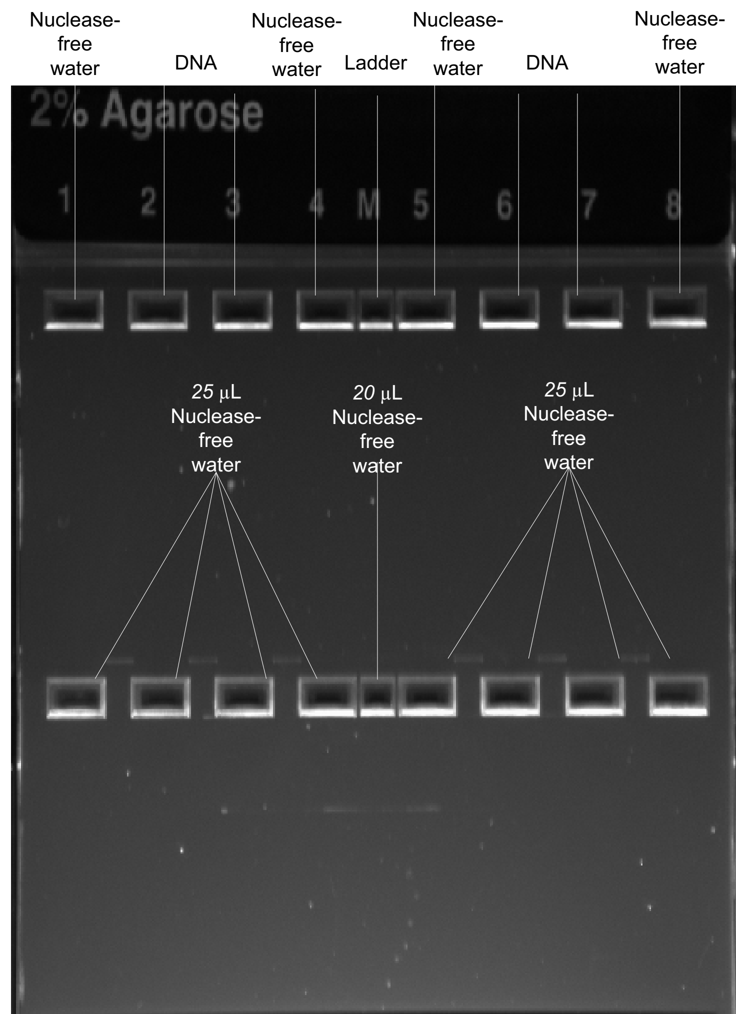
   a. Load 20 µL of the pooled library DNA into each well of the *top row* of wells. If the sample volume < 20 µL, add nuclease-free water to the well for a total volume of 20 µL. Skip the center well (smaller well in the top center of the gel for the ladder); and skip a single well to the right and left 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.

   b. Load 2 µL of 50-bp ladder at 0.1 µg/µL to the center top well. Add 15 µL of water to fill the well.

   c. Fill the empty wells in the top row with 20 µL of nuclease-free water.

d. 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. (See Figure 1.)

**Figure 1** Where to load DNA, ladder, and nuclease-free water in a Library Size Selection gel to size-select the DNA.




3. 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.

4. If needed during the run, fill the middle collection wells with nuclease-free water.

5. When the 250-bp band from the marker (ladder) lane is at the top of the collection well, stop the run if the run has not already stopped (see Figure 2).

   **Note:** After amplification, the total size of the adapter in a barcoded library is 93 bp. The elution size is ~240 to 270 bp.

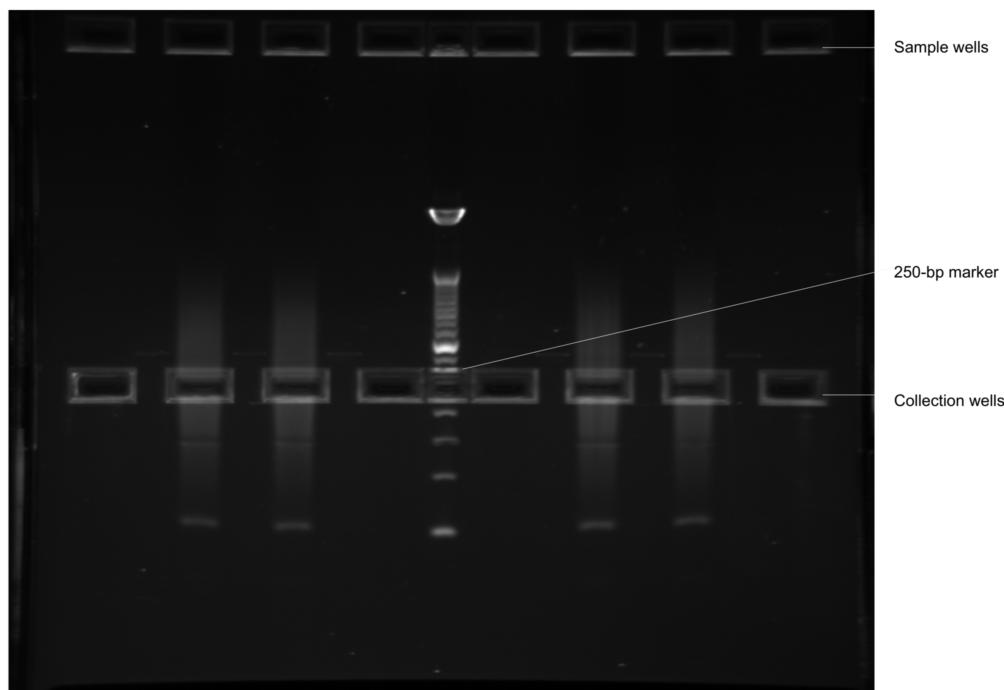
6. Collect the solution from the wells and pool according to samples.

7. Wash each collection well with 25 µL with nuclease-free water, then retrieve the wash solution with the solution collected in Step 6.

8. (Optional) Concentrate the DNA with a SOLiD™ Library purification column.

9. Pool any remaining libraries that will be combined into a single emulsion. Refer to the *Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378) [or to the *Applied Biosystems SOLiD™ 3 Plus System Templated Bead Preparation Guide* (PN 4442695)].

**Figure 2** Elution of ~240- to 270-bp region from a SOLiD™ Library Size Selection gel.




STOPPING POINT Store the purified DNA in Elution Buffer (E1) at –20 °C, or proceed directly to the *Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378) [or to the *Applied Biosystems SOLiD™ 3 Plus System Templated Bead Preparation Guide* (PN 4442695)].

# Troubleshooting

| Observation | Possible cause | Solution |
|---|---|---|
| Unexpected barcode and template association. | • Barcode adaptor cross-contamination.<br>• Samples were not in binding buffer when pooled before nick translation/amplification. | • Take extreme care to avoid mixing different barcode adaptor reagent(s). Between barcode preparations, change gloves frequently.<br>• Ensure that the libraries are in binding buffer to inactivate ligase. |
| Unequal barcoded library representation. | • Libraries were not mixed in equal molar amounts when libraries were pooled before nick translation and amplification/quantitation.<br>• Barcode adaptor cross-contamination. | • Pool the libraries in equal molar amounts after they have been individually amplified and quantified.<br>• Take extreme care to avoid mixing different barcode adaptor reagent(s). Between barcode preparations, change gloves frequently. |
| Low or unequal percentage of binned barcode reads that map to the reference sequence. | There may be a high amount of adaptor dimer in one or more of the barcoded libraries. | Gel-purify the libraries and/or ensure better gel separation between templated fragments and adaptor dimers. |

# Ordering Information

**Table 7** Required Applied Biosystems reagent kits

| Item (part number)‡ | Components | Kit components used in... |
|---|---|---|
| SOLiD™ Fragment Library Barcoding Kit 1–16 (4444836) | • Multiplex Library P1 Adaptor, 50 µM<br>• Multiplex Library PCR-1, 50 µM<br>• Multiplex Library PCR-2, 50 µM<br>• Barcodes 001–016, 50 µM<br>• Applied Biosystems SOLiD™ Fragment Library Barcoding Kit Module 1–16 Protocol (PN 4443045)<br>• Applied Biosystems SOLiD™ Fragment Library Barcoding Kit Module 1–16 Quick Reference Card (PN 4443044) | Preparation of 16 barcoded fragment libraries |
| SOLiD™ Fragment Library Barcoding Kit Module 1–16 (4444837) | • Multiplex Library P1 Adaptor, 50 µM<br>• Multiplex Library PCR-1, 50 µM<br>• Multiplex Library PCR-2, 50 µM<br>• Barcodes 001–016, 50 µM | Preparation of 16 barcoded fragment libraries |

**Table 7** Required Applied Biosystems reagent kits

| Item (part number)‡ | Components | Kit components used in... |
|---|---|---|
| SOLiD™ Fragment Library Construction Kit with Size Selection Gels (4443471)§ | SOLiD™ Fragment Library Enzyme/Core Kit:<br>• 5X End Polishing Buffer<br>• dNTP, 10 mM<br>• End Polishing Enzyme 1<br>• End Polishing Enzyme 2 | DNA end repair |
| | • 5X Ligase Buffer<br>• T4 DNA Ligase | Ligation of P1 and P2 Adaptors (not needed) |
| | Platinum® PCR Amplification Mix | Nick translation/library amplification |
| | SOLiD™ Library Column Purification Kit | DNA end repair, ligation of P1 and P2 Adaptors, and nick translation/library amplification |
| | SOLiD™ Library Size Selection Gels, 10 gels | Size selection |

‡ 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.

**Table 8** Optional Applied Biosystems reagent kits

| Item (part number)‡ | Components | Kit components used in... |
|---|---|---|
| SOLiD™ Fragment Library Construction Kit (4443473)§ | SOLiD™ Fragment Library Enzyme/Core Kit:<br>• 5X End Polishing Buffer<br>• dNTP, 10 mM<br>• End Polishing Enzyme 1<br>• End Polishing Enzyme 2 | DNA end repair |
| | • 5X Ligase Buffer<br>• T4 DNA Ligase | Ligation of P1 and P2 Adaptors |
| | Platinum® PCR Amplification Mix | Nick translation/library amplification |
| | SOLiD™ Library Column Purification Kit | DNA end repair, ligation of P1 and P2 Adaptors, and nick translation/library amplification |
| SOLiD™ Fragment Library Construction Kit Reagents (4443713) | SOLiD™ Fragment Library Enzyme/Core Kit:<br>• 5X End Polishing Buffer<br>• dNTP, 10 mM<br>• End Polishing Enzyme 1<br>• End Polishing Enzyme 2 | DNA end repair |
| | • 5X Ligase Buffer<br>• T4 DNA Ligase | Ligation of P1 and P2 Adaptors |
| | Platinum® PCR Amplification Mix | Nick translation/library amplification |

‡ 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.

**Table 9** Oligonucleotides in the SOLiD™ Barcoded Fragment Library Oligos Kit

| Component | Size | Quantity |
|---|---|---|
| Multiplex Library P1 Adaptor, 50 µM<br>5′ -ATCACCGACTGCCCATAGAGAGGTT-3′<br>5-CCTCTCTATGGGCAGTCGGTGAT-3′ | 1120 µL | 1 tube<br>(blue cap) |
| Multiplex Library PCR-1, 50 µM<br>5′ -CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT-3′ | 1280 µL | 1 tube<br>(blue cap) |
| Multiplex Library PCR-2, 50 µM<br>5′ -CTGCCCCGGGTTCCTCATTCT-3′ | 1280 µL | 1 tube<br>(blue cap) |
| Barcode-001, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGTAAGAGG**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(clear cap) |
| Barcode-002, 50 µM<br>5′-CGCCTTGGCCGTACAGCAG3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGGAGTGGT**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(clear cap) |
| Barcode-003, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**ATAGGTTATA**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(clear cap) |
| Barcode-004, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GGATGCGGTC**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(clear cap) |
| Barcode-005, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGGTGTAAG**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(red cap) |
| Barcode-006, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGAGGGACA**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(red cap) |
| Barcode-007, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGTTATGCC**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(red cap) |
| Barcode-008, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGCGAGGAT**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(red cap) |
| Barcode-009, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGTTGCGAC**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(black cap) |

**Table 9** Oligonucleotides in the SOLiD™ Barcoded Fragment Library Oligos Kit

| Component | Size | Quantity |
|---|---|---|
| Barcode-010, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGTAAGCT**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(black cap) |
| Barcode-011, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCGACACG**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(black cap) |
| Barcode-012, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGAGGAAAA**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(black cap) |
| Barcode-013, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGTAAGGC**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(green cap) |
| Barcode-014, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCGGCAGA**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(green cap) |
| Barcode-015, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGTTGAATG**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(green cap) |
| Barcode-016, 50 µM<br>5′ -CGCCTTGGCCGTACAGCAG-3′<br>5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGAGACGTT**CTGCTGTACGGCCAAGGCG-3′ | 70 µL | 1 tube<br>(green cap) |

**Table 10** Required equipment

| Item[‡] | Source |
|---|---|
| Covaris™ S2 System<br><br>(110 V for U.S. customers)<br>(220 V for international customers)<br><br>The system includes:<br>• Covaris™ S2 sonicator<br>• Latitude™ laptop from Dell® Inc.<br>• MultiTemp III Thermostatic Circulator<br>• Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube<br>• Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube<br>• Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube<br>• Covaris-2 Series Machine Holder for (one) microTUBE<br>• Covaris microTUBE Prep Station<br>• Covaris Water Tank Label Kit<br>• Covaris microTUBEs (1 pack of 25)<br>For system materials summary, see “Covaris™ S2 System Materials Summary,” the *Applied Biosystems SOLiD™ 3 Plus System Site Preparation Guide* (PN 4444009). | • Applied Biosystems 4387833 (110 V)<br>• Applied Biosystems 4392718 (220 V)<br>or<br>Covaris |
| Microcentrifuge 5417R, refrigerated, without rotor | • Eppendorf[§] 022621807 (120 V/60 Hz)<br>• Eppendorf[‡] 022621840 (230 V/50 Hz) |
| FA-45-24-11, fixed-angle rotor,<br>24 × 1.5/2 mL, including aluminum lid, aerosol-tight | Eppendorf[‡]<br>022636006 |
| 96-well GeneAmp® PCR System 9700 (thermal cycler) | • Applied Biosystems N8050200 (Base)<br>• Applied Biosystems 4314443 (Block)[‡] |
| NanoDrop® ND-1000 Spectrophotometer (computer required) | Thermo Scientific<br>ND-1000 |
| 6-Tube Magnetic Stand | Applied Biosystems<br>AM10055 |
| E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit | Invitrogen<br>G6465 |
| Vortexer | Major Laboratory Supplier (MLS) |

**Table 10** Required equipment

| Item‡ | Source |
|---|---|
| Picofuge | 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 equivalent but validation of the equipment for library preparation is required.

**Table 11** Optional equipment

| Item‡ | Source |
|---|---|
| 2100 Bioanalyzer | Agilent Technologies<br>G2938C |
| Qubit™ Quantitation Starter Kit | Invitrogen<br>Q32860 |

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

**Table 12** Required consumables

| Item‡ | Source |
|---|---|
| 1X Low TE Buffer§ | Applied Biosystems<br>4389764 |
| Nuclease-free Water, 1 L | Applied Biosystems<br>AM9932 |
| Covaris microTUBEs | Covaris<br>520045 |
| Isopropyl alcohol | Sigma-Aldrich<br>I9516 |
| Ethylene glycol | American Bioanalytical<br>AB00455-01000 |
| 50-bp ladder | Invitrogen<br>10416-014 |
| 0.5-mL LoBind Tubes | Eppendorf<br>022431005 |
| 1.5-mL LoBind Tubes | Eppendorf<br>022431021 |
| CF-1 Calibration Fluid Kit | Thermo Scientific<br>CF-1 |

**Table 12** Required consumables

| Item‡ | Source |
|---|---|
| PR-1 Conditioning Kit# | Thermo Scientific<br>PR-1 |
| Filtered pipettor tips | Major Laboratory Supplier (MLS) |
| PCR strip tubes | 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.

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

**Table 13** Optional consumables

| Product name‡ | Vendor |
|---|---|
| Agilent DNA 1000 Kit | Agilent Technologies<br>5067-1504 |

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

# Safety

This appendix covers: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  - General chemical safety . . . . . 32
  - SDSs . . . . . 33
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- General safety alerts for all chemicals . . . . . 35

# Chemical safety

## General chemical safety

### Chemical hazard warning

**WARNING! CHEMICAL HAZARD.** Before handling any chemicals, refer to the Material 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 Material 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 33.)
- 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, formerly Material Safety Data Sheets) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

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

### Obtaining SDSs

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

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

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

## Chemical waste safety

### Chemical waste hazards

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

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

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

## Chemical waste safety guidelines

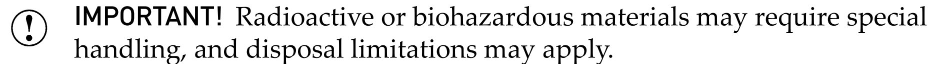
To minimize the hazards of chemical waste:

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

## Waste disposal

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

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.



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

# General safety alerts for all chemicals

Avoid contact with skin, eyes, and/or clothing. Read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

# Documentation and Support

## Related documentation

| Document | Part number | Description |
|---|---|---|
| *Applied Biosystems SOLiD™ Fragment Library Barcoding Kit 1–96 Quick Reference Card* | 4448275 | Describes in a quick reference format how to prepare a fragment barcoded library with 96 barcodes. |
| *Applied Biosystems SOLiD™ 4 System Library Preparation Guide* | 4445673 | Describes how to prepare fragment and mate-paired libraries for templated bead preparation and sequencing on the SOLiD™ 4 System. |
| *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™ SETS Software v4.0 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™ ICS Software v4.0 Help* | — | Describes the software and provides procedures for common tasks (see the Instrument Control Software). |

| Document | Part number | Description |
|---|---|---|
| *Applied Biosystems SOLiD™ 4 BioScope Software v1.2 User Guide* | 4448431 | Provides a bioinformatics analysis framework for flexible application analysis (data-generated mapping, SNPs, count reads) from sequencing runs. |
| *Applied Biosystems SOLiD™ 4 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™ Fragment Library Barcoding Kit Module 1–16 Protocol* | 4443045 | Describes how to prepare a fragment barcoded library with 16 barcodes. |
| *Applied Biosystems SOLiD™ Fragment Library Barcoding Kit Module 1–16 Quick Reference Card* | 4443044 | Describes in a quick reference format how to prepare a fragment barcoded library with 16 barcodes. |
| *Applied Biosystems SOLiD™ 3 Plus System Library Preparation Guide* | 4442697 | Describes how to prepare fragment and mate-paired libraries for templated bead preparation and sequencing on the SOLiD™ 3 Plus System. |
| *Applied Biosystems SOLiD™ 3 Plus System Library Preparation Quick Reference Card* | 4442698 | Provides brief, step-by-step procedures for preparing libraries. |
| *Applied Biosystems SOLiD™ 3 Plus System Templated Bead Preparation Guide* | 4442695 | Describes how to prepare templated beads by emulsion PCR (ePCR), required before sequencing on the SOLiD™ 3 Plus System. |
| *Applied Biosystems SOLiD™ 3 Plus System Templated Bead Preparation Quick Reference Card* | 4442696 | Provides brief, step-by-step procedures for preparing templated beads by emulsion PCR (ePCR), required before sequencing on the SOLiD™ 3 Plus System. |
| *Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Guide* | 4442357 | Describes how to load and run the SOLiD™ 3 Plus System for sequencing. |
| *Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Quick Reference Card* | 4442358 | Provides brief, step-by-step procedures for loading and running the SOLiD™ 3 Plus System. |
| *Applied Biosystems SOLiD™ 3 Plus System Site Preparation Guide* | 4444009 | Provides all the information that you need to set up the SOLiD™ 3 Plus System. |

| Document | Part number | Description |
|---|---|---|
| *Applied Biosystems SOLiD™ SETS Software v3.5 Getting Started Guide* | 4444007 | Provides an alternate platform to monitor runs, modify settings and reanalyze previous runs that are performed on the SOLiD System. |
| *Applied Biosystems SOLiD™ ICS Software v3.5 Help* | — | Describes the software and provides procedures for common tasks (see the Instrument Control Software). |
| *Applied Biosystems SOLiD™ Analysis Tools (SAT) v3.5 Reference Guide* | 4443929 | Provides advanced technical information on how to modify pipelines for in-depth sequencing analysis using the SOLiD System. |
| *Applied Biosystems SOLiD™ BioScope Software v1.0 Getting Started Guide* | 4442694 | Provides a bioinformatics analysis framework for flexible application analysis (data-generated mapping, SNPs, count reads) from sequencing runs. |

**Note:** For updated versions of the *SOLiD™ Fragment Library Barcoding Kit 1–96 Protocol* and *Quick Reference Guide*, go to:

**www.invitrogen.com**

# Obtaining support

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

**www.appliedbiosystems.com**

At the Applied Biosystems web site, you can:

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

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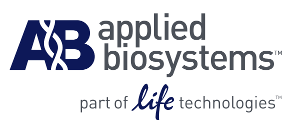


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