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Applied Biosystems SOLiD™ 4 System

Library Preparation Guide

April 2010

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

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

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Contents

Preface

Safety information

Note: For important instrument safety information, refer to the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* (PN 4448379). For general safety information, see this Preface and Appendix I, "Safety" on page 245. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

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

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

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

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

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

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 249.

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

How to use this guide

Text conventions

This guide uses the following conventions:

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

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

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

  Select **File ▸ Open ▸ Spot Set**.

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

User attention words

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

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

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

How to obtain support

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

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

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

1 Introduction

Library preparation overview

Note: If you are performing standard DNA sequencing analysis, prepare the library according to the procedures in this guide. For other applications of the SOLiD™ System, go to: **http://solid.appliedbiosystems.com**, then select your area of interest. On the selected page, click the library preparation step under Experimental Workflow to find application-specific information and protocols.

Library preparation is the first step in which samples are adapted for SOLiD™ System sequencing. During library preparation, forward and reverse adaptors are added to the ends of DNA inserts (see Figure 1).

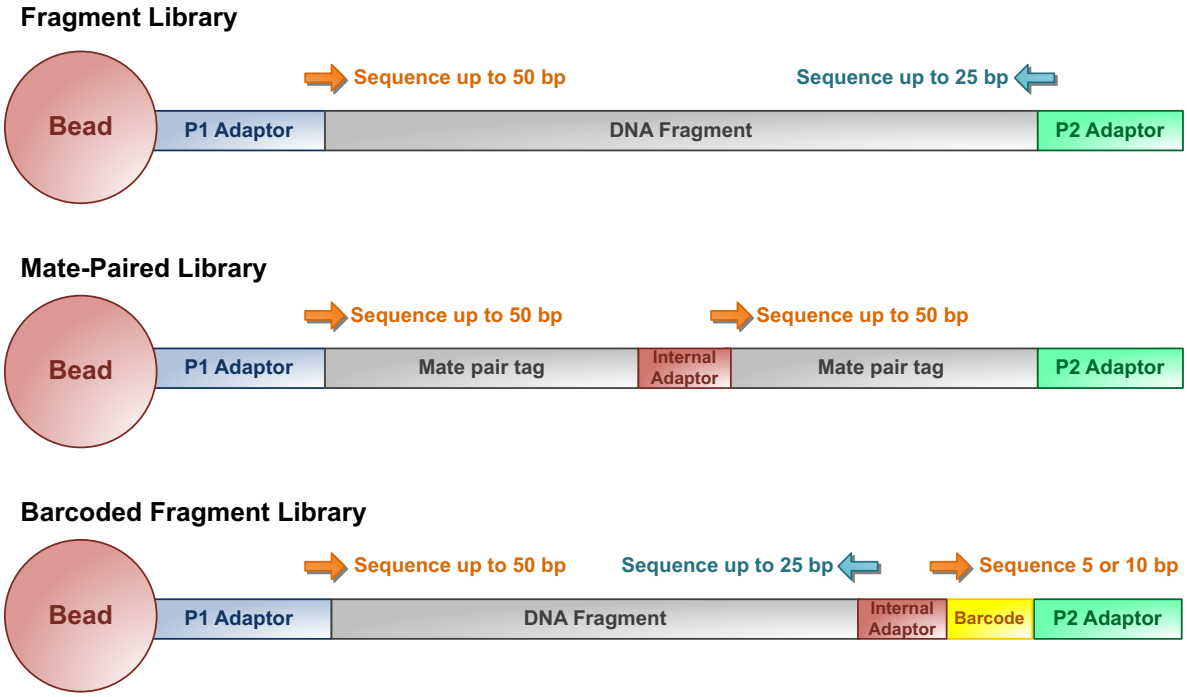


Figure 1 Fragment and mate-paired library construction.

Choose the appropriate library type

Table 1 Libraries that can be sequenced on the SOLiD™ 4 System

Library type	Features	Go to…
Fragment	• Adaptors on each end of sheared DNA insert (see Figure 1 on page 11) • Less input DNA required (10 ng to 5 µg) • Appropriate for sequence lengths ≤300 bp • Simpler library construction workflow • Higher recovery of unique molecules	Chapter 2, "Fragment Library Preparation" on page 13
Mate-paired	• Two DNA insert tags 600 bp to 6 kb apart (see Figure 1 on page 11) • More input DNA required (5 µg to 20 µg) • More even coverage of genome • Better ability for unique tag placement	Chapter 3, "Mate-Paired Library Preparation" on page 41
Barcoded fragment	• Same as fragment library, except with a barcode sequence located on one of the adaptors to enable multiplexed sequencing (see Figure 1 on page 11) • 500 ng to 5 µg input DNA required • Can be pooled for templated bead preparation	Chapter 4, "Barcoded Fragment Library Preparation" on page 127

The type 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, and shorter read lengths are adequate. For information about specific applications, go to the SOLiD System website:

http://solid.appliedbiosystems.com

or contact your field applications specialist.

2 Fragment Library Preparation

This chapter covers:

Overview

This chapter describes the method to generate a small-insert library (150 to 180 bp, before adaptor ligation). This method involves shearing DNA into small fragments and ligating P1 and P2 Adaptors (see Figure 2 and Figure 3).

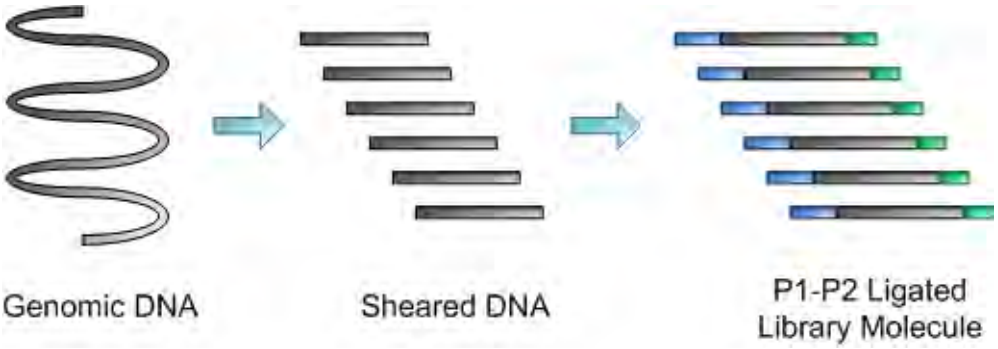


Figure 2 Basic fragment library preparation workflow overview.

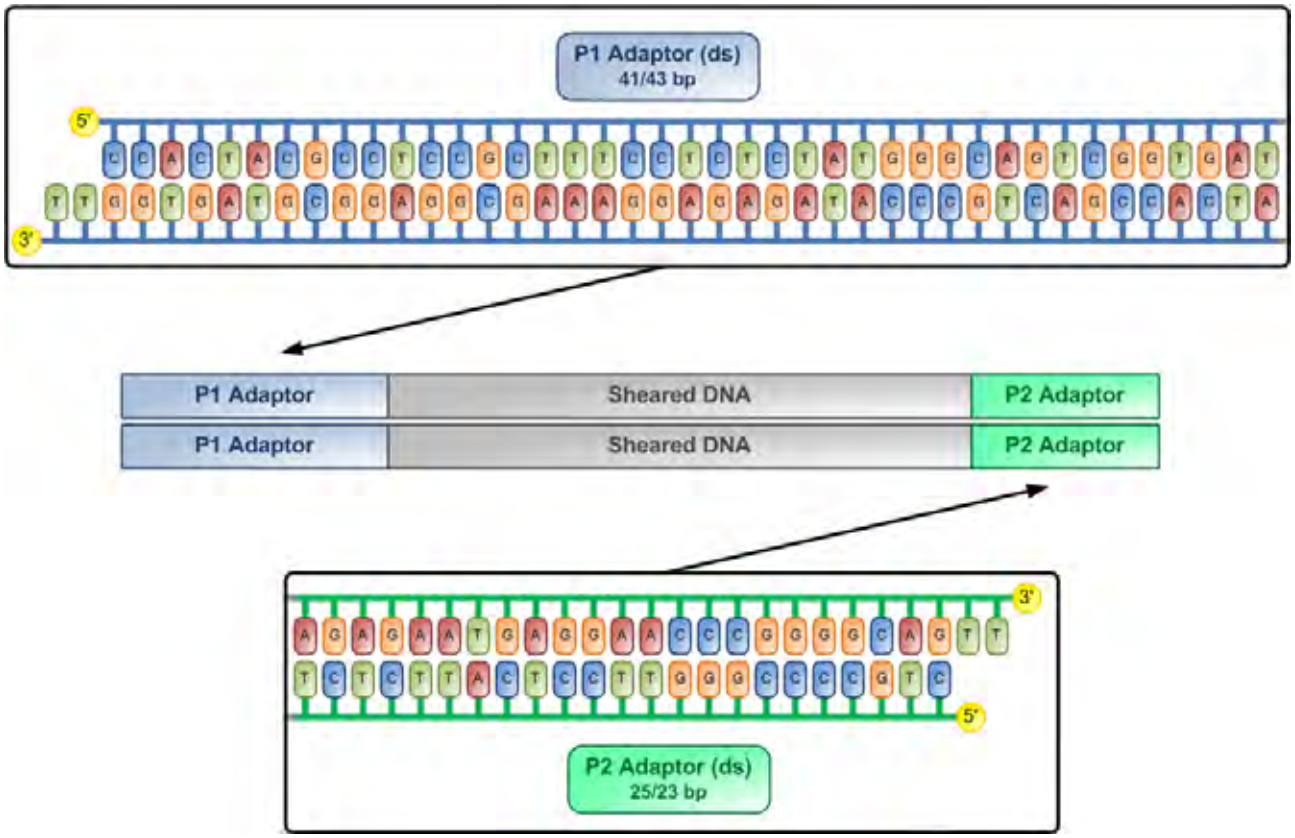


Figure 3 Fragment library design.

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 4). 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.

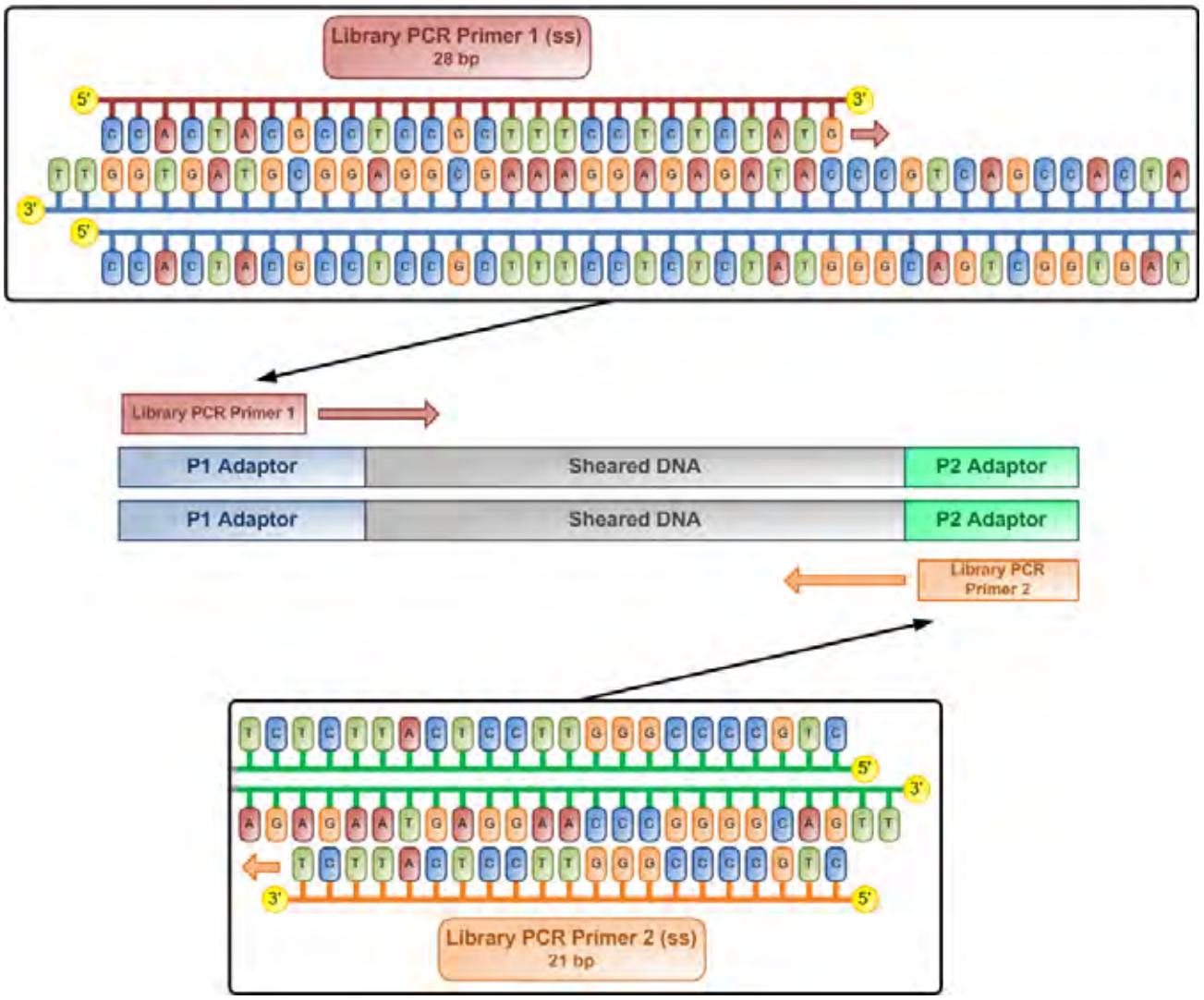


Figure 4 Fragment library amplification design.

This chapter is organized into two sections. Section 2.1 describes how to generate a fragment library using gel-based size-selection. Section 2.2 describes how to generate a fragment library without gel-based size-selection.

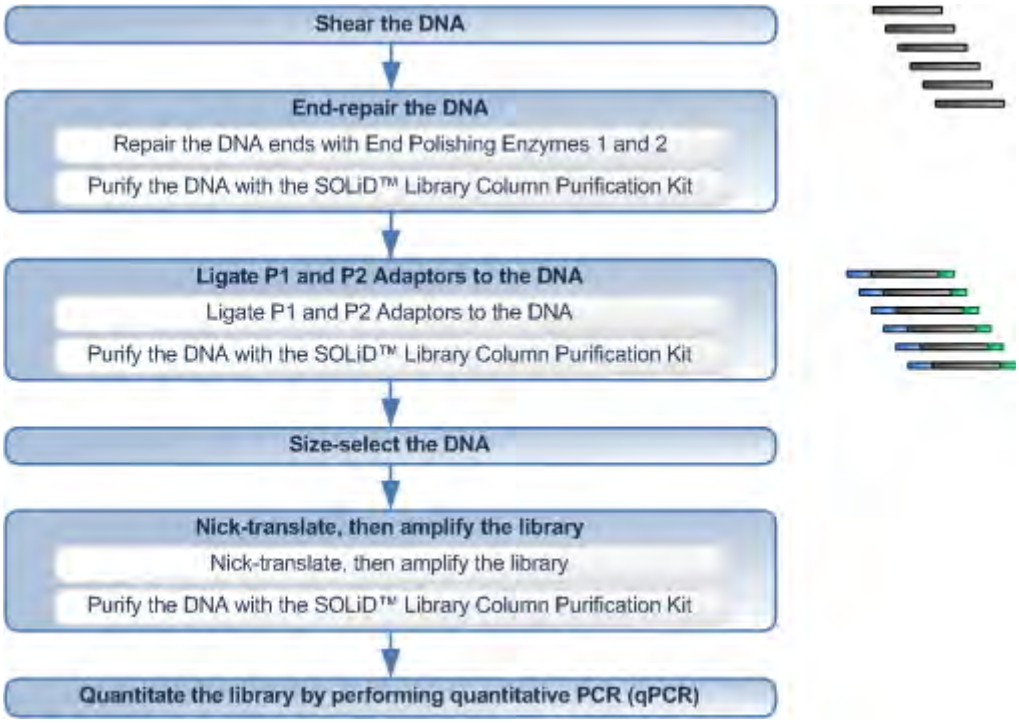
Section 2.1 Prepare a standard fragment library

This protocol is designed for 10 ng to 5 µg of genomic DNA or ligated PCR product. You should modify the protocol with any change in the starting amount of DNA. If you are constructing a targeted resequencing library with small-sized PCR products (≤500 bp), then you must perform a PCR-product ligation step. For a concatenation protocol, contact your field applications specialist.

Materials and equipment required

See Appendix A on page 145 for a list of equipment, kits, and consumables necessary for this procedure.

Workflow



Workflow overview

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) by using a Covaris™ S2 System. The conditions have been tested for shearing 10 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 ATP 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

P1 and P2 Adaptors are ligated to the ends of the end-repaired DNA. The P1 and P2 Adaptors are included in double-stranded form in the SOLiD™ Fragment Library Oligos Kit.

Size-select the DNA

The ligated, purified DNA is run on a SOLiD™ Library Size Selection gel. The correctly sized ligation products (200 to 230 bp) are electrophoresed to the collection wells of the Size Selection gel. The eluate in each collection well can be transferred directly to the nick translation reaction.

Nick-translate, then amplify the library

The eluates from the SOLiD Library Size Selection gel undergo nick translation and subsequently amplification using Library PCR Primers 1 and 2 and Platinum® PCR Amplification Mix. After amplification, PCR samples are purified with the SOLiD™ Library Column Purification Kit.

Quantitate the library by performing quantitative PCR (qPCR)

The library is quantitated by using the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), described in Appendix B, "SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit" on page 173.

Tips

- 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.

Shear the DNA

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 to 100 µL in 1X Low TE Buffer in a LoBind tube (see Table 2).

Table 2 Dilute the DNA for shearing

Component	Amount
DNA	10 ng to 5 µg
1X Low TE Buffer	Variable
Total	100 µL

2. 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.

3. 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 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. 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.

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 186.

4. 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
5× 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 approximately 700 µL of end-repaired DNA in the binding buffer to the column(s). The maximum yield of DNA can be achieved if the amount of DNA loaded to a PureLink™ 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 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 (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189). 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 ºC, or proceed directly to "Ligate the P1 and P2 Adaptors to the DNA" on page 22.

Ligate P1 and P2 Adaptors to the DNA

Ligate the P1 and 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, see "Ligation of P1 and P2 Adaptors" on page 216). 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):

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

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

Example:

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

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

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

$$= 5.5\ \text{µL adaptor needed}$$

2. Combine and mix the following components (see Table 4).

Table 4 Ligation mix

Component	Volume (µL)
P1 Adaptor (ds), 50 pmol/µL	*Y*
P2 Adaptor (ds), 50 pmol/µL	*Y*
5× T4 Ligase Buffer	40
DNA	48 to 50
T4 Ligase, 5 U/µL	10
Nuclease-free Water	Variable
Total	200

3. Incubate the mixture 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.
2. Apply approximately 700 µL of the ligated DNA in the binding buffer to the column(s). The maximum yield of DNA can be achieved if the amount of DNA loaded to a PureLink™ 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, 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 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 "Size-select the DNA" on page 24.

Size-select the DNA

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 gel on the E-Gel® iBase™ system linked with the E-Gel Safe Imager™ Real-time Transilluminator.

2. Load the gel as follows (for exact fill volumes of the wells, refer to the *Invitrogen E-Gel® SizeSelect™ Agarose Gels Quick Reference Card*):

   a. Load 20 µL of the ligated, purified 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 (see Figure 5 on page 25).

   b. 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 (see Figure 5 on page 25).

   c. Fill 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 5 on page 25).

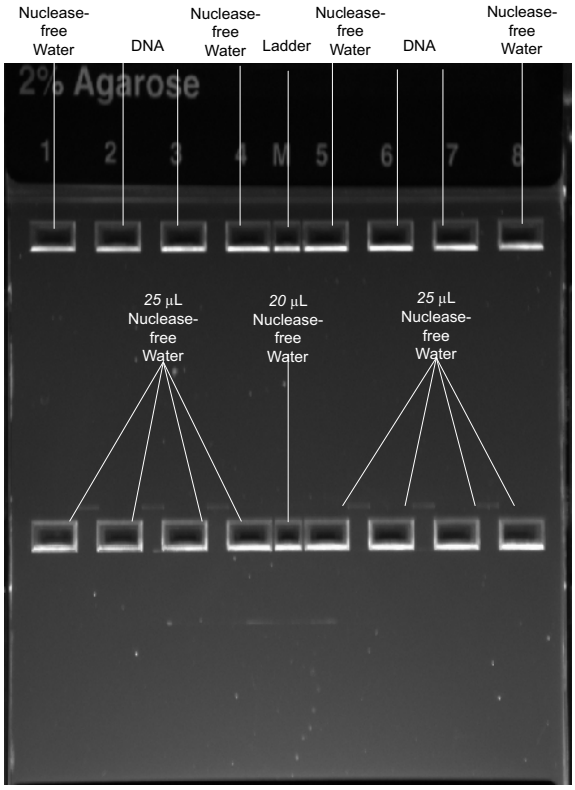


Figure 5 Where to load DNA, ladder, and Nuclease-free Water in a SOLiD™ Library Size Selection gel to size-select the DNA.

3. Run the gel:
   - iBase program: **Run SizeSelect 2%**
   - Run time: **11:40** (11 minutes and 40 seconds)

   Monitor the 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 200-bp band from the marker (ladder) lane is at the bottom but still within the collection well, stop the run if the run has not already stopped (see Figure 6 on page 26).

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

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

8. (Optional) Concentrate the DNA with a SOLiD™ Library Column Purification Kit. No concentration of the DNA is needed, however, if the DNA will be nick-translated or amplified according to the procedures below.

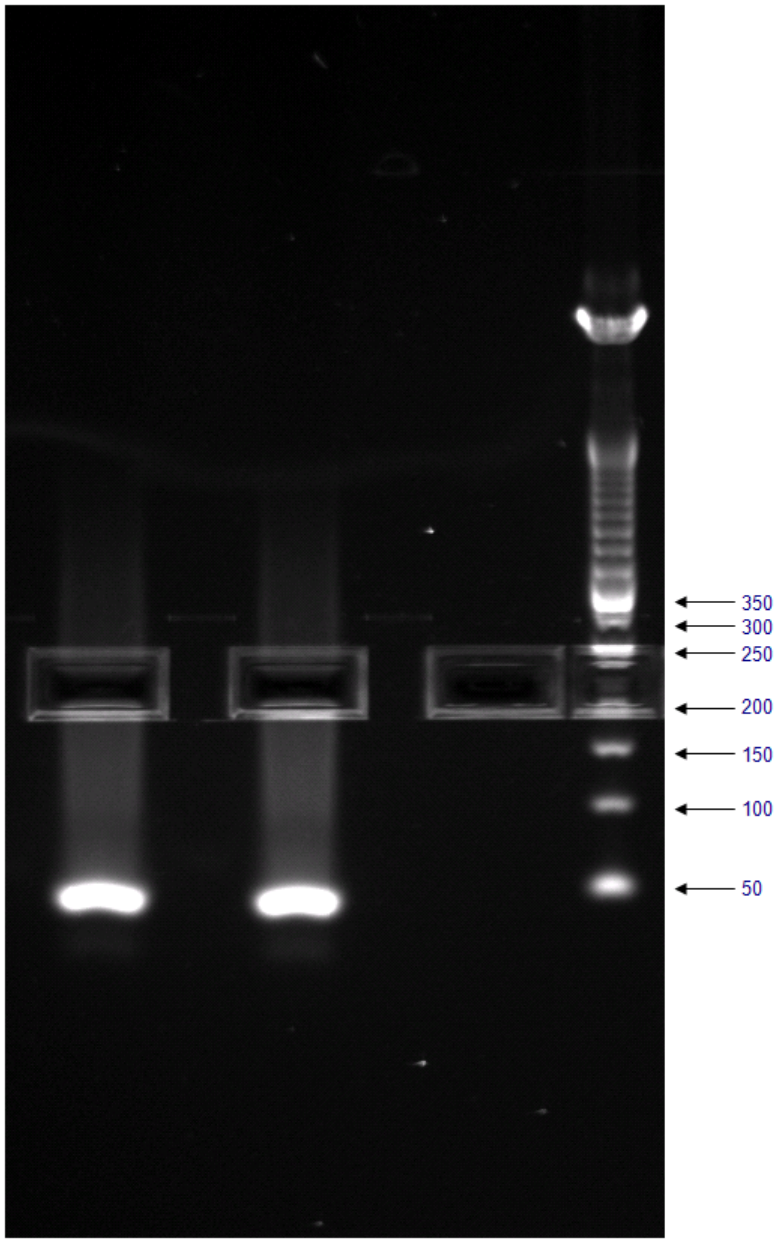


Figure 6 Elution of ~200- to 230-bp region from a SOLiD™ Library Size Selection gel.

Nick-translate, then amplify the library

Nick-translate, then amplify the library

1. Prepare a master mix for the number of reactions needed based on the amount of starting input DNA, plus one additional reaction for the negative control (see Table 5).

Table 5 Suspend the gel eluate according to starting input DNA

If the gel eluate DNA is…	>100 µL	≤100 µL
Component	**Volume (µL)** **F = volume of eluate/100**	**Volume (µL)**
Platinum® PCR Amplification Mix	380 × F	380
Library PCR Primer 1, 50 µM	10 × F	10
Library PCR Primer 2, 50 µM	10 × F	10
Total	400 × F	400

2. If the volume of the eluate is:
   - ≤100 µL, add 400 µL of master mix to the gel eluate, then distribute in 4 PCR reaction tubes.
   - > 100 µL, add 400 µL of master mix to every 100 µL of eluate, then distribute in 125-µL aliquots to PCR reaction tubes.

3. Run the PCR (Table 6 on page 28).

IMPORTANT! Minimize the number of cycles to avoid overamplification and production of redundant molecules. Determine the number of cycles based on the amount of starting input DNA.

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 10 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:
10 ng to 100 ng: 10 cycles
100 ng to 1 µg: 6 to 8 cycles
1 µg to 2 µg: 4 to 6 cycles
2 µg to 5 µg: 2 to 3 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 approximately 700 µL of PCR product in the binding buffer to the column(s). The maximum yield of DNA can be achieved if the amount of DNA loaded to a PureLink™ 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 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)

For accurate library quantitation, quantitative PCR is strongly recommended. For a protocol using the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), see Appendix B, "SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit" on page 173.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at –20 °C, or proceed directly to emulsion PCR in the *Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378) or the *Applied Biosystems SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (PN 4441486).

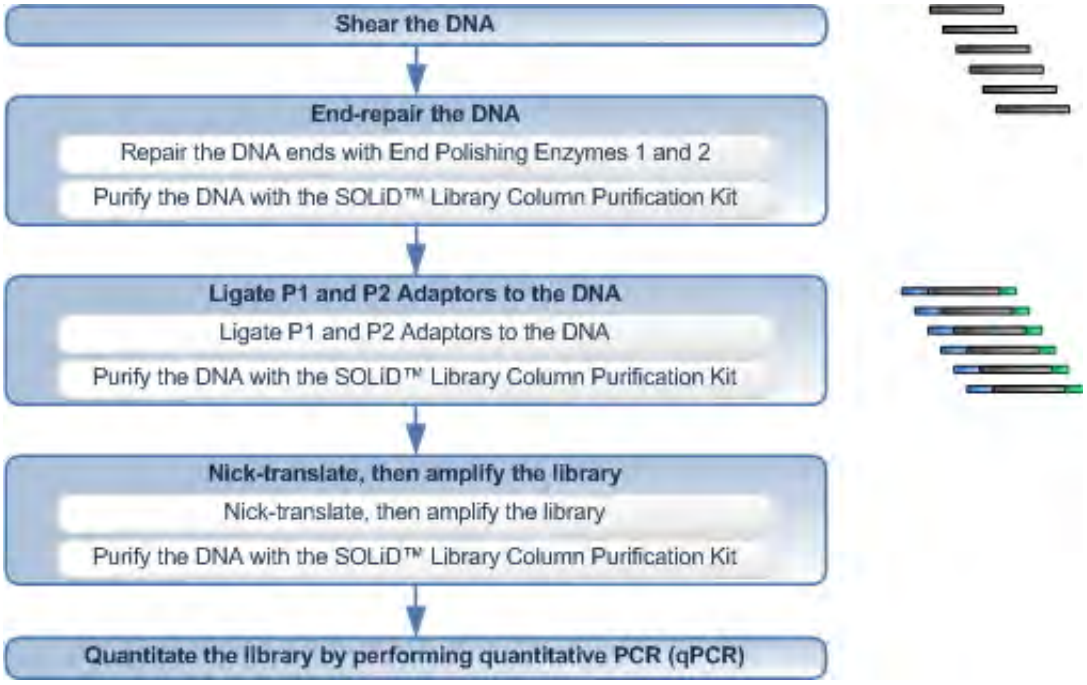
Section 2.2 Prepare an express fragment library

This protocol is designed for 10 ng to 5 µg of genomic DNA or ligated PCR product. You should modify the protocol with any change in the starting amount of DNA. If you are constructing a targeted resequencing library with small-sized PCR products (≤500 bp), then you must perform a PCR-product ligation step. For a concatenation protocol, contact your field applications specialist.

Materials and equipment required

See Appendix A on page 145 for a list of equipment, kits, and consumables necessary for this procedure.

Workflow



Workflow overview

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 10 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 ATP 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

P1 and P2 Adaptors are ligated to the ends of the end-repaired DNA. The P1 and P2 Adaptors are included in double-stranded form in the SOLiD™ Fragment Library Oligos Kit.

Nick-translate, then amplify the library

The adaptor-ligated, purified DNA undergoes nick translation, then amplification using Library PCR Primer 1, Library PCR Primer 2, and Platinum® PCR Amplification Mix. After amplification, the PCR samples are purified with the SOLiD™ Library Column Purification Kit.

Quantitate the library by performing quantitative PCR (qPCR)

The library is quantitated by using the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), described in Appendix B, "SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit" on page 173.

Tips

- 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.

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 1X Low TE Buffer in a LoBind tube (see Table 7).

Table 7 Dilute the DNA for shearing

Component	Amount
DNA	10 ng to 5 µg
1X Low TE Buffer	Variable
Total	100 µL

2. 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.

3. 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 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. 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.

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 186.

4. 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 8):

Table 8 Mix for end-repair of DNA

Component	Volume (µL)
Sheared DNA	100
5× 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 approximately 700 µL of end-repaired DNA in the binding buffer to the column(s). The maximum yield of DNA can be achieved if the amount of DNA loaded to a PureLink™ 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 (see Appendix C, "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189). 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 ºC, or proceed directly to "Ligate P1 and P2 Adaptors to the DNA" on page 37.

Ligate P1 and P2 Adaptors to the DNA

Ligate P1 and 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, see "Ligation of P1 and P2 Adaptors" on page 216). 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):

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

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

Example:

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

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

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

$$= 5.5\ \text{µL adaptor needed}$$

2. Combine (see Table 9):

Table 9 Ligation mix

Component	Volume (µL)
P1 Adaptor (ds), 50 pmol/µL	*Y*
P2 Adaptor (ds), 50 pmol/µL	*Y*
5X T4 Ligase 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.
2. Apply approximately 700 µL of the ligated DNA in the binding buffer to the column(s). The maximum yield of DNA can be achieved if the amount of DNA loaded to a PureLink™ 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, 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 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" on page 39.

Nick-translate, then amplify the library

Nick-translate, then amplify the library

1. Prepare a PCR reaction mix (see Table 10).

Table 10 PCR reaction mix: a mix for nick translation and amplification of the library

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

2. Pipet 125 µL of the PCR reaction mix into each of four PCR tubes.

3. Run the PCR (Table 11).

IMPORTANT! Minimize the number of cycles to avoid overamplification and production of redundant molecules. Determine the number of cycles based on the amount of starting input DNA.

Table 11 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 10 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:
10 ng to 100 ng: 10 cycles
100 ng to 1 µg: 6 to 8 cycles
1 µg to 2 µg: 4 to 6 cycles
2 µg to 5 µg: 2 to 3 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 approximately 700 µL of PCR product in the binding buffer to the column(s). The maximum yield of DNA can be achieved if the amount of DNA loaded to a PureLink™ column is ≤5 µg. Use more columns if necessary. Keep for now.
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 using the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), described in Appendix B, "SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit" on page 173.

STOPPING POINT. Store the DNA in Elution Buffer (E1) at –20 °C, or proceed directly to emulsion PCR in the *Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378) or the *Applied Biosystems SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (PN 4441486).

3 Mate-Paired Library Preparation

This chapter covers:

Overview

This chapter describes the method to make a mate-paired library with insert sizes ranging from 600 bp to 6 kb. A mate-paired library consists of pairs of DNA fragments that are "mates" because they originated from the two ends of the same genomic DNA fragment. CAP adaptors connect the DNA mate pair together through an internal adaptor.

For 2 × 50 bp mate-paired libraries, size-selected genomic DNA fragments are ligated to LMP CAP Adaptors and circularized with internal adaptors (see Figure 7). The resulting DNA circle has one nick in each strand because the LMP CAP Adaptor does not have the 5′ phosphate in one of its oligonucleotides. Nick translation using *E. coli* DNA polymerase I "pushes" the nick into the genomic DNA region in 5′ to 3′ direction. The length of nick-translated DNA can be controlled by adjusting reaction temperature and time. T7 exonuclease and S1 nuclease digestion cuts the DNA at the position opposite to the nick and releases the DNA mate pair. P1 and P2 Adaptors are then ligated to the ends of the mate-paired library for subsequent amplification by PCR (see Figure 9 on page 45).

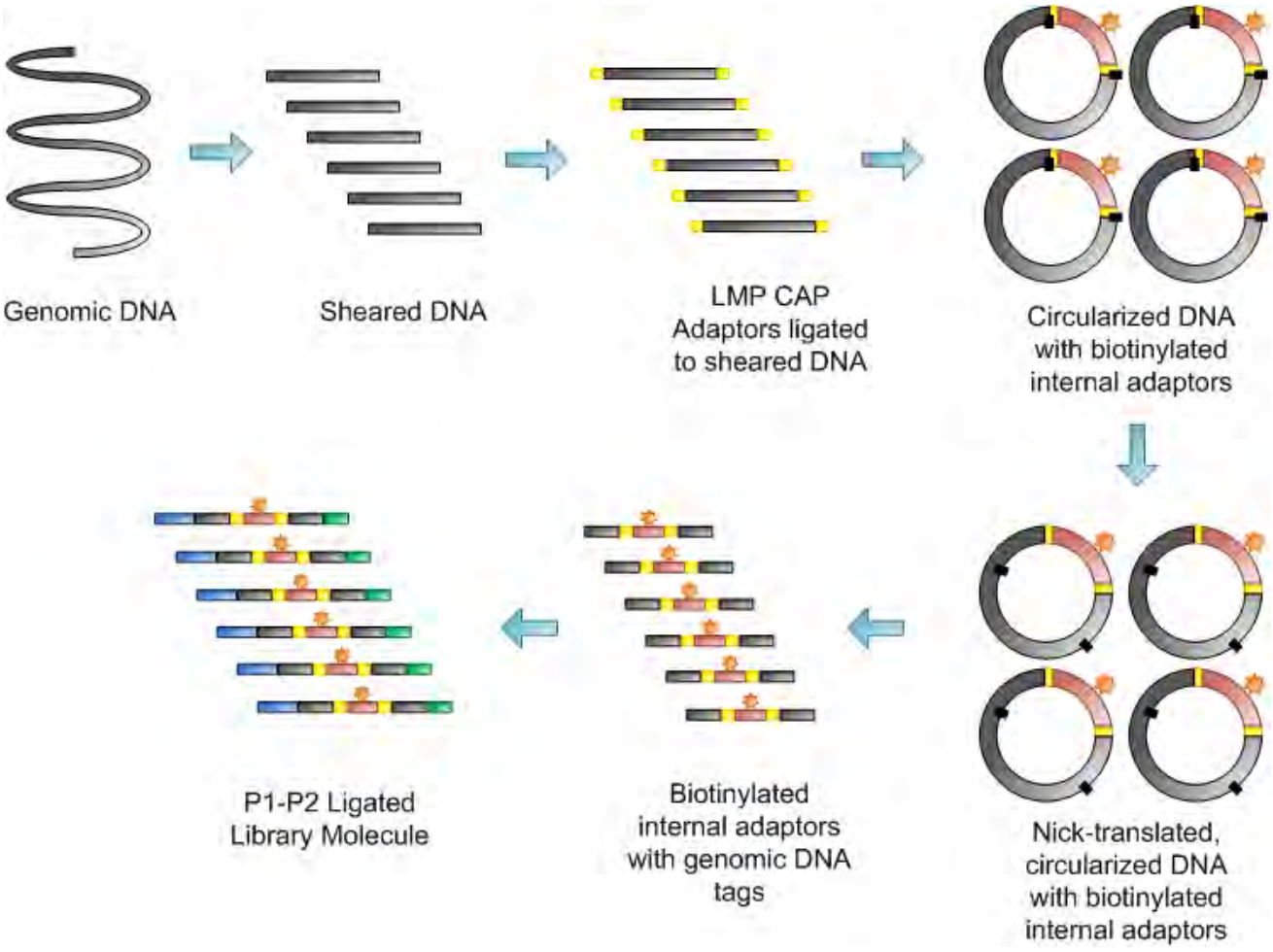


Figure 7 Basic 2 × 50 bp mate-paired library preparation workflow.

For 2 × 25 bp mate-paired libraries, EcoP15I CAP Adaptors are ligated to sheared, methylated DNA (see Figure 8). The EcoP15I restriction enzyme sites in the genomic DNA are methylated prior to EcoP15I CAP Adaptor ligation to ensure that only the unmethylated enzyme recognition sites in the CAP adaptor are recognized by EcoP15I during the restriction enzyme step. As a result, EcoP15I cleaves 25 to 27 bp away from the unmethylated enzyme recognition sites in the CAP linker, yielding mate-paired genomic DNA attached to either side of the internal adaptor. P1 and P2 Adaptors are then ligated to the ends of the mate-paired library for subsequent amplification by PCR (see Figure 9 on page 45).

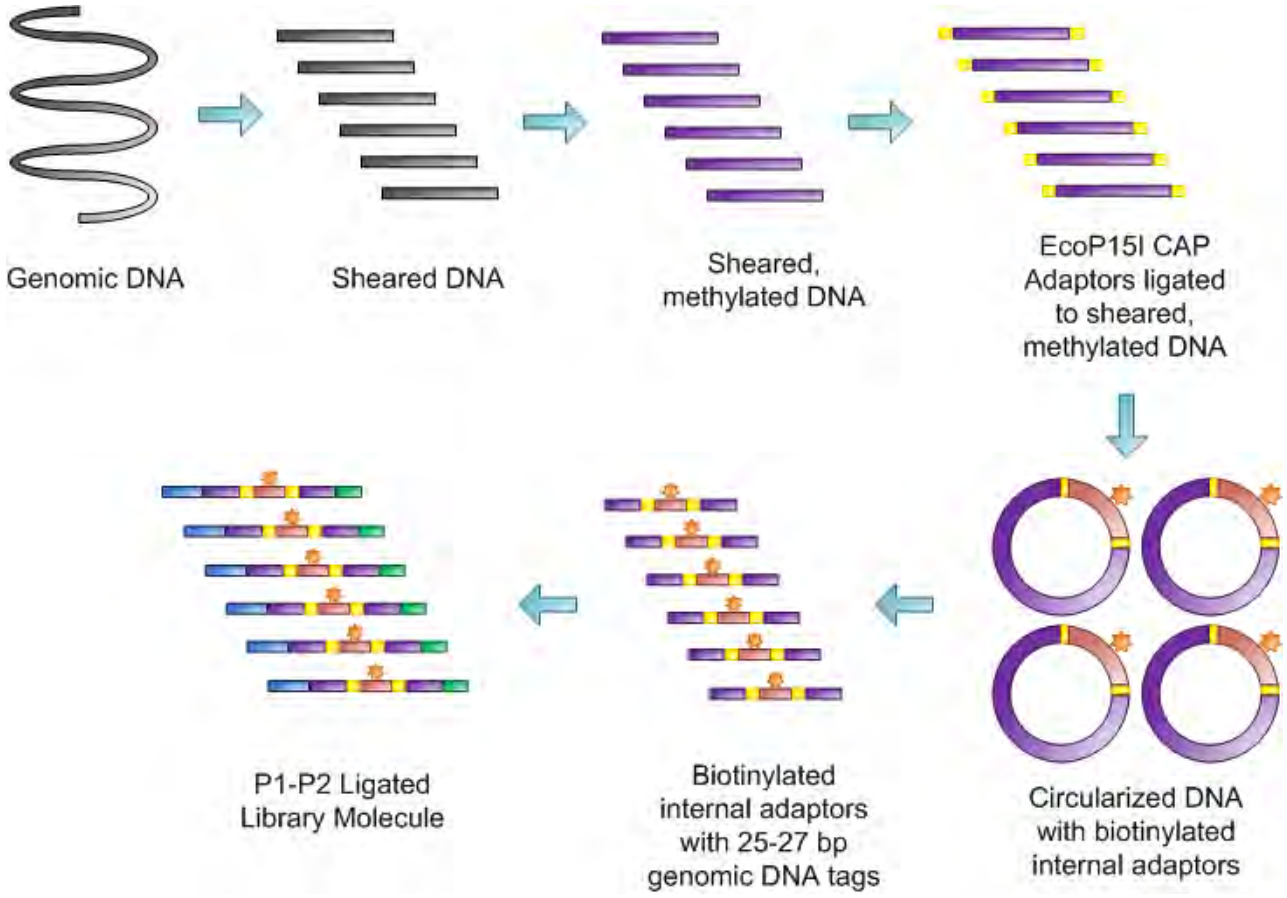


Figure 8 Basic 2 × 25 bp mate-paired library preparation workflow.

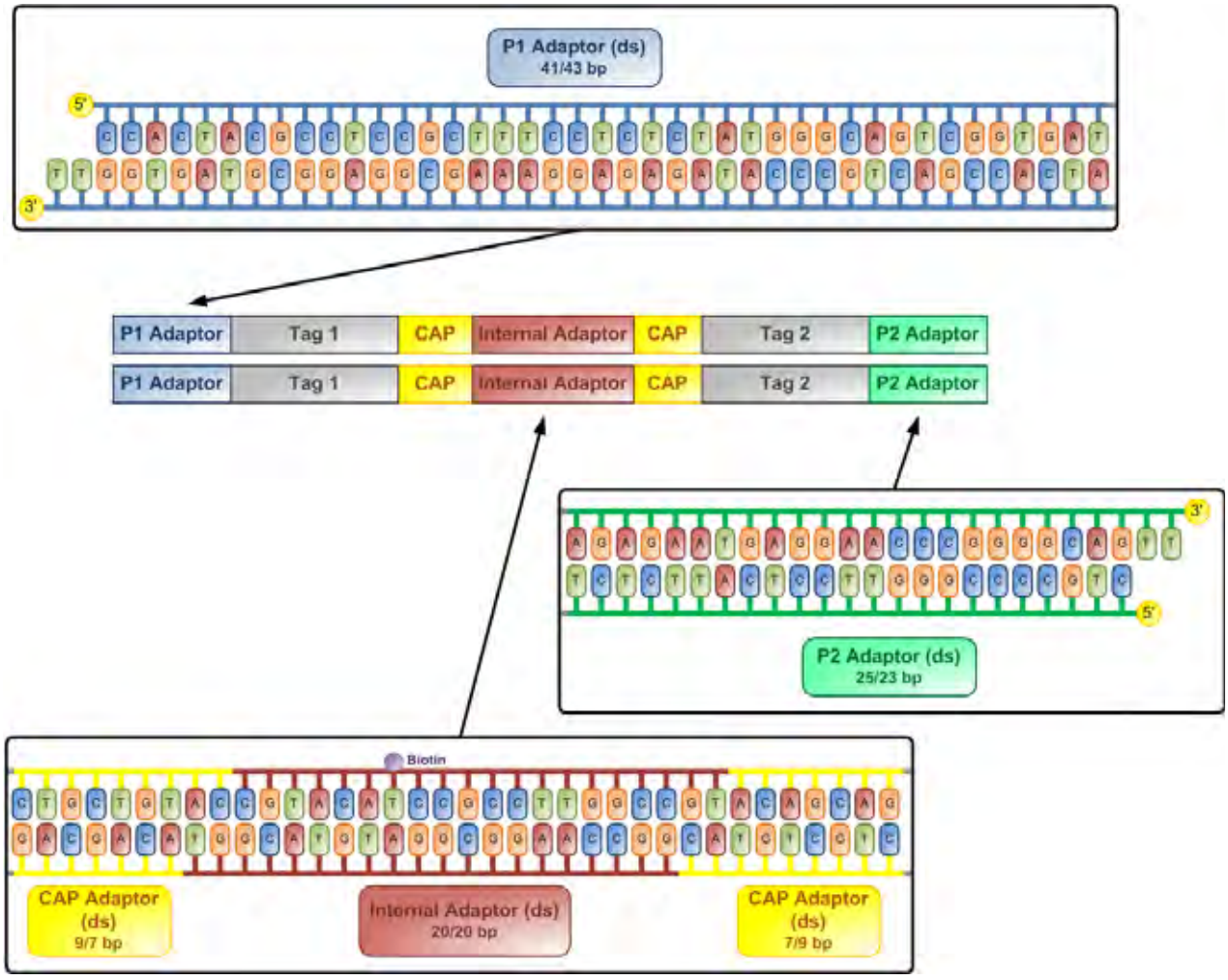


Figure 9 Mate-paired library design.

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 10 on page 46). 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, because they do not have 3′ sequences compatible with the sequencing primers.

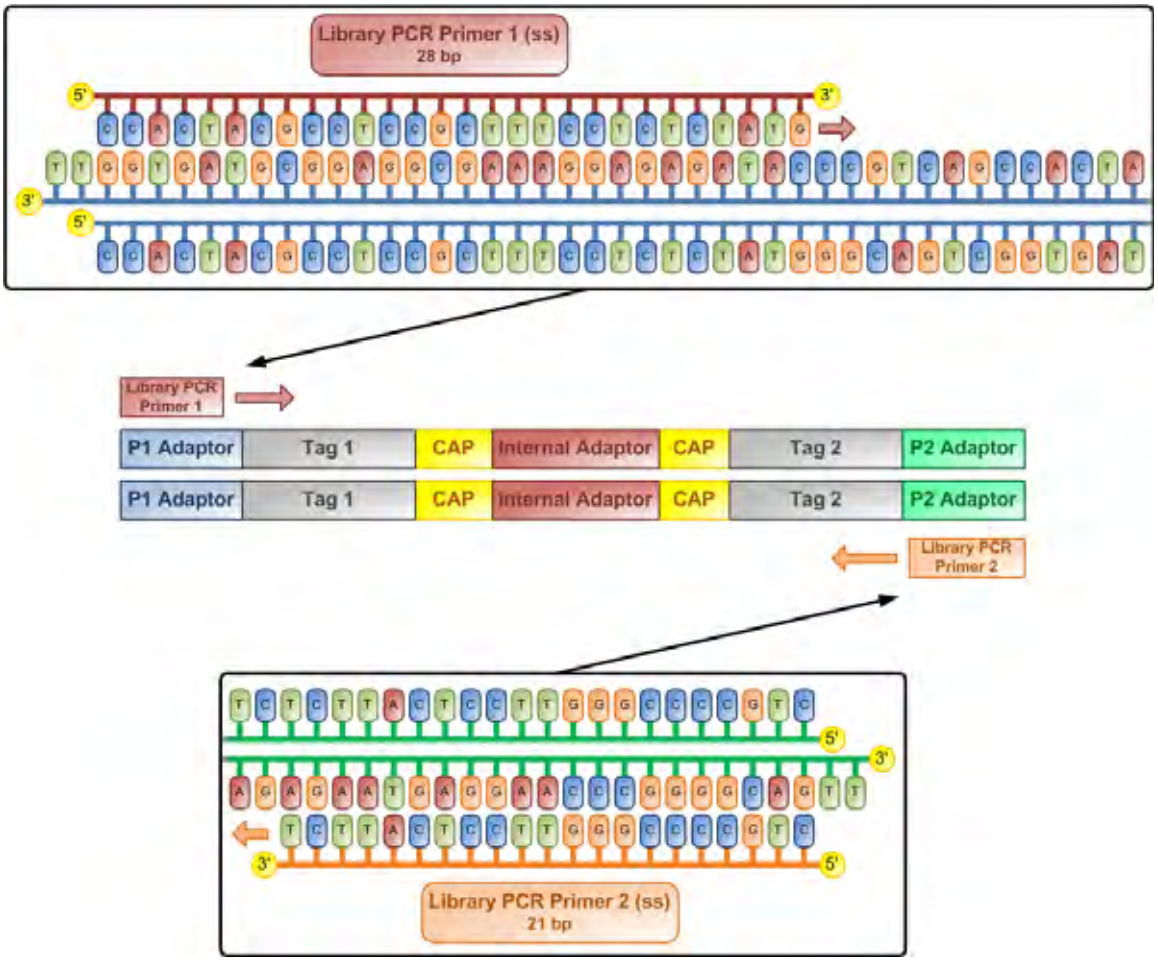


Figure 10 Mate-paired library amplification design.

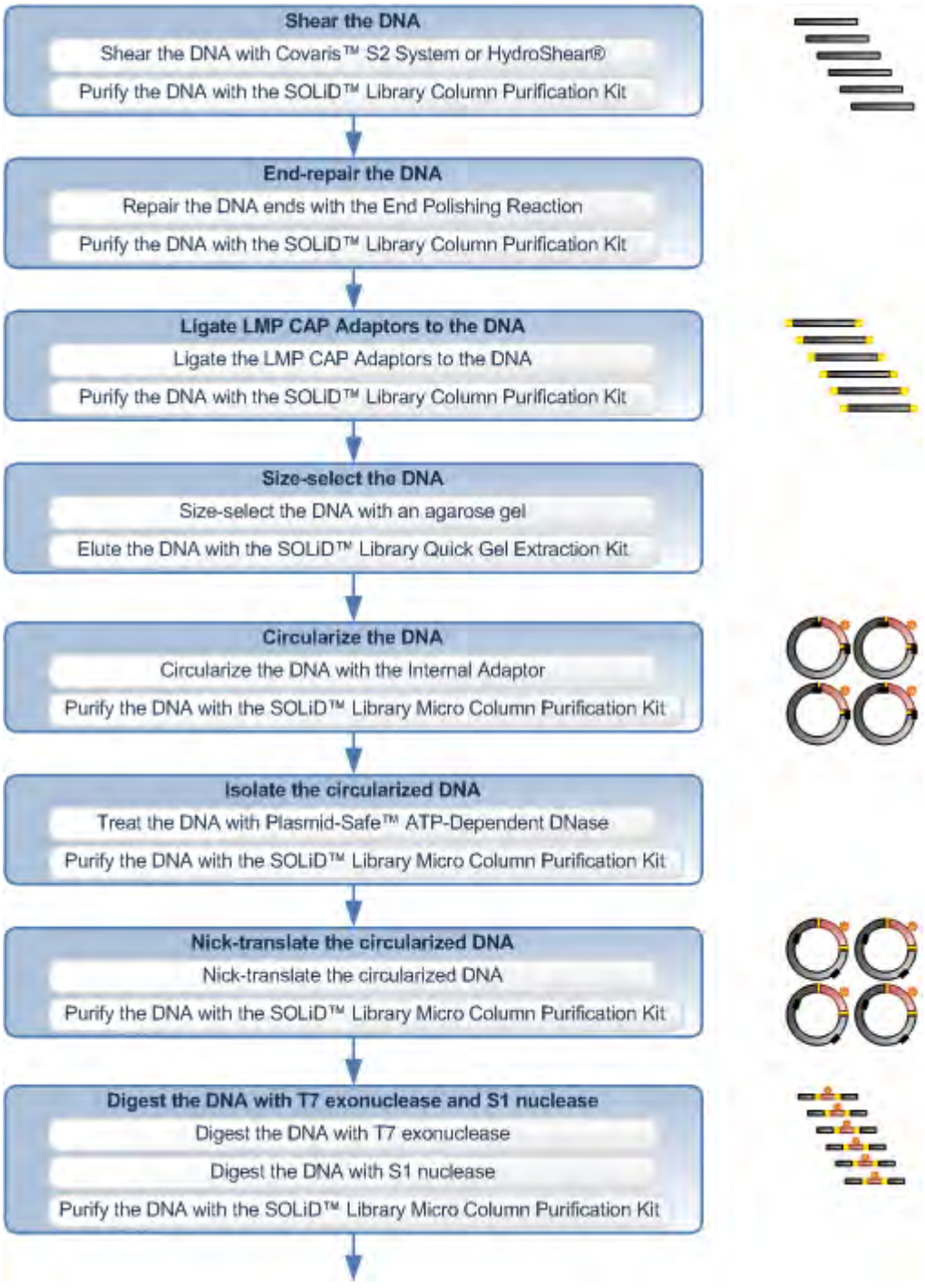
This chapter is organized into two sections. Section 3.1 describes how to generate a 2 × 50 bp mate-paired library. Section 3.2 describes how to generate a 2 × 25 bp mate-paired library.

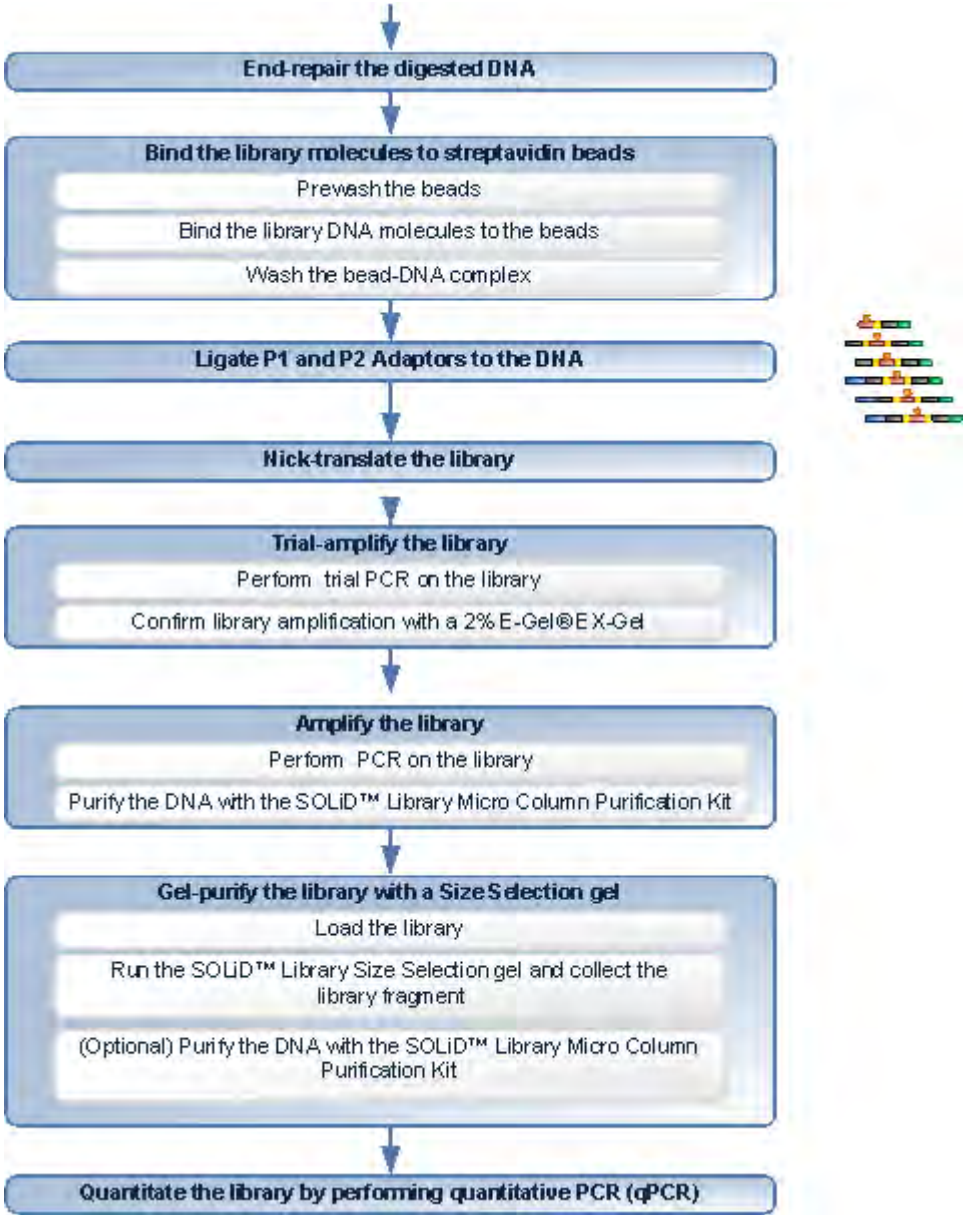
Section 3.1 Prepare a 2 × 50 bp mate-paired library

Materials and equipment required

See Appendix A on page 145 for a list of equipment, kits, and consumables necessary for this procedure.

Workflow


Shear the DNA
Shear the DNA with Covaris™ S2 System or HydroShear®
Purify the DNA with the SOLiD™ Library Column Purification Kit
End-repair the DNA
Repair the DNA ends with the End Polishing Reaction
Purify the DNA with the SOLiD™ Library Column Purification Kit
Ligate LMP CAP Adaptors to the DNA
Ligate the LMP CAP Adaptors to the DNA
Purify the DNA with the SOLiD™ Library Column Purification Kit
Size-select the DNA
Size-select the DNA with an agarose gel
Elute the DNA with the SOLiD™ Library Quick Gel Extraction Kit
Circularize the DNA
Circularize the DNA with the Internal Adaptor
Purify the DNA with the SOLiD™ Library Micro Column Purification Kit
Isolate the circularized DNA
Treat the DNA with Plasmid-Safe™ ATP-Dependent DNase
Purify the DNA with the SOLiD™ Library Micro Column Purification Kit
Nick-translate the circularized DNA
Nick-translate the circularized DNA
Purify the DNA with the SOLiD™ Library Micro Column Purification Kit
Digest the DNA with T7 exonuclease and S1 nuclease
Digest the DNA with T7 exonuclease
Digest the DNA with S1 nuclease
Purify the DNA with the SOLiD™ Library Micro Column Purification Kit



Workflow overview

Shear the DNA

The genomic DNA is sheared to yield 600 bp to 6 kb fragments. To shear for a mate-paired library with insert sizes between 600 bp and 1 kb, the Covaris™ S2 System is recommended. To shear for a mate-paired library with insert sizes between 1 kb and 6 kb, the HydroShear® DNA Shearing Device is recommended.

The Covaris S2 System shears the DNA into fragments through sonication. Follow the manufacturer's guidelines and test the recommended shearing conditions.

The HydroShear DNA Shearing Device uses hydrodynamic shearing forces to fragment DNA strands. Perform an initial standard run, and adjust for DNA from different organisms as needed.

Note: A calibration run to assess the shearing efficacy of your device prior to starting your first library preparation is highly recommended.

Sample purification is performed with PureLink™ columns supplied in the SOLiD™ Library Column Purification Kit and the SOLiD™ Library Micro Column Purification Kit. PureLink columns have a 40-µg capacity, and PureLink™ Micro columns have a 5-µg capacity. For maximum recovery, load <30 µg of DNA onto one PureLink column. Use multiple columns if necessary. All columns can be loaded multiple times if the volume of initial DNA and binding buffer mixture exceeds the volume capacity of the column. For more detailed information on purification of DNA with PureLink columns, see the manufacturer's instructions. *If you have larger amounts of DNA for library construction, you can substitute this step with phenol-chloroform-isoamyl alcohol extraction and isopropyl alcohol precipitation* (see Appendix C, "Supplemental Procedures" on page 185).

End-repair the DNA

For fast and efficient blunt-ended ligation, End Polishing Enzyme 1 and End Polishing Enzyme 2 are used to convert DNA with damaged or incompatible 5′ -protruding and/or 3′ -protruding ends to 5′ -phosphorylated, blunt-ended DNA. The conversion to blunt-end DNA is accomplished by exploiting the 5′ -to-3′ polymerase and the 3′ -to-5′ exonuclease activities of End Polishing Enzyme 2. End Polishing Enzyme 1 and ATP are also included for phosphorylation of the 5′ -ends of the blunt-ended DNA for subsequent ligation.

Ligate LMP CAP Adaptors to the DNA

LMP CAP ligation adds the LMP CAP Adaptors to the sheared, end-repaired DNA. The LMP CAP Adaptor is missing a 5′ phosphate from one of its oligonucleotides, which results in a nick on each strand when the DNA is circularized in a later step. The LMP CAP Adaptors are included in double-stranded form in the SOLiD™ Mate-Paired Library Oligos Kit.

Size-select the DNA

Size-selection is performed after CAP adaptor ligation to remove unbound CAP adaptors. Depending on the desired insert-size range, the ligated, purified DNA is run on a 0.8% or 1% agarose gel. The correctly sized ligation products are excised and purified using the SOLiD™ Library Quick Gel Extraction Kit.

IMPORTANT! *Size selection should not be skipped under any circumstances.* Contamination of unbound CAP adaptors can compromise the circularization reaction in the next step.

Circularize the DNA

Sheared DNA ligated to LMP CAP Adaptors is circularized with a biotinylated internal adaptor. To increase the chances that ligation will occur between two ends of one DNA molecule versus two different DNA molecules, a very dilute reaction is used. The circularization reaction products are purified using the SOLiD™ Library Quick Gel Extraction Kit. The Internal Adaptor is included in double-stranded form in the SOLiD™ Mate-Paired Library Oligos Kit.

Isolate the circularized DNA

Plasmid-Safe™ ATP-Dependent DNase is used to eliminate uncircularized DNA. After the Plasmid-Safe DNase-treated DNA is purified using the SOLiD Library Micro Column Purification Kit, the amount of circularized product is quantified.

To proceed with library construction, a minimum of 100 ng of circularized product, based on NanoDrop® Instrument's nucleic acid measurement, is recommended. For more complex genomes, more circularized DNA is recommended for a high-complexity library.

Nick-translate the circularized DNA

Nick translation using *E. coli* DNA polymerase I translates the nick into the genomic DNA region. The size of the mate-paired tags to be produced can be controlled by adjusting the reaction temperature and time.

Digest the DNA with T7 exonuclease and S1 nuclease

T7 exonuclease recognizes the nicks within the circularized DNA. With its 5′ -to-3′ exonuclease activity, T7 exonuclease digests the unligated strand away from the tags creating a gap in the sequence. This gap creates an exposed single-stranded region that is more easily recognized by S1 nuclease, so the library molecule can be cleaved from the circularized template.

End-repair the DNA

For fast and efficient blunt-ended ligation, End Polishing Enzyme 1 and End Polishing Enzyme 2 are used to convert DNA with damaged or incompatible 5′ -protruding and/or 3′ -protruding ends to 5′ -phosphorylated, blunt-ended DNA. The conversion to blunt-end DNA is accomplished by exploiting the 5′ -to-3′ polymerase and the 3′ -to-5′ exonuclease activities of End Polishing Enzyme 2. End Polishing Enzyme 1 and ATP are also included for phosphorylation of the 5′ ends of the blunt-ended DNA for subsequent ligation.

Bind the DNA molecules to the streptavidin beads

Dynabeads® MyOne™ Streptavidin C1 specifically bind to the biotin-labeled Internal Adaptor in the library molecules to purify the library from side products.

Ligate P1 and P2 Adaptors to the DNA

P1 and P2 adaptors are ligated to the ends of the end-repaired DNA. The P1 and P2 Adaptors are included in double-stranded form in the SOLiD™ Mate-Paired Library Oligos Kit.

Wash the DNA-bound streptavidin beads

Library molecules bound to streptavidin beads are washed and purified from ligation side products.

Nick-translate the library

The ligated, purified DNA undergoes nick translation with DNA polymerase I.

Trial-amplify the library

The library is trial-amplified using Library PCR Primers 1 and 2 with the Platinum® PCR Amplification Mix. The mix includes a proofreading enzyme for high-fidelity amplification, to determine the number of PCR cycles so that the amplified library is just visible on 2% E-Gel® EX Gel.

Amplify the library

The library is amplified using Library PCR Primers 1 and 2 with the Platinum® PCR Amplification Mix, which includes a proofreading enzyme for high-fidelity amplification. Reduce the number of cycles as much as possible and use the entire nick-translated product for amplification to get maximum representation of the library and to avoid PCR-related biases due to differential amplification of library molecules.

Gel-purify the library with a Size Selection gel

The library is run on an SOLiD™ Library Size Selection gel. The library band (250 to 350 bp) can be extracted and desalted using the SOLiD Library Micro Column Purification Kit.

Quantitate the library by performing quantitative (qPCR)

The library is quantitated by using the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), described in Appendix B, “SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit” on page 173).

Tips

- 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, 1.5-mL, and 2-mL tubes with Eppendorf LoBind Tubes.
- Thaw reagents on ice before use.

Shear the DNA

Prepare for shearing

IMPORTANT! For accuracy, determine sample DNA concentration using a double-stranded DNA-specific fluorescence assay. Assays recommended are the Invitrogen Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, P7589), the Invitrogen Quant-iT™ dsDNA HS Assay Kit (Invitrogen, Q32851 or Q32854); or the Invitrogen Quant-iT™ dsDNA BR Assay Kit (Invitrogen, Q32850 or Q32853).

1. Choose the appropriate shearing method based on the desired insert size of the mate-paired library (see Table 12 on page 54).

Note: These conditions are guidelines. A shearing trial prior to large-scale shearing is recommended if additional DNA is available.

Table 12 Recommended shearing conditions for mate-paired library insert sizes.

Insert size	Shearing method	Shearing conditions
600 to 800 bp	Covaris™ S2 shearing in 20% glycerol (13 mm × 65 mm borosilicate tube)	• Number of Cycles: **75** • Bath Temperature: **5** °C • Bath Temperature Limit: **12** °C • Mode: **Frequency sweeping** • Water Quality Testing Function: **Off** • Duty cycle: **2%** • Intensity: **7** • Cycles/burst: **200** • Time: **10 seconds**
800 to 1000 bp	Covaris™ S2 shearing in 20% glycerol (13 mm × 65 mm borosilicate tube)	• Number of Cycles: **30** • Bath Temperature: **5** °C • Bath Temperature Limit: **12** °C • Mode: **Frequency sweeping** • Water Quality Testing Function: **Off** • Duty cycle: **2%** • Intensity: **5** • Cycles/burst: **200** • Time: **10 seconds**
1 to 2 kb	HydroShear® Standard Shearing Assembly	• SC5‡ • 20 cycles
2 to 3 kb	HydroShear® Standard Shearing Assembly	• SC9 • 20 cycles
3 to 4 kb	HydroShear® Standard Shearing Assembly	• SC13 • 20 cycles
4 to 5 kb	HydroShear® Standard Shearing Assembly	• SC15 • 5 cycles
5 to 6 kb	HydroShear® Standard Shearing Assembly	• SC16 • 25 cycles

‡ Speed code (SC): 5.

IMPORTANT! If you are using the Covaris™ S2 System, 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.

2. If the DNA source is not limiting, ensure that the shearing conditions result in the desired insert sizes. Shear 5 µg DNA and run 150 ng sheared DNA on a 1% E-Gel® EX Gel according to the manufacturer's specifications.

Shear the DNA with the Covaris™ S2 System

1. In a round-bottomed 13-mm × 65-mm borosilicate tube, dilute 10 to 20 µg of DNA in 500 µL solution so that the final volume contains 20% glycerol in Nuclease-free Water (see Table 13).

   Note: To prepare a short-insert (≤1 kb) mate-paired library from a small genome, ≥5 µg of DNA is sufficient.

Table 13 Dilute the DNA for shearing

Component	Amount
UltraPure™ Glycerol	100 µL
DNA	10 to 20 µg
Nuclease-free Water	Variable
Total	500 µL

2. Shear the DNA using the Covaris™ S2 System shearing program described in Table 12 on page 54.
3. Transfer 500 µL of sheared DNA into a clean 1.5-mL LoBind tube.
4. Wash the borosilicate tube with 100 µL of Nuclease-free Water and transfer the wash to the 1.5-mL LoBind tube. Mix by vortexing and then proceed to "Purify the DNA with the SOLiD™ Library Column Purification Kit" on page 56.

Shear the DNA with the HydroShear® DNA Shearing Device

1. In 1.5-mL LoBind Tubes, dilute 10 to 20 µg of DNA to 150 µL with Nuclease-free Water. If you are starting with an input >20 µg, split the DNA into ≤20 µg aliquots and shear each aliquot in 150-µL volume. For better coverage of large and complex genomes, more DNA should be used if it is available.
2. On the Edit Wash Scheme tab, specify the solution and cycles:
   - 2 cycles of WS1 (0.2 N HCl)
   - 2 cycles of WS2 (0.2 N NaOH)
   - 3 cycles of Nuclease-free Water
3. Run the wash scheme on the HydroShear DNA Shearing Device.
4. Adjust the speed code (SC) and number of cycles according to Table 12 on page 54, and adjust the volume setting to 150 µL.
5. Begin shearing. Repeat the shearing for the other aliquot of DNA, if applicable. It is not necessary to run the wash cycle if both tubes contain the same DNA.
6. Run the wash scheme after DNA shearing is complete.
7. Pool the aliquots of sheared DNA, if applicable.

Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
2. Apply about 700 µL of the sample in the binding buffer to the PureLink™ column(s) in collection tube(s).
3. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through.
4. Repeat steps 2 and 3 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
5. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
6. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at *maximum speed* to remove residual wash buffer.
7. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
8. Add 50 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
9. Centrifuge the column(s) at *maximum speed* for 1 minute.
10. Add the eluate from step 9 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
11. Centrifuge the column(s) at *maximum speed* for 1 minute.
12. If necessary, pool the eluted DNA.
13. Quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189).

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "End-repair the sheared DNA" on page 57.

End-repair the sheared DNA

Repair the DNA ends with the end polishing reaction

Note: This reaction is optimal for ≤20 µg of starting material. If > 20 µg of starting material is used for shearing, scale up the reaction as needed.

1. Combine and mix the following components in a LoBind tube (see Table 14):

Table 14 Combine components for end-repair of DNA

Component	Amount
Sheared DNA	48 µL
5X End Polishing Buffer	20 µL
dNTP, 10 mM	2.5 µL
End Polishing Enzyme 1, 10 U/µL	3 µL
End Polishing Enzyme 2, 5 U/µL	8 µL
Nuclease-free Water	18.5 µL
Total	100 µL

2. Incubate the mixture at room temperature (20 to 25 °C) for 30 minutes.

Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
2. Apply about 700 µL of the sample in the binding buffer to the PureLink™ column(s) in collection tube(s).
3. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
4. Repeat steps 2 and 3 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
5. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
6. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at *maximum speed* to remove residual wash buffer.
7. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
8. Add 50 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
9. Centrifuge the column(s) at *maximum speed* for 1 minute.
10. Add the eluate from step 9 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
11. Centrifuge the column(s) at *maximum speed* for 1 minute.

12. If necessary, pool the eluted DNA.

13. Quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189).

    STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Ligate LMP CAP Adaptors to the DNA" on page 59.

14. (Optional) For structural variation studies where tighter size selection of fragments is required, perform an additional size selection: see "Size-select the DNA" on page 61. After size-selection and purification, proceed to "Ligate LMP CAP Adaptors to the DNA" on page 59. An additional size selection will reduce the yield significantly. If a narrow insert-size distribution is not critical, proceed directly to "Ligate LMP CAP Adaptors to the DNA" on page 59.

Ligate LMP CAP Adaptors to the DNA

Ligate the LMP CAP 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 (see "Ligation of LMP CAP Adaptors" on page 216).

   Example:

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

$$\textbf{\textit{Y}}\ \text{µL adaptor needed} = \#\ \text{µg DNA} \times \frac{\textbf{\textit{X}}\ \text{pmol}}{1\ \text{µg DNA}} \times 100 \times \frac{1\ \text{µL adaptor needed}}{50\ \text{pmol}}$$

Example:

For 12 µg of purified end-repaired DNA with an average insert size of 1.5 kb:

$$\textbf{\textit{X}}\ \text{pmol/µg DNA} = 1\ \text{µg DNA} \times \frac{10^6\ \text{pg}}{1\ \text{µg}} \times \frac{1\ \text{pmol}}{660\ \text{pg}} \times \frac{1}{1500} = 1.0\ \text{pmol/µg DNA}$$

$$\textbf{\textit{Y}}\ \text{µL adaptor needed} = 12\ \text{µg DNA} \times \frac{1.0\ \text{pmol}}{1\ \text{µg DNA}} \times 100 \times \frac{1\ \text{µL adaptor needed}}{50\ \text{pmol}}$$

$$= 24\ \text{µL adaptor needed}$$

2. Combine and mix the components below (see Table 15). If a larger reaction volume is required to incorporate all of the DNA, scale up the T4 DNA Ligase and 5✕ Ligase Buffer. Add 1 µL of T4 DNA Ligase per 20 µL of reaction volume. Add 1 µL of 5✕ Ligase Buffer per 5 µL of reaction volume.

   Table 15 Ligation mix

Component	Volume (µL)
LMP CAP Adaptor (ds), 50 µM	*Y*
5✕ Ligase Buffer	40
T4 DNA Ligase	10
DNA	Variable
Nuclease-free Water	Variable
Total	200

3. Incubate the reaction mixture at room temperature (20 to 25 °C) for 15 minutes.

Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
2. Apply about 700 µL of the sample in the binding buffer to the PureLink™ column(s) in collection tube(s).
3. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
4. Repeat steps 2 and 3 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
5. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
6. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at *maximum speed* to remove residual wash buffer.
7. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
8. Add 50 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
9. Centrifuge the column(s) at *maximum speed* for 1 minute.
10. Add the eluate from step 9 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
11. Centrifuge the column(s) at *maximum speed* for 1 minute.
12. If necessary, pool the eluted DNA.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Size-select the DNA" on page 61.

Size-select the DNA

Size-select the DNA with an agarose gel

1. Determine the appropriate percentage of agarose gel needed to size-select DNA (see Table 16).

Table 16 Percent agarose gel needed to size-select DNA

Desired insert size	Agarose gel needed (%)
600 to 3000 bp	1.0
3 to 6 kb	0.8

2. Prepare the appropriate percentage agarose gel in 1✕ TAE buffer with 10 µL of 1:10,000 SYBR® Safe gel stain (Invitrogen, S33102) per 100 mL gel volume. To prepare the gels, use either Agarose-LE (Applied Biosystems, AM9040) or UltraPure™ Agarose 1000 (Invitrogen, 10975-035).

3. Add 10✕ BlueJuice™ Gel Loading Buffer to the purified ligated DNA (1 µL of 10✕ Gel Loading Buffer for every 10 µL of mate-paired library).

4. Load the 1 Kb Plus DNA Ladder (Invitrogen, 10787-018) to one well. Load dye-mixed sample per well according to the well capacity into remaining wells. Use the minimum number of wells possible. There should be at least one lane between the ladder well and the sample wells to avoid contamination of the sample with ladder.

5. Run the gel at the appropriate voltage to achieve optimal separation of the size of interest.

IMPORTANT! To obtain maximum resolution of DNA fragments, agarose gels should be run at ≤ 5 V/cm. The distance is measured as the shortest path between the electrodes, not the agarose gel length itself.

6. Visualize the gel on a Safe Imager™ Blue Light Transilluminator with a ruler lying on top of the transilluminator.

IMPORTANT! Exposing DNA to UV light may damage the DNA. Using SYBR® Safe gel stain and the Safe Imager™ Blue Light Transilluminator eliminates the risk of UV damage to DNA during size selection.

7. Using the ladder bands and the ruler for reference, excise the band of the gel corresponding to the insert size range of interest with a clean razor blade (see Figure 11 on page 62). If desired, take a tighter cut for a tighter size selection. If the gel piece is large, slice it into smaller pieces.

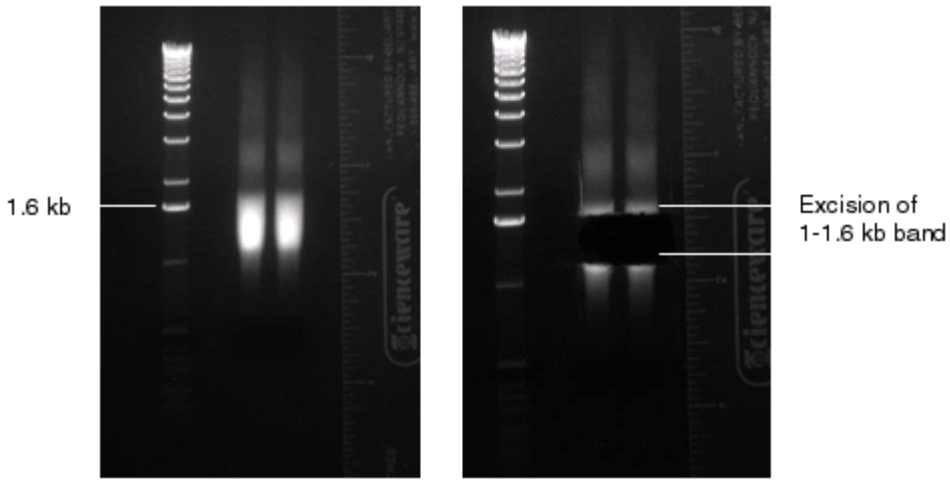


Figure 11 Excision of 1-1.6 kb range in a 1.0% agarose gel.

Elute the DNA using the SOLiD™ Library Quick Gel Extraction Kit

1. Weigh the gel slice in a 15-mL polypropylene conical colorless tube.
2. Add 30 µL of Gel Solubilization Buffer (L3) for every 10 mg of gel.
3. Dissolve the gel slice by vortexing the tube at *room temperature* until the gel slice has dissolved completely (~15 minutes).

> **IMPORTANT!** Do *not* heat the gel to dissolve the gel slice. When heated, the DNA denatures and short-insert libraries form heteroduplexes. Heteroduplexes are deleterious to the library.

4. Add 1 gel volume of isopropanol to the dissolved gel slice. For example, add 10 µL of isopropanol to 10 mg of gel. Mix well.
5. Apply the dissolved gel mixture to the Quick Gel Extraction column(s) in Wash Tube(s). Use one column per 400 mg agarose or load ≤2000 µL of dissolved gel mixture per column. Use more columns if necessary.
6. Centrifuge the column(s) at > 12,000 × g for 1 minute, then discard the flow-through and place the column back on the Wash Tube(s).
7. Add 700 µL of Wash Buffer (W1) with ethanol to the Quick Gel Extraction column(s).
8. Centrifuge the column(s) at > 12,000 × g for 1 minute, then discard the flow-through.
9. Centrifuge the Quick Gel Extraction column(s) again at *maximum speed* for 2 minutes to remove any residual Wash Buffer.
10. Transfer the Quick Gel Extraction column(s) to clean 1.5-mL LoBind tube(s).

11. Add 50 µL of Elution Buffer (E1, from the SOLiD™ Library Column Purification Kit, *not* Buffer E5 from the SOLiD™ Library Quick Gel Extraction Kit) to the center of the column(s) to elute the DNA, then let the column(s) stand for 5 minutes at room temperature.

Note: For large fragments, increasing the incubation time to 10 minutes will increase the yield.

12. Centrifuge the column(s) at > 12,000 × g for 1 minute. The 1.5-mL LoBind tube(s) contain the purified DNA.

13. Add the eluate from step 13 back to the Quick Gel Extraction column(s), then let the column(s) stand for 1 minute at room temperature.

14. Centrifuge the column(s) at > 12,000 × g for 1 minute.

15. If necessary, pool the eluted DNA into one 1.5-mL LoBind tube.

16. Quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189).

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 °C, or proceed directly to "Circularize the DNA with the Internal Adaptor" on page 64.

Circularize the DNA

Circularize the DNA with the Internal Adaptor

1. Prepare a circularization reaction by mixing the following components listed (in order) based on the desired insert size, where *X* is the number of micrograms of DNA to be circularized (see Table 17). If a larger reaction volume is required, scale up the T4 DNA Ligase and 5✕ Ligase Buffer. Add 1 µL of T4 DNA Ligase per 40 µL of reaction volume. Add 1 µL of 5✕ Ligase Buffer per 5 µL of reaction volume.

Table 17 Mix for DNA circularization by insert size

Components	600 to 800 bp	800 to 1000 bp	1 to 2 kb	2 to 3 kb	3 to 4 kb	4 to 5 kb	5 to 6 kb
Nuclease-free Water	Variable	Variable	Variable	Variable	Variable	Variable	Variable
DNA	*X* µg	*X* µg	*X* µg	*X* µg	*X* µg	*X* µg	*X* µg
5✕ Ligase Buffer	(*X* × 47) µL	(*X* × 54) µL	(*X* × 73) µL	(*X* × 100) µL	(*X* × 112) µL	(*X* × 125) µL	(*X* × 144) µL
Internal Adaptor (ds), 2 µM	(*X* × 3.75) µL	(*X* × 2.84) µL	(*X* × 1.5) µL	(*X* × 0.9) µL	(*X* × 0.65) µL	(*X* × 0.5) µL	(*X* × 0.4) µL
T4 DNA Ligase, 5 U/µL	(*X* × 6) µL	(*X* × 6.75) µL	(*X* × 9) µL	(*X* × 12.5) µL	(*X* × 14) µL	(*X* × 15.6) µL	(*X* × 18) µL
Total	(*X* × 235) µL	(*X* × 270) µL	(*X* × 365) µL	(*X* × 500) µL	(*X* × 560) µL	(*X* × 625) µL	(*X* × 720) µL

Example

For 2 µg of DNA in 1 to 2 kb size range to be circularized:

Mix for DNA circularization by insert size

Components	Amount
Nuclease-free Water	Variable
DNA	2 µg
5✕ Ligase Buffer	146 µL
Internal Adaptor (ds), 2 µM	3 µL
T4 DNA Ligase, 5 U/µL	18 µL
Total	730 µL

2. Incubate the reaction at room temperature (20 to 25 °C) for 30 minutes.

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

IMPORTANT! If > 6 µg DNA was used in the circularization reaction, use a SOLiD™ Library Column Purification Kit, then follow the steps in "Purify the DNA with the SOLiD™ Library Column Purification Kit" on page 60. Make these changes to the procedure:

- Use 1 column per 5 mL of sample in Binding Buffer (B2-L) with isopropanol (40%).
- Load ≤800 µL sample in Binding Buffer each time onto the column(s). Spin the column(s) for 15 seconds at 10,000 × g except for the last loading. After the last loading, spin the column(s) for 1 minute.
- Use 40 µL of Elution Buffer (E1) to elute DNA from the column(s).

Unless specified otherwise, use the SOLiD™ Library Micro Column Purification Kit for all other steps after circularization.

1. Pre-spin empty PureLink™ Micro columns in collection tubes at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-L) with isopropanol (40%) to 1 volume of sample. Mix well. Use one PureLink Micro column per 4 to 5 mL of sample in Binding Buffer (B2-L).
3. Apply ≤800 µL of sample in the binding buffer to the PureLink Micro column(s) in collection tube(s).
4. Centrifuge the column(s) at 10,000 × g for 15 seconds except for the last loading. After each spin, discard the flow-through. After the last loading, spin the column(s) for 1 minute. dsDNA is bound to the column.
5. Repeat steps 3 and 4 until the entire sample has been loaded onto the column(s). Place the PureLink Micro column(s) back into the same collection tube(s).
6. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
7. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
8. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
9. Add 25 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
10. Centrifuge the column(s) at 14,000 × g for 1 minute.
11. Add the eluate from step 10 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
12. Centrifuge the column(s) at 14,000 × g for 1 minute.

13. If necessary, pool the eluted DNA.

IMPORTANT! Proceed directly without stopping to "Isolate the circularized DNA".

Isolate the circularized DNA

Treat the DNA with Plasmid-Safe™ ATP-Dependent DNase

1. Combine and mix the following components, where *X* is the volume in µL of DNA and *Y* is the number of micrograms of DNA used in the circularization reaction (see Table 18).

Table 18 Mix for DNase treatment of DNA

Component	Volume (µL)
ATP, 25 mM	5
10× Plasmid-Safe™ Buffer	10
Plasmid-Safe™ DNase, 10 U/µL	(*Y* ÷ 3)‡
DNA	*X*
Nuclease-free Water	Variable
Total	100

‡ Use 1 µL of Plasmid-Safe™ DNase, 10 U/µL if $Y \leq 3$ µg.

If $X > 80$ µL, adjust the total reaction volume accordingly. The volume of ATP and 10× Plasmid-Safe™ Buffer should be proportional to the total reaction volume.

Example

For 2 µg DNA used in the circularization reaction:

Mix for DNase treatment of DNA

Component	Volume (µL)
ATP, 25 mM	5
10× Plasmid-Safe™ Buffer	10
Plasmid-Safe™ DNase, 10 U/µL	1
DNA	25
Nuclease-free Water	59
Total	100

2. Incubate the reaction mixture at 37 °C for 40 minutes.

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

1. Pre-spin empty PureLink™ Micro columns in collection tubes at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
3. Apply the sample in the binding buffer to the PureLink Micro column(s) in collection tube(s).
4. Let the column(s) stand for 1 minute at room temperature.
5. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
6. Add 500 µL of additional Binding Buffer (B2-S) with isopropanol (55%) to wash the column(s).
7. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
8. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
9. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
10. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
11. Add 25 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
12. Centrifuge the column(s) at 14,000 × g for 1 minute.
13. Add the eluate from step 12 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
14. Centrifuge the column(s) at 14,000 × g for 1 minute.
15. If necessary, pool the eluted DNA into one 1.5-mL LoBind tube.
16. Quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189).

IMPORTANT! DNA must be quantitated with the NanoDrop® ND-1000 Spectrometer, *not* by PicoGreen® or any other fluorescence assay.

If you have:

- ≥ 100 ng DNA (NanoDrop measurement), proceed directly without stopping to "Nick-translate the circularized DNA".
- < 100 ng DNA (NanoDrop measurement), prepare the library again with any necessary changes to improve yield. Restart the library preparation from "Shear the DNA" on page 53.

Nick-translate the circularized DNA

Nick-translate the circularized DNA

IMPORTANT! Incubate the nick translation reaction at 5 °C on a thermocycler using the "No heated lid" feature. DNA polymerase I is very sensitive to slight changes in temperature. Before adding enzyme to the reaction mix for nick translation, chill the enzyme and the reaction mix *separately* in a thermocycler at 5 °C according to the procedure below.

1. Calculate the amount of DNA polymerase I (10 U/µL) needed (*Y* µL) in the nick-translation reaction. For *X* ng of circularized DNA, use (*X* ÷ 100) µL of DNA polymerase I, where *X* is the number of nanograms of circularized DNA. If *X* < 400 ng (*Y*<4 µL), use 4 µL.

2. Prepare the reaction mix in a 0.2-mL PCR tube (see Table 19 on page 69):

Table 19 Reaction mix for nick translation of DNA

Component	Amount
dNTP, 10 mM	(*X* ÷ 80) µL‡
Nick Translation Buffer	10 µL
DNA	*X* ng§
Nuclease-free Water	Variable
Total	(100–*Y*) µL

‡ If *X* < 400 ng, use 5 µL.
§ If X > 1 µg, divide the DNA into <1 µg aliquots, then set up parallel nick-translation reactions.

Example

For 200 ng of circularized DNA, because *X* <400 ng, 4 µL DNA polymerase I (10 U/µL), is required.

Mix the reaction mix components:

Component	Amount
dNTP, 10 mM	5 µL
Nick Translation Buffer	10 µL
DNA	200 ng
Nuclease-free Water	Variable
Total	96 µL

3. Incubate the mix without DNA polymerase I at 5 °C in a thermocycler for for approximately 5 minutes.
4. In a 0.2-mL tube, add *Y* µL of DNA polymerase I, then pulse spin.
5. Incubate the DNA polymerase I at 5 °C in a thermocycler for at least 1 minute.
6. Set the timer to 9 minutes.

Note: Incubating the nick translation reaction for 8 to 10 minutes at 5 °C on a calibrated thermocycler generates a final library with the desired size (~300 bp). If necessary, adjust the reaction time on the same thermocycler according to the results from the first library.

7. Transfer all of the reaction mix to the tube containing the DNA polymerase I incubating at 5 °C, then pipet up and down the total reaction mix 5 times to mix.
8. Start the timer.
9. Prepare 400 µL of Binding Buffer (B2-S) with isopropanol (55%) in a 1.5-mL LoBind tube.

10. At the end of the incubation, *immediately* transfer the nick translation reaction to the 1.5-mL LoBind tube containing the binding buffer. Binding buffer denatures the enzyme and stops the reaction.

11. Proceed to "Purify the DNA with the SOLiD™ Library Micro Column Purification Kit".

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

1. Pre-spin empty PureLink™Micro columns in collection tubes at 10,000 × g for 1 minute before use.
2. Mix well the nick-translated DNA in Binding Buffer (B2-S) with isopropanol (55%).
3. Apply all of the mix to the PureLink Micro column(s) in collection tube(s).
4. Let the column(s) stand for 1 minute at room temperature.
5. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
6. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
7. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
8. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
9. Add 25 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
10. Centrifuge the column(s) at 14,000 × g for 1 minute.
11. Add the eluate from step 10 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
12. Centrifuge the column(s) at 14,000 × g for 1 minute.
13. If necessary, pool the eluted DNA into one 1.5-mL LoBind tube.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 °C or proceed directly to "Digest the DNA with T7 exonuclease and S1 nuclease" on page 71.

Digest the DNA with T7 exonuclease and S1 nuclease

Digest the DNA with T7 exonuclease

1. Combine (see Table 20):

Table 20 T7 exonuclease reaction mix from *X* ng circularized DNA

Component	Amount
DNA	From *X* ng circularized DNA
10× Buffer 4	(X ÷ 20) µL
T7 exonuclease, 10 U/µL	(*X* ÷ 80) µL
Nuclease-free Water	Variable
Total	(X ÷ 2) µL

Example

For 200 ng of circularized DNA:

Component	Amount
DNA	From 200 ng circularized DNA
10× Buffer 4	10 µL
T7 exonuclease, 10 U/µL	2.5 µL
Nuclease-free Water	Variable
Total	100 µL

2. Incubate the reaction mixture at 37 °C for 30 minutes.
3. Heat inactivate the T7 exonuclease at 70 °C for 20 minutes.
4. Chill the reaction on ice for 5 minutes.

Digest the circularized DNA with S1 nuclease

1. Freshly dilute 1 µL of S1 Nuclease to 25 U/µL with S1 Nuclease Dilution Buffer.
2. Combine (see Table 21):

Table 21 S1 nuclease reaction mix

Component	Amount
T7 exonuclease-digested DNA	(*X* ÷ 2) µL
3 M NaCl	(*X* ÷ 60) µL
S1 nuclease, 25 U/µL	(*X* ÷ 50) µL

Example

For T7 exonuclease-digested DNA from 200 ng of circularized DNA:

Component	Amount
T7 exonuclease-digested DNA	100 µL
3 M NaCl	3.3 µL
S1 nuclease, 25 U/µL	4 µL

3. Incubate the reaction mixture at 37 °C for 30 minutes. *Immediately* proceed to the next step, "Purify the DNA with the SOLiD™ Library Micro Column Purification Kit".

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

1. Pre-spin empty PureLink™ Micro columns in collection tubes at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
3. Apply the sample in the binding buffer to the PureLink Micro column(s) in collection tube(s).
4. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
5. Repeat steps 3 and 4 until the entire sample has been loaded onto the column(s). Place the PureLink Micro column(s) back into the same collection tube(s).
6. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
7. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
8. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
9. Add 25 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.

10. Centrifuge the column(s) at 14,000 × g for 1 minute.
11. Add the eluate from step 10 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
12. Centrifuge the column(s) at 14,000 × g for 1 minute.
13. If necessary, pool the eluted DNA into one 1.5-mL LoBind tube.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 °C or proceed directly to "End-repair the digested DNA".

End-repair the digested DNA

1. Combine (see Table 22):

Table 22 Reaction mix

Component	Amount
S1-digested DNA	*X* ng
5× End Polishing Buffer	20 µL
dNTP, 10 mM	2.5 µL
End Polishing Enzyme 1, 10 U/µL	1 µL
End Polishing Enzyme 2, 5 U/µL	2 µL
Nuclease-free Water	Variable
Total	100 µL

2. Incubate the reaction mix at room temperature (20 to 25 °C) for 30 minutes.
3. Stop the reaction by combining and mixing (see Table 23):

Table 23 Stop end-repair mix

Component	Volume (µL)
End-repaired DNA	100
0.5 M EDTA	5
Bead Binding Buffer	200
Nuclease-free Water	95
Total	400

Bind the library molecules to streptavidin beads

Prewash the beads

1. Combine (see Table 24):

Table 24 Prepare 1× BSA solution

Component	Volume (µL)
100× BSA	5
Nuclease-free Water	495
Total	500

2. Vortex the tube of Dynabeads® MyOne™ Streptavidin C1, then transfer 90 µL of the beads into a 1.5-mL LoBind Tube.
3. Add 500 µL of Bead Wash Buffer to the 90 µL of solution of beads, vortex the beads for 15 seconds, then pulse-spin.
4. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
5. Add 500 µL of 1× BSA and vortex for 15 seconds, then pulse-spin the tube.
6. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
7. Add 500 µL of Bead Binding Buffer. Vortex the beads for 15 seconds, then pulse-spin.
8. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

Bind the library DNA molecules to the beads

1. Add the entire 400 µL of solution of library DNA in Bead Binding Buffer (see Table 23 on page 73) to the pre-washed beads, then vortex.
2. Rotate the solution at room temperature (20 to 25 °C) for 30 minutes, then pulse-spin.

Wash the bead-DNA complex

1. Combine (see Table 25):

Table 25 Prepare 1× Ligase Buffer

Component	Volume (µL)
5× Ligase Buffer	120
Nuclease-free Water	480
Total	600

2. Place the tube with the bead-DNA complex in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
3. Resuspend the beads in 500 µL of Bead Wash Buffer, then transfer the beads to a new 1.5-mL LoBind tube. Vortex the beads for 15 seconds, then pulse-spin.
4. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
5. Resuspend the beads in 500 µL of Bead Wash Buffer. Vortex the beads for 15 seconds, then pulse-spin.
6. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
7. Repeat steps 5 and 6 once.
8. Resuspend the beads in 500 µL of 1× Ligase Buffer. Vortex the beads for 15 seconds, then pulse-spin.
9. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
10. Resuspend the beads in 94 µL of 1× Ligase Buffer.

Ligate P1 and P2 Adaptors to the DNA

1. Calculate the amount of P1 and P2 Adaptors needed for the ligation reaction based on the amount of circularized DNA from "Isolate the circularized DNA" on page 66 and the calculation below. For calculation details, see Appendix E, "Formulas and calculations" on page 215.

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

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

Example:
For 1 µg of purified circularized DNA with an average size of 1536 (1500 bp insert + 36 bp internal adaptor)

$$X \text{ pmol/µg DNA} = 1 \text{ µg DNA} \times \frac{10^6 \text{ pg}}{1 \text{ µg}} \times \frac{1 \text{ pmol}}{660 \text{ pg}} \times \frac{1}{1536} = 1 \text{ pmol/µg DNA}$$

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

$$= 0.6 \text{ µL adaptor needed}$$

2. Combine (see Table 26):

Table 26 Combine for ligation of end-repaired DNA to P1 and P2 Adaptors

Component	Volume (µL)
DNA-bead complex	94
P1 Adaptor (ds), 50 µM	*Y*
P2 Adaptor (ds), 50 µM	*Y*
T4 DNA Ligase, 5 U/µL	5
Total	Variable (~100)

3. Incubate the reaction mixture at room temperature (20 to 25 °C) on a rotator for 15 minutes.

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

5. Resuspend the beads in 500 µL of Bead Wash Buffer and transfer the beads to a new 1.5-mL LoBind tube. Vortex the beads for 15 seconds, then pulse-spin.

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

7. Resuspend the beads in 500 µL of Bead Wash Buffer. Vortex the beads for 15 seconds, then pulse-spin.

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

9. Repeat steps 7 and 8 once.

Nick-translate the library

1. Combine (see Table 27):

Table 27 Combine for nick translation

Component	Volume (µL)
Nuclease-free Water	83
Nick Translation Buffer	10
dNTP, 10 mM	5
DNA Polymerase I, 10 U/µL	2
Total	100

2. Add the mix for nick translation to the washed beads.

3. Incubate the mixture at room temperature (20 to 25 °C) for 15 minutes.

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

5. Resuspend the beads in 500 µL of Elution Buffer (E1) from the SOLiD™ Library Column Purification Kit. Vortex, then pulse-spin the beads.

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

7. Resuspend the beads in 30 µL of Elution Buffer (E1).

STOPPING POINT. Store the DNA-Bead complexes in Elution Buffer (E1) at 4 ºC, or proceed directly to "Trial-amplify the library" on page 78.

Trial-amplify the library

Perform Trial PCR on the library

1. Prepare a PCR master mix for amplification reactions (see Table 28):

Table 28 PCR master mix for amplification of the library

Component	Volume (µL)
Platinum® PCR Amplification Mix‡	70
Library PCR Primer 1, 50 µM	1.4
Library PCR Primer 2, 50 µM	1.4
Total	72.8

‡ Platinum® PCR Amplification Mix contains a proofreading enzyme for high-fidelity amplification.

2. Vortex the master mix. For the negative control, transfer 23 µL of the PCR master mix to a PCR tube. Label the tube "PCR #0".

3. Add 4 µL of DNA-bead complex solution to the remaining 49.8 µL of PCR master mix. Vortex the mix, then divide evenly (~25µL) between two PCR tubes labelled "PCR #1" and "PCR #2".

4. Run (see Table 29):

Table 29 PCR conditions to amplify the library

Stage	Step	Temp	Time
Holding	Denature	94 °C	3 min
Cycling‡	Denature	94 °C	15 sec
	Anneal	62 °C	15 sec
	Extend	70 °C	1 min
Holding	Extend	70 °C	5 min
Holding	—	4 °C	∞

‡ Tube #1: 10 cycles.
Tubes #0 and #2: 14 cycles.

Confirm library amplification with a 2% E-Gel® EX Gel

1. Mix 0.5 µL of 1 µg/µL 100-bp DNA Ladder (Invitrogen, 10628-050) with 40 µL of Nuclease-free Water.

2. Load 20 µL of PCR #0, PCR #1, and PCR #2 into separate wells of a 2% E-Gel® EX Gel. Load 20 µL of diluted 100-bp DNA Ladder in an adjacent well for reference. Do not add any loading dye to the samples or DNA Ladder.

3. Run the E-Gel EX Gel on an E-Gel® iBase™ Power System, according to the manufacturer's instructions, for 10 minutes.

4. Take a picture of the gel (see Figure 12). Ideally, the size of amplified library should be between 275 and 325 bp, but any size of amplified library ranging from 250 to 350 bp is acceptable. Choose a PCR cycle where amplified library products are just visible on the gel. Based on the intensity of the products, increase or reduce up to 2 cycles for final library amplification.

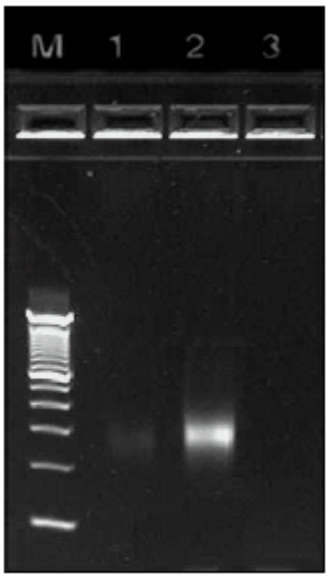


Figure 12 Mate-paired library trial amplification sample run on a 2% E-Gel® EX Gel. M: 100-bp DNA Ladder; Lane 1: PCR #1 (10 cycles); Lane 2: PCR #2 (14 cycles); Lane 3: PCR #0 (negative control). Based on this picture, use 10 cycles for final library amplification.

STOPPING POINT. Store the DNA-Bead complexes in Elution Buffer (E1) at 4 ºC, or proceed directly to "Amplify the library" on page 80.

Amplify the library

Perform PCR on the library

1. Prepare a master mix for amplification reactions (see Table 30):

Table 30 PCR master mix for amplification of the library

Component	Volume (µL)
Platinum® PCR Amplification Mix‡	350
Library PCR Primer 1, 50 µM	7
Library PCR Primer 2, 50 µM	7
Total	364

‡ Platinum® PCR Amplification Mix contains a proofreading enzyme for high-fidelity amplification.

2. For the negative control, aliquot 50 µL of PCR master mix to a PCR tube. Add 5 µL of Nuclease-free Water to the tube.

3. Add 26 µL of DNA-bead complex solution to the remaining 314 µL of PCR master mix. Vortex to mix, then divide evenly (~84 µL) among four PCR tubes.

4. Run (see Table 31):

Table 31 PCR conditions to amplify the library

Stage	Step	Temp	Time
Holding	Denature	94 °C	3 min
Cycling‡	Denature	94 °C	15 sec
	Anneal	62 °C	15 sec
	Extend	70 °C	1 min
Holding	Extend	70 °C	5 min
Holding	—	4 °C	∞

‡ Cycling number determined by trial amplification. See "Trial-amplify the library" on page 78.

5. Pool all of the PCR samples into a 1.5-mL LoBind tube.

6. Place the tube of beads in a magnetic rack, then transfer the supernatant to a fresh 2-mL LoBind tube. Discard the tube containing the beads.

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

1. Pre-spin an empty PCR Micro column in a collection tube at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-L) with isopropanol (40%) to 1 volume of sample.
3. Apply 750 µL of the PCR product in the binding buffer to the PureLink Micro column in a collection tube.
4. Centrifuge the column at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
5. Repeat steps 3 and 4 until the entire sample has been loaded onto the column. Place the PureLink Micro column back into the same collection tube.
6. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column.
7. Centrifuge the column at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
8. Transfer the column to a clean 1.5-mL LoBind tube.
9. Add 25 µL of Elution Buffer (E1) to the center of the column to elute the DNA, then let the column stand for 1 minute at room temperature.
10. Centrifuge the column at 14,000 × g for 1 minute.
11. Add the eluate from step 10 back to the column, then let the column stand for 1 minute at room temperature (20 to 25 °C).
12. Centrifuge the column at 14,000 × g for 1 minute.
13. Run 1 µL of the concentrated library on a DNA 1000 Chip on the Agilent Technologies 2100 Bioanalyzer (see Figure 13 on page 82). If the library size distribution is between 250 and 350 bp — and there *are no PCR by-products < 200 bp detectable* (see Figure 13B on page 82)— skip "Gel-purify the library with a Size Selection gel" on page 82. Proceed directly to "Quantitate the library by performing quantitative PCR (qPCR)" on page 87.

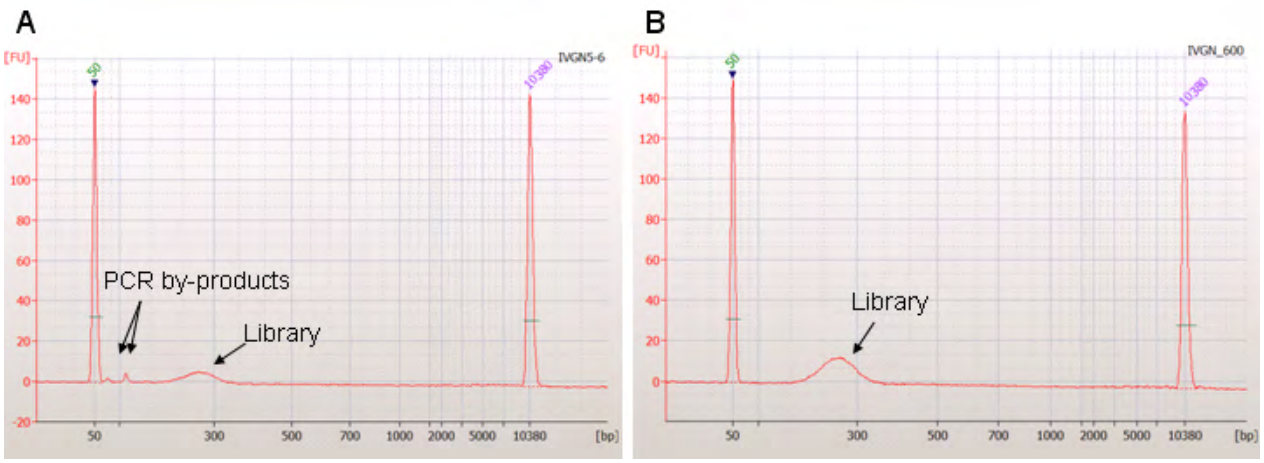


Figure 13 Bioanalyzer electropherograms of libraries that were amplified by PCR and were purified with the SOLiD™ Library Micro Column Purification Kit. 3A, A library with PCR by-products that needs to be gel-purified with a SizeSelect gel. 3B, A library without PCR by-products that can be quantitated by qPCR.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 °C or proceed directly to "Gel-purify the library with a Size Selection gel" or "Quantitate the library by performing quantitative PCR (qPCR)" on page 87 as required.

Gel-purify the library with a Size Selection gel

Load the library

1. Plug the adapter plug of the E-Gel® iBase™ Power System into an electrical outlet.
2. Remove the SOLiD™ Library Size Selection gel from its package, then insert the gel with its combs into the iBase system:
   a. Slide the gel into the two electrode connections on the iBase system. Ensure that the two electrodes on the right side of the cassette touch the two contacts on the iBase system. The Invitrogen logo should be at the bottom of the base.
   b. Press the gel firmly at the top and bottom to seat the gel in the iBase system. If the gel is correctly inserted, a red light glows.
3. Remove the combs.
4. Load ≤ 500 ng of sample in 25 µL volume without loading dye into the wells of the *top* row. Use Nuclease-free Water as diluent if necessary. 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 500 ng of DNA per lane (see Figure 14 on page 83).

5. Mix 0.5 µL of 1 µg/µL of 100 bp DNA Ladder (Invitrogen, 15628-050) with 20 µL of Nuclease-free Water. Load 10 µL of the diluted DNA ladder into the small middle well of the top row (marked "M"; see Figure 14).

6. Load 25 µL of Nuclease-free Water into remaining empty wells in the top row.

7. Load 25 µL of Nuclease-free Water into wells 1 to 8 in the *middle* of the gel and 10 µL of Nuclease-free Water in the middle marker well of the bottom row (see Figure 14).

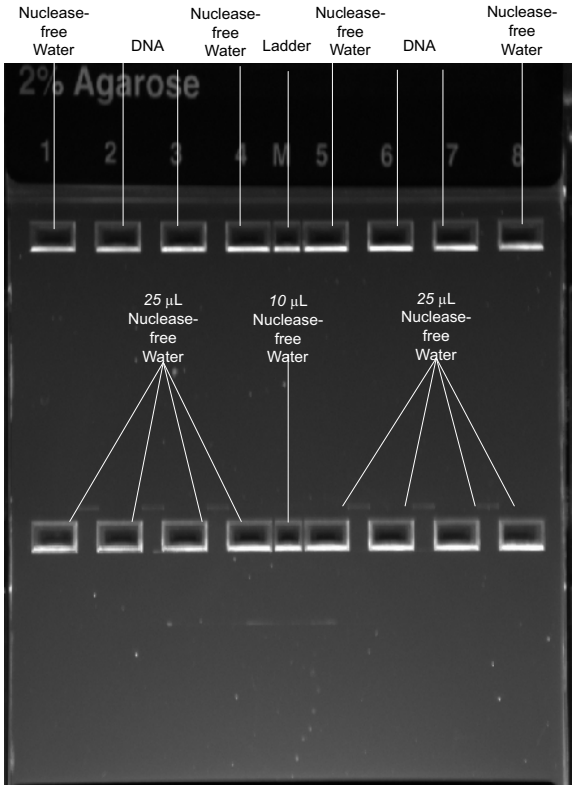


Figure 14 Where to load DNA, ladder, and Nuclease-free Water on a SOLiD™ Library Size Selection gel to size-select the DNA.

Run the SOLiD™ Library Size Selection gel and collect the library fragment

1. Place the E-Gel® iBase™ Power System over a Safe Imager™ Real-Time Transilluminator. Use the orange cover or orange goggles to view the bands and to avoid overexposure of your eyes to the blue light.

2. Run the gel. On the iBase system:

   a. Select **SizeSelect 2%** (refer to the iBase Power System manual for instructions).

   b. Press **Go**. The red light turns green.

3. Monitor the gel. At the end of a run, the iBase system flashes a red light and beeps rapidly:
   - If the front line of library products has not reached the reference line, run the gel for about 1 to 2 more minutes until the band reaches the line. The ideal size of a library is from 275 to 325 bp, but a library ranging from 250 to 350 bp is acceptable.
   - When the front line of library products reaches the reference line, press **Go** to stop the run. Proceed to step 4.

4. When the front line of library products reaches the reference line, refill the bottom row again with Nuclease-free Water until each well is full. Some pre-filled water is lost during the run.

5. Press **Go** to run the gel until the library products enter the collection well. For optimal results, monitor the run in a darkened room.

6. Collect all of the DNA from the collection well using a 20-µL pipette fitted with a tip (see Figure 15 on page 85). Do not perforate the bottom of the agarose collection well. Due to migration of the DNA into the bottom of the well, some residual DNA remains underneath the well.

IMPORTANT! If the library products overrun the collection well and reenter the gel, select **REVERSE E-Gel** on the iBase Power System to run the library products backward into the collection well. Collect all of the DNA.

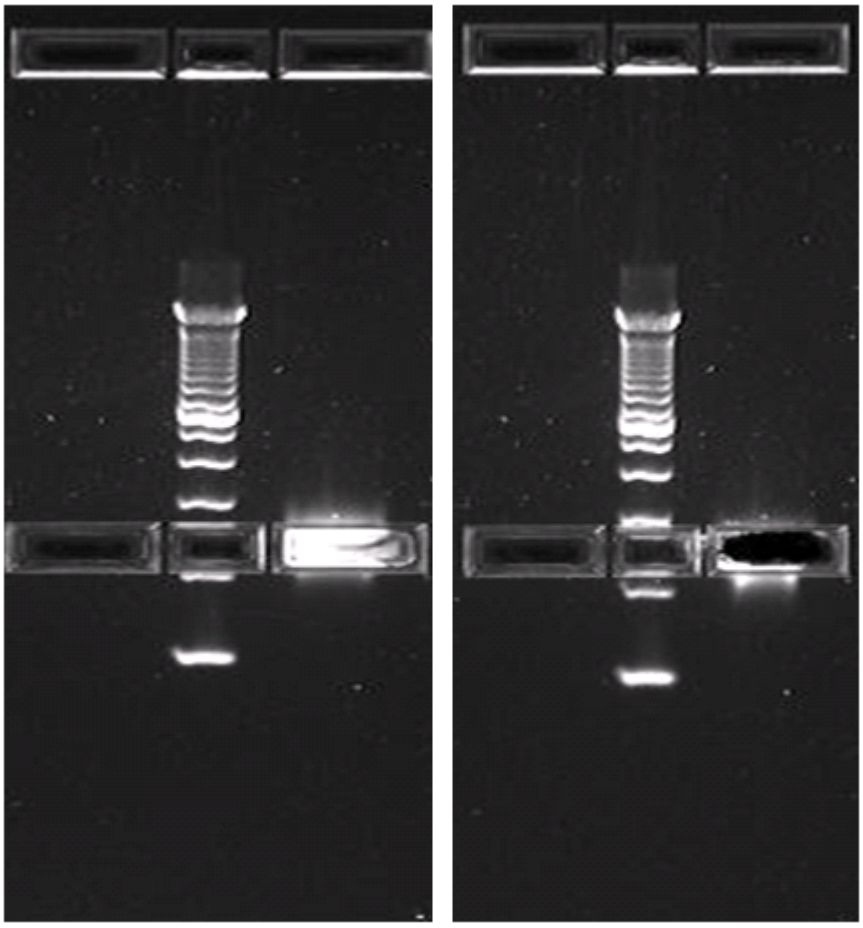


Figure 15 Collection of the of 250 to 325-bp library band from the SOLiD™ Library Size Selection gel.

(Optional) Purify the DNA with the SOLiD™ Library Micro Column Purification Kit

Note: If a concentrated sample is not necessary, skip this purification step, then proceed to "Quantitate the library by performing quantitative PCR (qPCR)" on page 87.

1. Pre-spin empty PureLink™ Micro column(s) in collection tube(s) at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample.
3. Apply the sample in the binding buffer to the PureLink Micro column(s) in a collection tube(s).
4. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
5. Repeat steps 3 and 4 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube.
6. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
7. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
8. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
9. Add 20 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column stand for 1 minute at room temperature.
10. Centrifuge the column(s) at 14,000 × g for 1 minute.
11. Add the eluate from step 10 back to the column(s), then let the column stand for 1 minute at room temperature.
12. Centrifuge the column(s) at 14,000 × g for 1 minute.
13. 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 to "Quantitate the library by performing quantitative PCR (qPCR)" on page 87.

Quantitate the library by performing quantitative PCR (qPCR)

For accurate library quantitation, quantitative PCR is strongly recommended. For a protocol using the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), see Appendix B, "SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit" on page 173.

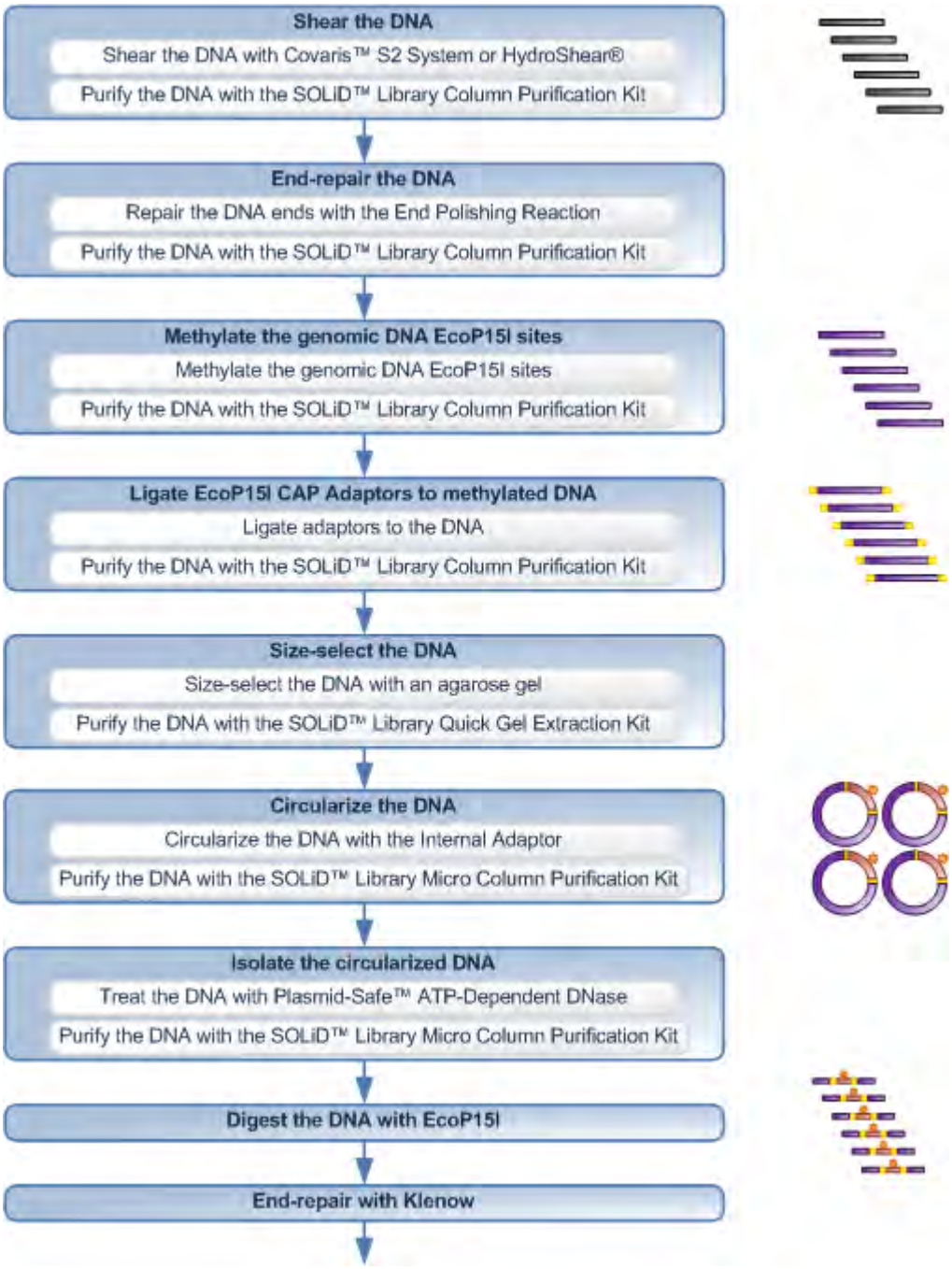
STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at −20 °C, or proceed to emulsion PCR in the *Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378) or the *Applied Biosystems SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (PN 4441486).

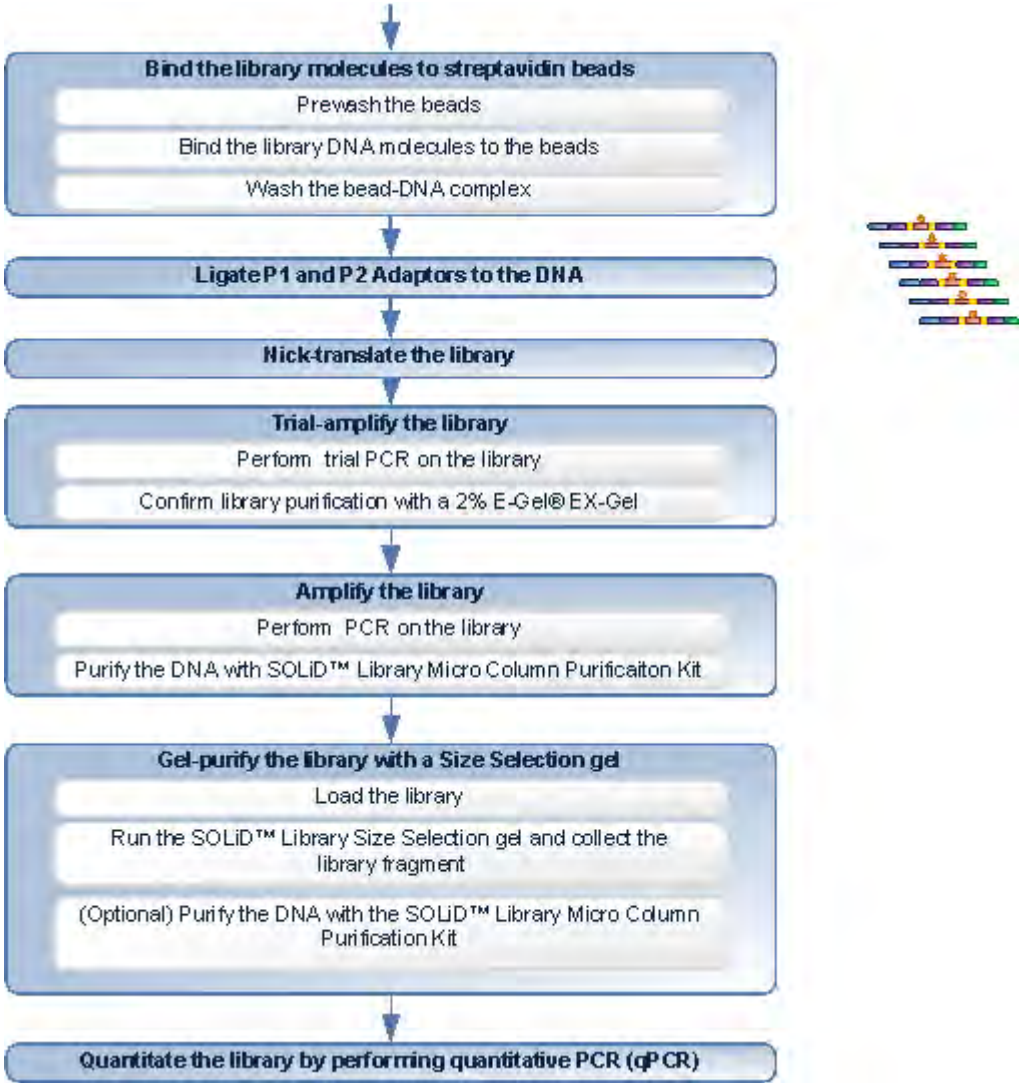
Section 3.2 Prepare a 2 × 25 bp mate-paired library

Materials and equipment required

See Appendix A on page 145 for a list of equipment, kits, and consumables necessary for this procedure.

Workflow


Shear the DNA
Shear the DNA with Covaris™ S2 System or HydroShear®
Purify the DNA with the SOLiD™ Library Column Purification Kit
End-repair the DNA
Repair the DNA ends with the End Polishing Reaction
Purify the DNA with the SOLiD™ Library Column Purification Kit
Methylate the genomic DNA EcoP15I sites
Methylate the genomic DNA EcoP15I sites
Purify the DNA with the SOLiD™ Library Column Purification Kit
Ligate EcoP15I CAP Adaptors to methylated DNA
Ligate adaptors to the DNA
Purify the DNA with the SOLiD™ Library Column Purification Kit
Size-select the DNA
Size-select the DNA with an agarose gel
Purify the DNA with the SOLiD™ Library Quick Gel Extraction Kit
Circularize the DNA
Circularize the DNA with the Internal Adaptor
Purify the DNA with the SOLiD™ Library Micro Column Purification Kit
Isolate the circularized DNA
Treat the DNA with Plasmid-Safe™ ATP-Dependent DNase
Purify the DNA with the SOLiD™ Library Micro Column Purification Kit
Digest the DNA with EcoP15I
End-repair with Klenow



Workflow overview

Shear the DNA

The genomic DNA is sheared to yield 600 bp to 6 kb fragments. To shear for a mate-paired library with insert sizes between 600 bp and 1 kb, the Covaris™ S2 System is recommended. To shear for a mate-paired library with insert sizes between 1 kb and 6 kb, the HydroShear® DNA Shearing Device is recommended.

HydroShear DNA Shearing Device uses hydrodynamic shearing forces to fragment DNA strands. The DNA in solution flows through a tube with an abrupt contraction. As it approaches the contraction, the fluid accelerates to maintain the volumetric flow rate through the smaller area of the contraction. During this acceleration, drag forces stretch the DNA until it snaps and until the pieces are too short for the shearing forces to break the chemical bonds. The flow rate of the fluid and the size of the contraction determine

the final DNA fragment sizes. While basic guidelines are given for shearing DNA using a HydroShear DNA Shearing Device, every HydroShear® instrument may need an initial standard run, and speed codes may need adjusting for DNA from different organisms.

Note: A calibration run to assess the shearing efficacy of your device prior to starting your first library preparation is highly recommended.

Sample purification is performed with PureLink™ columns supplied in the SOLiD™ Library Column Purification Kit and the SOLiD™ Library Micro Column Purification Kit. PureLink columns have a 40-µg capacity, and PureLink Micro columns have a 5-µg capacity. For maximum recovery, load <30 µg of DNA onto one PureLink column. Use multiple columns if necessary. All columns can be loaded multiple times if the volume of initial DNA and binding buffer mixture exceeds the volume capacity of the column. For more detailed information on purification of DNA with PureLink columns, see the manufacturer's instructions. *If you have larger amounts of DNA for library construction, you can substitute this step with phenol-chloroform-isoamyl alcohol extraction and isopropyl alcohol precipitation* (see Appendix C, "Supplemental Procedures" on page 185).

End-repair the DNA

For fast and efficient blunt-ended ligation, End Polishing Enzyme 1 and End Polishing Enzyme 2 are used to convert DNA with damaged or incompatible 5′ -protruding and/or 3′ -protruding ends to 5′ -phosphorylated, blunt-ended DNA. The conversion to blunt-end DNA is accomplished by exploiting the 5′ -to-3′ polymerase and the 3′ -to-5′ exonuclease activities of End Polishing Enzyme 2. End Polishing Enzyme 1 and ATP are also included for phosphorylation of the 5′ -ends of the blunt-ended DNA for subsequent ligation.

Methylate the genomic DNA EcoP15I sites

Methylation of the EcoP15I sites in the genomic DNA prevents digestion at the EcoP15I sites. EcoP15I is a type III restriction enzyme that recognizes the nucleotide sequence CAGCAG. For effective cleavage of a DNA molecule, EcoP15I needs two unmethylated, inversely-oriented EcoP15I recognition sites and cleaves the DNA 25/27 bp away from its binding site. The restriction activity requires ATP and in its absence, EcoP15I methylates only the fifth-base adenine in its binding site CAGC**A**G. This methylation is further boosted in the presence of exogenous S-adenosylmethionine, a methyl group donor. After methylation of genomic DNA EcoP15I sites, completion of methylation can be confirmed with a test digestion in the presence of ATP and its analysis on an agarose gel.

Ligate EcoP15I CAP Adaptors to the methylated DNA

EcoP15I CAP ligation adds the EcoP15I CAP Adaptors to the sheared, methylated DNA. The adaptors contain the EcoP15I restriction site that ultimately is used to make 25- to 27-bp genomic DNA mate-paired tags. The EcoP15I CAP Adaptors are included in double-stranded form in the SOLiD™ Mate-Paired Library Oligos Kit.

Size-select the DNA

Depending on the desired insert-size range, the ligated, purified DNA is run on a 0.8% or 1% agarose gel. The correctly sized ligation products are excised and purified using the SOLiD™ Library Quick Gel Extraction Kit.

Size-selection after CAP adaptor ligation removes unbound CAP adaptors. *Size selection should not be skipped under any circumstances.* Contamination of unbound CAP adaptors can compromise the circularization reaction in the next step.

Circularize the DNA

Sheared, methylated DNA ligated to EcoP15I CAP Adaptors is circularized with a biotinylated internal adaptor. To increase the chances that ligation will occur between two ends of one DNA molecule versus two different DNA molecules, a very dilute reaction is used. The circularization reaction products are purified using the SOLiD Library Micro Column Purification Kit. The Internal Adaptor is included in double-stranded form in the SOLiD™ Mate-Paired Library Oligos Kit.

Isolate the circularized DNA

Plasmid-Safe™ ATP-Dependent DNase is used to eliminate uncircularized DNA. After the Plasmid-Safe DNase-treated DNA is purified using the SOLiD Library Micro Column Purification Kit, the amount of circularized product is quantified. A ≥100 ng of circularized product is recommended to proceed with library construction. For more complex genomes, more circularized DNA is recommended for a high-complexity library.

Digest the DNA with EcoP15I

In the presence of sinefungin, EcoP15I digests the circularized DNA 25 to 27 bp away from the CAGCAG recognition site. The digestion creates two genomic DNA tags 25 to 27 bp long, connected with an internal adaptor between tags.

End-repair with Klenow

The Klenow fragment is used to convert 5′ -protruding and/or 3′ -protruding ends to 5′ -phosphorylated, blunt-ended DNA for blunt-end ligation.

Bind the DNA molecules to the streptavidin beads

Dynabeads® MyOne™ Streptavidin C1 specifically bind to the biotin-labeled Internal Adaptor in the library molecules to purify the library from side products.

Ligate P1 and P2 Adaptors to the DNA

P1 and P2 adaptors are ligated to the ends of the end-repaired DNA. The P1 and P2 Adaptors are included in double-stranded form in the SOLiD™ Mate-Paired Library Oligos Kit.

Nick-translate the library

The ligated, purified DNA undergoes nick translation with DNA polymerase I.

Trial-amplify the library

The library is trial-amplified using Library PCR Primers 1 and 2 with the Platinum® PCR Amplification Mix, which includes a proofreading enzyme for high-fidelity amplification, to determine the number of PCR cycles so that the amplified library is just visible on 2% E-Gel® EX Gel.

Amplify the library

The library is amplified using Library PCR Primers 1 and 2 with the Platinum® PCR Amplification Mix, which includes a proofreading enzyme for high-fidelity amplification. Reduce the number of cycles as much as possible and use the entire nick-translated product for amplification to get maximum representation of the library and to avoid PCR-related biases due to differential amplification of library molecules.

Gel-purify the library with a Size Selection gel

The library is run on an SOLiD™ Library Size Selection gel. The library band (154 to 156 bp) can be extracted and desalted using the SOLiD™ Library Micro Column Purification Kit.

Quantitate the library by performing quantitative (qPCR)

Quantitate the library by using the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), described in Appendix B, “SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit” on page 173).

Tips

- 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, 1.5-mL, and 2-mL tubes with Eppendorf LoBind Tubes.
- Thaw reagents on ice before use.

Shear the DNA

Prepare for shearing

IMPORTANT! For accuracy, determine sample DNA concentration using a double-stranded DNA-specific fluorescence assay. Assays recommended are: the Invitrogen Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, P7589), the Invitrogen Quant-iT™ dsDNA HS Assay Kit (Invitrogen, Q32851 or Q32854); or the Invitrogen Quant-iT™ dsDNA BR Assay Kit (Invitrogen, Q32850 or Q32853).

1. Choose the appropriate shearing method based on the desired insert size of the mate-paired library (see Table 32 on page 95).

Note: These conditions are guidelines. A shearing trial prior to large-scale shearing is recommended if additional DNA is available.

Table 32 Recommended shearing conditions for mate-paired library insert sizes

Insert size	Shearing method	Shearing conditions
600 to 800 bp	Covaris™ shearing in 20% glycerol (13 mm × 65 mm borosilicate tube)	• Number of Cycles: **75** • Bath Temperature: **5** °C • Bath Temperature Limit: **12** °C • Mode: **Frequency sweeping** • Water Quality Testing Function: **Off** • Duty cycle: **2%** • Intensity: **7** • Cycles/burst: **200** • Time: **10 seconds**
800 to 1000 bp	Covaris™ shearing in 20% glycerol (13 mm × 65 mm borosilicate tube)	• Number of Cycles: **30** • Bath Temperature: **5** °C • Bath Temperature Limit: **12** °C • Mode: **Frequency sweeping** • Water Quality Testing Function: **Off** • Duty cycle: **2%** • Intensity: **5** • Cycles/burst: **200** • Time: **10 seconds**
1 to 2 kb	HydroShear® Standard Shearing Assembly	• SC5‡ • 20 cycles
2 to 3 kb	HydroShear® Standard Shearing Assembly	• SC9 • 20 cycles
3 to 4 kb	HydroShear® Standard Shearing Assembly	• SC13 • 20 cycles
4 to 5 kb	HydroShear® Standard Shearing Assembly	• SC15 • 5 cycles
5 to 6 kb	HydroShear® Standard Shearing Assembly	• SC16 • 25 cycles

‡ Speed code (SC): 5.

IMPORTANT! If you are using the Covaris™ S2 System, 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.

2. If the DNA source is not limiting, ensure that the shearing conditions result in the desired insert sizes. Shear 5 µg DNA and run 150 ng sheared DNA on a 1% E-Gel® EX Gel according to the manufacturer's specifications.

Shear the DNA with the Covaris™ S2 System

1. In a round-bottomed 13 mm × 65 mm borosilicate tube, dilute 5 to 20 µg of DNA in 500 µL so that the final volume contains 20% glycerol in Nuclease-free Water (see Table 33).

Table 33 Dilute the DNA for shearing

Component	Amount
UltraPure™ Glycerol	100 µL
DNA	5 to 20 µg
Nuclease-free Water	Variable
Total	500 µL

2. Shear the DNA using the Covaris™ S2 System shearing program described Table 32 on page 95.
3. Transfer 500 µL of sheared DNA into a clean 1.5-mL LoBind tube.
4. Wash the borosilicate tube with 100 µL of Nuclease-free Water and transfer the wash to the 1.5-mL LoBind tube. Mix by vortexing and then proceed to "Purify the DNA with the SOLiD™ Library Column Purification Kit" on page 97.

Shear the DNA with the HydroShear® DNA Shearing Device

1. In 1.5-mL LoBind Tubes, dilute 5 to 20 µg of DNA to 150 µL with Nuclease-free Water. If you are starting with >20 µg, split the DNA into ≤20 µg aliquots and shear each aliquot in 150-µL volume. For better coverage of large and complex genomes, more DNA should be used if it is available.
2. On the Edit Wash Scheme tab, specify the solution and cycles:
   - 2 cycles WS1 (0.2 N HCl)
   - 2 cycles WS2 (0.2 N NaOH)
   - 3 cycles Nuclease-free Water
3. Run the wash scheme on the HydroShear.
4. Adjust the speed code (SC) and number of cycles according to Table 32 on page 95 and adjust the volume setting to 150 µL.
5. Begin shearing. Repeat the shearing for the other aliquot of DNA, if applicable. It is not necessary to run the wash cycle if both tubes contain the same DNA.
6. Run the wash scheme after DNA shearing is complete.
7. Pool the aliquots of sheared DNA, if applicable.

Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
2. Apply about 700 µL of the sample in the binding buffer to the PureLink™ column(s) in collection tube(s).
3. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through.
4. Repeat steps 2 and 3 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
5. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
6. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at *maximum speed* to remove residual wash buffer.
7. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
8. Add 50 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
9. Centrifuge the column(s) at *maximum speed* for 1 minute.
10. Add the eluate from step 9 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
11. Centrifuge the column(s) at *maximum speed* for 1 minute.
12. If necessary, pool the eluted DNA.
13. Quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189).

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "End-repair the sheared DNA" on page 98.

End-repair the sheared DNA

Repair the DNA ends with the End Polishing reaction

Note: This reaction is optimal for ≤ 20 µg of starting material. If > 20 µg of starting material is used for shearing, scale up the reaction as needed.

1. Combine and mix the following components in a LoBind tube (see Table 34):

Table 34 Combine components for end-repair of DNA

Component	Amount
Sheared DNA	48 µL
5X End Polishing Buffer	20 µL
dNTP, 10 mM	2.5 µL
End Polishing Enzyme 1, 10 U/µL	3 µL
End Polishing Enzyme 2, 5 U/µL	8 µL
Nuclease-free Water	18.5 µL
Total	100 µL

2. Incubate the mixture at room temperature (20 to 25 °C) for 30 minutes.

Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
2. Apply about 700 µL of the sample in the binding buffer to the PureLink™ column(s) in collection tube(s).
3. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
4. Repeat steps 2 and 3 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
5. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
6. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at *maximum speed* to remove residual wash buffer.
7. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
8. Add 50 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
9. Centrifuge the column(s) at *maximum speed* for 1 minute.
10. Add the eluate from step 9 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
11. Centrifuge the column(s) at *maximum speed* for 1 minute.

12. If necessary, pool the eluted DNA.

13. Quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189).

14. (Optional) For structural variation studies where tighter size selection of fragments is required, perform an additional size selection (see "Size-select the DNA" on page 104. After size-selection and purification, proceed to "Methylate the genomic DNA EcoP15I sites"). If tight insert size distribution is not critical, proceed directly to"Methylate the genomic DNA EcoP15I sites".

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Methylate the genomic DNA EcoP15I sites".

Methylate the genomic DNA EcoP15I sites

Methylate the genomic DNA EcoP15I sites

1. Combine and mix the following components, where *X* indicates the number of micrograms of end-repaired DNA. Use a final concentration of at least 360 µM S-adenosylmethionine and 10 U of EcoP15I enzyme per 1 µg of end-repaired DNA (see Table 35).

Table 35 Combine components for methylation of DNA

Component	Amount
Sheared, end-repaired DNA	*X* µg
10× NEBuffer 3	*X* µL
100× BSA	(*X* ÷ 10) µL
EcoP15I, 10 U/µL	*X* µL
S-adenosylmethionine, 32 mM	(*X* × 3 ÷ 25) µL
Nuclease-free Water	Variable
Total	(*X* × 10) µL

Example

For 12.5 µg of sheared, end-repaired DNA:

Combine components for methylation of DNA

Component	Amount
Sheared, end-repaired DNA	12.5 µg
10× NEBuffer 3	12.5 µL
100× BSA	1.25 µL
EcoP15I, 10 U/µL	12.5 µL
S-adenosylmethionine, 32 mM	1.5 µL
Nuclease-free Water	Variable
Total	125 µL

IMPORTANT! S-adenosylmethionine is an extremely labile molecule, sensitive to repeated freeze-thaw cycles. Always dispense S-adenosylmethionine into smaller aliquots at the first thaw to avoid multiple freeze-thaws. S-adenosylmethionine should not be used beyond its expiration date.

2. Incubate the methylation reaction mixture at 37 °C for 2 hours or overnight.

Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
2. Apply about 700 µL of the sample in the binding buffer to the PureLink™ column(s) in collection tube(s).
3. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
4. Repeat steps 2 and 3 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
5. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
6. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at *maximum speed* to remove residual wash buffer.
7. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
8. Add 50 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
9. Centrifuge the column(s) at *maximum speed* for 1 minute.
10. Add the eluate from step 9 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
11. Centrifuge the column(s) at *maximum speed* for 1 minute.
12. If necessary, pool the eluted DNA.
13. Quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189).
14. (Optional) To confirm DNA methylation, see "Confirm complete methylation of DNA fragments" on page 200.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Ligate EcoP15I CAP Adaptors to the methylated DNA" on page 102.

Ligate EcoP15I CAP Adaptors to the methylated DNA

Ligate the adaptors to the DNA

1. Calculate the amount of adaptor, *Y*, needed for the reaction based on the amount of DNA from the last purification step:

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

$$\textbf{\textit{Y}}\ \text{µL adaptor needed} = \#\ \text{µg DNA} \times \frac{\textbf{\textit{X}}\ \text{pmol}}{1\ \text{µg DNA}} \times 100 \times \frac{1\ \text{µL adaptor needed}}{50\ \text{pmol}}$$

Example:

For 12 µg of purified end-repaired DNA with an average insert size of 1.5 kb

$$\textbf{\textit{X}}\ \text{pmol/µg DNA} = 1\ \text{µg DNA} \times \frac{10^6\ \text{pg}}{1\ \text{µg}} \times \frac{1\ \text{pmol}}{660\ \text{pg}} \times \frac{1}{1500} = 1.0\ \text{pmol/µg DNA}$$

$$\textbf{\textit{Y}}\ \text{µL adaptor needed} = 12\ \text{µg DNA} \times \frac{1.0\ \text{pmol}}{1\ \text{µg DNA}} \times 100 \times \frac{1\ \text{µL adaptor needed}}{50\ \text{pmol}}$$

$$= 24\ \text{µL adaptor needed}$$

2. Combine and mix the following components (see Table 36). If a larger reaction volume is required to incorporate all of the methylated DNA, scale up the T4 DNA Ligase and 5✕ Ligase Buffer. Add 1 µL of T4 DNA Ligase per 20 µL of reaction volume. Add 1 µL of 5✕ Ligase Buffer per 5 µL of reaction volume.

Table 36 Ligation mix

Component	Volume (µL)
EcoP15I CAP Adaptor (ds), 50 pmol/µL	*Y*
5✕ Ligase Buffer	20
T4 DNA Ligase, 5 U/µL	5.0
DNA	Variable
Nuclease-free Water	Variable
Total	100

3. Incubate at room temperature (20 to 25 °C) for 15 minutes.

Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
2. Apply about 700 µL of the sample in the binding buffer to the PureLink™ column(s) in collection tube(s).
3. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
4. Repeat steps 2 and 3 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
5. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
6. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at *maximum speed* to remove residual wash buffer.
7. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
8. Add 50 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
9. Centrifuge the column(s) at *maximum speed* for 1 minute.
10. Add the eluate from step 9 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
11. Centrifuge the column(s) at *maximum speed* for 1 minute.
12. If necessary, pool the eluted DNA.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Size-select the DNA" on page 104.

Size-select the DNA

Size-select the DNA with an agarose gel

1. Determine the appropriate percentage of agarose gel needed to size-select DNA (see Table 37).

   Table 37 Percent agarose gel needed to size-select DNA

Desired insert size	Agarose gel needed (%)
600 to 3000 bp	1.0
3 to 6 kb	0.8

2. Prepare the appropriate percentage agarose gel in 1✕ TAE buffer with 10 µL of 1:10,000 SYBR® Safe gel stain (Invitrogen, S33102) per 100 mL gel volume. To prepare the gels, use either Agarose-LE (Applied Biosystems, AM9040) or UltraPure™ Agarose 1000 (Invitrogen, 10975-035).

3. Add 10✕ Blue Juice™ Gel Loading Buffer to the purified ligated DNA (1 µL of 10✕ Gel Loading Buffer for every 10 µL of mate-paired library).

4. Load the 1 Kb Plus DNA Ladder (Invitrogen, 10787-018) to one well. Load dye-mixed sample per well according to the well capacity into remaining wells. Use the minimum number of wells possible. There should be at least one lane in between the ladder well and the sample wells to avoid contamination of the sample with ladder.

5. Run the gel at the appropriate voltage to achieve optimal separation of the size of interest.

   IMPORTANT! To obtain maximum resolution of DNA fragments, agarose gels should be run at ≤ 5 V/cm. The distance is measured as the shortest path between the electrodes, not the agarose gel length itself.

6. Visualize the gel on a Safe Imager™ Blue Light Transilluminator with a ruler lying on top of the transilluminator.

   IMPORTANT! Exposing DNA to UV light may damage the DNA. Using SYBR® Safe gel stain and the Safe Imager Blue Light Transilluminator eliminates the risk of UV damage to DNA during size selection.

7. Using the ladder bands and the ruler for reference, excise the band of the gel corresponding to the insert size range of interest with a clean razor blade (see Figure 16 on page 105). If desired, take a tighter cut for a tighter size selection. If the gel piece is large, slice it into smaller pieces.

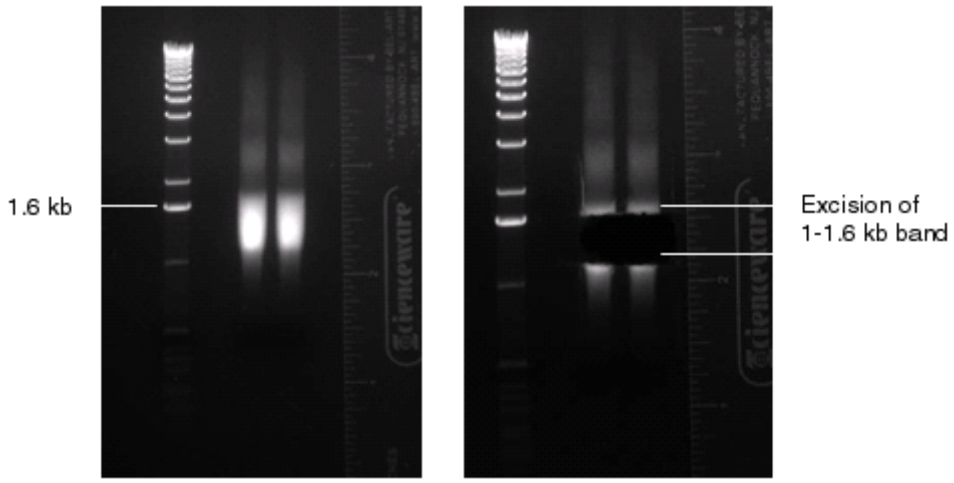


Figure 16 Excision of 1-1.6 kb range in a 1.0% agarose gel.

Purify the DNA using the SOLiD™ Library Quick Gel Extraction Kit

1. Weigh the gel slice in a 15-mL polypropylene conical colorless tube.
2. Add 30 µL of Gel Solubilization Buffer (L3) for every 10 mg of gel.
3. Dissolve the gel slice by vortexing the tube at *room temperature* until the gel slice has dissolved completely (~15 minutes).

> **IMPORTANT!** Do *not* heat the gel to dissolve the gel slice. When heated, the DNA denatures and short-insert libraries form heteroduplexes. Heteroduplexes are deleterious to the library.

4. Add 1 gel volume of isopropanol to the dissolved gel slice. For example, add 10 µL of isopropanol to 10 mg of gel. Mix well.
5. Apply the dissolved gel mixture to the Quick Gel Extraction column(s) in Wash Tube(s). Use one column per 400 mg agarose or load ≤2000 µL of dissolved gel mixture per column. Use more columns if necessary.
6. Centrifuge the column(s) at > 12,000 × g for 1 minute, then discard the flow-through and place the column back on the Wash Tube(s).
7. Add 700 µL of Wash Buffer (W1) with ethanol to the Quick Gel Extraction column(s).
8. Centrifuge the column(s) at > 12,000 × g for 1 minute, then discard the flow-through.
9. Centrifuge the Quick Gel Extraction column(s) again at *maximum speed* for 2 minutes to remove any residual Wash Buffer.
10. Transfer the Quick Gel Extraction column(s) to clean 1.5-mL LoBind tube(s).

11. Add 50 µL of Elution Buffer (E1, from the SOLiD™ Library Column Purification Kit, *not* Buffer E5 from the SOLiD™ Library Quick Gel Extraction Kit) to the center of the column(s) to elute the DNA, then let the column(s) stand for 5 minutes at room temperature.

12. Centrifuge the column(s) at > 12,000 × g for 1 minute. The 1.5-mL LoBind tube(s) contain the purified DNA.

IMPORTANT! For large fragments, increasing the incubation time to 10 minutes will increase the yield.

13. Add the eluate from step 12 back to the Quick Gel Extraction column(s), then let the column(s) stand for 1 minute at room temperature.

14. Centrifuge the column(s) at > 12,000 × g for 1 minute.

15. If necessary, pool the eluted DNA into one 1.5-mL LoBind tube.

16. Quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189).

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 °C, or proceed directly to "Circularize the DNA" on page 107.

Circularize the DNA

Circularize the DNA with the Internal Adaptor

1. Prepare a circularization reaction by mixing the following components listed (in order) based on the desired insert size, where *X* is the number of micrograms of DNA to be circularized (see Table 38). If a larger reaction volume is required, scale up the T4 DNA Ligase and 5✕ Ligase Buffer. Add 1 µL of T4 DNA Ligase per 40 µL of reaction volume. Add 1 µL of 5✕ Ligase Buffer per 5 µL of reaction volume.

Table 38 Mix for DNA circularization by insert size

Components	600 to 800 bp	800 to 1000 bp	1 to 2 kb	2 to 3 kb	3 to 4 kb	4 to 5 kb	5 to 6 kb
Nuclease-free Water	Variable	Variable	Variable	Variable	Variable	Variable	Variable
DNA	*X* µg	*X* µg	*X* µg	*X* µg	*X* µg	*X* µg	*X* µg
5✕ Ligase Buffer	(*X* × 47) µL	(*X* × 54) µL	(*X* × 73) µL	(*X* × 100) µL	(*X* × 112) µL	(*X* × 125) µL	(*X* × 144) µL
Internal Adaptor (ds), 2 µM	(*X* × 3.75) µL	(*X* × 2.84) µL	(*X* × 1.5) µL	(*X* × 0.9) µL	(*X* × 0.65) µL	(*X* × 0.5) µL	(*X* × 0.4) µL
T4 DNA Ligase, 5 U/µL	(*X* × 6) µL	(*X* × 6.75) µL	(*X* × 9) µL	(*X* × 12.5) µL	(*X* × 14) µL	(*X* × 15.6) µL	(*X* × 18) µL
Total	(*X* × 235) µL	(*X* × 270) µL	(*X* × 365) µL	(*X* × 500) µL	(*X* × 560) µL	(*X* × 625) µL	(*X* × 720) µL

Example

For 2 µg of DNA in 1 to 2 kb size range to be circularized:

Mix for DNA circularization by insert size

Components	Amount
Nuclease-free Water	Variable
DNA	2 µg
5✕ Ligase Buffer	146 µL
Internal Adaptor (ds), 2 µM	3 µL
T4 DNA Ligase, 5 U/µL	18 µL
Total	730 µL

2. Incubate the reaction at room temperature (20 to 25 °C) for 30 minutes.

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

IMPORTANT! If > 6 µg DNA was used in the circularization reaction, use the SOLiD™ Library Column Purification Kit, then follow the steps in "Purify the DNA with the SOLiD™ Library Column Purification Kit" on page 103. Make these changes to the procedure:

- Use 1 column per 5 mL of sample in Binding Buffer (B2-L) with isopropanol (40%).
- Load ≤800 µL sample in Binding Buffer each time onto the column(s). Spin the column(s) for 15 seconds at 10,000 × g except for the last loading. After the last loading, spin the column(s) for 1 minute.
- Use 40 µL of Elution Buffer (E1) to elute DNA from the column(s).

Unless specified otherwise, use the SOLiD™ Library Micro Column Purification Kit for all other steps after circularization.

1. Pre-spin empty PureLink™ Micro columns in collection tubes at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-L) with isopropanol (40%) to 1 volume of sample. Mix well.
3. Apply the sample in the binding buffer to the PureLink Micro column(s) in collection tube(s).
4. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
5. Repeat steps 3 and 4 until the entire sample has been loaded onto the column(s). Place the PureLink Micro column(s) back into the same collection tube(s).
6. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
7. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
8. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
9. Add 25 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
10. Centrifuge the column(s) at 14,000 × g for 1 minute.
11. Add the eluate from step 10 back to the column(s), then let the column(s) stand for 1 minute at room temperature.

12. Centrifuge the column(s) at 14,000 × g for 1 minute.

13. If necessary, pool the eluted DNA.

IMPORTANT! Proceed directly without stopping to "Isolate the circularized DNA" on page 110.

Isolate the circularized DNA

Treat the DNA with Plasmid-Safe™ ATP-Dependent DNase

1. Combine and mix the following components, where *X* is the volume in µL of DNA and *Y* is the number of micrograms of DNA used in the circularization reaction (see Table 39).

Table 39 Mix for DNase treatment of DNA

Component	Volume (µL)
ATP, 25 mM	5
10× Plasmid-Safe™ Buffer	10
Plasmid-Safe™ DNase, 10 U/µL	(*Y* ÷ 3)‡
DNA	*X*
Nuclease-free Water	Variable
Total	100

‡ Use 1 µL of Plasmid-Safe™ DNase, 10 U/µL if $Y \leq 3$ µg.

If $X > 80$ µL, adjust the total reaction volume, accordingly. The volume of ATP and 10× Plasmid-Safe™ Buffer should be proportional to the total reaction volume.

Example

For 2 µg DNA used in the circularization reaction:

Mix for DNase treatment of DNA

Component	Volume (µL)
ATP, 25 mM	5
10× Plasmid-Safe™ Buffer	10
Plasmid-Safe™ DNase, 10 U/µL	1
DNA	25
Nuclease-free Water	59
Total	100

2. Incubate the reaction mixture at 37 °C for 40 minutes.

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

1. Pre-spin empty PureLink™ Micro columns in collection tubes at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
3. Apply the sample in the binding buffer to the PureLink Micro column(s) in collection tube(s).
4. Let the column(s) stand for 1 minute at room temperature.
5. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
6. Add 500 µL of additional Binding Buffer (B2-S) with isopropanol (55%) to wash the column(s).
7. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
8. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
9. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
10. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
11. Add 25 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
12. Centrifuge the column(s) at 14,000 × g for 1 minute.
13. Add the eluate from step 12 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
14. Centrifuge the column(s) at 14,000 × g for 1 minute.
15. If necessary, pool the eluted DNA into one 1.5-mL LoBind tube.
16. Quantitate the purified DNA by using 2 µL of the sample on the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189).

IMPORTANT! Proceed directly without stopping to "Digest the circularized DNA with EcoP15I" on page 112.

Digest the circularized DNA with EcoP15I

1. Using 10 units of EcoP15I per 100 ng circularized DNA, calculate the amount of EcoP15I enzyme, *X*, needed to digest the circularized DNA:

$$X\ \mu L\ EcoP15I = \#\ ng\ DNA \times \frac{10\ U}{100\ ng\ DNA} \times \frac{1\ \mu L}{10\ U}$$

Example:

For 1000 ng circularized DNA

$$X\ \mu L\ EcoP15I = 1000\ ng\ DNA \times \frac{10\ U}{100\ ng\ DNA} \times \frac{1\ \mu L}{10\ U}$$

$$= 10\ \mu L\ EcoP15I$$

2. Combine in a LoBind tube (see Table 40):

Table 40 Mix to digest circularized DNA with EcoP15I

Component	Volume (µL)
Circularized DNA	Variable
10X NEBuffer 3	10
100X BSA	1
Sinefungin, 10 mM	1
10X ATP	10
EcoP15I, 10 U/µL	*X*
Nuclease-free Water	Variable
Total	100

3. Incubate the reaction mixtures at 37 °C overnight.

4. Combine (see Table 41):

Table 41 Mix to digest circularized DNA with EcoP15I

Component	Volume (µL)
EcoP15I-digested DNA	100
10 mM Sinefungin	1
10X ATP	2
EcoP15I, 10 U/µL	1
Total	104

5. Incubate the reaction mixture at 37 °C for 1 hour.

6. Denature the enzyme at 65 °C for 20 minutes and chill on ice for 5 minutes.

End-repair with Klenow

1. Combine (see Table 42):

Table 42 Combine to end-repair EcoP15I-digested DNA

Component	Volume (µL)
EcoP15I-digested DNA	104
dNTP, 10 mM	4
Klenow Fragment	1
Total	109

2. Incubate the reaction mixture at room temperature (20 to 25 °C) for 15 minutes.
3. Add 2.2 µL of Stop Buffer.
4. Denature the enzyme at 65 °C for 20 minutes, then chill on ice for 5 minutes.
5. Add Nuclease-free Water (~90 µL) to the stopped end-repair reaction to reach a final volume of 200 µL.
6. Put the tube of DNA on ice until you incubate the DNA with the pre-washed streptavidin beads (see "Prewash the beads" on page 114).

Bind the library molecules to streptavidin beads

Prewash the beads

1. Combine (see Table 43):

Table 43 Prepare 1× BSA solution

Component	Volume (µL)
100× BSA‡	5
Nuclease-free Water	495
Total	500

‡ A component of the EcoP15I Enzyme Kit from New England Biolabs® Inc.

2. Vortex the bottle of Dynabeads® MyOne™ Streptavidin C1, then transfer 90 µL of the beads into a 1.5-mL LoBind Tube.
3. Add 500 µL of Bead Wash Buffer to the 90 µL of solution of beads, vortex the beads for 15 seconds, then pulse-spin.
4. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
5. Add 500 µL of 1× BSA and vortex for 15 seconds, then pulse-spin the tube.
6. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
7. Add 500 µL of Bead Binding Buffer. Vortex the beads for 15 seconds, then pulse-spin.
8. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
9. Resuspend the beads in 200 µL of Bead Binding Buffer.

Bind the library DNA molecules to the beads

1. Add the entire 200 µL of solution of library DNA in Bead Binding Buffer to the 200 µL of pre-washed beads, then vortex.
2. Rotate the solution at room temperature (20 to 25 °C) for 30 minutes, then pulse-spin.

Wash the bead-DNA complex

1. Combine (see Table 44):

Table 44 Prepare 1× Ligase Buffer

Component	Volume (µL)
5× Ligase Buffer	120
Nuclease-free Water	480
Total	600

2. Place the tube with the bead-DNA complex in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
3. Resuspend the beads in 500 µL of Bead Wash Buffer, then transfer the beads to a new 1.5-mL LoBind tube. Vortex the beads for 15 seconds, then pulse-spin.
4. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
5. Resuspend the beads in 500 µL of Bead Wash Buffer. Vortex the beads for 15 seconds, then pulse-spin.
6. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
7. Repeat steps 5 and 6 once.
8. Resuspend the beads in 500 µL of 1× Ligase Buffer. Vortex the beads for 15 seconds, then pulse-spin.
9. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
10. Resuspend the beads in 94 µL of 1× Ligase Buffer.

Ligate P1 and P2 Adaptors to the DNA

1. Calculate the amount of P1 and P2 Adaptors needed for the ligation reaction based on the amount of circularized DNA from "Isolate the circularized DNA" on page 110 and the calculation below. For calculation details, see Appendix E, "Formulas and calculations" on page 215.

$$\textbf{\textit{X}}\ \text{pmol/µg DNA} = 1\ \text{µg DNA} \times \frac{10^6\ \text{pg}}{1\ \text{µg}} \times \frac{1\ \text{pmol}}{660\ \text{pg}} \times \frac{1}{\text{Circularized DNA size}}$$

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

Example:
For 1 µg of purified circularized DNA with an average size of 1536 (1500 bp insert + 36 bp internal adaptor)

$$\textbf{\textit{X}}\ \text{pmol/µg DNA} = 1\ \text{µg DNA} \times \frac{10^6\ \text{pg}}{1\ \text{µg}} \times \frac{1\ \text{pmol}}{660\ \text{pg}} \times \frac{1}{1536} = 1\ \text{pmol/µg DNA}$$

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

$$= 0.6\ \text{µL adaptor needed}$$

2. Combine (see Table 45):

Table 45 Combine for ligation of end-repaired DNA to P1 and P2 Adaptors

Component	Volume (µL)
DNA-bead complex	94
P1 Adaptor (ds), 50 µM	*Y*
P2 Adaptor (ds), 50 µM	*Y*
T4 DNA Ligase, 5 U/µL	5
Total	Variable (~100)

3. Incubate the reaction mixture at room temperature (20 to 25 °C) on a rotator for 15 minutes.

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

5. Resuspend the beads in 500 µL of Bead Wash Buffer and transfer the beads to a new 1.5-mL LoBind tube. Vortex the beads for 15 seconds, then pulse-spin.

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

7. Resuspend the beads in 500 µL of Bead Wash Buffer. Vortex the beads for 15 seconds, then pulse-spin.

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

9. Repeat steps 7 and 8 once.

Nick-translate the library

1. Combine (see Table 46):

Table 46 Combine for nick translation

Component	Volume (µL)
Nuclease-free Water	83
Nick Translation Buffer	10
dNTP, 10 mM	5
DNA Polymerase I, 10 U/µL	2
Total	100

2. Add the mix for nick translation to the washed beads.

3. Incubate the mixture at room temperature (20 to 25 °C) for 15 minutes.

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

5. Resuspend the beads in 500 µL of Elution Buffer (E1) from the SOLiD™ Library Column Purification Kit. Vortex, then pulse-spin the beads.

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

7. Resuspend the beads in 30 µL of Elution Buffer (E1).

STOPPING POINT. Store the DNA-Bead complexes in Elution Buffer (E1) at 4 ºC, or proceed directly to "Trial-amplify the library" on page 118.

Trial-amplify the library

Perform trial PCR on the library

1. Prepare a PCR master mix for amplification reactions (see Table 47):

Table 47 PCR master mix for amplification of the library

Component	Volume (µL)
Platinum® PCR Amplification Mix‡	70
Library PCR Primer 1, 50 µM	1.4
Library PCR Primer 2, 50 µM	1.4
Total	72.8

‡ Platinum® PCR Amplification Mix contains a proofreading enzyme for high-fidelity amplification.

2. Vortex the master mix. For the negative control, transfer 23 µL of the PCR master mix to a PCR tube. Label the tube "PCR #0".

3. Add 4 µL of DNA-bead complex solution to the remaining 49.8 µL of PCR master mix. Vortex the mix, then divide evenly (~25µL) between two PCR tubes labelled "PCR #1" and "PCR #2".

4. Run (see Table 48):

Table 48 PCR conditions to amplify the library

Stage	Step	Temp	Time
Holding	Denature	94 °C	3 min
Cycling‡	Denature	94 °C	15 sec
	Anneal	62 °C	15 sec
	Extend	70 °C	1 min
Holding	Extend	70 °C	5 min
Holding	—	4 °C	∞

‡ Tube #1: 10 cycles.
Tubes #0 and #2: 14 cycles
For starting DNA > 20 µg, you can use 8 and 12 cycles for the trial amplification.

Confirm library amplification with a 2% E-Gel® EX Gel

1. Mix 0.5 µL of 1 µg/µL 25-bp DNA Ladder (Invitrogen, 10597-011) with 40 µL of Nuclease-free Water.

2. Load 20 µL of PCR #0, PCR #1, and PCR #2 into separate wells of a 2% E-Gel® EX Gel. Load 20 µL of diluted 25-bp DNA Ladder in an adjacent well for reference. You do *not* need to add loading buffer to the samples.

3. Run the E-Gel EX Gel on an E-Gel® iBase™ Power System, according to the manufacturer's instructions, for 10 minutes.

4. Take a picture of the gel (see Figure 17). The expected size of amplified library is ~155 bp. Choose a PCR cycle where amplified library products are just visible on the gel. Based on the intensity of the products, increase or reduce up to 2 cycles for final library amplification.

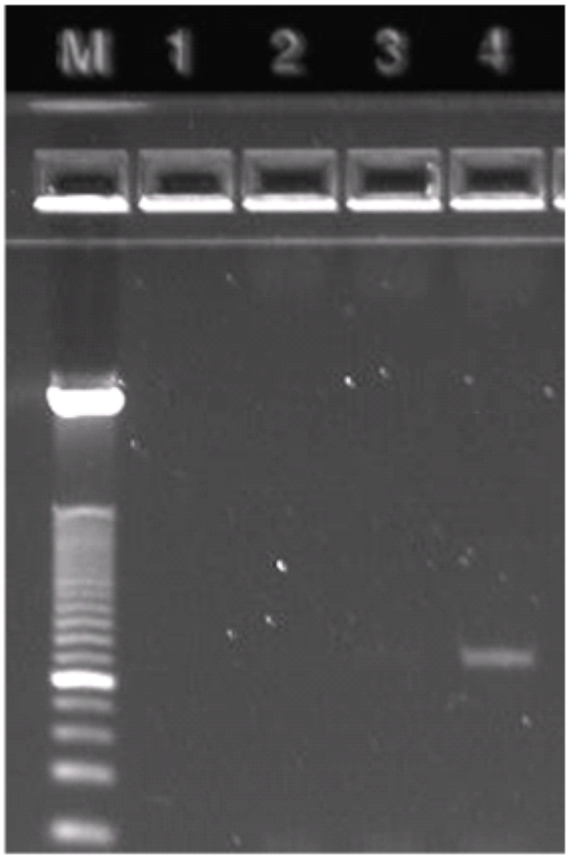


Figure 17 2x25 bp mate-paired library trial amplification sample run on a 2% E-Gel® EX gel. M: 25-bp DNA ladder. Lane1: empty; Lane 2: PCR #0 (negative control); Lane 3: PCR #1 (10 cycles); Lane 4: PCR #2 (14 cycles). Based on this picture, ~12 cycles should be used for final library amplification.

STOPPING POINT. Store the DNA-Bead complexes in Elution Buffer (E1) at 4 ºC, or proceed directly to "Amplify the library" on page 120.

Amplify the library

Perform PCR on the library

1. Prepare a master mix for amplification reactions (see Table 49):

Table 49 PCR master mix for amplification of the library

Component	Volume (µL)
Platinum® PCR Amplification Mix‡	350
Library PCR Primer 1, 50 µM	7
Library PCR Primer 2, 50 µM	7
Total	364

‡ Platinum® PCR Amplification Mix contains a proofreading enzyme for high-fidelity amplification.

2. For the negative control, aliquot 50 µL of PCR master mix to a PCR tube. Add 5 µL of Nuclease-free Water to the tube.

3. Add 26 µL of DNA-bead complex solution to the remaining 314 µL of PCR master mix. Vortex to mix, then divide evenly (~84 µL) among four PCR tubes.

4. Run (see Table 50):

Table 50 PCR conditions to amplify the library

Stage	Step	Temp	Time
Holding	Denature	94 °C	3 min
Cycling‡	Denature	94 °C	15 sec
	Anneal	62 °C	15 sec
	Extend	70 °C	1 min
Holding	Extend	70 °C	5 min
Holding	—	4 °C	∞

‡ Cycling number determined by trial amplification. See "Trial-amplify the library" on page 118.

5. Pool all of the PCR samples into a 1.5-mL LoBind tube.

6. Place the tube of beads in a magnetic rack, then transfer the supernatant to a fresh 2-mL LoBind tube. Discard the tube containing the beads.

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

1. Pre-spin empty PureLink™ Micro column in collection tubes at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-L) with isopropanol (40%) to 1 volume of sample.
3. Apply 750 µL of the PCR product in the binding buffer to the PureLink Micro column in a collection tube.
4. Centrifuge the column at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
5. Repeat steps 3 and 4 until the entire sample has been loaded onto the column. Place the PureLink Micro column(s) back into the same collection tube.
6. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column.
7. Centrifuge the column at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
8. Transfer the column to a clean 1.5-mL LoBind tube.
9. Add 25 µL of Elution Buffer (E1) to the center of the column to elute the DNA, then let the column stand for 1 minute at room temperature.
10. Centrifuge the column at 14,000 × g for 1 minute.
11. Add the eluate from step 10 back to the column, then let the column stand for 1 minute at room temperature (20 to 25 °C).
12. Centrifuge the column at 14,000 × g for 1 minute.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 °C or proceed directly to "Gel-purify the library with a Size Selection gel" on page 122.

Gel-purify the library with a Size Selection gel

Load the library

1. Plug the adapter plug of the E-Gel® iBase™ system into an electrical outlet.
2. Remove the SOLiD™ Library Size Selection gel from its package, then insert the gel with its combs into the iBase system:
   a. Slide the gel into the two electrode connections on the iBase system. Ensure that the two electrodes on the right side of the cassette touch the two contacts on the iBase system. The Invitrogen logo should be at the bottom of the base.
   b. Press the gel firmly at the top and bottom to seat the gel in the iBase system. If the gel is correctly inserted, a red light glows.
3. Remove the combs.
4. Load ≤ 500 ng of sample in 25 µL volume without loading dye into the wells of the *top* row. Use Nuclease-free Water as diluent if necessary. 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 500 ng of DNA per lane (see Figure 18 on page 123).
5. Mix 0.5 µL of 1 µg/µL of 25-bp DNA Ladder (Invitrogen, 10597-011) with 20 µL of Nuclease-free Water. Load 10 µL of the diluted DNA ladder into the small middle well of the top row (marked “M”; see Figure 18 on page 123).
6. Load 25 µL of Nuclease-free Water into any remaining empty wells in the top row.
7. Load 25 µL of Nuclease-free Water into wells 1 to 8 in the *middle* of the gel and 10 µL of Nuclease-free Water in the middle marker well of the bottom row (see Figure 18 on page 123).

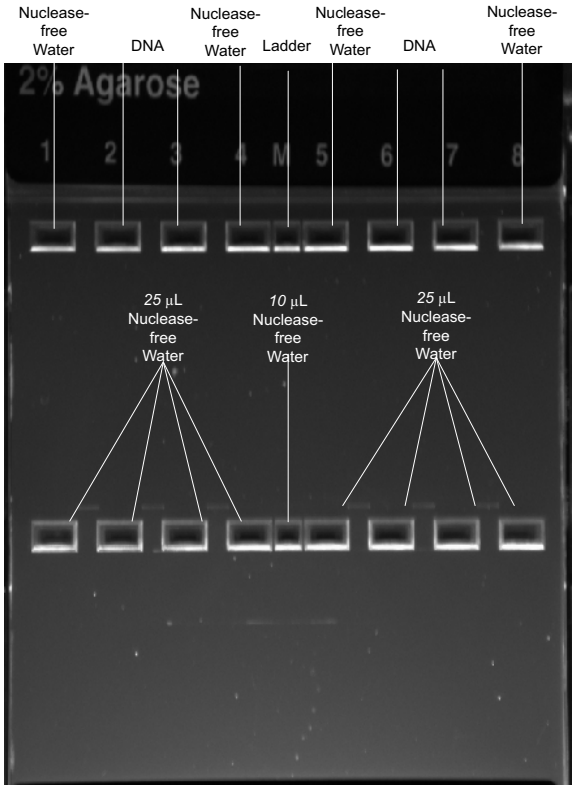


Figure 18 Where to load DNA, ladder, and Nuclease-free Water on a SOLiD™ Library Size Selection gel to size-select the DNA.

Run the SOLiD™ Library Size Selection gel and collect the library fragment

1. Place the E-Gel iBase Power System over a Safe Imager™ Real-Time Transilluminator. Use the orange cover or orange goggles to view the bands and to avoid overexposure of your eyes to the blue light.

2. Run the gel. On the iBase system:

   a. Select **SizeSelect 2%** (refer to the iBase Power System manual for instructions).

   b. Press **Go**. The red light turns green.

3. Monitor the gel. At the end of a run, the iBase system flashes a red light and beeps rapidly:
   - If the 155-bp band has not reached the reference line, run the gel for about 1 to 2 more minutes until the band reaches the line.
   - When the band of interest (~155 bp) reaches the reference line, press **Go** to stop the run. Proceed to step 4.

4. When the band reaches the reference line, refill the bottom row again with Nuclease-free Water until each well is full. Some pre-filled water is lost during the run.

5. Press **Go** to run the gel until the 155-bp band enters the collection well. For optimal results, monitor the run in a darkened room.

6. Collect the DNA from the collection well using a 20-µL pipette fitted with a tip (see Figure 19). Do not perforate the bottom of the agarose collection well. Due to migration of the DNA into the bottom of the well, some residual DNA remains underneath the well.

IMPORTANT! If the 155-bp band overruns the collection well and reenter the gel, select **REVERSE E-Gel** on the iBase Power System to run the band backwards into the collection well. Collect the DNA.

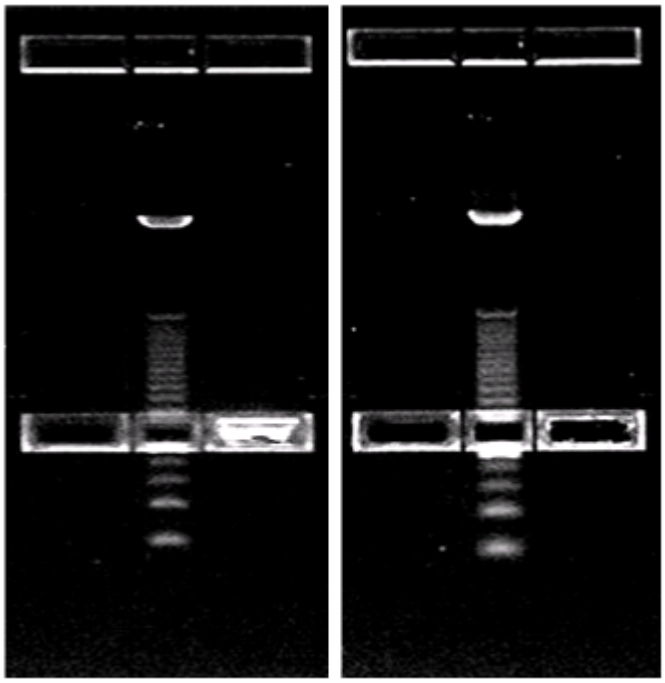


Figure 19 Collection of the of 155-bp library band from the SOLiD™ Library Size Selection gel.

(Optional) Purify the DNA with the SOLiD™ Library Micro Column Purification Kit

Note: If a concentrated sample is not necessary, skip this purification step, then proceed to "Quantitate the library by performing quantitative PCR (qPCR)" on page 126.

1. Pre-spin empty PureLink™ Micro column in collection tube(s) at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample.
3. Apply the sample in the binding buffer to the PureLink Micro column(s) in collection tube(s).
4. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
5. Repeat steps 3 and 4 until the entire sample has been loaded onto the column(s). Place the PureLink Micro column(s) back into the same collection tube.
6. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column.
7. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at 14,000 × g to remove residual wash buffer.
8. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
9. Add 20 µL of Elution Buffer (E1) to the center of the column to elute the DNA, then let the column stand for 1 minute at room temperature.
10. Centrifuge the column(s) at 14,000 × g for 1 minute.
11. Add the eluate from step 10 back to the column(s), then let the column stand for 1 minute at room temperature.
12. Centrifuge the column(s) at 14,000 × g for 1 minute.
13. 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 to "Quantitate the library by performing quantitative PCR (qPCR)" on page 126.

Quantitate the library by performing quantitative PCR (qPCR)

For accurate library quantitation, quantitative PCR is strongly recommended. For a protocol using the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), see Appendix B, "SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit" on page 173.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at – 20 °C, or proceed to emulsion PCR in the *Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378) or the *Applied Biosystems SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (PN 4441486).

4 Barcoded Fragment Library Preparation

This chapter covers:

Overview

This chapter describes the method to generate 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 20).

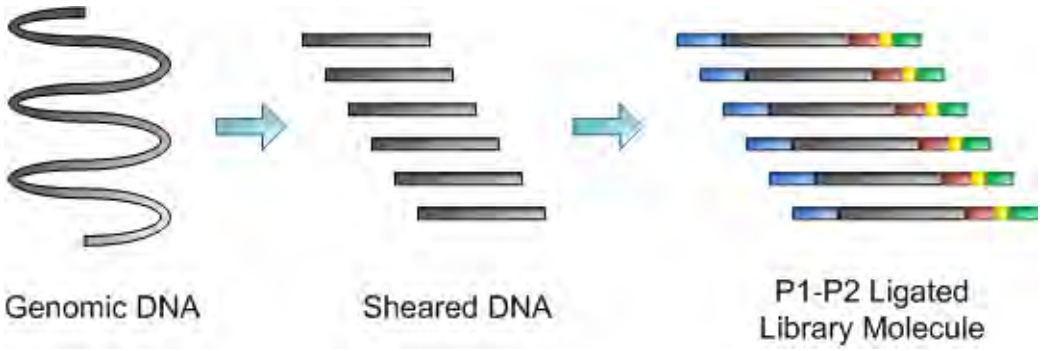


Figure 20 Basic barcoded fragment library preparation workflow overview.

The Multiplex P2 Adaptor consists of 3 segments of sequence:

1. Internal adaptor sequence, which is necessary for sequencing the barcode
2. Barcode sequence
3. 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 21 on page 129).

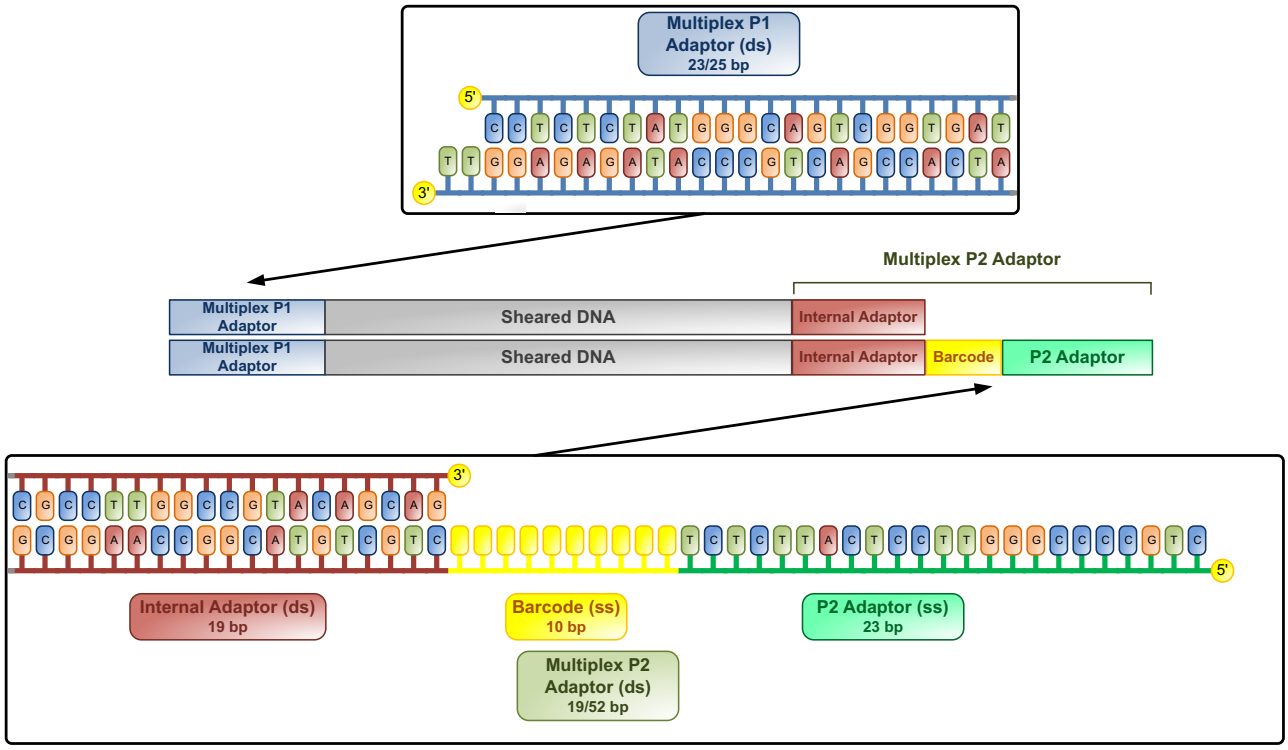


Figure 21 Barcoded fragment library design.

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 22 on page 130). 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.

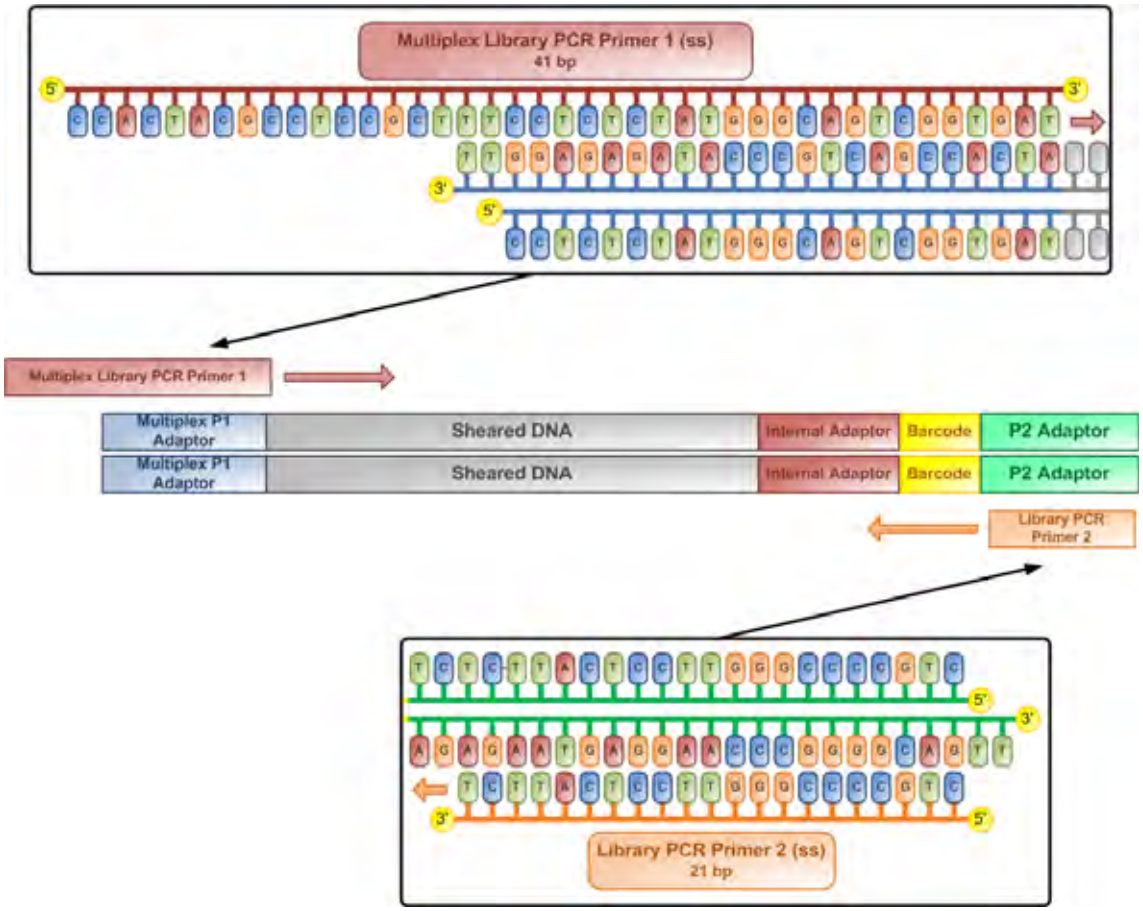


Figure 22 Barcoded fragment library amplification design.

This chapter describes how to generate a barcoded fragment DNA library. 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).

Prepare a barcoded fragment library

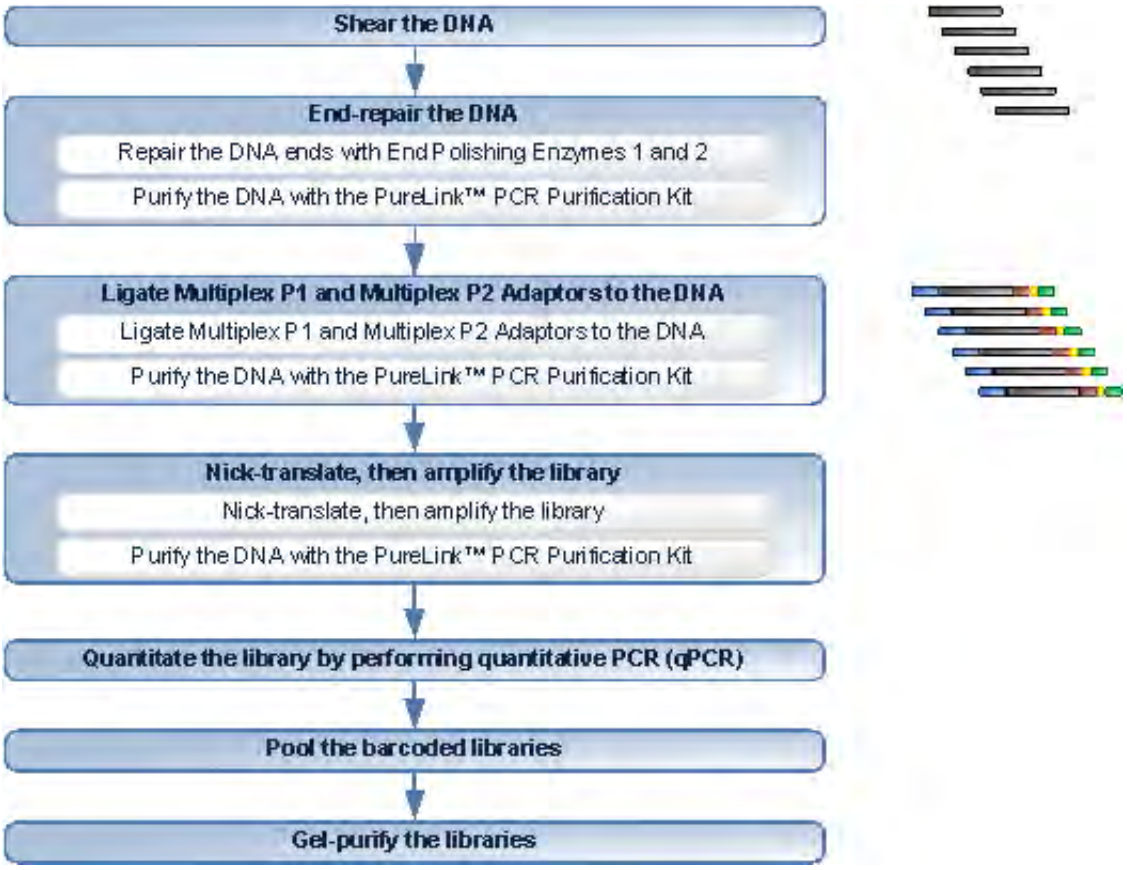
The protocol is designed for 500 ng to 5 µg of genomic DNA or ligated PCR product. If the starting amount of genomic DNA is outside the range of 500 ng to 5 µg, you should modify the protocol. For technical assistance, contact your local field application specialist.

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.

Materials and equipment required

The SOLiD™ Fragment Library Barcoding Kit should be used together with a SOLiD™ Fragment Library Construction Kit. See Appendix A on page 145 for a list of equipment, kits, and consumables necessary for this procedure.

Workflow



Workflow overview

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 ATP 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 Multiplex P1 and Multiplex P2 Adaptors to the DNA

Multiplex P1 and Multiplex 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, 13–16, 17–20, 21–24, 25–28, 29–32, 33–36, 37–40, 41–44, 45–48, 49–52, 53–56, 57–60, 61–64, 65–68, 69–72, 73–76, 77–80, 81–84, 85–88, 89–92, or 93–96.

Nick-translate, then amplify the library

The adaptor-ligated, purified DNA undergoes nick translation, then amplification using Multiplex Library PCR-1 and Multiplex Library PCR-2 primers 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™ Library TaqMan® Quantitation Kit (PN 4449639), described in Appendix B, "SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit" on page 173.

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" on page 142).

Equal molar amounts of each barcoded library are mixed together. This sample of combined barcoded libraries can be processed together through templated bead preparation [refer to the *Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378) or the *Applied Biosystems SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (PN 4441486)]. If you want to perform fewer steps and accurate quantitation of each barcoded library is not critical, then you can pool barcoded libraries at an earlier step, just prior to nick translation and amplification.

Gel-purify the libraries

The library is run on an SOLiD™ Library Size Selection gel. The correctly sized amplification 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.

Shear the DNA

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, 13–16, 17–20, 21–24, 25–28, 29–32, 33–36, 37–40, 41–44, 45–48, 49–52, 53–56, 57–60, 61–64, 65–68, 69–72, 73–76, 77–80, 81–84, 85–88, 89–92, or 93–96.

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 to 100 µL in 1X Low TE Buffer in a LoBind tube (see Table 51).

Table 51 Dilute the DNA for shearing

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

2. 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, see "Load and unload Covaris™ microTUBE vials from the Covaris™ microTUBE holder" on page 186.

3. 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 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. 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.

4. 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 52):

Table 52 Mix for end-repair of DNA

Component	Volume (µL)
Sheared DNA	100
5× 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 (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 189). 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 ºC, 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, see "Ligation of P1 and P2 Adaptors" on page 216). 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, 13–16, 17–20, 21–24, 25–28, 29–32, 33–36, 37–40, 41–44, 45–48, 49–52, 53–56, 57–60, 61–64, 65–68, 69–72, 73–76, 77–80, 81–84, 85–88, 89–92, or 93–96. 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 the next step).

2. For each library, combine (see Table 53):

Table 53 Ligation mix

Component	Volume (µL)
Multiplex Library P1 Adaptor, 50 µM	*Y*
Barcode-0XX, 50 µM	*Y*
5X T4 Ligase 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* (go to step 2). Individually purified barcoded libraries are then pooled after quantification by qPCR (see "Pool the barcoded libraries" on page 142). 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 (< 5 µg DNA/column) 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" on page 140.

Nick-translate, then amplify the library

Nick-translate, then amplify the library

1. Prepare a PCR reaction mix (see Table 54).

Table 54 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. Pipet 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 55).

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 55 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 new 1.5-mL LoBind Tubes.

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 a protocol using the SOLiD™ Library TaqMan® Quantitation Kit (PN 4449639), see Appendix B, "SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit" on page 173.

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" on page 142.

Pool the barcoded libraries

IMPORTANT! If you:

- *Pooled the libraries after ligation of the Multiplex P1 and Multiplex 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, 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

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 gel as follows (for exact fill volumes of the wells, refer to the *Invitrogen E-Gel® SizeSelect™ Agarose Gels Quick Reference Card*):

   a. Load 20 µL of the pooled library DNA into each well 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 (see Figure 23 on page 143).

   b. 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 (see Figure 23 on page 143).

   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 23 on page 143).

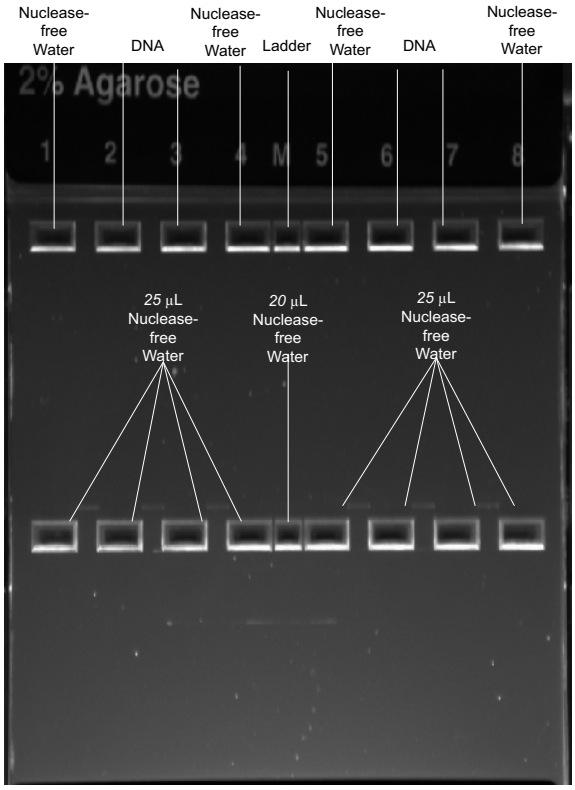


Figure 23 Where to load DNA, ladder, and Nuclease-free Water in a SOLiD™ Library Size Selection gel to size-select the DNA.

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 24 on page 144).

   Note: After amplification, the total size of the adaptors 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 *SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378) or the *Applied Biosystems SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (PN 4441486)].

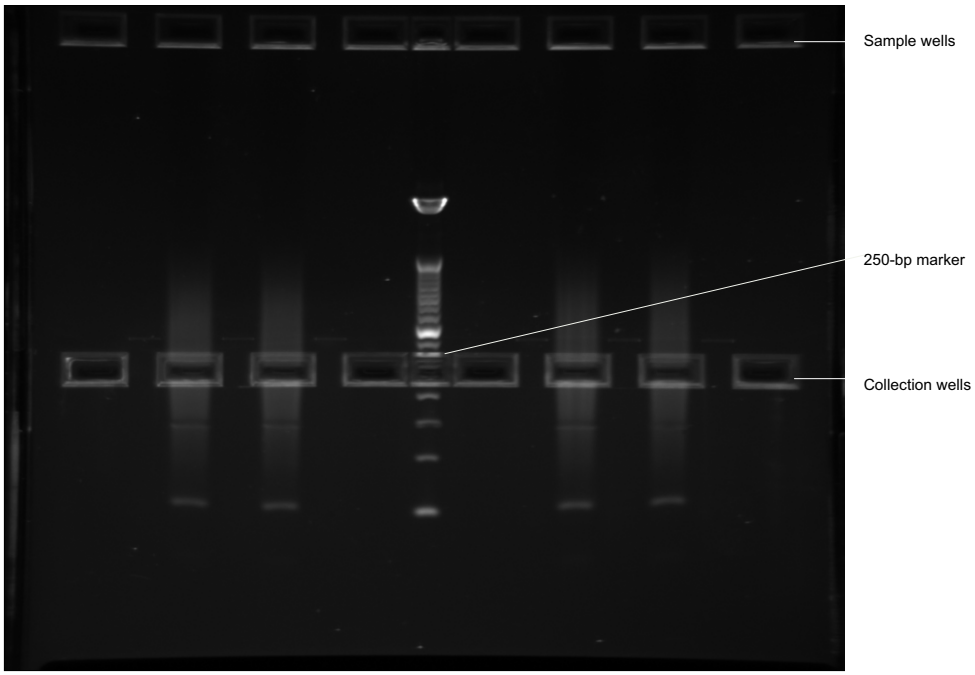


Figure 24 Elution of ~240- to 270-bp region from a SOLiD™ Library Size Selection gel.

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 the *Applied Biosystems SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (PN 4441486).

A Required Materials

This appendix covers:

Prepare a standard fragment library

Table 56 Required Applied Biosystems reagent kits

Item (part number)‡	Components	Kit components used in…
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 Construction Kit with Size Selection Gels (4443471)§	SOLiD™ Fragment Library Enzyme/Core Kit: • 5X End Polishing Buffer • dNTP, 10 mM • End Polishing Enzyme 1 • End Polishing Enzyme 2	DNA end repair
	• 5X Ligase Buffer • 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™ 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 57 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: • 5× End Polishing Buffer • dNTP, 10 mM • End Polishing Enzyme 1 • End Polishing Enzyme 2	DNA end repair
	• 5× Ligase Buffer • 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: • 5× End Polishing Buffer • dNTP, 10 mM • End Polishing Enzyme 1 • End Polishing Enzyme 2	DNA end repair
	• 5× Ligase Buffer • 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 58 Required equipment

Item‡	Source
Covaris™ S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris™ S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, refer to "Covaris™ S2 System Materials Summary," *SOLiD™ 4 System Site Preparation Guide* (PN 4448639).	• Applied Biosystems 4387833 (110 V) • Applied Biosystems 4392718 (220 V) or Covaris
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
E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit	Invitrogen G6465
Vortexer	Major Laboratory Supplier (MLS)

Table 58 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 59 Optional equipment

Item‡	Source
2100 Bioanalyzer	Agilent Technologies G2938C
Qubit™ Quantitation Starter Kit	Invitrogen Q32860

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

Table 60 Required consumables

Item‡	Source
1× Low TE Buffer	Applied Biosystems 4389764
Nuclease-free Water, 1 L	Applied Biosystems AM9932
Covaris microTUBEs	Covaris 520045
Isopropyl alcohol	Sigma-Aldrich I9516
Ethylene glycol	American Bioanalytical AB00455-01000
50-bp ladder	Invitrogen 10416-014
0.5-mL LoBind Tubes	Eppendorf 022431005

Table 60 Required consumables

Item‡	Source
1.5-mL LoBind Tubes	Eppendorf 022431021
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit§	Thermo Scientific 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.

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

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 61 Optional consumables

Item‡	Source
Agilent DNA 1000 Kit§	Agilent Technologies 5067-1504

‡ 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.

Prepare an express fragment library

Table 62 Required Applied Biosystems reagent kits

Item (part number)‡	Components	Kit components used in…
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 Construction Kit (4443473)§	SOLiD™ Fragment Library Enzyme/Core Kit: • 5X End Polishing Buffer • dNTP, 10 mM • End Polishing Enzyme 1 • End Polishing Enzyme 2	DNA end repair
	• 5X Ligase Buffer • 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

‡ 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 63 Optional Applied Biosystems reagent kits

Item (part number)‡§	Components	Kit components used in…
SOLiD™ Fragment Library Construction Kit Reagents (4443713)	SOLiD™ Fragment Library Enzyme/Core Kit: • 5X End Polishing Buffer • dNTP, 10 mM • End Polishing Enzyme 1 • End Polishing Enzyme 2	DNA end repair
	• 5X Ligase Buffer • 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.
§ Invitrogen products can be ordered at **www.invitrogen.com**.

Table 64 Required equipment

Item‡	Source
Covaris™ S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris™ S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, refer to "Covaris™ S2 System Materials Summary," *SOLiD™ 4 System Site Preparation Guide* (PN 4448639).	• Applied Biosystems 4387833 (110 V) • Applied Biosystems 4392718 (220 V) or Covaris
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
E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit	Invitrogen 6465
Vortexer	Major Laboratory Supplier (MLS)

Table 64 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 65 Required consumables

Item‡	Source
1× Low TE Buffer	Applied Biosystems 4389764
Nuclease-free Water, 1 L	Applied Biosystems AM9932
Covaris™ microTUBEs	Covaris™ 520045
Isopropyl alcohol	Sigma-Aldrich I9516
Ethylene glycol	American Bioanalytical AB00455-01000
0.5-mL LoBind Tubes	Eppendorf 022431005
1.5-mL LoBind Tubes	Eppendorf 022431021
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit§	Thermo Scientific 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.
§ The NanoDrop® Conditioning Kit is useful for “reconditioning” the sample measurement pedestals to a hydrophobic state if they become “unconditioned” (refer to the Nanodrop user’s manual for more information). The PR-1 kit consists of a container of specially formulated polishing compound and a supply of convenient applicators.
# 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.

Prepare a 2 × 50 bp mate-paired library

Table 66 Required Applied Biosystems reagent kits

Item (part number)‡	Components	Kit components used in…
SOLiD™ Mate-Paired Library Oligos Kit (4400468)	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™ Library Oligos Kit 2 – LMP CAP Adaptor (ds)	2 × 50 bp mate-paired library preparation
	SOLiD™ Library Oligos Kit 2 – Internal Adaptor (ds)	DNA circularization
	SOLiD™ Library Oligos Kit 2 – EcoP15I CAP Adaptor (ds)	2 × 25 bp mate-paired library preparation
SOLiD™ Long Mate-Paired Library Construction Kit (4443474)§#	SOLiD™ Long Mate-Paired Library Enzyme Kit 1: • 5X End Polishing Buffer • End Polishing Enzyme 1, 10 U/µL • End Polishing Enzyme 2, 5 U/µL • dNTP, 10 mM	DNA end repair
	• 5X Ligase Buffer • T4 DNA Ligase, 5 U/µL	Adaptor ligation, DNA circularization
	• DNA Polymerase I, 10 U/µL • Nick Translation Buffer	Nick translation
	• 0.5 M EDTA	Stop end-repair reaction
	• 100X BSA	Bead wash
	• Platinum® PCR Amplification Mix	Library amplification (*Table continued on next page...*

Table 66 Required Applied Biosystems reagent kits

Item (part number)‡	Components	Kit components used in…
	SOLiD™ Long Mate-Paired Library Enzyme Kit 2 (Exo/Nucleases): • T7 Exonuclease, 10 U/µL • 10X Buffer 4 • S1 Nuclease • 3 M NaCl • S1 Dilution Buffer • 10X Plasmid-Safe™ Buffer • Plasmid-Safe™ DNase • ATP, 25 mM	Gap creation at a nicked site Digestion at gap sites to release mate-paired fragments Uncircularized DNA removal
	SOLiD™ Mate-Paired Library Bead & Buffer Kit: • Dynabeads® MyOne™ Streptavidin C1 • Bead Wash Buffer • Bead Binding Buffer	Mate-paired library capture
	SOLiD™ Library Column Purification Kit SOLiD™ Library Micro Column Purification Kit	DNA purification
	SOLiD™ Library Quick Gel Extraction Kit SOLiD™ Library Size Selection Gels	DNA 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 or Invitrogen, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Invitrogen products can be ordered at **www.invitrogen.com**.

Table 67 Optional Applied Biosystems reagent kits

Item (part number)‡	Components	Kit components used in…
SOLiD™ Long Mate-Paired Library Construction Kit without Purification (4443711)§#	SOLiD™ Long Mate-Paired Library Enzyme Kit 1:	
	• 5X End Polishing Buffer • End Polishing Enzyme 1, 10 U/µL • End Polishing Enzyme 2, 5 U/µL • dNTP, 10 mM	DNA end repair
	• 5X Ligase Buffer • T4 DNA Ligase, 5 U/µL	Adaptor ligation, DNA circularization
	• DNA Polymerase I, 10 U/µL • Nick Translation Buffer	Nick translation
	• 0.5 M EDTA	Stop end-repair reaction
	• 100X BSA	Bead wash
	• Platinum® PCR Amplification Mix	Library amplification
	SOLiD™ Long Mate-Paired Library Enzyme Kit 2 (Exo/Nucleases):	
	• T7 Exonuclease, 10 U/µL • 10X Buffer 4 • S1 Nuclease	Gap creation at a nicked site
	• 3 M NaCl • S1 Dilution Buffer • 10X Plasmid-Safe™ Buffer	Digestion at gap sites to release mate-paired fragments
	• Plasmid-Safe™ DNase • ATP, 25 mM	Uncircularized DNA removal

(*Table continued on next page...*)

Table 67 Optional Applied Biosystems reagent kits

Item (part number)‡	Components	Kit components used in…
	SOLiD™ Mate-Paired Library Bead & Buffer Kit: • Dynabeads® MyOne™ Streptavidin C1 • Bead Wash Buffer • Bead Binding Buffer	Mate-paired library capture

‡ 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 or Invitrogen, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Invitrogen products can be ordered at **www.invitrogen.com**.

Table 68 Required equipment

Product Name‡	Vendor
HydroShear® DNA Shearing Device from Genomic Solutions®§	• Applied Biosystems 4392889 (115 V) • Applied Biosystems 4392890 (230 V)
Covaris™ S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris™ S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, refer to "Covaris™ S2 System Materials Summary," *SOLiD™ 4 System Site Preparation Guide* (PN 4448639).	• Applied Biosystems 4387833 (110 V) • Applied Biosystems 4392718 (220 V) or Covaris
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
Labquake Rotisserie Rotator, Barnstead/Thermolyne	VWR 56264-312

Table 68 Required equipment

Product Name‡	Vendor
6-Tube Magnetic Stand *or* DynaMag™– 2 Magnet	• Applied Biosystems AM10055 • Invitrogen 123-21D
E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit	Invitrogen G6465
Safe Imager™ 2.0 Blue Light Transilluminator *or* Safe Imager™ Blue Light Transilluminator	Invitrogen G6600 Invitrogen S37102
Gel imaging system	Major Laboratory Supplier (MLS)
Tabletop Centrifuge	MLS
Gel boxes and power supplies for agarose gels	MLS
Vortexer	MLS
Picofuge	MLS
Incubator (37 ºC)	MLS
Incubator (70 ºC)	MLS
Scale	MLS
Timer	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.
§ For more information on the HydroShear® DNA Shearing Device and materials, refer to the manufacturer's documentation.
# Or equivalent but validation of the equipment for library preparation is required.

Table 69 Optional equipment

Product name‡	Vendor
2100 Bioanalyzer	Agilent Technologies G2938C
Qubit® Fluorometer	Invitrogen Q32857

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

Table 70 Required consumables

Item ‡	Source
E-Gel® SizeSelect™ 2% Agarose (optional)	Invitrogen G6610-02
E-Gel® EX Gel, 2%, 10-Pak	Invitrogen G4010-02
UltraPure™ DNA Typing Grade 50X TAE Buffer	Invitrogen 24710-030
Agarose-LE *or* UltraPure™Agarose 1000	Applied Biosystems AM9040 Invitrogen 10975-035
SYBR® Safe DNA Gel Stain (10,000X)	Invitrogen S33102
10X BlueJuice™ Gel Loading Buffer	Invitrogen 10816-015
25 bp DNA Ladder	Invitrogen 10597-011
100 bp DNA Ladder	Invitrogen 15628-050
1 Kb Plus DNA Ladder	Invitrogen 10787-018
UltraPure™ Glycerol	Invitrogen 15514-011
Covaris Tubes and Caps, 125	Applied Biosystems 4399054
Ethanol	Sigma-Aldrich E7023
Isopropyl alcohol	Sigma-Aldrich I9516
Ethylene glycol	American Bioanalytical AB00455-01000
0.5-mL LoBind Tubes	Eppendorf 022431005
1.5-mL LoBind Tubes	Eppendorf 022431021
2.0-mL LoBind Tubes	Eppendorf 022431048
Hydrochloric Acid, 0.20 N	VWR VW8888-0

Table 70 Required consumables

Item ‡	Source
Sodium Hydroxide, 0.20 N	VWR VW8889-0
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit§	Thermo Scientific PR-1
Filtered pipettor tips#	Major Laboratory Supplier (MLS)
Razor blades	MLS
PCR strip tubes	MLS
15-mL conical polypropylene tubes	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.

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 71 Optional consumables

Product name‡	Vendor
Agilent DNA 1000 Kit	Agilent Technologies 5067-1504
1% E-Gel® EX Gel	Invitrogen G401001
Quanti-iT™ PicoGreen® dsDNA Assay Kit	Invitrogen P7589
Quant-iT™ dsDNA HS Assay Kit	Invitrogen Q32851 or Q32854
Quant-iT™ dsDNA BR Assay Kit	Invitrogen Q32850 or Q32853

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

Prepare a 2 × 25 bp mate-paired library

Table 72 Required Applied Biosystems reagent kits

Item (part number)‡	Components	Kit components used in…
SOLiD™ Mate-Paired Library Oligos Kit (4400468)	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™ Library Oligos Kit 2 – EcoP15I CAP Adaptor (ds)	2 × 25 bp mate-paired library preparation
	SOLiD™ Library Oligos Kit 2 – Internal Adaptor (ds)	DNA circularization
	SOLiD™ Library Oligos Kit 2 – LMP CAP Adaptor (ds)	2 × 50 bp mate-paired library preparation

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

Table 73 Required Applied Biosystems reagent kits — continued

Item (part number)‡	Components	Kit components used in…
SOLiD™ 2 × 25 bp Mate-Paired Library Construction Kit (4443472)§#	SOLiD™ 2 × 25 bp Mate-Paired Library Enzyme Kit: • 5X End Polishing Buffer • End Polishing Enzyme 1, 10 U/µL • End Polishing Enzyme 2, 5 U/µL • dNTP, 10 mM	DNA end repair
	• 5X Ligase Buffer • T4 DNA Ligase, 5 U/µL	Adaptor ligation, DNA circularization
	• Klenow Fragment • Stop Buffer	DNA end repair
	• DNA Polymerase I, 10 U/µL • Nick Translation Buffer	Nick translation
	• Sinefungin, 10 mM	EcoP15I digestion cofactor
	• 10X Plasmid-Safe™ Buffer • Plasmid-Safe™ DNase • ATP, 25 mM • Platinum® PCR Amplification Mix	Uncircularized DNA removal Library amplification
	SOLiD™ Mate-Paired Library Bead & Buffer Kit: • Dynabeads® MyOne™ Streptavidin C1 • Bead Wash Buffer • Bead Binding Buffer	Mate-paired library capture
	SOLiD™ Library Column Purification Kit SOLiD™ Library Micro Column Purification Kit	DNA purification
	SOLiD™ Library Quick Gel Extraction Kit SOLiD™ Library Size Selection Gels	DNA 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.

Invitrogen products can be ordered at **www.invitrogen.com**.

Table 74 Required equipment

Product Name‡	Vendor
HydroShear® DNA Shearing Device from Genomic Solutions®§	• Applied Biosystems 4392889 (115 V) • Applied Biosystems 4392890 (230 V)
Covaris™ S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris™ S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, refer to "Covaris™ S2 System Materials Summary," *SOLiD™ 4 System Site Preparation Guide* (PN 4448639).	• Applied Biosystems 4387833 (110 V) • Applied Biosystems 4392718 (220 V) or Covaris
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
Labquake Rotisserie Rotator, Barnstead/Thermolyne	VWR 56264-312

Table 74 Required equipment

Product Name‡	Vendor
6-Tube Magnetic Stand *or* DynaMag™– 2 Magnet	• Applied Biosystems AM10055 • Invitrogen 123-21D
E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit	Invitrogen G6465
Safe Imager™ 2.0 Blue Light Transilluminator *or* Safe Imager™ Blue Light Transilluminator	Invitrogen G6600 Invitrogen S37102
Gel imaging system	Major Laboratory Supplier (MLS)
Tabletop Centrifuge	MLS
Gel boxes and power supplies for agarose gels	MLS
Vortexer	MLS
Picofuge	MLS
Incubator (37 ºC)	MLS
Incubator (65 ºC)	MLS
Scale	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.
§ For more information on HydroShear® DNA Shearing Device and materials, refer to the manufacturer's documentation.
# Or equivalent but validation of the equipment for library preparation is required.

Table 75 Optional equipment

Product name‡	Vendor
2100 Bioanalyzer	Agilent Technologies G2938C
Qubit® Fluorometer	Invitrogen Q32857

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

Table 76 Required consumables

Item ‡	Source
E-Gel® SizeSelect™ 2% Agarose (optional)	Invitrogen G6610-02
E-Gel® EX Gel, 2%, 10-Pak	Invitrogen G4010-02
UltraPure™ DNA Typing Grade 50X TAE Buffer	Invitrogen 24710-030
Agarose-LE *or* UltraPure™ Agarose 1000	Applied Biosystems AM9040 Invitrogen 10975-035
SYBR® Safe DNA Gel Stain (10,000X)	Invitrogen S33102
10X BlueJuice™ Gel Loading Buffer	Invitrogen 10816-015
25-bp DNA Ladder	Invitrogen 10597-011
1 Kb Plus DNA Ladder	Invitrogen 10787-018
UltraPure™ Glycerol	Invitrogen 15514-011
Covaris Tubes and Caps, 125	Applied Biosystems 4399054
S-adenosylmethionine (SAM), 32 mM	New England BioLabs B9003S
EcoP15I	New England BioLabs R0646L
Ethanol	Sigma-Aldrich E7023
Isopropyl alcohol	Sigma-Aldrich I9516
Ethylene glycol	American Bioanalytical AB00455-01000
0.5-mL LoBind Tubes	Eppendorf 022431005
1.5-mL LoBind Tubes	Eppendorf 022431021
2.0-mL LoBind Tubes	Eppendorf 022431048

Table 76 Required consumables

Item ‡	Source
Hydrochloric Acid, 0.20 N	VWR VW8888-0
Sodium Hydroxide, 0.20 N	VWR VW8889-0
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit§	Thermo Scientific PR-1
Filtered pipettor tips#	Major Laboratory Supplier (MLS)
Razor blades	MLS
PCR strip tubes	MLS
15-mL conical polypropylene tubes	MLS

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

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

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 77 Optional consumables

Product name‡	Vendor
Agilent DNA 1000 Kit	Agilent Technologies 5067-1504
1% E-Gel® EX Gel	Invitrogen G401001
Quanti-iT™ PicoGreen® dsDNA Assay Kit	Invitrogen P7589
Quant-iT™ dsDNA HS Assay Kit	Invitrogen Q32851 or Q32854
Quant-iT™ dsDNA BR Assay Kit	Invitrogen Q32850 or Q32853

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

Prepare a barcoded fragment library

Table 25 Required Applied Biosystems reagent kits

Item (part number)‡	Components	Kit components used in…
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
SOLiD™ Fragment Library Barcoding Kit Module 1–16 (4444837) 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)	• Multiplex Library P1 Adaptor, 50 µM • Multiplex Library PCR-1, 50 µM • Multiplex Library PCR-2, 50 µM • Barcodes 0XX, 50 µM	Preparation of 16 barcoded fragment libraries
SOLiD™ Fragment Library Construction Kit with Size Selection Gels (4443471)§	SOLiD™ Fragment Library Enzyme/Core Kit: • 5X End Polishing Buffer • dNTP, 10 mM • End Polishing Enzyme 1 • End Polishing Enzyme 2	DNA end repair
	• 5X Ligase Buffer • 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™ 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 78 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: • 5× End Polishing Buffer • dNTP, 10 mM • End Polishing Enzyme 1 • End Polishing Enzyme 2	DNA end repair
	• 5× Ligase Buffer • 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: • 5× End Polishing Buffer • dNTP, 10 mM • End Polishing Enzyme 1 • End Polishing Enzyme 2	DNA end repair
	• 5× Ligase Buffer • 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 79 Required equipment

Item‡	Source
Covaris™ S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris™ S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, see "Covaris™ S2 System Materials Summary," *SOLiD™ 4 System Site Preparation Guide* (PN 4448639)	• Applied Biosystems 4387833 (110 V) • Applied Biosystems 4392718 (220 V) or Covaris
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
E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit	Invitrogen G6465
Vortexer	MLS

Table 79 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 80 Optional equipment

Item‡	Source
2100 Bioanalyzer	Agilent Technologies G2938C
Qubit™ Quantitation Starter Kit	Invitrogen Q32860

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

Table 81 Required consumables

Item‡	Source
1× Low TE Buffer	Applied Biosystems 4389764
Nuclease-free Water, 1 L	Applied Biosystems AM9932
Covaris microTUBEs	Covaris 520045
Isopropyl alcohol	Sigma-Aldrich I9516
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
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1

Table 81 Required consumables

Item‡	Source
PR-1 Conditioning Kit§	Thermo Scientific 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.

§ 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.

Table 82 Optional consumables

Product name‡	Vendor
Agilent DNA 1000 Kit	Agilent Technologies 5067-1504

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

B SOLiD™ 4 System Library Quantitation with the SOLiD™ Library TaqMan® Quantitation Kit

This appendix covers:

Overview

Purpose of the kit

A key component of SOLiD™ system sequencing is emulsion PCR (ePCR), which involves monoclonal amplification of individual species of template DNA from a complex library pool. During ePCR, multiple copies of a single DNA sequence are coated onto a single 1-µm magnetic bead within a water-in-oil emulsion droplet. For optimal monoclonal amplification, precise quantification of the input library is critical.

Quantitative PCR (qPCR) is the preferred method for determining the amount of amplifiable template in a SOLiD™ library. Quantitative PCR provides the high level of specificity required by ePCR, and can accurately measure extremely low quantities of DNA, allowing the user to dilute SOLiD™ libraries to very low concentrations for quantitation.

The SOLiD™ Library TaqMan® Quantitation Kit contains the following validated reagents for qPCR:

- SOLiD™ Library qPCR Mix, an optimized master mix of qPCR reagents including DNA polymerase and dNTPs
- TaqMan® Assay for SOLiD™ Library Quantification (Ac00010015a1), a fluorogenic probe-based qPCR detection assay
- SOLiD™ Library qPCR Standard, a validated, pre-quantified, ready-to-use standard specifically designed for quantifying SOLiD™ libraries in qPCR

The kit is designed to quantify libraries accurately regardless of size, can be used on any real-time instrument, and is compatible with both fast and standard cycling programs.

Advantages of the kit

- *The TaqMan® Assay* (Ac00010015a1) provides more specificity and accuracy in detecting amplifiable templates over non-probe-based quantitation methods.
- *The validated, ready-to-use qPCR standard* requires only a simple serial dilution for use in qPCR. It can also be used to determine the optimal concentration of template to use in ePCR.
- *The highly robust qPCR mix* can accommodate a wide range of cycling conditions and reaction volumes, and combines highly sensitive detection with a broad quantification range.

The TaqMan® Assay

In qPCR, the qPCR Standard and unknown library template are amplified using two sequence-specific primers with a TaqMan® fluorogenic probe labeled with FAM™ dye and a dye quencher. A fluorescent signal is generated by the FAM dye when it detaches from the probe as the DNA polymerase in the SOLiD™ Library qPCR Mix extends the 3′ strand (see Figure 26 on page 175).

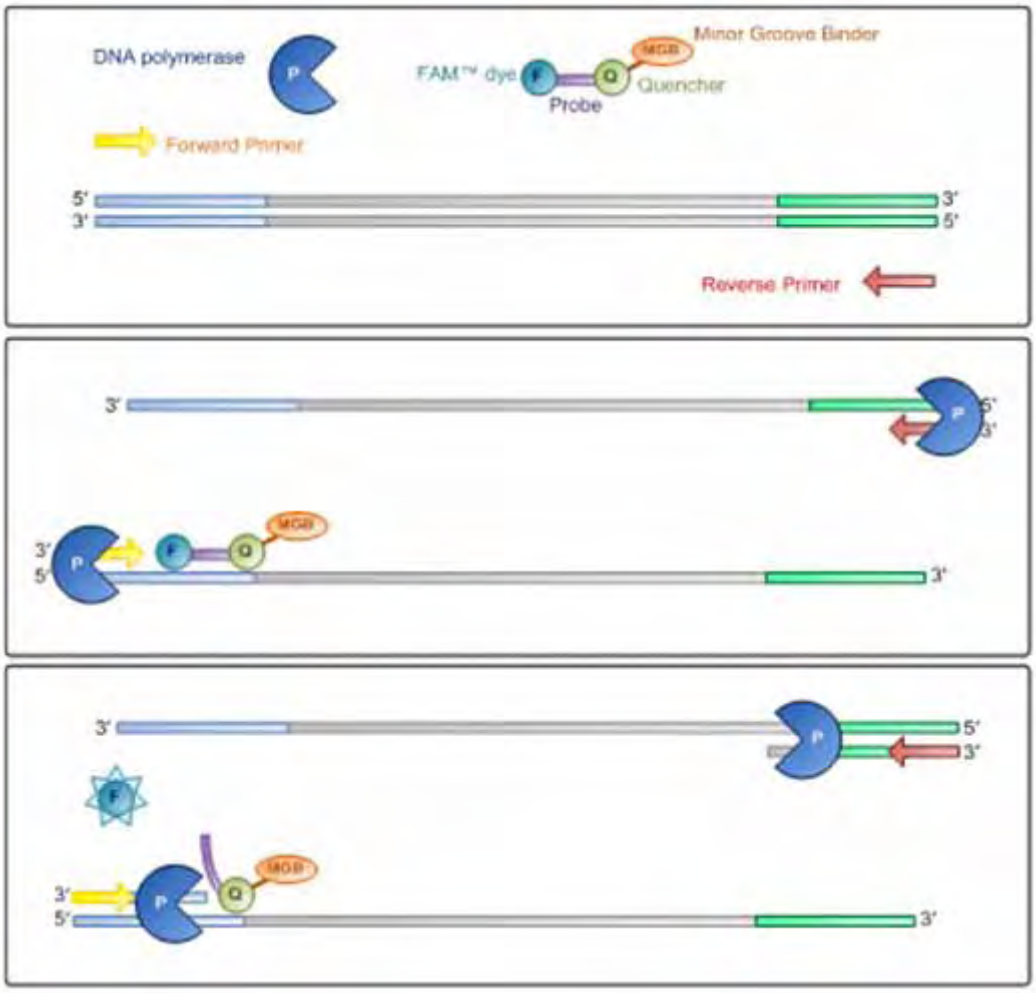


Figure 26 Generation of the FAM dye signal during 3′ extension.

Platinum® *Taq* DNA Polymerase

Platinum® *Taq* DNA Polymerase, included in the qPCR mix, is recombinant *Taq* DNA polymerase complexed with proprietary antibodies that block polymerase activity at ambient temperatures. Activity is restored after the initial denaturation step in PCR cycling, providing an automatic hot start in qPCR for increased sensitivity, specificity, and yield.

Uracil DNA Glycosylase (UDG)

Heat-labile UDG and dUTP in the qPCR mix prevent the reamplification of carryover products between qPCR reactions. dUTP ensures that any amplified DNA will contain uracil, while heat-labile UDG removes uracil residues from single- or double-stranded DNA.

The heat-labile form of UDG used in this kit is completely inactivated at temperatures of 50 °C and higher and will not degrade amplicons following qPCR, thus enabling their use for downstream applications such as cloning.

Instrument capability

The SOLiD™ Library TaqMan® Quantitation Kit can be used with a wide range of real-time instruments, including the Applied Biosystems 7900HT, 7300, 7500, StepOne™, and StepOnePlus™ Instruments.

Materials and equipment required

Table 83 Required Applied Biosystems reagent kits

Item (Part number)‡	Components
SOLiD™ Library TaqMan® Quantitation Kit (4449639)	SOLiD™ Library TaqMan® qPCR Module: • SOLiD™ Library qPCR Mix • ROX Reference Dye • Ac00010015a1 TaqMan® Assay SOLiD™ Library qPCR Standard

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

Note: The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on the Invitrogen website. Go to:
www.invitrogen.com/cofa
The CofA is searchable by product lot number, which is printed on each box.

Table 84 Required equipment

Item‡	Source
Applied Biosystems 7900HT, 7300, 7500, StepOne™, StepOnePlus™, PRISM® 7000, or PRISM® 7700 Instruments	Applied Biosystems
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
Vortexer	MLS (Major Laboratory Supplier)
Picofuge	MLS
Pipettors, 20 µL	MLS
Pipettors, 200 µL	MLS
Pipettors, 1000 µL	MLS

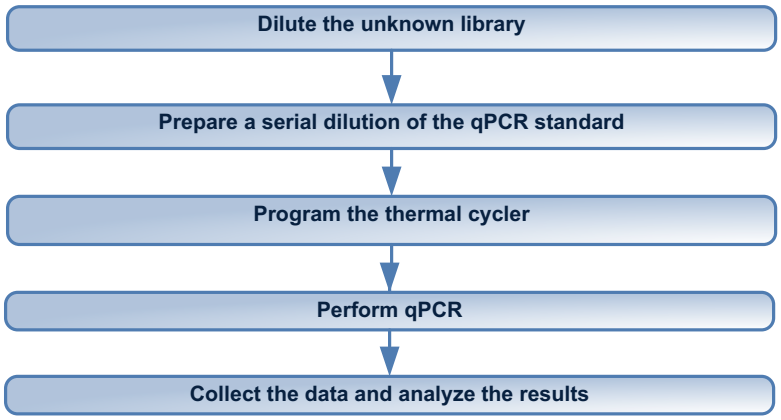
‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.
§ Or equivalent but validation of the equipment for library preparation is required.

Table 85 Required consumables

Item‡	Source
MicroAmp® Fast Optical 96-well reaction plate with barcode, 0.1 mL	Applied Biosystems 4346906
MicroAmp® Optical Adhesive Film	Applied Biosystems 4360954
0.5-mL LoBind Tubes	Eppendorf 022431005
1.5-mL LoBind Tubes	Eppendorf 022431021
Filtered pipettor tips	Major Laboratory Supplier (MLS)
DEPC-treated water	MLS

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

Workflow



For detailed instructions on each step of the workflow, see "The qPCR protocol" on page 179.

Tips

Kit components and storage

All components are shipped on dry ice. Store all components at –20 °C for long-term storage. The SOLiD™ Library qPCR Mix may be stored at 4–8 °C for up to one month.

Reaction setup and conditions

- Maintain a sterile environment when handling SOLiD™ libraries and the qPCR standard to avoid any contamination from DNases.
- Ensure that all equipment that comes in contact with DNA is sterile, including pipette tips and microcentrifuge tubes.
- qPCR reaction volumes can be scaled from 5 µL to 100 µL, depending on the plate size and instrument (for example, the 7500 Fast Real-Time PCR System uses 20 µL per well in both standard and fast mode).
- Set up all samples including no-template control (NTC) in triplicate to increase accuracy.
- Perform all steps requiring 0.5-mL and 1.5-mL tubes with Eppendorf LoBind Tubes.
- Avoid multiple thaws of samples.

384-well plate volumes

For 384-well plates, we recommend a maximum reaction volume of 10 µL per well.

ROX™ reference dye concentration

ROX™ dye is recommended for fluorescence normalization on Applied Biosystems instruments. ROX dye is composed of a glycine conjugate of 5-carboxy-X-rhodamine, succinimidyl ester and is supplied at a concentration of 25 µM.

Determine the amount of 25-µM ROX to use with a particular instrument (see Table 86).

Table 86 Amount of 25-µL ROX to use according to instrument

Instrument	ROX amount per 20-µL reaction	Final ROX concentration
AB 7300, 7900HT, StepOne™, StepOnePlus™, and ABI PRISM® 7000 and 7700 Instruments	0.4 µL	500 nM
AB 7500	0.04 µL	50 nM

The qPCR protocol

Dilute the unknown library

1. Dilute aliquots of your unknown library to a target concentration of 50 pg/µL in DEPC-treated water in LoBind Tubes on ice. The concentration may be determined by a method other than qPCR, such as a NanoDrop spectrophotometer. Prepare enough diluted sample for both qPCR quantification and subsequent ePCR reactions. These aliquots may be stored at –20 °C.

2. Prior to qPCR, further dilute the diluted library 1:1000 in DEPC-treated water in a LoBind tube on ice, to target a range within the standard curve. (For a standard 20-µL qPCR reaction, you will need 5 µL of diluted unknown per well.)

Prepare a serial dilution of qPCR standard

The SOLiD™ Library qPCR Standard is supplied in a volume of 10 µL at 5 nM. To prepare the standard for qPCR:

1. Thaw the SOLiD™ Library qPCR Standard on ice.

2. Dilute 1 µL of the standard in 49 µL of DEPC-treated water, to a concentration of 100 pM, then prepare four sequential 10-fold dilutions from the 100 pM dilution (see Table 87). Prepare sufficient volume of each dilution for the number and size of qPCR reactions (for example, for a 20-µL qPCR reaction volume, you will need 5 µL of diluted standard per well, or 15 µL per triplicate plus a small overage for pipetting errors).

Table 87 Example dilutions of standard (scale for your qPCR reaction volumes/replicates)

	Dilution from 5 nM stock	Components	Final concentration
Standard 1	1:50	1 µL of stock + 49 µL of DEPC-treated water	100 pM
Standard 2	1:500	5 µL of Standard 1 + 45 µL of DEPC-treated water	10 pM
Standard 3	1:5,000	5 µL of Standard 2 + 45 µL of DEPC-treated water	1 pM
Standard 4	1:50,000	5 µL of Standard 3 + 45 µL of DEPC-treated water	0.1 pM
Standard 5	1:500,000	5 µL of Standard 4 + 45 µL of DEPC-treated water	0.01 pM

Program the thermal cycler

Program your thermal cycler according to the instructions provided with the instrument. Enter the quantities for the 5 dilutions of the qPCR Standard from 100 to 0.01 pM. The quantity for the unknown will be calculated in pM.

The cycling programs have been developed as a general starting point when using the SOLiD™ Library qPCR Mix. The fast cycling program was developed using the AB 7500 in Fast mode (see Table 88).

Note: This mix is highly robust and can be used with a wide range of cycling programs on different instruments. If you have an alternative program that you want to use, test it with this mix.

Table 88 Suggested cycling programs to use with the SOLiD™ Library qPCR Mix

Cycles	*Fast* cycling program‡		Cycles	*Standard* cycling program	
	Temp	Time		Temp	Time
—	95 °C	20 sec	—	95 °C	2 min
40	95 °C	3 sec	40	95 °C	15 sec
	60 °C	30 sec		60 °C	1 min

‡ Developed using the AB 7500 in Fast mode.

Perform qPCR

Use the procedure below as a general starting point. Volumes for a standard 20-µL reaction are provided. Scale the reaction volume as needed for your real-time instrument.

1. Based on the number and size of your qPCR reactions (including no-template controls), prepare a master mix of all components except template on ice. Volumes for a single reaction are shown below; scale as needed (see Table 89).

   Table 89 Master mix for a single 20-µL qPCR reaction

Component	Volume (µL)
SOLiD™ Library qPCR Mix	10
Ac00010015_a1 TaqMan® Assay (20X mix)	1
ROX™ Reference Dye, 25 µM	0.4 µL/0.04 µL‡
DEPC-treated water	to 15 µL final volume

   ‡ See Table 86 on page 178 for the amount/concentration of ROX to use for your specific instrument.

2. For a 20-µL reaction, pipet 15 µL of the master mix into each well of the PCR plate.

3. Add 5 µL of diluted standard or unknown library to each appropriate well. Add 5 µL of sterile water to the no-template control (NTC).

4. Seal the plate. Make sure that all components are at the bottom of the well. Centrifuge the plate briefly, if needed.

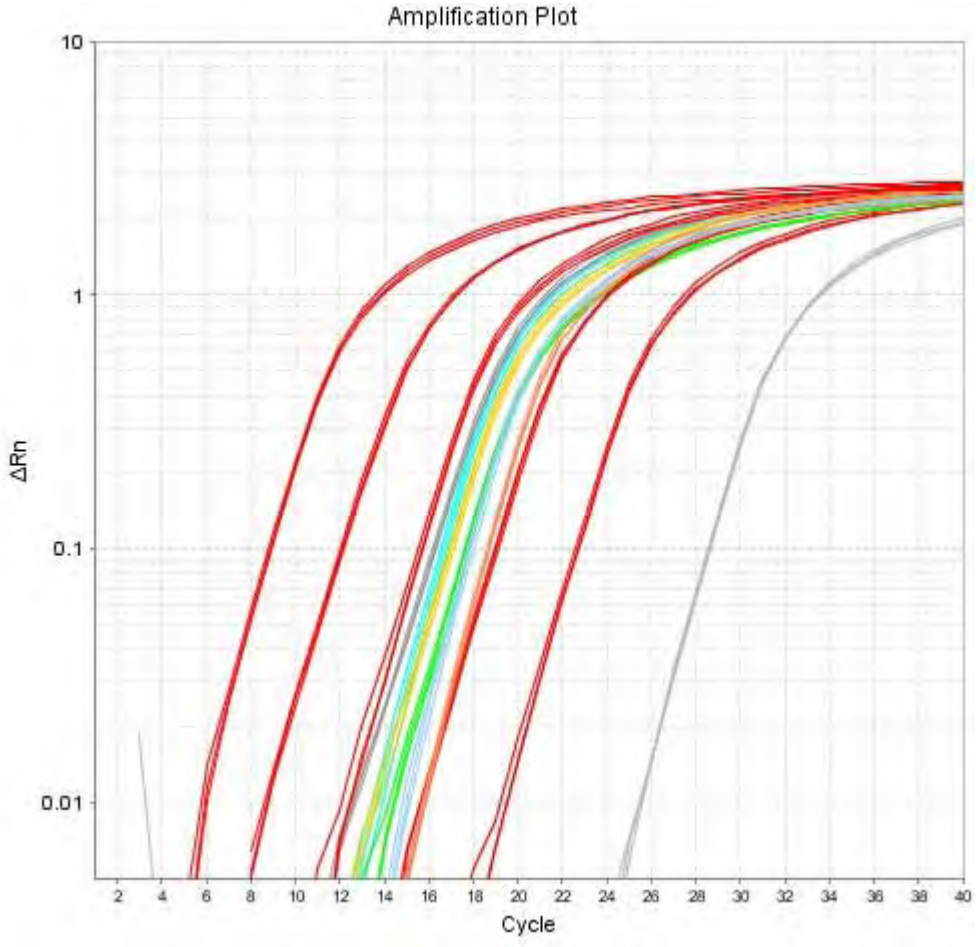
5. Place reactions in a real-time instrument programmed as described previously. Collect data and analyze results.

Example data

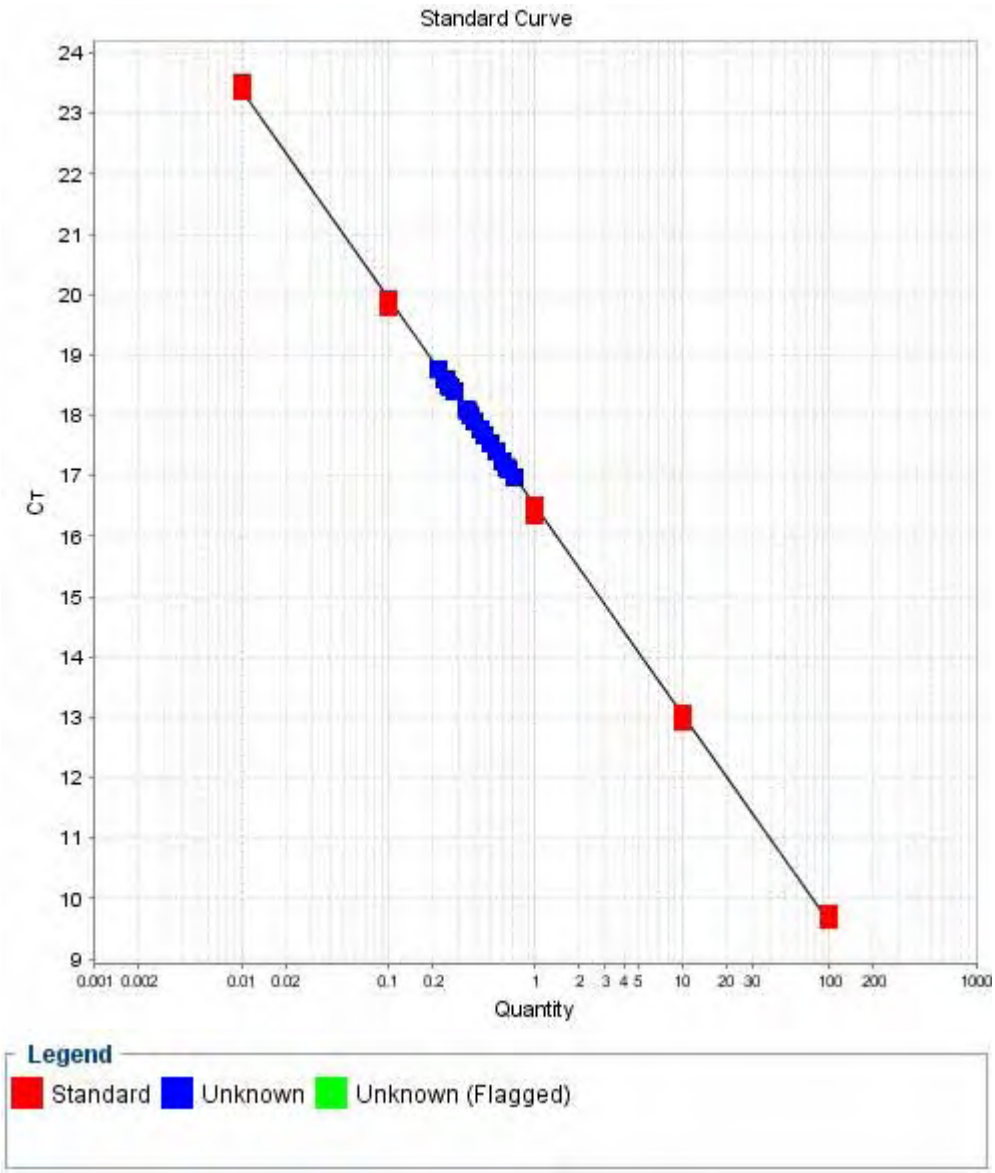
Amplification plot and standard curve

Below are an amplification plot and standard curve generated using the reagents supplied in this kit. Dilutions of the SOLiD™ Library qPCR Standard and multiple SOLiD™ libraries were prepared in triplicate, and the reactions were run on the Applied Biosystems StepOnePlus™ Real-Time PCR System.

Amplification plot



Standard curve



Determine the optimal library concentration for ePCR

Perform a WFA run with dilutions of the qPCR standard

The SOLiD™ Library TaqMan® Quantitation Kit will accurately quantify your unknown library; however, it will not tell you how much library to use in ePCR. To determine this, we recommend preparing a three-point titration of the SOLiD™ Library qPCR Standard in ePCR and performing a workflow analysis (WFA) run on the templated beads. You can then analyze the run to determine the optimal concentration of library to use in ePCR.

IMPORTANT! You only need to perform this assay once to determine the optimal titration point for all libraries quantitated by the qPCR standard. If the ePCR protocol is updated, we recommend running the assay again with the new protocol.

General procedure

For detailed information on preparing the SOLiD™ Library qPCR Standard for ePCR, refer to the *Applied Biosystems SOLiD 4 System Templated Bead Preparation Guide* (PN 4448378) or the *Applied Biosystems SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (PN 4441486). For information on performing and analyzing a WFA run, refer to the *Applied Biosystems SOLiD 4 System Instrument Operation Guide* (PN 4448379).

1. Perform ePCR and prepare templated beads from the SOLiD™ Library qPCR Standard. A 3-point titration of the standard is recommended to find the optimal titration point. We suggest using final ePCR library concentrations of 0.1, 0.3, and 0.5 pM as starting points; however, you may choose your own titrations if you have historical data that supports a different range.

2. Perform WFA runs on the titrated, templated beads. The titration point that provides the best results is the titration point to use for your unknown libraries. Because the concentrations used in ePCR are in picomolar (pM) units, there is no conversion necessary for library size.

Troubleshooting

Problem	Cause	Solution
Signal appears in no-template controls (NTCs)	Template or reagents may be contaminated by nucleic acids (DNA, cDNA)	Some spurious amplification may occur in NTCs at high CTs (for example, ~30). This is above the CT range for actual template, and thus has no effect on quantitation. Take standard precautions to avoid contamination when preparing your PCR reactions. Ideally, amplification reactions should be assembled in a DNA-free environment. We recommend using aerosol-resistant barrier tips.
No PCR product is evident, either in the qPCR graph or on a gel	The protocol was not followed correctly	Verify that all steps have been followed and the correct reagents, dilutions, volumes, and cycling parameters have been used.
	Template contains inhibitors, nucleases, or proteases, or has otherwise been degraded.	Purify or re-purify your template.
PCR product is evident on a gel, but not in the qPCR graph	qPCR instrument settings are incorrect	Confirm that you are using the correct instrument settings (dye selection, reference dye, filters, and acquisition points).
	There are problems with your specific qPCR instrument	See your instrument manual for tips and troubleshooting.
PCR efficiency is above 110%	Template contains inhibitors, nucleases, or proteases, or has otherwise been degraded	Purify or re-purify your template. Inhibitors in the template may result in changes in PCR efficiency between dilutions.
	Nonspecific products may be amplified	Run the PCR products on a 4% agarose gel after the reaction to identify contaminants.
PCR efficiency is below 90%	The PCR conditions are suboptimal	Verify that the reagents you are using have not been freeze-thawed multiple times and have not remained at room temperature for too long. Verify that the amount of primers you are using is correct.

C

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 (see Figure 27).

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.

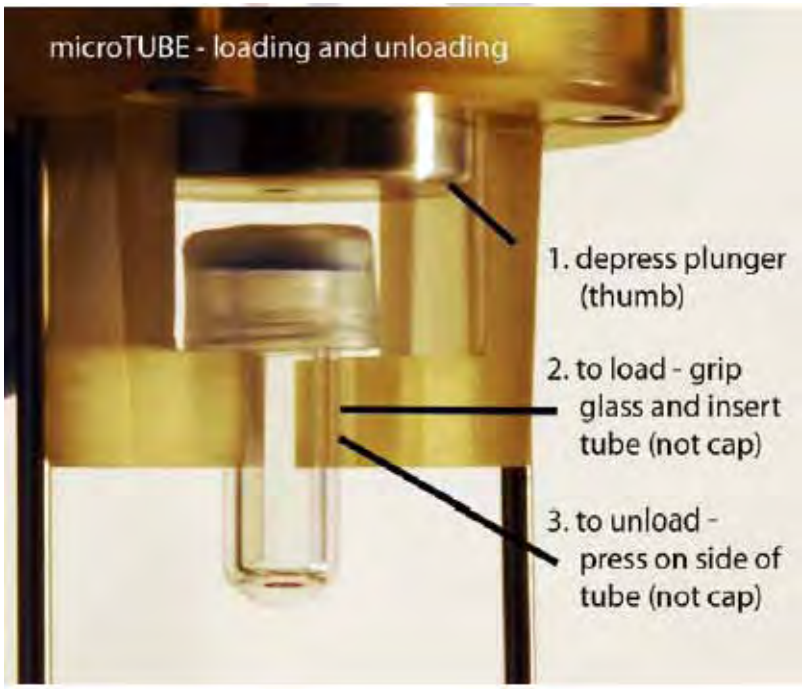


Figure 27 How to load and unload a microTUBE vial from the microTUBE holder.

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 (see Figure 27).

Hybridize oligonucleotides

Oligonucleotide hybridization is required to hybridize single-stranded oligonucleotides to create double-stranded oligonucleotides.

Materials and equipment required

Table 90 Required equipment

Item‡	Source
96-well GeneAmp® PCR System 9700 (thermal cycler)	• Applied Biosystems N8050200 (base) • Applied Biosystems 4314443 (block)
Pipettors	Major Laboratory Supplier (MLS)

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

Table 91 Required consumables

Item‡	Source
5× T4 DNA Ligase Buffer	Invitrogen Corporation 46300-018
SOLiD™ Buffer Kit – 1× Low TE Buffer	Applied Biosystems 4389764§
Oligonucleotides	Major Laboratory Supplier (MLS)#
PCR strip tubes	MLS
Filtered pipettor tips	MLS

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.
§ The part number for the complete SOLiD™Buffer Kit is 4387918.
# 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.

Procedure

1. Prepare 125 µM stock of individual oligonucleotides in 1× Low TE Buffer.
2. Mix equal volumes of 125 µM oligonucleotide A and B.
3. Add 1 part of 5× Ligase Buffer to 4 parts of the oligonucleotide mixture for a final concentration of 50 µM oligonucleotide A and 50 µM oligonucleotide B in 1× Ligase Buffer.
4. Hybridize the oligonucleotides by running the following program on a PCR machine (see Table 92 on page 188):

Table 92 Hybridization protocol

Temperature (°C)	Time (minutes)
95	5
72	5
60	5
50	3
40	3
30	3
20	3
10	3
4	∞

STOPPING POINT. After hybridization, store the 50-µM hybridized oligonucleotides at –20 °C until ready for use in library construction.

Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer

The Thermo Scientific NanoDrop® 1000 Spectrophotometer measures nucleic acid samples up to 3700 ng/µL without dilution.

Materials and equipment required

Table 93 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.

Table 94 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 (see Figure 28 on page 190).

Figure 28 NanoDrop® ND-1000 Spectrophotometer software dialog box (from http://nanodrop.com/nd-1000-software.html).

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 (see Figure 29 on page 191).

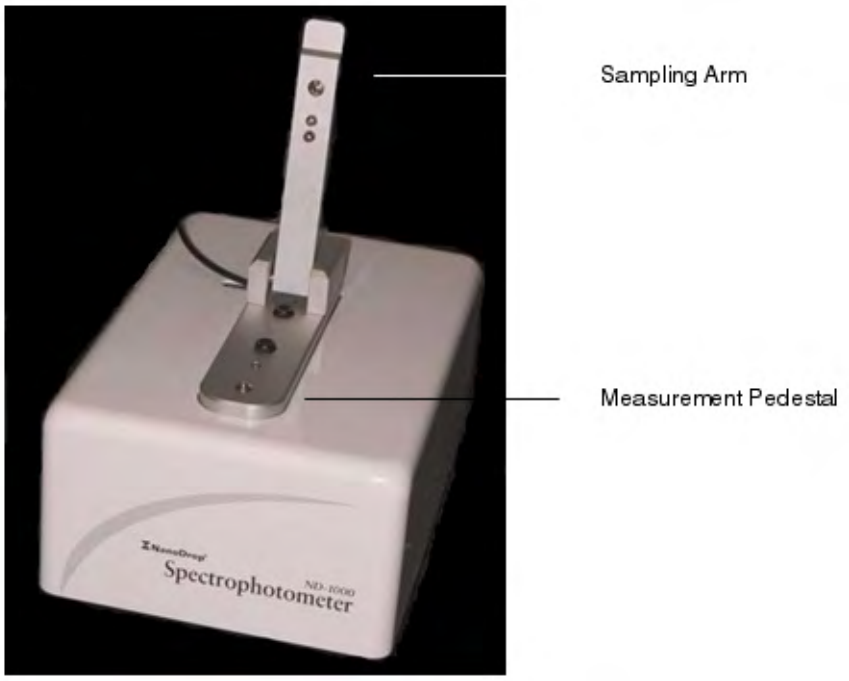


Figure 29 Components of the NanoDrop® ND-1000 Spectrophotometer.

5. In the dialog box, click **OK** and allow the instrument to initialize.
6. Lift the sampling arm and use a Kimwipe® 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 (see Figure 30 on page 192).

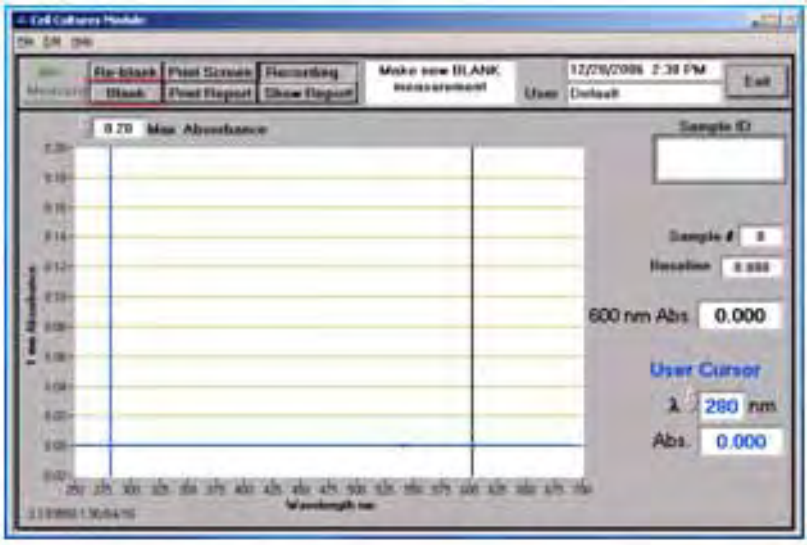


Figure 30 NanoDrop® ND-1000 Spectrophotometer software measurement dialog box.

9. Lift the sampling arm and wipe away the buffer from both the upper and lower measurement pedestals with a Kimwipe. 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.

Phenol-chloroform-isoamyl alcohol extraction

Phenol-chloroform-isoamyl alcohol extraction can be used to isolate DNA. Qiagen MaXtract High Density Tubes can be used in an alternative procedure (see "Phenol-chloroform-isoamyl alcohol extraction with MaXtract" on page 194).

Materials and equipment required

Table 95 Required equipment

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
Pipettors	Major Laboratory Supplier (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 96 Required consumables

Item‡	Source
Phenol:chloroform:isoamyl alcohol, with pH 7.9 buffer	Applied Biosystems AM9730
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.

Procedure

1. Add an equal volume of cold phenol-chloroform-isoamyl alcohol to the sample and vortex.
2. Centrifuge at room temperature at 21,000 × g (minimum 14,000 × g) for 5 minutes.
3. Transfer the upper aqueous layer to a new tube.
4. Discard the phenol:chloroform:isoamyl alcohol layer in hazardous waste.
5. Proceed to "Isopropanol precipitation" on page 198.

Phenol-chloroform-isoamyl alcohol extraction with MaXtract

Phenol-chloroform-isoamyl alcohol extraction can be used to isolate DNA. Qiagen MaXtract High Density Tubes can be used for increased recovery.

Materials and equipment required

Table 97 Required equipment

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
Pipettors	Major Laboratory Supplier (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 98 Required consumables

Item‡	Source
MaXtract High Density Tubes	Qiagen 129046
Phenol:chloroform:isoamyl alcohol, with pH 7.9 buffer	Applied Biosystems AM9730
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.

Procedure

1. Centrifuge the MaXtract tube at 21,000 × g (minimum 14,000 × g) for 20 seconds.
2. Add an equal volume of cold phenol:chloroform:isoamyl alcohol to the aqueous sample.
3. Mix by inversion.
4. Transfer the solution to the pre-spun MaXtract tube.
5. Centrifuge at room temperature at 21,000 × g (minimum 14,000 × g) for 5 minutes.
6. Transfer the upper aqueous layer to a new tube.
7. Discard the MaXtract tube with phenol:chloroform:isoamyl layer in hazardous waste.
8. Proceed to "Isopropanol precipitation" on page 198.

PAGE gel DNA elution

Materials and equipment required

Table 99 Required equipment

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
Pipettors	Major Laboratory Supplier (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 100 Required consumables

Item‡	Source
TE, pH 8.0	Applied Biosystems PN AM9858
7.5 M Ammonium acetate	Sigma-Aldrich A-2706
100X BSA	New England, Inc. B9001S
0.45 µm Nanosep® spin columns	VWR ODM45C34
0.5-mL LoBind Tubes	Eppendorf 022431005
1.5-mL LoBind Tubes	Eppendorf 022431021
21-gauge needle	Major Laboratory Supplier (MLS)
Razor blades	MLS
Filtered pipettor tips	MLS

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

Procedure

1. Excise the appropriate-sized band using a clean razor blade.
2. Using a 21-gauge needle, make a hole in the bottom of a 0.5-mL LoBind tube.
3. Place the gel piece(s) in the 0.5-mL LoBind tube.
4. Place the 0.5-mL LoBind tube with the gel in a 1.5-mL LoBind tube and centrifuge at ≥10,000 × g (13,000 rpm) for 3 minutes to shred the gel and collect in the bottom tube.
5. If some gel pieces remain in the 0.5-mL LoBind tube, repeat the centrifugation step using a new 1.5-mL LoBind tube and then pool the tubes.
6. Add enough PAGE Elution Buffer (1 volume of 7.5 M ammonium acetate in 5 volumes of 1✕ TE) to soak the gel pieces completely with a thin layer of liquid on top.
7. Incubate at room temperature for 20 minutes. The length of elution time can be increased 2 to 3 times for maximum elution.
8. Transfer supernatant to a new 1.5-mL LoBind tube.
9. Add 250 µL of PAGE Elution Buffer to the gel pieces.
10. Incubate at 37 °C for 40 minutes.
11. Pool all of the liquids, including the first elution, into a 0.45-µm filter Nanosep® spin column with a 1.5-mL LoBind tube as the collection tube. Centrifuge the column at ≥10,000 × g (13,000 rpm) for 5 minutes.
12. Proceed to "Isopropanol precipitation" on page 198.

Isopropanol precipitation

Isopropanol precipitation can be used to purify and/or concentrate DNA.

Materials and equipment required

Table 101 Required equipment

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
Pipettors	Major Laboratory Supplier (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 102 Required consumables

Item‡	Source
• 7.5 M Ammonium acetate, or • 3 M Sodium acetate, pH 5.5	• Sigma-Aldrich, A-2706 • Applied Biosystems, PN AM9740
Glycogen, 5 mg/mL	Applied Biosystems AM9510
Isopropyl alcohol	Sigma-Aldrich I9516
Ethanol	E7023
Filtered pipettor tips	Major Laboratory Supplier (MLS)

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

Procedure

1. Add either an equal volume of 7.5 M ammonium acetate or 1/10 volume of 3 M sodium acetate (pH 5.5) to the aqueous phase.
2. Add 1/100 volume of glycogen.
3. Add 0.7 volume of 100% isopropyl alcohol at room temperature. Vortex well.
4. Incubate at room temperature for 5 minutes to precipitate.
5. Centrifuge the solution at 21,000 × g (minimum 14,000 × g) for 15 minutes.
6. Remove the supernatant.
7. Wash the DNA pellet three times with 200 µL 70% ethanol to remove salts. Ensure all the isopropanol is completely removed. If the pellet is dispersed during the wash, then centrifuge at 21,000 × g (minimum 14,000 × g) for 2 minutes.
8. Completely remove the 70% ethanol by a short centrifugation step and a pipette tip.
9. Air-dry the sample for 2 to 5 minutes.

Confirm complete methylation of DNA fragments

To confirm complete methylation of DNA fragments, the following is compared on a quality control gel: (1) unmethylated, unsheared genomic DNA, (2) unmethylated, unsheared EcoP15I-digested genomic DNA, (3) methylated, sheared genomic DNA, and (4) methylated, sheared, EcoP15I-digested DNA.

Materials and equipment required

Table 103 Required kit

Item‡	Source
SOLiD™ Library Column Purification Kit	Applied Biosystems 4443744 (50 reactions)

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

Table 104 Required equipment

Item‡	Source
E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit	Invitrogen G6465
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
Incubator (37 °C)	Major Laboratory Supplier (MLS)
Pipettors	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 105 Required consumables

Item‡	Source
1% E-Gel® EX Gel	Invitrogen G401001
1 Kb Plus DNA Ladder	Invitrogen 10787-018
Gel Loading Solution, All-Purpose, Native Agarose	Applied Biosystems PN AM8556

Table 105 Required consumables

Item‡	Source
EcoP15I, 10 U/µL	New England Biolabs R0646L
100× BSA	New England Biolabs B9001S
Sinefungin	Sigma-Aldrich S8559
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.

Digest the DNA with EcoP15I

1. Combine and mix the following components in two separate microcentrifuge tubes (one for unmethylated, unsheared DNA and one for methylated, sheared DNA) (see Table 106):

Table 106 Mix to digest circularized DNA with EcoP15I

Component	Amount
DNA	0.5 µg
10× NEBuffer 3,	10
100× BSA	1
10 mM Sinefungin	1
10× ATP	20
EcoP15I Enzyme, 10 U/µL	1
Nuclease-free Water	Variable
Total	100

2. Incubate the digestion reaction mixtures at 37 °C for 2 hours.

Purify the DNA with the SOLiD™ Library Column Purification Kit

1. Add 4 volumes of Binding Buffer (B2-S) with isopropanol (55%) to 1 volume of sample. Mix well.
2. Apply about 700 µL of the sample in the binding buffer to the PureLink™ column(s) in collection tube(s).
3. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
4. Repeat steps 2 and 3 until the entire sample has been loaded onto the column(s). Place the column(s) back into the same collection tube(s).
5. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
6. Centrifuge the column(s) at 10,000 × g for 1 minute, then discard the flow-through. Repeat centrifugation at *maximum speed* to remove residual wash buffer.
7. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
8. Add 50 µL of Elution Buffer (E1) to the center of the column(s) to elute the DNA, then let the column(s) stand for 1 minute at room temperature.
9. Centrifuge the column(s) at *maximum speed* for 1 minute.
10. Add the eluate from step 9 back to the column(s), then let the column(s) stand for 1 minute at room temperature.
11. Centrifuge the column(s) at *maximum speed* for 1 minute.
12. If necessary, pool the eluted DNA.

STOPPING POINT. Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Confirm DNA methylation on a gel".

Confirm DNA methylation on a gel

1. Mix 1 µL of 1 Kb Plus DNA Ladder and 19 µL of Nuclease-free Water.
2. Load the diluted ladder onto a 1% E-Gel® EX Gel. Load 20 µL of sample per well. There should be at least one lane between the ladder well and the sample wells to avoid contamination of the sample with ladder.
3. Run the gel and confirm that the sheared, methylated DNA is the expected size relative to the controls (see Figure 31 on page 204).
4. Proceed directly to "Ligate EcoP15I CAP Adaptors to the methylated DNA" on page 102.

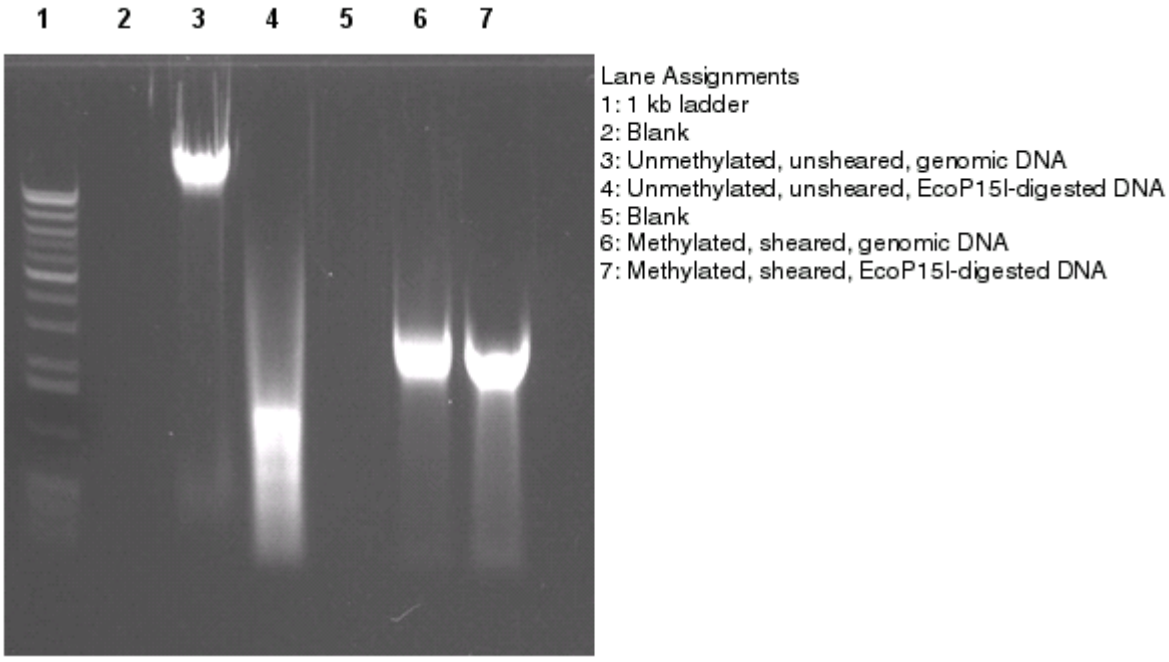


Figure 31 Methylation confirmation gel.

D Oligonucleotide Sequences

This appendix covers:

Library construction oligonucleotides

Adaptor sequences

Multiplex adaptor and barcode sequence	Sequence (bp)
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
EcoP15I CAP Adaptor, 50 µM	
5′ Phos-CTGCTGTAC-3′	9
5′ Phos-ACAGCAG-3′	7
LMP CAP Adaptor, 50 µM	
5′ Phos-CTGCTGTAC-3′	9
5′ -ACAGCAG-3′	7
Internal Adaptor, 2 µM Biotin ▼	
5′ Phos-CGTACATCCGCCTTGGCCGT-3′	20
5′ Phos-GGCCAAGGCGGATGTACGGT-3′	20

Multiplex adaptor and barcode sequences

Multiplex adaptor and barcode sequence	Sequence (bp)
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

Multiplex adaptor and barcode sequence	Sequence (bp)
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
Barcode-014, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCGGCAGA**CTGCTGTACGGCCAAGGCG-3′	52

Multiplex adaptor and barcode sequence	Sequence (bp)
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
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

Multiplex adaptor and barcode sequence	Sequence (bp)
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
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

Multiplex adaptor and barcode sequence	Sequence (bp)
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
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

Multiplex adaptor and barcode sequence	Sequence (bp)
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
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

Multiplex adaptor and barcode sequence	Sequence (bp)
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
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

Multiplex adaptor and barcode sequence	Sequence (bp)
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
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

Multiplex adaptor and barcode sequence	Sequence (bp)
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 Formulas and calculations

This appendix covers:

Fragment library

Ligation of P1 and P2 Adaptors

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

$$x_1 \text{ pmol DNA for adaptor ligation} = \#\text{ µg DNA} \times \frac{X \text{ pmol}}{1 \text{ µg DNA}}$$

$$x_2 \text{ pmol adaptor needed} = x_1 \text{ pmol} \times 30$$

$$Y \text{ µL adaptor needed} = x_2 \text{ pmol} \times \frac{1 \text{ µL}}{50 \text{ pmol}}$$

2 × 50 bp mate-paired library

Ligation of LMP CAP Adaptors

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

$$x_1 \text{ pmol DNA for adaptor ligation} = \#\text{ µg DNA} \times \frac{X \text{ pmol}}{1 \text{ µg DNA}}$$

$$x_2 \text{ pmol adaptor needed} = x_1 \text{ pmol} \times 100$$

$$Y \text{ µL adaptor needed} = x_2 \text{ pmol} \times \frac{1 \text{ µL}}{50 \text{ pmol}}$$

DNA circularization

The formula to determine dilution of ligation reaction and achieve intramolecular ligation [Francis S. Collins and Sherman M. Weissman, "Directional Cloning of DNA Fragments at a Large Distance from an Initial Probe: A Circularization method," *PNAS* **81** (1984): 6812-6816] is:

$$J = \frac{63.4}{(\text{DNA size in kb})^{1/2}}$$

$$I\ \text{ng/µL} = \frac{J}{\text{Targeted circularization efficiency}} - J$$

$$= \text{Final concentration of DNA for circularization}$$

Example:

For an insert size of 1 to 2 kb

$$J = \frac{63.4}{(1.5)^{1/2}} = 51.8$$

$$I\ \text{ng/µL} = \frac{51.8}{0.95} - 51.8 = 2.74\ \text{ng/µL}$$

The final concentration of DNA required for circularization can be calculated using the formula above. The circularization reaction volume per µg of DNA can then be calculated (see Table 107).

Table 107 Circularization reaction volumes

Insert Size	Final concentration of DNA for circularization	Calculation	Circularization reaction volume per 1 µg DNA
600 to 800 bp	4.3 ng/µL	1000 ng ÷ 4.3 ng/µL	235 µL
800 to 1000 bp	3.75 ng/µL	1000 ng ÷ 3.75 ng/µL	270 µL
1 to 2 kb	2.74 ng/µL	1000 ng ÷ 2.74 ng/µL	365 µL
2 to 3 kb	2.1 ng/µL	1000 ng ÷ 2.1 ng/µL	500 µL
3 to 4 kb	1.8 ng/µL	1000 ng ÷ 1.8 ng/µL	560 µL
4 to 5 kb	1.6 ng/µL	1000 ng ÷ 1.6 ng/µL	625 µL
5 to 6 kb	1.4 ng/µL	1000 ng ÷ 1.4 ng/µL	720 µL

The amount of Internal Adaptor (ds) needed for circularization can be calculated as follows:

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

$$x_1 \text{ pmol DNA for adaptor ligation} = \#\text{ µg DNA} \times \frac{X \text{ pmol}}{1 \text{ µg DNA}}$$

$$x_2 \text{ pmol adaptor needed} = x_1 \text{ pmol} \times 3$$

$$Y \text{ µL adaptor needed} = x_2 \text{ pmol} \times \frac{1 \text{ µL}}{2 \text{ pmol}}$$

Example:
For 20 µg of DNA with an insert size of 1 to 2 kb

$$X \text{ pmol/µg DNA} = 1 \text{ µg DNA} \times \frac{10^6 \text{ pg}}{1 \text{ µg}} \times \frac{1 \text{ pmol}}{660 \text{ pg}} \times \frac{1}{1500} = 1.0 \text{ pmol/µg DNA}$$

$$Y \text{ µL adaptor needed} = 20 \text{ µg DNA} \times \frac{1.0 \text{ pmol}}{1 \text{ µg DNA}} \times 3 \times \frac{1 \text{ µL adaptor needed}}{2 \text{ pmol}}$$

$$= 30 \text{ µL adaptor needed}$$

Ligation of P1 and P2 Adaptors

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

$$x_1 \text{ pmol DNA for adaptor ligation} = \#\text{ µg DNA} \times \frac{X \text{ pmol}}{1 \text{ µg DNA}}$$

$$x_2 \text{ pmol adaptor needed} = x_1 \text{ pmol} \times 30$$

$$Y \text{ µL adaptor needed} = x_2 \text{ pmol} \times \frac{1 \text{ µL}}{50 \text{ pmol}}$$

2 × 25 bp mate-paired library

Ligation of EcoP15I CAP Adaptors

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

$$x_1 \text{ pmol DNA for adaptor ligation} = \#\text{ µg DNA} \times \frac{X \text{ pmol}}{1 \text{ µg DNA}}$$

$$x_2 \text{ pmol adaptor needed} = x_1 \text{ pmol} \times 100$$

$$Y \text{ µL adaptor needed} = x_2 \text{ pmol} \times \frac{1 \text{ µL}}{50 \text{ pmol}}$$

DNA circularization

The formula to determine dilution of ligation reaction and achieve intramolecular ligation [Francis S. Collins and Sherman M. Weissman, “Directional Cloning of DNA Fragments at a Large Distance from an Initial Probe: A Circularization method,” *PNAS* **81** (1984): 6812-6816] is:

$$J = \frac{63.4}{(\text{DNA size in kb})^{1/2}}$$

$$I \text{ ng/µL} = \frac{J}{\text{Targeted circularization efficiency}} - J$$

$$= \text{Final concentration of DNA for circularization}$$

Example:

For an insert size of 1 to 2 kb

$$J = \frac{63.4}{(1.5)^{1/2}} = 51.8$$

$$I \text{ ng/µL} = \frac{51.8}{0.95} - 51.8 = 2.74 \text{ ng/µL}$$

The final concentration of DNA required for circularization can be calculated using the formula above. The circularization reaction volume per µg of DNA can then be calculated (see Table 108 on page 220).

Table 108 Circularization reaction volumes

Insert Size	Final concentration of DNA for circularization	Calculation	Circularization reaction volume per 1 µg DNA
600 to 800 bp	4.3 ng/µL	1000 ng ÷ 4.3 ng/µL	235 µL
800 to 1000 bp	3.75 ng/µL	1000 ng ÷ 3.75 ng/µL	270 µL
1 to 2 kb	2.74 ng/µL	1000 ng ÷ 2.74 ng/µL	365 µL
2 to 3 kb	2.1 ng/µL	1000 ng ÷ 2.1 ng/µL	500 µL
3 to 4 kb	1.8 ng/µL	1000 ng ÷ 1.8 ng/µL	560 µL
4 to 5 kb	1.6 ng/µL	1000 ng ÷ 1.6 ng/µL	625 µL
5 to 6 kb	1.4 ng/µL	1000 ng ÷ 1.4 ng/µL	720 µL

The amount of Internal Adaptor (ds) needed for circularization can be calculated as follows:

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

$$x_1 \text{ pmol DNA for adaptor ligation} = \#\text{ µg DNA} \times \frac{X \text{ pmol}}{1 \text{ µg DNA}}$$

$$x_2 \text{ pmol adaptor needed} = x_1 \text{ pmol} \times 3$$

$$Y \text{ µL adaptor needed} = x_2 \text{ pmol} \times \frac{1 \text{ µL}}{2 \text{ pmol}}$$

Example:
For 20 µg of DNA with an insert size of 1 to 2 kb

$$X \text{ pmol/µg DNA} = 1 \text{ µg DNA} \times \frac{10^6 \text{ pg}}{1 \text{ µg}} \times \frac{1 \text{ pmol}}{660 \text{ pg}} \times \frac{1}{1500} = 1.0 \text{ pmol/µg DNA}$$

$$Y \text{ µL adaptor needed} = 20 \text{ µg DNA} \times \frac{1.0 \text{ pmol}}{1 \text{ µg DNA}} \times 3 \times \frac{1 \text{ µL adaptor needed}}{2 \text{ pmol}}$$

$$= 30 \text{ µL adaptor needed}$$

Ligation of P1 and P2 Adaptors

$$X \text{ pmol/µg DNA} = 1 \text{ µg DNA} \times \frac{10^6 \text{ pg}}{1 \text{ µg}} \times \frac{1 \text{ pmol}}{660 \text{ pg}} \times \frac{1}{86 \text{ bp}} = 17.6 \text{ pmol/µg DNA}$$

$$x_1 \text{ µg DNA for linker ligation} = \#\text{ µg DNA} \div \text{Fold reduction}$$

$$x_2 \text{ pmol DNA available for adaptor ligation} = x_1 \text{ µg} \times \frac{17.6 \text{ pmol}}{1 \text{ µg DNA}}$$

$$x_3 \text{ pmol adaptor needed} = x_2 \text{ pmol} \times 60$$

$$Y \text{ µL adaptor needed} = x_3 \text{ pmol} \times \frac{1 \text{ µL}}{50 \text{ pmol}}$$

F Checklists and workflow tracking forms

This appendix covers:

Workflow checklists: prepare a standard fragment library

	Equipment	Reagents	Preparation steps
Shear the DNA	☐ Covaris™ S2 System ☐ Covaris™ microTube ☐ Covaris microTube adaptor ☐ Covaris microTube loading station ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 1× Low TE Buffer ☐ Ethylene glycol	☐ Degas the water in the Covaris™ S2 System 30 minutes prior to use. ☐ Supplement the circulated water chiller with 20% ethylene glycol.
End-repair the DNA	☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Vortexer ☐ Picofuge ☐ Pipettors ☐ 1.5-mL LoBind tubes ☐ Filtered pipettor tips	☐ 5× End-Polishing Buffer ☐ dNTP Mix ☐ End Polishing Enzyme 1 ☐ End Polishing Enzyme 2 ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw 5× End-Polishing Buffer and dNTP Mix on ice.
Ligate P1 and P2 Adaptors to the DNA	☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ P1 Adaptor (ds) (50 µM) ☐ P2 Adaptor (ds) (50 µM) ☐ 5× T4 Ligase Buffer ☐ T4 Ligase ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw P1 and P2 Adaptors on ice. ☐ Thaw 5×T4 Ligase Buffer on ice.
Size-select the DNA	☐ iBase™ System ☐ E-Gel Safe Imager™ instrument ☐ Pipettors ☐ Filtered pipettor tips	☐ E-Gel® 2% SizeSelect™ gel ☐ 50 bp DNA Ladder ☐ Nuclease-free Water	☐ Thaw 50 bp DNA Ladder on ice.
Nick-translate and amplify the library	☐ Thermal cycler ☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ Pipettors ☐ 1.5-mL LoBind tubes ☐ PCR strip tubes ☐ Filtered pipettor tips	☐ Library PCR Primer 1 ☐ Library PCR Primer 2 ☐ Platinum® PCR Amplification Mix ☐ SOLiD™ Library Column Purification Kit	☐ 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	

Workflow checklists: prepare an express fragment library

	Equipment	Reagents	Preparation steps
Shear the DNA	☐ Covaris™ S2 System ☐ Covaris™ microTube ☐ Covaris microTube adaptor ☐ Covaris microTube loading station ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 1× Low TE Buffer ☐ Ethylene glycol	☐ Degas the water in the Covaris™ S2 System 30 minutes prior to use. ☐ Supplement the circulated water chiller with 20% ethylene glycol.
End-repair the DNA	☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 5× End-Polishing Buffer ☐ dNTP Mix ☐ End Polishing Enzyme 1 ☐ End Polishing Enzyme 2 ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw 5× End-Polishing Buffer and dNTP Mix on ice.
Ligate P1 and P2 Adaptors to the DNA	☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ P1 Adaptor (ds) (50 µM) ☐ P2 Adaptor (ds) (50 µM) ☐ 5× T4 Ligase Buffer ☐ T4 Ligase ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw P1 and P2 Adaptors on ice. ☐ Thaw 5× T4 Ligase Buffer on ice.
Nick-translate, then amplify the library	☐ Thermal cycler ☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ PCR strip tubes ☐ Filtered pipettor tips	☐ Library PCR Primer 1 ☐ Library PCR Primer 2 ☐ Platinum® PCR Amplification Mix ☐ SOLiD™ Library Column Purification Kit	☐ 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	—

Workflow tracking: prepare standard and express fragment libraries

Sample:

Quantitation		Lot number	
Step	**Quantity of DNA**	**Step**	**Lot number**
Starting Amount		SOLiD™ Library Oligos Kit 1	
End-Repair		P1 Adaptor	
Quantitative PCR		P2 Adaptor	
		Library PCR Primer 1	
		Library PCR Primer 2	

Sample:

Quantitation		Lot number	
Step	**Quantity of DNA**	**Step**	**Lot number**
Starting Amount		SOLiD™ Library Oligos Kit 1	
End-Repair		P1 Adaptor	
Quantitative PCR		P2 Adaptor	
		Library PCR Primer 1	
		Library PCR Primer 2	

Sample:

Quantitation		Lot number	
Step	**Quantity of DNA**	**Step**	**Lot number**
Starting Amount		SOLiD™ Library Oligos Kit 1	
End-Repair		P1 Adaptor	
Quantitative PCR		P2 Adaptor	
		Library PCR Primer 1	
		Library PCR Primer 2	

Sample:

Quantitation		Lot number	
Step	**Quantity of DNA**	**Step**	**Lot number**
Starting Amount		SOLiD™ Library Oligos Kit 1	
End-Repair		P1 Adaptor	
Quantitative PCR		P2 Adaptor	
		Library PCR Primer 1	
		Library PCR Primer 2	

Workflow checklists: prepare a 2 × 50 bp mate-paired library

	Equipment	Reagents	Preparation steps
Shear the DNA with Covaris™ S2 System	☐ Covaris™ S2 System ☐ Covaris™ Tubes and Caps ☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 1 M Tris, pH 8.0 ☐ Nuclease-free Water ☐ Ethylene glycol ☐ UltraPure™ Glycerol ☐ SOLiD™ Library Column Purification Kit	☐ Degas the water in the Covaris™ S2 System 30 minutes prior to use. ☐ Supplement the circulated water chiller with 20% ethylene glycol.
Shear the DNA with HydroShear® DNA Shearing Device	☐ HydroShear® DNA Shearing Device ☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Pipettors ☐ Filtered pipettor tips	☐ Nuclease-free Water ☐ 0.2 N HCl ☐ 0.2 N NaOH ☐ 1.5-mL LoBind tubes ☐ SOLiD™ Library Column Purification Kit	—
End-repair the DNA	☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 5× End Polishing Buffer ☐ dNTP, 10 mM ☐ End Polishing Enzyme 1 ☐ End Polishing Enzyme 2 ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ End-repair reagents on ice.
Ligate LMP CAP Adaptors to the DNA	☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ LMP CAP Adaptor (ds) (50 µM) ☐ 5× Ligase Buffer ☐ T4 DNA Ligase ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw LMP CAP Adaptor on ice. ☐ Thaw ligation reagents on ice.
Size-select the DNA	☐ Gel box and power supply for agarose gel ☐ Safe Imager™ Blue Light Transilluminator ☐ Gel imaging system ☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ Pipettors ☐ Scale ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Razor blades ☐ 15-mL conical polypropylene tubes ☐ 1.5-mL LoBind tubes	☐ 1× TAE buffer ☐ Agarose ☐ BlueJuice™Gel Loading Buffer ☐ 1 Kb Plus DNA Ladder ☐ SYBR® Safe gel stain ☐ Nuclease-free Water ☐ SOLiD™ Library Quick Gel Extraction Kit ☐ Isopropyl alcohol	☐ Prepare 1× TAE buffer. ☐ Prepare a 0.8% or 1.0% agarose gel.
Circularize the DNA	☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 5× Ligase Buffer ☐ T4 DNA Ligase ☐ Internal Adaptor (ds) ☐ Nuclease-free Water ☐ SOLiD™ Library Micro Column Kit	☐ Thaw Internal Adaptor on ice. ☐ Thaw ligation reagents on ice.

	Equipment	Reagents	Preparation steps
Isolate the circularized DNA	☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Incubator (37 °C) ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ ATP, 25 mM ☐ 10× Plasmid-Safe™ Buffer ☐ Nuclease-free water ☐ SOLiD™ Library Micro Column Purification Kit	☐ Thaw Plasmid-Safe™ ATP-Dependent DNase reagents on ice.
Nick-translate the library	☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ Pipettors ☐ Thermal cycler ☐ Timer ☐ 1.5-mL LoBind tubes ☐ Filtered pipettor tips	☐ dNTP Mix (10 mM each) ☐ DNA Polymerase I (10 U/µL) ☐ Nick Translation Buffer ☐ Nuclease-free Water ☐ SOLiD™ Library Micro Column Purification Kit ☐ Isopropyl alcohol	☐ Thaw dNTP Mix and Nick Translation Buffer on ice.
Digest the DNA with T7 exonuclease and S1 nuclease	☐ Incubator (37 °C) ☐ Incubator (70 °C) ☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ T7 exonuclease, 10 U/µL ☐ 10× Buffer 4 ☐ S1 Nuclease Dilution Buffer ☐ S1 Nuclease, 25 U/µL ☐ 3 M Sodium chloride ☐ Nuclease-free Water ☐ SOLiD™ Library Micro Column Purification Kit ☐ Isopropyl alcohol ☐ Ice	☐ Thaw Buffer 4 and S1 Nuclease Dilution Buffer on ice.
End-repair the digested DNA	☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 0.5 M EDTA ☐ 5× End Polishing Buffer ☐ dNTP, 10 mM ☐ End Polishing Enzyme 1 ☐ End Polishing Enzyme 2 ☐ Bead Binding Buffer ☐ Nuclease-free Water	☐ Thaw end-repair reagents on ice.
Bind the library molecules to beads	☐ Vortexer ☐ Picofuge ☐ 6 Tube Magnetic Rack ☐ Rotator ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 100× BSA ☐ Dynabeads® MyOne™ Streptavidin C1 beads ☐ Bead Wash Buffer ☐ Bead Binding Buffer ☐ 5× Ligase Buffer ☐ Nuclease-free Water	☐ Thaw 100× BSA and 5× Ligase Buffer on ice.

	Equipment	Reagents	Preparation steps
Ligate P1 and P2 Adaptors the DNA	☐ Vortexer ☐ Picofuge ☐ Pipettors ☐ Rotator ☐ 6 Tube Magnetic Rack ☐ 1.5-mL LoBind tubes ☐ Filtered pipettor tips	☐ T4 DNA Ligase, 5 U/µL ☐ P1 Adaptor (ds) ☐ P2 Adaptor (ds) ☐ Bead Wash Buffer ☐ Nuclease-free Water	☐ Thaw P1 Adaptor (ds) and P2 Adaptor (ds) on ice.
Nick-translate library	☐ 6 Tube Magnetic Rack ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ dNTP Mix (10 mM) ☐ DNA Polymerase I (10 U/µL) ☐ Elution Buffer (E1)	☐ Thaw end-repair reagents on ice.
Trial-amplify the library	☐ Thermal cycler ☐ E-Gel® iBase™ Power System ☐ Gel imaging system ☐ PCR strip tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ Library PCR Primer 1 ☐ Library PCR Primer 2 ☐ Platinum® PCR Amplification Mix ☐ 2% E-Gel® EX-Gel ☐ 100-bp DNA ladder ☐ Nuclease-free Water	☐ Thaw Library PCR Primers 1 and 2 on ice. ☐ Thaw Platinum PCR Amplification Mix on ice.
Amplify the library	☐ Thermal cycler ☐ E-Gel® iBase™ Power System ☐ Microcentrifuge ☐ 6 Tube Magnetic Rack ☐ 2100 Bioanalyzer ☐ PCR strip tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ Library PCR Primer 1 ☐ Library PCR Primer 2 ☐ Platinum® PCR Amplification Mix ☐ Nuclease-free Water ☐ 1.5-mL LoBind tubes ☐ 2.0-mL LoBind tubes ☐ DNA 1000 Chip ☐ SOLiD™ Library Micro Column Purification Kit	☐ Thaw Library PCR Primers 1 and 2 on ice. ☐ Thaw Platinum PCR Amplification Mix on ice. ☐ Thaw DNA 1000 kit reagents on ice.
Gel-purify the library	☐ E-Gel® iBase™ Power System ☐ Safe Imager™ Blue Light Transilluminator ☐ Gel imaging system ☐ Microcentrifuge ☐ Vortexer ☐ Scale ☐ Picofuge ☐ Pipettors ☐ 1.5-mL LoBind tubes ☐ Filtered pipettor tips	☐ E-Gel® SizeSelect™ 2% Gel ☐ 100-bp DNA ladder ☐ Gel Loading Solution ☐ Nuclease-free Water ☐ SOLiD™ Library Micro Column Purification Kit	—
Quantitate	☐ Real-time PCR system	☐ SOLiD™ Library TaqMan® Quantitation Kit	—

Workflow tracking: prepare a 2 × 50 bp mate-paired library

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
End-Repair	
Size-Selection	
Plasmid-Safe™ DNase Treatment	
Quantitative PCR	

Lot number	
Step	**Lot number**
SOLiD™ Library Oligos Kit 1	
P1 Adaptor	
P2 Adaptor	
Library PCR Primer 1	
Library PCR Primer 2	
SOLiD™ Library Oligos Kit 2	
LMP CAP Adaptor	
Internal Adaptor	
Library PCR Master Mix	

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
End-Repair	
Size-Selection	
Plasmid-Safe™ DNase Treatment	
Quantitative PCR	

Lot number	
Step	**Lot number**
SOLiD™ Library Oligos Kit 1	
P1 Adaptor	
P2 Adaptor	
Library PCR Primer 1	
Library PCR Primer 2	
SOLiD™ Library Oligos Kit 2	
LMP CAP Adaptor	
Internal Adaptor	
Library PCR Master Mix	

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
End-Repair	
Size-Selection	
Plasmid-Safe™ DNase Treatment	
Quantitative PCR	

Lot number	
Step	**Lot number**
SOLiD™ Library Oligos Kit 1	
P1 Adaptor	
P2 Adaptor	
Library PCR Primer 1	
Library PCR Primer 2	
SOLiD™ Library Oligos Kit 2	
LMP CAP Adaptor	
Internal Adaptor	
Library PCR Master Mix	

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
End-Repair	
Size-Selection	
Plasmid-Safe™ DNase Treatment	
Quantitative PCR	

Lot number	
Step	**Lot number**
SOLiD™ Library Oligos Kit 1	
P1 Adaptor	
P2 Adaptor	
Library PCR Primer 1	
Library PCR Primer 2	
SOLiD™ Library Oligos Kit 2	
LMP CAP Adaptor	
Internal Adaptor	
Library PCR Master Mix	

Workflow checklists: prepare a 2 × 25 bp mate-paired library

	Equipment	Reagents	Preparation Steps
Shear the DNA with Covaris™ S2 System	☐ Covaris™ S2 System ☐ Covaris™ Tubes and Caps ☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 1 M Tris, pH 8.0 ☐ Nuclease-free Water ☐ Ethylene glycol ☐ UltraPure™ Glycerol ☐ SOLiD™ Library Column Purification Kit	☐ Degas the water in the Covaris™ S2 System 30 minutes prior to use. ☐ Supplement the circulated water chiller with 20% ethylene glycol.
Shear the DNA with HydroShear® DNA Shearing Device	☐ HydroShear® DNA Shearing Device ☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Pipettors ☐ Filtered pipettor tips	☐ Nuclease-free Water ☐ 0.2 N HCl ☐ 0.2 N NaOH ☐ 1.5-mL LoBind tubes ☐ SOLiD™ Library Column Purification Kit	—
End-repair the DNA	☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 5× End Polishing Buffer ☐ dNTP, 10 mM ☐ End Polishing Enzyme 1 ☐ End Polishing Enzyme 2 ☐ Nuclease-free water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw end-repair reagents on ice.
Methylate the genomic EcoP15I sites	☐ Incubator (37 °C) ☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 10× NEBuffer 3 ☐ 100× BSA ☐ EcoP15I, 10 U/µL ☐ S-adenosylmethionine ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw 10× NEBuffer 3, 100× BSA, and S-adenosylmethionine on ice.
Ligate EcoP15I Adaptors to methylated DNA	☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ EcoP15I CAP Adaptor (ds) (50 µM) ☐ 5× Ligase Buffer ☐ T4 DNA Ligase ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw EcoP15I CAP Adaptor on ice. ☐ Thaw ligation reagents on ice.
Size-select the DNA	☐ Gel box and power supply for agarose gel ☐ Safe Imager™ Blue Light Transilluminator ☐ Gel imaging system ☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ Pipettors ☐ Scale ☐ Razor blades ☐ 15-mL conical polypropylene tubes ☐ 1.5-mL LoBind tubes ☐ NanoDro® ND-1000 Spectrophotometer	☐ 1× TAE buffer ☐ Agarose ☐ 10× BlueJuice™ Gel Loading Buffer ☐ 1 Kb Plus DNA Ladder ☐ SYBR® gel stain ☐ Nuclease-free Water ☐ SOLiD™ Library Quick Gel Extraction Kit	☐ Prepare 1× TAE buffer. ☐ Prepare a 0.8% or 1.0% agarose gel.

	Equipment	Reagents	Preparation Steps
Circularize the DNA	☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ Pipettors ☐ Filtered pipettor tips	☐ 5× Ligase Buffer ☐ T4 DNA Ligase, 5 U/µL ☐ Internal Adaptor (ds) ☐ Nuclease-free Water ☐ 1.5-mL LoBind tubes ☐ SOLiD™ Library Micro Column Purification Kit	☐ Thaw Internal Adaptor on ice. ☐ Thaw ligation reagents on ice.
Isolate the circularized DNA	☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Incubator (37 °C) ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ ATP, 25 mM ☐ 10× Plasmid-Safe(TM) Buffer ☐ Plasmid-Safe(TM) DNase ☐ Nuclease-free Water ☐ SOLiD™ Library Micro Column Purification Kit	☐ Thaw Plasmid-Safe™ ATP-Dependent DNase reagents on ice.
Digest the DNA	☐ Incubator (37 °C) ☐ Incubator (65 °C) ☐ Vortexer ☐ Scale ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 10× NEBuffer 3 ☐ 100× BSA ☐ Sinefungin ☐ 10× ATP ☐ EcoP15I Enzyme (10 U/µL) ☐ Nuclease-free Water ☐ Ice	☐ Prepare 10 mM Sinefungin ☐ Thaw 10× NEBuffer 3, 100× BSA, 10× ATP on ice
End-repair with Klenow	☐ Incubator (65 °C) ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ dNTP Mix (10 mM) ☐ DNA polymerase, Klenow large fragment ☐ Stop Buffer ☐ Nuclease-free Water ☐ Ice	☐ Thaw dNTP Mix on ice
Bind the library molecules to beads	☐ Vortexer ☐ Picofuge ☐ 6 Tube Magnetic Rack ☐ Rotator ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 100× BSA ☐ Dynabeads® MyOne™ Streptavidin C1 beads ☐ 5× Ligase Buffer ☐ Bead Wash Buffer ☐ Bead Binding Buffer ☐ Nuclease-free Water	☐ Thaw 100× BSA and 5× Ligase Buffer on ice.
Ligate P1 and P2 Adaptors the DNA	☐ Vortexer ☐ Picofuge ☐ Pipettors ☐ 6 Tube Magnetic Rack ☐ Rotator ☐ 1.5-mL LoBind tubes ☐ Filtered pipettor tips	☐ T4 DNA Ligase, 5 U/µL ☐ P1 Adaptor (ds) ☐ P2 Adaptor (ds) ☐ Bead Wash Buffer ☐ Nuclease-free Water	☐ Thaw P1 Adaptor (ds) and P2 Adaptor (ds) on ice.
Nick-translate library	☐ 6 Tube Magnetic Rack ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ dNTP Mix (10 mM) ☐ DNA Polymerase I (10 U/µL) ☐ Nick Translation Buffer	☐ Thaw dNTP Mix on ice.

	Equipment	Reagents	Preparation Steps
Trial-amplify the library	☐ Thermal cycler ☐ E-Gel® iBase™ Power System ☐ PCR strip tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ Library PCR Primer 1 ☐ Library PCR Primer 2 ☐ Platinum® PCR Amplification Mix ☐ Nuclease-free Water ☐ 2% E-Gel® EX-Gel	☐ Thaw Library PCR Primers 1 and 2 on ice. ☐ Thaw Platinum® PCR Amplification Mix
Amplify the library	☐ Thermal cycler ☐ E-Gel® iBase™ Power System ☐ 6 Tube Magnetic Rack ☐ PCR strip tubes ☐ 1.5-mL LoBind tubes ☐ 2.0-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ Library PCR Primer 1 ☐ Library PCR Primer 2 ☐ Platinum® PCR Amplification Mix ☐ 25-bp DNA Ladder ☐ Nuclease-free Water ☐ SOLiD™ Library Micro Column Purification Kit	☐ Thaw Library PCR Primers 1 and 2 on ice. ☐ Thaw Platinum® PCR Amplification Mix
Gel-purify the library	☐ E-Gel® iBase™ Power System ☐ Safe Imager™ Real-Time Transilluminator ☐ Gel imaging system ☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ Scale ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ E-Gel® SizeSelect™ 2% Gel ☐ 25-bp DNA ladder ☐ Nuclease-free Water ☐ SOLiD™ Library Micro Column Purification Kit	—
Quantitate	☐ Real-time PCR system	☐ SOLiD™ Library TaqMan® Quantitation Kit	—

Workflow tracking: prepare a 2 × 25 bp mate-paired library

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
End-Repair	
Methylation of Genomic EcoP15I	
Size-Selection	
Plasmid-Safe™ DNase Treatment	
Quantitative PCR	

Lot number	
Step	**Lot number**
SOLiD™ Library Oligos Kit 1	
P1 Adaptor	
P2 Adaptor	
Library PCR Primer 1	
Library PCR Primer 2	
SOLiD™ Library Oligos Kit 2	
EcoP15I CAP Adaptor	
Internal Adaptor	

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
End-Repair	
Methylation of Genomic EcoP15I	
Size-Selection	
Plasmid-Safe™ DNase Treatment	
Quantitative PCR	

Lot number	
Step	**Lot number**
SOLiD™ Library Oligos Kit 1	
P1 Adaptor	
P2 Adaptor	
Library PCR Primer 1	
Library PCR Primer 2	
SOLiD™ Library Oligos Kit 2	
EcoP15I CAP Adaptor	
Internal Adaptor	

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
End-Repair	
Methylation of Genomic EcoP15I	
Size-Selection	
Plasmid-Safe™ DNase Treatment	
Quantitative PCR	

Lot number	
Step	**Lot number**
SOLiD™ Library Oligos Kit 1	
P1 Adaptor	
P2 Adaptor	
Library PCR Primer 1	
Library PCR Primer 2	
SOLiD™ Library Oligos Kit 2	
EcoP15I CAP Adaptor	
Internal Adaptor	

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
End-Repair	
Methylation of Genomic EcoP15I	
Size-Selection	
Plasmid-Safe™ DNase Treatment	
Quantitative PCR	

Lot number	
Step	**Lot number**
SOLiD™ Library Oligos Kit 1	
P1 Adaptor	
P2 Adaptor	
Library PCR Primer 1	
Library PCR Primer 2	
SOLiD™ Library Oligos Kit 2	
EcoP15I CAP Adaptor	
Internal Adaptor	

Workflow checklists: prepare a barcoded fragment library

	Equipment	Reagents	Preparation steps
Shear the DNA	☐ Covaris™ S2 System ☐ Covaris microTube adaptor ☐ Covaris microTube loading station ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 1× Low TE Buffer ☐ Ethylene glycol ☐ Covaris™ microTube ☐ Ethylene glycol	☐ Degas the water in the Covaris™ S2 System 30 minutes prior to use. ☐ Supplement the circulated water chiller with 20% ethylene glycol.
End-repair the DNA	☐ Microcentrifuge ☐ NanoDrop® ND-1000 Spectrophotometer ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 5× End-Polishing Buffer ☐ dNTP Mix ☐ End Polishing Enzyme 1 ☐ End Polishing Enzyme 2 ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw 5× End-Polishing Buffer and dNTP Mix on ice.
Ligate P1 and P2 Adaptors to the DNA	☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ Multiplex Library P1 Adaptor (ds) (50 µM) ☐ Barcode-0XX (50 µM) ☐ 5× T4 Ligase Buffer ☐ T4 Ligase ☐ Nuclease-free Water ☐ SOLiD™ Library Column Purification Kit	☐ Thaw P1 and P2 Adaptors on ice. ☐ Thaw5× T4 Ligase Buffer on ice.
Nick-translate, then amplify the library	☐ Thermal cycler ☐ Microcentrifuge ☐ Vortexer ☐ Picofuge ☐ PCR strip tubes ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ Multiplex Library PCR-1 ☐ Multiplex Library PCR-2 ☐ Platinum® PCR Amplification Mix ☐ SOLiD™ Library Column Purification Kit	☐ 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	—
Pool the barcoded libraries	☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	—	—
Gel-purify the libraries	☐ iBase™ System ☐ E-gel Safe Imager™ instrument ☐ Pipettors ☐ Filtered pipettor tips	☐ E-Gel® 2% SizeSelect™ gel ☐ 50 bp DNA Ladder ☐ Nuclease-free Water	☐ Thaw 50 bp DNA Ladder on ice.

Workflow tracking: prepare a barcoded fragment library

Sample:			**Barcode:**
Quantitation		**Lot number**	
Step	**Quantity of DNA**	**Step**	**Lot number**
Starting Amount		Multiplex Library P1 Adaptor	
End-Repair		Multiplex Library PCR-1	
Quantitative PCR		Multiplex Library PCR-2	
		Barcode-0XX	

Sample:			**Barcode:**
Quantitation		**Lot number**	
Step	**Quantity of DNA**	**Step**	**Lot number**
Starting Amount		Multiplex Library P1 Adaptor	
End-Repair		Multiplex Library PCR-1	
Quantitative PCR		Multiplex Library PCR-2	
		Barcode-0XX	

Sample:			**Barcode:**
Quantitation		**Lot number**	
Step	**Quantity of DNA**	**Step**	**Lot number**
Starting Amount		Multiplex Library P1 Adaptor	
End-Repair		Multiplex Library PCR-1	
Quantitative PCR		Multiplex Library PCR-2	
		Barcode-0XX	

Sample:			**Barcode:**
Quantitation		**Lot number**	
Step	**Quantity of DNA**	**Step**	**Lot number**
Starting Amount		Multiplex Library P1 Adaptor	
End-Repair		Multiplex Library PCR-1	
Quantitative PCR		Multiplex Library PCR-2	
		Barcode-0XX	

Sample:			**Barcode:**
Quantitation		**Lot Number**	
Step	**Quantity of DNA**	**Step**	**Lot number**
Starting Amount		Multiplex Library P1 Adaptor	
End-Repair		Multiplex Library PCR-1	
Quantitative PCR		Multiplex Library PCR-2	
		Barcode-0XX	

G Covaris ™ S2 System

This appendix covers:

Operation notes

Note: For important instrument safety information, refer to the Covaris™ S2 System manual.

Fill the tank

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

Degas the water

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

Set the chiller

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

Perform required maintenance of the Covaris™ S2 System

The Covaris S2 System requires regular maintenance to work properly. Perform the tasks in the table below (see Table 109):

Table 109 Required maintenance of the Covaris™ S2 System

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

Covaris™ S2 System Programs

Fragment library preparation (standard, express, and barcoded)

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

Program the Covaris™ S2 System:

- 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! 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.

Mate-paired library preparation

Table 110 Recommended shearing conditions or desired mate-paired library insert sizes.

Insert size	Shearing method	Shearing conditions
600 to 800 bp	Covaris™ Shearing in 20% glycerol (13 mm × 65 mm borosilicate tube)	• Number of Cycles: **75** • Bath Temperature: **5** °C • Bath Temperature Limit: **12** °C • Mode: **Frequency sweeping** • Water Quality Testing Function: **Off** • Duty cycle: **2%** • Intensity: **7** • Cycles/burst: **200** • Time: **10 seconds**
800 to 1000 bp	Covaris™ Shearing in 20% glycerol (13 mm × 65 mm borosilicate tube)	• Number of Cycles: **30** • Bath Temperature: **5** °C • Bath Temperature Limit: **12** °C • Mode: **Frequency sweeping** • Water Quality Testing Function: **Off** • Duty cycle: **2%** • Intensity: **5** • Cycles/burst: **200** • Time: **10 seconds**

H Instrument Warranty Information

This appendix covers:

Computer configuration

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

Limited product warranty

Applied Biosystems warrants that all standard components of the SOLiD™ 4 Analyzer, IKA® ULTRA-TURRAX® Tube Drive, the Covaris™ S2 System, APC UPS, and the recirculating chiller will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. Applied Biosystems warrants the Genomic Solutions HydroShear® DNA Shearing Device will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will replace a defective Hydroshear DNA Shearing Device during the warranty period. The following parts of the Hydroshear are user-replaceable and not covered by the warranty on the HydroShear DNA Shearing Device: shearing assembly, syringes, syringe adapters, syringe shields, and output tubing.

Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its SOLiD 4 Analyzer, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days from the date the warranty begins. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

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

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

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

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

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

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

Warranty period effective date

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

Warranty claims

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

Warranty exceptions

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

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

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

Warranty limitations

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REPRESENTATION, OR WARRANTY MADE BY ANY AGENT, EMPLOYEE, OR REPRESENTATIVE OF APPLIED BIOSYSTEMS WILL NOT BE BINDING ON APPLIED BIOSYSTEMS, UNLESS IN A WRITING SIGNED BY AN EXECUTIVE OFFICER OF APPLIED BIOSYSTEMS.

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Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or willful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Damages, claims, and returns

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

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

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

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

I Safety

This appendix covers:

Instrumentation safety

Note: For important instrument safety information, refer to the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* (PN 4448379) and the Covaris™ S2 System manual. For general safety information, see the "Preface" on page 9.

General instrument safety

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See "About SDSs" on page 249.

Cleaning or decontaminating the instrument

CAUTION! Using a cleaning or decontamination method other than that specified by the manufacturer may result in damage to the instrument.

Physical hazard safety

Solvents and pressurized fluids

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

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

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

Chemical safety

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 249.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

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

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

Obtaining SDSs

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

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

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

Chemical waste safety

Chemical waste hazards

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

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

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

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

Waste disposal

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

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

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

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

Biological hazard safety

General biohazard

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

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.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

Glossary

barcode	Unique sequence identifier added to the sample during library construction
barcoded fragment library	Fragment library with a barcode sequence appended to the 3′ end of the sheared DNA fragments
EcoP15I CAP Adaptor	Double-stranded oligonucleotide 7 to 9 bases long containing the EcoP15I restriction sequence that is ligated to a sheared DNA insert during 2 × 25 bp mate-paired library construction
fragment library	Library consisting of a sheared DNA fragment with P1 and P2 Adaptors ligated to the 5′ end and 3′ end, respectively
Internal Adaptor	Double-stranded oligonucleotide 20 bases long, used to circularize DNA during mate-paired library construction
library	Set of DNA tags prepared from the same biological sample, to be sequenced 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
LMP CAP Adaptor	Double-stranded oligonucleotide 7 to 9 bases long, with a phosphate missing from one of the ends. The adaptor is ligated to a sheared DNA insert during 2 × 25 bp mate-paired library construction.
mate-paired library	Library consisting of two DNA tags a known distance apart linked by an internal adaptor with P1 and P2 Adaptors ligated to the 5′ end and 3′ end, respectively
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

multiplexing	Method to analyze multiple biological samples in a single spot using barcodes
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	A length of DNA to be sequenced
templated bead preparation	Process of adding library template to beads by emulsion PCR, enriching the beads to remove beads without template, and modifying the 3′ end of the template on the beads to prepare for bead deposition and sequencing

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.
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.

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

Note: For additional documentation, see "How to obtain support" on page 10.

Send us your comments

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IMPORTANT! The e-mail address above is for submitting comments and suggestions relating *only* to documentation. To order documents, download PDF files, or for help with a technical question, see "How to obtain support" on page 10.

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