USER GUIDE

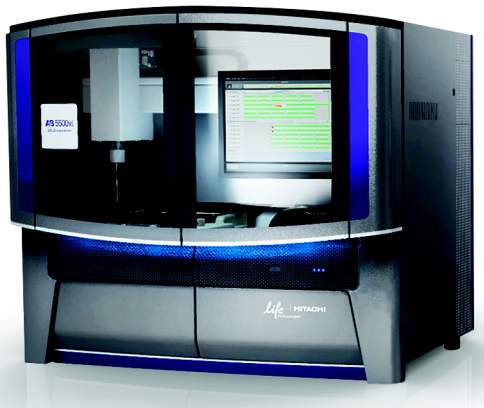
applied biosystems® by life technologies™

Mate-Paired Library Preparation

5500 Series SOLiD™ Systems

Publication Part Number 4460958 Rev. A
Revision Date March 2011

- **prepare libraries**
- prepare beads
- run sequencer
- analyze data



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Part Number 4460958 Rev. A

03/2011

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About This Guide

Safety information

Note: For important instrument safety information, refer to the *5500 Series SOLiD™ Sequencers User Guide* (Part no. 4456991). For general safety information, see this section and Appendix F, "Safety" on page 75. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

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

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

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

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

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

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Applied Biosystems instruments*.

SDSs

The SDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 76.

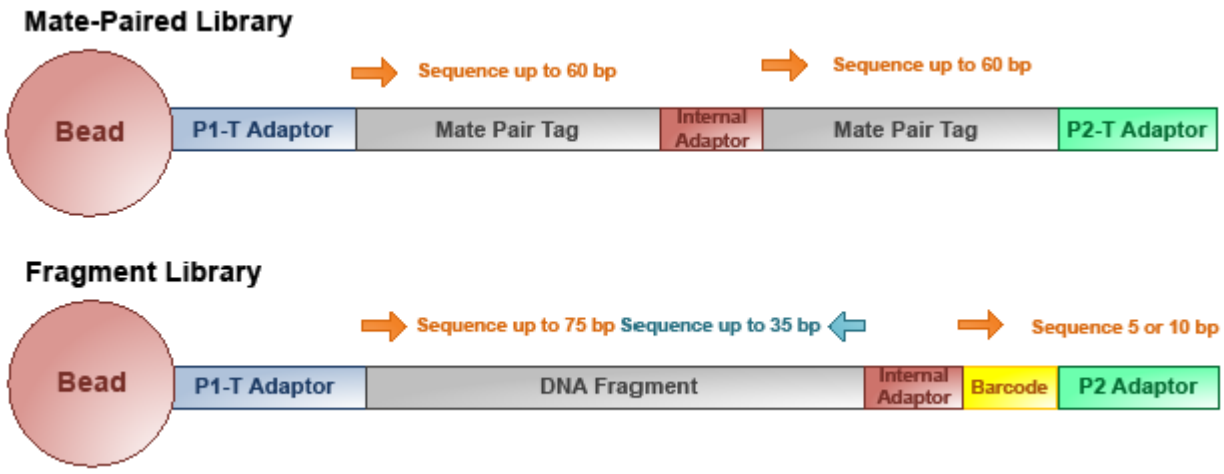
IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

About the Products

For a more detailed overview of library types that can be sequenced on the 5500 Series SOLiD™ Sequencers, see "Choose the appropriate library type" on page 61.

Library preparation

Library preparation is the first step in which samples are adapted for sequencing on the 5500 Series SOLiD™ Sequencers. During library preparation, forward and reverse adaptors are added to the ends of DNA inserts (The bead is for illustration purposes only and is not added until the bead preparation step):



Product information

Purpose of the product

To prepare mate-paired libraries for sequencing on the 5500 Series SOLiD™ Sequencers or SOLiD™ 4 System, use the 5500 SOLiD™ Mate-Paired Library Construction Kit (Part no. 4464418).

Use the 5500 SOLiD™ Mate-Paired Library Construction Kit and the adaptors to:

- *Prepare a 2 × 60 bp mate-paired DNA library.* The mate-paired library is sequenced on the SOLiD™ 3 and 4 Systems and the 5500 Series SOLiD™ Sequencers. 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. Mate-paired adaptors MPR and MPL adaptors form an internal adaptor to connect the DNA mate pair together.
- *Prepare mate-paired libraries with shorter mate tags, such as 2 × 35 bp and 2 × 50 bp.* For shorter mate-paired libraries, use shorter nick translation times. The longer 2 × 60 bp mate-paired library can, however, be sequenced as a 2 × 35 bp or 2 × 50 bp mate-paired library.
- Prepare mate-paired libraries with 1–3 kb insert size. Mate-paired libraries with a 600 bp-1 kb insert size can also be prepared *without* modification. But mate-paired libraries with >3 kb inserts may need modification for optimal library construction.
- Increase mapping specificity over standard fragment library sequencing.
- Detect large structural variations in the genome.
- Bridge sequencing gaps.

Kit contents and storage temperatures

Kit contents

The 5500 SOLiD™ Mate-Paired Library Construction Kit contains materials sufficient to prepare 12 mate-paired libraries:

Part	Description	Storage temperature
5500 SOLiD™ Mate-Paired Library Enzyme Module	One each	–20°C
5500 SOLiD™ Mate-Paired Library Amplification Module	One each	–20°C
5500 SOLiD™ Mate-Paired Library Bead & Buffer Module	One each	4°C
SOLiD™ Mate-Paired Library Oligo Module	One each	–20°C
SOLiD™ Library Micro Column Purification Kit	One each	Room temperature
SOLiD™ Library Quick Gel Extraction Kit	One each	Room temperature

2 × 60 bp Mate-Paired Library Preparation

For an overview of library types that can be sequenced on the 5500 Series SOLiD™ Sequencers, see "Choose the appropriate library type" on page 61.

Workflow

This chapter describes the method to prepare a mate-paired library with a 1–3 kb insert size. Mate-paired libraries with a 600 bp-1 kb insert size can also be prepared *without* modification. But mate-paired libraries with >3 kb inserts may need modification for optimal library construction.

For a graphical overview of mate-paired library preparation, see "Preparation of mate-paired libraries" on page 62.

Steps	Total estimated time
Quantitate and assess sample DNA quality	
Quantitate the DNA with a fluorescence assay (page 16) ⬇ Run an agarose gel to assess sample DNA quality (page 18) ⬇	Varies with laboratory practice

Shear the DNA with the HydroShear® DNA Shearing Device Shearing conditions (page 17) ↓ Shear the DNA (page 17) ↓	
Size-select the DNA Prepare an agarose gel (page 18) ↓ Run the agarose gel (page 18) ↓ Excise the sample from the agarose gel (page 18) ↓ Elute the DNA using the SOLiD™ Library Quick Gel Extraction Kit (page 19) ↓	~3 h
Quantitate the sheared, purified DNA (page 20) Stopping point ↓	
End-repair the DNA (page 20) ↓	
Ligate MP Adaptors to the DNA Calculate the amount of adaptor to use (page 21) ↓ Ligate the MP Adaptors to the DNA (page 22) ↓ Purify the DNA using Agencourt AMPure® XP Reagent (page 22) ↓	~2 h
Quantitate the ligated, purified DNA (page 23) ↓	
Assess the recovery of DNA (page 23) Stopping point ↓	

Circularize the DNA by intra-molecular hybridization(page 23) ⬇ **Isolate the circularized DNA** Treat the DNA with Plasmid-Safe™ DNase (page 24) ⬇ Purify the DNA using Agencourt AMPure® XP Reagent (page 25) ⬇ **(Optional) Quantitate the circularized DNA (page 26)** ⬇ **Nick-translate the circularized DNA** Nick-translate the circularized DNA (page 26) ⬇ Purify the DNA with the SOLiD™ Library Micro Column Purification Kit (page 27) ⬇ Stopping point ⬇	~2 h
Digest the DNA with T7 Exonuclease and S1 Nuclease Digest the DNA with T7 exonuclease (page 28) ⬇ Digest the circularized DNA with S1 Nuclease (page 28) ⬇ Purify the DNA using Agencourt AMPure® XP Reagent (page 29) ⬇ Stopping point ⬇	<2 h

Add a dA-Tail to the digested DNA (page 30) ↓ **Bind the library molecules to streptavidin beads** Prewash the beads (page 30) ↓ Bind the library DNA molecules to the beads (page 31) ↓ Wash the bead-DNA complex (page 31) ↓ **Ligate P1-T and P2-T Adaptors to the DNA (page 32)** Stopping point ↓	~2 h
Nick translate and trial-amplify the library Perform trial PCR on the library (page 33) ↓ Confirm library amplification with a 2% E-Gel® EX Gel (page 34) ↓ Stopping point ↓	<1.5 h
Nick-translate and amplify the library Perform PCR on the library (page 36) ↓ Purify the DNA with the SOLiD™ Library Micro Column Purification Kit (page 36) ↓ Stopping point ↓ **Evaluate the library (page 37)** Stopping point ↓	1.5 h

(Optional) Size-select the library with a SOLiD™ Library Size Selection gel Load the library (page 28) ↓ Run the SOLiD™ Library Size Selection gel and collect the library fragment (page 40) ↓ Purify the DNA with the SOLiD™ Library Micro Column Purification Kit (page 42) ↓ **Check the size distribution of the library (page 43)** ↓ Stopping point ↓	~1 h
Quantitate the library by performing quantitative PCR (qPCR) (page 43) ↓ Stopping point	Varies with laboratory practice

Procedural guidelines

- The protocol is designed for 1–5 µg starting genomic DNA. For optimal complexity, the required amount of starting DNA depends on the size of the genome and the required sequencing depth. For *high* (30× or more) sequence coverage of a human genome (3 Gb), use ~5 µg starting DNA. For *low* sequence coverage, such as 2–3× of a human genome or 30× sequence coverage of a <500 Mb genome, use 1–2 µg DNA and about the same number of amplification cycles as for 5 µg input.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols. Applied Biosystems recommends the Eppendorf 5417R tabletop microcentrifuge.
- Perform all steps requiring 0.5-mL and 1.5-mL tubes with 0.5-mL Eppendorf LoBind Tubes (Eppendorf Part no. 022431005) and 1.5-mL Eppendorf LoBind Tubes (Eppendorf Part no. 022431021).
- Thaw reagents on ice just before use.

Quantitate and assess sample DNA quality

Quantitate the DNA with a fluorescence assay

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

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

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

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

Run an agarose gel to assess sample DNA quality

1. Run a fraction of the sample DNA on an agarose gel.
2. Inspect the gel bands for sample DNA quality:

Observation	Possible cause	Recommended actions or consequences
Smear at bottom of gel	RNA	Remove excess RNA.
Long smear below high molecular weight band	Severe damage to sample DNA	• High risk of library preparation failure, • Lower library yield • Low coverage during sequencing Use another, undamaged DNA sample.
Ladder pattern for genomic DNA sample		

Shear the DNA

The DNA is sheared to yield 700 bp to 3 kb fragments. To shear for a mate-paired library with insert sizes between 700 bp and 1 kb, the Covaris® System is recommended (see "Shear the DNA for inserts ≤1 kb with the Covaris® System" on page 55). To shear for a mate-paired library with insert sizes between 1 kb and 6 kb, the HydroShear® DNA Shearing Device is recommended.

Shear the DNA with the Covaris® System

(*Optional*) Use the Covaris® System instead of the HydroShear® DNA Shearing Device to generate kb DNA fragments (see "Shear the DNA for inserts ≤1 kb with the Covaris® System" on page 55). Follow the manufacturer's guidelines for shearing. Use the appropriate tubes for the targeted size fragments. Concentrate the sheared DNA to 40–60 µL with a SpeedVac® Concentrator or SOLiD™ Library Micro Column Purification Kit, to load the concentrated DNA into a single, 1-cm lane of a size selection gel (see the volume-reducing conditions in "Shear the DNA").

Shear the DNA with the HydroShear® DNA Shearing Device

Shearing guidelines

- Perform a small-scale shearing trial before large-scale shearing, if DNA is available.
- Adjust shearing conditions as needed, especially when working with organisms whose DNA have high- or low-GC content.
- Calibrate the shearing run to assess the shearing efficacy of the device before starting the first library preparation.

Shearing conditions

Insert size	Shearing method	Shearing conditions
<1 kb	Follow the shearing conditions in "Shear the DNA for inserts ≤1 kb with the Covaris® System" on page 55	—
~1 kb	HydroShear® Standard Shearing Assembly	• SC2† • 20 cycles
~2 kb	HydroShear® Standard Shearing Assembly	• SC9 • 20 cycles
~3 kb	HydroShear® Standard Shearing Assembly	• SC13 • 20 cycles

† Speed code (SC): 2.

Shear the DNA

1. In 1.5-mL LoBind Tubes, dilute 1–5 µg of DNA to 150 µL with Nuclease-free Water. For better coverage of large and complex genomes, use ~5 µg of DNA.
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 the table in "Shearing conditions" on page 17, and adjust the volume setting to 150 µL.
5. Shear the DNA.
6. Run the wash scheme after DNA shearing is complete to clean the shearing device.
7. Reduce the volume of the sheared DNA to ~40–60 µL in order to load the sample into a 1-cm wide well on a size selection gel. Use a SpeedVac® Concentrator or equivalent instrument.

   Note: If a SpeedVac® Concentrator is *not* available, and there is ≥2 µg of DNA to concentrate, then use the SOLiD™ Library Micro Column Purification Kit with B2-S Buffer. After the wash, elute the purified, sheared DNA with 50 µL Elution Buffer (E1).

Size-select the DNA

The correctly sized products are excised and purified using the SOLiD™ Library Quick Gel Extraction Kit.

Prepare an agarose gel

1. Prepare a 1% agarose gel, with 1× SYBR® Safe Gel Stain and 1× TAE buffer:

Component	Volume
1× TAE	100 mL
Agarose†	1 g
10,000× SYBR® Safe gel stain‡	10 µL
Total	**100 mL**

† Use either Agarose-LE (Applied Biosystems, AM9040) or UltraPure™ Agarose 1000 (Invitrogen, 10975-035)

‡ Invitrogen Part no. S33102

2. Add 10× BlueJuice™ Gel Loading Buffer to the purified sheared DNA (1 µL of 10× Gel Loading Buffer for every 10 µL of mate-paired library).
3. Load the 1 Kb Plus DNA Ladder (Invitrogen, 10787-018) to one well.

   Use these guidelines:
   - 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.

Run the agarose gel

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

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

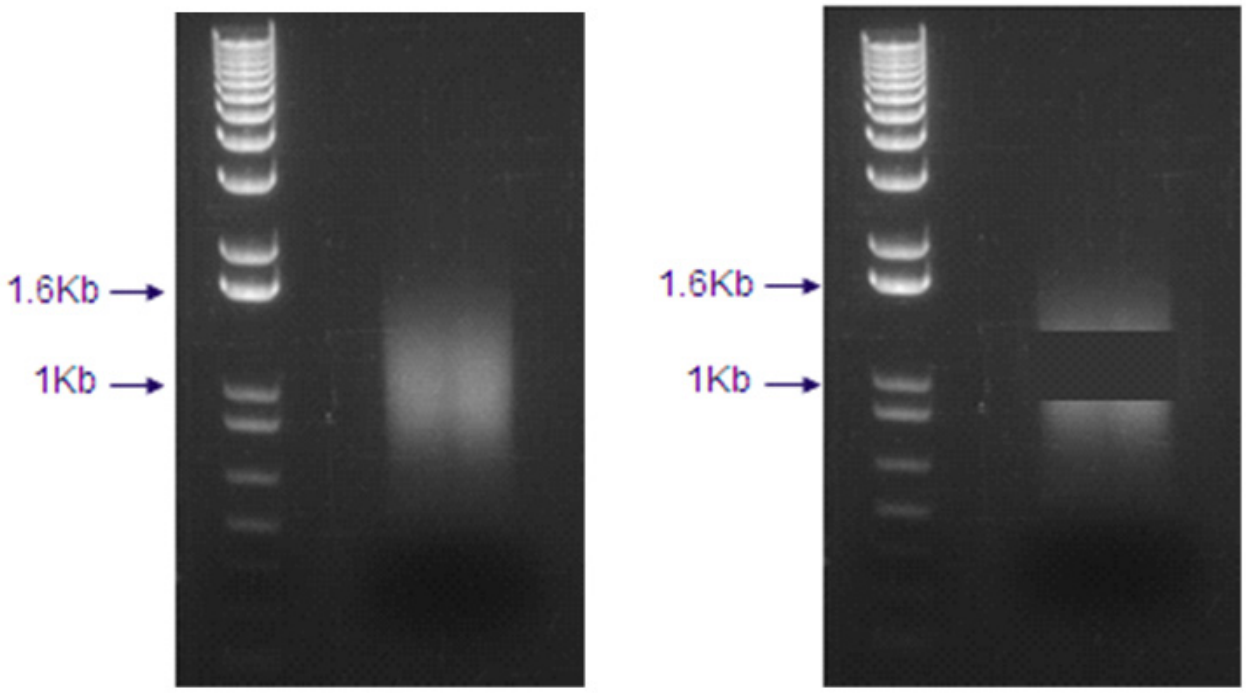
Excise the sample from the agarose gel

1. 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.

2. Using the ladder bands and the ruler for reference, cut the band from the gel with a clean razor blade.

For example, cut a band 0.9–1.3 kb for a 1-kb insert from a 1% agarose gel:



For a *narrower* size selection, make a tighter cut. For a *wider* size distribution, make a broader cut, such as 0.8–1.4 kb.

3. If the gel piece is large, then slice it into smaller pieces.

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

1. Weigh the gel slice. If the gel slice ≥ 200 mg, elute the gel slice in a 15-mL polypropylene conical colorless tube. If the gel slice is < 200 mg, elute the gel slice in a 1.5-mL LoBind Tube.
2. Dissolve the gel:
   a. Add 30 µL of Gel Solubilization Buffer (L3) for every 10 mg of gel.
   b. Dissolve the gel slice by vortexing the tube a few times during incubation at *room temperature* until the gel slice has dissolved completely. The gel slice dissolves in ~15 minutes.

      IMPORTANT! Do *not* heat the gel to dissolve the gel slice. At 50°C, the DNA denatures, and short-insert libraries form heteroduplexes. Heteroduplexes are deleterious to the library.

   c. 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.
3. Load the DNA onto the column(s):
   a. 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.

   b. Spin the column(s) at > 12,000 × g for 1 minute at room temperature, then discard the flow-through and place the column back on the Wash Tube(s).

4. Wash the column(s):
   a. Add 500 µL of Wash Buffer (W1) with ethanol to the Quick Gel Extraction column(s).
   b. Spin the column(s) at > 12,000 × g for 1 minute at room temperature, then discard the flow-through.
   c. Spin the Quick Gel Extraction column(s) again at *maximum speed* for 2 minutes to remove any residual Wash Buffer.
5. Elute the DNA:
   a. Transfer the Quick Gel Extraction column(s) to clean 1.5-mL LoBind tube(s).
   b. Add 50 µL of Elution Buffer (E5) and incubate the sample at room temperature for 5 minutes.
   c. Spin the column(s) at > 12,000 × g for 1 minute at room temperature. The 1.5-mL LoBind tube(s) contain the purified DNA.
   d. Add the eluate from the last spin back to the Quick Gel Extraction column(s), then let the column(s) stand for 5 minutes.
   e. Spin the column(s) at > 12,000 × g for 1 minute at room temperature.
6. If more than one gel extraction column is used, pool the eluted DNA, then reduce the total volume to <70 µL with a SpeedVac® Concentrator or equivalent method.

Quantitate the sheared, purified DNA

Quantitate the purified DNA using 1 µL of sample with the Qubit™ dsDNA HS Assay Kits (Invitrogen Part no. Q32851) and the Qubit® 2.0 (Invitrogen Part no. Q32866).

STOPPING POINT Store the purified DNA in Elution Buffer (E5) at 4 °C, or proceed directly to "End-repair the DNA".

End-repair the DNA

For fast and efficient blunt-ended ligation, End Polishing E2 enzyme is used to convert DNA with damaged or incompatible 5′ -protruding and/or 3′ -protruding ends to 5′ -phosphorylated, blunt-ended DNA. End Polishing E1 enzyme and ATP are also included for phosphorylation of the 5′ -ends of the blunt-ended DNA for subsequent ligation.

1. For ≤5 µg of starting material, combine and mix the components below in a LoBind tube. If >5 µg of starting material for mate-paired libraries with 1–3 kb inserts, scale up or set up parallel reactions:

Component	Volume
Nuclease-free Water	17.0 µL
5✕ Reaction Buffer	20 µL
10 mM dNTP	4.0 µL
End Polishing E1	4.0 µL
End Polishing E2	5.0 µL
Size-selected DNA	50.0 µL
Total	**100 µL**

2. Incubate the mixture at room temperature (20–25°C) for 30 minutes.
3. Heat inactivate the enzymes at 75°C for 20 minutes.
4. Put the DNA on ice.

Ligate MP Adaptors to the DNA

This ligation step adds the MP Adaptors to the sheared, end-repaired DNA. The MP Adaptors are missing a 5′ phosphate at the non-joining end; as a result, there is a nick on each strand when the DNA is circularized. The MP Adaptors are included in double-stranded form in the SOLiD™ Mate-Paired Library Standard Adaptors module.

Calculate the amount of adaptor to use

Calculate the amount of adaptor needed, *Y*, for the reaction based on the amount of DNA before the end-repair step:

$$\text{µg-to-pmol conversion factor} = \frac{10^6 \text{ pg}}{1 \text{ µg}} \times \frac{1 \text{ pmol}}{660 \text{ pg}} \times \frac{1}{\text{Average insert size}}$$

$$\textbf{\textit{Y}} \text{ µL adaptor needed} = \text{\# µg DNA} \times (\text{µg-to-pmol conversion factor}) \times 50 \times \frac{1 \text{ µL adaptor needed}}{25 \text{ pmol}}$$

For example, if you have 1 µg of purified, size-selected DNA and an average insert size of 1.5 kb:

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

$$\textbf{\textit{Y}} \text{ µL adaptor needed} = 1 \text{ µg DNA} \times 1.0 \text{ pmol/µg DNA} \times 50 \times \frac{1 \text{ µL adaptor needed}}{25 \text{ pmol}}$$

$$= 2 \text{ µL adaptor needed}$$

Note: If Y<1 µL, use 1 µL in the reaction.

Ligate the MP Adaptors to the DNA

1. Combine:

Component	Volume
End-repaired DNA	100 µL
5X Reaction Buffer	10 µL
ATP, 100 mM	1 µL
MPR Adaptor (ds), 25 µM	*Y* µL†
MPL Adaptor (ds), 25 µM	*Y* µL†
T4 DNA Ligase, 5U/µL	15 µL
Nuclease-free Water	Variable µL
Total	**150 µL**

† If *Y*<1 µL, use 1 µL.

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

Purify the DNA using Agencourt AMPure® XP Reagent

1. Resuspend the Agencourt AMPure® XP Reagent beads.
2. Bind the DNA to the Agencourt AMPure® XP Reagent:
   a. Prepare the bead suspension in the sample reaction:

Component	Volume
Sample reaction	150 µL
Nuclease-free Water	150 µL†
Agencourt AMPure® XP Reagent	240 µL‡
Total	**540 µL**

† Equal to 1 volume of sample reaction.
‡ Equal to 1.6 volumes of sample reaction.

   b. Vortex the beads for 15 seconds, then pulse-spin.
   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.
   d. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
3. Wash the DNA 2 times. For each wash, keep the tube in the DynaMag™-2 magnetic rack:
   a. Add 600 µL of 70% ethanol to the tube, without disturbing the beads.
   b. Keep the tube in a DynaMag™-2 magnetic rack for at least 1 minute, then remove and discard the supernatant without disturbing the beads.
4. Remove the tube from the DynaMag™-2 magnetic rack, pulse-spin the tube, return the tube to the magnetic rack, then remove and discard the supernatant with a 20-µL pipettor.
5. Open the tube, then dry the beads at room temperature (20–25°C) for 3 minutes.

6. Elute the DNA:
   a. Remove the tube from the DynaMag™-2 magnetic rack, then add 50 µL Elution Buffer. (E1)
   b. Vortex the beads for 15 seconds, pulse-spin, then incubate the beads at room temperature (20–25°C) for 3 minutes.
   c. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears.
   d. Transfer the supernatant to a new 1.5-mL LoBind Tube.

Quantitate the ligated, purified DNA

Quantitate the purified DNA using 1 µL of sample with the Qubit™ dsDNA HS Assay Kits (Invitrogen Part no. Q32851) and the Qubit® 2.0 Fluorometer (Invitrogen Part no. Q32866).

Assess the recovery of DNA

If the recovery compared to starting unsheared genomic DNA is...	Then go to...
>5%	"Circularize the DNA by intra-molecular hybridization".
<5%	"Circularize the DNA by intra-molecular hybridization". Minimize loss in the following purification steps and evaluate the stringency of the first size selection. Life Technologies recommends >+/-10% of the target insert size.
≤ 50 ng	"Quantitate and assess sample DNA quality" on page 16 to start mate-paired library preparation again. If the starting material is 1 µg and the recovery remains low, use more starting material.

STOPPING POINT Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Circularize the DNA by intra-molecular hybridization".

Circularize the DNA by intra-molecular hybridization

The mate-paired adaptor contains a blocking oligonucleotide to protect the 3′ overhangs of the MP Adaptors from self-annealing. At circularization, heat denaturation removes the blocking oligonucleotide. The DNA circularizes through intramolecular hybridization at low concentrations.

1. Fill all of the holes to be used in a heat block with water, then pre-heat the block to 70°C
2. Calculate the total volume of the circularization reaction (*T*, µL), so that for a known concentration of DNA [DNA] (ng/µL) and known volume of DNA (*V*), the final concentration of DNA in the reaction is 0.5 ng/µL:

   $T = [DNA] \times V / 0.5$

   Example

   If [DNA] = 5 ng/µL and *V* = 50 µL, then *T* = 500 µL.

   Note: If the total volume of hybridization is >1000 µL, use *T* = 1000 µL.
3. Combine:

Component	Volume
DNA	*V* µL
10× Plasmid-Safe™ Buffer	*T*/10 µL
Nuclease-free Water	*T*-(*T*/10)-*V* µL
Total	***T* µL**

4. Incubate the reaction in the heat block at 70°C for 5 minutes, then place the reaction on ice for 5 minutes.

IMPORTANT! The incubation time is *critical*. Keep the time as close to 5 minutes as possible, then proceed to the next *immediately* to "Isolate the circularized DNA".

Isolate the circularized DNA

Plasmid-Safe™ DNase is used to eliminate uncircularized DNA. After Plasmid-Safe™ DNase-treatment, the DNA is purified using the Agencourt AMPure® XP Reagent.

Treat the DNA with Plasmid-Safe™ DNase

1. Combine the following, where *T* = the total volume of the circularization reaction (µL):

Component	Volume
Circularized DNA	*T* µL
ATP, 100 mM	*T*/100 µL
Plasmid-Safe™ DNase, 10 U/µL	*T*/100 µL

Example

If T = 800 µL, then:

Component	Volume
ATP, 100 mM	8.0 µL
Plasmid-Safe™ DNase, 10 U/µL	8.0 µL

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

Purify the DNA using Agencourt AMPure® XP Reagent

1. Resuspend the Agencourt AMPure® XP Reagent beads.
2. Bind the DNA to the Agencourt AMPure® XP Reagent:
   a. Prepare the bead suspension in the sample reaction:

Component	Volume
Sample reaction	*T* µL
Bead Dilution Buffer	0.7 × *T* µL
Agencourt AMPure® XP Reagent	0.3 × *T* µL

   b. Vortex the beads for 15 seconds, then pulse-spin.
   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.
   d. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
3. Wash the DNA 2 times. For each wash, keep the tube in the DynaMag™-2 magnetic rack:
   a. Add 600 µL of 70% ethanol to the tube, without disturbing the beads.
   b. Keep the tube in a DynaMag™-2 magnetic rack for at least 1 minute, then remove and discard the supernatant without disturbing the beads.
4. Remove the tube from the DynaMag™-2 magnetic rack, pulse-spin the tube, return the tube to the magnetic rack, then remove and discard the supernatant with a 20-µL pipettor.
5. Open the tube, then dry the beads at room temperature (20–25°C) for 3 minutes to dry the sample.
6. Mix:

Component	Volume
Nuclease-free Water	84 µL
Nick Translation Buffer	10 µL

7. Elute the DNA:
   a. Remove the tube from the DynaMag™-2 magnetic rack, then add the 94 µL pre-mixed solution of Nick Translation Buffer to the tube of DNA.
   b. *Gently* vortex the beads for 15 seconds, pulse-spin, then incubate the beads at room temperature (20–25°C) for 3 minutes.

c. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears.

d. Transfer the *supernatant* to a new 0.2-mL PCR tube.

IMPORTANT! Proceed to the next step *immediately*.

(*Optional*) Quantitate the circularized DNA

IMPORTANT! If the starting material is 1–2 µg, skip this step and proceed *immediately* to "Nick-translate the circularized DNA".

Quantitate the purified DNA using 1 µL of sample with the Qubit™ dsDNA HS Assay Kits (Invitrogen Part no. Q32851) and the Qubit® 2.0 Fluorometer (Invitrogen Part no. Q32866).

IMPORTANT! Proceed to the next step *immediately*.

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. For convenience, for different mate-tag sizes, change the reaction time but keep the temperature constant.

Nick-translate the circularized DNA

IMPORTANT! Incubate the nick translation reaction at 5°C on a thermal cycler using the "No heated lid" feature. DNA polymerase I is very sensitive to slight changes in temperature. If your thermocycler does not have a "No heated lid" feature, leave the lid off. Before adding enzyme to the reaction mix for nick translation, chill the enzyme and the reaction mix *separately* in a thermocycler at 5°C for several minutes.

1. Combine in the 0.2-mL PCR tube:

Component	Volume
DNAse-treated, purified DNA	93–94 µL
10 mM dNTP	5 µL

2. Vortex the mix, then pulse-spin.
3. Incubate the mix without DNA polymerase I at 5°C in a thermocycler for for 2–3 minutes. Use the "no heated lid" feature or leave the lid off.
4. In another 0.2-mL PCR tube, add 3 µL of DNA polymerase I, then pulse spin.
5. Incubate the DNA polymerase I at 5°C in a thermocycler for ≥1 minute. Use the "no heated lid" feature or leave the lid off.

6. Set the timer to 10–11 minutes.
7. Transfer all of the reaction mix to the tube containing the DNA polymerase I incubating at 5°C, then pipet the total reaction mix up and down 5 times to mix. Use the "no heated lid" feature or leave the lid off.
8. Start the timer.

   Note: The time for nick-translation depends on laboratory practice and thermocycler conditions. The ideal library peak for 2 × 60 bp mate-paired library sequencing is just over 300 bp.
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 Binding Buffer (B2-S). Binding Buffer (B2-S) denatures the enzyme and stops the reaction.

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

1. Pre-spin an empty PureLink® Micro columns in collection tubes at 10,000 × g for 1 minute. Verify the column membranes are intact and are not lifted or folded after the spin.
2. Load the DNA onto the PureLink® Micro columns:
   a. Mix the nick-translated DNA well in Binding Buffer (B2-S) with isopropanol (55%).
   b. Apply all of the mix to the PureLink® Micro column(s) in collection tube(s).
   c. Spin the column(s) at 10,000 × g for 1 minute at room temperature, then discard the flow-through. dsDNA is bound to the column.
3. Wash the column(s):
   a. Return the PureLink® Micro column(s) to the same collection tube(s).
   b. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
   c. Spin the column(s) at 10,000 × g for 1 minute at room temperature, then discard the flow-through.
   d. Spin the column(s) at 14,000 × g at room temperature to remove residual wash buffer.
4. Elute the DNA:
   a. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
   b. 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.
   c. Spin the column(s) at 14,000 × g for 1 minute at room temperature.
   d. Add the eluate from the last spin back to the column(s), then let the column(s) stand for 1 minute.
   e. Spin the column(s) at 14,000 × g for 1 minute at room temperature.
5. 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 ºC, or proceed directly to "Digest the DNA with T7 Exonuclease and S1 Nuclease".

Digest the DNA with T7 Exonuclease and S1 Nuclease

T7 exonuclease recognizes the nicks within the circularized DNA. With its 5′ -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 mate-paired tags can be cleaved from the circularized template.

Digest the DNA with T7 exonuclease

1. Combine:

Component	Amount
DNA	25 µL
10X Buffer 4	5.0 µL
T7 Exonuclease	2.0 µL
Nuclease-free Water	18.0 µL
Total	**50 µL**

2. Incubate the reaction mixture at 37°C for 15 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 50 U/µL with S1 Nuc (nuclease) Dilution Buffer.
2. Combine:

Component	Amount
DNA	50 µL
3 M NaCl	1.7 µL
S1 Nuclease	2.0 µL
Total	**53.7 µL**

3. Incubate the reaction mixture at 37°C for 45 minutes.

IMPORTANT! Proceed to the next step *immediately*.

Purify the DNA using Agencourt AMPure® XP Reagent

1. Resuspend the Agencourt AMPure® XP Reagent beads.
2. Bind the DNA to the Agencourt AMPure® XP Reagent:
   a. Prepare the bead suspension in the sample reaction:

Component	Volume
Sample reaction	53 µL
Agencourt AMPure® XP Reagent	95 µL†
Total	**148 µL**

† Equal to 1.8 volumes of sample.

   b. Vortex the beads for 15 seconds, then pulse-spin.
   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.
   d. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
3. Wash the DNA 2 times. For each wash, keep the tube in the DynaMag™-2 magnetic rack:
   a. Add 600 µL of 70% ethanol to the tube, without disturbing the beads.
   b. Keep the tube in a DynaMag™-2 magnetic rack for at least 1 minute, then remove and discard the supernatant without disturbing the beads.
4. Remove the tube from the DynaMag™-2 magnetic rack, pulse-spin the tube, return the tube to the magnetic rack, then remove and discard the supernatant with a 20-µL pipettor.
5. Open the tube of DNA, then dry the beads at room temperature (20–25°C) for 3 minutes to dry the sample.
6. Elute the DNA:
   a. Remove the tube from the DynaMag™-2 magnetic rack, then add the 50 µL of Elution Buffer (E1) to the tube of DNA.
   b. Vortex the beads for 15 seconds, pulse-spin, then incubate the beads at room temperature (20–25°C) for ≥3 minutes.
   c. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears.
   d. Transfer the supernatant to a new 1.5-mL LoBind Tube.

STOPPING POINT Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Add a dA-Tail to the digested DNA" on page 30.

Add a dA-Tail to the digested DNA

Adding a dA tail to the S1-nuclease-treated DNA by A-Tailing Enzyme II increases the efficiency of ligation to P1-T and P2-T Adaptors.

1. Combine to prepare the dA-tailing mix:

Component	Amount
T7/S1-digested DNA	50 µL
Nick Translation Buffer	10 µL
dA + dNTP Mix	1.0 µL
A-Tailing Enzyme II	3.0 µL
Nuclease-free Water	36.0 µL
Total	**100 µL**

2. Incubate the reaction mix at 37°C for 30 minutes.

   Note: During incubation, you can pre-wash the streptavidin beads (see "Prewash the beads").

3. Add 5.0 µL of 0.5 M EDTA to the dA-tailing mix to stop the reaction.
4. Combine:

Component	Volume
Stopped dA-tailing mix	105 µl
Bead Binding Buffer	200.0 µL
Nuclease-free Water	95.0 µL
Total	**400 µL**

Bind the library molecules to streptavidin beads

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

Prewash the beads

1. Prepare 1× BSA solution:

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

2. Vortex the tube of Dynabeads® MyOne™ Streptavidin C1, then transfer 50 µL of the beads into a 1.5-mL LoBind Tube.

3. Add 500 µL of Bead Wash Buffer to the 50 µL of solution of beads, vortex the beads for 15 seconds, then pulse-spin.
4. Place the tube in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
5. Add 500 µL of 1X BSA and vortex for 15 seconds, then pulse-spin the tube.
6. Place the tube in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
7. Add 500 µL of Bead Binding Buffer. Vortex the beads for 15 seconds, then pulse-spin.

IMPORTANT! Proceed to step 8 only after the A-tailing of the DNA is stopped (see step 4 of "Add a dA-Tail to the digested DNA" on page 30).

8. Place the tube in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then 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 "Add a dA-Tail to the digested DNA" on page 30) to the pre-washed beads, then vortex for 15 seconds.
2. Rotate the solution at room temperature (20–25°C) for 30 minutes, then pulse-spin.

Wash the bead-DNA complex

1. Prepare 1X Reaction Buffer. For one sample:

Component	Volume
5X Reaction Buffer	120 µL
Nuclease-free Water	480 µL
Total	**600 µL**

2. Place the tube with the bead-DNA complex in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
3. Wash the beads ***3*** times. For each wash:
   a. Resuspend the beads in 500 µL of Bead Wash Buffer. Vortex the beads for 15 seconds, then pulse-spin.
   b. Place the tube in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
4. Wash and resuspend the beads:
   a. Resuspend the beads in 500 µL of 1X Reaction Buffer. Vortex the beads for 15 seconds, then pulse-spin.
   b. Place the tube in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
   c. Resuspend the beads in 86 µL of 1X Reaction Buffer.

Ligate P1-T and P2-T Adaptors to the DNA

Note: P1-T and P2-T Adaptors have a 3' T overhang. Previous SOLiD™ P1 and P2 Adaptors do not. The adaptor sequences in the final ligated library with the P1-T and P2-T Adaptors are the same as the SOLiD™ P1 and P2 Adaptors, which are used for preparing the library for sequencing with the SOLiD™ 4 System.

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

The ligated library molecules are bound to streptavidin beads, washed, and purified from ligation by-products.

1. Ligate the P1-T and P2-T Adaptors to the bead-bound DNA:
   a. Combine:

Component	Volume
DNA-bead complex	86 µL
P1-T Adaptor (ds), 10 µM	2.0 µL
P2-T Adaptor (ds), 10 µM	2.0 µL
T4 DNA Ligase, 5 U/µL	10.0 µL
Total	**100 µL**

   b. Rotate the reaction mixture at room temperature (20–25°C) for 30 minutes.
   c. Place the tube in the DynaMag™-2 magnetic rack for ≥1 minute until the solution clears, then remove and discard the supernatant.
2. Wash the DNA-beads ***3*** times. For each wash:
   a. Resuspend the beads in 500 µL of Bead Wash Buffer. Vortex the beads for 15 seconds, then pulse-spin.
   b. Place the tube in the DynaMag™-2 magnetic rack for ≥1 minute until the solution clears, then remove and discard the supernatant.
3. Wash and resuspend the DNA-bead complex:
   a. Resuspend the DNA-bead complex in 500 µL of Elution Buffer (E1). Vortex the DNA-bead complex for 15 seconds, then pulse-spin.
   b. Place the tube in the DynaMag™-2 magnetic rack for ≥1 minute until the solution clears, then remove and discard the supernatant.
   c. Resuspend the DNA-bead complex in 30 µL of Elution Buffer (E1).

STOPPING POINT Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Nick translate and trial-amplify the library" on page 33.

Nick translate and trial-amplify the library

The ligated, purified DNA undergoes nick translation during PCR with Platinum® PCR Amplification Mix.

Next, the library is trial-amplified using Library PCR Primers 1 and 2 with the Platinum® PCR Amplification Mix. Trial amplification determines the number of PCR cycles to be used for final library amplification without overamplification. Choose the number of PCR cycles from the trial PCR so that the amplified library is just visible on 2% E-Gel® EX Gel.

Perform trial PCR on the library

1. Prepare a PCR master mix for amplification reactions:

Component	Volume
Platinum® PCR Amplification Mix†	70.0 µL
Library PCR Primer 1, 50 µM	1.4 µL
Library PCR Primer 2, 50 µM	1.4 µL
Total	**72.8 µL**

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

2. Vortex the PCR 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 (~25 µL) between two PCR tubes labelled "PCR #1" and "PCR #2".
4. Use two different thermocyclers or run PCR sequentially for these numbers of cycles as follows:

Sample no.	Number of cycles
0	14 cycles
1	10 cycles
2	14 cycles

5. Run:

Stage	Step	Temp	Time
Holding	Nick translation	72°C	20 min
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

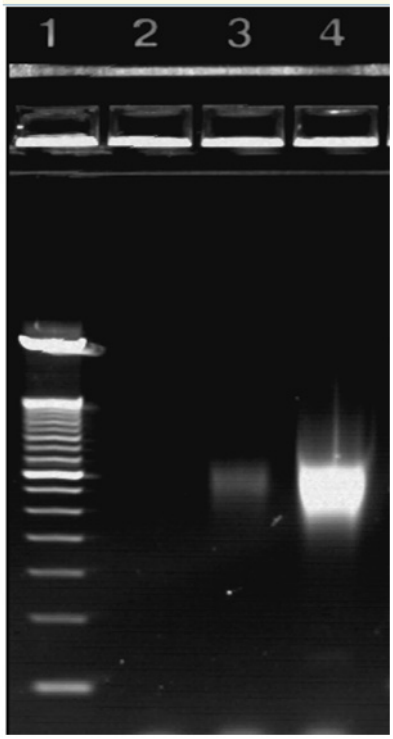
1. Mix 0.5 µL of 1 µg/µL 100-bp DNA Ladder (Invitrogen Part no. 10628-050) or 0.5 µL of 1 µg/µL 50-bp DNA Ladder (Invitrogen Part no. 10416-014) with 40 µL of Nuclease-free Water.
2. Load 20 µL of PCR #0, PCR #1, and PCR #2 into separate wells of a 2% E-Gel® EX Gel. Load 20 µL of diluted 100-bp DNA Ladder 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 1 on page 35). Choose a PCR cycle where amplified library products are faintly visible on the trial-PCR gel:

   Determine the number of PCR cycles for final library amplification:

Size-select the DNA after amplification?	Then determine the number of PCR cycles based on...
No	The intensity of the products on the gel *only*.
Yes	The intensity of the products on the gel *and* add at least one more PCR cycle to compensate for sample loss.

Note: For a 2x60 mate paired library, the ideal peak size of an amplified library is just over ~300 bp. A peak size of amplified library from 280–350 bp is acceptable.

Figure 1 Mate-paired library trial amplification sample run on a 2% E-Gel® EX Gel. Lane 1: 50-bp DNA Ladder; Lane 2: PCR #0 (negative control); Lane 3: PCR #1 (10 cycles); Lane 4: PCR #2 (14 cycles). Based on this picture, if there is *no* size selection after amplification, use 9 cycles for final library amplification. If there *is* size selection after amplification, use 10 or 11 cycles.



STOPPING POINT Store the DNA-Bead complexes in Elution Buffer (E1) at 4 ºC, or proceed directly to "Nick-translate and amplify the library".

Nick-translate and 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 DNA-complex for amplification to get maximum representation of the library and to avoid PCR-related biases due to differential amplification of library molecules.

Perform PCR on the library

1. Prepare a master mix for amplification reactions:

Component	Volume
Platinum® PCR Amplification Mix†	100.0 µL
Library PCR Primer 1, 50 µM	2.0 µL
Library PCR Primer 2, 50 µM	2.0 µL
Total	**104 µL**

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

2. Place the tube of P1-T/P2-T-ligated DNA-beads in the DynaMag™-2 magnetic rack for ≥1 minute until the solution clears (see "Ligate P1-T and P2-T Adaptors to the DNA" on page 32).
3. With a 20-µL pipettor, *carefully* remove and dispose of the supernatant until ~5 µL of Elution Buffer (E1) remains above the beads. Do not draw beads into the pipettor tip.
4. Add the master mix from step 1 to the beads.
5. Vortex the beads for 15 seconds. then transfer the suspension to a new PCR tube.
6. Run:

Stage	Step	Temp	Time
Holding	Nick translation	72°C	20 min
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 "Nick translate and trial-amplify the library" on page 33.

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

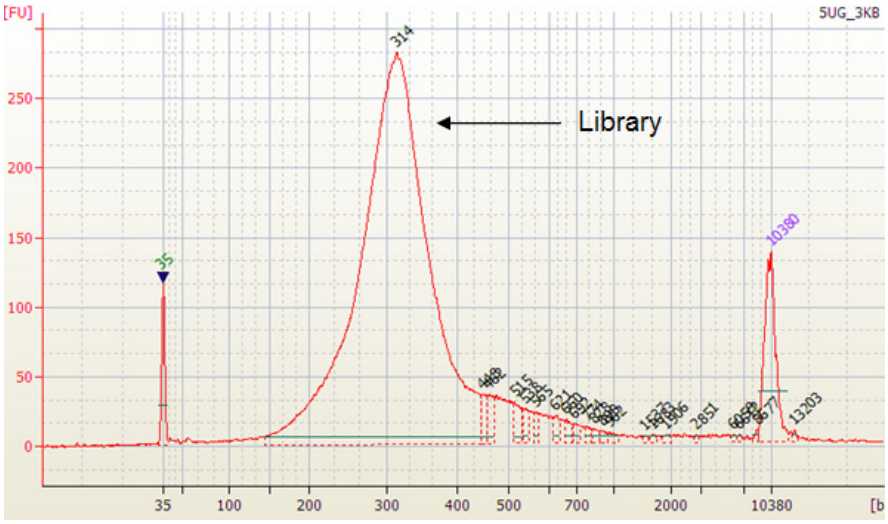
1. Pre-spin empty PureLink® Micro column in collection tubes at 10,000 × g for 1 minute before use.
2. Add 4 volumes of Binding Buffer (B2-L) with isopropanol (40%) to 1 volume of sample. Mix well.
3. Load the DNA onto the PureLink® Micro column:
   a. Apply all of the PCR sample with beads to the PureLink® Micro column in collection tube.
   b. Spin the column at 10,000 × g for 1 minute at room temperature, then discard the flow-through. dsDNA is bound to the column.
   c. Ensure that the entire PCR sample has been loaded onto the column(s).

4. Wash the column(s):
   a. Return the PureLink® Micro column to the same collection tube.
   b. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column.
   c. Spin the column at 10,000 × g for 1 minute at room temperature, then discard the flow-through.
   d. Spin the column at 14,000 × g to remove residual wash buffer.
5. Elute the DNA:
   a. Transfer the column to clean 1.5-mL LoBind Tube.
   b. 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.
   c. Spin the column at 14,000 × g for 1 minute at room temperature.
   d. Add the eluate from the last spin back to the column, then let the column stand for 1 minute.
   e. Spin the column(s) at 14,000 × g for 1 minute at room temperature.

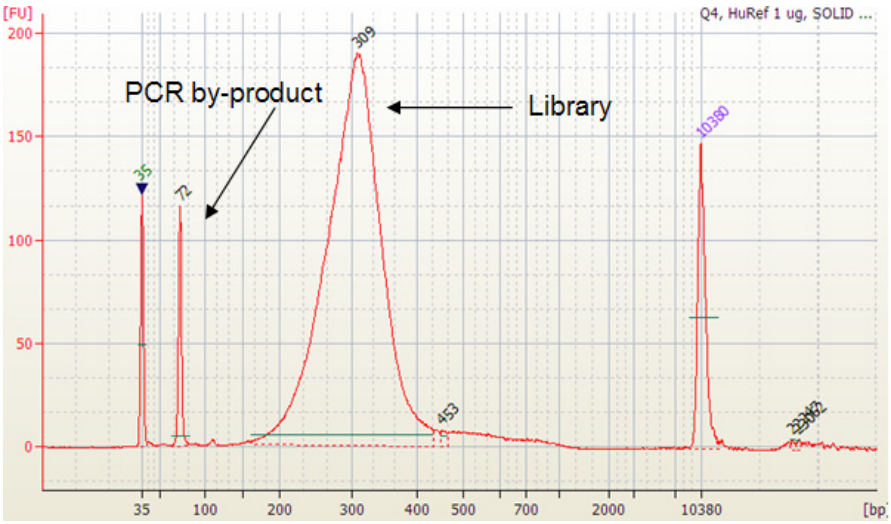
STOPPING POINT Store the purified DNA in Elution Buffer (E1) at 4°C or proceed to "Evaluate the library".

Evaluate the library

1. Run 1 µL of the concentrated library on a High Sensitivity DNA Chip in the Agilent Technologies 2100 Bioanalyzer™ to confirm amplification quality:
   Bioanalyzer™ electropherogram of library without PCR by-products that can be quantitated by qPCR:



Bioanalyzer™ electropherogram of library with PCR by-products that needs to be gel-purified with a SizeSelect gel:



2. Proceed as follows:

If you need...	Then...
Greatest sequencing output for 2 × 60-bp mate-paired sequencing run	Remove fragments <240 bp. Proceed to "(Optional) Size-select the library with a SOLiD™ Library Size Selection gel". **IMPORTANT!** Size-selection reduces library yield by 3–5 fold.
Maximum number of mate-paired molecules, *and* there are no PCR by-products in a library of acceptable size	Skip size selection and proceed to "Quantitate the library by performing quantitative PCR (qPCR)" on page 43. Size-selection is optional.

STOPPING POINT Store the purified DNA in Elution Buffer (E1) at 4°C or proceed to "(Optional) Size-select the library with a SOLiD™ Library Size Selection gel" or "Quantitate the library by performing quantitative PCR (qPCR)" on page 43, as required.

(*Optional*) Size-select the library with a SOLiD™ Library Size Selection gel

Choosing to size-select the library or not depends on the purity and size of the amplified library. There must be no visible small PCR by-products in the final amplified library after column purification. The library peak size must be acceptable for the type of sequencing (to see acceptable library peak sizes for 2 × 60 bp libraries, see "Evaluate the library" on page 37).

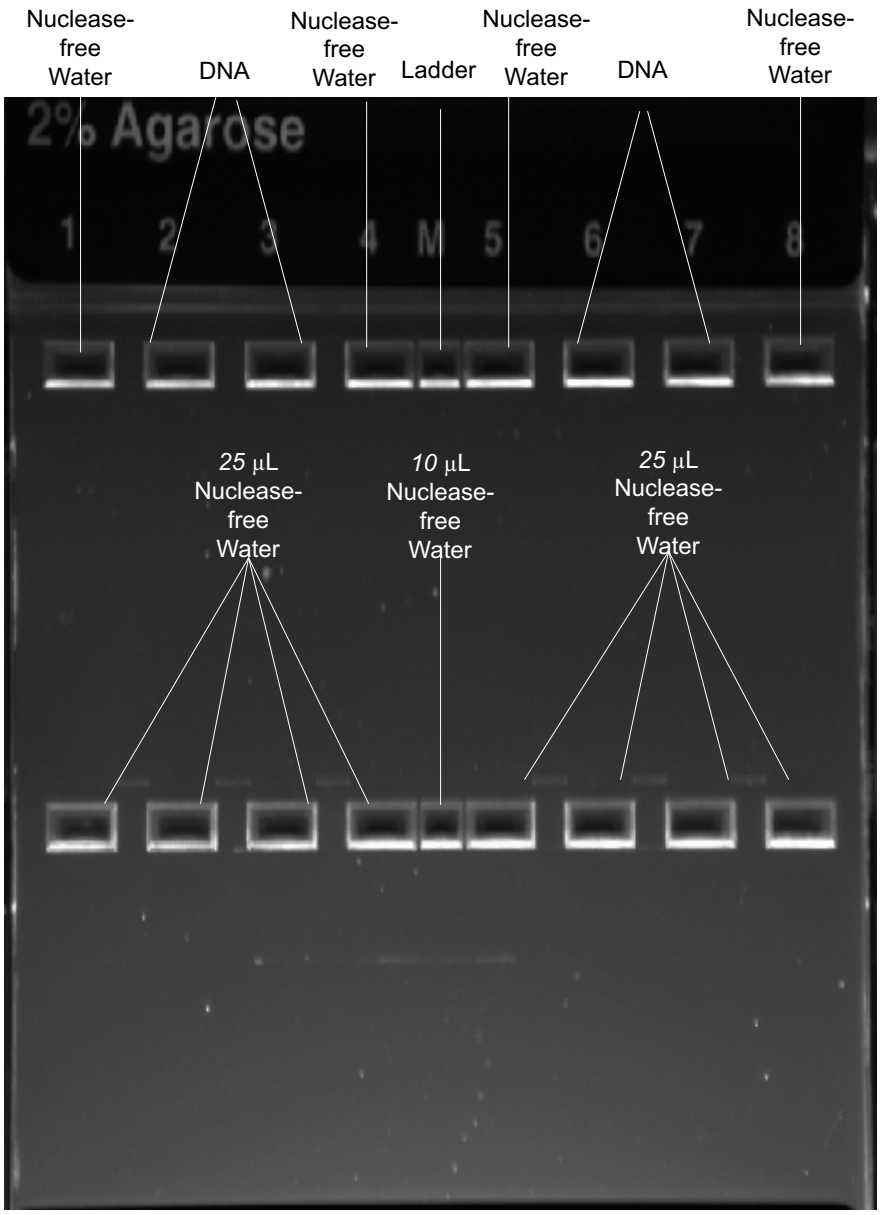
When a full-length sequencing tag is not critical, such as for clonal coverage, then the size-selecting the library is not necessary. Proceed to "Check the size distribution of the library" on page 43.

If you intend to maximize sequencing coverage, then you may prefer to size-select the library. For sequencing of 2 × 60 bp libraries, size-select the library to remove fragments <240 bp. For sequencing of 2 × 50 bp libraries, size-select the library to remove fragments <220 bp. Limit the size of the library to ≤350 bp. Size-selection may reduce the final library yield over not size-selecting the library by 3–4-fold. You must take into account the reduced yield when determining the optimal number of PCR cycles for final library amplification.

The library is run on an SOLiD™ Library Size Selection gel. Extract and desalt the library band (250 –350 bp) using the SOLiD Library Micro Column Purification Kit.

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.
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.
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 1–8 in the *middle* of the gel and 10 µL of Nuclease-free Water in the middle marker well of the bottom row:



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

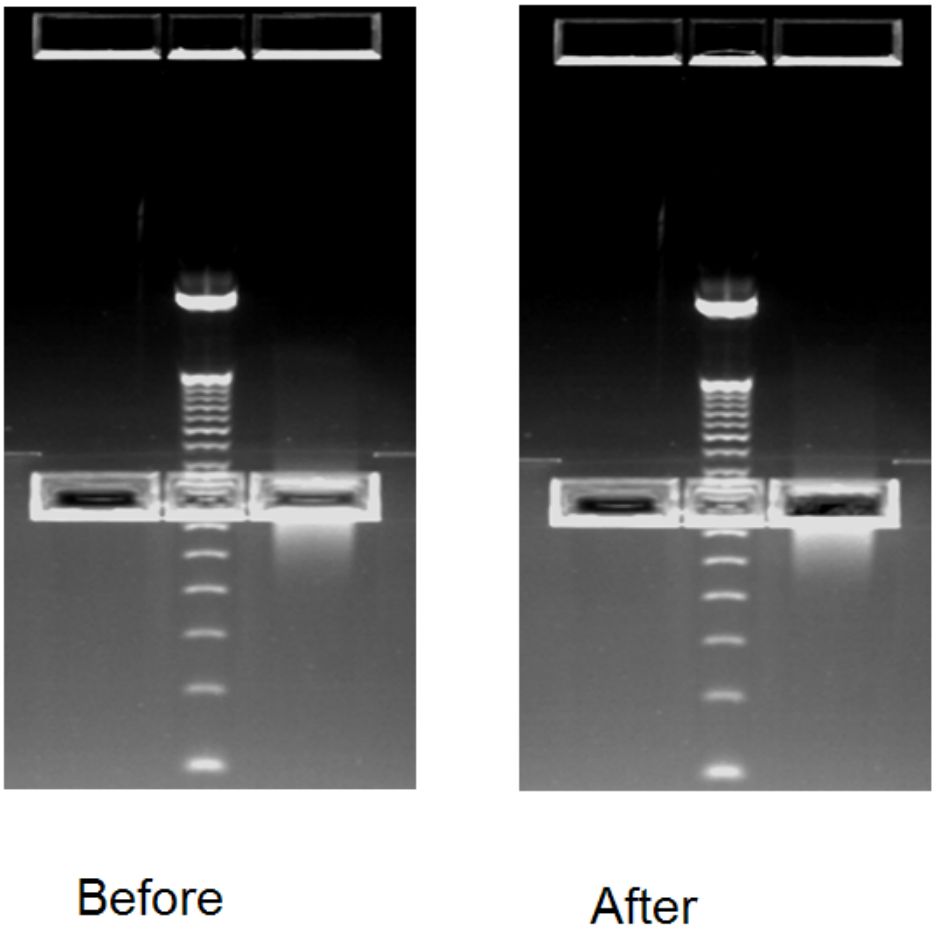
1. Place the E-Gel® iBase™ Power System over a Safe Imager™ Real-Time Transilluminator. Use the orange cover or orange goggles to view the bands and to avoid overexposure of your eyes to the blue light.

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 1–2 more minutes until the band reaches the line.

     IMPORTANT! The ideal size of a library is from 275–325 bp, but a library ranging from 240–380 bp is acceptable. To generate an optimal number of full-length mate tags in 2 × 60 bp sequencing, the minimum library size must be ≥240 bp.

   - When the front line of library products reaches the reference line, press **Go** to stop the run.
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 the sample (250 bp–350 bp) from the collection well every 10 seconds using a 20-µL pipettor fitted with a tip. After each collection, flush the collection well with 20 µL of Nuclease-Free Water, then collect this wash. For example:



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.

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

Note: If a concentrated sample is not necessary, skip this purification step and proceed to "Quantitate the library by performing quantitative PCR (qPCR)" on page 43.

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. Load the DNA onto the PureLink® Micro columns:
   a. Apply the sample in the binding buffer to the PureLink® Micro column(s) in collection tube(s).
   b. Spin the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
   c. If necessary, apply the sample in the binding buffer to the PureLink® Micro column(s) in collection tube(s).
   d. Spin the column(s) at 10,000 × g for 1 minute, then discard the flow-through. dsDNA is bound to the column.
   e. Ensure that the entire sample has been loaded onto the column(s).
4. Wash the column(s):
   a. Return the PureLink® Micro column(s) to the same collection tube(s).
   b. Add 650 µL of Wash Buffer (W1) with ethanol to wash the column(s).
   c. Spin the column(s) at 10,000 × g for 1 minute, then discard the flow-through.
   d. Spin the column(s) at 14,000 × g to remove residual wash buffer.
5. Elute the DNA:
   a. Transfer the column(s) to clean 1.5-mL LoBind tube(s).
   b. Add 20 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.
   c. Spin the column(s) at 14,000 × g for 1 minute.
   d. Add the eluate from the last spin back to the column(s), then let the column(s) stand for 1 minute.
   e. Spin the column(s) at 14,000 × g for 1 minute.
6. 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 for short-term storage or at –20°C for long-term storage. Or, proceed to "Check the size distribution of the library".

Check the size distribution of the library

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

STOPPING POINT Store the DNA in Low TE Buffer at 4°C for short-term storage or at –20°C for long-term storage.

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 (Part no. 4449639), refer to the *Applied Biosystems SOLiD™ Library TaqMan® Quantitation Kit* protocol (Invitrogen Part no. A12120).

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

Troubleshooting

Observation	Possible cause	Recommended action
<50 ng DNA recovered before circularization (see "Assess the recovery of DNA" on page 23)	Mass of sample DNA too low	• Use a fluorescence assay specific for dsDNA to measure the starting DNA (see "Quantitate and assess sample DNA quality" on page 16). • Use ≥1 µg DNA sample for library construction.
	Initial size-selection too narrow	Size-select DNA in the gel >+/-10% of the targeted insert size. For example, for a 1-kb insert, cut at least 0.9–1.1 kb. A gel band of 0.9–1.3 kb is cut routinely.
	Poor genomic DNA quality	Check the integrity and purity of the DNA sample by running a small fraction on an agarose gel.
	Need to be familiar with the procedure or refine techniques	Practice the protocol with 5 µg of high quality DNA sample.
	Substantial loss of DNA during concentration or purification	• If micro-columns are used, check the column filters to ensure they are seated in the columns, and pre-spin the columns before use. • If the Agencourt AMPure® XP Reagent Kit is used, avoid vortexing the DNA-bound beads vigorously during 70% ethanol washes.
	Unknown	Repeat the protocol with more DNA sample. For a 1–3 kb insert, use as close to 5 µg as possible.
After 14-cycle amplification, no trial PCR product visible	Circularized DNA break before nick-translation	Do not stop between the DNase reaction and the nick-translation.
	Mate-paired library insert size is >>3 kb	Use >5 µg genomic DNA, or amplify the library beads for >14 cycles.
	Need to be familiar with the procedure or refine techniques	Practice the protocol with 5 µg of high quality DNA sample.
	Substantial loss of DNA during concentration or purification	• If micro-columns are used, check the column filters to ensure they are seated in the columns and pre-spin the columns before use. • If the Agencourt AMPure® XP Reagent Kit is used, avoid vortexing the DNA-bound beads vigorously during 70% ethanol washes.
	Unknown	Repeat the protocol with more DNA sample. For a 1–3 kb insert, use as close to 5 µg as possible.

Observation	Possible cause	Recommended action
A library from a trial PCR shows large molecular weight (>1 kb) products in addition to mostly the expected-size PCR products	Carry-over of linear fragment or of non-mate-paired fragments by streptavidin beads	Proceed to final PCR amplification. The large molecular weight by-products usually are reduced in the final PCR, and a small fraction of large molecular weight PCR by-products does not affect emulsion PCR (ePCR) and sequencing results.
Trial PCR library size is too small (<250 bp)	Nick-translation did not work as expected	• If the PCR library size is 200–250 bp, consider 2 × 50 mate-paired sequencing. • Increase the nick-translation time. • Ensure that the nick-translation incubation is at 5°C.
	Genomic DNA sample is severely damaged; for example, genomic DNA from formalin-fixed paraffin embedded sample (FFPE)	Increase the nick-translation time.
	Too much sample DNA used for a 1–3 kb insert library	Use as close to 5 µg sample DNA as possible. If >5 µg is used, scale up the the end-repair and nick-translation reactions.
Trial PCR library size is too big (>350 bp)	Nick-translation did not work as expected	• Ensure that the nick-translation incubation is at 5°C. • Shorten the time for nick-translation. • A library peak of up to 400 bp can still be sequenced. Limited sequencing performance data is available for a long mate-paired library with a peak >400 bp.
Bioanalyzer™ electropherogram displays multiple peaks in the library after final size selection	Incomplete or mis-timed sample collection from the size selection collection well	Collect the sample every 10 seconds, then flush the well with 20 µL of Nuclease-Free Water and collect the wash after each collection.
After gel extraction, volume of sample for end repair is too large	Used >1 gel extraction column and/or did not concentrate the sheared DNA sample sufficiently in the SpeedVac® Concentrator	• Use a SOLiD™ Library Micro Column to micro-concentrate the sample before loading on the size selection gel, or • With a SpeedVac® Concentrator or equivalent, concentrate the sheared DNA sample sufficiently to load in 1 well of a size-selection gel so that 1 gel extraction column is sufficient for purification.

A Ordering Information

This appendix covers materials for *2 × 60 bp* mate-paired library preparation:

Sufficient reagents are supplied in the 5500 Series SOLiD™ System kits to prepare up to 12 libraries at 5 µg input DNA for high-throughput sequencing with the 5500 Series SOLiD™ System.

Upon receipt of the 5500 Series SOLiD™ System kits, immediately store each components at the temperature specified on the label.

Required Applied Biosystems reagent kit

Item (part no.)†	Components
5500 SOLiD™ Mate-Paired Library Kit (4464418)	• 5500 SOLiD™ Mate-Paired Library Enzyme Module • 5500 SOLiD™ Amplification Module • 5500 SOLiD Mate-Paired Bead & Buffer Module • SOLiD™ Mate-Paired Library Oligo Module • SOLiD™ Library Micro Column Purification Kit • SOLiD™ Library Quick Gel Extraction Kit

Item (part no.)[†]	Components
5500 SOLiD™ Mate-Paired Library Enzyme Module (4464419)[‡§]	• 10 mM dNTP • dA + dNTP Mix • End Polishing E1 • End Polishing E2 • A-tailing Enzyme II • 5X Reaction Buffer • T4 DNA Ligase, 5 U/µL • DNA Polymerase I • Nick Translation Buffer • EDTA, 0.5 M • 100X BSA • 10X Plasmid-Safe™ Buffer • Plasmid-Safe™ DNase, 10 U/µL • ATP, 100 mM • T7 Exonuclease • 10X Buffer 4 • S1 Nuclease • 3 M NaCl • S1 Nuc Dilution Buffer
5500 SOLiD™ Mate-Paired Amplification Module (4464421)	Platinum® PCR Amplification Mix
SOLiD™ Mate-Paired Library Bead & Buffer Module (4464420)	• Dynabeads® MyOne™ Streptavidin C1 • Bead Wash Buffer • Bead Binding Buffer • Bead Dilution Buffer
SOLiD™ Mate-Paired Library Oligo Module (4464422)	• P1-T Adaptor (ds), 10 µM • P2-T Adaptor (ds), 10 µM • Library PCR Primer 1, 50 µM • Library PCR Primer 2, 50 µM • MPR Adaptor (ds), 25 µM • MPL Adaptor (ds), 25 µM
SOLiD™ Library Micro Column Purification Kit (4443751)	• Binding Buffer (B2-L) • Binding Buffer (B2-S) • Wash Buffer • Elution Buffer • Micro Spin Columns • Elution Tubes

Item (part no.)†	Components
SOLiD™ Library Quick Gel Extraction Kit (4443733)	• Wash Buffer (W1) • Elution Buffer (E5) • Gel Solubilization Buffer (L3) • Quick Gel Extraction Columns • Wash Tubes • Recovery Tubes

† 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**.

Required equipment

Product Name†	Vendor
HydroShear® DNA Shearing Device from Genomic Solutions®‡§	• Applied Biosystems 4392889 (115 V) • Applied Biosystems 4392890 (230 V)
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)‡
Labquake Rotisserie Rotator, Barnstead/ Thermolyne	VWR 56264-312
6-Tube Magnetic Stand *or* DynaMag™– 2 Magnet (magnetic rack)	• Applied Biosystems AM10055 • Invitrogen 123-21D

Product Name†	Vendor
Safe Imager™ 2.0 Blue Light Transilluminator *or* Safe Imager™ Blue Light Transilluminator	Invitrogen G6600 Invitrogen S37102
Qubit® 2.0 Fluorometer	Invitrogen Q32866
Gel imaging system	Major Laboratory Supplier (MLS)‡‡
Tabletop Centrifuge	MLS
Sample concentrator (SpeedVac® Concentrator)	MLS
Gel boxes and power supplies for agarose gels	MLS
Vortexer	MLS
PicoFuge® Microcentrifuge	MLS
Incubator (37ºC)	MLS
Incubator (70ºC)	MLS
Incubator (75ºC)	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.

§ Optional if used for all DNA fragmentation.

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

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

Optional equipment

Product name†	Vendor
Covaris® S220 System‡ (110 V for U.S. customers) (220 V for international customers) The Covaris® S220 System includes: • Covaris® S220 sonicator • Universal Voltage Kit • Latitude® laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris®-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris®-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris® microTUBE Prep Station • Covaris® Water Tank Label Kit • Covaris® microTUBEs (1 pack of 25)	Applied Biosystems 4465653
Covaris® S2 System§ (110 V for U.S. customers) (220 V for international customers)	**Note:** Mate-paired libraries can be prepared with the Covaris® S2 System. New users should purchase the Covaris® S220 System.
E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit	Invitrogen G6465
2100 Bioanalyzer™	Agilent Technologies G2938C

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

‡ Or the Covaris® S2 System.

§ Or the Covaris® S220 System.

Required consumables

Item †	Source
Agencourt AMPure® XP 5 mL Kit or Agencourt AMPure® XP 60 mL Kit	Beckman Coulter Genomics A63880 or A63881
Invitrogen Qubit™ dsDNA HS Assay Kit *or* Invitrogen Qubit™ dsDNA BR Assay Kit *or* Invitrogen Quant-iT™ PicoGreen® dsDNA Assay Kit	Invitrogen Q32851 or Q32854 Invitrogen Q32850 or Q32853 Invitrogen P7589
E-Gel® EX Gel, 2%, 10-Pak	Invitrogen G4010-02
UltraPure™ DNA Typing Grade 50× TAE Buffer	Invitrogen 24710-030
Agarose-LE *or* UltraPure™Agarose 1000	Applied Biosystems AM9040 Invitrogen 10975-035
SYBR® Safe DNA Gel Stain (10,000×)	Invitrogen S33102
10× BlueJuice™ Gel Loading Buffer	Invitrogen 10816-015
50 bp DNA Ladder	Invitrogen 10416-014
100 bp DNA Ladder	Invitrogen 15628-050
1 Kb Plus DNA Ladder	Invitrogen 10787-018
Covaris® Tubes and Caps, 125	Applied Biosystems 4399054
Ethanol	Sigma-Aldrich E7023

Item †	Source
2-Propanol	Sigma-Aldrich I9516
Ethylene glycol	American Bioanalytical AB00455-01000
1.5-mL LoBind Tubes	Eppendorf 022431021
2.0-mL LoBind Tubes	Eppendorf 022431048
MicroAmp® Optical 8-Tube Strip, 0.2 mL	Applied Biosystems 4316567
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
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.

Optional consumables

Product name†‡	Vendor
1X TE Buffer	Invitrogen 12090-015
SOLiD™ Library Size Selection Gel	Applied Biosystems 4443733
Agilent High Sensitivity DNA Kit	Agilent Technologies 5067-4626

† 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.

B Supplemental Procedures

This appendix covers:

Shear the DNA for inserts ≤1 kb with the Covaris® System

Shearing guidelines

- Perform a small-scale shearing trial before large-scale shearing, if DNA is available.
- Adjust shearing conditions according to different organisms.
- Calibrate the shearing run to assess the shearing efficacy of the device before starting the first library preparation.

Quantitate the DNA

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

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

  or

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

  or

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

Choose the shearing system

IMPORTANT! If you are using the Covaris® System, set the chiller temperature to 2–5 °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.

You can shear the DNA with two supported shearing systems:

- The Covaris® S220 System (see "Shear the DNA with the Covaris® S220 System" on page 56).

  or

- The Covaris® S2 System (see "Shear the DNA with the Covaris® S2 System" on page 57.

Shear the DNA with the Covaris® S220 System

1. In a Covaris® microTUBE, mix:

Component	Amount
DNA	1–5 µg
1X TE Buffer	Variable µL
Total	**120 µL**

2. Shear the DNA using the Covaris® S220 System shearing according to these conditions:

IMPORTANT! Ensure that the Bath Temperature Limit is set to 15°C, and keep the bath temperature to ≤10°C.

Target insert (peak)	Covaris® S220 System shearing conditions
700 bp	• Duty Factor: **5%** • Peak Incident Power (PIP): **105 Watts** • Cycles per burst: **200** • Time: **60 seconds** • Number of cycles: **1** • Waterbath temperature: **6–8°C** • Bath Temperature Limit: **15°C** • Power mode: **Frequency sweeping** • Degassing mode: **Continuous** • Water level: **12** • Water Quality Testing Function: **Off** • AFA intensifier: **Yes**
1000 bp	• Duty Factor: **1%** • Peak Incident Power (PIP): **175 Watts** • Cycles per burst: **200** • Time: **45 seconds** • Number of cycles: **2** • Waterbath temperature: **6–8°C** • Bath Temperature Limit: **15°C** • Power mode: **Frequency sweeping** • Degassing mode: **Continuous** • Water level: **12** • Water Quality Testing Function: **Off** • AFA intensifier: **Yes**

3. Transfer 120 µL of sheared DNA into a clean 1.5-mL LoBind tube.

4. Reduce the volume of the sheared DNA to ~40–60 µL in order to load the sample into a 1-cm wide well on a size selection gel. Use a SpeedVac® Concentrator or equivalent instrument.

   Note: If a SpeedVac® Concentrator is *not* available, and there is ≥2 µg of DNA to concentrate, then use the SOLiD™ Library Micro Column Purification Kit with B2-S Buffer. After the wash, elute the purified, sheared DNA with 50 µL Elution Buffer (E1).

5. Proceed to "Measure the amount of sheared, purified DNA" on page 59.

Shear the DNA with the Covaris® S2 System

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

      The water should cover the visible glass part of the tube.

   b. Set the chiller temperature to 2–5°C to ensure that the temperature reading in the water bath displays 5°C.

   c. Supplement the circulated water chiller with 20% ethylene glycol.

2. In a Covaris® microTUBE, mix:

Component	Amount
DNA	1–5 µg
1× TE Buffer	Variable µL
Total	**120 µL**

3. Shear the DNA using the Covaris® S2 System shearing according to these conditions:

IMPORTANT! Ensure that the Bath Temperature Limit is set to 15°C, and keep the bath temperature to ≤10°C.

Target insert (peak)	Covaris® S2 System shearing conditions
700 bp	• Duty cycle: **5%** • Intensity: **3** • Cycles per burst: **200** • Time: **60 seconds** • Number of cycles: **1** • Waterbath temperature: **6–8°C** • Bath Temperature Limit: **15°C** • Power mode: **Frequency sweeping** • Degassing mode: **Continuous** • Water level: **12** • Water Quality Testing Function: **Off** • AFA intensifier: **Yes**
1000 bp	• Duty cycle: **1%** • Intensity: **5** • Cycles per burst: **200** • Time: **45 seconds** • Number of cycles: **2** • Waterbath temperature: **6–8°C** • Bath Temperature Limit: **15°C** • Power mode: **Frequency sweeping** • Degassing mode: **Continuous** • Water level: **12** • Water Quality Testing Function: **Off** • AFA intensifier: **Yes**

4. Transfer 120 µL of sheared DNA into a clean 1.5-mL LoBind tube.

5. Reduce the volume of the sheared DNA to ~40–60 µL in order to load the sample into a 1-cm wide well on a size selection gel. Use a SpeedVac® Concentrator or equivalent instrument.

   Note: If a SpeedVac® Concentrator is *not* available, and there is ≥2 µg of DNA to concentrate, then use the SOLiD™ Library Micro Column Purification Kit with B2-S Buffer. After the wash, elute the purified, sheared DNA with 50 µL Elution Buffer (E1).

Check the insert sizes

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.

Measure the amount of sheared, purified DNA

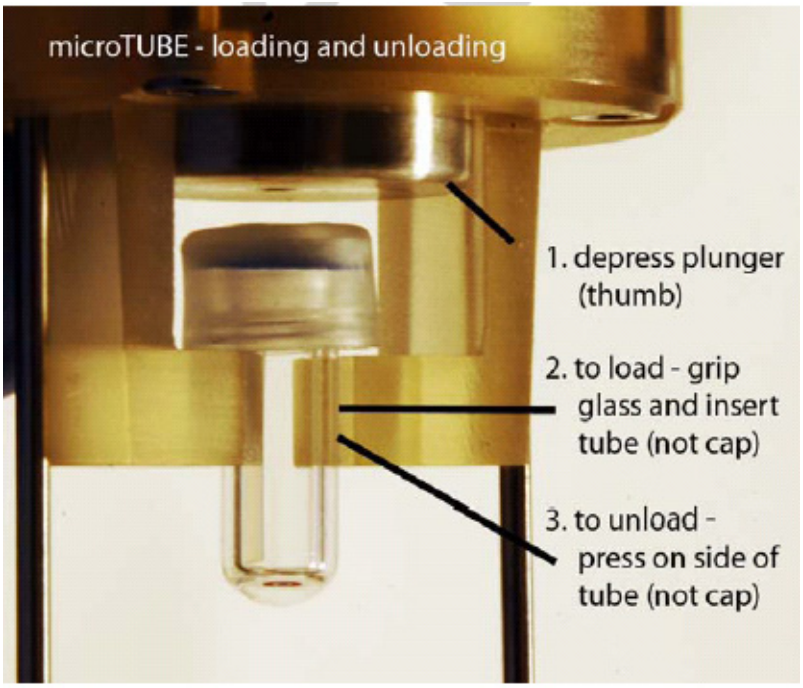
Quantitate the purified DNA using 1 µL of sample with the Quant-iT™ dsDNA HS Assay Kits (Invitrogen Part no. Q32851) and the Qubit™2.0 Fluorometer (Invitrogen Part no. Q32866).

STOPPING POINT Store the purified DNA in Elution Buffer (E1) at 4 ºC, or proceed directly to "Size-select the DNA" on page 18.

Load and unload Covaris® microTUBE vials

Load Covaris® microTUBE vials

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



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

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

Unload Covaris® microTUBE vials

1. Use a thumb to push the stainless steel plunger up into the body of the microTUBE holder to relieve pressure on the cap.

2. Press against the side of the glass tube (*not* against the cap) to free the microTUBE from the grip of the holder.

C

Overview

This appendix covers:

Choose the appropriate library type

These are the types of libraries that can be sequenced on the 5500 Series SOLiD™ Sequencers:

Library type	Features	Applications	Go to...
Mate-paired	• Two DNA insert tags 700 bp–3 kb apart. • Separated by an internal adaptor. • More input DNA required (1–5 µg). • Paired reads enable unique mapping in regions not accessible to single read sequencing. • Information on tag orientation and apparent distance between tags. • Increase mapping specificity over standard fragment library sequencing. • Detect large structural variations in the genome. • Bridge sequencing gaps.	• *De novo* sequencing, primary library • Genomic resequencing, primary library • Methylation analysis	"2 × 60 bp Mate-Paired Library Preparation" on page 11

Library type	Features	Applications	Go to...
Fragment	• Appropriate for sequence lengths ≤300 bp. • Adaptors on each end of sheared DNA insert. • Multiplexed sequencing. • The protocol is designed for 10 ng–5 µg of genomic DNA or ligated PCR product. • Compared to mate-paired libraries, fragment libraries yield a higher recovery of unique molecules, when normalized to the same input amount.	• Targeted resequencing, primary library • Genomic resequencing • Methylation analysis	Refer to the *Fragment Library Preparation: 5500 Series SOLiD™ Systems User Guide* (Part no. 4460960)

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:

www.appliedbiosystems.com/solid5500

or contact your field applications specialist.

Preparation of mate-paired libraries

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. The mate paired ends are connected together through an internal adaptor (MP Adaptor) to form a circle. For the 5500 Series SOLiD™ Sequencers, the preparation of a mate-paired library involves use of a circularization method that is different from the circularization method used for mate-paired library preparation with previous versions of the SOLiD™ System. The new circularization method improves circularization efficiency by several fold.

For long mate-paired libraries (for example to produce sequencing reads, 2 × 60 nt), size-selected genomic DNA fragments are ligated to new intramolecular circularization (MPR and MPL) adaptors then self-circularized by hybridization in a very dilute solution. (see Figure 2 on page 63). The resulting DNA circle has one nick in each strand because the MP 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′ –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-T and P2-T Adaptors are then ligated to the ends of the mate-paired library for subsequent amplification by PCR (see Figure 3 on page 64).

Figure 2 Basic 2 × 60 bp mate-paired library preparation workflow.

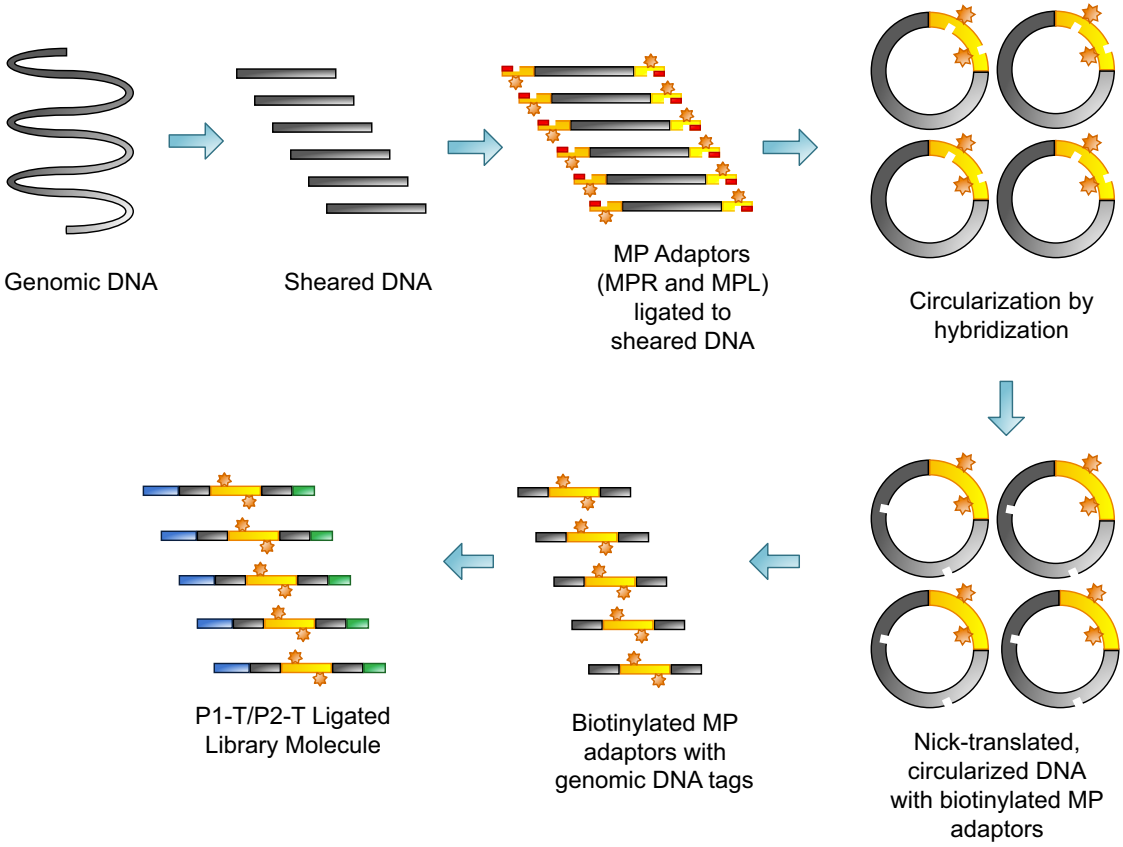
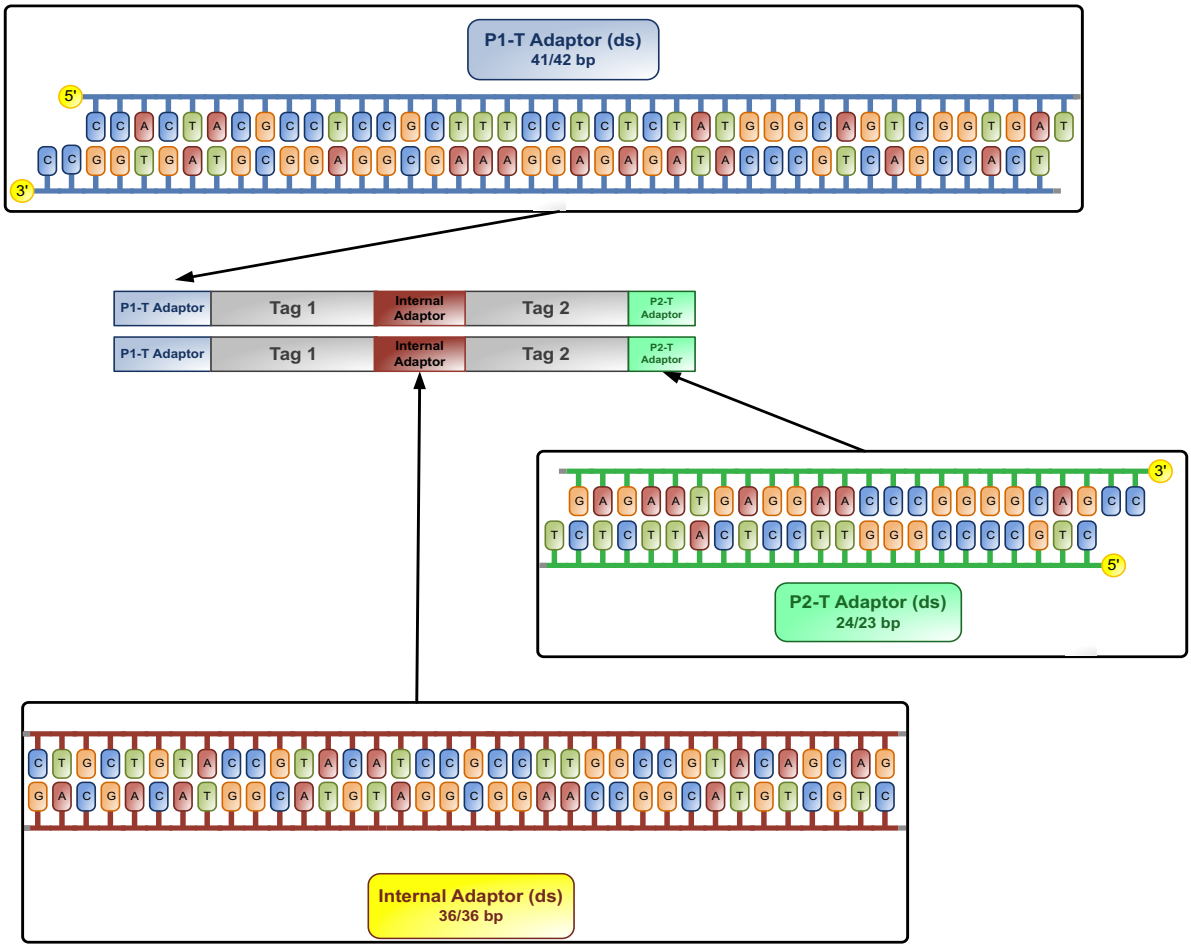
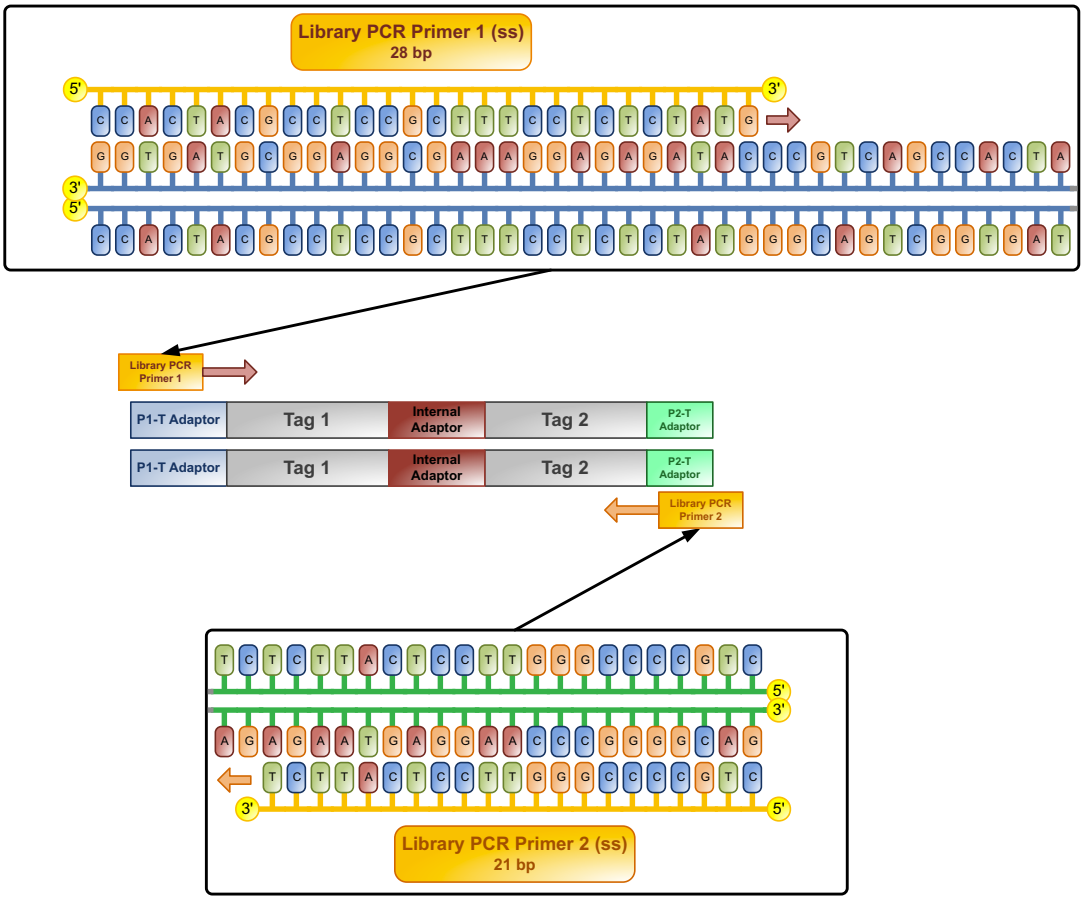


Figure 3 Mate-paired library design.



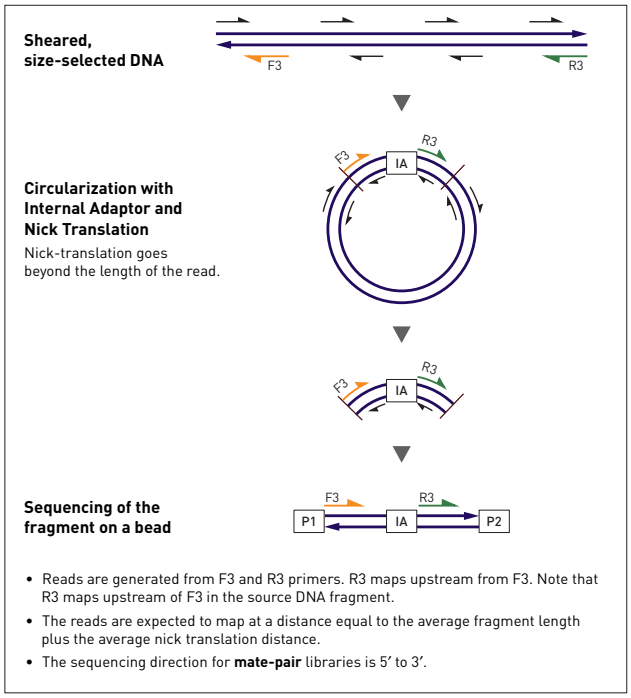
After P1-T and P2-T Adaptors are ligated to the sheared DNA, the library is amplified using primers specific to the P1-T and P2-T Adaptors (see Figure 4 on page 65). These primers can be used only for library amplification and not for alternative or modified library construction adaptor design, because they do not have 3′ sequences compatible with the sequencing primers.

Figure 4 Mate-paired library amplification design.



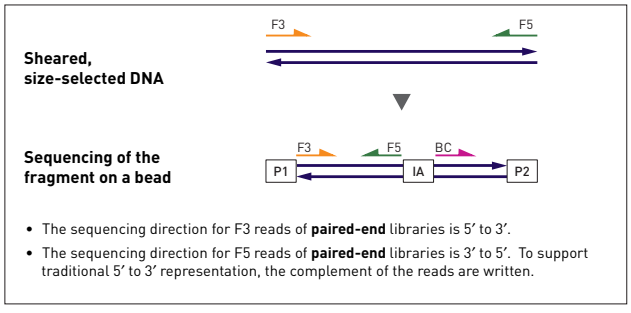
Sequence orientation from source DNA to sequence map

Mate-pair



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

Paired-end



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

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

D Oligonucleotide Sequences

Library construction oligonucleotides

Adaptor sequences

Note: The internal adaptor used for DNA mate-paired libraries is different from the internal adaptor used for RNA libraries.

Adaptor and primer sequences	Length (nt)
P1-T Adaptor, 50 µM	
5′ -CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT-3′	41
5′ -TCACCGACTGCCCATAGAGAGGAAAGCGGAGGCGTAGTGGCC-3′	42
P2-T Adaptor, 50 µM	
5′ - GAGAATGAGGAACCCGGGGCAGCC-3′	24
5′ -CTGCCCCGGGTTCCTCATTCTCT-3′	23
Library PCR Primer 1, 50 µM	
5′ -CCACTACGCCTCCGCTTTCCTCTCTATG-3′	28
Library PCR Primer 2, 50 µM	
5′ -CTGCCCCGGGTTCCTCATTCT-3′	21

E Checklist and workflow tracking form

Workflow checklists: prepare a 2 × 60 bp mate-paired library

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

	Equipment	Reagents	Preparation steps
Shear the DNA with the Covaris® S220 System	☐ Covaris® S220 System ☐ Covaris® Tubes and Caps ☐ Qubit® 2.0 Fluorometer ☐ SpeedVac® Concentrator	☐ 1 M Tris, pH 8.0 ☐ Ethylene glycol ☐ UltraPure™ Glycerol	☐ Degas the water in the Covaris® S220 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 ☐ Qubit® 2.0 Fluorometer	☐ Nuclease-free Water ☐ 0.2 N HCl ☐ 0.2 N NaOH ☐ 1.5-mL LoBind tubes ☐ SOLiD™ Library Column Purification Kit	—
Size-select the DNA	☐ Gel box and power supply for agarose gel ☐ Safe Imager™ Blue Light Transilluminator ☐ Gel imaging system ☐ Qubit® 2.0 Fluorometer ☐ Razor blades ☐ 15-mL conical polypropylene tubes ☐ DynaMag™-2 Magnetic Rack	☐ 1× TAE buffer ☐ Agarose ☐ BlueJuice™Gel Loading Buffer ☐ 1 Kb Plus DNA Ladder ☐ SYBR® Safe gel stain ☐ Invitrogen Library Quick Gel Extraction Kit ☐ Isopropyl alcohol	☐ Prepare 1× TAE buffer. ☐ Prepare 1.0% agarose gel.
End-repair the DNA	☐ Qubit® 2.0 Fluorometer	☐ 5× T4 DNA Ligase Buffer ☐ dNTP, 10 mM ☐ T4 Polynucleotide Kinase, 10 U/µL ☐ T4 DNA Polymerase 5 U/µL ☐ Nuclease-free Water	☐ End-repair reagents on ice.
Ligate NIC Adaptors to the DNA	☐ DynaMag™-2 Magnetic Rack	☐ MPR Adaptor , 25 µM ☐ MPL Adaptor, 25 µM ☐ T4 DNA Ligase, 5U/µL ☐ 5× T4 DNA Ligase Buffer ☐ T4 DNA Ligase ☐ AMPure XP Beads ☐ Ethanol, Absolute ☐ Elution Buffer (E1)	☐ Thaw adaptors on ice.
Quantitate	☐ Qubit® 2.0 Fluorometer	☐ Quant-iT™ dsDNA HS Assay Kit	☐
Circularize the DNA by intra-molecular hybridization	—	☐ 10× Plasmid-Safe™ Buffer	☐ Thaw buffer on ice.

	Equipment	Reagents	Preparation steps
Isolate the circularized DNA	☐ Qubit® 2.0 Fluorometer ☐ Incubator (37 ºC)	☐ ATP, 25 mM ☐ 10× Plasmid-Safe™ Buffer ☐ Agencourt AMPure® XP Kit ☐ Ethanol, Absolute ☐ Elution Buffer (E1)	☐ Thaw Plasmid-Safe™ ATP-Dependent DNase reagents on ice.
Nick-translate the circularized DNA	☐ Thermal cycler ☐ Timer	☐ dNTP Mix (10 mM each) ☐ DNA Polymerase I (10 U/µL) ☐ Nick Translation Buffer ☐ 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 ºC) ☐ Incubator (70 ºC)	☐ T7 exonuclease, 10 U/µL ☐ 10× Buffer 4 ☐ S1 Nuclease Dilution Buffer ☐ S1 Nuclease, 25 U/µL ☐ Agencourt AMPure® XP Kit ☐ Ethanol, Absolute ☐ Elution Buffer (E1)) ☐ Ice	☐ Thaw Buffer 4 Buffer on ice.
Blunt-end the digested DNA with an A-tail	—	☐ 0.5 M EDTA ☐ 1x Nick Translation Buffer ☐ dA + dNTP, 50 mM ☐ Klenow Exo-, 5U/µL ☐ Bead Binding Buffer	☐ Thaw end-repair reagents on ice.
Bind the library molecules to streptavidin beads	☐ 6 Tube Magnetic Rack ☐ Rotator	☐ 100× BSA ☐ Dynabeads® MyOne™ Streptavidin C1 beads ☐ Bead Wash Buffer ☐ Bead Binding Buffer ☐ 5× Ligase Buffer	☐ Thaw 100× BSA and 5× Ligase Buffer on ice.

	Equipment	Reagents	Preparation steps
Ligate P1-T and P2-T Adaptors to the DNA	☐ Rotator ☐ DynaMag™-2 Magnetic Rack	☐ T4 DNA Ligase, 5 U/µL ☐ P1-T Adaptor (ds) ☐ P2-T Adaptor (ds) ☐ Bead Wash Buffer	☐ Thaw P1-T Adaptor (ds) and P2-T Adaptor (ds) on ice.
Nick – translate and trial-amplify the library	☐ Thermal cycler ☐ E-Gel® iBase™ Power System ☐ Gel imaging system ☐ PCR strip tubes	☐ Library PCR Primer 1 ☐ Library PCR Primer 2 ☐ Platinum® PCR Amplification Mix ☐ 2% E-Gel® EX-Gel ☐ 100-bp DNA ladder	☐ Thaw Library P1 and P2 PCR Primers on ice. ☐ Thaw Platinum PCR Amplification Mix on ice.
Nick –translate and Amplify the library	☐ Thermal cycler ☐ E-Gel® iBase™ Power System ☐ Microcentrifuge ☐ DynaMag™-2 Magnetic Rack ☐ 2100 Bioanalyzer™ ☐ PCR strip tubes	☐ Library P1 PCR Primer ☐ Library P2 PCR Primer ☐ Platinum® PCR Amplification Mix ☐ DNA 1000 Chip ☐ SOLiD™ Library Micro Column Purification Kit	☐ Thaw Library P1 and P2 PCR Primers on ice. ☐ Thaw Platinum PCR Amplification Mix on ice. ☐ Thaw DNA 1000 kit reagents on ice.
(*Optional*) Size-select the library	☐ E-Gel® iBase™ Power System ☐ Safe Imager™ Blue Light Transilluminator ☐ Gel imaging system	☐ E-Gel® SizeSelect™ 2% Gel ☐ 100-bp DNA ladder ☐ Gel Loading Solution ☐ SOLiD™ Library Micro Column Purification Kit	—
Quantitate the library by qPCR	☐ Real-time thermal cycler	☐ SOLiD™ Library TaqMan® Quantitation Kit	—

Workflow tracking: prepare a 2 × 60 bp mate-paired library

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
Size selection	
Ligation of NIC Adaptors	
Circularization	
Quantitative PCR	

Lot number	
Step	**Lot number**
5500 SOLiD™ Mate-Paired Library Standard Adaptors	
5500 SOLiD™ Mate-Paired Library Kit	
Platinum® PCR Amplification Mix	

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
Size selection	
Ligation of NIC Adaptors	
Circularization	
Quantitative PCR	

Lot number	
Step	**Lot number**
5500 SOLiD™ Mate-Paired Library Standard Adaptors	
5500 SOLiD™ Mate-Paired Library Kit	
Platinum® PCR Amplification Mix	

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
Size selection	
Ligation of NIC Adaptors	
Circularization	
Quantitative PCR	

Lot number	
Step	**Lot number**
5500 SOLiD™ Mate-Paired Library Standard Adaptors	
5500 SOLiD™ Mate-Paired Library Kit	
Platinum® PCR Amplification Mix	

Sample:

Quantitation	
Step	**Quantity of DNA**
Starting Amount	
Shearing the DNA	
Size selection	
Ligation of NIC Adaptors	
Circularization	
Quantitative PCR	

Lot number	
Step	**Lot number**
5500 SOLiD™ Mate-Paired Library Standard Adaptors	
5500 SOLiD™ Mate-Paired Library Kit	
Platinum® PCR Amplification Mix	

F Safety

This appendix covers:

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 76.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.

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

SDSs

About SDSs

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

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

Obtaining SDSs

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

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

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

Chemical waste safety

Chemical waste hazards

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

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

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

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

Waste disposal

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

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

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

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories*; **http://www.cdc.gov/biosafety/publications/index.htm**).
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Documentation and Support

Related documentation

For related documents, refer to the *5500 Series SOLiD™ Systems User Documentation Quick Reference*
(Part no. 4465102).

Obtaining support

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

www.appliedbiosystems.com

At the Applied Biosystems website, you can:

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

Glossary

fragment library	A library that has a single insert prepared from genomic DNA for sequencing on the SOLiD™ System. Fragment libraries compatible with the 5500 Series SOLiD™ Sequencers can be sequenced with a forward-only run or with a paired-end run.
internal adaptor (IA)	The internal adaptor sequence is incorporated into the template during library construction and provides a common hybridization target for SOLiD™ sequencing primers. See the *5500 Series SOLiD™ Systems Sequencing Products Ordering Guide* for a schematic of sequencing primers compatible with each type of SOLiD™ library. • The IA sequence is different in DNA-source libraries and RNA-source libraries, therefore sequencing primers specific for RNA and DNA libraries must be used for reverse reads (F5 tag). • The IA-containing adaptors used during mate-paired library preparation are different from the adaptors used for fragment library preparation, but the SOLiD™ FWD2 Seq. Primers are used for all forward reads originating in the IA sequence, generating the R3 and BC tags.
library	A set of DNA or cDNA molecules prepared from the same biological specimen and prepared for sequencing on the SOLiD™ System.
Library PCR Primer 1	Single-stranded oligonucleotide used in library amplification and corresponding to the P1-T Adaptor sequence.
Library PCR Primer 2	Single-stranded oligonucleotide used in library amplification and corresponding to the P2-T Adaptor sequence.
mate-paired library	Library consisting of two DNA segments that reside a known distance apart in the genome, linked by an internal adaptor, and with P1 and P2 Adaptors ligated to the 5' and 3' ends of the template strand, respectively.
MP Adaptor (Adaptors MPR and MPL)	The double-stranded oligonucleotides that are ligated to a sheared DNA insert to form the internal adaptor sequence during mate-paired library construction.
P1-T Adaptor	A T-tailed double-stranded oligonucleotide containing the P1 sequence that is ligated to A-tailed DNA segments during library construction; the result is that the P1 sequence is attached to the 5' end of the template strand.
P2-T Adaptor	A T-tailed double-stranded oligonucleotide containing the P2 sequence that is ligated to A-tailed DNA segments during library construction; the result is that the P2 sequence is attached to the 3' end of the template strand.

tag	There are two uses for this term. • Sequencing data from a single bead with a single primer set; sometimes used interchangeably with *read*. • A length of DNA or cDNA to be sequenced; especially, a relatively short stretch of DNA or cDNA that is used to infer information about the longer native molecule from which it is derived, such as in mate-paired library sequencing and SAGE™ analysis, respectively.
templated bead preparation	Process of covalently attaching and clonally amplifying template strands to beads by emulsion PCR, enriching the beads to remove beads without template, then modifying the 3' end of the template on the beads to prepare for bead deposition and sequencing

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