USER GUIDE

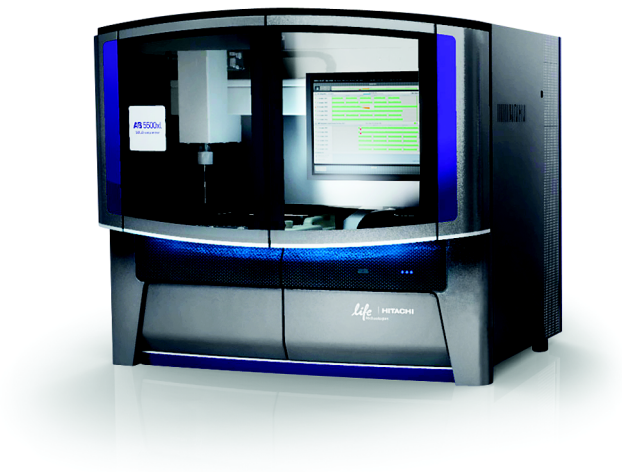
applied biosystems® by life technologies™

Fragment Library Preparation

5500 Series SOLiD™ Systems

Publication Part Number 4460960 Rev. A

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



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Part Number 4460960 Rev. A

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Contents

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 "Safety" on page 87. 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 88.

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

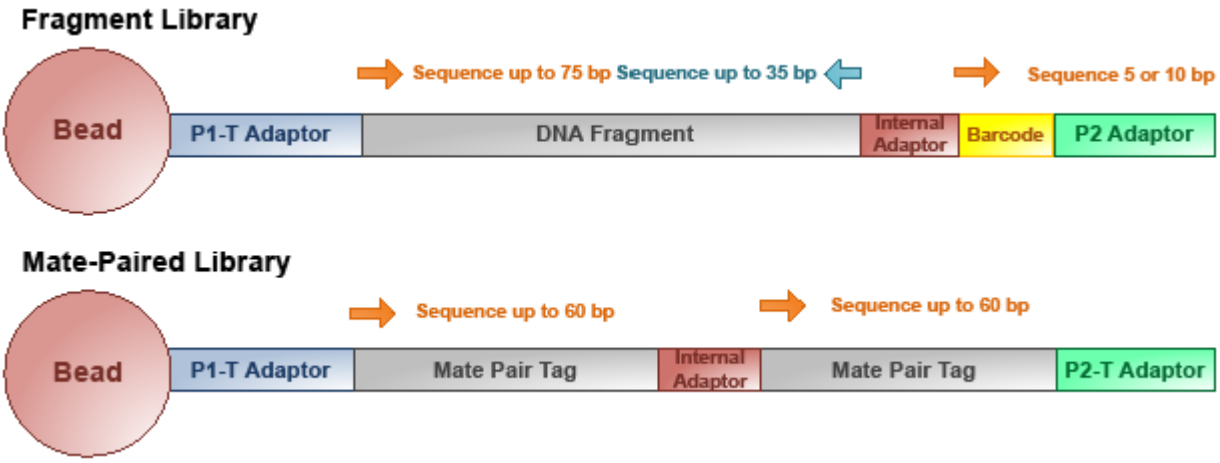
About the Products

IMPORTANT! If you have purchased the AB Library Builder™ System and you want to prepare a fragment library with an automated system, refer to the *Fragment Library Preparation Using the AB Library Builder™ System: 5500 Series SOLiD™ Systems User Guide* (Part no. 4460965).

For a more detailed overview of library types and the library preparation workflows, see "Overview" on page 67.

Library preparation

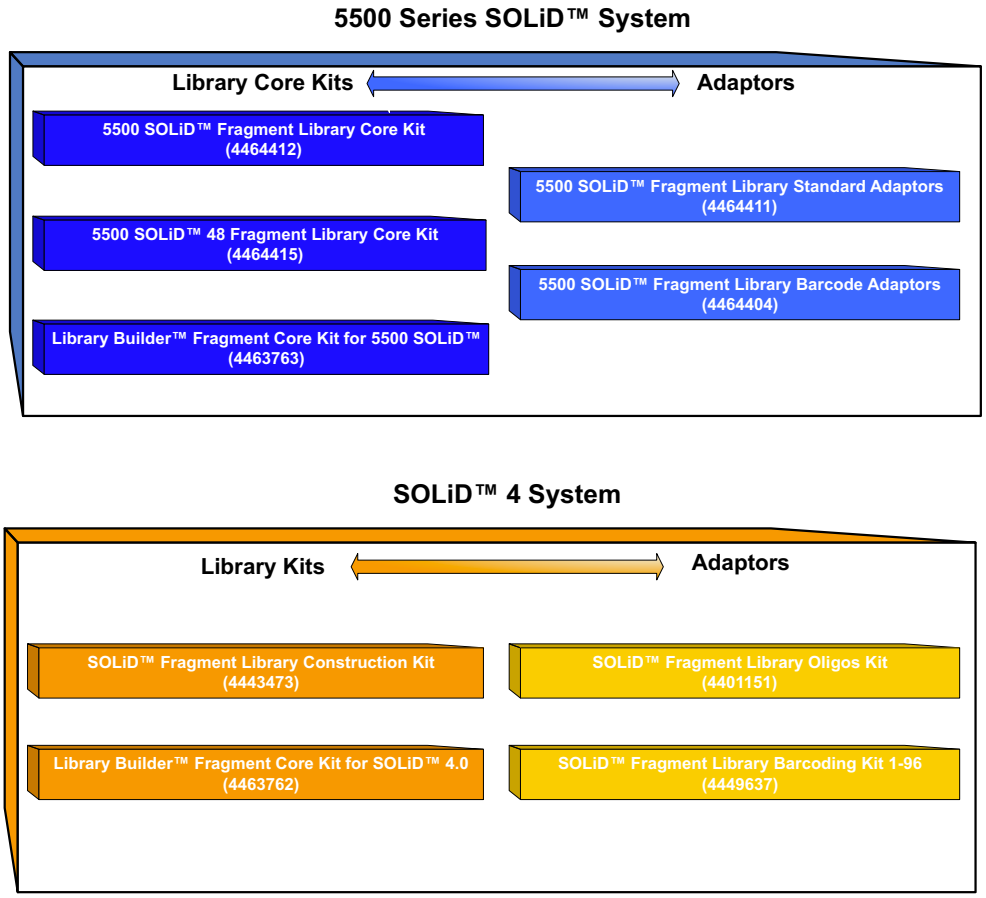
Library preparation is the first step in which samples are adapted for sequencing on the 5500 Series SOLiD™ Sequencers. During library preparation, forward and reverse adaptors are added to the ends of sheared DNA fragments (The bead is for illustration purposes only and is not added until the bead preparation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



Product information

Purpose of the product

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



How to use library core kits with adaptors

This user guide describes how to use the 5500 SOLiD™ Fragment Library Core Kit with the 5500 SOLiD™ Fragment Library Standard Adaptors or the 5500 SOLiD™ Fragment Library Barcoding Adaptors. Use the 5500 SOLiD™ 48 Fragment Library Core Kit with the adaptors for automated liquid-handling systems such as the Beckman Coulter Biomek® FXP and Tecan Freedom EVO® instruments. To use the Library Builder™ Core Kit for 5500 SOLiD™ with the adaptors, refer to *Fragment Library Preparation Using the AB Library Builder™ System: 5500 Series SOLiD™ Systems User Guide*.

Use the 5500 SOLiD™ Fragment Library Core Kit and the adaptors to:

- Prepare a *single* fragment library for forward and reverse reads (100–250 bp, before adaptor ligation) for sequencing on the 5500 Series SOLiD™ Sequencers. A fragment library consists of a sheared DNA fragment with a P1 Adaptor and a Standard Adaptor, ligated to the 5′ end and 3′ end, respectively.
- Prepare *multiple* fragment libraries in parallel for multiplex or non-multiplex sequencing.

Kit contents and storage conditions

Kit contents

The 5500 SOLiD™ Fragment Library Core Kit (Part no. 4464412) contains materials sufficient to prepare 12 fragment libraries:

Part (part no.)	Description	Storage temperature
5500 SOLiD™ Fragment Library Enzyme Module (4464413)	One each	–20°C
5500 SOLiD™ Fragment Library Amplification Module(4464414)	One each	–20°C

The adaptor kits contain materials sufficient to prepare 12 fragment libraries when using 5 µg of input DNA:

Part	Description	Storage temperature
5500 SOLiD™ Fragment Library Standard Adaptors (4464411)	One each	–20°C
5500 SOLiD™ Fragment Library Barcode Adaptors (4464404)	One each	–20°C

Prepare a Single Fragment Library

IMPORTANT! Customers who have purchased the AB Library Builder™ System and who wish to prepare a fragment library with an automated system, refer to the *Fragment Library Preparation Using the AB Library Builder™ System: 5500 Series SOLiD™ Systems User Guide* (Part no. 4460965)

For an overview of library types that can be sequenced on the 5500 Series SOLiD™ Sequencers, see "Choosing the appropriate library type" on page 67. For a graphical overview of fragment library preparation, see "Overview" on page 67.

Workflow

Preparing a single fragment library takes ~4 h without amplification and ~5 h with amplification:

Quantitate the DNA (page 15)

↓

Shear the DNA (page 15)

↓

End-polish the DNA (page 18)

↓

Size-select the DNA by Agencourt AMPure® XP Reagent (page 18)

Stopping point

↓

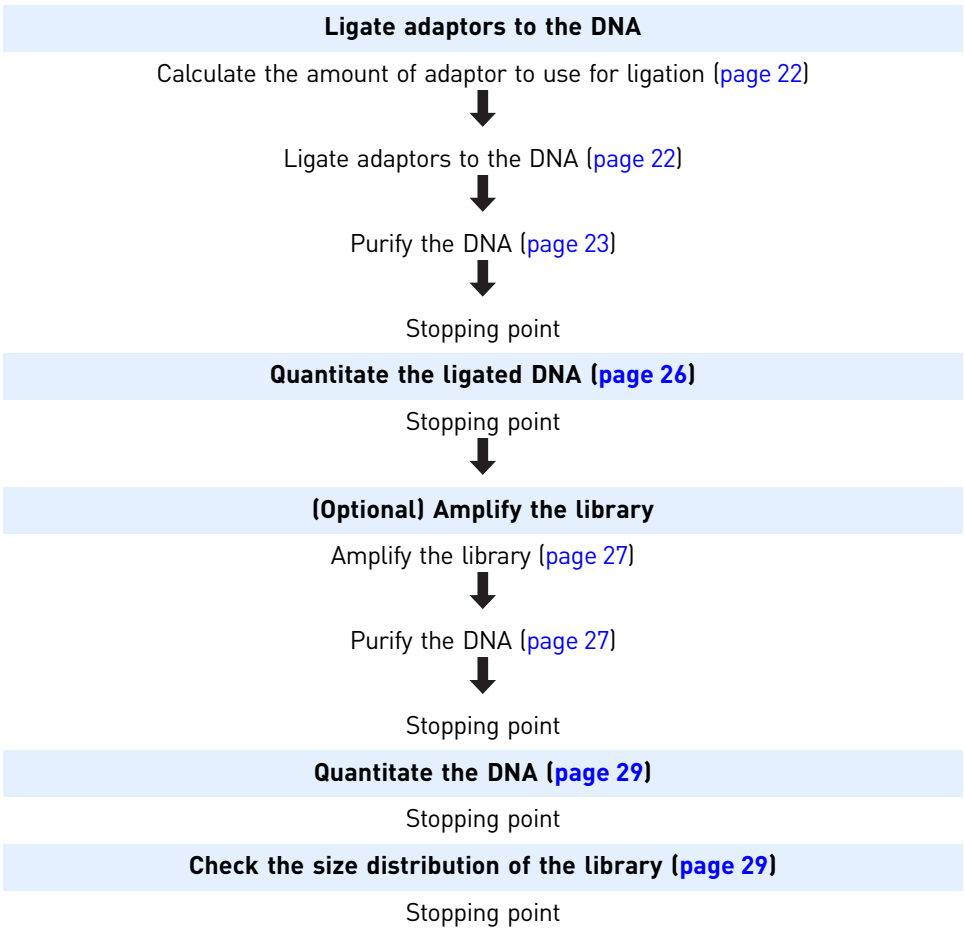
Quantitate the size-selected DNA (page 21)

Stopping point

↓

Add a dA-tail to the size-selected DNA (page 21)

↓



Procedural guidelines

- The protocol is designed for 10 ng–5 µg of genomic DNA.
- To construct a targeted resequencing library with small-sized PCR products (≤500 bp), first perform a PCR-product ligation step to concatenate the DNA. For a concatenation protocol, contact your field application specialist.
- Use good laboratory practices to minimize cross-contamination of products.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols. Applied Biosystems recommends the Eppendorf 5417R tabletop microcentrifuge.
- Perform all steps requiring 0.5-mL and 1.5-mL tubes with 0.5-mL Eppendorf LoBind Tubes (Eppendorf Part no. 022431005) and 1.5-mL Eppendorf LoBind Tubes (Eppendorf Part no. 022431021).
- Thaw reagents on ice before use, but thaw Shear Buffer at room temperature.

Quantitate the DNA

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

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

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

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

Shear the DNA

This step involves sonicating the input DNA into small fragments with a mean fragment size of 160 bp and a fragment size range of 100–250 bp (before adaptor ligation) using the Covaris® System. The conditions have been tested for shearing 10 ng–5 µg DNA in a total volume of 120 µL. For certain DNA samples, optimizing the shearing protocol may be necessary.

You can shear the DNA with two supported shearing systems:

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

  or
- The Covaris® S2 System (see "Shear the DNA with the Covaris® S2 System" on page 16.

Shear the DNA with the Covaris® S220 System

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

1. Dilute the components below in a 1.5-mL LoBind Tube. Shear Buffer reduces DNA damage from sonication:

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

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

      The water should cover the visible glass part of the tube.
   b. Set the chiller temperature to 2–5 °C to ensure that the temperature reading in the water bath displays 5°C.

The circulated water chiller should be supplemented with 20% ethylene glycol.

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

IMPORTANT! Do not introduce a bubble into the bottom of the tube.

Note: To load and unload the Covaris® microTUBE correctly from the microTUBE holder, see "Load and unload Covaris® microTUBE vials from the Covaris® microTUBE holder" on page 61.

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

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

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

5. Remove the sheared DNA:
   a. Place the Covaris® microTUBE into the loading station.
   b. With the snap-cap on, insert a pipette tip through the pre-split septa, then slowly remove the sheared DNA.
   c. Transfer the sheared DNA into a new 1.5-mL LoBind tube.

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. Dilute the desired amount of DNA to 100 µL in 1X Low TE Buffer in a LoBind tube:

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

3. Load the DNA into the Covaris® S2 System:
   a. Place a Covaris® microTUBE into the loading station.
   b. Keeping the snap-cap on the tube, use a tapered pipette tip to slowly transfer the 100 µL of DNA sample through the pre-split septa.

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

      To load and unload the Covaris® microTUBE correctly from the microTUBE holder, see "Load and unload Covaris® microTUBE vials from the Covaris® microTUBE holder" on page 61.

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

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

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

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

End-polish the DNA

End Polishing E1 and E2 enzymes convert DNA with incompatible 5′ -protruding and/or 3′ -protruding ends to blunt-ended, 5′ -phosphorylated DNA.

The end polishing process converts DNA with overhangs to blunt-ended DNA by exploiting the 5′ -to-3′ polymerase and the 3′ -to-5′ exonuclease activities of the enzymes used in the procedure below. A kinase phosphorylates the 5′ ends of the DNA.

1. Combine in a new 1.5-mL LoBind Tube:

Component	Amount
Sheared DNA	120 µL
5✕ Reaction Buffer	40 µL
10 mM dNTP	8.0 µL
End Polishing E1	8.0 µL
End Polishing E2	10 µL
Nuclease-Free Water	14 µL
Total	**200 µL**

2. Vortex the reaction for 5 seconds, then pulse-spin.
3. Incubate the mixture at room temperature (20–25°C) for 30 minutes.

Size-select the DNA by Agencourt AMPure® XP Reagent

Use Agencourt AMPure® XP Reagent (purchased separately) to size-select the library with magnetic beads. The first incubation with the AMPure® XP beads selectively captures DNA >250 bp on the beads, and DNA ≤250 bp is retained in the supernatant. The second incubation with the retained supernatant and new beads selectively captures DNA >100 bp in the beads. Therefore, the retained beads contain DNA between 100–250 bp.

Bead-based size selection provides, on average, higher yields over gel-based size selection. The first size selection with Agencourt AMPure® XP reagent removes the longest DNA in the pellet. The second size selection with Agencourt® AMPure® XP reagent removes the shortest DNA in the supernatant.

1. Resuspend the Agencourt AMPure® XP Reagent beads and allow the mixture to come to room temperature.

2. Prepare 70% ethanol:

Component	Volume
Nuclease-Free Water	300 µL
Ethanol, Absolute	700 µL
Total	**1000 µL**

3. Size-select the DNA with Agencourt AMPure® XP Reagent:
   a. Combine sheared DNA and resuspended, ambient Agencourt AMPure® XP Reagent in a 1.5-mL LoBind Tube:

Component	Volume
End-polished DNA	200 µL
Agencourt AMPure® XP Reagent	100 µL†
Total	**300 µL**

† Equal to 0.5 volume of end-polished DNA.

   b. Vortex the beads for 10 seconds, then pulse-spin.
   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.
   d. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears. The solution is clear of brown tint when viewed at an angle, as shown below:

4. Without disturbing the pellet, carefully transfer the ***supernatant***, which contains the DNA of the desired size, to a new 1.5-mL LoBind Tube. Discard the ***pellet***.

5. Bind the size-selected DNA in the supernatant to the Agencourt AMPure® XP Reagent:
   a. Combine in a 1.5-mL LoBind Tube:

Component	Volume
Supernatant	~300 µL
Agencourt AMPure® XP Reagent	60 µL†
Total	**~360 µL**

† Equal to 0.3 volume of the end-polish reaction volume of 200 µL.

   b. Vortex the beads for 10 seconds, then pulse-spin.
   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.
   d. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the ***supernatant***. Save the ***pellet***, which contains the DNA.
6. Wash the DNA-bead complex ***3*** times. For each wash:
   a. Add 200 µL of *freshly prepared* 70% ethanol to the tube, mix by inverting the tube a few times, then pulse-spin.
   b. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
7. Remove the tube from the DynaMag™-2 magnetic rack, pulse-spin the tube, return the tube to the magnetic rack, then remove and discard the supernatant with a 20-µL pipettor.
8. Open the tube, then dry the beads at room temperature (20–25°C) for 5–10 minutes.
9. Elute the DNA:
   a. Remove the tube from the DynaMag™-2 magnetic rack, then add 36 µL Low TE Buffer directly to the pellet to disperse the beads.
   b. Pipette the suspension up and down to mix.
   c. Vortex the beads for 10 seconds, then pulse-spin.
   d. Place the tube in a magnetic rack for at least 1 minute until the solution clears.
   e. Transfer the *supernatant* containing the size-selected DNA to a new 1.5-mL LoBind Tube.

STOPPING POINT Store the purified DNA in Low TE Buffer at 4 ºC, or proceed directly to "Quantitate the size-selected DNA" on page 21.

Quantitate the size-selected DNA

Measure the DNA concentration using:

- 2 µL of sample with the Qubit™ dsDNA HS Assay Kit (Invitrogen Part no. Q32851) and the Qubit® 2.0 Fluorometer (Invitrogen Part no. Q32866)

  or

- 2 µL of sample in the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 62)

  or

- 1 µL of sample in the Agilent Technologies 2100 Bioanalyzer™.

IMPORTANT! The average yield of size-selected DNA is 30% of input quantity. If the yield is substantially <20%, troubleshoot the low yield, then repeat the procedure from "Shear the DNA" on page 15.

STOPPING POINT Store the purified DNA in Low TE Buffer at 4 ºC, or proceed directly to "Add a dA-tail to the size-selected DNA".

Add a dA-tail to the size-selected DNA

A thermostable polymerase adds non-templated dA to the 3′ ends of the DNA. The thermostable polymerase lacks 3′ –5′ exonuclease activity at higher temperatures.

1. Combine in a 1.5-mL LoBind Tube:

Component	Amount
Size-selected DNA	34 µL
5✕ Reaction Buffer	10 µL
10 mM dATP	1.0 µL
A-Tailing Enzyme I	5.0 µL
Total	**50 µL**

2. Incubate the mixture at 68°C for 30 minutes, then cool to room temperature.

Note: While the reaction is incubating, calculate the amount of adaptors needed for ligation (see "Calculate the amount of adaptor to use for ligation" on page 22).

Ligate adaptors to the DNA

Calculate the amount of adaptor to use for ligation

If the *input* DNA before shearing is...

- <100 ng: Use 0.06 µL of each adaptor or an equivalent amount of adaptor after dilution. For example, use 0.6 µL of a 10-fold dilution of an adaptor or 1.2 µL of a 20-fold dilution.
- ≥100 ng: Calculate the amount of adaptor needed, Y, for the reaction based on the amount of DNA from the last purification step. If DNA fragments were sheared using the standard protocol for fragment library preparation, the average insert size should be approximately 165 bp before adaptor ligation, as shown in the calculation and example below:

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

$$\textbf{\textit{Y}} \text{ µL adaptor needed} = \text{\# µg DNA} \times \text{(µg-to-pmol conversion factor)} \times 10 \times \frac{\text{1 µL adaptor needed}}{\text{50 pmol}}$$

Example

For 1 µg of purified end-repaired DNA with an average insert size of 165 bp and 30% yield after size selection (0.3 µg of size-selected DNA):

$$\text{µg-to-pmol conversion factor} = \frac{10^6 \text{ pg}}{1 \text{ µg}} \times \frac{1 \text{ pmol}}{660 \text{ pg}} \times \frac{1}{165} = 9.2 \text{ pmol/µg DNA}$$

$$\textbf{\textit{Y}} \text{ µL adaptor needed} = 0.3 \text{ µg DNA} \times 9.2 \text{ pmol/µg DNA} \times 10 \times \frac{\text{1 µL adaptor needed}}{\text{50 pmol}}$$

$$= 0.55 \text{ µL adaptor needed}$$

Ligate adaptors to the DNA

IMPORTANT! Do not use P1 and P2 Adaptors that are designed for fragment library preparation and sequencing on the SOLiD™ 4 System. These adaptors are *not* compatible with reverse-read sequencing on the 5500 Series SOLiD™ Sequencers. Use only P1-T and Barcode-T-0XX Adaptors that are designed for the 5500 Series SOLiD™ Sequencers.

1. In a new 1.5-mL LoBind Tube, combine for a ligation master mix:

Component	Volume
5X Reaction Buffer	3.0 µL
P1-T Adaptor, 50 µM	*Y µL*
Barcode-T-0XX, 50 µM†	*Y µL*
T4 DNA Ligase, 5 U/µL	6.5 µL
10 mM dNTP	1.2 µL
Nuclease-free Water	Variable µL
Total	**15 µL**

† If 5500 SOLiD™ Fragment Library Standard Adaptors are used, Barcode-T-0XX is Barcode-T-001.

2. Add the entire 15 µL of ligation master mix from step 1 above to the reaction mixture from "Add a dA-tail to the size-selected DNA" on page 21 for a total of 61 µL total volume.
3. Vortex the reaction for 5 seconds, then pulse-spin.
4. Incubate the reaction in a thermocycler with the lid heater *on*:

IMPORTANT! Incubation nick-translates the DNA.

Stage	Temp	Time
Holding	20°C	30 min
Holding	72°C	20 min
Holding	4°C	∞

Purify the DNA

If you want to...	Then...
Purify the ligated DNA quickly with high yield	Proceed to "Purify the DNA using Agencourt AMPure® XP Reagent". Use 39 µL of Agencourt AMPure® XP Reagent with 65 µL of ligated DNA, equal to 0.6× of Agencourt AMPure® XP Reagent per sample volume.
Purify the ligated DNA with a column for convenience	Proceed to "Purify the DNA with the SOLiD™ Library Micro Column Purification Kit" on page 25.

Purify the DNA using Agencourt AMPure® XP Reagent

1. Resuspend the Agencourt AMPure® XP Reagent beads, and allow the mixture to come to room temperature.
2. Prepare 70% ethanol:

Component	Volume
Nuclease-Free Water	300 µL
Ethanol, Absolute	700 µL
Total	**1000 µL**

3. Bind the DNA to the resuspended, ambient Agencourt AMPure® XP Reagent:
   a. Prepare the bead suspension in the sample reaction:

Component	Volume
Ligation reaction	65 µL
AMPure® XP Reagent	39 µL†

† Equal to 0.6 volumes of sample reaction.

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

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

d. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle; then, carefully remove and discard the supernatant:

4. Wash the DNA *3* times. For each wash:

   a. Add 200 µL of *freshly prepared* 70% ethanol to the tube, mix by inverting the tube a few times, 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.

5. 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.

6. Open the tube, then dry the beads at room temperature (20–25°C) for 5–10 minutes.

7. Elute the DNA:

   a. Remove the tube from the DynaMag™-2 magnetic rack, then add 22 µL Low TE Buffer directly to the pellet to disperse the beads.

   b. Pipette the suspension up and down to mix.

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

   d. Place the tube in the magnetic rack for at least 1 minute until the solution clears.

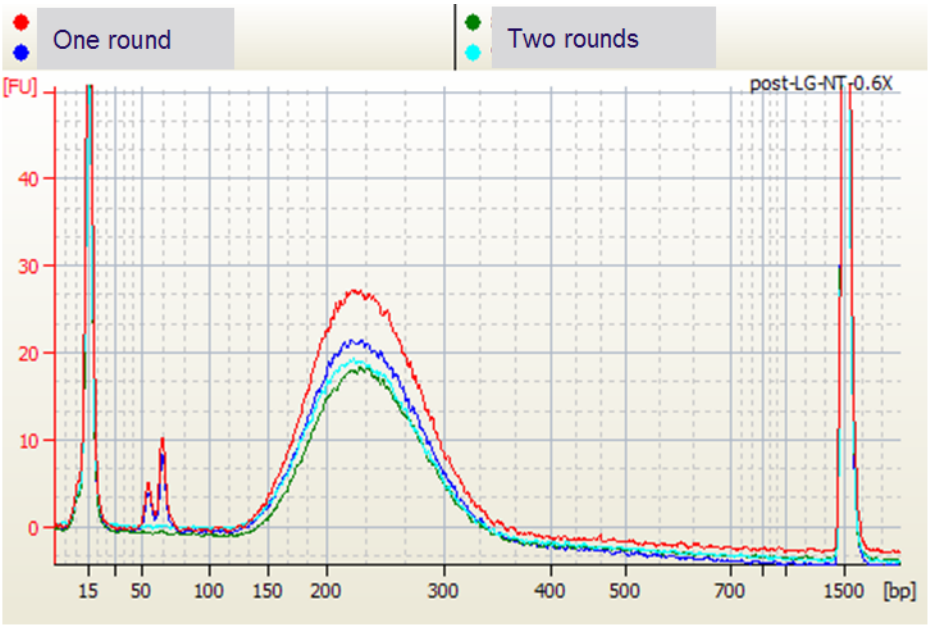
   e. Transfer the *supernatant* containing the size-selected DNA to a new 1.5-mL LoBind Tube.

   STOPPING POINT Store the purified DNA in Low TE Buffer at 4 ºC.

8. Proceed as follows:

If you want to...	Then...
Further remove residual adaptors the DNA	Repeat "Purify the DNA using Agencourt AMPure® XP Reagent" on page 23. Use 30 µL of Agencourt AMPure® XP Reagent with 20 µL of bead-purified sample, equal to 1.5× of Agencourt AMPure® XP Reagent per sample volume (see figure below).
Quantitate the DNA without additional purification	Proceed to "Quantitate the ligated DNA" on page 26

A second purification of the ligated DNA with the Agencourt AMPure® XP Reagent substantially removes unligated adaptors:



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

1. Pre-spin an empty PureLink® Micro column in a collection tube at 10,000 × g for 1 minute before use.
2. If not already prepared, prepare the Binding and Wash Buffers:
   a. Add sufficient 100% isopropanol to Binding Buffer B2-L to prepare Binding Buffer B2-L with 40% isopropanol.
   b. Add sufficient 100% ethanol to the Wash Buffer (W1) to prepare Wash Buffer (W1) with 80% ethanol.
3. To 1 volume (65 µL) of ligation reaction, add 4 volumes (260 µL) of Binding Buffer (B2-L) with isopropanol (40%). Mix well.
4. Load the DNA onto the PureLink® Micro column:
   a. Apply all of the sample from step 3 to the PureLink® Micro column in a 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 sample has been loaded onto the column.
5. Wash the column:
   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 for 1 minute at room temperature to remove residual wash buffer and dry the silica membrane, then discard the flow-through and collection tube.

6. Elute the DNA:
   a. Transfer the column to a clean Elution Tube.
   b. Add 22 µL of Elution Buffer (E1) to the center of the column to elute the DNA, then let the column stand for 1 minute at room temperature.
   c. Spin the column at 14,000 × g for 1 minute at room temperature.
7. (*Optional*) To potentially improve recovery of DNA:
   a. Add the eluate from the last spin back to the column, then let the column stand for 1 minute.
   b. 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 ºC, or proceed directly to "Quantitate the ligated DNA".

Quantitate the ligated DNA

Measure the DNA concentration using:

- 2 µL of sample with the Qubit™ dsDNA HS Assay Kit (Invitrogen Part no. Q32851) and the Qubit® 2.0 Fluorometer (Invitrogen Part no. Q32866)

  or
- 2 µL of sample in the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 62)

  or
- 1 µL of sample in the Agilent Technologies 2100 Bioanalyzer™

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

STOPPING POINT Store the purified DNA in its current buffer at 4 ºC, or proceed directly to "(Optional) Amplify the library".

(*Optional*) Amplify the library

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

Amplify the library

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

1. In a 0.2-mL PCR tube, prepare the PCR mixture:

Component	Volume
Adaptor-ligated, purified DNA	20 µL†
Platinum® PCR Amplification Mix	100 µL
Library PCR Primer 1, 50 µM	2.5 µL
Library PCR Primer 2, 50 µM	2.5 µL
Total	**125 µL**

† <20 µL is acceptable. Do not adjust the PCR volume.

2. Vortex the reaction for 5 seconds, then pulse-spin.
3. Determine the number of PCR cycles:

Starting amount of DNA	Number of cycles
10–100 ng	10 cycles
100 ng–1 µg	6 to 8 cycles
1–2 µg	4 to 6 cycles
2–5 µg	0 to 3 cycles

4. Run the PCR:

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

Purify the DNA

1. Resuspend the Agencourt AMPure® XP Reagent beads, and allow the mixture to come to room temperature.

2. Prepare 70% ethanol:

Component	Volume
Nuclease-Free Water	300 µL
Ethanol, Absolute	700 µL
Total	**1000 µL**

3. Transfer the PCR reaction from step 4 in "Amplify the library" to a new 1.5-mL LoBind Tube.

4. Bind the DNA to the Agencourt AMPure® XP Reagent:

   a. Prepare the bead suspension in the sample reaction:

Component	Volume
Amplified library	125 µL
Agencourt AMPure® XP Reagent	187.5 µL†

† Equal to 1.5 volumes of sample reaction.

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

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

   d. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle; then, remove and discard the supernatant.

5. Wash the DNA *3* times. For each wash:

   a. Add 200 µL of *freshly prepared* 70% ethanol to the tube, mix by inverting the tube a few times, 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.

6. 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.

7. Open the tube, then dry the beads at room temperature (20–25°C) for 5–10 minutes.

8. Elute the DNA:

   a. Remove the tube from the DynaMag™-2 magnetic rack, then add 30 µL Low TE Buffer directly to the pellet to disperse the beads.

   b. Pipette the suspension up and down to mix.

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

   d. Place the tube in a magnetic rack for at least 1 minute until the solution clears.

   e. Transfer the *supernatant* containing the size-selected DNA to a new 1.5-mL LoBind Tube.

Quantitate the DNA

Measure the DNA concentration by using:

- 2 µL of sample with the Qubit™ dsDNA HS Assay Kit (Invitrogen Part no. Q32851) and the Qubit® 2.0 Fluorometer (Invitrogen Part no. Q32866)

  or

- 2 µL of sample in the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 62)

  or

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

  and/or

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

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

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.

Troubleshooting

See "Troubleshooting" on page 51.

Prepare Multiple Fragment Libraries

Follow this chapter if you are preparing libraries by the non-automated method:

- Pooled, barcoded libraries for multiplexed sequencing

  or

- Multiple libraries in parallel that are not pooled.

For an overview of library types that can be sequenced on the 5500 Series SOLiD™ Sequencers, see "Choosing the appropriate library type" on page 67. For a graphical overview of fragment library preparation, see "Overview" on page 67.

IMPORTANT! Customers who have purchased the AB Library Builder™ System and who wish to prepare a fragment library with an automated system, refer to the *Fragment Library Preparation Using the AB Library Builder™ System: 5500 Series SOLiD™ Systems User Guide* (Part no. 4460965)

Customers who have access to automated liquid-handling systems such as the Beckman Coulter Biomek® FXP and Tecan Freedom EVO® instruments can use the SOLiD 48-library core kits. For more information, contact your local representative.

Workflow

To prepare 6–12 fragment libraries, it takes ~5–6 h without amplification and ~6–8 h with amplification:

Quantitate the DNA (page 33)

↓

Shear the DNA (page 33)

↓

End-polish the DNA (page 36)

↓

Size-select the DNA by Agencourt AMPure® XP Reagent (page 37)

Stopping point

↓

Quantitate the size-selected DNA (page 39)

Stopping point

Add a dA-tail to the size-selected DNA (page 40)

Ligate adaptors to the DNA

Calculate the amount of adaptor to use for ligation (page 41)

Ligate adaptors to the DNA (page 41)

Purify the DNA (page 42)

Stopping point

Quantitate the ligated DNA (page 45)

Stopping point

(Optional) Amplify the libraries

Amplify the libraries (page 46)

Purify the DNA (page 47)

Stopping point

Quantitate the DNA (page 48)

Stopping point

Check the size distribution of the libraries (page 48)

Stopping point

(Optional) Pool equal molar barcoded libraries (page 49)

Stopping point

Procedural guidelines

- The protocol is designed for 10 ng–5 µg of genomic DNA or ligated PCR product.
- If you are trying to construct a targeted resequencing library with small-sized PCR products (≤500 bp), then you must first perform a PCR-product ligation step to concatenate the DNA. For a concatenation protocol, contact your field application specialist.
- Use good laboratory practices (change gloves frequently) to minimize cross-contamination of products.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols. Applied Biosystems recommends the Eppendorf 5417R tabletop microcentrifuge.
- Perform all steps requiring 0.5-mL and 1.5-mL tubes with Eppendorf LoBind Tubes.
- Thaw Shear Buffer at *room temperature* just before use.
- Thaw all other reagents on *ice* just before use.

Quantitate the DNA

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

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

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

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

Shear the DNA

This step involves sonicating the input DNA into small fragments with a mean fragment size of 160 bp and a fragment size range of 100–250 bp (before adaptor ligation) using the Covaris® System. The conditions have been tested for shearing 10 ng–5 µg DNA in a total volume of 120 µL. For certain DNA samples, optimizing the shearing protocol may be necessary.

You can shear the DNA with two supported shearing systems:

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

  or
- The Covaris® S2 System (see "Shear the DNA with the Covaris® S2 System" on page 35.

Shear the DNA with the Covaris® S220 System

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

1. Dilute the components below in a 1.5-mL LoBind Tube. Shear Buffer reduces DNA damage from sonication:

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

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

      The water should cover the visible glass part of the tube.
   b. Set the chiller temperature to 2–5 °C to ensure that the temperature reading in the water bath displays 5°C.

      The circulated water chiller should be supplemented with 20% ethylene glycol.
3. Load the DNA:
   a. Place a Covaris® microTUBE into the loading station.
   b. With the snap-cap on the tube, use a tapered pipette tip to slowly transfer the 120 µL of DNA sample through the pre-split septa.

      IMPORTANT! Do not introduce a bubble into the bottom of the tube.

      Note: To load and unload the Covaris® microTUBE correctly from the microTUBE holder, see "Load and unload Covaris® microTUBE vials from the Covaris® microTUBE holder" on page 61.

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

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

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

5. Remove the sheared DNA:
   a. Place the Covaris® microTUBE into the loading station.
   b. With the snap-cap on, insert a pipette tip through the pre-split septa, then slowly remove the sheared DNA.
   c. Transfer the sheared DNA into a new 1.5-mL LoBind tube.

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. Dilute the desired amount of DNA to 100 µL in 1× Low TE Buffer in a LoBind tube:

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

3. Load the DNA into the Covaris® S2 System:
   a. Place a Covaris® microTUBE into the loading station.

b. Keeping the snap-cap on the tube, use a tapered pipette tip to slowly transfer the 100 µL of DNA sample through the pre-split septa.

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

   To load and unload the Covaris® microTUBE correctly from the microTUBE holder, see "Load and unload Covaris® microTUBE vials from the Covaris® microTUBE holder" on page 61.

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

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

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

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

End-polish the DNA

End Polishing E1 and E2 enzymes convert DNA with incompatible 5′ -protruding and/or 3′ -protruding ends to blunt-ended 5′ -phosphorylated, blunt-ended DNA.

The end polishing process converts DNA with overhangs to blunt-ended DNA by exploiting the 5′ -to-3′ polymerase and the 3′ -to-5′ exonuclease activities of the enzymes used in the procedure below. A kinase phosphorylates the 5′ ends of the DNA.

1. In a new 1.5-mL LoBind Tube, combine to prepare the end-polishing master mix:

Component	Amount per library	Master mix for *N* libraries
5× Reaction Buffer	40 µL	40 µL × (1.1 × *N*)
10 mM dNTP	8.0 µL	8.0 µL × (1.1 × *N*)
End Polishing E1	8.0 µL	8.0 µL × (1.1 × *N*)
End Polishing E2	10 µL	10 µL × (1.1 × *N*)
Nuclease-Free Water	14 µL	14 µL × (1.1 × *N*)
Total	**80 µL**	**80 µL × (1.1 × *N*)**

2. Label 1.5-mL LoBind Tubes, one tube for each library.
3. Transfer 120 µL of each sheared DNA to the appropriately labeled tube.
4. Pipette 80 µL of the master mix from step 1 into each labeled tube. While pipetting the master mix, take care not to cross-contaminate libraries.
5. Vortex each reaction for 5 seconds, then pulse-spin.
6. Incubate each reaction at room temperature (20–25°C) for 30 minutes.

Size-select the DNA by Agencourt AMPure® XP Reagent

Use Agencourt AMPure® XP Reagent (purchased separately) to size-select the library with magnetic beads. The first incubation with the AMPure® XP beads selectively captures DNA >250 bp on the beads, and DNA ≤250 bp is retained in the supernatant. The second incubation with the retained supernatant and new beads selectively captures DNA >100 bp in the beads. Therefore, the retained beads contain DNA between 100–250 bp.

Bead-based size selection provides, on average, higher yields over gel-based size selection. The first size selection with Agencourt AMPure® XP reagent removes the longest DNA in the pellet. The second size selection with Agencourt AMPure® XP reagent removes the shortest DNA in the supernatant.

Follow the protocol for each library to size-select ≤12 libraries. Follow the manufacturer's instructions for handling the beads.

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

Component	Volume
Nuclease-Free Water	300 µL × *N*
Ethanol, Absolute	700 µL × *N*
Total	**1000 µL × *N***

3. Size-select the DNA with resuspended, ambient Agencourt AMPure® XP Reagent:
   a. Combine sheared DNA and Agencourt AMPure® XP Reagent:

Component	Volume
End-polished DNA	200 µL
Agencourt AMPure® XP Reagent	100 µL†
Total	**300 µL**

† Equal to 0.5 volume of end-polished DNA.

   b. Vortex the beads for 10 seconds, then pulse-spin.
   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.
   d. Place the tube in a DynaMag™-2 Magnetic Rack for at least 1 minute until the solution clears. The solution is clear of brown tint when viewed at an angle, as shown below:

4. Without disturbing the pellet, carefully transfer the ***supernatant*** of each library, which contains the DNA of the desired size, to a new 1.5-mL LoBind Tube, labelled with the library name. Discard the ***pellet***.
5. Bind the size-selected DNA in the supernatant to the Agencourt AMPure® XP Reagent:
   a. Combine:

Component	Volume
Supernatant	~300 µL
Agencourt AMPure® XP Reagent	60 µL†
Total	**~360 µL**

† Equal to 0.3 volume of the end-polish reaction volume of 200 µL.

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

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

d. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the ***supernatant***. Save the ***pellet***, which contains the DNA.

6. Wash the DNA-bead complex *3* times. For each wash:

   a. Add 200 µL of *freshly prepared* 70% ethanol to the tube, mix by inverting the tube a few times, 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.

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

8. Open the tube, then dry the beads at room temperature (20–25°C) for 5–10 minutes.

9. Elute the DNA:

   a. Remove the tube from the DynaMag™-2 magnetic rack, then add 36 µL Low TE Buffer directly to the pellet to disperse the beads.

   b. Pipette the suspension up and down to mix.

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

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

   e. Transfer the *supernatant* containing the size-selected DNA to a new 1.5-mL LoBind Tube.

STOPPING POINT Store the purified DNA in Low TE Buffer at 4 ºC, or proceed directly to "Quantitate the size-selected DNA".

Quantitate the size-selected DNA

Measure the DNA concentration of each library using:

- 2 µL of sample with the Qubit™ dsDNA HS Assay Kit (Invitrogen Part no. Q32851) and the Qubit® 2.0 Fluorometer (Invitrogen Part no. Q32866)

  or

- 2 µL of sample in the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 62)

  or

- 1 µL of sample in the Agilent Technologies 2100 Bioanalyzer™

IMPORTANT! The average yield of size-selected DNA is 30% of input quantity. If the yield is substantially <20%, troubleshoot the low yield, then repeat the procedure from "Shear the DNA" on page 33.

STOPPING POINT Store the purified DNA in Low TE Buffer at 4 ºC, or proceed directly to "Add a dA-tail to the size-selected DNA".

Add a dA-tail to the size-selected DNA

A thermostable polymerase adds non-templated dA to the 3′ ends of the DNA. The thermostable polymerase lacks 3′ –5′ exonuclease activity at higher temperatures.

1. In a new 1.5-mL LoBind Tube, combine to prepare the master mix to add an A tail to each library:

Component	Amount per library	Master mix for *N* libraries
5X Reaction Buffer	10 µL	10 µL × (1.1 × *N*)
10 mM dATP	1.0 µL	1.0 µL × (1.1 × *N*)
A-Tailing Enzyme I	5.0 µL	5.0 µL × (1.1 × *N*)
Total	**16 µL**	**16 µL × (1.1 × *N*)**

2. Label 1.5-mL LoBind Tubes, one tube for each library.
3. Transfer 34 µL of each size-selected DNA to the appropriately labeled tube.
4. Pipette 16 µL of the master mix from step 1 into each labeled tube. While pipetting the master mix, take care not to cross-contaminate libraries.
5. Incubate each reaction at 68°C for 30 minutes, then cool to room temperature.

Note: While the reaction is incubating, calculate the amount of adaptors needed for ligation (see "Calculate the amount of adaptor to use for ligation" on page 41).

Ligate adaptors to the DNA

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

Use the barcodes according to these conditions:

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

Calculate the amount of adaptor to use for ligation

If the *input* DNA before shearing is...

- <100 ng: Use 0.06 µL of each adaptor or an equivalent amount of adaptor after dilution. For example, use 0.6 µL of a 10-fold dilution of an adaptor or 1.2 µL of a 20-fold dilution.
- ≥100 ng: Calculate the amount of adaptor needed, Y, for the reaction based on the amount of DNA from the last purification step. If DNA fragments were sheared using the standard protocol for fragment library preparation, the average insert size should be approximately 165 bp before adaptor ligation, as shown in the calculation and example below:

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

$$\textbf{\textit{Y}} \text{ µL adaptor needed} = \text{\# µg DNA} \times (\text{µg-to-pmol conversion factor}) \times 10 \times \frac{1 \text{ µL adaptor needed}}{50 \text{ pmol}}$$

Example

For 1 µg of purified end-repaired DNA with an average insert size of 165 bp and 30% yield after size selection (0.3 µg of size-selected DNA):

$$\text{µg-to-pmol conversion factor} = \frac{10^6 \text{ pg}}{1 \text{ µg}} \times \frac{1 \text{ pmol}}{660 \text{ pg}} \times \frac{1}{165} = 9.2 \text{ pmol/µg DNA}$$

$$\textbf{\textit{Y}} \text{ µL adaptor needed} = 0.3 \text{ µg DNA} \times 9.2 \text{ pmol/µg DNA} \times 10 \times \frac{1 \text{ µL adaptor needed}}{50 \text{ pmol}}$$

$$= 0.55 \text{ µL adaptor needed}$$

Ligate adaptors to the DNA

IMPORTANT! Do not use P1 and P2 Adaptors that are designed for fragment library preparation and sequencing on the SOLiD™ 4 System. These adaptors are *not* compatible with reverse-read sequencing on the 5500 Series SOLiD™ Sequencers. Use only P1-T and Barcode-T-0XX Adaptors that are designed for the 5500 Series SOLiD™ Sequencers.

1. In a new 1.5-mL LoBind Tube, combine for a ligation master mix:

Component	Volume per library	Master mix for *N* libraries
5✕ Reaction Buffer	3.0 µL	3.0 µL × (1.1 × *N*)
P1-T Adaptor, 50 µM	*Y µL*	*Y* µL × (1.1 × *N*)
Barcode-T-0XX, 50 µM	*Y µL*	*Y* µL × (1.1 × *N*)
T4 DNA Ligase, 5 U/µL	6.5 µL	6.5 µL × (1.1 × *N*)
10 mM dNTP	1.2 µL	1.2 µL × (1.1 × *N*)
Nuclease-free Water	Variable	Variable
Total	**15 µL**	**15 µL × (1.1 × *N*)**

2. Add 15 µL of the ligation master mix from step 1 above to the reaction mixture from "Add a dA-tail to the size-selected DNA" on page 40 for a total of 61 µL total volume in each tube.

3. Vortex each reaction for 5 seconds, then pulse-spin.
4. Incubate each reaction in a thermocycler with the lid heater *on*:

IMPORTANT! Incubation nick-translates the DNA.

Stage	Temp	Time
Holding	20°C	30 min
Holding	72°C	20 min
Holding	4°C	∞

Purify the DNA

If you want to...	Then...
Purify the ligated DNA quickly with high yield	Proceed to "Purify the DNA using Agencourt AMPure® XP Reagent". Use 39 µL of Agencourt AMPure® XP Reagent with 65 µL of ligated DNA, equal to 0.6✕ of Agencourt AMPure® XP Reagent per sample volume.
Purify the ligated DNA with a column for convenience	Proceed to "Purify the DNA with the SOLiD™ Library Micro Column Purification Kit" on page 44

Purify the DNA using Agencourt AMPure® XP Reagent

1. Resuspend the Agencourt AMPure® XP Reagent beads, and allow the mixture to come to room temperature.
2. Prepare 70% ethanol:

Component	Volume
Nuclease-Free Water	300 µL
Ethanol, Absolute	700 µL
Total	**1000 µL**

3. Bind the DNA to the resuspended, ambient Agencourt AMPure® XP Reagent:
   a. Prepare the bead suspension in the sample reaction:

Component	Volume
Ligation reaction	65 µL
AMPure® XP Reagent	39 µL†

† Equal to 0.6 volumes of sample reaction.

   b. Vortex the beads for 10 seconds, then pulse-spin.
   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.

d. Place the tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle; then, carefully remove and discard the supernatant.

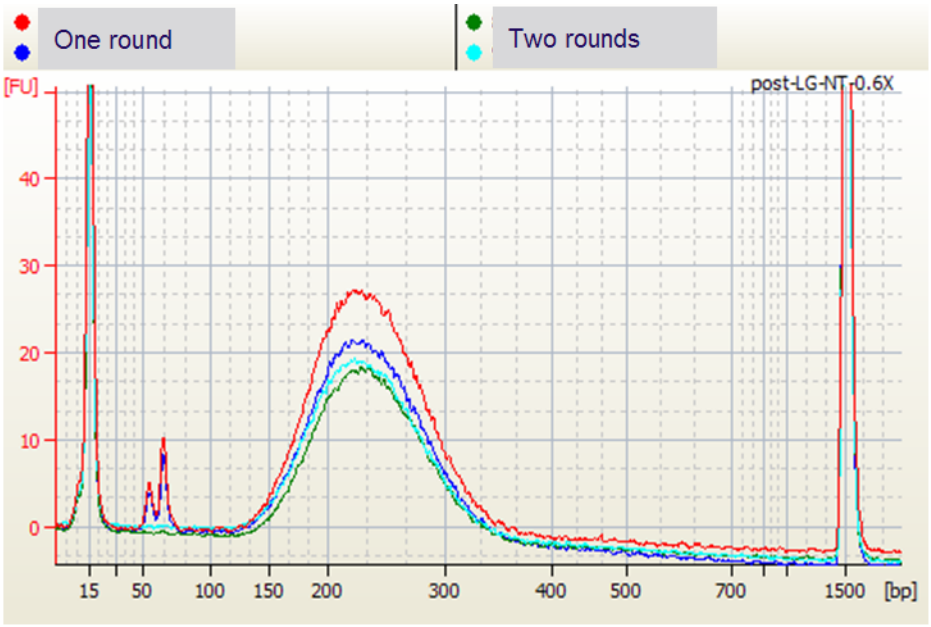
4. Wash the DNA *3* times. For each wash:
   a. Add 200 µL of *freshly prepared* 70% ethanol to the tube, mix by inverting the tube a few times, then pulse-spin.
   b. Place the tube in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
5. 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.
6. Open the tube, then dry the beads at room temperature (20–25°C) for 5–10 minutes.
7. Elute the DNA:
   a. Remove the tube from the DynaMag™-2 magnetic rack, then add 22 µL Low TE Buffer directly to the pellet to disperse the beads.
   b. Pipette the suspension up and down to mix.
   c. Vortex the beads for 10 seconds, then pulse-spin.
   d. Place the tube in the magnetic rack for at least 1 minute until the solution clears.
   e. Transfer the *supernatant* containing the size-selected DNA to a new 1.5-mL LoBind Tube.

   STOPPING POINT Store the purified DNA in Low TE Buffer at 4 ºC.

8. Proceed as follows:

If you want to...	Then...
Further remove residual adaptors from the DNA	Repeat "Purify the DNA using Agencourt AMPure® XP Reagent" on page 42. Use 30 µL of Agencourt AMPure® XP Reagent with 20 µL of bead-purified sample, equal to 1.5X of Agencourt AMPure® XP Reagent per sample volume (see figure below).
Quantitate the DNA without additional purification	Proceed to "Quantitate the ligated DNA" on page 45

A second purification of the ligated DNA with the Agencourt AMPure® XP Reagent substantially removes unligated adaptors:



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

1. Pre-spin an empty PureLink® Micro column in a collection tube at 10,000 × g for 1 minute before use.
2. If not already prepared, prepare the Binding and Wash Buffers:
   a. Add sufficient 100% isopropanol to Binding Buffer B2-L to prepare Binding Buffer B2-L with 40% isopropanol.
   b. Add sufficient 100% ethanol to the Wash Buffer (W1) to prepare Wash Buffer (W1) with 80% ethanol.
3. To 1 volume (65 µL) of ligation reaction, add 4 volumes (260 µL) of Binding Buffer (B2-L) with isopropanol (40%). Mix well.
4. Load the DNA onto the PureLink® Micro column:
   a. Apply all of the sample from step 3 to the PureLink® Micro column in a 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 sample has been loaded onto the column.
5. Wash the column:
   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 for 1 minute at room temperature to remove residual wash buffer and dry the silica membrane, then discard the flow-through and collection tube.

6. Elute the DNA:
   a. Transfer the column to a clean Elution Tube.
   b. Add 22 µL of Elution Buffer (E1) to the center of the column to elute the DNA, then let the column stand for 1 minute at room temperature.
   c. Spin the column at 14,000 × g for 1 minute at room temperature.
7. (*Optional*) To potentially improve recovery of DNA:
   a. Add the eluate from the last spin back to the column, then let the column stand for 1 minute.
   b. 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 ºC, or proceed directly to "Quantitate the ligated DNA".

Quantitate the ligated DNA

Measure the DNA concentration by using:

- 2 µL of sample with the Qubit™ dsDNA HS Assay Kit (Invitrogen Part no. Q32851) and the Qubit® 2.0 Fluorometer (Invitrogen Part no. Q32866)

  or
- 2 µL of sample in the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 62)

  or
- 1 µL of sample in the Agilent Technologies 2100 Bioanalyzer™

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

STOPPING POINT Store the purified DNA in its current buffer at 4 ºC, or proceed directly to "(Optional) Amplify the libraries".

(*Optional*) Amplify the libraries

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

Amplify the libraries

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

1. Transfer 20.0 µL of each purified library from "Purify the DNA" on page 42 to a labelled 0.2-mL PCR tube. Less than 20 µL volume is acceptable. Do not adjust the volume to 20µL.
2. In a new 1.5-mL LoBind Tube, combine for a PCR master mix:

Component	Volume per amplification	Master mix for *N* libraries
Platinum® PCR Amplification Mix	100 µL	100 µL × (1.1 × *N*)
Library PCR Primer 1, 50 µM	2.5 µL	2.5 µL × (1.1 × *N*)
Library PCR Primer 2, 50 µM	2.5 µL	2.5 µL × (1.1 × *N*)
Total	**105 µL**	**105 µL × (1.1 × *N*)**

3. Pipette 105 µL of the master mix from step 2 into each labelled tube. While pipetting the master mix, take care not to cross-contaminate libraries.
4. Vortex the reaction for 5 seconds, then pulse-spin.
5. Determine the number of PCR cycles:

Starting amount of DNA	Number of cycles
10–100 ng	10 cycles
100 ng–1 µg	6–8 cycles
1–2 µg	4–6 cycles
2–5 µg	0–3 cycles

6. Run the PCR:

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

Purify the DNA

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

Component	Volume
Nuclease-Free Water	300 µL × *N*
Ethanol, Absolute	700 µL × *N*
Total	**1000 µL × *N***

3. For every amplified library, label a new 1.5-ml LoBind Tube.
4. Transfer each PCR reaction (125 µL) from "(Optional) Amplify the libraries" on page 45 to the appropriately labeled 1.5-mL LoBind Tube.
5. Bind the DNA to the resuspended, ambient Agencourt AMPure® XP Reagent:
   a. For each library, prepare the bead suspension:

Component	Volume
Amplified library	125 µL
Agencourt AMPure® XP Reagent	187.5 µL†

† Equal to 1.5 volumes of sample reaction.

   b. Vortex the beads for 10 seconds, then pulse-spin.
   c. Incubate the mixture at room temperature (20–25°C) for 5 minutes.
   d. Place each tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle; then, remove and discard the supernatant.
6. Wash the DNA ***3*** times. For each wash:
   a. Add 200 µL of *freshly prepared* 70% ethanol to each tube, mix by inverting the tube a few times, then pulse-spin.
   b. Place each tube in the DynaMag™-2 magnetic rack for at least 1 minute until the solution clears, then remove and discard the supernatant.
7. Remove the tube from the DynaMag™-2 magnetic rack, pulse-spin the tube, return the tube to the DynaMag™-2 magnetic rack, then remove and discard the supernatant with a 20-µL pipettor.
8. Open each tube, then dry the beads at room temperature (20–25°C) for 5–10 minutes.
9. Elute the DNA:
   a. Remove each tube from the DynaMag™-2 magnetic rack, then add 30 µL Low TE Buffer directly to the pellet to disperse the beads.
   b. Pipette the suspension up and down to mix.

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

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

e. Transfer the *supernatant* containing the size-selected DNA to a new 1.5-mL LoBind Tube.

Quantitate the DNA

Measure the DNA concentration by using:

- 2 µL of sample with the Qubit™ dsDNA HS Assay Kit (Invitrogen Part no. Q32851) and the Qubit® 2.0 Fluorometer (Invitrogen Part no. Q32866)

  or

- 2 µL of sample in the NanoDrop® ND-1000 Spectrophotometer (see "Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer" on page 62)

  or

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

  and/or

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

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

Check the size distribution of the libraries

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

STOPPING POINT Store the DNA in Low TE Buffer at 4°C for short-term storage or at –20°C for long-term storage; or proceed to "(Optional) Pool equal molar barcoded libraries" on page 49.

(Optional) Pool equal molar barcoded libraries

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

Use the barcodes according to these conditions:

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

1. Quantitate the libraries to be pooled by qPCR [refer to the *Applied Biosystems SOLiD™ Library TaqMan® Quantitation Kit* protocol (Invitrogen Part no. A12120)].
2. Mix together equal molar amounts of each barcoded library of *similar* size in an appropriately sized LoBind Tube. Vortex the tube.

STOPPING POINT Store the library DNA in Elution Buffer (E1) at 4°C, or proceed directly to emulsion PCR, as describe in the *SOLiD™ EZ Bead™ Emulsifier Getting Started Guide* (PN 4441486).

Troubleshooting

See "Troubleshooting" on page 51.

Troubleshooting

Observation	Possible cause	Recommended action
Quantities of DNA for the same sample do not match between different quantitation methods	Quantities differ due to different properties of DNA measured according to method	• Measure duplicates of sample or take replicate measurements of the same sample for an average. • Use more than one quantitation method. **Note:** The NanoDrop® ND-1000 Spectrophotometer measures UV absorption of ssDNA, dsDNA, and free nucleotides. Free nucleotides and ssDNA have higher extinction coefficients than dsDNA. The Qubit® 2.0 Fluorometer measures the fluorescence from probes bound to dsDNA. The NanoDrop® ND-1000 Spectrophotometer tends to overestimate DNA and the Qubit® 2.0 Fluorometer tends to underestimate DNA. The Agilent Technologies 2100 Bioanalyzer™ measures sample peak area against to a reference peak, which could be subject to some error.

Observation	Possible cause	Recommended action
Library yields lower than expected	DNA loss during purification	Purification with the Agencourt AMPure® XP Reagent: • Ensure that the Agencourt AMPure® XP Reagent beads are fresh and used before the expiration date. • Prepare fresh 70% ethanol. • Elute the DNA with a solution of low ionic strength such as Low TE Buffer. Purification with the SOLiD™ Library Micro Column Purification Kit:® • Pre-spin the columns. ® • Add 2-propanol to buffers B2-S and B2-L and ethanol to Wash Buffer (W1) as instructed.
	Adaptor ligation suboptimal	If the DNA yield after end-repair and *input* DNA before shearing is <100 ng, use ≥0.06 µL of adaptor or an equivalent quantity of diluted adaptor.
	Incomplete nick-translation	Ensure that the incubation temperature after ligation is raised to 72°C for 20 minutes.
After adaptor ligation, bioanalyzer trace shows multiple peaks in the library	Narrow peaks shorter than library peaks are adaptors	Repeat purification with the Agencourt AMPure® XP Reagent using 1.5× volume of beads to sample volume or repeat purification with the SOLiD™ Micro Column Purification Kit.
	Two broad peaks or a peak and a shoulder in the main library population of peaks (100–300 bp) are due to lower ligation efficiency	Quantitate the library by qPCR. If needed, amplify the library [see "(Optional) Amplify the library" on page 26 or "(Optional) Amplify the libraries" on page 45].

A Ordering Information

This appendix covers:

Sufficient reagents are supplied in the 5500 Series SOLiD™ System kits to prepare up to 12 or up to 48 libraries 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 kits for library preparation

Item (part no.)†	Components
5500 SOLiD™ Fragment Library Core Kit (4464412)	• 5500 SOLiD™ Fragment Library Enzyme Module • 5500 SOLiD™ Fragment Library Amplification Module
5500 SOLiD™ Fragment Library Enzyme Module (4464413)	• 10 mM dNTP • End Polishing E1 • End Polishing E2 • 5✕ Reaction Buffer • A-Tailing Enzyme I • T4 DNA Ligase, 5 U/µL • 10 mM dATP • Shear Buffer
5500 SOLiD™ Fragment Library Amplification Module (4464414)	Platinum® PCR Amplification Mix

Item (part no.)†	Components
5500 SOLiD™ Fragment Library Standard Adaptors (4464411)	• Barcode-T-001, 50 µM • P1-T Adaptor, 50 µM • Library PCR Primer 1, 50 µM • Library PCR Primer 2, 50 µM
5500 SOLiD™ Fragment Library Barcode Adaptors 1–96 (4464404)	• 5500 SOLiD™ Fragment Library Barcode Adaptors 1–16, 50 µM each • 5500 SOLiD™ Fragment Library Barcode Adaptors 17–32, 50 µM each • 5500 SOLiD™ Fragment Library Barcode Adaptors 33–48, 50 µM each • 5500 SOLiD™ Fragment Library Barcode Adaptors 49–64, 50 µM each • 5500 SOLiD™ Fragment Library Barcode Adaptors 65–80, 50 µM each • 5500 SOLiD™ Fragment Library Barcode Adaptors 81–96, 50 µM each
5500 SOLiD™ Fragment Library Barcode Adaptors 1–16 (4464405)	• Barcode adaptors T-001–T-016 • P1-T Adaptor, 50 µM • Library PCR Primer 1, 50 µM • Library PCR Primer 2, 50 µM
5500 SOLiD™ Fragment Library Barcode Adaptors 17–32 (4464406)‡	• Barcode adaptors T-017–T-032 • P1-T Adaptor, 50 µM • Library PCR Primer 1, 50 µM • Library PCR Primer 2, 50 µM

Item (part no.)†	Components
5500 SOLiD™ Fragment Library Barcode Adaptors 33–48 (4464407)‡	• Barcode adaptors T-033–T-048 • P1-T Adaptor, 50 µM • Library PCR Primer 1, 50 µM • Library PCR Primer 2, 50 µM
5500 SOLiD™ Fragment Library Barcode Adaptors 49–64 (4464408)‡	• Barcode adaptors T-049–T-064 • P1-T Adaptor, 50 µM • Library PCR Primer 1, 50 µM • Library PCR Primer 2, 50 µM
5500 SOLiD™ Fragment Library Barcode Adaptors 65–80 (4464409)‡	• Barcode adaptors T-065–T-080 • P1-T Adaptor, 50 µM • Library PCR Primer 1, 50 µM • Library PCR Primer 2, 50 µM
5500 SOLiD™ Fragment Library Barcode Adaptors 81–96 (4464410)‡	• Barcode adaptors T-081–T-096 • P1-T Adaptor, 50 µM • Library PCR Primer 1, 50 µM • Library PCR Primer 2, 50 µM

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

Required Applied Biosystems reagent kits for automated liquid-handling systems

Note: Customers who have access to an automated liquid-handling system such as the Beckman Coulter Biomek® FXP and Tecan Freedom EVO® instruments, can choose from the kits below:

Item (part no.)†	Components
5500 SOLiD™ Fragment 48 Library Core Kit (4464415)	• 5500 SOLiD™ 48 Fragment Library Enzyme Module • 5500 SOLiD™ 48 Fragment Library Amplification Module

Item (part no.)†	Components
5500 SOLiD™ 48 Fragment Library Enzyme Module (4464416)	• 10 mM dNTP • End Polishing E1 • End Polishing E2 • 5X Reaction Buffer • A-tailing Enzyme I • T4 DNA Ligase, 5 U/µL • 10 mM dATP • Shear Buffer
5500 SOLiD™ 48 Fragment Library Amplification Module (4464417)	Platinum® PCR Amplification Mix

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

Optional Applied Biosystems Reagent Kits

Item (part no.)†	Components
SOLiD™ Library Micro Column Purification Kit (4443751)	• Binding Buffer (B2-L) • Binding Buffer (B2-S) • Wash Buffer • Elution Buffer • Micro Spin Columns • Elution Tubes

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

Required equipment

Item†	Source
Covaris® S220 System‡ (110 V for U.S. customers) (220 V for international customers) The Covaris® S220 System includes: • Covaris® S220 sonicator • Universal Voltage Kit • Latitude® laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris®-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris®-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris®-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris®-2 Series Machine Holder for (one) microTUBE • Covaris® microTUBE Prep Station • Covaris® Water Tank Label Kit • Covaris® microTUBEs (1 pack of 25)	Applied Biosystems 4465653
Covaris® S2 System§ (110 V for U.S. customers) (220 V for international customers)	**Note:** Fragment libraries can be prepared with the Covaris® S2 System. New users should purchase the Covaris® S220 System.
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)‡

Item†	Source
DynaMag™- 2 Magnet (magnetic rack)	Invitrogen 123-21D
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Qubit® 2.0 Fluorometer	Invitrogen Q32866
Vortexer	Major Laboratory Supplier (MLS)‡‡
PicoFuge® Microcentrifuge	MLS
Pipettors, 2 µL	MLS
Pipettors, 20 µL	MLS
Pipettors, 200 µL	MLS
Pipettors, 1000 µL	MLS

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

‡ Or the Covaris® S2 System.

§ Or the Covaris® S220 System.

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

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

Optional equipment

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

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

Required consumables

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

Item†	Source
MicroAmp® Optical 8-Tube Strip, 0.2 mL	Applied Biosystems 4316567
Invitrogen Qubit™ dsDNA HS Assay Kit *or* Invitrogen Qubit™ dsDNA BR Assay Kit *or* Invitrogen Quant-iT™ PicoGreen® dsDNA Assay Kit	Invitrogen Q32851 or Q32854 Invitrogen Q32850 or Q32853 Invitrogen P7589
Agencourt AMPure® XP 5 mL Kit or Agencourt AMPure® XP 60 mL Kit	Beckman Coulter Genomics A63880 or A63881
Covaris® microTUBEs	Covaris 520045
2-Propanol	Sigma-Aldrich I9516
Ethylene glycol	American Bioanalytical AB00455-01000
0.5-mL LoBind Tubes	Eppendorf 022431005
1.5-mL LoBind Tubes	Eppendorf 022431021
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit‡	Thermo Scientific PR-1
Ethanol, absolute	Sigma-Aldrich E7023
Filtered pipettor tips	Major Laboratory Supplier (MLS)§

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

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

§ 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
Agilent DNA 1000 Kit	Agilent Technologies 5067-1504

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

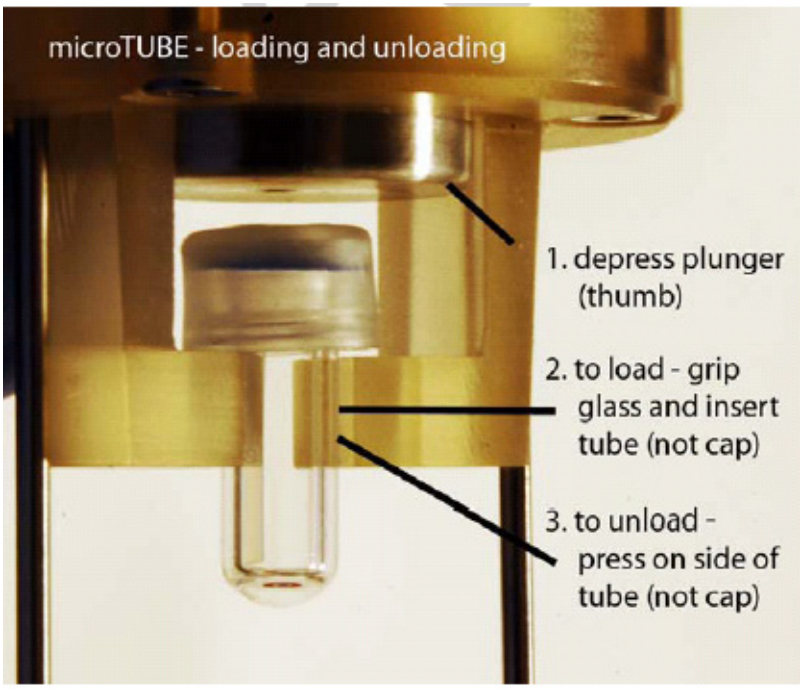
B Supplemental Procedures

This appendix covers:

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

Load Covaris® microTUBE vials

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



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

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

Unload Covaris® microTUBE vials

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

Quantitate the DNA with the NanoDrop® ND-1000 Spectrophotometer

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

Materials and equipment required

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

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

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

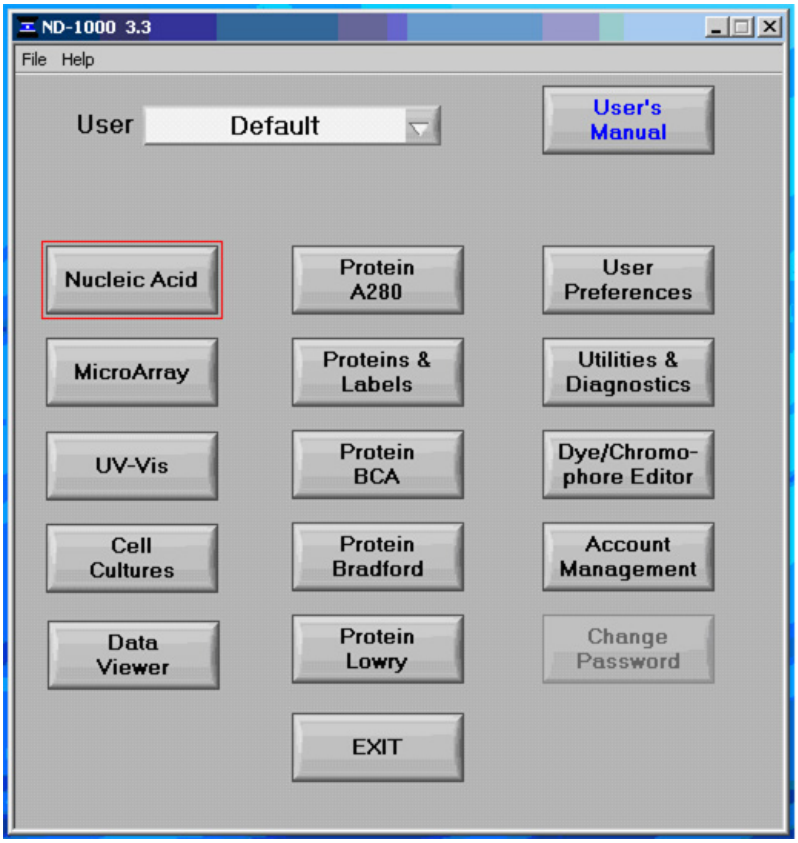
Required consumables	
Item†	**Source**
Nuclease-free Water (1 L)	Applied Biosystems AM9932
CF-1 Calibration Fluid Kit‡	Thermo Scientific CF-1
PR Conditioning Kit	Thermo Scientific PR-1
Filtered pipettor tips	Major Laboratory Supplier (MLS)

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

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

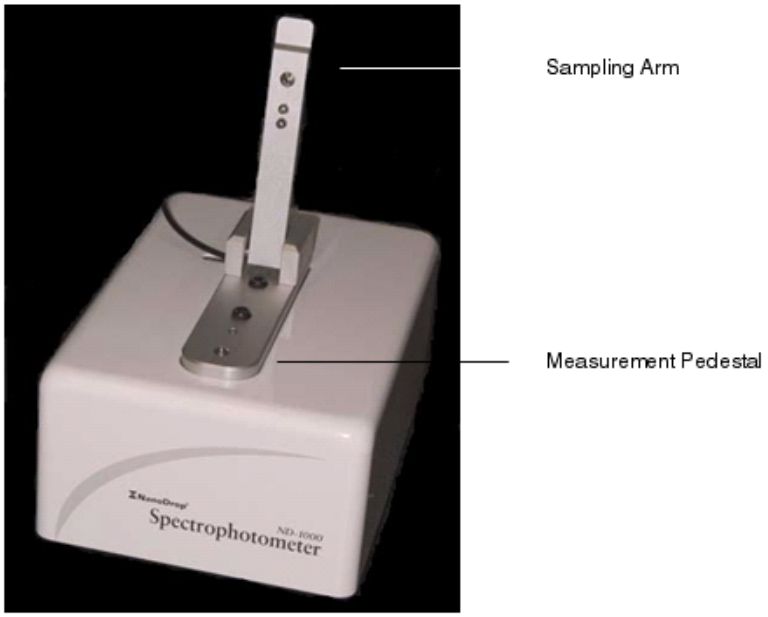
Procedure

1. Ensure that the NanoDrop® ND-1000 Spectrophotometer is properly calibrated. Use the CF-1 Calibration Fluid Kit if necessary.
2. Open the NanoDrop® ND-1000 Spectrophotometer software to display a dialog box:

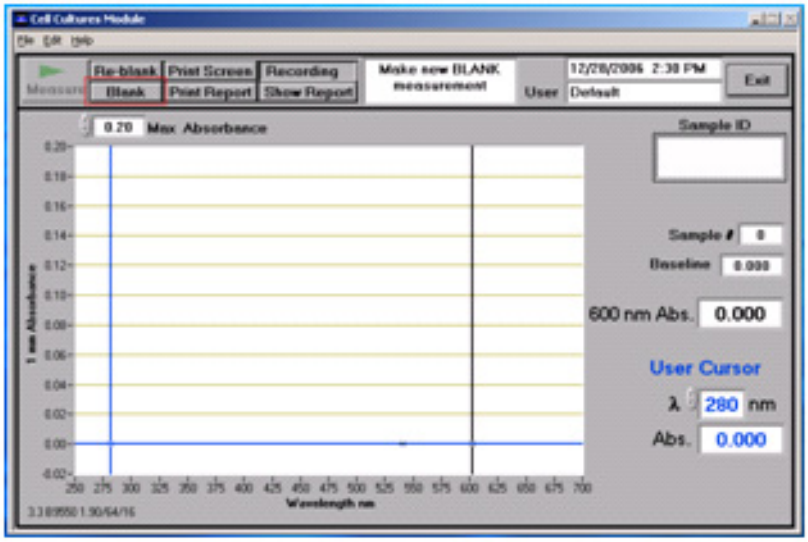


3. Select the **Nucleic Acid** button.

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



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



9. Lift the sampling arm and wipe away the buffer from both the upper and lower measurement pedestals with Kimwipes®. The instrument is now ready to take readings.

10. Load 2 µL of DNA sample onto the lower measurement pedestal and lower the sampling arm.

C Overview

This appendix covers:

Choosing 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...
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	Chapter 2, "Prepare a Single Fragment Library" on page 13 *or* Chapter 3, "Prepare Multiple Fragment Libraries" on page 31

Library type	Features	Applications	Go to...
Mate-paired	• Two DNA insert tags 600 bp–6 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	*Mate-Paired Library Preparation: 5500 Series SOLiD™ Systems User Guide* (Part no. 4460958)

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 5500 Series SOLiD™ Sequencers website:

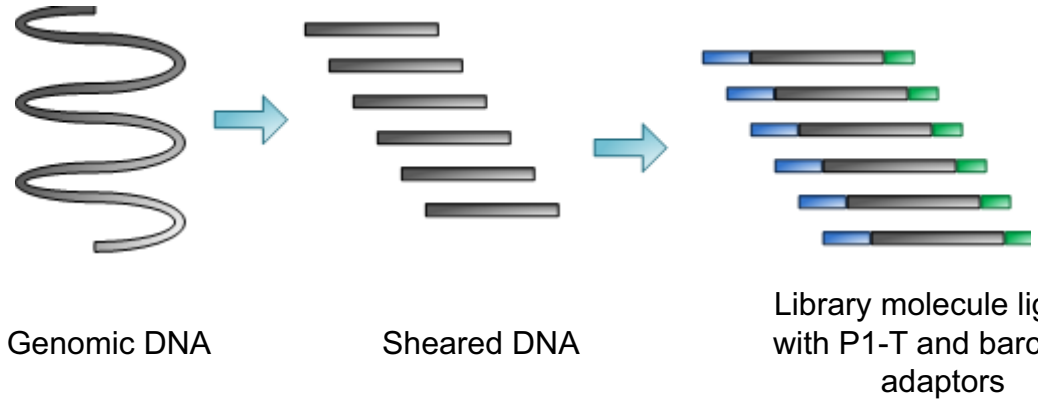
www.appliedbiosystems.com/solid5500

Or, contact your field applications specialist.

Preparing fragment libraries

Fragment library preparation involves shearing DNA into small fragments and ligating P1-T and barcoded adaptors specific for fragment library preparation (see Figure 1).

Figure 1 Fragment library preparation workflow overview

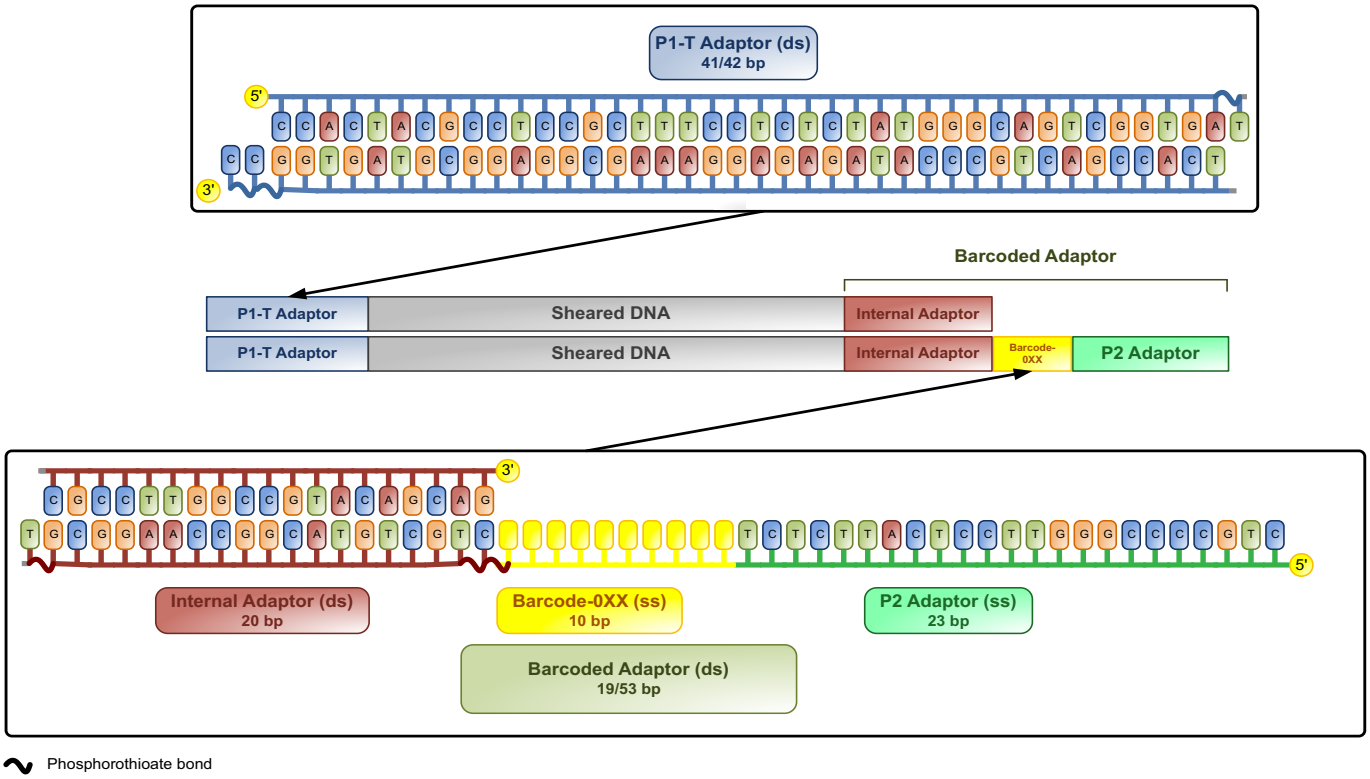


The barcoded adaptor consists of 3 segments of sequence:

1. Internal adaptor sequence, which is necessary for sequencing the barcode
2. Barcode sequence
3. P2 Adaptor sequence, which is used for library amplification and emulsion PCR

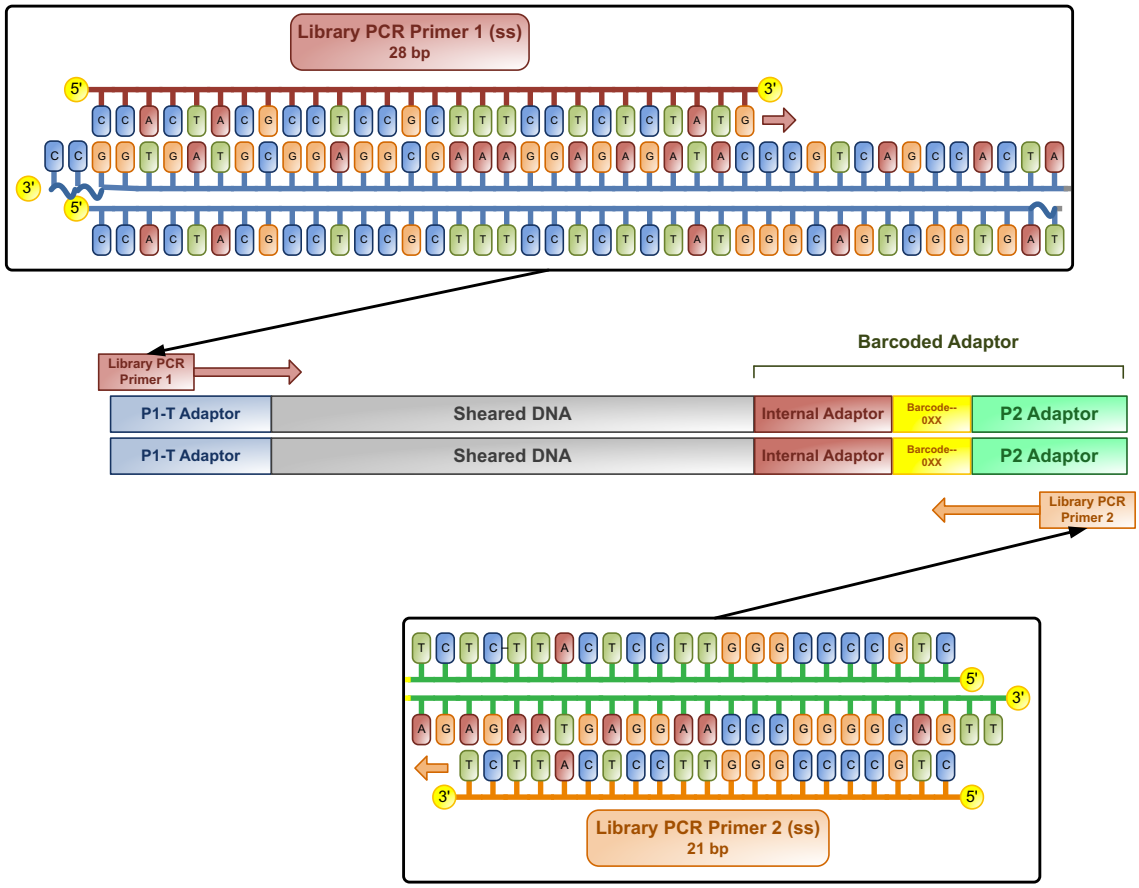
Different libraries to be multiplexed in the same sequencing run are ligated to barcoded adaptors with different barcode sequences. Ninety-six barcode sequences are available to tag different libraries (see Figure 2 on page 70).

Figure 2 Fragment library design



After P1-T and barcoded adaptors are ligated to the sheared DNA, the library is amplified using Library PCR Primers 1 and 2, specific to the P1 and barcoded adaptors (see Figure 3 on page 71). 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 necessary for the sequencing chemistry.

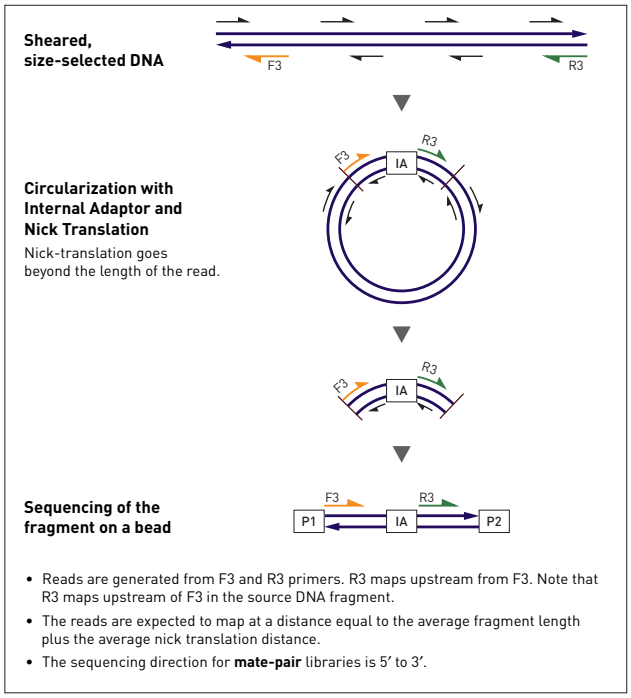
Figure 3 Fragment library amplification design



For RNA applications, an alternative method to generate barcoded libraries is described in the protocols for the SOLiD™ RNA Barcode Module 1-16 (Part no. 4427046), SOLiD™ RNA Barcode Module 17-32 (Part no. 4453189), and SOLiD™ RNA Barcode Module 33-48 (Part no. 4453191).

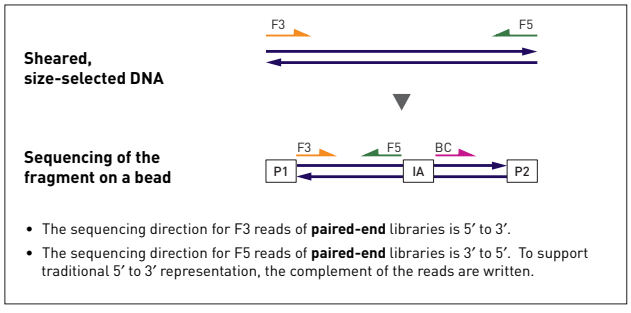
Sequence orientation from source DNA to sequence map

Mate-pair



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

Paired-end



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

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

D Oligonucleotide Sequences

Library construction oligonucleotides

PCR Primer and adaptor sequences

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

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

Adaptor and primer sequences	Length (nt)
P1-T Adaptor, 50 µM	
5′ -CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGA~T-3′	41
5′ -TCACCGACTGCCCATAGAGAGGAAAGCGGAGGCGTAGTGG~C~C-3′	42
Standard Adaptor, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGTAAGAGG**CTGCTGTACGGCCAAGGCGT-3′	53
Library PCR Primer 1, 50 µM	
5′ -CCACTACGCCTCCGCTTTCCTCTCTATG-3′	28
Library PCR Primer 2, 50 µM	
5′ -CTGCCCCGGGTTCCTCATTCT-3′	21

Barcoded adaptor sequences

Barcoded adaptor sequence	Length (nt)
Barcode-T-001, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGTAAGAGG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-002, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGGAGTGGT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-003, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATAGGTTATA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-004, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGATGCGGTC**CTGCTGTACGGCCAAGGCGT-3′	53

Barcoded adaptor sequence	Length (nt)
Barcode-T-005, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGGTGTAAG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-006, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGAGGGACA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-007, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGTTATGCC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-008, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGCGAGGAT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-009, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGTTGCGAC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-010, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGTAAGCT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-011, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCGACACG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-012, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGAGGAAAA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-013, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGTAAGGC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-014, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCGGCAGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-015, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGTTGAATG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-016, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGAGACGTT**CTGCTGTACGGCCAAGGCGT-3′	53

Barcoded adaptor sequence	Length (nt)
Barcode-T-017, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGCTCACCGC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-018, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGCGGATGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-019, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATGGTAACTG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-020, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTCAAGCTTT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-021, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCGGTTCC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-022, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGAAGATGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-023, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGTGCTTG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-024, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGTCGGTAT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-025, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AACATGATGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-026, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CGGGAGCCCG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-027, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CAGCAAACTT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-028, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGCTTACTAC**CTGCTGTACGGCCAAGGCGT-3′	53

Barcoded adaptor sequence	Length (nt)
Barcode-T-029, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAATCTAGGG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-030, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTAGCGAAGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-031, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCTGGTGCGT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-032, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGTTGGGTG**CCTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-033, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CGTTGGATAC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-034, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**TCGTTAAAGG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-035, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGCGTAGGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-036, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTTCTCACAT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-037, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CTGTTATACC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-038, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTCGTCTTAG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-039, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**TATCGTGAGT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-040, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAAAGGGTTA**CTGCTGTACGGCCAAGGCGT-3′	53

Barcoded adaptor sequence	Length (nt)
Barcode-T-041, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**TGTGGGATTG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-042, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAATGTACTA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-043, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CGCTAGGGTT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-044, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGGATGATC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-045, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTACTTGGCTC**TGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-046, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGTCGTCGAA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-047, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGGGATGG**CCTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-048, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCCGTAAGTG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-049, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATGTCATAAG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-050, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAAGGCTTGC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-051, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGCAGGAGT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-052, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTAATTGTAA**CTGCTGTACGGCCAAGGCGT-3′	53

Barcoded adaptor sequence	Length (nt)
Barcode-T-053, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTCATCAAGT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-054, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAAAGGCGGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-055, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGCTTAAGCG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-056, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCATGTCACC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-057, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**CTAGTAAGAA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-058, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**TAAAGTGGCG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-059, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGTAATGTC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-060, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCCTCGGT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-061, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGATTATCG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-062, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGTGAGGGT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-063, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGGTTCGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-064, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGCTACACC**CTGCTGTACGGCCAAGGCGT-3′	53

Barcoded adaptor sequence	Length (nt)
Barcode-T-065, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGGATCAAGC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-066, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GATGTAATGT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-067, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTCCTTAGGG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-068, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCATTGACGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-069, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GATATGCTTT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-070, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCCCTACAGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-071, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ACAGGGAACG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-072, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGTGAATA**CCTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-073, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCAATGACGT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-074, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGACGCTGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-075, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTATCTGGGC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-076, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGTTTTAGG**CTGCTGTACGGCCAAGGCGT-3′	53

Barcoded adaptor sequence	Length (nt)
Barcode-T-077, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATCTGGTCTT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-078, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGCAATCATC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-079, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGTAGAATTA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-080, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTTTACGGTG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-081, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAACGTCATT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-082, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGAAGGGAG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-083, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGATGGCGTA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-084, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGGATGAAC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-085, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GGAAAGCGTT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-086, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGTACCAGGA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-087, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATAGCAAAGC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-088, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTTGATCATG**CTGCTGTACGGCCAAGGCGT-3′	53

Barcoded adaptor sequence	Length (nt)
Barcode-T-089, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AGGCTGTCTA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-090, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTGACCTACT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-091, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GCGTATTGGG**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-092, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AAGGGATTAC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-093, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GTTACGATGC**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-094, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**ATGGGTGTTT**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-095, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**GAGTCCGGCA**CTGCTGTACGGCCAAGGCGT-3′	53
Barcode-T-096, 50 µM	
5′ -CGCCTTGGCCGTACAGCAG-3′	19
5′ -CTGCCCCGGGTTCCTCATTCTCT**AATCGAAGAG**CTGCTGTACGGCCAAGGCGT-3′	53

E Checklist and workflow tracking form

This appendix covers:

Workflow checklists: prepare a fragment 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
Quantitate the DNA	☐ Qubit® 2.0 Fluorometer	☐ Quant-iT™ kit	—
Shear the DNA	☐ Covaris® S220 System ☐ Covaris® microTube adaptor ☐ Covaris® microTube loading station ☐ Covaris® microTube	☐ 1× Low TE Buffer ☐ (Optional) 100× Shear Buffer ☐ Ethylene glycol	☐ Degas the water in the Covaris® S2 or S220 System 30 minutes prior to use. ☐ Supplement the circulated water chiller with 20% ethylene glycol. ☐ Thaw Shear Buffer at room temperature.
End-polish the DNA	☐ NanoDrop® ND-1000 Spectrophotometer ☐ DynaMag™-2 Magnetic Rack	☐ 5× Reaction Buffer ☐ dA + dNTP Mix, 10 mM ☐ End Polishing E1 ☐ End Polishing E 2 ☐ A-Tailing Enzyme 1	☐ Thaw buffers on ice.
Size-select the DNA by AMPure XP Reagent	—	☐ Agencourt AMPure® XP Reagent ☐ Ethanol, Absolute	☐ Warm AMPure® XP Bead mixture to room temperature.
Quantitate the size-selected DNA	☐ Qubit® 2 Fluorometer or ☐ NanoDrop® ND-1000 Spectrophotometer or ☐ Agilent Technologies 2100 Bioanalyzer™	☐ Quant-iT™ kit	—
Add an A-tail to the size-selected DNA	☐ NanoDrop® ND-1000 Spectrophotometer	☐ 5× Reaction Buffer ☐ 10 mM dATP ☐ A-Tailing Enzyme 1 ☐ Nuclease-free Water	☐ Thaw buffers on ice.
Ligate Adaptors to the DNA	☐ DynaMag™-2 magnetic rack	☐ 5× Reaction Buffer ☐ P1-T Adaptor, 50 µM ☐ Barcoded-T-0XX adaptor, 50 µM ☐ 10 mM dNTP ☐ Agencourt AMPure® XP Reagent ☐ Ethanol, Absolute	☐ Thaw adaptors on ice. ☐ Thaw 5× T4 Ligase Buffer on ice.
(Optional) Amplify the library	☐ Thermocycler	☐ Library PCR Primer 1 ☐ Library PCR Primer 2 ☐ Platinum® PCR Amplification Mix ☐ Agencourt AMPure® XP Reagent ☐ Ethanol, Absolute	☐ Thaw Library PCR Primers 1 and 2 on ice. ☐ Thaw Platinum® PCR Amplification Mix on ice.
Quantitate the DNA	☐ Qubit® 2.0 Fluorometer or ☐ NanoDrop® ND-1000 Spectrophotometer or ☐ Agilent Technologies 2100 Bioanalyzer™ or ☐ Real-time thermal cycler	☐ Quant-iT™ dsDNA HS Assay Kit or ☐ Applied Biosystems SOLiD™ Library TaqMan® Quantitation Kit	—

Workflow tracking: prepare a fragment library

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

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

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

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

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

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 88.)
- 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

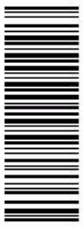
barcode	A short, unique sequence that is incorporated into a library that enables identification of the library during multiplex sequencing.
Barcoded Adaptor	During fragment library preparation, the double-stranded oligonucleotide that is ligated to the genomic DNA fragment such that the internal adaptor, barcode sequence, and the P2 Adaptor are at the 3' end of the sequencing template.
barcoded library	A library that has a unique barcode sequence incorporated that enables identification of the library during multiplex sequencing.
fragment library	A library that has a single insert prepared from genomic DNA for sequencing on the SOLiD™ System. Fragment libraries compatible with the 5500 Series SOLiD™ Sequencers can be sequenced with a forward-only run or with a paired-end run.
internal adaptor (IA)	The internal adaptor sequence is incorporated into the template during library construction and provides a common hybridization target for SOLiD™ sequencing primers. 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 Adaptor sequence.
mate-paired library	Library consisting of two DNA segments that reside a known distance apart in the genome, linked by an internal adaptor, and with P1 and P2 Adaptors ligated to the 5' and 3' ends of the template strand, respectively.
multiplex sequencing	Sequencing runs in which multiple barcoded libraries are simultaneously sequenced in a single flowchip lane. Each bead is assigned to the correct library after the sequencing run according to the sequence of its barcode.

P1-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.
Standard Adaptor	During fragment library preparation, the double-stranded oligonucleotide that is ligated to the genomic DNA fragment such that the internal adaptor, barcode sequence BC-001, and the P2 Adaptor are at the 3' end of the sequencing template.
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

Index

T

W



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