USER GUIDE

applied biosystems by life technologies™

AB Library Builder™ Whole Transcriptome Core Kit for 5500 Genetic Analysis Systems

Catalog Numbers 4472690 and 4473926

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Revision Date 12 December 2011

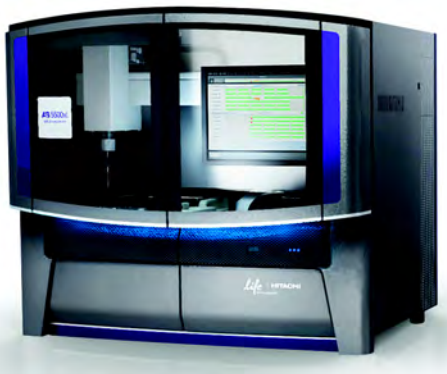
design experiment

▶ **prepare libraries**

prepare beads

run sequencer

analyze data



life technologies™

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About This Guide

Safety information

Note: For important instrument safety information, refer to the *AB Library Builder™ System User Guide* (Part no. 4463421). For general safety information, see this section and Appendix A. 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 Life Technologies are available from our web sites at **www.lifetechnologies.com** or **www.appliedbiosystems.com**.

IMPORTANT! For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

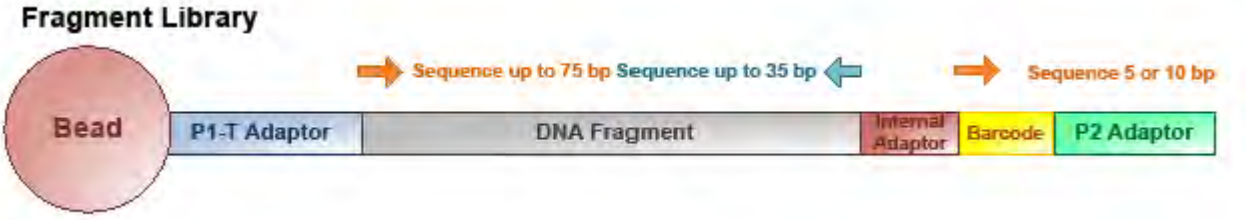
About This Product

Product information

The AB Library Builder™ Whole Transcriptome Core Kit for 5500 Genetic Analysis Systems provides reagents and materials for using the AB Library Builder™ System to prepare RNA transcripts expressed in cells or tissues for analysis on 5500 Genetic Analysis Systems. This user guide provides instructions for preparing whole transcriptome libraries starting from standard input amounts of poly(A) RNA (100–500 ng) or rRNA-depleted total RNA (200–500 ng).

Library preparation

Library preparation is the first step in which samples are adapted for sequencing on the 5500 Genetic Analysis Systems. During library preparation, RNA fragments are transcribed into cDNA and forward and reverse adaptors are added to the ends of each cDNA segment (the bead is for illustration purposes only and is not added until the bead preparation step):



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

For a more detailed overview of library types and the library preparation workflows, see "Appendix C: Supplemental Background Information" in the *Fragment Library Preparation Using the AB Library Builder™ System User Guide*.

How to use the core kits and adaptors

This user guide describes how to use the AB Library Builder™ Whole Transcriptome Core Kit for 5500 Genetic Analysis Systems, which contains reagents for preparing cDNA libraries from poly(A) RNA or rRNA-depleted total RNA on the AB Library Builder™ System with the Protocol Card: Library Builder™ Whole Transcriptome. The protocol card directs the instrument to hybridize and ligate adaptors to the RNA and then generate, purify, and size select the cDNA.

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

Preparing barcoded libraries

When you use the AB Library Builder™ Whole Transcriptome Core Kit for 5500 Genetic Analysis Systems with one of the SOLiD® RNA Barcoding Kits, you can prepare barcoded libraries to enable sequencing of multiple samples in a single, multiplexed sequencing run. Sequencing of multiplexed libraries is fully supported by the 5500 Genetic Analysis Systems. Instructions for using the SOLiD® RNA Barcoding Kits are included in product inserts that come with the kits and in this protocol.

Kit contents and storage conditions

Kit contents

The AB Library Builder™ Whole Transcriptome Core Kit for 5500 Genetic Analysis Systems (Catalog no. 4472690) contains materials sufficient to prepare up to 13 cDNA libraries. Catalog no. 4473926 includes four complete kits.

AB Library Builder™ Whole Transcriptome Cartridges

Part	Description	Storage
AB Library Builder™ Whole Transcriptome Cartridges for 5500 Genetic Analysis Systems	13 cartridges, each containing ready-to-use reagents	–20°C

AB Library Builder™ WT Amplification Reagents

Part	Description	Storage
Platinum® PCR SuperMix High Fidelity	3 tubes (1 mL each)	–20°C
SOLiD® 5' PCR Primer	100 µL	–20°C
SOLiD® 3' PCR Primer	100 µL	–20°C

AB Library Builder™ RNA Fragmentation and Ligation Reagents

Part	Description	Storage
RNase III	20 µL	–20°C
10X RNase III Reaction Buffer	20 µL	–20°C
2X Ligase Buffer	13 tubes (40 µL each)	–20°C

Fragmented RNA Concentrator Module

Part	Description	Storage
Binding Buffer (L3)	3.3 mL	–20°C
Wash Buffer (W5)	1.5 mL	–20°C
RNase-Free Water	1.75 mL	–20°C
Spin Columns	13 columns	Ambient

Part	Description	Storage
Wash Tubes	13 tubes	Ambient
Recovery Tubes	13 tubes	Ambient

Plastics Module

Part	Description	Storage
Sample and elution tubes	52 tubes	Ambient
Tips and holders	26 tips and holders	Ambient

Materials and equipment required but not included

Catalog numbers are Life Technologies unless otherwise noted. To order Life Technologies products, visit **www.lifetechnologies.com**. For widely available products, go to major laboratory suppliers (MLS).

Item	Catalog no.
Protocol Card: Library Builder™ 5500 RNA Whole Transcriptome	4472736
Applied Biosystems thermal cycler with heated lid, capable of holding 0.2-mL tubes: • Veriti® 96-Well Thermal Cycler • GeneAmp® PCR System 9700	Visit www.appliedbiosystems.com
Agilent® 2100 Bioanalyzer™ Instrument	Agilent G2938A
Microcentrifuge	MLS
NanoDrop® Spectrophotometer	Thermo Scientific
Pipettors, positive displacement or air-displacement	MLS
8-strip PCR Tubes & Caps, RNase-free, 0.2-mL	AM12230
Non-Stick RNase-free Microfuge Tubes (0.5 mL), 500	AM12350
Ambion® Non-Stick RNase-free Microfuge Tubes (1.5 mL), 250	AM12450
Ethanol, 100%, ACS reagent grade or equivalent	MLS
Pipette tips, RNase-free	MLS
ATP Soln (optional)	AM8110G
T4 Polynucleotide Kinase (Cloned) 10 U/µL (optional)	AM2310
Qubit® 2.0 Fluorometer	Q32866
Centrifugal vacuum concentrator (for example, SpeedVac)	MLS
Agencourt® RNAClean® XP Reagent	Beckman Coulter A63987
DynaMag™-2 Magnetic Rack	12321D
Quant-iT™ RNA Assay Kit, 100 assays	Q32852
Agilent® DNA 1000 Kit	Agilent 5067-1504

Item	Catalog no.
Agilent® RNA 6000 Pico Kit	Agilent 5067-1513
Agencourt® AMPure® XP Reagent, 5mL	Beckman Coulter Genomics A63880
Optional materials:	
ERCC RNA Spike-In Mix	4456740
ERCC ExFold RNA Spike-In Mixes	4456739
MicroPoly(A)Purist™ Kit	AM1919
RiboMinus™ Eukaryote Kit for RNA-Seq	A10837
RiboMinus™ Plant Kit for RNA-Seq	A10838
FirstChoice® Total RNA	Various; visit www.lifetechnologies.com
iPrep™ Purelink® Virus Kit	IS10008
iPrep™ Trizol® Plus RNA Kit	IS10007
iPrep™ Purelink® Total RNA Kit	IS10006

Optional: SOLiD® RNA Barcoding Kits

For more information on SOLiD® RNA Barcoding Kits, visit **www.appliedbiosystems.com**.

Item	Catalog/Part no.
SOLiD® RNA Barcoding Kit, Modules 1-16	4427046
SOLiD® RNA Barcoding Kit, Modules 17-32	4453189
SOLiD® RNA Barcoding Kit, Modules 33-48	4453191
SOLiD® RNA Barcoding Kit, Modules 49-64	4456501
SOLiD® RNA Barcoding Kit, Modules 65-80	4456502
SOLiD® RNA Barcoding Kit, Modules 81-96	4456503
SOLiD® RNA Barcoding Kit, Modules 1-48	4461565
SOLiD® RNA Barcoding Kit, Modules 49-96	4461566
SOLiD® RNA Barcoding Kit, Modules 1-96	4461567

Build the Library

Procedural guidelines

- The protocol is designed for 100–500 ng poly(A) RNA or 200–500 ng rRNA-depleted total RNA.
- 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 Ambion® Non-Stick RNase-free Microfuge Tubes.
- Thaw reagents on ice or at room temperature before use.

Fragment the whole transcriptome RNA (standard input)

Start with 100–500 ng poly(A) RNA or 200–500 ng rRNA-depleted total RNA
(We strongly recommend that you add ERCC RNA Spike-In Control Mixes to the input total RNA see page 13)

↓

Fragment the RNA:

Fragment the RNA using RNase III (page 14) OR
Fragment the RNA by chemical hydrolysis (page 15)

↓

Clean up the fragmented RNA (page 15)

↓

Assess the yield and size distribution of the fragmented RNA (page 16)

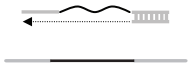
↓

Construct the amplified whole transcriptome library (standard input)

Set up the AB Library Builder™ System for cDNA library preparation (page 19))

↓

Start the run (page 26)



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Amplify the library (page 29))

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Purify the amplified library (page 30)

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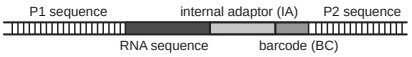
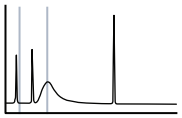
Assess the yield and size distribution of the amplified DNA (page 31)





Proceed with templated bead preparation

Refer to the *SOLiD® EZ Bead Emulsifier Getting Started Guide* (Part no. 4441486)

Guidelines for RNA sample type and amount

Isolate high-quality RNA

Use highest-quality RNA available as your starting material. RNA extraction from a variety of sources may be automated on the Library Builder™ System using the iPrep™ Purelink® Virus Kit (IS10008), iPrep™ Trizol® Plus RNA Kit (IS10007) or iPrep™ Purelink® Total RNA Kit (IS10006) from Life Technologies. Alternatively, the Ambion® FirstChoice® Total RNA provides high-quality, intact RNA isolated from a variety of sources. Best results are obtained when using RNA with an RNA integrity number (RIN) greater than 7.

Use 100–500 ng poly(A) RNA or 200–500 ng rRNA-depleted total RNA.

- For poly(A) RNA, we recommend performing two rounds of oligo(dT) selection of the poly(A) RNA; for example, use the Ambion MicroPoly(A)Purist™ Kit. Also, confirm the absence of 18S and 28S rRNA; for example, check the profile of the poly(A) RNA on an Agilent® 2100 Bioanalyzer™ Instrument.
- For rRNA-depleted total RNA, we recommend that you remove rRNA from total RNA for transcriptome analysis using the RiboMinus™ Eukaryote Kit for RNA-Seq or the RiboMinus™ Plant Kit for RNA-Seq, both from Life Technologies. Also, confirm the absence of 18S and 28S rRNA; for example, check the profile of the rRNA-depleted RNA on an Agilent® 2100 Bioanalyzer™ Instrument.

Ambion® Spike-In Control Mixes

We highly recommend adding Ambion® ERCC Spike-In Control Mixes (Part nos. 4456739 or 4456740, from Life Technologies) to the input RNA at the earliest step possible, preferably at the total RNA stage following the guidelines below, prior to whole transcriptome library preparation.

- The Ambion® ERCC RNA Spike-In Control Mixes provide a set of external RNA controls that enable performance assessment of a variety of technology platforms used for gene expression experiments. Add one Spike-In Mix to each RNA sample, and run the Spike-In Mix-containing samples on your platform. Then compare the Spike-In Mix data to known Spike-In Mix concentrations and ratios to assess the dynamic range, lower limit of detection, and fold-change response of your platform.
- The Ambion® ERCC RNA Spike-In Control Mixes are pre-formulated sets of 92 polyadenylated transcripts from the ERCC plasmid reference library. The transcripts are traceable through the manufacturing process to the NIST plasmid reference material. For detailed information, please refer to the ERCC Spike-In Control protocol. For your convenience, we have included the following table as reference during experimental design.

Amount of sample RNA	Volume of Spike-In Mix 1 or Mix 2 (dilution)†	
	Total RNA	Poly(A) RNA
20 ng	4 µL (1:10000)	2 µL (1:100)
50 ng	1 µL (1:1000)	5 µL (1:100)
100 ng	2 µL (1:1000)	1 µL (1:10)
500 ng	1 µL (1:100)	5 µL (1:10)
1000 ng	2 µL (1:100)	
5000 ng	1 µL (1:10)	

† ERCC RNA Spike-In Mix 1, ExFold Spike-In Mix 1, or ExFold Spike-In Mix 2.

Fragment the whole transcriptome RNA

This section provides procedures for two methods of RNA fragmentation: enzymatic and chemically induced. For more information and to help you select the best RNA fragmentation method for your experiment, see "Amplified library construction concepts" on page 45. Use information about your experimental goals along with this information to choose the optimal fragmentation method for your study.

Fragmentation of the whole transcriptome RNA involves the following procedures:

1. Fragment the RNA:
   - Fragment the RNA using RNase III
     OR
   - Fragment the RNA by chemical hydrolysis
2. Clean up the fragmented RNA
3. Assess the yield and size distribution of the fragmented RNA

Fragment the RNA using RNase III

For this procedure, use the following components from the kit:

- RNase-free Water
- 10X RNase III Reaction Buffer
- RNase III

1. For each RNA sample, assemble the following reaction components on ice:

Component (add in order shown)	Volume
RNA sample and RNase-free Water: • Poly(A) RNA: 100–500 ng • rRNA-depleted total RNA: 200–500 ng • WT Control RNA: 500 ng	8 µL
10X RNase III Reaction Buffer	1 µL
RNase III	1 µL
Total volume	10 µL

IMPORTANT! To reduce fragmentation variability, accurately pipet 1 µL of 10X RNase III Reaction Buffer and 1 µL of RNase III to each sample. **Do not make a master mix that contains only 10X RNase III Reaction Buffer and RNase III**.

2. Flick the tube or pipet up and down a few times to mix, then spin briefly.
3. Incubate the reaction in a thermal cycler at 37°C for 10 minutes.
4. *Immediately* after the incubation, add 90 µL of RNase-free Water, then place the fragmented RNA on ice.

Note: Be careful not to contaminate the tube of RNase-free water with RNA or RNase from the sheared samples.

5. Proceed with cleaning up the fragmented RNA immediately, or leave the fragmented RNA on ice for less than 1 hour.

Fragment the RNA by chemical hydrolysis

If you have fragmented the RNA using the RNase III procedure, skip this procedure and proceed to the next section.

For this procedure, use the following components from the kit:

- RNase-free Water
- 10X RNase III Reaction Buffer

For the kinase reaction, use the following components not included in this kit:

- Ambion® T4 Polynucleotide Kinase (Cloned) 10 U/µL
- Ambion® ATP Soln.

1. For each RNA sample, assemble the reaction on ice:

Component (add in order shown)	Volume
RNA sample and RNase-free Water: • Poly(A) RNA: 100–500 ng • rRNA-depleted total RNA: 200–500 ng • WT Control RNA: 500 ng	9 µL
10X RNase III Reaction Buffer	1 µL
Total volume	10 µL

2. Flick the tube or pipet up and down a few times to mix, then spin briefly.
3. Incubate the reaction in a thermal cycler at 95°C for 10 minutes.

   IMPORTANT! Incubate exactly 10 minutes. Shortening or lengthening the incubation time can result in suboptimal size distribution of the fragmented RNA.
4. *Immediately* after the incubation, snap-cool the tube on ice.

   IMPORTANT! Proceed with the next step immediately.
5. To each 10 µL fragmented RNA sample, add the kinase reaction components:

Component (add in order shown)	Volume
Ambion® T4 Polynucleotide Kinase (Cloned) 10 U/µL	1 µL
Ambion® ATP Soln	1 µL

6. Flick the tube or pipet up and down a few times to mix, then spin briefly.
7. Incubate the reaction in a thermal cycler at 37°C for 30 minutes.
8. *Immediately* after the incubation, add 88 µL of RNase-free Water, then place the fragmented RNA on ice.

Proceed with cleaning up the fragmented RNA immediately, or leave the fragmented RNA on ice for less than 1 hour.

Clean up the fragmented RNA

Use the Fragmented RNA Concentrator Module included in the kit.

1. Add Binding Buffer (L3) and ethanol to the fragmented RNA, then mix well:
   - 100 µL of Binding Buffer (L3)
   - 250 µL of 100% ethanol

2. Bind the RNA sample containing Binding Buffer (L3) and ethanol to the Spin Column:
   a. Place the Spin Column in a clean 1.5-mL Wash Tube.
   b. Load 450 µL of the RNA sample containing Binding Buffer (L3) and ethanol onto the Spin Column.
   c. Spin the column at 12,000 × g for 1 minute.
   d. Discard the flow through.
3. Prepare the Wash Buffer (W5) with ethanol as shown below, mix, then store at room temperature:

Component	Volume
100% ethanol	6 mL
Wash Buffer (W5)	1.5 mL

4. Wash the RNA:
   a. Return the Spin Column to the Wash Tube.
   b. Add 500 µL of Wash Buffer (W5) with ethanol to the Spin Column.
   c. Spin the column at 12,000 × g for 1 minute.
   d. Discard the flow through.
   e. Return the Spin Column in the Wash Tube.
   f. Spin the column at maximum speed for 2 minutes.
5. Elute the RNA in a clean Recovery Tube:
   a. Place the Spin Column in a clean Recovery Tube.
   b. Add 12 µL of RNase-Free water to the center of the Spin Column.
      IMPORTANT! Use the RNase-free water from this kit. Other nuclease-free water may contain DEPC which will affect downstream enzymatic reactions.
   c. Wait 1 minute, then spin the column at maximum speed for 1 minute.

   Note: You should recover approximately 10 µL of fragmented RNA from the column.

Assess the yield and size distribution of the fragmented RNA

Use the Quant-iT™ RNA Assay Kit with the Qubit® Fluorometer and the Agilent® RNA 6000 Pico Chip Kit with the Agilent® 2100 Bioanalyzer™ Instrument.

Note: You can use a NanoDrop® Spectrophotometer in place of the Quant-iT™ RNA Assay Kit and Qubit® Fluorometer. However, RNA eluted from spin columns may contain extra salts or other components that affect readings on the NanoDrop® Spectrophotometer. For increased accuracy, quantitate the RNA concentration using the Quant-iT™ RNA Assay Kit on the Qubit® Fluorometer.

1. Quantitate the yield of the fragmented RNA using the Quant-iT™ RNA Assay Kit on the Qubit® Fluorometer.

   Refer to the *Quant-iT™ RNA Assay Kit Protocol* or the *Qubit® Fluorometer Instruction Manual* for instructions.
2. Assess the size distribution of the fragmented RNA:

a. Dilute 1 µL of the sample 1:10 with RNase-Free Water.

b. Run the diluted sample on an Agilent® 2100 Bioanalyzer™ Instrument with the RNA 6000 Pico Chip Kit. Follow the manufacturer's instructions for performing the assay.

c. Using the 2100 expert software, review the size distribution.

   The fragmentation procedure should produce a distribution of RNA fragment sizes from 35 nt to several hundred or a few thousand nt, depending on your sample type. The median size is normally 125–200 nt. See Figures 1-5 on the following pages.

   Note: For instructions on how to review the size distribution, refer to the *Agilent® 2100 Bioanalyzer™ 2100 Expert User's Guide* by Agilent®.

If the profile for the fragmented RNA does not meet the typical results, see "Troubleshooting" on page 35 for guidance.

3. Proceed according to the amount of fragmented RNA you have in 5 µL:

Amount of fragmented RNA in 5 µL	Instructions
• ≥50 ng poly(A) RNA • ≥100 ng rRNA-depleted total RNA • ≥100 ng WT Control RNA	Proceed with "Set up the AB Library Builder™ System for cDNA library preparation" on page 19. Store the remaining RNA at –80°C.
• <50 ng poly(A) RNA • <100 ng rRNA-depleted total RNA	1. Dry 50–100 ng of the RNA completely in a centrifugal vacuum concentrator at low or medium heat (≤ 40°C); this should take 10–20 minutes. 2. Resuspend in 5 µL RNase-free water, then proceed with "Set up the AB Library Builder™ System for cDNA library preparation" on page 19.

Typical results of fragmentation of whole transcriptome RNA

Figures 1-3 show profiles from an Agilent® 2100 Bioanalyzer™ Instrument after RNase III fragmentation and cleanup. Figure 1 shows results with HeLa poly(A) RNA. Figure 2 shows results with rRNA-depleted HeLa RNA. Figure 3 shows results with WT control total RNA. Figures 4-5 show profiles from an Agilent 2100 Bioanalyzer Instrument after chemical hydrolysis and cleanup. Figure 4 shows results with Hela poly(A) RNA. Figure 5 shows results with rRNA-depleted Hela RNA.

Figure 1 Size distribution of RNAse III fragmented HeLa poly(A) RNA (median size is 165 nt)

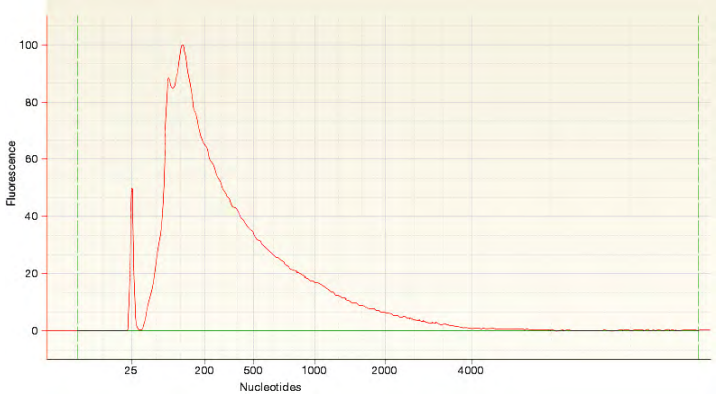


Figure 2 Size distribution of RNAse III fragmented rRNA-depleted HeLa RNA (median size is 140 nt)

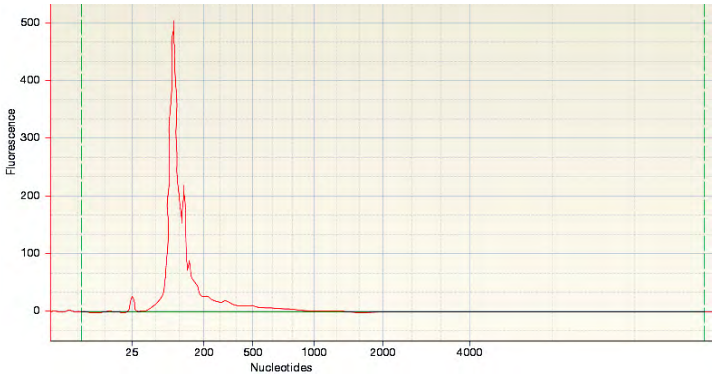


Figure 3 Size distribution of RNAse III fragmented WT Control total RNA (median size is 195 nt)

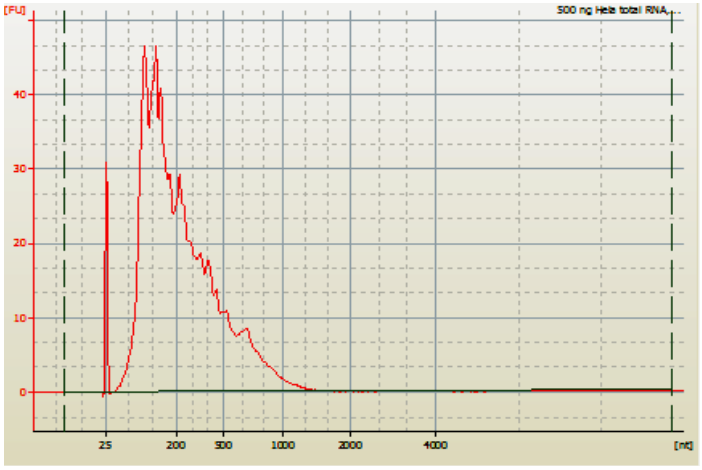


Figure 4 Size distribution of chemical fragmented Hela poly(A) RNA (median size is 165 nt)

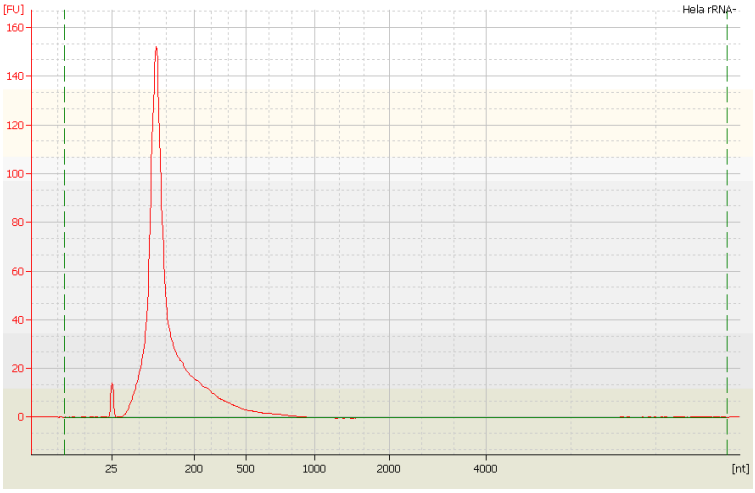
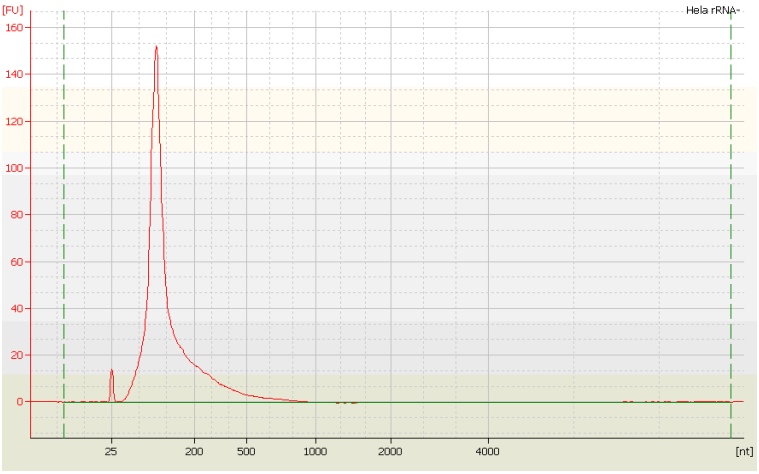


Figure 5 Size dist. of chemical fragmented rRNA-depleted Hela RNA (median size is 138 nt)



Set up the AB Library Builder™ System for cDNA library preparation

Overview

Use the AB Library Builder™ System with the AB Library Builder™ Whole Transcriptome Core Kit for 5500 Genetic Analysis Systems to generate adaptor-ligated and purified cDNA libraries from the fragmented whole transcriptome RNA.

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

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

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

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

1. "Thaw the reagents" on page 19.
2. "Insert or change the protocol card and power ON the instrument" on page 20.
3. "Load the racks and tubes" on page 21.
4. "Insert the racks into the AB Library Builder™ Device" on page 24.

Thaw the reagents

Before starting, thaw the following reagents at room temperature, at 4°C, or on ice. Thaw an appropriate number of cartridges and 2X Ligase Buffer tubes for your experiment.

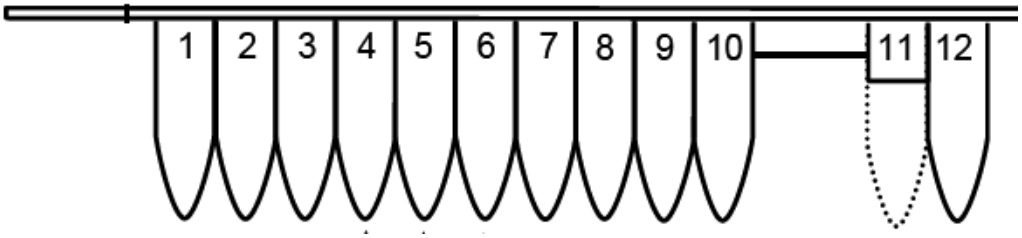
1. Thaw the AB Library Builder™ Whole Transcriptome Cartridge at room temperature or on ice for ≤2 hours or until completely thawed.

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

2. Thaw one tube of 2X Ligase Buffer for each cartridge you are using. Thaw at room temperature or on ice.

Cartridge configuration

Each cartridge has 12 compartments for reagents:



Cartridge compartment numbers	Volumes
1	1200 µL
2–3	1000 µL
4–6	20 µL
7	empty
8	20 µL
9–10	empty
11: Tube of 2X Ligase Buffer to be inserted	40 µL
12: Compartment for Agencourt® RNAClean® XP beads	400 µL

Insert or change the protocol card and power ON the instrument

IMPORTANT! Do not remove the protocol card while the instrument is on. Removing the card stops the run, and it may cause instrument data file loss. To remove the card, see step 3.

If you accidentally remove the protocol card during a run, power off the instrument immediately to minimize potential for instrument data loss.

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

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

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

2. Open the card slot.

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

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

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

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

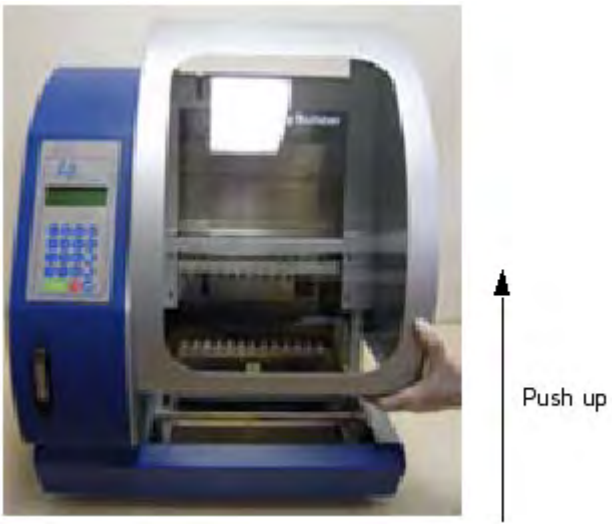
Load the racks and tubes

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

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

Remove the racks from the instrument

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



Load the cartridge rack

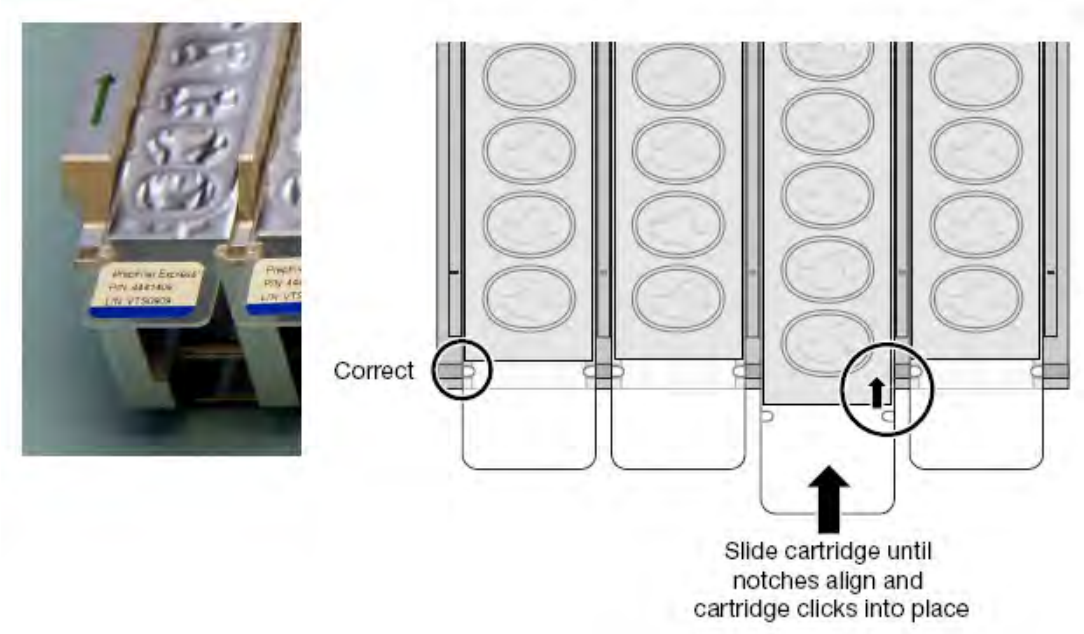
IMPORTANT! One cartridge is required per sample. Use only AB Library Builder™ Cartridges.

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

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

1. After each cartridge has thawed, gently tap the cartridge on the laboratory bench until any liquid droplets that might be underneath the foil seal or on the sides of the tube are deposited into the bottom of the well.
2. Load the cartridges into the cartridge rack by sliding each cartridge along the groove in the direction of the arrow until the cartridge clicks into place. Make sure that the notches in the cartridge align with the notches in the cartridge rack.

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



Load 2X Ligase Buffer in the cartridges

1. Pulse-spin a thawed tube of 2X Ligase Buffer.
2. Remove and discard the lid, and place the tube in the open position 11 of the cartridge.

Add Agencourt® RNAClean® XP Reagent to the cartridge

1. Thoroughly resuspend the Agencourt® RNAClean® XP Reagent by pipetting up and down.
2. Carefully peal the seal from compartment 12 on the cartridge, or pierce the seal with an unused pipette tip.

   IMPORTANT! Do not remove or pierce the foil seals covering compartments 1–10.
3. Carefully transfer 400 µL of Agencourt® RNAClean® XP Reagent to the unsealed compartment 12 on the cartridge.

Load the tip and tube rack

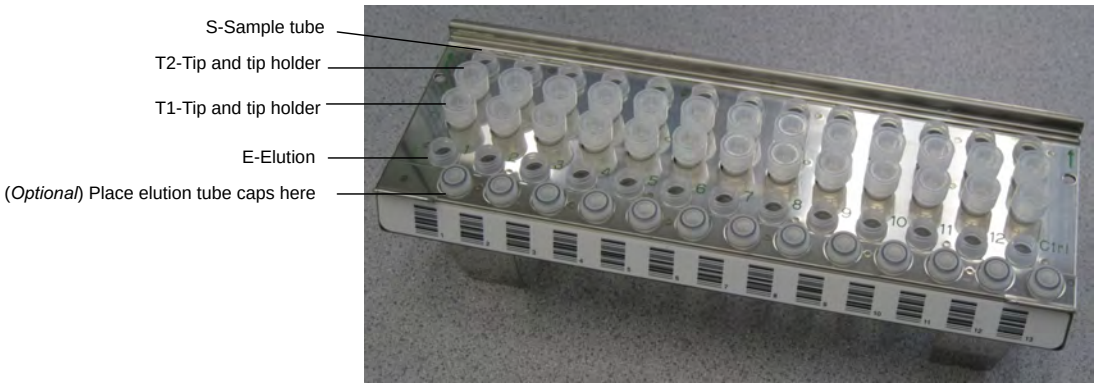
IMPORTANT! If you are processing fewer than 13 samples, make sure to load the tips and tubes in the same positions as the reagent cartridges that are loaded in the cartridge rack.

Load the tip and tube rack in the following order:

1. **Row S** (fourth row): Load with sample/elution tubes containing 5 µL of fragmented RNA. Make sure that the cap is off.
2. **Row T1 and T2** (second and third rows): Load with AB Library Builder™ Tips inserted into tip holders.

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

3. **Row E** (first row): Label the sample/elution tubes with the sample names for each library, and load into the rack with the caps removed and secured:



Insert the racks into the AB Library Builder™ Device

IMPORTANT!

Insert the cartridge rack before the tip and tube rack. Changing the order of loading the racks may cause the instrument to stop during a run.

Use only AB Library Builder™ Sample Tubes (sample/elution tubes). Other tubes may be picked up by the nozzle tips due to differences in tube height and shape, stopping the run.

Insert the cartridge rack

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

Insert the loaded cartridge rack into the instrument as shown below:

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

Insert the tip and tube rack

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

For additional instructions on instrument operation, see the *AB Library Builder™ System User Guide* (Part no. 4463421).

Start the run

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

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

   IMPORTANT! Do not open the door during a protocol run. To pause or cancel the run, see "AB Library Builder™ System operation" on page 48.

   Note: If you lose power or the power cord is unplugged, the run stops. When the power resumes, the digital display shows the Main menu. You cannot resume the run. If the tips are still on the syringe unit when the power resumes, return the tips to the original positions.
7. At the end of the run (the instrument beeps briefly and the digital display shows "Finished Protocol"). To unload the instrument:

   a. Press  to return to the Main menu, then open the instrument door.
   b. Remove the elution tubes. Confirm that they are properly labeled, then cap the elution tubes containing the library in ~24–48 µL.
   c. Place each elution tube in a DynaMag™-2 Magnetic Rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle; then transfer the ***supernatant*** to a new labeled tube.
   d. Remove the tip and tube rack and cartridge rack.

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

f. Close the instrument door.

g. Clean the tip and tube rack as needed.

Note: The heat block in the AB Library Builder™ must cool to <35°C before initiating another run. If the heat block temperature is >35°C, the instrument will signal the user to place the cartridges and samples at 4°C and wait until the instrument indicates that it is ready.

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

Set up for a new run

WARNING! Do not clean the instrument with acids, or bases (such as bleach). Acids and bases can react with the guanidine thiocyanate from iPrep™ kits and generate toxic gas.

Note: To set up for a new run using the *same* protocol card, leave the instrument on. To set up for a new run with a *different* protocol card, power off the instrument, then change the protocol card.

Amplify and quantitate the library

Amplify and purify the library

Note: For the following protocol, you may use either Agencourt® RNAClean® XP Reagent *or* AMPure® XP Reagent.

Prepare the reagents

1. Thaw the Platinum® High Fidelity SuperMix and SOLiD® PCR Primers on ice.

   Note: You may use either the PCR primers provided in the kit, or to create barcoded libraries, use the barcoded primers provided in any of the SOLiD® RNA Barcoding Kits listed on page 10.

2. Approximately 30 minutes before starting, resuspend the Agencourt® RNAClean® XP Reagent *or* AMPure® XP Reagent and allow the beads to come to room temperature.

Amplify the library

1. In a new 1.5-mL non-stick RNase-free tube, prepare the following master mix per library:

Component	Volume per amplification
Platinum® High Fidelity SuperMix	200 µL
SOLiD® 5' PCR Primer	5 µL
SOLiD® 3' PCR Primer (or barcoded PCR primer mix from a SOLiD® RNA Barcoding Kit, e.g SOLiD® 3' Primers: BC001–BC016)	5 µL
Total	210 µL

2. Divide the master mix into two 0.2-mL PCR tubes, and add half of the cDNA library (~12–24 µL) to each tube.
3. Vortex the reaction for 5 seconds, then pulse-spin.
4. Transfer the tubes to a thermal cycler and run the following program (see **Note** below on cycling conditions):

Stage	Step	Temp	Time
Holding	Denature	95°C	5 min
Cycling (12-15 cycles)	Denature	95°C	30 sec
	Anneal	62°C	30 sec
	Extend	72°C	30 sec
Holding	Extend	72°C	7 min
Holding	—	4°C	∞

Note: Run 15 cycles if you started with 50–100 ng of fragmented RNA. If necessary, adjust the number of cycles according to the amount of fragmented RNA, but for optimal results run between 12 and 18 cycles. Too many cycles will result in overamplification and change the expression profile.

Purify the amplified library

Note: For highest yield and greatest purity, we recommend performing two sequential cleanups as described below. Some users may find the results from a single cleanup to be acceptable for their specific application. In this case, to minimize adaptor-dimers in the final library that can interfere with emPCR, we recommend using a 1X volume (250 µL) of Agencourt® RNAClean® XP Reagent or AMPure® XP Reagent instead of the 1.6X volume in step 4 below. The second cleanup described in step 17 may then be omitted.

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

Component	Volume
RNase-Free Water	480 µL × *N*
Ethanol, Absolute	1120 µL × *N*
Total	**1600 µL × *N***

2. For every amplified library, label two new 1.5-mL non-stick RNase-free tubes.
3. Pool the two PCR reactions per library into one of the labeled 1.5-mL non-stick tubes. The total combined volume of the amplified library is ~260 µL.
4. Resuspend the RNAClean® XP Reagent or AMPure® XP Reagent (at room temperature) by pipetting up and down, and add 400 µL of Reagent (~1.6X) to the amplified library. (If you are performing a single cleanup, add 1X of Reagent. See the **Note** above.)
5. Vortex the mixture for 10 seconds, then pulse-spin.
6. Incubate the mixture at room temperature (20–25°C) for 5 minutes.
7. Place each tube in a DynaMag™-2 Magnetic Rack for at least 3 minutes until the solution is clear of brown tint when viewed at an angle; then, remove and discard the supernatant.
8. Wash the DNA 2 times with 70% ethanol. For each wash:
   a. Without removing the tube from the magnet, add 400 µL of *freshly prepared* 70% ethanol and incubate for 30 seconds. Do not disturb the pellet.
   b. Aspirate and discard ethanol.
9. Remove the tube from the magnet, pulse-spin the tube, and return the tube to the magnet for at least 1 minute until the solution clears.
10. Carefully remove and discard the supernatant with a 20-µL pipettor.
11. Open the tube and dry the beads on the magnet at room temperature (20–25°C) for ≤5 minutes.
12. Remove the tube from the magnet and add 50 µL of RNase-free water directly to the pellet to disperse the beads.
13. Vortex the beads for 10 seconds, then pulse-spin.

14. Incubate the beads for 2 minutes at room temperature.
15. Place the tube in the magnet for at least 1 minute until the solution clears.
16. Transfer the *supernatant* containing the amplified library to the second labeled 1.5-mL non-stick RNase-free tube.
17. Clean up the cDNA a second time: Add 80 µL (1.6X) of resuspended RNAClean® XP Reagent or AMPure® XP Reagent to each library, and then repeat steps 5–16 above. This removes adaptor-dimers that can interfere with emPCR.

Quantitate the DNA

Assess the yield and size distribution of the amplified DNA

Use a NanoDrop® Spectrophotometer, and the Agilent® 2100 Bioanalyzer™ Instrument with the DNA 1000 Kit (Agilent®).

1. Measure the concentration of the purified DNA with a NanoDrop® Spectrophotometer, and if necessary, dilute the DNA to <50 ng/µL for accurate quantitation with the DNA 1000 Kit.
2. Run 1 µL of the purified DNA on an Agilent® 2100 Bioanalyzer™ Instrument with the DNA 1000 Kit. Follow the manufacturer's instructions for performing the assay.
3. Using the 2100 expert software, perform a smear analysis to quantify the percentage of DNA that is 25–200 bp.

   Note: In general, we see a positive correlation between RNA-Seq mapping statistics and the percent of DNA library inserts that are above 200 bp in length. We recommend minimizing the percent of inserts that fall in the 25–200 bp range. Please use your judgement when deciding the best way to proceed with libraries from precious samples that fall close to these recommended quality values of more than 50% DNA in 25–200 bp range.

Percent of DNA in the 25–200 bp range	Next steps
Less than 50%	Proceed with templated bead preparation for the 5500 Genetic Analysis System.

Percent of DNA in the 25–200 bp range	Next steps
50-60%	You may perform another round of purification and size-selection on the amplified DNA using Agencourt® RNAClean® XP Reagent or AMPure® XP Reagent: 1. Bring the sample volume to 50 µL with RNase-free Water. 2. Use RNAClean® XP Reagent or AMPure® XP Reagent to decrease the % of amplified DNA in the 25–200 bp range. Start with step 17 at the end of the "Purify the amplified library" above, but elute with 35 µL of RNase-free Water (instead of the 50 µL used in step 12). OR Alternatively, you may proceed with templated bead preparation and expect to see slightly higher % of filtered reads in your sequencing data when compared to libraries with less than 50% in the 25–200 bp range.
More than 60%	We recommend performing another round of purification and size-selection. See step 1 and step 2 above.

Note: For instructions on how to perform the smear analysis, refer to the *Agilent® 2100 Bioanalyzer™ Expert User's Guide*.

4. Determine the median peak size (bp) and molar concentration (nM) of the cDNA library using the Agilent® software.

   Note: The mass concentration of the cDNA must be <50 ng/µL for accurate quantitation with the DNA 1000 Kit.

   Alternatively, obtain the mass concentration by another method, and convert the mass concentration to molar concentration.

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

Proceed with templated bead preparation

When the amplified DNA in the 25–200 bp range is minimal, you can proceed with templated bead preparation for the 5500 Genetic Analysis System, in which each library template is clonally amplified on SOLiD™ P1 DNA Beads by emulsion PCR. Refer to the *SOLiD™ EZ Bead Emulsifier Getting Started Guide* (Part no. 4441486).

Barcoded libraries are pooled prior to templated bead preparation. For more information refer to the product insert for your barcode kit.

Note: When optimizing library concentrations (singleplex or multiplex sequencing pools) by workflow analysis (WFA), library concentrations of 0.4 pM and 0.8 pM for ePCR are recommended.

Typical size profiles of amplified libraries

Typical size distributions (Agilent® 2100 Bioanalyzer™ Instrument profiles) of amplified libraries prepared from HeLa poly(A) RNA (Figure 6), HeLa WT Control total RNA (Figure 7), and rRNA-depleted HeLa RNA (Figure 8) using the AB Library Builder™ Whole Transcriptome Core Kit are shown.

Figure 6 Size distribution of amplified library prepared from HeLa poly(A) RNA

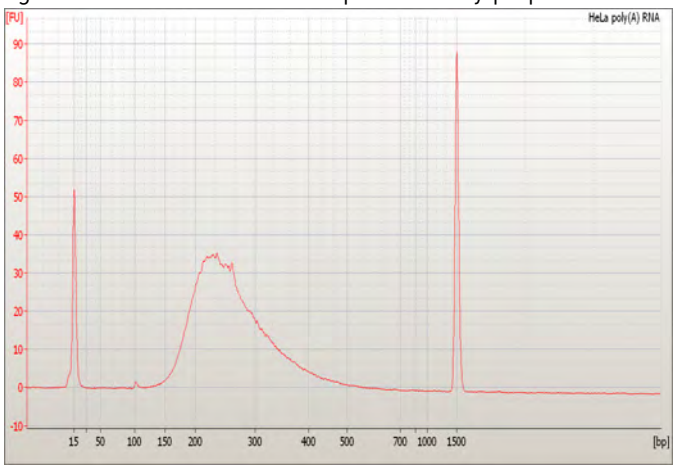


Figure 7 Size distribution of amplified library prepared from WT control total RNA

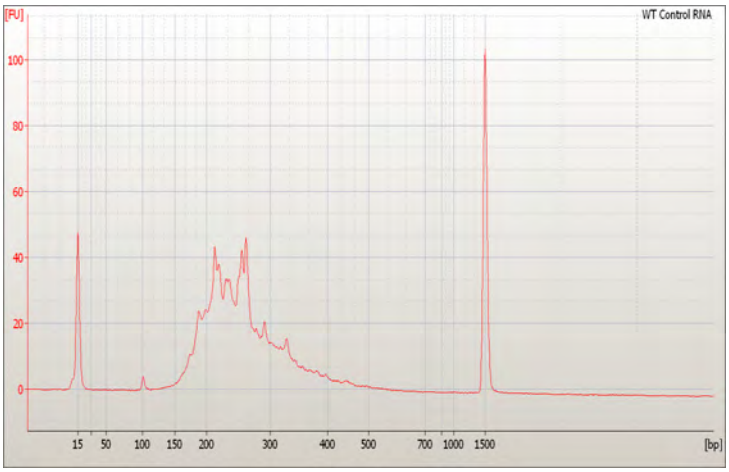
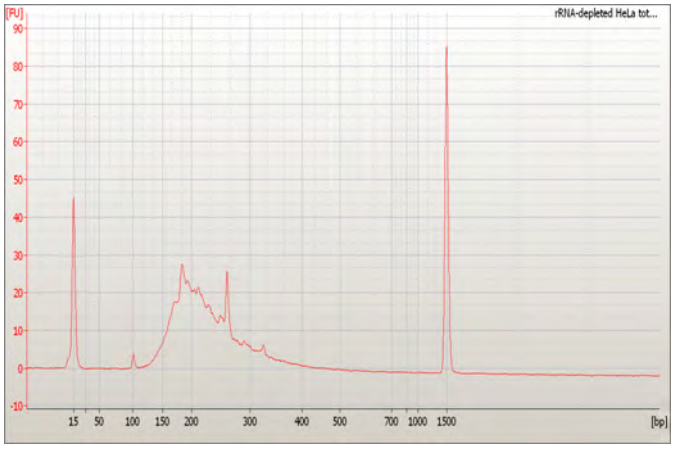


Figure 8 Size distribution of amplified library prepared from rRNA-depleted HeLa total RNA



Expected yields

The recovery of your experimental RNA will depend on its source and quality. The following results are typically seen with Human Brain Reference and HeLa RNAs.

Workflow	Input amount	Typical recovery amount
Fragment the whole transcriptome RNA	500 ng poly(A) RNA, total RNA, or rRNA-depleted total RNA	300–400 ng RNA
Amplify and purify the library	50–100 ng fragmented RNA	>100 ng cDNA

Troubleshooting

For symptoms other than those listed in this section, contact Technical Support ("Documentation and Support" on page 51).

Observation	Possible Cause	Recommended action
Before the run		
Check D-rings on instrument	—	Periodically grease or replace the D-rings on the instrument as needed.
During the automated run		
No power (the digital display is blank and the fan does not turn on when you power on)	AC power cord is not connected	Check AC power cord connections at both ends. Use the correct cords.
	Fuse has blown	Check the integrity of the fuse and replace it if necessary (refer to the *AB Library Builder™ System User Guide*). If the problem persists after connecting the correct power cord and replacing the fuse, contact Technical Support.
The digital display is blank, but the fan turns on when you power on.	Protocol card is not inserted correctly	Power off the instrument and re-insert the protocol card in the proper orientation into the card slot. Insert it completely into the slot by manually pushing the card.
	Protocol card was inserted when the instrument was powered on	Power off the instrument, then power on the instrument.
Error code displayed	—	See "Instrument error codes" on page 38.
Reagent cartridges, tips, or tubes are not inserted in the correct positions.	—	Press **STOP** to pause the run. Open the door, add the missing items, then press **START** to resume the run. Do not open the door without pausing the run.

Observation	Possible Cause	Recommended action
Run stops after an initial start (you may also see an error code).	• Instrument door opened during the run • Reagent cartridges, tips, or tubes incorrectly loaded in the rack • Racks incorrectly loaded on the instrument	IMPORTANT! If you open the instrument door while the instrument is running, the run stops, and it cannot be restarted. If you need to open the instrument door during a run, first press **Stop** to pause the run, then open the door. 1. Follow the procedure in "Instrument error codes" on page 38. 2. Before starting a new run, make sure that the reagent cartridges, tips, and tubes are correctly loaded: • Slide the reagent cartridges into the cartridge rack as described in "Load the racks and tubes" on page 21. • Load the cartridge rack before the tip and tube rack for proper positioning. • Do not cap the tubes. 3. If the instrument continues to stop during the run, contact Applied Biosystems Technical Support.
	Reagent cartridges not completely thawed	1. Stop the run. 2. Remove the tip and tube rack, then remove the cartridge rack. 3. Inspect cartridge wells 2 and 3 for ice. 4. If any well is frozen, close the door to the AB Library Builder™ Device, then thaw the cartridges completely. 5. Replace the tips in position T2. 6. Insert the cartridge rack then the tip and tube rack onto the AB Library Builder™ Device. 7. Restart the run.
No DNA yield	No sample added to tube	Add samples to tubes, load new reagent cartridges, then perform the run again.
No liquid in tip, or liquid in tip not moving	No sample added to tube, leading to wet filter barrier on the tip and blockage of nozzles	Add samples to tubes, load new reagent cartridges, then perform the run again.
Buffer in the bottom tray	Motor movements are not smooth	Schedule preventive maintenance annually to ensure proper motor movements.
	Reagent cartridges, tips, or tubes incorrectly loaded in the rack	If you are processing fewer than 13 samples, make sure to load the tips and tubes in the same positions as the reagent cartridges that are loaded in the cartridge rack. See below for leakage from tips.
Leakage from tips or uneven liquid handling between nozzles	D-Rings are not greased regularly or they need replacement	You can continue the run, but maintain the D-rings as scheduled. To prevent leakage, maintain or replace the D-rings (refer to the (*AB Library Builder™ System User Guide*).
Blockage of tips	Too much starting material causing clumps or aggregates	Contact Technical Support. In future runs, use the sample volume recommended in the user guide for the kit you are using.

Observation	Possible Cause	Recommended action
After the automated run		
No elution volume	Sample volume is lower than the recommended volume, leading to wet filter barrier on the tip and blockage of nozzles.	In future runs, use the recommended sample volume for the protocol you are using. Long-term operation with lower-than-recommended sample volumes can lead to issues with liquid handling performance.
Observed DNA peak size is significantly different from the expected DNA peak size	Enzymes or buffer not at bottom of wells	Tap the wells down against a hard surface to move enzymes and buffer to bottom of wells, then inspect the wells.
Final library is brownish	Beads in final library	1. Place the tube with the final library in a DynaMag™-2 Magnetic Rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle. 2. Without disturbing the pellet, carefully transfer the supernatant, which contains the final library, to a new 1.5-mL non-stick RNase-free tube.
Agilent® software doesn't calculate one concentration and peak size	The software detects multiple peaks in the amplified cDNA profile	Refer to "Analyze multiple peaks as one peak" on page 48.
Low yield and poor size distribution in the amplified library	You recovered <20% of the input RNA after you fragmented and cleaned up the RNA	Decrease the RNase III digestion from 10 minutes to 5 minutes (see "Fragment the RNA using RNase III" on page 14).
Low yield in the amplified library and very few differences in the Agilent® 2100 Bioanalyzer™ Instrument traces before and after you fragment the RNA	RNA fragmentation failed	Purify the RNA sample again to remove the extra salts that may affect the RNase III activity. If RNA fragmentation still fails, increase the RNase III digestion from 10 minutes to 20 minutes (see "Fragment the RNA using RNase III" on page 14). For chemical fragmentation procedure, slowly increase the fragmentation time to 12–15 minutes.
Low yield and no PCR products	Too much ethanol introduced into PCR reaction.	Use optional drying step in bead clean up protocol.

Observation	Possible Cause	Recommended action
	An enzymatic reaction or purification procedure failed	1. Dilute the cDNA 1:10, then use 1 µL in a 100-µL PCR. 2. Check the yield before and after purification. 3. If you get the same results, repeat the AB Library Builder™ procedure with more fragmented RNA, and run a parallel procedure with fragmented Control RNA.
Normal or high yield but PCR products larger than 300 bp	Too many PCR cycles resulted in overamplification	Decrease the number of PCR cycles (see "Amplify the library" on page 29)

Instrument error codes

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

If you observe an error code:

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

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

2. Press **ESC** to return to the Main menu.
3. If there are tips attached to the nozzles, press **1** to select the Manual screen, then press **2** to return the tips to the original position.
4. Power OFF the instrument, remove the protocol card, wait 5 minutes, insert the protocol card, then power on the instrument.
5. Run the axis test (refer to the *Fragment Library Preparation Using the AB Library Builder™ System*).

6. If the axis test:
    - Is successful, start a new extraction run. Use new samples and plastics where required.
    - Is *not* successful, contact Technical Support.

Safety

General chemical safety

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

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

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 42.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

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

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

Obtaining SDSs

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

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

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

Chemical waste safety

Chemical waste hazards

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

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

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

Chemical waste safety guidelines

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

To minimize the hazards of chemical waste:

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

Waste disposal

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

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

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

Biological hazard safety

General biohazard

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

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

Additional information about biohazard guidelines is available at:
www.cdc.gov

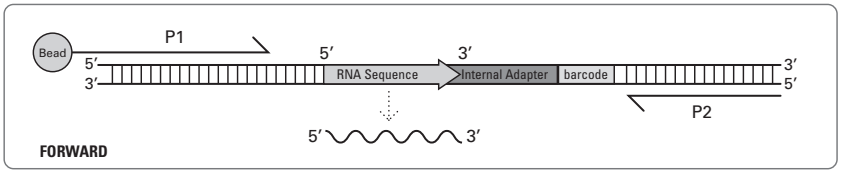
Supplemental Information

Amplified library construction concepts

Hybridization and ligation to the Adaptor Mix

The RNA samples are hybridized and ligated with the SOLiD™ Adaptor Mix. The SOLiD™ Adaptor Mix is a set of oligonucleotides with a single-stranded degenerate sequence at one end and a defined sequence required for SOLiD™ System sequencing at the other end. The SOLiD™ Adaptor Mix constrains the orientation of the RNA in the ligation reaction such that hybridization with the SOLiD™ Adaptor Mix yields template for SOLiD System sequencing from the 5′ end of the sense strand. The downstream emulsion PCR primer alignment and the resulting products of templated bead preparation for SOLiD System sequencing are illustrated below.

Figure 9 Strand-specific RNA sequence information



Sequences of the SOLiD® primers included in the kit

SOLiD® 5′ PCR primer

The 5' PCR primer is the SOLiD® emulsion PCR primer P1.

5′- CCA CTA CGC CTC CGC TTT CCT CTC TAT GGG CAG TCG GTG AT -3′

SOLiD® 3′ PCR primer

5′- CTG CCC CGG GTT CCT CAT TCT CTG TGT AAG AGG CTG CTG TAC GGC CAA GGC G -3′

About the RNA fragmentation methods

To generate whole transcriptome cDNA libraries with insert sizes suitable for SOLiD™ sequencing, you need to fragment the poly(A) RNA or rRNA-depleted total RNA to 100–200 nt before proceeding to ligation.

In this guide, we provide procedures for two methods of RNA fragmentation. Select the fragmentation method from the two options presented by using the guidance below:

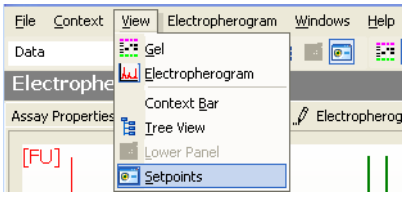
- **RNase III fragmentation**: Provides convenience and robustness for comprehensive transcriptome analysis. RNase III digestion leaves 5′ phosphate and 3′ hydroxyl groups on the RNA fragments, and the resulting RNA fragments can be directly used in the hybridization and ligation reactions. The RNase III fragmentation method produces more consistent sequencing results because this method is effective across a wide range of enzyme activities, incubation times, and input amounts. We have observed RNase III fragmentation results in lower amounts of rRNA fragments being sequenced than when chemical fragmentation is used. This is assumed to be related to the role RNase III has in processing rRNAs. This method is useful for performing transcript-level quantification.
- **Chemical fragmentation**: The resulting RNA fragments need to be repaired using T4 polynucleotide kinase before proceeding to ligation. Because RNA fragmentation by chemical hydrolysis occurs rapidly, we recommend that you determine the optimal conditions for your experimental system. As noted above, we have observed a higher percentage of rRNA sequences are present in libraries constructed from chemically fragmented RNA than with RNase III fragmented RNA. Therefore, if minimizing rRNA sequences in the final library is very important, RNase III should be considered for fragmentation. The chemical fragmentation method produces a more uniform distribution of sequence tags across the transcripts. This method is useful for quantifying exon levels, identifying novel splicing, and discovering fusion genes.

Using 2100 expert software to assess whole transcriptome libraries

Perform a smear analysis

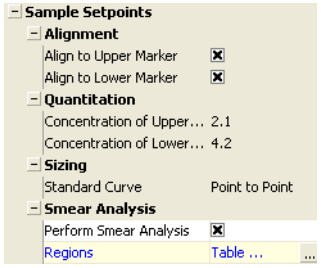
Perform a smear analysis to quantify the percentage of DNA in the 25–200 bp size range.

1. In the 2100 expert software, select **View** ▸ **Setpoints**.



2. On the **Global** tab, select **Advanced** settings.

3. In the Sample Setpoints section of the Advanced settings, select the **Perform Smear Analysis** checkbox, then double-click **Table**.

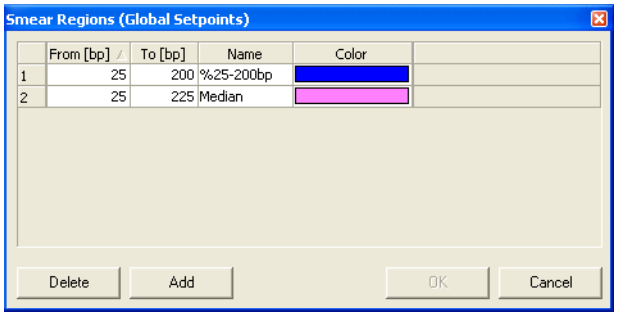


4. Set the smear regions in the Smear Regions dialog box:
   a. Click **Add**, then enter 25 bp and 200 bp for the lower and upper limits, respectively.

      These settings are used to determine the percentage of total product that is 25–200 bp in length.

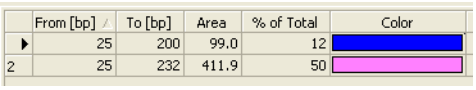
   b. Click **Add**, enter 25 bp and 225 bp, then click **OK**.

      This is an arbitrary upper limit which is used to determine the median size.



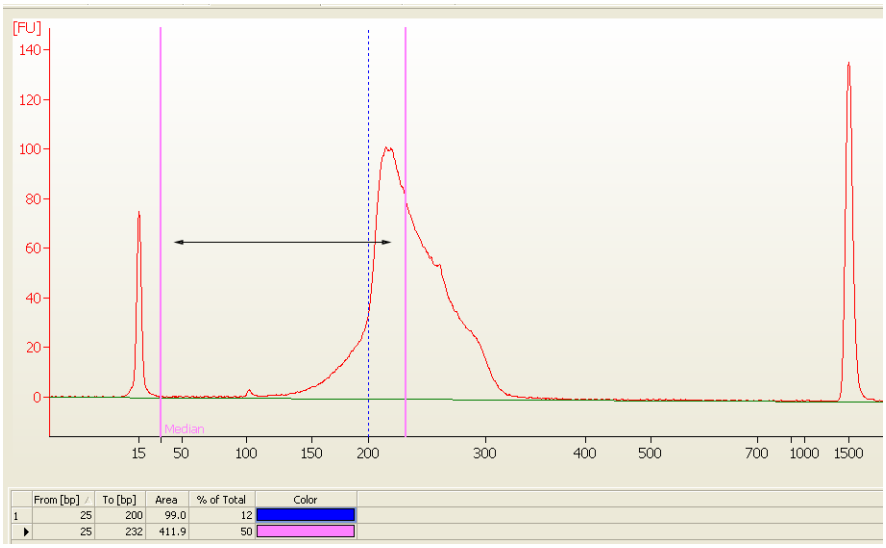
5. Select the **Region Table** tab.

6. In the Region Table, review the percentage of the total product in the size ranges you set.



Determine the median size

On the Region Table tab, drag the upper limit line that you set to the left or right until the Region Table indicates 50% of Total.

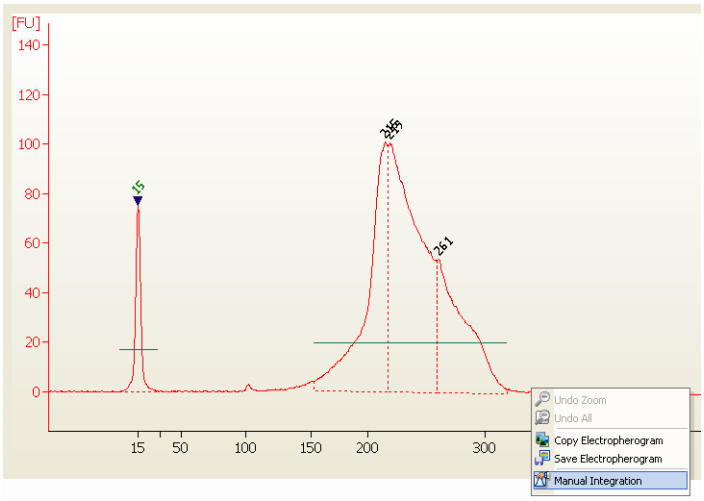


Analyze multiple peaks as one peak

On the Peak Table tab, you may observe that the bioanalyzer software identified multiple peaks in a region that you want to consider as one peak. To obtain one concentration and automatically determine median size for a peak region, manually set the size range of the desired peak region.

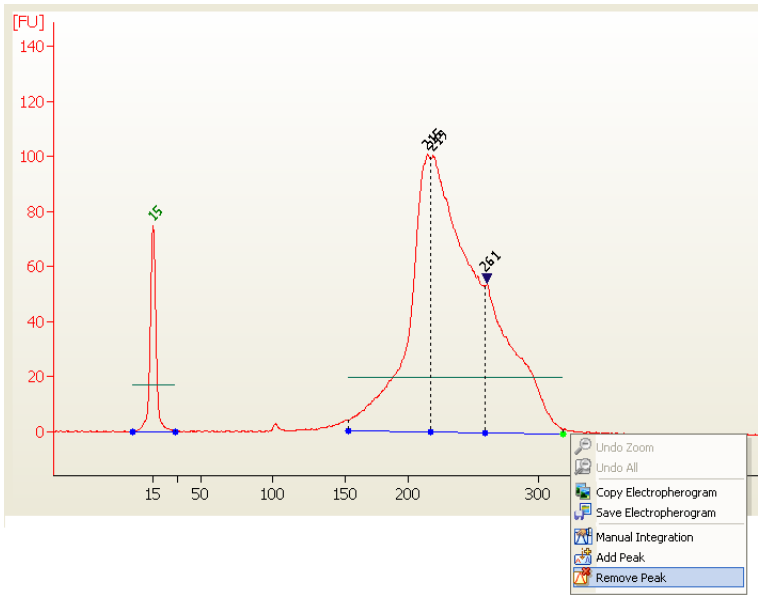
1. In the bottom left corner of the software window, select the **Peak Table** tab.

2. Right-click anywhere on the electropherogram, then select **Manual Integration**.



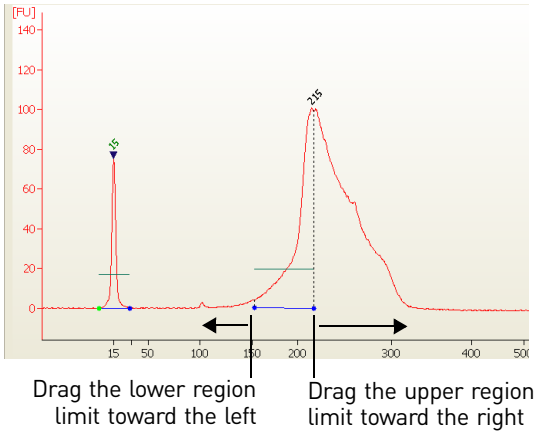
3. To remove multiple peaks:

a. Place the cursor on the peak to remove, right-click, then select **Remove Peak**.

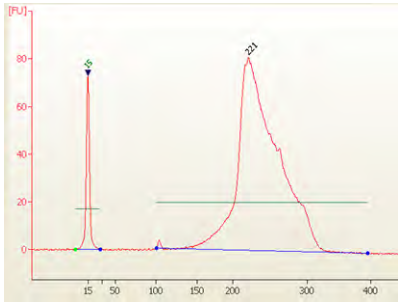


b. Repeat until one peak remains within the region of interest

c. Drag the lower and upper region limits of the region until the entire library is included.



4. The software recalculates the median size (bp), concentration (ng/µL), and molarity (nM) of the peak region and displays the values in the Peak Table.



		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
▶	◀	15	4.20	424.2	Lower Marker
2		221	28.18	193.3	
3	▶	1,500	2.10	2.1	Upper Marker

Documentation and Support

Related documentation

AB Library Builder™ System User Guide (Part no. 4463421)

Fragment Library Preparation Using the AB Library Builder™ System (Part no. 4460965)

SOLiD® Total RNA-Seq Kit Protocol (Part no. 4452437)

Obtaining support

For the latest services and support information for all locations, visit:
www.lifetechnologies.com or **www.appliedbiosystems.com.**

At the web sites, you can:

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

Glossary

barcode	A short, unique sequence that is incorporated into a library that enables identification of the library during multiplex sequencing.
barcoded library	A library that has a unique barcode sequence incorporated that enables identification of the library during multiplex sequencing.
fragment library	A library that has a single insert prepared from genomic DNA for sequencing. Fragment libraries compatible with the 5500 Series Genetic Analysis System can be sequenced with a forward-only run or with a paired-end run.
internal adaptor (IA)	The internal adaptor sequence is incorporated into the template during library construction and provides a common hybridization target for SOLiD® sequencing primers. The IA sequence is different in DNA-source libraries and RNA-source libraries, therefore sequencing primers specific for RNA and DNA libraries must be used for reverse reads (F5 tag). The IA-containing adaptors used during mate-paired library preparation are different from the adaptors used for fragment library preparation, but the sequencing primers used for forward reads originating in the IA sequence (R3 and BC tags) are the same. See the 5500 Series SOLiD® Systems Sequencing Products Ordering Guide for a schematic of sequencing primers compatible with each type of SOLiD® library.
library	A set of DNA or cDNA molecules prepared from the same biological specimen and prepared for sequencing on the 5500 Series Genetic Analysis System.
SOLiD® 5' PCR Primer	Single-stranded oligonucleotide used in library amplification.
SOLiD® 3' PCR Primer	Single-stranded oligonucleotide used in library amplification.
barcoded PCR primers	A set of 3' PCR primers each with one of 96 unique barcode sequences. To be used with the standard SOLiD 5' PCR Primer.
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.

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

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