Applied Biosystems

SOLiD™ Whole Transcriptome Analysis Kit

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Contents

Safety information

Note: For general safety information, see Appendix B, "Safety" on page 35.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "MSDSs" on page 36.

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

How to use this guide

Text conventions

This guide uses the following conventions:

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

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

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

  Select **File ▸ Open ▸ Spot Set**.

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

User attention words

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

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

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

How to obtain support

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

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

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

SOLiD™ Whole Transcriptome Analysis Kit

Product information

Purpose of the product

Use the SOLiD™ Whole Transcriptome Analysis Kit (PN 4425680) to convert the full set of RNA transcripts expressed in a cell or tissue into a cDNA library for transcriptome analysis on the Applied Biosystems SOLiD™ Sequencing System.

High throughput sequencing of the transcriptome using the SOLiD System enables genome-wide expression profiling with high sensitivity and a wider dynamic range than microarray technology. Also, whole transcriptome library preparation performed as described in this protocol preserves the strandedness of the RNA transcripts. Preserving the strandedness simplifies data analysis, allows determination of the directionality of transcription and gene orientation, and facilitates detection of opposing and overlapping transcripts. Refer to "Overview of the SOLiD™ Whole Transcriptome Analysis Kit procedure" on page 25 and the workflow on page 4 for further details.

When used with the SOLiD™ Transcriptome Multiplexing Kit (PN 4427046), the SOLiD Whole Transcriptome Analysis Kit can be used to prepare "barcoded" libraries to enable sequencing of multiple transcriptome samples in a single, multiplexed, SOLiD System sequencing run. Sequencing of multiplexed libraries is fully supported by the SOLiD 3 System.

Kit contents

Sufficient reagents are supplied in the SOLiD Whole Transcriptome Analysis Kit to prepare cDNA libraries from 12 samples for high-throughput sequencing with the SOLiD System.

Table 1 SOLiD™ Whole Transcriptome Analysis Kit components

Component	Amount	Cap
10× RNase III Buffer	20 µL	red
RNase III	20 µL	red
2× Ligation Buffer	150 µL	green
Adaptor Mix A	30 µL	green
Adaptor Mix B	30 µL	purple
Hybridization Solution	40 µL	green
Ligation Enzyme Mix	30 µL	green
10× RT Buffer	50 µL	yellow
ArrayScript™ Reverse Transcriptase	20 µL	yellow
10×PCR Buffer	660 µL	white
AmpliTaq® DNA Polymerase	110 µL	white

Table 1 SOLiD™ Whole Transcriptome Analysis Kit components *(continued)*

Component	Amount	Cap
dNTP Mix	500 µL	white
SOLiD™ 5′ PCR Primer	100 µL	white
SOLiD™ 3′ PCR Primer	100 µL	blue
Control RNA (1 µg/µL)‡	50 µL	clear
Nuclease-free Water	1.75 mL	clear

‡ HeLa Total RNA

Storage

Upon receipt of the SOLiD Whole Transcriptome Analysis Kit, immediately store the components at –20 °C.

You may store the Nuclease-free Water at room temperature, 4 °C, or –20 °C.

Materials and equipment not included

Equipment

Item	Source
Thermal cycler with heated lid, capable of holding 0.2-mL tubes: • Veriti® 96-Well Thermal Cycler • GeneAmp® PCR System 9700	Applied Biosystems
Qubit® Fluorometer	Invitrogen PN Q32857
XCell *SureLock*™ Mini-Cell	Invitrogen PN EI0001
Agilent 2100 Bioanalzyer	Agilent PN G2938A
NanoDrop™ Spectrophotometer **Note:** May be used instead of the Qubit Fluorometer, but the results from the NanoDrop Spectrophotometer are less accurate.	Thermo Scientific
Centrifugal vacuum concentrator (for example, SpeedVac)	MLS
Microcentrifuge	MLS
Pipettors, positive displacement or air-displacement	MLS
Transilluminator	MLS

Supplies

Item	Source
8-strip PCR Tubes & Caps, RNase-free, 0.2-mL	Applied Biosystems PN AM12230
Non-Stick RNase-free Microfuge Tubes (0.5 mL), 500	Applied Biosystems PN AM12350
Non-Stick RNase-free Microfuge Tubes (1.5 mL), 250	Applied Biosystems PN AM12450
Pipette tips, RNase-free	MLS

Reagents

Item	Source
Nuclease-free Water (not DEPC-treated), 100 mL	Applied Biosystems PN AM9938
50 bp DNA Ladder‡	Invitrogen PN 10416-014
Novex® 6% TBE-Urea Gels 1.0 mm, 10 well‡	Invitrogen PN EC6865BOX
Novex® TBE-Urea Sample Buffer (2×), 10 mL‡	Invitrogen PN LC6876
Novex® TBE Running Buffer (5×), 1 L‡	Invitrogen PN LC6675
PureLink™ PCR Micro Kit, 50 preps‡	Invitrogen PN K310050
PureLink™ RNA Micro Kit, 50 preps‡ **Note:** This kit is required only if the RiboMinus Concentration Module is not available.	Invitrogen PN 12183016
Quant-iT™ RNA Assay Kit, 100 assays‡	Invitrogen PN Q32852
RiboMinus™ Concentration Module, 6 preps‡ **Note:** The RiboMinus™ Concentration Module is not equivalent to the RiboMinus™ Eukaryote Kit for RNA-Seq or to the RiboMinus™ Plant Kit for RNA-Seq.	Invitrogen PN K1550-05
SYBR® Gold nucleic acid gel stain, 10,000× concentrate in DMSO, 500 µL‡	Invitrogen PN S-11494
Agilent DNA 1000 Kit‡	Agilent PN 5067-1504
Agilent RNA 6000 Pico Chip Kit‡	Agilent PN 5067-1513
MinElute® PCR Purification Kit (50)‡	Qiagen PN 28004
Ethanol, 100%, ACS reagent grade or equivalent‡	MLS

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

(Optional) Barcoded primers for multiplex SOLiD™ System sequencing

The Applied Biosystems SOLiD™ Transcriptome Multiplexing Kit (PN 4427046) includes a set of 16 SOLiD 3′ PCR Primers that are individually "barcoded" for multiplex SOLiD System sequencing. Instructions for using the barcoded primers are included in this protocol.

Workflow

Fragmentation of whole transcriptome RNA

0.1–1 µg poly(A) RNA or 0.2–1 µg total RNA or rRNA-depleted total RNA

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Fragment the RNA (page 5)

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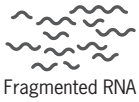
Clean up the RNA (page 6)



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Assess the yield and size distribution of the fragmented RNA (page 7)

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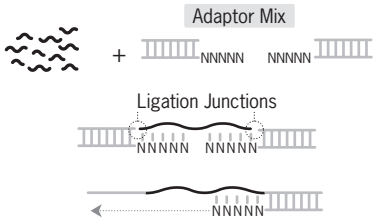
Amplified library construction

Hybridize and ligate the RNA (page 10)



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Perform reverse transcription (page 11)

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Purify the cDNA (page 12)

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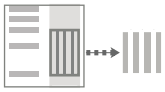
Size select the cDNA (page 13)



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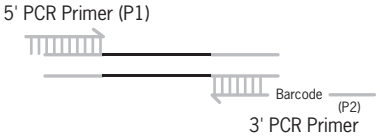
Amplify the cDNA (page 17)



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Purify the amplified DNA (page 18)

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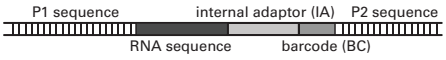
Assess the yield and size distribution of the amplified DNA (page 19)



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SOLiD™ System templated bead preparation and sequencing

Proceed with the *Applied Biosystems SOLiD™ 3 System Templated Bead Preparation Guide* (PN 4407421)

Fragmentation of whole transcriptome RNA

Overview

Fragmentation of the whole transcriptome RNA involves the following procedures:

1. Fragment the RNA (below)
2. Clean up the RNA (page 6)
3. Assess the yield and size distribution of the fragmented RNA (page 7)

RNA sample type and amount

Use 0.1–1 µg poly(A) RNA or 0.2–1 µg total RNA or rRNA-depleted total RNA.

- For poly(A) RNA, Applied Biosystems recommends performing two rounds of oligo(dT) selection of the poly(A) RNA; for example, use the Applied Biosystems Poly(A)Purist™ Kit (PN AM1916). Also, confirm the absence of 18S and 28S rRNA; for example, check the profile of the poly(A) RNA on an Agilent 2100 Bioanalyzer.
- For rRNA-depleted total RNA, Applied Biosystems recommends that you remove rRNA from total RNA for transcriptome analysis using the Invitrogen RiboMinus™ Eukaryote Kit for RNA-Seq (PN A1083708) or the Invitrogen RiboMinus™ Plant Kit for RNA-Seq (PN A1083808).

Use only high-quality RNA as your starting material. FirstChoice® Total RNA and Poly(A) RNA provide high-quality, intact RNA isolated from a variety of sources.

Fragment the RNA

Use components from the SOLiD™ Whole Transcriptome Analysis Kit:

- Nuclease-free Water
- 10× RNase III Buffer
- RNase III

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

Component	Volume
RNA sample: • Poly(A) RNA: 0.1–1 µg • Total RNA: 0.2–1 µg • rRNA-depleted total RNA: 0.2–1 µg	≤8 µL
Nuclease-free Water	to 8 µL
10× RNase III Buffer	1 µL
RNase III	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 37 °C for 10 minutes.

4. *Immediately* after the incubation, add 90 µL of Nuclease-free Water, then place the fragmented RNA on ice. Go to the next step immediately, or leave the fragmented RNA on ice for less than 1 hour.

Clean up the RNA

Use the RiboMinus™ Concentration Module (Invitrogen).

Alternatively, you can use the PureLink™ RNA Micro Kit (Invitrogen) (page 27).

1. Prepare the Wash Buffer (W5) with ethanol, then store at room temperature:

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

2. Add to the fragmented RNA, then mix well:

Component	Volume
Binding Buffer (L3)	100 µL
100% ethanol	250 µL

3. 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 flowthrough.
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 flowthrough.
   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 20 µL of RNase-Free Water to the center of the Spin Column.
   c. Wait 1 minute, then spin the column at maximum speed for 1 minute.

Assess the yield and size distribution of the fragmented RNA

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

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* by Invitrogen for instructions.

   Note: The Quant-iT RNA Assay Kit requires more than 5 ng RNA in each assay for accurate quantitation. If you started with less than 200 ng RNA, use 2–3 µL for the Quant-iT RNA Assay or concentrate your sample with a centrifugal vacuum concentrator.

2. Assess the size distribution of the fragmented RNA:

   a. Dilute the RNA to less than 5 ng/µL.

   b. Run 1 µL on an Agilent 2100 bioanalyzer 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 average size should be 100–200 nt. See Figures 1 and 2 on page 8.

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

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

Amount of fragmented RNA in 3 µL	Instructions
• ≥50 ng poly(A) RNA • ≥100 ng rRNA-depleted total RNA	Proceed with "Amplified library construction" on page 9. 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 3 µL Nuclease-free Water, then proceed with "Amplified library construction" on page 9.

Typical results of fragmentation of whole transcriptome RNA

Figure 1 and Figure 2 show profiles from an Agilent 2100 Bioanalyzer after RNase III fragmentation and cleanup. Figure 1 shows results with HeLa poly(A) RNA. Figure 2 shows results with rRNA-depleted HeLa RNA.

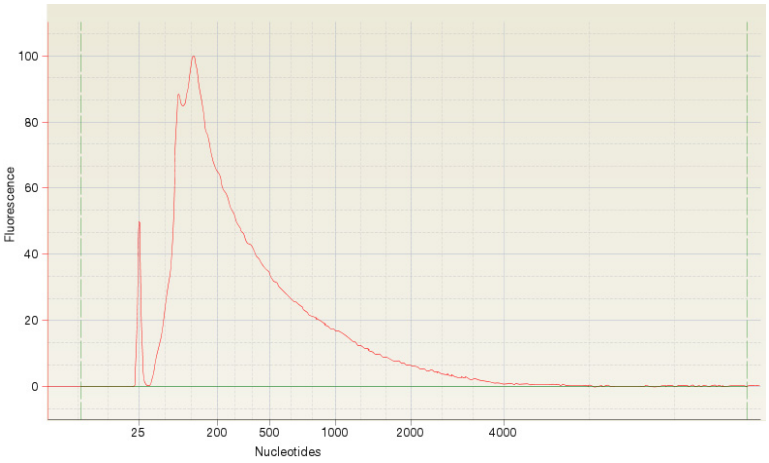


Figure 1 Size distribution of fragmented HeLa poly(A) RNA

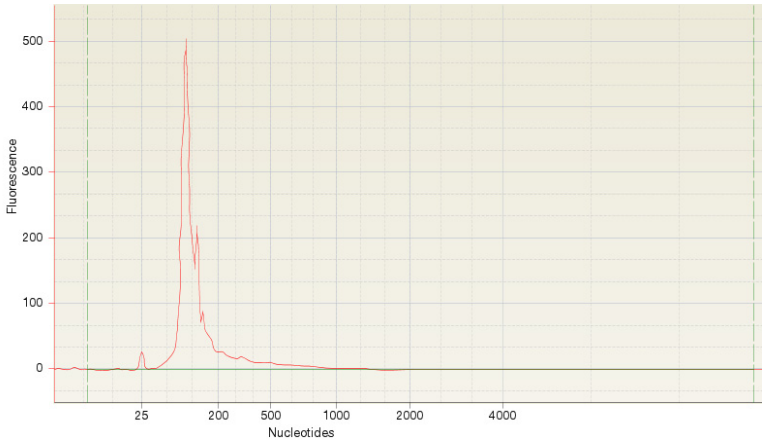


Figure 2 Size distribution of fragmented rRNA-depleted HeLa RNA

Amplified library construction

Overview

Amplified library construction involves the following procedures:

1. Hybridize and ligate the RNA (page 10)
2. Perform reverse transcription (page 11)
3. Purify the cDNA (page 12)
4. Size select the cDNA (page 13)
5. Amplify the cDNA (page 17)
6. Purify the amplified DNA (page 18)
7. Assess the yield and size distribution of the amplified DNA (page 19)
8. Proceed with SOLiD™ System templated bead preparation (page 20)

Choose Adaptor Mix A or Adaptor Mix B for use

- If you are preparing only one whole transcriptome library, use Adaptor Mix A; this will prepare template for SOLiD™ System sequencing from the 5′ end of the sense strand of the RNA.
- To achieve higher confidence in the complete sequence of larger RNAs in the whole transcriptome, prepare two separate whole transcriptome RNA libraries, using Adaptor Mix A in the hybridization reaction for one library and Adaptor Mix B in the hybridization reaction for the other library. (Do not mix the libraries for SOLiD System sequencing unless they are barcoded.)

  Adaptor Mix B yields template for sequencing the reverse complement of the RNA, providing sequencing information from the 3′ end of the sense strand.

For further information, refer to "Overview of the SOLiD™ Whole Transcriptome Analysis Kit procedure" on page 25.

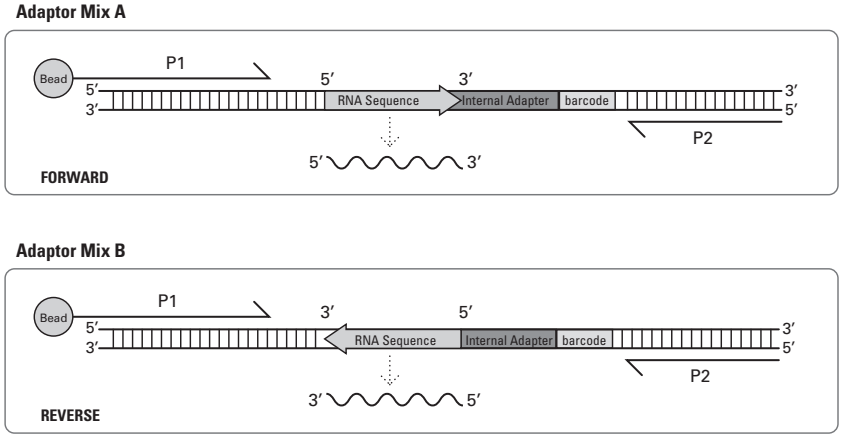


Figure 3 Adaptor Mix choice and RNA sequence

Hybridize and ligate the RNA

Use components from the SOLiD™ Whole Transcriptome Analysis Kit:

- Adaptor Mix A or Adaptor Mix B
- Hybridization Solution
- Nuclease-free Water
- 2× Ligation Buffer
- Ligation Enzyme Mix

1. On ice, prepare the hybridization mix in 0.2 mL PCR tubes:

Component	Volume
Adaptor Mix A or B‡	2 µL
Hybridization Solution	3 µL
Fragmented RNA sample: • Poly(A) RNA: 50 ng • Total RNA: 100 ng • rRNA-depleted total RNA: 100 ng	3 µL
Total volume per reaction	8 µL

‡ Use Adaptor Mix A for SOLiD System sequencing from the 5′ end. Use Adaptor Mix B for sequencing from the 3′ end. To sequence the RNAs from both the 5′ and 3′ ends, set up two ligation reactions, each with one Adaptor Mix.

2. Slowly pipet up and down a few times to mix well, then spin briefly.

3. Run the hybridization reaction in a thermal cycler:

Temperature	Time
65 °C	10 min
16 °C	5 min

4. Add the RNA ligation reagents to the 8-µL hybridization reactions:

Component (add in order shown)	Volume
2× Ligation Buffer‡	10 µL
Ligation Enzyme Mix	2 µL

‡ 2× Ligation Buffer is very viscous; pipet slowly to dispense it accurately.

5. Flick the tube or slowly pipet up and down a few times to mix well, then spin briefly.

6. Incubate the 20-µL ligation reaction in a thermal cycler at 16 °C for 16 hours.

Note: If possible, set the temperature of the thermal cycler lid to match the block temperature. Otherwise, incubate the reaction with the heated lid turned off, or do not cover the reaction tubes with the heated lid.

Perform reverse transcription

Use components from the SOLiD™ Whole Transcriptome Analysis Kit:

- Nuclease-free Water
- 10✕ RT Buffer
- dNTP Mix
- ArrayScript™ Reverse Transcriptase

1. On ice, prepare 20 µL of RT Master Mix for each sample:

Component	Volume
Nuclease-free Water	13 µL
10X RT Buffer	4 µL
dNTP Mix	2 µL
ArrayScript™ Reverse Transcriptase	1 µL
Total volume per reaction	**20 µL**

Note: Include 5–10% excess volume in the master mix to compensate for pipetting error.

2. On ice, add 20 µL of RT Master Mix to each 20-µL ligation reaction.
3. ***Gently*** vortex to mix thoroughly, then spin briefly.
4. Incubate the 40-µL RT reaction in a thermal cycler with a heated lid at 42 °C for 30 minutes.

Note: The cDNA can be stored at –20 °C for a few weeks, stored at –80 °C for long-term storage, or used immediately.

Purify the cDNA

Use the MinElute® PCR Purification Kit (Qiagen).

Note: The kit may be supplied with Buffer PB (without pH Indicator) or Buffer PBI (with pH Indicator). Either buffer can be used as is; it is not necessary to add pH Indicator to Buffer PB before use.

1. Add Nuclease-free Water and Buffer PB or Buffer PBI to the cDNA:
   a. Transfer all of the cDNA (40 µL) to a clean 1.5-mL microcentrifuge tube.
   b. Add 60 µL of Nuclease-free Water.
   c. Add 500 µL of Buffer PB or Buffer PBI, then mix well.
2. Load the cDNA onto the MinElute column:
   a. Load 600 µL of the sample containing Buffer PB or Buffer PBI onto the MinElute column.
   b. Spin the column at 13,000 × g for 1 minute.
   c. Discard the flowthrough.
3. Wash the cDNA:
   a. Return the MinElute column to the microcentrifuge tube.
   b. Add 750 µL of Buffer PE to the MinElute column.
   c. Spin the column at 13,000 × g for 1 minute.
   d. Discard the flowthrough.
   e. Return the MinElute column to the microcentrifuge tube.
   f. Spin the column at 13,000 × g for 1 minute.
4. Elute the cDNA in a clean microcentrifuge tube:
   a. Place the MinElute column in a clean microcentrifuge tube.
   b. Add 10 µL of Buffer EB to the center of the MinElute column.
   c. Wait 1 minute, then spin the column at 13,000 × g for 1 minute.

Size select the cDNA

Use Novex® pre-cast gel products, a 50 bp DNA Ladder, and SYBR® Gold nucleic acid gel stain (Invitrogen):

- Novex® 6% TBE-Urea Gel 1.0 mM, 10 Well
- Novex® TBE Running Buffer (5×)
- Novex® TBE-Urea Sample Buffer (2×)
- XCell *SureLock*™ Mini-Cell
- 50 bp DNA Ladder
- SYBR® Gold nucleic acid gel stain

For more instructions on running Novex gels, refer to the *Novex® Pre-Cast Gel Electrophoresis Guide* by Invitrogen.

For more instructions on staining the gel, refer to the *SYBR® Gold Nucleic Acid Gel Stain* manual by Invitrogen.

1. Prepare the gel as described in the *Novex® Pre-Cast Gel Electrophoresis Guide* by Invitrogen:
   a. Prepare 1000 mL of 1× TBE Running Buffer:

Component	Volume
Novex® TBE Running Buffer (5×)	200 mL
Deionized water	800 mL
Total volume	1000 mL

   Prepare 1000 mL of 1× TBE Running Buffer using Novex® TBE Running Buffer (5×).
   b. Place the Novex® 6% TBE-Urea Gel in the XCell *SureLock*™ Mini-Cell.
   c. Add 1× TBE Running Buffer to the Upper Buffer Chamber and the Lower Buffer Chamber.
2. Dilute the 50 bp DNA Ladder:

Component	Volume	Concentration
50 bp DNA Ladder	1 µL	1 µg/µL
RNase-free water	24 µL	–
Total volume	**25 µL**	**40 ng/µL**

3. Prepare the cDNA and the DNA ladder:
   a. Mix 5 µL of the cDNA with 5 µL of 2× Novex TBE-Urea Sample Buffer.
   b. Mix 5 µL of the 40 ng/µL 50 bp DNA Ladder with 5 µL of 2× Novex TBE-Urea Sample buffer.
   c. Heat the cDNA and the DNA Ladder at 95 °C for 3 minutes.

d. Snap-cool the tubes on ice. Leave the tubes on ice for less than 30 minutes.

Note: Do not leave denatured samples on ice for longer than 30 minutes. If the denatured samples are left on ice for longer than 30 minutes, repeat step 3c before loading the samples.

4. Before you load the samples, flush the wells of the gel several times with 1X TBE Running Buffer to remove urea from the wells.

Note: Flushing the wells is important to obtain sharp bands.

5. Load the cDNA samples and the DNA Ladder, avoiding the lanes next to the edges of the gel.

Note: Load the DNA Ladder to the left of the cDNA sample. If you have multiple samples, load the DNA Ladder to the left of each cDNA sample.

6. Run the gel at 180 V for ~17 minutes or until the leading dye front reaches the middle of the gel.

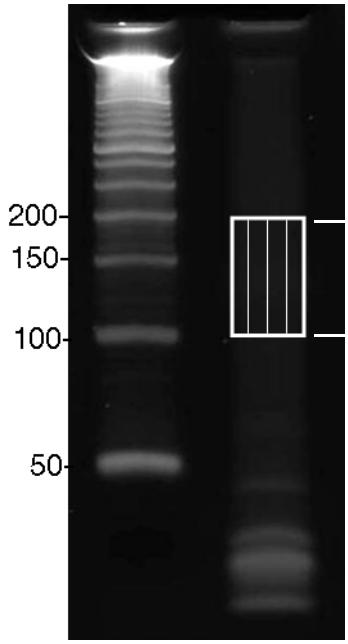
IMPORTANT! Do not run the gel too long.

7. Stain the gel with SYBR® Gold nucleic acid gel stain for 5–10 minutes.
   - Add 5 µL of the SYBR Gold nucleic acid gel stain to 50 mL of 1✕ TBE Running Buffer.

8. Illuminate the stained gel, then excise the gel containing 100–200 nt cDNA:

Note: Be careful not to include extra gel that does not contain any cDNA.

a. Using a clean razor blade, make horizontal cuts to excise the gel containing 100–200 nt cDNA.

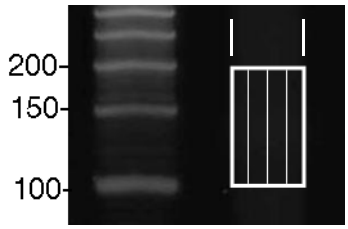


Make the horizontal cuts first to obtain the desired insert length

Note: If you are using a UV transilluminator to visualize the reaction products, work quickly to limit their exposure to UV radiation.

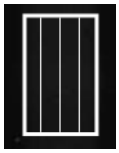
Note: To obtain the desired insert length, you can adjust the cuts. However, too much gel will severely inhibit the PCR reactions. See Table 2 on page 16 for the expected lengths of the insert and PCR product according to the length of the cDNA that is excised from the gel.

b. Reduce the width of the gel piece by making vertical cuts on both edges of the smear.



Make the vertical cuts on the edges of the smear

9. Transfer the gel piece to a clean working area, maintaining the orientation of the gel, then cut the gel vertically into 4 pieces using a clean razor blade. Each gel slice should be about 1 mm × 6 mm.



Cut the gel vertically into 4 pieces

10. Place the two gel slices from the middle of the lane individually into clean 0.2-mL PCR tubes, and place the outside gel slices into a clean 1.5-mL microcentrifuge tube for storage.

    To generate sufficient cDNA for accurate quantitation, you need to run 2 amplification reactions using 2 gel pieces, 1 gel piece in each reaction. You may store the other 2 pieces for 2 weeks at –20 °C.

    Note: To maximize the yield for SOLiD System sequencing, use the 2 gel pieces from the middle of the lane.

Example of size selection

Figure 4 shows 5 µL of purified cDNA from HeLa poly(A) RNA run on a Novex 6% TBE-Urea Gel with the Invitrogen 50 bp DNA Ladder. The white rectangle indicates the area of the gel to excise. Each vertical slice can be used for one 100-µL PCR.

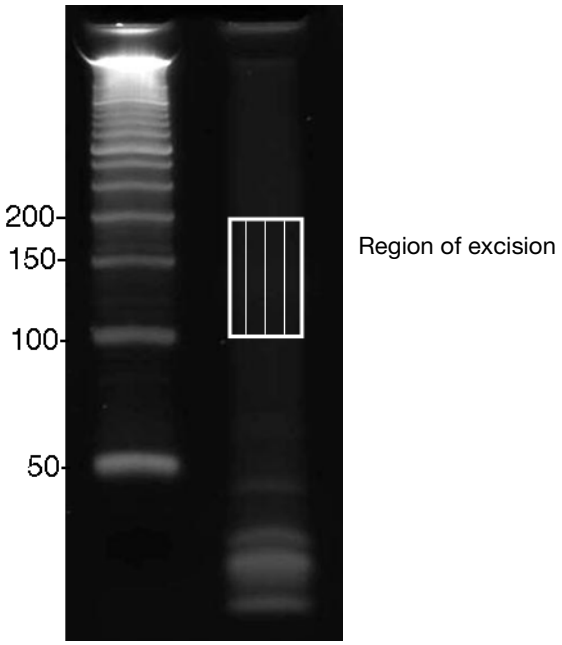


Figure 4 Example of size selection of cDNA from HeLa poly(A) RNA

Expected lengths of the insert and PCR product according to excised cDNA length

Table 2 Expected lengths of the insert and PCR product according to excised cDNA length

Excised cDNA length (nt)	Insert length (bp)	PCR product length (bp)
50	~0	~100
100	~50	~150
150	~100	~200
200	~150	~250
250	~200	~300

Amplify the cDNA

Use components from the SOLiD™ Whole Transcriptome Analysis Kit:

- Nuclease-free Water
- 10✕ PCR Buffer
- 2.5 mM dNTP Mix
- SOLiD™ 5′ PCR Primer
- AmpliTaq® DNA Polymerase
- SOLiD™ 3′ PCR Primer

(Optional) To prepare cDNA libraries for multiplex SOLiD System sequencing, substitute barcoded SOLiD™ 3′ Primers from the SOLiD™ Transcriptome Multiplexing Kit.

Note: Plan your experiments to include multiples of four different barcoded libraries in every multiplex sequencing pool, to preserve color balance on the SOLiD System sequencing run. Use the SOLiD 3′ Primers from the SOLiD Transcriptome Multiplexing Kit in these color-balanced groups: BC1–BC4, BC5–BC8, BC9–BC12, or BC13–BC16. For further information, refer to *SOLiD™ Transcriptome Multiplexing Kit Product Insert* (PN 4441076) and *SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302).

1. For each cDNA sample, prepare duplicate in-gel amplification reactions to generate sufficient cDNA for emulsion PCR:
   a. Ensure that each gel slice from step 10 on page 16 is placed in a 0.2-mL PCR tube. If necessary, transfer the gel slice to the PCR tube using a clean pipette tip.
   b. Prepare 98 µL PCR master mix for each gel slice:

Component	Volume	
	One 100-µL reaction	Two 100-µL reactions‡
Nuclease-free Water	76.8 µL	169.0 µL
10✕ PCR Buffer	10 µL	22.0 µL
dNTP Mix	8 µL	17.6 µL
SOLiD™ 5′ PCR Primer	2 µL	4.4 µL
AmpliTaq® DNA Polymerase	1.2 µL	2.6 µL
Total volume	**98 µL**	**215.6 µL**

‡ When preparing PCR master mix for multiple reactions, it is best to include 5–10% excess volume to compensate for pipetting error. The table shows 10% excess.

   c. Transfer 98 µL PCR master mix into each 0.2-mL PCR tube.
   d. Add 2 µL SOLiD 3′ PCR Primer to each tube.
   (Optional) Substitute 2 µL of barcoded SOLiD 3′ Primer from the SOLiD Transcriptome Multiplexing Kit for each sample, as described above.

2. Run the PCR reactions in a thermal cycler:

Stage	Temp	Time
Hold	95 °C	5 min
Cycle (15 cycles)	95 °C	30 sec
	62 °C	30 sec
	72 °C	30 sec
Hold	72 °C	7 min

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 input fragmented RNA, but for optimal results run between 12 and 18 cycles. Too many cycles results in overamplification and changes the expression profile.

Purify the amplified DNA

Use the PureLink™ PCR Micro Kit (Invitrogen):

- PureLink™ Micro Kit Column
- Collection Tube
- Binding Buffer (B2)
- Wash Buffer (W1)
- PureLink™ Elution Tube

IMPORTANT! Do not use other PCR purification kits. Other purification kits are not as effective in the removal of unincorporated primers. Unincorporated primers can affect the final quantitation and emulsion PCR.

1. Prepare the sample:
   a. Combine the two 100-µL PCR reactions in a new 1.5-mL tube.

      IMPORTANT! If you used two different barcoded SOLiD 3′ Primers for your sample, do not combine the PCRs at this step.

   b. Add 800 µL of Binding Buffer (B2) to the tube, then mix well.
2. Load the sample onto the PureLink™ Micro Kit Column:
   a. Place the PureLink™ Micro Kit Column in a clean Collection Tube.
   b. Load 500 µL of the sample containing Binding Buffer (B2) onto the column.
   c. Spin the column at 10,000 × g for 1 minute.
   d. Discard the flowthrough.
   e. Load the remaining 500 µL of the sample containing Binding Buffer (B2) onto the column.

f. Spin the column at 10,000 × g for 1 minute.

g. Discard the flowthrough.

3. Wash the DNA:

a. Return the column to the Collection Tube.

b. Add 600 µL of Wash Buffer (W1) to the column.

c. Spin the column at 10,000 × g for 1 minute.

d. Discard the flowthrough.

e. Return the column to the Collection Tube.

f. Spin the column at 14,000 × g for 1 minute.

4. Elute the DNA in a clean PureLink™ Elution Tube:

a. Place the column in a clean PureLink™ Elution Tube.

b. Add 10 µL of Elution Buffer to the center of the membrane.

c. Wait 1 minute, then spin the column at 14,000 × g for 1 minute.

d. Repeat step 4b through step 4c for a total elution volume of 20 µL.

Assess the yield and size distribution of the amplified DNA

Use a NanoDrop spectrophotometer, and the Agilent 2100 Bioanalyzer 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 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 25–150 bp.

Percent of DNA in the 25–150 bp range	Next steps
Less than 20%	Proceed with SOLiD™ System templated bead preparation (see page 20).
Greater than 20%	Follow the troubleshooting instructions for "Normal yield and bad size distribution in the amplified library" on page 22.

Note: For instructions on how to perform the smear analysis, refer to "Setting up a smear analysis" on page 29, and to the *Agilent 2100 Bioanalyzer 2100 Expert User's Guide* by Agilent.

4. Determine the median peak size (bp) and molar concentration (nM) of the cDNA library using the Agilent software. For more information, refer to "Selected settings in Agilent bioanalyzer software" on page 29.

   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. A concentration conversion calculator is available at:

www4.appliedbiosystems.com/techlib/append/concentration_calculator.html

Proceed with SOLiD™ System templated bead preparation

When less than 20% of the amplified DNA is in the 25–150 bp range, you can proceed with the SOLiD™ System templated bead preparation stage, in which each library template is clonally amplified on SOLiD™ P1 DNA Beads by emulsion PCR. Refer to the *Applied Biosystems SOLiD™ 3 System Templated Bead Preparation Guide* (PN 4407421).

Barcoded libraries are pooled prior to templated bead preparation. For more information refer to *SOLiD™ Transcriptome Multiplexing Kit Product Insert* (PN 4441076).

Note: When optimizing SOLiD Whole Transcriptome Analysis Kit 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 profiles) of amplified libraries prepared from HeLa poly(A) RNA (Figure 5) and rRNA-depleted HeLa RNA (Figure 6) using the SOLiD™ Whole Transcriptome Analysis Kit are shown. Figure 7 is an example of a sub-optimal size distribution with >20% DNA 25–150 bp in length, prepared from rRNA-depleted FirstChoice® Human Brain Reference RNA (PN AM6050).

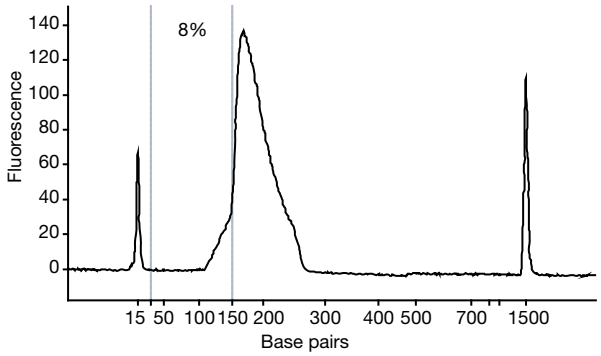


Figure 5 Size distribution of amplified library prepared from HeLa poly(A) RNA

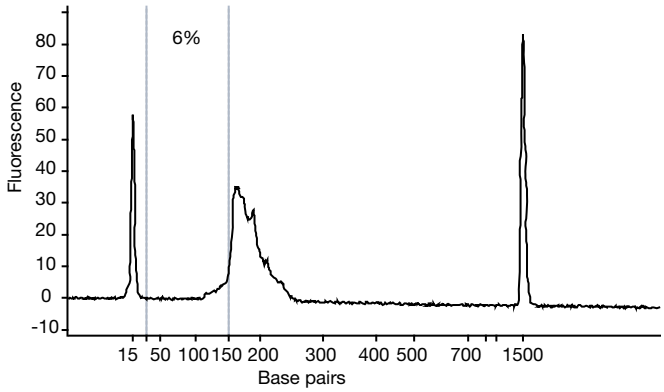


Figure 6 Size distribution of amplified library prepared from rRNA-depleted HeLa total RNA

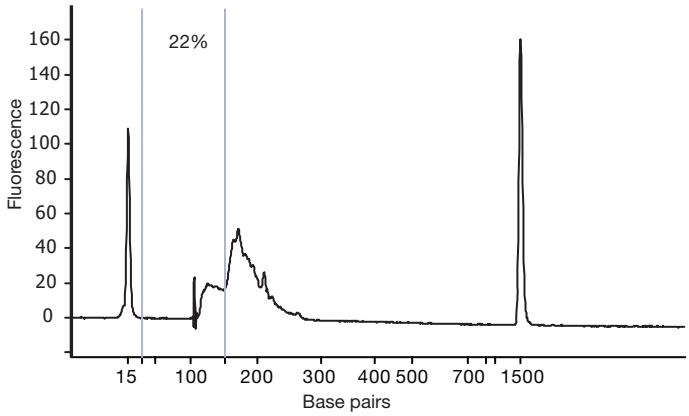


Figure 7 Sub-optimal size distribution of amplified library

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
Fragmentation of whole transcriptome RNA (page 5)	1 µg poly(A) RNA, total RNA, or rRNA-depleted total RNA	500–800 ng RNA
Amplified library construction (page 9)	50–100 ng fragmented RNA	>200 ng cDNA

Troubleshooting

Observation	Possible cause	Solution
Agilent software doesn't calculate one concentration and peak size	The software detects multiple peaks in the amplified cDNA profile	Refer to "Manual adjustment for multiple peaks" on page 31.
Low yield and bad size distribution in the amplified library	You recovered <20% of the input RNA after you fragmented the RNA and cleaned up the RNA	Decrease the RNase III digestion from 10 minutes to 5 minutes (step 3 on page 5).
Low yield in the amplified library and very few differences in the 2100 bioanalyzer traces before and after you fragment the RNA	RNA fragmentation failed	Fragment the RNA again with a positive control, then if RNA fragmentation is successful with the positive control, increase the RNase III digestion from 10 minutes to 20 minutes (step 3 on page 5).
Low yield and no PCR products	The gel ran too long or too much gel was added to the PCR	Reduce the running time (step 6 on page 14) and add less gel to the PCR (step 1 on page 17).
	An enzymatic reaction or column purification performed after RNase III treatment failed	1. Dilute the cDNA 1:10, then use 1 µL in a 100-µL PCR. 2. Check the yield before and after purification using the PureLink™ PCR Micro Kit. 3. If you get the same results, repeat the ligation with more fragmented RNA, and run a parallel ligation reaction with fragmented Control RNA.
Normal or high yield but the purified amplified cDNA shows one or more sharp peaks between 100 and 150 bp in the Agilent 2100 Bioanalyzer trace	Nonspecific amplification	Increase the PCR annealing temperature to 68–72 °C (step 2 on page 18).
Normal or high yield but PCR products larger than 300 bp	Too many PCR cycles resulted in overamplification	Decrease the number of PCR cycles (step 2 on page 18).
Normal yield and bad size distribution in the amplified library	Too much sample was loaded on the Novex® TBE-Urea Gel	Decrease the volume of sample loaded to less than 10 µL (step 5 on page 14).
	The wells of the Novex TBE-Urea Gel contained urea	Before you load the samples, flush the wells of the gel several times with 1X TBE Running Buffer to remove urea from the wells and to obtain sharp bands (step 4 on page 14).
You decreased the volume of sample loaded on the Novex® TBE-Urea Gel, but smear analysis of the purified amplified cDNA shows that >20% of the cDNA is in the 25–150 bp range.	Fragmented RNA sample contains too many small fragments	Follow "Second-round size selection of amplified cDNA" on page 32.
	Size selection was not successful	

Positive control reaction

A general troubleshooting strategy is to perform the SOLiD™ Whole Transcriptome Analysis Kit procedure using the Control RNA (HeLa total RNA) provided with the kit.

- Use 0.2-1 µg Control RNA for the fragmentation procedure starting on page 5.
- Use 100 ng fragmented Control RNA in the amplified library construction procedure starting on page 9.
- The expected yields for the Control RNA are listed on page 21.

Supplemental Information

This appendix contains:

Overview of the SOLiD™ Whole Transcriptome Analysis Kit procedure

The procedure is based on Applied Biosystems Ligase-Enhanced Genome Detection (Ambion LEGenD™) technology (patent pending); an overview is shown in the workflow on page 4.

Fragmentation of whole transcriptome RNA

Poly(A) RNA, total RNA, or ribosomal RNA-depleted total RNA is first fragmented using RNase III, to convert the whole transcriptome sample to RNA of a size appropriate for SOLiD™ System sequencing. After cleanup using the RiboMinus™ Concentration Module, fragmented RNA samples with sufficient yield and an appropriate size distribution are ready for preparation of amplified cDNA libraries.

Amplified library construction

Hybridization and ligation to Adaptor Mix

The fragmented RNA sample is next hybridized and ligated with either Adaptor Mix A or Adaptor Mix B. These Adaptor Mixes are sets of oligonucleotides with a single-stranded degenerate sequence at one end and a defined sequence required for SOLiD System sequencing at the other end. Each Adaptor Mix constrains the orientation of the RNA in the ligation reaction such that hybridization with Adaptor Mix A yields template for SOLiD System sequencing from the 5′ end of the sense strand, while hybridization with Adaptor Mix B yields template for sequencing the reverse complement (yielding sequence starting from the 3′ 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 in Figure 8 on page 26.

If you are preparing only one whole transcriptome library, use Adaptor Mix A. To achieve higher confidence in the complete sequence of larger RNAs in the whole transcriptome library, prepare two ***separate*** libraries, using Adaptor Mix A in the hybridization reaction for one library and Adaptor Mix B in the hybridization reaction for the other library.

Note: These libraries should not be mixed for the SOLiD System sequencing run unless you also use different barcoded SOLiD 3′ Primers to amplify each library (see "In-gel cDNA library amplification" below).

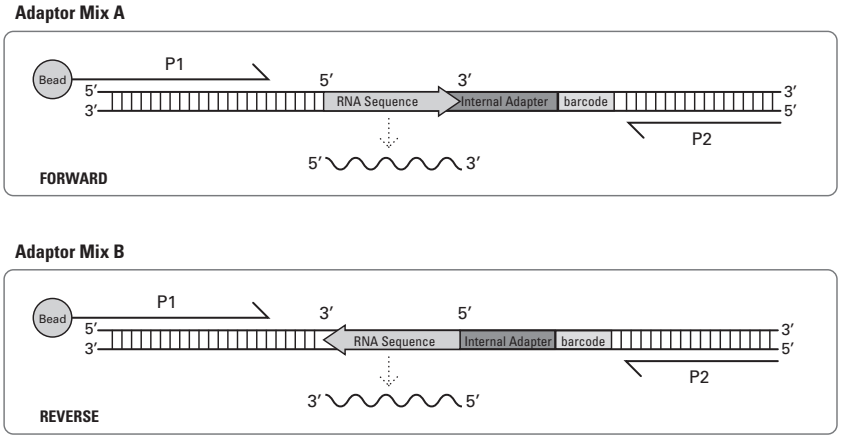


Figure 8 Strand-specific RNA sequence information from SOLiD™ Whole Transcriptome Analysis Kit products

Reverse transcription and size selection

Next, the RNA population with ligated adaptors is reverse transcribed to generate single-stranded cDNA copies of the fragmented RNA molecules. After a cleanup step using the MinElute® PCR Purification Kit, the sample is subjected to denaturing gel electrophoresis, and gel slices containing cDNA in the desired size range are excised.

In-gel cDNA library amplification (single- or multiplex) and final cleanup

The size-selected cDNA is next amplified using ~15 cycles of PCR that take place in the gel slices. This step appends required terminal sequences to each molecule and generates sufficient template for SOLiD System sequencing. Limiting the cycle number minimizes the synthesis of spurious PCR products and better preserves the RNA profile of the sample.

- To prepare template for singleplex SOLiD System sequencing, use the PCR primers included in the SOLiD Whole Transcriptome Analysis Kit.
- For multiplex SOLiD System sequencing, use the 3′ PCR primers supplied in the SOLiD Transcriptome Multiplexing Kit.

  The 3′ (reverse) PCR primers in the SOLiD Transcriptome Multiplexing Kit contain the P2 sequence required for SOLiD emulsion PCR, a unique barcode sequence, and an internal adaptor sequence necessary for sequencing the barcode (Figure 8). Use a different 3′ PCR primer in the

amplification reaction for each cDNA sample to generate a barcoded cDNA library that can be mixed with other barcoded libraries for multiplex SOLiD System sequencing. Plan the in-gel PCRs so that barcoded libraries are generated using multiples of the color-balanced groups of four 3′ primers, to preserve color balance during multiplexed sequencing on the SOLiD System. For further information, refer to *SOLiD™ Transcriptome Multiplexing Kit Product Insert* (PN 4441076) and *SOLiD™ 3 System SETS Software Getting Started Guide* (PN 4389302).

The 5′ PCR primer is identical in each kit; its sequence corresponds to SOLiD emulsion PCR primer 1 (P1 in Figure 8).

After the PCR, the amplified cDNA is cleaned up using the PureLink™ PCR Micro Kit. The yield and size distribution of each cDNA library is assessed; it is important to have sufficient cDNA for accurate quantitation prior to SOLiD System templated bead preparation, and to use only libraries with sufficiently long inserts for SOLiD System sequencing.

SOLiD™ System templated bead preparation (not included)

The amplified cDNA library generated with the SOLiD Whole Transcriptome Analysis Kit is ready for attachment to beads at the emulsion PCR step of the SOLiD System sample preparation workflow. Refer to the *Applied Biosystems SOLiD™ 3 System Templated Bead Preparation Guide* (PN 4407421).

Barcoded libraries are combined into color-balanced sequencing pools (in multiples of four) just prior to templated bead preparation.

Sequences of the SOLiD™ PCR primers included in the kit

Two SOLiD PCR primers are provided in the SOLiD Whole Transcriptome Analysis Kit. The concentration of each primer is 25 µM. The primer sequences are listed below.

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′

Fragmented RNA cleanup using the PureLink™ RNA Micro Kit

After you fragment the RNA (page 5), you can use the PureLink™ RNA Micro Kit (Invitrogen) to clean up the RNA instead of the RiboMinus™ Concentration Module.

Note: It is not necessary to add 2-mercaptoethanol or DTT to the Lysis Buffer.

1. Before using Wash Buffer II for the first time:
   a. Add 60 mL of 96–100% ethanol directly to the bottle.
   b. Check the box on the Wash Buffer II label to indicate that ethanol was added.
   c. Store Wash Buffer II with ethanol at room temperature.
2. To the RNase III digestion and water, add 100 µL of Lysis Buffer and 250 µL 100% ethanol, then mix well.
3. Bind the RNA to the PureLink™ Micro Kit Spin Column:
   a. Load 450 µL of the sample containing Lysis Buffer and ethanol onto the Spin Column (with a Collection Tube).
   b. Spin the column at 13,000 × g for 15 seconds at room temperature.
   c. Discard the flowthrough.
4. Wash the sample:
   a. Return the Spin Column to the Collection Tube.
   b. Add 500 µL of Wash Buffer II with ethanol to the Spin Column.
   c. Spin the column at 13,000 × g for 15 seconds.
   d. Discard the flowthrough.
5. Wash the sample again:
   a. Return the Spin Column to the Collection Tube.
   b. Add another 500 µL of Wash Buffer II with ethanol to the Spin Column.
   c. Spin the column at 13,000 × g for 1 minute.
   d. Discard the flowthrough.
   e. Return the Spin Column to the Collection Tube.
   f. Using a pipette, carefully remove any buffer adhering to the top of the o-ring inside the column.
   g. Spin the column at 14,000 × g for 1 minute.
6. Elute the RNA:
   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.
   c. Wait 1 minute.
   d. Spin the Column at 14,000 × g for 1 minute.
7. Proceed with "Assess the yield and size distribution of the fragmented RNA" on page 7.

Selected settings in Agilent bioanalyzer software

Setting up a smear analysis

Perform a smear analysis to quantify the percentage of DNA in the 25–150 bp size range.

1. Select **View** ▸ **Setpoints** tab.
2. Select the **Global** tab ▸ **Advanced** settings.
3. Select **Smear Analysis**.
4. Double-click **Table**.
5. In the Smear Regions Window, select **Add** a region, and enter 25 bp and 150 bp for the lower and upper limits, respectively.

   These settings are used to determine the percentage of total product that is 25–150 bp in length.
6. **Add** a second region, enter 25 bp and 180 bp, and select **OK**.

   This is an arbitrary upper limit which will be used to determine the median size.
7. View the **Region Table** tab, which will display the percentage of the total product in the size ranges you have set.

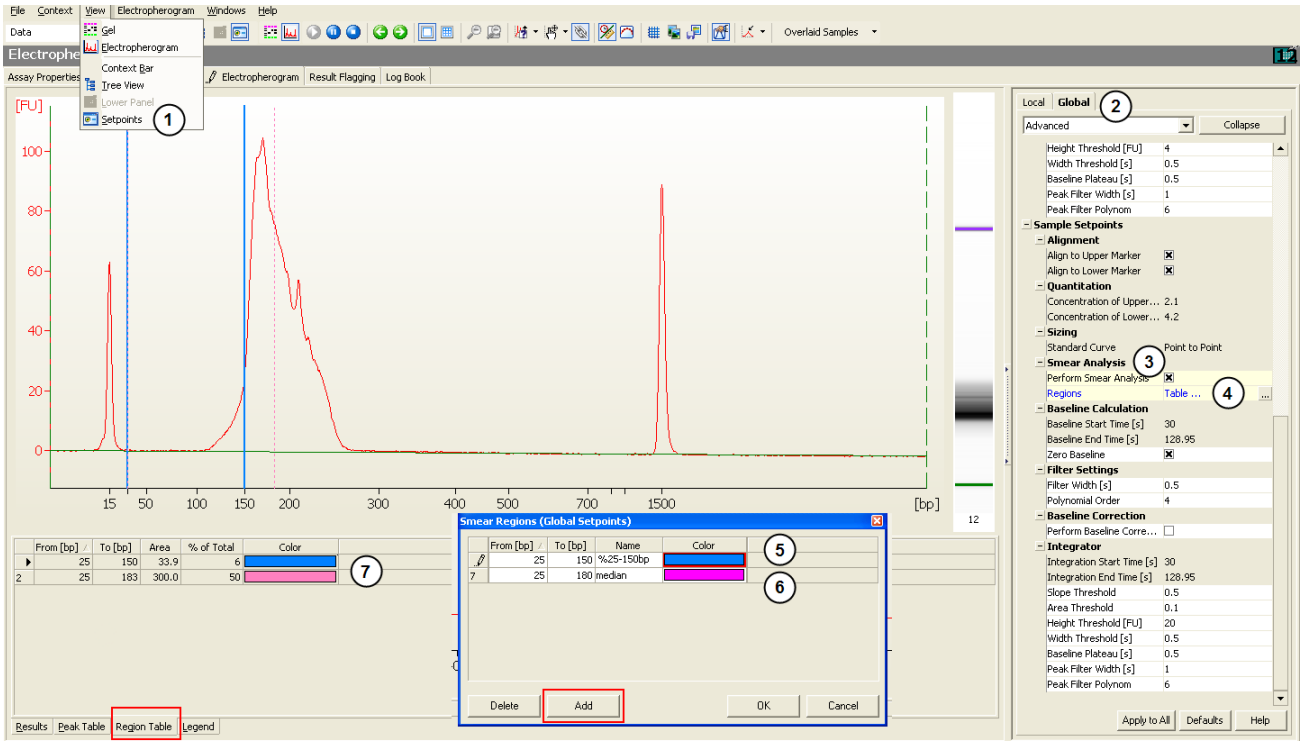


Figure 9 Smear analysis region settings

Manual determination of median size

Under the Region Table tab, drag the upper limit line set in step 6 on page 29 to the left or right until the Region Table indicates 50% of Total (Figure 10).

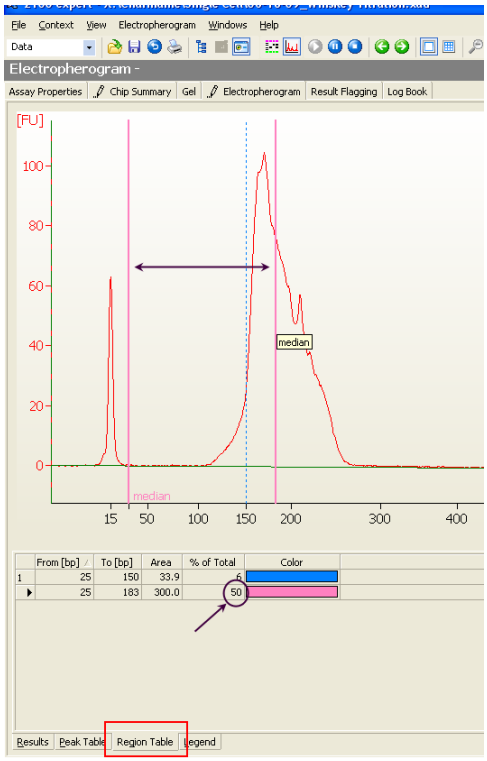


Figure 10 Manual determination of median size

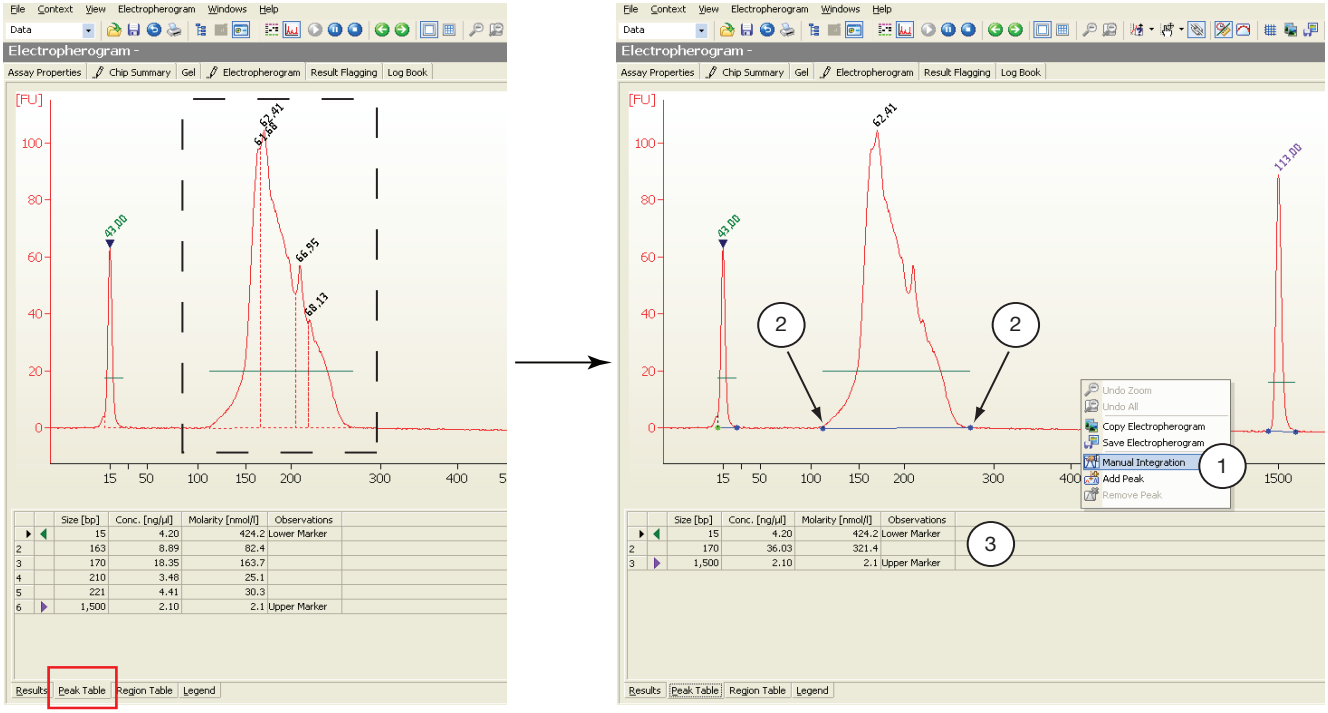
Manual adjustment for multiple peaks

In the Peak Table tab, you may see that the bioanalyzer software has 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 (Figure 11):

1. Under the **Peak Table** tab, right-click anywhere on the electropherogram and select **Manual Integration**.

2. Drag the lower and upper region limits of the region with multiple peaks to consolidate multiple peaks into one region.

3. The median size (bp), concentration (ng/µL), and molarity (nM) of each peak region will be automatically calculated and displayed in the Peak Table.



Multiple peaks

One peak

Figure 11 Manual adjustment for multiple peaks

Second-round size selection of amplified cDNA

If >20% of the amplified cDNA library from step 4 on page 19 is in the size range 25–150 bp, or if you see distinct bands in the size range 100–150 bp, perform a second round of size selecion of the amplified, double-stranded cDNA using ***nondenaturing*** polyacrylamide gel electrophoresis.

Run the amplified cDNA on a nondenaturing 6% polyacrylamide gel.

1. Add 4 µL Gel Loading Solution (AM8556) to the eluted cDNA (~20 µL) from step 4 on page 19.

2. Set up a 6% nondenaturing acrylamide gel.

   a. Prepare gel mix; 5 mL is enough gel solution for one minigel.

for 5 mL	Component
0.5 mL	10X TBE (for example, Ambion® P/N AM9863)
0.75 mL	40% acrylamide (acryl:bis-acryl = 19:1; for example, Ambion P/N AM9022)
to 5 mL	deionized water

   b. Stir at room temperature, then add:

50 µL	10% ammonium persulfate in dH_2O
5 µL	TEMED

   Mix briefly after adding the last two ingredients, which catalyze polymerization, then pour the gel immediately.

   c. Set up the gel following the manufacturer's instructions for the details of attaching gels to the running apparatus.

   Use 1✕ TBE as the gel running buffer.

   IMPORTANT! Do not heat the samples before loading.

3. Load the sample into 3 adjacent wells (8 µL per well) of a nondenaturing 6% TBE polyacrylamide gel.

   Note: Include a separate well with 5 µL 50 bp Ladder (40 ng/µL).

4. Run the gel at ~140 V for ~30 minutes.

   Note: Nondenaturing gels must be run slowly to avoid heat denaturation of the samples.

5. Stain the gel with SYBR® Gold dye, following the manufacturer's instructions.

Excise the gel in the size range 150–250 bp

Illuminate the stained gel, then excise the gel in the size range 150–250 bp.

Note: If you are using a UV transilluminator to visualize the nucleic acid, work quickly to limit its exposure to UV radiation.

Purify the amplified DNA from the gel

Use PAGE Elution Buffer (recipe below) and Spin Columns and Tubes (Applied Biosystems PN AM10065).

1. Prepare ~600 µL PAGE Elution Buffer for each sample.

Component	Volume
TE Buffer, pH 8 (10 mM Tris-HCl, ph 8, 1 mM EDTA)	5 mL
5 M ammonium acetate (2.5 M final concentration)	5 mL
Final volume	**10 mL**

2. Shred the gel piece:
   a. Use a 21-gauge needle to puncture through the bottom-center of a 0.5-mL microcentrifuge tube.
   b. Place the gel piece in the punctured 0.5-mL tube, then place the 0.5-mL tube into a larger, 1.5-mL, nuclease-free microcentrifuge tube.
   c. Spin for 3 minutes at 13,000 x g to shred the gel.
   d. Place the 1.5-mL tube containing the shredded gel piece on ice.
   e. Inspect the 0.5-mL tube, and if any gel pieces remain, repeat the centrifugation step into a fresh 1.5-mL tube. Pool the gel pieces into one collection tube using a pipette tip.
3. Elute the DNA in PAGE elution buffer:
   a. Add 300 µL of PAGE Elution Buffer to the shredded gel pieces.
   b. Incubate the mixture overnight at room temperature, with gentle agitation.
   c. Transfer the buffer, which contains eluted DNA, to a fresh tube, leaving the gel fragments behind.
      Store the DNA on ice during the second elution (step 3e).
   d. Add another 300 µL of PAGE Elution Buffer to the shredded gel pieces.
   e. Incubate the buffer and gel pieces for 1–2 hr at 37°C, with gentle agitation.
4. Remove the gel pieces from the sample using a filter spin column:
   a. Combine the PAGE elution buffer from step 3c with the buffer plus gel slurry from step 3e.

b. Cut a pipette tip to make a larger opening and use it to transfer the combined PAGE elution buffer and gel slurry from each sample to a Spin Column.

c. Spin the Spin Column at top speed for 5 minutes to remove gel pieces. The DNA is now in the flowthrough.

Alternatively, you can use a 0.45 µm-filter spin column from another manufacturer for this step, following the manufacturer's instructions for the maximum centrifugation speed.

5. Precipitate the DNA, then resuspend in 20 µL of Nuclease-free Water:

a. Add 1/100 volume of glycogen and 0.7 volume of isopropanol to each sample.

b. Mix thoroughly, then incubate at room temperature for 5 minutes.

c. Spin the sample at 13,000 x g for 20 minutes at room temperature.

d. Carefully remove and discard the supernatant, then air dry the pellet.

e. Resuspend the DNA pellet in 20 µL of Nuclease-free Water.

Note: Accurate quantitation of the DNA is important for the downstream SOLiD™ System emulsion PCR titration step. The resuspension volume should yield DNA sufficiently concentrated for accurate measurements (~10 ng/µL).

Safety

This appendix covers:

General chemical safety

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- 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 MSDS.
- 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 MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

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

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

Obtaining MSDSs

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At **www.appliedbiosystems.com**, select **Support** ▸ **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At **www.ambion.com**, go to the web catalog page for the product of interest. Select **MSDS**, then right-click to print or download.
- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com), telephone (650-554-2756; USA), or fax (650-554-2252; USA) your request, specifying the catalog or part number(s) and the name of the product(s). The associated MSDSs will be e-mailed unless you request fax or postal delivery. Requests for postal delivery require 1 to 2 weeks for processing.

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

Biological hazard safety

General biohazard

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

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)

- 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

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