

SOLiD™ Whole Transcriptome Analysis Kit

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Safety information


Note: For general safety information, see this Preface and [Appendix B, “Safety” on page 27](#). 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.

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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 27](#).

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**.

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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.

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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.

Kit contents

Sufficient reagents are supplied in the SOLiD™ Whole Transcriptome Analysis Kit (PN 4425680) to prepare cDNA libraries from 12 samples for high throughput sequencing with the SOLiD system. [Table 1](#) lists the reagents supplied in the kit.

Table 1 Reagents for whole transcriptome library preparation

Component	Amount	Cap
2X Ligation Buffer	150 µL	green
10X RNase III Buffer	20 µL	red
10X RT Buffer	50 µL	yellow
10X PCR Buffer	660 µL	white
Adaptor Mix A	30 µL	green
Adaptor Mix B	30 µL	purple
AmpliTaq® DNA Polymerase	110 µL	white
ArrayScript™ Reverse Transcriptase	20 µL	yellow
Control RNA (1 µg/µL)	50 µL	clear
dNTP Mix	500 µL	white
Hybridization Solution	40 µL	green
Ligation Enzyme Mix	30 µL	green
Nuclease-free Water	1.75 mL	clear
RNase III	20 µL	red
SOLiD™ 3' PCR Primer	100 µL	blue
SOLiD™ 5' PCR Primer	100 µL	white

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: <ul style="list-style-type: none"> 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 Bioanalyzer	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

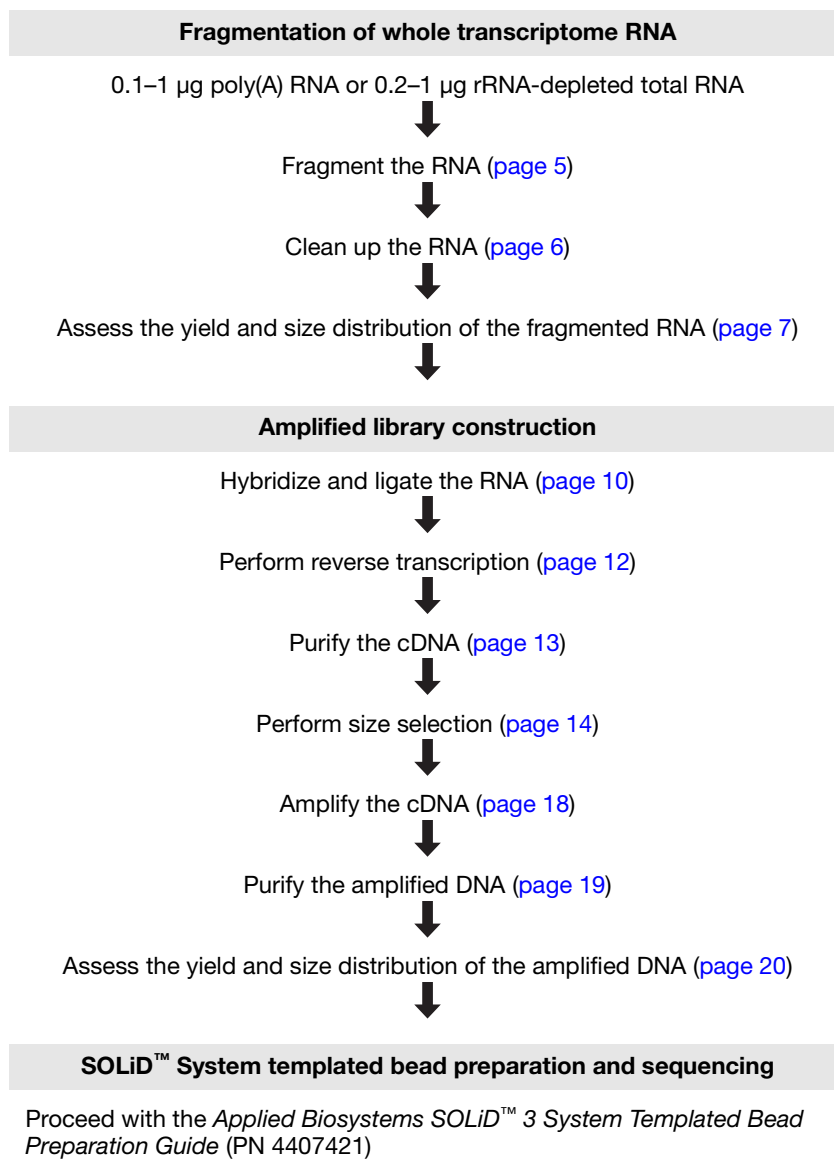
Reagents

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

Item	Source
Novex® TBE-Urea Sample Buffer (2X), 10 mL‡	Invitrogen PN LC6876
Novex® TBE Running Buffer (5X), 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, 10,000X 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.

Workflow



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 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 large 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
- 10X RNase III Buffer
- RNase III

For the following hazards, see the complete safety alert descriptions in “[Chemical alerts](#)” on page 29:



WARNING! CHEMICAL HAZARD. 10X RNase III Buffer and RNase III.

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

Component	Volume
0.1–1 µg poly(A) RNA or 0.2–1 µg rRNA-depleted RNA and Nuclease-free Water	8 µL
10X 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 25](#)).

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 and the Qubit® Fluorometer (Invitrogen) and the RNA 6000 Pico Chip Kit and the Agilent® 2100 Bioanalyzer (Agilent).

For instructions on how to use the Quant-iT™ RNA Assay Kit or the Qubit® Fluorometer, refer to the *Quant-iT™ RNA Assay Kit Protocol* or the *Qubit™ Fluorometer Instruction Manual* by Invitrogen.

Note: Alternatively, you can use a NanoDrop™ Spectrophotometer. 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.

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 and/or the RNA concentration is less than 5 ng/μL, 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.

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
<ul style="list-style-type: none"> • ≥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.
<ul style="list-style-type: none"> • <50 ng poly(A) RNA • <100 ng rRNA-depleted total RNA 	<ol style="list-style-type: none"> 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.

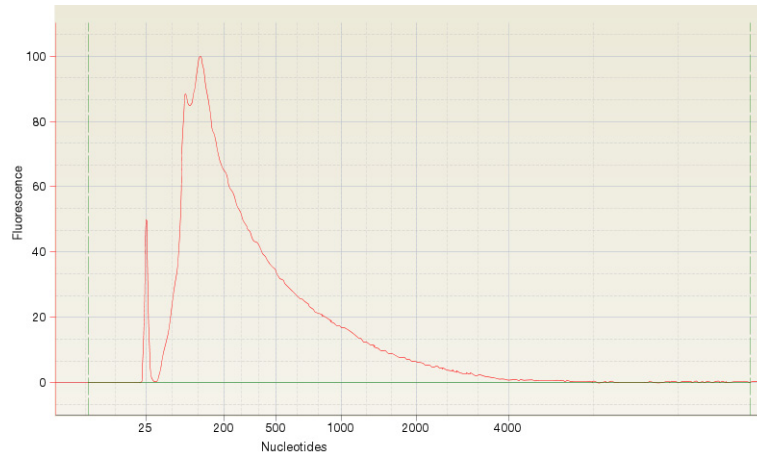


Figure 1 HeLa poly(A) RNA run on an Agilent 2100 Bioanalyzer after RNase III fragmentation and cleanup

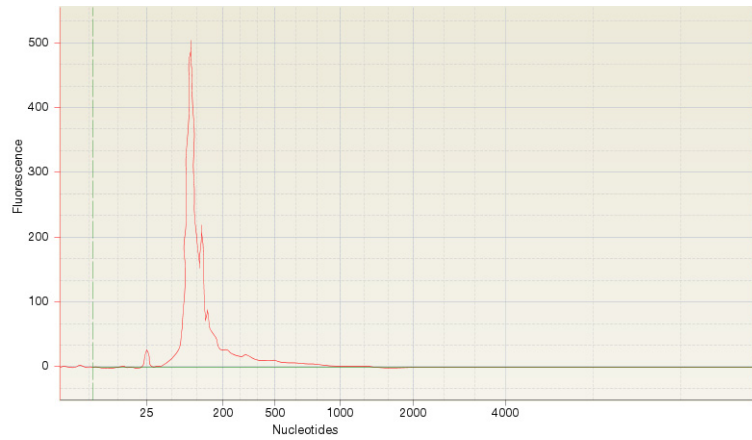


Figure 2 rRNA-depleted HeLa RNA run on an Agilent 2100 Bioanalyzer after RNase III fragmentation and cleanup

Amplified library construction

Overview

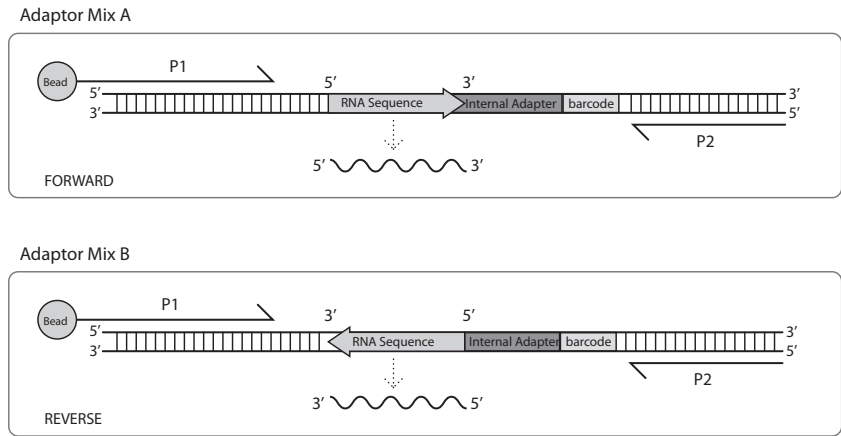
Amplified library construction involves the following procedures:

1. [Hybridize and ligate the RNA \(page 10\)](#)
2. [Perform reverse transcription \(page 12\)](#)
3. [Purify the cDNA \(page 13\)](#)
4. [Perform size selection \(page 14\)](#)
5. [Amplify the cDNA \(page 18\)](#)
6. [Purify the amplified DNA \(page 19\)](#)
7. [Assess the yield and size distribution of the amplified DNA \(page 20\)](#)
8. [Proceed with SOLiD™ System templated bead preparation \(page 20\)](#)

Select whether to use Adaptor Mix A or Adaptor Mix B

During the hybridization reaction, the RNA is hybridized to sets of oligonucleotides with a single-stranded degenerate sequence at one end of the oligonucleotide and a defined sequence required for SOLiD™ sequencing at the other end.

The oligonucleotides in Adaptor Mix A yield template for SOLiD™ sequencing from the 5' end of the sense strand. The oligonucleotides in Adaptor Mix B yield template for sequencing the reverse complement, providing sequencing information from the 3' end of the sense strand.



Note: 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, 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.

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

For the following hazards, see the complete safety alert descriptions in “[Chemical alerts](#)” on page 29.

CAUTION! CHEMICAL HAZARD. Hybridization Solution, 2× Ligation Buffer, and 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:	3 µL
• poly(A) RNA: 50 ng	
• rRNA-depleted total RNA: 100 ng	
Total volume per reaction	8 µL

[‡] Use Adaptor Mix A for SOLiD 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- μ L hybridization reactions:

Component (add in order shown)	Volume
2X Ligation Buffer [‡]	10 μ L
Ligation Enzyme Mix	2 μ L

[‡] 2X 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- μ L 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
- 10X RT Buffer
- dNTP Mix
- ArrayScript™ Reverse Transcriptase

For the following hazards, see the complete safety alert descriptions in [“Chemical alerts” on page 29](#).



WARNING! CHEMICAL HAZARD. ArrayScript™ Reverse Transcriptase and 10X RT Buffer.

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 centrifuge 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.

Perform size selection

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 (5X)
- Novex® TBE-Urea Sample Buffer (2X)
- 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 1X TBE Running Buffer:

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

- b. Place the Novex® 6% TBE-Urea Gel in the XCell SureLock™ Mini-Cell.
- c. Add 1X 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 2X Novex TBE-Urea Sample Buffer.
- b. Mix 5 µL of the 40 ng/µL 50 bp DNA Ladder with 5 µL of 2X 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.

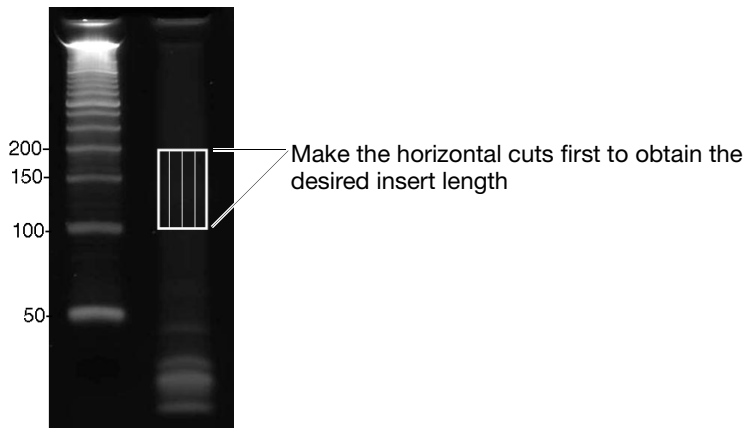
IMPORTANT! Do not run the gel too long.

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 1X TBE Running Buffer.

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

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

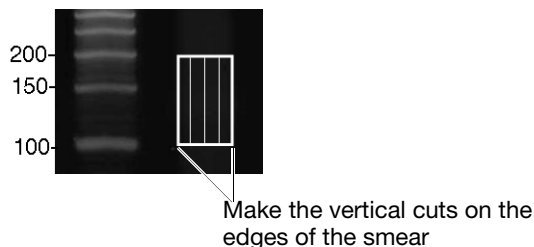
- a. Using a clean razor blade, make horizontal cuts to excise the 100–200-nt cDNA.



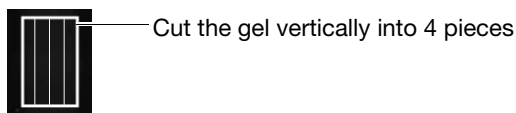
Note: If you are using a UV transilluminator to visualize the reaction products, work quickly to limit their exposure to UV radiation.

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 17](#) 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.



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.



10. Place the gel pieces into a clean 1.5-mL microcentrifuge tube. To generate sufficient cDNA for emulsion PCR, you need to run 2 amplification reactions using 2 gel pieces – 1 gel piece in each tube. You may store the other 2 pieces for 2 weeks at -20°C .

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

Example of size selection

Figure 3 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.

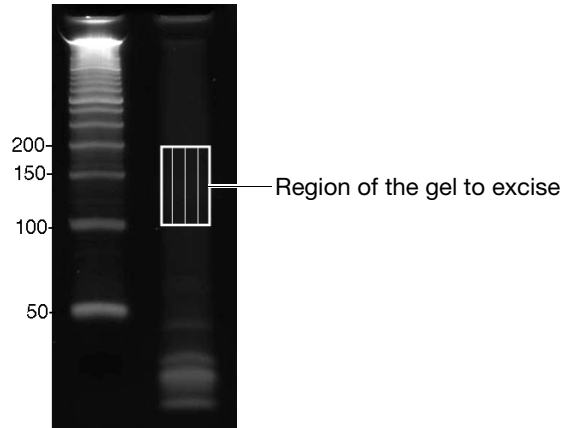


Figure 3 Example of size selection of cDNA from HeLa poly(A) RNA

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 (nts)	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
- 10X PCR Buffer
- SOLiD™ 3' PCR Primer
- SOLiD™ 5' PCR Primer
- 2.5 mM dNTP Mix
- AmpliTaq® DNA Polymerase

For the following hazards, see the complete safety alert descriptions in “[Chemical alerts](#)” on page 29:



WARNING! CHEMICAL HAZARD. AmpliTaq® DNA Polymerase.

1. Prepare 2 reactions to generate sufficient cDNA to perform emulsion PCR:
 - a. Using a clean pipette tip, transfer 1 gel piece to each of two 0.2-mL PCR tubes.
 - b. Prepare 100 µL of PCR master mix for each 0.2-mL PCR tube:

Component	Volume	
	One 100-µL reaction	Two 100-µL reactions (including 10% excess)
Nuclease-free Water	76.8 µL	169.0 µL
10X PCR Buffer	10 µL	22.0 µL
SOLiD™ 3' PCR Primer	2 µL	4.4 µL
SOLiD™ 5' PCR Primer	2 µL	4.4 µL
2.5 mM dNTP Mix	8 µL	17.6 µL
AmpliTaq® DNA Polymerase	1.2 µL	2.64 µL
Total volume	100 µL	220.04 µL

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

- c. Transfer 100 µL of PCR master mix into each 0.2-mL PCR tube.
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.
 - 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 the DNA 1000 Kit and the Agilent® 2100 Bioanalyzer (Agilent).

1. 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.
2. Using the 2100 expert software, perform a smear analysis to quantify the percentage of DNA in the 25–150 bp range.

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 the *Agilent 2100 Bioanalyzer 2100 Expert User’s Guide* by Agilent.

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).

Typical results of amplified library construction

Figure 4 and Figure 5 show profiles from an Agilent 2100 Bioanalyzer after amplified library construction using the SOLiD™ Whole Transcriptome Analysis Kit. Figure 4 shows results with HeLa poly(A) RNA. Figure 5 shows results with rRNA-depleted HeLa RNA.

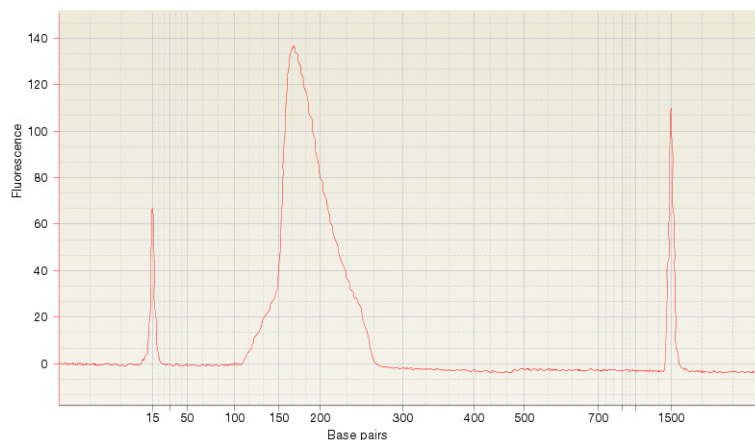


Figure 4 HeLa poly(A) RNA run on an Agilent 2100 Bioanalyzer after amplified library construction

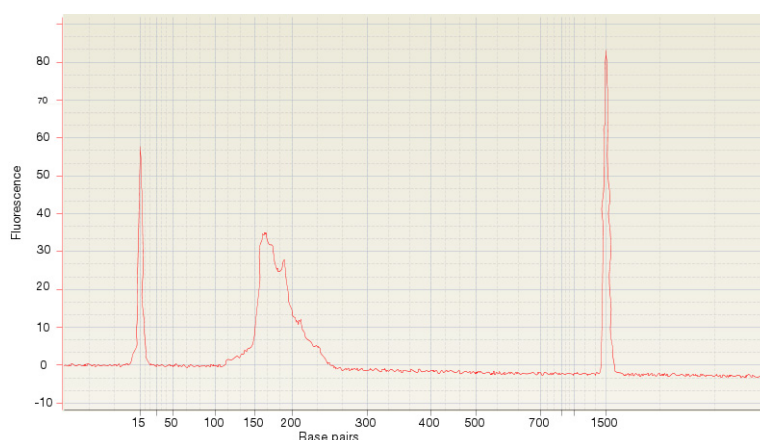


Figure 5 rRNA-depleted HeLa RNA run on an Agilent 2100 Bioanalyzer after amplified library construction

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 or rRNA-depleted total RNA	500–800 ng RNA
Amplified library construction (page 9)	50–100 ng fragmented RNA	200–800 ng cDNA

Troubleshooting

Observation	Possible cause	Solution
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 6).
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 6).
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 15) and add less gel to the PCR (step 1 on page 18).
	An enzymatic reaction or column purification performed after RNase III treatment failed	<ol style="list-style-type: none"> 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.
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 (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 15).
	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 15).
You decreased the volume of sample loaded on the Novex® TBE-Urea Gel, but smear analysis of the purified amplified DNA shows that greater than 20% of the DNA is in the 25–150 bp range.	Fragmented RNA sample contains too many small fragments	Load PCR products onto a native PAGE gel, recover the 150–250 bp fragments, then purify the DNA from the gel following the purification procedure on page 23 .
	Size selection was not successful	

If you repeat the size selection after you purify the amplified DNA

If you repeat the size selection after you purify the amplified cDNA, you need to purify the DNA from the gel before proceeding. Use PAGE Elution Buffer (recipe below) and Spin Columns and Tubes (Applied Biosystems PN AM10065).

Prepare PAGE Elution Buffer

Prepare ~600 µL 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

Purify the amplified DNA from the gel

1. 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.
2. 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 2e](#)).
 - 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.
3. Remove the gel pieces from the sample using a filter spin column:
 - a. Combine the PAGE elution buffer from [step 2c](#) with the buffer plus gel slurry from [step 2e](#).

- 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.

4. 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).

Supplemental Information

This appendix contains:

- Clean up the RNA using the PureLink™ RNA Micro Kit. 25
- Sequences of the SOLiD™ PCR primers included in the kit. 26

Clean up the RNA using the PureLink™ RNA Micro Kit

After you fragment the RNA, you can use the PureLink™ RNA Micro Kit (Invitrogen) to clean up the RNA.

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 μ L of Lysis Buffer and 250 μ L 100% ethanol, then mix well.
3. Bind the RNA to the PureLink™ Micro Kit Spin Column:
 - a. Load 450 μ L of the sample containing Lysis Buffer and ethanol onto the Spin Column (with a Collection Tube).
 - b. Spin the column at $13,000 \times g$ 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 μ L of Wash Buffer II with ethanol to the Spin Column.
 - c. Spin the column at $13,000 \times g$ 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 at 13,000 × g for 1 min.
 - 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 at 14,000 × g for 1 min.
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 min.
 - d. Spin the Column at 14,000 × g for 1 min.
7. Proceed with [“Assess the yield and size distribution of the fragmented RNA” on page 7.](#)

Sequences of the SOLiD™ PCR primers included in the kit

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

SOLiD™ 5' PCR primer	The P1 adapter is the 5' PCR primer in each SOLiD PCR Primer Set. The sequence is shown in the 5' to 3' orientation: 5' - CCA CTA CGC CTC CGC TTT CCT CTC TAT GGG CAG TCG GTG AT -3'
SOLiD™ 3' PCR primer	The 3' PCR primer in each SOLiD PCR Primer Set. The sequence is shown in the 5' to 3' orientation: 5' - CTG CCC CGG GTT CCT CAT TCT CTG TGT AAG AGG CTG CTG TAC GGC CAA GGC G -3'

Safety

This appendix covers:

■ General chemical safety	27
■ MSDSs	27
■ Biological hazard safety	28
■ Chemical alerts	29

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**, then **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. Click **MSDS**, then right-click to print or download.
- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.
- 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; bmbi.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

Chemical alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see “Safety alert words” on page v.

General alerts for all chemicals

Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Specific chemical alerts



WARNING! 2× Ligation Buffer may cause eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.



WARNING! 10× PCR Buffer causes eye, skin, and respiratory tract irritation. Exposure may cause nervous system depression. May be harmful if swallowed. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.



WARNING! 10× RNase III Buffer causes eye, skin, and respiratory tract irritation. May be harmful if absorbed through the skin, inhaled, or swallowed. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.



WARNING! 10× RT Buffer contains dithiothreitol. Exposure may cause nervous system depression. May be harmful if swallowed. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.



WARNING! AmpliTaq® DNA Polymerase may cause eye, skin, and respiratory tract irritation. May be harmful if swallowed. Avoid breathing vapor. Use with adequate ventilation.



WARNING! ArrayScript™ Reverse Transcriptase may cause eye, skin, and respiratory tract irritation. May be harmful if swallowed. Avoid breathing vapor. Use with adequate ventilation.



WARNING! Hybridization Solution may cause eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.



WARNING! Ligation Enzyme Mix may cause eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.





WARNING! RNase III may cause eye, skin, and respiratory tract irritation. May be harmful if absorbed through the skin. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.

Part Number 4409491 Rev. D 05/2009



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