SOLiD™ Whole Transcriptome Analysis Kit

Note: For safety and biohazard guidelines, refer to the “Safety” section in the *SOLiD™ Whole Transcriptome Analysis Kit Protocol* (PN 4409491). For every chemical, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

For detailed instructions, refer to the *SOLiD™ Whole Transcriptome Analysis Kit Protocol* (PN 4409491).

Fragmentation of whole transcriptome RNA

1 Fragment the RNA

a. 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
10X RNase III Buffer	1 µL
RNase III	1 µL
Total volume	**10 µL**

b. Incubate the reaction in a thermal cycler at 37 °C for 10 minutes.

c. *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.

2 Clean up the RNA

Use the RiboMinus™ Concentration Module (Invitrogen).

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

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

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

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

c. Clean up the RNA:

Step	Load	Spin	Flowthrough
Bind the RNA to the Spin Column	450 µL of RNA sample containing Binding Buffer (L3) and ethanol	12,000 × g for 1 min	Discard
Wash the RNA	500 µL of Wash Buffer (W5) with ethanol	12,000 × g for 1 min	Discard
	–	Max speed for 2 min	Discard
Elute the RNA in a clean Recovery Tube	20 µL of RNase-Free Water	Wait 1 min, then max speed for 1 min	Save the RNA

3 Assess the yield and size distribution of the fragmented RNA

a. Quantitate the yield of the fragmented RNA using the Quant-iT™ RNA Assay Kit on the Qubit® Fluorometer (Invitrogen).

b. Assess the size distribution of the fragmented RNA:

1. Dilute the RNA to less than 5 ng/µL.
2. Run 1 µL on an Agilent® 2100 bioanalyzer with the RNA 6000 Pico Chip Kit (Agilent).
3. Using the 2100 expert software, review the size distribution. The average size should be 100–200 nt.

c. 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. 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.

Amplified library construction

1 Hybridize and ligate the RNA

a. 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.

b. Run the hybridization reaction in a thermal cycler:

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

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

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

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

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

2 Perform reverse transcription

a. 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.

b. On ice, add 20 µL of RT Master Mix to each 20-µL ligation reaction.

c. *Gently* vortex to mix thoroughly, then spin briefly.

d. Incubate the 40-µL RT reaction in a thermal cycler with a heated lid at 42 °C for 30 minutes.

3 Purify the cDNA

Use the MinElute® PCR Purification Kit (Qiagen).

a. Add Nuclease-free Water and Buffer PB or Buffer PBI to the cDNA:

1. Transfer all of the cDNA (40 µL) to a clean 1.5-mL microcentrifuge tube.
2. Add 60 µL of Nuclease-free Water.
3. Add 500 µL of Buffer PB or Buffer PBI, then mix well.

b. Purify the cDNA:

Step	Load	Spin	Flowthrough
Load the cDNA onto the MinElute column	600 µL of sample containing Buffer PB or Buffer PBI	13,000 × g for 1 min	Discard
Wash the cDNA	750 µL of Buffer PE	13,000 × g for 1 min	Discard
	–	13,000 × g for 1 min	Discard
Elute the cDNA in a clean microcentrifuge tube	10 µL of Buffer EB	Wait 1 min, then 13,000 × g for 1 min	Save the cDNA

4 Size select the cDNA

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

a. Prepare and assemble a Novex® 6% TBE-Urea Gel in a XCell *SureLock*™ Mini-Cell, using 1X TBE Running Buffer, as described in the *Novex® Pre-Cast Gel Electrophoresis Guide* by Invitrogen.

b. Dilute the 50 bp DNA Ladder to 40 ng/µL (1 µL DNA Ladder + 24 µL RNase-free water).

c. Prepare the cDNA and the DNA ladder:

1. Mix 5 µL of the cDNA with 5 µL of 2X Novex TBE-Urea Sample Buffer.
2. Mix 5 µL of the 40 ng/µL 50 bp DNA Ladder with 5 µL of 2X Novex TBE-Urea Sample buffer.
3. Heat the cDNA and the DNA Ladder at 95 °C for 3 minutes.
4. Snap-cool the tubes on ice. Leave the tubes on ice for less than 30 minutes.

d. Before you load the samples, flush the wells of the gel several times with 1X TBE Running Buffer.

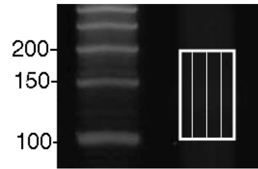
e. Load the cDNA samples and the DNA Ladder, avoiding the lanes next to the edges of the gel.

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

g. Stain the gel with SYBR® Gold nucleic acid gel stain for 5–10 minutes. (5 µL of SYBR Gold nucleic acid gel stain with 50 mL of 1X TBE Running Buffer.)

h. Illuminate the stained gel, then use a clean razor blade to excise the gel containing 100–200 nt cDNA.

i. On a clean work surface, cut the excised gel piece vertically into 4 slices using a clean razor blade.



Excise the region of the gel containing the desired size range, then cut the gel vertically into 4 pieces. To minimize the risk of too much gel in the PCR reaction, limit the gel slices to about 1 mm × 6 mm.

j. 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.

Use the 2 middle gel slices for the in-gel PCR to maximize the yield.

5 Amplify the cDNA

(Optional) For multiplex SOLiD™ System sequencing, use barcoded SOLiD™ 3′ Primers from the SOLiD™ Transcriptome Multiplexing Kit, in color-balanced groups of four. For more information refer to *SOLiD™ Transcriptome Multiplexing Kit Product Insert* (PN 4441076).

a. For each cDNA sample, prepare duplicate in-gel amplification reactions to generate sufficient cDNA for emulsion PCR:

1. Prepare and add 98 µL PCR master mix to each gel piece (1 gel piece per 0.2 mL PCR tube):

Component	Volume	
	One 100-µL reaction	**Two 100-µL reactions‡**
Nuclease-free Water	76.8 µL	169.0 µL
10X 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**

‡ Includes 10% excess volume in the master mix to compensate for pipetting error.

2. Add 2 µL SOLiD 3′ PCR Primer or barcoded SOLiD 3′ Primer to each tube.

b. Run the PCR reactions in a thermal cycler for 12 to 18 cycles:

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

6 Purify the amplified DNA

Use the PureLink™ PCR Micro Kit (Invitrogen).

a. Combine the two 100-µL PCR reactions in a new 1.5-mL tube, add 800 µL of Binding Buffer (B2) to the tube, then mix well.

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

b. Purify the amplified DNA:

Step	Load	Spin	Flowthrough
Load half of the sample onto the PureLink™ Micro Kit Column	500 µL of sample containing Binding Buffer (B2)	10,000 × g for 1 min	Discard
Load the remaining half of the sample onto the PureLink™ Micro Kit Column	500 µL of sample containing Binding Buffer (B2)	10,000 × g for 1 min	Discard
Wash the DNA	600 µL of Wash Buffer (W1)	10,000 × g for 1 min	Discard
	–	14,000 × g for 1 min	Discard
Elute the DNA into a clean PureLink™ Elution Tube (1)	10 µL of Elution Buffer	Wait 1 min, then 14,000 × g for 1 min	Save the DNA
Elute the DNA into a clean PureLink™ Elution Tube (2)	10 µL of Elution Buffer	Wait 1 min, then 14,000 × g for 1 min	Save the DNA

7 Assess the yield and size distribution of the amplified DNA

a. 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.

b. Run 1 µL of the purified DNA on an Agilent® 2100 Bioanalyzer with the DNA 1000 Kit (Agilent).

c. Using the 2100 expert software, perform a smear analysis to quantify the percentage of DNA that is 25–150 bp.

Proceed with SOLiD System templated bead preparation only if <20% of the amplified DNA is in the size range 25–150 bp.

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

8 Proceed with SOLiD™ System templated bead preparation

Emulsion PCR library concentrations (singleplex or multiplex sequencing pools) of 0.4 pM and 0.8 pM for ePCR are recommended for workflow analysis. Refer to the *Applied Biosystems SOLiD™ 3 System Templated Bead Preparation Guide* (PN 4407421).

Materials

Item	Source
SOLiD™ Whole Transcriptome Analysis Kit	Applied Biosystems PN 4425680
SOLiD™ Transcriptome Multiplexing Kit	Applied Biosystems PN 4427046
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
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‡	Invitrogen PN 12183016
Quant-iT™ RNA Assay Kit, 100 assays‡	Invitrogen PN Q32852
RiboMinus™ Concentration Module, 6 preps‡§	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
Pipette tips, RNase-free	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.
§ The RiboMinus™ Concentration Module is not equivalent to the RiboMinus™ Eukaryote Kit for RNA-Seq or to the RiboMinus™ Plant Kit for RNA-Seq.

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