

## Alternative Fragmentation Protocol for SOLiD™ Total RNA-Seq Kit

### Required materials

- SOLiD™ Total RNA-Seq Kit (Applied Biosystems 4445374)
- T4 Polynucleotide Kinase (T4 PNK, Cloned, 10U/μL) (Applied Biosystems AM2310) and ATP Soln. (10 mM) (Applied Biosystems AM8110G)
- Invitrogen RiboMinus Concentration Module (Invitrogen K1550-05)
- Agilent RNA 6000 pico chip (Agilent 5067-1511)

### Fragment the whole transcriptome RNA using alternative method

Fragmentation the whole transcriptome RNA involved the following procedures:

1. Fragment the RNA
2. Kinasing fragmented RNA
3. Clean up fragmented RNA
4. Assess the yield and size distribution of fragmented RNA

### Guidelines for RNA sample type and amount

Please refer to “Guidelines for RNA sample type and amount” in SOLiD™ Total RNA-Seq kit manual, page 14.

### Fragment the RNA

Use components from the SOLiD™ Total RNA-Seq kit

- Nuclease-free water
- RNase III reaction buffer

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

Component	Volume
RNA sample and nuclease-free water:	9 μL
<ul style="list-style-type: none"> <li>• Poly(A) RNA: 100-500 ng</li> <li>• rRNA-depleted total RNA: 200-500 ng</li> <li>• WT control RNA: 500 ng</li> </ul>	
RNase III reaction buffer	1 μL
<b>Total volume</b>	<b>10 μL</b>

2. Flick the tube or pipet up and down a few times to mix, then spin briefly.
3. Incubate the reaction in a thermal cycler at 95°C for 10 minutes. Snap cool on ice **immediately** after the incubation. Go to the next step immediately.

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**IMPORTANT!** Incubate exactly 10 minutes. Shortening or elongating incubation time could cause suboptimal size distribution of fragmented RNA.

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### **Kinasing fragmented RNA**

4. Set up kinasing reaction by adding T4 Polynucleotide Kinase and ATP to each PCR tube:

<b>Component</b>	<b>Volume</b>
Fragmentation reaction from step 3	10 $\mu$ L
T4 PNK, 10U/ $\mu$ L	1 $\mu$ L
ATP, 10 mM	1 $\mu$ L
<b>Total volume</b>	<b>12 <math>\mu</math>L</b>

5. Flick the tube or pipet up and down a few times to mix, then spin briefly.
6. Incubate at 37°C for 30 minutes. Snap cool on ice. Go to next step immediately or leave the fragmented RNA on ice for less than 1 hour.

### **Clean up fragmented RNA**

7. Add 88  $\mu$ L of Nuclease-free Water to each reaction.
8. Add 100 $\mu$ L binding buffer from Invitrogen RiboMinus Concentration Module (Invitrogen K1550-05) and 250 $\mu$ L 100% EtOH, mix well.
9. Load 450  $\mu$ L sample to the column and spin at 12,000 \*g for 1 minute, discard flow-through and put column back into the same tube.
10. Add 500 $\mu$ L Wash Buffer (W5) with ethanol to the column and centrifuge for 1 min at 12,000 \*g.
11. Discard flow-through and place the column in a clean wash tube. Centrifuge the column for an additional 2 min at maximum speed.
12. Place the column in a clean 1.5 ml recovery tube.
13. To elute RNA, add 12  $\mu$ L RNase-free water to the center of the column. Let the column stand for 1 min, and then centrifuge for 1 minute at maximum speed. ~10  $\mu$ L fragmented RNA will be recovered from the column.

### **Assess the yield and size distribution of fragmented RNA**

Please refer to SOLiD™ Total RNA-Seq kit manual, Chapter 2, page 16.

## Typical results of fragmentation of whole transcriptome RNA

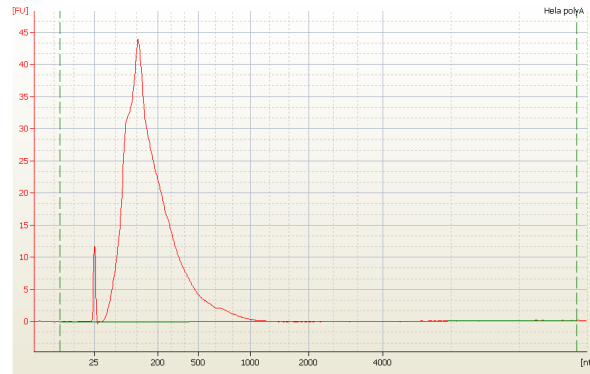


Figure 1: Size distribution of fragmented Hela poly(A) RNA

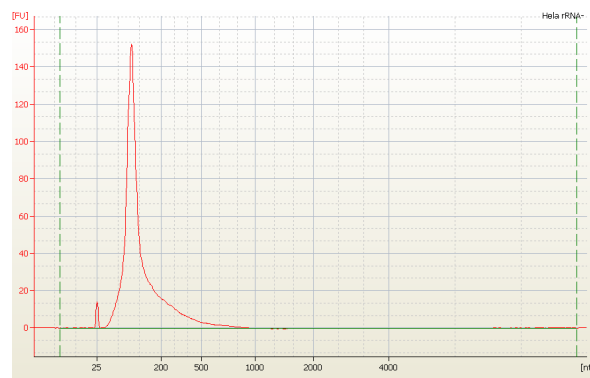


Figure 2: Size distribution of fragmented Hela rRNA-depleted total RNA

The fragmentation procedure should produce a distribution of RNA fragment sizes from 35 nts to several hundred or a few thousand nts, depending on your sample type. To get optimal results, the median size should be 100-200 nts, ideally between 130-170 nts. Percent of RNA fragments in the 100-200 nts range should be greater than 50%.

Proceed with **“Construct the amplified whole transcriptome library”** (page 18, SOLiD™ Total RNA-Seq Kit protocol). Use the input recommendation in the kit protocol.