Whole transcriptome analysis using Agilent Strand Specific RNA Library Preparation Kits

Quick Start Guide

RNA sequencing, or RNA-seq, has enabled the study of coding and non-coding RNAs thereby providing a deeper understanding of gene regulation and the transcriptome. Agilent Technologies’ SureSelect Strand Specific RNA Library Prep kit protocol uses oligo-dT methods to purify poly(A) RNA from total RNA, resulting in RNA-seq libraries comprised of mRNA. In some instances, researchers may be interested in creating libraries that are comprised of both poly(A) and non-poly(A) transcripts. This application note describes how the SureSelect Strand Specific RNA Library Prep kit for Illumina paired-end multiplexed sequencing can be used in combination with the Epicentre Ribo-Zero™ family of ribosomal RNA (rRNA) depletion kits. Removal of the abundant rRNAs from a total RNA sample using the information outlined in this application note will facilitate the generation of RNA-seq libraries that contain both poly(A) and non-poly(A) transcripts for downstream target enrichment and/or whole transcriptome RNA-seq studies.

Quick Tip!

When performing this alternative library preparation protocol, be sure to select the appropriate Epicentre Ribo-Zero Magnetic Kit (from their Selection Guide) based on the sample type that will be processed.

Protocol for using Ribo-Zero kits with the SureSelect Strand Specific RNA Library Prep Kit:


2. Follow the Ribo-Zero protocol to purify the protocol to purify the rRNA-depleted sample (3.D).
   The Ribo-Zero Magnetic kit manual offers three methods:
   3.D.1. Ethanol Precipitation of the rRNA-depleted sample
   3.D.2. Agencourt® RNAClean™ XP Kit
   3.D.3. RNeasy® MinElute® Cleanup Kit
3.D.1. Ethanol Precipitation

1. Follow the protocol and dissolve the dry pellet in 19 ul of RNA Seq Fragmentation Mix.

2. Follow the “SureSelect Strand-Specific RNA Library Prep for Illumina Multiplexed Sequencing kit” or “SureSelect XT RNA Target Enrichment for Illumina Multiplexed Sequencing kit” protocol to fragment the RNA: Step 2 in “Sample Preparation”.

3.D.2. Agencourt RNAClean XP Kit

1. Follow the protocol from steps 1 to 8 to purify the rRNA-depleted RNA.

2. Add 20 ul of RNase Free water to the beads, mix well and incubate for 2 minutes at room temperature.

3. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.

4. Remove the supernatant (~20 ul) to a 1.5-mL LoBind tube and lyophilize it in a speed vacuum.

5. Dissolve the dry pellet in 19 ul of RNA Seq Fragmentation Mix.

6. Follow the “SureSelect Strand-Specific RNA Library Prep for Illumina Multiplexed Sequencing kit” or “SureSelect XT RNA Target Enrichment for Illumina Multiplexed Sequencing kit” protocol to fragment the RNA: Step 2 in “Sample Preparation”.

3.D.3. RNeasy MinElute Cleanup Kit

1. Follow the protocol from steps 1 to 10 to purify the rRNA-depleted RNA.

2. Place the RNeasy MinElute spin column in a new 1.5 –mL tube (supplied in the Qiagen kit). Add 20 ul of RNase free water directly to the center of the spin column membrane. Centrifuge for 1 minute at full speed to elute the RNA.

3. Lyophilize eluate in a speed vacuum.

4. Dissolve the dry pellet in 19 ul of RNA Seq Fragmentation Mix.

5. Follow the “SureSelect Strand-Specific RNA Library Prep for Illumina Multiplexed Sequencing kit” or “SureSelect XT RNA Target Enrichment for Illumina Multiplexed Sequencing kit” protocol to fragment the RNA: Step 2 in “Sample Preparation”.

Ordering Information

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<th>Product Description</th>
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www.agilent.com/genomics/NGS

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