Rapid and Efficient Methods for Ribosomal RNA Removal from Plant and Metatranscriptome Samples

Cindi A. Hoover1 and Cris Kinross2
1DOE Joint Genome Institute, Walnut Creek, CA, USA, 2Epicentre (an Illumina® company), Madison, WI, USA

Introduction

Deep sequencing of cDNA prepared from total RNA (RNA-Seq) or mRNA (mRNA-Seq) has become the method of choice for transcript discovery, discovery of novel transcripts, and identification of alternative splicing events. However, standard whole-transcriptome approaches to RNA-Seq face a significant challenge, as the vast majority of reads map to rRNA. One solution—poly(A) enrichment—does not capture several biologically relevant RNA species, such as microRNA and other noncoding RNAs, and is ineffective for prokaryote samples. To overcome these challenges, Epicentre developed Ribo-Zero™ rRNA removal technology for mammalian, plant, and bacterial total RNA samples. The technology provides excellent removal of rRNA, even from degraded and archived FFPE RNA samples. Here we present preliminary rRNA removal data from two prokaryotic metatranscriptome samples, cow rumen and a sample of mixed prokaryotes. The data show effective rRNA removal and an increase in mapped reads compared to nondepleted control samples. Additionally, we present a comparison of Ribo-Zero kits for prokaryotic samples.

Results

Comparison of Ribo-Zero kits on Rice Stem Sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Reads (million)</th>
<th>% rRNA</th>
<th>% Map</th>
<th>% Adap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mettr_1 no depletion</td>
<td>6.08</td>
<td>72.1</td>
<td>4.5</td>
<td>21.5</td>
</tr>
<tr>
<td>Mettr_1 Ribo-Zero A</td>
<td>7.96</td>
<td>5.3</td>
<td>67.5</td>
<td>9.4</td>
</tr>
<tr>
<td>Mettr_1 Ribo-Zero B</td>
<td>6.82</td>
<td>6.1</td>
<td>70.2</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Rice stem sample was treated with either the Ribo-Zero Kit for Plant Leaf (nonmagnetic) or the Magnetic Ribo-Zero Kit for Plant Seeds/Roots. ScriptSeq v2 libraries were prepared and sequenced on an Illumina HiSeq 2000 at JGI. Ribosomal reads were mapped in bowtie using -v 6 by Epicentre.

Methods Overview

Figure 1. Schematic overview of the Ribo-Zero™ rRNA removal method.

- Total RNA
- Inclusion of fragmented 3′ end, 5′ end
- Add Ribo-Zero™ rRNA Removal Reagents
- Incubation 15 min
- Add Magnetic Beads
- Incubation 10 min
- Remove Magnetic Beads
- Purify RNA-depleted RNA

The process is completed in less than 1.5 hours.

Figure 2. Schematic overview of the ScriptSeq™ v2 directional, di-tagged library preparation method.

- Add Di-Tagged 5′ TTO
- Incubate 15 min
- Add Di-Tagged 3′ TTO
- Incubate 15 min
- Purify cDNA
- Add Adapter: 5′ tag
- Add Ribo-Zero™ rRNA Removal Reagents
- Incubation 15 min
- Add Adapter: 3′ tag
- Add Ribo-Zero™ rRNA Removal Reagents
- Incubation 15 min
- Purify cDNA
- Amplify with Illumina TruSeq libraries

The process is completed in less than 4 hours, with no intermediate purification steps from RNA to di-tagged cDNA fragments.

Figure 3. Schematic overview of metatranscriptome library preparation

- Ribo-Zero™ rRNA Depletion
- mRNA fragmentation (250 nt)
- Double-stranded cDNA synthesis (MnCl2)
- Fragmentation
- Adapter Ligation
- Illumina library amplification

The process is completed in 7 hours.

Table 1. Summary of RNA removal.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Reads (million)</th>
<th>Nuclear (# Mapped Reads)</th>
<th>Mitochondrial (# Mapped Reads)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribo-Zero Plant Leaf</td>
<td>150199353</td>
<td>1659</td>
<td>1206</td>
</tr>
<tr>
<td>Ribo-Zero Seed/Root</td>
<td>246010394</td>
<td>959</td>
<td>587</td>
</tr>
</tbody>
</table>

Figure 4. Profile of RNA-Seq library after treatment with Ribo-Zero kits.

A rice stem sample was treated with either the Ribo-Zero Kit for Plant Leaf (nonmagnetic) or the Magnetic Ribo-Zero Kit for Plant Seeds/Roots. ScriptSeq v2 libraries were prepared and sequenced on an Illumina HiSeq 2000. Reads were analyzed by Picard Tools CollectRNASeqMetrics.

Table 2. Synthetic metatranscriptome composition.

- Organisms in ‘Synthetic’ metatranscriptome, Mettr_1 and cow rumen. All data presented here used the Ribo-Zero Meta-Bacteria kit and/or the Human/Mouse/Rat kit. Library construction began with 1 μg of total RNA, unless otherwise specified. After cDNA synthesis, samples were processed as nonstranded, amplified Illumina TruSeq libraries.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Amount of total RNA in pool (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa CM778</td>
<td>0.1</td>
</tr>
<tr>
<td>Pedicoccus pentosae</td>
<td>6.0</td>
</tr>
<tr>
<td>Acinetobacter sp. ADP1</td>
<td>2.5</td>
</tr>
<tr>
<td>Synechococcus elongatus PCC 7942</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Table 3. Summary of cow rumen metatranscriptome sequencing metrics.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Adapter</th>
<th>% RNA</th>
<th>% Map</th>
<th>% Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow rumen no depletion control</td>
<td>3.7</td>
<td>82.4</td>
<td>7.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Ribo-Zero Meta-Bacteria 1</td>
<td>1.2</td>
<td>15.9</td>
<td>27.3</td>
<td>55.2</td>
</tr>
<tr>
<td>Ribo-Zero Meta-Bacteria 2</td>
<td>3.9</td>
<td>13.0</td>
<td>27.3</td>
<td>55.7</td>
</tr>
<tr>
<td>Ribo-Zero B</td>
<td>12.1</td>
<td>4.9</td>
<td>26.7</td>
<td>56.3</td>
</tr>
<tr>
<td>Ribo-Zero A</td>
<td>1.2</td>
<td>67.8</td>
<td>10.6</td>
<td>56.3</td>
</tr>
</tbody>
</table>

Figure 6. Final cow rumen RNA-Seq libraries with rRNA removal.

Agilent DNA HS QC of final cow rumen libraries. Two separate rounds of Ribo-Zero Meta-Bacteria + H/M/R = red trace. Single round of mixture of Ribo-Zero Meta-Bacteria + H/M/R removal solutions = blue trace. Inset shows total rumen RNA vs. Ribo-Zero depleted RNA.

Table 4. Summary of synthetic metatranscriptome sequencing metrics.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Adapter</th>
<th>% RNA</th>
<th>% Map</th>
<th>% Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic metatranscriptome no depl.</td>
<td>15%</td>
<td>86%</td>
<td>0%</td>
<td>46%</td>
</tr>
<tr>
<td>Synthetic metatranscriptome 1</td>
<td>15%</td>
<td>86%</td>
<td>0%</td>
<td>46%</td>
</tr>
<tr>
<td>Synthetic metatranscriptome 2</td>
<td>15%</td>
<td>86%</td>
<td>0%</td>
<td>46%</td>
</tr>
</tbody>
</table>

Summary

- Ribo-Zero rRNA Removal
  - Efficient "single-pass" removal of rRNA from both intact and fragmented total RNA samples in <1.5 hours.
  - Highly effective on complex metatranscriptome samples.
  - Kits for human/mouse/rat (mammalian), bacteria, and plant are now available in a magnetic format for improved ease of use.

ScriptSeq v2 Library Preparation

- Simple ligation-free, directional RNA-Seq library preparation method in under 4 hours from rRNA-depleted RNA or poly(A)+ mRNA
- High-quality RNA-Seq libraries from either intact or fragmented total RNA samples.
- Excellent strand preservation (>98%) and transcription coverage.
- Compatible with Illumina instruments with barcoding option available.

Acknowledgement

Special thanks to Jeff Martin of the Joint Genome Institute for his help with data transfer and analysis.

*Contact: cahoover@bl.gov