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

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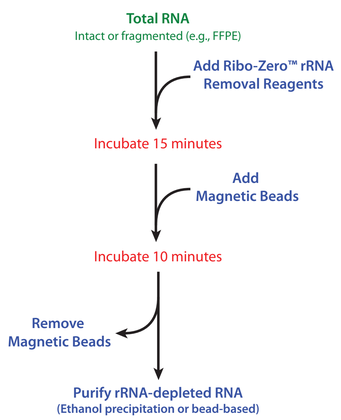
Introduction

Deep sequencing of cDNA prepared from total RNA (RNA-Seq) or mRNA (mRNA-Seq) has become the method of choice for transcript profiling, 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 removal of rRNA from Plant Leaf or Plant Seeds/Roots on the same rice-stem sample to illustrate the difference in reads mapped to rRNA between these kits. Sequence data were generated using an Illumina® HiSeq, but the Ribo-Zero technology is compatible with many downstream applications.

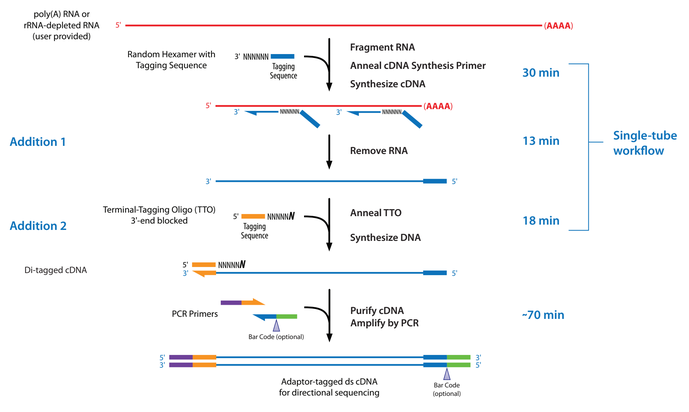
Methods Overview

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



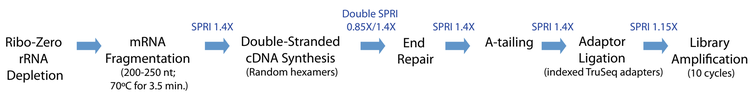
The process is completed in less than 1.5 hours.

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



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



The process is completed in 7 hours.

Results

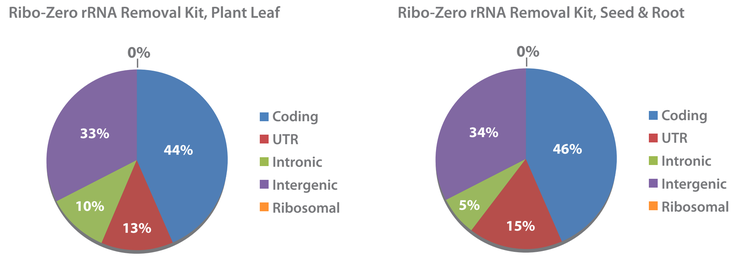
Comparison of Ribo-Zero kits on Rice Stem Sample

Table 1. Summary of rRNA removal.

	Total Reads (million)	Nuclear (# Mapped Reads)			Mitochondrial (# Mapped Reads)		
		25S	18S	5.8S	23S	16S	5S
Ribo-Zero Plant Leaf (nonmagnetic)	150199353	1659	1206	1110	1123	11544	50
Ribo-Zero Seed/ Root (Magnetic)	246010394	959	587	745	1508	19990	4

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 0 by Epicentre.

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 (Plant Leaf) or Ribo-Zero Kit (Plant Seed/Root). ScriptSeq v2 libaries were prepared and sequenced on an Illumina HiSeq 2000. Reads were analyzed by Picard Tools CollectRnaSeqMetrics.

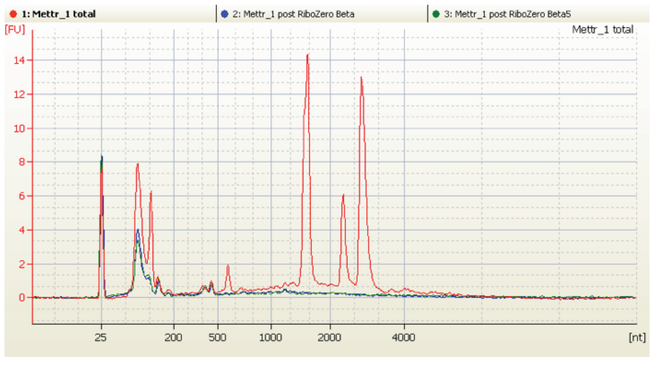
Metatranscriptome Samples

Ribo-Zero kits have been tested at the JGI on several metatranscriptome samples, including '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 2. Synthetic metatranscriptome composition.

Organisms in 'Synthetic' Metatranscript sample, Mettr_1	Amount of total RNA in pool (ug)
Prochlorococcus marinus pastoris CMP1986	0.1
Pediococcus pentosaceus	6.0
Acinetobacter sp. ADP1	2.5
Cyanobacterium synechocystis PCC 6803	3.0
Synechococcus elongates PCC 7942	0.5

Figure 5. rRNA removal from synthetic metatranscriptome.

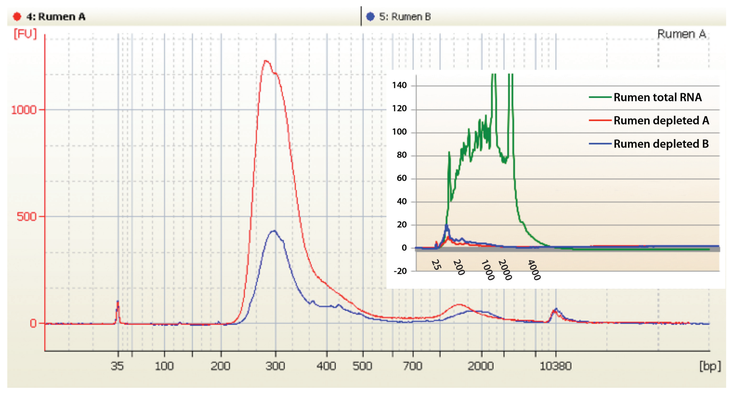


Metatranscriptome samples treated with Ribo-Zero Meta-Bacteria kit (Mettr_1; blue and green traces) show depletion of 16S and 23S rRNA bands compared to total RNA (red trace, RIN 8.4); Agilent 6000 Total Nano.

Table 3. Summary of synthetic metatranscriptome sequencing metrics.

Sample	Total Reads (million)	% rRNA	% Map	% Adap
Mettr_1 no depletion	6.08	72.1	4.5	21.5
Mettr_1 Ribo-Zero A	7.96	5.3	67.5	9.4
Mettr_1 Ribo-Zero B	6.82	6.1	70.2	7.9

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 cow rumen metatranscriptome sequencing metrics.

Sample	% Adapter	% rRNA	% Map (rumen)	% Other
Cow rumen no depletion control	3.7	82.4	3.4	10.5
Ribo-Zero Meta-Bacteria 1	1.2	15.9	27.7	55.2
Ribo-Zero Meta-Bacteria 2	3.9	13.0	27.3	55.7
Ribo-Zero A Meta-Bacteria/Human/Mouse/Rat (3 µg, 1 rd. of mixed depletion)	1.2	67.8	10.6	56.3
Ribo-Zero B Meta-Bacteria/Human/Mouse/Rat (3 µg, 2 separate rds. of depletion)	12.1	4.9	26.7	56.3

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 transcript coverage.
- Compatible with Illumina instruments with barcoding option available.

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