Bacterial RNA sequencing workflow for PGM™

Over the years, we at Ambion receive a number of requests for information about the best way to isolate and ribo-deplete prokaryotic total RNA for next-gen sequencing. I just wanted to give a brief overview and some suggestions on RNA bacterial sequencing using the Ion platform (specifically, the PGM™). After trying several methods to extract and purify total RNA from *E. coli* dh10b, we found that lysing and extracting using TRIzol® Reagent and isolating/purifying with the mirVana™ miRNA isolation glass-fiber filter results in the most complete recovery of total RNA. Specifically, we followed the TRIzol® Reagent protocol and homogenized using trizol then phase separated with chloroform. We then transferred the aqueous phase containing the RNA into a new RNase-free 1.5mL microcentrifuge tube and followed the mirVana™ miRNA Isolation Kit protocol starting with the Total RNA Isolation Procedure section and eluted with 100uL of Elution Solution. This method is able to recover both large and small RNA molecules including miRNA, siRNA, snRNA, and other small RNA transcripts of yet unknown functions. The total RNA went through DNase I digestion to remove any genomic DNA contamination. Before proceeding to rRNA removal we checked the quality of the total RNA on an Agilent 2100 Bioanalyzer using the RNA 6000 Nano kit (see Figure 1). It is important that the RNA be high quality for maximum rRNA removal efficiency.

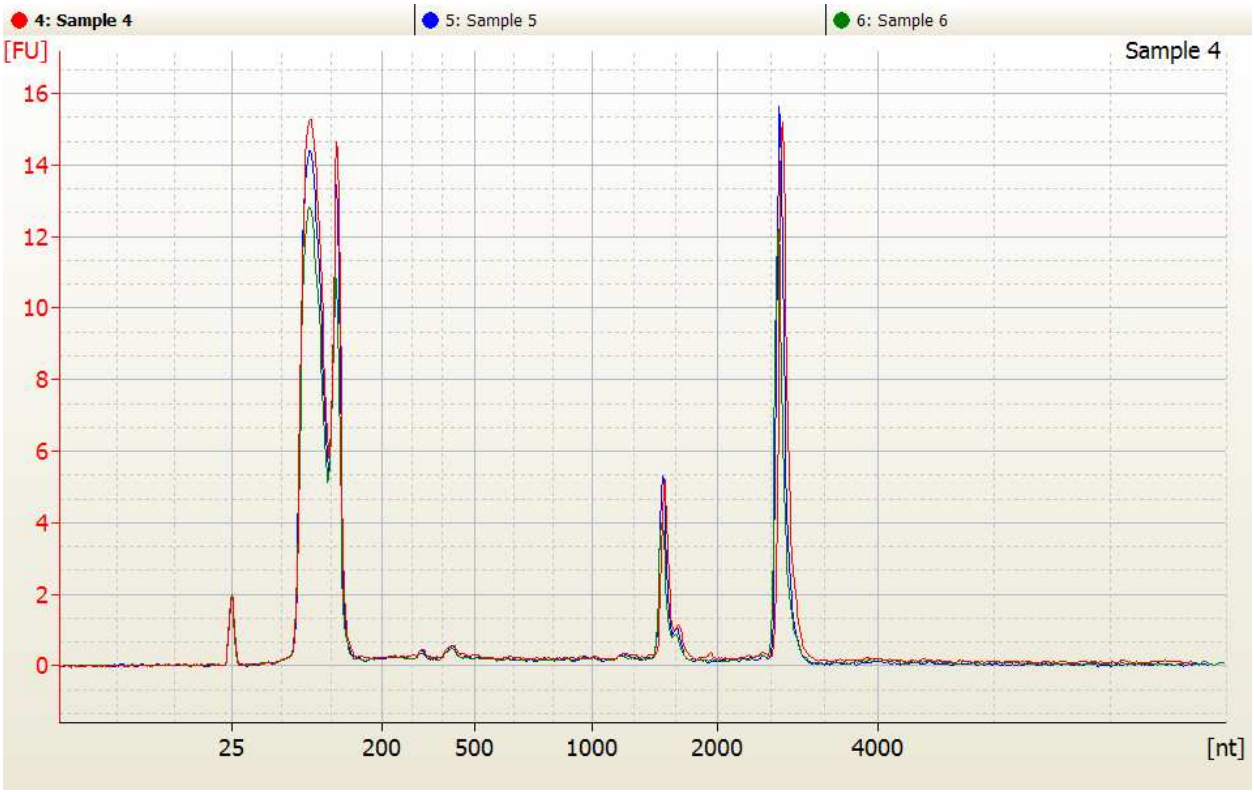

Figure 1. Agilent 2100 Bioanalyzer electropherogram of total RNA using TRIzol® Reagent for lysis/extraction and mirVana™ miRNA isolation glass-fiber filter for purification.

We started with 3µg of total RNA and performed rRNA depletion by following the RiboMinus™ Eukaryote System v2 protocol but modified by replacing the RiboMinus™ Eukaryote probe in the kit with the bacterial probe set described below. The bacterial probe set used was designed by Zhoutao Chen and contains two 16S rRNA and three 23S rRNA sequence-specific 5'-biotin labeled oligonucleotides (12.5pmol/µL). Each probe is single-stranded containing three Locked Nucleic Acid (LNA™) monomers and targets many prokaryotic species (a complete list can be found in Table 1 in *Chen and Duan*[1]). Please contact us to obtain probes or probe sequences. Following hybridization and removal of the rRNA-probe complex the rRNA-depleted RNA was cleaned and concentrated using the RiboMinus™ Magnetic Bead Clean Up Module. 1µl of the rRNA-depleted RNA was quantified using the Qubit® RNA Assay Kit and the Qubit® 2.0

Fluorometer. rRNA depletion was verified using the Agilent 2100 Bioanalyzer with the Agilent RNA 6000 Pico Kit (see Figure 2).

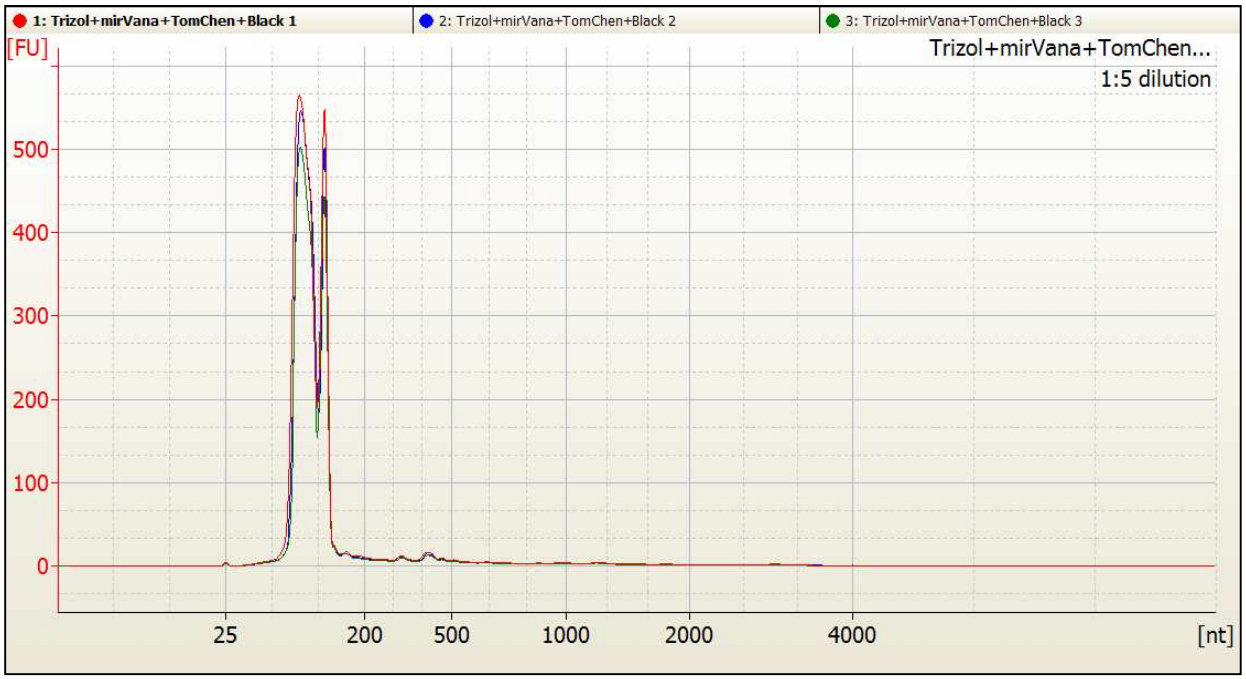

Figure 2. Agilent 2100 Bioanalyzer electropherogram of rRNA-depleted RNA using RiboMinus™ Eukaryote System v2 protocol with bacterial probes.

Libraries were constructed using the Ion Total RNA-seq v2 for Whole Transcriptome Kit and templated with the Ion OneTouch™ 200 Template Kit v2 DL. Sequencing was performed on the Ion PGM™ using the Ion 318™ Chip and Ion PGM™ 200 Sequencing v2 Kits. The sequencing runs were analyzed using Torrent Suite 3.2 and aligned to *E. coli* dh10b reference using Bowtie2. See 16S and 23S mapping results below.

Percent of mapped reads aligned to 16S and 23S rRNA			
rRNA	**Rep1**	**Rep2**	**Rep3**
16S	3.49%	3.21%	3.59%
23S	9.75%	8.95%	9.89%

Libraries can also be templated using the Ion PI™ Template OT2 200 Kit and sequenced on the Ion Proton™ Sequencer using the Ion PI™ Chip Kit v2 and Ion PI™ Sequencing 200 Kit for deeper sequencing.

1. Chen, Zhoutao, and Xiaoping Duan. "Ribosomal RNA depletion for massively parallel bacterial RNA-sequencing applications." In *High-Throughput Next Generation Sequencing*, pp. 93-103. Humana Press, 2011.