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Evaluation of bacterial ribosomal RNA (rRNA) depletion methods for sequencing microbial community transcriptomes

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Microbial community (metagenomics) structure and function are known to play a significant role in human health, development and disease. Shotgun sequencing of metagenomes provides a catalog of genes in the microbial community. However, to understand function, it is necessary to interrogate the community transcriptome. Bacterial RNA content is >95% rRNA, and unlike eukaryotic mRNA, bacterial mRNA is not polyadenylated for easy isolation using oligo-dT selection. Therefore, it is essential to develop rRNA depletion protocols to make RNAseq cost effective. We are comparing different rRNA depletion methods: a hybrid subtraction-based approach using the MICROBExpress Kit (Ambion) and a 5'-phosphate-dependent exonuclease-based approach using the mRNA-ONLY™ Prokaryotic mRNA Isolation Kit (Epicentre). These depletion methods are being tested on well characterized genomes with a range of GC composition: *P. marinus* (30%), *E. coli* (51%), and *R. sphaeroides* (69%).

MICROBExpress rRNA capture oligos were designed to work on intact *E.coli* 5S, 16S and 23S rRNA, however, all microbes do not have intact 16S and 23S: in *R. sphaeroides,* the 23S molecule is processed into separate 1.4kb and 1.6kb molecules, and in *P.marinus,* some of the 23S appears as smaller 0.7kb and 2.3kb molecules. Hence, different removal efficiencies have been observed. Visual inspection of the rRNA molecules on the Agilent Bioanalyzer shows almost complete depletion of 16S and 23S peaks for *E.coli,* depletion of the 16S peak for both *R. sphaeroides* and *P. marinus* but not the smaller 23S processed molecules in *R. sphaeroides* and *P. marinus.* By comparison, mRNA-ONLY removes roughly 2/3 of the rRNA transcripts in both *E.coli and R. sphaeroides.*

To determine efficiency of mRNA enrichment using these methods, the mRNA was transcribed to cDNA and sequenced by Illumina. rRNA reads decreased by 25% in *E.coli* and 4.5% in *R. sphaeroides* with MICROBExpress and ~17% in both with mRNA-ONLY. As a result, a larger fraction of sequenced bases align to the annotated transcriptome with both methods: ~30-40% with MICROBExpress) and ~13-28% with mRNA-ONLY. We are evaluating additional depletion methods that will increase the amount of non-rRNA reads. These will also be applied to *P. marinus.*

Determining enrichment efficiency by sequencing is very costly, thus we are developing qPCR assays for high, mid, and low expressed genes and the 16S and 23S rRNA genes for these organisms, and we will assess changes in rRNA/mRNA ratios with different depletion rRNA methods. The optimal method will be applied to microbe community transcriptomes.

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