CRISPR mediated depletion of abundant rRNA molecules from host and bacteria improve sensitivity in microbial and metagenomic RNA Sequencing

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Introduction

Abundant ribosomal RNA (rRNA) from host organelles and bacterial species inhibit the sensitivity of RNA-sequencing to detect and characterize low expressed transcripts when performing shotgun RNA-Seq. The overabundance of rRNA in stool samples can interfere with the efficient identification of biologically significant transcripts, meaning genes or bacterial species with low expression are often barely detectable. In addition, much of the transcriptomic data captured is uninformative, thus hindering discovery. While fecal samples comprise a mixture of both human and bacterial cells, they typically have a higher bacterial to human ratio. Removal of abundant cDNA library molecules enables greater sensitivity of detection of lower expressed transcripts in microbiome sample, thus enabling greater detection of bacterial species as compared to current methods. Here we present the application of CRISPRclean® Plus technology to samples prepared from ZymoBIOMICS Fecal Reference control and compare the performance against Illumina Ribo-Zero Plus.

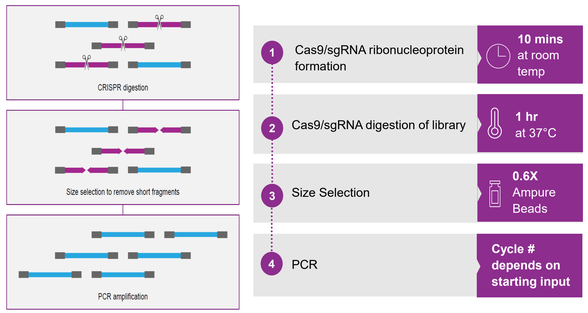

Figure 1. Overview of CRISPRclean technology. Double stranded cDNA library products are cleaved, following adapter ligation, using CAS9 and specifically designed guide RNAs. Cleaved fragments do not contain adapters on both ends and will not amplify in subsequent PCR reactions. The cleaved fragments are removed from the library pool during short fragment cleanup.

Methods

CRISPRclean Plus and Illumina Ribo-Zero Plus kits were tested under three conditions with three replicates each. A total of 18 libraries were prepared from a ZymoBIOMICS Fecal Reference sample and sequenced on a NextSeq 2000. The sequence data from each sample was down sampled to 64M paired end reads and aligned using BWA to a custom database containing ribosomal data from SILVA, Rfam and human. Unmapped reads (non-ribosomal) were processed through Kraken2[1] to analyze the microbiome composition (Figure 2).

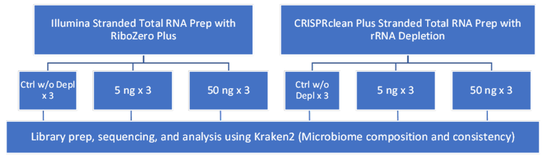

Figure 2. Experimental overview of comparison between CRISPRclean Plus and Illumina Ribo-Zero Plus kits.

1. Wood, D.E., Lu, J. & Langmead, B. Improved metagenomic analysis with Kraken 2. Genome Biol 20, 257 (2019).

Results

CRISPRclean Plus is more efficient at removing rRNA

To compare the rRNA depletion efficiencies of CRISPRclean Plus with Illumina Ribo-Zero Plus, the percent alignment to rRNA at 5 ng and 50 ng total RNA input was assessed. CRISPRclean Plus was more efficient at rRNA depletion with a lower percent alignment to rRNA after depletion compared to both non-depleted control and Illumina Ribo-Zero Plus (Figure 3).

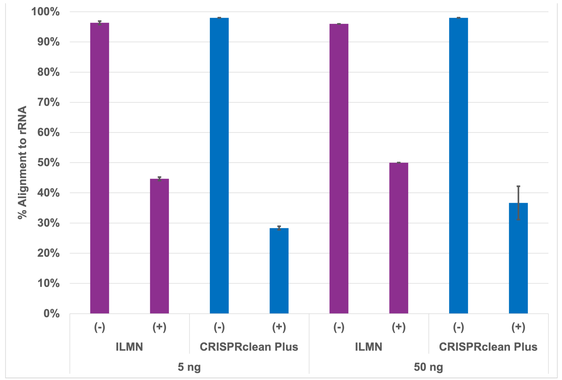

Figure 3: Percent alignment to rRNA

CRISPRclean Plus depletion provides increased sensitivity for greater bacterial species detection

We next set out to evaluate the bacterial detection sensitivity of CRISPRclean Plus compared to Illumina Ribo-Zero Plus. The data revealed that CRISPRclean Plus produced a 4–5 fold increase in bacterial species identified using CRISPRclean Plus compared to Illumina Ribo-Zero Plus after depletion of rRNA at 5 ng and 50 ng input (Figure 5). The cut-off is set at greater than or equal to 10 RPM (reads per million).

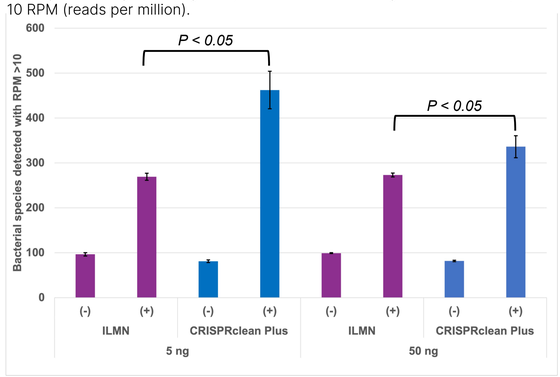

Figure 4. Increased sensitivity for detection of bacterial species

CRISPRclean Plus depletes more bacterial rRNA and provides deeper insight into the taxonomic composition of the microbiome

We surmised that assessing the depletion rate alone did not provide a comprehensive picture and that further insight could be gained from a more in-depth analysis of the depleted library content. Thus, we set out to evaluate the taxonomic composition of the CRISPRclean Plus and Illumina Ribo-Zero Plus depleted libraries as compared to non-depleted libraries.

A closer examination taxonomic composition of all three kingdoms of life revealed that while bacterial rRNA accounted for most of the overall remaining coverage following depletion (Figure 5, light purple), the presence of relatively more eukaryota rRNA (Figure 5, dark purple) and archaeal rRNA transcripts (Figure 5, blue) were observed in the CRISPRclean Plus depleted libraries compared to Illumina Ribo-Zero Plus.

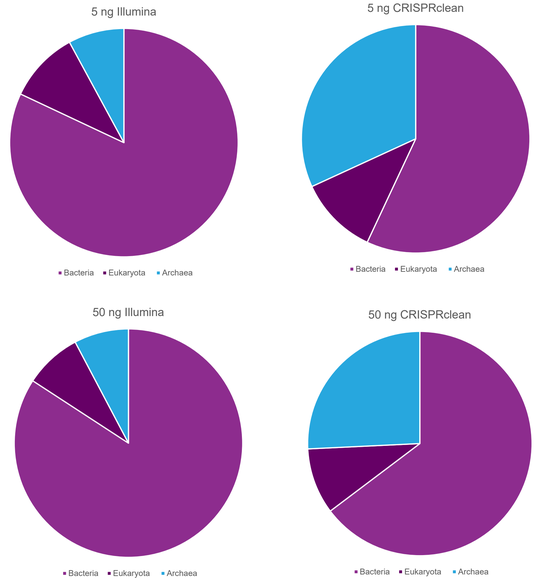

Figure 5: Taxonomic composition of non-depleted or depleted samples.

CRISPRclean Plus retains more bacterial species read counts

To further assess bacterial detection performance following depletion, we evaluated the impact of the CRISPRclean Plus and Illumina Ribo-Zero Plus methods on the retention of bacterial species in depleted vs. non-depleted samples. Read counts from replicates before and after depletion were higher for CRISPRclean Plus depleted samples (50-fold) compared to Illumina Ribo-Zero Plus for depleted samples (9-fold). The data revealed that at 5 ng input, the CRISPRclean Plus method retained more bacterial species read counts than the Illumina Ribo-Zero Plus method (50 vs. 9-fold increased sensitivity, respectively; (Figure 6). These data provide researchers with further confidence in the performance of CRISPRclean Plus rRNA depletion for providing increased bacterial detection.

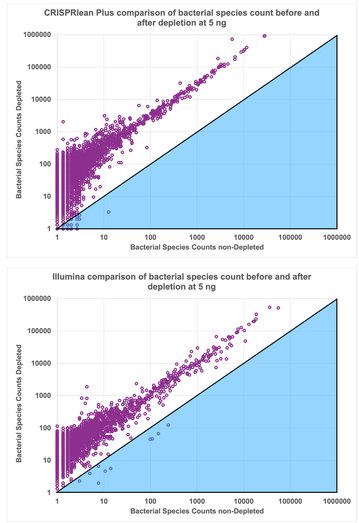

Figure 6. Read counts to bacterial species

Conclusions

CRISPRclean Plus offers more comprehensive and accurate insights into complex microbial communities. CRISPRclean Plus applied to fecal samples increases the number of identified bacterial species by 4-5 fold, providing researchers with greater biological insight.

When compared to Illumina Ribo-Zero Plus, CRISPRclean Plus:

- Outperforms Illumina Ribo-Zero Plus in depletion rates.
- Is a more accurate method for detecting bacterial species in fecal samples compared to Illumina Ribo-Zero Plus.
- Provides deeper insight into the taxonomic composition of the microbiome in fecal samples than Illumina Ribo-Zero Plus.
- Retains more bacterial species after depletion than Illumina Ribo-Zero Plus.
- Read counts are more highly correlated with the Zymo control and produced extremely low library bias.

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