High yield depletion for an RNA-seq application using an innovative benchtop platform for workflow automation and miniaturization

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INTRODUCTION

Transcriptomic analysis by RNA sequencing is hindered by the abundance of ribosomal RNA (rRNA), representing ≥ 80% of molecules in total RNA. Removal of rRNA prior to RNA-Seq enables researchers and clinicians to focus on analyzing high-value, informative portions of the transcriptome while lowering sequencing costs. In an effort to optimize, miniaturize and automate rRNA removal, Inorevia has added Illumina's Ribo-Zero Plus rRNA Depletion Kit to its NGS applications portfolio.

HOW DOES IT WORK?

Magelia, Inorevia's multi-OMICs platform is ideal for automating Illumina's Ribo-Zero Plus rRNA Depletion Kit. Its precise handling of magnetic beads paired with input/volume reduction are a great match for treating precious samples. This bolsters sample input reduction, as Illumina's depletion kit is already compatible with low RNA quantities (≤ 100 ng). Improved reaction kinetics assist efficient rRNA removal from total RNA by targeted hybridization to DNA probes and subsequent RNase H cleavage. The pairing of Magelia with Illumina's Ribo-Zero Plus rRNA Depletion Kit meets or exceeds performance metrics in all tested samples. This study summarizes the advantages of using Magelia for rRNA depletion on human standard total RNA.

PLATFORM FEATURES

- **High quality on low input samples**
- **Integrated heating/cooling/thermal cycling**
- **Full automation of complex workflows**
- **Compact benchtop platform**
- **Connected instrument**
- **Proprietary cartridges prevent inter and intra run cross-contamination**

METHODS

Total control human RNA was processed for ribodepletion in parallel in the Magelia and manually (Figure 1). Ribodepleted RNAs were prepared for sequencing using the Illumina Stranded Total RNA Prep, Ligation kit. Libraries were pooled and sequenced on a NextSeq 500 Mid Output Kit v2 (150 cycles), using a 2 × 76 bp paired-end read length.
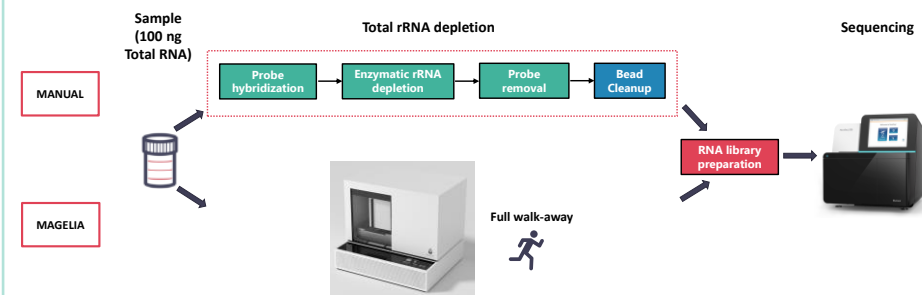

Figure 1 : Magelia rRNA depletion workflow for standard human control total RNA

SUPERIOR RRNA DEPLETION IN MAGELIA

rRNA count revealed that manually treated samples showed 27,5x more rRNA transcripts than Magelia treated ones (Figure 2). This implies higher depletion efficiency, a key performance feature impacting transcriptome resolution.

Significantly higher (p= 0,0001) overall absolute average expression was found for Magelia treated samples (Figure 3), confirming improved depth for informative parts of the transcriptome.
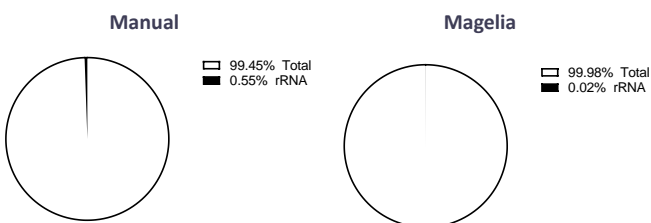

Figure 2 : Proportion of rRNA transcripts in manually and Magelia treated samples
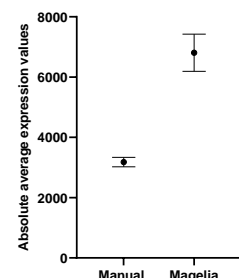
Figure 3: Absolute average expression

IMPROVED TRANSCRIPTOMIC RESOLUTION FOR MAGELIA TREATED SAMPLES

A 7,6- and 8,3-fold difference was found for Magelia and manually treated samples respectively when comparing the top and bottom 20 transcribed genes reported in a compilatory transcriptomics database (Figure 4).
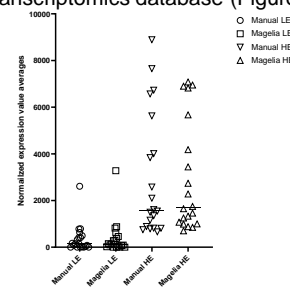

Figure 4: Normalized expression values for manually and Magelia treated samples. LE designates lowly expressed genes, HE designates highly expressed genes.

Low expression genes were below the overall normalized expression value average by at least 4-fold, supporting biological relevance for the obtained data. All previously described gene fusion events were detected. As shown on the example below (Figure 5), average coverage was 210x and 634x for manually and Magelia treated samples respectively, **highlighting improved depth/transcriptome resolution for Magelia treated samples, linked to superior depletion.**
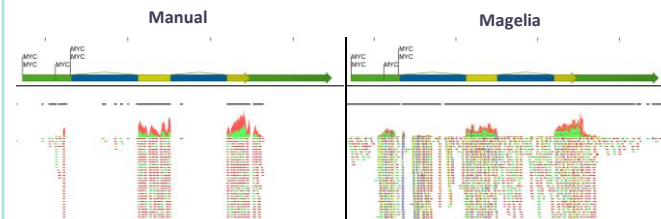

Figure 5: Mappings to the MYC gene region of Chr. 8 for manually and Magelia treated samples. Red and green lines represent mapped reads. Red and green histograms represent read depth.

CONCLUSION

rRNA depletion in Magelia, embodies a robust, effective, and simple method to secure high-quality transcriptome data. It facilitates automated analysis of low biomass samples which has recently been applied to provide unprecedented resolution into a bat virome study (manuscript under preparation).