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Efficient rRNA depletion using the new RiboMinus Bacteria 2.0 Kit to uncover the complexity of Bacterial transcriptomes

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ABSTRACT

Global transcriptomic analysis is often hindered by the high abundance of ribosomal RNA (rRNA) content in bacterial cells, comprising up to 98% of RNA in cells. To remove unwanted rRNA and enrich for valuable protein coding transcripts of interest, rRNA depletion by capture probe hybridization has been the method of choice for RNA-seq, because it maintains relative transcriptome levels. The existing RiboMinus™ Bacteria design has had limited success with partially degraded total RNA, due to an abundance of fragmented rRNA contamination in compromised RNA that the current design of eight locked nucleic acid (LNA) probes does not address. Presently, we evaluate the efficiency of a new RiboMinus™ Bacteria 2.0 Kit in depleting rRNA from various inputs (100ng-5ug) of *E. coli* total RNA, which utilizes a new probe mix design. The RiboMinus™ procedure entails hybridization of a new set of 3′-biotin labeled ssDNA oligonucleotide probes (called RiboMinus™ Pan-Prokaryote Probe Mix) targeting sequence-specific regions of 16S, 23S rRNA and 5S rRNA. This RNA/probe hybrid is then removed from the sample using streptavidin-coated magnetic beads, leaving behind rRNA-depleted RNA. Sequencing results indicate that the newly developed RiboMinus™ Pan-Prokaryote Probe Mix in the RiboMinus™ Bacteria 2.0 workflow exhibits superior rRNA depletion efficiency compared to the existing kit, with less than 10% of total reads mapping to 16S and 23S rRNA vs. 90-95% of total reads mapping to rRNA using the existing kit. Removal of rRNA also results in a 10-fold increase of protein coding transcripts, from 5% in the existing kit to 55% in RiboMinus™ Bacteria 2.0. In addition, rRNA removal increases sensitivity for detection of noncoding transcripts. Finally, we also demonstrate that the newly developed RiboMinus™ Bacteria 2.0 Kit utilizing the new RiboMinus™ Pan-Prokaryote Probe Mix exhibits high ribosomal removal efficiency when *Salmonella enterica* RNA samples were tested.

MATERIALS AND METHODS

rRNA-depleted RNA was obtained from E.Coli DH5α total RNA using the new RiboMinus™ Bacteria 2.0 Kit. Input amounts of 100ng-5μg total RNA were hybridized with the RiboMinus™ Pan-Prokaryote Probe Mix. The probe mix is a mixture of biotinylated DNA oligos designed against conserved regions of rRNA across a wide variety of pan-prokaryote species (see Table 1). Next, the rRNA-probe complexes are removed from the total RNA by capture with streptavidin-conjugated RiboMinus™ Magnetic Beads. The resulting rRNA depleted RNA is concentrated and purified with magnetic Nucleic Acid Binding Beads (see Figure 2). Approximately, 50ng of rRNA-depleted RNA was used in the whole transcriptome library workflow as described in the Ion Total RNA-Seq Kit v2 manual. Libraries were sequenced using the Ion GeneStudio™ S5 Systems.

RNA-Seq data analysis. FastQC (Babraham Bioinformatics, [http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/]) used to evaluate the quality of raw reads. All samples were aligned to reference genomes using universal RNA-seq aligner STAR and alignment metrics collected (alignment rate, chimeric re(https://www.ncbi.nlm.nih.gov/pubmed/23104886) ads fraction, duplication rates, etc). Biotype distribution was calculated based on QORTS read counts (https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-015-0670-5).

FIGURE 1. WHOLE TRANSCRIPTOME RNA-SEQ WORKFLOW

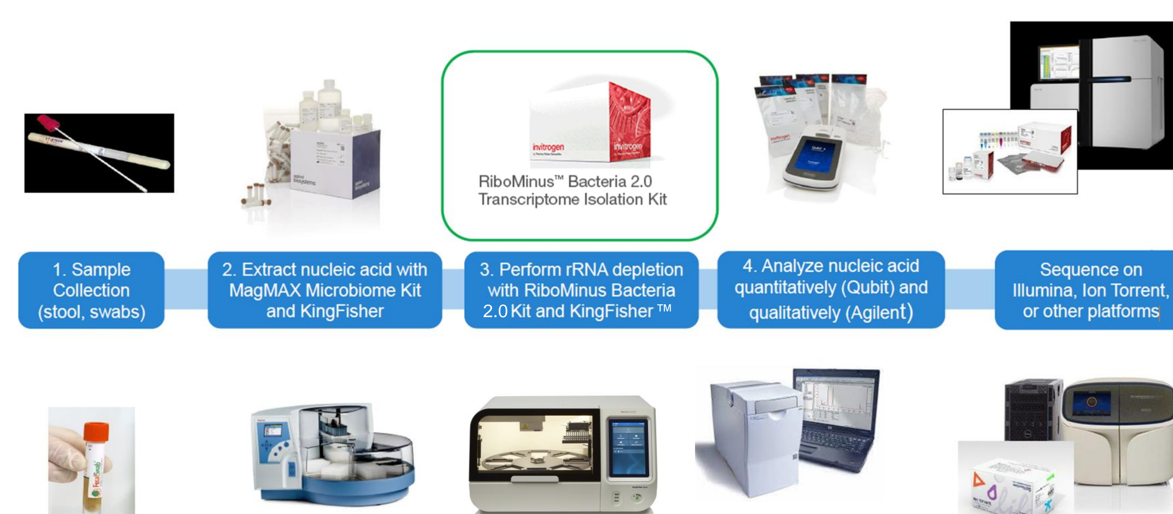


TABLE 1 – LIST OF PHYLA & SUB-PHYLA REPRESENTATIVE SPECIES IN PROBE DESIGN

Domain	Phylum	Sub-phyla / representative
ARCHAEA	Proteoarchaeota	Thaumarchaeota Crenarchaeota
	Euryarchaeota	Pyrococcus Halobacteria Methanopyri Methanococci Thermoplasma
BACTERIA	Proteobacteria	α β γ δ ε
	Gram-positives	Actinobacteria Firmicutes Photosynthetic Bacteria
	Cyanobacteria	
	Spirochaetes	Spriochaetes Leptospiras
	Green Sulphur Bacteria	Chlorobi
	Bacteroidetes	Bacteroides Flavobacterium
	Planctomycetes	
	Chlamydiae	
	Radioresistant Micrococci	Thermophiles Deinococcus
	Green Non-Sulphur Bacteria	Chloroflexi
	Thermotogae	
	Aquificae	Aquifex
	Acidobacteria	
	Fibrobacteres	

Table 1. RiboMinus™ Pan-Prokaryote Probe Mix is designed against 76 representative species of bacteria and archaebacteria from the common phyla and sub-phyla listed in Table 1. The Probe Mix consists of 3′ biotinylated ssDNA oligos designed to target 5S, 16S, and 23S rRNA. The probe mix contains over 100 designs and has been tested against *E. coli, Bifidobacterium longum subsp. infantis, Staphylococcus aureus, Bacteroides thetaiotaomicron* and *Salmonella enterica.*

FIGURE 2 – RIBOMINUS™ BACTERIA 2.0 DEPLETION WORKFLOW

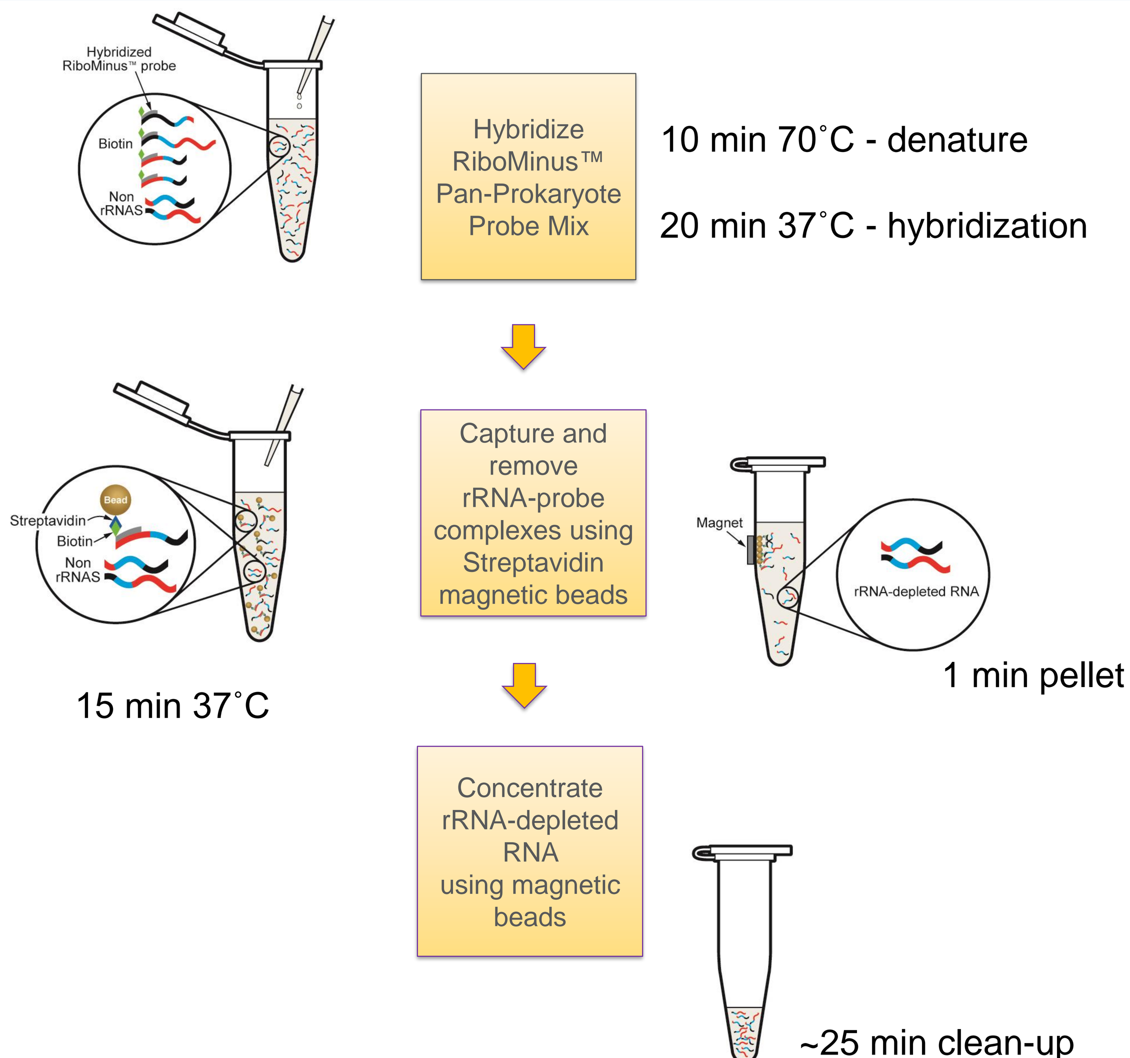


FIGURE 3 – ANALYSIS OF rRNA-DEPLETED RNA

A.

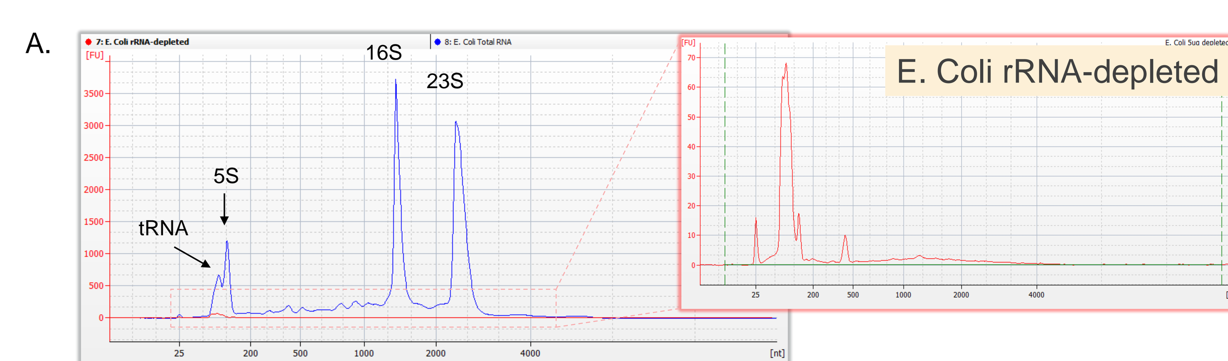
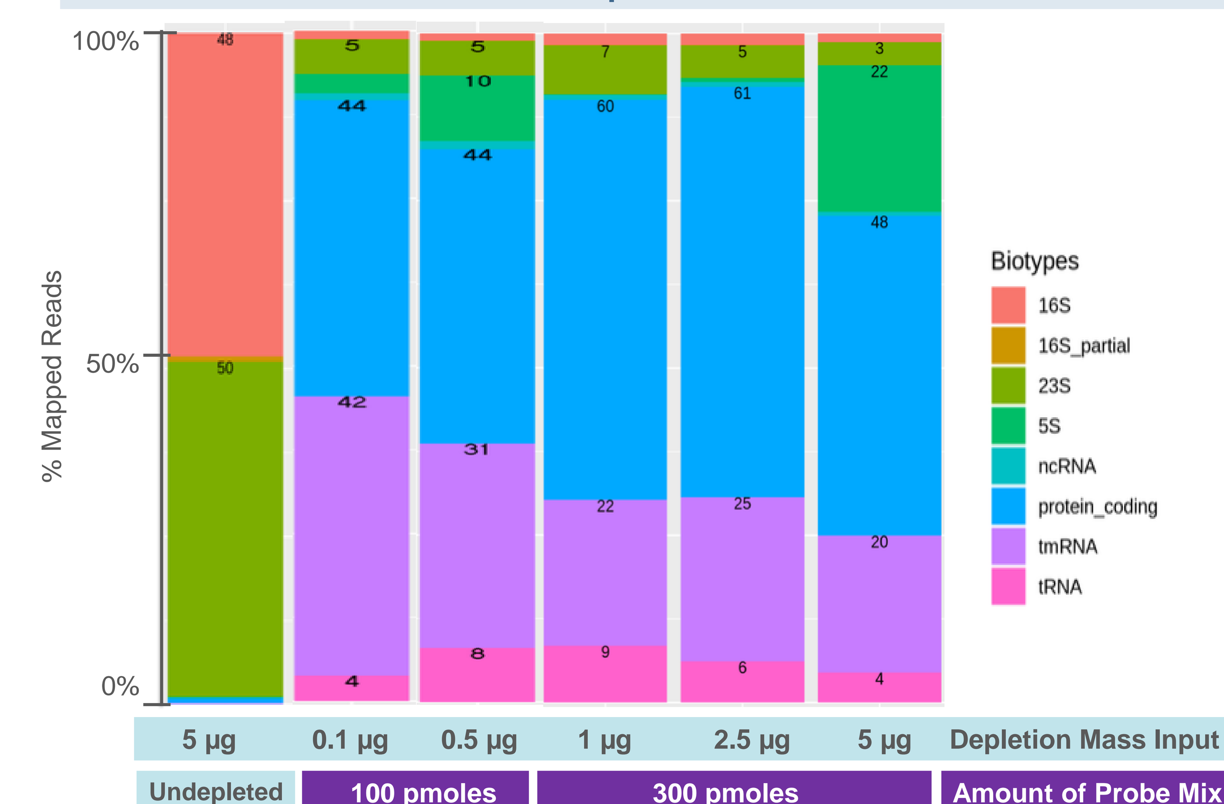


FIGURE 3A. Agilent® 2100 Bioanalyzer® profiles using the RNA 6000 Pico Assay showing an overlay of E. Coli rRNA-depleted RNA with undepleted total RNA (left) and an enlarged detailed trace of rRNA-depleted RNA (aka RiboMinus RNA) (right). rRNA-depleted RNA is shown in red and undepleted total RNA in blue. 5 μg of E. Coli (RIN 8.7) and 300 pmoles of RiboMinus™ Pan-Prokaryote Probe Mix was used to perfrom the RiboMinus™ Bacteria 2.0 workflow.

B.

RNA-Seq detected transcripts by biotypes for E. Coli rRNA-depleted libraries



C.

RNA-Seq detected transcripts by biotypes for E. Coli Manual vs Automated KingFisher rRNA depletion workflow

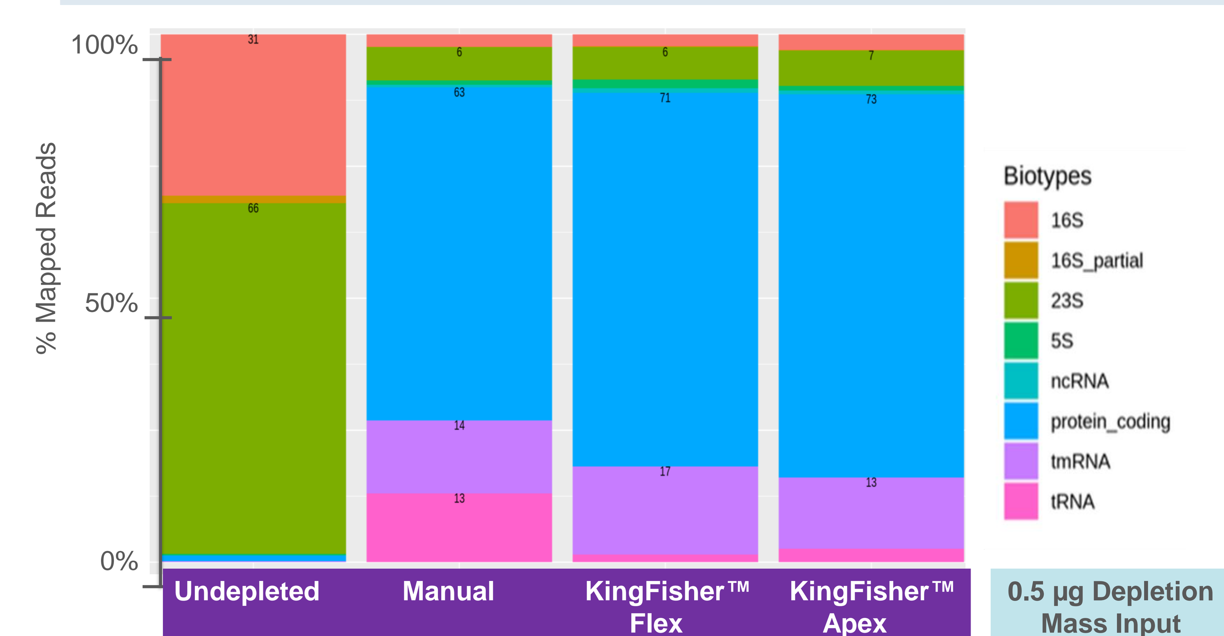


FIGURE 3B. Mapping Statistics Performance of RiboMinus Bacteria 2.0 Kit with E. Coli using 100 ng to 5 μg mass input total RNA into depletion. Compared to undepleted, samples treated with the new Pan-Prokaryote Probe Mix show a much greater percentage of reads mapping to protein coding genes, from <2% in undepleted samples to >44% in rRNA-depleted samples. **3C.** Mapping Statistics comparing manual and automated depletion performance using KingFisher™ instruments on E. Coli total RNA.

D.

RNA-Seq detected transcripts by biotypes for Salmonella enterica rRNA-depleted libraries

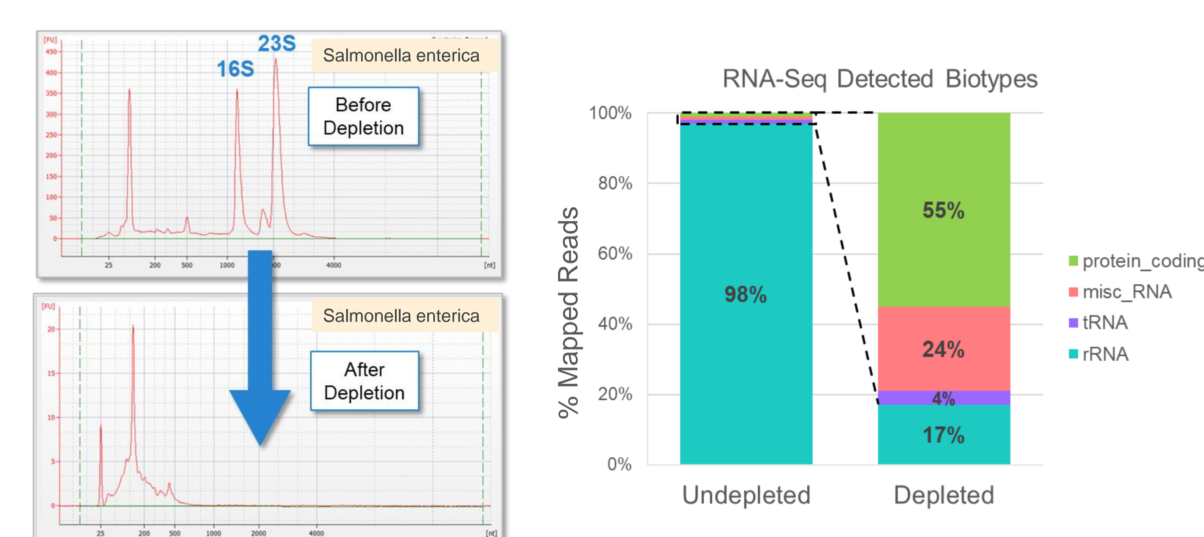


FIGURE 3D. Mapping Statistics Performance of RiboMinus Bacteria 2.0 Kit with Salmonella enterica using 1μg mass input total RNA into depletion. Compared to undepleted, samples treated with the new Pan-Prokaryote Probe Mix show a much greater percentage of reads mapping to protein coding genes, from <2% in undepleted samples to >55% in rRNA-depleted samples.

FIGURE 4 – COMPARISON TO RIBOZERO

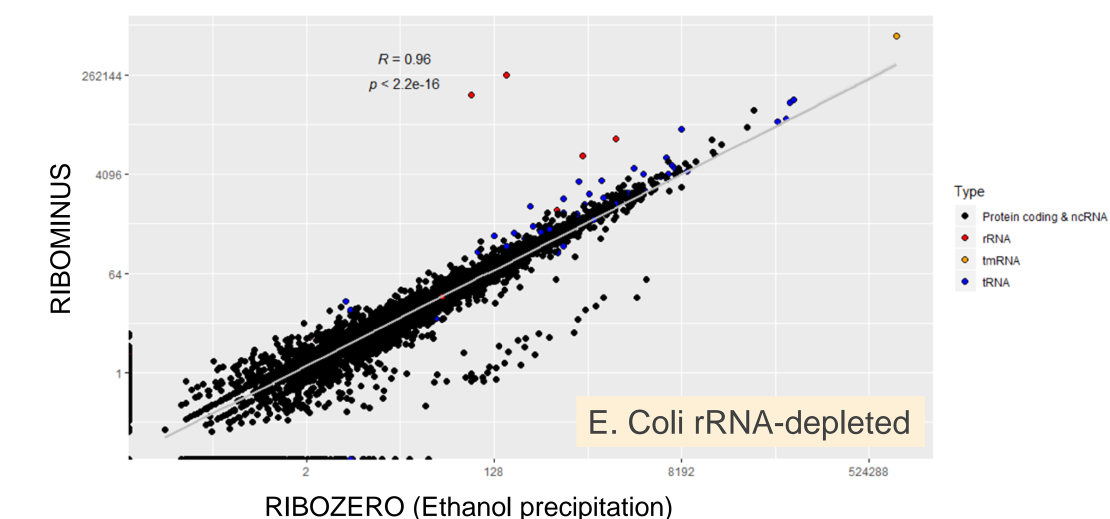


FIGURE 4A. Scatter plot showing pairwise count comparisons with high correlation between RiboZero and RiboMinus Bacteria 2.0. R=0.96.

BENEFITS OF NEW RIBOMINUS BACTERIA 2.0 KIT

- Efficient depletion of ribosomal RNA: 5S, 16S, and 23S targets
- Compatible with a wide range of pan-prokaryote species
- Broad input range: 100ng - 5μg total RNA
- < 2 hours manual workflow for 12 depletion samples
- Workflow is automatable on KingFisher instruments
- Compatible with meta-transcriptomics studies

CONCLUSIONS

- We developed a fast and efficient workflow for depletion of ribosomal RNA from Bacterial total RNA samples towards transcriptome analysis.
- Ribosomal RNA is consistently and efficiently removed.
- RNA-Seq data indicates that recovered rRNA-depleted RNA (aka RiboMinus™ RNA) is enriched for protein coding genes.
- This new kit is also applicable to microbiome preps

REFERENCES

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2. Hartley SW, Mullikin JC (2015) QoRTs: A comprehensive toolset for quality control and data processing of RNA-Seq experiments. BMC Bioinformatics 16:224.

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TRADEMARKS/LICENSING

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