

Ribo-Zero™ Plus Microbiome Depletion Kit

Fast, efficient depletion
of host and bacterial
rRNA for high-quality
metatranscriptomics
applications

- Streamlined RNA-to-analysis solution features capture probes for efficient human and microbiome-specific 5S, 16S, and 23S rRNA depletion
- Fast and flexible workflow supports a wide range of input RNA, from 25 ng to 1000 ng, for robust ribodepletion from complex microbial samples (eg, stool)
- Easy-to-use BaseSpace™ Sequence Hub Microbiome Metatranscriptomics App enables simplified data analysis and accurate visualization of results

illumina®

Introduction

Metatranscriptomics is a powerful technique for the characterization of microbial function, gene regulation, and dynamic host-microbiome interactions in real time. However, the high relative abundance of ribosomal (rRNA) sequences in bacterial samples reduces RNA sequencing (RNA-Seq) capacity, making it challenging to detect lower expressing but biologically relevant transcripts. Further, the lack of poly(A) tails on expressed bacterial RNA samples also prevents specific enrichment of bacterial messenger RNA (mRNA) for transcriptomics analyses. For these reasons, elimination of rRNA prior to RNA-Seq is crucial for obtaining high-quality metatranscriptomics libraries.

The Ribo-Zero Plus Microbiome Depletion Kit provides fast and efficient removal of undesirable rRNA from complex microbial samples. Robust rRNA depletion coupled with access to the BaseSpace™ Microbiome Metatranscriptomics App allows researchers to focus their analyses on high-value, informative portions of the transcriptome in a streamlined RNA-to-analysis workflow.

Proven ribodepletion technology

The Ribo-Zero Plus Microbiome Depletion Kit applies proven Ribo-Zero Plus rRNA depletion technology to remove pan-bacterial rRNA, including 5S, 16S, and 23S rRNA, from total RNA isolated from complex microbial communities. Ribodepletion using the Ribo-Zero Plus workflow involves targeted hybridization to DNA probes and subsequent ribonuclease H (RNase H)-mediated cleavage (Figure 1). RNA is first hybridized with DNA probes complementary to rRNA. These probes direct RNase H to digest rRNA. Next, the DNA probes are digested with DNase I, followed by bead purification of the remaining intact RNA. Ribodepleted samples then undergo library preparation, sequencing, and data analysis.

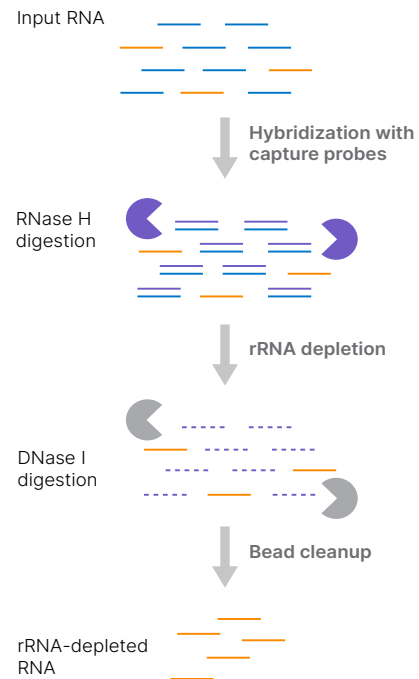


Figure 1: Ribo-Zero Plus Microbiome ribodepletion protocol—Input RNA is hybridized to rRNA-complementary oligonucleotide capture probes. The hybridized rRNA is then enzymatically degraded by RNase H followed by DNA probe digestion with DNase I. After bead cleanup to remove remaining enzymes and oligonucleotides, the enriched RNA samples are ready for subsequent library preparation steps.

Broad microbial species coverage

The Ribo-Zero Plus Microbiome Depletion Kit features an expanded capture probe set comprising probes designed against 5S, 16S, and 23S rRNA from diverse microbial species, including common adult and infant gut bacteria, and ATCC MSA-2002, MSA-2005, and MSA-2006. The broad coverage of this kit effectively eliminates unwanted RNA from thousands of bacterial strains commonly found in the gut from complex samples, such as stool. In addition, capture probes for human rRNA included in the Ribo-Zero Plus Microbiome Depletion Kit eliminate host and microbial rRNA in a single reaction (Figure 2).

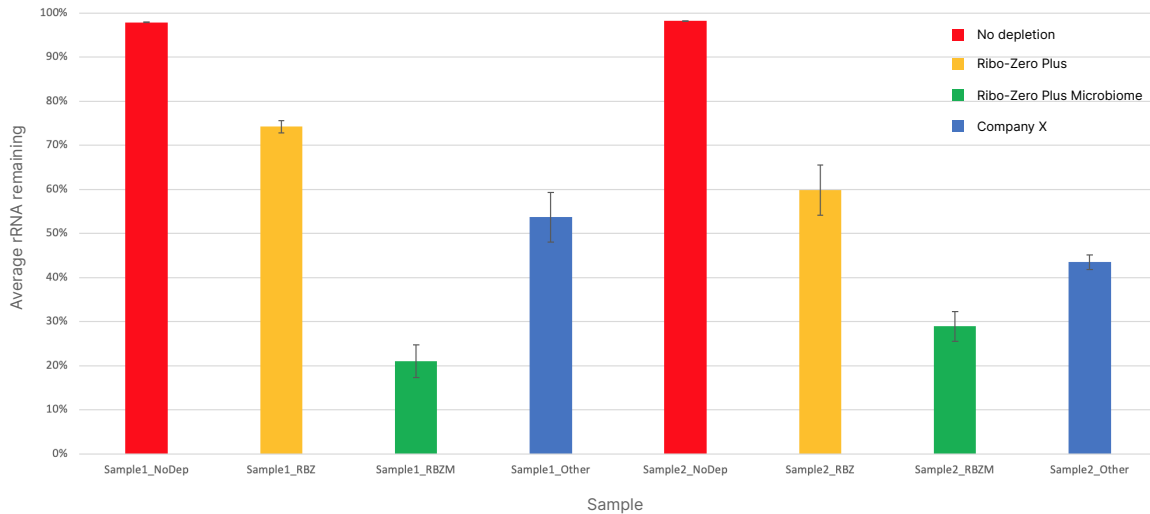


Figure 2: Robust ribodepletion with Ribo-Zero Plus Microbiome Depletion Kit— The Ribo-Zero Plus Microbiome Depletion Kit effectively depletes rRNA (5S, 16S, and 23S) levels for human and bacterial species in a single-tube reaction. Results are compared to Ribo-Zero Plus rRNA Depletion Kit and a commercially available ribodepletion kit from Company X. Two stool mixtures were tested. Sample 1 is a mixture containing both adult and infant human stool samples, while Sample 2 is a commercially available stool sample from Zymo (Cat no. 6331).

The Ribo-Zero Plus Microbiome Depletion Kit delivers robust depletion of contaminating rRNA in as little as 25 ng of high-quality total RNA. The low input sample requirements expand the pool of sample types that can be processed for sensitive metatranscriptomics applications.

Integrated RNA-to-analysis workflow

The Ribo-Zero Plus Microbiome Depletion Kit integrates seamlessly with existing automation and next-generation sequencing (NGS) workflows without the need for adjustments to existing protocols (Figure 3). The kit is available either as a depletion-only standalone product, or as part of a complete workflow including Illumina Stranded Total RNA Prep with Ribo-Zero Plus and Ribo-Zero Plus Microbiome Depletion Kit. This flexible solution can accommodate 96 samples and is compatible with NextSeq™ 500, NextSeq 550, NextSeq 1000, NextSeq 2000, and NovaSeq™ 6000 sequencing systems, depending on your specific research needs.

Simplified data analysis and visualization

Bioinformatic analysis of metatranscriptomics data is complicated and computationally intensive. The Ribo-Zero Plus Microbiome Depletion Kit includes access to the BaseSpace Microbiome Metatranscriptomics App, offering a streamlined RNA-to-analysis workflow. This cloud-based app is optimized for data generated using Illumina Stranded Total RNA with Ligation and Ribo-Zero Plus Microbiome Depletion Kit (Figure 4). The intuitive user interface allows researchers to analyze complex microbial communities and uncover novel insights into metabolic profiles.

Input FASTQ files can easily be manipulated to generate high-quality data and visuals for taxonomy, reads of interest, relative abundance of microbial species, and predicted genes involved in bacterial-host interactions (Figure 5).

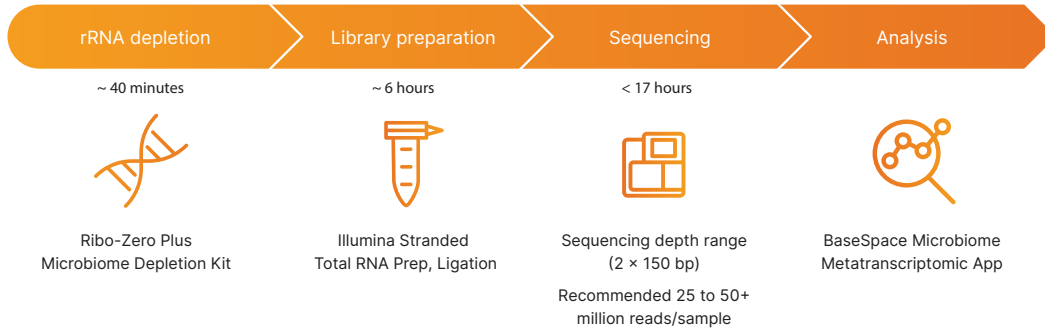


Figure 3: Ribo-Zero Plus Microbiome Depletion Kit in NGS-based RNA-Seq workflows—The Ribo-Zero Plus Microbiome Depletion Kit can be incorporated into existing workflows for RNA-Seq to effectively eliminate undesirable rRNA from total RNA samples in ~40 minutes.

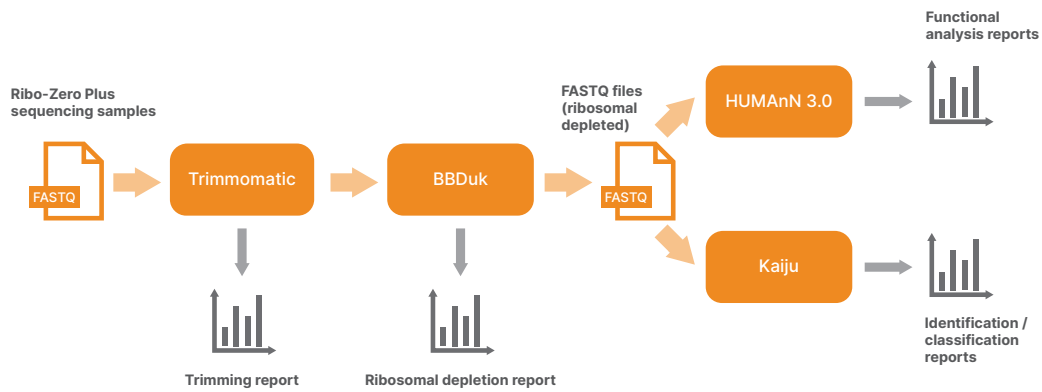


Figure 4: Functional data analysis pipeline using the BaseSpace Microbiome Metatranscriptomics App—The first step in data analysis uses Trimmomatic to filter out low quality and short reads from input FASTQ files. Next, BBduk is used to remove host reads, if a host genome is selected on the input form, and any residual rRNA reads. Finally, the filtered reads are processed with Kaiju to provide an estimate of the taxonomic composition of the sample and with HUMANn 3 to quantify expression across functional groups of genes, including gene families, metabolic pathways, and gene ontology (GO) terms.

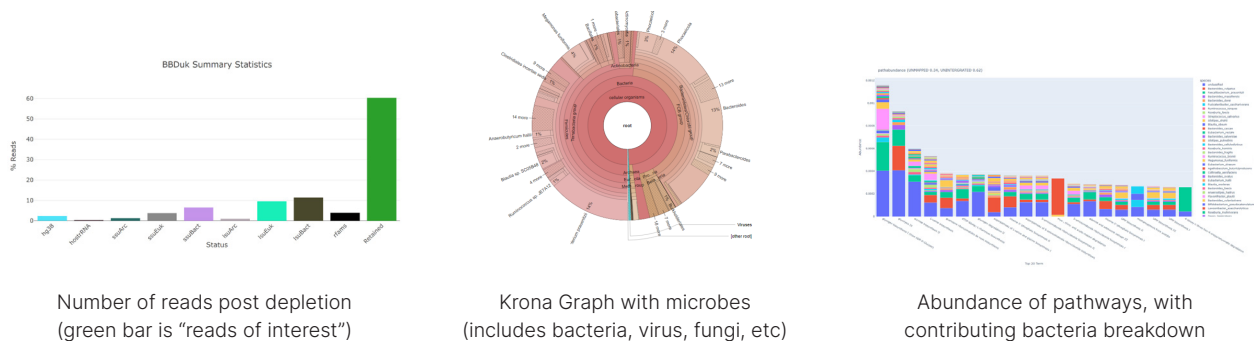


Figure 5: Data visualization using the BaseSpace Microbiome Metatranscriptomics App—(A) Bar graph of read distribution post-ribodepletion, where the green bar indicates reads of interest; (B) Krona graph showing relative taxonomic abundance of microbial species in the metagenome; (C) Graphical representation of active metabolic pathways broken down according to contribution of individual bacterial species to each of these pathways.

Summary

The Ribo-Zero Plus Microbiome Depletion Kit enables researchers working with the human microbiome to optimize their metatranscriptomic research by eliminating host and bacterial rRNA prior to RNA-Seq. The expanded capture probe sets included in this kit target thousands of bacterial strains as well as human rRNA, efficiently removing undesirable rRNA found in complex samples in approximately 40 minutes. Access to the BaseSpace Microbiome Metatranscriptomics App simplifies data analysis and visualization, delivering a streamlined RNA-to-analysis workflow for metatranscriptomics research.

Learn more

Illumina Ribo-Zero Plus rRNA Depletion Kit, [illumina.com/products/by-type/accessory-products/ribo-zero-plus-rna-depletion](https://www.illumina.com/products/by-type/accessory-products/ribo-zero-plus-rna-depletion)

Illumina Stranded Total RNA Library Prep with Ligation, [illumina.com/products/by-type/sequencing-kits/library-prep-kits/stranded-total-rna-prep](https://www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/stranded-total-rna-prep)

illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

© 2022 Illumina, Inc. All rights reserved. All trademarks are the property of Illumina, Inc. or their respective owners. For specific trademark information, see www.illumina.com/company/legal.html.
M-GL-00916 v1.0

Ordering information

Product	Catalog no.
Ribo-Zero Plus Microbiome Depletion Kit (Standalone)	20072062
Total RNA Library Prep with Ligation, Ribo-Zero Plus Microbiome Depletion Kit	20072063