NEW

LEXOGEN

Enabling complete transcriptome sequencing

RiboCop rRNA Depletion Kits for Bacteria

- Efficient depletion of 23S, 16S, and 5S rRNA from monocultures and mixed bacterial samples
- Fast and simple protocol: 1.5 hours with only 30 minutes hands-on time
- Workflow without enzymatic reactions preserves full-length RNA
- 1 ng - 1 µg total RNA input
- Compatible with high and low quality RNA
- Sophisticated probe design to eliminate off-target effects

Introduction

RNA extracted from bacterial species comprises up to 98 % of the ribosomal RNAs (rRNA) presenting a unique challenge especially when analyzing the transcriptome capacity from complex bacterial communities. Lexogen's RiboCop rRNA Depletion Kits for Bacteria efficiently remove 23S, 16S, and 5S rRNA from mixed bacterial samples and monocultures. Intact as well as degraded material may be processed using a time efficient and simple workflow. Resulting rRNA-free samples are suitable for NGS library preparation and similar applications affording a comprehensive view of bacterial transcriptome composition.

RiboCop uses a set of affinity probes designed for specific and efficient depletion of rRNA sequences from intact as well as fragmented input RNA and is also compatible with FFPE derived samples. Lexogen's sophisticated probe design minimizes off-target effects that can distort NGS data quality. Input amounts as low as 1 ng and up to 1 µg total RNA are applicable depending on sample composition and the chosen depletion Probe Mix. No enzymatic reactions or mechanical shearing steps are involved, leaving full-length transcripts intact for downstream processing. The entire protocol is automation-friendly as magnetic beads are utilized for depletion and purification probes.

Workflow

Affinity probes and total RNA are mixed and denatured, facilitating access of probes to target sequences. Hybridization is performed at elevated temperature. During the hybridization step, depletion beads are washed and ultimately used to remove affinity-tagged probes along with hybridized ribosomal RNA from solution.

The final purification step uses magnetic beads to clean up remaining RNA rounding off the procedure (Fig. 1).

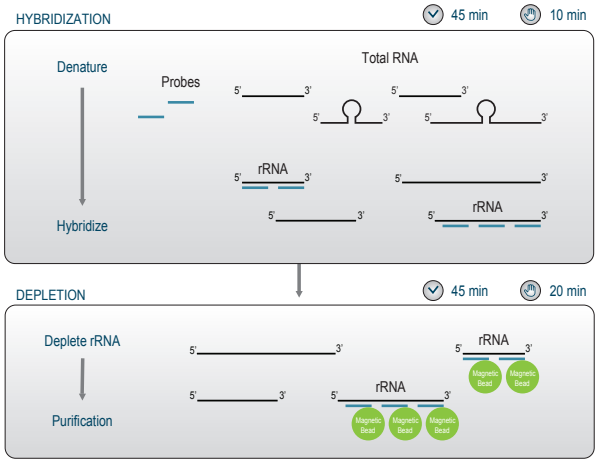

Figure 1 | Schematic overview of the RiboCop workflow.

Within 1.5 hours of total processing time samples excluding rRNA are obtained. Resulting material may be directly channeled into NGS library preparation, for example using CORALL Total RNA-Seq Library Prep Kits (Cat. No. 095) or adapter ligation protocols such as Lexogen's Small RNA-Seq Library Prep Kit (Cat. No. 052).

RiboCop is offered as a stand-alone kit with the option to choose from three optimized Probe Mixes for depletion of rRNA from Gram Positive (Cat. No. 127) and Gram Negative Bacteria (Cat. No. 126) grown in monoculture or for Mixed Bacterial Samples (Cat. No. 125).

Ordering Information

Catalog Numbers:
125 (RiboCop rRNA Depletion Kit for Mixed Bacterial Samples (META))
126 (RiboCop rRNA Depletion Kit for Gram Negative Bacteria (G-))
127 (RiboCop rRNA Depletion Kit for Gram Positive Bacteria (G+))
Associated Products:
095 (CORALL Total RNA-Seq Library Prep Kit)
052 (Small RNA-Seq Library Prep Kit for Illumina)

RiBO COP™
Select and Deplete

Robust Performance Over a Wide Range of Input Amounts

Ribosomal RNA accounts for up to 98 % of bacterial transcripts. Efficient removal of rRNA substantially decreases sequencing costs and enables comprehensive analyses of bacterial transcriptomes. To demonstrate the performance of RiboCop for Bacteria ribosomal RNA was depleted from *E. coli* total RNA using RiboCop for Gram Negative Bacteria over a wide range of input amounts (1 ng to 1 µg). RiboCop for Bacteria efficiently reduces rRNA reads from 98 % to 1-3 % over a broad range of input amounts (Fig. 2).

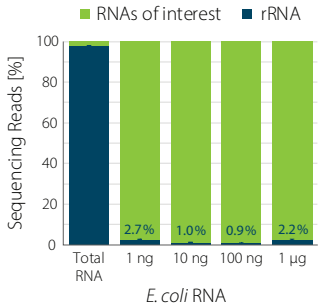

Figure 2 | RiboCop rRNA Depletion for Bacteria efficiently removes rRNA across a wide range of input amounts. NGS libraries were prepared using Lexogen's CORALL Total RNA-Seq Library Prep Kit and successful depletion was monitored by sequencing and subsequent analyses of remaining rRNA reads from untreated (total RNA) and depleted *E. coli* RNA, sequenced on Illumina NextSeq (1x75 bp) and analyzed by mapping to the MG1655 reference using BBMap. The percentage of reads mapping to rRNA is plotted in blue.

Performance on Meta-transriptome Samples

The RiboCop rRNA Depletion Kit for Mixed Bacterial Samples (META) is specifically designed for depletion of complex, mixed populations, such as environmental communities or microbiome samples. Low quality microbiome samples from stool extractions were used for characterization of the Probe Mix designed for meta-transcriptomics (Tab. 1). The META Probe Mix can also be used for efficient and robust depletion of monocultures up to 100 ng total RNA input (Fig. 3).

Sample	% rRNA reads
stool microbiome 10 ng	14.75 (± 1.4)
stool microbiome 100 ng	15.32 (± 1.4)
stool microbiome 1 µg	23.37 (± 1.6)

Table 1 | Depletion rates for meta-transcriptome analyses. Stool microbiome RNA was treated with RiboCop for Mixed Bacterial Samples (META). NGS-Libraries were prepared, sequenced and analyzed as described in Fig. 2. Reads were mapped to ~60 annotations.

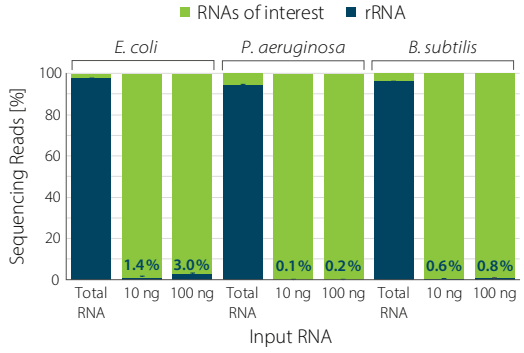

Figure 3 | RiboCop rRNA Depletion for Mixed Bacterial Samples (META) efficiently removes rRNA from various bacterial species. Two RNA amounts from monocultures of the indicated species were subjected to rRNA depletion using the META Probe Mix. Library preparation and sequencing were performed as described in Fig. 2. Reads were mapped against the respective genomes of *E. coli* MG1655, *P. aeruginosa* PAO1, and *B. subtilis* 168. The percentage of reads mapping to rRNA is plotted in blue.

Depletion of All Bacterial rRNA Subclasses

All rRNA subclasses, including 5S rRNA, are efficiently removed by all RiboCop for Bacteria Kits. Table 2 lists the reads obtained per rRNA subclass.

	E. coli			*B. subtilis*		
rRNA	Total	G-	META	Total	G+	META
23S	64.2 %	0.6 %	1.1 %	55.4 %	0.5 %	0.4 %
16S	32.2 %	0.3 %	0.4 %	40.9 %	0.4%	0.4 %
5S	0.5 %	0.02 %	0.06 %	0.01 %	<0.01 %	<0.01 %
Overall	**96.9 %**	**0.9 %**	**1.6 %**	**96.3 %**	**0.9 %**	**0.8 %**

Table 2 | Efficient depletion of all rRNA subclasses. Depletion rates comparing RiboCop rRNA Depletion Kits for Gram Negative (G-) and Gram Positive (G+) Bacteria and Mixed Bacterial Samples (META). 100 ng total RNA of *E. coli*, and *B. subtilis* were subjected to depletion using the indicated Probe Mixes. CORALL libraries were prepared from depleted samples, sequenced on NextSeq (1 x 75 bp) and mapped to the respective references using BBMap to assess the percentage of rRNA reads per class. Untreated total RNA served as control. Determined rRNA percentages are the mean of at least two experiments.

Excellent Reproducibility and Elimination of Off-target Effects

RiboCop ensures consistent transcript correlations after depletion across a wide input range. Fig. 4 shows excellent reproducibility between replicates and a high correlation between genes in depleted vs. untreated samples, highlighting exceptional specificity for rRNA sequences.

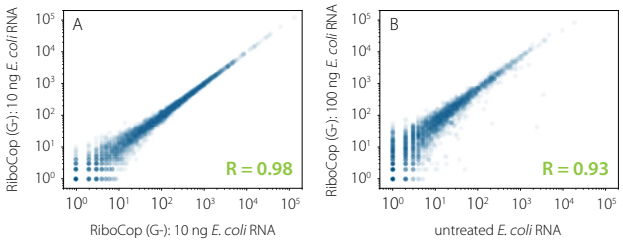

Figure 4 | RiboCop maintains unbiased expression profiles while efficiently removing undesired rRNA. A) Correlation of replicates for two independent depletion reactions using 10 ng *E. coli* RNA. **B)** Correlation plot comparing transcript expression in untreated vs. ribo-depleted samples for 100 ng of *E. coli* RNA. Total RNA was depleted with RiboCop for Gram Negative Bacteria. CORALL libraries were prepared and sequenced as described for Fig. 2, reads were mapped against the MG1655 reference genome using STAR Aligner and counted (FeatureCounts) prior to correlating gene counts between RiboCop-treated and untreated samples.

Summary

Lexogen's RiboCop rRNA Depletion Kits for Bacteria efficiently removes 23S, 16S, and 5S rRNAs from bacterial samples derived from monocultures and complex, mixed bacterial communities. The protocol is compatible with a wide range of input RNA amounts starting at 1 ng. The correlation analyses of bacterial transcriptome data demonstrate excellent reproducibility and consistent transcript expression regardless of treatment or input. The protocol is automation-friendly and compatible with intact and degraded RNA, including FFPE-derived samples. RiboCop-treated RNA is suitable for all random-primed total RNA library prep and adapter ligation protocols.

Find out more about RiboCop at www.lexogen.com
Contact us at info@lexogen.com or +43 1 345 1212-41, or find your local contact at www.lexogen.com/distributors.