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RiboCop rRNA Depletion Kits for Bacteria

Lexogen's RiboCop rRNA Depletion Kits for Bacteria remove undesired rRNA using an enzyme-free, automation-friendly workflow. RiboCop is applicable to a broad input range (1 ng - 1 µg), and suitable for intact and fragmented RNA. Sophisticated probe design for Gram negative, Gram positive, and mixed bacterial species ensures maintenance of unbiased transcription profiles while efficiently removing 23S, 16S, and 5S rRNA.

Introduction

RNA extracted from bacterial species comprises up to 98 % of 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 depleted RNA is suitable for NGS library preparation and similar applications affording a comprehensive view of bacterial transcriptome composition.

RiboCop Removes Undesired RNA by Hybridization and Capture

RiboCop uses a set of affinity probes designed for specific and efficient depletion of rRNA sequences from intact as well as fragmented input RNA. Lexogen's sophisticated probe design minimizes off-target effects that can distort NGS data. 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. Depletion kits with dedicated probe mixes are available for Gram negative (G-, Cat. No. 126), and Gram positive bacteria (G+, Cat. No. 127), and mixed bacterial species (META, Cat. No. 125). No enzymatic reactions or mechanical shearing steps are involved, leaving full-length transcripts intact for downstream processing (Fig. 1).

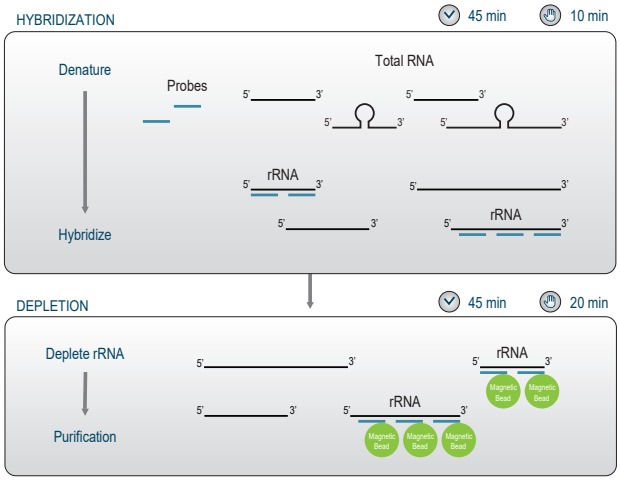

Figure 1 | Schematic overview of the RiboCop workflow. *Affinity probes and total RNA are mixed and denatured. Hybridization is performed at elevated temperature. Depletion beads are used to remove affinity-tagged probes along with hybridized ribosomal RNA from solution. The final purification step uses magnetic beads to clean up the depleted RNA.*

The entire protocol is automation-friendly as magnetic beads are utilized for depletion and purification (Fig. 1). 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 RNA-Seq V2 Library Prep Kits (Cat. No. 171 - 176) or adapter ligation protocols.

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 % for all tested input amounts (Fig. 2).

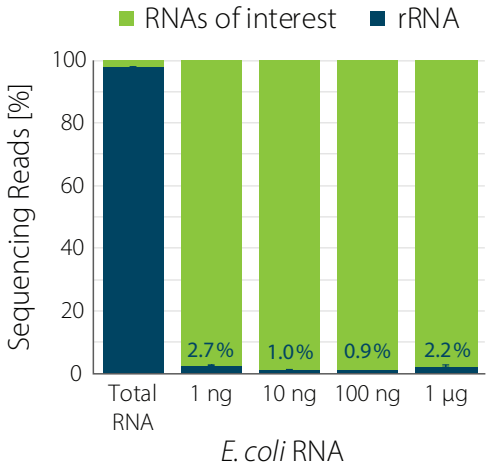

Figure 2 | RiboCop rRNA Depletion for Bacteria efficiently removes rRNA across a wide range of input amounts. *NGS libraries were prepared using Lexogen's CORALL Total RNA-Seq Library Prep Kit. Successful depletion was monitored by sequencing and subsequent analyses of remaining rRNA reads from untreated (Total RNA) and depleted E. coli RNA, sequenced on Illumina NextSeq500 (1x75 bp). Reads were mapped 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).

Table 1 | Depletion rates for meta-transcriptome analyses.

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)

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.

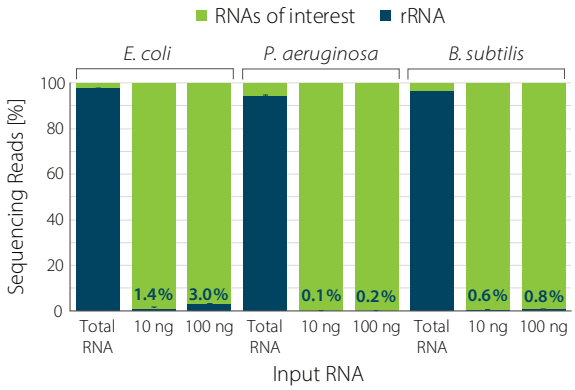

Figure 3 | RiboCop rRNA Depletion for Mixed Bacterial Samples (META) efficiently removes rRNA from various bacterial species. *Two RNA amounts from monocultures of the indicated species were subjected to rRNA depletion using the META Probe Mix. Library preparation and sequencing were performed as described 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.

Table 2 | Efficient depletion of all rRNA subclasses.

	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 %**

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 NextSeq500 (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.

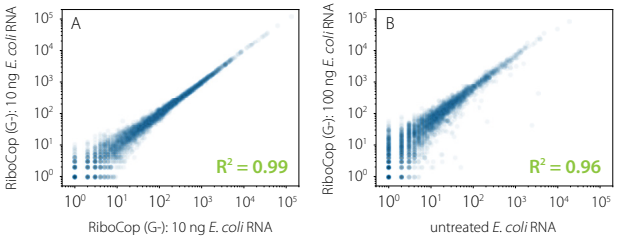

Figure 4 | RiboCop maintains unbiased expression profiles while efficiently removing undesired rRNA. A) *Correlation of replicates for two independent depletion reactions using 10 ng E. coli RNA.* ***B)*** *Correlation plot comparing transcript expression in untreated vs. ribo-depleted samples for 100 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 remove 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.

Key Features

- **Performance:** Remove undesired 23S, 16S, and 5S rRNA from monocultures and mixed bacterial samples to save sequencing space and increase multiplexing capacity.
- **Broad Range of Species:** Apply RiboCop for Bacteria to Gram negative, Gram positive or mixed bacterial samples using specifically designed mixes for highest performance.
- **Broad Input Range:** Deplete rRNA from as low as 1 ng input RNA. RiboCop for Bacteria performs robustly over a broad input range from 1 ng to 1 µg, and with intact and fragmented RNA.
- **Easy-to-use:** The enzyme-free protocol preserves full-length RNA and is automation-friendly.
- **Consistent:** RiboCop for Bacteria maintains unbiased transcript expression through excellent reproducibility and innovative probe design that eliminates off-target effects.
- **Convenient:** Combine RiboCop Kits for Bacteria and Human/Mouse/Rat for simultaneous depletion of host and bacterial rRNA.

Ordering Information

Cat. №	Product Name
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	
144	RiboCop rRNA Depletion Kit for Human/Mouse/Rat (HMR) V2
171 - 176	CORALL RNA-Seq V2 Library Prep Kits

Find out more about RiboCop at www.lexogen.com

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RiBO COP™ Select and Deplete