LEXOGEN
The RNA Experts

RiboCop rRNA Depletion Kits for Human/Mouse/Rat V2

Lexogen's RiboCop rRNA Depletion Kits for Human/Mouse/Rat 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, including FFPE material. Sophisticated probe design ensures maintenance of unbiased transcription profiles upon depletion while efficiently removing 28S, 18S, 5.8S, 5S, and 45S as well as mt16S and mt12S rRNA.

Introduction

Total RNA from mammalian species is comprised of large amounts of undesired RNA, such as ribosomal RNA (rRNA) constituting ~80 - 90 % of the total RNA sample, and globin mRNA, accounting for ~35 - 80 % of mRNA in blood samples. Lexogen's RiboCop rRNA Depletion Kits for Human/Mouse/Rat remove undesired cytoplasmic (28S, 18S, 5.8S, 5S, and 45S rRNA) and mitochondrial (mt16S, mt12S) from intact as well as degraded material, including formalin-fixed, paraffin-embedded (FFPE) samples. Combined depletion of rRNA and globin mRNA with RiboCop (HMR+Globin) allows highly convenient processing of blood samples. The resulting depleted RNA is suitable for NGS library preparation and other demanding applications affording a comprehensive view of the transcriptome.

RiboCop Removes Undesired RNA by Hybridization and Capture

RiboCop uses a set of affinity probes designed for specific and efficient depletion of rRNA and globin mRNA 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. 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 (Fig. 1).

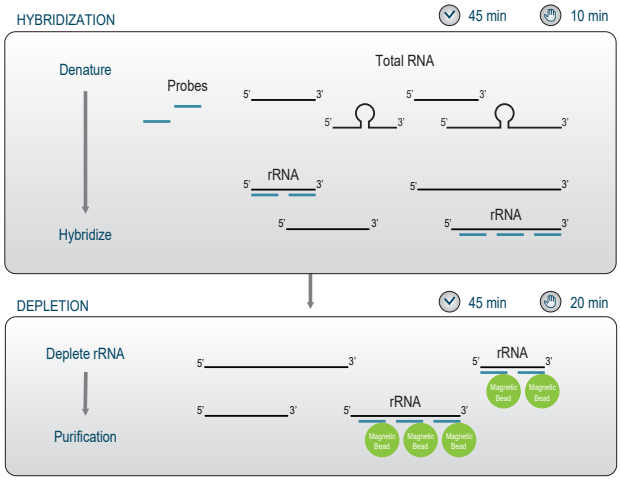

Figure 1 | Schematic overview of the RiboCop workflow. Affinity probes and total RNA are mixed and denatured. Hybridization is performed at elevated temperature. Depletion beads are used to remove affinity-tagged probes along with hybridized ribosomal RNA from the solution. The final purification step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

Within 1.5 hours of total processing time samples free from rRNA (and globin mRNA) are obtained. Resulting material may be directly channeled into NGS library preparation, for example using CORALL RNA-Seq V2 Library Prep Kits with UDIs (Cat. No. 171 - 176) bundles with RiboCop are available as Cat. No. 183 - 186.

Robust Performance Over a Wide Range of Input Amounts

Efficient removal of rRNA substantially decreases sequencing costs and drastically increases sensitivity of transcriptome analysis. To demonstrate the performance of RiboCop, ribosomal RNA was depleted from total Universal Human Reference RNA (UHRR) using RiboCop for Human/Mouse/Rat (HMR) V2 with 1 ng, 100 ng, and 1 µg UHRR. RiboCop efficiently reduces rRNA reads from 84 % to <1 % over a broad range of input amounts (Fig. 2).

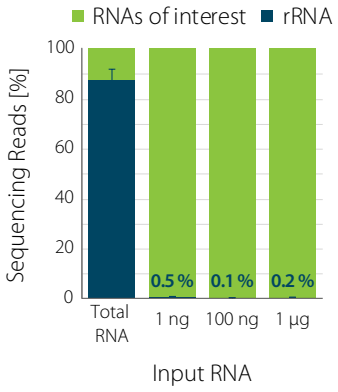

Figure 2 | RiboCop rRNA Depletion for Human/Mouse/Rat (HMR) V2 efficiently removes rRNA across a wide range of input amounts. NGS libraries were prepared using Lexogen's CORALL Total RNA-Seq Library Prep Kit. Successful depletion was monitored by sequencing (Illumina NextSeq500, 1x75 bp) and subsequent analysis of remaining rRNA reads from untreated (Total RNA) and depleted UHRR (1 ng - 1 µg). Reads were mapped to the GRCh38.95* human reference using BBMap. The percentage of reads mapping to rRNA is plotted in blue. (Asterisks denotes manual curation of annotations).

RiboCop Performance Across Species

RiboCop enriches RNAs of interest across a wide range of total RNA input amounts by removing all subclasses of undesired rRNA from human, mouse, and rat samples (Fig. 3 and Tab. 1).

RiBOCOP™
Select and Deplete

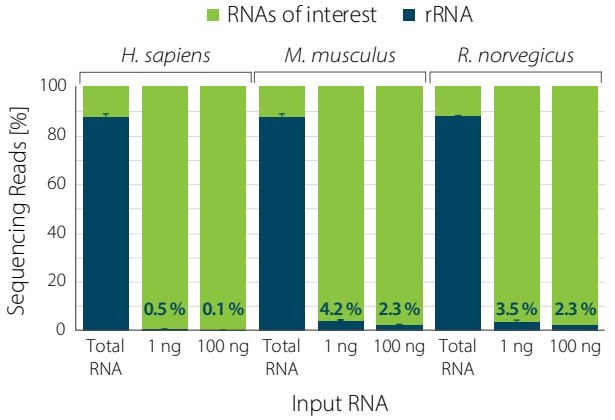

Figure 3 | RiboCop rRNA Depletion for Human/Mouse/Rat (HMR) V2 efficiently removes rRNA from human (UHR), mouse (liver) and rat (liver) RNA. Two RNA amounts of the indicated species were subjected to rRNA depletion using the HMR V2 Probe Mix. Library preparation and sequencing were performed as described in Fig. 2. Reads were mapped against the respective reference genomes for human (GRCh38.95*), mouse (mmu_GRCm38.95*), and rat (rno_Rnor_6.0.95*).The percentage of reads mapping to rRNA is plotted in blue. (Asterisks denotes manual curation of annotations).

Table 1 | Efficient depletion of all rRNA subclasses.

	H. sapiens		*M. musculus*		*R. norvegicus*	
rRNA	**Total**	**HMR V2**	**Total**	**HMR V2**	**Total**	**HMR V2**
28S	38.12 %	0.05 %	37.71 %	1.86 %	40.50 %	1.79 %
18S	39.20 %	0.02 %	46.33 %	0.35 %	44.47 %	0.27 %
mt16S	2.77 %	0.02 %	3.64 %	0.04 %	1.50 %	0.01 %
mt12S	1.47 %	0.01 %	2.85 %	0.02 %	1.27 %	-
5.8S	0.01 %	-	0.09 %	-	0.13 %	-
5S	0.07 %	-	0.08 %	-	0.03 %	-
Overall	**81.6 %**	**0.1 %**	**90.7 %**	**2.3 %**	**87.9 %**	**2.1 %**

Reads mapping to rRNA for the RiboCop rRNA Depletion Kit for Human/Mouse/Rat (HMR) V2 for 100 ng total RNA from the indicated species (human: UHRR, mouse liver and rat liver RNA for *M. musculus* and *R. norvegicus*, respectively). CORALL libraries were prepared and analyzed as described in Fig. 2. Untreated total RNA served as control. Determined rRNA percentages are the mean of at least three experiments.

Excellent Reproducibility and Elimination of Off-target Effects

RiboCop ensures consistent transcript correlations of depleted and non-depleted samples, as well as excellent reproducibility between replicates (Fig. 4), highlighting exceptional specificity for rRNA sequences.

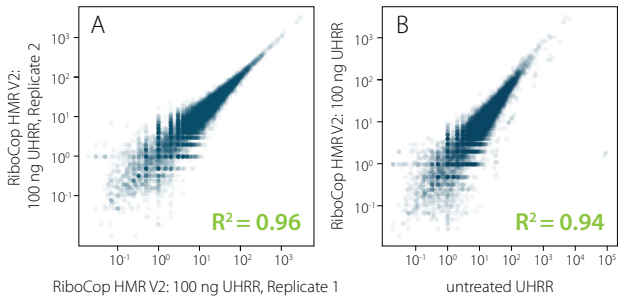

Figure 4 | RiboCop HMR V2 maintains unbiased expression profiles while efficiently removing undesired rRNA. A) Correlation of replicates for two independent depletions using 100 ng UHRR. **B)** Correlation plot comparing transcript expression in untreated vs. ribo-depleted samples for 100 ng UHRR. CORALL libraries were prepared and sequenced as described for Fig. 2, reads were mapped to the human reference genome using STAR Aligner and counted (FeatureCounts) prior to correlating gene counts between RiboCop-treated and untreated samples.

Combined rRNA and Globin Depletion from Whole Blood RNA

Globin constitutes ~35 - 80 % of all blood mRNA. The RiboCop HMR+Globin Probe Mix simultaneously removes rRNA and globin mRNA, providing a highly convenient workflow for blood samples and freeing up sequencing space for RNAs of interest (Fig. 5).

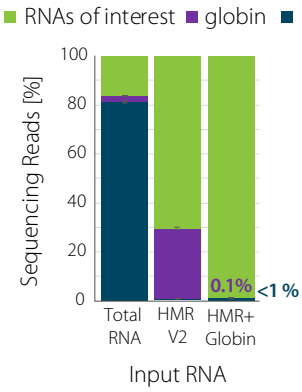

Figure 5 | RiboCop rRNA Depletion for Human/Mouse/Rat plus Globin (HMR+Globin) efficiently removes rRNA and globin mRNA from human blood RNA. 10 ng human whole blood RNA were used as input for RiboCop using either the HMR V2 or the HMR+Globin Probe Mix. CORALL library preparation, sequencing, and data analysis were performed as described in Fig. 2, rRNA reads are plotted in blue, globin reads in purple.

Summary

Lexogen's RiboCop rRNA Depletion Kits for Human/Mouse/Rat (HMR) V2 and (HMR+Globin) remove undesired rRNA and globin mRNA, ultimately focusing sequencing reads on RNAs of interest. The protocol is compatible with a wide range of input RNA amounts starting at 1 ng. The RiboCop method is automation-friendly and suitable for intact and degraded RNA, including FFPE-derived samples. RiboCop-treated RNA is compatible with all standard random-primed total RNA library preparation kits, including CORALL Total RNA-Seq Library Prep Kits.

Key Features

- **Performance:** Remove undesired rRNA (28S, 18S, mt16S, mt12S, 5.8S, 5S, and 45S rRNA) to save sequencing space and increase multiplexing capacity. **NEW!** Combined rRNA and globin mRNA depletion offers a convenient workflow solution for analysis of blood samples.
- **Broad Input Range:** Deplete rRNA from as low as 1 ng input RNA. RiboCop performs robustly over a broad input range up to 1 µg and is suitable for intact and fragmented RNA, including FFPE material.
- **Easy-to-use:** The enzyme-free protocol preserves full-length RNA and is automation-friendly.
- **Consistent:** RiboCop maintains unbiased transcript expression through excellent reproducibility and innovative probe design that eliminates off-target effects.
- **Convenient:** Combine RiboCop HMR and Bacteria kits for simultaneous depletion of host and bacterial rRNA.

Ordering Information

Catalog Numbers:
144 (RiboCop rRNA Depletion Kit for Human/Mouse/Rat (HMR) V2)
145 (RiboCop rRNA Depletion Kit for Human/Mouse/Rat plus Globin (HMR+Globin)
146 - 147 (CORALL Total RNA-Seq Library Prep Kit Version 1 with RiboCop)
183 -186 (CORALL Total RNA-Seq V2 Bundles with RiboCop rRNA depletion)
Associated Products:
125 - 127 (RiboCop rRNA Depletion Kits for Bacteria)
095, 117-119, 132-134 (CORALL Total RNA-Seq Library Prep Kits Version 1)
171- 176 (CORALL RNA-Seq V2 Library Prep Kits)

Find out more about RiboCop at www.lexogen.com
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