NEW

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The RNA Experts

RiboCop rRNA Depletion Kit for Yeast

Lexogen's RiboCop rRNA Depletion Kit for Yeast removes undesired rRNA using an enzyme-free, automation-friendly workflow. RiboCop is applicable to a broad input range (10 ng - 1 µg), and suitable for intact and degraded RNA. Sophisticated probe design ensures maintenance of unbiased transcription profiles while efficiently removing cytoplasmic and mitochondrial rRNA.

Introduction

Total RNA from yeast species is comprised of large amounts of undesired RNA, such as ribosomal RNA (rRNA) constituting up to ~97 % of the total RNA sample. Lexogen's RiboCop rRNA Depletion Kits for Yeast remove undesired cytoplasmic (25S, 18S, 5.8S, 5S, and 35S) and mitochondrial (21S, 15S and ACI60_gr01, ACI60_gr02) rRNA from intact as well as degraded input RNA. The resulting depleted RNA is suitable for NGS library preparation and other demanding applications affording a comprehensive view of the transcriptome.

RiboCop for Yeast 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 heavily degraded input RNA. Lexogen's sophisticated probe design minimizes off-target effects that can distort NGS data. Input amounts as low as 10 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).

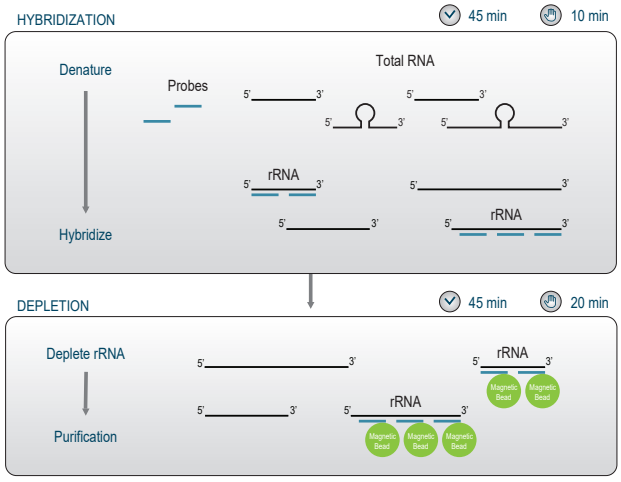

Figure 1 | Schematic overview of the RiboCop workflow. Affinity probes and total RNA are mixed and denatured. Hybridization is performed at elevated temperature. Depletion beads are used to remove affinity-tagged probes along with hybridized ribosomal RNA from the solution. The final purification step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

Within 1.5 hours of total processing time, samples free from rRNA are obtained and can be directly used for NGS library preparation, for example with CORALL RNA-Seq V2 (Cat. No. 171 - 176).

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 *S. cerevisiae* total RNA using RiboCop for Yeast with 10 ng, 100 ng, and 1 µg input RNA. RiboCop efficiently reduces rRNA reads from ~97 % to <1.5 % over a broad range of input amounts (Fig. 2).

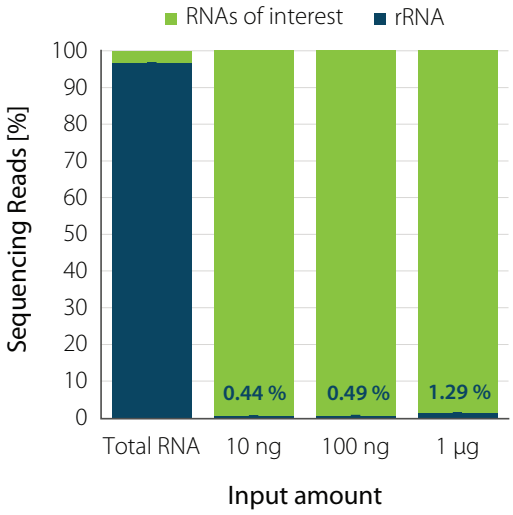

Figure 2 | RiboCop rRNA Depletion for Yeast efficiently removes rRNA across a wide range of input amounts. NGS libraries were prepared using Lexogen's CORALL RNA-Seq V2 Library Prep Kit. Successful depletion was monitored by sequencing (Illumina NextSeq500, 1x50 bp) and subsequent analysis of remaining rRNA reads from untreated (Total RNA) and depleted *S. cerevisiae* RNA (10 ng - 1 µg). The percentage of reads mapping to rRNA is plotted in blue.

RiboCop Maintains Unbiased Expression Profiles

RiboCop for Yeast enriches RNAs of interest while maintaining unbiased transcriptome profiles as indicated by high correlation between the transcript abundance of untreated and ribo-depleted samples for two different input amounts (Fig. 3).

In addition, sequencing reads are focused on RNAs of interest and exonic reads are significantly increased upon depletion with RiboCop for Yeast regardless of the RNA input amount (Fig. 4).

RiBO COP™
Select and Deplete

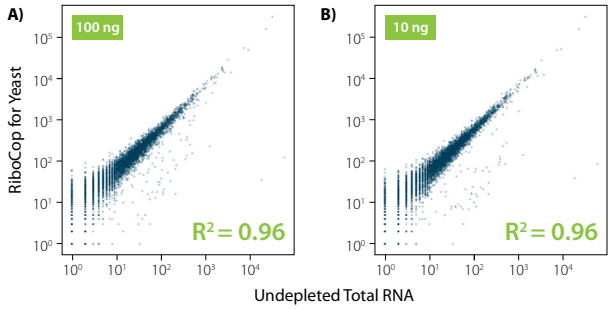

Figure 3 | RiboCop for Yeast maintains unbiased expression profiles while efficiently removing undesired ribosomal RNA. Correlation of transcript abundance in untreated samples vs. samples depleted with RiboCop for Yeast using 100 ng (A) and 10 ng *S. cerevisiae* RNA input (B). Libraries were prepared using Lexogen's CORALL RNA-Seq V2 Library Prep Kit and sequenced on Illumina NextSeq (1×50 bp). Reads were mapped against the *S. cerevisiae* reference genome using STAR aligner and counted with FeatureCounts. Correlations are shown for uniquely mapping reads.

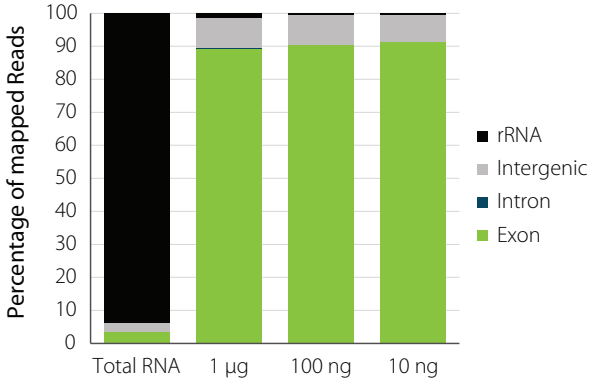

Figure 4 | Feature distribution of mapped reads for *S. cerevisiae* with and without ribo-depletion. RiboCop for Yeast rRNA depletion significantly increases the fraction of reads mapped to exons. Yeast RNA was processed and sequenced as described in Fig. 3.

Increased Gene Detection Upon rRNA Depletion

RiboCop for Yeast affords an unobstructed view of the yeast transcriptome and increases gene detection through efficient rRNA removal (Fig. 5).

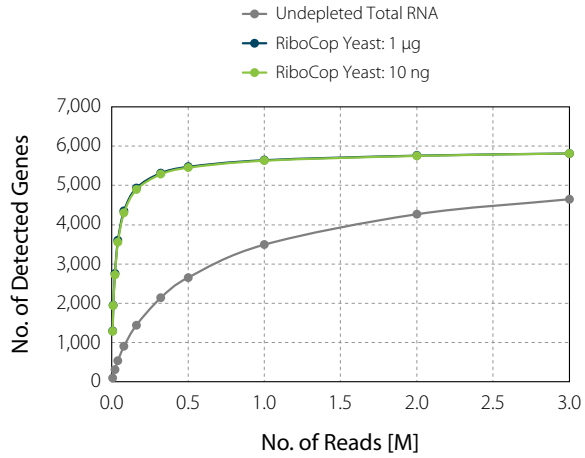

Figure 5 | Increased gene detection upon rRNA depletion. The number of detected genes per number of reads uniquely mapping to exons (FeatureCounts) was plotted for undepleted total RNA as well as depleted *S. cerevisiae* RNA across different input amounts. Libraries were prepared using Lexogen's CORALL RNA-Seq V2 Library Prep Kit and sequenced on Illumina NextSeq (1×50 bp).

Excellent Reproducibility Between Replicates

RiboCop protocols are robust and highly reproducible. Correlation plots show excellent reproducibility between replicates independent of the input amount used for the depletion reaction (Fig. 6).

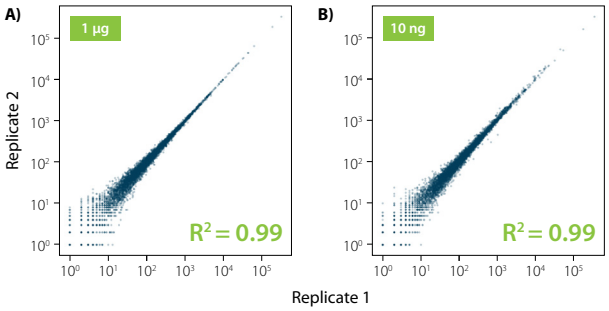

Figure 6 | Excellent reproducibility between replicates. Correlation of replicates for two independent depletion reactions using 10 ng (A) and 1 µg *S. cerevisiae* total RNA as input (B). Total RNA was ribo-depleted with RiboCop for Yeast. Libraries were prepared using Lexogen's CORALL RNA-Seq V2 Library Prep Kit and sequenced on Illumina NextSeq (1×50 bp). Correlations are shown including multimapping reads.

Summary

Lexogen's RiboCop rRNA Depletion Kit for Yeast removes undesired rRNA, ultimately focusing sequencing reads on RNAs of interest. The protocol is compatible with a wide range of input RNA amounts from 10 ng to 1 µg. The RiboCop method is automation-friendly and suitable for intact and degraded RNA. RiboCop-treated RNA is compatible with all standard random-primed total RNA library preparation kits, including CORALL RNA-Seq V2 Library Prep Kits.

Key Features

- **Performance:** Save sequencing space and increase multiplexing capacity by removing undesired cytoplasmic (25S, 18S, 5.8S, 5S, and 35S) and mitochondrial (21S, 15S and ACI60_gr01, ACI60_gr02) rRNA.
- **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.
- **Broad Input Range:** Deplete rRNA from as low as 10 ng input RNA. RiboCop performs robustly over a broad input range up to 1 µg and is suitable for intact and degraded RNA.

Ordering Information

Catalog Numbers:
190 (RiboCop rRNA Depletion Kit for Yeast)
Associated Products:
125 - 127 (RiboCop rRNA Depletion Kits for Bacteria)
144 - 145 (RiboCop rRNA Depletion Kits for Human/Mouse/Rat)
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
Contact us at support@lexogen.com or +43 1 345 1212-41, or find your local contact at www.lexogen.com/distributors.