LEXOGEN

Enabling complete transcriptome sequencing

RiboCop rRNA Depletion Kit V1.2

- Efficient elimination of rRNA
- Simple workflow – no enzymatic reactions
- Output ready for NGS library prep

Introduction

Current Next Generation Sequencing (NGS) technologies are routinely applied in the context of RNA-Seq. RNA is extracted from complex biological sources and known to comprise informative molecular species as well as large amounts of abundant RNA, most notably ribosomal RNAs (rRNA). These generally are of little interest. Techniques for depletion of rRNA molecules prior to sequencing are required for the design of efficient experimental workflows. Enhanced sequencing depth for relevant RNA species can thus be obtained.

Lexogen's RiboCop rRNA Depletion Kit enables removal of rRNA from human, mouse, and rat total RNA samples. Resulting material is suited for Next Generation Sequencing as well as other demanding applications. Total RNA samples are treated using a set of affinity probes for specific depletion of rRNA sequences. Number and positioning of probes are designed for applicability towards intact as well as fragmented input RNA. Even FFPE-derived RNA samples are amenable to RiboCop rRNA depletion.

Hybridization probes specifically bind ribosomal RNA and serve as affinity tags for efficient removal of duplexes using magnetic beads. Samples void of 28S, 18S, 5.8S, 45S, 5S, mt16S and mt12S ribosomal sequences are obtained, affording a comprehensive view of transcriptome composition.

Input amounts as low as 1 ng and up to 1 µg total RNA are applicable. Depending on sample composition and experimental requirements, extending the RiboCop protocol beyond these boundaries is possible. No enzymatic reactions or mechanical shearing steps are involved, leaving full-length transcripts intact for downstream processing. The entire protocol is automation friendly by utilizing magnetic beads for depletion and purification purposes.

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 conditioned 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 and rounds off the procedure.

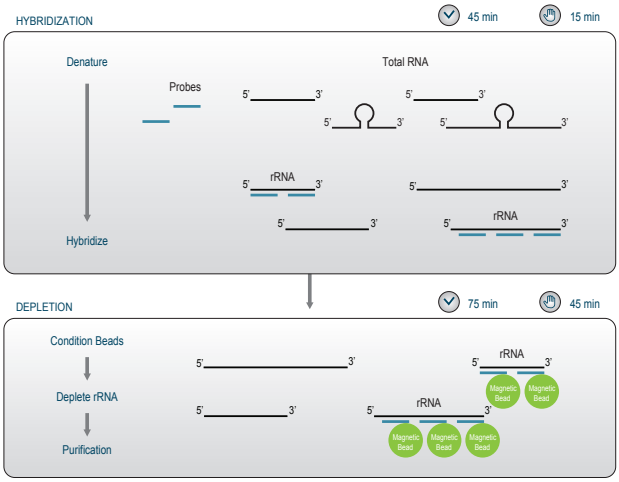

Figure 1 | Schematic overview of the RiboCop workflow.

Within 2 hours of total processing time the depletion protocol is finished and samples excluding rRNA are obtained. Resulting material may be directly fed into optional RNA quantification and NGS library preparation.
RiboCop is offered in different kit sizes as a stand-alone kit (Cat. No. 037) as well as in combination with the SENSE Total RNA-Seq Libray Prep Kit (Cat. No. 042).

Ordering Information

Catalog Numbers:
037.24 (RiboCop rRNA Depletion Kit V1.2 (Human/Mouse/Rat), 24 preps)
037.96 (RiboCop rRNA Depletion Kit V1.2 (Human/Mouse/Rat), 96 preps)
042.08 (SENSE Total RNA-Seq Library Prep Kit for Illumina with RiboCop, 8 preps)
042.24 (SENSE Total RNA-Seq Library Prep Kit for Illumina with RiboCop, 24 preps)
042.96 (SENSE Total RNA-Seq Library Prep Kit for Illumina with RiboCop, 96 preps)

RiboCop Characterization

Ribosomal RNA was depleted from total RNA samples using Lexogen's RiboCop rRNA Depletion Kit V1.2 (Human/Mouse/Rat). Universal Human Reference RNA (UHRR), Human Brain Reference RNA (HBRR), RNA extracted from formalin-fixed, paraffin embedded (FFPE) human liver, mouse RNA (obtained using Lexogen's SPLIT RNA Extraction Kit, Cat. No. 008), as well as rat liver samples served as starting material for performance characterization. Input amounts spanned more than two orders of magnitude, starting from below 10 ng and 1µg total RNA. Depleted material was characterized both by micro-capillary electrophoresis as well as Next Generation Sequencing.

Results - Microcapillary electrophoresis

Successful rRNA depletion results in removal of, amongst others, 28S and 18S rRNA. These rRNA peaks usually dominate electropherograms of intact total RNA and should be absent after RiboCop depletion.

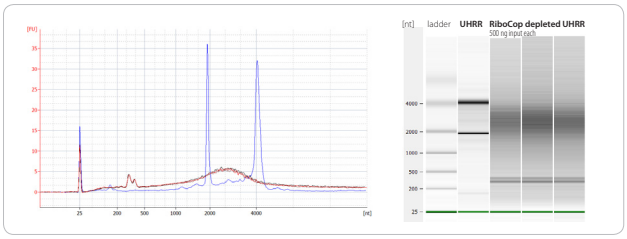

Figure 2 | Overlay of RNA Bioanalyzer traces before and after RiboCop depletion. Blue trace: diluted total Universal Human Reference RNA, intact (RIN 9.1). Red, brown, and black traces: RiboCop-depleted material.

The three reactions shown in Figure 2 were performed using 500 ng UHRR as input each. 28S and 18S rRNA peaks are absent, indicating successful depletion. Depleted RNA is detected as a broad smear reaching beyond 4 kb.

Results - NGS

Depleted material was library-prepped using Lexogen's SENSE Total RNA-Seq Library Prep Kit for Illumina. Barcoded libraries were pooled and sequenced on a HiSeq device. Resulting reads were de-multiplexed, mapped to rRNA reference sequences and quantified against non-depleted libraries. Measured depletion efficiencies for individual rRNAs are reproduced in Table 1.

	UHRR	HBRR	FFPE	Mouse	Rat
18S rRNA	99.29 %	98.98 %	89.36 %	99.60 %	98.76 %
28S rRNA	98.70 %	97.40 %	86.38 %	98.14 %	97.01 %
5.8S rRNA	99.55 %	99.51 %	99.34 %	99.85 %	98.73 %
5S rRNA	99.39 %	96.49 %	98.75 %	99.24 %	97.74 %
mt_rRNA	99.79 %	99.50 %	96.49 %	99.12 %	99.55 %
Overall	**99.05 %**	**98.22 %**	**88.51 %**	**98.77 %**	**97.97 %**

Table 1 | Depletion efficiency. Depletion using RiboCop. Resulting material was library prepped and sequenced, corresponding non-depleted samples served as reference for quantification. Values listed are for 100 ng total RNA input per reaction.

Observed depletion efficiencies in sequencing experiments routinely exceed 98 %. Even highly degraded samples, exemplified by the FFPE sample in column 3 are amenable to RiboCop rRNA depletion. Non-rRNA reads were mapped to Ensembl-annotated genomes and assessed for gene expression and class-attributions.

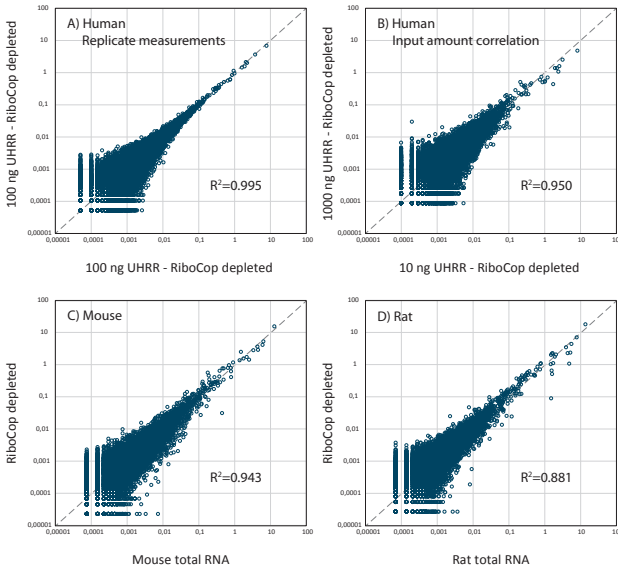

Figure 3 | A) Reproducibility: RiboCop-depleted human RNA samples (100 ng input each) **B) Input amounts:** Ribocop-depleted human RNA samples (10 ng vs 1 µg input RNA) **C) Mouse:** RiboCop-depleted mouse RNA (100 ng input) vs 100 ng mouse total RNA, **D) Rat:** RiboCop-depleted rat RNA (100 ng input) vs 100 ng rat total RNA

Excellent reproducibility of the RiboCop protocol is evidenced in Figure 3A). Replicate depletion of 100 ng human input material each yields R^2 exceeding 0.99. In Figure 3B) relative gene expression values from 10 ng input total RNA are plotted against 1 µg input. Thus, input amounts spanning two orders of magnitude are reproduced in this correlation. Figures 3C) and 3D) highlight applicability of mouse and rat input material. Plots are against respective non-depleted total RNA.

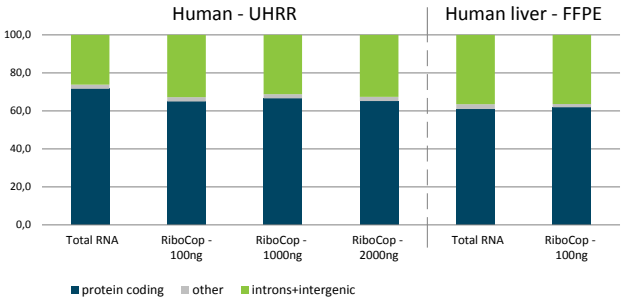

Figure 4 | RiboCop - mapping class-attribution: relative amounts of non-rRNA reads are reproduced before and after RiboCop rRNA depletion.

Quantification of mapped non-rRNA reads according to annotation classes confirms stable detection rates for different input amounts and two different samples of human origin.

RiBO COP™ Select and Deplete

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