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.

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 RRNA 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 1 μg 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. 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 samples. Measured depletion efficiencies for individual rRNAs are reproduced in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>UHRR</th>
<th>HBRR</th>
<th>FFPE</th>
<th>Mouse</th>
<th>Rat</th>
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<tr>
<td>28S rRNA</td>
<td>99.29%</td>
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<td>89.36%</td>
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<td>18S rRNA</td>
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<td>86.38%</td>
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<td>5.8S rRNA</td>
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<td>99.51%</td>
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<tr>
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<td>96.49%</td>
<td>98.75%</td>
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<tr>
<td>mt_rRNA</td>
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<td>Overall</td>
<td>99.05%</td>
<td>98.22%</td>
<td>88.51%</td>
<td>98.77%</td>
<td>97.97%</td>
</tr>
</tbody>
</table>

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.

Find more about RiboCop at www.lexogen.com

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