riboPOOL: Affordable Custom/Ribosomal RNA Depletion Against Any Species for RNA-Seq

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With the rising use of RNA Sequencing (RNA-Seq) with Next-Generation Sequencing (NGS), there is a growing demand for reagents that increase output and reliability of sequencing data. Ribosomal RNAs (rRNAs) occupy more than half of total RNA sequencing reads. For sensitive detection of scientifically relevant RNAs, rRNAs are removed either by physical depletion methods using rRNA-specific probes or by isolating relevant coding RNAs through poly-A-tail affinity enrichment. Current commercial rRNA removal kits are costly and limited to well-studied species. riboPOOLs developed by siTOOLs Biotech in partnership with IMGM Laboratories and scientists from University of Würzburg and Bayreuth, present an affordable and flexible solution that gives scientists the freedom to deplete rRNAs or other custom abundant RNAs from any species. Composed of high complexity pools of optimally-designed biotinylated DNA probes, riboPOOLs specifically associate with cytoplasmic and mitochondrial rRNAs, enabling their removal with magnetic streptavidin-coated beads. Human and mouse riboPOOLs were demonstrated to outperform current commercial rRNA depletion kits (Ribo-Zero, Illumina) in RNA-Seq experiments, depleting ~10% more rRNA while leaving other RNA intact. riboPOOLs have been made against a diverse array of organisms including Planarian, Silkworm, Drosophila, Arabidopsis and Bacteria. Furthermore, riboPOOLs can be tailored to deplete abundant tissue-specific mRNAs, such as globin in red blood cells, making it applicable for NGS-based clinical diagnostics.

Objective

Design, produce and test performance of rRNA depletion probes (riboPOOL) against current commercial solution (Ribo-Zero by Illumina) for human and mouse total RNA samples.

Methods

Design and Production of riboPOOLs

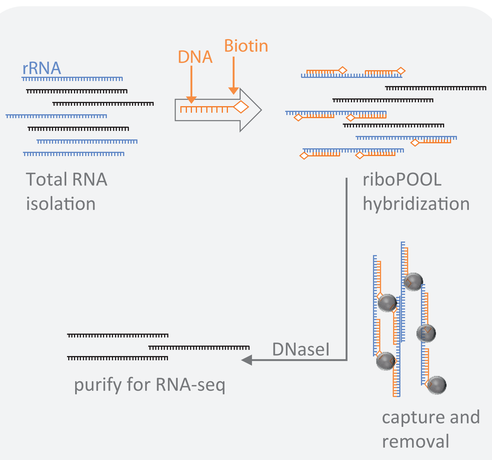
Candidate probes were evenly distributed across cytoplasmic 5S, 5.8S, 18S, 28S ribosomal RNAs and 12S and 16S mitochondrial ribosomal RNAs from human and mouse.

Key criteria for probe selection:

- Meet mismatch criteria against genome-wide sequences
- Within GC% range for optimal thermodynamic hybridization
- Avoidance of low complexity regions and repetitive elements

Biotinylated probes were HPLC-purified and dissolved in nuclease-free water at 100 pmol/µl.

rRNA Depletion



rRNA depletion workflow

riboPOOLs are high complexity pools of 3'-biotinylated single-stranded DNA.

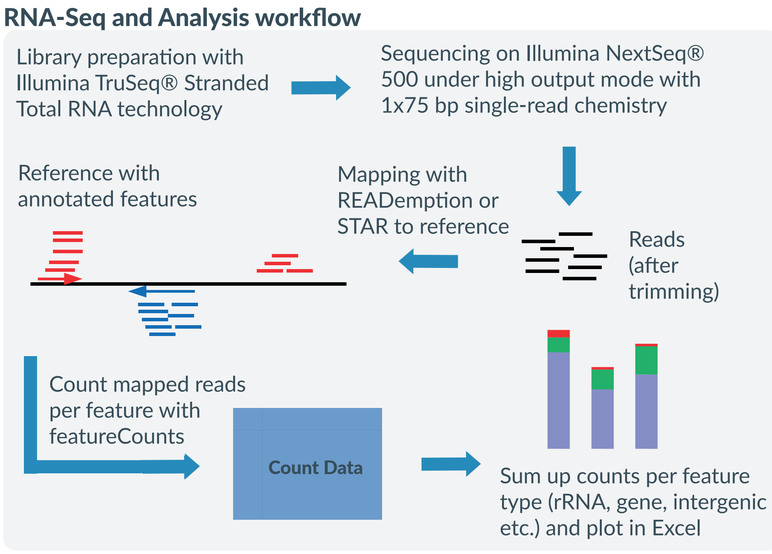
riboPOOLs hybridize selectively to rRNA, enabling their removal using streptavidin-coated magnetic beads.

Total RNA (1 µg, RIN ≥ 7.5) was isolated from HeLa cells or mouse embryonic fibroblasts and subject to rRNA depletion with 100 pmol of human or mouse riboPOOL or Ribo-Zero Gold (Human/Mouse/Rat) Kit (Illumina) according to manufacturer's instructions. Two biological replicates were performed per reagent.

RNA-Seq and Data Analysis

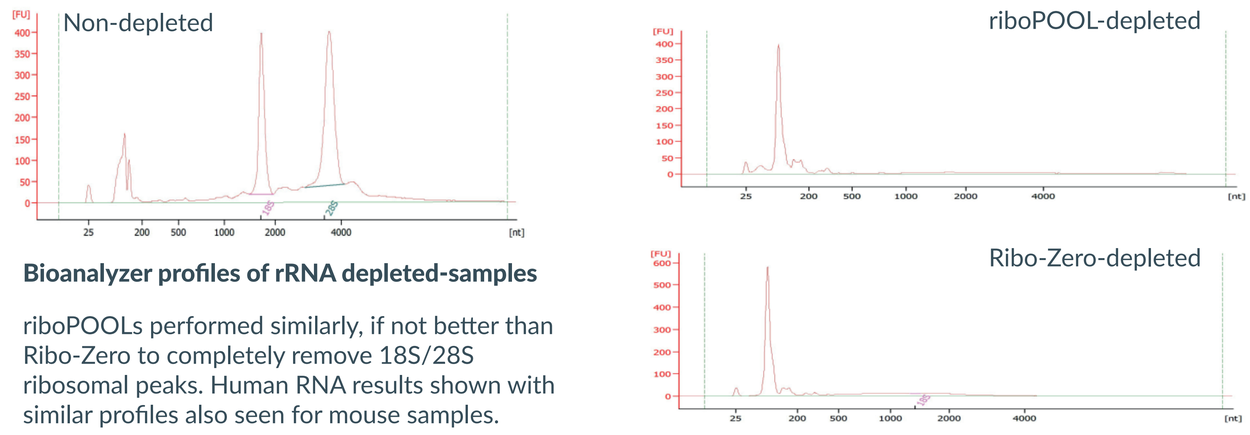
Samples were first analyzed on the Agilent 2100 Bioanalyzer.

RNA-Seq and Analysis workflow



Results

Complete removal of 18S/28S rRNA peaks by riboPOOL (Bioanalyzer)

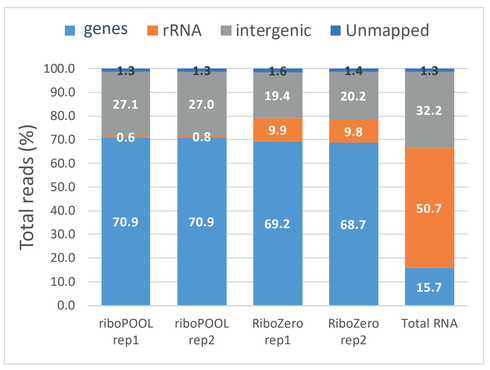


Bioanalyzer profiles of rRNA depleted-samples

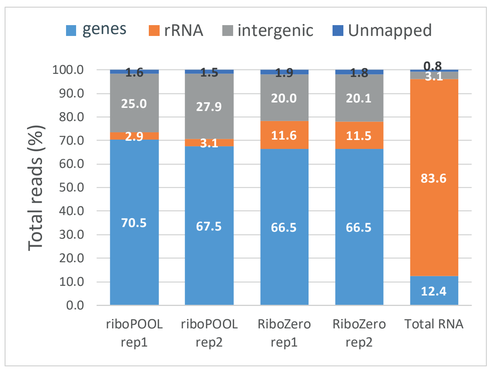
riboPOOLs performed similarly, if not better than Ribo-Zero to completely remove 18S/28S ribosomal peaks. Human RNA results shown with similar profiles also seen for mouse samples.

More efficient removal of rRNAs by riboPOOL compared to Ribo-Zero (RNA-Seq)

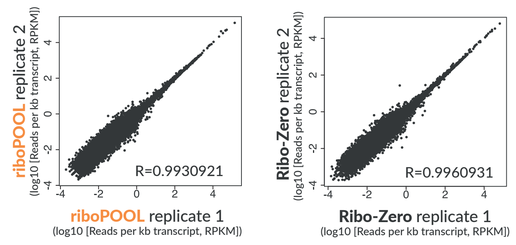
Human riboPOOL depleted 98.6% of ribosomal RNA, compared to 80.6% by Ribo-Zero



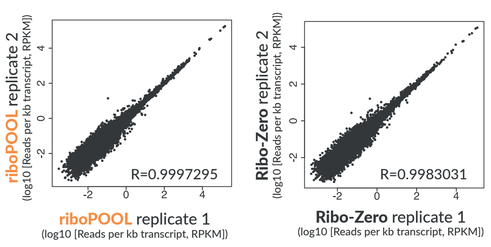
Mouse riboPOOL depleted 96.4% of ribosomal RNA, compared to 86.1% by Ribo-Zero



Comparable reproducibility between biological replicates for human riboPOOL and Ribo-Zero



Comparable reproducibility between biological replicates for mouse riboPOOL and Ribo-Zero



Both human and mouse riboPOOLs depleted ~10% more ribosomal RNA compared to Ribo-Zero. This resulted in more reads assigned to relevant RNAs from genes (introns + exons) and intergenic regions for riboPOOL-depleted samples. Both reagents performed with similar experimental reproducibilities.

Conclusion

Human and mouse riboPOOLs provided more efficient depletion of ribosomal RNAs with similar reproducibility when tested against the current commercial solution, Ribo-Zero from Illumina.