Application Note

riboPOOL

Efficient ribosomal RNA depletion across multiple species and input amounts in 70 min

- Effective RNA input range with riboPOOL: 100 ng - 5 µg
- Combination riboPOOLs can be applied for multiple species
- Fast riboPOOL workflow time: 70 min

Introduction

For ribosomal RNA depletion prior to RNA-Seq, **riboPOOLs** by **siTOOLs Biotech** provides a flexible and affordable solution that can be efficiently applied to any species.

Here, we established efficient riboPOOL-mediated rRNA depletion across a broad RNA input range (100 ng -5 µg) in 70 min with one capture and removal step. The Combination riboPOOL (Human/Mouse/Pan-Prokaryote) was also tested on human, mouse and E. coli samples, showing riboPOOLs can be applied for samples across multiple species.

Materials & Methods

RNA was extracted from SW48 cells (human), NIH3T3 cells (mouse), and DH10B E. coli respectively using Nucleospin® RNA (Macharey Nagel).

Ribosomal RNA depletion with Human riboPOOL, Mouse/Rat riboPOOL, and Pan-Prokaryote riboPOOL or Combination riboPOOL was performed with Nucleospin RNA Clean-up XS (Machery Nagel) according to **rRNA Depletion Protocol with riboPOOL** available on website under Resources. Streptavidin-magnetic beads were either Dynabeads MyOne C1 (Thermo Fisher) or siTOOL beads.

Depletion efficiency was assessed with Bioanalyzer® using RNA pico-chip (Agilent Technologies) or by real-time quantitative PCR (rtqPCR) using primers specific to ribosomal RNA or GAPDH.

Tips for working with riboPOOLs

- Use highest quality DNA-free RNA
- Use DNA low-binding tubes and low-retention tips
- Avoid leaving tubes open or at r.t. over long periods
- Due to rRNA abundance, expect to lose ~90% of RNA

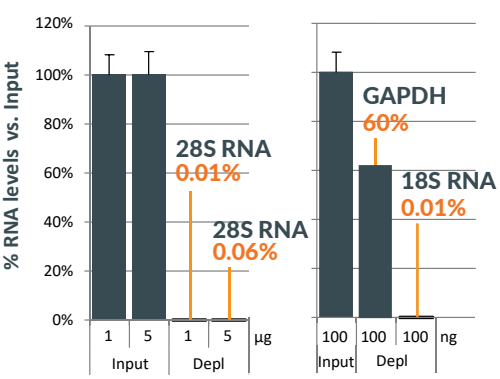
Results

riboPOOL is effective over a broad RNA input range (100 ng - 5 µg)

Loss of human rRNA (18S/28S) at RNA inputs of 100 ng, 1 µg and 5 µg was observed by Bioanalyzer and rtqPCR after rRNA depletion with human riboPOOL. *M: 25nt RNA marker present in every sample*

Bioanalyzer Analysis

rtqPCR Analysis

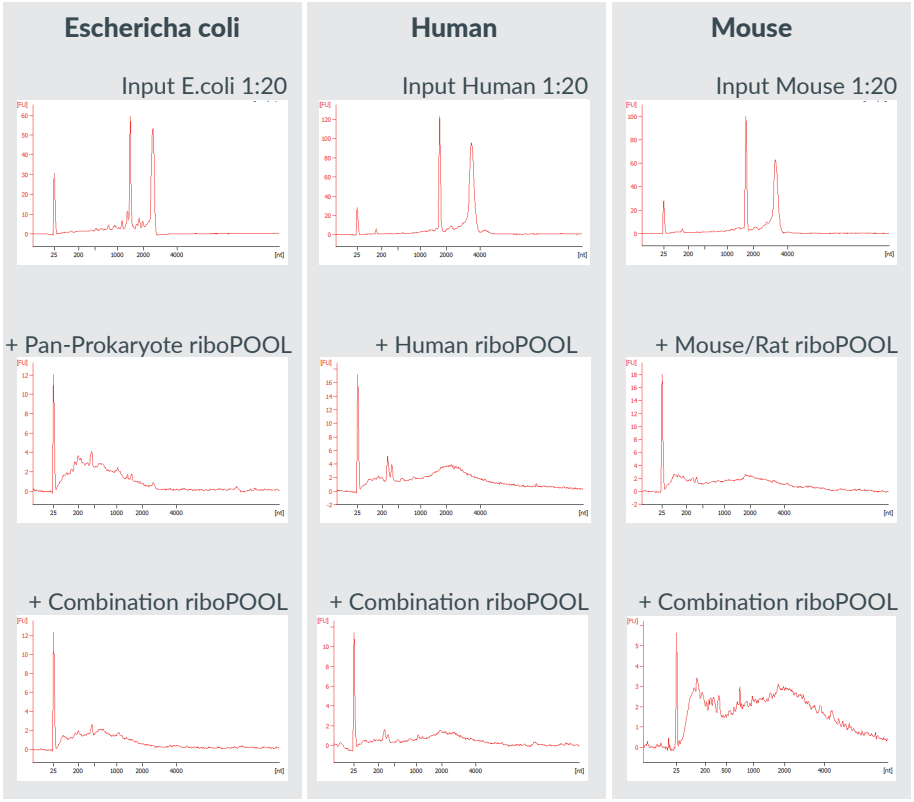


Results

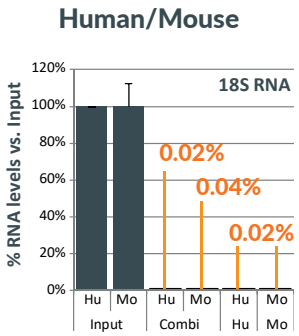
Combination riboPOOL (Human/Mouse/Pan-Prokaryote) efficiently depletes rRNA from Human, Mouse and Escherichia coli RNA

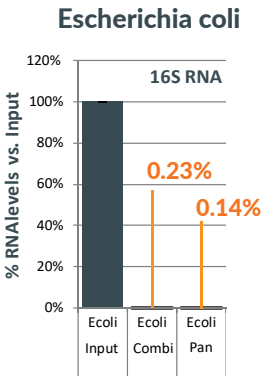
Efficient depletion of human, mouse and E. coli RNA with the Combination riboPOOL consisting of a 1:1:1 mix of Human, Mouse/Rat and Pan-Prokaryote riboPOOL was observed for 1 µg RNA input.

Bioanalyzer Analysis



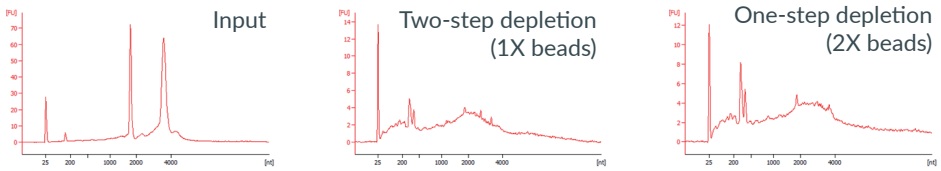
rtqPCR Analysis



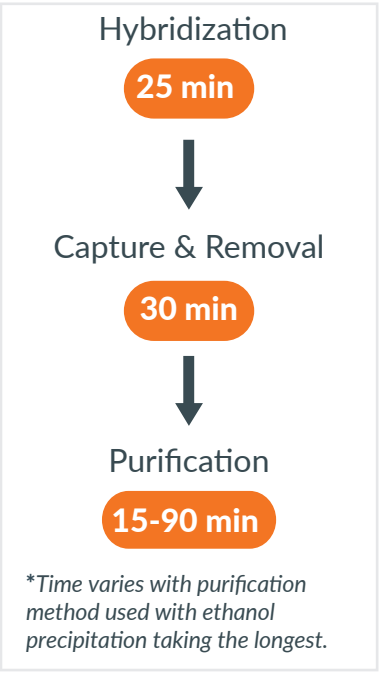


Fast workflow time of 70 min with riboPOOL

Depletion of 1 µg RNA with the human riboPOOL was accomplished in ~70 min with one capture and removal step. Similar depletion efficiencies were achieved by doubling the volume of streptavidin-coated magnetic beads used. *Previous protocol (v1_5) had two capture and removal steps.*



riboPOOL Workflow



**Time varies with purification method used with ethanol precipitation taking the longest.*

Conclusion

By Bioanalyzer/rtqPCR assessment, we show riboPOOL-mediated rRNA depletion is efficient across a broad RNA input range (100 ng - 5 µg), when applied in combination and with a fast workflow time of 70 min.

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