A customizable approach for selective removal of abundant RNAs enhances the sensitivity of transcript detection across species

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INTRODUCTION

The large dynamic range of transcript expression within total RNA presents a challenge to whole-transcriptome sequencing. Highly expressed transcripts with minimal biological interest can dominate readouts, masking detection of more informative lower abundance transcripts. Here, we present a customizable approach to enrich for RNAs of interest by eliminating unwanted RNAs. This method is based on hybridization of probes to the targeted RNA and subsequent enzymatic degradation of the selected RNAs. The probe sequences confer RNA removal specificity and can be designed to deplete unwanted RNA from any organism.

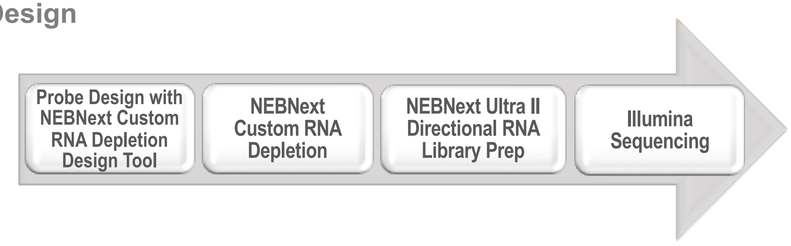
We developed a user-friendly web tool to enable custom depletion of any RNAs of interest. We used this web tool and depletion method to remove rRNA from total RNA of various species, including the mosquito *Aedes aegypti as well as* archaea *Thermococcus kodakarensis* and *Pyrococcus furiosus*. Additionally, we used this approach to target coding RNAs in human total RNA, and supplemented an existing anti-rRNA probe set for depletion of both rRNA and the selected coding RNAs.

Using strand-specific RNA sequencing we measured depletion efficiency and transcript expression. We achieved high depletion efficiency (up to 99%) for all targeted RNAs across species, while maintaining transcript abundance of non-targeted RNA. This translated into an enrichment of RNAs of interest and an increased depth of sequencing coverage.

The method described here is a simple and reliable solution to improve sensitivity in RNA-Seq studies. The design tool offers flexibility and control over the probe design process facilitating a customized and economical RNA sequencing experience. Importantly, the depletion and library construction method are amenable to high throughput sample preparation and robotic automation.

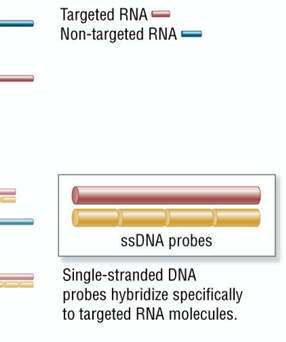
METHODS

Experimental Design



Probe Design with the web-based NEBNext® Custom RNA Depletion Design Tool

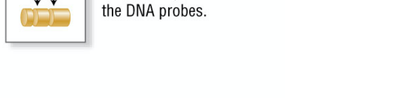
Step 1. Enter the sequence of the RNA you want to deplete in FASTA format, 5 to 3' orientation.
Press the "Design Probes" button



Step 2. The NEBNext® Custom RNA Depletion Design Tool outputs probe sequences spanning the input RNA.
An email is also sent with the probe sequences

Step 3. Order ssDNA probes from your trusted oligo provider. No modifications needed.

Step 4. Use the probes with the NEBNext® RNA Depletion Core Reagent Set or to supplement an existing NEBNext® RNA Depletion Kit .

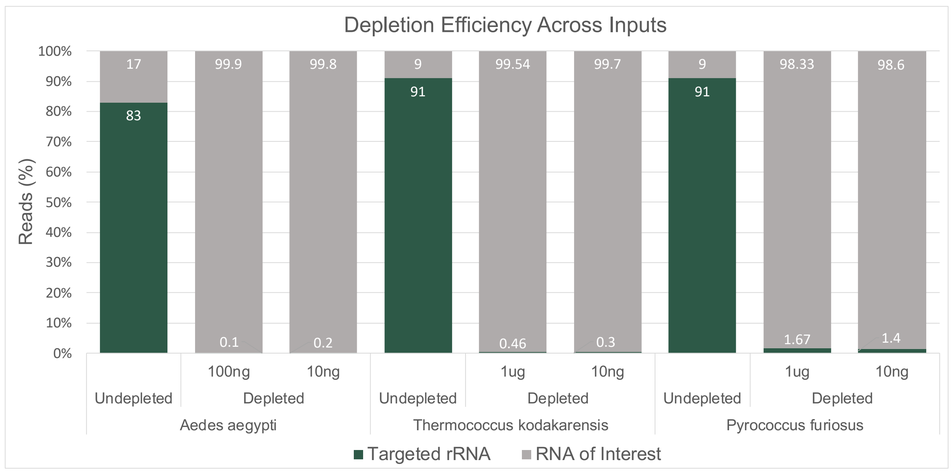


Depletion of the Targeted RNA using the NEBNext® RNA Depletion Workflow

Total RNA is hybridized with ssDNA probes targeting rRNA, followed by RNase H digestion to degrade targeted RNA. The DNA probes are then digested with DNase I, and the reaction is cleaned using magnetic beads. The entire workflow can be done in <2 hours with only 8 minutes of hands-on time. Ribosomal RNA depletion can be immediately followed by RNA-seq library preparation, and sequencing on an Illumina instrument.

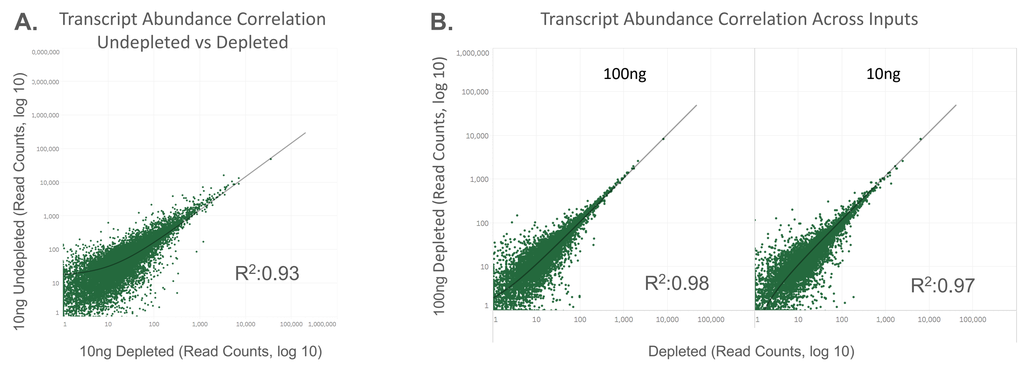
RESULTS

Figure 1. The NEBNext® custom RNA depletion approach enriches for RNAs of interest by efficiently removing targeted RNA from total RNA across species and a wide range of inputs.



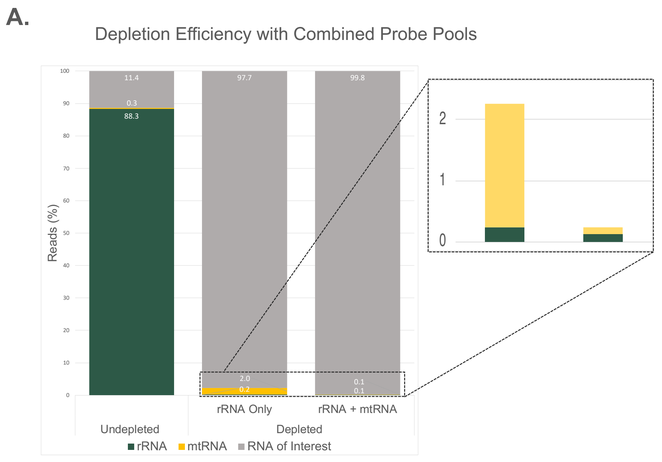
The NEBNext® Custom RNA Depletion Design Tool was used to design probes against *Aedes aegypti, Thermococcus kodakarensis and Pyrococcus furiosus* rRNA. Total RNA (1ug or 100ng and 10ng) was used as input for rRNA depletion using the NEBNext® RNA Depletion Core Reagent Set with the designed probes. RNA-seq libraries were prepared using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (NEB #E7760) followed by paired-end sequencing on a NextSeq ® instrument (2 x 75 bp). Reads were down sampled to 20 million (seqtk) from each library. Read pairs were identified as ribosomal using mirabait (6 or more shared 25-mers), and levels of rRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering. The data represents an average of 3 replicates. The method efficiently depletes targeted rRNA across species and a wide range of total RNA input amount (1ug-10ng).

Figure 2. Depletion of targeted RNA does not affect non-targeted transcripts.



The NEBNext® Custom RNA Depletion Design Tool was used to design probes against *Aedes aegypti* rRNA. Adult *Aedes aegypti* mosquitos where purchased from Benzon Research. Total RNA was extracted using the Monarch® Total RNA Miniprep Kit (NEB #T2010S) and used as input (100ng and 10ng) for rRNA depletion using the NEBNext® RNA Depletion Core Reagent Set with the designed probes. RNA-seq libraries were prepared using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (NEB #E7760) followed by paired-end sequencing on a NextSeq ® instrument (2 x 75 bp). Reads were down sampled to 20 million (seqtk) from depleted libraries and 200 million from undepleted libraries. Transcript abundance were estimated using Salmon and transcripts from Vectorbase (AaegL5.2 assembly). Read counts and R^2 values for the linear fit are shown. A) The average of three replicates is shown. rRNA Depletion does not affect abundances of non-targeted transcripts. B) Transcript abundances are maintained between replicates and across input amounts.

Figure 3. Probe pools are combined to efficiently deplete human rRNA and mitochondrial mRNA using the NEBNext® custom RNA depletion approach.



The NEBNext® Custom RNA Depletion Design Tool was used to design probes against human mitochondrial mRNA (mtRNA). The probes were combined with the NEBNext® rRNA Depletion Kit V2 (Human/Mouse/Rat) depletion solution. Total universal human reference RNA (1ug) was depleted of mtRNA and rRNA using the NEBNext® RNA Depletion Core Reagent Set with the combined pool. RNA-seq libraries were prepared using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (NEB #E7760) followed by paired-end sequencing on a NextSeq® instrument (2 x 75 bp). Reads were downsampled to 20 million (seqtk) from each library. A) Read pairs were identified as ribosomal and mitochondrial using mirabait (6 or more, 25-mers), and levels of rRNA and mtRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering. Both rRNA and mtRNA are efficiently depleted. B) Integrative Genome Viewer (IGV) visualization of read coverage across the human mitochondrial genes.

CONCLUSIONS

- The NEBNext® Custom RNA Depletion Design Tool facilitates the design of probes to remove unwanted RNA in any organism of interest.
- The probes are used in conjunction with the NEBNext® RNA Depletion Core Reagent Set to efficiently remove unwanted RNA.
- The method is amenable for a wide range of inputs (10ng-1ug total RNA), and compatible with any RNA library prep kit.
- Successful depletion was achieved using this method on total RNA from *Aedes aegypti*, *Thermococcus kodakarensis*, *Pyrococcus furiosus*, and *Homo sapiens*.
- Depletion of highly abundant transcripts, such as rRNA, greatly increased the number of reads mapping to RNAs of interest.
- Depletion does not affect transcript abundance of RNA species not targeted.
- Designed probes can be combined with existing depletion solutions for a more customized experimental setup.

ACKNOWLEDGEMENTS

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