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


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 reads, 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.

METHODS

Experimental Design

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

Step 1. Enter the sequence of the RNA you want to deplete in the FASTA format. Click on the “Search” button.

Step 2. Select the “Design Probes” button.

Step 3. Order the probes from the provided template.

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

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.

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

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

CONCLUSIONS

• The NEBNext® Custom RNA Depletion 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 protocol.

• 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 rRNAs 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.

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