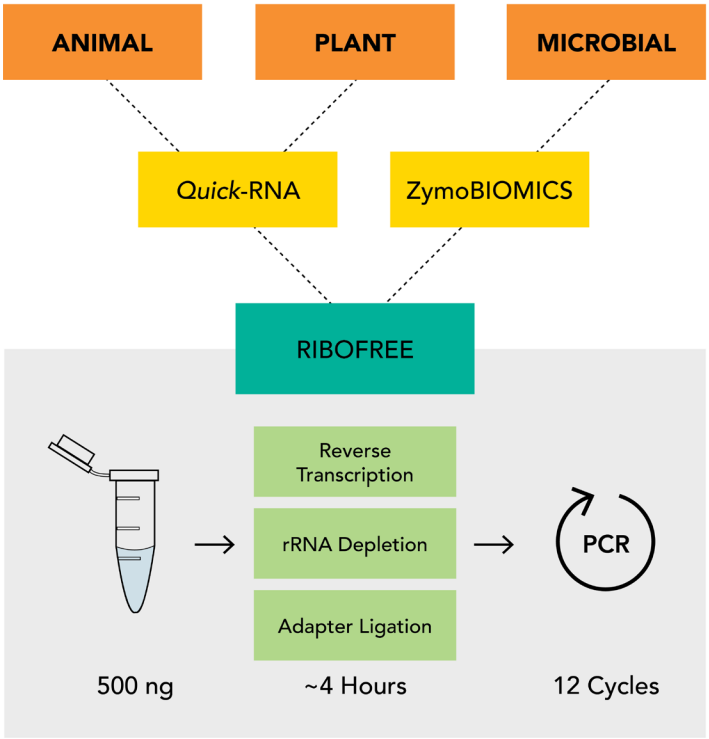
Multi-Organism Transcriptomics with Zymo-Seq RiboFree® Total RNA Library Kit

Introduction

RNA sequencing (RNA-seq) has become an essential tool in many research fields, covering a wide range of organisms and systems[1, 2]. However, generating accurate and informative RNA-seq data for transcriptomic study can be difficult due to the exceedingly high proportion of ribosomal RNA (rRNA). Although several rRNA removal strategies have been developed to aid in RNA-seq library preparation, probe-free rRNA depletion has been reported as more versatile compared to other methods, especially for its potential universal compatibility with different classes of organisms[3]. Zymo-Seq RiboFree® Total RNA Library Kit has been developed and optimized based on this novel rRNA depletion strategy, which has been quickly adopted by researchers for transcriptomic studies in animals, plants, and microorganisms[4-7]. In this application note, we describe a whole-transcriptome analysis on several model organisms using the RiboFree® kit to further validate its cross-species compatibility. We also introduce a complete workflow from RNA extraction to bioinformatic analysis that serves as a quick guide for researchers to kick off their own transcriptomic studies in one or multiple organisms.



Zymo-Seq RiboFree® Total RNA Library Kit Workflow. Zymo Research provides a wide collection of RNA extraction kits to meet various research needs[6]. Cow (*B. taurus*), chicken (*G. gallus*), corn (*Z. mays*), wheat (*T. aestivum*), grape (*V. vinifera*), and yeast (*C. albicans*) total RNA were extracted and used as input for RiboFree® depletion. Identical reverse transcription, depletion incubation (2 hours), and indexing PCR steps were used for each sample.

Methods

RNA Extraction

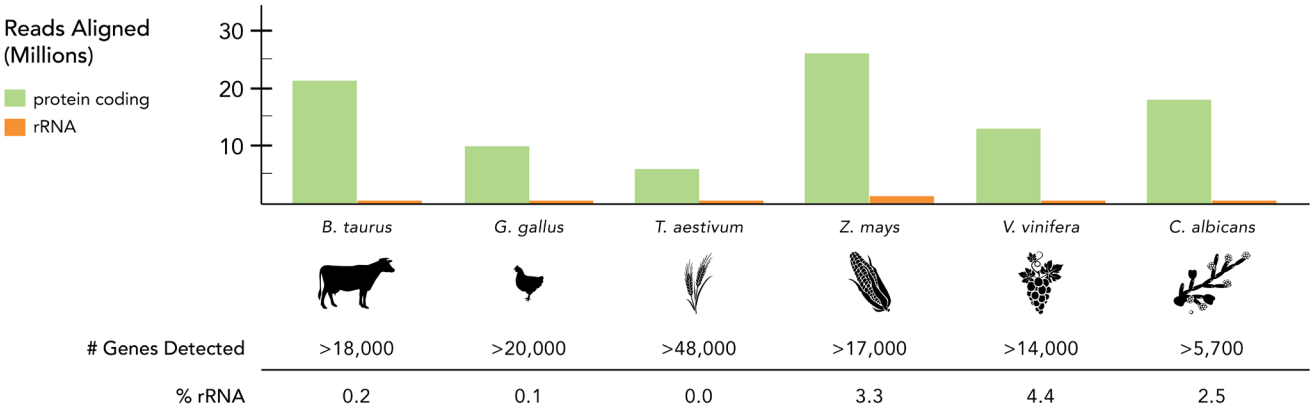
For animal samples, total RNA was extracted from Chicken (*Gallus gallus*) and Bovine (*Bos taurus*) spleen tissues with the *Quick*-RNA™ Miniprep Plus Kit (R1057) according to the standard protocol with proteinase K digestion and DNase I treatment performed. For plant samples, total RNA was extracted from Corn (*Zea mays*), Wheat (*Triticum aestivum*), and Grape (*Vitis vinifera*) leaf tissues with the *Quick*-RNA™ Plant Kit (R2024) according to the standard protocol. The Precellys® 24 homogenizer (Bertin Corp) was used to homogenize the leaf tissues. Total RNA was extracted from 15 mL of in-house grown Yeast (*Candida albicans*) cultures with the ZymoBIOMICS® RNA Miniprep Kit (R2001) according to the standard protocol.

Library Preparation and Sequencing

500 ng of total RNA was used as input per sample for library preparation with the Zymo-Seq RiboFree® Total RNA Library Kit (R3000) according to the standard protocol. Specifically, rRNA depletion time was 2 hours and 12 cycles of Indexing PCR were performed. Libraries were characterized on TapeStation® 2200 using the D1000 ScreenTape® (Agilent) to confirm the size distribution. Libraries were then quantified with the KAPA® Library Quantification Kit (Roche, KK4824), normalized and pooled at 2 nM for 100-bp, paired-end sequencing on the Illumina® HiSeq® 1500 platform. At least 27 million reads were obtained for each library.

Bioinformatic Analysis

A custom Nextflow pipeline adapted from nf-core/rnaseq pipeline version 1.4.2. was applied for bioinformatic analysis. In short, reads were trimmed according to the recommendation in the RiboFree® kit protocol and aligned to reference genomes using STAR. Reads overlapping with exons were assigned to genes using featureCounts. Classification of rRNA genes/exons and their reads were based on Ensembl annotations and RepeatMasker rRNA



RiboFree® RNA-seq Multi-Organism Coverage. Ribosomal RNA depletion performed with Zymo-Seq RiboFree® demonstrated > 95% depletion across vertebrates, plant, and microbial species. "# Genes Detected" accounted for the number of unique gene IDs with FPKM ≥ 1.

tracks from UCSC genome browser where applicable. The reference genomes used for analysis are assemblies GRCg6a (*G. gallus*), ARS-UCD1.2 (*B. taurus*), B73 RefGen_v4 (*Z. mays*), IWGSC (*T. aestivum*), and 12X (*V. vinifera*) from Ensembl, and ASM18296v3 (*C. albicans*) from RefSeq.

Results

To test the species compatibility of Zymo-Seq RiboFree® Total RNA Library Kit, two technical replicates each for cow, chicken, corn, wheat, grape, and yeast total RNA were extracted and processed with the RiboFree® workflow. 500 ng of input RNA was used for each sample. The resulting libraries produced high-quality reads where an average of >75% of reads were uniquely aligned across all samples, and as high as 95% uniquely aligned in chicken. Protein coding regions comprised the dominant biotype in each sample, with rRNA effectively depleted to below 5% in all organisms. Genes with FPKM ≥1 were counted as detected. The successful depletion of rRNA from the total RNA samples allowed for high resolution detection of protein coding genes as well as non-coding RNAs. For example, in yeast over 5,700 genes were detected of the ~6,100 annotated genes.

Conclusion

The ability to deplete rRNA from a total RNA sample vastly improves the sequencing efficiency for profiling RNA molecules of interest, thus making transcriptomic research more financially feasible. By employing the Zymo-Seq RiboFree® Total RNA Library Kit, we successfully depleted rRNA in multiple species with a single standard protocol. The produced RiboFree® libraries provide robust transcriptomic coverage in a species-independent manner, allowing researchers to study the transcriptome of any organism of interest. Moreover, this simple, same-day workflow maximizes benchwork efficiency, saving precious time for experiments during a period when researchers may have limited lab access.

Tips for High-Quality RiboFree® Libraries and Bioinformatics:

- *RNA quality:* Determine the RNA Integrity Number (RIN) by a preferred method such as TapeStation. For RNA of low integrity (RIN < 4), use a minimum of 250 ng of RNA as input and use RNA Clean and Concentrator – 5 (R1013) instead of the Magbeads for the first clean up after depletion.
- *Use DNA-free RNA:* Treat RNA samples with DNase I to eliminate contaminating DNA. The presence of DNA may reduce the quality of the resulting library and introduce bias.
- *Bead cleanup considerations:* Allow the Select-a-Size MagBeads to equilibrate to room temperature for at least 30 minutes prior to use. Homogenize the bead solutions by inverting and vortexing. For efficient nucleic acid recovery, ensure that any residual wash buffer is completely removed and the beads are optimally dried. Optimally dried beads are matte in appearance; not glossy and not cracked.
- *Bioinformatics:* The sequencing reads from RiboFree® libraries can be analyzed by publicly available packages. No customized scripts are needed. Please refer to Appendix F: Bioinformatics in the Kit Protocol for information. Contact tech@zymoresearch.com if you need bioinformatic support for RiboFree® data analysis.
- *Large sample numbers:*
  - Multichannel pipette: To reduce hands-on time, reagents may be pre-aliquoted into strip tubes for sample addition using a multichannel pipette. Visually confirm that the volumes dispensed are uniform.
  - Automation: The RiboFree® workflow is automation ready! Contact automation@zymoresearch.com for details.

Citations

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