DATASHEET

A simplified approach to count ribosome-associated polyadenlyated transcripts

Introduction

Determining the composition of the proteome and relative abundance of proteins in biological and clinical samples is a fundamental component of biomedical research. Methodologies that quantify transcripts that are associated with ribosomes yield a more accurate and unbiased representation of protein production than present proteomic efforts; however, current strategies are time-consuming and laborious. eRibo Count is a method that quantitates ribosome association transcriptome-wide by targeting a key ribosomal protein with CLIP (crosslinking and immunoprecipitation)[1]. eRibo Count is a streamlined CLIP protocol by which libraries of the sample's transcriptome and translatome can be generated within two days. The ability to quantitate ribosome occupancy on transcripts can yield insight into translational efficiency. This provides a means to measure differences in expression between samples (e.g. normal vs. disease tissue or drug treated vs. untreated samples).

Highlights

Capture what you've been missing

Prepare libraries in less than 2 days and obtain information missed by RNA-Seq alone

Define the translatome

Detect ribosome-associated RNAs across the transcriptome

Quantify ribosome occupancy

Determine relative quantitation of ribosome occupancy across a multitude of sample conditions

Specifications

Sample Input Range	5-10 million cells
Starting Material	Tissue or crosslinked cells
Sequencing Platform	Illumina
Read Depth	30 million reads
Run Parameters	SE 50

eRibo Count Workflow

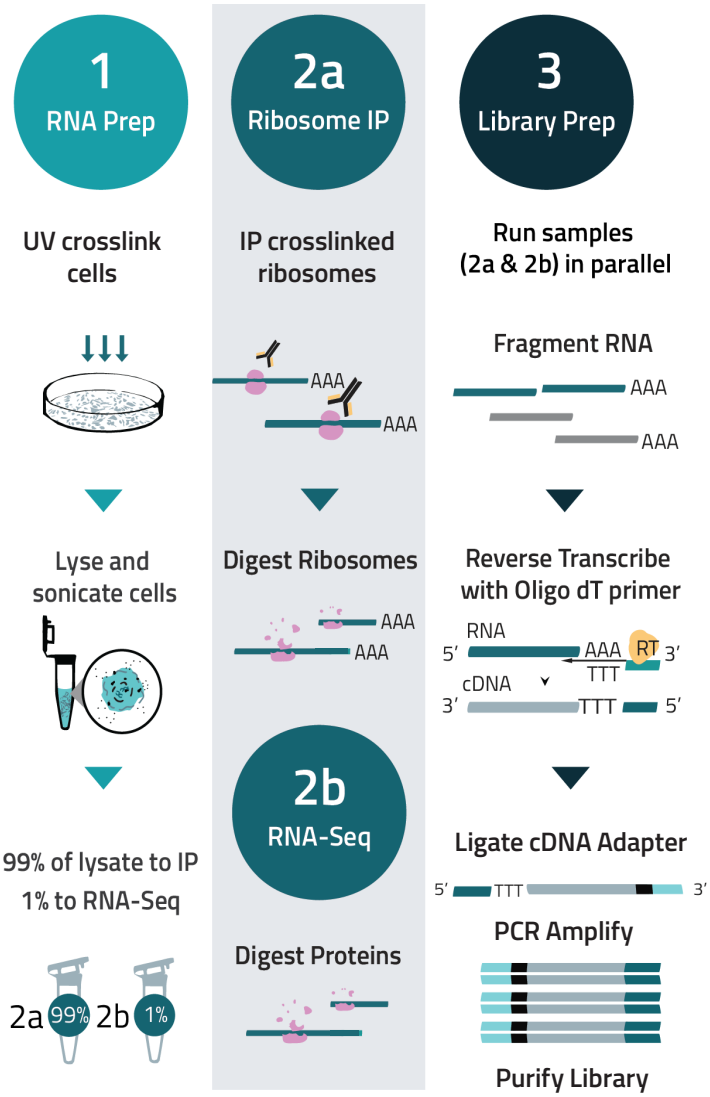

Figure 1. Ribosome-RNA interactions are captured by UV crosslinking. Following cell lysis, a key ribosomal protein is immunoprecipitated (IP) to capture ribosome-associated transcripts, while 1% of the lysate is used for RNA-Seq. The RNA-Seq and ribosome IP fractions use Oligo dT priming for reverse transcription (RT) and are processed in parallel to generate libraries for sequencing.

References

1. Van Nostrand EL et al., Nature. 2020 Jul;583(7818):711-719.
2. Li BB et al., Proc Natl Acad Sci. 2018;115(40):E9325-E9332.1.

Detect changes in translation that would be missed by RNA-Seq alone

eRibo Count can quantitate changes in ribosome occupancy in response to treatment.

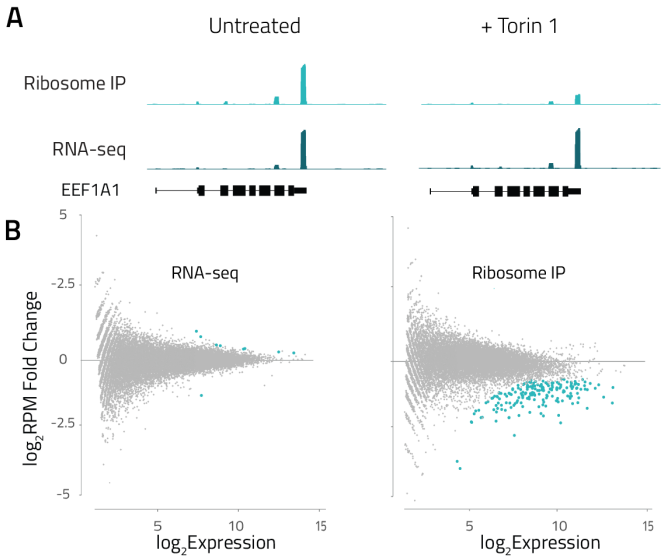

Figure 2. Torin 1, a well-characterized translation inhibitor, causes reduced ribosome occupancy on TOP- and TOP-like motif-containing genes in response to acute treatment[2]. (A) Ribosome IP and RNA-Seq reads mapped on the EEF1A1 gene before and after Torin 1. RNA-Seq levels are constant while ribosome IP levels are reduced upon treatment. (B) MA plots of genes in ribosome IP (right) and RNA-Seq (left); blue dots represent significant differences in response to Torin 1.

Calculate ribosome occupancy

eRibo Count can measure changes in ribosome association on transcripts. Ribosome occupancy (RO) is a ratio of ribosome IP to RNA-Seq thus normalizing to changes in transcript levels.

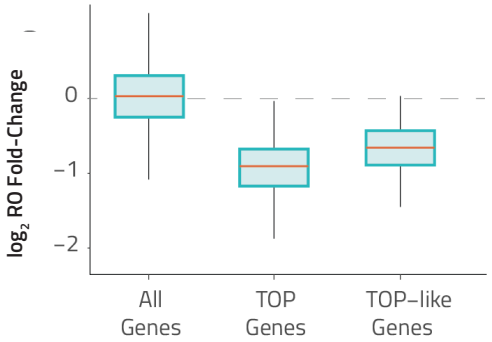

Figure 3. Fold-change of the Ribosome Occupancy (Ribosome IP/RNA-seq) between Torin 1-treated and untreated cells. Reduction of RO is shown for TOP motif-containing genes and TOP-like motif-containing genes after acute Torin 1 treatment compared to all genes detected.

Generate competitive RNA-Seq data

eRibo Count provides highly useful mapping information when compared to service providers offering only RNA-Seq

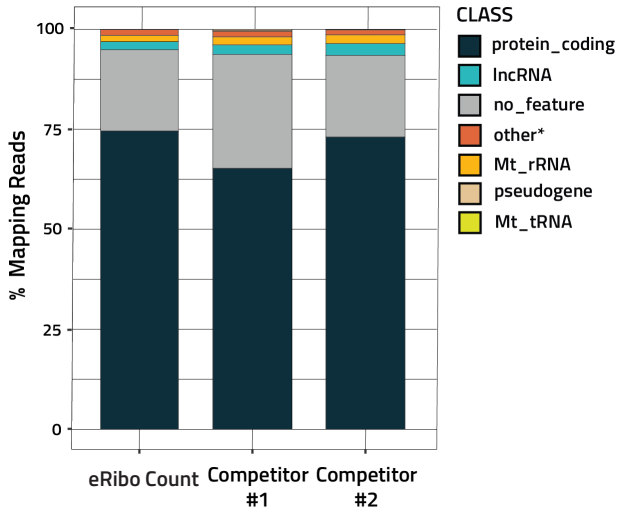

Figure 4. Proportion of gene transcript biotypes in uniquely mapped reads from eRibo Count RNA-Seq libraries compared to leading service providers. Biotypes were assigned based on the Gencode v35 annotation. *Other includes rRNA, miRNA, non-coding RNA, snRNA, snoRNA, IG and TR genes, and ncRNA-related pseudogenes.

More data in less time

Our NGS technology quantitates ribosome association transcriptome-wide by targeting a key ribosomal protein, and supplies two comprehensive data sets

eRibo Count Benefits

- Interrogate both transcriptional and translational changes in a single experiment
- Understand how gene expression varies between conditions
- Calculate ribosome occupancy
- Gain a deeper understanding of how regulation and mis-regulation of translation impact physiology and disease progression

More information about eRibo Count online at **eclipsebio.com** or contact us at **info@eclipsebio.com.**