Obtain ribosomal, RNA-Seq, and splicing data, transcriptome-wide from a single experiment

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 quantitate transcripts that are associated with ribosomes and being translated yield a more accurate and unbiased representation of protein levels. eRibo Total is a method that maps and quantitates ribosome occupancy transcriptome-wide by targeting a key ribosomal protein with eCLIP (enhanced crosslinking and immunoprecipitation)[1]. The ability to quantitate the ribosome occupancy on transcripts can yield insight into translational efficiency and provides a means to measure the differences in expressions between samples (e.g. normal vs. disease tissue or drug treated vs. untreated samples).

Highlights

Report changes in ribosome occupancy

Reveal association of ribosomes with RNA and detect changes in translation missed by RNA-Seq

Calculate translational efficiency

Use ribosome occupancy data to infer the rate of translation between genes

Identify splicing variants

Generate data on potential splicing events at both the RNA-Seq and ribosome-associated RNA levels

Specifications

Sample Input Range	5-10 million cells
Starting Material	Tissue or crosslinked cells
Sequencing Depth	30 million reads
Run Parameters	PE 150

eRibo Total Workflow

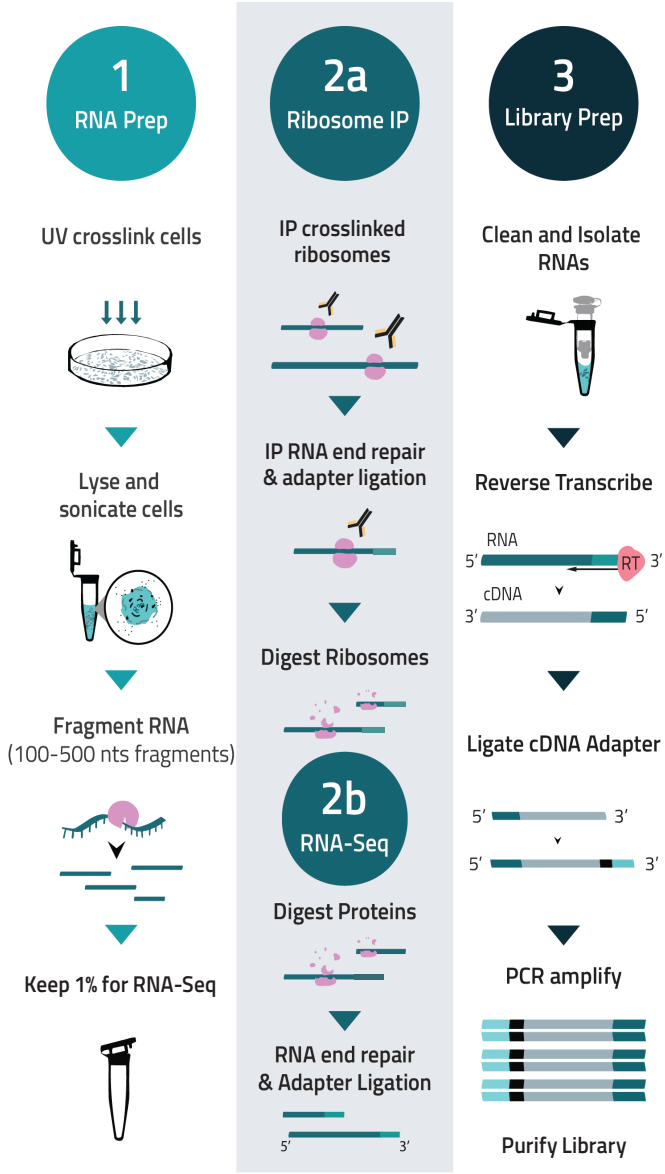

Figure 1. The eRibo Total workflow outlines the use of crosslinked materials through ribosome immunoprecipitation (IP), RNA-Seq and library preparation. Crosslinking of ribosomal proteins to the RNA ensures capture of direct interactions. After crosslinking and lysis, RNA is fragmented, and a specific ribosomal protein is immunoprecipitated. 1% of this sample is used for RNA-Seq. After library generation, samples are amplified by PCR to obtain sufficient material for high-throughput 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

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

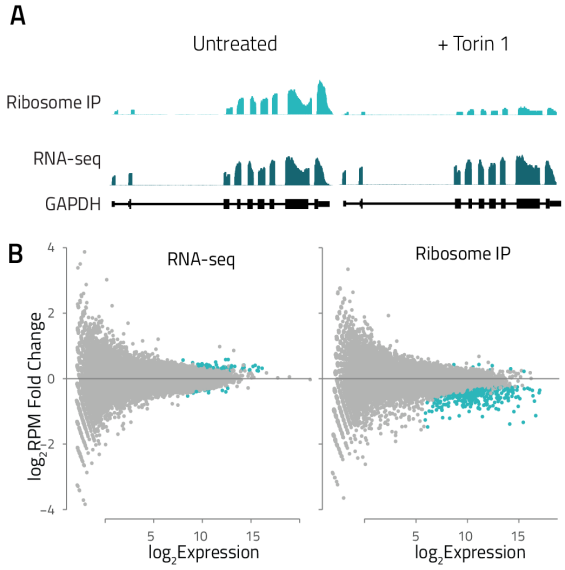

Figure 2. Acute Torin 1 treatment impacts ribosome association without changing RNA transcript levels[2]. (A) Ribosome IP and RNA-Seq reads mapped on the TOP motif-containing gene GAPDH, before and after acute Torin 1 treatment. RNA-Seq levels are constant while ribosome IP levels are reduced upon treatment. (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 Total can measure changes in ribosome association on transcripts. Ribosome occupancy (RO) is a ratio of ribosome IP over RNA-Seq, thus normalizing to changes in transcript levels.

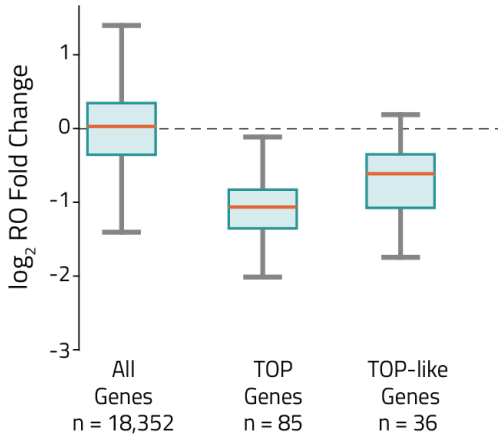

Figure 3. Fold-change of the Ribosome Occupancy (Ribosome IP/RNA-Seq) between Torin 1-treated and untreated samples. Reduction of RO is shown for TOP motif genes and TOP-like motif-containing genes after acute Torin 1 treatment compared to all genes detected.

Splicing analysis from eRibo Total

The Ribosome IP and RNA-Seq libraries both have long inserts that allow for quantitation of splicing changes and inference of spliced isoforms being translated.

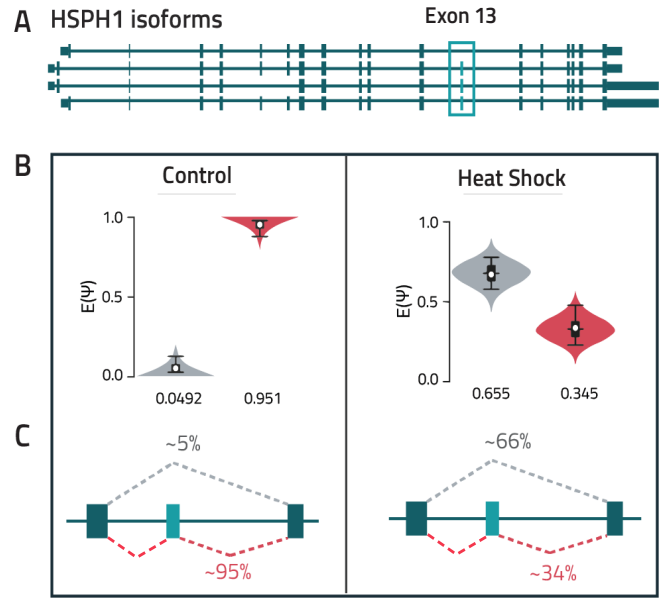

Figure 4. eRibo Total libraries quantifying significant exclusion of an exon upon heat shock. (A) HSPH1 annotated transcript isoforms. Exon 13 contains a nuclear localization sequence which results in cytosolic localitzation of the protein when the exon is excluded from transcripts. (B) Violin plots showing change in percent junction usage. Grey indicates percent junction exclusion, while red indicates percent junction inclusion. (C) Splicing event schematic. Splicing event quantification was performed with MAJIQ (Vaquero et al. 2016).

eRibo Total benefits

- Reveal the translatome of cells or tissues
- Detect transcriptome-wide changes in ribosome-association on RNAs
- Identify differential gene expression from both RNA-Seq and ribosome-associated RNA data

More data from one sample

Obtain two comprehensive data sets to quantitate ribosome association transcriptome-wide

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