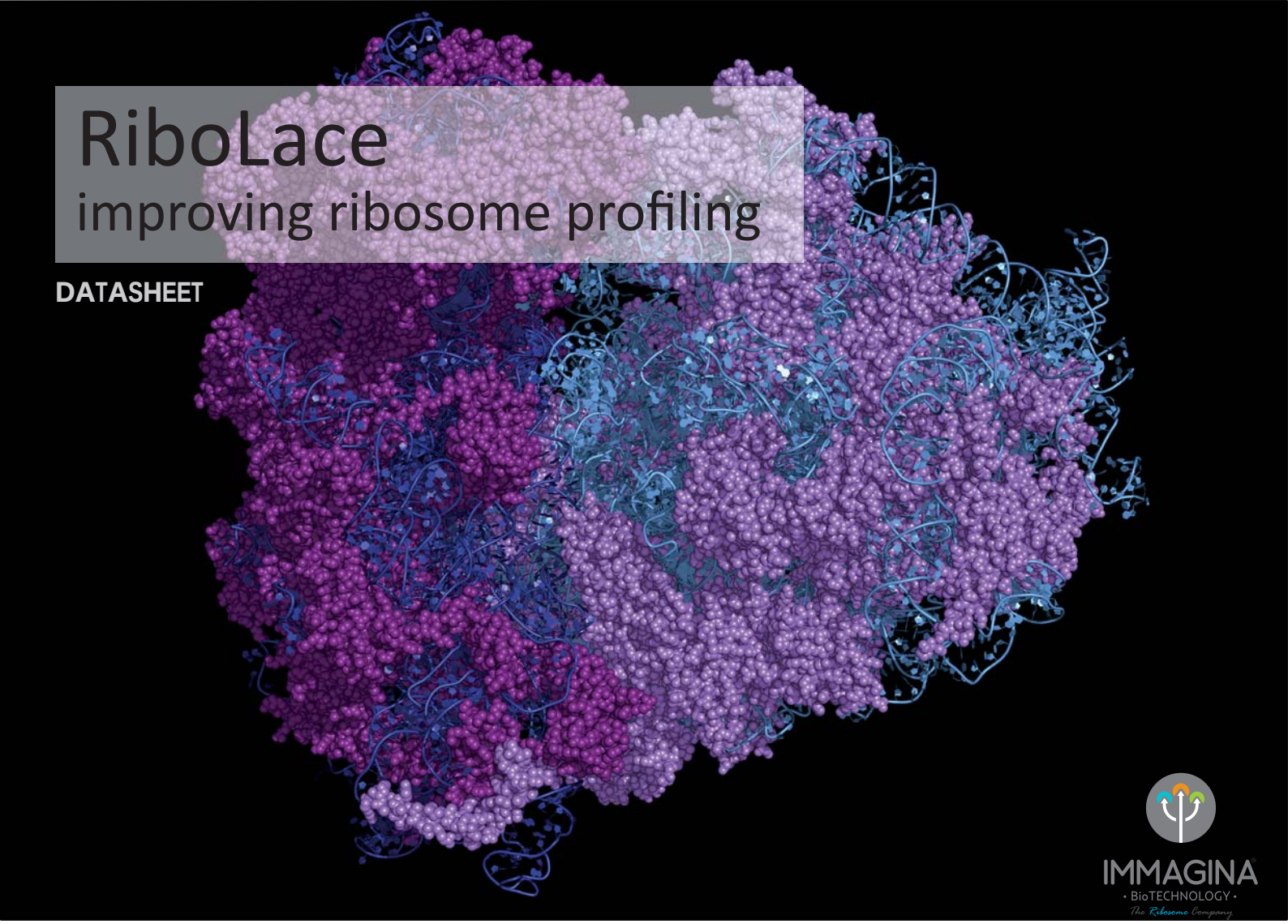
RiboLace
improving ribosome profiling

DATASHEET



IMMAGINA
• BioTECHNOLOGY •
The Ribosome Company

Introduction

IMMAGINA BIOTECHNOLOGY provides an innovative solution for ribosome profiling with RiboLace. Classical ribosome profiling approaches do not distinguish between fragments protected either by actively translating or by inactive ribosomes.

IMMAGINA's proprietary RiboLace technology allows 1-day selective extraction of ribosomes in active translation and purification of ribosome protected fragments (RPFs). Suitable starting material can be lysates of flash-frozen tissues or immortalized/primary cell cultures (>10.000 cells).

Downstream library preparation for NGS-ILLUMINA sequencing is possible with our RiboLace Ribo-seq Module 2 kit or any other protocol for small RNA sequencing.

Highlights

Low RNA input requirements

- 30 times less input material than current availble Ribo-seq protocols.

Only active ribosomes captured

- positional data of active ribosomes with nucleotide resolution;
- translation levels estimation and protein levels accurate prediction;
- works reliably both *in vitro* and *in vivo*.

Short and simple workflow

- antibody-free and tag-free pull-down;
- no ultracentrifugation step.

Simple, fast and efficient workflow for magnetic purification of active ribosomes.

RiboLace isolates RNA fragments protected by puromycin-trapped translating ribosomes by simply incubating endonuclease digested cell/tissue lysates with magnetic beads.

The combination of a puromycin analog (RiboLace probe) and cycloheximide, which clamps ribosomes on mRNA fragments, allows to easily trap and purify actively translating ribosomes and their RNA protected fragments.

By applying dedicated computational tools (e.g. RiboWaltz; Lauria et al., 2018), it is possible to selectively portray the position of *bona fide* active ribosomes at single nucleotide resolution.

RiboLace Workflow

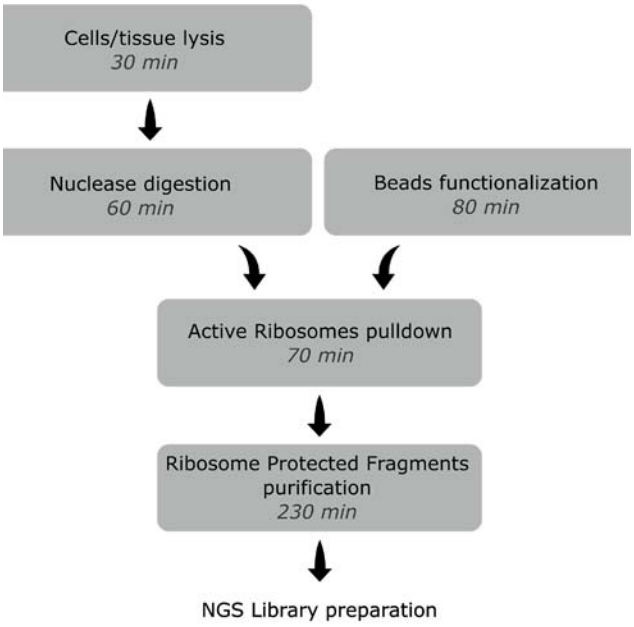


Fig. 1 The RPFs purified by RiboLace Module 1 kit can be used as input for RiboLace Module 2 library preparation kit or any other kits suitable for small RNAs library construction.

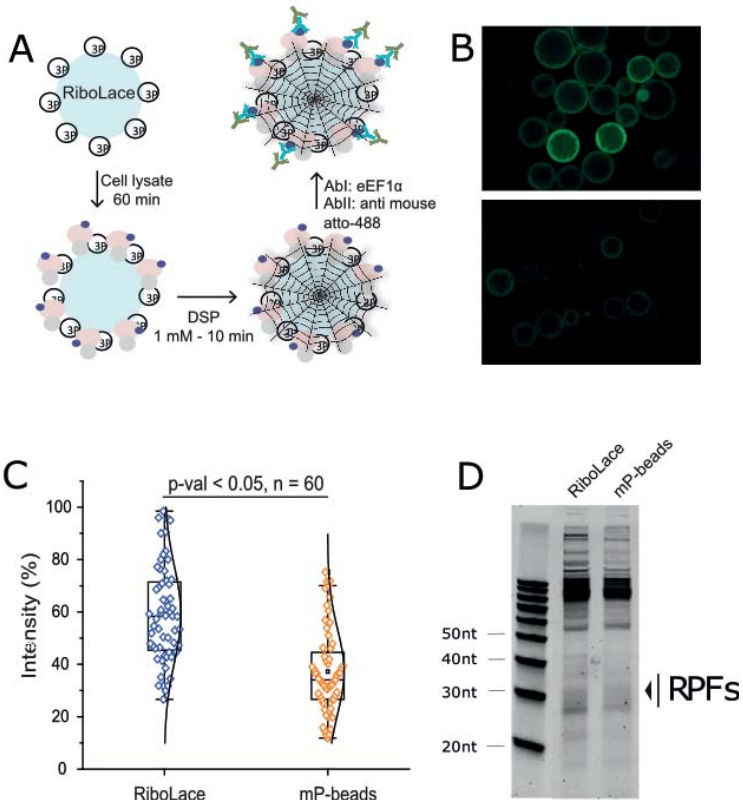


Fig. 2 A) Experimental design for testing the performance of RiboLace in capturing ribosomes B) Fluorescence detection and quantification (C) of eEF1A on RiboLace beads compared with control (mP beads). D) Denaturing polyacrylamide TBE-urea gel of RPFs recovered with RiboLace and mP-beads RNA after ribosomal RNA depletion. RPF enrichment is shown with a black arrow.

RiboLace enriches for factors associated with active translation and enhances the recovery of Ribosome Protected Fragments.

The performance of RiboLace in capturing ribosomes was evaluated first by measuring the fluorescence emission of an active translation-associated factor, eIF1A, on RiboLace probe crosslinked beads compared with control beads (mP beads). The results, showed in Fig. 2A, B and C, confirmed the enrichment of eIF1a, demonstrating that RiboLace is indeed able to capture bona fide active ribosomes. Second, we confirmed that RiboLace was able to enhance ribosome-protected fragments recovery after lysates endonuclease digestion (Fig. 2D).

RiboLace provides an improved estimation of protein level with respect to the use of total RNA or polysomal RNA.

To demonstrate that RiboLace provides an improved estimation of protein level with respect to the classical transcriptome and translatome analysis, total RNA, polysomal RNA, and RiboLace were compared to the proteome (Fig. 3A).
RiboLace displayed the highest correlation with protein levels (0.48), significantly improving the correlation obtained with polysomal RNA (0.41) and total RNA (0.18) (Fig. 3B).

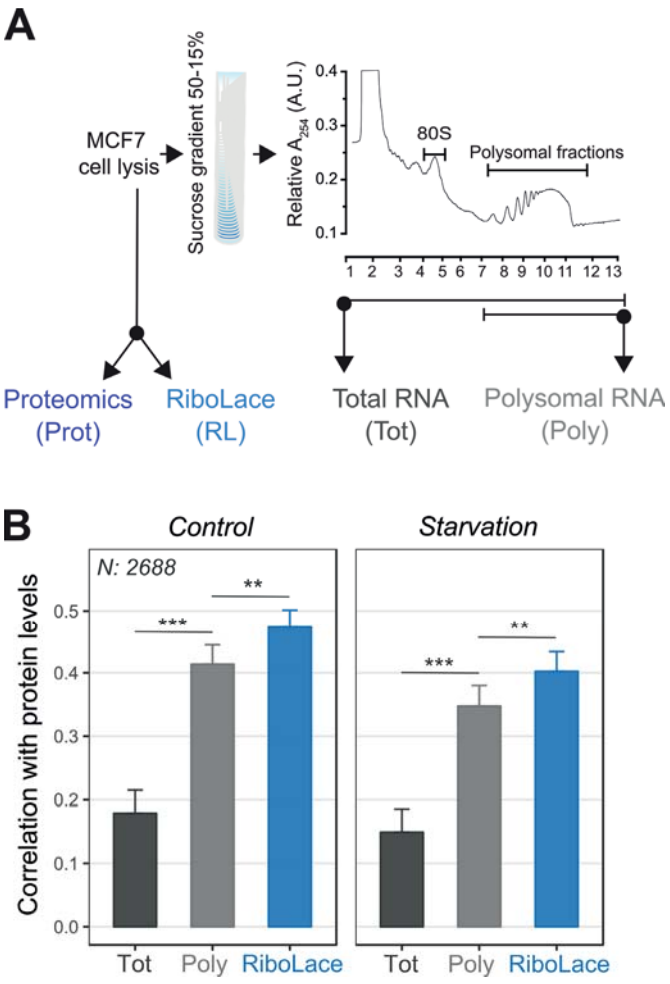


Fig. 3 (A) Experimental design for comparing the global RNA repertoire of RNAs associated with RiboLace by next-generation sequencing, total RNA sequencing (RNAseq), and polysomal sequencing (POL-seq) to the cellular proteome. (B) Correlation analysis between proteome, determined by mass spectrometry, and total RNA, polysomal RNA, and RiboLace RNA, respectively, determined by deep sequencing.

Reproducible and reliable sequencing results

To evaluate the reproducibility and reliability of sequencing results, we performed standard Ribo-seq (Ingolia, 2009) and RiboLace Ribo-seq on the same samples in duplicates (without rRNA depletion) (Fig. 4A). RiboLace reduced the rRNA contamination by about 40 %, although a slight increase of tRNA percentage was observed. Reads mapping to mRNA/ncRNA sequences accounted for about 20 % of total reads. As expected the distribution of read lengths obtained with RiboLace showed a peak at about 30 nt (Fig. 4B).

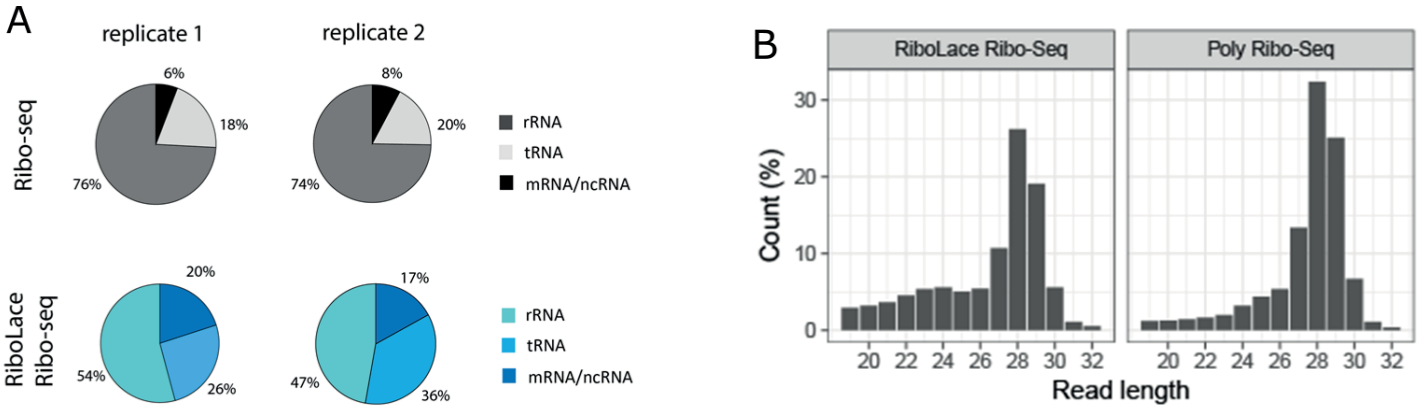


Fig. 4 (A) Pie chart representing the percentage of mapping reads on coding and non-coding RNAs obtained from standard Ribo-seq and RiboLace Ribo-seq. (B) Ribosome protected fragments (RPFs) length distribution obtained with standard Ribo-seq and RiboLace Ribo-seq.

Active Ribosome Profiling with RiboLace: high quality sequencing data

The possiblity to perform Ribo profiling with RiboLace was evaluated on different mammalian cell lines and mouse tissues. As for canonical Ribo-seq, an enrichment of signal from mapped reads along coding sequence regions was observed (Fig. 5A), demonstrating that RiboLace is indeed able to capture bona fide ribosomes. Occupancy meta-profiles, derived from the aggregation of signals on single genes, presented the typical trinucleotide periodicity of the ribosome P-site along coding sequences, which is suggestive of signal derived from ribosomes moving along transcripts (Fig. 5B and 5C).

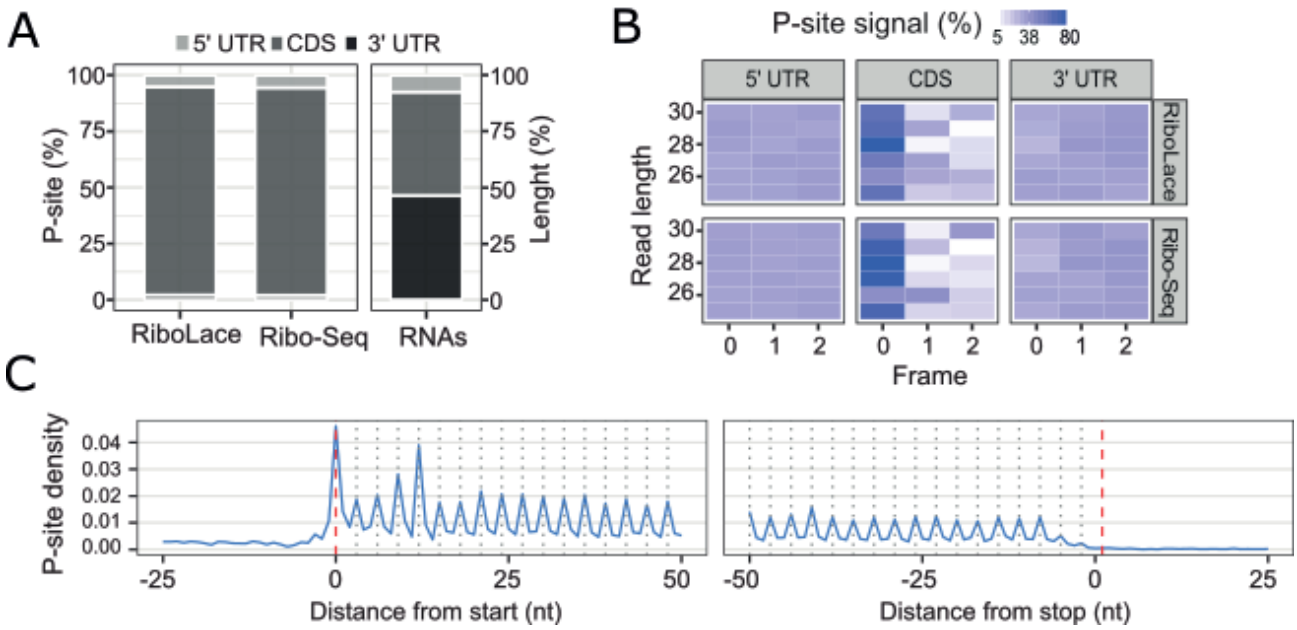


Fig. 5: (A) Percentage of P-sites mapping to the 5′ UTR, coding sequence (CDS), and 3′ UTR of mRNAs from RiboLace and Ribo-seq data. Right, length percentage of each mRNA region. (B) Percentage of P-sites corresponding to the three possible reading frames in RiboLace (top) and Ribo-seq (bottom) along the 5′ UTR, CDS, and 3′ UTR, stratified for read length. (C) Meta-gene profiles showing the density of P-sites around translation initiation sites (TISs) and translation termination sites (TTSs) for RiboLace.

Positional Analysis with RiboWaltz

IMMAGINA uses a pipeline called RiboWaltz, an R package that integrates quality controls of the ribosome profiling data, P-site identification for improved interpretation of positional information and a variety of graphical representations.
(Lauria et al., 2018)

https://github.com/LabTranslationalArchitectomics/riboWaltz

The benefits

Clamer et al., Active Ribosome Profiling with RiboLace.
Cell Rep., 2018, Oct 23;25(4):1097-1108.e5.

LOWER INPUT REQUIREMENTS: 30-40x lower than standard Riboseq methods

STRONG ENRICHMENT of translated transcripts, which are functionally relevant for biological pathways of interest

Capturing the active ribosomes is IMPROVING greatly **THE DATA** to noise ratio and **INCREASING THE CONCORDANCE** of transcriptomics data with the actual proteome

Workflow improvements significantly **REDUCING LAB TIME** and allowing for higher sample throughput

Based on beads separation: possibility to run MULTIPLE SAMPLES in parallel for automated high-throughput (HT) experiments

Product specialist
Paola Bernabò, PhD
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Ordering information

Product name	Catalog no.	No. of reactions
RiboLace Ribo-Seq - Module 1	#RL001_mod1	9
RiboLace Ribo-Seq - Module 2	#RL001_mod2	9
Tissue Lysis Buffer	#RL001-2	9 *(6 mL)*

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