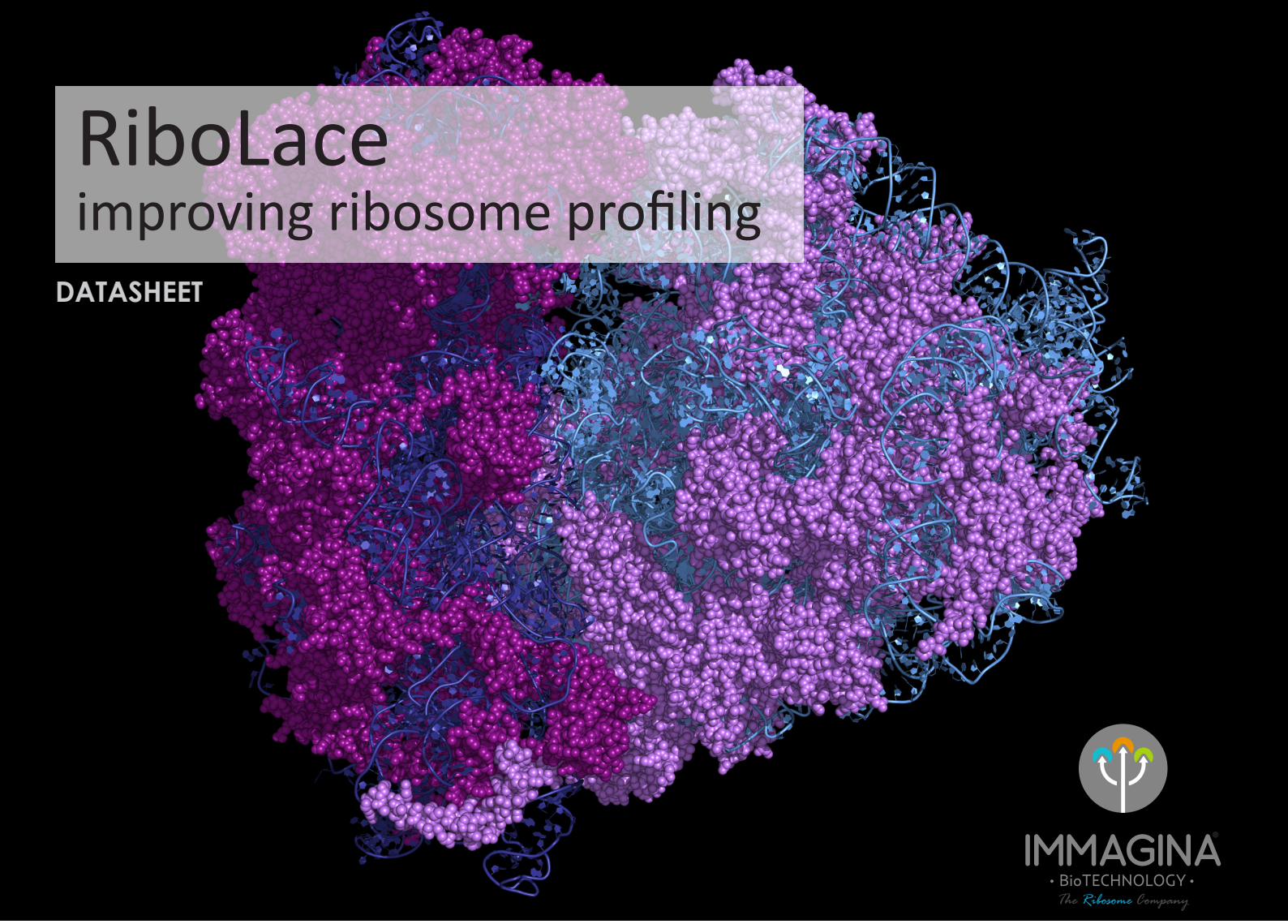
RiboLace
improving ribosome profiling

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 (>300.000 cells).

Downstream library preparation for NGS-ILLUMINA sequencing is possible with our LACEseq kit or any other protocol for small RNA sequencing.

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

RiboLace isolates RNA fragments protected by puromycin-trapped translating ribosomes by incubating endonuclease digested cell/tissue lysates with magnetic beads (Fig.1).

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

By applying dedicated computational tools (e.g. Martian pipeline from IMMAGINA), it is possible to selectively portray the position of *bona fide* active ribosomes at single nucleotide resolution.

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.

Version 3 13/11/2023

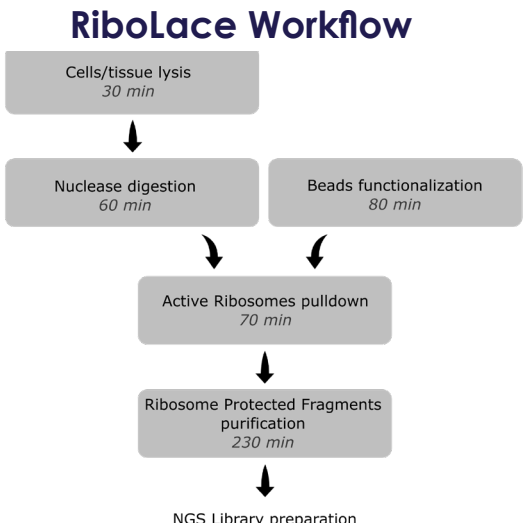


Fig. 1 The RPFs purified by RiboLace kit can be used as input for LACEseq library preparation kit or any other kits suitable for small RNAs library construction.

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

The performance of RiboLace in capturing active 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).

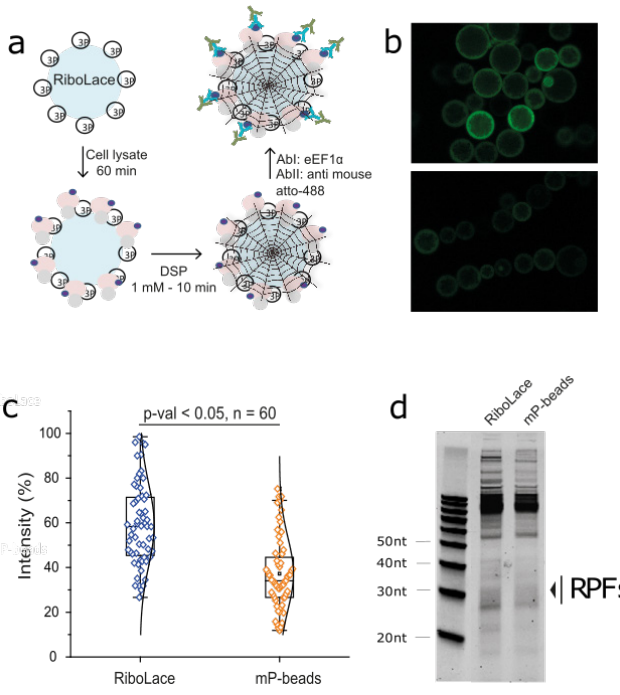


Fig. 2 A) Experimental design for assessing the performance of RiboLace in capturing ribosomes B) Fluorescence detection and quantification (C) of eEF1A on RiboLace beads compared with control (mP beads). D) Denaturing polyacrylamide TBE-urea gel of RPFs recovered with RiboLace and mP-beads RNA after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

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).

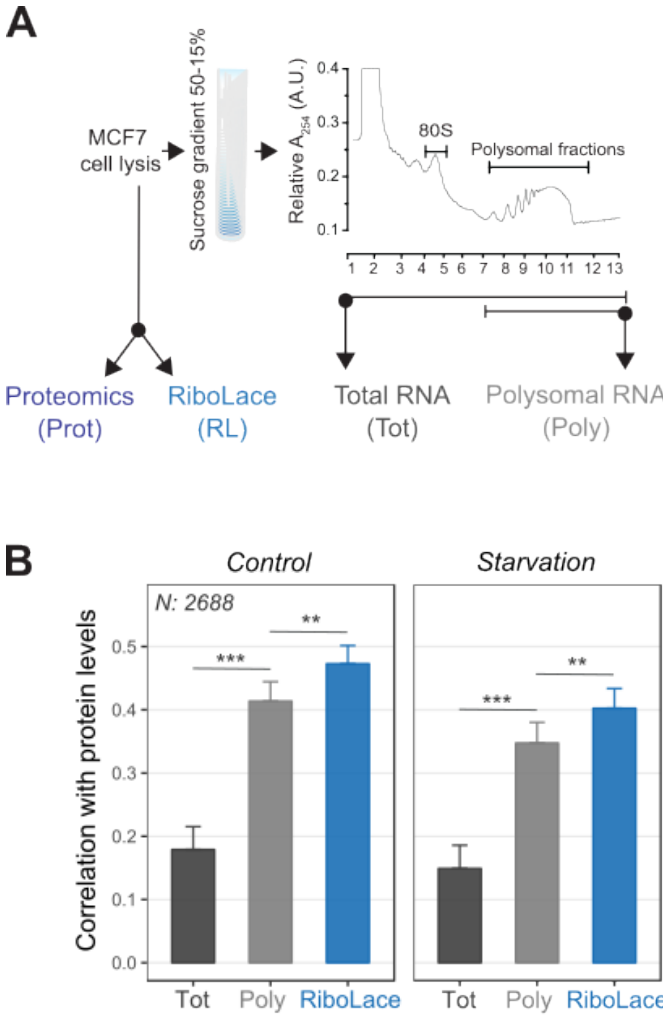


Fig. 3 (A) Experimental design for comparing the global RNA repertoire of RNAs associated with RiboLace by next-generation sequencing, total RNA sequencing (RNAseq), and polysomal sequencing (POL-seq) to the cellular proteome. (B) Correlation analysis between proteome, determined by mass spectrometry, 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 Riboseq (Ingolia, 2009) and RiboLace Riboseq on the same samples in duplicates (without rRNA depletion) (Fig. 4). In addition, downstream of RiboLace, we tested a competitor and our LaceSeq libraries to assess yield recovery. RiboLace reduced the rRNA contamination by about 50 %, while together with our LaceSeq library, this value drop by about 20%. Reads mapping to mRNA/ncRNA sequences accounted for about 70 % of total reads, providing a higher reads yield compare to both canonical Riboseq and other commercially available libraries.

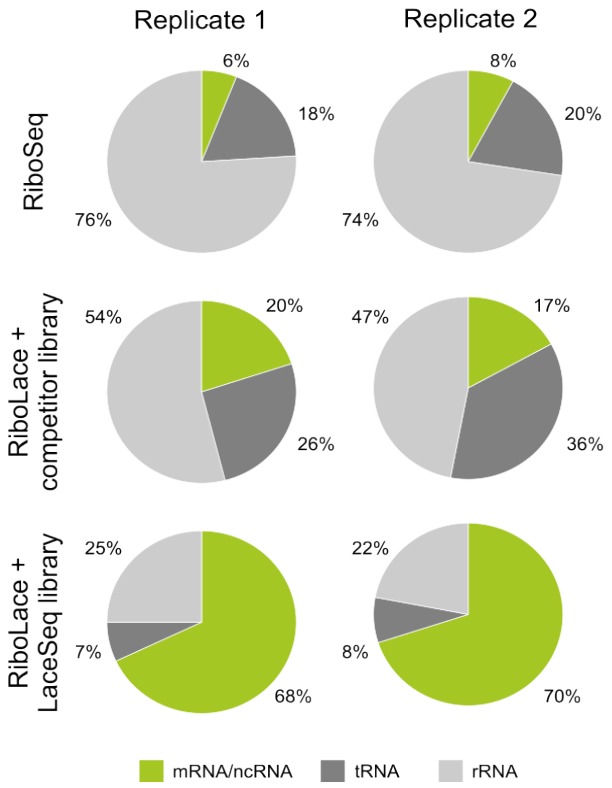


Fig. 4 Pie chart representing the percentage of mapping reads on coding and non-coding RNAs obtained from standard Riboseq, RiboLace Riboseq together with a competitor library, and RiboLace Riboseq coupled with our LaceSeq library preparation kit.

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. 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. 5). In particular, the usage of RiboLace and LaceSeq together displayed a better signal/noise ratio thanks to the higher quantity of reads mapping on coding sequences retrieved.

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

RiboSeq Analysis with Martian

Martian is a fully automated IMMAGINA newly developed bioinformatics package tool able to process data from sequencing output originated with the RiboLace and LaceSeq kits combined.
Important feature is the interactive graphical user interface, that enable the user to visualize different details from the data thanks to the integration of ribosome profiling data with other RNA features such as coverage information for each transcript and codon usage.

Martian
by IMMAGINA Biotechnology

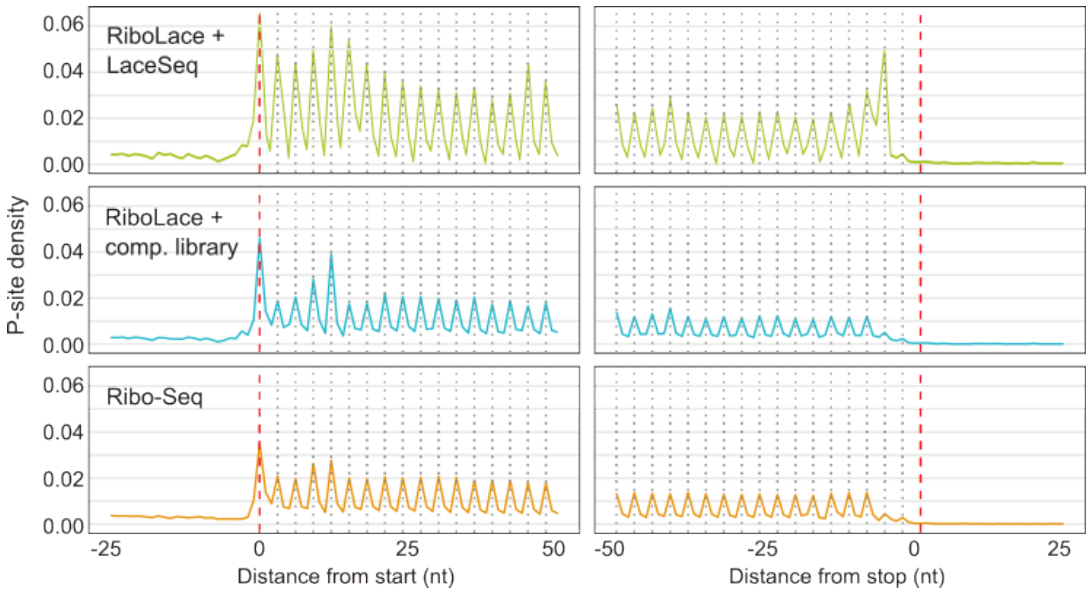


Fig. 5: Meta-gene profiles showing the density of P-sites around translation initiation sites (TISs) and translation termination sites (TTSs) for standard Riboseq, RiboLace Riboseq together with a competitor library, and RiboLace Riboseq coupled with our LaceSeq library preparation kit.

Product specialist
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Ordering information

Product	Nr of rxns	Cat. no
RiboLace Starter Kit	12 rxns	RL00S-04
RiboLace Pro	12 rxns	RL00P-12
ALL-IN-ONE RiboLace Pro	12 rxns	RS0XL-12
ALL-IN-ONE RiboLace Gel Free	12 rxns	GF001-12
RiboLace 360 Gel Free *	12 rxns	360SQ-12
PAGExt	24 rxns (12 RNA + 12 DNA extractions)	KGE00_12
LACEseq	12 rxns	LS001_12
iUDIs	12 pair unique dual indexes	UDI0Z-12

* Only available in Europe

For Research Use Only. Not for use in diagnostic procedures.

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