DATASHEET

LACEseq for ribosome profiling.

Your RIBO-seq NGS library prep

Introduction

LACEseq kit uses IMMAGINA's proprietary technology for selective sequencing of ribosome footprints produced by the action of a nuclease generating RNA fragments with a 3'-phosphorylated end.

The kit is designed for a quick and accurate library preparation and sequencing of Ribosome Protected Fragments (RPFs).

LACEseq is the only ALL INCLUSIVE solution for ribosome profiling experiments when combined with *RiboLace module 1* for active ribosome isolation and *PAGExt™* for optimal RPFs recover.

Highlights

- Selective sequencing of Ribosome protected fragments (RPFs)
- Reduced rRNA contamination;
- Reduced sequencing deep needed for optimal Ribo-seq analysis
- Quick (2-day) high quality library preparation;
- The only ALL INCLUSIVE solution when combined with RiboLace™ and PAGExt™

LACEseq is an integrated IMMAGINA solution

LACEseq can be used after ribosome purification with RiboLace™ , or after RPF extracted from whole ribosome separation with standard sucrose gradient/cushioning or column-mediated size selection.

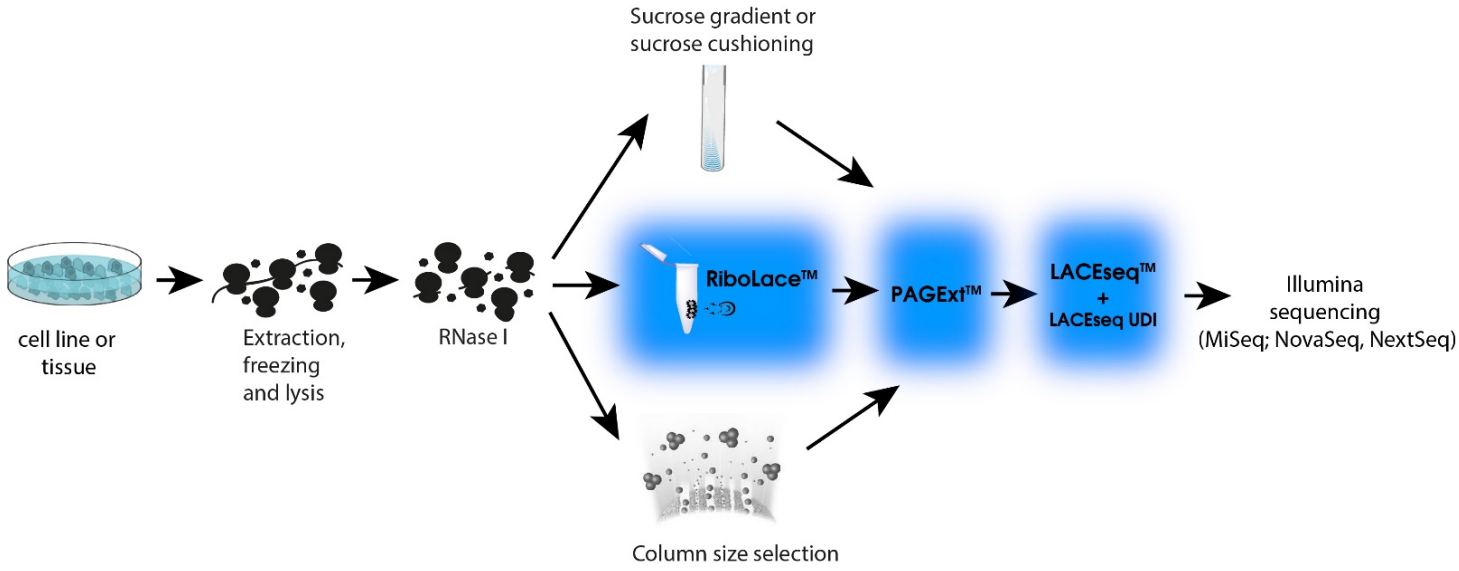

Fig. 1: Overview IMMAGINA's modules for ribosome profiling experiments

LACEseq workflow

LACEseq was developed to selectively sequence RPFs, for high quality Ribo-seq analysis. The final library is 220 nt and Illumina adapter and index sequences are incorporated during library amplification.

As illustrated in fig. 2, the complete LACEseq workflow comprises six steps: (1) RPF phosphorylation, (2) adaptor ligation, (3) circularization, (4) Reverse transcription and (5,6) two PCR steps.

Indexing primers are provided with the LACEseq UDI plate™ (cat. number #iUD-12), for unique dual index sequencing.

Following PCR amplification, purification, and validation, sequencing libraries may require size selection. Size selection can be performed using PAGExt™ kit (cat. number #KGE-002) by IMMAGINA.

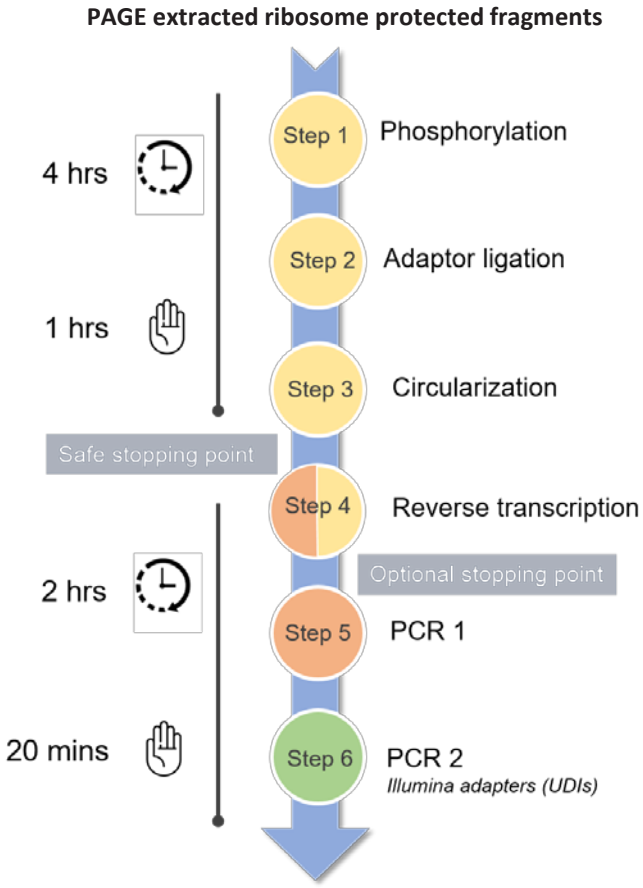

Fig. 2: schematic wokflow for LACEseq.

Active Ribosome Profiling with RiboLace: high quality sequencing data

The possiblity to perform Ribosome profiling with LACEseq was evaluated on different mammalian cell lines. As for canonical Ribo-seq obtained with *RiboLace module 2* or other methods, an enrichment of signal from mapped reads along coding sequence regions was observed for both RPF extracted by sucrose cushioning or RiboLace™ (Fig. 3 and Fig. 4), demonstrating that LACEseq is indeed able accurately seqeuce ribosome protected fragments. Occupancy meta-profiles, derived from the aggregation of signals on single genes, shows the typical trinucleotide periodicity of the ribosome P-site along coding sequences, which indicate signal from ribosomes moving along transcripts (Fig 3 and Fig 4).

RPF from sucrose cushion: sequencing output

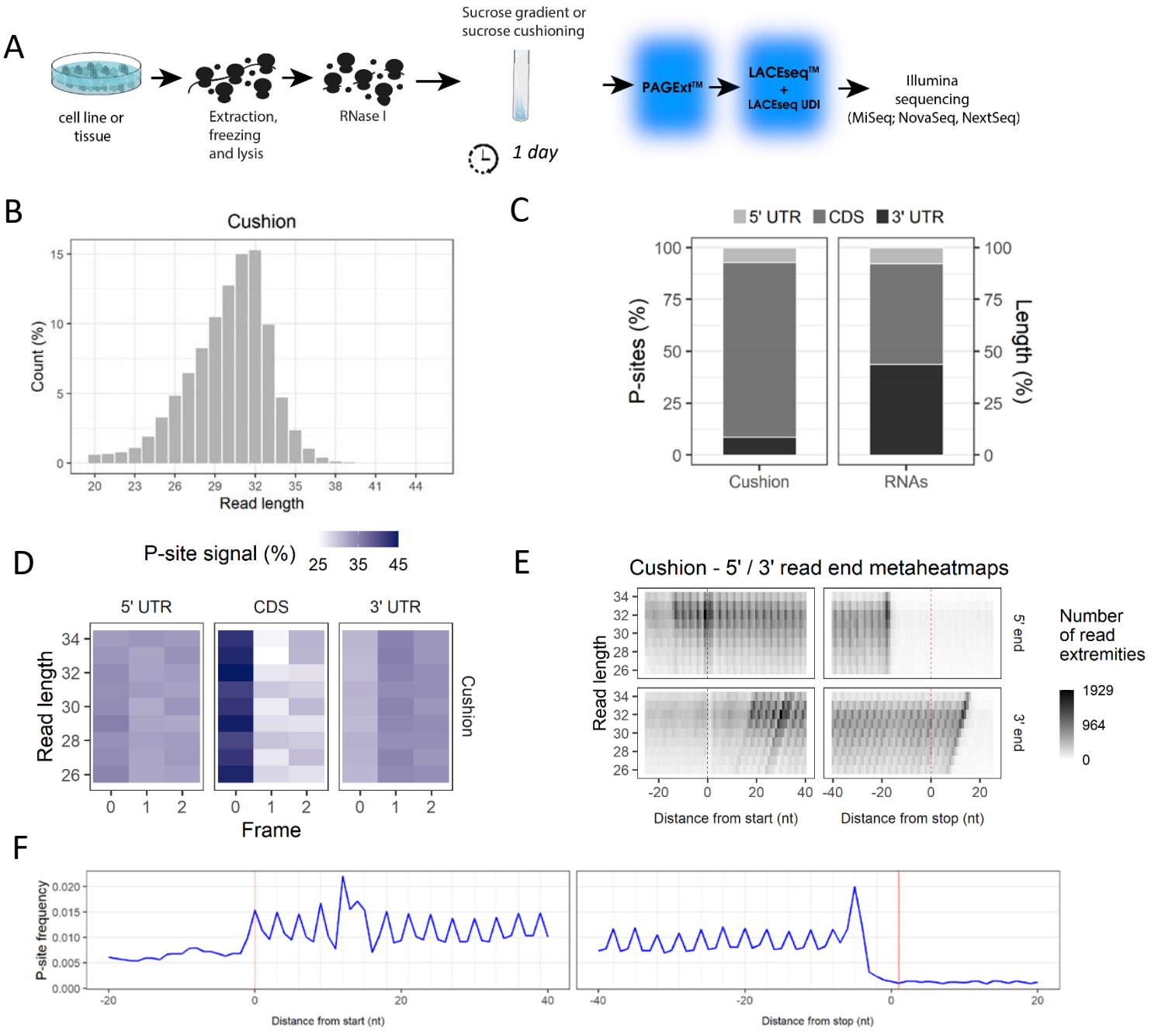

Fig. 3: (A) Overview of the ribosome profiling experiment with separation of ribosomes by cushioning or sucrose gradient. (B) RPF length distribution. (C) Percentage of P-sites mapping to the 5′ UTR, coding sequence (CDS), and 3′ UTR of mRNAs from LACE-seq. Right, length percentage of each mRNA region. (D) Percentage of P-sites corresponding to the three possible reading along the 5′ UTR, CDS, and 3′ UTR, stratified for read length. (E) Metaheatmap showing the accumulation of reads for each RPF length around the start and stop codons after P-site identification. Sequencing performed on NovaSeq6000(F) Meta-gene profiles showing the density of P-sites around translation initiation sites (TISs) and translation termination sites (TTSs) for RiboLace + LACEseq. PCR duplicates are comparable with other commercial kit for small RNA sequencing and with the data reported in Ingolia et al., 2012.

RPF from RiboLace: sequencing output

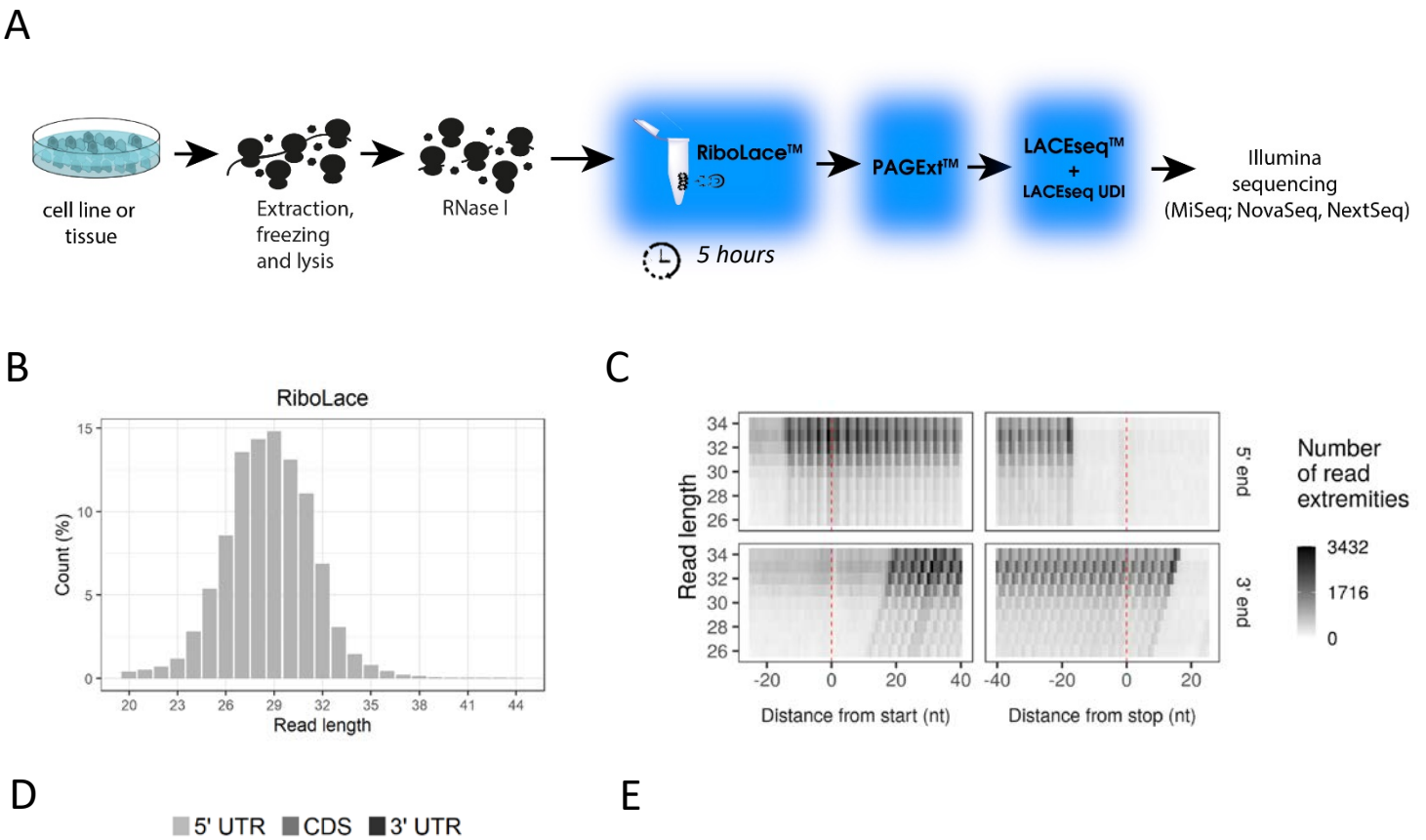

Fig. 4: (A) Overview of the ribosome profiling experiment with separation of ribosomes by RiboLace™. (B) RPF length distribution. (C). Metaheatmap showing the accumulation of reads for each RPF length around the start and stop codons after P-site identification. Sequencing performed on NovaSeq6000. (D) Percentage of P-sites mapping to the 5′ UTR, coding sequence (CDS), and 3′ UTR of mRNAs from LACE-seq. Right, length percentage of each mRNA region (E) Meta-gene profiles showing the density of P-sites around translation initiation sites (TISs) and translation termination sites (TTSs) for RiboLace + LACEseq. PCR duplicates are comparable with other commercial kit for small RNA sequencing and with the data reported in Ingolia et al., 2012.

Bioinformatic pipeline

IMMAGINA uses a pipeline called RiboWaltz *(Lauria et al., 2018). A Dedicated bioinformatic R-based pipeline is available upon registration on our website at www.immaginabiotech.com*

ALL IN ONE SOLUTION FOR YOUR RIBO-seq EXPERIMENTS WITH IMMAGINA's products

LOWER INPUT REQUIREMENTS: 20x lower number of cellst han standard Ribo-seq methods (Ingolia et al.,2012)

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

IMPROVED ACCURACY over existing methods: only active ribosomes and only real RNase I generated fragments

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

The Ribosome Company

	LACEseq™	RiboLace™ module 2
TIME	2 days	10 days
Input requirements	> 5 ng RPF	> 10 ng RPF
Specificity	3’ phosphate RNAs	3’ OH RNAs
% rRNA output	30-70	50-90
Platform	NextSeq; HiSeq, NovaSeq 6000	Hiseq 2500
Unique Dual Index	Yes, LACEseq iUDI plate	No
Million raw reads needed	20-80	70-100
Compatible products	iUDI; RiboLace; PAGExt	RiboLace; PAGExt
All-in-one solution?	Yes, no other reagents needed	No, other enzymes to be supplied

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Ordering information

Product name	Catalog no.	No. of reactions
LACEseq	#LS-001	9

Complementary products

Product name	Catalog no.	No. of reactions
RiboLace module 1	#RL001_mod1	9
PAGExt kit	#KGE-002	18
iUDIs plate LACEseq	#LS-UDI-001-16	16

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