RiboLace Gel Free kit

DATASHEET

Introduction

Ribosome profiling is an essential method to tackle questions on protein synthesis underlying many diseases and to retrieve accurate information on gene expression in clinical and preclinical projects. This method generates a snapshot of which proteins are synthesized by the cells at a certain time, and how they are produced. Traditionally, ribosome profiling experiments are limited by complex wet lab procedures and informatician analysis.

IMMAGINA makes ribosome profiling as easy as RNAseq. Our RiboLace Gel Free kit is a complete solution for an easy and fast gel-free Ribosome Profiling experiment with a dedicated bioinformatics tool.

Highlights

Short and simple workflow

- Short protocol: 2-3 days sample to sequencing
- No gel extraction required
- No specialized equipment needed
- Magnetic beads based

Requirements and output

- Low input requirement
- Only active ribosomes
- Disomes/Trisomes detection
- Better coverage with higher read density
- Determine periodicity and codon usage
- Define uORF and cryptic ORF

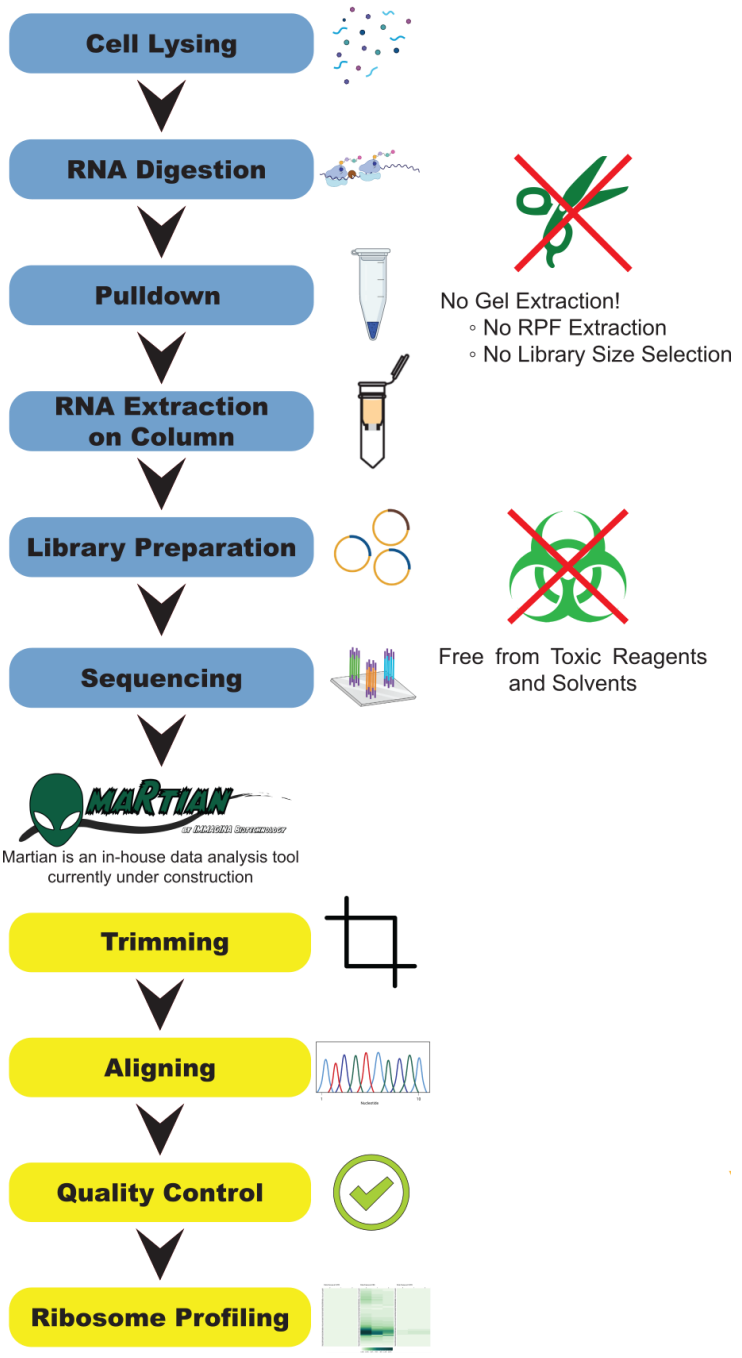


Fig.1 Workflow for RiboLace Gel-Free kit (blue) and Martian pipeline (yellow)

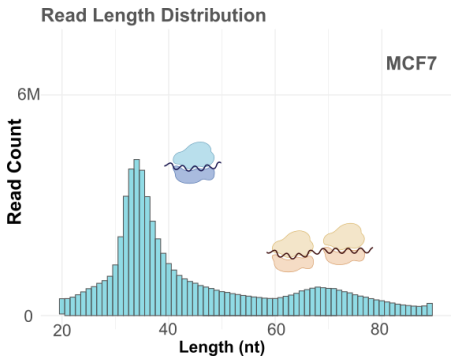
Simple, fast and efficient workflow

The RiboLace Gel Free workflow (Fig.1) is based on a simple affinity purification and magnetic separation of translating ribosomes. The Ribosome Protected Fragments (RPFs) are then extracted and inserted in a new library preparation suitable for Illumina sequencing.
The entire protocol is performed without RNA / DNA gel purification steps enabling a fast and easy workflow. This method has been validated on different cell lines and tissues (e.g., immortalized and primary cell lines, mouse brain and liver tissues).

Martian: the new powerful pipeline for RiboSeq data analysis

Martian is a fully automated IMMAGINA newly developed bioinformatics package tool able to process data from sequencing output originated with the RiboLace gel free kit.
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.

RiboLace Gel Free provides and improved snapshot of the protein translation at a transcript level



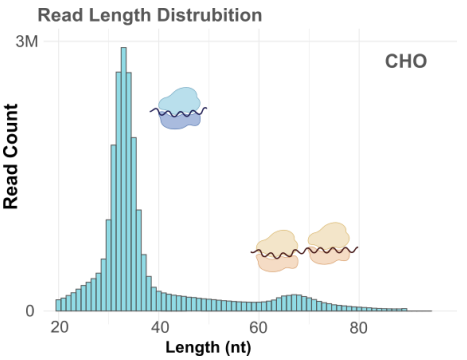
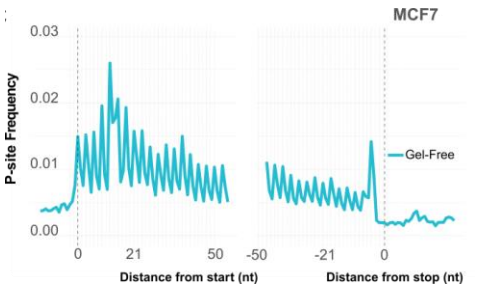


Fig.2 Read Length Distributions of RiboLace Gel-Free Riboseq analysed with Martian package, MCF7 cells, left and CHO cells, right.



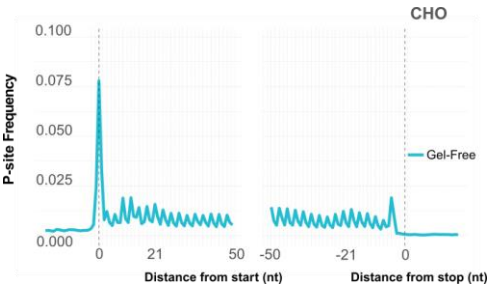


Fig.3 Meta-gene profiles showing the density of P-sites around translation initiation sites (TISs) and translation termination sites (TTSs) for RiboLace Gel-Free.

Reads belonging both from monosomes and disomes (Fig.2) were mainly present in the coding sequence (Fig.4), highlighting the efficacy of the kit in pulling down actively translating ribosomes. The occupancy meta-profiles (Fig.3) show the typical trinucleotide periodicity of the ribosome P-site along coding sequences, which indicate signal from ribosomes moving along transcripts.
Compared to the classical RiboSeq method (Ingolia et. al, 2012 Fig.5), the workflow takes fewer days (2-3 days) thanks to the removal of gel extraction of RPFs, couple with an improved library preparation.

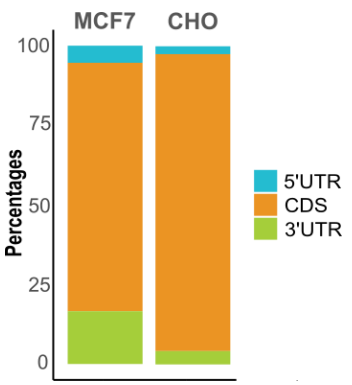


Fig.4 Percentage of P-sites mapping to the 5' UTR, coding sequence (CDS), and 3' UTR of mRNAs.

	RiboSeq Ingolia et.al., 2012	RiboLace Gel Free
	Gel-Cut	**Gel-Free**
Input Requirements (Immortalized Cancer Cells)	High (>3M cells)	Low (>0.3M cells)
Accuracy	All Ribosomes	Active in Translation
Workflow Time	10 Days	2-3 Days
Gel Extraction	Yes	No
RNA Extraction Method	Phenol Chloroform	Column Based

Fig.5 Comparison with current RiboSeq method

Ordering information

Product name	Catalog no.	No. of reactions
RiboLace Gel Free	#GF001	12

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

Complementary products

Product name	Catalog no.	Volume
Tissue lysis buffer	#RL001-2	15 mL

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