

# Ribosome Profiling

**Commonly used acronym:** Ribo-seq

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## Organisation

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**Geographical Area** Flemish Region

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Animal health, Human health
<b>The Method is situated in</b>	Translational - Applied Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Specify the type of cells/tissues/organs</b>	This method can be adapted to any cell type and tissue

## DESCRIPTION

### Method keywords

Translational

Ribo-seq

Translatomics

illumina

ORF-delineation

ribosome profiling footprints  
Gene expression  
translatome

### **Scientific area keywords**

cancer research  
Translational Regulation  
human diseases  
Peptide Folding

### **Method description**

These are the general guidelines on sample input requirements to successfully start our RIBO-seq procedures:

Cell lines in suspension: pellet of 10 – 50 M snap frozen cells. The pre-treatment and collection specifications are also mentioned in our pre-processing protocol.

Adherent cell lines: lysate with minimum concentration of 50 ng/ l (fluorescence measurement: Qubit HS RNA or Ribogreen).

Hard / Soft tissue types: input amount has to be discussed.

Workflow: \*RNase I digestion, sucrose gradient fractionation\*, and RNA isolation, as per Supplier's ribosome profiling protocol \*Depletion of rRNA species\*\* (i.e., ribodepletion) \*Preparation of ribosome profiling libraries (strand-specific) \*Quality control\*\*\* (QC) of libraries (e.g., Bioanalyzer analysis or similar, small sequencing run) \*Deep sequencing of libraries To measure translation efficiency, normalized ribosomal footprint read counts must be normalized for gene expression. Therefore, total RNA-seq is to be performed. Specifically, CGTC requires: #Total RNA extraction from the same material as that used for ribosome profiling #Ribodepletion #Library preparation for strand-specific total RNA-seq #Quality control (QC) of libraries (e.g., Bioanalyzer analysis, small sequencing run) #Deep sequencing of libraries

### **Lab equipment**

iSeq100 Illumina PCR machines Thermomixer

### **Method status**

History of use  
Internally validated

Published in peer reviewed journal

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

- Ribosome profiling is highly beneficial as NGS alternative to or complementary to MS-based protein and peptide identification and will develop into a common practice for next-generation proteomics.
- With ribosome profiling the translational control is investigated and the gene expression is measured at the translational level.
- It allows to determine the rate of protein synthesis over a large dynamic range.

### **Challenges**

- The price is high.
- It is an elaborate and sensitive protocol that requires qualified personnel to perform.
- Limiting factor: High concentration/quantity of material is needed to produce good/ reliable results.

### **Modifications**

The method can be modified to study Microbiome communities (MetaRibo-Seq) which is a very promising area of research related to human health.

### **Future & Other applications**

Applications:

- Identification of translated sequences within the complex transcriptome,
- Mapping sites of translation initiation (TIS),
- Measurement of differential gene expression at the level of mRNA translation,
- Identification of novel protein coding genes and ribosome pausing.

## **REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION**

### **References**

Verbruggen, S., Ndah, E., Van Criekinge, W., Gessulat, S., Kuster, B., Wilhelm, M., ... & Menschaert, G. (2019). PROTEOFORMER 2.0: further developments in the ribosome

profiling-assisted proteogenomic hunt for new proteoforms. *Molecular & Cellular Proteomics*, 18(8), S126-S140.

Van Damme, P., Gawron, D., Van Crielinge, W., & Menschaert, G. (2014). N-terminal proteomics and ribosome profiling provide a comprehensive view of the alternative translation initiation landscape in mice and men. *Molecular & Cellular Proteomics*, 13(5), 1245-1261.

### **Associated documents**

[PIIS1535947620331005.pdf](#)

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