Ribosome Profiling

Commonly used acronym: *Ribo-seq*
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SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Translational - Applied Research
Type of method	In vitro - Ex vivo
This method makes use of	Human derived cells / tissues / organs
Specify the type of cells/tissues/organs	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 Criekinge, 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

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