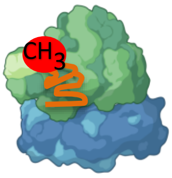
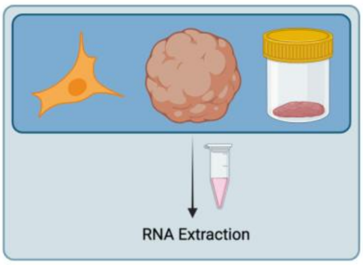
RibosOMICS platform – Dec 2023
Technical sheet – RiboMethSeq V3

RiboMethSeq



Objective: Analysis of 2’O-ribose methylation of ribosomal RNA used for (1) biomarker or (2) molecular mechanism involved in translational regulation.

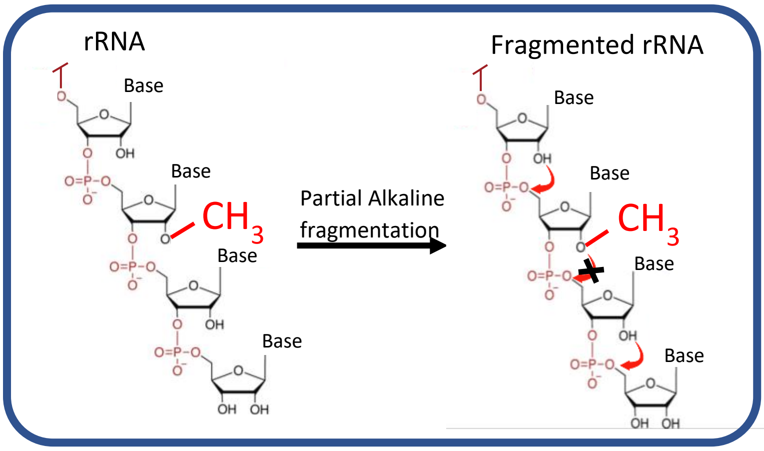
Sample

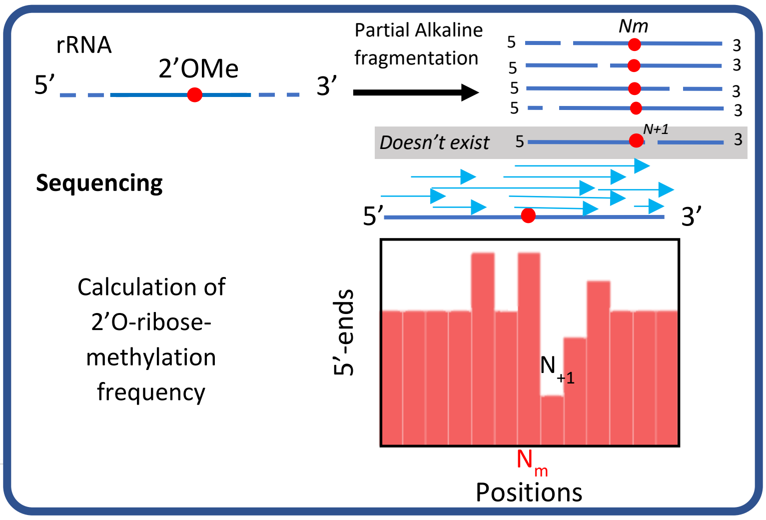


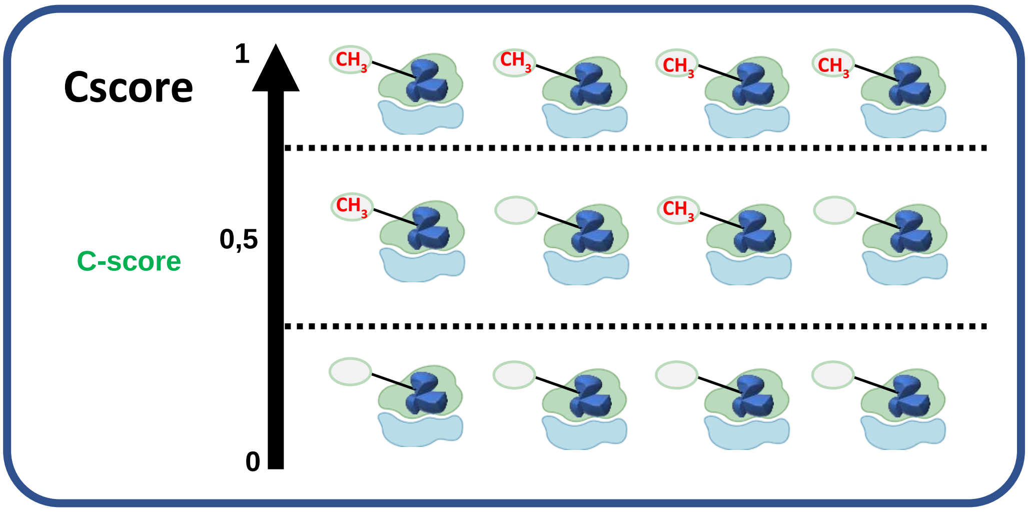
- **To provide:** 200 ng RNA (Trizol, kit Qiagen, Zymo, Macherey…) in 10 µL (20ng/µL)
- **Run organisation:** design for Illumina NovaSeq sequencer
  1 librarie = 23 samples + 1 reference RNA (normalization)

  1 run = 2 libraries

Principle: Approach based on RNAseq to quantify the level of 2’Ome at the known rRNA sites. Presence of 2’O-ribose methylation (2’OMe) protects the phosphodiester bond located at the 3’ of the 2’O-methylated nucleotide from alkaline hydrolysis (Fig.1). Thus, the presence of 2’OMe at the given nucleotide *n* induces under-representation of RNA fragments starting at the nucleotide *n+1* and ending at position *n* allowing to calculate a 2’OMe level at the corresponding nucleotide position (Fig.2), or C-score varying from 0 to 1 (Fig.3).







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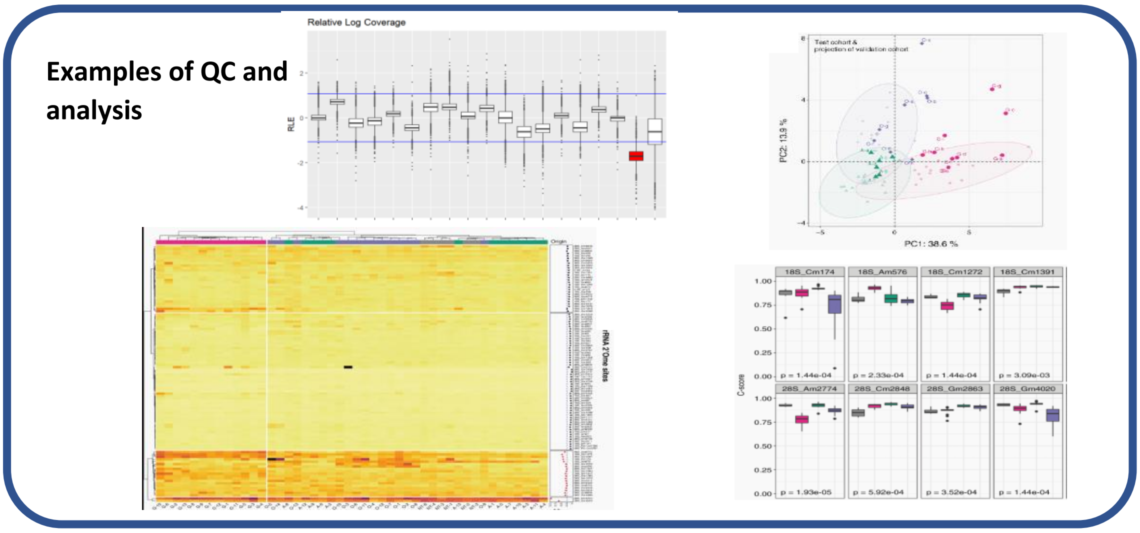
Analysis: Analyses are standardized thanks to our in-house bioinformatic tools (github/RibosomeCRCL/ribomethseq-nf and rRMSAnalyzer). Three steps are required:

- Alignment and counting: perform by PGT platform using ribomethseq-nf
- QC: performed by RibosOMICS using rRMSAnalyzer
- Analysis: can be performed either by the demander or RibosOMICS using rRMSAnalyzer. It includes unsupervised and differential analyses


Examples of QC and analysis

Reporting: Several items can be provided at different time points of the projects:

- **Raw data**: are accessible upon request: fastQ, counts
- **Cscore**: for each sample will be transmitted as a *.csv file
- **QC report:** includes explanation of the different representations and the interpretation of the results to identify putative batch effect or outlier sample as .html file
- **Analytic report**: includes analyses of rRNA 2’Ome profiles (i.e., unsupervised methods) and comparison of rRNA 2’Ome level at each site (i.e., mean comparison) as .html files.