Product data sheet

RNA Sequencing

Product Overview

Traditional RNA sequencing allows gene expression analysis of either the coding regions (mRNAseq), the whole transcriptome (total RNAseq) including non-coding RNAs, or small RNAs (small RNAseq) in total RNA extracted from whole tissue or isolated cells/nuclei.

The mRNAseq workflow begins with an enrichment of polyA-tailed. Either polyA enriched RNA or **total RNA** are then fragmented followed by a double-stranded cDNA synthesis using a mixture of random and oligo(dT) priming. Libraries are generated by ligating unique dual index (UDI) adaptors to the RNA fragments followed by strand selection, a ribosomal depletion step (optional for mRNAsq) and PCR amplification.

The small RNAseq workflow begins with 3' and 5' adapter ligation to the RNA (either total RNA or isolated small RNA). Following cDNA synthesis, libraries are generated.

Sequencing is performed on the NovaSeq X Plus platform (Illumina) with tailored sequencing settings based on the project design and the user's needs.

What is Included

- RNA QC
- Library preparation
- Quality control of library
- Library pooling and sequencing on Illumina NovaSeq X Plus
- FASTQ files
- Standard data processing, QC and basic bioinformatics analyzes (optional).

Input and quality requirements

RNA extraction

Extraction of RNA is the responsibility of the user. During the extraction protocol, it is important to include a DNase treatment to prevent genomic DNA contamination. If performing small RNAseq, ensure to capture small RNAs during the extraction. Contact SCOP to consult.

Quality

The quality of the RNA will reflect the downstream process – poor quality may lead to compromised data.

- RIN> 7 (strongly recommended).

Sample drop-off

Extracted RNA for both QC and libraries should be delivered at a specific concentration which depends on the assay type.
In general, SCOP recommends:

- mRNAseq: ≥5ng/µl
- total RNAseq: ≥25 ng/µl
- small RNAseq: ≥20 ng/µl

Contact SCOP for more information.

Data Deliverables

The data will be processed with the NF-core pipeline. The data will be transferred to the project folder including:

- FASTQ files
- Alignment and read-counts (optional)
- Extensive QC report (optional)
- Differential expression analysis (optional)
- Data upload to repository prior publication.