

## RNA-seq Service Guidelines

RNA should be isolated in a high-quality manner. Qiagen portfolio kits for RNA extraction and purification are recommended.

Required quantities:

- **mRNA-seq services:** minimum 10 ng of total RNA.
- **Long non-coding RNA and whole transcriptome sequencing services (Total RNA-seq):** minimum 10 ng of total RNA (Human, Mouse, Rat).

### RNA-seq Service Workflow

Three biological replicates are recommended.

#### 1. RNA quality control

Measurement of concentration as well as determination of RIN value (RNA integrity number).

#### 2. Library preparation

- Poly-A selection for mRNA-seq or rRNA depletion for total RNA-seq (with lncRNA)
- Stranded library preparation technology
- Final QC prior to sequencing (quantification and fragment size)

#### 3. Sequencing

- Paired-End 50 bp. Please contact us should you have different requirements.

**mRNA-seq:** 25-30 million raw reads/sample.

**Total RNA-seq:** 45-50 million raw reads/sample.

#### 4. Bioinformatics analysis

Alignment to reference genome and quantification of known genes and/or transcripts is included.

Provided files:

- Report with sequencing statistics
- Raw data in FASTQ format
- FastQC report
- Alignment in BAM format

- Un-normalized counts matrix
- DESeq2 normalized counts matrix

These files will be available for download for 1 month and stored for 12 months after project completion. Please request additional long-term storage if needed.

## 5. Additional bioinformatics analysis on request

- **Differential expression:** differential expression testing between samples for genes and/or transcripts.
- **Gene ontology terms analysis:** analysis of differentially expressed gene sets. Gene Ontology terms that are overrepresented in differentially expressed genes may indicate the underlying biological processes involved.
- **Pathway analysis:** identification of biochemical pathways in which genes associated with differential expression may be overrepresented.

## 6. Additional information

For sample preparation and sample shipment it is mandatory to follow Diagenode's guidelines. If customer samples do not meet Diagenode's quality requirements, any additional QC of new samples will be charged to the customer. Any delay in sample shipment to Diagenode's facilities might result in delaying customer's project.

Generated files will be available for download for 1 month and stored for an additional period of 3 months on Diagenode's servers. Additional long-term storage of data is available upon request. This offer includes a one-hour call to walk you through the results if needed.

Original samples are stored at Diagenode during 4 months after project completion but will be discarded once this time is exceeded. Return shipment of samples is available upon request.

Any additional service which is beyond the current project scope will be charged to the customer.