Alithea Genomics

MERCURIUS

High Throughput Transcriptomics service

Sample submission guideline

v.0.1.220404

Please read this guideline and contact us at **info@alitheagenomics.com** if you have any questions.

Sample submission protocol at glance

1. Transfer the RNA samples in the 96 well plate following the instructions provided below
2. Fill in the Sample Submission Form and send it to **info@alitheagenomics.com**
3. Ship the samples on dry ice and forward to us the shipment tracking number

Requirements for the samples

To ensure high quality and fast turnaround of our service it is important that we receive purified total RNA samples in a 96- well plate with concentrations as uniform as possible.

Please pay attention to the following requirements and let us know if any of those does not comply with your submission.

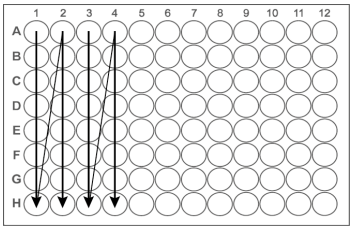
RNA sample requirements

- Volume per well: ≥10 µL
- RIN value: ≥6
- 260/230 value: >1.5
- Required concentration: 50-100 ng/µL
- Uniform concentration across samples (e.g. 50 or 100 ng/µl for all samples).

NOTE: variation in concentration across samples will translate to variation in the number of sequencing reads across the same samples.

96 well plate requirements

- **Label 96-well plates** with the date and user name or project ID **on the short side (e.g., YYYY-MM-DD Name)**
- Layout of samples in 96 well plate:



Important considerations for RNA samples preparation

Samples quality check

The success of the library preparation and quality of the sequencing data largely depends on the quality of the RNA samples.

To ensure the best outcome the quality of at least some (10% recommended) RNA samples from every extraction batch should be assessed as following:

- **RNA purity.** Residuals of phenol or guanidine in RNA samples are considerably decreasing the yield of cDNA after reverse transcription. The level of impurity should be estimated by taking 260/230 ratio with **Nanodrop** spectrophotometer (ThermoFisher) or alike and provided in the **Sample Submission Form.** The expected values are >1.5.
- **RNA integrity.** The integrity should be assessed by capillary electrophoresis system (i.e. Fragment Analyzer, Bioanalyzer or Tapestation, Agilent). The RNA integrity is quantified with RIN (RNA integrity number) or **DV200** values and should be provided in the **Sample Submission Form**.

Samples quantification

RNA concentrations should be assessed using the one of the following methods. It is recommended to re-measure the concentration of highly concentrated samples after dilution.

- The **dye-based** RNA quantification assays (e.g., Qubit™ RNA (#Q33224, ThermoFisher); Quant-it™ RiboGreen (#R11490, ThermoFisher) or similar) ensure the most accurate measurement of RNA concentration, specifically for low concentrated samples (<50 ng/µL)
- **Nanodrop** instrument can be used for quantification, but it is prone to error when low-concentration samples (below 50 ng/µL) are measured.
- The concentrations and the method used should be provided in the **Sample Submission Form**.

Batch-effect and sample replicates

- RNA extraction protocol can produce considerable technical variation across the samples that might become evident in the RNA-seq data. This is why it's recommended to preform RNA extraction in a single batch following the same protocol by produced by the same person. If RNA for a project is isolated in several batches the samples should be randomized between the isolation batches.
- All the RNA samples intended to be used for the differential expression (DE) analysis should be included in the same library, up to 96 samples. Due to batch effects, it might be difficult to perform DE analysis across samples from different libraries.
- Including at least 3 (or more) biological replicates is strongly recommended as it makes the analysis more reliable.