ALITHEA GENOMICS

MERCURIUS™

Multiplexed RNA-seq (mRNA) Service

Sample Submission Guidelines

January 2025

Sample submission guidelines at a glance

1. Transfer the RNA samples to a 96-well PCR plate following the instructions below and store it at -80°C before shipment.
2. Fill in the Sample Submission Form **(SSF)** and **check all the boxes** in the Sample submission checklist below; send both files to **orders@alitheagenomics.com**.

   Please be aware that any inconsistency may lead to delays or additional fees.
3. Ship the samples on dry ice and send us the shipment tracking number.

Sample submission checklist

- ☐ **Sample Submission Form (SSF)** is filled properly with a **unique sample ID** Consider adding a suffix for technical replicates (e.g., XX_rep1, XX_rep2, etc.). Make sure that information about **all the samples shipped is provided in the SSF**.
- ☐ The sample volume should be **20 µL** per well **and identical for all samples** (!).
- ☐ The concentration of all samples is uniform, and the A260/230 ratio is assessed and provided in the SSF.
- ☐ The **minimum number** of samples in each group (to be pooled together) is **16**.
- ☐ Samples are provided in the 96-well RNase/DNase-free PCR plate. **Samples in tubes cannot be processed.**
- ☐ Plates with samples are clearly labeled with the same Plate ID as in the sample submission form.
- ☐ Plates are well sealed with adhesive and temperature-resistant seal (aluminum is ideal).

Essential considerations for input material

Samples quantity and integrity

- The tested range of total RNA amount is 10 – 1000 ng (per well).
- The recommended amount is 500 ng of total RNA per well.
- The volume should be 20µL/sample and identical across all samples.
- The recommended RIN number is > 7

Samples purity

- RNA samples, extracted with TRIzol, phenol, chloroform, or guanidine are prone to residual contamination with organic solvents that considerably decrease cDNA yield. Make sure to follow the washing steps of the used protocol.
- To ensure the high purity of RNA, assess the 260/230 ratio for all samples.
- Samples should be free of salts and DNA.
- The 260/230 ratio values should be between 1.8 and 2.2.

Samples uniformity

- To ensure an even distribution of reads after sequencing, the RNA amount, integrity, and 260/230 values of the starting RNA samples must be as uniform as possible, with a max 10% variation.
- To obtain such uniform amounts, we recommend the following:
    - Use dye-based methods for RNA quantification (e.g., Qubit Quant-iT or RiboGreen).
    - Dilute samples to obtain the same RNA concentration in all wells (±10%).
    - Re-measure the RNA concentration of all samples to confirm uniform concentration.
    - Ensure the 260/230 ratio is between 1.8 and 2.2.

Batch-effect and sample replicates

- RNA extraction protocol can produce considerable technical variation across the samples; therefore, performing RNA extraction in a single batch is strongly recommended.
- If the differential expression (DE) analysis is planned, respective RNA samples should be included in the same library. Comparison of samples from different libraries can be biased.
- Including at least 3 (or more) biological replicates is highly recommended for the reliability of the experimental setup.

Samples preparation

1. Label a new 96-well RNase/DNase-free PCR plate.
2. Pipette the RNA samples to the new 96-well PCR plate according to the filled Sample Submission Form. Follow the column-based direction (column 1, then column 2, etc.).
3. Seal the 96-well PCR plate with an aluminum seal and briefly spin it down.
4. Store the samples at -80°C before shipment.

Alithea Genomics SA

Phone +41 78 830 31 39
Email info@alitheagenomics.com
Web www.alitheagenomics.com

Address
Route de la Corniche, 8
1066 Épalinges
VD, Switzerland