ALITHEA GENOMICS

MERCURIUS™

FFPE-seq Service

Sample Submission Guidelines

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Sample submission guidelines at a glance

1. Transfer the RNA samples to a 96- or 384-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 minimum sample volume is **10 µL** per well.
- ☐ 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- or 384-well RNase/DNase-free PCR plates. **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 100 – 1000 ng (per well).
- The minimum volume should be 10 µL.
- The accepted RIN values are 1-10, but it is crucial that all samples have a similar range of RIN (i.e., 2 - 7 or >7).

Samples purity

- RNA samples containing traces of formalin or paraffin will result in lower cDNA yield. Make sure to follow the washing steps of the protocol used.
- To ensure the high purity of RNA, assess the 260/230 ratio for all samples.
- 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 >1.5.

Batch-effect and sample replicates

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

Samples preparation

1. Label a new 96- or 384-well RNase/DNase-free PCR plate.
2. Pipette the RNA samples to the new 96- or 384-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- or 384-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