



MERCURIUS™

Targeted BRB-seq Service

Project and Sample Submission Guidelines

Phase I - Project submission guidelines at a glance

1. Please complete the **Gene Panel Submission Form (GPSF)** with the required information about genes of interest and send it to orders@alitheagenomics.com.
2. Ship representative purified RNA samples (one per different condition, in quantity 2-5 µg/sample) to Alithea to validate the custom detection panel.

Phase II - 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 **20 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).

Requirements for gene panel development and validation (Phase I)

1. The total gene number should be in the range of 5-100 genes per panel.
2. The minimum representative number of RNA samples for gene panel validation (i.e., for control and three types of treatment, four RNA samples must be sent—one from each group).
3. The RNA amount should be 2-5 µg/sample, with a minimum RIN number > 6.

Essential considerations for input material (Phase I and II)

Samples quantity and integrity

- The tested range of total RNA amount is 200 – 1000 ng (per well).
- The minimum volume should be 20 µL.
- The recommended RIN number is > 6.

Samples purity

- RNA samples extracted with TRIzol, phenol, chloroform, or guanidine are prone to residual contamination with organic solvents, considerably decreasing cDNA yield.
- Assess the 260/230 ratio for all samples (it should be >1.5) to ensure the high purity of RNA.

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 maximum variation 10%.
- 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 and the RIN value is > 6 across the samples.

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.
- For the experimental setup's reliability, it is highly recommended that at least 3 (or more) biological replicates be included.

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.

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