



MERCURIUS™

# High sensitivity low-input BRB-seq Service

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## 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 **15 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 pg – 500 ng (per well).
- The minimum volume should be 15 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 that considerably decrease cDNA yield.
- To ensure the high purity of RNA, assess the 260/230 ratio for all (or at least for 10% of samples) from the same RNA purification batch.
- The 260/230 ratio values should be >1.5.

### 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 ( $\pm 10\%$ ).
  - Re-measure the RNA concentration of all samples to confirm uniform concentration.
  - Ensure that the 260/230 ratio is >1.5 and the RIN value is > 6 across the samples.

### 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- 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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