ALITHEA GENOMICS

MERCURIUS™

Full-Length Plant BRB-seq Service

Sample Submission Guidelines

November 2025

Full-Length Plant BRB-seq for 96-well Plate Format

Sample submission guidelines at a glance

1. Transfer the RNA samples to a 96-well PCR plate following the instructions below and store them 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. Request the **shipping address** from your sales specialist.
4. Ship the samples on dry ice, ensuring the plates are placed between layers of dry ice to maintain a consistent freezing temperature throughout transit. Please provide us with the shipment tracking number.

Sample submission checklist

- ☐ The Sample Submission Form (SSF) must be filled out correctly with a unique sample ID. Consider adding a suffix for technical replicates (e.g., XX_rep1, XX_rep2, etc.). Ensure that the SSF provides information about all the shipped samples.
- ☐ Avoid having randomly distributed samples across the plate layout (e.g., in A01, A06, B03, G10-12, etc.).
- ☐ 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 are labeled with the same Plate ID as indicated in the SSF.
- ☐ Plates are well sealed with an adhesive and a 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 20 µL/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, the 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 into 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.

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