GENOMICS

MERCURIUS™

# Total Blood BRB-seq Service

## Sample Submission Guidelines

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# Total Blood 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

- ☐ Total Blood BRB-seq service is optimized for efficient rRNA and globin transcripts (HBB, HBA1, and HBA2) depletion in **human blood** RNA samples. The used mix also targets embryonic and fetal globin transcripts. For more details and to discuss compatibility with other species, please contact us at info@alitheagenomics.com.
- ☐ The Sample Submission Form (SSF) must be completed correctly, including 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 **15 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 per group (to be pooled together in the same library) 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.

# Essential considerations for input material

## Sample quantity and integrity

- The tested range of total RNA amount is 100 pg – 100 ng (per well).
- The recommended amount is  a concentration between 2-25 ng/ µL of total RNA per well.
- The volume should be 15 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.
- 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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