ALITHEA GENOMICS

Technical Note

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Assessing BRB-seq library prep kit performance with inline control RNA sample

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Introduction

This note describes the use of Control Human RNA (Ctrl_RNA) provided in the BRB-seq kit to assess the kit performance. Here we demonstrate the expected cDNA and library yield after using the various amount of input control RNA.

The tube provided in the kit contains 5 µg of human total RNA from HEK293T cells. It's recommended to use 1 µg of RNA per RT reaction in 5 wells and pool them after the RT step following the detailed protocol in the MERCURIUS Kit User Guide (v.0.2.1).

cDNA preparation

The RT was performed in 20 µL per well using 1-5 µg of total RNA. Each sample was either directly purified using DNA Clean and concentrator kit (Zymo, D4014) or wells were pooled (RT from 5 wells with 1 µg of RNA each) before column purification. After elution each sample was treated with Exo and subjected to the second strand synthesis. Then samples were purified using AMPure beads (0.6x ratio, Beckman Coulter CNGS-0050) and eluted in 20 µL with water. cDNA was quantified using Qubit and profiled with a Fragment Analyzer (Agilent, model 5200). **Figure 1Error! Reference source not found.** shows the estimation of cDNA yield and **Error! Reference source not found.Figure 2** the expected cDNA profile.

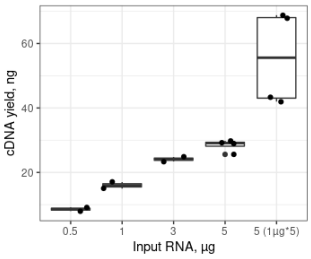

Figure 1 | Expected cDNA yield from total human RNA used in a single RT reaction (0.5-5 µg/reaction) or 5 RT reactions with 1 µg/well (n= 4).

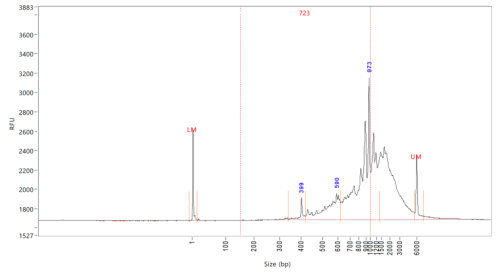

Figure 2 | Expected cDNA profile.

Library preparation

Various amounts (5-20 ng) of cDNA prepared from Control RNA were used for the library preparation following the tagmentation, purification with AMPure beads (0.6x), PCR amplification and two rounds of purification with AMPure beads (0.6x). The amount of cDNA for tagmentation can vary, and we recommend keeping some cDNA for a backup. Typically, 10 ng of cDNA is sufficient to obtain 20-40 ng of DNA library after 13-14 cycles of amplification. **Table 1** shows the approximate amount of amplification cycles and expected library yield and the **Figure 3** shows the fragment size distribution.

The final yield of the library and the profile may vary depending on RNA quality and species of origin as well as pipetting manipulations during purification steps.

Table 1 | Expected yield of BRB-seq libraries from different amount of tagmented cDNA.

Tagmented cDNA, ng	# PCR cycles	Expected Library yield, ng
5	14-15	20-40
10	13-14	
20	11-12	

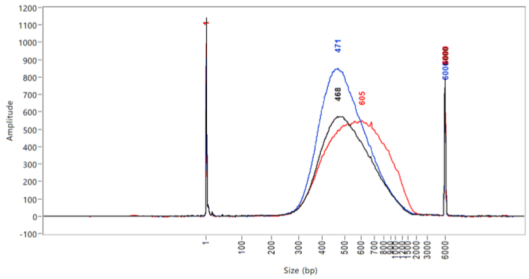

Figure 3 | BRB-seq library profiles prepared by tagmentation of 5 (black), 10 (blue) or 20 (red) ng of cDNA.

Conclusions

The tube with a sample of human total RNA in BRB-seq kits provides an inline control to ensure the kit's optimal performance which can be assessed with the metrics from the current technical note.