Procedure for the evaluation of amplification after emPCR.

Instead of discarding supernatants after addition of Melt Solution as stated in step 3.6.1.4 of emPCR Amplification Method Manual, amendments to be performed are as follows:

At this point, the supernatant contains the denatured single stranded complementary DNA templates of the amplified library fragments.

1. Carefully remove and transfer the **supernatant from step 3.6.1.4** to a new 2.0 ml collection tube.

2. Repeat previous step (second Melt Solution wash of the beads), and pool the two Melt Solution washes together in the same tube, for a total volume of ~2.0 ml.

3. In a separate 50 ml Falcon tube, prepare the neutralization solution by mixing 5.0 ml of Qiagen's PB buffer with 300 µl of sodium acetate 3M.

4. Transfer 1.0 ml of the recovered supernatant pool to the freshly-prepared neutralization solution from step 3, and vortex to mix.

5. Purify the neutralized single stranded template DNA library using one column from a MinElute PCR Purification Kit (Qiagen). Follow the manufacturer's instructions for using spin columns and a microcentrifuge, with the following exceptions:

   a. Because the volume recovered is large (after addition of Qiagen's Buffer PB), apply the sample to each column in nine aliquots of ~750 µl each, spinning after each addition.
   b. After the PE dry spin, rotate the columns 180° and spin an additional 30 seconds to ensure complete removal of the ethanol.
   c. Elute each column with 30 µl of Buffer EB or TE Buffer (from the Rapid Library Preparation Kit; room temperature).

6. Dilute 1:10 the eluate (e.g. 2 ul eluate + 18 ul EB/TE Buffer), and run a 1 ul aliquot on an Agilent Bioanalyzer RNA 6000 Pico chip, to assess the quality of the amplified library for the characteristics listed on corresponding Library Prep Manual.