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QC Check and Size Selection Using Pippin Prep - NEBNext Multiplex Small RNA Sample Prep Set for Illumina (E7300)

Overview

Note: There are several different methods for performing size selection. It is recommended to choose the appropriate method based on the QC check of the library using the Bioanalyzer. Size selection using AMPure XP Beads does not remove small fragments. If you perform the QC check and your sample contains Adaptor dimer (127 bp peak) or excess primers (70-80 bp) it is recommended to use gel or Pippin Prep for size selection.

Size selection of the Small RNA library (147 bp) can done on Pippin Prep instrument using the 3% Agarose, dye free gel with internal standards (Sage Science # CDF3010).

1. Purify the PCR amplified cDNA construct (100 µl) using a QIAQuick PCR Purification Kit.

   IMPORTANT: Before eluting the DNA from the column, centrifuge the column with the lid of the spin column open for 5 minutes at 13,200 rpm. Centrifugation with the lid open ensures that no ethanol remains during DNA elution

2. Elute amplified DNA in 32 µl nuclease-free water.

It is recommended to QC your library before performing size selection:

1. Load 1 µl of the purified PCR reaction on the Bioanalyzer using a DNA 1000 chip according to the manufacturer's instructions (Figure 1).

2. miRNA library should appear as a peak at 147 bp peak (that correspond for 21 nucleotide insert).

Program the protocol for size selection on Pippin Prep Instrument as follow:

1. In the Pippin Prep software, go to the Protocol Editor Tab.

2. Click “Cassette” folder, and select “3% DF Marker F”.

3. Select the collection mode as “Range” and enter the size selection parameters as follow: BP start (105) and the BP end (155). BP Range Flag should indicate “broad”. *Note: This protocol is optimized to select for 147–149 bp peak.*

4. Click the “Use of Internal Standards” button.

5. Make sure the “Ref Lane” values match the lane numbers.

6. Press “Save As” and name and save the protocol.

Prepare sample for size selection as follow:

1. Bring loading solution to room temperature.

2. For each sample, combine 30 µl sample with 10 µl of DNA marker F (labeled F).

3. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

4. Load 40 µl (DNA plus marker) on one well of the 3% agarose cassette.

5. Run the program with the settings indicated above.

6. After sample has been eluted, collect 40 µl sample from elution well. Run 1 µl in a Bioanalzyer using the high sensitivity chip.

   Note: If the Ethidium Bromide free cassettes was used, no purification is required before running sample on the bioanalyzer.

Figure 1: Typical results from (A) human brain and (B) rat testis total RNA libraries before size selection.
The 143 and 153 bp bands correspond to miRNAs and piRNAs, respectively. The bands on the Bionalyzer electropherograms resolve in sizes ~ 6-8 nucleotides larger than sizes observed on PAGE gels and can shift from sample to sample due to an incorrect identification of the marker by the bioanalyzer software. miRNA peak should be ~ 143-146 bp.

Figure 2: Electropherogram trace of pippin prep size selected library from human brain total RNA.