Size-selection for libraries generated from low quality RNA

RealSeq®-AC libraries prepared from high quality RNA samples are efficiently purified with AMPure beads as recommend in the RealSeq®-AC protocol. For most libraries, this purification protocol successfully removes short inserts, adapter dimers, and other contaminants. However, libraries prepared from low quality RNA samples may contain an excess of adapter dimers or short inserts which lowers the quality of the sequencing results if not removed before sequencing. Excess adapter dimers or short inserts increase the percentage of reads of sizes < 15 bp, reducing the overall percentage of usable reads.

Gel purifying libraries is an effective method to remove adapter dimers and short inserts and there are several methods that can be employed. Below we show results from a standard gel-purification using precast gels as well as results from an automatic gel-purification using the Pippin Prep instrument (Sage Science).

Option 1. Size-selection with Novex gels

RealSeq-AC libraries can be purified using standard PAGE gels. Figure 1 shows an example of a size-selected library using 6% Novex TBE gels (ThermoFisher). A complete protocol for theis procedure can be found on the SomaGenics' website (www.somagenics.com) in the RealSeq®-AC Application Notes.

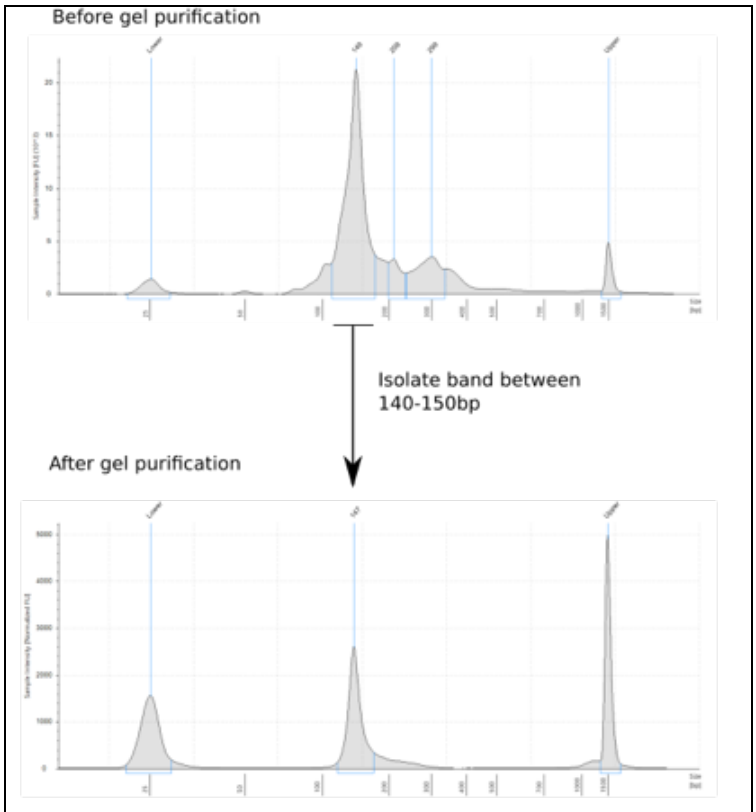

Figure 1. TapeStation profile of a RealSeq®-AC sequencing library before and after gel purification with 6% Novex TBE gels.

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Option 2. Size-selection with Pippin Prep

To evaluate automated size selection using the Pippin Prep instrument (Sage Science), RealSeq®-AC libraries were prepared from both low quality synthetic RNA and total RNA samples (RIN<7). Bioanalyzer profiles of libraries before and after Pippin Prep purification are shown in Figure 2. Data analysis of libraries with or without Pippin Prep purification are shown in Figure 3. Libraries prepared from low quality synthetic RNA contain a high percentage of reads with inserts smaller than 15 nt (left panel). Data from two different input amounts are shown. Libraries purified using the Pippin Prep system demonstrate an increase in the percentage of usable reads (over 15 nt) compared to those purified using AMPure beads alone. The right panel of Figure 3 shows sequencing data analysis from libraries prepared with a total RNA sample with a RIN value < 7. The percentage of sequencing reads that map to the target genome are shown. At every input amount, size-selection of libraries using the Pippin Prep increases the percentage of reads that map to the target genome.

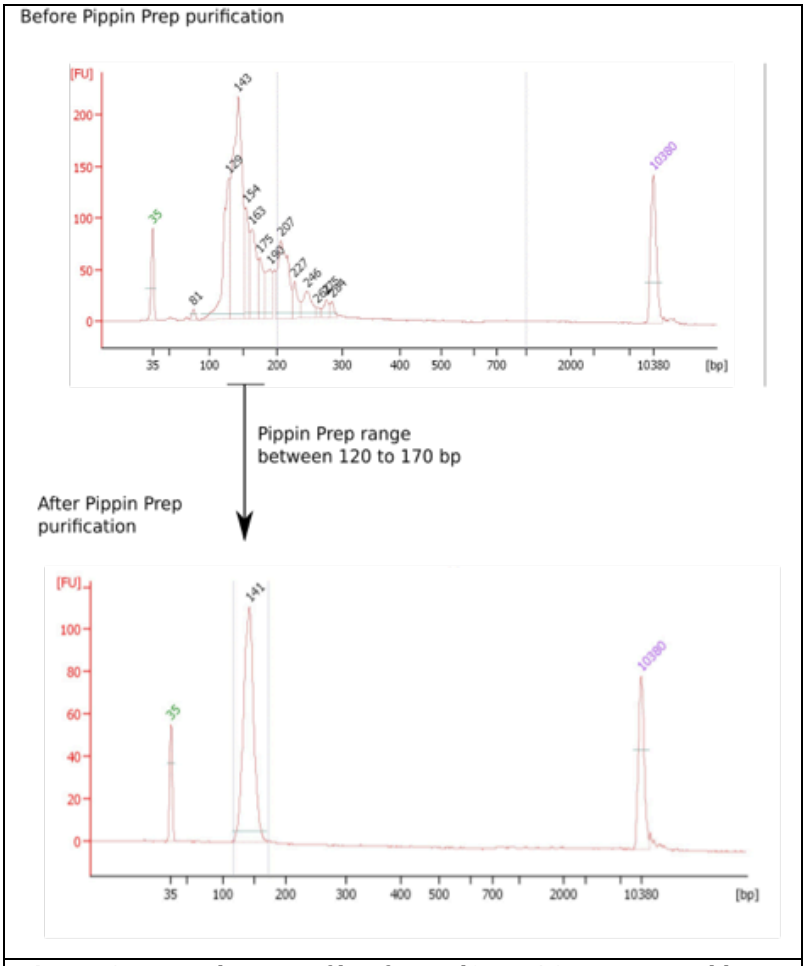

Figure 2. Bioanalyzer profile of a RealSeq®-AC sequencing library before and after Pippin Prep purification with a 3% Agarose gel cassette.

Figure 3. Low quality sequencing libraries were purified with AMPure Beads as recommended in the RealSeq®-AC protocol or with Pippin Prep (Sage Sciences). The left panel shows libraries generated with low quality synthetic RNAs using two different input amounts. Size selection using Pippin Prep increased the number of reads larger than 15 nt (21% for 100 fmoles, and 9% for 10 fmoles). The right panel shows libraries generated from low quality total RNA using two different input amounts. Size selection using Pippin Prep increased the number of reads mapping to the target genome.

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