DNA cleanup and size selection using sparQ PureMag Beads

Keywords: sparQ PureMag Beads, size selection, purification, cleanup, DNA, RNA

ABSTRACT

sparQ PureMag Beads are nucleic acid binding, magnetic beads. This application note details the usage of sparQ PureMag Beads for DNA cleanup and size selection. This work builds on previous application notes demonstrating the use of sparQ PureMag Beads for RNA cleanup and size selection, and their stability at room temperature for extended periods of time.

INTRODUCTION

DNA or RNA size selection is the process by which fragments of a specific size or size range are captured and unwanted fragments or contaminants are removed. In DNA library preparation protocols for next generation sequencing (NGS), size selection maximizes sequencing efficiency and avoids sequencing of unwanted fragments (those too long or short for the sequencing protocol and adapter dimers). Size selection can be single or double-sided, removing longer and/or shorter fragments.

sparQ PureMag Beads are paramagnetic beads that can reversibly bind nucleic acids. Unlike other magnetic beads, sparQ PureMag Beads have very broad utility and have previously been shown to be suitable for cleanup and size selection for both DNA and RNA. In addition, sparQ PureMag Beads have been demonstrated to be stable at room temperature for up to 6 months, increasing procedural flexibility. In this application note, we further demonstrate the use of sparQ PureMag Beads specifically for cleanup and size selection of DNA, testing an accelerated binding protocol for a range of target fragment size ranges.

MATERIALS AND METHODS

DNA cleanup

For DNA cleanup experiments, input was 475 ng of DNA library prepared from hgDNA (Roche) using sparQ DNA Frag and Library Prep Kit according to standard instructions. DNA cleanup experiments were carried out in triplicate as follows with either sparQ PureMag Beads or Beckman Coulter SPRIselect™:

1. 0.8X beads were added to the DNA sample.
2. The DNA/beads mixture was incubated at room temperature (RT) for 1 min or 5 min.
3. The beads were pelleted on a magnet and the supernatant removed and discarded.
4. The bead pellet was washed twice in 80% EtOH, then air-dried.
5. The pellet was resuspended in 35 µl 10 mM Tris-HCl pH 8.0 and incubated at RT for 1 min or 5 min. The beads were pelleted and the eluted DNA collected in a fresh tube.

DNA size selection

For size selection experiments, DNA was fragmented using sparQ DNA Frag and Polishing reagent. DNA Frag and Polishing Buffer (5 µl) and DNA Frag and Polishing Enzyme Mix (10 µl) were added to 500 ng hgDNA for a total volume of 50 µl. Two independent reactions were incubated at 32°C for 6 min and 10 min, respectively, followed by incubation at 65°C for 30 min. The two reactions were combined to generate a wide size distribution pattern.

Input of 200 ng of fragmented DNA for each experiment was then purified with a 0.8X beads-to-sample ratio as detailed in "DNA cleanup" above. Then, the DNA was subjected to size selection as follows, using either sparQ PureMag Beads or SPRIselect (Beckman Coulter). Three sets of size selection ratios were tested, as specified in Table 1.

1. Eluted DNA was mixed with the 1st binding beads volume and incubated for 1 min at RT.
2. The beads were pelleted and the supernatant transferred to a new tube. The 2nd binding beads volume was added to the supernatant, mixed thoroughly and incubated for 1 min at RT.
3. The beads were pelleted and the supernatant discarded.

4. The bead pellet was washed twice in 80% EtOH, then air-dried.
5. The pellet was resuspended in 35 µl 10 mM Tris-HCl pH 8.0 and incubated at RT for 1 min or 5 min. The beads were pelleted and the eluted DNA collected in a fresh tube.

Final DNA fragment range (bp)	250–500 bp	450–600 bp	500–800 bp
	Ratio	Ratio	Ratio
Initial cleanup	0.8X	0.8X	0.8X
1st binding	0.65X	0.55X	0.50X
2nd binding	0.85X	0.7X	0.65X

Table 1 **Bead ratios used for size selection experiments.**

RESULTS AND DISCUSSION

Equivalent performance for DNA cleanup

A key application of sparQ PureMag Beads is DNA cleanup during the NGS library preparation process. Magnetic bead cleanup provides a reliable and accurate method to cleanup post-ligation and post-PCR DNA products. To investigate performance for DNA cleanup, a library prepared using sparQ DNA Frag & Library Prep Kit was used as input. DNA was purified with either 0.8X sparQ PureMag Beads or SPRIselect. Two incubation times were used for DNA binding to beads and DNA elution from beads: 5 min as specified in the sparQ PureMag Bead product manual and 1 min as specified in instructions for SPRIselect.

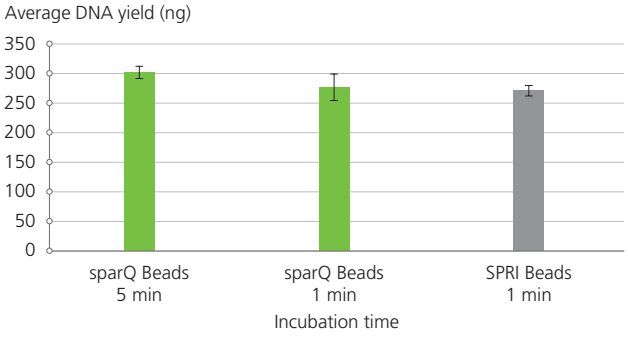

Figure 1 **Equivalent DNA cleanup using sparQ PureMag Beads and SPRIselect.** Cleanup was carried out on 475 ng of DNA library with sparQ PureMag Beads (green) or SPRIselect (gray). Either 1 or 5 min bead binding and elution incubation times were used based on the SPRIselect and sparQ PureMag Beads product manuals, respectively.

Quantification of eluted DNA from each condition demonstrated equivalent performance between sparQ PureMag Beads (58.1% recovery) and SPRIselect (56.8% recovery) 1 min incubation. When incubation time was extended to 5 min, DNA recovery increased to 63.5% with sparQ PureMag Beads. These results suggest that sparQ PureMag Beads can be used in place of SPRIselect for DNA cleanup with no change in incubation time. However, increasing incubation time to 5 min can further increase DNA yield with sparQ PureMag Beads.

Equivalent size selection

Accurate size selection of DNA libraries for next generation sequencing can be vital for maximum data output from sequencing. hgDNA (500 ng) was fragmented then subjected to size selection using sparQ PureMag Beads or SPRIselect, using 1 min incubation time for DNA binding and elution steps. Three different sets of sample-to-beads ratios were used to select for various peak fragment sizes.

For all three conditions, sparQ PureMag Beads gave equivalent size selection to SPRIselect. For example, for selection of fragments in the region of 450–600 bp, sparQ PureMag Beads gave a peak fragment size of 477 bp with range of 753 bp and SPRIselect gave a peak fragment size of 474 bp with range of 745 bp. This demonstrates comparable performance in size selection with either beads in this experiment.

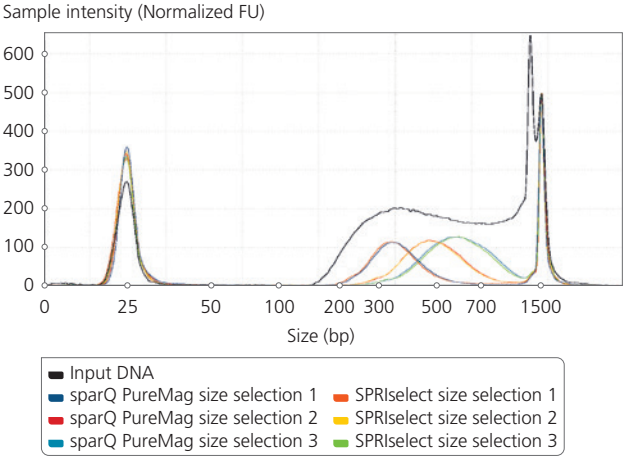

Figure 2 **Equivalent size selection.** Fragmented DNA was size selected using the ratios of beads detailed in Table 1 to select for fragment ranges: (1) 250–500 bp, (2) 450–600 bp or (3) 500–800 bp, using either sparQ PureMag Beads or SPRIselect. Peaks at 25 and 1500 bp represent low and high MW markers.

CONCLUSION

In this application note, we have demonstrated comparable DNA cleanup and size selection with sparQ PureMag Beads and SPRIselect. For DNA cleanup, using sparQ PureMag Beads gave high recovery with just 1 min incubation time, effectively removing DNA fragments >100 bp and retaining longer fragments. A further increase in DNA yield was achieved with sparQ PureMag Beads by increasing the incubation time to 5 min. Additionally, accurate and reproducible size selection was demonstrated with sparQ PureMag Beads at varying sample-to-beads ratios, highlighting their wide utility for various sequencing applications. Coupled with previous results demonstrating stability of sparQ PureMag Beads at room temperature for 6 months, this data suggests that sparQ PureMag Beads are a cost-effective alternative for cleanup and size selection.

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