**SPRI Beads-based Size Selection in the Range of 2-10kb**

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Application of solid-phase reversible immobilization (SPRI) beads for size selection in molecular biology should be expanded, in light of the property of the beads to accommodate to high MW intervals of DNA fragment size selection, depending on composition of bead-suspension buffer. Here we show how the conventional size selection interval of 150–800 bp be shifted to 1.5–7 Kbp with by adjusting the concentration of NaCl in the stock suspension buffer. The MW capacity of SPRI beads also change when NaCl replaced with other cations and when the concentration of polyethylene glycol (PEG) 8000 is decreased. Testing the limits of SPRI beads revealed cuts as high as 10 Kbp are possible for some salt/PEG combinations of modified SPRI beads-suspension buffers.

**KEY WORDS:** sizing, long read, sequencing, DNA

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**INTRODUCTION**

Solid-phase reversible immobilization (SPRI) beads, introduced in 1998, are essential components in high throughput DNA/RNA cleanup and size selection. Typical application of commercial beads has been limited to the interval between 150 and 800 bp of fragmented DNA. Gel-based selections have been used instead for targeting longer DNA fragments, for example, in mate-pair sequencing NGS libraries production (3–6 Kbp), 10x Genomics-linked reads (10 Kbp), and in the third generation sequencing methods such as Pacific Biosciences (Menlo Park, CA) (>6 Kbp) and Oxford Nanopore Technologies (Oxford, United Kingdom) (>10 Kbp). Gel-based solutions are limited by long duration, expensive equipment, and poor sample recovery.

Here we offer a simple, adjustable, high-throughput/high recovery method of “enhancement” of SPRI beads capabilities to long (2–10 Kbp) DNA fragment selection, which can be used instead of gel-based lower MW elimination, for potential applications in PacBio, Oxford Nanopore, and 10x Genomics-linked reads processing.

**MATERIALS AND METHODS**

**Materials**

PCRCleanTM-DX beads (C-1003-250; Aline Biosciences, Woburn, MA, USA), Agencourt AMPureXP beads (A63881; Beckman Coulter, Brea, CA, USA), polyethylene glycol (PEG) 8000 (HR2-535; Hampton Research, Aliso Viejo, CA, USA), NaCl (AM9759; Thermo Fisher Scientific, Burlington, MA, USA), MgCl₂ (AM9530G; Ambion, Austin, TX, USA), MgSO₄ (230391-500G; MilliporeSigma), CaCl₂ (C5670-100G; MilliporeSigma), Nuclease-Free Water (02-0201-0500; Avantor, Radnor, PA, USA). Aline Biosciences and Beckman Coulter products were tested in parallel using NaCl_750-Peg20 and found to perform similarly. The displayed data were obtained with PCRCleanTM-DX beads of Aline Biosciences.

**Methods**

**Preparation of fragmented gDNA for size selection**

Murine gDNA (Fig. 1A, lane 1) was used as a substrate for all data. Similar results were observed with DNA sheared by Covaris gTubes (520079; Covaris, Woburn, MA, USA), Covaris miniTube Red (520066; Covaris), and NEB Fragmentase (M0348S; New England Biolabs, Ipswich, MA, USA) (unpublished results).

Fifty microliters of 1–100 ng/μl genomic DNA (Fig. 1A, lane 1), was initially precipitated using 50 μl of SPRI beads following the standard 1:1 v/v protocol, with the exception that the beads were not removed from the final eluent [elution buffer (EB), 10 mM TrisHCl pH 8.0]. Material was quantified using either a nanodrop or qubit and suspension volume adjusted to 100 μl at 0.5–50 ng/μl. This bead/DNA mixture was used for subsequent purifications (Supplemental Tables S1 and S2).

**Size selection of fragmented gDNA with high MW-selection buffer**

Different buffer compositions and fractions were added to the bead/DNA mixture (Table 1). Liquid phases were carefully mixed by gently pumping through a wide bore pipet tip (12–15 times) and incubated without shaking at RT for at least 10 min (10–30 min was tested). Bead pellet collected on magnetic stand and supernatant carefully
transferred. Beads were washed twice with 70% ethanol and eluted in 20–30 μl EB. DNA from the supernatant (containing shorter material) was recovered by addition of 50 μl regular SPRI beads in standard buffer and purified as in regular protocol [2].

Analysis of DNA smears/traces before and after size selection

The gDNA shear and the ladder pre- and post-beads treatment, derived from bead pellets and bead supernatants, were analyzed using FEMTO Pulse genomic and/or NGS DNA gel (Agilent Technologies, Santa Clara, CA, USA), by loading 2 μl/lane of dilutions: 10–100 pg/μl for FEMTO Pulse gDNA and 1–10 ng/μl for next generation sequencing (NGS) DNA applications. Threshold was determined as the lowest MW where DNA is observed on the trace using Agilent’s PROsize 3.0 software (example in Supplemental Fig. S2).

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Pulse gDNA and 1–10 ng/μl for next generation sequencing (NGS) DNA applications. Threshold was determined as the lowest MW where DNA is observed on the trace using Agilent’s PROsize 3.0 software (example in Supplemental Fig. S2).

gDNA size selection for PacBio SMRTbell, using NaCl_Peg20 buffer

The semidegraded gDNA was diluted in 50 μl TE and precipitated with 50 μl of SPRI beads; bead pellet eluted with 101 μl of TE, 1 μl taken for qubit quality control (QC); the DNA from 100 μl of beads suspension was precipitated by 65 μl of NaCl-Peg20 buffer, bead pellet collected on magnet, washed 2 times with 200 μl of 70% ethanol and eluted in 39 μl of water; 2 μl taken QC by Qubit and FEMTO-Pulse, the remaining 37 μl used for SMARTbell library preparation according to the manual,3 with the exception that all subsequent AMPureXP cleanups were 0.8 times and no Pippin size selection was applied.

RESULTS

In developing new buffers for SPRI beads, there are 3 key parameters that must be tracked: 1) sizing threshold, the cutoff for binding; 2) yield, the amount of material lost in the selection; and 3) sizing sensitivity, how rapidly the sizing threshold changes as salt concentration changes. The third parameter is critical because if very small changes in concentration result in large changes in either cutoff or yield, the ability to control the reaction will be very limited. For example, typical SPRI beads suspension buffer is composed of 20% PEG8000 and 2.5 M NaCl and allows for size selection in the 150–800 bp, 800 bp reached at v/v ~0.4×, and DNA binding lost at v/v ~0.38×,1,2

FIGURE 1

Size selection with different NaCl-Peg20 buffers. A) FEMTO pulse gel image of samples size-selected with different NaCl-Peg20 buffers at different bead-to-sample ratios. MW cutoff detection in gel, NaCl molarity and v/v of the corresponded buffer shown at the top and gDNA recovery at the bottom. B) FEMTO pulse traces for select samples from Fig. 1A. NaCl 1250-Peg20 (red), NaCl 1000-Peg20 (blue), NaCl 750-Peg20 (yellow), and NaCl 500-Peg20 (green); gDNA prior to size selection (black) has lower MW border of ~1.3 Kbp. C) Plotting the percentage of SPRI buffer vs. the resulting size cutoff reveals poor size sensitivity of HMW buffers. The NaCl 750-Peg20 (yellow) shows the least size sensitivity among HMW buffers: NaCl 1250-Peg20 (red), NaCl 1000-Peg20 (blue), NaCl 500-Peg20 (green). Shown in black is the conventional 2.5 M NaCl buffer.

TABLE 1

Simplified names of buffers tested

| Buffer composition | Buffer working name |
|--------------------|---------------------|
| 2.5 M NaCl, 20% PEG8000 | Regular SPRI |
| 1.25 M NaCl, 20% PEG8000 | NaCl 1250-Peg20 |
| 1.0 M NaCl, 20% PEG8000 | NaCl 1000-Peg20 |
| 0.75 M NaCl, 20% PEG8000 | NaCl 750-Peg20 |
| 0.5 M NaCl, 20% PEG8000 | NaCl 500-Peg20 |
| 0.25 M NaCl 20% PEG8000 | NaCl 250-Peg20 |
| 0.75 M LiCl, 20% PEG8000 | LiCl 750-Peg20 |
| 0.5 M MgCl2, 20% PEG8000 | MgCl2 500-Peg20 |
| 0.5 M CaCl2, 20% PEG8000 | CaCl2 500-Peg20 |
| 0.5 M MgSO4, 20% PEG8000 | MgSO4 500-Peg20 |
| 0.75 M NaCl, 10% PEG8000 | NaCl 750-Peg30 |
| 0.75 M NaCl, 5% PEG8000 | NaCl 750-Peg5 |
| 0.75 M LiCl, 10% PEG8000 | LiCl 750-Peg10 |
| 0.75 M LiCl, 5% PEG8000 | LiCl 750-Peg5 |
| 0.5 M MgCl2, 10% PEG8000 | MgCl2 500-Peg10 |
| 0.5 M MgCl2, 5% PEG8000 | MgCl2 500-Peg5 |
To test if we could identify conditions where a higher MW cutoff could be achieved, we modified the standard NaCl PEG buffer to reduce the NaCl concentration (0.25–1.25 M) while keeping PEG concentrations constant (Fig. 1A, B). High MW breakpoints up to 7 kbp between the beads pellet/supernatant could be achieved with all the buffers tested except 0.25 M NaCl/20%PEG, which did not support DNA binding (Supplemental Table S1). Plotting MW cutoff vs. relative buffer volume revealed very sharp slope of HMW buffers compared to the traditional 2.5 M buffer (Fig. 1C). The 0.75 M NaCl had the lowest sizing sensitivity among the high molecular weight (HMW) buffers, tested.

We also tested whether cations other than NaCl might be able to shift the sizing threshold while maintaining a low enough sizing sensitivity to be used routinely for library sizing. To address this, we tested buffers containing 20% PEG and 0.5–0.75 M other salts (Fig. 2). We observed that other salts also have HMW range similar to NaCl (Supplemental Table S2), but the precipitation border is still hard to control due to small shifts in buffer concentration having large impacts on size distribution (Supplemental Table S2). Among the salts, tested, LiCl_750-Peg20 buffer had the most amenable sizing sensitivity, surpassing even that of 0.75 M NaCl.

Still, none of the SPRI buffers capable of creating robust thresholds higher than 7 Kbp. Both, the salts and the PEG in SPRI buffer play critical role in forming DNA precipitate on magnetic beads. Salts provide ion “bridging” of negatively charged molecules such as DNA and SPRI beads, PEG8000 “crowds” solutes, and water-suspended substances together by shrinking water-soluble space. In attempt to expand the threshold, we turned to the strategy of reducing PEG8000 in suspension buffer; keeping the salt concentration constant at 0.5–0.75 M, the PEG concentration was reduced to 5–10%. Using these solutions, we were able to achieve an HMW cutoff of >10 Kbp using 0.5 M MgCl2, 5% PEG8000 (Fig. 2, black line). For monovalent salts, reducing the concentration of PEG was not efficient and lead to complete loss of DNA immobilization on beads (Supplemental Fig. S1, lane 8).

The use of size selection for large inserts is increasingly important for amplicon sequencing using Pacific Biosciences and Oxford Nanopore instruments. To test the use of SPRI beads for sizing large inserts, we used an amplicon

FIGURE 2
Effect of varying cations in SPRI buffers. A) Summary of size-selection thresholds for different buffers: NaCl 750-Peg20 (yellow), MgCl 500-Peg20 (gray), CaCl 500-Peg20 (red), MgSO 500-Peg20 (blue), LiCl 750-Peg20 (green), MgCl 500-Peg5 (black). B) FEMTO pulse gel image of the MgCl 500-Peg5 precipitation of large DNA at varying bead:samples ratios. Peak length, cutoff length, and yield are indicated.

FIGURE 3
Sizing large insert for Pacific Biosciences sequencing. A) FEMTO pulse gel image of the original DNA having 1 and 8 Kbp peaks (lane 1), bead bound material after 0.65× NaCl 750-Peg20 cleanup (lane 2), and unbound after the cleanup following standard isolation (lane 3). B) FEMTO pulse gDNA trace image of the original DNA. C) FEMTO pulse gDNA trace image of size-selected DNA. D) Insert length of Pacific Biosciences library as determined by circular consensus sequencing.
library with a large amount of low MW material as a test case (Fig. 3A). In this instance, a 1.1-Kbp contaminant was observed with an 8-Kbp primary product for sequencing. Using 0.65× NaCl 750-PEG20, we were able to cleanly separate the high MW product from the 1.1-Kbp contaminant while maintaining ~30% yield (Fig. 3B, C). The 8-kbp product was prepared for single molecule real time (SMRT) sequencing using a Pacific Biosciences Sequel, and the consensus circular sequence (CCS) product was the expected size with minimal low MW contamination (Fig. 3D).

In summary, changing the cation and its concentration can be used to significantly increase the sizing threshold of SPRI beads into the kb range. The selection of condition can be optimized for specific selections needed. In our lab, we use 0.75 M NaCl 750-PEG20 for selections of 6 Kbp and MgCl 500-PEG5 for selections of 10 Kbp. Other salts/PEG concentrations can be considered if other selections are desired.

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