Extended Ability of the Axygen® AxyPrep MAG PCR Clean-up Kit for Effective DNA Fragment Selection

Application Note

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Adapting the Axygen AxyPrep MAG PCR Clean-up Kit for next generation sequencing sample preparation

The process of eliminating dimers, Taq DNA polymerase, metal ions, as well as other impurities in a post-PCR setting using the Axygen AxyPrep MAG PCR Clean-up kit has been well validated and applied. Indeed, this product has proven to be effective for DNA purification and concentration determination owing to the benefits conferred from the introduction of paramagnetic beads and a stable binding buffer. However, as more customers demand products that can effectively and efficiently select DNA fragments of specified lengths from a uniform smear sample to conduct next-generation sequencing (NGS) analyses, a new protocol was devised to meet this need. Using the optimized protocol, DNA fragments can be effectively selected from sheared DNA samples in defined interval with a size distribution ranging from 100 to 1,000 bp, required for most NGS platforms and molecular approaches requiring DNA fragments.

Demonstration of sample preparation and the updated protocol

Previous studies have demonstrated an apparent inverse correlation between the concentration of polyethylene glycol (PEG) and the size of DNA fragments that can bind to the surface of paramagnetic beads[1,2]. At a high concentration, all DNA fragments are bound to the beads; whereas when the concentration is too low, only the larger genomic DNA molecules will bind. The PCR Clean-up kit buffer has an optimized concentration of PEG that allows for the effective size-selection of DNA fragments ranging from 100 to 1,000 bp.

To demonstrate this ability, a DNA sample from *Escherichia coli* was prepared using the Axygen AxyPrep Plasmid DNA extraction kit. The nucleic acids were eluted in standard TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and sheared via sonication in a Covaris S220 ultrasonicator with the designated peaks of 300, 500, and 800 bp. Finally, the separate sheared plasmid DNAs were mixed to obtain uniform smears of 100 to 1,000 bp samples.

Simple and convenient DNA fragment selection using the Axygen AxyPrep MAG PCR Clean-up kit

A detailed version of the procedure can be found in the standard protocol provided with the kit. In brief, a sheared DNA sample ranging from 100 to 1,000 bp is used as the input material. The input volume is 50 µL and the elution volume is 20 µL. Because the elution volume is lower than the starting volume, the process also concentrates the DNA solution. The PCR Clean-up kit reagent can be added to the sample DNA according to the desired size range (Table 1). The addition ratio is interpreted as the ratio of the PCR Clean-up kit needed versus the initial input volume such that PCR Clean-up Solution volume added = _____ (Initial Ratio) x Initial DNA Sample volume[3].

After binding and incubating for 5 minutes at room temperature, the beads are separated from the suspension by placing the mixture on a magnetic rack. (The desired DNA fragments are still in the solution, while the larger fragments than the target DNA length are on the beads.) Next, the supernatant is removed and placed in a new tube, and additional reagent is added: Additional PCR Clean-up Solution volume added = [_____ (Final Ratio) - _____ (Initial Ratio)] x Initial DNA Sample volume[3]. (The selected DNA fragments are on the beads.) The supernatant is subsequently removed by placing the tube on a magnetic rack again. The beads are then washed twice with 200 µL of 70% ethanol to remove traces of salt and PEG. Finally, an elution buffer (Tris-EDTA) or biomolecular-grade water is used to elute the selected DNA fragments from the beads. Following this protocol, the resultant DNA size range was analyzed by an Agilent 2100 Bio-analyzer (Figure 1).

Table 1. Recommend Ratio Combination for Double-sided DNA Size Selection

Lane	Initial Ratio	Final Ratio	Selection Range (bp)	Peak (bp)
1	0.9x	1.1x	220 - 280	232
2	0.8x	1.0x	250 - 320	252
3	0.7x	0.9x	270 - 350	304
4	0.6x	0.8x	300 - 500	352
5	0.5x	0.7x	330 - 700	457
6	0.4x	0.6x	500 - 1,500	1,100

Axygen AxyPrep MAG PCR Clean-up kit shows successful DNA fragment selection and adaptability

Using a step-wise input ratio, the Axygen AxyPrep MAG PCR Clean-up kit demonstrated excellent selection capability for sheared DNA fragments ranging from 100 to 1,000 bp. Although the selection range is more refined for smaller fragments, a more precise selection protocol of fragments between 600 and 1,000 bp can be optimized based on the user's need. This can be done by fine-tuning the input ratios to 0.55 to 0.66, for example. In general, the yield is better for the selection of larger fragments (≥500 bp). However, this phenomenon could have resulted by uneven size mix in sheared DNA sample, because the Covaris system does not generate a perfectly uniform smear.

This procedure is highly automatable and can be easily applied to high throughput platforms. Moreover, the yield rate can be increased by increasing the incubation temperature to 50°C, as well as by employing preventive measures to eliminate bead loss (such as gentle pipetting).

Furthermore, the PCR Clean-up kit can be used to size-select DNA fragments up to 2,000 bp in length by simply lowering the initial input ratio. However, the smallest value possible is approximately 0.4 for a one-step selection process; lower values would result in a lower yield of DNA fragments.

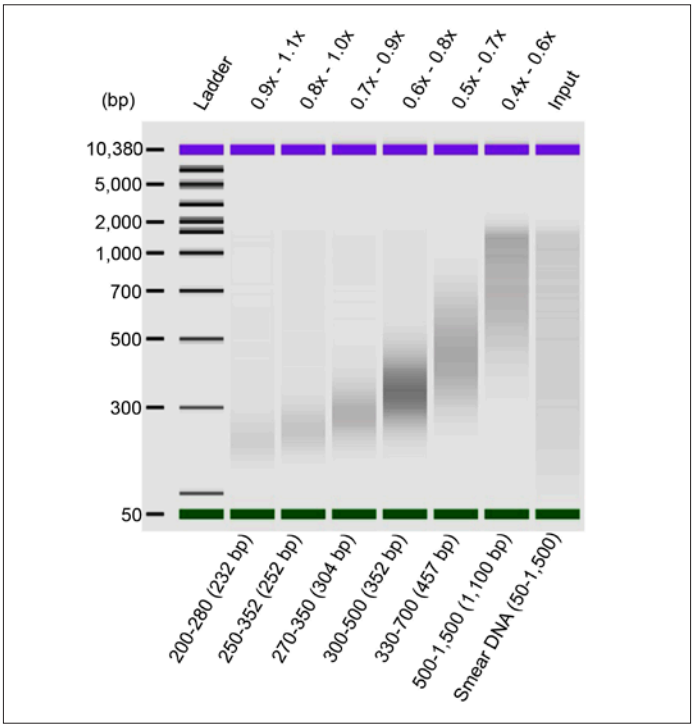

Figure 1. The resultant DNA size range was analyzed by an Agilent 2100 Bio-analyzer. Range of fragments were selected by increasing the amount of PCR Clean-up solution according to Table 1. The DNA size markers are indicated on the left. The size of the input smear of the DNA samples ranged from 50 to 1,500 bp.

References

1. Lis JT and Schleif R. (1975). Size fractionation of double-stranded DNA by precipitation with polyethylene glycol. Nucleic Acids Research, 383-389.
2. DeAngelis MM, Wang DG, and Hawkins TL. (1995). Solid-phase reversible immobilization for the isolation of PCR products. Nucleic Acids Research, 4742-4743.
3. Effective DNA Fragment Selection Using the Axygen AxyPrep MAG PCR Clean-up Kit Protocol (CLS-A-AN-433).

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