Purification of PCR Products for Sanger Sequencing Using ADS™ PCR Cleaning Beads

ABSTRACT

PCR product purification or cleanup for downstream Sanger sequencing is essential to get high-quality sequencing results. While several methods are available for PCR cleanup, magnetic beads provide a fast and cost-effective solution. Here we demonstrate that the cleanup of 1.2 kb and 0.4 kb PCR products using ADS™ PCR Cleaning Beads shows better or similar DNA recovery and similar sequencing results compared to the performance using spin column purification (using Vendor N products) and magnetic beads purification (using Vendor O products).

INTRODUCTION

Sanger sequencing has been widely used for identifying DNA mutations such as point mutations, insertions and deletions over the past 40 years. The templates used for sequencing can be single-stranded or double stranded DNA, big templates (genomic DNA, cosmid or bacterial artificial Chromosome, BAC) or small templates (PCR products), circular DNA (plasmid) or rolling cycle amplified (RCA) templates. Among these, PCR products, along with plasmids, are currently the most common and preferred ones.

PCR templates should be a single product (single product band on gel) and purification of the PCR product is necessary. Apart from PCR products, other reaction components, such as the unincorporated dNTPS, primers and DNA polymerase, can change the sequencing reaction or interfere with the sequencing reactions.

There are multiple methods for cleaning up the PCR products, such as using spin columns, magnetic beads or ethanol purification. Among these, magnetic beads can be fast and cost-effective. Here we compare the performance in terms of DNA recovery and Sanger sequencing results of two magnetic beads with that of spin column for purification of PCR products.

MATERIAL AND METHODS

PCR Reactions: A 1.2 kb and a 0.4 kb DNA fragment were amplified using ADS™ Taq DNA polymerase, ddNTPs and 5xPCR Green buffer (AdvancedSeq, Pleasanton, CA). The two fragments from PCR reactions were cleaned up and sequenced as described below.

PCR Reaction Cleanup: A spin column (from Vendor B), magnetic beads (from Vendor O) and ADS PCR Cleaning Beads (AdvancedSeq) were used in parallel to purify 10 µl of the two PCR products mentioned above, following instructions from the manufactures. The ADS™ PCR Cleaning Beads and DNA ratios (volume) was 1.5X. The eluted DNA was quantified using the NanoDrop ND-1000 Spectrotrophotometer (Thermo Fisher Scientific).

Sequencing Reactions: The SupreDye™ BD3 Cycling Sequencing kit (AdvancedSeq) was used to set up the sequencing reactions with the M13 forward primer following the instruction manual. The input for the template amounts for the 0.4kb and 1.2 kb fragments was 10 ng and 40 ng, respectively.

Sequencing Reaction Cleanup: The sequencing reactions were cleaned up using ADS™ Sequencing Reaction Cleaning Beads (AdvancedSeq) following the instruction manual. The samples were eluted into 65µl elution buffer.

Capillary Electrophoresis: The samples were directly loaded on an ABI 3130xl Genetic Analyzer (Applied Biosystems) and run on the 50 cm capillary array filled with PwrPOP™ P7 polymer (AdvancedSeq) for 60 minutes.

RESULTS AND DISCUSSION

Comparison of PCR Recovery from Different Methods

ADS PCR Cleaning Beads at 1.5X beads to PCR reaction volume ratio, spin column purification from Vendor N, and magnetic beads from Vendor O were used to purify 10 µl of the 1.2 kb PCR reaction. The recovery yield comparison is shown in Figure 1. The ADS PCR Cleaning Beads (1.5X) showed the best recovery (100%); spin column from Vendor N had about 83% recovery and the magnetic beads from Vendor O showed 45% recovery. All the methods showed similar yield with only marginal differences for the recovery of the 0.4 kb fragment (data not shown).

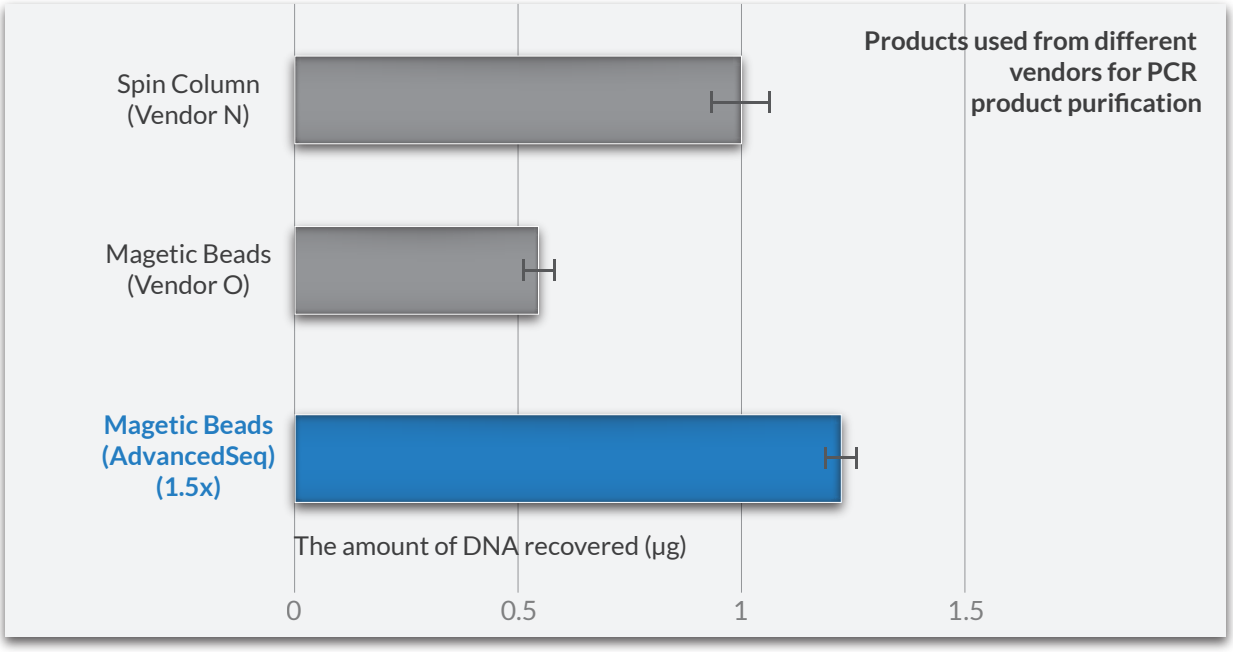

Figure 1. Relative recovery rate comparison for the 1.2 kb PCR product purified using various methods from different vendors

Sequencing Comparison Using the PCR Products Purified from Different Methods

Sequencing results obtained for the 1.2 kb (Figure 2) and 0.4 kb (data not shown) PCR templates purified using different methods were compared. All the four purified PCR products generated similar sequencing results in terms of the peak intensity and signal uniformity as seen from the electropherograms and raw data files.

In conclusion, we have demonstrated that:

(1) ADS™ PCR Cleaning Beads provide good recovery of 1.2kb and 0.4 kb fragment (using 1.5X beads and PCR product volume ratio) when compared to spin column purification (Vendor N) and magnetic beads purification (vendor O);

(2) PCR products purified from ADS™ PCR cleaning beads generate similar quality sequencing data compared to the data from the above two vendors.

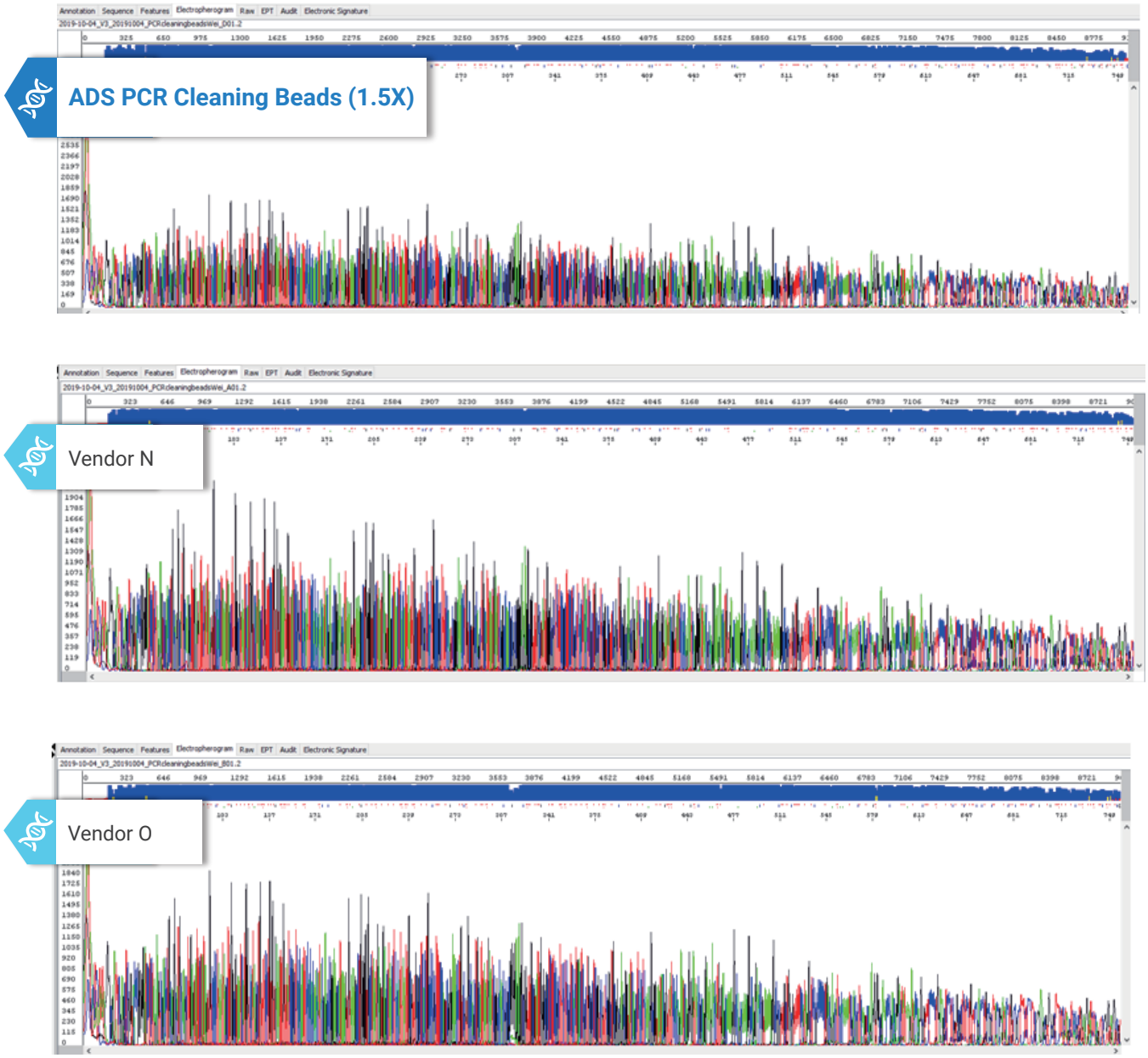

Figure 2. Electropherogram data from Sanger sequencing of the 1.2 kb PCR fragment.