

Axygen® AxyPrep™ Mag PCR Clean-Up Effectively and Efficiently Isolates PCR Contaminants from Product



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SnAPPShots

A brief technical report from the Corning Applications Group

*Katherine E. Strathearn Ph.D.,
and Mark E. Rothenberg Ph.D.
Corning Incorporated,
Life Sciences
Kennebunk, Maine 04043*

Introduction

The efficient removal of unincorporated deoxynucleotide triphosphates (dNTPs), salts and enzymes after any PCR reaction is essential for the success of downstream applications such as genotyping, Sanger, and Next Generation Sequencing. The AxyPrep Mag PCR Clean-Up Kit utilizes a unique paramagnetic bead technology for rapid high-throughput purification of PCR products. Additionally, the AxyPrep Mag PCR Clean-Up Kit employs an optimized buffer to selectively bind PCR amplicons 60 bp and larger to paramagnetic beads, providing end users flexibility and high recovery options. The protocol comprises binding, washing and eluting steps, where primers, nucleotides, salts and enzymes in the reaction mixture are efficiently removed leaving a purified PCR product that is essentially free of contaminants. No centrifugation or filtration steps are required, allowing the process to be adaptable to multiple commercially available automation platforms.

Materials and Methods

Plasmid DNA Preparation

GC10 competent cells (Sigma-Aldrich®, Cat. No. G2544) were heat shocked with gWIZ™ GFP plasmid DNA (Genewiz®) and cultured in LB-Broth (Sigma-Aldrich, Cat. No. L3022) at 37°C for 16 h at 250 rpm. Plasmid DNA was purified using the AxyPrep Plasmid Mini-prep Kit (Corning, Cat. No. AP-MN-P-50) and quantified with the EnVision® multimode plate reader (PerkinElmer®).

Polymerase Chain Reaction (PCR)

PCR reactions contained 200 ng gWIZ GFP DNA, TAQ Polymerase Buffer with 2 mM of Mg²⁺ (Corning, Cat. No. PCR-TAQ-R-5), 20 μM of each dNTP (Corning, Cat. No. PCR-DNTP-S-254), TAQ Polymerase (Corning, Cat. No. PCR-TAQ-R-5), and 250 nM of each primer. Primers were generated by Integrated DNA Technologies Inc., to anneal to GFP (~ 700 base pairs [bp]). Samples were added to a PCR micro-

plate (Corning, Cat. No. PCR-96-FLT-C) and the following program was applied in a MultiGene™ Thermal Cycler (Labnet): 1 cycle of 2 min. at 95°C for denaturation, followed by 25 cycles (30sec. at 95°C for denaturation, 1 min. at 59.5°C for annealing, 3 min. at 72°C for extension) and a final 10 min. extension at 72°C.

PCR Clean-Up

PCR products were isolated from contaminants using the AxyPrep Mag PCR Clean-Up Kit (Corning, MAG-PCR-CL-5) or a similar competitor version using the IMAG™-96P magnetic beads separation device (Corning, IMAG-96P) as described in each manufacturer's protocols. Briefly, a portion of the PCR products was added to either the Corning paramagnetic beads or the competitor's beads. The samples were washed with 70% ethanol and eluted with 40 μL of 10 mM Tris-HCl (pH 8.0). All of the samples were loaded onto a 1% agarose gel (Corning, Cat. No. AGR-LE-100) containing ethidium bromide. The gel was transferred to an electrophoresis unit and the unit was operated under constant voltage at 90 volts for 60 minutes. Following electrophoresis the gel was imaged and quantified using the Dolphin Viewer II (Wealtec®) imager and software. DNA quality was evaluated using the EnVision multimode plate reader (PerkinElmer).

Results

PCR Clean-Up

To evaluate the ability of the AxyPrep Mag PCR Clean-Up Kit to effectively purify and recover PCR products, PCR reactions were performed to amplify the GFP gene encoded in the plasmid gWIZ GFP. After amplification, 10 μL of the reaction mixture was added to either the AxyPrep PCR Clean-Up paramagnetic beads or the competitor's version of the paramagnetic beads. Both kits were able to effectively remove contaminants (e.g. primer dimers and template DNA) from the PCR products (Figure 1A). Following electrophoresis, the pixel intensities of the PCR products were evaluated pre and post-purification (Figure 1B). The AxyPrep Mag PCR Clean-Up Kit recovered 90% of the PCR sample, whereas the competitor's version recovered only 80% (Figure 1C). To evaluate whether there were protein (Figure 1D) or other (Figure 1E) contaminants in the sample, the A260:A280 and A260:A230 ratios were obtained, respectively. Both kits performed equally. The results presented in Figure 1 demonstrate the ability of the AxyPrep™ Mag PCR Clean-Up Kit to isolate PCR products from the reaction mixture with a higher yield recovery than a comparable competitor product.

Summary

- ▶ The Axygen® AxyPrep™ Mag PCR Clean-Up Kit effectively and efficiently purifies PCR products within 20 minutes.
- ▶ The AxyPrep Mag PCR Clean-Up Kit significantly recovers a higher percentage of PCR product (~90%) compared to a competitor (~80%).

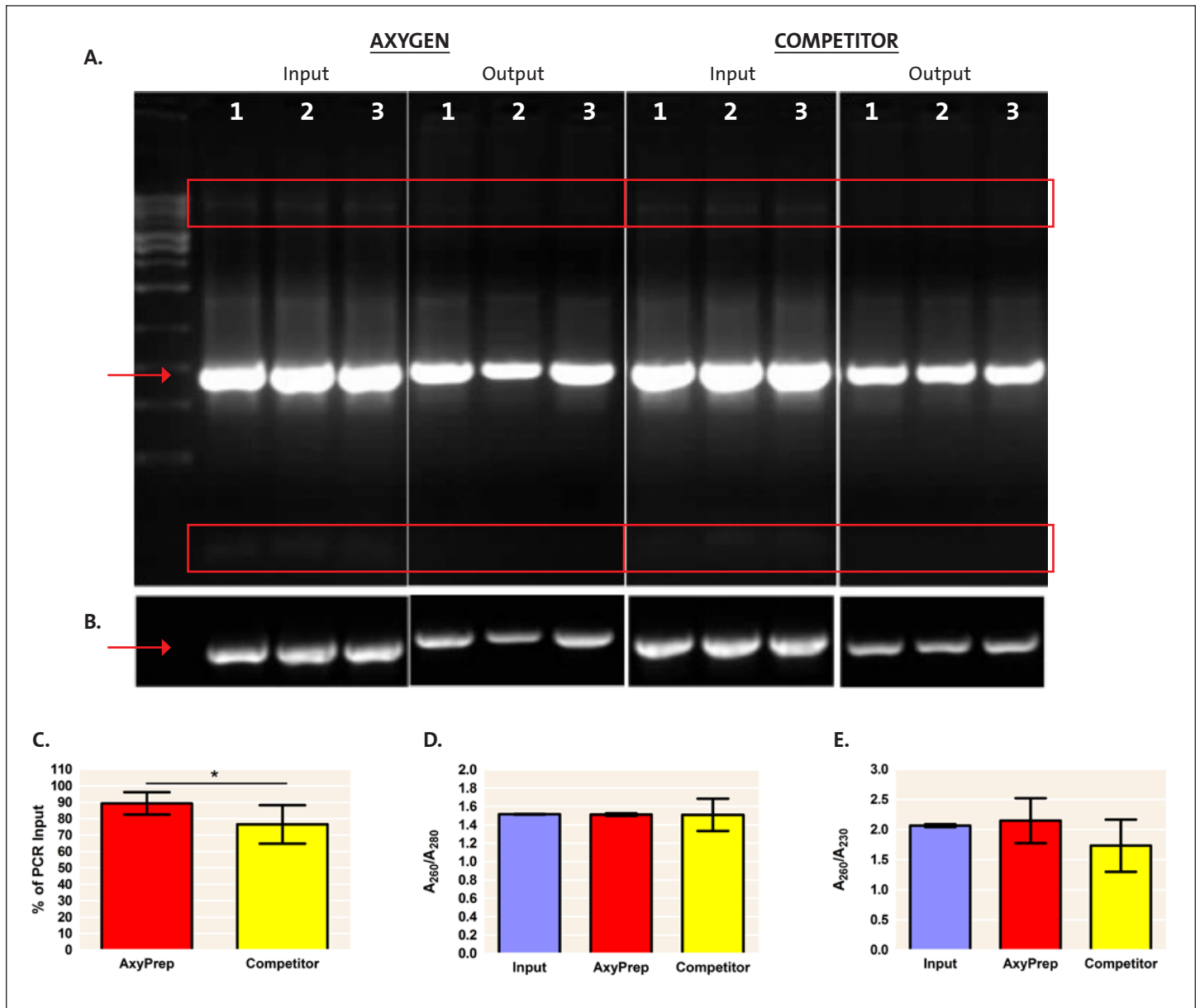
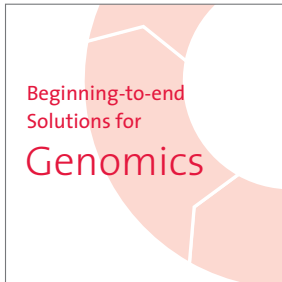


Figure 1. Axygen AxyPrep Mag PCR Clean-Up Kit purifies and recovers approximately 90% of the PCR product. (A) Representative gel depicting the ability of the AxyPrep Mag PCR Clean-Up Kit to effectively remove template DNA (top rectangle) and primer dimers (bottom rectangle) from PCR products (~700 bp). (Input, Purified, Output) (B) The same gel represented in (A) but less exposed. This image was used for the quantification of the PCR amplicons. (C) Quantification of the PCR amplicons using the Dolphin Viewer II Software. The AxyPrep Mag PCR Clean-Up Kit is able to significantly recover a higher percentage of the PCR product (~90%) compared to a competitor (~80%). This experiment was performed in triplicate, three individual times. In the paired t-test, the *p-value < 0.05. To evaluate whether there were protein (D) or other (E) contaminants in the sample the A₂₆₀:A₂₈₀ and A₂₆₀:A₂₃₀ ratios were obtained, respectively. Both kits performed equally.



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Corning Incorporated
Life Sciences

836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476

www.corning.com/lifesciences

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