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

A Corning Brand

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 Miniprep 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 Mg^{2+} (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 microplate (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%).

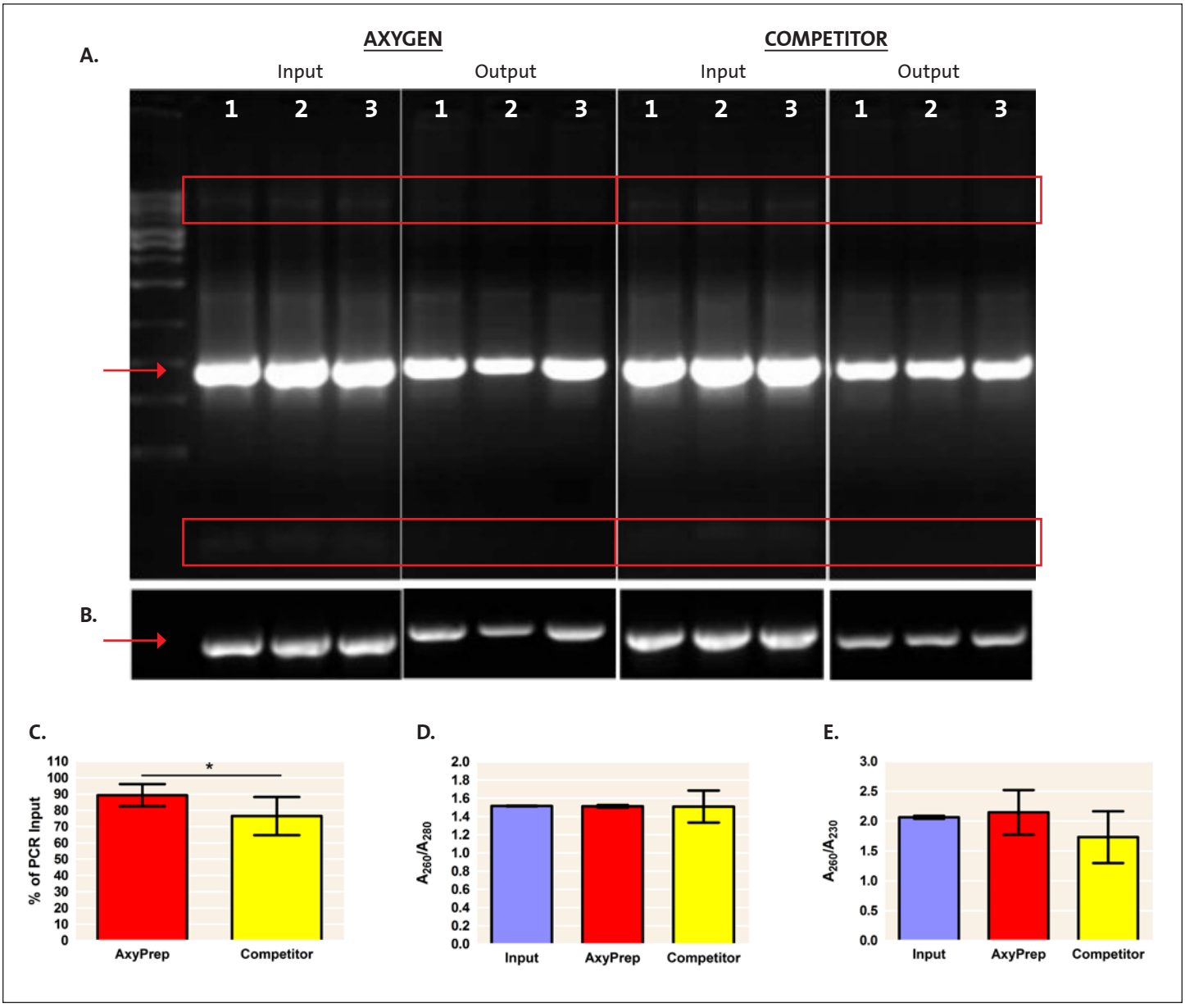
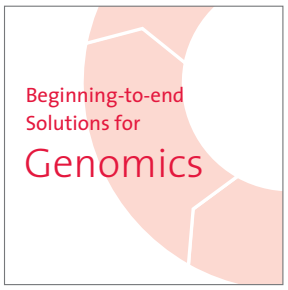


Figure 1. Axygen AxyPrep Mag PCR Clean-Up Kit purifies and recovers approximately 90% of the PCR product. (A) Representative gel depicting the ability of the AxyPrep Mag PCR Clean-Up Kit to effectively remove template DNA (top rectangle) and primer dimers (bottom rectangle) from PCR products (~700 bp). (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.



www.corning.com/lifesciences/solutions

In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs doing what you do every day, across the globe. From collection to analysis, our technical experts understand your challenges and your need for simplified, efficient, low- to high-throughput genomics processes.

A combination of global manufacturing expertise, extensive use of in-house automation, an unsurpassed commitment to product innovation and a thorough understanding of your processes enables Corning to offer a beginning-to-end portfolio of high-quality, reliable consumables and reagents for genomics applications.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

For additional Axygen product or distributor information, please e-mail us at CLSCustServ@Corning.com, visit our website at www.corning.com/lifesciences/axygen or call 1.800.492.1110. Outside the United States, call 978.442.2200.

For Axygen technical information, please e-mail us at AxgSupport@Corning.com or call 1.800.429.9436. Outside the United States, call 510.494.8900.

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

CORNING | FALCON AXYGEN GOSSELIN PYREX