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TECH NOTE

A new optimized PCR clean up method using the NUNC™ glass fiber filter plate

Abstract

Using the NUNC™ Glass Fiber Filter Plate, we have developed an optimized protocol for clean up of PCR products. Briefly, PCR mix is adjusted with potassium acetate and guanidine hydrochloride for DNA binding on the Glass Fiber Filter Plate. After impurities are washed out, purified PCR product is eluted into a NUNC DeepWell™ reception plate. Optimal concentrations of potassium acetate and guanidine hydrochloride in the PCR-KAc-GuHCl mix were used to isolate nine different sizes of PCR products (103-2799 bp). The wash strategy produces high quality and yield of PCR product. Purified PCR product contains no protein or short dsDNA, and less than 1% primer ssDNA. We report a high recovery rate of PCR product DNA, particularly for small PCR products. Well-to-well reproducibility is confirmed by the recovery in a 96 well plate. Restricted digestion and sequencing tests demonstrate the high quality of purified PCR product, which is suitable for down-stream applications. The combination of a NUNC Glass Fiber Filter Plate with this optimized protocol leads to efficient isolation of high quality PCR-amplified DNA.

Introduction

The Polymerase Chain Reaction (PCR) is a popular and powerful tool in molecular biology, and PCR products have been used in various down-stream applications (Sambrook et al, 1989; Dieffenbach and Dveksler, 1995). Successful purification of PCR product relies upon the preservation of PCR product and the removal of all impurities, such as unincorporated nucleotides, primers, buffers, enzymes and short dsDNA. PCR products range in size from 100 bp to a few thousand bp. Small DNA, such as primers, dimers and other short DNA, share similar properties with small PCR products. Thus, high yield and high purity are particularly important for purification of small PCR products. Low recovery of small PCR product has been reported using the PCR clean up products from several suppliers. Previous work shows that the NUNC Glass Fiber Filter Plate successfully isolates plasmid DNA (Lu et al, 2002). Based on the properties of glass fiber, we have now developed a new PCR clean up protocol with the Glass Fiber Filter Plate. In this new protocol, buffer concentrations were optimized and selected for high yield and purity of PCR products from 100 bp to a few thousand bp.

Materials and Methods

PCR primers: Nine primers, 20-21 oligonucleotides in length, were designed with a Tm at 58.7-60.4°C. Amplification resulted in nine different sizes of PCR products (104-2799 bp).

PCR clean up: Following PCR amplification, 10 mM EDTA was added to the PCR mix in order to stop the reaction. 3M potassium, 5M acetate buffer (KAc), and 6M guanidine hydrochloride (GuHCl) were added to adjust the PCR-KAc-GuHCl mix for DNA binding to the glass fiber filter. The mix was loaded into wells of the Glass Fiber Filter Plate and vacuum was applied. Impurities were sequentially washed with 50 µl of wash buffer 1 (0.3M potassium, 0.5M acetate and 2M GuHCl) and twice with 1 ml of wash buffer 2 (10 mM Tris-HCl, 1 mM EDTA, 80% ethanol, pH 7.4). Finally, the PCR product was eluted from the filter with elution buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.5).

Protein, ssDNA and dsDNA assays: Protein (including enzyme), ssDNA and dsDNA levels were examined before and after clean up. The NanoOrange™ protein quantitation kit (Molecular Probes, Eugene, OR), which detects protein down to 10 ng/ml, was used for protein detection. To assay the primer levels, the OliGreen® ssDNA quantita-

tion kit (Molecular Probes) was used. The PicoGreen® dsDNA quantitation kit (Molecular Probes) was used to measure dsDNA levels.

Results

Protocol optimization: Data indicates that 0.5M KAc in the PCR-KAc-GuHCl mix results in high recovery of all nine PCR products. Recovery of the smallest PCR products (<300 bp) can be increased by lowering the concentration of KAc to 0.25M (Figure 1).
2M GuHCl in the PCR-KAc-GuHCl mix resulted in high recovery levels for all sizes of PCR products. Thus, 0.5M KAc and 2M GuHCl in PCR-KAc-GuHCl mix were selected for the PCR clean up protocol. For small PCR products (<300 bp), 0.25M KAc is optional for recovery (Figure 2).
High quality PCR product was obtained using a small volume (50 µl) of wash buffer 1 (same concentration of KAc and GuHCl as in the PCR-KAc-GuHCl mix) plus 2 x 1 ml of wash buffer 2 (10mM Tris-HCl, 1mM EDTA, 80% ethanol and 2 x 1 ml volumes of wash buffer 2, pH 7.4). This new wash strategy reduces primer residue, produces excellent PCR product for sequencing, and does not significantly affect the recovery of PCR product. Thus, two optimized wash buffers are used in the new PCR clean up protocol.

High quality of PCR product: The high quality of PCR product was confirmed using agarose gel electrophoresis. Single bands were clearly visible for each of the 9 different PCR products (103, 153, 222, 305, 629, 863, 1339, 2089 and 2799 bp) (Figure 3).

Using the NanoOrange™ protein quantitation kit, no protein was detected in the purified PCR product, suggesting that the clean up procedure removes any trace of Taq polymerase (Figure 4).

To examine the primer ssDNA residue in the PCR product, a primer mixture of 9 primers (0.2-4 µM for each primer) was added into the PCR mix without template and Taq polymerase. Following clean up, more than 99% of ssDNA was eliminated (Figure 5).
In order to assess short dsDNA synthesized during the PCR reaction, a mimic PCR mix containing 9 primers and no template, was run through the PCR reaction and clean up procedure. Due to the lack of template DNA, large PCR products are not expected to be synthe-

Figure 3 Agarose gel electrophoresis of nine PCR products, 103 to 2799 bp

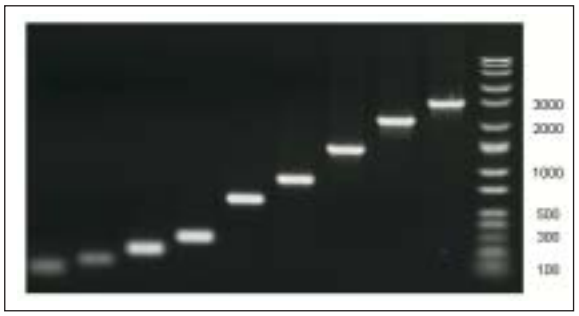

Figure 4 Removal of protein by clean up method

Figure 5 Removal of ssDNA by clean up method

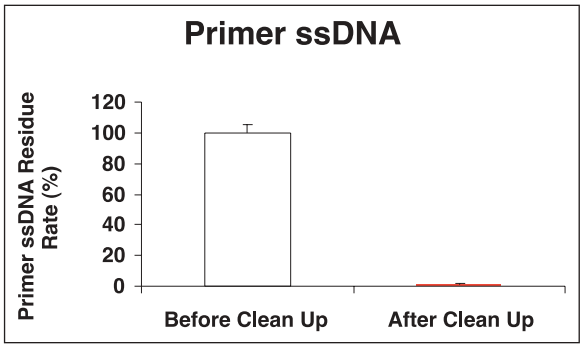

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Figure 1 Optimization of Potassium Acetate concentration

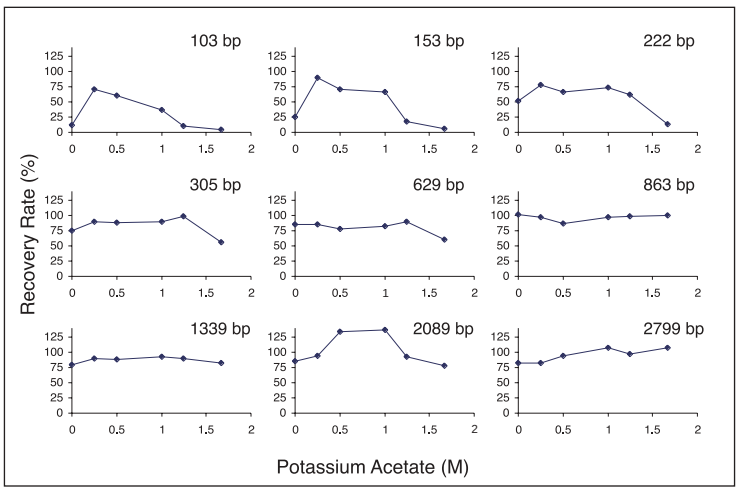
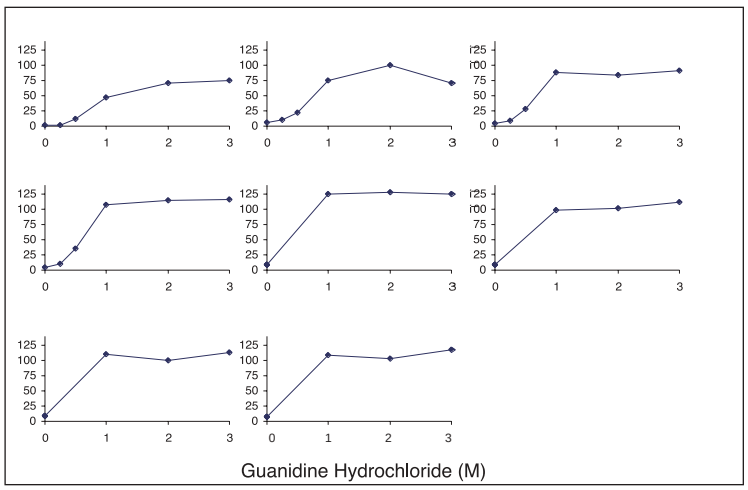


Figure 2 Optimization of Guanidine Hydrochloride concentration



sized. Because PicoGreen reagent can weakly stain ssDNA, a primer control group was employed to exclude the background reading from primers. The difference between the primer group and the primer + Taq group presents the specific reading from dsDNA synthesized by Taq polymerase, which may be dimer or other kinds of short dsDNA according to property of Taq polymerase. Data reveal that a very small amount of dsDNA had been synthesized in the PCR mix, and that the newly synthesized short dsDNA had been cleaned up in the final elution (Figure 6).

High yield and reproducibility: The optimized PCR clean up protocol yielded high recovery rates for all PCR products (103 – 2799 bp). The small PCR products (103, 153 and 222 bp) were cleaned up with 0.25M KAc (Figure 7).

High reproducibility was demonstrated after recovery of PCR products in a 96 well plate. The average recovery rate was 94.0 + 8.15% (mean ± SD) for a 1339 bp PCR product (Figure 8).

Down-stream applications: Using our optimized clean up protocol, the purified PCR products are suitable for down stream applications. The digestion of the 926 bp PCR product by EcoR 1 induces two small bands in an agarose gel (Figure 9). The cleaned up PCR product also is excellent for sequencing (Figure 10 - on back).

Figure 6 Removal of short dsDNA

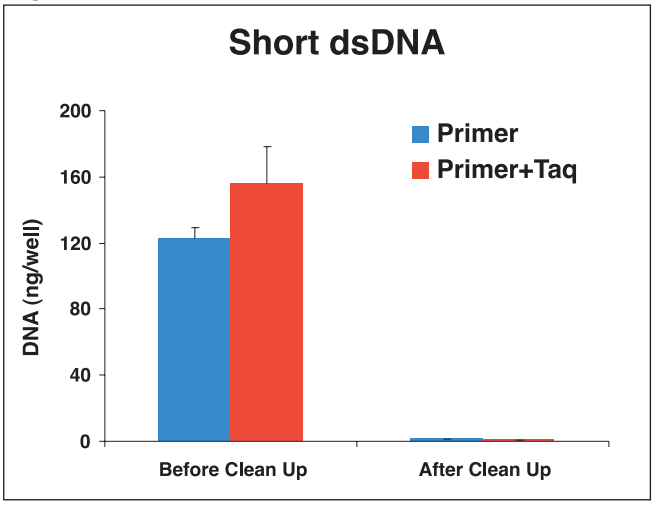


Figure 7 High recovery of PCR product after clean up

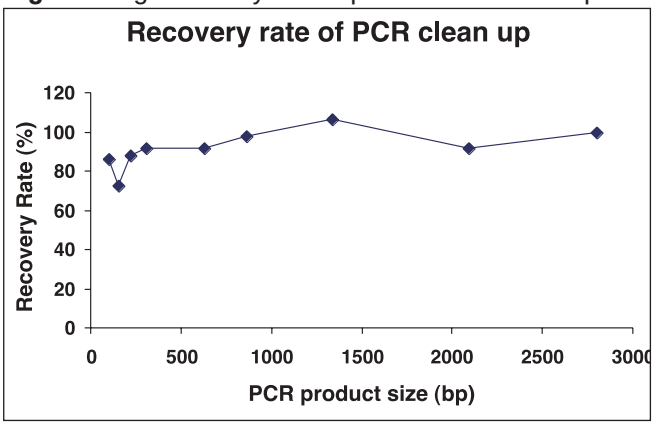


Figure 8 High well-to-well reproducibility of 1339 bp product

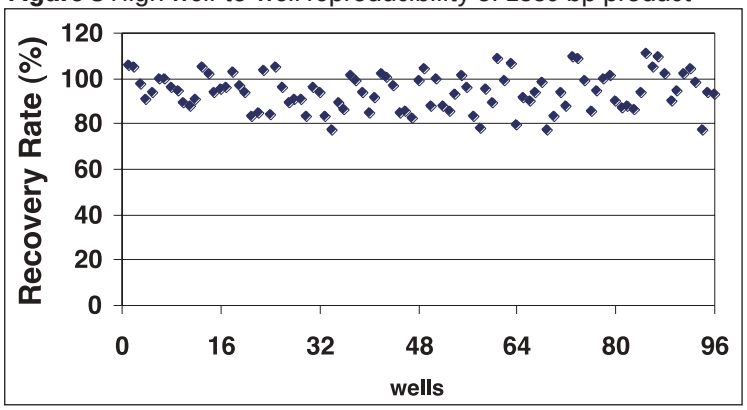
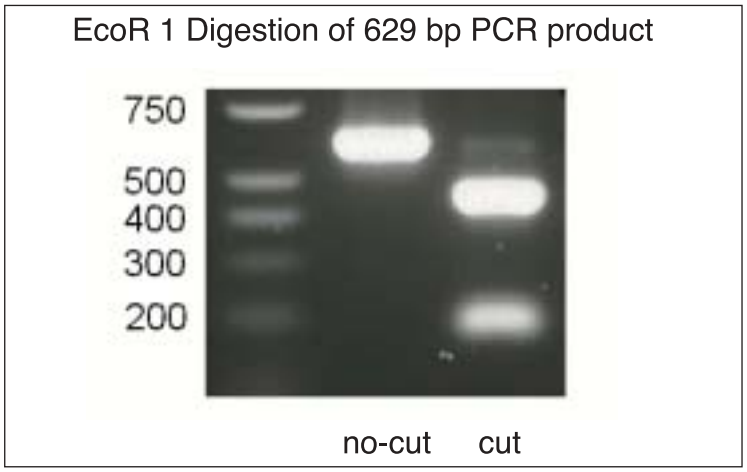


Figure 9 Purified PCR product is suitable for restriction digestion



Conclusion

Using the NUNC™ Glass Fiber Filter Plate, an optimized PCR product clean up protocol has been developed, which achieves both high quality and high yield for purification of various sizes of PCR products.

Credits

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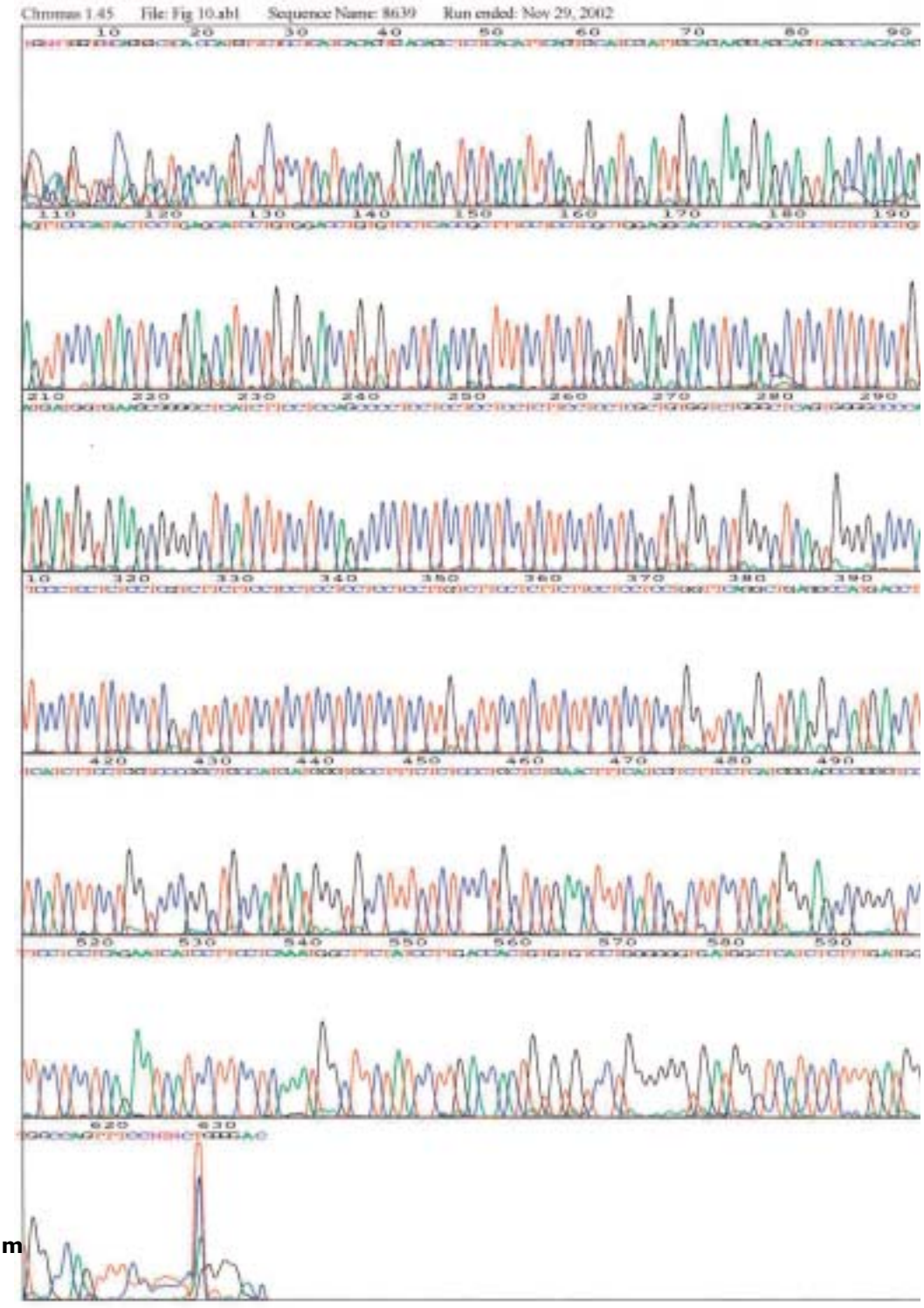
Figure 10 Purified PCR product is suitable for sequencing



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Lu, W., D. Dwyer, D. Schroen and T. Cummins, (2002) A cost-efficient application for plasmid DNA purification using a 96 deepwell DNA binding filter plate, Program No. 609.9, Abstract view/Itinerary Planner, Washington, DC: Society for Neuroscience, Online.

Sambrook, J., E. F. Fritsch and T. Maniatis, (1989) Molecular cloning – a laboratory manual, p14.5-14.13, Cold Spring Harbor Laboratory

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