High Throughput PCR Cleanup Using 384 Well Glass Fiber Filter Plates and Positive Pressure on an Automated Workstation

James Batchelor, Lynn Jordan, and Ed Alderman, Zymark Corporation, 68 Elm Street, Hopkinton, MA 01757
Helen L. Krasnow and Debra S. Hoover, Corning Life Sciences, 2 Alfred Road, Kennebunk, ME 04043

INTRODUCTION

Separation of PCR products from residual primers can be accomplished in a high throughput manner using a glass fiber matrix. This procedure relies upon the differential binding of primers and PCR products to the glass fiber under high salt conditions. After washing the PCR products are eluted from the matrix.

Filter plates combined with a Positive Pressure Filtration Unit allow these procedures to be easily automated. Positive pressure is an attractive option for use with filter plates, as it can generate higher pressures than vacuum filtration, reduces liquid hold up volume and maximizes sample recovery, can minimize the potential for aerosol formation, and simplifies eluate collection.

Materials

Instrumentation

Liquid transfers were performed with a Sciclone ALH 3000 (Zymark) equipped with a 100µL Automation Certified Pipette Tips (Zymark) and a Bulk Reagent Dispenser. A Positive Pressure Filtration Unit (Zymark) was installed on the deck along with a Deck Mounted Shaker (Zymark).

Reagents

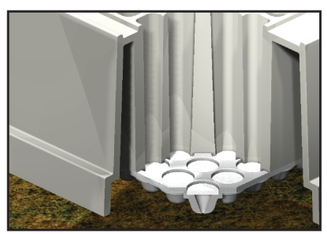
De-ionized Water
4M Potassium Iodide
10mM Tris (pH 8.0)
80% (v/v) Ethanol
Human beta-Actin cDNA

Microplates

384 well FiltrEX™ Glass Fiber Filter Plate (Corning # 3533)
384 well Polypropylene Microplate (Corning #3656)

Figure 1 - FiltrEX™ 384 well filter plate construction.

Patented nozzle design and individually integrally sealed filter disks prevents filtrate cross contamination. Rigid design and wide skirt allows for robotic handling and bar-coding.



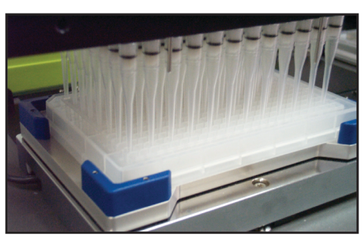
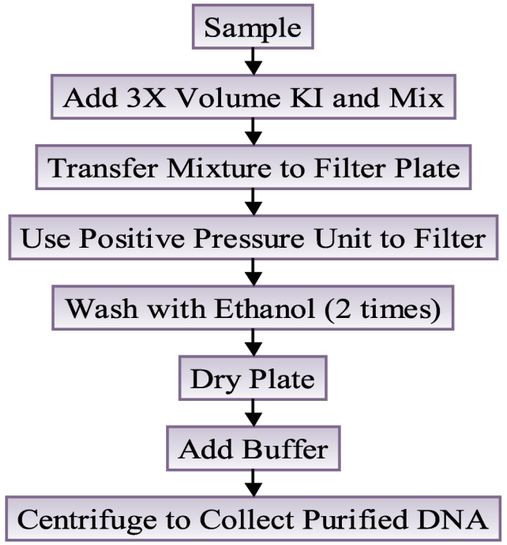


Figure 2 - 384 well microplate on the deck mounted shaker.

This device is a variable speed, orbital shaker which holds a standard SBS footprint plate.



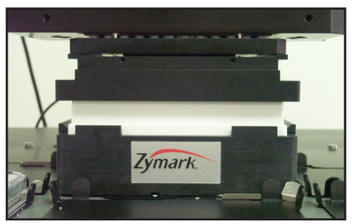


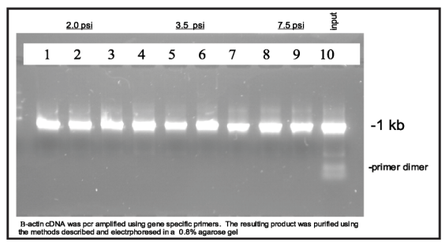
Figure 3. FiltrEX™ 384 well Filter Plate in the Positive Pressure Filtration Unit

The upper portion attaches to the head like disposable tips. The bottom portion has a Drain line leading to a carboy to collect waste.

Results

Gel electrophoresis of the purified PCR products is shown in Figure 4. Lanes 1-3 were purified using 2.0 psi positive pressure filtration, lanes 4-6 with 3.5 psi positive pressure filtration, and lanes 7-9 are with 7.5 psi positive pressure filtration. 400ng of input PCR product (unfiltered control) is shown in lane10. Purification of PCR product resulted in an average recovery of 82% at all filtration pressures tested (Figure 5). DNA purified using this method can be readily sequenced and > 600 bases read; typical results are shown in Figure 6.

Figure 4. Recovery of Purified PCR Products



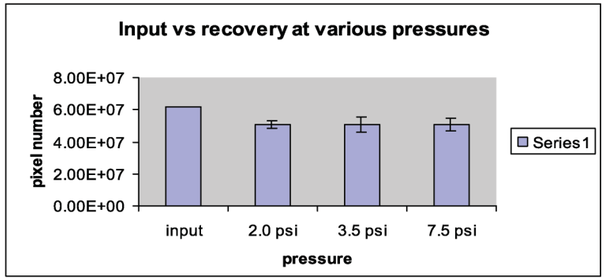


Figure 5 - Amplified pcr products (400ng input) were purified by positive pressure filtration at the indicated pressures. DNA recovery was measured by densitometry. The average recovery was 82%.

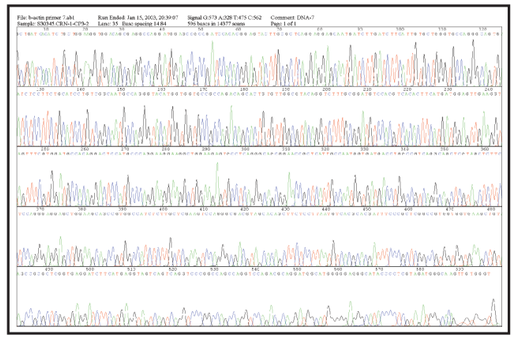


Figure 6 - A 1 kb fragment of B-actin was pcr amplified and purified using positive pressure. (see methods)

The purified fragment was sequenced by cycle sequencing using the Big Dye v. 3.1 sequencing kit (ABI). The sample was run on an ABI 3700 sequencer.

Discussion

Automated purification of PCR products can be achieved using a FiltrEX™ Glass Fiber Filter Plate and automated liquid handler equipped with a Deck Mounted Shaker, Bulk Dispenser, and a Positive Pressure Filtration Unit. All liquid transfer, mixing, and filtration steps were performed without the need for microplate transport.

The results demonstrate that positive pressure filtration and glass fiber filter plates successfully separates the PCR product from the unincorporated primers and nucleotides and results in high yields and long sequence reads. The combination of the automated liquid handler and FiltrEX™ filter plates results in a system that can purify PCR products in a high throughput environment.

CORNING
Discovering Beyond Imagination

Zymark