# Rapid Efficient Purification of Both Plasmid and PCR DNA Using Corning Glass Fiber Filter Plates

Hong Jiang, M.A., Larry Vaughan, Ph.D. and Linda Belkowski, Ph.D.\* Corning Incorporated, Life Sciences, Kennebunk, ME and \*Acton, MA

# Abstract

Most commercially available approaches to purifying DNA rely on selective binding of DNA to a matrix followed by washing and elution. The recent trend has been away from single tube methods toward higher throughput formats. Although several commercial methods exist, they often lack robotic friendliness or cost effectiveness for high throughput applications. We have addressed these requirements with a low cost glass fiber filter plate, which conforms to the SBS standard footprint and is therefore compatible with fluid handling and robotic devices. Each well consists of an integrally sealed glass fiber disk



with a high flow membrane beneath to eliminate fiber shedding. This construction and the plate's proprietary nozzle design eliminate well to well cross-talk both in the filter plate as well as the receiver. In this communication, we report the development of simple, rapid methods for PCR fragment and plasmid DNA purification using this plate. In both cases superior quality DNA was obtained with low CVs and high yields. Quality was confirmed by sequencing, and exceeded that of many commercially available kits at a fraction of their cost. This approach is ideally suited to large scale DNA purification for both sequencing and array content preparation.

# Methods

## Plasmid Purification:

1. Grow overnight cultures (1 mL) of Escherichia coli in individual wells of a 2 mL block (Corning #3960), harvest by centrifugation (1500 x g for 5 minutes) and carefully decant the media.

2. Add 50 µL of Solution I (50 mM Glucose, 10 mM Tris, pH 7.5, 1mM EDTA, pH 8, 90 µg/mL RNase A) to each well. Vortex to resuspend the cells .

3. Add 100 µL of Solution II (0.2 N NaOH, 1% SDS) to each well. Mix gently.

4. Add 150 µL of Solution III (4 volumes of 5 M potassium acetate, 1 volume of 10 M (57% v/v) glacial acetic acid) per well, mix, and incubate at room temperature for 5 minutes. 5. Add 380 µL of 6 M potassium iodide to the lysate and mix thoroughly.

6. Transfer 400 uL of lysate from each well of the block to a well of a 0.2 um PVDF Filter Plate

(Corning # 3504). Place a glass fiber Filter Plate (Corning #3511) beneath the PVDF plate in a vacuum filtration manifold. Apply vacuum for 1 minute to bind the DNA to the glass fiber Filter Plate.

7. Wash the wells twice with 200 µL of 80% ethanol. After the second wash, dry the plate under vacuum for 2 minutes. Tap the plate to remove any residual ethanol droplets in the nozzle. Place the plate on its side and air dry at room temperature for 90 minutes.

8. Elute the DNA with 80 µL of 10 mM Tris, pH 8.5. Place a clean collection plate (Corning #3363) under the filter plate in the vacuum manifod and apply vacuum for 2 minutes to elute the bound DNA.

# PCR Clean-up:

1. Combine the PCR products with 3 volumes 6M KI.

2. Apply to a glass fiber filter plate (Corning # 3511) and filter under vacuum for 1 minute. 3. Wash the bound DNA twice with 100 µL of 80% ethanol. After the second wash, dry the plate under vacuum for 2 minutes. Tap the plate to remove any residual ethanol droplets in the nozzle.

4. Place the plate on its side and air dry at room temperature for 90 minutes.

5. Elute the DNA by adding 80 µL of 10 mM Tris pH 8.5 per well and applying vacuum for 2 minutes.

# Average yield: 4 µg of supercoiled plasmid DNA from a 1 ml culture.

2 3 4 5 6 7 8

Figure 1. Plasmid DNA samples. Plasmid DNA was recovered in ~ 55 µL/well. 2 µL plasmid DNA was loaded per lane. Lanes 2-8 contain DNA from 7 randomly selected wells of the Filter Plate.

### >85% Fragment recovery >95% Primer removal



Figure 3. PCR samples purified using Corning Glass Fiber Filter Plate. Lane 4 contains the PCR reaction before purification (an excessive amount of primer was added to demonstrate the efficiency of purification).

A

B

Figure 5. Sequence analysis of purified (B) and unpurified (A) PCR products. DNA was sequenced using a PE 3700 Sequencer and a Big Dye<sup>™</sup> sequencing kit.

# Conclusions



•Plasmid prep and PCR clean-up with Corning filter plates result in consistent yields of high quality DNA as evidenced by sequencing •Optimized plasmid prep protocol yields a less viscous lysate that is reproducibly transferred either manually or using robotics •Procedures require minimal working time, utilize common lab reagents, and offer significant cost savings (>90%) over commercial kits Integrally sealed wells and proprietary nozzle design eliminate sample cross contamination.

•Automation friendly - plates meet proposed industry standards

# **Results - Plasmid DNA purification**

Results - PCR clean-up

Sequencing of 580 error-free bases is routinely obtained. Sector Contractions adde in thick there have done a. allowed all the state of the state of the

## Figure 2. Sequencing Analysis of Plasmid DNA prepared with Corning Plates.

Plasmid DNA was purified as described and its sequence determined using an ABI Prism®377 Sequencer and a Big Dye<sup>™</sup> sequencing kit.

# Size fractionation can be achieved by varying [KI]



1 2 3 4 5 6 7 8 9

Figure 4. Recovery of DNA fragments of different sizes by varying [KI]. 10 µg DNA ladder was loaded in each well of the plate after mixing with KI.