

High Throughput Plasmid DNA Preparation Using PVDF Filter Plates On An Automated Workstation

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Introduction

Filter plates are a versatile and essential component for high throughput DNA isolation. Liquid handlers and centrifuges have been the instruments of choice for these purification procedures. Today's liquid handlers can perform multiple steps of the process, and interface with other external devices for down stream applications such as sequencing through the use of a robotic plate handler.

In this communication, we demonstrate a single step plasmid purification using a commercially available 96 well filter plate with an automated liquid handler. This method, based on a modified alkaline lysis protocol, enables rapid DNA purification with consistent yields of high quality DNA. Developments in automated liquid handlers and filter plate design and construction are significantly improving high throughput genomic sample preparation.

Materials

Instrumentation:

Liquid transfers were performed with a Sciclone ALH 3000 equipped with 100µL Automation Certified Pipette Tips, Bulk Reagent Dispenser, and a Deck Mounted Shaker (Zymark).

Centrifugation was performed using an Eppendorf Centrifuge Model 5804.

Plate drying was done using a VWR vacuum Oven Model 1430.

Microplates:

96 well FiltrEX PVDF filter plate (Corning # 3505)

96 well microplate (Corning #3359)

Reagents:

Solution I: 0.25% glucose/10 mM EDTA/22.5 mM Tris/ pH 8.0

Solution II: 0.2 N NaOH/0.7% SDS/0.1% Triton X100

Solution III: 3M KoAC

Isopropanol

70% EtOH

10 mM Tris, pH 8.0

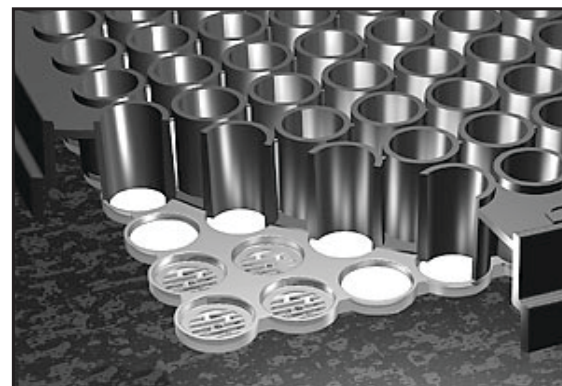


Figure 1. FiltrEX™ 96 well filter plate construction

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.

Method

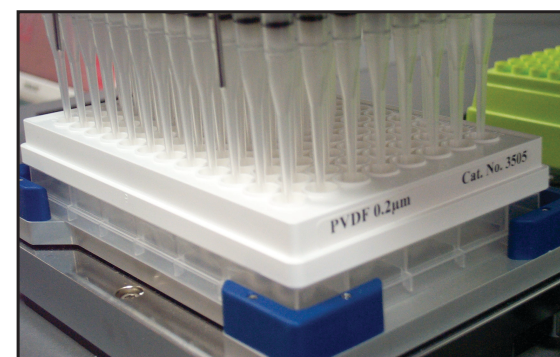
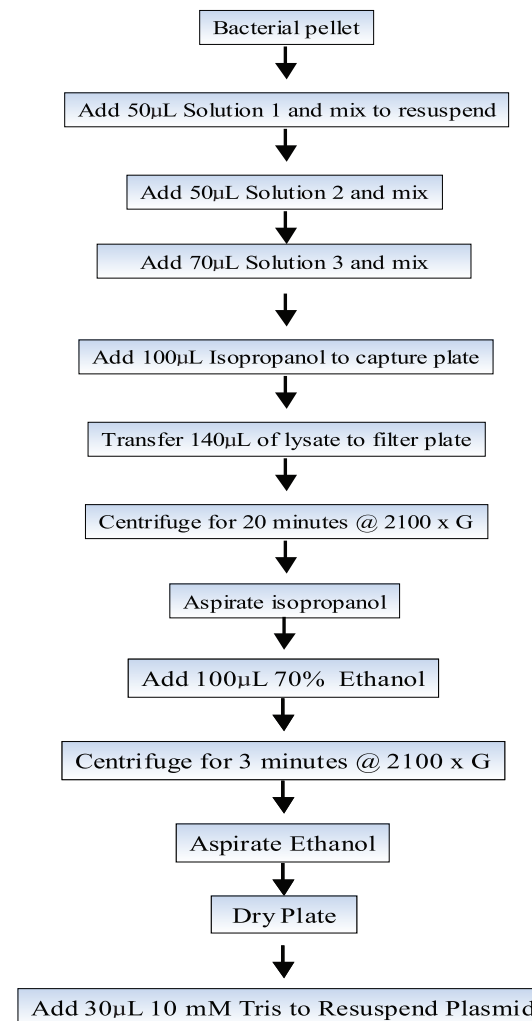


Figure 2. 96 well microplate on the deck mounted shaker.

This device is a variable speed, orbital shaker which holds a standard SBS footprint plate. The shaker has a self-centering motion to assure it stops in the same location everytime.

Results

The purified plasmid is verified through a plasmid digest (Figure 3) and through sequence analysis on an ABI 3700 sequencer (Figure 4).

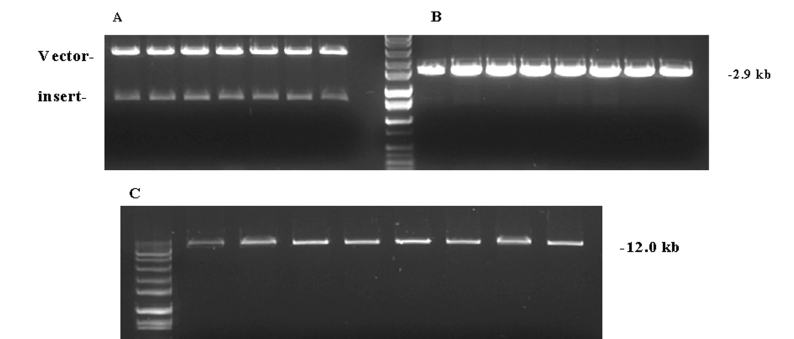


Figure 3. Plasmid Digest.

DNA generated using a modified alkaline lysis protocol (see methods) was digested with Eco RI to produce either a 2 kb fragment (A) or linearized plasmid (B and C).

The plasmid sizes are: A. 4.0 kb, B. 2.9 kb, C. 12 kb.

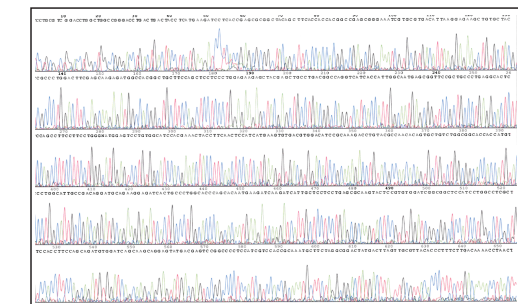


Figure 4. Sequence analysis

Representative chromatogram generated using DNA purified with the Corning 96 well FiltrEX PVDF filter plate and the Zymark Sciclone ALH 3000 workstation. DNA was sequenced with Big Dye version 3.0 on an ABI 3700.

Discussion

Automated purification of plasmid DNA can be achieved with a FiltrEX 96 well PVDF filter plate using a Sciclone ALH 3000 equipped with a Deck Mounted Shaker and a Bulk Dispenser. All liquid transfer and mixing steps were performed without the need for microplate transport. Throughput can be readily increased by incorporating an on deck centrifuge and adding plate moving capabilities.

Our results demonstrate that high yields of sequencing quality plasmid DNA can be produced using FiltrEX PVDF filter plates and the Sciclone ALH 3000 liquid handling system.

