Video Article

Streamlined Purification of Plasmid DNA From Prokaryotic Cultures

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Abstract

We describe the complete process of AcroPrep Advance Filter Plates for 96 plasmid preparations, starting from prokaryotic culture and ending with high purity DNA. Based on multi-well filtration for bacterial lysate clearance and DNA purification, this method creates a streamlined process for plasmid preparation. Filter plates containing silica-based media can easily be processed by vacuum filtration or centrifuge to yield appreciable quantities of plasmid DNA. Quantitative analyses determine the purified plasmid DNA is consistently of high quality with average $OD_{260/280}$ ratios of 1.97. Overall, plasmid yields offer more pure DNA for downstream applications, such as sequencing and cloning. This streamlined method of using AcroPrep Advance Filter Plates allows for manual, semi-automated or fully-automated processing.

Video Link

The video component of this article can be found at http://www.jove.com/video/2407/

Protocol

1. Lysate Clearance

1. Grow *E. coli* cultures transformed with desired plasmid DNA in deep well plates (1 mL of culture/well) in Luria Broth with appropriate antibiotic overnight at 37 °C with shaking.
2. Pellet E. *coli* in culture plate at 5,000 x g for 10 minutes, then decant supernatant.
3. Resuspend each pellet in 100 µL Resuspension Buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA,100 µg/mL RNase A).
4. Add 100 µL/well Lysis Buffer (200 mM NaOH, 1% SDS) and shake using plate shaker for 2 minutes.
5. Add 100 µL/well Neutralization Buffer (3.0 M Potassium Acetate, pH 5.5) and shake for 2 minutes.
6. Transfer cell lysate to lysate clarification plate.
7. Place DNA binding plate on top of 350 µL collection plate and place into bottom of vacuum manifold. Assemble vacuum manifold (see Figure 1).
8. Place lysate clarification plate on top of vacuum apparatus.
9. Apply vacuum at 10 in. Hg (0.34 bar) and collect filtrate into DNA binding plate. Do not use vacuum greater than 12 in. Hg. (0.41 bar).
10. Disassemble vacuum manifold. Place 2 mL waste collection plate in bottom of apparatus.

2. DNA Binding

1. Move the DNA binding plate to the top of the manifold (see Figure 2).
2. Add 300 µL/well Binding Buffer (6 M Guanidine-HCl) and pipette up and down to mix.
3. Apply vacuum [~ 5 in. Hg (0.17 bar) for slow vacuum] and discard filtrate. DNA is now bound to membrane.
4. Wash with 400 µL/well of Wash Buffer (80% Ethanol).
5. Apply vacuum, then discard the filtrate. Discard unnecessary if using 2 mL collection plate or filtering directly to waste.
6. Repeat wash once.
7. Blot bottom of filter plate on absorbent towel to dry.
8. Apply vacuum once more for 5-10 minutes to ensure removal of residual alcohol.
9. Blot bottom to ensure removal of ethanol droplets.

3. DNA Elution

1. Add 70 µL/well Elution Buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). If higher DNA concentration is desired, volume can be reduced to 50 µL/well, but the average total yield will be lower.
2. Incubate the plate at room temperature for one minute.
3. Purified DNA can be eluted by either vacuum or centrifugation.
4. Vacuum method: Place clean collection plate (DNase, RNase free) into vacuum manifold. Place filter plate on top of vacuum manifold, apply vacuum at 15 in. Hg (0.5 bar) for 1 minute until all elution buffer has passed through the DNA binding plate. Collect purified DNA (see Figure 3).

5. Centrifugation method: Place purification filter plate on top of clean collection plate and centrifuge at 1,000 x g for 5 minutes.

4. Quantitative and Qualitative Analyses

1. Measure $OD_{260/280}$, calculate concentration of purified DNA.
2. Perform agarose gel analysis of the purified pDNA.

5. Representative Results

E. coli lysate clarified using traditional centrifugation and Pall AcroPrep Advance lysate clarification Filter Plates showed appreciable reduction of debris from starting material. (Figure 4).

Plasmid DNA at three concentrations was spiked into TE buffer and passed through the AcroPrep Advance lysate clarification filter plate. A comparison of plasmid DNA concentration, pre- and post-filtration, shows ~100% recovery (Figure 5) at all three concentrations.

To demonstrate the recovery of DNA from clarified *E. coli* lysate, plasmid DNA was spiked into crude lysate and then clarified by filtration. The same plasmid DNA was added to cell lysate following filtration. Electrophoresis was performed on aliquots from each sample (Figure 6). A single, clear band of similar intensity from each sample demonstrates little if any DNA loss during the filtration-based clarification step.

Cultures of *E. coli* containing pCAT plasmid DNA were purified from *E. coli* lysates using Pall's 350 µL and 1 mL DNA binding Filter Plates, as well as two other brands of 350 µL DNA binding plates. All purifications were performed as specified in the manufacturer's recommended protocol. The highest DNA concentration and total yield are seen from Pall's 1 mL AcroPrep Advance DNA purification filter plate (Figure 7 and Table 2).

Although Competitor W also shows high DNA concentration (155 ng/µL), total DNA yield is the lowest (4.7 µg/well) due to the low recovery volume, ~30 µL. Competitor M gave the lowest concentration of DNA at 108 ng/µL with a total yield of 5.6 µg/well.

Agarose gel analysis of purified pCAT DNA from the three different DNA binding plates is shown in Figure 8. Each collected sample volume was adjusted to 65 µL to correct for differences in recovery volume prior to electrophoresis. All samples show supercoiled plasmid DNA, but DNA yield from each of the Filter Plates varies.

Figure 9 shows the prepared DNA is all in supercoiled form and has similar concentration.

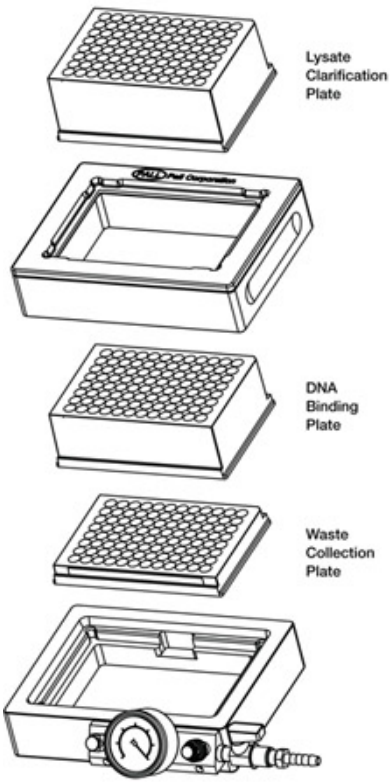

Figure 1. Assembly of Vacuum Manifold for Lysate Clarification.

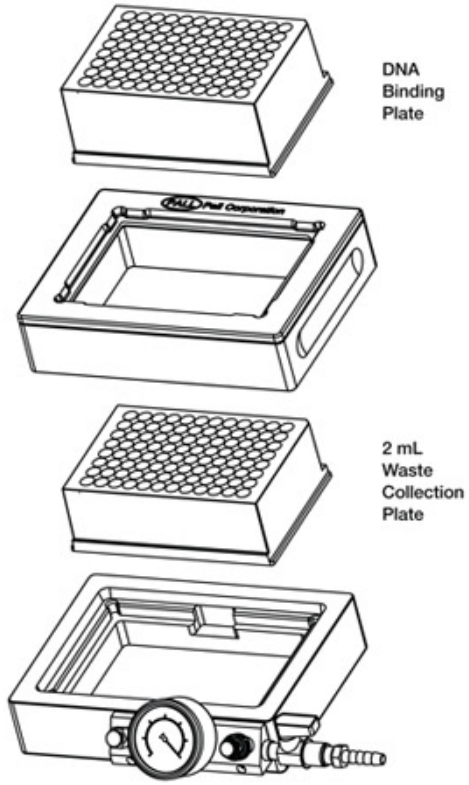

Figure 2. Assembly of Vacuum Manifold for DNA Binding.

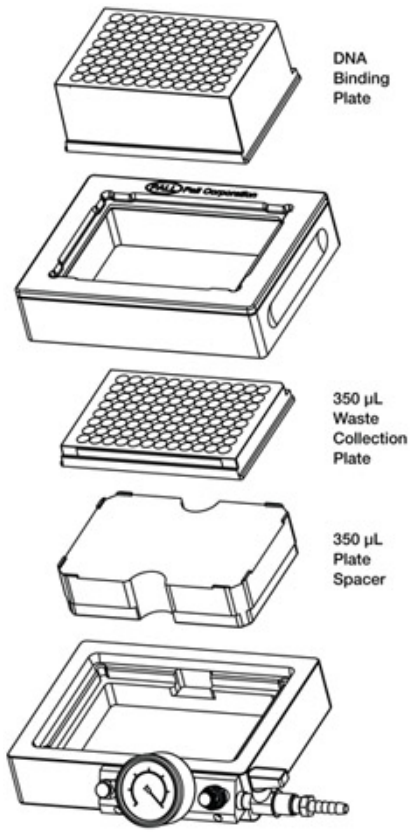

Figure 3. Assembly of Vacuum Manifold for Vacuum Elution.

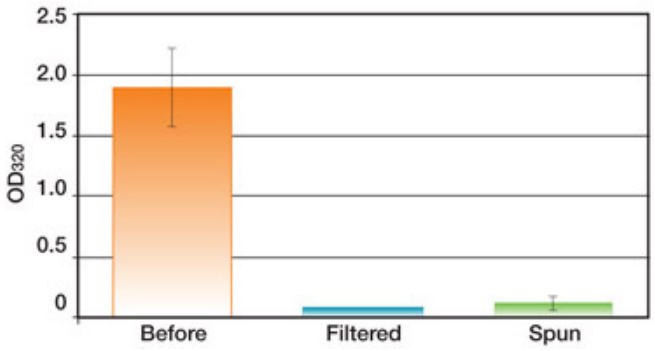

Figure 4. Filtration simplifies sample clarification. Triplicate samples of 300 µL of crude lysate clarified by vacuum filtration (350 µL AcroPrep Advance lysate clarification filter plate) or traditional centrifugation. OD_{320} measured before and after clarification.

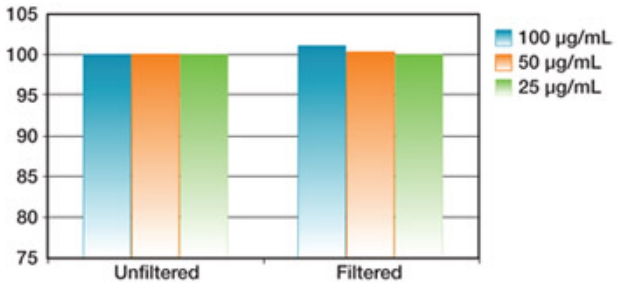

Figure 5. Full recovery of pDNA after passage through AcroPrep Advance lysate clarification filter plate. 300 µL TE buffer spiked with pCAT at 25, 50 and 100 µg/mL. DNA concentration and recovery calculated from OD_{260} before and after filtration, N = 2.

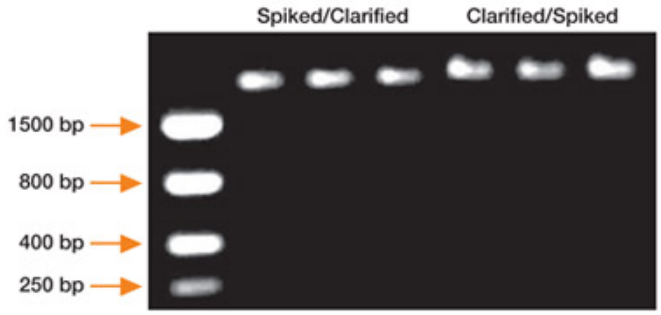

Figure 6. Recovery of pCAT DNA after clarification. Equal amounts of pUC19 spiked into lysate before or after clarification, then diluted 1 to 10 in TE buffer. Loaded 2 µL/lane on 1.2% Agarose gel.

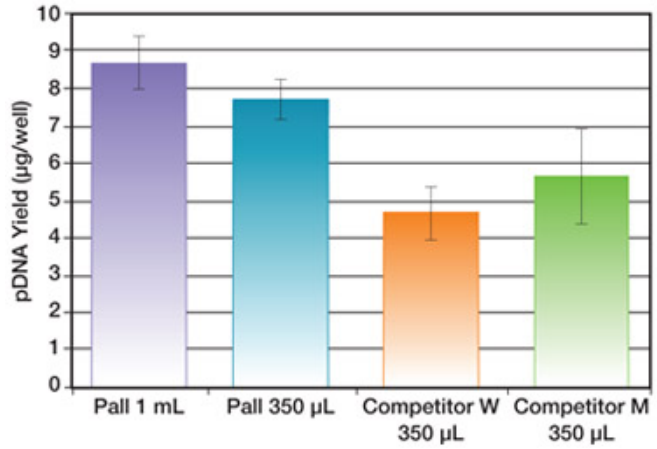

Figure 7. Higher plasmid DNA yield per well with pall Acroprep Advance DNA binding filter plate than competitor plates. pCAT plasmid DNA yield (OD_{260}) using indicated DNA purification plates with 1 mL (Pall and Competitor M) or 1.5 mL (Competitor W) overnight culture of DH5α. Purification using plate manufacturer's recommended protocol. Error bars indicate standard error (n > 6).

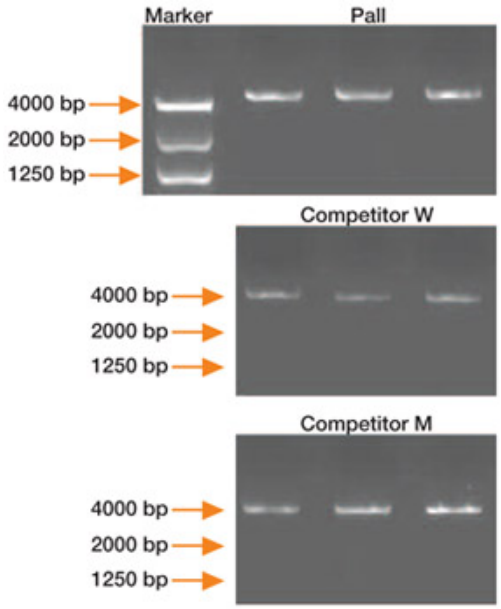

Figure 8. Agarose gel analysis of pCAT DNA purified on indicated DNA binding plate. 1.2% agarose gel electrophoresis of the purified pDNA. Pooled samples from three separate purifications, eluate adjusted to 65 µL after purification, diluted 1:10. Loaded 2 µL per lane.

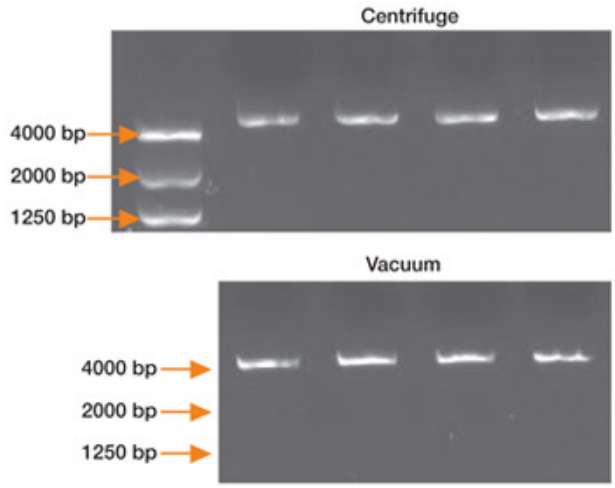

Figure 9. Quality and recovery of pure dna similar when centrifuge or vacuum methods are used for final eluate collection agarose gel electrophoresis of the purified pDNA. The manufacturer's recommended protocol for AcroPrep Advance Filter Plates was followed for pDNA purification. Elution was performed by centrifuge at 1,000 x g for 5 minutes or vacuum with 15 in. Hg (0.5 bar) for 2 minutes.

Discussion

We describe a facile, streamlined protocol of AcroPrep Advance Filter Plates that affords several advantages. This method provides maximum plasmid DNA yields when processed in manual, semi-automated, or fully-automated AcroPrep Advance formats. An integrated prefilter on the lysate clearance plate provides consistent filtration of samples, even those containing high levels of particulates. The well geometry of the plate results in faster, more uniform filtration rates across the plate with minimal hold-up volume; and the outlet tip geometry provides direct flow of samples into receiver plate without concerns of cross contamination. Importantly, the high binding capacity membrane in DNA binding plate of AcroPrep Advance Filter Plates is optimized for maximum recovery of high quality plasmid DNA.

Disclosures

The authors of this video-article are employees of Pall Life Sciences who produce the instrument and reagents featured in the video and text.

References