Purification of Plasmid DNA Using Corning® Glass Fiber Filter Plates

Application Report

Innovative Techniques in Drug Discovery

CORNING

Life Sciences

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Automated manipulation and analysis of cloned DNA requires plasmid purification methods that can be readily adapted to 96 well plate formats. In addition to being compatible with robotic plate and liquid handling systems, these methods must generate sufficient quantities of high purity DNA for subsequent applications such as sequencing. While quantity and quality of DNA prepared by these methods are critical, the method must also be cost effective.

Methods for plasmid purification generally involve alkaline bacterial lysis followed by separation of the plasmid from cellular debris and chromosomal DNA. Older methods that employed organic extraction, alcohol precipitation, and density gradient centrifugation have largely been replaced by chromatographic separation using silica or ion exchange. These newer methods take advantage of the differential binding of plasmid DNA to the resin, enabling impurities to be washed away prior to elution of the plasmid.

Corning has incorporated DNA adsorbing glass fiber into a 96 well Filter Plate. The plate design meets the recommendations of the Society for Biomolecular Screening, assuring compatibility with automated plate handling and liquid dispensing equipment. In addition they offer substantial time and cost savings and can be adapted to automated format.

In this report, we describe a method for the isolation of plasmid DNA (pCR3.1 containing a 1.5 kb insert) from *Escherichia coli* strain Top10F' using Corning glass fiber (Cat. No. 3511) filter plates that is a simple, economical alternative to the use of plasmid preparation kits. The entire procedure can be completed in approximately 30 minutes. It is also well suited to automation as the entire process, beginning with growth of individual bacterial cultures, is carried out in a 96 well format. This method routinely yields 4 µg of supercoiled plasmid DNA per mL of bacterial culture. Purity of DNA prepared in this manner is confirmed by OD_{260}/OD_{280}

which is typically ≥1.9 and the ability to sequence and read 550 bases or more.

Procedure

1. Inoculate 1 mL of Luria Broth (LB) plus appropriate antibiotic with a single colony of *E. coli* into each well of a Corning® 2 mL block (Cat. No. 3960) and grow for 16 h with shaking (150 rpm).
2. Harvest the cells by centrifugation (1500 x g) for 5 minutes and carefully decant the media. Remove residual traces of media by inverting the block and tapping it gently on tissue paper.
3. 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. Seal the block with aluminum tape (Corning Cat. No. 6569) and vortex to resuspend the cells. *Ensure that cells are thoroughly resuspended and that no clumps remain.*
4. Remove the seal from the block and add 100 µL of Solution II (0.2 N NaOH, 1% SDS) to each well. Shake gently by hand to mix and incubate at room temperature until the solution in the wells is completely clear (*do not exceed 5 minutes* or DNA denaturation may occur).
5. 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 thoroughly by shaking gently and incubate at room temperature for 5 minutes.
6. Add 380 µL of 6 M potassium iodide to the lysate and shake by hand to mix thoroughly. *Inadequate mixing will result in low yields.*
7. Insert pipette tips to the bottoms of the wells and carefully transfer 400 µL of lysate from each well of the block to a well of a Corning 0.2 µm PVDF Filter Plate (Cat. No. 3504). While transfer of small amounts of cell debris will not affect results, try to leave as much behind as possible.
8. Place a Corning 0.66 mm glass fiber Filter Plate (Cat. No. 3511) beneath the 0.2 µm PVDF plate in a vacuum filtration manifold. Apply vacuum for 1 min to collect the DNA solution into the wells of the glass fiber Filter Plate.
9. Wash the wells twice with 200 µL of 80% ethanol, applying the vacuum briefly to remove the alcohol. Following the second wash, run the vacuum for 3 minutes in order to remove as much ethanol as possible. Remove the plate from the manifold and tap it firmly (in the upright position) on a clean tissue to remove any residual alcohol from the filter nozzles. Repeat the vacuum and blotting steps to remove any remaining alcohol. Place the plate on its side and air dry for 60 to 90 minutes.
10. Add 80 µL of 10 mM Tris, pH 8.5 to each well of the plate. Place a clean collection plate (Corning Cat. No. 3363) under the filter plate in the vacuum manifod and apply vacuum for 2 minutes to elute the bound DNA.
11. Carefully remove the filter plate. Seal the collection plate (Corning Cat. No. 6569) and store the DNA at -20°C until ready for use.

Results and Discussion

Bacterial colonies were grown in standard media (Luria Broth) prior to lysis. It is important not to use rich media (such as Terrific Broth or 2X LB) as *E. coli* cultures grown under these conditions tend to contain high quantities of proteins that make plasmid isolation more difficult.

Gel electrophoresis of plasmids purified using a Corning glass fiber Filter Plate is shown in Figure 1. For this experiment, individual colonies were grown up and DNA isolated as described above. Plasmids were recovered in a total volume of approximately 55 µL/well; 2 µL of purified plasmid was loaded into each lane of the gel.

Plasmid yield was determined by UV absorbance using a Corning UV Plate (Cat. No. 3635). Average yields were 4 µg of supercoiled plasmid DNA from a 1 mL culture. A_{260}/A_{280} for DNA prepared in this manner is typically ≥ 1.9

In order to further ascertain the purity of the plasmid samples they were subjected to automated sequencing using an ABI Prism® 377 instrument. Typical results are shown in Figure 2. In this case, 580 error-free bases were read prior to stopping the instrument.

We have modified the traditional alkaline lysis method so that the two critical steps in the process; clarification of the bacterial lysate (by the 0.2 µm PVDF filter plate) and final purification of plasmid DNA (by binding to and elution from the glass fiber plate) are optimized. The small pore size of the PVDF membrane ensures that the bacterial lysate is effectively clarified so that microaggregates do not interfere with DNA binding to the glass fiber plate or contaminate the final DNA preparation. The composition and proportions of solutions I, II and III used here result in a non-viscous lysate that is easy to pipette. This ensures consistent transfer of the desired volume to the filter plate.

Effective binding of plasmid DNA to the glass fiber plate is achieved when the lysate is combined with the binding (KI) solution. The binder-free borosilicate glass in the glass fiber filter plate has a high binding capacity and excellent elution profile for plasmid DNA. Most commonly used methods recommend mixing the binding solution and the lysate in the glass fiber plate. This requires multiple

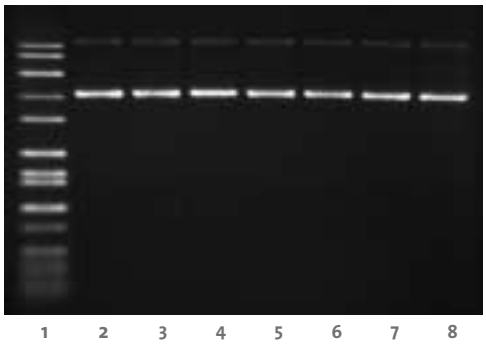

Figure 1. Agarose gel electrophoresis of Plasmid DNA prepared using Corning Filter Plates. Plasmid DNA samples were prepared as described in the text and separated in a 1% agarose gel in 1X TAE buffer. Lane 1 contains 10 µL of Hi-Lo™ markers (total DNA = 1 µg). Lanes 2-8 contain plasmid preparations isolated using 7 different wells of the Filter Plate. Plasmid DNA was recovered in approximately 55 µL total volume. 2 µL of recovered plasmid were loaded in each lane of the gel.

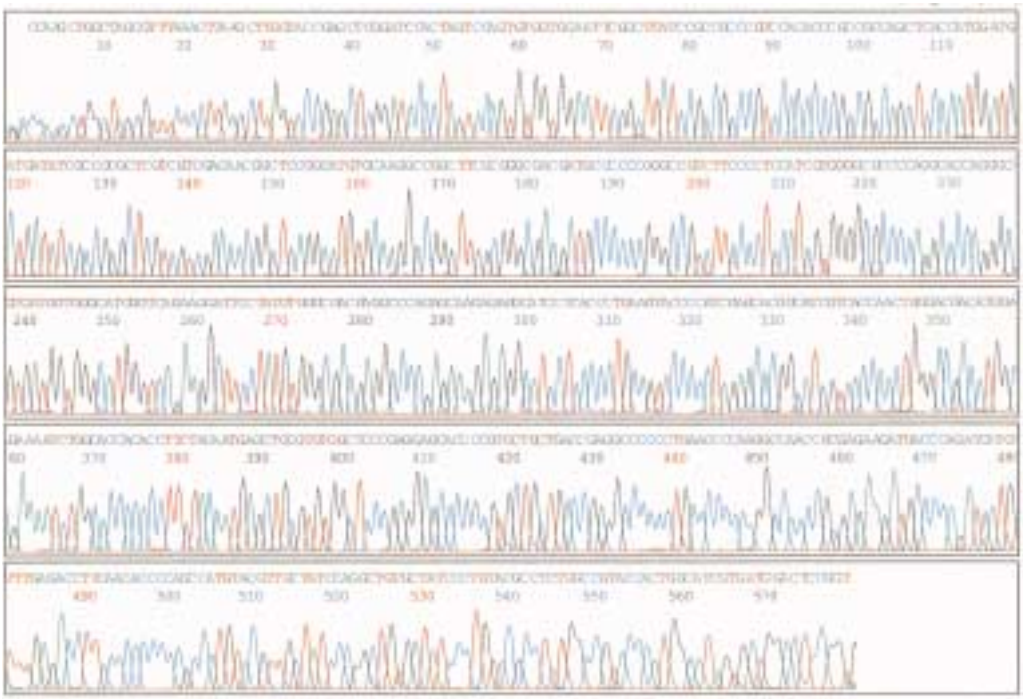

Figure 2. Sequence Analysis of Plasmid DNA prepared with Corning Plates. Plasmid DNA was purified as described and its sequence determined using an ABI Prism® 377 Sequencer and a BigDye™ sequencing kit.

pipetting of solutions to ensure thorough mixing and can lead to inconsistent binding of the DNA to the glass fiber. We have found that addition of the binding solution directly to the lysate mix (Step 6 in the procedure) confers a number of advantages. First, it makes the process more convenient and easier to automate (multiple blocks can be processed at the same time and placed on a table shaker to mix the contents). Second, addition of the high salt binding solution to the neutralized lysate promotes protein precipitation and results in further clarification of the DNA solution (by the PVDF filter plate) prior to binding to the glass fiber. This results in higher yields and purity of the final DNA preparation. In addition, more DNA lysate can be loaded onto the glass fiber plate after the first 400 µL has been filtered through. This is of particular benefit if low copy number plasmids are being purified.

It is important that all residual ethanol be removed from the wells of the plate prior to elution of the DNA. Incomplete removal will make it difficult to recover all of the eluate from the filter plate nozzles and residual ethanol can interfere with further manipulations. The drying step can be shortened by incubating the plate at 37°C for 20 minutes.

Plasmid preparation with Corning glass fiber Filter Plates is simple and easily automated. All of the Corning® plates used (blocks for bacterial culture, filter plates, collection plates and UV plates) are built to the SBS footprint to assure compatibility with robotic equipment. The procedure gives good yields of DNA which can be reliably sequenced. In our laboratory we are routinely able to read >550 bases of sequence from plasmid DNA prepared using this method. Quantitative yield and DNA quality are comparable to DNA prepared using commercial reagent kits that have a significantly higher cost per prep.

Corning Product Ordering Information

Cat. No.	Description	Qty/Pk	Qty/Cs
3363	V-bottom Polypropylene 96 well plate	25	100
3504	96 well white polystyrene filter plate, 0.2 µm PVDF hydrophilic membrane	10	50
3511	96 well white polystyrene filter plate, 0.66 mm Glass Fiber	10	50
3960	Polypropylene 96 well block, 2mL, V-bottom (sterile)	5	25
6569	Aluminum sealing tape	Bulk	100

For customer service or technical support, please call **1.800.492.1110**. For additional product information, visit our website at **www.corning.com/lifesciences**