Volume 6 No. 39

TECH NOTE

Cost-efficient plasmid DNA purification using the NUNC™ 96 DeepWell™ DNA binding filter plate

Abstract

NUNC™ has developed a 96 DeepWell™ DNA binding filter plate and method for the rapid purification of high quality plasmid DNA. Bacterial cultures were incubated in 2 ml sterile DeepWell reception plates. Following standard alkaline lysis and neutralization, plasmid DNA was clarified using filter plates containing high retention, fast flow frits. Plasmid DNA was then isolated using a 96 DeepWell filter plate containing a plasmid DNA-binding filter matrix. Following optimization of the wash buffer components, up to 12.16±0.72 µg/well of purified plasmid DNA was recovered. An A_{260}/A_{280} ratio of 1.97 confirmed the high purity of isolated plasmid DNA. Well-to-well reproducibility was also demonstrated following restriction digestion and agarose gel electrophoresis. The combination of NUNC 96 DeepWell DNA binding plates along with optimized, standard reagents leads to a cost-efficient approach toward large scale, high purity plasmid DNA preparation.

Introduction

Plasmid purification using glass fiber is a popular, simple and efficient method, involving an initial alkaline lysis procedure (Birnboim and Doly, 1979; Sambrook et al, 1989; Engelstein et al, 1998; Skowronski et al, 2000). Plasmid purification kits, including 96 well-format microplates and related solutions, have been developed by several suppliers. However, the price of these kits makes plasmid purification cost-ineffective, particularly when processing a large number of samples.

We have shown it is possible to improve the plasmid purification procedure and reduce the cost per prep. NUNC has developed a cost-efficient plasmid purification system, in which a fritted plate is used for lysate clarification. A DNA purification procedure was optimized for both high quality and quantity of plasmid DNA. The amount of purified plasmid DNA obtained from our glass fiber membrane is similar to that from silica resin, but with a lower risk of mechanical shearing and at a reduced cost.

Material and Methods

Bacterial culture and harvest: *E. Coli* strain HB101, containing pGEM-4 plasmid with a histidine-rich calcium-binding protein cDNA insert, was grown overnight at 37°C with rotating agitation in NUNC sterile 2 ml deepwell plate (NUNC #278743), and pelleted by centrifugation (2000 x g, 5 min.).

Alkaline lysis: After removing the supernatant, the bacterial pellet was resuspended in 100 µl of GTE solution (50 mM glucose, 25 mM Tris-HCl, 10 mM EDTA, 100 µg/ml RNase A, pH 8.0), then treated by 200 µl of lysis solution (0.2 N NaOH, 1% SDS), 200 µl of neutralization buffer (3M potassium, 5M acetate, pH 4.8) and 500 µl of 6M guanidine hydrochloride, sequentially.

Clarification of plasmid DNA: The soluble plasmid DNA was clarified from genomic DNA and lysed cell debris by means of a fritted DeepWell filter plate (NUNC #278011). All buffers and plates were maximized for yield and purity of isolated DNA.

Plasmid DNA binding: The plasmid DNA in clarified lysate was bound to glass fiber membranes in DeepWell filter plates (NUNC™ #278010), optimized for yield and purity.

Wash and elute: After plasmid DNA binding, any impurities were removed by an optimized wash buffer. Finally, the pure plasmid DNA was eluted from the filter with TE buffer.

Results

Following bacterial alkaline lysis, we optimized conditions for lysate clarification, DNA binding, and DNA elution. For lysate clarification, several different frits and filter combinations were assembled into DeepWell filter microplates and compared. The data reveal that fritted plates result in high levels of purity and yield of plasmid DNA. Thus, the fritted plate was selected for plasmid DNA clarification. In the DNA binding plate, four different glass fiber membranes (various pore sizes and different sources) were examined for their DNA binding capability. A_{260}/A_{280} data show that one glass fiber membrane results in higher levels of plasmid DNA in final eluent than other glass fiber membranes, so this filter membrane was employed in the NUNC DNA binding plate.

For the wash step, different wash solutions, containing varying concentration of sodium and ethanol, various pHs (4.6-7.4) and other reagents were compared. The wash solutions containing 80% ethanol yielded the highest levels of plasmid DNA. Thus, 80% ethanol with 10 mM Tris-HCl and 1 mM EDTA (pH 7.4) was selected. This buffered formulation with low concentration of EDTA protects DNA in process and provides favorable conditions for the downstream applications.

Our optimized microplates and protocol reproducibly yielded plasmid DNA of high quality and quantity. The average ratio of A_{260}/A_{280} OD units in 96 wells is 1.97 ± 0.02 (means ± SD) (Fig. 2). The average amount of purified plasmid DNA from 96 wells can be as high as 12.16 ± 0.72 µg/well from 5 ml of bacteria culture (Fig. 3). This means that the total capacity of the binding filter exceeds what could realistically be expected from a standard "miniprep" procedure. Further demonstration of reproducibility from well to well is obtained following agarose gel electrophoresis, which shows equal intensity bands of plasmid DNA.

The purified plasmid DNA is suitable for common downstream applications such as restriction digestion and sequencing. Figure 4 shows equal single bands in agarose gel, after purified plasmid DNAs from different wells were cut by the restriction nuclease Hind III at one site. The purified plasmid DNA is also suitable for sequencing (Fig. 5), (Hofmann et al, 1991).

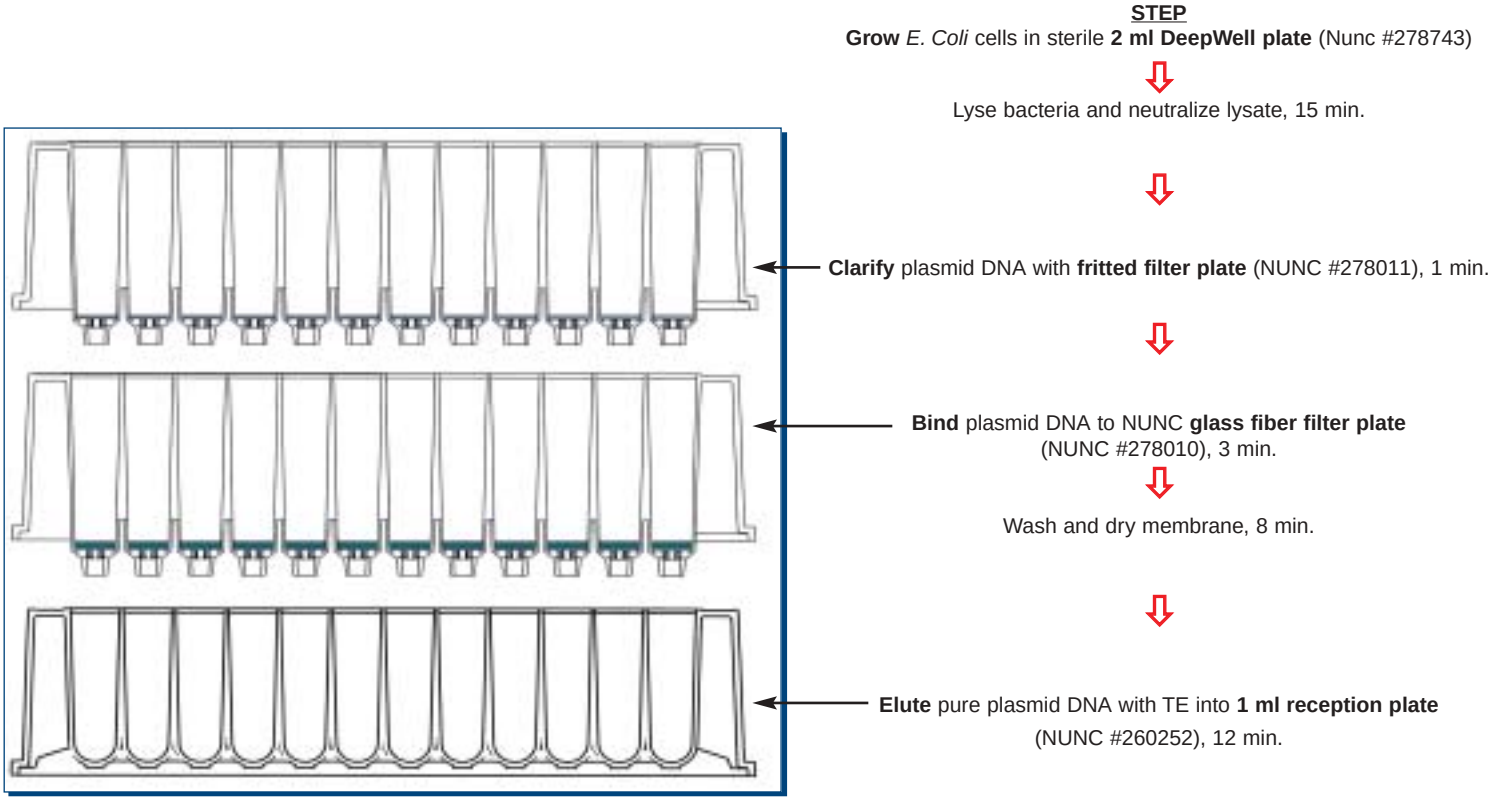

Figure 1

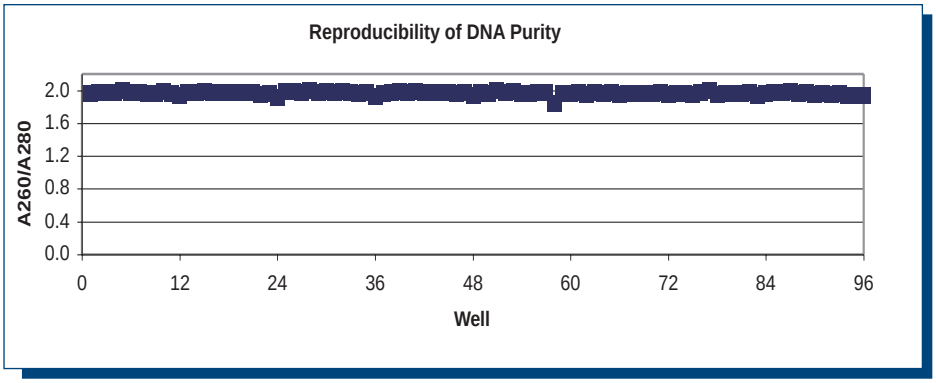

Figure 2 High quality plasmid DNA is confirmed by A_{260}/A_{280} ratio determination. The average A_{260}/A_{280} ratio is 1.97 ± 0.02 (means ± SD) across 96 wells.

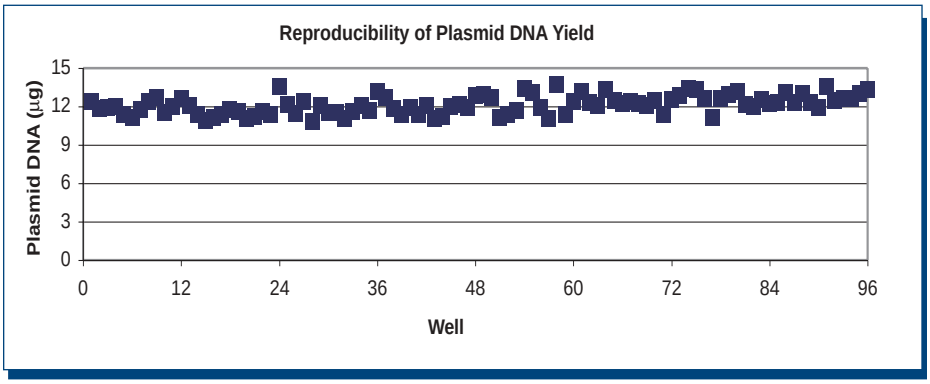

Figure 3 Reproducibility of purified plasmid DNA from 96 wells. The average amount of plasmid DNA from 96 wells is as high as 12.16 ± 0.72 µg/well (means ± SD) from (pooled) 5 ml of bacteria culture using the NUNC plasmid purification procedure.

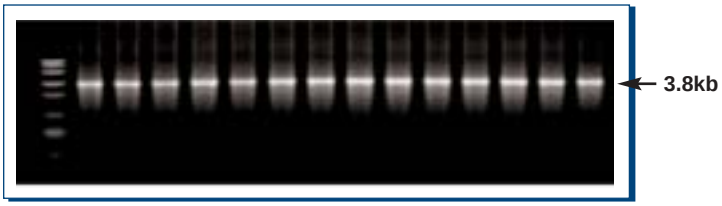

Figure 4 Restriction digestion of purified plasmid DNA. The purified plasmid DNA is suitable for down-stream applications. The plasmid DNA was digested by restriction nuclease Hind III prior to agarose gel electrophoresis.

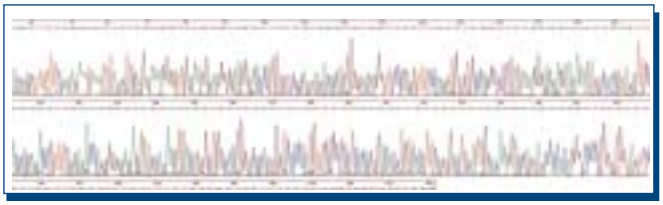

Figure 5 Typical sequencing data. The purified plasmid DNA is also suitable for sequencing.

Conclusion

High purity and yield of plasmid DNA with low cost is critical for successful downstream applications. NUNC™ Brand plasmid purification microplates with the accompanying detailed optimized protocol provide excellent tools for plasmid purification. Furthermore, NUNC plates are not bundled with expensive reagents, thus providing more flexibility for the researcher to formulate their own. These features are particularly beneficial for frequent and high volume plasmid purification laboratories and centers.

Credits

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