Application Note

NORGEN BIOTEK CORPORATION

Plasmid DNA Purification using Norgen Preparative Chromatography Columns

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INTRODUCTION

In the field of biotechnology, it is extremely important to be able to isolate high quality, biologically active plasmid DNA. This DNA can be used in a number of applications including transformations, transfections, and *in vivo* studies. Traditionally, plasmid DNA isolation has been performed using lengthy ethanol precipitation procedures and expensive CsCl banding.

In the interest of providing a quick, simple and cost-effective method for the isolation of high quality plasmid DNA from bacterial cultures, Norgen Biotek has introduced Preparative Chromatography Columns packed with DNA-Select™ resin. This resin exhibits a number of superior features in chromatography purification of plasmid DNA in comparison to commonly used matrices. The columns come in 4 sizes, and can yield 5 – 22 mg of plasmid DNA.

Norgen's plasmid DNA isolation is based on a modified alkaline/SDS cell lysis procedure, followed by purification with Preparative Chromatography Columns. The plasmid DNA will bind to the column while degraded RNA, cellular proteins, and metabolites are washed off. The purified plasmid DNA is then eluted from the column into a salt-free buffer. This plasmid DNA has a functional purity equivalent to CsCl-banded DNA and is suitable for a number of downstream applications including subcloning, enzymatic manipulations, labeling, transformation, and DNA sequencing.

In this application note, the 25x100 Norgen Preparative Chromatography Column was used to purify the plasmid pCMVβ from 500 mL of bacterial culture. The plasmid was then tested for purity and functionality through its use in a number of downstream applications.

MATERIALS AND METHODS

Plasmid DNA Isolation

E.coli strain DH5α was used to propagate the plasmid pCMVβ. A single colony was picked with a sterile wooden stick and inoculated into 2-5 mL of Luria Broth (LB) with antibiotics. This culture was grown for 6-8 hr at 37°C with rapid agitation, and then used to inoculate 500 mL of LB (with antibiotic). The culture was incubated overnight (14-16 hr), until an OD_{600} of 1.5 to 2.0 was reached. The bacterial culture was then harvested at 3000 xg for 20 minutes. The resulting bacterial pellet was resuspended in 25 mL of Resuspension Solution (50mM Tris-Cl pH 7.5; 10mM EDTA pH 8.0; 100ug/ml RNase A). After a 30 minute incubation at 37°C, 25 mL of Lysis Solution (1%SDS; 0.2N NaOH) was added and the mixture incubated at room temperature for not more than 5 minutes. Next, 25 mL of Neutralization Solution (2M Potassium acetate pH 4.8) was added and the mix was incubated at 4°C for 30 minutes. The lysate was then spun down at 10,000 x g for 20 minutes. The supernatant was filtered through an 8 μm filter and then through a 0.45 μm filter. Guanidine hydrochloride was added to the filtered lysate to a final concentration of 0.7M.

The filtered bacterial lysate was then applied to the pre-packed Norgen column of 25 x 100 at 1ml/min using a peristaltic pump. The column was then moved to the chromatography system AKTAprime, and washed with 6 BV (Bed Volumes) of a low concentration of guanidine solution at 2ml/min and eluted with TE solution of pH 11 (1mM EDTA & 50mM Tris) at 1ml/min. Fractions were collected in each stage, and the elution fractions were pooled together and tested in various downstream applications.

Restriction Enzyme Digestion

One μg of purified plasmid DNA was digested with the appropriate amount of *Hind*III and *Eco*RI for 2 hours at 37°C.

Polymerase Chain Reaction

PCR was performed in a 50 μl reaction volume using 3 μl of purified pCMVβ (diluted 1:100 in distilled water) as the template. A typical PCR cycle was run in the MJ Research Minicycler, and 10 μl of the reaction was analyzed with agarose gel electrophoresis.

Transfections

For transfections, approximately 3 x 10^5 Hela cells were seeded in 6-well plates. Calcium-phosphate transfections were performed (Graham and van der Eb, 1973) using 1 μg of the column-purified plasmid or 1 μg of CsCl-banded DNA. After sufficient time, the cells were stained and assayed for *lacZ* activity, in order to determine the transfection efficiency.

RESULTS

Purified plasmid DNA was tested for a number of downstream biological applications. First, the DNA was tested for its ability to be digested by restriction enzymes. A double digest of the column-purified plasmid DNA was performed using *Eco*RI and *Hind*III, and Figure 1 is a gel picture of the result. It can be seen that the pCMVβ could be fully digested with the enzymes, indicating that the plasmid eluted from the column is sufficiently pure and free of contaminants.

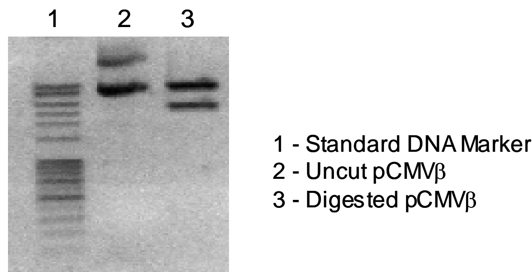

Figure 1. Agarose gel picture of column-purified DNA digested with *Eco*RI and *Hind*III.

Norgen purified DNA was then tested for its ability to be used as a template in a PCR reaction. Column-eluted plasmid DNA was diluted and used in the reaction in order to amplify an 800 base pair product. As can be seen in Figure 2, the PCR reaction was successful and a sharp band of the expected size was produced. These results again indicate that plasmid DNA purified using the column is sufficiently pure and free of contamination.

Lastly, the biological activity of the purified plasmid DNA was tested. Calcium-phosphate transfections were carried out on Hela cells using 1 µg of column-purified DNA, as well as CsCl-banded DNA as a control. Figure 3 indicates that Norgen column-purified DNA was found to show a higher transfection efficiency than the CsCl purified DNA, indicating that the purified plasmid is able to maintain a high degree of biological activity.

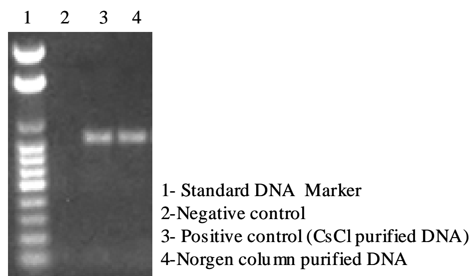

Figure 2. Agarose gel picture of PCR results for column-purified plasmid DNA

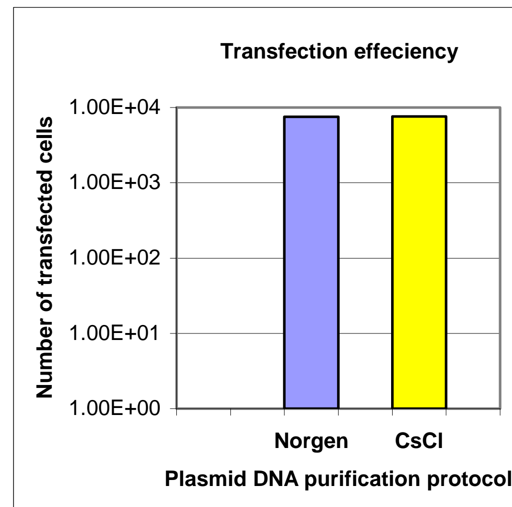

Figure 3. Transfection efficiencies of Hela cells using Norgen column-purified DNA and CsCl-banded DNA

DISCUSSION AND CONCLUSIONS

The plasmid pCMVβ was isolated from a bacterial culture using Norgen's modified alkaline/SDS cell lysis procedure, and then further purified on a 25x100 Preparative Chromatography Column. The plasmid is eluted from the column into a salt-free buffer, and no further purification or concentration steps are required prior to downstream applications.

The purified plasmid was evaluated for its use in a number of downstream applications. The plasmid was found to be sufficiently pure to allow for a double-restriction enzyme digestion with *Hind*III and *Eco*RI, and was able to be used as the template in a PCR reaction. The biological activity of the plasmid was tested through transfection of Hela cells, and it was found to retain a high level of biological activity.

Purification of plasmids using Norgen's Preparative Chromatography columns eliminates the need for expensive equipment (e.g. ultracentrifuges) and reagents (e.g. CsCl), as well as toxic and mutagenic substances (e.g. phenol, chlorofom and ethidium bromide). The procedure is both simple and user-friendly, and results in a plasmid DNA preparation that can be used directly in various downstream applications.