Plasmid Purification Using CHT™ Ceramic Hydroxyapatite Support

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Introduction

Plasmid DNA is being used successfully as a gene delivery vector in a variety of clinical applications (Smith et al. 1999). Plasmids for gene therapy are usually produced in an *E. coli* host. One of the technical challenges associated with producing plasmid DNA of gene therapy grade is the removal of contaminants such as bacterial chromosomal DNA, RNA, host proteins, and endotoxin.

Chromatography plays a key role in the large-scale purification of plasmids, both as a process step and as an analytical tool. Different types of chromatography such as ion exchange, gel filtration, reverse phase, and affinity have been used for the separation of plasmid DNA. Chromatography is often preceded by RNase treatment, diafiltration, precipitation, dilution, and other steps, which can increase process costs, decrease productivity and product recovery. Thus, there is a significant need for an alternative plasmid purification method.

We have developed a very effective and rapid method for preparing plasmid DNA from *E. coli* that should be readily scalable. Use of our modified alkaline lysis procedure (patent pending) allows direct loading of the clarified lysate to CHT ceramic hydroxyapatite, thus eliminating the need for any other sample preparation or handling steps.

Methods and Results

Cell Lysis

A plasmid DNA sample (5,955 base pairs, derived from pUC19) was grown in *E. coli* strain DH5α. Cells were grown in Terrific Broth medium supplemented with 100 µg/ml ampicillin. The cell pellets were resuspended in 40 mM sodium phosphate buffer, pH 8.0, containing 25 mM EDTA in an ice bath. AquaPure™ lysis buffer (catalog #732-6541) was added to the suspension, mixed immediately, and stored on ice for 3–5 min. Potassium chloride (1 ml, 3.0 M) was added immediately and mixed, and the resulting suspension was adjusted to pH 4.5 with 1.0 N HCl, then neutralized to pH 7.0 with NaOH solution. The crude lysate was centrifuged at 15,000 x g for 20 min at 4°C. The precipitate was removed by filtering on a 70 µm nylon cell strainer.

Purification

The clear *E. coli* lysate was injected onto a CHT column (Econo-Pac® CHT Type II, 5 ml cartridge, catalog #732-0081) with a particle size of 20 µm and a nominal pore diameter of 800–1,000 Å. The column was washed with 5 column volumes of 10 mM sodium phosphate buffer, pH 7.0, containing 1 mM EDTA, followed by a linear gradient to 0.4 M sodium phosphate buffer for 10 column volumes at a flow rate of 1.5 ml/min. The elution process was monitored at 254 nm (Figure 1). The fractions collected were analyzed spectrophotometrically at 260 and 280 nm to determine DNA and protein content.

DNA purity of fractions 35 to 37 (Figure 1, peak 3) was analyzed by electrophoresis in a 0.8% agarose gel (Figure 2, lanes 13–15). Fractions 35, 36, and 37 were found to contain pure plasmid DNA and some of the nicked species. These fractions were digested with *Eco*RI restriction enzyme for 1.5 hr at 37°C. The digested DNA was analyzed by 0.8% agarose gel electrophoresis. The gel results indicated that the supercoiled DNA (undigested) migrated faster than the linearized (digested) DNA (Figure 3, lanes 6–11), confirming the identity and purity of the supercoiled plasmid DNA.

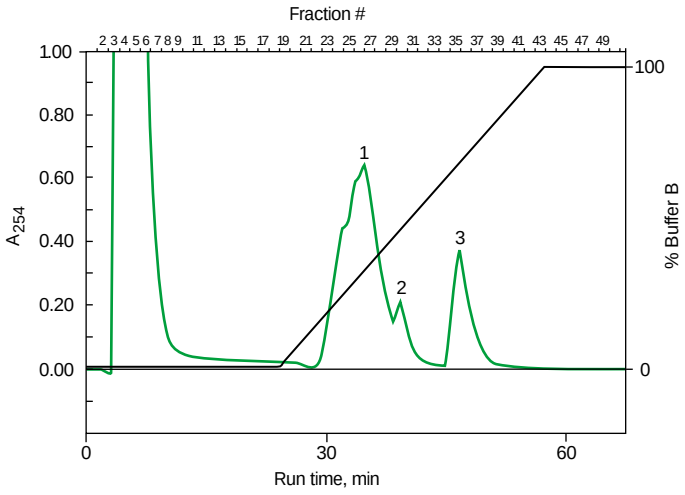

Fig. 1. Purification of plasmid DNA on CHT II support.
Buffer A: 10 mM sodium phosphate + 1 mM EDTA, pH 7.0
Buffer B: 0.4 M sodium phosphate + 1 mM EDTA, pH 7.0
Flow rate: 1.5 ml/min
Gradient: 0–100% buffer B for 10 column volumes
Fraction size: 2.0 ml

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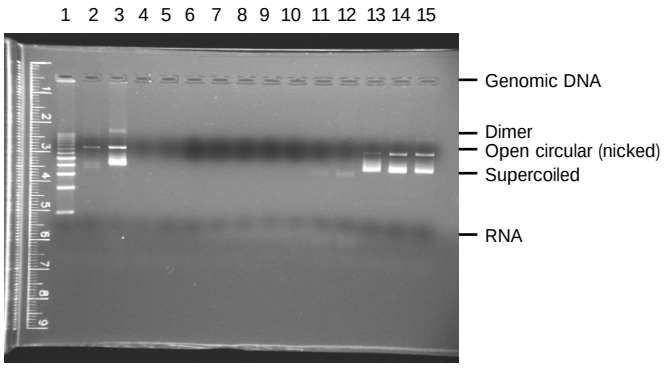

Fig. 2. Electrophoretic analysis of purified plasmid DNA. Lane 1, 1 kb molecular mass ruler; lane 2, control plasmid DNA; lane 3, clear lysate; lanes 4, 5, and 6, flowthrough fraction number 3, 5, and 7; lanes 7, 8, 9, and 10, peak 1; lanes 11 and 12, peak 2; lane 13, fraction 35; lane 14, fraction 36; lane 15, fraction 37.

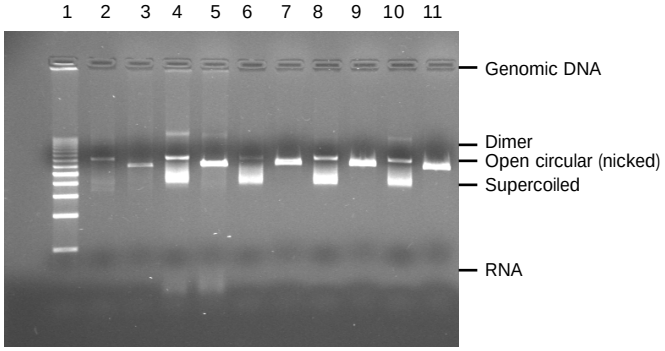

Fig. 3. Restriction enzyme analysis of purified plasmid DNA. Lane 1, 1 kb molecular ruler; lanes 2 and 3, undigested and digested plasmid DNA (control); lanes 4 and 5, undigested and digested lysate; lanes 6 and 7, undigested and digested fraction 35; lanes 8 and 9, undigested and digested fraction 36; lanes 10 and 11, undigested and digested fraction 37.

Fractions from the CHT column were analyzed for nucleic acid content, genomic DNA, endotoxin, and protein. The results are in Table 1.

Table 1. Analysis of CHT II fractions.

Sample	Total DNA (µg/ml)*	Volume (ml)	Genomic DNA (µg/ml)**	Endotoxin (EU/µg)***	Protein (µg/ml)†
Clarified lysate (load)	2025	5	33.01	33.3	1,812
Flowthrough	617.5	16	undetectable	12.3	564
Peaks 1 and 2	29.28	26	1.18	5.51	40
Peak 3	18.67	20	1.45	0.15	9

* A_{260}/A_{280}; **PCR assay; ***LAL assay; †BCA assay

No RNA contamination (<0.1 µg/sample) was detected by agarose gel electrophoresis (data not shown).

Plasmid Binding Capacity

The dynamic binding capacity of CHT was tested using purified plasmid DNA (pS3, 10 kb) and a 1.0 ml Econo-Pac cartridge. Using the buffer system described in Figure 1 at a flow rate of 0.5 ml/min, we loaded pure plasmid to saturation and determined recovery of the eluted DNA by spectroscopy at 260 nm. The recoverable dynamic binding capacity was 0.23 mg/ml, representing a recovery of 55.0%. Different plasmids and buffer systems may exhibit different binding capacities or recoveries.

Discussion

The method we devised for cell lysis coupled with chromatography on CHT was shown to provide pure plasmid DNA in a single step. The elimination of sample manipulation prior to loading the CHT column increases productivity and should result in improved recovery. Addition of a polishing or capture step to the process should further enhance the clearance of host cell contaminants.

Reference

Smith GJ III et al., Fast and accurate method for quantitating *E. coli* host-cell DNA contamination in plasmid DNA preparations, Biotechniques 26, 518–526 (1999)

The polymerase chain reaction (PCR) process is covered by patents owned by Hoffman-LaRoche. Use of the PCR process requires a license.

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