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## HPLC '90

### Poster Presentation

#### Large Scale Purification of Plasmids from Crude Cell Lysates by Anion Exchange HPLC

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# Optimization of HPLC Separation

The purification of plasmid DNA from contaminating RNA contained in the alkaline lysed/ RNase Proteinase K digested/ PEG precipitated sample (i.e. crude cell lysate) was performed on the Waters Protein-Pak DEAE 8HR anion exchange column ( 10 x 100 mm glass column). The mechanism of the separation is based upon the difference of the overall charge of the various nucleic acid species contained in the solution. The smaller, less charged RNA species elute prior to the larger Plasmid DNA via the use of the simple sodium chloride gradient in a 25mM Tris/Cl, 1mM EDTA, pH 8.0 buffer. Ethidium bromide and cesium chloride are not required to affect the separation.

Optimization of the separation of the plasmid from the contaminating RNA fragments was accomplished by performing a series of experiments where 36 $\mu$ g ( determined off line where 1.0 OD<sub>260</sub> = 50 $\mu$ g/ml ) of crude lysate material was injected and separated on the column using various gradient programs. For the initial separation ( Figure 1 ) a linear gradient from 250mM to 600mM NaCl was performed in 40 minutes at 1.5ml/min. Excellent resolution was observed between the desired plasmid DNA and contaminating RNA.

In order to maximize the amount of plasmid which could be purified in a single run, the gradient conditions were modified so as to minimize RNA retention. Note: To eliminate the possibility of sample carryover from previously injected samples, the column is cleaned by injection of 0.1N phosphoric acid ( 200 $\mu$ l ) immediately following plasmid elution from the column. This procedure removes residual plasmid without adversely affecting this polymer-based, anion exchange packing.

# Introduction

The rapid purification of microgram to milligram quantities of plasmid DNA for use as vectors or substrates in biological assays is of growing importance. The isolation from crude cell lysates by classical methods is time consuming and often does not adequately purify the plasmid from other bacterial cell components such as chromosomal DNA, RNA and proteins. Traditionally, the purification of plasmid DNA from crude cell lysates is performed using cesium chloride gradient centrifugation in the presence of ethidium bromide. Typical centrifugation times range from 5 to 48 hours, depending on rotor design and must frequently be repeated to affect complete plasmid separation from bacterial cell contaminants. Additional steps are subsequently required to remove the intercalated ethidium bromide, a potent mutagen, and cesium chloride from the preparation.

High performance liquid chromatographic (HPLC) separation techniques offer a cost effective and labor saving alternative to gradient centrifugation for plasmid purifications. The separation is based on high resolution anion exchange chromatography with Waters Protein-Pak<sup>TM</sup> DEAE 8HR column. This chemistry utilizes a DEAE functionalized hydrophilic polymer base material having a large internal pore size that is ideal for the rapid separation of biological macromolecules. HPLC separations may be completed in a fraction of the time required for centrifugation with quantitative recovery of biologically active plasmids.

# Large Scale Purification and Analysis

Having optimized the sample preparation and chromatographic conditions, 3.8 mg of crude lysate containing a 2939 base pair plasmid, pRNH124, was loaded and separated on the Protein-Pak DEAE 8HR column (Figure 5). The amount of isolated plasmid during this separation was 2.4 mg. As much as 10 mg of total nucleic acid material containing 5 mg of plasmid DNA has been successfully chromatographed on this 10 x 100mm column without exceeding the column's capacity ( 4 ). It should be noted, however, that variations in the absolute yield of purified plasmid varies depending upon the efficiency of plasmid replication within the bacterial host cells.

The biological activity of the HPLC purified plasmid versus plasmids purified by cesium chloride centrifugation was evaluated by restriction endonuclease, bacterial transformation ( 5 ) and transfection experiments ( 6 ). Based upon agarose gel electrophoresis (Figure 6), the HPLC purified crude lysate containing the pRNH124 plasmid was free of contaminating host RNA. Furthermore, the monomer, dimer and concatamer plasmid forms were completely linearized when digested with appropriate restriction endonucleases. Achieving this degree of purity generally requires two gradient centrifugations. In addition, the elimination of ethidium bromide in the HPLC procedure significantly reduces plasmid nicking resulting in transformation efficiencies in excess of  $10^7$  colonies per microgram DNA. Transfection efficiencies of HPLC purified plasmids are also consistently better, compared to cesium chloride gradient purified material. As such, the Protein-Pak DEAE 8HR purified plasmid has proven to be effective vectors as well as excellent substrates for enzymatic and/or hybridization experiments.

The subsequent separation ( Figure 2 ) utilized a linear gradient from 400mM to 600mM NaCl over 40min at 1.5ml/min. This gradient resulted in minimal retention of the RNA while yielding even greater resolution of the plasmid DNA from the contaminants.

Utilizing these conditions, 9.0 mg of crude lysate was chromatographed on the Protein-Pak DEAE 8HR column with results shown in Figure 3. Fractions 5 - 20 min. and 22.5 - 26.5 min. were collected for analysis. Chromatography of the 5 - 20 min. fraction indicated that there was no plasmid DNA breakthrough during the 9.0mg crude lysate run (Fig 4). 1.0mg of plasmid DNA was contained in the 22.5 - 26.5 min. fraction (Fig 3). Although the maximum capacity for the plasmid DNA had not been established during the 9.0 mg crude lysate separation, it was thought that a slower flow rate (e.g. 0.8 ml/min) would enhance mass transfer enabling higher plasmid masses to be purified in a single chromatographic run.

## References

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## Summary

High performance anion exchange chromatography using the Waters Protein-Pak DEAE 8HR chemistry is ideal for rapid large scale purifications of biologically active plasmid DNA. Purifications are achieved in less than 60 minutes using a simple NaCl gradient in a TRIS-EDTA, pH 8.0 buffer system. A 10 x 100mm column will easily purify as much as 5 milligrams of Plasmid DNA in a single run. Cesium chloride and ethidium bromide are not required. Plasmid DNA recoveries from this polymer-based chemistry consistently exceed 95% and the possibility of sample carryover from previously HPLC purified material is eliminated by the inclusion of a 0.1N phosphoric acid cleaning procedure.

Figure 2 Optimized separation of plasmid DNA from contaminating lysed RNA.

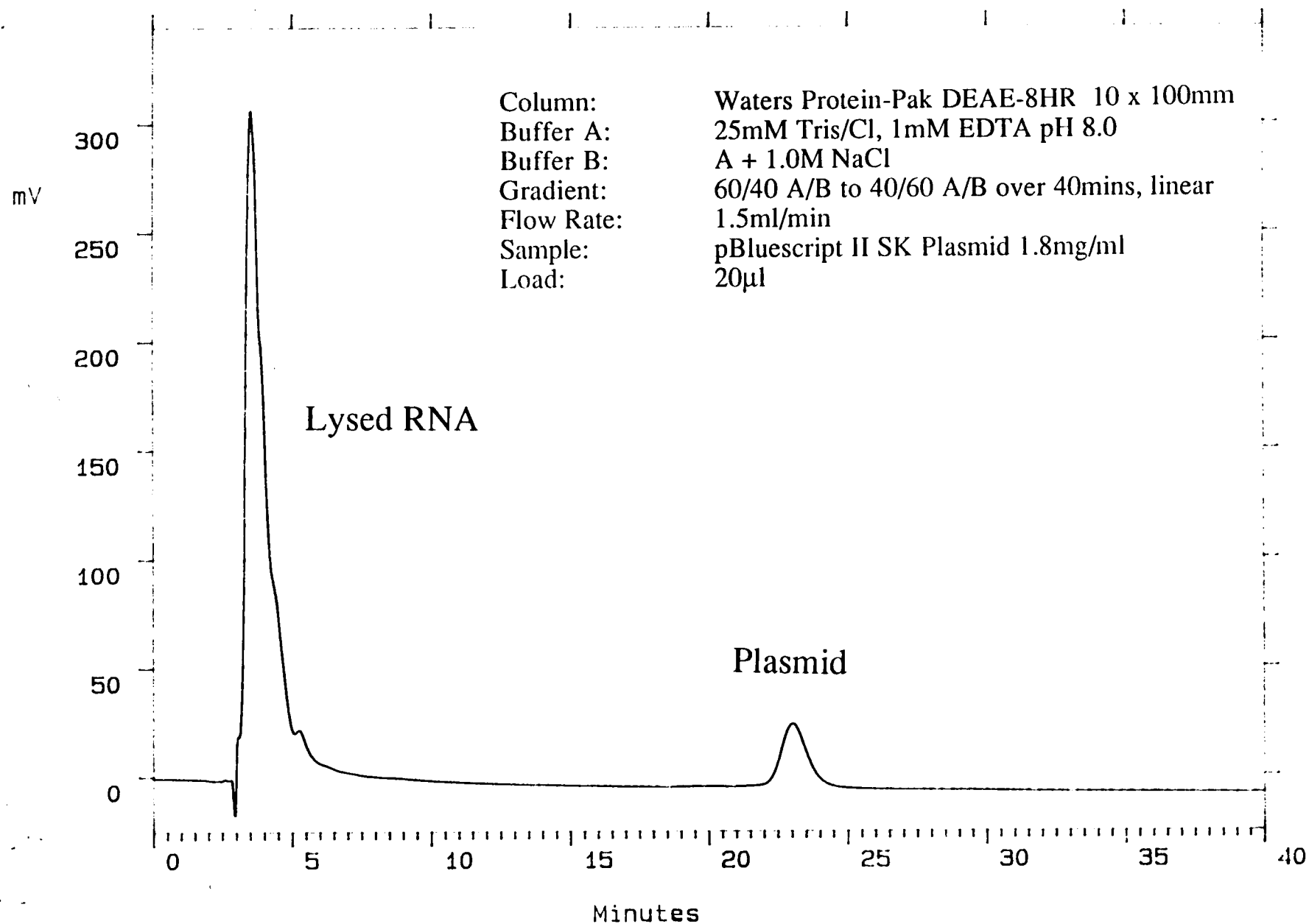
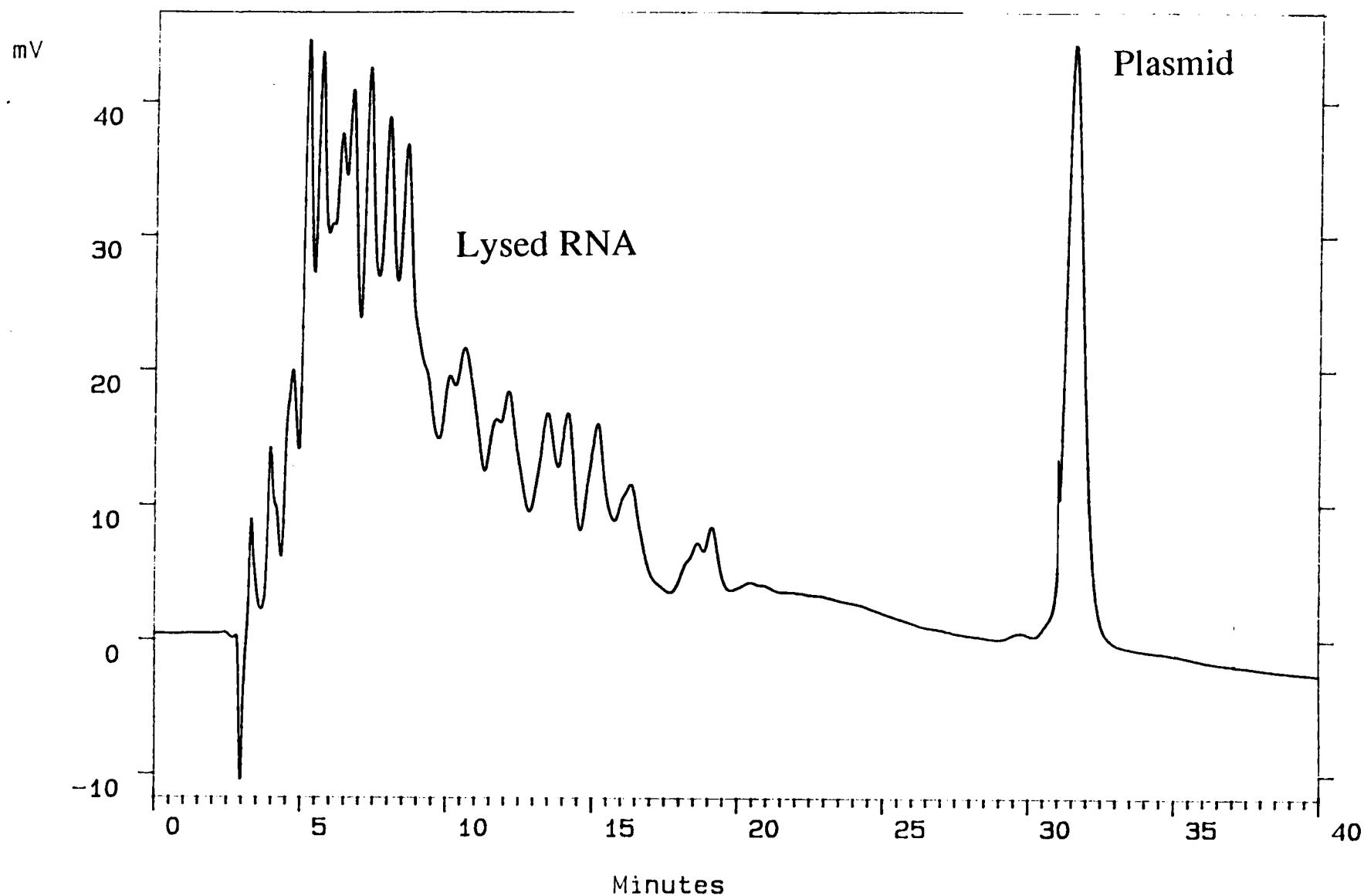




Figure 1 Initial separation of plasmid DNA from contaminating lysed RNA.



Column:	Waters Protein-Pak DEAE-8HR 10 x 100mm
Buffer A:	25mM Tris/Cl, 1mM EDTA pH 8.0
Buffer B:	A + 1.0M NaCl
Gradient:	75/25 A/B to 40/60 A/B over 40mins, linear
Flow Rate:	1.5ml/min
Sample:	pBluescript II Plasmid 1.8mg/ml
Load:	20µl