Waters
Lab Highlights
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HPLC Purification of Oligonucleotides, Restriction Fragments and Plasmids[1]

Highlight from the Sixth World Wide Technical Meeting

The isolation and purification of nucleic acids (e.g. oligonucleotides, restriction fragments and plasmids) is of growing importance in the life sciences. These molecules have classically been purified using a variety of non-HPLC techniques such as electrophoresis and gradient ultracentrifugation. Unfortunately, these techniques are often time and labor intensive and can result in low sample recovery[2,3]. Furthermore, the final product can be contaminated with substances derived from the separation matrix necessitating an additional purification step[4].

Recently, a new high performance column (Waters Gen-Pak™FAX) has been shown to be highly effective in purifying a variety of nucleic acids. Synthetic oligonucleotides,[5,6] restriction fragments,[6-9] and plasmids[6,10] can be purified on this polymer-based anion exchanger, utilizing a simple NaCl gradient in an aqueous buffer system (e.g. 20mM Tris/Cl, pH 8.0). Organic solvents or chaotropic agents (e.g. 6M urea) are not required. Separations on this column are easily automated, and can result in the rapid purification of high yields of biologically active material (e.g. > 97% for synthetic oligonucleotides[4]). This chemistry should prove beneficial to those working in the area of molecular biology or associated disciplines. Representative chromatograms for synthetic oligonucleotide (Figure 1), restriction fragment (Figure 2) and plasmid (Figure 3) separations are shown below.

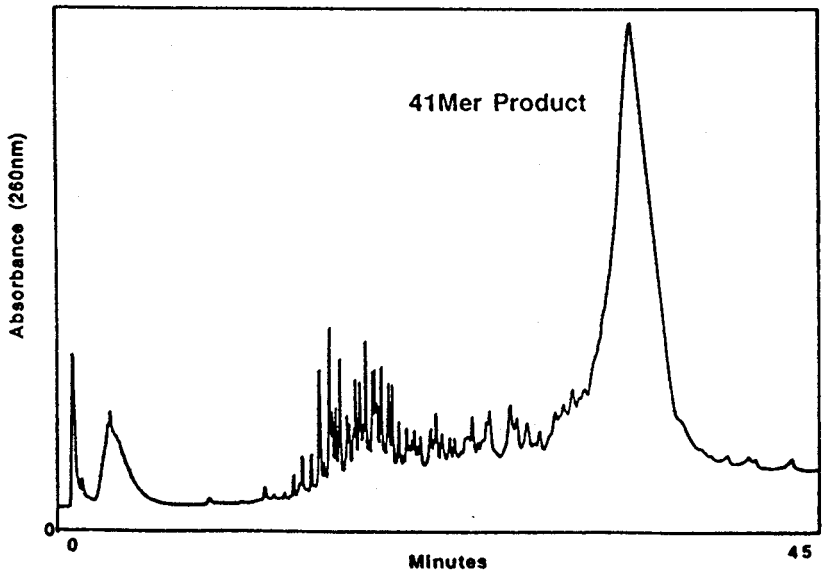

Figure 1: Gen-Pak™ FAX separation of synthetic oligonucleotide (41mer) from failure sequences. For conditions see Reference 4.

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Figure 2: Gen-Pak™ FAX separation of crude PBR plasmid preparation. For conditions see Reference 9.

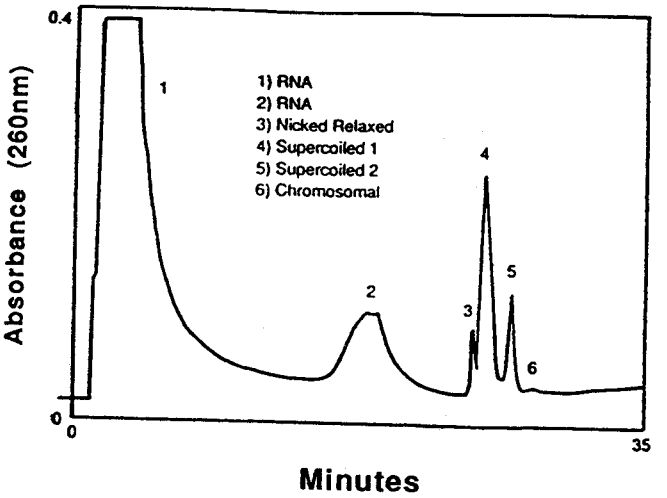
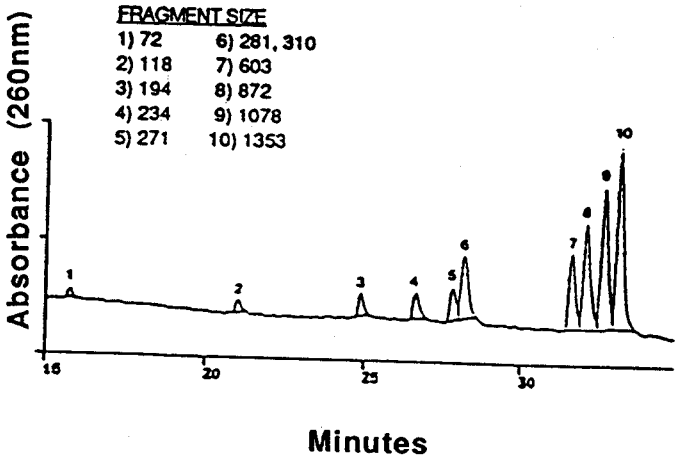


Figure 3: Gen-Pak™ FAX separation of restriction fragments of øX produced by Hae III digestion. Chromatogram courtesy of Dr. George Jackowski, Hospital for Sick Children, Toronto, Canada. For conditions see Reference 6.



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