Essentials in biore

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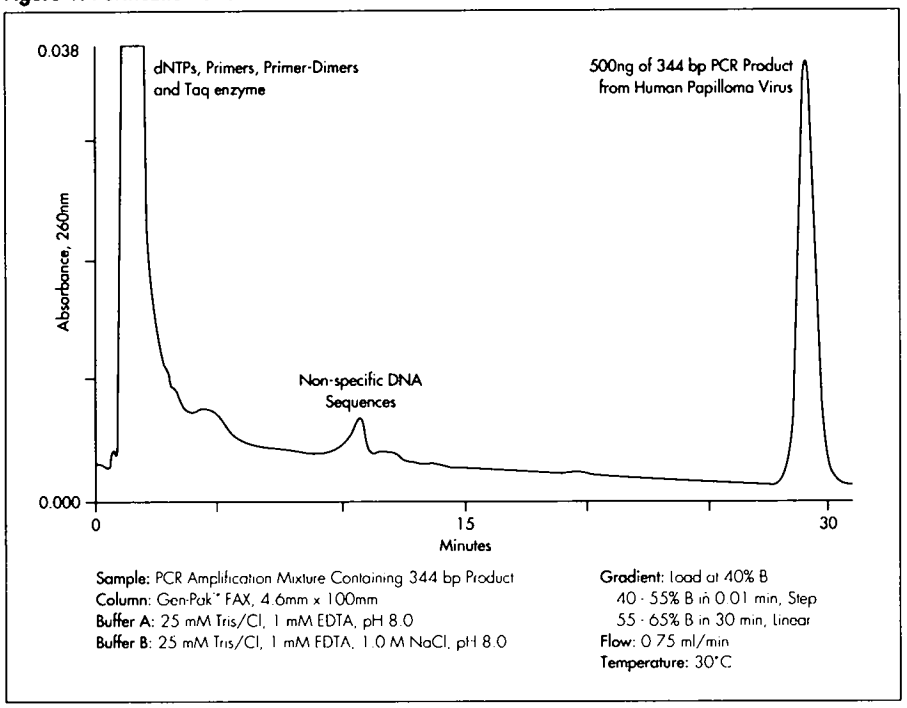
Purification of Polymerase Chain Reaction* Products for Direct Sequencing.

Chromatography eliminates contaminants prior to sequencing.

The identification of mutations and polymorphisms in genomic DNA is important in studies of the molecular basis of disease and has a variety of practical applications in disease diagnostics. The selective amplification of specific DNA segments by polymerase chain reaction (PCR) has proven to be an extremely valuable tool for this research[1]. Via this technique, specific DNA regions for sequence analysis are available within hours compared to the use of laborious and time consuming vector construction and cloning experiments. However, difficulties have been encountered when attempting to obtain direct double stranded dideoxynucleotide sequence analysis of amplified target DNA due to the presence of excess deoxynucleotide triphosphates (dNTPs), primers and primer-dimers in the PCR reaction mixture[2]. These additional molecules often result in undesirable background noise on the sequencing autoradiograms making portions of the sequence, and sometimes the entire sequence, unreadable. Although numerous purification strategies (e.g. gel electrophoresis and ultrafiltration) are easy to perform, they are plagued by low sample recoveries or incomplete separation of the contaminants from the desired target DNA. Investigations, in collaboration with Dr. Jay Doniger of the Georgetown University Medical Center, have validated the use of High Performance Liquid Chromatography (HPLC) for target DNA purification from PCR amplification mixtures yielding sequencing results comparable to those obtained using M13 templates[3].

Figure 1: Purification of 344 Base Pair PCR Product.



Purification of a 344 base pair PCR product from contaminating dNTPs, primers, primer-dimers and other contaminating molecules on the Gen-Pak FAX column is accomplished using a simple salt gradient in a Tris/EDTA buffer. This makes direct sample collection for immediate DNA sequencing possible.

PCR target DNA isolated within 30 minutes.

HPLC using the Waters Gen-Pak™ FAX anion exchange column is ideally suited for PCR product purifications. Figure 1 illustrates the purification of a 344 base pair DNA fragment produced from a PCR amplification of a human papilloma virus integrated within a human exocervical epithelial cell line. The target DNA is easily isolated from contaminating dNTPs, primers, primer-dimers and non-specific DNA sequences contained in the PCR reaction mixture using a simple NaCl gradient in a Tris/EDTA buffer system. Since the separation is based upon the overall charge of the molecules in the sample, the smaller contaminating species elute prior to the larger, more negatively charged PCR product. The isolated target DNA can thus be collected directly into a microfuge tube with sample recoveries consistently exceeding 95%. In addition, this chromatographic technique can be used to isolate multiplex PCR products present in a multiple amplification mixture having several sets of PCR primers. This cannot be achieved by ultrafiltration. Furthermore, on-line 260 nm monitoring eliminates the need for ethidium bromide staining making direct quantitation of the collected PCR product possible.

**See U.S. Patent No. 4683202 to Cetus Corporation.*

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Cleaner DNA sequencing gel autoradiograms.

The 344 base pair DNA fragment from a human papilloma virus purified on the Waters Gen-Pak FAX column was successfully sequenced using the Sequenase® 77 DNA Polymerase protocol (United States Biochemical) of the dideoxynucleotide chain termination reaction. A segment of the autoradiogram from the sequencing gel starting at 20 bases downstream of the primer is shown in Figure 2. Note the absence of bands across all four lanes. Such bands are often observed when sequencing non-purified PCR products. Because the chromatographically purified product is free from contaminating primers or primer-dimers present in the PCR reaction mixture, the need to prepare and use a prelabelled primer for the sequencing reaction is eliminated[3]. Thus, utilizing this time saving chromatographic technique, sequencing results comparable to those obtained using M13 subcloned DNA are easily achieved.

Versatile column chemistry.

Chromatography on the polymer-based Gen-Pak FAX anion exchange column is well suited not only for the purification of PCR products but also for the purification and analysis of DNA restriction fragments, synthetic oligonucleotides and plasmids. In each case, simple easily implemented protocols have been developed to aid you in applying chromatographic technology. The high resolution separations and quantitative recoveries achieved offer an alternative to gel electrophoretic, centrifugation and ultrafiltration techniques for separating DNA for molecular biology applications.

Increase utilization of instrumentation.

In addition to applications involving PCR products, Waters, an established leader in chromatographic chemistries and instrumentation, offers a comprehensive assortment of documented purification techniques for proteins, peptides, amino acids and other biomolecules. The versatility of HPLC instrumentation makes chromatography a cost effective purification and analysis tool.

Summary.

Same day PCR amplification and sequencing is desired in many investigatory or diagnostic situations. Prior to sequencing, contaminating nucleic acid species must be removed from the PCR target DNA. The rapid purification and quantitative recovery of the desired PCR product on the Waters Gen-Pak FAX anion exchange column enables one to obtain sequencing results comparable to techniques requiring laborious cloning procedures. This application extends the use of HPLC for the purification and analysis of biomolecules.

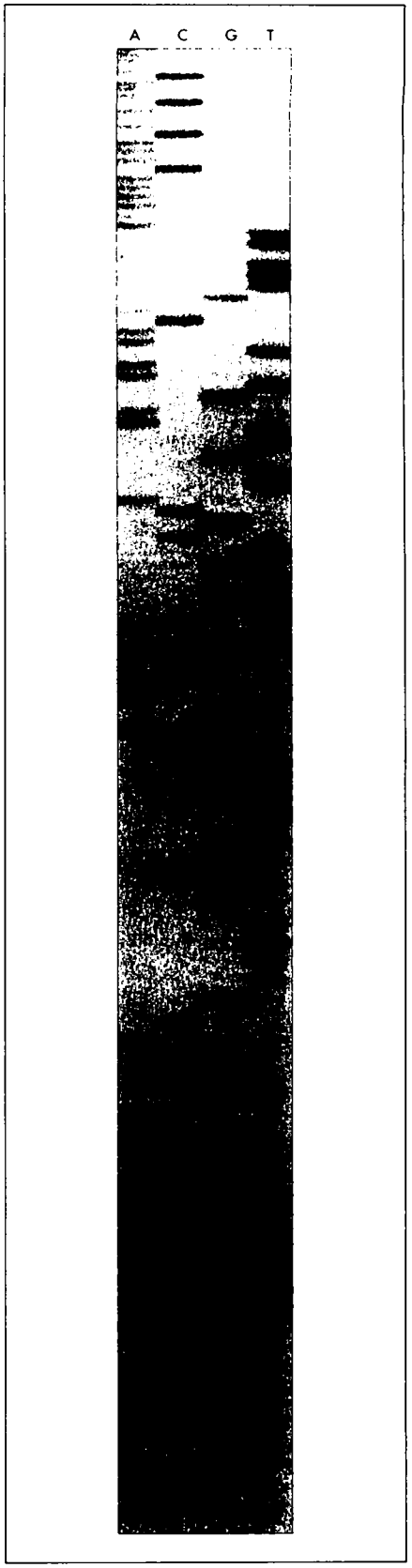
References:
1. Engelke, D.R. P.A. Hoener and F. Collins. 1988 *Proc. Nat. Acad. Sci. U.S.A.* 85. 544-548
2. Mihovilovic, M. and J.E. Lee. 1989. *Biotechniques* 7. 14. 16.
3. Warren, W. and J. Doniger, (manuscript in preparation).

Ordering Information.

Waters Gen-Pak FAX Column, 4.6mm x 100mm	PN 15490
Waters Temperature Control System	PN 38039
Waters DNA Purification System	*

*Contact your local Waters technical representative to configure the optimal system for your laboratory.

Figure 2: Autoradiogram of Sequencing Gel.



Autoradiogram of sequencing gel shows partial sequence of the 344 base pair PCR amplified DNA purified on the Gen-Pak FAX column. The sequence shown starts 20 bases downstream of the primer. Subcloning or the use of a pre-labelled sequencing primer are not required.