Bill Warren
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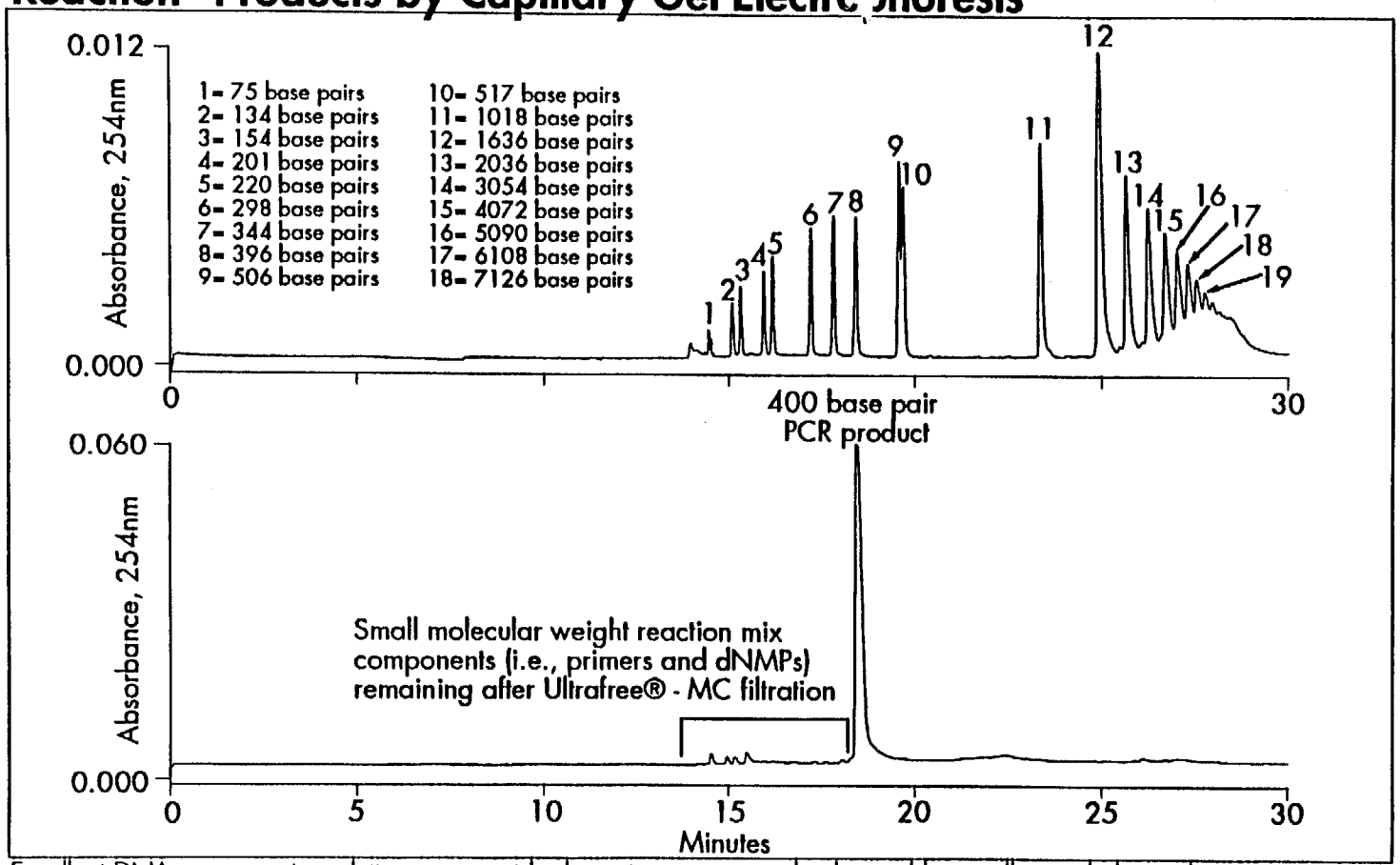
Rapid Analysis of DNA Restriction Fragments and Polymerase Chain Reaction* Products by Capillary Gel Electrophoresis



Excellent DNA component resolution over a wide dynamic range is easily obtained by capillary gel electrophoresis using "entangled polymer networks".

Conditions:

Capillary: Waters™ Accusep™ (100µm x 60cm)

Electrolyte: 100mM Sodium Phosphate, pH 5.0 with 0.5% Hydroxypropylmethylcellulose (4,000 centipoises)

Samples: Top: 1 kb DNA Ladder (Gibco BRL) at 250 ng/ml

Bottom: PCR Sample filtered through Millipore Ultrafree® -MC (30,000 NMWL) Membrane

Injection: - 5 kV for 15 sec.

Run: - 10 kV

Temperature: 22°C

* See U.S. Patent # 4683202 to Cetus Corporation

Objective:

The objective of this application note is to demonstrate the use of capillary gel electrophoresis for the analysis of DNA restriction fragments and Polymerase Chain Reaction (PCR) products.

Details:

The characterization of double stranded DNA molecules has traditionally been performed using agarose or polyacrylamide slab gel electrophoresis. While this technique remains very popular, it is time-consuming, labor intensive, and difficult to automate. Furthermore, precise quantitation of the DNA species contained in a sample (e.g., in quantitative PCR analysis) is indirect, requiring post-electrophoresis staining, visualization and detection techniques.

Capillary electrophoresis using "entangled polymer networks" overcomes many of the drawbacks inherent with classical slab gel or other capillary electrophoresis techniques. These classical methods use *in-situ*, cross- linked polymers that can become plugged by large DNA fragments (i.e., $>10^5$ base pairs) making repetitive use difficult (1, 2). By comparison, capillary electrophoresis using entangled polymers is not plagued by column fouling because a fresh solution of the size-sieving buffer (i.e., 50mM sodium phosphate, pH 5.0 with 0.5% hydroxypropylmethylcellulose) is used prior to each run. This helps ensure good run-to-run migration time and area count reproducibility (e.g., relative standard deviations <1.5% and 5.0% respectively for the analyses (n=6) of a Hae III digest of Phi X DNA). Furthermore, specially coated capillaries are not required because separations are performed at a pH that minimizes electroosmotic flow. PCR samples can also be readily analyzed by this technique although sample processing through an Ultrafree-MC 30,000 NMWL regenerated cellulose device is highly recommended to both desalt and remove small reaction mixture contaminants.

System:

The system used consisted of a Waters™ Quanta 4000 Capillary Electrophoresis System with a negative power supply and a Millennium™ 2010 ChromatographyManager.

References:

1) Heiger, D.N., A.S. Cohen and B.L. Karger. 1990. Separation of DNA Restriction Fragments by High Performance Capillary Electrophoresis with Low and Zero Crosslinked Polyacrylamide Using Continuous and Pulsed Electric Fields. J. Chromatogr. 516: 33-48.

2) MacCrehan, W.A., H.T. Rasmussen and D.M. Northrop. 1992. Size-Selective Capillary Electrophoresis (SSCE) Separation of DNA Fragments. J. of Liq. Chrom. 15: 1063-1080.