

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

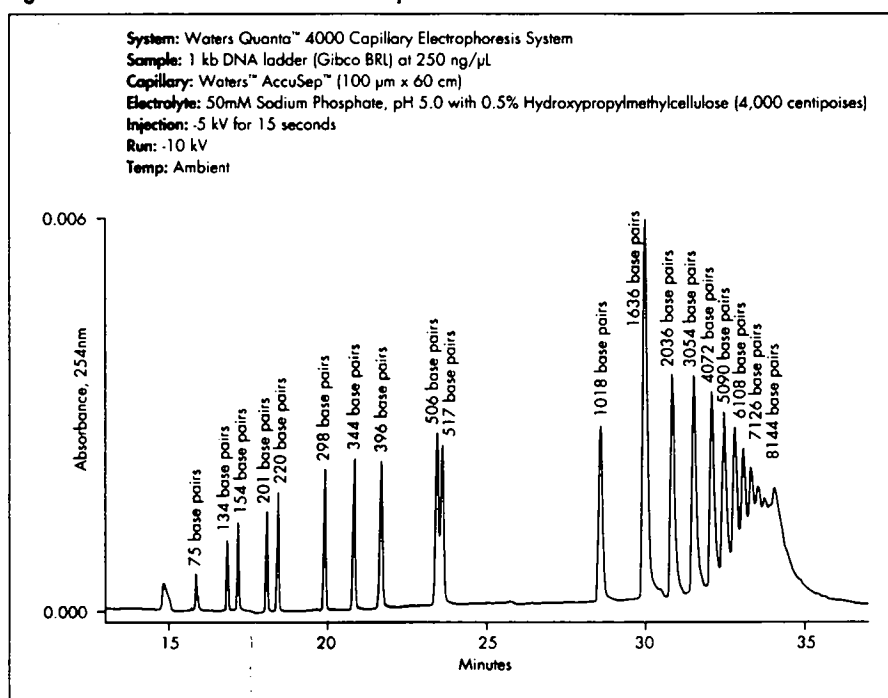
An alternative to agarose or polyacrylamide slab gel electrophoresis.

Characterization of double stranded DNA molecules arising from restriction endonuclease digestions or polymerase chain reaction (PCR) is an important part of many molecular biology investigations. Traditionally, DNA fragment size and concentration determinations have been performed using agarose or polyacrylamide slab gel electrophoresis¹. While this classic methodology provides useful information, the technique is time-consuming, labor intensive, and difficult to automate. Furthermore, precise quantitation of the various size DNA fragments contained in the sample is indirect, requiring post-electrophoresis staining, visualization and detection techniques.

Capillary electrophoresis (CE) overcomes many of the drawbacks inherent with slab gel methodologies. Agarose and polyacrylamide gel preparation techniques are eliminated since high resolution separations occur in an easy to prepare liquid matrix containing a linear polymer. In addition, accurate quantitation, run-to-run reproducibility and convenient data documentation of double stranded DNA separations makes this methodology an attractive alternative to slab gel techniques.

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

Figure 1: Double Stranded DNA Ladder Separation



Electropherogram of a double stranded DNA ladder separation shows the dynamic range of the technique.

High resolution separations with turn-key automation.

Capillary electrophoresis of double stranded DNA species on the Waters™ Quanta™ 4000 System offers excellent component resolution as demonstrated by the analysis of a DNA fragment ladder (Figure 1). Separation methods development is not required using this automated, turn-key technique since one electrolyte formulation can be used to analyze fragments ranging from 50 to 12,000 base pairs in length. The use of commercially prepared, gel-filled capillaries is not required. Furthermore, UV detection of the DNA species as they migrate through the capillary permits

direct fragment quantitation and eliminates the need for post-run staining and visualization techniques.

Sample Preparation for CE Analysis.

Although the PCR technique amplifies very small amounts of DNA, the final concentration of actual PCR product may be quite low and difficult to detect by the described capillary electrophoretic technique with on-line, UV absorbance detection. Since the PCR reaction mixture has a much higher electrolyte concentration than the CE buffer, longer electrokinetic or hydrostatic injection times cannot be satisfactorily used to increase the amount

of sample introduced into the capillary. An alternative approach is to process the reaction mixture through a Millipore Ultrafree™ MC filter unit (30,000 NMWL, regenerated cellulose) to effectively desalt the sample prior to CE analysis.⁵ Detection of low concentrations of PCR products via electrokinetic injection are thus possible. In addition, this easy to use, ultrafiltration technique significantly reduces the amount of small molecular weight reaction mixture components (e.g. dNTPs, oligonucleotide primers, primer-dimers) which can co-migrate with larger size PCR products.

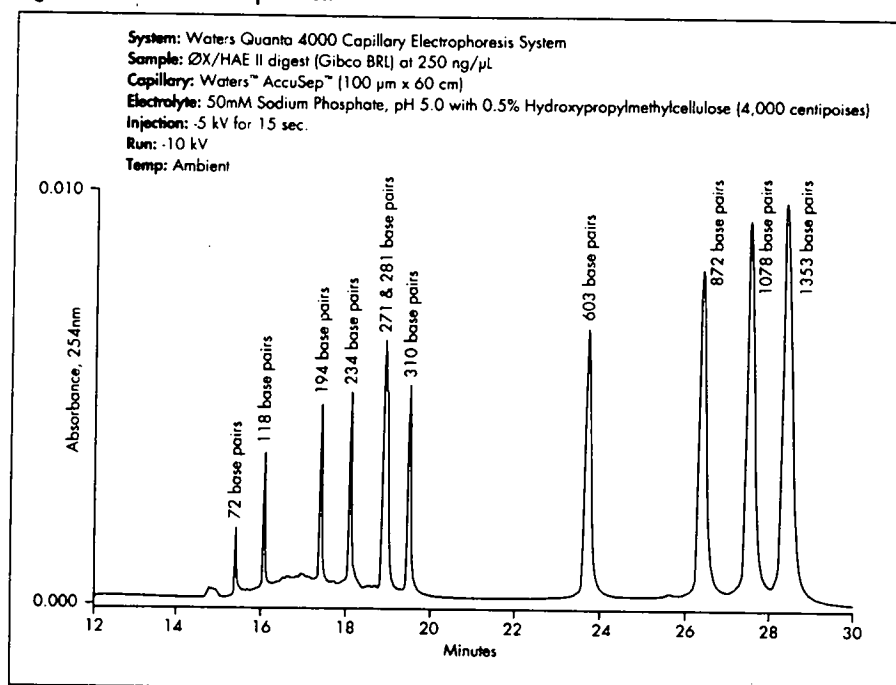
Rapid analysis with reproducible results.

Capillary electrophoresis analysis of a sample containing DNA fragments can be performed within 45 minutes. Good run-to-run reproducibility is maintained by filling the capillary with fresh electrolyte between sample analyses. Migration time and area count relative standard deviations of repetitive analysis of a Hae III digest of a Phi X DNA sample (Figure 2) were 1.3% and < 5% respectively (N=4).

Easy data archival.

As with the other Waters HPLC and CE techniques for DNA fragment analysis^{2,3,4}, each capillary electrophoresis separation is permanently recorded via a chart recorder or on a data station such as the Waters Millennium™ 2010 Chromatography Manager. The Millennium database lets users search through CE files using any criteria, so sample counts, tracking and statistical analyses are obtained quickly and easily.

Figure 2: Plasmid DNA Separation



Electropherogram of a restriction enzyme digest of plasmid DNA using a capillary filled with electrolyte and a hydrophilic cellulose-based linear polymer. Separations are performed at pH 5.0 which eliminates the need to use specially prepared, coated capillaries for this application.

Millipore: For all your DNA research needs.

Millipore offers a complete range of instruments and nucleic acid synthesis reagents for construction of DNA, RNA and modified DNA oligomers. A complete listing of chemical products for bioresearch (Directory of Chemical Products) may be obtained by contacting your Waters Technical Sales Representative.

References:

- 1) Rickwood, D. and B.B. Hames, Eds 1990 "Gel Electrophoresis of Nucleic Acids: A Practical Approach, 2nd Ed." IRL Press, Oxford, England
- 2) Waters Gen-Pak™ FAX Column HPLC Separation of DNA Restriction Fragments and PCR Products. (See Millipore Application Note T74 and T75)
- 3) Rapid Purification and Quantitation of Polymerase Chain Reaction Products. (See Millipore Application Note N95)
- 4) Rapid, High Resolution Analysis and Purification of Synthetic Oligonucleotides. (See Millipore Application Note T110)
- 5) Ultrafree™ MC Application Notes PC006 and TB002.

Ordering Information:

	Part Number
Quanta 4000E CE System	
60 Hz	250000
50 Hz	250001
254nm Filter	250454
AccuSep Capillary Assembly (100 µm x 60 cm)	250-08
Hydroxypropylmethylcellulose	H-7509
Sigma Chemical Company (Viscosity of 2% aqueous solution at 25°C = @4,000 centipoises)	
Ultrafree™ MC Filter Unit	UFC3 LTK 25
30,000 NMWL low-binding PLTK membrane, 25/pk	

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