94-0481 **Essentials in biore**

Rapid Analysis of Synthetic Oligonucleotides by Capillary Gel Electrophoresis

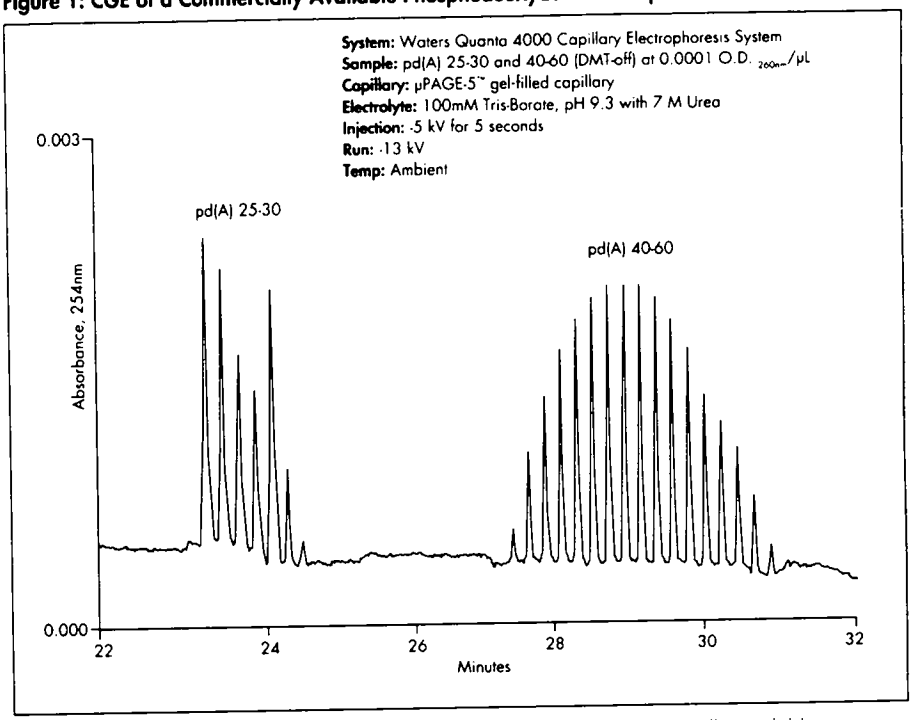
An alternative to slab gel techniques.

Biotechnology research involving the use of synthetic oligonucleotides has significantly grown during the past several years. Today, these bio-reagents are routinely used as primers in the polymerase chain reaction and in DNA sequencing. Utilization of these molecules as therapeutic agents is also being actively investigated which will further increase the demand for product.

Traditionally, product quality has been established using slab gel electrophoresis performed under denaturing conditions[1]. While this classic methodology provides useful information, the technique is time consuming, labor intensive, and difficult to automate. Furthermore, precise quantitation of the amount of full length product is indirect, requiring post-electrophoresis staining, visualization and detection techniques.

Capillary gel electrophoresis (CGE) overcomes many of the drawbacks inherent with slab gel methodologies. As required, excellent component resolution is maintained with this rapid method of analysis. Accurate quantitation, run-to-run reproducibility and convenient data documentation makes CGE an attractive alternative to slab gel techniques.

Figure 1: CGE of a Commercially Available Phosphodeoxyadenine Preparation.



Capillary gel electrophoresis yields single nucleotide resolution of a commercially available phosphodeoxyadenine preparation.

Reproducible, high resolution separations with turnkey automation.

Capillary gel electrophoresis of synthetic oligonucleotides on the Waters™ Quanta™ 4000 system offers reproducible separations with excellent component resolution as demonstrated by the analysis of a phosphodeoxyadenine standard mixture {pd(A)} (Figure 1). Methods development is not required using this automated, turnkey system which can successfully analyze samples up to two hundred nucleotides in length. UV detection of the DNA species as they migrate through the capillary permits direct oligonucleotide quantitation and eliminates the need for post-run staining and visualization techniques. In addition, run-to-run reproducibility is significantly better than that obtained using slab gels. Migration time and area count relative standard deviations of repetitive analysis of a pd(A) 25-30 and 40-60 sample were 0.78% and 2.0% respectively (N=6) attesting to the reproducibility of this method.

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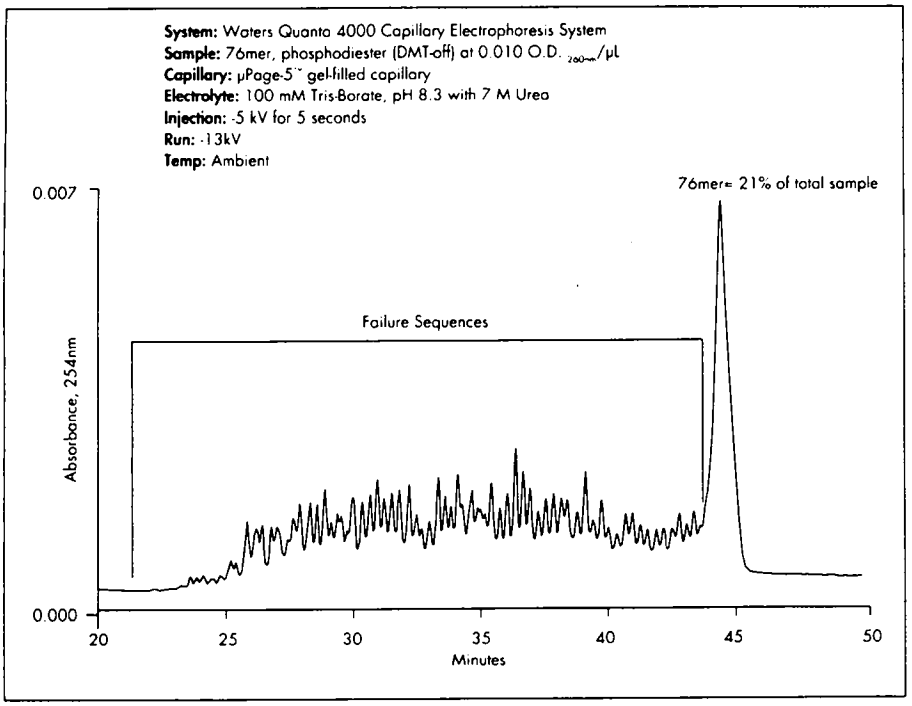
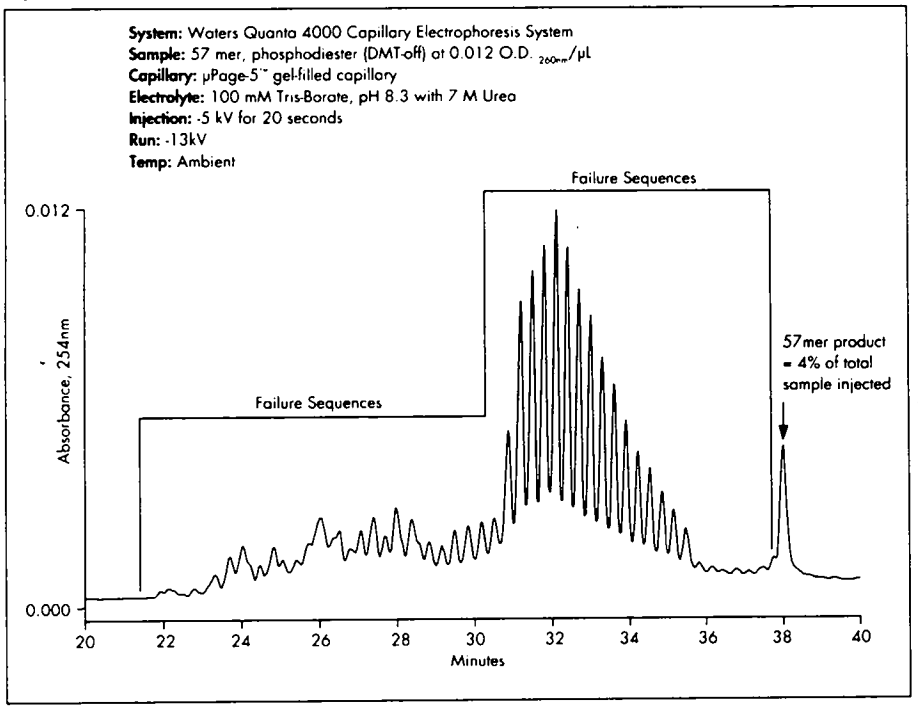
Figure 2: Quantitation of Detritylated 76mer Product



Precise quantitation of the amount of detritylated 76mer product contained in a synthesis reaction mixture is easily obtained using capillary gel electrophoresis. Compared to slab gel techniques, post-run staining, visualization and recording techniques can be eliminated.

Figure 3: CGE Analyzes Quality of a Synthesis Reaction



Capillary gel electrophoresis can help establish the quality of a synthesis reaction prior to investing time and energy in purification efforts. Furthermore, this easy to use technique can help monitor the day-to-day performance of your automated DNA synthesizer.

Rapid sample analysis.

Within 45 minutes, capillary gel electrophoresis can be used to determine the quality of a synthesized oligonucleotide. As seen with the analysis of phosphodiester oligonucleotides, excellent resolution of oligonucleoti desynthesis mixtures following detritylation is obtained (Figures 2 and 3). In addition, because only nanogram amounts of sample are required for each capillary analysis, excessive amounts of valuable material are not sacrificed.

Easy data archival.

As with the other Waters HPLC techniques for nucleic acid analysis[3,4,5], each capillary gel electrophoresis separation is permanently recorded via a chart recorder or on a data station such as the Waters Millennium™ 2010. The Millennium database lets users search through CE files using any criteria, so sample counts, tracking and statistical analyses are obtained quickly and easily.

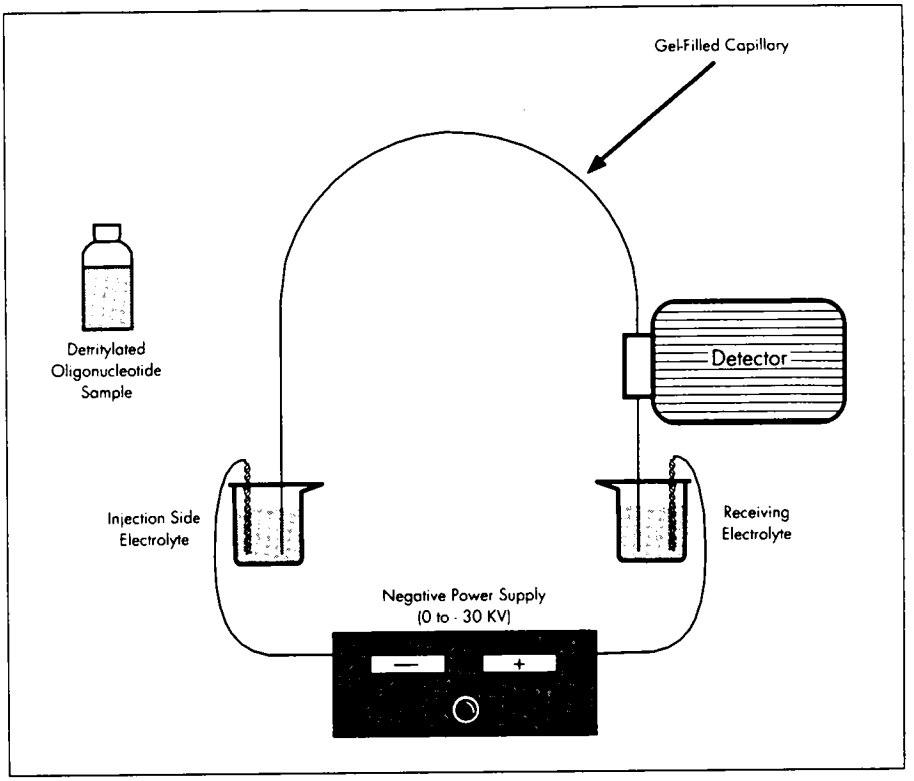
Principles and practice of capillary gel electrophoresis.

The use of capillary gel electrophoresis for the analysis of synthetic oligonucleotide mixtures has been recently documented[2]. The basis of the separation involves the differential migration of the various sized DNA species as they are electrophoretically driven through the gel matrix in a manner similar to that employed with conventional slab gels. During the separation, the smaller length failure sequences move more rapidly through the gel-filled capillary, due to their relatively smaller Stokes radii, than the larger full length oligonucleotide product.

Because gel-filled capillaries are of small internal diameter (e.g. 100 µm), excellent heat dissipation from the gel matrix occurs. Therefore, CE separations can be performed at higher field strengths (250 Volts/cm) than are possible using conventional slab gels resulting in efficient separations and relatively short analysis times.

Waters Quanta™ 4000 system configured for synthetic oligonucleotide analysis, uses an on-line UV detector for real-time monitoring of the separated DNA species as they migrate through the capillary (Figure 4). The detector flow cell is comprised of a discrete section of the capillary from which the protective polyimide coating has been removed. This design allows for the detection of relatively low amounts of oligonucleotides (i.e. low nanogram quantities) with substantially improved accuracy and reproducibility over gel staining detection methods. Gel-filled capillaries containing crosslinked polyacrylamide together with the necessary electrolyte (e.g. Tris/Borate/Urea buffer), and oligonucleotide standards can be conveniently purchased. The utilization of these devices is straightforward and fully described in the care and use manuals provided with these kits.

Figure 4: CE for Analyzing DNA Restriction Fragment.



Design overview of Waters™ Quanta™ 4000 Capillary Electrophoresis System used for the analysis of synthetic DNA. Direct, on-line 254 nm detection eliminates post-run staining and visualization techniques required with slab gel methodologies.

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) Turner, K. 1991. "New Dimensions in Capillary Electrophoresis Columns." LC/GC 9: 350-353.
3) Essentials in bioresearch (T74 and T75): "Waters Gen-Pak FAX Column HPLC Separation of DNA Restriction Fragments and PCR Products."
4) Essentials in bioresearch (N95): "Rapid Purification and Quantitation of Polymerase Chain Reaction Products."
5) Essentials in bioresearch (T10): "Rapid, High Resolution Analysis and Purification of Synthetic Oligonucleotides."

Ordering Information:	**Part Number**
Quanta 4000E CE System	
60 Hz	250000
50 Hz	250001
254nm Filter	250454
Capillary holders	250640
μPAGE™-5 (5%T/5%G) gel-filled capillary starter kit includes 2 capillaries, buffer, standards	054549
μPAGE™-5 (5%T/5%G) gel-filled capillary replacement kit includes 2 capillaries, standards	054559

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