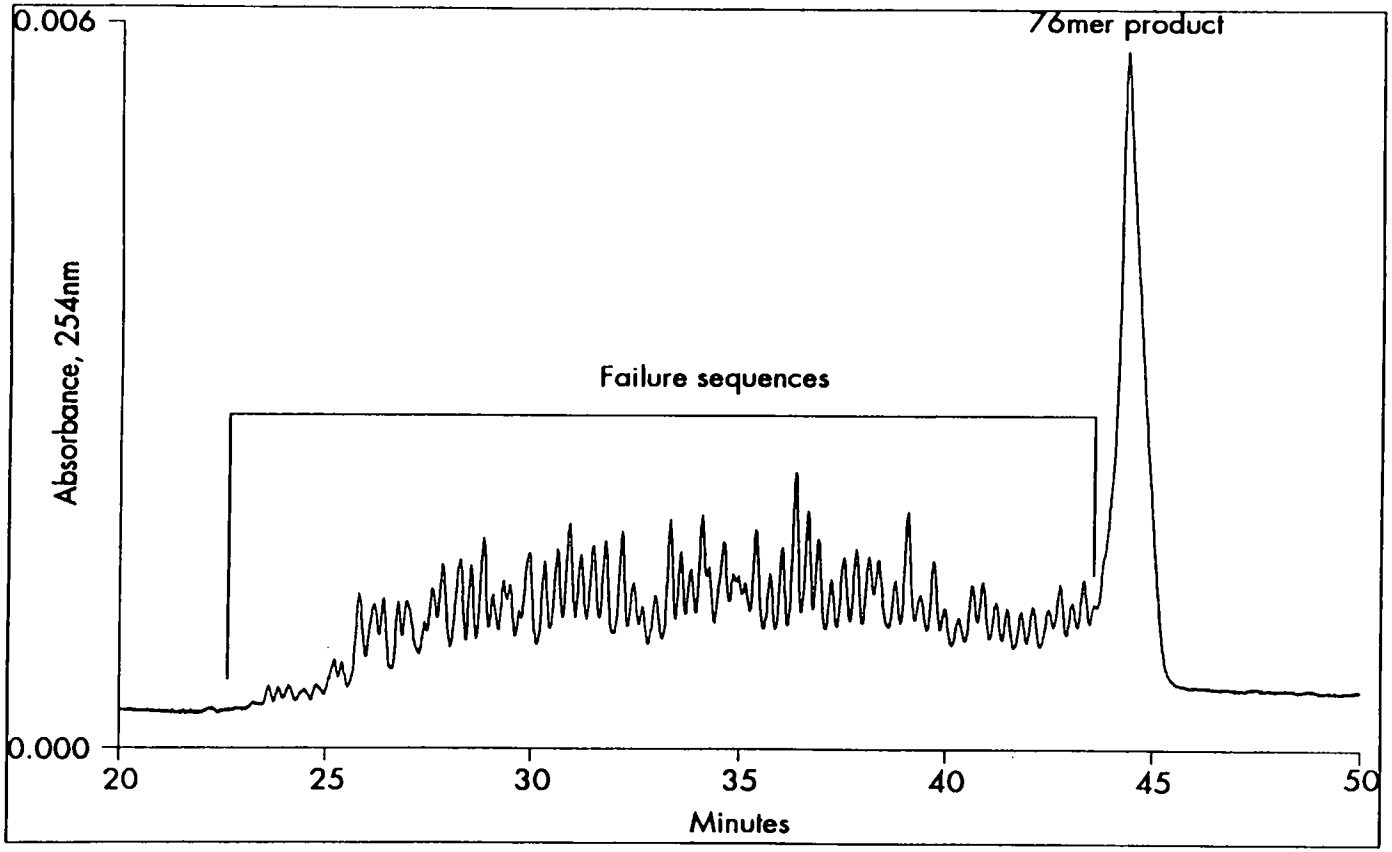
Analysis of Synthetic Oligonucleotides by Capillary Electrophoresis



Capillary electrophoresis on the Water's Quanta 4000 using gel filled capillaries is an efficient technique for the analysis of synthesized DNA. Via this easy to use automated technique, excellent resolution of the desired oligonucleotide product from shorter length, failure sequences is easily accomplished .

Conditions:

Capillary: µPage-5™ gel-filled (J & W Scientific, 91 Blue Ravine Road, Folsom, CA)

Electrolyte: 100mM Tris-Borate, pH 8.3 with 7M Urea

Sample: Detritylated 76mer at 0.010 O.D. $_{260nm}$ / µl

Injection: -5 kV for 20 sec

Run: -13 kV.

Detection: 254nm

Objective:

The objective of this application note is to demonstrate the use of capillary electrophoresis for the analysis of synthetic oligonucleotides.

Details:

Capillary electrophoresis using gel-filled capillaries is an efficient new analytical technique for the evaluation of synthetic oligonucleotide mixtures (8,9). The basis of the separation involves the molecular sieving 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 gel electrophoresis. During the separation, the smaller length failure sequences contained in the reaction mixture move more rapidly through the gel-filled capillary, due to their relatively smaller stokes radii, than the larger full length oligonucleotide product. The presence of the DMT group on a full length product will increase its migration rate through the capillary compared to the same detritylated sequence. For this reason, analysis of fully detritylated samples is recommended to simplify electropherogram interpretation.

By design, gel-filled capillaries are of small internal diameter (e.g. 0.75μm) which promotes efficient heat dissipation from the gel matrix during the separation. As a result, CE separations can be performed at higher field strengths (250 Volts/cm) than are possible using conventional slab gels. This translates into very efficient separations in relatively short run times. In addition, the Quanta 4000 uses an on-line UV detector for real-time monitoring of the separated DNA species as they migrate through the capillary. (Note: The actual detector flow cell is comprised of a discrete section of the capillary in which the protective polyamide coating has been removed.) This design allows for the detection of low amounts of oligonucleotides (i.e. low nanogram quantities) during each separation.

System:

The system used consisted of a Waters Quanta 4000 Capillary Electrophoresis System and an 860 Expert Ease Data System (signal via SIM/Lace).

References:

1) Cohen, A.S., D.R. Najarian, A. Paulus, A.Gutterman, J.A. Smith and B.L. Karger. 1988. Rapid separation and purification of oligonucleotides by high-performance capillary gel electrophoresis. Proc. Natl. Acad. Sci. U.S.A. 85: 9660-9663.

2) Guttman, A. and N. Cooke. 1991. Capillary Gel Affinity Electrophoresis of DNA Fragments. Anal. Chem. 63: 2038-2042.