INTEGRATED DNA TECHNOLOGIES

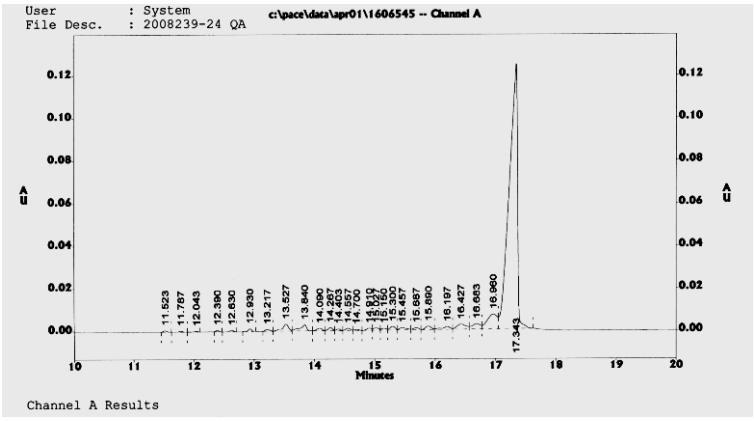
Capillary Electrophoresis of Oligonucleotides
Quick Look

This is a modified, quick look version of the full Technical Report "Capillary Electrophoresis of Oligonucleotides." Please see the full version for a more comprehensive explanation.

The technique of capillary gel electrophoresis (CGE) has become and industry standard for assessing the purity of biochemical syntheses. It is designed to delivery accurate quantitative information in an automated, high throughput environment.

Very small volumes of sample are introduced into a fused silica capillary that has an inner diameter of 10 □m and is filled with a sieving matrix (gel) containing 7 M urea. The capillary is heated to 30°C which, along with the urea, denatures the oligonucleotide. In the presence of a high voltage electrical field, the oligonucleotides migrate through the gel and the different species present in the injected sample are separated by size; the resolving power of the capillary is determined by the voltage. Absorbance is measured at 254 nm as the zones migrate past an optical window. The optical density of each zone is plotted as a function of time in the capillary to create an electropherogram, or trace. Oligonucleotide purity is simply the ratio of the area of the peak corresponding to the full-length synthesis product to the total area of the trace.

Figure 1. A capillary electrophoresis trace of an oligonucleotide synthesis. The axes are uV absorbance units (AU) versus time on the column in minutes. The ratio of the main peak to the total indicates purity greater than 95%.



The final, main peak is the full-length synthesis product. The penultimate peak usually represents the population of products in which a single nucleotide is missing (n-1mers). Since chemical reactions are never 100% efficient, not all of the available couplings will occur at each step in the synthesis. While

the majority of these failed couplings are capped, a small population of the growing oligonucleotides will remain reactive. Other peaks to the left of the main peak are composed primarily of capped oligonucleotides from n-2 to n-(n-1). Occasionally, peaks will appear to the right of the main peak and represent oligonucleotide species that were not completely deprotected or have extra bases (n+1mers).

The IDT High Throughput Analysis Department has the capacity to perform thousands of CGE analyses daily. Currently, IDT provides CGE on all purified oligonucleotides <61 bases free of charge. Both CGE and mass spectrometry results are available on the web site.