Analysis of Amplified Nucleic Acids by Capillary Electrophoresis Using Polymer Solutions

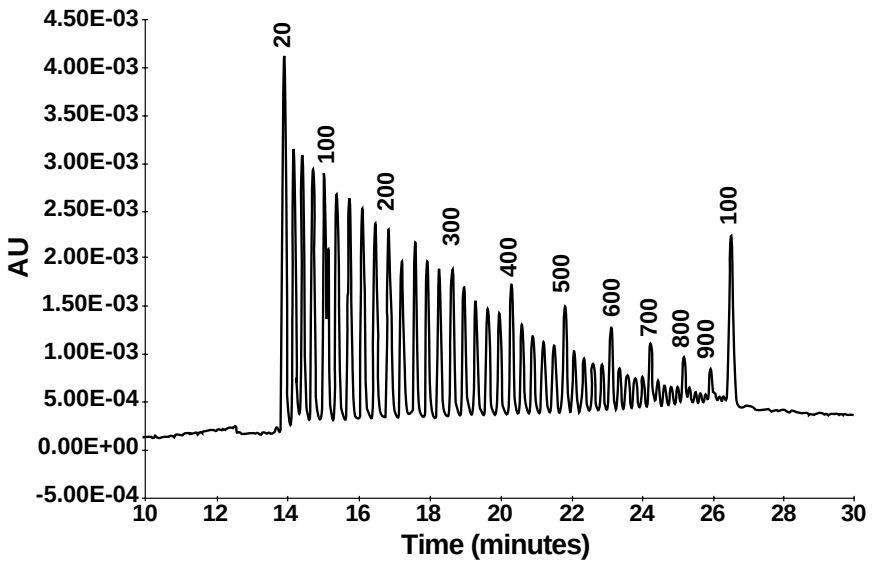

Fig. 1. Analysis of 20+100 bp DNA ladder by capillary electrophoresis. Conditions: PCR* Product Analysis Buffer (catalog number 148-5024), 36 cm x 50 µm, LPA coated capillary (catalog number 148-3070), detection at 260 nm, electrophoretic injection 10 sec. at 10 kV, run voltage was set at 5kV. Capillary and reagents compartment were thermostated to 27 °C.

Introduction

Nucleic acids amplification simplifies and transforms experimental medical and biological sciences. The main purpose of these techniques is the generation of a DNA molecule from a particular template, and the increment in concentration of the product.

Amplification methods are widely used in forensic sciences, molecular evolution, DNA sequencing, genetic analysis, genetic expression, genomic mapping, clinical diagnosis of cancer, detection of infectious diseases, and gene therapy. Due to its resolving power, slab gel electrophoresis has traditionally been the method of choice for analysis of nucleic acids, but the limitations and drawbacks of slab gel electrophoresis have stimulated the search for alternative electrophoretic methods. Traditional electrophoresis is labor intensive, lacks quantitative results, and is difficult to automate.

Recently, the performance of electrophoresis in capillaries of reduced internal diameter (5 to 200 µm) has proven to be an alternative technique to slab gel electrophoresis. Capillary electrophoresis (CE) is commonly performed in fused silica capillaries that allow on-line detection of the analytes. This mode of detection is rapid and produces quantitative results, opening another source of information (not only the compound of interest is detected, but also the amount present becomes known) that can be exploited by the analyst.

Nucleic acid analyses by electrophoresis are typically performed under the influence of a sieving matrix. In the capillary format, sieving can be obtained using a gel filled capillary or through the employment of sieving polymers. The advantages of polymer solutions include simplicity (the capillary content is easily replenished in between runs by flushing with the polymer solution), capillary longevity, cost per analysis, reproducibility, and the ability to use multiple chemistries in a single capillary. Also polymer solutions are compatible with hydrodynamic injection, thus allowing the analysis of samples containing high levels of salt without the need for desalting.

Results

Figure 1 shows an electropherogram of a 20 mer ladder spiked with a 100 mer ladder. The migration times of the ladder components can be used to determine the size of DNA fragments of unknown size. Figure 2 displays the analysis of a PCR* product. By comparison with the ladder, it was estimated that the PCR* product was a 320 mer. The peaks that appear before the product are the components of the reaction mixture (*e.g.* dNTPs, primers). Figure 3 depicts a standard curve constructed by plotting the migration times of the DNA ladder vs. chain length.

DNA amplification and its multiple applications have increased the need for fast, accurate analytical methods. These results demonstrate that CE is a viable alternative to slab gel electrophoresis.

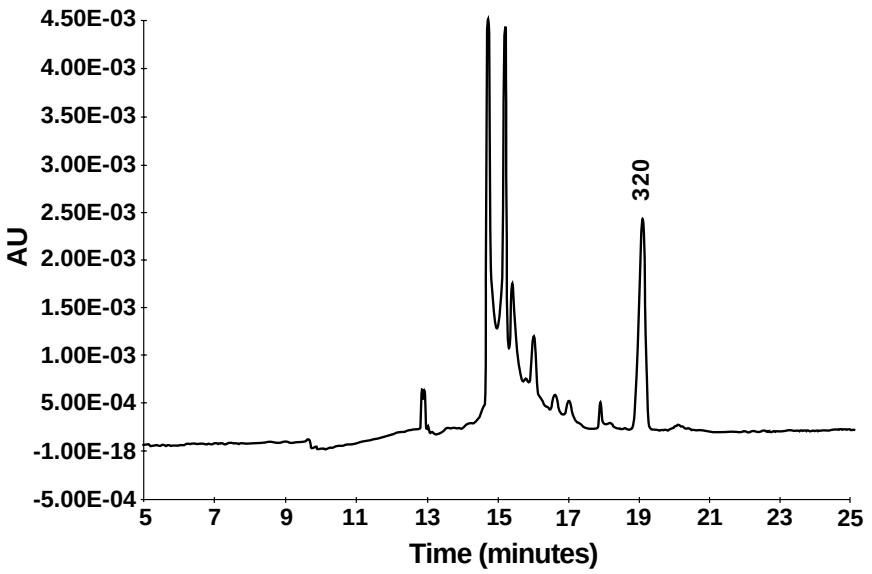

Fig. 2. Analysis of PCR* product by capillary electrophoresis. Conditions identical to those described in Figure 1.

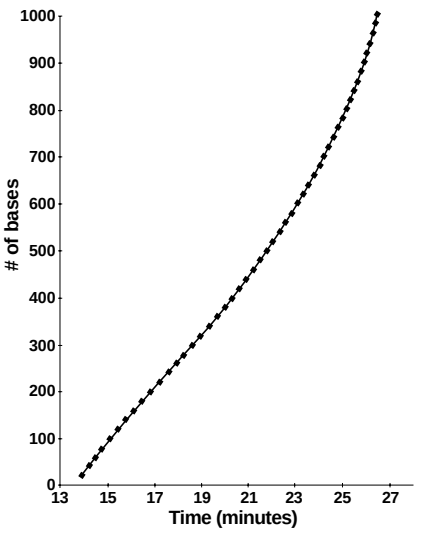

Fig. 3. Standard curve constructed by plotting the migration times of the DNA ladder vs. chain length.

References

1. Zhu, M., Hansen, D. L., Burd, S. and Gannon, F., *J. of Chromatography*, **480**, 311, 1989.

2. Mullis, K. B., Ferre, F. and Gibbs, R. A., **The Polymerase Chain Reaction**, Birkhauser Boston, 1994.

* The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffman-La Roche. Use of the PCR process requires a license.

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