366c New Approaches to Genotyping with End-Labeled Free Solution Electrophoresis

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The size-based separation of DNA molecules is a critical step in modern molecular biology techniques, including DNA sequencing and genotyping as well as "DNA fingerprinting" for forensic applications. Conventional techniques for accomplishing DNA separation rely on electrophoresis in crosslinked gels or entangled polymer solutions to provide molecular sieving. Electrophoresis in sieving matrices has important drawbacks, including a fundamental limit in the ability to separate large DNA fragments, poor performance under high electric fields, and practical difficulties with handling crosslinked gels or viscous polymer solutions. End-Labeled Free-Solution Electrophoresis (ELFSE) is an alternative and potentially very powerful technique for separating DNA fragments according to size in the absence of a sieving matrix, via the creation of novel bioconjugate moieties. Since no sieving matrix is necessary, the difficulties associated with loading viscous polymer solutions into narrow microchannels are eliminated. In ELFSE, each DNA molecule in a sample is covalently modified with a unique frictional modifier or "drag-tag" that serves to modify the electrophoretic mobility of the DNA in a size-dependent fashion. In addition to DNA sequencing, ELFSE has recently been demonstrated for highly multiplexed mutation detection by single-base extension (SBE). A family of differently sized polypeptoid drag-tags was conjugated to SBE primers specific to 10 loci on p53 exons 5-9. An optimized thermal cycling protocol was used to generate the SBE products in less than 10 minutes, and the differently sized drag-tags allowed easy separation of each of the SBE products in free solution in capillaries. Rapid separations were possible in glass microchips, with excellent resolution of the SBE products achieved in less than 30 seconds. This SBE-ELFSE assay is an excellent candidate for implementation on an integrated microfluidic device. By performing the thermal cycling, reaction cleanup, and separation on a multichannel device, very high throughput is possible for detection of mutations and single-nucleotide polymorphisms. ELFSE has also been employed for separation of PCR products in free-solution. Thiolated primers allow drag-tags to be attached to one or both ends of the PCR product either before or after thermal cycling. With a drag-tag attached to one end only, the PCR products are denatured prior to analysis, allowing high-resolution separation of the drag-tag labeled, single-stranded DNA. Even higher resolving power can be achieved by attaching drag-tags to both ends of the PCR product, and analyzing the DNA in double-stranded form. This type of analysis is well-suited for STR sizing and other PCR-based genotyping assays. PCR sizing with ELFSE separation is another promising candidate for implementation on an integrated microfluidic device, and could be the breakthrough required for a truly high-throughput, high-impact lab-on-a-chip device for genotyping and DNA fingerprinting.