485d DNA Sequencing by Free-Solution Conjugate Electrophoresis with Genetically Engineered Protein Polymer Drag-Tags

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The separation of DNA according to size, for applications in DNA sequencing and genotyping, is typically performed using electrophoresis in crosslinked gels or entangled polymer solutions. In recent years, high-throughput sequencing and genotyping is carried out using automated capillary electrophoresis, in microchannels with 50-75 μm inner diameters. Further miniaturization of gel electrophoresis is being pursued actively, and promises further increases in the speed and throughput, as well as a reduction in costs. However, gel-based DNA electrophoresis in microchannels has its shortcomings. One drawback in particular is the difficulty associated with loading high-viscosity polymeric sieving matrices into microchannels. This is a major obstacle in the implementation of microfluidic chip-based sequencing and genotyping. Free-Solution Conjugate Electrophoresis (FSCE) is an alternative method for DNA separation that is performed without any gel or sieving matrix but through the covalent attachment of a frictional "drag-tag" to each DNA fragment. This "drag-tag" modifies the electrophoretic mobility of DNA molecules in a size-dependent fashion. Since no sieving matrix is needed, the difficulties associated with loading viscous polymer solutions into narrow microchannels are eliminated. Instead, size-based DNA separation can be accomplished in free aqueous solution. The drag-tag must meet several criteria. It must be a large polymer that is also water-soluble and totally monodisperse, and which can be attached end-on to DNA molecules. To meet these challenging requirements, a series of non-natural polypeptide drag-tags or "protein polymers" has been created by genetic engineering. Artificial genes encoding custom-designed repetitive polypeptides were constructed using a controlled cloning technique and expressed in E. Coli. After immobilized metal affinity chromatographic purification, these larger protein polymer drag-tags have been successfully conjugated to an oligonucleotide primer, and used to prime the enzymatic Sanger reaction to generate drag-tag-labeled DNA sequencing fragments. Using this novel bioconjugate sample, we have been able to demonstrate 4-color sequencing of ~ 150 bases of M13 DNA by capillary electrophoresis, in the absence of a sieving matrix and in under 15 minutes. Both linear and branched drag-tag architectures are being explored, leading to interesting design possibilities. With further work, we hope to replace the present methods of DNA sequencing with an FSCE method that yields long read lengths in short chip electrophoresis-based separations.