Gel and Polymer-Solution Mediated Separation of Biopolymers by Capillary Electrophoresis

András Guttman*
Torrey Mesa Research Institute, 3115 Merryfield Row, La Jolla, CA 92121

Abstract

In the age of genomics and proteomics, high-resolution separation techniques are routinely utilized in an integrated and automated fashion to solve formidable separation problems and provide the means for large-scale analysis of biological samples with excellent resolution. By automating the current manual procedures, capillary gel (CGE) and polymer-solution mediated electrophoresis greatly enhance the productivity of biopolymer analysis while also reducing both analysis time and the human intervention necessary from sample loading to data processing. The advent of this novel and high-performance bioseparation technique has made it possible to sequence the human genome and revealed global changes in the genome and proteome level, bringing about a revolutionary transition in our views of living systems on the molecular basis. CGE and polymer-solution mediated electrophoresis and related microseparation methods (e.g., electrophoresis microchips) are quickly becoming important separation and characterization tools in analytical biochemistry and molecular biology. This review gives an overview of the key application areas of DNA, protein, and complex carbohydrate analysis, and summarizes the latest developments on CGE column technology, including capillary coatings and sieving polymer matrices. Micropreparative aspects and related microseparation techniques are also discussed.

Introduction

In biological sciences, each decade has a major theme. It was genomics and DNA sequencing in the last decade of the 20th century, and it is functional genomics and proteomics for the first decade of the 21st century. These major fields also have major challenges in separation science. Electrophoresis, a differential migration method based on the size-to-charge ratio of ions in the presence of an applied electric field, had, from its beginning, a strong biological focus. In the 1930s, Nobel Laureate, Arne Tiselius, enumerated many of the important principles of electrophoresis (1). Later, in order to contain the bulk fluids, electrophoresis was practiced using supporting (i.e., anticonvective) media (e.g., gels made of cross-linked polyacrylamide and agarose) (2). When the pore sizes of such gels are optimally controlled, the electrophoretic migration of polyionic biopolymers migrate as a function of their size (instead of their charge-to-mass ratio), because of the sieving effect of the gel. It was discovered that denatured proteins in the presence of the surfactant sodium dodecyl sulfate (SDS) yielded migration based on molecular weight, as a result of a constant mass-to-charge ratio of the SDS–protein complexes (3). Similar phenomena occur in the gel separation of single- and double-stranded (ds) DNA in which each base or base pair yields a constant increment of mass and charge. Size separation proved to be very important for biopolymer separation in genomics and proteomics.

In the last decade of the 20th century, an automated and high-performance electric field mediated differential migration technique, capillary electrophoresis (CE), was introduced in almost every aspect of the basic and applied biomedical and clinical research. Using narrow bore fused-silica capillaries filled with cross-linked gels or noncross-linked linear polymer solutions, unprecedented high resolving power was achieved in separation of biologically important macromolecules. As an instrumental approach to electrophoresis, CE offers online detection and full automation. The method is ideally suited for handling microliter amounts of sample material and the throughput is superior to conventional approaches. Capillary gel electrophoresis (CGE) separates complex mixtures in just minutes with excellent reproducibility, generating a large amount of data. Another important aspect is the multicapillary operation that currently represents the basis of DNA sequencing machines. The availability of high-capacity computer systems capable of rigorous qualitative and quantitative analysis of the separation profiles enables the establishment, storage, and operation with large databases. Recent implementation of contemporary microfabrication technology to electrophoresis microchips initiated a fast moving interdisciplinary field in the size separation of biopolymers (4).

Gels and polymer solution mediated CE was one of the supported technologies by the Human Genome project to be developed for DNA sequencing. With linear polymer solutions, enormous column efficiencies were found, up to 3×10^7 plates/m (5) that were required to separate consecutive Sanger fragments for which the mobility differences were extremely small. As the advent of CGE has already made possible it to sequence the

* Current address: Diversa, 4955 Directors Place, San Diego, CA 92121.

human genome (6), it is anticipated that this technique will also reveal global changes at the proteome level, bringing about the revolutionary transition in our views of living systems on the molecular basis. CGE began the drive towards miniaturization (lab-on-a-chip), as microfluidic devices can be manufactured similar to that of semiconductor devices, and existing CE methods can be readily transferred from the capillary to the chip (4).

Experimental

Capillary coating technology

To eliminate the distortive effects of electroosmotic flow (EOF) and minimize wall adsorption of the analyte molecules, special coating techniques have been developed. As early as the mid 1980s, Hjerten suggested the use of bifunctional reagent γ-methacryloxypropyl-trimethoxysilane and subsequent cross-linking the surface-bound methylacryl groups with linear polyacrylamide to suppress EOF (7). Later, similar polyacrylamide-coated capillary columns were introduced for SDS CGE separation of proteins (8). Small carbohydrates were also attempted to covalently coat fused-silica capillaries through the same bifunctional reagent (9). Unfortunately, at higher pHs the Si–O–Si bond is susceptible to hydrolysis; therefore, a more stable direct Si–C coating was introduced by Cobb et al. (10). The Si–C bond was assumed to be more hydrolytically stable, resulting in improved stability over a wide pH range of 2–10. For protein analysis by SDS–CGE, covalent dextran coating featured good run-to-run migration time reproducibility when the capillary was also filled with similar dextran polymer (11). Various existing liquid chromatography (LC) and gas chromatography coatings were also attempted with gel or polymer solution filled capillary columns (12,13). Covalently coated capillaries also offer high-speed separations with little or no requirement for preseparation equilibration.

Because of the extra efforts in chemistry and labor involved in the fabrication of covalently coated capillary surfaces, noncovalent so-called "dynamic coatings" quickly emerged (14). Dynamic coatings create a thin layer of adsorbed material at the inner surface of the capillary. It can be applied either prior to or during the electrophoresis separation. Such coatings can be readily regenerated by flushing the coating solution through the column whenever necessary. Both dynamic and covalently coated capillaries have proven useful in CE with gels or polymer solutions, as summarized in two recent reviews discussing the current state of the art of covalent and dynamic coatings (14,15). Tian et al. compared various surface derivatization agents in conjunction with coating polymers for DNA heteroduplex analysis of the breast-cancer susceptibility gene (BRCA1) (16) and found that the combination of chlorodimethyloctylsilane with polyvinylpyrrolidone (PVP) polymer that was most effective was used to separate the relevant DNA fragments in the hydroxyethylcellulose (HEC) matrix within 10 min. Chiari et al. applied absorbed polydimethylacrylamide-co-allyl glycidyl ether coating to CE analysis of DNA fragments that were found to be stable even under such harsh conditions as highly alkaline pHs, elevated temperatures, and denaturing conditions that usually rapidly deteriorate most other coatings (17). Some of the dynamic coating materials, such as short-chain polydimethylacrylamide, may also serve as sieving medium for mutation analysis of DNA fragments in uncoated capillaries (18). Recent efforts combine the advantages of dynamic and covalent coatings (19); that is, the thin layer of polyacrylamide is dynamically coated to the inner surface of the capillary followed by allylamine treatment (20).

Sieving gels and polymer solutions

The capillaries were filled first with cross-linked gels similar to those used in traditional polyacrylamide gel electrophoresis (PAGE), and they have proven to be particularly promising in the analysis of single-stranded DNA (primers and probes) and small protein molecules (21). The first report on single nucleotide resolution of the $pdA_{40–60}$ DNA ladder (Figure 1) by Guttman et al. (22) opened up new horizons in modern CGE-based DNA sequencing analysis, which actually made possible the faster-than-anticipated completion of the Human Genome project (6). The three-dimensional structure of the cross-linked polyacrylamide gel creates a molecular sieve and, therefore, results in size separation of biopolymers under an electric field. Similar to traditional slab gel electrophoresis, the monomers of the separation matrix are filled into the narrow bore capillary and polymerized in situ inside the capillary. As gels may vary from viscous fluids to solids (23), less viscous polymers can be filled into a capillary and replaced by applying pressure on a polymer-filled container connected to the separation column. Today, both types of high-viscosity cross-linked gels and low-viscosity polymer solutions are extensively employed for the separation of various biopolymers (24,25).

Polyacrylamide, the most widely used cross-linked gel, is usually covalently attached to the inner capillary surface via a bifunctional reagent; that is, γ-methacryloxypropyltrimeth-

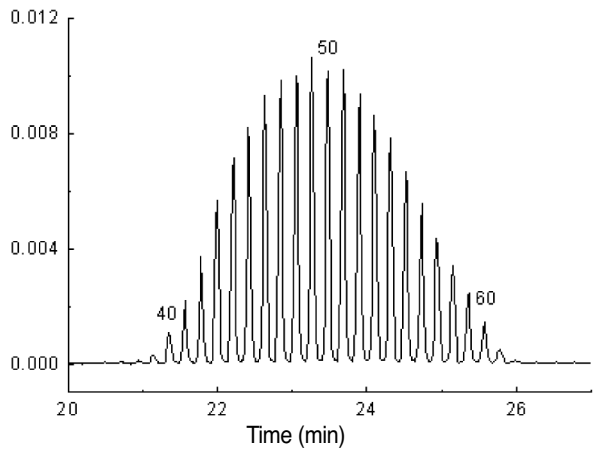

Figure 1. The first CGE-based single-stranded DNA separation ($pdA_{40–60}$) that opened up the possibility of large-scale DNA sequencing in microbore systems. Conditions: polyacrylamide gel (7.5% T–3.3% C) in 7M urea; 100mM Tris and 250mM borate (pH = 8.3); E = 350 V/cm; detection, 260 nm; capillary, l = 30 cm (effective); and 75-µm i.d.

oxysilane. The pore size of the gel is determined by the relative concentration of monomer (acrylamide) and cross-linker (*N,N*-methylene-bisacrylamide) used in the polymerization reaction (26). Samples are introduced into cross-linked gel-filled capillaries by electrokinetic injection method only (i.e., starting the electrophoresis run from the sample vial). Cross-linked gels are sensitive to temperature, changes in pH, and high voltage (23) during both polymerization and separation, usually leading to bubble formation (i.e., termination of the current flow).

The use of linear polymer solutions became very popular and extensively used recently for biopolymer analysis (27–30). These noncross-linked polymers feature very flexible dynamic pore structure, defined only by dynamic interactions between the polymer chains. Thus, their sieving capability can be adjusted by changing such separation variables as capillary temperature, separation voltage, salt concentration, or pH. Linear polymer solutions are not heat sensitive, and even if a thin layer of the polymer is attached to the capillary wall (see dynamic coating), the separation matrix can usually be simply replaced in the capillary by pressure. Noncross-linked sieving matrices support both electrokinetic and pressure injection methods, also enable sample stacking, and offer excellent run-to-run peak area reproducibility, supporting routine quantitative analysis. In the early report of Karger et al., linear polyacrylamide was introduced into coated capillary columns and applied to the separation of dsDNA fragments ranging up to several thousand base pairs (Figure 2) (27). Later, Widhalm et al. demonstrated the usefulness of linear

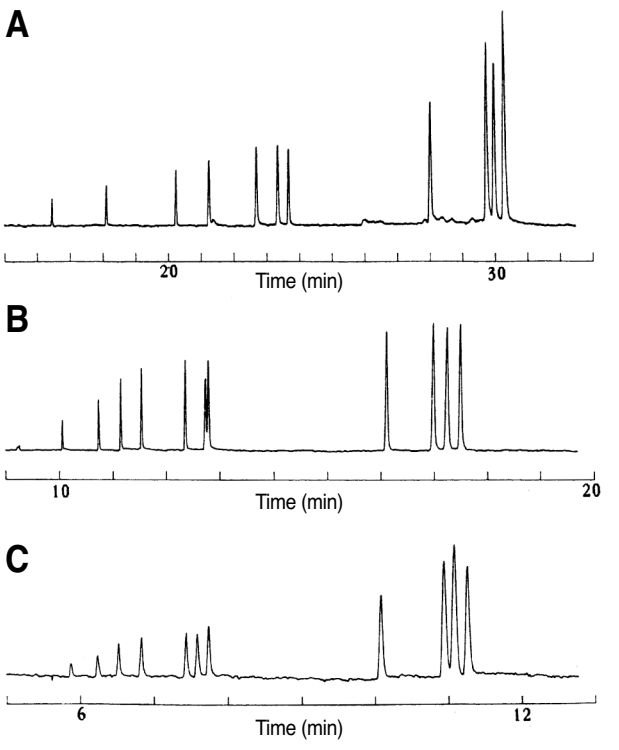

Figure 2. Separation of Hae III restriction fragment digest of ϕX 174 DNA on linear polyacrylamide gel compositions of (A) 6%, (B) 9%, and (C) 12% T. Peaks in increasing migration order: 72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, 1353, 4353, and 7253 bp. Conditions: l = 35 cm, 15 cm, and 10 cm, respectively; E = 300 V/cm; buffer, 100mM TBE; and injection, 150 V/cm for 3 s.

polyacrylamide gels for the separation of SDS-protein complexes ranging from 17.8 to 77 kD in 60 min (31). Others successfully employed linear polyacrylamide material for the analysis of protein samples of biological origin (32–34).

Alternative matrices (such as agarose) and low concentration entangled linear polymers (such as derivatized celluloses, dextran, polyethylene oxide, hydroxyethyl cellulose, pollulane, and polyvinyl alcohol) have also been attempted in CE separation of biopolymers. Melted agarose gel-filled capillaries were studied in the separation of dsDNA molecules (35). Separations of DNA fragments using derivatized celluloses were demonstrated in the early 1990s by different research groups (36,37). de Carmejane et al. investigated the migration of dsDNA molecules in semidilute HEC solution and suggested the segmental DNA motion, allowing quantitative description of the changing shape of the DNA as it interacts with the sieving polymer (38). With a three-dimensional view of the migrating analyte molecules, they observed U-shape conformations oriented at an angle to the microscope plane, as well as ambiguities and artifacts resulting from loss of information from DNA segments not in focus. Barron's group extensively studied the impact of polymer hydrophobicity on the properties and performance of DNA sequencing matrices for CE (39), emphasizing the importance of polymer hydrophobicity to obtain high-performance DNA sequencing matrices as they form robust highly entangled polymer networks. The same group introduced an interesting new sequencing media, featuring a thermocontrollable viscosity switch (40). Polyethylene oxide (PEO) and PVP solutions both proved to be excellent separation matrices also featuring self-coating properties for CE-based DNA sequencing (41–45). PVP sieving and wall coating matrix was used for rapid molecular diagnostics of 21-hydroxylase deficiency, detecting the most common mutations in the 21-hydroxylase gene using primer extension technique and CE (46). The Cy5-labeled primer and the two possible primer extension products (mutant and wild type) were completely separated in 90 s on a 10-cm effective-length capillary. A versatile low-viscosity polyethylene-oxide-based sieving matrix was developed for nondenaturing DNA separations in capillary array electrophoresis (47).

Recently introduced novel thermoresponsive copolymers, consisting of hydrophobic and hydrophilic blocks [such as pluronics (48–50)] and polyisopropylacrylamide grafted with PEO chains (51), have offered promising opportunities. The grafted copolymer of poly(*N*-isopropylacrylamide)-γ-polyethyleneoxide (51) with self-coating ability and slightly adjustable viscosity properties was applied for rapid and high-resolution separation of dsDNA fragments. In particular, thermoresponsive polymers can offer some practical advantages in CE, such as easier handling and loading of the viscous polymer solutions without the requirement of a high-pressure manifold. Sudor et al. introduced a novel block copolymer thermoassociating matrix for DNA sequencing (52). These comb polymers are made of hydrophilic polyacrylamide backbone, grafted with poly-*N*-isopropylacrylamide side chains, and characterized by lower critical solution temperature. Thermothickening properties of these polymers, attributable to the formation of transient intermolecular cross-links at higher temperatures, offer clear advantages for DNA sequencing [e.g., easy handling of low viscosity solu-

tions at low temperatures and excellent sieving characteristics of high viscosity solutions at elevated temperatures (sequencing read lengths up to 800 bases)].

Other low-concentration polymer solutions, such as of natural polysaccharide glucomannan, were also attempted as a sieving additives for the separation of DNA fragments up to 1400 bp (53). Nakatani et al. studied the effect of temperature and viscosity of pullulan-based sieving medium on electrophoretic behavior of SDS proteins in CE (54). TreviSol, a low-viscosity polysaccharide matrix, has been characterized in CE for the separation of DNA fragments and exhibited good separation ability for larger DNA fragments (55). The same group evaluated composite agarose–HEC matrices for the separation of DNA fragments by CE (56). Relative to homogenous gels, these composite matrices provided enhanced separation selectivity, especially for DNA fragments larger than 100 bp. In another approach, a viscosity adjustable block copolymer (PEO99/PEO69/PEO99) (49) and a triblock polymer of polyethyleneoxide–polypropyleneoxide–polyethyleneoxide (48) was successfully applied to obtain high-speed separation of nucleic acids by CE. Recently, Chiari et al. synthetized sugar-bearing polyacrylamide copolymers as sieving matrices and capillary coatings for DNA electrophoresis (57) and also copolymerized poly(*N*,*N*-dimethylacrylamide) with hydrophilic monomers to improve separation performance (58). A copolymer of acrylamide and β-D-glucopyranoside was used as a low-viscosity and high-capacity sieving matrix for the separation of dsDNA molecules. The chain length was inversely proportional to the number of glucose residues incorporated into the copolymer (59). Purified galactomannans from guaran, tara gum, and locust bean gum were attempted as sieving medium for CE-based DNA sequencing. Separation efficiency exceeded one million theoretical plates for DNA fragments less than 600 bases long (60). Pluronic copolymer liquid crystals were successfully used as unique, replaceable media in CGE (50). Separation of peptide/protein model mixtures was attempted in surface-modified capillaries filled with Pluronic liquid crystals acting in secondary partition mechanism (61,62). Solutions of monomeric nonionic surfactants (*n*-alkyl polyoxyethylene ethers) behave as dynamic polymer structures and therefore can be used as sieving matrices for DNA fragment analysis in capillary columns (44). Separation of dsDNA fragments were also successfully attempted in a transient interpenetrating network of PVP with polyacrylamide (63) and poly(*N*,*N*-dimethylacrylamide) (64). Viscosity measurements revealed that this interpenetrating network had significantly higher viscosity than that of the simple mixture containing the same amount of components. Other additives, such as mannitol, were attempted to enhance the sieving ability of hydroxy-propylmethylcellulose (HPMC) for dsDNA fragment analysis (65,66), suggesting that chains are formed through hydrogen bondings among mannitol, HPMC, and borate, reshaping the network and decreasing the pore size. Addition of glycerol to entangled solution of HPMC-enhanced separation performance on DNA fragments in CE, which was probably caused by the formation of dimeric 1:2 borate–didiol complexes with both glycerol and HPMC (67).

Separation of nucleic acids, DNA sequencing

CE with polymer matrices is the method of choice for the large-scale and high-throughput separation of nucleic acids, such as genotyping, polymerase chain reaction (PCR) product analysis, and DNA sequencing. Nondenaturing gels or polymer networks are suggested to recognize size and shape (secondary structure) differences of nucleic acids. Denaturing CGE is utilized mainly in pure size separation of relatively short single-stranded oligonucleotides (primers and probes) and in DNA sequencing. As a general rule of thumb, lower concentration nondenaturing gels or polymer solutions accommodate several base-pair resolving power for PCR products and larger dsDNA molecules, and high-concentration denaturing gels enable single-base resolution, possibly up to 1000 bases or higher (i.e., useful for DNA sequencing). Major applications on size-sieving CE of nucleic acids are discussed in detail for diagnostics (68), genotyping (69), antisense DNA analysis (70–73), linear and supercoiled DNA separation (74), single-stranded conformation polymorphism study (75), DNA sequencing (76–79), and DNA restriction fragments (80). Comprehensive reviews on specific molecular diagnostics applications are given (81–83).

Large-scale genotyping by precise sizing of multiplexed short tandem repeat loci was reported using energy-transfer fluorescent primers by high-resolution capillary array electrophoresis (84). Analysis of a polyadenine tract, the $(A)_{10}$ repeat within the cysteine-rich domain of the transforming growth factor-beta type II receptor gene in colorectal cancer, was attempted by nongel-sieving CE (85). Optimized conditions enabled the determination of one nucleotide difference in 8–32 nucleotides. Stellwagen et al. (86) studied DNA conformation and structure using CGE and found that counter ions preferentially bound to DNA oligomers with A-tracts, especially in A_nT_n sequence motif. Genetic profiling of grape plant variants and clones were analyzed using dynamic size-sieving CE in conjunction with random amplified polymorphic DNA analysis (87). Relative to slab-gel electrophoresis using ethidium bromide staining, CGE with laser-induced fluorescence (LIF) detection provided superior separation efficiency and detection limits in revealing polymorphic differences.

An example of selectivity manipulation by soluble affinity ligand interactions was evaluated by the addition of intercalating agents to the separation gel-buffers system in dsDNA separations (37,88). In this instance, the ligand (ethidium bromide) intercalated between the two strands of the DNA double helix during the separation process. As it is positively charged at the separation pH, the complexation reduces the migration times of all the DNA fragments and, therefore, increases the separation time window (i.e., enhanced resolution).

Constant denaturant CE (CDCE) permits high-resolution separation of single-base variations occurring in an approximately 100 bp isomelting DNA sequence based on their differential melting temperatures [single-nucleotide polymorphism (SNP) hunting]. By coupling CDCE for highly efficient enrichment of mutants with high-fidelity polymerase chain reaction, Thilly et al. (89) developed an analytical approach to detecting point mutations at frequencies equal to or greater than 10^{-6} in human genomic DNA. The same group used the CDCE for pooled blood samples to identify SNPs in Scnn1a and Scnn1b genes (90), and they introduced a two-point LIF detection method to improve novel mutation identification and precise fraction collection (91).

The separation of DNA restriction fragments was studied in CE with polymer solutions under alkaline conditions in epoxy-coated capillaries and found that at pH 11 the theoretical plate numbers exceeded several million (92). At pH 12, single-stranded DNA molecules were still well separated in entangled HEC solutions, however, the resolution significantly decreased in dilute polymer solutions. Heller (93,94) thoroughly studied the separation of double- and single-stranded DNA in the linear poly-*N,N*-dimethylacrylamide matrix and found significant differences between the experimental data and predicted scaling laws. Analysis of gamma-radiation-induced damage to plasmid DNA was evaluated by using dynamic size sieving CE (95). Oncolumn sample preconcentration and separation of DNA samples was demonstrated in an open tubular capillary system utilizing EOF with PEO sieving matrix (96). This polymer also featured wall coating capability, especially in low ionic strength buffer systems. CE of RNA in dilute and semidilute polymer solutions of HEC and the dependence of solute mobility on its chain length were consistent with separation by transient entanglement mechanism in dilute solutions (97). Another interesting application employed 1% PVP in 1× tris-boric acid-ethylenediaminetetraacetic acid buffer with 4M urea and 0.5µM ethidium bromide separation medium for automated and quantitative RNA screening by CE, using commercially available instrumentation and reagents and enabling high throughput and large-scale analysis of RNA samples (98). Recently, Hjerten's group introduced a novel polyacrylamide-gel formulation with cross-linked allyl-β-cyclodextrin for the separation of DNA fragments (99).

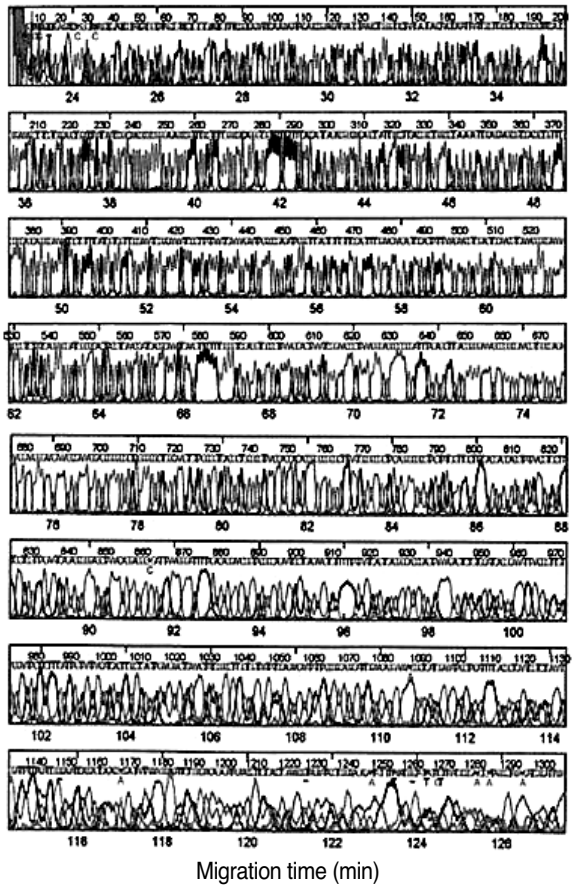

Figure 3. Electrophoretic separation of DNA sequencing fragments generated on ssM13mp18 with BigDye-labeled universal (–21) primer and AmpliTaq FS at the optimum experimental conditions: 2.0% (w/w) LPA 9 Mda–0.5% (w/w) 50 kDa LPA and 200 V/cm and 60°C. Conditions: effective length l = 30 cm, (L = 45 cm), 75-µm i.d., 365-µm o.d., polyvinylalcohol coated capillary. Running buffer: (both cathode and anode) 50mM Tris–50mM TAPS–2mM EDTA. The cathode running buffer also contained 7M urea, the same as in the separation matrix. The samples were injected at a constant electric field of 25 V/cm (0.7 µA) for 10 s and electrophoresed at 200 V/cm (10.2 µA) at 60°C.

One of the most important applications of denaturing CGE is DNA sequencing. During the last decade, the lack of adequate stability of cross-linked polyacrylamide gels within microbore columns initiated a rapid development to find novel, more capillary friendly sieving matrices. Karger et al. (76) demonstrated the usefulness of noncross-linked polymeric solutions to attain rapid separation of single-stranded DNA molecules, also enabling the application of high temperatures during electrophoresis. Choosing the appropriate electrophoresis parameters (e.g., separation voltage, temperature, etc.) for the analysis of DNA sequencing fragments plays an important role to obtain high speed and long read-length separations. Temperatures as high as 80°C were successfully applied to attain sequencing speed of more than 1000 bases/h (Figure 3) (100,101). The high salt content of the DNA sequencing reaction mixture was another important issue to overcome. Sample preparation and cleanup turned out to be a significant part of CGE-based DNA sequencing using electrokinetic injection. Conventionally, DNA sequencing samples are processed by ethanol precipitation that is rather labor intensive and also unreliable in regard to the remaining salt concentration in the samples. Combination of ultrafiltration membranes and spin columns resulted in a significant and consequent decrease in salt concentration (101).

Analysis of proteins and complex carbohydrates

Analysis of protein molecules by capillary SDS gel electrophoresis is a rapid automated separation and characterization technique, and it is considered as a modern instrumental approach to SDS–PAGE. First Cohen et al. demonstrated the usefulness of cross-linked SDS polyacrylamide gels in narrow-bore capillaries and revealed the excellent separation power of this system by resolving the two chains of insuline in less than 8 min using a 7.5% T–3.3% C polyacrylamide gel containing 8M urea and 0.1% SDS (8). Size separation of SDS-protein complexes can be readily attained in coated capillaries filled with cross-linked gels (102) or noncross-linked polymer networks (103). Figure 4 depicts one of the early applications of the technique for the analysis of a standard protein test mixture ranging in size from 14.2 to 205 kD (103). Capillary SDS gel electrophoresis also proved to be a very important separation tool for rapid molecular weight estimation (104) and purity check of recombinant proteins (105). Applicability of CE with SDS nongel sieving in the biotechnology industry was demonstrated by the analysis of a therapeutic recombinant monoclonal antibody, using precolumn fluorophore labeling of recombinant monoclonal antibody (rMAb) to obtain low nanomolar detection limits (106). This assay illustrated the advantages of enhanced preci-

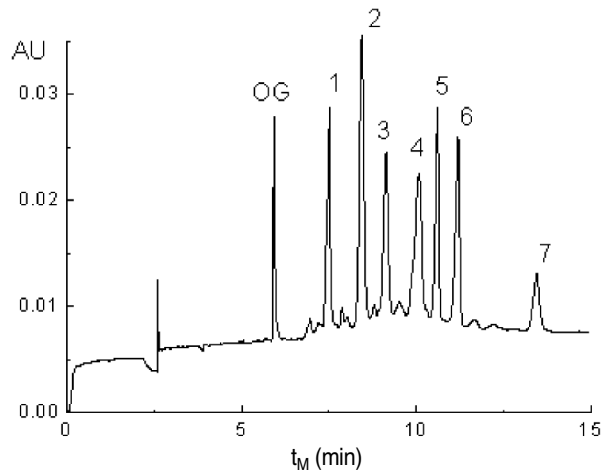

Figure 4. Capillary SDS gel electrophoresis trace of a protein testmixture. (1) β-lactalbumin, molecular weight = 14,200; (2) carbonic anhydrase, molecular weight = 29,000; (3) ovalbumin, molecular weight = 45,000; (4) bovine serum albumin, molecular weight = 66,000; (5) phosphorylase B, molecular weight = 97,400; (6) β-galactosidase, molecular weight = 116,000; (7) myosin, molecular weight = 205,000; and (OG) tracking dye Orange-G. Conditions: E = 300 V/cm; injection, 100-ng protein mix; and detection, 214 nm.

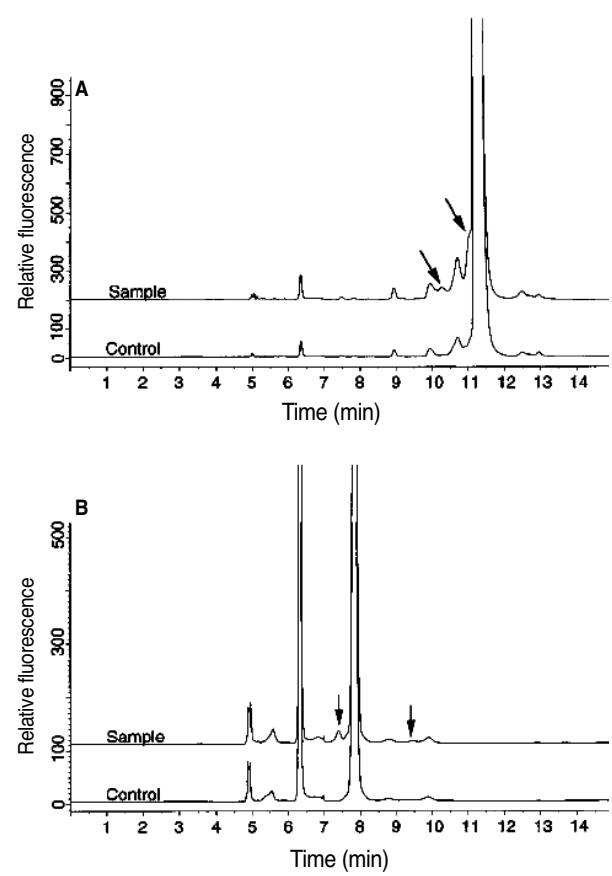

Figure 5. Capillary SDS electrophoresis nongel sieving separations of nonreduced (A) and reduced (B) preparations of rMAb control sample that was labeled with 5-TAMRA.SE and a sample that exhibited evidence of a microbial infection during cell culture fermentation. Arrows indicate the appearance of new peaks in the infected sample preparation.

sion and robustness, speed, ease of use, and online detection in monitoring bulk manufacture of protein pharmaceuticals (Figure 5) (106). Another interesting approach utilized SDS–CGE and LIF detection with a two-spectral channel detector that resolved fluorescence from the samples and standards covalently labeled by two different dyes (107). Others found picomolar detection limits with noncovalent fluorogenic labeling of proteins using Sypro Red dye (108). Dovichi et al. reported rapid and efficient capillary SDS gel electrophoresis separation of proteins by characterizing HT29 human colon cancer adenocarcinoma cells (109). The cells were lysed inside a capillary, followed by protein denaturation with SDS and fluorophore labeling with 3-(2-furoyl)-quinoline-2-carboxyaldehide, and separated using an 8% pollulan sieving matrix. Typical resolution was approximately 30 protein components of a single HT29 cell, similar to the band capacity of SDS–PAGE. However, fluorescent detection provided high sensitivity, ranging from 10^{-10}–10^{-11}M, and single-cell level analysis was completed in 45 min. Other research groups reported the separation and comparative analysis of apolipoproteins by capillary zone and capillary SDS gel electrophoresis (110,111). A comprehensive review on capillary SDS gel electrophoresis of proteins was published (29).

The field also moved towards biomedical and biotechnology applications. Kustos et al. (112) successfully studied outer membrane proteins, lipopolysaccharides, hemolysin, and the in vivo and in vitro virulence of wild type Proteus penneri 357 and its two isogenic mutant variants (a transposon and a spontaneous mutant) using CE with dynamic sieving. CE was found to be suitable for comparative analysis of bacterial protein patterns of genetic variants also providing valuable insights in connection with bacteriological virulence. Other interesting applications of capillary SDS gel electrophoresis have been reported for protein

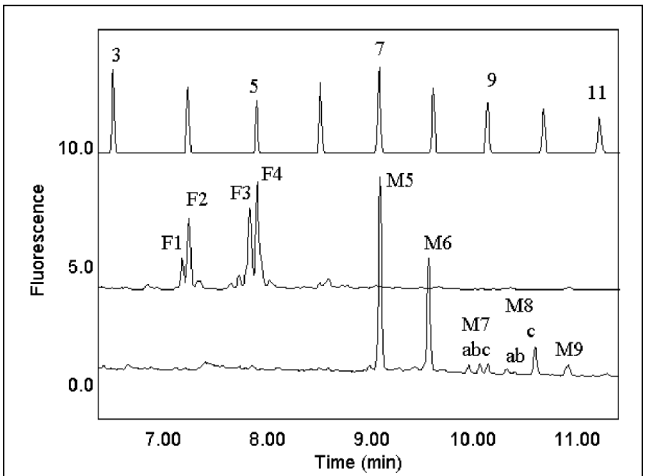

Figure 6. Electropherograms of APTS-labeled glycans from bovine fetuin (middle trace) and bovine ribonuclease B (lower trace) compared with the maltooligosaccharide ladder standard (upper trace). Numbers on the upper trace correspond to the degree of polymerization of the glucose oligomers. Peaks: F1 = tetrasialo–triantennary–2xα2,6; F2 = tetrasialo–triantennary–2xα2,3; F3 = trisialo–triantennary–2xα2,6; and F4 = trisialo–triantennary–2xα2,3. M5–M9: Mannose 5–Mannose 9. Conditions: capillary, l = 40 cm (effective) neutrally coated capillary (eCAP N–CHO) with 50-µm i.d.; buffer, 25mM acetate, pH 4.75; detection, LIF; excitation, 488 nm; emission, 520 nm; applied field strength, 500 V/cm; and temperature, 20°C.

characterization of bacterial lysates (113) and profiling of human serum proteins (114).

Recognizing the importance of such posttranslational modifications as glycosylation, in the early 1990s Horváth's group started extensively investigating the separation and characterization of electrophoretic migration behavior of complex carbohydrates, derivatized via their reducing end with 8-aminonaphthalene-1,3,6-trisulfonic acid by reductive amination (115). Bonn et al. compared CE and micellar electrokinetic chromatography of 4-aminobenzonitrile carbohydrate derivatives (116). Complex carbohydrates, released from glycoproteins, were readily profiled by CGE–LIF-based detection of the 1-aminopyrene-3,6,8-trisulfonic acid (APTS) labeled sugar molecules (Figure 6) (117). High-mannose-type oligosaccharides of ribonuclease B were derivatized by APTS and separated by CE using PEO separation medium (118).

Electrophoresis in microfabricated devices

Recently emerging microfluidics-based analytical techniques brought the promise of further increasing the speed and throughput of electric-field-mediated separations in capillary dimensions (119). Electrophoresis microchips were fabricated by the well-matured techniques from the semiconductor industry, demonstrating that channels and other functional elements can be readily fabricated in glass substrates using photolithography and chemical etching. Early feasibility experiments proved the usefulness of electrophoresis in microfabricated devices more than a decade ago (120). With the advent of the so-called simple cross and double T injector structure, well-defined amounts of samples could be readily analyzed on electrophoresis microchips (121). Samples are typically loaded electrokinetically into the injector cross region, then the analyte molecules are separated by means of applying the electric field not only along the separation channel but in a smaller extent to the sample and waste reservoirs as well, to prevent bleeding of the sample into the separation channel (Figure 7) (133). The separated solute molecules are then most frequently visualized by confocal microscopy with LIF detection (122). Short injection plugs, high electric field strengths, and short effective channel lengths result in separations in seconds with extremely high efficiencies because of minimized extracolumn broadening effects (123). Electrophoresis microchips have already been applied to the analysis of amino acids (124), DNA restriction fragments (125–127), PCR products (128), DNA sequencing (129,130), genotyping (131) and rapid PCR, preconcentration, and DNA analysis (132). Ronai et al. (133) used PVP-filled glass microchips to evaluate the influence of operational variables on the separation of dsDNA molecules. Effects of sieving matrix concentration (Ferguson plot), migration characteristics (reptation plot), separation temperature (Arrhenius plot), electric field strength, and intercalator dye concentration were all thoroughly examined. Parallelization is readily applicable to microfluidics devices, as Sassi et al. (134) demonstrated the possibility of rapid (< 10 min) separations of alleles of the D1S80 locus in a 16-channel plastic multichannel microdevice. The chip was replicated from a microfabricated master and laminated with a plastic film. Shi et al. (135) introduced a pressurized capillary array system to simultaneously load 96 samples into 96 sample wells of a radial microchannel array electrophoresis microplate for high-throughput DNA sizing. As a result, 96 samples were analyzed in less than 90 s/microplate, demonstrating the power of microfabricated devices for large-scale and high-performance nucleic acid characterization. Another approach of miniaturization is ultrathin-layer gel electrophoresis that is a combination of slab gel electrophoresis and CGE (136), providing a multilane separation platform (a plurality of virtual channels) with excellent heat dissipation characteristics allowing the application of high voltages necessary to obtain rapid and efficient analysis of biopolymers. Detection of the separated bands is usually accomplished in real time by continuous LIF scanning of the separation lanes. Baba et al. reviewed array electrophoresis microchip and onchip integration with other systems, in addition to the application of microchip electrophoresis in DNA sizing, genetic analysis, and DNA sequencing (137). Microfabrication also offers a novel approach to high-performance micro- and nanovolume LC through the application of micromachined chromatographic phase support (138). In a comprehensive review, Landers et al. highlighted the progress towards CE and microchip electrophoresis as clinical diagnostic tools, with literature coverage from 1996 to 2000 (139).

Micropreparative fraction collection following microchip-based electrophoretic analysis of biomolecules is of major importance for a variety of biomedical applications. Various size DNA fragments can be separated and collected by simply redirecting

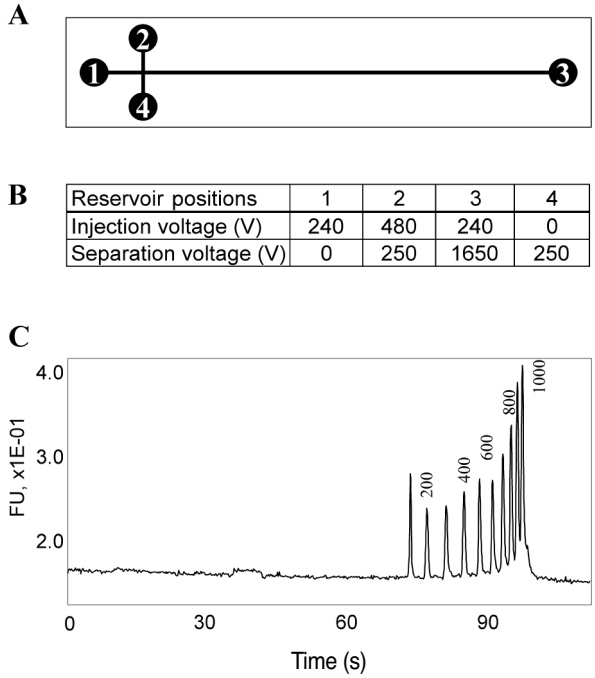

Figure 7. Reservoir assignment of the electrophoresis microchip (A), applied injection and separation voltages (B), and a typical and rapid separation of the 100-bp sizing ladder (C). Numbers on peaks correspond to the size of DNA fragments in base pairs. Conditions: sieving matrix, 2% polyvinylpyrrolidone (molecular weight = 1,300,000 Da) solution in 1× TBE buffer; effective separation length, 30 mm; applied electric field strength, 200 V/cm; room temperature; sample, 100-bp dsDNA ladder labeled with Sytox Orange (0.5µM final concentration); and injection, 100-µm double T (35 fg DNA/injection).

the desired portions of the detected sample zones to corresponding collection wells using appropriate voltage manipulations (140). The efficiency of sampling and collection of the fractions was enhanced by placing a cross channel at/or downstream of the detection point. Following the detection of the band of interest, the potentials were reconfigured to sampling/collection mode so that the selected sample zone migrated to the appropriate collection well of the microdevice. The potential distribution assured that the rest of the analyte components in the separation column were retarded, stopped, or reversed, increasing in this way the spacing between the sample zone being collected and the one immediately following. By these means, precise collection of spatially close consecutive bands could be facilitated. Once the target sample fraction reached the corresponding collection well, the potentials were switched back to separation mode. Alternation of the separation/detection and sampling/collection cycles was repeated until all required sample zones were physically isolated. The integrated device consisted of a sample introduction, separation, fraction sampling, and fraction collection compartments. The amounts of DNA collected in this way were enough for further downstream sample processing, such as conventional PCR-based analysis.

Conclusion

Electrophoresis in narrow-bore channels, such as CE and integrated microfabricated device technology, opened up new horizons in bioseparations. Entering the era of proteomics from genomics, we expect to see a paradigm shift towards miniaturized and multiplexed high-resolution separation techniques used in integrated and automated fashion to solve formidable separation problems and provide means for ultrahigh-throughput analysis. Most current separation protocols for DNA and protein analysis, already in use in molecular biology and biotechnology labs, can be readily transferred to capillary array or microchannel devices. The real strength of miniaturization is the possibility of integrating existing methods/functionalities in a way that allows sample preparation, reactions, analysis, and even fraction collections to be carried out on a single microdevice (lab-on-a-chip) (140). Capillary and microchip electrophoresis devices are intrinsically acquiescent to full automation, enabling large-scale analyses with considerably less human intervention than conventional techniques, resulting in significant savings in time, labor, and expenses.

References

1. A. Tiselius. Moving boundary electrophoresis. *Trans. Faraday. Soc.* **33:** 524 (1937).
2. A.T. Andrews. *Electrophoresis.* Claredon Press, Oxford, U.K., 1986.
3. A. Chrambach. *The Practice of Quantitative Gel Electrophoresis.* VCH Publishers, Deerfield Beach, FL, 1985.
4. J. Khandurina and A. Guttman. Bioanalysis in microfluidic devices. *J. Chromatogr.* **943:** 159–83 (2002).
5. A. Guttman, A.S. Cohen, D.N. Heiger, and B.L. Karger. Analytical and micropreparative ultrahigh resolution of oligonucleotides by polyacrylamide gel high performance capillary electrophoresis. *Anal. Chem.* **62:** 137–41 (1990).
6. J.C. Venter et al. The sequence of the human genome. *Science* **291:** 1304–51 (2001).
7. S. Hjerten. High-performance electrophoresis: elimination of electroendosmosis and solute adsorption. *J. Chromatogr.* **347:** 191–98 (1985).
8. A.S. Cohen and B.L. Karger. High-performance sodium dodecyl sulfate polyacrylamide gel capillary electrophoresis of peptides and proteins. *J. Chromatogr.* **397:** 409–517 (1987).
9. G. Bruin, R. Huisden, J. Kraak, and H. Poppe. Performance of carbohydrate-modified fused-silica capillaries for the separation of proteins by zone electrophoresis. *J. Chromatogr.* **480:** 339–49 (1989).
10. K.A. Cobb, V. Dolnik, and M. Novotny. Electrophoretic separations of proteins in capillaries with hydrolytically stable surface structures. *Anal. Chem.* **62:** 2478–83 (1990).
11. K. Ganzler, K.S. Greve, A.S. Cohen, B.L. Karger, A. Guttman, and N. Cooke. High-performance capillary electrophoresis of SDS-protein complexes using UV-transparent polymer network. *Anal. Chem.* **64:** 2665–71 (1992).
12. S.F.Y. Li. *Capillary Electrophoresis.* Elsevier, Amsterdam, the Netherlands, 1993.
13. C. Barta, M. Sasvari-Szekely, and A. Guttman. Simultaneous analysis of various mutations on the 21-hydroxylase gene by multi-allele specific amplification and capillary gel electrophoresis. *J. Chromatogr. A* **817:** 281–86 (1998).
14. P.G. Righetti, C. Gelfi, B. Verzola, and L. Castelletti. The state of the art of dynamic coatings. *Electrophoresis* **22:** 603–11 (2001).
15. J. Horvath and V. Dolnik. Polymer wall coatings for capillary electrophoresis. *Electrophoresis* **22:** 644–55 (2001).
16. H. Tian, L.C. Brody, D. Mao, and J.P. Landers. Effective capillary electrophoresis-based heteroduplex analysis through optimization of surface coating and polymer networks. *Anal. Chem.* **72:** 5483–92 (2000).
17. M. Chiari, M. Cretich, and J. Horvath. A new absorbed coating for DNA fragment analysis by capillary electrophoresis. *Electrophoresis* **21:** 1521–26 (2000).
18. J. Ren, A. Ulvik, H. Refsum, and P.M. Ueland. Applications of short-chain polydimethylacrylamide as sieving medium for the electrophoretic separation of DNA fragments and mutation analysis in uncoated capillaries. *Anal. Biochem.* **276:** 188–94 (1999).
19. P. Shieh, D. Hoang, A. Guttman, and N. Cooke. Capillary SDS gel electrophoresis of proteins: I. reproducibility and stability. *J. Chromatogr. A* **676:** 219–26 (1994).
20. P. Shieh. Coated Capillary Columns and Electrophoretic Separation Methods for Their Use. U.S. Patent 5,462,646, October 31, 1995.
21. B.L. Karger, A.S. Cohen, and A. Guttman. High performance capillary electrophoresis in the biological sciences. *J. Chromatogr.* **492:** 585–614 (1989).
22. A. Guttman, A. Paulus, A.S. Cohen, B.L. Karger, H. Rodriguez, and W.S. Hancock. *Electrophoresis '88.* C. Schafer-Nielsen, Ed. VCH, Weinheim, Germany, 1988, pp. 151–59.
23. T. Tanaka. Gels. *Sci. Am.* **244:** 124–38 (1981).
24. P.G. Righetti and C. Gelfi. Electrophoresis gel media: the state of the art. *J. Chromatogr. B* **699:** 63–75 (1997).
25. P.G. Righetti and C. Gelfi. Recent advances in capillary electrophoresis of DNA fragments and PCR products in poly(n-substituted acrylamides). *Anal. Biochem.* **244:** 195–207 (1997).
26. S. Hjerten. Molecular sieve chromatography on polyacrylamide gels, prepared according to a simplified method. *Arch. Biochem. Biophys. Suppl.* **1:** 147–51 (1962).
27. D.N. Heiger, A.S. Cohen, and B.L. Karger. Separation of DNA restriction fragments by high performance capillary electrophoresis with low and zero crosslinked polyacrylamide using continuous and pulsed electric fields. *J. Chromatogr.* **516:** 33–48 (1990).

28. B.L. Karger, Y.H. Chu, and F. Foret. Capillary electrophoresis of proteins and nucleic acids. *Annu. Rev. Biophys. Biomol. Struct.* **24:** 579–610 (1995).
29. A. Guttman. Capillary sodium dodecyl sulfate-gel electrophoresis of proteins. *Electrophoresis* **17:** 1333–41 (1996).
30. A. Guttman. Capillary Electrophoresis using Replacement Gels. U.S. Patent 5,332,481, July 26, 1994.
31. A. Widhalm, C. Schwer, D. Blass, and E. Kenndler. Capillary zone electrophoresis with a linear, noncrosslinked polyacrylamide gel: separation of proteins according to molecular mass. *J. Chromatogr.* **546:** 446–51 (1991).
32. D. Wu and F. Regnier. Sodium dodecyl sulfate-capillary gel electrophoresis of proteins using non-cross-linked polyacrylamide. *J. Chromatogr.* **608:** 349–56 (1992).
33. W. Werner, D. Demorest, and J.E. Wictorowicz. Automated Ferguson analysis of glycoproteins by capillary electrophoresis using a replaceable sieving matrix. *Electrophoresis* **14:** 759–63 (1993).
34. K. Habenbrock, K. Schugerl, and R. Freitag. Analysis of plasmid-DNA and cell protein of recombinant *Escherichia coli* using capillary gel electrophoresis. *Electrophoresis* **14:** 753–58 (1993).
35. P. Bocek and A. Chrambach. Capillary electrophoresis of DNA in agarose solutions at 40 degrees C. *Electrophoresis* **12:** 1059–61 (1991).
36. H.E. Schwartz, K. Ulfelder, F.J. Sunzeri, M.P. Busch, and R.G. Brownlee. Analysis of DNA restriction fragments and polymerase chain reaction products towards detection of the AIDS (HIV-1) virus in blood. *J. Chromatogr.* **559:** 267–83 (1991).
37. S. Nathakarnkitkool, P.J. Oefner, G. Bartsch, M.A. Chin, and G.K. Bonn. High-resolution capillary electrophoretic analysis of DNA in free solution. *Electrophoresis* **13:** 18–31 (1992).
38. O. de Carmejane, Y. Yamaguchi, T.I. Todorov, and M.D. Morris. Three-dimensional observation of electrophoretic migration of dsDNA in semidilute hydroxyethylcellulose solution. *Electrophoresis* **22:** 2433–41 (2001).
39. M.N. Albarghouthi, B.A. Buchholz, E.A. Doherty, F.M. Bogdan, H. Zhou, and A.E. Barron. Impact of polymer hydrophobicity on the properties and performance of DNA sequencing matrices for capillary electrophoresis. *Electrophoresis* **22:** 737–47 (2001).
40. B.A. Buchholz, E.A. Doherty, M.N. Albarghouthi, F.M. Bogdan, H. Zhou, and A.E. Barron. Microchannel DNA sequencing matrices with a thermally controlled "viscosity switch". *Anal. Chem.* **73:** 157–64 (2001).
41. Y. Kim and E.S. Yeung. Separation of DNA sequencing fragments up to 1000 bases by using poly(ethylene oxide)-filled capillary electrophoresis. *J. Chromatogr. A* **781:** 315–25 (1997).
42. Q. Gao and E.S. Yeung. A matrix for DNA separation: genotyping and sequencing using poly(vinylpyrrolidone) solution in uncoated capillaries. *Anal. Chem.* **70:** 1382–88 (1998).
43. W. Wei and E.S. Yeung. Improvements in DNA sequencing by capillary electrophoresis at elevated temperature using poly(ethylene oxide) as a sieving matrix. *J. Chromatogr. B* **745:** 221–30 (2000).
44. W. Wei and E.S. Yeung. DNA capillary electrophoresis in entangled dynamic polymers of surfactant molecules. *Anal. Chem.* **73:** 1776–83 (2001).
45. J.M. Song and E.S. Yeung. Optimization of DNA electrophoretic behavior in poly(vinyl pyrrolidone) sieving matrix for DNA sequencing. *Electrophoresis* **22:** 748–54 (2001).
46. C. Barta, Z. Ronai, M. Sasvari-Szekely, and A. Guttman. Rapid single nucleotide polymorphism analysis by primer extension and capillary electrophoresis using polyvinyl pyrrolidone matrix. *Electrophoresis* **22:** 779–82 (2001).
47. R.S. Madabhushi, M. Vainer, V. Dolnik, S. Enad, D.L. Barker, D.W. Harris, and E.S. Mansfield. Versatile low-viscosity sieving matrices for nondenaturing DNA separations using capillary array electrophoresis. *Electrophoresis* **18:** 104–11 (1997).
48. D. Liang and B. Chu. High speed separation of DNA fragments by capillary electrophoresis in poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock polymer. *Electrophoresis* **19:** 2447–53 (1998).
49. C. Wu, T. Liu, and B. Chu. Viscosity-adjustable block copolymer for DNA separation by capillary electrophoresis. *Electrophoresis* **19:** 231–41 (1998).
50. R.L. Rill, Y. Liu, D.H. Van Winkle, and B.R. Locke. Pluronic copolymer liquid crystals: unique, replaceable media for capillary gel electrophoresis. *J. Chromatogr. A* **817:** 287–95 (1998).
51. D. Liang, L. Song, S. Zhou, V.S. Zaitsev, and B. Chu. Poly(N-isopropylacrylamide)-γ-poly(ethyleneoxide) for high resolution and high speed separation of DNA by capillary electrophoresis. *Electrophoresis* **20:** 2856–63 (1999).
52. J. Sudor, V. Barbier, S. Thirot, D. Godfrin, D. Hourdet, M. Millequant, J. Blanchard, and J.L. Viovy. New block-copolymer thermoassociating matrices for DNA sequencing: effect of molecular structure on rheology and resolution. *Electrophoresis* **22:** 720–28 (2001).
53. T. Izumi, M. Yamaguchi, K. Yoneda, T. Isobe, T. Okuyama, and T. Shinoda. Use of glucomannan for the separation of DNA fragments by capillary electrophoresis. *J. Chromatogr. A* **652:** 41–46 (1993).
54. M. Nakatani, A. Shibukawa, and T. Nakagawa. Effect of temperature and viscosity of sieving medium on electrophoretic behavior of sodium dodecyl sulfate-proteins on capillary electrophoresis in presence of pullulan. *Electrophoresis* **17:** 1210–13 (1996).
55. B.A. Siles and G.B. Collier. The characterization of a new size-sieving polymeric matrix for the separation of DNA fragments using capillary electrophoresis. *J. Capillary Electrophor.* **3:** 313–21 (1996).
56. B.A. Siles, D.E. Anderson, N.S. Buchanan, and M.F. Warder. The characterization of composite agarose/hydroxyethylcellulose matrices for the separation of DNA fragments using capillary electrophoresis. *Electrophoresis* **18:** 1980–89 (1997).
57. M. Chiari, M. Cretich, S. Riva, and M. Casali. Performances of new sugar-bearing poly(acrylamide) copolymers as DNA sieving matrices and capillary coatings for electrophoresis. *Electrophoresis* **22:** 699–706 (2001).
58. M. Chiari, M. Cretich, and R. Consonni. Separation of DNA fragments in hydroxylated poly(dimethylacrylamide) copolymers. *Electrophoresis* **23:** 536–41 (2002).
59. M. Chiari, F. Damin, A. Melis, and R. Consonni. Separation of oligonucleotides and DNA fragments by capillary electrophoresis in dynamically and permanently coated capillaries, using a copolymer of acrylamide and beta-D-glucopyranoside as a new low viscosity matrix with high sieving capacity. *Electrophoresis* **19:** 3154–59 (1998).
60. V. Dolnik, W.A. Gurske, and A. Padua. Galactomannans as a sieving matrix in capillary electrophoresis. *Electrophoresis* **22:** 707–19 (2001).
61. I. Miksik and Z. Deyl. Application of Pluronic copolymer liquid crystals for the capillary electrophoretic separation of collagen type I cyanogen bromide fragments. *J. Chromatogr. B* **739:** 109–16 (2000).
62. I. Miksik, Z. Deyl, and V. Kasicka. Capillary electrophoretic separation of proteins and peptides using Pluronic liquid crystals and surface-modified capillaries. *J. Chromatogr. B* **741:** 37–42 (2000).
63. L. Song, T. Liu, D. Liang, D. Fang, and B. Chu. Separation of double-stranded DNA fragments by capillary electrophoresis in interpenetrating networks of polyacrylamide and polyvinylpyrrolidone. *Electrophoresis* **22:** 3688–98 (2001).
64. Y. Wang, D. Liang, J. Hao, D. Fang, and B. Chu. Separation of double-stranded DNA fragments by capillary electrophoresis using polyvinylpyrrolidone and poly(N,N-dimethylacrylamide) transient interpenetrating network. *Electrophoresis* **23:** 1460–66 (2002).
65. Y. Shen, Q. Xu, F. Han, K. Ding, F. Song, Y. Fan, N. Zhu, G. Wu, and B. Lin. Application of capillary nongel sieving electrophoresis for gene analysis. *Electrophoresis* **20:** 1822–28 (1999).
66. F. Xu, M. Jabasini, and Y. Baloa. DNA separation by microchip

electrophoresis using low-viscosity hydroxypropylmethylcellulose—50 solutions enhanced by polyhdroxy compounds. *Electrophoresis* **23:** 3608–14 (2002).
67. J. Cheng and K.R. Mitchelson. Glycerol-enhanced separation of DNA fragments in entangled solution capillary electrophoresis. *Anal. Chem.* **66:** 4210–14 (1994).
68. B.A. Siles, K.A. O'Neil, D.L. Tung, L. Bazar, G.B. Collier, and C.I. Lovelace. The use of dynamic size-sieving capillary electrophoresis and mismatch repair enzymes for mutant DNA analysis. *J. Capillary Electrophor.* **5:** 51–58 (1998).
69. A.R. Isenberg, B.R. McCord, B.W. Koons, B. Budowle, and R.O. Allen. DNA typing of a polymerase chain reaction amplified D1S80/amelogenin multiplex using capillary electrophoresis and a mixed entangled polymer matrix. *Electrophoresis* **17:** 1505–11 (1996).
70. C. Gelfi, M. Perego, and P.G. Righetti. Capillary electrophoresis of oligonucleotides in sieving liquid polymers in isoelectric buffers. *Electrophoresis* **17:** 1470–75 (1996).
71. I. Barme, G.J. Bruin, A. Paulus, and M. Ehrat. Preconcentration and separation of antisense oligonucleotides by on-column isotachophoresis and capillary electrophoresis in polymer-filled capillaries. *Electrophoresis* **19:** 1445–51 (1998).
72. K. Khan, A. Van Schepdael, T. Saison-Behmoaras, A. Van Aerschot, and J. Hoogmartens. Analysis of antisense oligonucleotides by on-capillary isotachophoresis and capillary polymer sieving electrophoresis. *Electrophoresis* **19:** 2163–68 (1998).
73. S.H. Chen and J.M. Gallo. Use of capillary electrophoresis methods to characterize the pharmacokinetics of antisense drugs. *Electrophoresis* **19:** 2861–69 (1998).
74. H. Oana, R.W. Hammond, J.J. Schwinefus, S.C. Wang, M. Doi, and M.D. Morris. High-speed separation of linear and supercoiled DNA by capillary electrophoresis. Buffer, entangling polymer, and electric field effects. *Anal. Chem.* **70:** 574–79 (1998).
75. J. Ren, A. Ulvik, P.M. Ueland, and H. Refsum. Analysis of single-strand conformation polymorphism by capillary electrophoresis with laser-induced fluorescence detection using short-chain polyacrylamide as sieving medium. *Anal. Biochem.* **245:** 79–84 (1997).
76. M.C. Ruiz-Martinez, J. Berka, A. Belenkii, F. Foret, A.W. Miller, and B.L. Karger. DNA sequencing by capillary electrophoresis with replaceable linear polyacrylamide and laser-induced fluorescence detection. *Anal. Chem.* **65:** 2851–58. (1993).
77. J. Zhang, Y. Fang, J.Y. Hou, H.J. Ren, R. Jiang, P. Roos, and N.J. Dovichi. Use of non-cross-linked polyacrylamide for four-color DNA sequencing by capillary electrophoresis separation of fragments up to 640 bases in length in two hours. *Anal. Chem.* **67:** 4589–93 (1995).
78. J. Bashkin, M. Marsh, D. Barker, and R. Johnston. DNA sequencing by capillary electrophoresis with a hydroxyethylcellulose sieving buffer. *Appl. Theor. Electrophor.* **6:** 23–28 (1996).
79. M.A. Quesada. Replaceable polymers in DNA sequencing by capillary electrophoresis. *Curr. Opin. Biotechnol.* **8:** 82–93 (1997).
80. M.H. Kleemiss, M. Gilges, and G. Schomburg. Capillary electrophoresis of DNA restriction fragments with solutions of entangled polymers. *Electrophoresis* **14:** 515–22 (1993).
81. P.G. Righetti and C. Gelfi. Capillary electrophoresis of DNA for molecular diagnostics. *Electrophoresis* **18:** 1709–14 (1997).
82. P.G. Righetti and C. Gelfi. Capillary electrophoresis of DNA for molecular diagnostics: an update. *J. Capillary Electrophor.* **6:** 119–24 (1999).
83. S. Hu and N.J. Dovichi. Capillary electrophoresis for the analysis of biopolymers. *Anal. Chem.* **74:** 2833–50 (2002).
84. Y. Wang, J.M. Wallin, J. Ju, G.F. Sensabaugh, and R.A. Mathies. High-resolution capillary array electrophoretic sizing of multiplexed short tandem repeat loci using energy-transfer fluorescent primers. *Electrophoresis* **17:** 1485–90 (1996).
85. M. Oto, A. Koguchi, and Y. Yuasa. Analysis of a polyadenine tract of the transforming growth factor-beta type II receptor gene in colorectal cancers by non-gel-sieving capillary electrophoresis. *Clin. Chem.* **43:** 759–63 (1997).
86. N. Stellwagen, C. Gelfi, and P.G. Righetti. The use of gel and capillary electrophoresis to investigate some of the fundamental physical properties of DNA. *Electrophoresis* **23:** 167–75 (2002).
87. B.A. Siles, K.A. O'Neil, M.A. Fox, D.E. Anderson, A.F. Kuntz, S.C. Ranganath, and A.C. Morris. Genetic fingerprinting of grape plant (*Vitis vinifera*) using random amplified polymorphic DNA (RAPD) analysis and dynamic size-sieving capillary electrophoresis. *J. Agric. Food Chem.* **48:** 5903–12 (2000).
88. A. Guttman and N. Cooke. Capillary gel affinity electrophoresis of DNA fragments. *Anal. Chem.* **63:** 2038–42 (1991).
89. X.C. Li-Sucholeiki, K. Khrapko, P.C. Andre, L.A. Marcelino, B.L. Karger, and W.G. Thilly. Applications of constant denaturant capillary electrophoresis/high-fidelity polymerase chain reaction to human genetic analysis. *Electrophoresis* **20:** 1224–32 (1999).
90. M.Z. Xue, O. Bonny, S. Morgenthaler, M. Bochud, V. Mooser, W.G. Thilly, L. Schild, and P.M. Leong-Morgenthaler. Use of constant denaturant capillary electrophoresis of pooled blood samples to identify single-nucleotide polymorphisms in the genes (Scnn1a and Scnn1b) encoding the alpha and beta subunits of the epithelial sodium channel. *Clin. Chem.* **48**: 718–28 (2002).
91. P.O. Ekstrom, R. Wasserkort, M. Minarik, F. Foret, and W.G. Thilly. Two-point fluorescence detection and automated fraction collection applied to constant denaturant capillary electrophoresis. *Biotechniques* **29:** 582–89 (2000).
92. Y. Liu and W.G. Kuhr. Separation of double- and single-stranded DNA restriction fragments: capillary electrophoresis with polymer solutions under alkaline conditions. *Anal. Chem.* **71:** 1668–73 (1999).
93. C. Heller. Separation of double-stranded and single-stranded DNA in polymer solutions: I. mobility and separation mechanism. *Electrophoresis* **20:** 1962–77 (1999).
94. C. Heller. Separation of double-stranded and single-stranded DNA in polymer solutions: II. separation, peak width and resolution. *Electrophoresis* **20:** 1978–86 (1999).
95. S.A. Nevins, B.A. Siles, and Z.E. Nackerdien. Analysis of gamma radiation-induced damage to plasmid DNA using dynamic size-sieving capillary electrophoresis. *J. Chromatogr. B* **741:** 243–55 (2000).
96. M.M. Hsieh, W.L. Tseng, and H.T. Chang. On-column preconcentration and separation of DNA fragments using polymer solutions in the presence of electroosmotic flow. *Electrophoresis* **21:** 2904–10 (2000).
97. T.I. Todorov, O. de Carmejane, N.G. Walter, and M.D. Morris. Capillary electrophoresis of RNA in dilute and semidilute polymer solutions. *Electrophoresis* **22:** 2442–47 (2001).
98. J. Khandurina, H.S. Chang, B. Wanders, and A. Guttman. Automated high throughput RNA analysis by capillary electrophoresis. *BioTechniques* **32:** 1226–28 (2002).
99. A. Vegvari and S. Hjerten. Stable homogeneous gel for molecular-sieving of DNA fragments in capillary electrophoresis. *J. Chromatogr. A* **960:** 221–27 (2002).
100. O. Salas-Solano, E. Carrilho, L. Kotler, A.W. Miller, W. Goetzinger, Z. Sosic, and B.L. Karger. Routine DNA sequencing of 1000 bases in less than one hour by capillary electrophoresis with replaceable linear polyacrylamide solutions. *Anal. Chem.* **70:** 3996–4003 (1998).
101. B.L. Karger and A. Guttman. From capillaries to the Genome. *Gen. Prot. Techn.* **3:** 12–16 (2003).
102. K. Tsuji. High-performance capillary electrophoresis of proteins. Sodium dodecyl sulphate-polyacrylamide gel-filled capillary column for the determination of recombinant biotechnology-derived proteins. *J. Chromatogr.* **550:** 823–30 (1991).
103. A. Guttman, P. Shieh, J. Lindahl, and N. Cooke. Capillary SDS gel electrophoresis of proteins: II. automated Ferguson analysis. *J. Chromatogr.* **676:** 227–31 (1994).
104. K. Benedek and A. Guttman. Ultrafast high performance capillary SDS gel electrophoresis of proteins. *J. Chromatogr.* **680:** 375–81 (1994).

105. A. Guttman, P. Shieh, and B.L. Karger. *Gel Electrophoresis of Proteins: A Practical Approach.* B.D. Hames, Ed. Oxford University Press, Oxford, U.K.,1998, pp. 105–26.
106. G. Hunt and W. Nashabeh. Capillary electrophoresis sodium dodecyl sulfate nongel sieving analysis of a therapeutic recombinant monoclonal antibody: a biotechnology perspective. *Anal. Chem.* **71:** 2390–97 (1999).
107. D.B. Craig, R.M. Polakowski, E. Arriaga, J.C. Wong, H. Ahmadzadeh, C. Stathakis, and N.J. Dovichi. Sodium dodecyl sulfate-capillary electrophoresis of proteins in a sieving matrix utilizing two-spectral channel laser-induced fluorescence detection. *Electrophoresis* **19:** 2175–78 (1998).
108. M. Harvey, D. Bandilla, and P.R. Banks. Subnanomolar detection limit for sodium dodecyl sulfate-capillary gel electrophoresis using a fluorogenic, noncovalent dye. *Electrophoresis* **19:** 2169–74 (1998).
109. S. Hu, L. Zhang, L.M. Cook, and N.J. Dovichi. Capillary sodium dodecyl sulfate-DALT electrophoresis of proteins in a single human cancer cell. *Electrophoresis* **22:** 3677–82 (2001).
110. S.D. Proctor and J.C. Mamo. Separation and quantification of apolipoprotein B-48 and other apolipoproteins by dynamic sieving capillary electrophoresis. *J. Lipid Res.* **38:** 410–14 (1997).
111. J. Stocks, M.N. Nanjee, and N.E. Miller. Analysis of high density lipoprotein apolipoproteins by capillary zone and capillary SDS gel electrophoresis. *J. Lipid Res.* **39:** 218–27 (1998).
112. I. Kustos, V. Toth, B. Kocsis, I. Kerepesi, L. Emody, and F. Kilar. Capillary electrophoretic analysis of wild type and mutant Proteus penneri outer membrane proteins. *Electrophoresis* **21:** 3020–27 (2000).
113. I. Kustos, B. Kocsis, I. Kerepesi, and F. Kilar. Protein profile characterization of bacterial lysates by capillary electrophoresis. *Electrophoresis* **19:** 2317–23 (1998).
114. T. Manabe, H. Oota, and J. Mukai. Size separation of sodium dodecyl sulfate complexes of human plasma proteins by capillary electrophoresis employing linear polyacrylamide as a sieving polymer. *Electrophoresis* **19:** 2308–16 (1998).
115. C. Chiesa and C. Horváth. Capillary zone electrophoresis of malto-oligosaccharides derivatized with 8-aminonaphthalene-1,3,6-trisulfonic acid. *J. Chromatogr. A* **45:** 337–52 (1993).
116. H. Schwaiger, P.J. Oefner, C. Huber, E. Grill, and G.K. Bonn. Capillary zone electrophoresis and micellar electrokinetic chromatography of 4-aminobenzonitrile carbohydrate derivatives. *Electrophoresis* **15:** 941–52 (1994).
117. A. Guttman. Carbohydrate profiling by capillary gel electrophoresis. *Nature* **380:** 461–62 (1996).
118. A. Guttman and T. Pritchett. Capillary gel electrophoresis separation of high-mannose type oligosaccharides derivatized by 1-aminopyrene-3,6,8-trisulfonic acid. *Electrophoresis* **16:** 1906–11 (1995).
119. A. Manz and H. Becker. *Microsystem Technology in Chemistry and Life Sciences.* Springer-Verlag, Berlin, Germany, 1999.
120. A. Manz, N. Graber, and H.M. Widmer. Miniaturized total chemistry analysis systems: a novel concept for chemical sensing. *Sens. Actuators B* **1:** 244–48 (1990).
121. C.S. Effenhauser, G.J.M. Bruin, and A. Paulus. Integrated chip-based CE—a review. *Electrophoresis* **18:** 2203–13 (1997).
122. S.C. Jacobson and J.M. Ramsey. Microchip electrophoresis with sample stacking. *Electrophoresis* **16:** 481–86 (1995).
123. C.S. Effenhauser. *Microsystem Technology in Chemistry and Life Sciences.* A. Manz, H. Becker, Eds. Springer, Heidelberg, Germany, 1999.
124. D.J. Harrison, Z.H. Fan, K. Seiler, A. Manz, and H.M. Widmer. Rapid separation of fluorescein derivatives using a micromachined capillary electrophoresis system. *Anal. Chim. Acta* **283:** 361–66 (1993).
125. S.C. Jacobson, R. Hergenroder, L.B. Koutny, and J.M. Ramsey. High speed separations on a microchip. *Anal. Chem.* **66:** 1114–18 (1994).
126. A.T. Wooley and R.A. Mathies. Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 11348–52 (1994).
127. A.T. Wooley, G.F. Sensabaugh, and R.A. Mathies. High-speed DNA genotyping using microfabricated capillary array electrophoresis chips. *Anal. Chem.* **69:** 2181–86 (1997).
128. C.S. Effenhauser, A. Paulus, A. Manz, and H.M. Widmer. High speed separation of antisense oligonucleotides on a micromachined capillary electrophoresis device. *Anal. Chem.* **66:** 2949–53 (1994).
129. A. Paulus. CE of DNA using capillaries and micromachined chips. *Amer. Lab.* **30:** 59–62 (1998).
130. D. Schmalzing, A. Adourian, L. Koutny, L. Ziaugra, P. Matsudaira, and D. Ehrlich. DNA sequencing on microfabricated electrophoretic devices. *Anal. Chem.* **70:** 2303–10 (1998).
131. C. Barta, Z. Ronai, Z. Nemoda, A. Szekely, E. Kovacs, M. Sasvari-Szekely, and A. Guttman. Analysis of dopamine D4 receptor gene polymorphism using microchip electrophoresis. *J. Chromatogr. A* **924:** 285–90 (2001).
132. J. Khandurina, T.E. McKnight, S.C. Jacobson, L.C. Waters, R.S. Foote, and J.M. Ramsey. Integrated system for rapid PCR-based DNA analysis in microfluidic devices. *Anal. Chem.* **72:** 2995–3000 (2000).
133. Z. Ronai, C. Barta, M. Sasvari-Szekely, and A. Guttman. DNA analysis on electrophoretic microchips: effect of operational variables. *Electrophoresis* **22:** 294–99 (2001).
134. A. Sassi, A. Paulus, I.D. Cruzado, T. Bjornson, and H.H. Hooper. Rapid, parallel separations of d1S80 alleles in a plastic microchannel chip. *J. Chromatogr. A* **894:** 203–17 (2000).
135. Y. Shi, P.C. Simpson, J.R. Schere, D. Wexler, C. Skibola, M.T. Smith, and R.A. Mathies. Radial Capillary array electrophoresis microplate and scanner for high-performance nucleic acid analysis. *Anal. Chem.* **71:** 5354–61 (1999).
136. A. Guttman and Z. Ronai. Ultra-thin-layer gel electrophoresis of biopolymers. *Electrophoresis* **21:** 3952–64 (2000).
137. L. Zhang, F. Dang, and Y. Baba. Microchip electrophoresis-based separation of DNA. *J. Pharm. Biomed. Anal.* **30:** 1645–54 (2003).
138. B. He, N. Tait, and F. Regnier. Fabrication of nanocolumns for liquid chromatography. *Anal. Chem.* **70:** 3790–97 (1998).
139. L.J. Jin, J. Ferrance, and J.P. Landers. Miniaturized electrophoresis: an evolving role in laboratory medicine. *BioTechniques* **31:** 1332–42 (2001).
140. J. Khandurina, T. Chovan, and A. Guttman. Micropreparative fraction collection in microfluidic devices. *Anal. Chem.* **74:** 1737–40 (2002).

Manuscript accepted August 8, 2003.