The potent identification power of DNA typing makes the establishment of databases for the storage of DNA profiles possible and desirable. New profiles can be compared with stored profiles to identify criminals, link unsolved cases, etc. This necessitates a high level of standardization among the laboratories participating in the information exchange networks. They must at least analyse the same polymorphic loci and, for RFLP typing, they must use the same enzymes and similar analytical protocols. Because of the large choice of polymorphic loci and analytical methods, the minimum degree of coherence is not at all warranted unless a strong effort is made between the laboratories to reach a consensus. And a consensus is difficult to reach in a fast moving field where new technologies are constantly being developed.

There is no doubt that DNA typing is the 'safest' identification technique ever used in forensic biology. But, because of its power and its consequent impact in a courtroom, it is and will remain under intense scrutiny by the forensic and legal communities. Consequently, a lot of attention has been paid to quality control and proficiency testing. There is certainly the opportunity for future improvements and developments in this field.

See Colour Plate 75.

See also: **II/Electrophoresis:** Blotting; Deoxyribonucleic Acid, Theory of Techniques for Separation; One-dimensional Polyacrylamide Gel Electrophoresis; One-dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis.

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Capillary Electrophoresis

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Introduction

One of the earliest reports on DNA capillary electrophoresis (CE) was by Cohen *et al*. who demonstrated the high resolution of nucleosides and oligonucleotides, in the absence of a sieving gel, by simply trapping the analyte into sodium dodecyl sulfate (SDS) micelles. One year later, in 1988, the same author discussed the separation of DNA restriction fragments in a gel-free environment. The first reports on the use of polyacrylamide gel capillary columns to separate DNA with remarkable efficiency and resolution were presented the same year by Guttman *et al*. and Cohen *et al*. By 1989, gel-filled CE was already a well-established technique. These preliminary reports were then followed by a flurry of articles describing applications of capillary gel electrophoresis (CGE) to the separation of DNA restriction fragments. In spite of the high resolving power and efficiency offered by CGE in the analysis of nucleic acids (15–30 million plates per metre and a single-base resolution for fragments ranging from 15 to more than 500 bases were reported), the difficulties of producing adequate gel-filled capillaries hindered their greater application. Heiger *et al*. proposed, as early as 1990, the use of polyacrylamide cross-linked with a very low or zero concentration of *N*,*N*-methylenebisacrylamide. Strangely enough, the revolution brought about by the use of polymer solutions as sieving matrices was only evident in 1991 as a result of the work of Guttman and Cooke and Grossman and Soane. Since then, hundreds of reports have demonstrated the advantages of performing DNA separations in a narrow fused silica capillary. Due to its high resolving power and quantitative capability, CE has been successfully applied to different kinds of DNA analysis, including the following: DNA sequencing, separation of restriction fragments, polymerase chain reaction (PCR) products and synthetic oligonucleotides.

Advantages of Capillary over Slab Gel Electrophoresis

For many years electrophoretic separations of DNA were carried out in slab gels. One of the main

Table 1 Typical field strengths applied in different DNA electrophoretic systems and analysis time

Separation system	*Typical field strength (V cm^{-1})*	*Time*
Agarose slab gel	5	Hours
Conventional polyacrylamide slab gel	8	Hours
Sequencing in thin polyacrylamide DNA slab gel	40	Hours
Capillary electrophoresis	300–700	Minutes or seconds

problems in electrophoresis is the dissipation of Joule heat created by the passage of current through the gel and the buffer solution. When the gel is not sufficiently and uniformly cooled, significant Joule heating occurs and temperature gradients are formed in the gel. The existence of a thermal gradient within the gel can cause band broadening and, eventually, DNA denaturation. An efficient way to overcome this problem is to carry out the separation in a fused silica capillary. At present, a typical capillary used for DNA electrophoresis has a 50–100 μm internal diameter (i.d.), a fused silica wall about 150 μm thick and a thin exterior coating (10 μm) of polyimide to provide flexibility. The high surface-to-volume ratio of the capillary leads to an efficient dissipation of Joule heat, which eliminates thermal and gravitational convection and allows the use of electric fields up to 700 V cm^{-1}. **Table 1** shows typical values of field strengths applied in slab gel and CE and analysis times required by the different systems. As a result of the high field strengths typically used in CE, DNA separation 25 times faster than in slab gel electrophoresis is easily achieved.

Besides high speeds, another great advantage of CE is that DNA samples can be automatically loaded and the separated zones can be detected online by fluorescence or UV absorbance. Furthermore, the ease of online detection renders CE suitable for automation. The amount of samples typically loaded in CE is about three orders of magnitude lower (2–100 nL) than that used in slab gel electrophoresis, making the technique useful as a tool for the analysis of molecular biology products, which are often available only in minute amounts. Two types of online injection modes are performed in CE: electrokinetic injection and hydrodynamic injection. Electrokinetic injection is accomplished by placing one end of the capillary in the sample vial (which may contain as little as 2 μL of sample) along with a platinum wire electrode. The application of an electric field for a short time forces charged analytes to enter into the capillary through electrophoretic migration. DNA molecules, being negatively charged, are injected at the cathode end of the capillary with the detector located at the anode end. In hydrodynamic injection, the capillary is placed in the sample vial and a sample plug is forced to enter into the capillary by applying, for a given amount of time, a differential pressure across the capillary. Both injection systems present advantages and disadvantages. Hydrodynamic injection avoids bias in the amount of the sample injected with the faster analytes injected in greater quantities and is more suitable for quantitation.

DNA Sieving Matrices

At neutral pH, DNA molecules being negatively charged migrate towards the anode when placed in a potential field. Their electrophoretic mobility is given by this simple expression:

$$\mu_e = \frac{q}{f} \qquad [1]$$

where q is the net charge on the molecule and f is the molecular friction coefficient. DNA molecules in free solution adopt an open, extended conformation that allows solvent molecules to stream around each segment of the biopolymer equally. This implies that electrophoretic mobility of double-stranded DNA in free solution is independent of molecular size as both the net charge on the DNA molecule and its frictional coefficient increase linearly with the number of base pairs. A size-dependent separation of DNA requires the addition of a sieving medium to the running buffer. In slab gel electrophoresis, cross-linked polyacrylamide and agarose became commonly adopted in DNA work as support matrices due to their ability to reduce thermal and gravitational convection which would suppress the resolution. In addition, porous gels allow the passage of analytes providing the size-based separation of free-draining polyelectrolytes with nearly equal free solution electrophoretic mobility.

In a fused silica capillary the anticonvective role and the DNA-separating role of the electrophoresis matrix can be decoupled. In CE, even in the absence of a dense gel matrix, only very minimal thermal convection and diffusion of analyte molecules occur during electrophoresis due to the high electrical resistance of fused silica, resulting in low current generation. This feature allows the replacement of the gel with a solution of a linear neutral polymer. Most of the problems resulting from difficulties in the production of good quality gel-filled columns and with their limited lifetime have simply been overcome with the introduction of polymer solutions. Capillaries filled with linear polymers are relatively easy to prepare and use. Due to their low viscosity, solutions of polymers can be automatically pumped into the

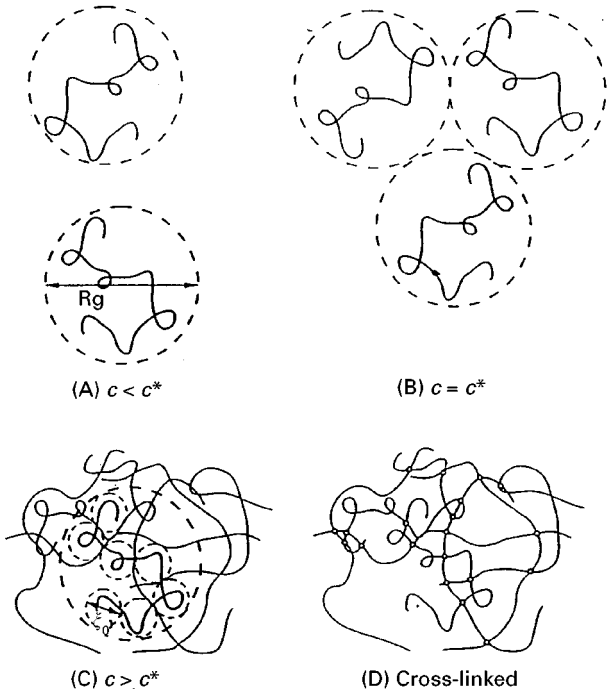

Figure 1 Schematic representation of flexible polymer in solution. (A) dilute solution; (B) $c = c^*$; (C) semidiluted solution; (D) cross-linked chains. (Reproduced with permission from Heller C (1997) *Analysis of Nucleic Acids by Capillary Electrophoresis*. Viesbaden: Vieweg.)

capillary before each run and removed at the end of the separation. The automatic replacement of the sieving matrix increases the capillary lifetime dramatically while reducing the need for sample clean-up.

Polymer Solutions as DNA Sieving Matrices

A number of polymers with different properties (such as molecular weight, hydrophilicity, chain stiffness) have been tested for their ability to sieve DNA molecules. Cellulose derivatives (hydroxyethyl, hydroxypropyl and hydroxypropylmethyl cellulose), uncross-linked polyacrylamide and polyacrylamide derivatives, dextran, polyvinyl alcohol, polyethylene oxide and polyethylene glycol represent some examples of sieving matrices used in CE with varying degrees of success.

According to Grossman and Soane, as size-dependent DNA electrophoretic separation requires the use of an entangled polymer solution. Coils of linear polymers, above a critical concentration, c^*, defined as the entanglement threshold concentration, begin to interact physically and form a physical gel with transient pores (**Figure 1**). The c^* concentration, characteristic for each polymer, can be experimentally estimated by measuring viscosity at different polymer concentrations and finding the point of deviation from linearity on the viscosity vs. concentration plot. In an entangled polymer solution the DNA migration mechanism is similar to that observed in chemical gels (cross-linked gels), complicated by the dynamic nature of the DNA–polymer and polymer–polymer interactions. A high resolution can only be achieved in a rather narrow DNA size range (from 100 to 2000 bp).

When a polymer solution is far below the entanglement threshold concentration, polymer chains remain isolated in solution and, even if they transiently interact with DNA molecules, they do not form a porous network. However, DNA molecules larger than 2 kbp can be separated in ultradilute hydroxyethyl cellulose (HEC) solutions, although with a lower resolution. A new separation mechanism, transient entanglement coupling mechanism, was proposed to explain why the separation occurs in the absence of a sieving effect. When DNA and isolated polymer chains encounter each other, they become transiently entangled. DNA molecules, more stiff and extended in solution than polymer chains, drag the latter until they escape, slowing down their electrophoretic mobility. Large DNA fragments experience stronger entanglement interactions, therefore the time required to escape is size-dependent and DNA mobility becomes size-dependent. Below c^*, high molecular weight polymer chains provide a high speed separation of large molecules with a moderate resolution.

A polymer, in order to be a good sieving matrix for DNA in capillary electrophoresis, should meet the following requirements:

1. high water solubility;
2. low viscosity when used above its entanglement threshold concentration;
3. high chemical stability;
4. good sieving capacity;
5. UV transparency.

Most studies have demonstrated that the molecular mass of the polymer dissolved in the electrophoresis buffer influences the resolution and the interval of DNA sizes which can be separated. Polyacrylamide has, for a long time, been the matrix of choice for DNA sequencing and for dsDNA fragments up to 600 bp. Recently, several authors have replaced polyacrylamide with polymers obtained by radical polymerization of *N*-mono and di-substituted acrylamido derivatives. These polymers offer the advantage of being more resistant to alkaline hydrolysis than polyacrylamide. One of the most promising derivatives of polyacrylamide that has found application in CE is the *N*,*N*-dimethylacrylamide (DMA). Short chain poly(DMA) is produced by carrying out polymerization in organic solvents such as dioxane and *t*-

butanol or in the presence of a chain transfer agent. Besides optimal sieving capacity, this polymer possesses the unique feature of suppressing electroosmotic flow (EOF) even at 0.001% w/v concentration, allowing DNA separation to be carried out in uncoated capillaries. For large DNA (600 bp to 23 kbp) cellulose ethers, hydroxyethyl, hydroxypropyl, hydroxypropylmethyl (HPMC) and methyl cellulose (MC) have been used as separation media. Chemically modified cellulose derivatives are typically used to separate DNA fragments at concentrations of 0.2–1% w/v. Due to the fact that stiff and extended chains become entangled more easily than flexible random coil polymers, the operative concentration of cellulosic polymers is considerably lower than that used with polyacrylamides.

Influence of Electroosmotic Flow on DNA Separation

The most important characteristic of CZE capillary columns is associated with the chemical structure of the fused silica. The presence of several types of silanol groups (SiOH) on the inner surface of the capillary column which are weakly acid in character dramatically influences the CZE separation mechanism by inducing an electroosmotic component to the transport of ions during the analysis. Negative immobile charges on the wall surface generate an electric double layer at the silica–buffer interface consisting of an immobile layer of positive ions strongly adsorbed on to the surface and a diffuse layer extending into the liquid, which is made up of hydrated ions loosely and aspecifically bonded to the surface. When an electric field is applied tangentially to the surface, electrical forces acting on the mobile part of the diffuse double layer cause the movement of ions toward the oppositely charged electrode. The migration of these ions drags the surrounding solvent molecules; this overall movement of solution is known as EOF.

The control of the EOF and the suppression of wall adsorption is a key aspect in the achievement of successful DNA separations for two reasons: firstly, EOF can cause the extrusion of the sieving matrix during electrophoresis and secondly, immobile charges on the wall can electrostatically interact with ions which are oppositely charged. DNA molecules, being negatively charged under typical separation conditions, e.g. pH 8.0, 100 mmol L^{-1} Tris–borate or acetate–EDTA buffer, are generally repelled by the wall. However, DNA sample contaminants and cationic intercalating dyes adsorb onto the wall, causing peak broadening and a reduction in efficiency.

There are a number of approaches to eliminate EOF and wall adsorption that act on the composition of the conducting medium. Most of these methods cannot be used for DNA since the operative pH and buffer ionic strengths that are typically used in the separation are chosen to maintain DNA molecules in their native, double-stranded helical conformation. Therefore there are severe limitations in the choice of buffer composition.

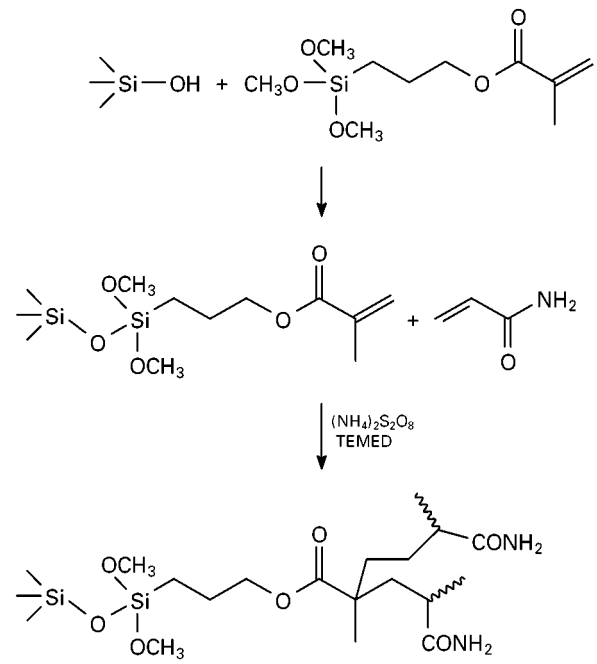

Figure 2 Synthetic scheme for a polyacrylamide coating.

An effective way to suppress EOF which is fully compatible with DNA separation conditions is by coating the capillary inner surface with a neutral polymer. This can be accomplished in several ways:

1. by dynamic adsorption of water-soluble neutral polymers. These modifiers, generally contained in the running buffer, are strongly adsorbed to the wall by hydrogen bonding or hydrophobic interactions. Methylcellulose and poly(vinyl alcohol), for instance, have been used to produce a layer of high viscosity on the capillary wall which suppresses EOF.
2. permanent coating with small or large molecules chemically bonded to the surface silanols or immobilized as films of various thicknesses on the capillary wall. The simplest way to prepare these coatings is by bonding an organosilane bearing a reactive group that can be used, in a second step, to incorporate the organosilane into the polymer (**Figure 2**). Polyacrylamide, polyvinylpyrrolidone, and epoxy polymer coatings have been successfully prepared in this way.

Independently of the type of neutral polymer and of the mechanism which the polymer uses to bond to the wall, the viscosity in the thick layer of the double layer appears to govern EOF suppression. However, the nature of the interaction with the wall and the structure of the polymer play an important role on the coating stability.

Detection

One of the major advantages of CE is that the separated zones can be detected online. A short stretch (1–2 mm) of the polyimide coating is removed from the outside of the capillary and the UV transparent (UV cut-off of 170 nm) fused silica 'window' is directly placed inside the detector. Online detection prevents diffusion of separated zones resulting from flowing through the connections required by an off-column detection system. Nucleic acids are detected by UV absorbance at 260 nm or fluorescence. UV absorbance is simple and inexpensive; furthermore, this detection system allows the detection of nucleic acids based on their intrinsic UV absorbance without any need for intercalating dyes that can alter DNA electrophoretic mobility. The main drawback of UV absorbance detection in CE is its limited sensitivity resulting from the short path length available across the 50 μm capillaries typically used for DNA separation. Because of this limitation, laser-induced fluorescence (LIF) is currently employed for applications that require a higher sensitivity. An example is DNA sequencing where separated zones contain 1–10 attomoles of material.

Although an LIF detection system is more expensive and complicated to build than a UV absorbance detector, its remarkable sensitivity renders this system the most popular method for DNA detection. The use of efficient fluorophores and laser excitation gives sensitivities up to six orders of magnitude greater than that typically achieved with UV absorbance. It has been demonstrated that bands containing 1 pg of DNA can be detected using ethidium bromide as fluorescent label. Several different labels with distinct spectral properties are available (FAM, JOE, TAMRA and ROX are four fluorophores used for DNA sequencing which has relatively widely spaced absorbance and emission spectra). These fluorophores are excited by the 488 nm and 514 nm lines of a common argon laser. Their $\lambda_{ex,max}$ varies from 550 to 610 nm whereas their $\lambda_{em,max}$ are between 520 and 605 nm.

Most of the efforts in developing LIF detection were carried out in the area of DNA sequencing by CE. The availability of good labelling strategies is at the heart of LIF detection development. Recently, energy transfer primers have been developed to increase sensitivity. These primers contain a common donor dye at the 5′ end and an acceptor dye about 8–10 nucleotides away. The presence of a common donor allows the use of a single laser at 488 nm to excite all four fluorophore pairs efficiently.

Applications

The advantages of DNA CE over slab gel are such that an increasing number of molecular biologists around the world are beginning to apply this rapid and efficient technique to DNA mapping and sizing separations of medical and genetic interest.

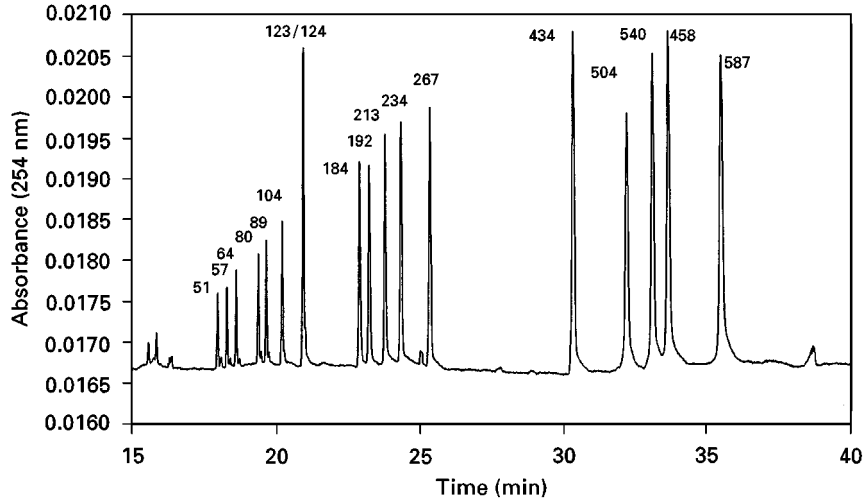

Figure 3 Electropherogram demonstrating the efficient separation of a DNA molecular mass marker containing a mixture of fragments from cleavage of plasmid pBR322 with restriction endonuclease HAE III. Buffer 100 mmol L^{-1} Tris–borate, 2 mmol L^{-1} EDTA, pH 8.2. Temperature: 25°C; injection: 5 s at 1 kV; separation 200 V cm^{-1}.

Restriction Endonucleases and DNA Digests

Most work in CE literature has involved separating DNA restriction digests. The discovery of enzymes capable of recognizing and cutting a particular short sequence of base pairs (usually 4–8 bp long) in a double-stranded DNA molecule has been of fundamental importance in molecular biology. Restriction enzymes are used to compare near-similar DNA molecules by cutting them into smaller fragments which differ in length or sequence. Restriction digests such as Φ174-HaeI-II, pBR322 HaeIII and pBR322 MspI, containing fragments in the size range from 50 to 1550 bp, have been separated by CE. Larger DNA fragments, from 125 to 23 130 bp, are usually generated with λ-HindIII. These restriction digests are relatively inexpensive, can be obtained at high concentration in a purified form and contain fragments of known size. They are often used in CE to test the separation efficiency of a given separation system (**Figure 3**).

Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a molecular copying process that allows the amplification of the quantity of DNA available for a given test. Using a three-step temperature cycle, PCR allows specific regions of DNA to be amplified to a detectable level. The analysis of PCR products requires the ability to separate the target sequence from nonspecific products that may result from the use of nonoptimized conditions or from overamplification. CE has been successfully used for quantification and sizing of amplified products. Given its small sample requirements, CE can detect PCR products at a low cycle number using LIF, providing an efficient tool to evaluate PCR reaction parameters. Many of the separations which can be carried out on a slab gel have been successfully performed in CE. Some of the most significant papers on separation of PCR products by CE are reported in **Table 2**.

Capillary Sequencing

The first separations of DNA sequencing fragments by CE were reported in 1990 by groups at DuPont, Utah, Wisconsin, Northeastern and Alberta. DNA sequencing involves the separation of a set of single-stranded fragments differing by one nucleotide in length which share a common starting point and terminate randomly at a particular base. Since 1977, two methods have been developed and refined to sequence DNA fragments up to 500 bp. They are based on nucleotide-specific chemical cleavage or on the use of chain-terminating inhibitors of DNA polymerases (dideoxynucleotides -ddNTP-). Almost all DNA sequencing reactions nowadays are carried out according to the enzymatic protocol of Sanger. DNA sequencing provides the ultimate tool in the detection of DNA sequence variation. However, for measuring variations in large DNA regions it is necessary first to

Table 2 Application of PCR product analysis by CE

Type	*System examined*	*PCR product size (bp) (resolution needed)*
Diagnostic	Androgen insensitivity syndrome	136, 139, 160 (3 bp)
Diagnostic	Congenital adrenal hyperplasia	127, 135 (8 bp)
Diagnostic	Cystic fibrosis ΔF508	95, 98 (3 bp)
Diagnostic	Cystic fibrosis, GATT microsatellites (for linkage)	111, 115 (4 bp)
Diagnostic	Down's syndrome, D21S11	220, 224 (4 bp)
Diagnostic	Duchenne and Becker muscular dystrophy (18) exon	88, 547 (3 bp)
Diagnostic	Dystrophin: DXS 164 locus	740 bp → 220, 520 bp
Diagnostic	ERBB2 oncogene	1.1 kb bp → 500, 520 bp
Diagnostic	Factor V mutation	115, 138, 202
Diagnostic	Hepatitis C virus	380, 187, 289
Diagnostic	HIV-1 virus (*gag* gene)	115
	gag, *pol* and *env* genes	142, 394, 442
Diagnostic	Kennedy's disease	480, 540
Diagnostic	Medium-chain acyl-coenzyme A dehydrogenase deficiency	175, 202
Diagnostic (SSCP)	p53 gene mutation clusters A and B	372
Diagnostic	Polio virus	53, 71, 97, 163
Diagnostic	VNTR: aboB locus	600–1000 (16 bp)
Diagnostic	ZFY gene, Y-chromosome	307
Forensic	HLA-DQa	242
Forensic	Mitochondrial DNA	402, 437, 1021
Forensic	STR: HUMTH01 locus	179–203 (4 bp)
Forensic	VNTR: D1S80	401–801 (16 bp)
Genotyping	Soybean plant simple sequence repeats (SSRs)	401–801 (16 bp)

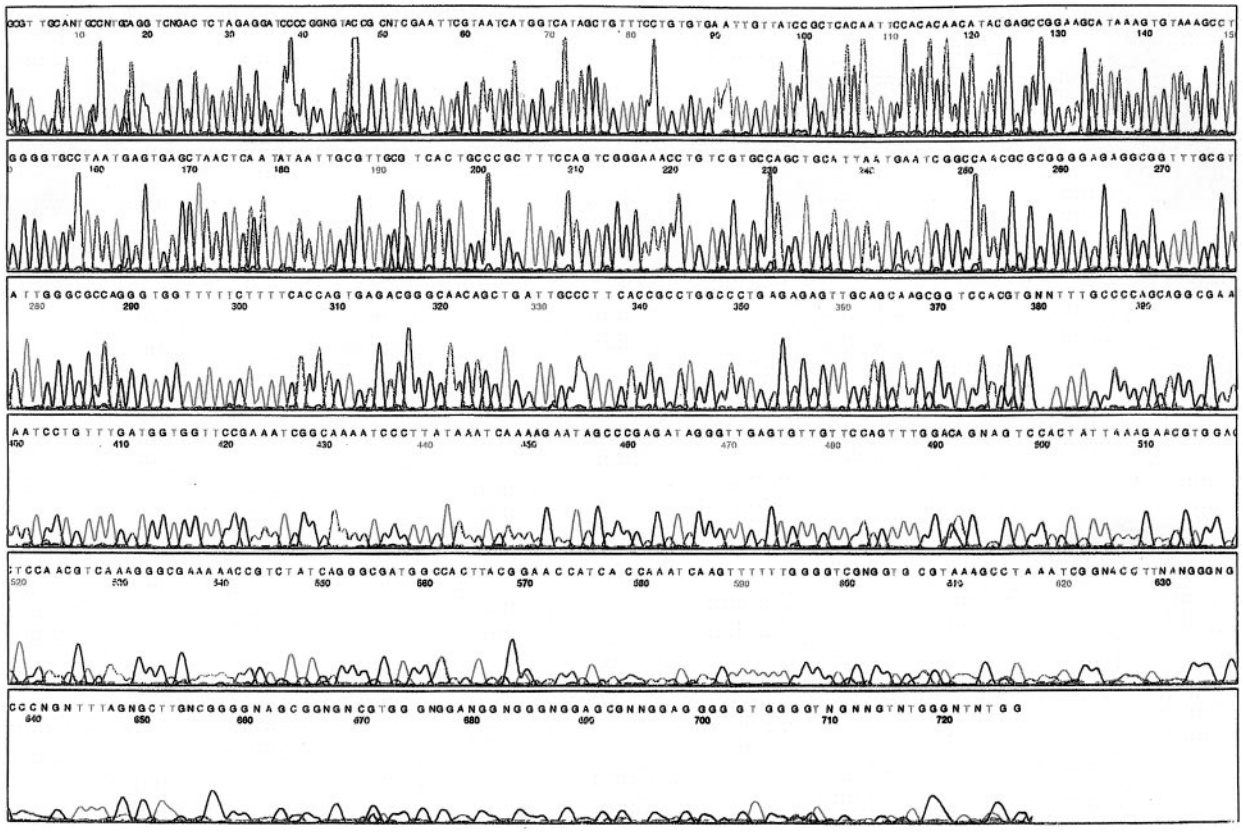

Figure 4 (See Colour Plate 76). Four-colour M13mp18 sequencing separation at 42°C. The separation of DNA sequencing fragments was performed in a 6.5% poly(dimethylacrylamide) (98 kDa) solution (75 cP) in 100 mmol L^{-1} TAPS buffer (pH 8.0) containing 8 mol L^{-1} urea. A 50 μm i.d. bare silica capillary, 51 cm long (40 cm to the window) was used. DNA was electrokinetically injected for 20 s at 60 V cm^{-1}, and the fragments were separated for 125 min at 160 V cm^{-1}. Reproduced from Madabhushi (1998), *Electrophoresis* 19: 224–230, with permission Wiley–VCH.

subdivide the region into smaller sections of about 400 bp. Hence, large numbers of sequencing reactions must be performed in each sample. To become applicable for screening purposes, DNA sequencing requires dramatic improvements in speed. CE provides a tool to increase sequencing rates significantly; at present, over 700 bases of sequence are routinely generated in a 2 h separation, and occasionally runs of 1.000 in 135 min have been generated. **Figure 4** shows a four-colour M13mp18 DNA sequencing separation.

Unlike in slab gel, where the four sets of A, C, G and T terminated fragments run in parallel in four different lines, in CE it is more convenient to run the four sets of fragments in the same capillary because this eliminates capillary-to-capillary variation in the migration velocity of different fragments. Different strategies have been developed to accomplish this goal, making use of four different labels with distinct spectral properties. Several labels, such as FAM, JOE, TAMRA and ROX, are available for sequencing. Some authors have reported the use of primers marked with different fluorophores whereas others have suggested the association of a particular dye to a specific ddNTP chain terminator. In one approach, fluorescence was excited with two lasers, collected with a single microscope objective and discriminated with a filter wheel. Alternatively, a single laser-excited fluorescence and a two-channel directly reading fluorescence detector were used to discriminate fluorescence between the dyes.

A number of capillary array electrophoresis instruments with UF detection have recently become commercially available. These instruments have great potential for all those involved in the human genome project. In capillary array electrophoresis, many capillaries (typically six arrays of 16 capillaries) are set in parallel allowing parallel separations to be run as in slab gel but offering the advantages of analysis speed and automation typical of capillary electrophoresis.

Mutational Analysis

Detection and localization of single-base differences in specific regions of genomic DNA are of primary importance in the analysis of mutations associated

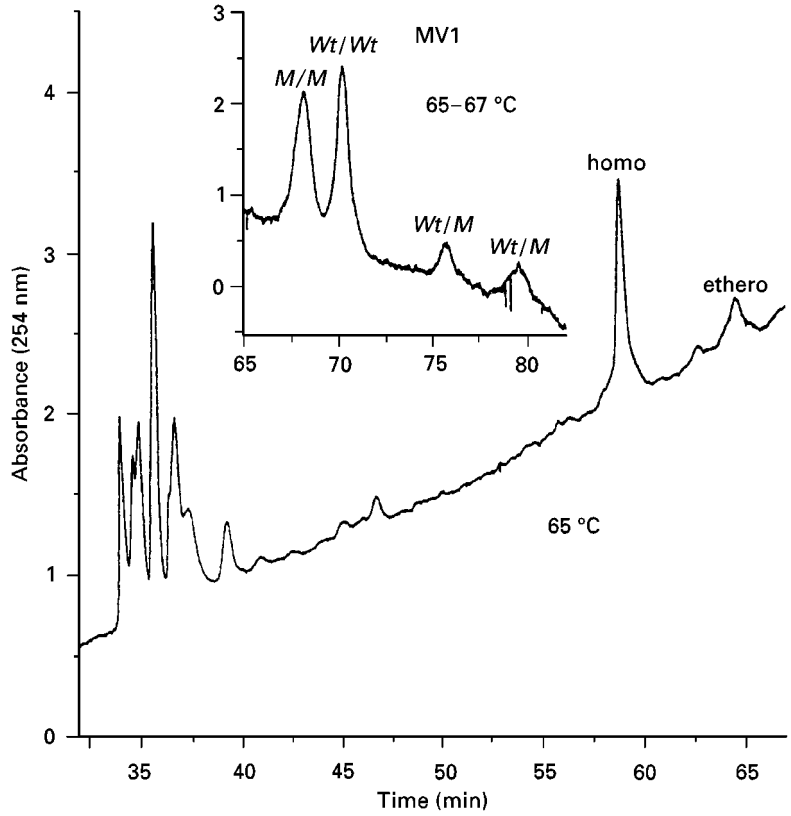

Figure 5 CZE analysis of mutant MV1 in exon 1 of the CFTR gene. The panel shows a run at a constant temperature plateau of 65°C. Inset: run in a 65–67°C temperature gradient, with a slope of 0.1°C min. Sample injection: electrokinetic, 3 s at 4 kV. Reproduced with permission from Heller (1997) *Analysis of Nucleic Acids by Capillary Electrophoresis*. Viesbaden: Vieweg.)

with human disease. The methods used to detect point mutations exploit sequence-dependent variations in base-pairing which cause differences in conformation and lead to altered electrophoretic mobilities. Differences in melting behaviour of duplex molecules are the bases for separation of DNA sequence variants by electrophoresis. By running the separation under a temperature-programmed gradient (TGCE or thermal gradient capillary electrophoresis), a local loss of interstrand base paring is induced, generating DNA molecules with a conformation very different from the normal worm-like rods. This allows the separation of molecules according to their melting characteristics and the detection of deviations from a wild-type sequence (**Figure 5**). In CE, the denaturing temperature gradients can be generated internally via ohmic heat produced by voltage ramps. The temperature increase linked to voltage ramps inside the capillary can be predicted as it depends on capillary diameter, its total length, the electric current values linked to a given applied voltage, the buffer electric conductivity and its coefficient of thermal conductivity. With the help of appropriate software, given these input parameters it is easy to assess dependence of temperature on voltage ramps. A voltage gradient is then generated during the run, taking into account the melting profiles of the amplified fragments that can be predicted by the dedicated software of Lerman and Silverstein.

Future Developments

Looking to the future, we can expect new developments in instrumentation and technology. Another promising area is micromachining technology which has found widespread use in electronic and mechanic engineering, and is now increasingly applied to analytical chemistry and biotechnology. Photolithography and chemical etching techniques have been combined to create the field of micromachining in which three-dimensional microstructures have been fabricated on a micrometer scale. Electrophoresis on a chip has been successfully used for the separation of oligonucleotides, DNA restriction fragments and PCR products. In these microchip systems, fluid flow and reagent mixing are achieved using electrokinetic transport phenomena. Future developments can also be expected in this last area.

See Colour Plate 76.

See also: **II/Electrophoresis:** Capillary Electrophoresis; Capillary Electrophoresis–Mass Spectrometry; Capillary Electrophoresis-Nuclear Magnetic Resonance. **III/ Deoxyribonucleic Acid Profiling:** Overview.

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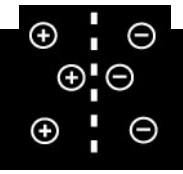
DETERGENT FORMULATIONS: ION EXCHANGE



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Introduction

The primary ingredients of a detergent are the surfactants, whereas *builders* provide the necessary backbone. The maximum efficiency of surfactants is achieved when the hardness in water is removed. Builders provide this essential function of water softening, primarily by means of ion exchange. Although the performance of surfactants is directly dependent on the efficacy of the builder, the consumer rarely notices the importance of the latter. The development in detergent technology during the last decade has been to improve the property of ion exchange that can lead to enhanced washing power of the detergent, while considering other factors such as environmental concerns and cost. This has been largely due to the development of ion exchange materials and molecular sieve type materials that have been used in detergent formulations as builders. Laundry detergents have a yearly $4.4 billion market alone in the United States, shared equally by the liquid and powder detergents. A typical detergent composition is shown in **Table 1**. In today's detergents, builders constitute about 6–25 wt% of liquid detergents and about 20–55 wt% of powder detergent formulations. Thus builders play a significant role in the detergents market.

Until the early twentieth century, cleaning products were essentially soaps, i.e. sodium salts of natural fatty acids. The surfactants in the first synthetic detergents were short chain alkylnaphthalenesulfonates, which were followed by long chain alkylbenzenesulfonates (ABS). ABS were prepared by alkylation of benzene with propylene tetramer followed by sulfonation. Although ABS had very good cleaning properties, they were non-biodegradable and their accumulation in the environment caused foaming in sewage treatment plants and rivers. Hence ABS were replaced by their straight chain analogues, linear alkylarylsulfonates (LAS) in 1965. Surfactants can be broadly classified as anionic, cationic and non-ionic. Today's detergent formulations contain mainly the anionic surfactants and a lesser amount of non-ionic surfactants. Cationic surfactants have very small market share compared to the anionic and non-ionic materials. The use of non-ionics is increasing in liquid detergents as they offer greater stability and formulation flexibility. Whereas the main function of builders is to provide water softening capability and alkalinity, they also serve other important functions as dispersants, antiredeposition agents, and anticorrosion, bleach stabilization and processing aid. **Figure 1** illustrates the various important functions played by zeolites in the washing process. Hard water, if not

Table 1 Typical detergent composition

Ingredient	*Powder detergent*	*Heavy-duty liquid detergent*
Surfactants		
Anionic	15–20	10–40
Non-ionic	0–3	0–10
Builders		
Zeolite	20–30	0–25
Citrate	0–5	0–10
Polycarboxylates	0–3	–
Carbonate	8–12	0–25
Sodium silicates	1–3	–
Sodium sulfate	20–25	–
Enzymes	0–2	0–1.5
Perborate bleach	0–5	0–10
Polymer stabilizer	–	0–1
Enzyme stabilizer	–	0–5