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Review

Analysis of disease-causing genes and DNA-based drugs by capillary electrophoresis Towards DNA diagnosis and gene therapy for human diseases

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Abstract

Rapid progress in the Human Genome Project has stimulated investigations for gene therapy and DNA diagnosis of human diseases through mutation or polymorphism analysis of disease-causing genes and has resulted in a new class of drugs, i.e., DNA-based drugs, including human gene, disease-causing gene, antisense DNA, DNA vaccine, triplex-forming oligonucleotide, protein-binding oligonucleotides, and ribozyme. The recent development of capillary electrophoresis technologies has facilitated the application of capillary electrophoresis to the analysis of DNA-based drugs and the detection of mutations and polymorphism on human genes towards DNA diagnosis and gene therapy for human diseases. In this article the present state of studies on the analysis of DNA-based drugs and disease-causing genes by capillary electrophoresis is reviewed. The paper gives an overview of recent progress in the Human Genome Project and the fundamental aspects of polymerase chain reaction-based technologies for the detection of mutations and polymorphism on human genes and capillary electrophoresis techniques. Attention is mainly paid to the application of capillary electrophoresis to polymerase chain reaction analysis, restriction fragment length polymorphism, single strand conformational polymorphism, variable number of tandem repeat, microsatellite analysis, hybridization technique, and monitoring of DNA-based drugs. Possible future trends are also discussed.

Keywords: Reviews; Human Genome Project; Polymerase chain reaction; DNA sequencing; DNA-based drugs

Contents

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1. Introduction

In vivo gene therapy is a strategy in which nucleic acid, usually in the form of DNA, is administered to modify the genetic repertoire of target cells for therapeutic purposes [1–3]. The excitement associated with the potential for in vivo gene therapy is based on two concurrent technological developments: methods to transfer and express genes in experimental animals in vivo [2,3], and the blossoming of new and powerful strategies to identify and sequence the estimated 80 000 genes comprising the human genome [4–10]. Together, these two technologies have the potential to make a quantum leap in human therapeutics, as it soon will be theoretically possible to administer any human gene to any organ in vivo. The era of "the gene as the drug" is clearly upon us. Additionally, technical advances over the past decade in the synthesis and molecular biology of nucleic acid analogues have spurred a research effort dedicated to the discovery of gene-targeted drugs [11–13], because of their potential as therapeutic agents which act by selectively modulating gene expression or inhibiting protein synthesis. The finding and identification of many disease-causing genes in the Human Genome Project [14–16] has also stimulated research for the development of new types of gene-targeted drugs.

These novel classes of drugs, including human gene [1–3], human disease-causing gene [2,3], DNA vaccine [17], antisense DNA [11–13], triplex-forming oligonucleotides [18], protein-binding oligonucleotides [11], and ribozyme [19,20], are called DNA-based drugs or RNA-based drugs. Development of the analytical technologies for DNA-based drugs are urgently required for quality control of these drugs, pharmacokinetic analysis, and therapeutic drug monitoring as is usually done in the analysis of general drugs. Capillary electrophoretic technologies coupled with the polymerase chain reaction (PCR) [21–23] have been rapidly developing as high-performance techniques for the analysis of DNA-based drugs [24–32]. Analytical technologies for DNA-based drugs including disease-causing genes are applicable not only to drug analysis of DNA-based drugs but also to DNA diagnosis for human diseases [33–86]. This article reviews the basis of the methods for gene therapy by DNA drugs and DNA diagnosis for human diseases and the application of capillary electrophoresis to the analysis of DNA-based drugs including disease-causing genes.

2. Human Genome Project and identification of disease-causing genes

The purpose of the Human Genome Project is to generate a set of information, material, and technology resources that will be readily available to the entire scientific community [4,5]. Specifically, the Human Genome Project is intended to construct detailed genetic and physical maps of the human genome, determine the complete nucleotide sequence of human genomic DNA consisting of 3000 mega base pairs (Mbp) (Table 1), and develop the new technology necessary to achieve these ambitious goals [5]a. The goals of the Human Genome Project also include the acquisition of DNA sequence information characterizing the genome of several non-

Table 1
Organisms under investigation through the Human Genome Project [5]a

Organism	Genome size, (bp)	Estimated number of genes	Genes/Mb
Escherichia coli (bacteria)	4.2×10^{6}	4 000	950
Saccharomyces cerevisiae (yeast)	1.5×10^{7}	6 000	400
Caenorhabditis elegans (roundworm)	1.0×10^{8}	13 000	130
Drosophila melanogaster (fruitfly)	1.2×10^{8}	10 000	83
Mus musculus (mouse)	3×10^{9}	80 000	27
Homo sapiens (human)	3×10^{9}	80 000	27

human organisms (Table 1) used extensively in research laboratories as model systems [5]a.

2.1. Mapping and the complementary DNA (cDNA) project

Indeed, from the beginning, progress in genomic research has been remarkably rapid. In late 1994, the human genetic linkage map became the first of the major goals of the Human Genome Project to be reached. The map contains more than 5000 loci covering 4000 centimorgans (cM) as listed in Table 2 [6], representing an average marker density of 0.7 cM. This is well beyond the initial Human Genome Project goal of a 2- to 5-cM map [4]. Considerable progress has also been made in constructing physical

Table 2
Genetic and physical mapping of human genome [6]b

Chromosome	Total STSs	Random STS[a]	Genes		Genetic markers	
			ESTs	GenBank	Généthon	CHLC
1	1374	252	275	106	460	153
2	1275	307	181	67	452	146
3	1097	269	181	64	353	134
4	919	210	112	45	281	121
5	858	196	125	30	312	97
6	858	181	114	39	312	108
7	781	168	141	39	272	83
8	739	183	104	35	248	104
9	577	132	106	30	188	68
10	719	154	131	26	281	60
11	706	122	140	42	272	64
12	707	132	104	64	250	91
13	418	102	48	13	164	54
14	489	106	95	27	163	53
15	428	97	97	22	145	30
16	435	87	79	18	180	32
17	447	66	97	39	186	34
18	403	91	46	18	136	64
19	246	23	45	20	121	15
20	386	84	68	26	144	32
21	156	28	18	12	61	13
22	274	19	38	17	67	12
X	587	145	63	28	216	28
Y	207	0	0	0	0	0
Total	15 086	3154	2408	827	5264	1595

[a] Unbiased STSs, generated by sequencing from a random genomic library.

maps of the human genome. The current five year Human Genome Project has set a target of a physical map consisting of 30 000 sequence-tagged site (STS) markers, with an average spacing of 100 kilo bases (kb) [4]. Already, a physical map has been constructed of the human genome containing more than 15 000 STSs as listed in Table 2, with an average spacing of about 200 kb, distributed over all the human chromosomes [6]b. This is about half the goal of 30 000 STS markers set by the Human Genome Project for a physical map, but it is already good enough to begin large-scale genome sequencing. A more detailed map, with STSs at an average resolution of 100 kb, was originally expected no later than the end of 1998 but may be achieved sooner.

An important parallel effort for most of the organisms listed in Table 1 has been the sequencing of the ends of large numbers of cDNA clones. Such sequences are commonly referred to as expressed sequence tags (ESTs). The term "EST" has been used to refer to a short DNA sequence derived from a cDNA clone and used to identify a genomic coding sequence. A considerable number of ESTs, more than 180 000, have been collected [7]. STSs derived from these ESTs will rapidly be incorporated into physical maps through the efforts of an international mapping consortium.

2.2. DNA sequencing of human and other genomes

The beginning phase of the Human Genome Project has been remarkably successful, especially for genetic and physical mapping, and for the cDNA project. Large-scale DNA sequencing of human and other organisms is now in progress [5,8,9]. In 1995, the first complete sequence of a genome for a free-living organism, namely *Haemophilus influenzae* [1.8 mega bases (Mb)] was determined [10]a. More recently, the entire genome of 3.5 Mb *Cyanobacterium* (a blue–green algae) has been sequenced in less than a year by one research group [10]b. Progress for *Saccharomyces cerevisiae* has been dramatic and the determination of the complete sequence (15 Mb) of the genome was achieved [10]c. Other complete prokaryotic DNA sequences are expected to follow soon, with the sequence of *Escherichia coli* (4.2 Mb) on track for completion in 1997 and that of *Bacillus subtilis* (4.2 Mb) also expected to be finished that year. The highly successful *Caenorhabditis elegans* program has completed almost 20 Mb of DNA sequence and continues at a current rate of 25 Mb per year, with the eventual goal of completing the entire 100 Mb genome in late 1998. Three Mb of *Drosophila melanogaster* sequence have also been generated. Sequencing the mouse genome has not yet been begun in earnest. The mouse DNA sequence in GenBank total only 15 Mb, with most being cDNA sequences and only a minority being genomic sequence. A total of only 35 Mb of human DNA have been sequenced as shown in Fig. 1A, but the rate of sequencing for human DNA is becoming larger and larger, because of maximizing the capacity of current sequencing technology. Order-of-magnitude improvements in DNA sequencing capability are promised by the next generation of electrophoresis-based automated sequencing instruments including capillary array electrophoresis and its microfabricated system now under development [5]b [26,28,87,88]. Given the current progress in these various genome projects, it is not too soon to begin to think about what biomedical research will look like after the completion of the first human genome sequence (currently estimated to occur around 2002 or 2003) [5]a.

2.3. Identification of disease-causing genes

Rapid progress in the Human Genome Project will lead to the identification of the estimated 80 000 genes within the human genome. It can be seen in Fig. 1B that the rate of identification of new genes is still relatively low, but the cDNA project and physical mapping lend confidence that the slope of the curve in Fig. 1B will soon increase significantly and that half or more of the genes in the human genome will be localized on physical maps within the next few year [5]b. The term "gene identification" is also used more broadly to describe the isolation of the gene underlying a particular phenotype or trait, such as a disease. In this sense, gene identification is a biological problem with considerable medical significance that the Human Genome Project resources are already helping to solve [14–16]. It is one measure of the success of the Human Genome Projects to date that, through the use of

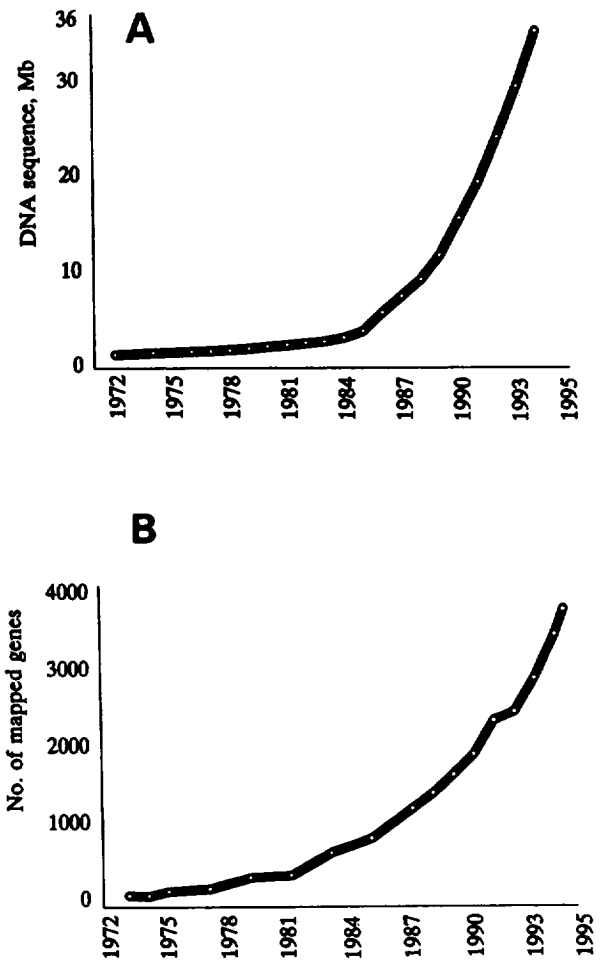

Fig. 1. Genomic information in public data bases, 1972–1995. (A) Mapped genes (B) DNA sequences. (From Ref. [5]b with permission).

these new tools, the speed with which disease genes are being found is rapidly increasing. New disease genes are being found and reported at a rate of several per month as listed in Table 3, compared with a few per year not so long ago. During the past two years, many genes for human diseases have been isolated by using the powerful methods of positional cloning, where no prior knowledge of the gene's function is available. In contrast to positional cloning, functional cloning or candidate-gene strategies make use of information that is known about the gene product and/or the function of the gene of interest that suggest it as a particularly good candidate for the affected gene in a particular disease.

Table 3
Disease-causing genes identified by positional cloning and positional candidate methods

Disease	Year	Gene symbol	Genetic locus
Retinoblastoma	1986	*RB*	13q14.2
Cystic fibrosis	1989	*CFTR*	7q
Myotonic dystrophy	1992	*MD*	19q13.3
Huntington disease	1993	*HD*	4p16.3
Alzheimer's disease	1993	*AD2(APOE)*	19q13.2
Breast cancer	1994	*BRCA1*	17q21
Colon cancer	1994	*hMLH1*	3p21.3
Osteoporosis	1994	*VDR*	-
Diabetes	1994	*IDDM4*	11q
Alzheimer's disease	1995	*AD3(S182)*	14q24.3
Hypertension	1995	*ACE*	17q23
Alzheimer's disease	1995	*AD4(STM2)*	1q31–42
Breast cancer	1995	*BRCA2*	13q

3. PCR and capillary electrophoretic techniques for the analysis of disease-causing genes and DNA-based drugs

Small alterations in DNA sequence of the disease-causing genes lead to many human diseases, such as cancer, diabetes, heart disease, myocardial infarction, atopy, atherosclerosis, cystic fibrosis, and Alzheimer's disease. The alterations in DNA sequence include many types of mutation and polymorphism on disease-causing genes, such as one or several nucleotides substitutions, deletion or insertion of some sequence, differences in a variable number of tandem repeat (VNTR) locus, and the genomic instability of microsatellite repeat. DNA diagnosis for human diseases is, therefore, realized by the analysis of the alterations in the DNA sequence of disease-causing genes. The rapid growth in the identification of new disease genes has increased the need to accurately characterize disease-causing mutations and identify DNA polymorphism. Since a large portion of sequence variation in the human genome is caused by single base changes, any method used to detect mutations or polymorphism must be capable of detecting a single-base substitution. Additionally, the analysis of mutations or polymorphism on disease-causing genes provides valuable information for designing some DNA-based drugs including DNA vaccine, antisense DNA, triplex-forming oligonucleotides, and ribozyme.

Several PCR based technologies have been developed for the highly sensitive and selective detection of mutation or polymorphism of human genes [21–23,89–93]. Slab gel electrophoresis has usually been used for the analysis of the PCR product in the conventional PCR based technologies, but the technique is time-consuming, labor-intensive, and non-quantitative. Capillary electrophoresis is rapidly becoming an important tool for the analysis of PCR product [24–78,94–111]. Rapid and precise genotyping of disease-causing genes using capillary electrophoresis results in high-performance DNA diagnosis for human diseases. Several separation media have been developed and the separation conditions have been optimized for the separation of DNA restriction fragments and PCR products with high-speed and high-resolution [94–106]. Two main detection schemes, UV and laser-induced fluorescence (LIF), have been developed for the detection of DNA fragments. The LIF detector becomes an important technique for the highly sensitive detection of DNA fragments, obtained at low concentration even by using PCR techniques [107]. For this purpose, new efficient intercalating dyes and fluorescently labeling agents have recently been synthesized and applied to the detection of low concentration PCR products [107–111].

3.1. PCR based technologies for the analysis of disease-causing genes

The currently available methods for mutation and polymorphism analysis include some modified PCR techniques [32], restriction fragment length polymorphism (RFLP) [21,22], single-strand conformation polymorphism (SSCP) [21,89], VNTR [90,91], microsatellite analysis [92,93], and hybridization techniques [22,23] as well as PCR analysis itself [21–23]. Some of them are shown in Fig. 2.

PCR is an in vitro method for amplifying specific DNA sequences using a heat-stable polymerase and two 20-base primers, one complementary to the (+)-strand at one end of the sequence to be amplified and the other complementary to the (−)-strand at the other end. Starting with trace amounts of a particular nucleic acid sequence from any source, PCR enzymatically generates millions or billions of exact copies, thereby making genetic analysis of tiny samples a relatively simple process, and is now a widely used technique in molecular biology, with direct applications in the fields of genetic research medical diagnosis, and forensic science [21–23]. RNA molecules can also be analyzed with PCR by first converting them to cDNA by reverse transcription using the enzyme reverse transcriptase. The resulting DNA transcript can then be amplified by the normal PCR process. With this approach, even rare mRNA molecules can be detected and monitored. Such PCR technique is called reverse transcription PCR (RT-PCR). PCR or RT-PCR also can be used to detect the existence of the defined sequence in a DNA or a RNA sample.

With regard to detection of viral injections, a test for human immunodeficiency virus type 1 (HIV-1) has been developed which can detect the presence of the virus earlier than any discernible antibody re-

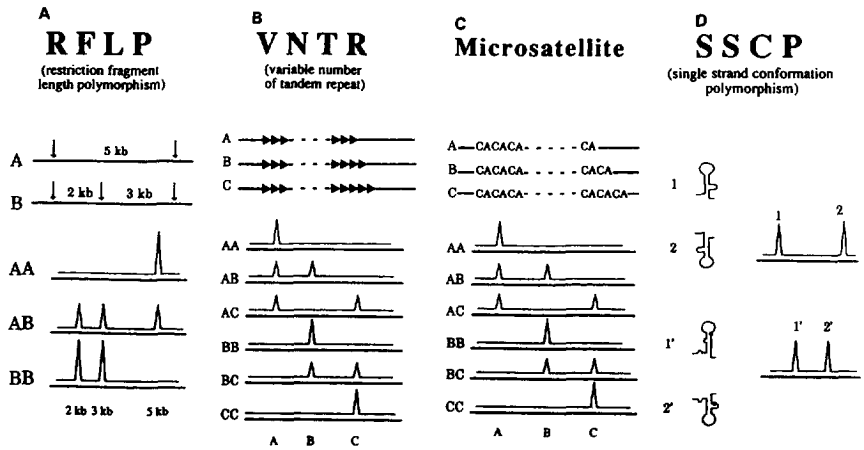

Fig. 2. Typical methods for the detection of polymorphism for human genome. (A) Restriction fragment length polymorphism (RFLP), (B) variable number of tandem repeat (VNTR), (C) microsatellite, (D) single strand conformation polymorphism (SSCP).

sponse in the injected person [32]. Likewise, PCR has been used in the detection of herpes, hepatitis, and cytomegaloviruses, as well as the human papilloma viruses which are associated with cervical cancer.

The amplification refractory mutation system (ARMS) or allele-specific amplification, which are modified PCR techniques, uses the PCR for detection of known point mutations without requiring endonuclease digestion or Southern hybridization. The PCR was first reported as a method for deletion screening for use with the dystrophin gene. Several PCRs are performed simultaneously (a "multiplex" reaction) and the absence of one or more products within a multiplex PCR is taken to indicate a deletion in that portion of the gene.

Ligation chain reaction is another PCR alternative. A thermostable ligase is used to specifically link two adjacent oligonucleotides which hybridize to a complementary target with perfect base-pairing at the junction. These oligonucleotide dimers present a second set of adjacent primers complementary to the first. This approach enables single base pair differences to be detected at the ligation junction and the entire process can be automated.

The RFLP analysis is a method for the detection of a DNA polymorphism which results from a loss or creation of a site at which a particular restriction enzyme cuts as shown in Fig. 2A. RFLPs are usually caused by mutation at a cutting site. DNA carrying the different "allelic" forms will give different sizes of DNA fragments on digestion with the appropriate restriction enzyme. Where a RFLP is closely linked to the defective allele at a disease locus, it can sometimes be used to detect the presence of the defective gene in a carrier or affected foetus even if the disease locus itself has not yet been accurately mapped or cloned [21,22].

The SSCP method is another PCR-based technology for the analysis of DNA mutations [21,89] as shown in Fig. 2D. The SSCP method is based on the principle that the electrophoretic mobility of single-stranded DNA, in a nondenaturing condition, depends not only on its size but also on its sequence. Single-stranded DNA in a nondenaturing gel has a folded structure that is determined by intramolecular interactions. These sequence-based secondary structures (conformers) affect the mobility of the DNA during electrophoresis on a nondenaturing polyacrylamide gel. A DNA molecule containing a mutation, even a single-base substitution, will have a different secondary structure than the wild type, resulting in a different mobility shift during electrophoresis than that of the wild type. The SSCP analysis has gained wide acceptance because of its simplicity, and has been used to detect sequence changes in various contexts.

The human genome contains large amounts of repetitive DNA sequences, some of which are arrayed as tandem repeat units. Polymorphic tandem

repeats occur in two general families, VNTR or microsatellite as shown in Fig. 2B,C. VNTR or minisatellites show variation within populations in the number of repeat units that range from 8 to 50 base pairs in length [90,91]. Microsatellites or short tandem repeats are genetically similar to VNTR at the molecular level, with the distinction that the core repeat unit of microsatellite ranges from two to six bases [92,93]. For example, two bases repeat (CA repeat) is illustrated in Fig. 2C. Both types of markers typically show some degree of heterogeneity in the core repeat unit itself, e.g., base substitutions, insertions, or deletions.

Using primers complementary to conserved sequences flanking the tandem repeat regions and PCR to amplify the VNTR or microsatellite produces a product whose length is directly proportional to the number of repeat units present. Allelic variation at VNTR or microsatellite loci, amplified by PCR, consists of different numbers of the same short DNA sequence (represented triangles) arranged in a tandem continuous array. These amplification products can be resolved as individual alleles by gel electrophoresis. Thus a person can have at most two different alleles, one from each chromosome, and the variable number of repeat units constitute a fragment-length polymorphism.

In 1991, it was discovered that the mutation responsible for the fragile X syndrome is the expansion of an unstable triplet repeat. It soon transpired that myotonic dystrophy, spinal and bulbar muscular atrophy, spinocerebellar atasia type 1, and Huntington disease were also caused by similar repeat expansions, and it is probable that other diseases result from the same mechanism of mutation [92,93]. These repeat regions are polymorphic in unaffected individuals, that is the number of repeated triplets varies within a certain range. If the number of repeats exceeds the normal range, the phenomenon is called a "permutation" because there is a strong possibility of further expansion to a "full mutation" during inheritance from parent to child. Both the PCR and Southern analysis are currently used for the detection of expanded unstable repeats. The PCR has the advantage of speed, convenience, and accurate measurement of normal and permutation alleles, but cannot reliably amplify full mutations. Failure to amplify a repeat region suggests that the number of repeats exceeds the limit of the PCR.

One of the forensic PCR tests is amplification of loci which vary in length according to the number of repeated sequences they contain: VNTR loci or microsatellite loci. Analysis simply involves resolving and visualizing the products of PCR on agarose or acrylamide gel electrophoresis.

Nucleic acid hybridization may be used to measure the degree of similarity (degree of homology) between two nucleic acid sequences as hybridization can occur between sequences which contain some complementarily, but which are not identical; that is, hybrids can tolerate mismatched base pairs (mismatches) in the double helix. DNA–DNA hybridization of this sort has been used to measure the broad similarity between different genomes for taxonomic and evolutionary studies.

A duplex of complementary DNA strands normally exists as a tightly associated double helix. In increasing concentrations of denaturant, for example, urea, the two strands begin to dissociate ("melt") starting with the region(s) most loosely held together. Since the bond between an AT nucleotide pair is weaker than that between a GC pair, AT-rich regions tend to separate first under such denaturing conditions. If a DNA duplex is being subjected to electrophoresis in a gradient of denaturant, at the point where the strands begin to separate the rate of migration is abruptly reduced because of the looser configuration of the duplex. If the fragment of DNA contains a mismatch in a region of the molecule that denatures early, the electrophoresis profile will be altered compared to a homoduplex of perfectly complementary strands. These properties form the basis of the denaturing gradient gel electrophoresis (DGGE) mutation detection assay.

3.2. Separation media and detection method for capillary electrophoresis

Separation media mainly used for the capillary electrophoretic separation of DNA-based drugs and polymorphism analysis for human genes are gel or polymer solutions as listed in Table 4. The separation of single-stranded oligonucleotide based drugs, such as antisense DNA, is achieved by using crosslinked polyacrylamide gel, in which concentrations vary from 3 to 8% T [g acrylamide + g N,N′-methylenebisacrylamide (Bis)/100 ml solution] and the degree of crosslinking vary from 3 to 5% C

Table 4
Proposed concentration range of the polymer-type separation matrix for the separation of DNA fragments

Effective DNA size range of separation (bp)	Concentration of polymer (%, w/v)		
	LPAA	HEC, HPMC,MC	PEG,PEO
1– 100	8.0–12	1.0 –3.0	6.0–8.0
100– 300	7.0– 8.0	0.7 –1.0	3.0–6.0
300– 1 000	5.0– 7.0	0.5 –0.7	2.0–3.0
1 000–10 000	3.0– 5.0	0.3 –0.5	0.5–2.0
10 000–30 000	2.0– 3.0	0.01–0.3	–

(g Bis/% T). More recently, high concentration linear polyacrylamides (8–10%) have been successfully applied to the separation of some oligonucleotide based drugs [80–86] as listed in Table 4. Since linear polyacrylamide is replaceable from the capillary, gels are now increasingly being replaced by polymer solution. For the separation of double-stranded DNA, electric fields between 200 and 500 V/cm have been used.

For the separation of double-stranded DNA-based drugs, including human gene, human disease-causing gene, and DNA vaccine, low crosslinked polyacrylamide gel (3% T and 0.5% C) and linear polyacrylamide are used [94,95]. High resolution separation of a PCR product was demonstrated as shown in Fig. 3 using low crosslinked polyacrylamide gel [51]. Fig. 3 clearly demonstrates that DNA fragments ranging from 75 to 5090 bp are

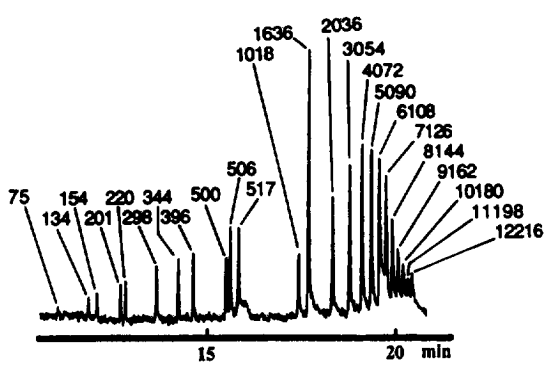

Fig. 3. Capillary gel electrophoretic separation of a mixture of a 500 bp PCR product and a 1 kbp DNA ladder. Capillary: 3% T, 0.5% C polyacrylamide gel-filled, 100 μm I.D., 360 μm O.D., total length 50 cm, effective length 30 cm. Running buffer: 100 m*M* Tris-borate–2 m*M* EDTA (pH 8.3). Field: 200 V/cm. Current: 25 μA. Temperature: 30°C. Injection: 100 V/cm for 5 s. UV detection: 260 nm. (From Ref. [51] with permission).

baseline resolved and larger fragments ranging from 6108 to 12 216 bp are almost completely resolved, yet the separation was completed within 20 min. It is noteworthy that the 500-bp PCR product is completely separated from the 506-bp DNA fragment, which differs by only 6 bp. Such separation cannot be obtained by the slab gel electrophoresis. The plate number achieved was 6×10^5 (2×10^6 plates per meter). High-speed separation of double-stranded DNA fragments was realized by using 3% T linear polyacrylamide and an applied electric field of 700 V/cm [95]. In this instance, less than 1.5 min is required for the complete resolution of DNA fragments ranging from 72 to 1353 bp.

Recently, other polymer networks are mainly used for the separation of double-stranded DNA. The materials currently used are methyl cellulose (MC) [96,101,104], hydroxyethyl cellulose (HEC) [97,99,102,103,106], hydroxypropyl cellulose [101], hydroxypropylmethyl cellulose (HPMC) [101], poly(ethylene glycol) (PEG) [33], poly(ethylene oxide) (PEO) [100], poly(vinyl alcohol) [99], and agarose [98]. For the separation of PCR products and restriction DNA fragments, less concentrated solutions of polymers can be used. Typical polymer concentrations are about 0.1–1.0% [101–106] as listed in Table 4 and most commercially available capillary electrophoretic instrumentations are now able to fill and empty the capillaries with such solutions automatically. For the separation of DNA fragments ranging from 300 to 5000 bp, 0.5% cellulose derivative solution would be selected [96,101,104,105]. Concentrations of cellulose derivative solution should be higher (0.7–1.0%) for the separation of shorter DNA fragments less than 300 bp [101,105,106]. Application of dilute polymer solution (less than 0.3%) will be appropriate for the separation of lager DNA fragments ranging from 5000 to 50 000 bp [102,103,105]. When low concentrated polymer solutions are applied, inner-wall coated capillaries are usually used for eliminating electroosmotic flow. Such capillaries are prepared by the chemical attachment of linear polyacrylamide to the capillary inner wall or some commercially available coated capillaries are used, e.g., J&W DB-17. For the separation of double-stranded DNA, electric fields between 50 and 800 V/cm have been used. For the optimization of the separation of double-stranded DNA fragments, some systematic studies on the

separation conditions have been published [29,102–106]. We can easily reach the optimum conditions for our own separations by choosing appropriate length, diameter, and coating for the capillary, electric field, temperature, pH of the buffer, type of polymer network, and its concentration. Fig. 4 illustrates the separation of DNA restriction fragments using coated capillary filled with 0.5% methyl cellulose solution [104]. Use of a high electric field (700 V/cm) significantly diminished the time required for the complete resolution of DNA fragments as shown in Fig. 4.

DNA fragments separated by polymer network are detected by UV detector or LIF detector. UV detection of DNA fragments is based on the UV absorption of the DNA bases, i.e., the wavelength and the molar absorption coefficient for the UV absorption maxima of DNA bases are 260 nm and 1.52×10^4 for deoxyadenine, 254 nm and 1.30×10^4 for deoxyguanosine, 267 nm and 9.65×10^3 for thymidine, and 271 nm and 9.0×10^3 for deoxycytidine, respectively.

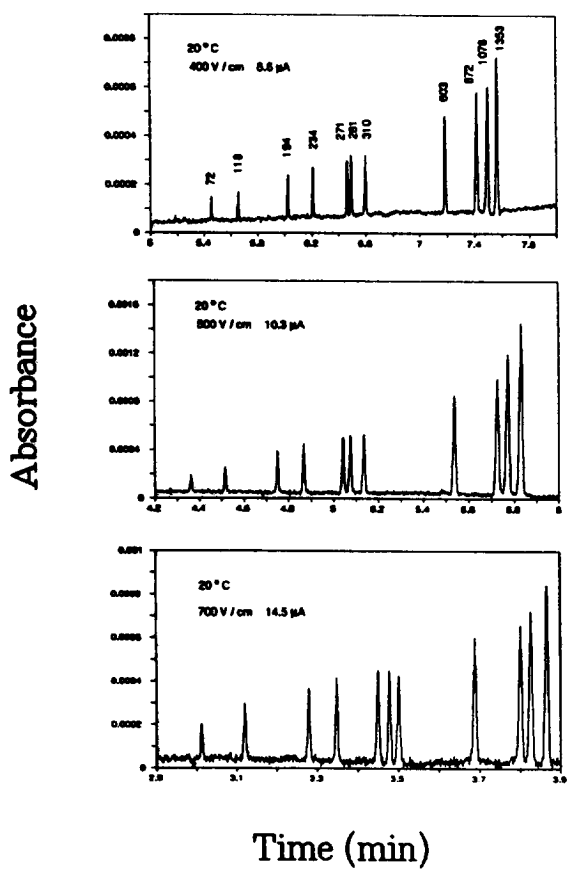

Fig. 4. Capillary electrophoretic separation of ϕX174RF DNA/*Hae* III restriction fragments. Capillary: J&W DB-1, 50 μm I.D., 360 μm O.D., total length 43 mm, effective length 35 cm. Running buffer: 200 m*M* Tris-borate–2 m*M* EDTA (pH 8.2) including 0.5% methyl cellulose (MC). Field, current, and temperature are as shown in the figure. Injection: 200 V/cm for 1 s. UV detection: 260 nm. (From Ref. [104] with permission).

The LIF detection of DNA fragments requires some fluorescence dyes, e.g., intercalating dyes and chemically labeling dyes. The intercalating dye as listed in Table 5 are currently used for the LIF detection of DNA fragments. Ethidium bromide has been the most widely used as an intercalating dye for capillary electrophoresis as well as slab gel electrophoresis, but it has some disadvantages, e.g., high background and low sensitivity. More recently, some new efficient intercalating dyes have been developed [107–109]. Some monomeric dye including thiazole orange (TO, benzothiazolinm-4-quinolinium dye), TO-PRO-1, and oxazole yellow (YO, benzoxazolium-4-quinolinium dye)-PRO-1 and dimeric dyes including TOTO-1, YOYO-1, YOYO-3, and POPO-3

Table 5
Fluorescent dyes useful for labeling PCR products by noncovalent binding and intercalation

Fluorophore[a]	Ex[b]	Em[c]	$(\varepsilon\times10^{-3})$[d]	QY[e]
Propidium iodide	536	617	5.8	–
Ethidium bromide	510	595	5.5	–
Ethidium homodimer-1	528	617	7.4	0.1
Acridine orange	502	526	62	–
TO	509	525	54	0.2
YO-PRO-1	491	509	52	0.44
TO-PRO-1	515	531	62	0.25
YOYO-1	491	509	84	0.52
TOTO-1	514	533	112	0.34
YOYO-3	612	631	167	0.15
POPO-3	534	570	146	0.46
SYBR Green I	494	521	–	–

[a] YO represents oxazole yellow (benzoxazolium-4-quinolinium dye); TO represents thiazole orange (benzothiazolium-4-quinolinium dye); PO represents benzoxazolium-4-pyridinium dye.
[b] Ex, wavelength (nm) at the excitation of absorption maximum.
[c] Em, wavelength (nm) at the emission maximum.
[d] $(\varepsilon\times10^{-3})$, extinction coefficient (cm^{-1} M^{-1}) multiplied by 1000.
[e] QY, quantum yield.

(benzoxazolium-4-pyridinium dye) are applied to the highly sensitive detection of double-stranded DNA fragments. These dyes have several advantages including low background and high sensitivity. Especially monomeric dyes, TO, TO-6, and YO-PRO-1 are better in detection sensitivity than dimeric dyes [107,108]. Recently, another monomeric dye, SYBR Green I, has been developed as a fluorescent dye well suited for efficient separation and quantitative, sensitive, and precise determination of double-stranded DNA using capillary electrophoresis [109]. Almost all the monomeric dyes listed in Table 5, including ethidium bromide, acridine orange, TO, YO-PRO-1, TO-PRO-1, SYBR Green I, are optimally excited by the argon ion laser (488 nm and/or 514 nm). Some dimeric dyes, YOYO-1 and TOTO-1, are suitable for excitation by argon ion laser too, and others, YOYO-3 and POPO-3, are excited by the He–Ne laser (543 nm and/or 633 nm). YOYO-1 is a benzoxazolium-4-quinolinium dimer that has one carbon atom bridging the aromatic rings of the unsymmetrical cyanines. YOYO-3, which differs from YOYO-1 only in the number of bridging carbon atoms (three), has longer wavelength spectral properties. The addition of intercalating dye also improves the resolution of DNA fragments [29,108].

Other LIF detection schemes for DNA involve direct labeling of the analyte with a suitable fluorophore. Fluorescently labeled probes and primers are used in many molecular biology applications involving hybridization, PCR, DNA sequencing, and multicolor detection for accurate SSCP analysis. Most used fluorescent labeling agents are so-called ABI dyes, including FAM, JOE, TAMRA, and ROX. Recently, new types of labeled agents called energy-transfer (ET) primer have been developed [110,111]. DNA primers and probes are usually synthesized with a fluorescent label attached to the 5′ end of the molecule.

4. DNA diagnosis through polymorphism analysis of disease-causing genes

Slab gel electrophoresis has been widely used as a standard method for DNA diagnosis by using PCR-based technologies. Many investigations [24–78], however, demonstrate that capillary electrophoresis is the automated technique offering the benefits of rapid separation, high resolution, and highly sensitive detection in comparison with time-consuming and labor-intensive slab gel electrophoresis. Capillary electrophoresis coupled with PCR-based technologies has been applied to the analysis of human genes including disease-causing genes and DNA diagnosis through mutation or polymorphism analysis of disease-causing genes. Table 6 and Table 7 list all applications of capillary electrophoresis for DNA diagnosis of human diseases, detection of specific gene, and polymorphism analysis of some genes and their separation and detection conditions.

4.1. PCR

Understanding and the diagnosis of infectious diseases caused by viruses have kept pace with advances in molecular virology. For this purpose, the PCR technique is becoming a powerful tool for the detection of the presence of the virus earlier than any discernible antibody response in the person infected with a virus through the detection of actual nucleic acids that are part of particular virus genome. However, post-PCR analysis using slab gel electrophoresis has severely limited the application of the PCR technique in DNA diagnosis. More recently, capillary electrophoresis has become an attractive alternative to the slab gels for the post-PCR analysis and has been applied to the PCR analysis for the detection of some viruses, e.g., HIV [32]a [33], polio virus [45], and hepatitis C virus (HCV) [72]. The family Retroviridae are enveloped single-stranded RNA viruses infecting humans. They are unique among RNA viruses in that their multiplication involves the synthesis of a DNA copy of the RNA which is then integrated into the genome of the injected cell. In these instances, the RT-PCR technique has been applied to the detection of viral infection.

Fig. 5 shows the DNA diagnosis for AIDS through the detection of multiplex PCR amplified HIV-1 *gag*, *pol*, *env* sequences by using capillary electrophoresis with LIF detector [32]a. Capillary electrophoresis with LIF realized accurate and sensitive detection of three targets of HIV-1 genome simultaneously. The relative standard deviation for

Table 6
DNA diagnosis of human diseases, detection of specific gene, and polymorphism analysis by capillary electrophoresis with LIF detector

Disease	Gene or genetic locus	Method	Capillary coating	Separation media	Field (V/cm)	Effective length (cm)	Dye	Ex/Em (nm)	Temp (°C)	Ref.
AIDS	HIV-1	multiplex PCR	coated	LPAA	200	30	TO	488/520	25	[32]a
Polio	polio virus	RT-PCR	coated	LPAA	200	30	TO	488/530	20	[45]
Hepatitis C	HCV	RT-PCR	DB-17	1% HEC	260	20	YO-PRO-1	488/510	25	[72]
-	*NQO1*	RT-PCR	coated	LPAA	107	58	TO	488/520	20	[73]
-	Tx	PCR	coated	3% LPAA	158	50	TO	488/520	25	[74]
MCAD deficiency	*MCAD*	allele specific PCR	non-coated	3% T 0.5% C PAG	200	30	TO	488/530	30	[53]
CF	*CFTR*	PCR/OLA	LPAA	6.2% LPAA	218	40	FAM	488/530	room	[57]
Alzheimer's	*APOE*	RFLP	coated	LPAA	200	40	TO	488/530	25	[76]
Alzheimer's	*APOE*	RFLP	DB-17	0.7% MC	180	40	YO-PRO-1	488/540	30	[78]
-	*IGF1-BP3*	SSCP	LPAA	7% LPAA	200	14	FAM, JOE	488/520, 560	30	[65]
-	*M. tuberculosis*	SSCP	non-coated	3% T, 3% C PAG	150	50	YO-PRO-1	488/520	25	[70]
-	*APOB*, D1S80	VNTR	DB-17	0.5% MC	150	50	TOTO-1	488/530	25	[38]
-	*APOB*, D1S80	VNTR	DB-17	0.5% MC	150	50	TOTO-1	488/530	25	[39]
-	HUMTHO1	microsatellite$(AATG)_n$	DB-17	1% HEC	38 μA[a]	60	YO-PRO-1	488/520	25	[37]
-	HUMTHO1	microsatellite$(AATG)_n$	DB-17	1% HEC	270	30	YO-PRO-1	488/520	25	[56]
-	HUMTHO1	microsatellite$(AATG)_n$	LPAA	0.8% HEC	80	25	ET-dyes	488/525, 600	22	[60]
-	HUMTHO1	microsatellite$(AATG)_n$	DB-17	1% HEC	185	20	YO-PRO-1	488/520	25	[63]
-	mitochondrial	hybridization (CDCE)	LPAA	6% LPAA	250		fluorescein	488/520	36	[50]

[a] Constant current experiment.

Table 7
DNA diagnosis of human diseases, detection of specific gene and polymorphism analysis by capillary electrophoresis with UV detector

Disease	Gene or genetic locus	Method	Capillary coating	Separation media	Field (V/cm)	Effective length (cm)	Detection wavelength	Temp. (°C)	Ref.
AIDS	HIV-1	multiplex PCR	DB-17	0.5% HPMC	175	50	260	25	[33]
DMD	dystrophin	multiplex PCR	PAAEE	6% LPAA	165	30	254	25	[68]
Polio	polio virus	RT-PCR	coated	LPAA	200	30	254	20	[45]
-	*bEGF*	RT-PCR	PAAEE	6% LPAA	100	30	254	25	[62]
21-OH deficiency	P450c21B	PCR	LPAA	6% LPAA	165	30	254	25	[47]
CF	*CFTR*	PCR	LPAA	6% LPAA	140	30	254	25	[48]
CF	*CFTR*	PCR	coated	LPAA	300	30	254	30	[61]
PKU	*PAH*	ARMS	non-coated	3% T, 0.5% C PAG	200	30	260	30	[44]
Cancer	ERBB2	RFLP	DB-17	0.5% HPMC	175	50	260	25	[34]
DMD	dystrophin	RFLP	coated	0.5% HPMC	160	50	260	room	[36]
Alzheimer's	*APOE*	RFLP	non-coated	3% T, 0.5% C PAG	200	30	260	40	[51]
CF	*CFTR*	RFLP	PAA	6% LPAA	165	30	254	25	[55]
Cancer	K-*ras*	RFLP	DB-17	polymer	272	22	260	30	[58]
Cancer	*p53*	SSCP	non-coated	4% LPAA	250	31	260	25	[35]
-	*H. annosum*	SSCP	DB-17	0.5% HPMC	228	50	260	22	[69]
Cancer	N-*ras*	SSCP	non-coated	8% LPAA	200	30	260	25	[75]
Cancer	Ki-*ras*	SSCP	non-coated	polymer	15 μA[a]	48	260	5	[77]
Heart disease	*APOB*	VNTR	DB-17	0.7% MC	200	30	260	30	[66]
-	SE33	microsatellite	DB-17	0.5% HEC	37.8 μA[a]	70	260	25	[40]
CF	*CFTR*	microsatellite $(GATT)_n$	LPAA	6%LPAA	140	30	254	25	[42]
Kennedy's	*AR*	microsatellite $(CAG)_n$	PAAEE	8% LPAA	100	31	254	room	[43]
Cancer	D2S123	microsatellite $(CA)_n$	coated	polymer	222	30	260	30	[59]
PAIS	*AR*	microsatellite $(CAG)_n$	LPAA	6% LPAA	140	30	254	25	[64]
Down's	D21S11	microsatellite	LPAA	8% LPAA	100	30	254	25	[71]
AIDS	HIV-1	hybridization	coated	0.5% HPMC	255	40	254	20	[41]
AIDS	HIV-1	hybridization	coated	0.5% HPMC	255	40	254	20	[46]
Hepatitis C	HCV	hybridization	coated	0.5% HPMC	255	40	254	25	[49]
-	*H.annosum*	hybridization	DB-17	0.5% HPMC	228	50	260	22	[52]
CF	*CFTR*	hybridization (TGCE)	LPAA	8% PAAEE	100	53	254	45–49	[54]
Cancer	*p53*	hybridization	coated	polymer	222	30	260	30	[67]

[a] Constant current experiment.

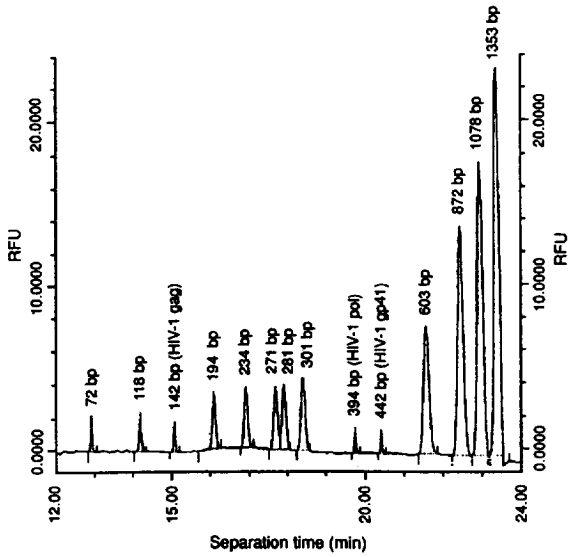

Fig. 5. Detection of multi-target PCR-amplified HIV-1 *gag*, *pol*, *env* sequences and ϕX174RF DNA/*Hae* III restriction fragments by capillary electrophoresis with LIF detector. Capilllary: 100 μm I.D., 360 μm O.D., total length 37 cm, effective length 30 cm. Running buffer containing intercalating dye is LIFluor dsDNA 1000 kit (Beckman). Field: 200 V/cm. Injection: 135 V/cm for 90 s. Temperature: 25°C. LIF detection: 488 nm (excitation), 520 nm (emission). (From Ref. [32]a with permission).

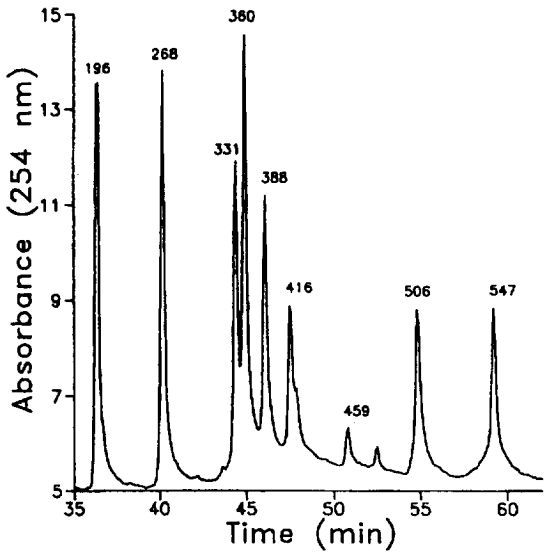

Fig. 6. Separation of nine dystrophin gene exons amplified by multiplex PCR by capillary electrophoresis with UV detector. Capillary: poly(N-acryloylaminoethyoxyethanol) (AAEE) coated, 75 μm I.D., 360 μm O.D., total length 37 cm, effective length 30 cm. Capillary is filled with 6% linear polyacrylamide. Running buffer: 89 m*M* Tris-borate–2 m*M* EDTA (pH 8.3). Field: 100 V/cm. Injection: 100 V/cm for 20–30 s. Temperature: 25°C. UV detection: 254 nm. (From Ref. [68] with permission).

replicate injection reached 1%. The detection limit was about 10^{-18} mol (amol), which is comparable to that of radioactive detection. Thus, the multiplex PCR yields (2–5 fmol) of the 10-copy target DNA template were readily detected.

Capillary electrophoresis with LIF has also been successfully applied to the post-amplification detection of a specific region for an HCV genome [72]. The detection sensitivity is comparable to or better than Southern blot analysis with chemiluminescence detection. Analysis of 39 patient samples by both capillary electrophoresis with LIF and Southern blot showed a 100% correlation. Concentration of PCR-amplified target region of HIV genome or polio viral genome was easily measured by capillary electrophoresis using internal standard co-amplified by PCR and the calibration curve [32]a [45]. This technique also allowed quantitative analysis of some specific gene expression, including basic fibroblast growth factor gene (*bEGF*) [62], NAD(P)H:quinone acceptor oxidoreductase gene (*NQO1*) [73], and Tx gene [74].

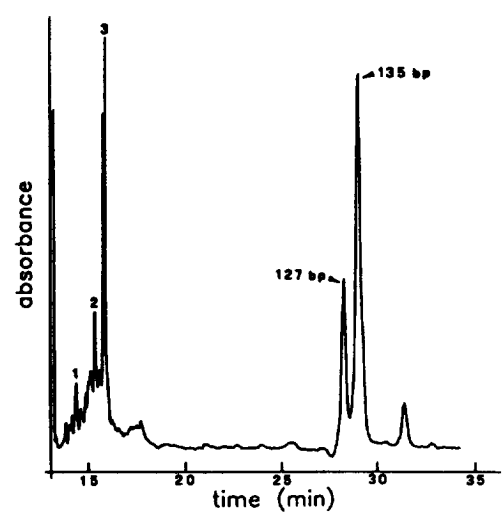

Fig. 7. Capillary electrophoresis analysis of the 8 bp deletion of P450c21B gene. Capillary: polyacrylamide coated, 100 μm I.D., 360 μm O.D., total length 37 cm, effective length 30 cm. Capillary is filled with 6% linear polyacrylamide. Running buffer: 100 m*M* Tris-borate–2 m*M* EDTA (pH 8.3). Field: 165 V/cm. Injection: 165 V/cm for 6–10 s. Temperature: 25°C. UV detection: 254 nm. (From Ref. [47] with permission).

Duchenne muscular dystrophy (DMD) was diagnosed by the simultaneous analysis of nine dystrophin gene exons using multiplex PCR and capillary electrophoretic post-amplification analysis as shown in Fig. 6 [68]. Nine main peaks are separated and detected in the electropherogram and each peak corresponds to each exon, i.e., 196 bp fragment for exon 4, 268 bp for exon 44, 331 bp for exon 12, 360 bp for exon 8, 388 bp for exon 51, 416 bp for exon 17, 459 bp for exon 19, 506 bp for exon 48, and 547 bp for exon 45, respectively. The multiplex PCR technique allows the detection of over 98% of DMD deletions. Capillary electrophoresis coupled with the multiplex PCR technique is an efficient technology for the direct identification of DMD deletions with high speed and easily applicable to the DNA diagnosis for DMD. Multiplex PCR coupled with oligonucleotide ligation assay (OLA) has been applied to the multiplex detection of the mutations on the different exons of cystic fibrosis transmembrane conductance regulator gene (*CFTR*) using capillary electrophoresis for the DNA diagnosis of cystic fibrosis [57].

21-Hydroxylase (21-OH) deficiency is recessively inherited and accounts for over 90% of the genetic disorders of steroidogenesis (congenital adrenal hyperplasia) [47]. In the affected individuals of 21-OH deficiency, the 8 bp deletion in exon 3 of P450c21B gene was found. Fig. 7 shows the capillary electrophoretic analysis of the 8 bp deletion in P450c21B gene. PCR is designed to produce a 135 bp DNA fragment for the wild-type, whereas a 127 bp fragment will be amplified by the PCR using the 8 bp deleted template of an affected individual. The electropherogram in Fig. 7 clearly illustrates that this individual would be affected. Mutation in *CFTR* results in the 3 bp deletion of *CFTR* in the case of the mutation, ΔF508. PCR amplification of the target

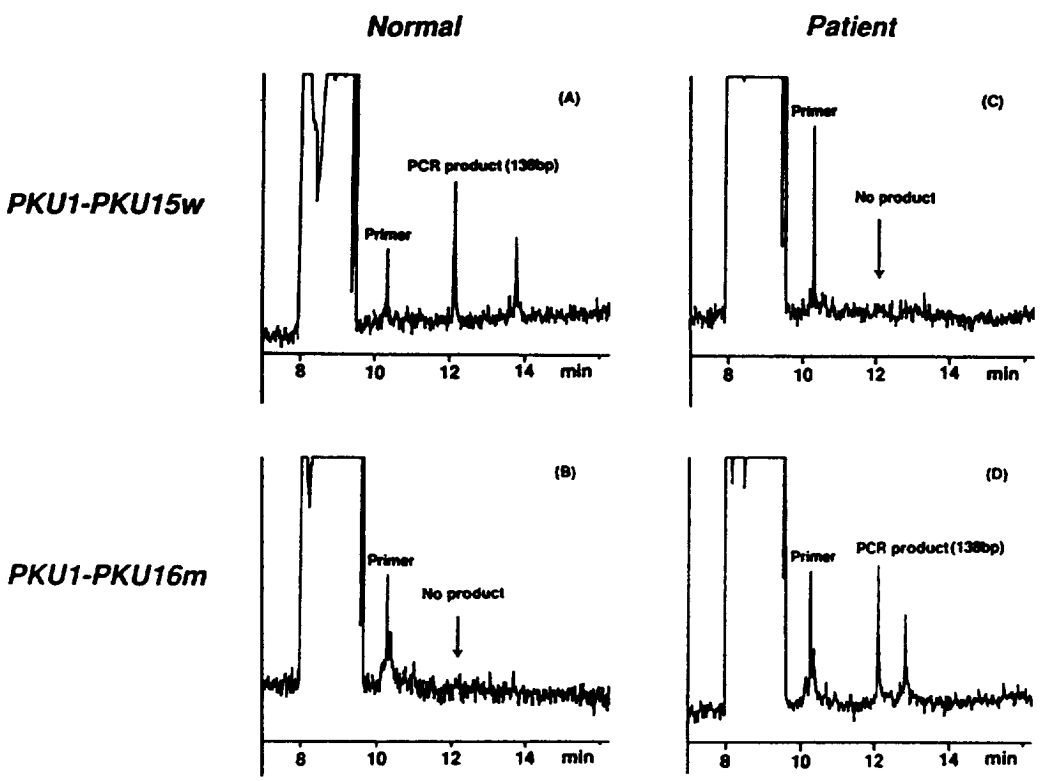

Fig. 8. Amplification refractory mutation system (ARMS) analysis of phenylalanine hydroxylase gene (*PAH*) by capillary electrophoresis with UV detector. Capillary: 3% T, 0.5% C polyacrylamide gel-filled, 100 μm I.D., 360 μm O.D., total length 50 cm, effective length 30 cm. Running buffer: 100 m*M* Tris-borate (pH 8.3). Field: 200 V/cm. Injection: 100 V/cm for 2 s. Temperature: 30°C. UV detection: 260 nm. (From Ref. [44] with permission).

region including the 3 bp deletion and the post-amplification analysis by capillary electrophoresis has been successfully applied to the accurate DNA diagnosis for cystic fibrosis [48,61].

Capillary electrophoresis has been successfully applied to the detection of point mutation for the medium-chain acyl-coenzyme A dehydrogenase gene (*MCAD*) by the PCR amplification of *MCAD* using allele specific primer [53] and the detection of point mutation of phenylalanine hydroxylase gene (*PAH*), which is the susceptibility gene of phenylketonuria (PKU) by the ARMS using two sets of allele specific primers [44]. Fig. 8 represents the ARMS analysis of *PAH* for the DNA diagnosis of PKU. Use of one allele specific primer, PKU15w, produces a 138 bp DNA fragment by the PCR using wild-type *PAH* as a template, but forms no PCR product using mutated *PAH* as a template. In contrast, the PCR product (138 bp) is amplified by using another allele specific primer, PKU16m, and mutated *PAH* as a template, but no product is formed from the wild-type *PAH*. Capillary electrophoresis, shows a sensitive detection of the PCR products and is successfully applied to the DNA diagnosis for PKU.

4.2. RFLP

PCR-RFLP is a powerful tool for the detection of known mutation and polymorphism of specific genes by the combination of appropriate design of a set of primers and the selection of a suitable restriction enzyme. Capillary electrophoresis has been successfully applied to the PCR-RFLP analysis for the DNA diagnosis of cancer [34,58], Alzheimer's disease [51,76,78], DMD [36], and cystic fibrosis [55]. Fig. 9 shows capillary electrophoretic separation of PCR-RFLP samples demonstrating *Mbo*I polymorphism of ERBB2 locus of human genome [34]. Digestion of the PCR product (1.1 kb) derived from the ERBB2 oncogene locus on chromosome 17q11 by *Mbo*I restriction enzyme yields a constant fragment of 550 bp and two polymorphic fragments of 520 and 500 bp, corresponding to alleles A1 and A2, respectively. Fig. 9 clearly illustrates that capillary electrophoretic analysis easily distinguishes three genotypes of homozygous A1/A1, homozygous A2/A2, and heterozygous A1/A2 by the comparison of the electrophoretic pattern of polymorphic fragments. A similar technique is applied to the detection of K-*ras*

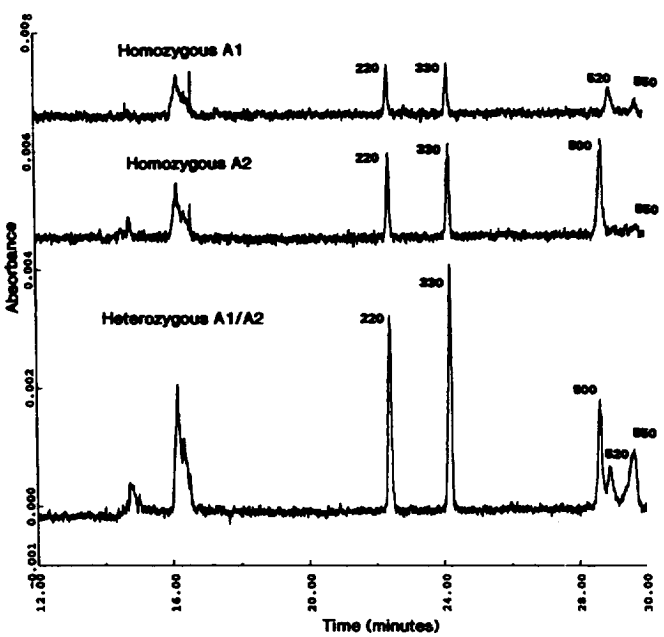

Fig. 9. PCR-RFLP analysis of ERBB2 locus by capillary electrophoresis with UV detector. Capillary: J&W DB-17, 100 μm I.D., 360 μm O.D., total length 57 cm, effective length 50 cm. Running buffer: 89 m*M* Tris-borate–2 m*M* EDTA (pH 8.5) including 0.5% hydroxypropylmethyl cellulose (HPMC). Field: 175 V/cm. Injection: 175 V/cm for 10 s. Temperature: 25°C. UV detection: 260 nm. (From Ref. [34] with permission).

oncogene codon 12 mutations by the PCR and subsequent digestion of PCR product by *Bst*NI restriction enzyme [58] for the DNA diagnosis of cancer.

More recently, apolipoprotein E gene (*APOE*) type 4 allele was proven to be a major risk factor for late-onset familial and sporadic Alzheimer's disease (age of onset after 60) and early-onset sporadic Alzheimer's disease (age of onset before 60). These important findings indicate that the *APOE* genotyping leads to DNA diagnosis for Alzheimer's disease. Common *APOE* polymorphisms are determined by type 2, 3, and 4 alleles. This polymorphism results in six *APOE* genotypes (E2/E2, E3/E3, E4/E4, E2/E3, E2/E4, and E3/E4). Risk for Alzheimer's disease increased from 20% to 90% with increasing number of *APOE* E4 alleles. The DNA samples for *APOE* genotyping was prepared by PCR amplification of *APOE* from human genomic DNA and subsequent digestion with *Hha*I restriction enzyme to yield constant fragments of 16, 18, 35 bp and four main polymorphic fragments of 48, 72, 83, and 91 bp, corresponding to alleles E2, E3, and E4. Fig. 10 illustrates the PCR-RFLP analysis of *APOE* by capillary electrophoresis with LIF detector [78]. The result demonstrates that *APOE* genotyping is achieved within only 10 min by the separation and sensitive detection of DNA fragments, that is, both 48- and 72-bp DNA fragments (homozygous E4/E4 genotype), both 48- and 91-bp fragments (homozygous E3/E3 genotype), and both 48-, 72-, and 91-bp fragments (heterozygous E3/E4 genotype). Other genotypes for *APOE* are determined in a similar manner [78]. Based on the genetic linkage study of *APOE*, PCR-RFLP analysis by capillary electrophoresis in Fig. 10 predicts that risk for Alzheimer's disease for the individual having E4/E4 genotype is about 90%, risk for the individual having E3/E3 genotype is 20%, and risk for the individual having E3/E4 genotype is 47%. Other RFLP studies on *APOE* genotyping have also been reported [51,76].

DNA diagnosis for DMD has been reported by the PCR amplification of DXS 164 locus and subsequent digestion by *Xmn*I restriction enzyme using capillary electrophoresis [36]. Simultaneous detection of four mutations on *CFTR* has been achieved by using multiplex PCR of target regions of four typical mutations on *CFTR*, including ΔF508, G542X, N1303K, and 1717-G→A mutations, and subsequent digestion of each PCR product by the specific restriction enzyme [55]. Capillary electrophoresis can separate and detect all resultant DNA fragments simultaneously within 40 min, owing to its high resolving power. This technique allows the accurate DNA diagnosis for cystic fibrosis through multiple detection of different mutations on *CFTR*.

4.3. SSCP

Capillary electrophoresis has been applied to SSCP analysis of *p53* tumor suppressor gene for DNA diagnosis of cancer [35]. A PCR-amplified DNA fragment of 372 bp was denatured into single-stranded DNA fragments and analyzed by capillary

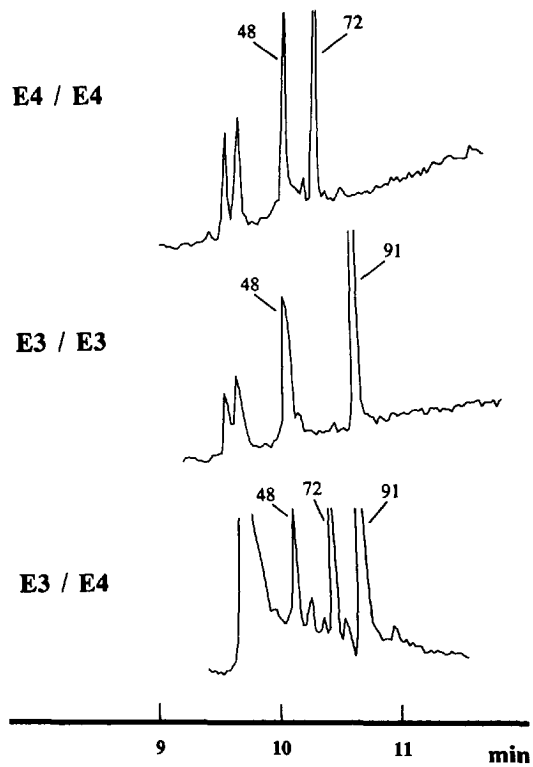

Fig. 10. PCR-RFLP analysis of apolipoprotein E gene (*APOE*) by capillary electrophoresis with LIF detector. Capillary: J&W DB-17, 100 μm I.D., 360 μm O.D., total length 56 cm, effective length 40 cm. Running buffer: 50 m*M* Tris-borate–2.5 m*M* EDTA (pH 8.3) including 0.7% methyl cellulose (MC). Field: 180 V/cm. Injection: 180 V/cm for 10 s. Temperature: 30°C. LIF detection: 488 nm (excitation), 540 nm (emission). (From Ref. [78] with permission).

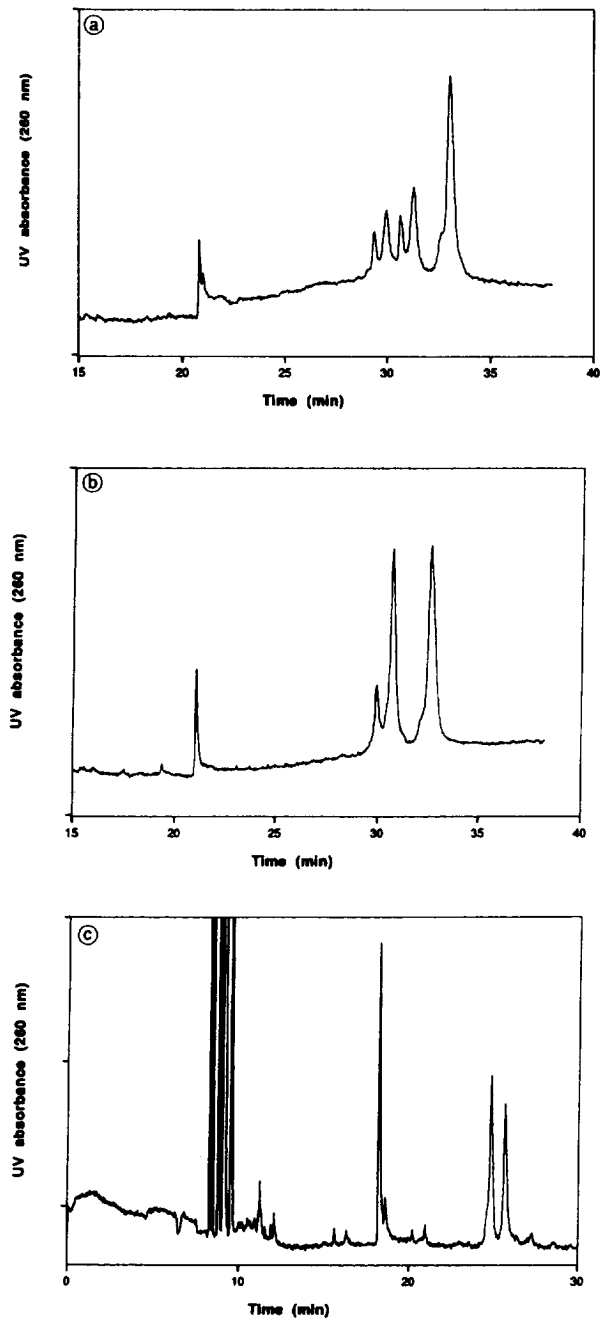

Fig. 11. SSCP analysis of *p53* tumor suppressor gene by capillary electrophoresis with UV detector. (a) Patient 1, (b) patient 2, (c) normal person. Capillary: 75 μm I.D., 360 μm O.D., total length 36 cm, effective length 31 cm. Capillary is filled with 4% linear polyacrylamide. Running buffer: 40 m*M* Tris-acetate–2 m*M* EDTA (pH 8.3). Field: 250 V/cm. Injection: 167 V/cm for 10 or 20 s. Temperature: 25°C. UV detection: 260 nm. (From Ref. [35] with permission).

electrophoresis using 4% linear polyacrylamide as a separation matrix. Fig. 11c shows the electropherogram of the single-stranded PCR product of normal white blood cells after denaturation. Since the complementary DNA sequence of the two single-stranded fragments have their own conformation, each single-stranded DNA fragment migrates into the capillary at the different mobilities and leads to two different peaks as shown in Fig. 11c. Fig. 11a,b show the separation of the PCR products of multiple myeloma patients 1 and 2 known to have a point mutation in the *p53* tumor suppressor gene. In these instances, peaks corresponding single-stranded DNA fragments show quite a difference in their mobility compared with those of wild-type *p53* tumor suppressor gene shown in Fig. 11c. Additionally, more than three peaks appeared in these cases, because single-stranded DNA fragments amplified from mutated *p53* gene have some metastable conformers as well as the most stable conformers. Comparison of SSCP analyses of Fig. 11c and Fig. 11a,b gives important information on the mutation of *p53* tumor suppressor gene and realizes the genetic screening for cancer.

A two-dye LIF detection system has been developed for the accurate analysis of SSCP using capillary electrophoresis and the separation conditions were extensively investigated for the optimization of the conditions for highly sensitive SSCP analysis [65]. SSCP analysis using slab gel electro-

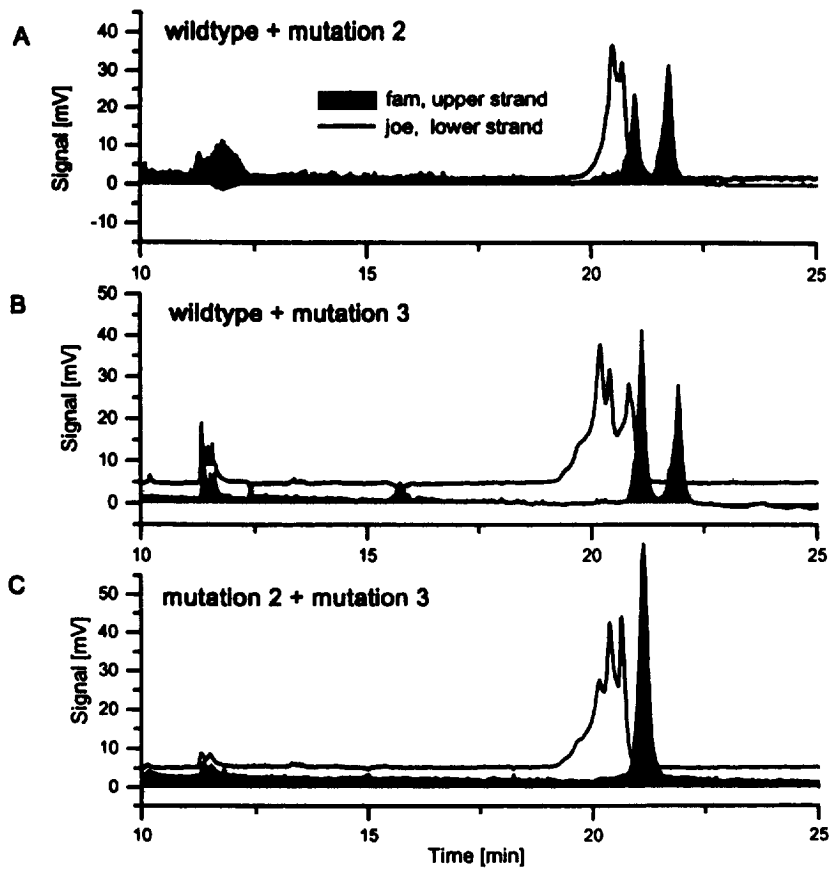

Fig. 12. SSCP analysis of insulin-like growth factor 1-binding protein 3 gene by capillary electrophoresis with LIF detector. Capillary: linear polyacrylamide coated, 100 μm I.D., 360 μm O.D., total length 20 cm, effective length 14 cm. Capillary is filled with 7% linear polyacrylamide. Running buffer: 89 m*M* Tris-borate–2 m*M* EDTA (pH 8.3). Field: 200 V/cm. Injection: 200 V/cm for 10 s. Temperature: 30°C. LIF detection: 488 nm (excitation), 520 nm (emission for FAM-labeled fragment) and 560 nm (emission for JOE-labeled fragment). (From Ref. [65] with permission).

phoresis usually requires the addition of 5–10% glycerol to the non-denaturing polyacrylamide gel and the gel maintenance at low temperature (5–10°C) for highly sensitive analysis of polymorphism and mutation [89]. Fig. 12 demonstrates that SSCP of a specific gene is detectable with high sensitivity by capillary electrophoresis using 7% linear polyacrylamide as a separation matrix without glycerol even at relative high temperatures (30°C). Similar results have been reported on the SSCP analysis for *divE* 42 gene, of which a mutant is clearly distinguished from its wild-type by capillary electrophoresis using 8% linear polyacrylamide without glycerol at 25°C [75].

Different fluorescent dyes are used for the labeling of both single-stranded fragments, FAM-labeled upper strands and JOE-labeled lower strands for wild-type and mutant [65], and use of two-dye labeling is demonstrated to give valuable information on the polymorphism analysis of the genes and genetic screening of mutated genes as illustrated in Fig. 12.

SSCP analysis using capillary electrophoresis has also been successfully applied to the detection of the mutation on Ki-*ras* oncogene [77], *Mycobacterium tuberculosis*-specific amplified DNA fragment [70], and *Heterobasidion annosum*-specific amplified DNA fragment [69].

4.4. VNTR

Human apolipoprotein B gene (*APOB*) maps to the short arm of chromosome 2, spans 42 kbp, and contains VNTR located immediately downstream from the *APOB*. The *APOB* VNTR alleles generally contain 25–52 repeats of the basic 16-bp unit. These alleles differ with respect to the number of 16-bp repeat units. Genetic linkage study of the *APOB* locus concludes that larger alleles containing more than 38 repeat units are more common in myocardial infarction patients and show an significant association with coronary heart disease. These alleles were also associated with elevated serum levels of total cholesterol and *APOB* among patients and with elevated serum levels of total triglycerides among controls.

Capillary electrophoresis has been applied to the analysis of a PCR-amplified *APOB* VNTR locus [38,39,66]. Analysis of the *APOB* PCR-amplified DNA fragments from three different individuals is shown in Fig. 13 [66]. Using the capillary electrophoresis technique all three allelic variations could be distinguished within only 17 min. All individuals were proven to have heterozygous alleles, because two bands are observed for PCR-amplification products from each individual. In DNA typing applications it is important to differentiate heterozygous from homozygous individuals. The capillary electrophoresis technique is demonstrated to be quite useful for this purpose, due to its high resolving power.

Based on their relative migration with respect to DNA size standards as shown in Figs. 3 and 4, the observed numbers of repeat units for all alleles are estimated and labeled on the peaks, and identical to the values estimated by the conventional method. The reproducibility of the migration time was in the range of 2–3% relative standard deviation (R.S.D.) (n=3). The products from one individual shown in the top electropherogram has two bands four repeat units (64 bp) apart. Other electropherograms also show heterozygous alleles containing both 35 and 47

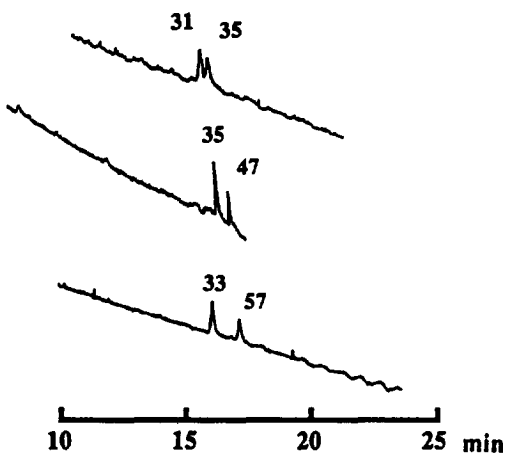

Fig. 13. Capillary electrophoresis separations of PCR-amplified DNA fragments for the *APOB* VNTR alleles of three different heterozygous individuals; both 31 (595 bp) and 35 (659 bp) repeat units (top), both 35 (659 bp) and 47 (851 bp) repeat units (middle), and both 33 (627 bp) and 57 (1011 bp) repeat units (bottom). Capillary: J&W DB-17, 100 μm I.D., 360 μm O.D., total length 50 cm, effective length 30 cm. Running buffer: 50 m*M* Tris-borate–2.5 m*M* EDTA (pH 8.3) including 0.7% methyl cellulose (MC). Field: 200 V/cm. Injection: 200 V/cm for 10 s. Temperature: 30°C. UV detection: 260 nm. (From Ref. [66] with permission).

repeat units (middle) and both 33 and 57 repeat units (bottom), respectively. Thus, the individuals shown in the middle and the bottom electropherogram have a risk factor for coronary heart disease, but a individual shown in the top electropherogram does not have a risk factor. The larger fragment in the top electropherogram is the same size as the smaller fragment from amplification products of another individual shown in the middle electropherogram. It is noteworthy that all smaller DNA fragments (31, 33, and 35 repeat units), which differ by two repeat units from each other, are resolved completely. This result illustrates that the *APOB* VNTR alleles differing by only one repeat unit are distinguishable from one another by using the capillary electrophoresis technique. The conventional analysis distinguishes alleles that differ by two or more repeat units and does not distinguish alleles if they differ by one unit. PCR amplification of D1S80 VNTR locus and the post-amplification analysis by capillary electrophoresis has been reported [38,39] and shows the complete resolution of the allelic variation of this locus and accurate determination of the number of repeat units (15 or 16 bp) with high speed.

4.5. Microsatellite analysis

Microsatellite or short tandem repeat instability has been shown to be relevant to various human diseases, including cancers. An increase or decrease in the number of $(CA)_n$ repeat units between lymphocyte and tumor DNA from the same patient was found. The D2S123 microsatellite loci [$(CA)_n$ repeat] of colorectal cancer patients were amplified by PCR and analyzed by capillary electrophoresis as shown in Fig. 14 [59]. The electrophoretic profile of the amplified products was compared between tumor and normal DNA from the same patient. In case A, the DNA fragment of the PCR product amplified from the tumor cell is shorter than that of the PCR product amplified from the normal cell, because the mobility of tumor DNA is larger than that of normal DNA. In case B, the larger DNA fragment shown in the PCR product for the normal cell has disappeared in the PCR products for the tumor cell, whereas other PCR products are almost identical for both cells. These are caused by the decrease in $(CA)_n$ repeat units in the D2S123 locus for cases A and B.

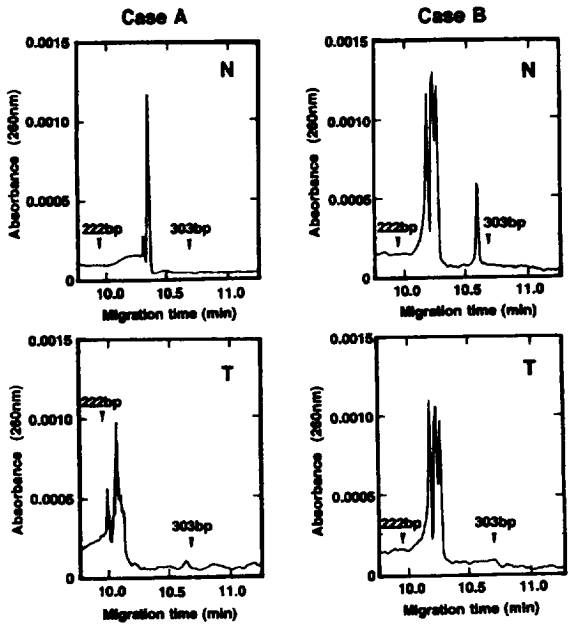

Fig. 14. Microsatellite instability analysis of $(CA)_n$ repeat of D2S123 locus by capillary electrophoresis with UV detector. (N) Normal cell, (T) tumor cell. Capillary: linear polyacrylamide coated, 100 μm I.D., 360 μm O.D. total length 36 cm, effective length 30 cm. Running buffer: PCR product analysis buffer (Bio-Rad). Field: 222 V/cm. Injection: 414 kPa for 1 s. Temperature: 30°C. UV detection: 260 nm. (From Ref. [59] with permission).

The results as shown in Fig. 14 illustrate that capillary electrophoresis will become a powerful tool for rapid DNA diagnosis of the cancer through the analysis of genomic instability of specific microsatellite loci in the human genome.

Some microsatellite repeats can undergo an increase in copy number by a process of dynamic mutations. The list of diseases associated with dynamic mutations due to repeat expansion is growing [92,93]. Several triplet expansions at different loci have been associated with a specific disease; fragile X syndrome, myotonic dystrophy, spinoulbar muscular atrophy, Huntington disease, spinocerebellar ataxia type 1, Kennedy disease, and so on. The CAG repeat expansion leads to some of these diseases, whereas the X syndrome and myotonic dystrophy are caused by the CGG/CCG expansion and the CTG/CAG expansion, respectively.

Capillary electrophoresis has been successfully applied to the analysis of triplet repeat expansion and DNA diagnosis for Kennedy disease [43,64]. The high resolving power of capillary electrophoresis allows the separation of the heterozygous microsatellite bands differing by only one CAG triplet (136 vs. 139 bp) within only 22 min and accurate diagnosis for Kennedy disease. Such separation is more difficult and could only be performed by using gradient gels and long running times using slab gel electrophoresis. Cystic fibrosis, of which the most common mutation is a 3-bp detection, ΔF508, is associated with the polymorphism for some microsatellite repeats located within several introns for the *CFTR*. Of these, the GATT polymorphism, at the junction of intron 6a and exon 6b, was amplified by PCR and analyzed by capillary electrophoresis [42]. The six-repeat allele has been reported to be strongly associated with the ΔF508 mutation on *CFTR*. Capillary electrophoresis using 6% linear polyacrylamide as a separation matrix completely resolved two heterozygous bands of the hexameric (111 bp) fragment and heptameric (115 bp) fragment within 30 min. In the particular case of cystic fibrosis, the analysis of microsatellite repeat by capillary electrophoresis results in DNA diagnosis for cysteic fibrosis with high-speed and high-accuracy.

Microsatellite marker is an important tool for the highly polymorphic marker for Down's syndrome, too [71]. Free trisomy 21 accounts for about 95% of all cases of Down's syndrome. A highly polymorphic (90% heterozygosity) microsatellite marker, chromosome 21-specific D21S11 locus, was amplified, analyzed by capillary electrophoresis as shown in Fig. 15, and applied to pre-natal DNA diagnosis for Down's syndrome. In the analysis of this microsatellite marker, trisomic individuals are expected to fall into two major groups: those with three bands of similar intensities or those with two bands with a ratio of 2:1. Fig. 15 clearly demonstrates that the PCR product from the affected individual induced three bands with a characteristic 1:1:1 ratio and is a case we have to evaluate for a pregnancy at risk. Capillary electrophoresis, therefore, is an easy and efficient screening tool in both post- and pre-natal diagnosis of Down's syndrome.

Microsatellite markers have shown sufficient variability among individuals to warrant application to forensic DNA typing [37,40,56,60,63]. Fig. 16 shows the high-speed separation of microsatellite marker [$(AATC)_n$ repeat] at the human tyrosine hydroxylase (HUMTHO1) locus and DNA typing using amplified-microsatellite repeat of HUMTHO1 [63]. In this instance, a dual internal standard (150 and 300 bp) approach is applied to the accurate DNA typing. In the analysis of the seven HUMTHO1 alleles, a correlation coefficient of 0.9999 between migration time and DNA size was obtained with a standard deviation of 0.3 bp about the regression line. In a three-day period, almost 100 samples containing PCR-amplified HUMTHO1 alleles could to be analyzed, owing to high-speed separation using capillary electrophoresis within 10.5 min per analysis. The capillary electrophoretic technique is applied to some population studies by the analysis of HUMTHO1 allele frequencies. The results are in good agreement with previously observed allele frequencies determined by slab gel electrophoresis [63]. The two dye approach, in which the microsatellite marker is labeled by one dye and the specific HUMTHO1

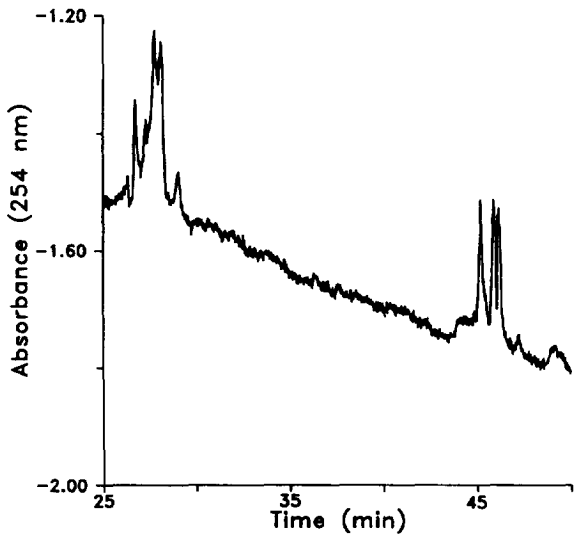

Fig. 15. Pre-natal diagnosis of Down's syndrome through PCR amplification of microsatellite marker by capillary electrophoresis with UV detector. Capillary: linear polyacrylamide coated, 75 μm I.D., 360 μm O.D., total length 37 cm, effective length 30 cm. Capillary is filled with 8% linear polyacrylamide. Running buffer: 89 m*M* Tris-borate–2 m*M* EDTA (pH 8.3). Field: 100 V/cm. Injection: 100 V/cm for 15–20 s. Temperature: 25°C. UV detection: 254 nm. (From Ref. [71] with permission).

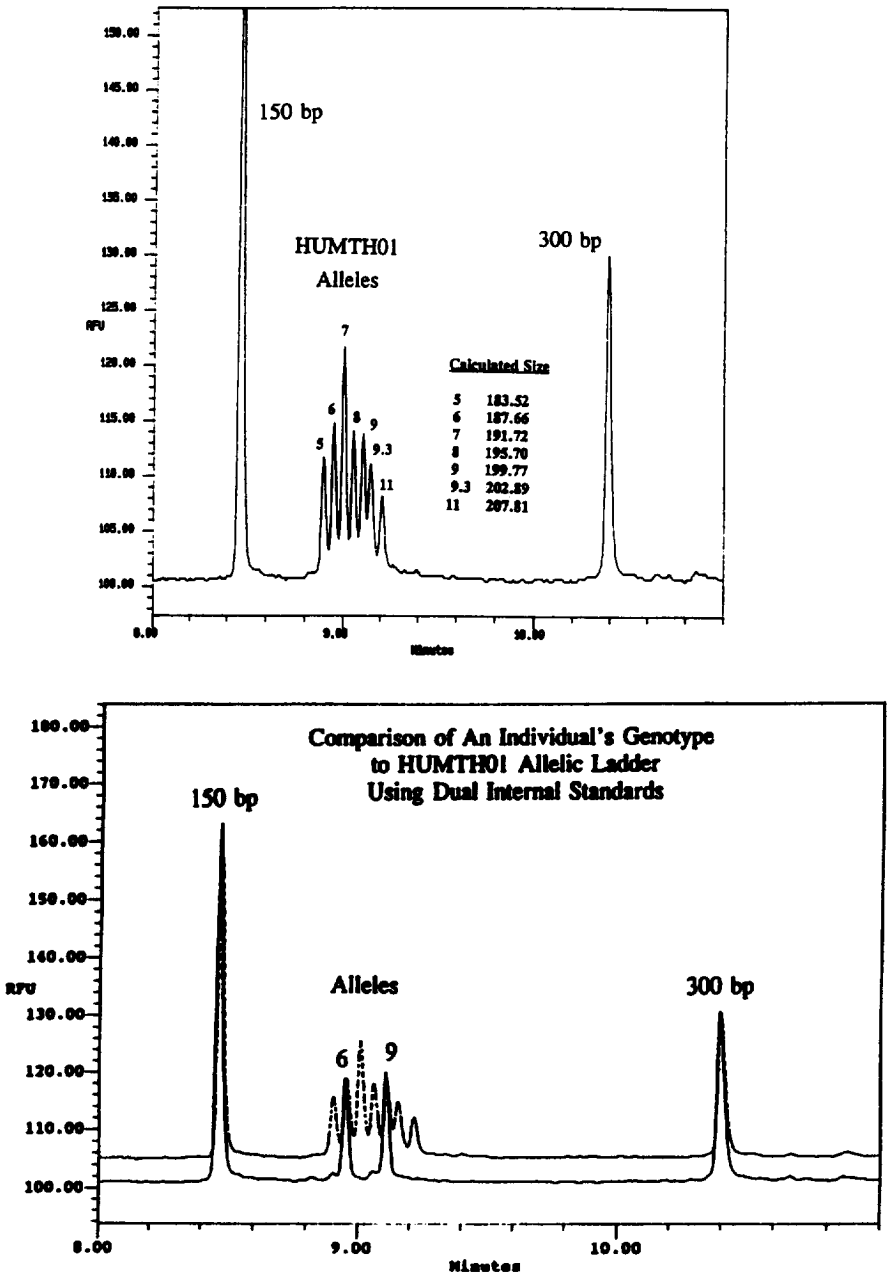

Fig. 16. Forensic DNA typing through microsatellite analysis of HUMTHO1 locus by capillary electrophoresis with LIF detector. Capillary: J&W DB-17, 50 μm I.D., 360 μm O.D., total length 27 cm, effective length 20 cm. Running buffer: 100 m*M* Tris-borate–2.5 m*M* EDTA (pH 8.2) including 1% hydroxyethyl cellulose (HEC). Field: 185 V/cm. Injection: 37 V/cm for 5 s. Temperature: 25°C. LIF detection: 488 nm (excitation), 520 nm (emission). (From Ref. [63] with permission).

allele amplified by PCR is labeled by another dye, is also demonstrated to be powerful for the accurate determination of HUMTHO1 fragment size [60] as well as the dual internal standard approach.

4.6. Hybridization technique

Hybridization is another powerful technique for the mutation detection of human disease-causing genes [41,46,49,52,67]. Capillary electrophoresis is applied to the detection of hybridization between synthetic oligonucleotides and HIV-1 genomic DNA amplified by PCR [41,46]. The PCR amplification of HIV-1 genome by unbalanced PCR using the primers SK145 and SK39 (10:1) produced both single-stranded and double-stranded DNA fragments as shown in Fig. 17A. Fig. 17B shows the analysis of the sample by the treatment of the sample as shown in Fig. 17A with proteinase K for the complete deactivation of DNA polymerase used for PCR and subsequent hybridization of the SK150 oligonucleotide probe, which is complementary to the SK145–SK39 *gag* region of the HIV-1 genome. The comparison of Fig. 17A,B clearly demonstrates that the hybridization of the SK150 probe to the single-stranded PCR product induces the drastic shift of the single-stranded SK145/SK39 PCR product. The results suggest that the capillary electrophoretic technique can be used to identify HIV-1 PCR products and to demonstrate the specificity of the amplification reaction, on the basis of a shift in the electrokinetic mobility by a synthetic oligonucleotide complementary to HIV-1 single-stranded DNA obtained by PCR. Similar technologies using capillary electrophoresis have been successfully applied to the detection of HCV [49], *Heterobasidion annosum* genotypes [52], and the mutation on the *p53* tumor suppressor gene [67].

In DGGE, which is an efficient technology for the detection of point mutation on the genes, the mobility of a partially melted DNA on the slab gel is reduced compared to an unmelted DNA molecule. A variant of DGGE, termed constant denaturant gel electrophoresis (CDCE) [50] and temperature gradient capillary electrophoresis (TGCE) [54] have recently been developed. In the case of CDCE as shown in Fig. 18, capillary electrophoresis was performed in capillaries filled with 6% linear polyacrylamide and a denaturant of both 3.3 *M* urea and 20% formamide). In a 10 cm portion of the capillary, which is the "denaturing zone", the temperature was elevated; in the rest of the capillary, ambient temperature conditions existed. Fig. 18 demonstrates the critical role of temperature in CDCE. Homoduplexes differed by a single base pair substitution (GC and AT) and heteroduplexes (GT and AC) obtaining from human mitochondria could be separated by the tuning the temperature. With an increase in temperature, the duplexes started to separate in the order of their melting stability, whereas all the duplexes were

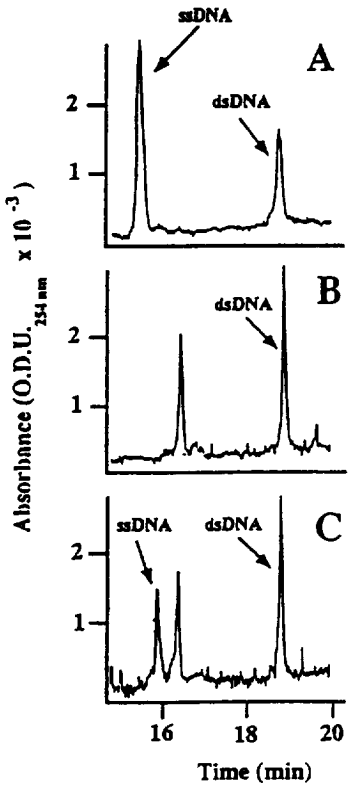

Fig. 17. Detection of hybridization between oligonucleotide probe and PCR-amplified HIV-1 genomic DNA by capillary electrophoresis with UV detector. (A) Unbalanced SK145/SK39 PCR products, (B) unbalanced SK145/SK39 PCR products treated for 15 min with proteinase K and further annealed with the SK150 probe, (C) a mixture of (A) and (B). Capillary: coated, 100 μm I.D., 360 μm O.D., total length 47 cm, effective length 40 cm. Running buffer: 100 m*M* Tris-borate (pH 8.3) including 0.5% hydroxypropylmethyl cellulose (HPMC). Field: 255 V/cm. Injection: 85 V/cm for 20 s. Temperature: 20°C. UV detection: 254 nm. (From Ref. [46] with permission).

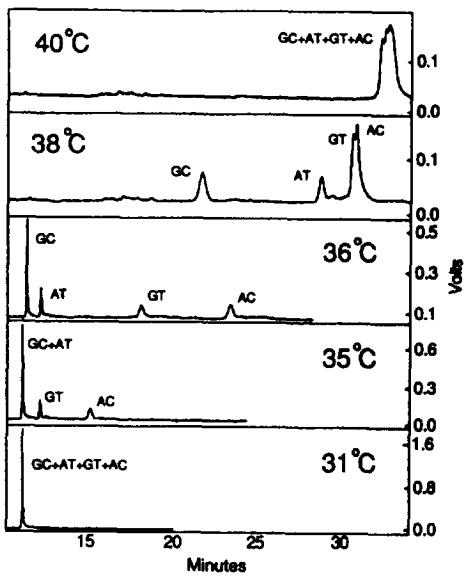

Fig. 18. Constant denaturant capillary electrophoretic separation. Capillary: linear polyacrylamide coated, 75 μm I.D., 360 μm O.D. Capillary is filled with 6% linear polyacrylamide. Running buffer: 89 m*M* Tris-borate–2 m*M* EDTA, 3.3 *M* urea, and 20% (v/v) formamide (pH 8.3). Field: 250 V/cm. LIF detection: 488 nm (excitation), 520 nm (emission). (From Ref. [50] with permission).

in the unmelted form at 31°C. CDCE, though useful for the identification of inherited mutations and polymorphisms, should be particularly suitable for detection of low fraction somatic mutants, known and unknown at specific DNA loci and for screening of tissues for mutant cancer cells. TGCE has been also reported as a powerful tool for the detection of point mutations [54]. The method is successfully applied to the identification of three point mutations located in exon 17b and of two polymorphisms located in exon 14a of the *CFTR*.

5. Analysis of DNA-based drugs

5.1. Gene therapy and DNA-based drugs

Recent progress in the identification of disease-causing genes in the Human Genome Project leads to realization of gene therapy for human diseases and opens up the era of the gene as the drug. For example, the first gene therapy trial for cystic fibrosis was initiated just 3.5 years after the discovery of the *CFTR*. In the gene therapy, the specific human genes, human disease-causing genes, and their cDNAs have been usually used as the drugs as listed in Table 8. Therefore, the drugs for gene therapy can be easily separated and detected by capillary electrophoresis as described in the previous section. There are some other DNA-based drugs, including DNA vaccine, antisense DNA, triplex-forming oligonucleotides, protein-binding oligonucleotides, and ribozyme. Of these, only antisense DNA has been analyzed by capillary electrophoresis. Therefore, this section is focused on the technology and the methodology for the analysis of antisense DNA.

Table 8
Gene therapy for human disease

Disease	Gene
ADA[a] defiency	ADA
FH[b]	LDL[c] receptor
CF[d]	*CFTR*[e]
Melanoma	TNF[f]
Melanoma	IL-2[g]
Neuroblastoma	IL-2
Breast cancer	IL-2
Liver metastasis from colon	*p53*
Non-small cell lung cancer	*p53*
AIDS[h]	HIV[i] ribozyme

[a] ADA, aenosine deaminase.
[b] FH, familial hypercholesterolemia.
[c] LDL, low density lipoprotein.
[d] CF, cystic fibrosis.
[e] CFTR, cystic fibosis transmembrane conductance regulator gene.
[f] TNF, tumor necrosis factor.
[g] IL-2, interleukin-2.
[h] AIDS, acquired immunodeficiency syndrome.
[i] HIV, human immunodeficiency virus.

The terms "oligonucleotides" and "antisense" are cropping up with increasing frequency in mainstream discussions of therapeutic drug development as listed in Table 9 [112]. Along with gene therapy, the concept of using oligonucleotide analogues to block gene expression or to modify a nucleotide sequence has moved from the realm of science fiction to the reality of clinical trials. Preclinical animal studies have begun to demonstrate proof-of-principle of a true antisense mechanism, and more than a handful of companies currently have oligonucleotide therapeutics in the clinic. Several more drug candidates will likely enter the clinic in 1996. Traditionally, the concept of therapeutic oligonucleotides has been

Table 9
Oligonucleotide-based drugs under development [112]

Drugs	Target	Mechanism	Clinical trial
AR132[a]	CMV[i]	Antisense	Preclinical
GPs0193[b]	HIV[j]	Antisense	Phase I
Anticode[c]	cancer	Antisense	Phase I/II
GS504[d]	CMV[i]	Antisense	Phase III
GS840[d]	HIV[j]	Antisense	Phase I/II
GS522[d]	Thrombin	Antisense	Preclinical
GEM91[e]	HIV[j]	Antisense	Phase I/II
GEM92[e]	HIV[j]	Antisense	IND in 1996
GEM93[e]	HIV[j]	Antisense	Oral formulation
ISIS 2922[f]	CMV[i]	Antisense	Phase III
ISIS 2105[f]	HPV[k]	Antisense	Phase II
ISIS 2302[f]	ICAM-1[l]	Antisense	Phase II
ISIS 3521[f]	Protein kinase C	Antisense	Phase I
ISIS 5132[f]	c-raf Kinase	Antisense	IND by March 1996
GMO[g]	Herditary disease	Triplex	Research stage
Code Marker[h]	cancer	Triplex	Research stage
AR177[a]	HIV[j]	Protein binding	Phase I

[a] Aronex Pharmaceuticals.
[b] Chugai Biopharmaceuticals.
[c] Genta.
[d] Gilead Sciences.
[e] Hybridon.
[f] Isis Pharmaceuticals.
[g] Epoch Pharmaceuticals.
[h] OncorPharm.
[i] CMV, cytomegalovirs.
[j] HIV, human immunodeficiency virus.
[k] HPV, human papilloma virus.
[l] ICAM-1, intercellular adhesion molecule 1.

synonymous with antisense, i.e., synthetic oligomers that bind a target mRNA, either triggering its destruction or blocking its translation into a peptide. The field has broadened, however, and now includes the use of single-strand oligonucleotides that from triple helices to block gene expression at the level of transcription or to stimulate site-specific repair of genetic mutations. First generation oligonucleotides may become a commercial reality by the year 2000, but the true potential of this technology will likely not be realized until second- and third generation products come to market in the next century. Companies are still grappling with issues of drug delivery, nuclease resistance, specificity and high-affinity binding.

5.2. Monitoring of antisense DNA

Capillary electrophoresis using linear polyacrylamide as a separation matrix has been mainly used as an analytical technique for antisense DNA oligomer [80–86]. Among several DNA analogues proposed as antisense DNA drugs [13], antisense DNA, in which the backbone is modified into phosphorothioate, is the most promising for therapeutic application. The highest resolutions for antisense DNA, greater than 20 bases, were obtained using capillary electrophoresis compared with reversed-phase and ion-exchange HPLC, and slab gel electrophoresis [80]. Capillary gel electrophoresis has also been successfully applied to high resolution of antisense DNA with different lengths [81]. Micellar electrokinetic chromatography was applied to resolve diasteromers of oligonucleotides possessing several chiral phosphoramidate bridges [79].

One of the antisense based drugs under clinical trial, ISIS 2922, was analyzed by capillary gel electrophoresis as shown in Fig. 19 [83]. Capillary gel electrophoresis is primarily a length-based separation yielding excellent resolution of the deletion

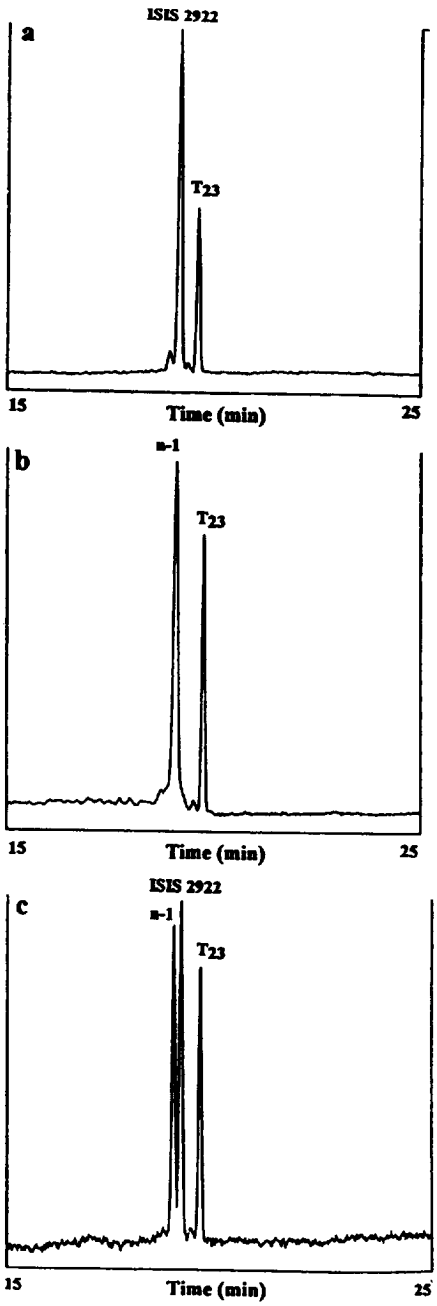

Fig. 19. Analysis of antisense DNA ISIS 2922 and its $n-1$ deletion sequence in addition to phosphorothioate thymidine oligonucleotide 23mer (T_{23}) by capillary electrophoresis. Capillary: 75 μm I.D., 360 μm O.D., total length 47 cm, effective length 40 cm. Capillary is filled with polyacrylamide gel (U100P, Beckman). Running buffer: Tris-borate–urea. Field: 300 V/cm. Injection: 149 V/cm for 20–30 s. Temperature: 30°C. UV detection: 254 nm. (From Ref. [83] with permission).

sequences of ISIS 2922 with high reproducibility. The R.S.D. of migration time for ISIS 2922 is 1.39% and that for the relative migration time to the internal standard, T_{23}, is 0.06%. However, "thiation failures" (mono or higher order partial phosphodiesters) are not separable by capillary electrophoresis.

Another antisense DNA under clinical trial, GEM, is analyzed by capillary electrophoresis based on the hybridization between GEM and its target DNA, COM, within the capillary as shown in Fig. 20 [82]. Three different mixtures (1,2,3) of GEM and COM were injected under non-denaturing conditions. Three peaks are observed and identified to COM, duplex, and GEM as shown in Fig. 20. Since hybridization is an equilibrium process, increase in duplex peak area is well correlated with the increase

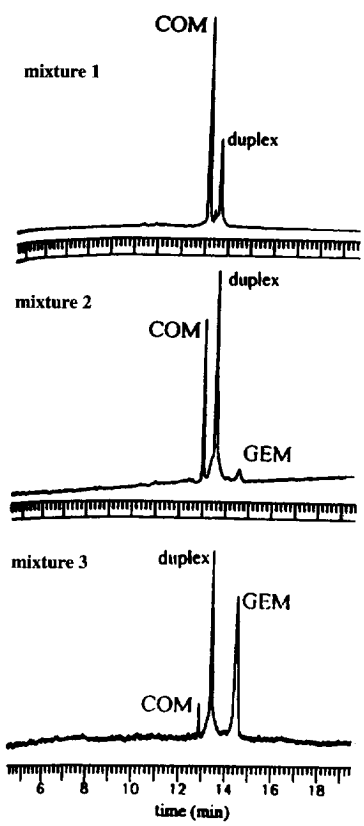

Fig. 20. Analysis of antisense DNA, GEM, its complementary sequence, COM, and duplex by capillary electrophoresis. Capillary: 75 μm I.D., 375 μm O.D., total length 60 cm, effective length 20 cm. Capillary is filled with 9% linear polyacrylamide. Running buffer: 0.1 *M* Tris-borate–2 m*M* EDTA and 7 *M* urea (pH 8.3). Field: 200 V/cm. Temperature: room. UV detection: 270 nm. (From Ref. [82] with permission).

in GEM concentration at a constant COM content in the injected sample mixture. GEM was completely hybridized in excess of COM (mixture 1) and therefore not observed in Fig. 20. The peak height for the duplex increases with an increase in the GEM concentration, while the COM peak height decreases as shown in Fig. 20. The calibration curve obtained by the hybridization technique has wide range of linearity in the range of GEM concentration from 10 to 1300 ppb. The technique, therefore, becomes a powerful tool for the analysis and quantification of antisense DNA to modulate HIV gene expression in vitro to support clinical studies.

Stability measurement of antisense DNA for en-

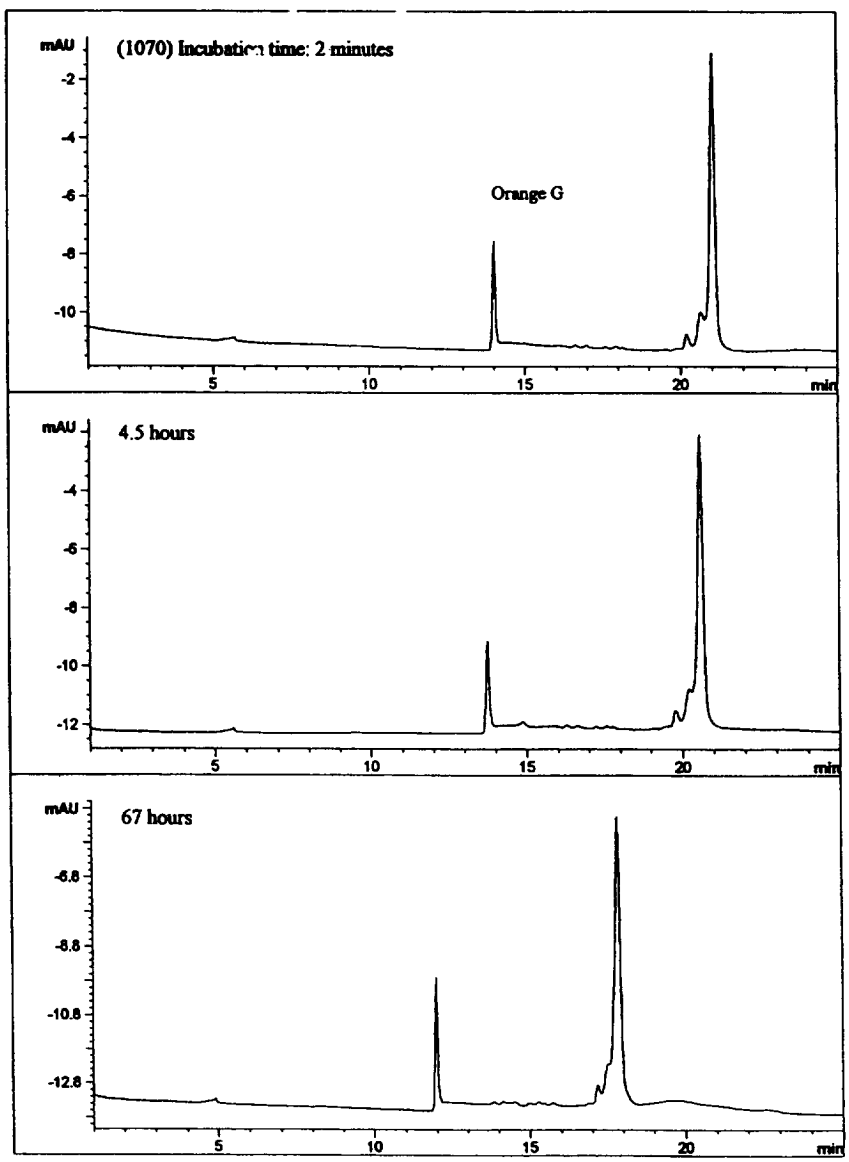

Fig. 21. Analysis of antisense DNA after various incubation times with snake venom nuelease by capillary electrophoresis. Capillary: 75 μm I.D., 37 μm O.D., total length 32 cm, effective length 24 cm. Capillary is filled with 10% linear polyacrylamide. Running buffer: 0.1 *M* Tris-borate (pH 8.5). Field: 313 V/cm. Injection: 156 V/cm for 5 s. Temperature: 30°C. UV detection: 260 nm (From Ref. [86] with permission).

zymatic degradation has been realized by capillary electrophoresis as shown in Fig. 21 [86]. Antisense DNA was treated with snake venom nuclease and its enzymatic degradation was monitored by capillary electrophoresis at the different incubation times. The antisense DNA was not changed in the course of the reaction as shown in Fig. 21, whereas natural oligonucleotides were quickly degraded over several hours by various nucleases. Capillary electrophoresis is also applicable to the analysis of antisense DNA in biological fluids, including serum, plasma, and urine [84]. The capillary electrophoretic technology will be useful for the therapeutic drug monitoring analysis of antisense DNA in the clinical trials. The sequencing of antisense DNA is achieved by capillary electrophoresis with laser-induced fluorescence detection [85].

6. Future trends

Many successful applications of capillary electrophoresis as described in this article undoubtedly demonstrate that the capillary electrophoretic technologies are the promising technology for DNA diagnosis for human diseases and quality control, pharmacokinetic analysis, and therapeutic drug monitoring of DNA-based drugs, including antisense DNA and several genetic drugs for gene therapy. Nowadays, the total throughput of capillary electrophoresis is almost comparable or less than that of slab gel electrophoresis. However, the use of capillary array electrophoresis [26,28,113–115] instead of capillary electrophoresis results in the drastic improvement in the total throughput of capillary electrophoretic technology, that is, capillary array electrophoresis equipped with 100 capillaries analyzes 100 DNA samples in less than 10 min. Additionally, development of microfabricated capillary array electrophoresis chips [87,88] will lead to a further increase in the separation lanes and order-of-magnitude improvement in the speed and throughput.

7. Note added in proof

After this manuscript was accepted I became aware of an article which has appeared and which will be briefly mentioned here. This paper deals with the separation of antisense DNA by capillary electrophoresis in comparison with those by slab gel electrophoresis and HPLC. The separation of antisense oligonucleotide was performed in 18% polymer solution. The results demonstrate the baseline resolution down to fragments as small as 7mer.

C. Gelfi, M. Perego, S. Morelli, A. Nicolin and P.G. Righetti, Antisense Nucl. Acid Drug Develop., 6 (1996) 47–53.

8. List of abbreviations

ACE	angiotensin conversion enzyme gene
AD	Alzheimer's disease gene
AIDS	acquired immunodeficiency syndrome
APOB	apolipoprotein B gene
APOE	apolipoprotein E gene
AR	androgen receptor gene
ARMS	amplification refractory mutation system
bEGF	basic fibroblast growth factor gene
bp	base pair(s)
BRCA	breast cancer gene
cDNA	complementary DNA
CDCE	constant denaturant capillary electrophoresis
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator gene
cM	centimorgan
DGGE	denaturing gradient gel electrophoresis
DMD	Duchenne muscular dystrophy
EST	expressed sequence tag
HCV	hepatitis C virus
HD	Huntington disease gene
HEC	hydroxyethyl cellulose
HIV	human immunodeficiency virus
hMLH	human mutL homolog gene
HPMC	hydroxypropylmethyl cellulose
HUMTHO1	human tyrosine hydroxylase
IDDM	insulin dependent diabetes mellitus gene
IGF1-BP3	insulin-like growth factor 1-binding protein 3 gene
kb	kilo bases

LIF	laser-induced fluorescence
LPAA	linear polyacrylamide
Mb	mega bases
MC	methyl cellulose
MCAD	medium-chain acyl-coenzyme A dehydrogenase gene
MD	muscular dystrophy gene
NQO1	NAD(P)H:quinone acceptor oxidoreductase gene
OLA	oligonucleotide ligation assay
PAAEE	poly(acryloylaminoethoxyethanol)
PAG	polyacrylamide gel
PAH	phenylalanine hydroxylase gene
PAIS	partial androgen insensitivity syndrome
PCR	polymerase chain reaction
PEG	poly(ethylene glycol)
PEO	poly(ethylene oxide)
PKU	phenylketonuria
RB	retinoblastoma gene
RFLP	restriction fragment length polymorphism
RT-PCR	reverse transcription-polymerase chain reaction
SSCP	single strand conformation polymorphism
STS	sequence-tagged site
TGCE	temperature gradient capillary electrophoresis
VDR	vitamin D receptor gene
VNTR	variable number of tandem repeat
21-OH	21-hydroxylase

Acknowledgments

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