8.1 INTRODUCTION

Temperature gradient gel electrophoresis (TGGE) and the related method denaturing-gradient gel electrophoresis (DGGE) are both based on the principle that the electrophoretic mobility of double-stranded DNA fragments is significantly reduced by their partial denaturation. Owing to the sequence dependence of the melting properties of DNA fragments, sequence variations can be detected. Although the sensitivity of TGGE and DGGE in detecting point mutations in genetic disorders and other settings has been reported to be close to 100%, these methods have never become as popular as other mutation detection methods such as SSCP (see Chapter 6), which may be related to the perception that it is difficult to design adequate PCR primers and set up the assays.

In this chapter, the basic principles of TGGE/DGGE will be discussed and procedures for setting up assays will be described, including how to design and test PCR primers suitable for TGGE/DGGE analysis. Furthermore, studies on the sensitivity of TGGE/DGGE for mutation analysis of genetic disorders will be reviewed and an overview of variations on the basic TGGE/DGGE method will be provided. TGGE and DGGE are robust and highly sensitive methods for mutation screening of genetic disorders that have many advantages that counterbalance the extra effort required in establishing the method.

8.2 THE THEORY OF TEMPERATURE-GRADIENT GEL ELECTROPHORESIS

8.2.1 Melting Behavior of Short Double-stranded DNA Fragments

Myers and colleagues (1985b) originally developed a method of separating DNA fragments differing by single nucleotide substitutions in denaturing gradient gels. The method was based on the notion that the denaturation (melting) of DNA fragments can be regarded as an equilibrium for each base pair (bp) between two distinct states: 1) double helical, and 2) a more random state in which bases are neither paired nor stacked on adjacent bases in any orderly way (Myers et al., 1987). The change from the first to the second state is caused by increasing temperature or increasing concentration of denaturing agents.

In the case of single-nucleotide substitutions, the replacement of an A:T bp (two hydrogen bonds) by a G:C pair (three hydrogen bonds) generally will be expected to increase the temperature at which the corresponding DNA sequence melts. The context of the nucleotide substitution also plays a role, and substitutions of A:T by T:A pairs, or...
G: C by C: G pairs, also can affect the temperature at which a DNA sequence dissociates.

Furthermore, a DNA fragment dissociates in a stepwise fashion as the temperature is gradually increased. Dissociation occurs nearly simultaneously in distinct, approximately 50 to 300 nucleotide long regions, termed “melting domains.” All nucleotides in a given melting domain dissociate in an all-or-nothing manner within a narrow temperature interval.

The melting temperature ($T_m$) indicates the temperature at which 50% of the individual molecules are dissociated in the given melting domain, and 50% are double helical. As indicated earlier, the $T_m$ is strongly dependent on the individual DNA sequence and can be altered significantly by small changes in the DNA sequence including single nucleotide substitutions.

### 8.2.2 Electrophoretic Mobility and the Melting State of DNA Fragments

TGGE is based on detecting differences in the electrophoretic mobility between molecules that may differ only at a single position. DNA fragments produced by the polymerase chain reaction (PCR) are subjected to electrophoresis through a linearly increasing gradient of temperature (or concentration gradient of denaturing agents such as urea and formamide for DGGE). Nucleotide substitutions and other small changes in the DNA sequence are associated with additional bands following TGGE.

The electrophoretic mobility of DNA fragments differs according to whether the fragment is completely double helical, if one or more melting domains is dissociated, or if complete dissociation to two single-stranded molecules has occurred. Each of these states can be visualized using a perpendicular TGGE experiment, as will be discussed further in Section 8.3.2.

The electrophoretic mobility of a double helical (nondenatured) DNA fragment is not significantly altered by single nucleotide substitutions within it, but is primarily dependent on the length and perhaps the curvature of the fragment (Haran et al., 1994). Therefore, assuming that PCR products contain a mixture of two DNA fragments that differ at a single position, as would be the case for a heterozygous point mutation, both fragments initially will progress through the gel at the same speed.

When the molecules reach that point in the gel where the temperature equals their $T_m$, the molecules will experience a decrease in mobility owing to a transition from a completely duplex (double helical) conformation to a partially denatured one. Dissociation of the first or first few melting domains generally results in a dramatic reduction in the mobility of the DNA fragment, because the fragment takes on a complex, branched conformation.

Due to the strong sequence dependence of the melting temperature, branching (dissociation) and consequent retardation of electrophoretic mobility occurs at different levels of the temperature gradient associated with bands at different positions in the gel (Myers et al., 1987). In addition to the two homoduplex molecules (wt/wt and mt/mt), two different heteroduplex molecules (wt/mt and mt/wt) can be formed by dissociating and reannealing DNA fragments containing a heterozygous mutation prior to performing TGGE (see Fig. 8.1). In practice, it is also possible to perform 40 cycles of PCR; the activity of the *Taq* polymerase is exhausted in the final cycles of PCR, such that heteroduplexes are formed as efficiently as is one performed denaturation and reannealing following PCR. Heteroduplex fragments then contain unpaired bases or “bulges” in the otherwise double helical DNA, resulting in a significant reduction in the $T_m$ of the affected melting domain (Ke and Wartell, 1995). The melting temperatures of the two heteroduplex molecules are generally different from one another, so that each heteroduplex is separately visible in the gel. A heterozygous point mutation will thus be visualized by the appearance of four bands: a band representing the normal allele (homoduplex), a band representing the mutant homoduplex that will lie above or underneath the wild-type homoduplex band, depending on the effect of the mutation on the $T_m$, and two heteroduplex bands that are always above the homoduplex bands (see Fig. 8.2; Myers et al. 1987). Mutant and wild-type homoduplex bands generally are separated by 2–10 mm, and the heteroduplex bands are often three or more cm above the homoduplex bands.

### 8.2.3 Mutations Are Detectable Only in the Lowest Melting Domain(s)

In the preceding discussion, a significant issue is that mutations are detectable only in the melting domain(s) with the lowest melting temperature. If, however, a DNA molecule contains several melting domains with different melting...
temperatures, it is generally not possible to visualize mutations located elsewhere than in the melting domain with the lowest $T_m$. Once the DNA fragments reach the temperature at which the first melting domain dissociates, the mobility of the fragment is greatly reduced so that it may not reach temperatures relevant for the higher $T_m$ domains under the conditions of the experiment. Also, dissociation of the highest $T_m$ domain results in complete dissociation of the DNA fragment into two single-stranded DNA molecules. Single-stranded DNA, like completely double helical DNA, does not demonstrate differences in electrophoretic mobility, owing to small sequence changes, and hence there is no possibility of distinguishing two sequences once complete dissociation has occurred.

The consequence of these observations is that only mutations in the lowest $T_m$ domain can be detected reliably by TGGE or DGGE (Myers et al., 1987).

### 8.2.4 GC- and Psoralen Clamps Extend the Usefulness of TGGE

Myers and colleagues (1985a) presented an extension of the original DGGE protocol that allowed mutations in every region of the DNA fragment under analysis to be detected. These researchers attached a 135 bp, GC-rich sequence, known as GC-clamp, to the $\beta$-globin promoter region in which mutations were being sought. The $\beta$-globin promoter region was found to contain two melting domains; without the GC-clamp, only mutations in the domain with the lower $T_m$ could be visualized in the gel. Owing to its high GC content, the GC-clamp has a significantly higher melting temperature than most naturally occurring sequences. The attachment of the GC-clamp was found to significantly alter the melting properties of the $\beta$-globin sequence and mutations in the entire $\beta$-globin sequence could be experimentally detected (Myers et al., 1985a). By adding a 40 nt G+C rich sequence to one of the two PCR primers, a GC-clamp can conveniently be added to any DNA fragment produced by PCR (Sheffield et al., 1989). It is also possible to use a universal GC-clamp that is incorporated into amplified DNA fragments during PCR, thereby avoiding the expense of synthesizing long primers (Top, 1992).

Psoralen-modified PCR primers are an alternative to GC-clamps. One of the two PCR primers is 5’ modified by 5-(octoxy)psoralen. The 5’ terminus of the primer should have two adenosine residues; if the natural sequence does not have AA, this sequence should be appended to the specific DNA sequence of the primer. Psoralens are bifunctional photoreagents that can form covalent bonds with pyrimidine bases (especially thymidine). If intercalated at 5’-TpT in double helical DNA (this will be the complementary sequence of the 3’ terminus of the other strand following PCR), psoralen forms a covalent bond with thymidine after photoinduction (Costes et al., 1993b). Photoinduction can be performed by exposing to the PCR products to a source of UV light (365) for 5 to 15 minutes, which can be done conveniently in the original PCR tubes or 96-well plates.

In general, psoralen clamping provides comparable results to GC clamping, except that cross-linking of the PCR fragments is only approximately 85% efficient, so that one observes single-stranded, denatured DNA fragments running below the main bands in the TGGE. Psoralen clamping sometimes is preferred over GC-clamping because the PCR is often easier to optimize, and bipolar clamping is possible if necessary (see section 8.3.4). Psoralen modification of primers is available from many commercial oligonucleotide sources.
8.3 THE PRACTICE OF TEMPERATURE-GRADIENT GEL ELECTROPHORESIS

Detailed protocols for TGGE and DGGE are available elsewhere (Kang et al., 1995; Murdaugh and Lerman, 1996). In the following sections, the most important issues concerning how to set up TGGE or DGGE assays successfully are discussed, including especially the issues related to primer design and optimization procedures. Several points that apply only to DGGE are discussed in Section 8.4.

8.3.1 Primer Design for TGGE/DGGE

One of the first and most widely used computer programs to design primers for TGGE was the Melt87 package by Lerman and Silverstein (1987). An updated version of this program (Melt94) is available at http://web.mit.edu/osp/www/melt.html. The Melt87 program calculates the Tm for each bp in the DNA fragment; that is, the temperature at which 50% of the individual molecules are double helical and 50% of the molecules are in a fully disordered, melted state. The results of such a calculation are termed “melting map” (see Fig. 8.3). DNA fragments typically are divided into distinct melting domains of about 50 to 300 bp in length, in which all base pairs have nearly an identical Tm. The melting map demonstrates the lowest melting domain in the DNA fragment; as mentioned earlier, only mutations in this region will be visible by TGGE analysis.

A further useful program in the Melt87 package is SQHTX. This program calculates the expected displacement in the gradient for a single-nucleotide mismatch (as would be the case for a heteroduplex molecule with a single-nucleotide substitution) at every position in the fragment. This analysis provides the clearest indication of the position in the fragment, where mutations will be detectable by TGGE analysis (Lerman and Silverstein, 1987). Figure 8.4 provides an example of a displacement map calculated with SQHTX.

The Melt87 programs are DOS-based and difficult to use for those with little experience with DOS and menu-based programs. Melt87 has no graphic capabilities of its own, and users need to process its output with a graphics program of their choice. For this reason, several freely available and proprietary programs have become available, which are significantly easier to use (see Table 8.1).

Users should load a DNA sequence encompassing the DNA fragment to be analyzed (e.g., an exon with flanking intron sequences) together with about 100 nucleotides’ “extra” sequence to either side of the fragment of interest.

**FIGURE 8.3** Melting map. This graphic represents a fragment from exon 14 of the NF1 gene and was produced using TGGE-Star. Each tick on the x-axis represents a base pair. The base pairs are numbered from 1 to 195. The y-axis shows the temperature where the probability for a bp to be melted has the value 0.95, 0.75, 0.5, 0.25, and 0.05, respectively. The 5’-terminus of the fragment corresponds to a GC-clamp. Additionally, one can distinguish two further melting domains: from the 5’-terminus to the 50th bp and from the 50th bp to the 3’-terminus. The difference between these two melting domains is small and the sensitivity of TGGE is not disturbed. If the difference between these two plateaus in the curve were higher, both regions would need to be tested in two different PCR-TGGE steps. Mutations were detected in both regions of this fragment: three asterisks above the x-axis mark positions of mutations detected with this assay (See color plate).

**FIGURE 8.4** Displacement maps calculated using the program SQHTX, and graphic created with TGGE-Star. In the case of a heterozygous mutation, two heteroduplex bands occur. Heteroduplexes do not migrate as far as the wild type fragments because they melt at lower temperatures. The distance of heteroduplex bands and wild type bands depends on the electrophoretic duration (x-axis) and the base position (y-axis). A mutation can be detected only when the displacement is higher than the resolution of the gel. The color codes indicate different electrophoretic times, and the width of each band of color indicates the expected displacement (in arbitrary units) in the gel for a point mutation at the corresponding position in the sequence (See color plate).
The previously mentioned programs can be used to find primers that result in a DNA fragment with melting properties adequate for TGGE or DGGE. In general, some amount of trial and error is needed to find optimal primers for any given sequence. Users need to decide both the position of the forward and reverse primers as well as whether the GC-clamp is to be placed on the 5′ or 3′ PCR primer or both (see later for a discussion of bipolar clamping). Programs such as TGGE-Star and MELTingeny facilitate this process by allowing users to easily shift primer positions and recalculate the melting maps. It should be mentioned that a 40-nucleotide GC-clamp can be substituted for a psoralen clamp in the computer analysis.

8.3.2 Perpendicular TGGE for the Determination of the Tm

In most cases in which TGGE is used for mutation analysis, parallel electrophoresis with simultaneous analysis of multiple samples will be performed. For each such assay, the optimal temperature gradient and run time must be determined experimentally. The procedures used for this purpose are described in this and the following section. The optimization process begins with a perpendicular TGGE experiment, in which electrophoresis is performed perpendicularly to the temperature gradient (see Fig. 8.5). Perpendicular TGGE is used to verify the reversible melting behavior of the DNA fragment and to determine its T_m under the experimental conditions. Perpendicular TGGE is run with a gradient of 20°C–60°C, which will be adequate for the vast majority of PCR fragments. Electrophoresis is initially performed at room temperature for 10–15 minutes to run the sample into the gel. Then, electrophoresis is stopped while a temperature gradient of 20°C–60°C is established, after which electrophoresis should be continued for 90–120 minutes. Figure 8.5 demonstrates the use of this analysis to determine the T_m of the DNA fragment being analyzed.

8.3.3 Travel Schedule Experiments

Up to three novel bands are observed upon TGGE/DGGE analysis of a heterozygous mutation or polymorphism. The separation will begin to become apparent when the heteroduplex molecules have reached their T_m, as their mobility will be retarded by partial denaturation. Separation of the homoduplex molecules will occur in a region of the gradient surrounding the T_m of the lowest melting domain of the DNA fragment. Therefore, TGGE assays are set up to avoid a long running time before the samples reach the effective range of separation. One should choose the temperature gradient such that the effective range of separation is approximately in the middle or somewhat above the middle of the gel, and that the upper and lower temperature ranges are separated by about 15°C from the T_m of the DNA fragment.

Once an appropriate temperature gradient has been chosen, the optimal running time can be determined by a travel-schedule experiment—a parallel TGGE experiment in which samples are applied every 30 minutes for three hours (or longer), such that the last sample to be loaded has run 30 minutes, and the first sample, three hours. Usually, one will see a reduction on electrophoretic mobility of samples after a certain period of time (generally 60 to 90 minutes if the temperature gradient was chosen correctly). Samples often do not continue to wander in the gel with any significant velocity once their melting temperature has been reached. These gels generally are run for about 30 minutes longer than the time determined in this manner (see Fig. 8.6). Different choices of the range and starting point of the temperature gradient affect both the range in the gel at which mutations will be visible as well as the optimal running time (see Fig. 8.7).

8.3.4 Bipolar Clamping

Occasionally, TGGE analysis will result in fuzzy bands that are difficult to evaluate, despite apparently adequate melting behavior, as predicted by Melt94 or other programs. Bipolar clamping of PCR products, by means of attaching a psoralen clamp to each of the two PCR primers rather than just one, is an efficacious method to improve melting characteristics of PCR fragments that are otherwise not amenable to TGGE/DGGE analysis (Gille et al., 1998). Bipolar clamping is a simple procedure that can significantly improve
results of TGGE analysis in cases where analysis with only one clamp has yielded suboptimal results. Programs such as TGGE-Star (Gille and Gille, 2002) offer the possibility of computer analysis with two clamps, and may suggest the use of bipolar clamping for amplicons whose predicted melting properties are otherwise not satisfactory.

8.4 DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)

The theory of DGGE/TGGE is described in detail in the first part of this chapter. Parallel DGGE is a form of polyacrylamide gel electrophoresis in which a double-stranded DNA fragment migrates into a gradient of linearly increasing denaturing conditions. The denaturing gradient is functionally equivalent to the temperature gradient of TGGE. The denaturants used are heat (a constant temperature of generally 60°C) and a fixed ratio of formamide (ranging from 0–40%) and urea (ranging from 0–7 M). The temperature of 60°C was empirically chosen to exceed the melting temperature of an AT-rich DNA fragment in the absence of a denaturant. For extremely GC-rich DNA sequences, higher temperatures (e.g., 75°C) can be used. To achieve a uniform temperature distribution the electrophoresis unit is attached to a circulating water bath.
8.4.1 Optimization of Gel Running Conditions

The computer programs (e.g., Melt94) described earlier reduce the number of preliminary experiments required for optimization of the gel running conditions. However, it is still necessary to run some preliminary gels to determine the optimal electrophoresis conditions and running times and to confirm that the optimal denaturing gradient has been chosen. The aim of these travel schedule gels is to have well-separated bands (normal and mutation positive control are simultaneously loaded on the gels) that are focused by the gradient. PCR products with two low-melting domains require different gel conditions for the analysis of each domain.

The choice of the denaturant concentration range can be determined as follows. The differences in gradient depth (the displacement) between a fragment and the same fragment with a change at a specified bp are calculated by the program SQHTX (Lerman and Silverstein, 1987) as described in Section 8.3.1. SQHTX calculates the displacement as the difference in temperature at which the wild-type homoduplex and the heteroduplex molecules partially melt (see Fig. 8.4). To convert between the temperature values and the denaturant concentration, a difference of 1°C is converted to a difference of 3% denaturant concentration (approximately equivalent to 1 cm distance within a 20% urea gradient gel). An experimental determination of gradient behavior can be achieved by perpendicular gel electrophoresis. Data from the perpendicular gels help to estimate the denaturant concentration range to use in parallel gel electrophoresis. For parallel gels, the gradient initially should be chosen with a 25% to 30% difference in denaturant concentration centered around the melting temperature of the domain (Myers et al., 1987). Once optimized gel running conditions have been established, the method can be used for mutation screening.

FIGURE 8.6 Travel schedule experiment. This experiment is used to determine the optimal running time of a TGGE experiment. Fragments, corresponding to exon 19a of the NF1 gene, in which one of the primers was modified with psoralen (see Section 8.2.4), were applied at intervals of 30 minutes, such that the first fragments had a total running time of 5 hours, and the last fragments to be applied had a running time of 60 minutes. Lanes labeled U contain PCR fragments that were not UV-irradiated to effect psoralen-mediated crosslinking, and lanes labeled N (two lanes were loaded for each timepoint) contain irradiated PCR fragments. One sees that the fragments initially are completely double-helical (1 h), such that irradiated and nonirradiated fragments display the same band pattern. Starting at the Tm of this fragment (40°C), the nonirradiated fragments (U) undergo complete dissociation so that only a single-strand band running well below the main band of the irradiated (cross-linked) fragments is visible (compare the time points at 2.5 and 3 hours. Additionally, the irradiated fragments (N) show a large reduction in electrophoretic mobility following partial denaturation at about 40°C. Under these conditions, an optimal running time would be 3 hours, although the running time could be reduced by adjusting the temperature gradient (see also Fig. 8.7). (See color plate)
specificity of TGGE/DGGE is exquisitely high. In other words, a false-positive four-band pattern occurs rarely if at all.

8.6 DETECTION RATE AND SENSITIVITY

By using DGGE Myers and colleagues (1985b) detected an estimated 40% of the sequence variants in a DNA fragment up to 500 bp in their initial study. The use of GC-clamps, psoralen clamps, or bipolar clamping, which aid the formation of uniform low melting domains, significantly improved the detection rate of TGGE/DGGE, which in many cases approaches nearly 100%.

The sensitivity of TGGE/DGGE for detecting known mutations is generally reported to be nearly 100%, generally performing as well as or better than other mutation detection methods (Abrams et al., 1990; Ferec et al., 1992; Gelfi et al., 1997; Gejman et al., 1998; Techenitchko et al., 1999; Zschocke et al., 2000; Breton et al., 2003). In one study with a panel of known mutations, DGGE detected 201 of 201 known mutations in the CFTR gene (Macek et al., 1997). The reasons for lower reported detection rates of unknown mutations in some studies has been speculated to be due to genetic heterogeneity (Ferec et al., 1999), clinical overdiagnosis (Katzke et al., 2002), or location of mutations in intronic or promoter regions that were not included in the screening program. Optimization of the TGGE/DGGE assay conditions and primers, perhaps including the use of bipolar clamping (Gille and Gille, 2002), may increase sensitivity.

In summary, the sensitivity of TGGE/DGGE, when properly used, is close to 100%.

TGGE/DGGE also has been shown to be very sensitive in the detection of mutations in situations where the mutation sequence is present in proportions less than 50% (as is generally the case when heterozygous mutations are sought in genomic DNA). This has proved useful in detection of heteroplasmy in mitochondrial disorders with heteroplasmic proportions as low as 1% (Tully et al., 2000), as well as in testing for residual disease in cancer (Ahnheut et al., 2001; Alkan et al., 2001).
8.7 RELATED TECHNIQUES AND VARIANTS

A wide range of improvements and further developments of the principles underlying DGGE and TGGE have appeared in the last decade, the most important of which are briefly summarized here.

**Broad range DGGE.** A single gel and a single set of conditions is used to screen all the exons of one gene (Guldberg and Guttler, 1994; Hayes et al., 1999).

**Multiplex DGGE.** Several exons are simultaneously analyzed in one DGGE gel (Costes et al., 1993a).

**Genomic DGGE (gDGGE).** Genomic DNA is digested with a restriction enzyme, electrophoresed by DGGE, transferred to nylon membrane, and hybridized to a unique DNA probe (Borresen et al., 1988).

**Constant DGGE (cDGGE).** Gels contain constant concentrations of denaturants. This allows an increased resolution of mutant fragments since they will constantly migrate with a different electrophoretic mobility through the whole length of the gel (Hovig et al., 1991).

**Constant denaturant capillary electrophoresis (CDCE).** DNA migrates through a 30 cm quartz capillary of 75 μm inner diameter, filled with a viscous polyacrylamide solution. A 10 cm part of the capillary, prior to the detector, is heated to a temperature permitting partial melting (see also previous chapter). Usually the DNA is fluorescein-labeled and detected by laser-induced fluorescence (Khrapko et al., 1994). Separation of DNA fragments is achieved by the differential velocity of partly melted DNA in a medium with uniform denaturant concentration.

**Temporal temperature gradient gel electrophoresis (TTGE).** A constant concentration of urea or formamide is used as in cDGGE, but the temperature during the run is increased gradually (Yoshino et al., 1991; Wiese et al., 1995). The denaturant concentration (usually 6–8% urea) used in TTGE can be determined either from the theoretical melting curve or experimentally from aper- pendicular DGGE.

**Microtemperature-gradient gel electrophoresis (μTGGE).** A minimized gel (20 × 20 × 0.5 mm) leads to the reduction of the amount of DNA required and to shorter running times (approximately 12 min at 100 V, 10 mA). The method was used in microbial ecology and epidemiology (Biyani and Nishigaki, 2001).

**Double-gradient, denaturing gradient gel electrophoresis (DG-DGGE).** In addition to the chemical denaturing gradient (formamide and urea) a second sieving gradient (e.g., 6%–12% polyacrylamide gradient) is used (Cremonesi et al., 1997).

**Two-dimensional DNA fingerprinting/two-dimensional gene scanning (TDGS, 2D-DNA typing).** Combines size fractionation of DNA fragments in the first dimension with their sequence-specific separation through DGGE in the second dimension (see also next chapter).

**Denaturing HPLC (dHPLC).** Uses an ion-pair chromatography separation principle, combined with a precise control of the column temperature and optimized mobile phase gradient for separation of mutant DNA molecules (reviewed in Xiao and Oefner, 2001).

**Cycling gradient capillary electrophoresis (CGCE).** DNA sequence variants are detected based on their differential migration in a polymer-filled capillary system. A cycling (oscillating) temporal temperature gradient

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**FIGURE 8.9** Mutation detection using DGGE analysis. a) Screening for the Gγ–158 C → T polymorphism in the promoter region of the human Gγ-globin gene. Lanes 3, 4, and 5 correspond to homozygous samples for that polymorphism; lanes 2 and 7 correspond to heterozygous samples; and lanes 1 and 6 correspond to samples that do not carry this polymorphism to either of the two alleles (photo courtesy of Dr. George P. Patrinos). b) DGGE analysis of the promoter region of the human Aγ-globin gene. Lanes 2 and 4 correspond to heterozygous cases for the Aγ–117 G → A mutation, leading to the Greek type of nondeletional Hereditary Persistence of Fetal Hemoglobin (nd-HPFH); lane 3 corresponds to a homozygous case for the same mutation; lane 1 corresponds to a wild-type control (photo courtesy of Dr. George P. Patrinos). c) Mutation analysis of exons 11 and 13 of the CFTR gene. Lanes 1, 2, 4, and 5 correspond to wild-type cases; lane 3 corresponds to a heterozygous case for the 8822X nonsense mutation; lane 6 corresponds to a heterozygous case for the G542X nonsense mutation, leading to cystic fibrosis (photo courtesy of Dr. Angeliki Balassopoulou, Athens, Greece). The gradient of denaturing agents is depicted at the left side of each gel.
is applied. This improvement enables utilization of a multiple injection technique, in which multiple samples are injected into the same capillary (or set of capillaries) separated by predefined time intervals of partial electrophoresis. A 96-capillary system is able to screen over 15,000 samples in 24 h (Minarik et al., 2003).

8.8 TECHNICAL EQUIPMENT FOR TGGE/DGGE

In general, for DGGE, preexisting vertical electrophoresis equipment with buffer-tank and combined heater/stirrer thermostat can be adapted. For TGGE, special equipment to achieve a constant temperature gradient is necessary.

The Biometra TGGE (Goettingen, Germany; www.biometra.de) system uses a temperature block powered by Peltier technology, which enables a strictly linear gradient that may allow more reproducible conditions than with conventional chemical gradients or temperature gradients using water baths. The Biometra TGGE system is available in two formats: A TGGE “mini” system operates small gels and is therefore suitable for fast, serial experiments. A TGGE maxi system provides a large separation distance and allows high parallel sample throughput.

The DCode mutation detection system (Bio-Rad Laboratories, Hercules USA) can be used to screen mutations by DGGE, TGGE, CDGE, TTGE, and other techniques. The system performs TTGE by controlling the buffer temperature during the electrophoresis run. A temperature control module regulates the rate of temperature increase in a uniform and linear fashion.

Sooner Scientific (Garvin, USA, www.soonersci.com) offers five different-sized DGGE Systems variants (for 2, 4, or 8 smaller gels or one large gel).

The INGENYphorU system (Ingeny International, GP Goes, The Netherlands; www.ingeny.com) is suitable for DGGE, TGGE, CDGE, and other techniques.

8.9 APPLICATIONS OF TGGE/DGGE AND RELATED METHODS

TGGE/DGGE has been applied in an increasing number of studies. A recent search in PubMed database found over 1,100 citations. The following applications have been described:

- Screening for polymorphisms in human genes; for example, COL1A2 gene (Borresen et al., 1988), alpha-1-antitrypsin (Hayes, 2003), human γ-globin genes (Patrinos et al., 1998; 2001; Fig. 8.9a,b)
- Mutation detection in human genes; for example, p53 (Pignon et al., 1994), FBN1 (Tiecke et al., 2001; Katzke et al., 2002; Robinson et al., 2002), NF1 (Peters et al., 1999; Fahsold et al., 2000), dystrophin gene (Hofstra et al., 2004), β-globin (Papadakis et al., 1997) and β-globin genes (Losekoot et al., 1990), CFTR gene (Fig. 8.9c), and so on
- Mutation and polymorphism detection in mitochondrial DNA (Hanekamp et al., 1996; Chen et al., 1999)
- Analysis in microbial ecology, determination of biodiversity of bacterial populations in soil, fresh, or salt water (Muyzer and Smalla, 1998; van Elsas et al., 2002)
- Genome profiling and provisiononal microbial species identification on the basis of random PCR and TGGE (Watanabe et al., 2002)
- Determination of biodiversity in fecal or intestinal microflora (Tannock, 2002)
- HLA typing (Uhrberg et al., 1994)
- Analysis of proteins and antibody binding (Riesner et al., 1991; Arakawa et al., 1993)
- Clonality analysis of T-cell or T-cell receptors (Plonquet et al., 2002; Lukowsky, 2003)
- Mutation detection and detection of variation between genomes of viral strains (Lu et al., 2002; Motta et al., 2002)
- Analysis of biodiversity and polymorphisms in plants (Gomes et al., 2003; Nikolcheva et al., 2003)
- Examination of the fidelity of DNA polymerases (Keohavong and Thilly, 1989)

8.10 CONCLUSIONS

TGGE/DGGE and related methods provide a very high sensitivity and are relatively easy and cheap to perform once the assays have been designed and optimized. The main advantages are in the high detection rate and specificity and improved heterozygote detection. The methodology is simple, nonradioactive, and relatively nontoxic. The disadvantages of TGGE and DGGE include mainly the limitation of PCR fragment length to about 500 nucleotides, the difficulties of analyzing GC-rich fragments, and the need for computer analysis of potential PCR fragments (which on the other hand can save time and money by eliminating the use of inadequate primers). However, once primers and conditions have been chosen, TGGE/DGGE is a robust and easy-to-perform mutation screening method. It is particularly well suited for the detection of known and unknown mutations in large genes, where high sensitivity is required and when large numbers of samples are to be tested.

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