Temporal Temperature Gradient Gel Electrophoresis of Cystic Fibrosis Samples on the DCode™ System

Paul Zoller and Theresa Redila-Flores, Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

Temporal temperature gradient gel electrophoresis (TTGE) is one of several methods that can be used to screen DNA fragments for small sequence changes or point mutations (Wiese et al. 1995, Yoshino et al. 1991). TTGE exploits the principle upon which DGGE is based, without the requirement for a chemical denaturing gradient. Amplified mutant and wild-type DNA from the gene of interest is loaded onto a polyacrylamide gel containing a constant concentration of urea. During electrophoresis, the temperature is increased gradually and uniformly. The result is a linear temperature gradient over the length of the electrophoresis run. Thus, the denaturing environment is formed by a constant concentration of urea in the gel in combination with the temporal temperature gradient.

The separation principle of TTGE is based on the melting behavior of DNA molecules. In a denaturing acrylamide gel, double-stranded DNA is subjected to conditions that will melt it in discrete segments called melting domains. The melting temperature (T_m) of these domains is sequence-specific. When the T_m of the lowest melting domain is reached, the DNA will become partially melted, creating branched molecules. Partial melting of the DNA reduces its mobility in a polyacrylamide gel. Since the T_m of a particular melting domain is sequence-specific, the presence of a mutation will alter the melting profile of that DNA in comparison to the wild-type DNA. Thus, the DNA containing the mutation will have a different mobility compared to the wild type.

In this experiment, we show that the TTGE analysis on the DCode universal mutation detection system can be used to analyze mutations in the cystic fibrosis gene.

Methods

Fourteen genomic DNA samples from exons 10 and 11 of the cystic fibrosis gene were amplified by the polymerase chain reaction (PCR) to create end products of 369 bp for exon 10 and 289 bp for exon 11. One of the primers contained a 40 bp GC clamp. Samples were provided by Dr L Silverman, University of North Carolina School of Medicine (Chapel Hill, NC USA).

A 16 x 16 cm, 1 mm thick 6 M urea/6% acrylamide/bis (37.5:1) gel in 1.25x TAE buffer (50 mM Tris, 25 mM acetic acid, 1.25 mM EDTA) was used. Approximately 200–300 ng of each amplified sample was mixed with 5 µl of 2x gel loading dye (70% glycerol, 0.05% Bromophenol Blue, 0.05% Xylene Cyanole, 2 mM EDTA) and electrophoresed on the DCode system at 130 V for 5 hr with a temperature range of 50–60°C and a temperature ramp rate of 2°C/hr. After electrophoresis, the gel was stained in 50 µg/ml ethidium bromide in 1.25x TAE buffer for 5 min and destained in buffer for 10 min. The gels were imaged on the Gel Doc™ 1000 system.

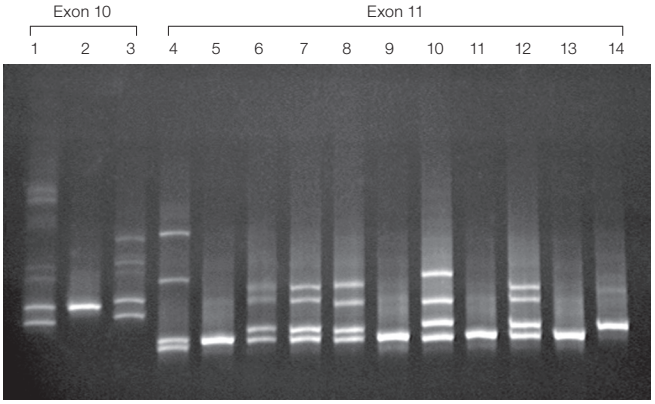

Fig. 1. Separation of amplified mutant and wild-type alleles of exons 10 and 11 from the cystic fibrosis gene on the DCode system. Lane 1, mutant allele (ΔF508); lane 2, wild-type allele; lane 3, mutant allele (V520F); lane 4, mutant allele (R560T); lanes 5, 9, 11, 13, wild-type allele; lane 6, exon 11 mutant allele (1717-1G-A); lane 7, mutant allele (G551S); lane 8, mutant allele (G542X); lane 10, mutant allele (R553X); lane 12, mutant allele (G551D); lane 14, mutant allele (S549N).

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Results and Discussion

The temperature range used in the TTGE separation was calculated using MacMelt™ DNA melting software. A melting profile of the amplified wild-type DNA sequence containing the 40 bp GC clamp was generated with MacMelt software, and the temperature range for TTGE was calculated by identifying the lowest and the highest non-GC-clamp melting temperature of the DNA sequence. Twelve degrees was subtracted from the calculated low and high theoretical melting temperatures, because every mole of urea used in the gel reduces the melting temperature by 2°C (Gelfi et al. 1994, Steger 1994). TTGE analysis of the 14 samples identified two mutant samples in exon 10 and seven mutant samples in exon 11 (Figure 1). The two heterozygous mutations resolved in exon 10 were ΔF508 (–CTT), a three-base deletion mutation in lane 1, and V520F (G→T), a single-base deletion mutation in lane 3. The heterozygous mutant samples resolved into two heteroduplex and two homoduplex bands. The heteroduplex bands migrated slower than the corresponding homoduplex bands. Six single-base heterozygous mutations and one single-base homozygous mutation were resolved in exon 11 (Figure 1). The heterozygous mutations from exon 11 were R560T (G→C) in lane 4, 1717-1 (G→A) in lane 6, G551S (G→A) in lane 7, G542X (G→T) in lane 8, R553X (C→T) in lane 10, and G551D (G→A) in lane 12. All four bands in the heterozygous mutations were resolved and can be distinguished from the wild-type samples by having a different migration pattern. Sample 14 in exon 11 was the homozygous mutation S549N (G→A), which migrates slower than the wild-type sample.

TTGE can be used as a method for screening mutations in the cystic fibrosis gene using the DCode system. The ability to control the temperature ramp rate during electrophoresis with the DCode system allows TTGE to be a reproducible technique.

References

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