Heteroduplex Analysis of Cystic Fibrosis Samples on the DCode™ System

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Introduction

Heteroduplex analysis (HA) is a commonly used mutation screening method due to its simplicity. The technique is based on conformational differences in double-stranded DNA caused by the formation of heteroduplex molecules (Nagamine et al. 1989). Heteroduplex molecules have a mismatch in the double strand, causing a distortion in its usual conformation. This distortion or altered conformation can be detected on polyacrylamide gels due to slower migration than the corresponding homoduplex molecules. Heteroduplexes are generated during the polymerase chain reaction (PCR) of a heterozygous individual or by adding mutant and wild-type DNA in the same PCR reaction or by denaturing and renaturing a mixture of mutant and wild-type DNA. Both mutant and wild-type samples are run on the same gel to analyze differences in mobility. Heteroduplex molecules with as little as one mismatch can show a different mobility in a gel from homoduplex molecules. Polyacrylamide analogs have been developed, such as DEM™ (detection-enhancing matrix) from Bio-Rad, which enhance the ability to detect mutations in heteroduplex samples when compared to conventional polyacrylamide gels (White et al. 1992).

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

Methods

The test samples consist of wild-type and mutant DNA samples from the cystic fibrosis gene exons 7 and 10. The heterozygous mutations from exon 7 were 1154insTC, two base insertion (+TC) and ΔF311, three base deletion (–TTC). The mutations from exon 10 were a heterozygous ΔF508, three base deletion (–CTT), a heterozygous compound sample Q493 (C to T) and ΔF508 (–CTT), a homozygous ΔF508 (–CTT), and a heterozygous ΔI507, three base deletion (–ATC). Samples were provided by Dr L Silverman, University of North Carolina School of Medicine (Chapel Hill, NC). Genomic DNA from both wild-type and mutant samples were amplified by PCR to create end products of 289 bp for exon 7 and 369 bp for exon 10.

A 16 cm x 20 cm, 0.75 mm thick 1x DEM gel made up in 0.6x TBE buffer (54 mM Tris, 54 mM boric acid, 1.2 mM EDTA) was used. A 1.5 L volume of 0.6x TBE buffer was added to the lower electrophoresis tank, and 350 ml of 0.6x TBE buffer was added to the upper chamber in the DCode system. Five µl (200–300 ng) of each amplified sample was mixed with 5 µl 2x gel loading dye (70% glycerol, 0.05% Bromophenol Blue, 0.05% Xylene Cyanol, 2 mM EDTA) and electrophoresed on the DCode system at 100 V for 16 hr at room temperature. After electrophoresis, the gel was stained in 50 µg/ml ethidium bromide in 0.6x TBE buffer for 5 min and destained in buffer for 10 min. The gel was imaged under ultraviolet (UV) transillumination.

Results and Discussion

Figure 1 shows the mutant and wild-type samples from the cystic fibrosis gene run on the DCode system. The mutant samples from exon 7 (Figure 1A) in lanes 2 and 3 resolve into two heteroduplex bands (upper bands) and a homoduplex band (lower band). The two heteroduplex bands in the mutant samples from exon 10 (Figure 1B) in lanes 2, 3, and 5 are resolved from the homoduplex bands. The ΔF508 homozygous mutant in lane 4 has no heteroduplex bands, and the homoduplex bands migrate the same as the wild-type. Under these conditions, it was not possible to resolve the two homoduplex bands of the ΔF508 mutant. This mutation may be detected if heteroduplexes are formed by mixing the mutant and wild-type samples together, then denaturing and renaturing the DNA.

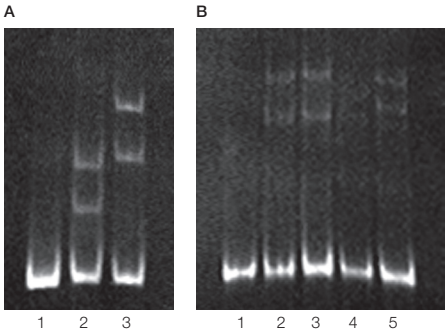

Fig. 1. Cystic fibrosis samples run on 1x DEM gels on the DCode system. **A**, Exon 7 samples. Lane 1, wild-type DNA; lane 2, mutant sample 1154insTC; lane 3, mutant sample ΔF311. **B**, Exon 10 samples. Lane 1, wild-type DNA; lane 2, mutant sample ΔF508; lane 3, compound mutant sample Q493/DF508; lane 4, mutant sample ΔF508; lane 5, mutant sample ΔI507.

The ability to resolve the heteroduplex bands in the mutant samples makes it possible to distinguish between the mutant and wild-type samples. Heteroduplex analysis can be used as a method for screening heterozygous mutations in the cystic fibrosis gene using the DCode system.

References

Nagamine CM et al., A PCR artifact: generation of heteroduplexes, Hum Genet 45, 337–339 (1989)

White MB et al., Detecting single base substitutions as heteroduplex polymorphisms, Genomics 12, 301–306 (1992)

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