Denaturing Gradient Gel Electrophoresis of β-Thalassemia Samples on the DCode™ System

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Introduction

Denaturing gradient gel electrophoresis (DGGE) is one of several methods that can be used to screen DNA fragments for small sequence changes or point mutations. In DGGE, the denaturing environment is created by a combination of uniform temperature and a linear denaturant gradient formed with urea and formamide (Fischer and Lerman 1983). A 100% denaturant solution consists of 7 M urea and 40% formamide. The denaturing gradient may be formed perpendicular or parallel to the direction of electrophoresis. When running a denaturing gradient gel, both the amplified mutant and wild-type DNA fragments are run on the same gel. This way, one can detect a mutation by seeing a band shift between the mutant and wild-type samples.

The separation principle of DGGE is based on the melting behavior of DNA molecules. In a denaturing gradient acrylamide gel, double-stranded DNA is subjected to a denaturant environment and will melt in discrete segments called melting domains. The melting temperature (T_m) of these domains is sequence-specific. When the T_m of the lowest domain is reached, the DNA will become partially melted, creating branched molecules. Partial melting reduces the DNA's 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 when compared 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 parallel DGGE analysis on the DCode universal mutation detection system can be used to analyze mutations in the β-globin gene.

Methods

The test samples consisted of a wild-type and three mutant DNA samples from the β-globin gene. The three single-base heterozygous mutations were IVS1-1 (G→A), IVS1-6 (T→C), and IVS1-110 (G→A). Genomic DNA from both wild-type and mutant samples was amplified by the polymerase chain reaction (PCR), creating an end product of 281 base pairs.

A 16 x 16 cm, 1 mm thick 8% acrylamide/bis (37.5:1) gel with a parallel denaturing gradient range of 35–55% in 1x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) was used. The gradient gel was cast using Bio-Rad's Model 475 gradient delivery system. Mutant sample (200–300 ng) 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 3 hr at a buffer temperature of 56°C. After electrophoresis, the gel was stained in 50 µg/ml ethidium bromide in 1x TAE 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 β-thalassemia samples run on a parallel DGGE gel. The three heterozygous samples in lanes 1–3 were resolved into two heteroduplex and two homoduplex bands. The heteroduplex bands (upper bands) migrated slower than the corresponding homoduplex bands (lower bands). The wild-type sample in lane 4 was resolved as one band, and it migrated to the same distance as the wild-type band in the mutant samples. The ability to resolve the heteroduplex fragments and the mutant homoduplex fragment in the mutant samples makes it possible to distinguish between samples that are mutant or wild type. From the results, it was possible to detect single-base substitutions in the mutant samples from the wild-type sample using parallel DGGE.

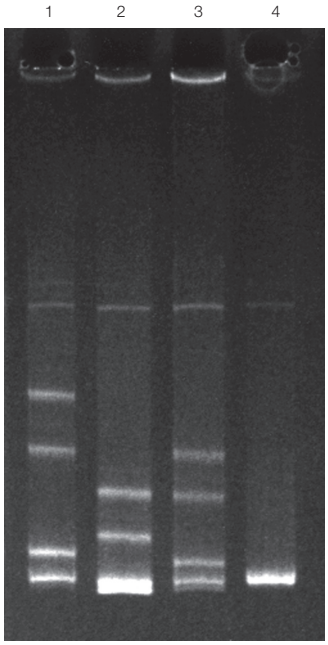

Fig. 1. Parallel DGGE separation of β-thalassemia mutants and wild-type samples run on the DCode system. Lane 1, mutant sample IVS1-1; lane 2, mutant sample IVS1-6; lane 3, mutant sample IVS1-110; lane 4, wild-type DNA.

Parallel DGGE can be used as a method for screening mutations in the β-globin gene using the DCode system. The DCode system can precisely control the buffer temperature due to a large buffer volume, electronic heat control, a stirrer, and a circulating pump.

Reference

Fischer SG and Lerman LS, DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory, Proc Natl Acad Sci USA 80, 1579–1583 (1983)

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