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

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Introduction

Constant denaturing gel electrophoresis (CDGE) is one of several methods that can be used to screen DNA fragments for small sequence changes or point mutations. CDGE is a modification of denaturing gradient gel electrophoresis (DGGE). In CDGE, the denaturing environment is created by a combination of uniform temperature and a constant denaturant formed with urea and formamide (Børresen et al. 1999). The optimal concentration of denaturant to use for CDGE is determined from a perpendicular or parallel denaturing gel at the point of maximum split between wild-type and mutant DNA. After a mutation has been identified by previously run DGGE gels, a CDGE gel can be used to rapidly screen samples for the presence of a mutation.

The separation principle of CDGE is based on the melting behavior of DNA molecules. In a denaturing acrylamide gel, double-stranded DNA is subjected to conditions that will melt the DNA 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 when compared to the wild type. Thus, the DNA containing the mutation will have a different mobility compared to the wild-type DNA.

In this experiment, we show that CDGE analysis on the DCode universal mutation detection system can be used to analyze mutations in the β-globin gene.

Methods

The test samples consist of a wild-type and four mutant DNA samples from the β-globin gene. The three single-base heterozygous mutations are IVS1-1 (G→A), IVS1-6 (T→C), IVS1-110 (G→A), and a compound mutation containing IVS1-1 and IVS1-6. Genomic DNA from both wild-type and mutant samples was amplified by the polymerase chain reaction (PCR) to create end products of 281 base pairs (bp). One of the primers contained a 40 bp GC clamp.

A 16 x 16 cm, 1 mm thick 8% acrylamide/bis (37.5:1) gel with a perpendicular denaturant gradient range of 20–70% in 1x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) was used. For DGGE, a 100% denaturant solution consists of 7 M urea and 40% formamide. The gradient gel was cast using Bio-Rad's Model 475 gradient delivery system. To find the optimum concentration of denaturant to use, approximately 1 µg of each amplified sample was mixed with 50 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 2.5 hr with a buffer temperature of 56°C.

After the optimum concentration of denaturant was determined, 200–300 ng of each amplified sample was mixed with 5 µl of 2x gel loading dye and electrophoresed on a 45% constant denaturant, 8% acrylamide/bis (37.5:1) gel in 1x TAE buffer on the DCode system at 130 V for 3 hr with a buffer temperature of 56°C.

After electrophoresis, gels were stained in 50 µg/ml ethidium bromide in 1x TAE buffer for 5 min and destained in buffer for 10 min. The gels were imaged under ultraviolet (UV) transillumination.

Results and Discussion

The β-thalassemia samples were first run on perpendicular DGGE gels to determine the optimum concentration of denaturant to use with CDGE. Figure 1 shows the heterozygous mutant IVS1-6 run on a 20–70% perpendicular DGGE gel. A split between the heterozygous and homozygous alleles is seen at approximately 45% denaturant concentration. In this gel, the two heteroduplex bands are located on the left side of the S-shaped curve and the remaining two bands are homoduplex. The denaturant concentration to use for the CDGE gel was determined by first measuring the distance along the gradient where the maximum split is seen between bands. Using the fluorescent ruler, the split is 4.2 cm from the start of the gradient. This distance is divided by the length of the gel and multiplied by the denaturant range. For example, (4.2 cm ÷ 8.4 cm) x (70% – 20%) = 25%. This number is added to the starting denaturant concentration to give the optimum concentration to use for CDGE (20% + 25% = 45%). Perpendicular gels were run for the other three mutations and the optimum denaturant calculated was approximately 45% (data not shown).

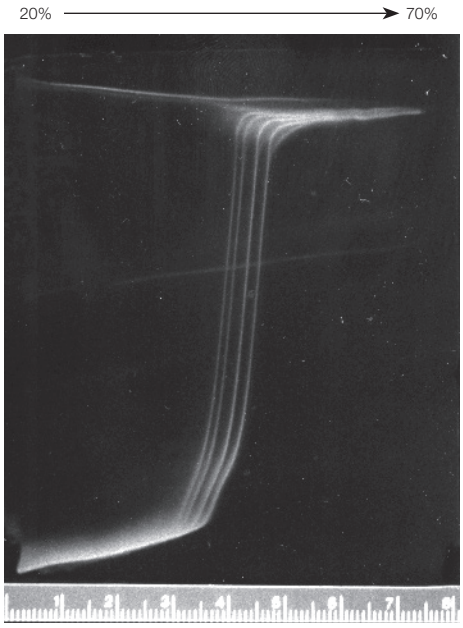

Fig. 1. Perpendicular DGGE separation of β-thalassemia mutant IVS1-6 run on the DCode system. The optimum denaturant concentration to use in a CDGE gel was determined at the location where the maximum split occurred between the heterozygous and homozygous alleles.

A 45% constant denaturing gel is shown in Figure 2. Under this condition, it is possible to resolve the homoduplex bands from the heteroduplex bands in the four mutant samples, making it possible to distinguish between the mutant and wild-type samples. The wild-type fragment migrated at the same distance as the wild-type fragment in the IVS1-1, IVS1-6 and IVS1-110 mutant samples. In the compound mutant sample, one allele contains the mutation IVS1-1, and the other allele contains the IVS1-6 mutation; therefore, no wild-type fragment is seen.

CDGE can be used as a method for screening for mutations in the β-globin gene using the DCode system. The DCode system has the flexibility to run both DGGE and CDGE on one electrophoresis unit.

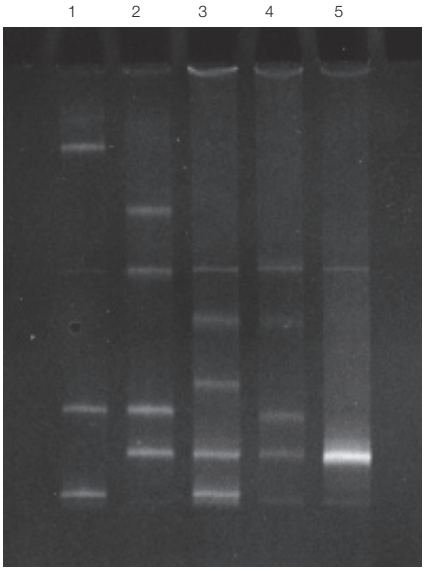

Fig. 2. CDGE separation of β-thalassemia samples run on the DCode system. Lane 1, compound mutant sample IVS1-1 + IVS1-6; lane 2, mutant sample IVS1-1; lane 3, mutant sample IVS1-6; lane 4, mutant sample IVS1-110; lane 5, wild-type DNA.

Reference

Børresen AL et al., Constant denaturant gel electrophoresis as a rapid screening technique for *p53* mutations, Proc Natl Acad Sci USA 88, 8405–8409 (1991)

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