Single-Stranded Conformational Polymorphism of β-Thalassemia Samples on the DCode™ System

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Introduction

Single-stranded conformational polymorphism (SSCP) is one of several methods that can be used to screen DNA fragments for small sequence changes or point mutations. The SSCP technique is based on the fact that single-stranded DNA has a defined secondary structure. Sequence differences as small as a single base change can affect this secondary structure and can be detected by electrophoresis in a nondenaturing polyacrylamide gel (Orita et al. 1989). Double-stranded mutant and wild-type samples are first denatured into single strands and then loaded onto the gel. Differences in mobility of the single strands between the wild-type DNA and the other samples indicate a mutation. SSCP is a widely used mutation screening method because of its simplicity. In this experiment, we show that SSCP 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 one wild-type and two mutant DNA samples from the β-globin gene. The two mutations from the β-globin gene are: heterozygous IVS1-6, a single base mutation from T to C, and homozygous β-sickle, a single base substitution from A to T. Genomic DNA from both wild-type and mutant samples was amplified by the polymerase chain reaction (PCR), creating an end product of 293 base pairs. For each amplified product, 5 µl was mixed with 5 µl 2x SSCP gel loading dye (95% formamide, 20 mM EDTA, 0.05% Xylene Cyanole, and 0.05% Bromophenol Blue) and heated at 95°C for 5 min to denature the double-stranded DNA into single-stranded fragments. The samples were chilled on ice before loading into the gel.

The denatured samples were loaded in a 16 x 20 cm, 0.75 mm thick 8% acrylamide/bis (37.5:1) gel that contained 7% glycerol and 1x TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA). The buffer was chilled by connecting the DCode electrophoresis cooling tank to an external chiller (Lauda RM20). The coolant used in the chiller was 50% ethylene glycol. The chiller was set to –20°C and the DCode temperature controller was set to 8°C. The fragments were electrophoresed at 30 W (constant) for 3.5 hr at 8°C.

After electrophoresis, the gel was stained in a 1:10,000 dilution of SYBR Green II (Molecular Probes, Inc.) in 1x TBE buffer for 45 min. The gel was imaged under ultraviolet (UV) transillumination.

Results and Discussion

Figure 1 shows the mutant and wild-type samples run at 8°C on the DCode system. Under these conditions, it is possible to resolve three distinct bands representing the two single strands (upper bands) and the reannealed double-stranded DNA (lower band). The middle single-stranded DNA of the mutant samples migrates to a different location than the wild-type single strand. The ability to resolve this difference in the single strands makes it possible to distinguish between mutant and wild-type samples. From the results, it was possible to detect single base substitutions between the mutant and wild-type samples using SSCP.

SSCP can be used as a method for screening mutations in the β-globin gene using the DCode system. The DCode system has a small gel format, which allows easy gel handling and can precisely control the buffer temperature due to a large buffer volume, cooling fingers in the electrophoresis tank, electronic heat control, a stirrer, and a circulating pump.

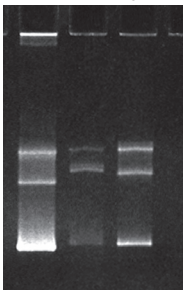

Fig. 1. SSCP samples run at 8°C on the DCode system. Lane 1, heterozygous mutant IVS1-6; lane 2, wild type; lane 3, homozygous mutant β-sickle.

Reference

Orita M et al., Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms, Proc Natl Acad Sci USA 86, 2766–2770 (1989)

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