

Non-radioactive single strand conformation polymorphism (SSCP) using the Pharmacia 'PhastSystem'

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Single Strand Conformation Polymorphism (SSCP) was first described by Orita and colleagues (1). In this technique, double stranded (ds) DNA is denatured to single stranded (ss) DNA and the products are electrophoresed in a non denaturing polyacrylamide gel. This technique is capable of detecting single point mutations in PCR amplified products (2) and has been used to define genetic alterations in certain human diseases (2, 3, 4). As described, however, the technique is relatively cumbersome requiring the use of radioactive nucleotides and large gels. These requirements compromise the routine use of SSCP in clinical laboratories. We, therefore, investigated the feasibility of using a semi-automated electrophoresis system for SSCP. The 'PhastSystem' (Pharmacia) has the advantages of preformed gels which can be rapidly electrophoresed in a controlled environment coupled with an extremely sensitive silver stain to visualize both ds and ss DNA.

In our experiments, we studied PCR amplified products of different exons in the human p53 gene. The PCR was run using primer-pairs, specific for each exon. The template DNA included plasmids (kindly provided by Dr B.Vogelstein), and cell lines containing defined point mutations. Using five known mutant sequences from exons 5, 7 and 8, we were able to demonstrate a mobility shift in at least one of the mutant ss DNAs in comparison to wild type. Electrophoretic mobility patterns obtained from the 5th exon of wild-type (php53B) and mutant (p53SCX3) are shown in Figure 1. Positive and negative ssDNA (obtained by denaturation of ds DNA) were identified by comparison with the migration of products obtained by asymmetric PCR (6).

The PCR product, diluted 1:25 in water, was heated to 95°C for 5 minutes and quenched on ice to produce almost complete denaturation. Strand separation was obtained using a 20% gel, at a temperature of 4°C. Running conditions were: (i) Pre-run: 400 V, 5.0 mAmp, 1.0 W, 4°C, 100 Vh; (ii) Sample application: 25V, 5.0 mAmp, 1.0 W, 4°C, 2 Vh; (iii) Run: 100–400 V, 5.0 mAmp, 1.0 W, 4°C, 350 Vh. The total electrophoresis and staining time was approximately 2 hours. In the 'PhastSystem', the conditions of electrophoresis (temperature, voltage and amperage) can be adjusted as needed to give optimal separations for individual sequences.

The 43×50×0.45 mm stained gels can be directly mounted and stored permanently as a projection slide.

These results confirm that the Pharmacia 'PhastSystem' may be helpful in applying SSCP in the Clinical Molecular Diagnostic Laboratory.

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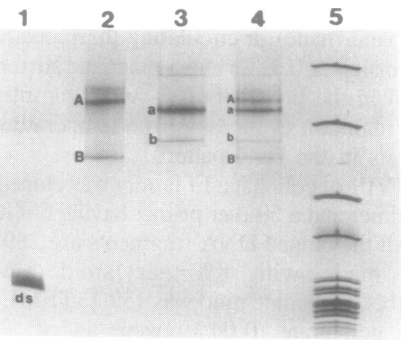

Figure 1. SSCP showing mobility shifts between wild type and mutant single strand DNA derived from PCR amplified products of the 5th exon of P53. **1:** ds, double strand DNA. **2:** positive (**A**) and negative (**B**) single strand DNA, wild type, php53B. **3:** positive (**a**) and negative (**b**) single strand DNA, mutant, p53SCX3. **4:** single strand DNA from wild type and mutant mixed together. **5:** MspI cut pBR322 size marker.

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