Protocols for an improved detection of point mutations by SSCP

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Efficient and reliable detection of single base substitutions has proven increasingly important in biomedical research. A variety of methods have been proposed among which Single Strand Conformation P olymorphism (SSCP) quickly gained success due to its simplicity and versatility (1, 2). SSCP is based on the relation between the electrophoretic mobility of a single-stranded DNA and its folded conformation, which, in turn, reflects its nucleotide sequence. Therefore, any change in the sequence theoretically causes a shift in the mobility of the analyzed molecule upon electrophoresis in a neutral polyacrylamide gel. Practically a standard PCR–SSCP analysis requires the production of a labelled PCR fragment which is diluted 1/10 and heat denatured in 80% formamide. Subsequently the denatured product is quickly loaded onto a non-denaturing polyacrylamide gel.

Using the original SSCP conditions (1, 2) which call for variations of two parameters: plus or minus 10% glycerol and running at 4°C or room temperature we were in some instances, faced with imcomplete separations of wild type and variant conformers. This prompted us to study the effect, on the mobility of four different sequences bearing characterized mutations, of four parameters likely to affect the conformation of single-stranded DNA: (i) temperature, (ii) percentage of acrylamide, (iii) ionic strength of the electrophoretic buffer and (iv) glycerol concentration. Sequences used were five variants of a 60 bp fragment of human papilloma virus (HPV) differing from each other by a single point mutation (see legend of Figure 1) as well as three PCR amplified portions of the p53 coding sequences where point mutations had been identified (Table I).

Increasing the ionic strength of the running buffer (1.5×TBE, 135 mM Tris-Borate pH 8.3, 6 mM EDTA), raising the glycerol concentrations above 10% or increasing the polyacrylamide concentration above 6% did not result in any improvement. In contrast running the gels in 0.5×TBE often resulted in sharper bands and mobility shifts were enhanced in 5% glycerol (Table I and Figure 1E). As a general rule we found gels run at room temperature in 0.5×TBE and 5% glycerol (Figure 1E, Table IE) more discriminative. However, for certain sequences a second run at 4°C without glycerol was informative (condition B, Table I, Figure 1B). This set of experiments indicates how much the mobility of single stranded DNA molecules can be affected by variations in electrophoretic conditions and, therefore, that it is possible to take advantage of them to detect single nucleotide substitutions.

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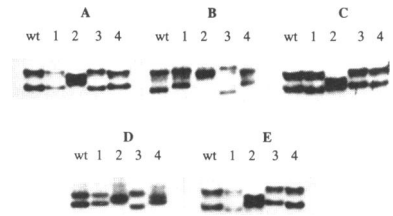

Figure 1. Effect of different SSCP electrophoretic conditions on the mobility of five sequences differing from each other by a single point mutation. These sequences, derived from HPV, are 60 bp long and are defined as follows: wt, wild type; 1, A_{33} is changed into G; 2, T_{34} to A; 3, A_{32} to T; 4, C_{35} to A. Running conditions are the following A: 1×TBE, no glycerol, 4°C; B: 0.5×TBE, no glycerol, 4°C; C: 0.5×TBE, 5% glycerol, 4°C; D: 1×TBE, 10% glycerol, room temperature; E: 0.5×TBE, 5% glycerol, room temperature.

Table I. Success in the detection of point mutations using different experimental conditions for SSCP.

	A 1×TBE 0% Gly 4°C	B **0.5×TBE** **0% Gly** **4°C**	C 0.5×TBE 5% Gly 4°C	D 1×TBE 10% Gly RT	E **0.5×TBE** **5% Gly** **RT**
p53 exon 5	+	+++	+	+	+++
p53 exon 5′	−	+	+	−	+++
p53 exon 7	+++	++	++	+	++
HPV-60 bp	+	+++	++	+	+++

+ or − signs indicate the quality of the discrimination between wild type and mutated conformers. Electrophoresis was done using a 6% polyacrylamide gel on an S2 BRL sequencing gel apparatus. Gels were run at 40 W constant power for 3 to 7 hours, depending on the conditions used. The most informative conditions are presented in bold characters. Four different sequences were analyzed using five alternative conditions. Results obtained with the HPV-60 bp sequences are those presented in Figure 1.

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