Single-Strand Conformation Polymorphism (SSCP) Analysis

PCR

SSCP analysis is generally considered to be most suitable for the detection of mutations in short stretches of DNA. Hence, the size of PCR fragments investigated are usually in the range of 175-250 bp. It is important to optimise the PCR reaction to minimise unwanted products which may interfere with gel analysis. The PCR products should be evaluated for purity by agarose gel electrophoresis before being loaded onto an SSCP gel.

Sample preparation

1. 150-300 ng of amplified DNA (usually about 10% of the total PCR volume) can be loaded per well. Aliquot proper amount of sample into separate tubes and add an equal volume of 2 x SSCP gel loading dye (0.05% bromophenol blue, 0.05% xylene cyanol, 95% formamide, 20 mM EDTA).
2. Prior to loading, denature the samples at 95^{o}C for 5 min and then place on ice.

Gel electrophoresis

Note: every primer set will require different conditions. Important variables includes run temperature (generally between 4C and room temp, although cooler is usually better; first testing 10C is recommended) and whether or not glycerol is added to the gel.

Electrophoresis was carried out on a Hoefer SE 600 system with 18 x 16 cm plates and 0.75 mm spacers *(short gels—as small as 8 cm—have also worked although every new locus may have different requirements).*

1. Prepare 6 % polyacrylamide gels (37.5:1 acrylamide to bisacrylamide) with and without 5% glycerol.

	+ glycerol	- glycerol
40% Acrylamide/Bis stock solution	4.5 mL	4.5 mL
50% Glycerol	3.0 mL	-
10 x TBE	1.5 mL	1.5 mL
distilled water up to	30 mL	30 mL

2. De-gas the acrylamide solution under vacuum until bubbles no longer appear (5-10 min).

3. Add 27 uL 10% ammonium persulfate (freshly made up) and 27 uL TEMED to the acrylamide solution (a final concentration of 0.09% (v/v) each of ammonium persulfate and TEMED solutions), mix by gentle swirling.
4. Cast the gel and leave to polymerise for about 2-3 h.
5. Remove the comb and wash the sample wells with 0.5 x TBE buffer to remove the unpolymerised polyacrylamide.
6. Run the gel in 0.5 x TBE buffer that has been pre-cooled to 10^{o}C. Place the gel tank in a tray filled with ice. Set the voltage to 200 V and run the gel for about 5 h. The temperature at the end of the run is about 17 ^{o}C.

Staining SSCP gels with SYBR® Gold

Alternatively, PCR products can be radiolabelled, or gels can be silver stained. We find SYBR Gold to be the easiest solution.

1. Remove the gel from the glass plate.
2. Place the gel into a dish containing 200 mL of running buffer and 1: 10 000 dilution SYBR® Gold (Molecular Probes, Inc.). Stain for about 30 min. *Note: this dye is rather expensive and bleaches easily. Avoid exposure to light during staining and use minimal volumes.*
3. Place the gel on a UV transilluminator and photograph using a SYBR Gold gel stain photographic filter (S-7569). *This filter is yellow in color; red-orange filters used to photograph ethidium bromide-stained gels can also be used, but sensitivity is reduced at least five-fold.*