Polymerase chain reaction—single strand conformational polymorphism (PCR–SSCP) is a simple and powerful technique for identifying sequence changes in amplified DNA. It is based on the observation that the mobility of single-stranded DNA in nondenaturing polyacrylamide gels is very sensitive to primary sequence, probably because slight sequence changes have major effects on conformation. In the absence of a robust theory for predicting mobility based on conformation and conformation based on sequence, SSCP remains largely empirical. Since SSCP techniques were last reviewed in this journal, there have been several improvements and new applications, which are discussed here.

SENSITIVITY

Much of the literature on SSCP deals with factors affecting sensitivity to detect single-base changes. Because reported results are always particular to specific fragments and sequence changes, generalizations can be problematic. Mutations that show no mobility shift under one set of conditions may be revealed under different conditions. Ranges of concentrations of acrylamide (usually from 4% to 12%) and of cross-linker bis-acrylamide (usually from 2% to 3.4% of the concentration of acrylamide) have been reported to be beneficial in particular circumstances as have additives such as 5–10% glycerol, 5% urea or formamide, and 10% dimethylsulfoxide or sucrose. Alterations in gel running temperature from 4°C to 37°C and changes in buffer concentration (possibly leading to changes in gel running temperature) may also help. Purine-rich strands may be more sensitive to base changes than pyrimidine-rich strands. Smaller fragments (<300 bp) are, in general, more likely to reveal single-base changes although fragment size and sequence context (the sequence of adjacent DNA) can have unpredictable effects on mobility shifts associated with particular base changes. RNA may be more sensitive to base-change-induced mobility shifts than DNA, and RNA can be made from amplified DNA by incorporating RNA polymerase promoters at the 5' ends of amplifying primers. At high concentration, denatured PCR products may reanneal quickly, complicating or preventing SSCP analysis; this may be prevented by including 33 mM methyl mercury (II) hydroxide in the denaturing sample buffer or by adding a “stacking” gel containing 75% formamide to keep complementary strands denatured until they separate in the gel.

Estimates of the proportion of mutations detectable by SSCP are affected by a number of SSCP conditions tested (temperature, glycerol concentration, acrylamide and bis-acrylamide concentrations, buffer concentration, etc.) but, in general, are in the 70% to >95% range for studies using two or three SSCP conditions. Thus, SSCP must still be considered a screening technique rather than a definitive means of identifying mutations.

The power of SSCP to detect mutations may be increased by performing SSCP analysis on dideoxy sequencing ladders derived from test fragments rather than on the fragments themselves. If there is a mutation, all dideoxy-terminated fragments greater than a certain size (corresponding to the position of the mutation) have non-wild-type sequence, and thus there are multiple chances to detect a mobility shift for each mutation.

ARTIFACTS

Free oligonucleotides present in a sample analyzed by SSCP can anneal to PCR product strands and alter their mobility. Even at concentrations as low as 150–6 nM, free oligonucleotides have been reported to lead to mobility shifts and subspecies. This problem can be avoided by diluting PCR products (~10−2 or greater) or by removing oligonucleotides prior to SSCP analysis.
For PCR products >200 bp, oligonucleotides are removed easily and quickly by passage over Sephacryl S-300 columns. A high concentration of PCR products often leads to the reannealing of complementary single strands, which can complicate the SSCP pattern. Although this can be avoided by diluting PCR products prior to denaturation for SSCP, use of dilute products necessitates sensitive detection methods such as autoradiography or silver staining. Temperature variation during electrophoresis can cause artifacts; temperature control with a special electrophoresis apparatus can solve this problem but results in added cost and complexity.

**NEW DETECTION METHODS**

Silver staining is nearly as sensitive as radiolabeling and provides a permanent record.\(^{(13-15)}\) Fluorescent dyes attached to oligonucleotides, in conjunction with sensitive fluorescence detectors, offer advantages in terms of automated data acquisition. Also, by running a marker DNA labeled with a different fluorophore in all lanes, corrections can be made for lane-to-lane variation in mobility.\(^{(16,17)}\)

**APPLICATIONS**

SSCP has been used most extensively to screen for inherited mutations\(^{(18-20)}\) or detect somatic mutations in cancer cells.\(^{(21,22)}\) Because of its sensitivity to single-base changes, SSCP has been used to search for polymorphisms in cloned or amplified DNA that can then be used as genetic markers. Extensive genetic maps using SSCP markers have been constructed in the mouse.\(^{(23,24)}\) SSCP can be used to purify different alleles amplified from a heterozygous individual to facilitate sequencing.\(^{(25)}\) SSCP can also be used to quantitate input DNA in a PCR, by adding known amounts of a sequence variant that is presumed to amplify with equal efficiency as the PCR target but migrates differently in SSCP (competitive PCR).\(^{(26)}\) In microbiology, SSCP has been used to classify virus strains.\(^{(27)}\)

SSCP can aid in the identification of mutations that are selected for in various bacteriological or viral systems.\(^{(28,29)}\) For example, we used SSCP to identify changes in human immunodeficiency virus (HIV-1) associated with adaptation to growth in T-cell lines.\(^{(29)}\) An advantage of SSCP in this setting is that non-wild-type SSCP bands correspond to mutations affecting a significant proportion of viral genomes, reflecting biological selection. We amplified overlapping segments of ~300 bp spanning the HIV envelope gene and found SSCP changes in one group of overlapping fragments. These fragments were cloned and sequenced to identify common sequence changes. We found that SSCP was also useful in identifying desired clones, as these clones contained inserts with SSCP patterns that matched the non-wild-type SSCP species seen on analysis of total proviral DNA. Variant species identified by SSCP were reinserted into a wild-type infectious viral clone and tested to determine whether they conferred enhanced ability to grow in T-cell lines. When SSCP is used to identify sequence changes associated with a selected phenotype, it is very important to have a confirmatory test of the biological significance of identified sequence changes. Several completely in vitro systems have been described for "molecular evolution" under selective pressure;\(^{(30,31)}\) SSCP analysis might be useful in these systems to identify strongly selected molecular variants.

SSCP is a powerful method for identifying sequence variation in amplified DNA. Recent refinements and new applications discussed here add significantly to its utility.

**SSCP PROTOCOL**

The following is an example of an SSCP protocol that we used for detection
of mutations in HIV-1 DNA. PCR conditions may need to be changed depending on target and primer sequences.

1. Amplification and Labeling of DNA by PCR
A typical 10-µl reaction mixture contains 1 µg of DNA, 5 pmol of each primer, 0.5 units of Taq polymerase, each deoxynucleoside triphosphate at 0.2 mM, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 0.01% gelatin. DNA is labeled by adding 0.5 µCi of [³²P]dCTP (3000 Ci/mmol) to the PCR mixture or by using ³²P-end-labeled primer(s). PCR conditions are 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec.

2. Denaturation of PCR Product
The amplified fragments are mixed with an equal volume of sample buffer [95% formamide, 20 mM EDTA (pH 8.0), 0.05% xylene cyanol, and 0.05% bromophenol blue], denatured at 85°C for 5 min, and cooled on ice. If possible artifacts owing to the presence of free primer are of concern, PCR-amplified DNA is diluted 1:100 with TE buffer or passed over a Sephacryl S-300 column before denaturation.

3. Gel Conditions
One hundred milliliters of a 5% nondenaturing gel containing 17 ml of 30% polyacrylamide–0.8% bisacrylamide mixture, 30 µl of TEMED, and 0.8 ml of 10% ammonium persulfate in 1× TBE (90 mM Tris-borate at pH 8.3, 4 mM EDTA) is prepared and cast in standard sequencing gel plates using 0.4-mm spacers and a sharkstooth comb. The cast gel is cooled in a cold room for at least 30 min prior to sample loading. Denatured DNA is loaded (2 µl/well) and electrophoresed at 4°C at a constant voltage of 500 V for 12–15 hr or 1100 V for 3–5 hr in 1× TBE electrophoresis buffer. After electrophoresis, the gel is transferred onto a sheet of Whatmann 3MM paper and vacuum-dryed before autoradiography.

REFERENCES