Single-strand Conformational Polymorphism

Kazunobu Fujita and
Jonathan Silver

Laboratory of Molecular
Microbiology, National Institute of
Allergy and Infectious Diseases,
National Institutes of Health,
Bethesda, Maryland 20892

Polymerase chain reaction–single strand conformational polymorphism (PCR–SSCP) is a simple and powerful technique for identifying sequence changes in amplified DNA.⁽¹⁾ 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,⁽²⁾ 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,^(3–5) as have additives such as 5–10% glycerol, 5% urea or formamide, and 10% dimethylsulfoxide or sucrose.^(1,4) 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.⁽⁴⁾ Smaller fragments (<300 bp) are, in general, more likely to reveal single-base changes,^(2,5,6) although fragment size and sequence context (the sequence of adjacent DNA) can have unpredictable effects on mobility shifts associated with particular base changes.⁽⁷⁾ 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.⁽⁸⁾ 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⁽⁹⁾ or by adding a "stacking" gel containing 75% formamide⁽¹⁰⁾ 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.^(4–7) 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.⁽¹¹⁾ 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.⁽¹²⁾ This problem can be avoided by diluting PCR products ($\sim 10^{-2}$ 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- μ l reaction mixture contains 1 μ g of DNA, 5 pmoles 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 μ Ci 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 μ l of TEMED, and 0.8 ml of 10% ammonium persulfate in 1 $\times$ 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 sharktooth comb. The cast gel is cooled in a cold room for at least 30 min prior to sample loading. Denatured DNA is loaded (2 μ l/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 $\times$ TBE electrophoresis buffer. After electrophoresis, the gel is transferred onto a sheet of Whatmann 3MM paper and vacuum-dried before autoradiography.

REFERENCES

1. Orita, M., Y. Suzuki, T. Sekiya, and K. Hayashi. 1989. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* **5**: 874–879.
2. Hayashi, K. 1991. PCR-SSCP: A simple and sensitive method for detection of mutations in genomic DNA. *PCR Methods Applic.* **1**: 34–38.
3. Savov, A., D. Angelicheva, A. Jordanova, A. Eigel, and L. Kalaydjieva. 1992. High percentage acrylamide gels improve resolution in SSCP analysis. *Nucleic Acids Res.* **20**: 6741–6742.
4. Glavac, D. and M. Dean. 1993. Optimization of the single-strand conformation polymorphism (SSCP) technique for detection of point mutations. *Hum. Mutat.* **2**: 404–414.
5. Hayashi, K. and D.W. Yandell. 1993. How sensitive is PCR-SSCP? *Hum. Mutat.* **2**: 338–346.
6. Sheffield, V.C., J.S. Beck, A.E. Kwitek, D.W. Sandstrom, and E.M. Stone. 1993. The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. *Genomics* **16**: 325–332.
7. Fan, E., D.B. Levin, B.W. Glickman, and D.M. Logan. 1993. Limitations in the use of SSCP analysis. *Mutat. Res.* **288**: 85–92.
8. Sarkar, G., H.-S. Yoon, and S.S. Sommer. 1992. Screening for mutations by RNA single-strand conformation polymorphism (rSSCP): Comparison with DNA-SSCP. *Nucleic Acids Res.* **20**: 871–878.
9. Weghorst, C.M. and G.S. Buzard. 1993. Enhanced single-strand conformation polymorphism (SSCP) detection of point mutations utilizing methylmercury hydroxide. *BioTechniques* **15**: 397–400.
10. Yap, E.P.H. and J.O'D. McGee. 1993. Nonisotopic discontinuous phase single strand conformation polymorphism (DP-SSCP): Genetic profiling of D-loop of human mitochondrial (mt) DNA. *Nucleic Acids Res.* **21**: 4155.
11. Sarkar, G., H.-S. Yoon, and S.S. Sommer. 1992. Dideoxy fingerprinting (ddF): A rapid and efficient screen for the presence of mutations. *Genomics* **13**: 441–443.
12. Cai, Q.-Q. and I. Touitou. 1993. Excess PCR primers may dramatically affect SSCP efficiency. *Nucleic Acids Res.* **21**: 3909–3910.
13. Ainsworth, P.J., L.C. Surh, and M.B. Coulter-Mackie. 1991. Diagnostic single strand confor-

- mational polymorphism, (SSCP): A simplified non-radioisotopic method as applied to a Tay-Sachs B1 variant. *Nucleic Acids Res.* **19**: 405–406.
14. Mohabeer, A.J., A.L. Hiti, and W.J. Martin. 1991. Non-radioactive single strand conformation polymorphism (SSCP) using the Pharmacia "PhastSystem." *Nucleic Acids Res.* **19**: 3154.
 15. Oto, M., S. Miyake, and Y. Yuasa. 1993. Optimization of nonradioisotopic single strand conformation polymorphism analysis with a conventional minislabs gel electrophoresis apparatus. *Anal. Biochem.* **213**: 19–22.
 16. Makino, R., H. Yazyu, Y. Kishimoto, T. Sekiya, and K. Hayashi. 1992. F-SSCP: Fluorescence-based polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis. *PCR Methods Applic.* **2**: 10–13.
 17. Ellison, J., M. Dean, and D. Goldman. 1993. Efficacy of fluorescence-based PCR-SSCP for detection of point mutations. *BioTechniques* **15**: 684–691.
 18. Michaud, J., L.C. Brody, G. Steel, G. Fontaine, L.S. Martin, D. Valle, and G. Mitchell. 1992. Strand-separating conformational polymorphism analysis: Efficacy of detection of point mutations in the human ornithine δ -aminotransferase gene. *Genomics* **13**: 389–394.
 19. Dean, M., M.B. White, J. Amos, B. Gerrard, C. Stewart, K.-T. Khaw, and M. Leppert. 1990. Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. *Cell* **61**: 863–870.
 20. Leren, T.P., K. Solberg, O.K. Rodningen, L. Ose, S. Tonstad, and K. Berg. 1993. Evaluation of running conditions for SSCP analysis: Application of SSCP for detection of point mutations in the LDL receptor gene. *PCR Methods Applic.* **3**: 159–162.
 21. Jacquemier, J., J. Moles, F. Penault-Llorca, J. Adelaide, M. Torrente, P. Viens, D. Birnbaum, and C. Theillet. 1994. p53 immunohistochemical analysis in breast cancer with four monoclonal antibodies: Comparison of staining and PCR-SSCP results. *Br. J. Cancer* **69**: 846–852.
 22. Condie, A., R. Eeles, A.-L. Borresen, C. Coles, C. Cooper, and J. Prosser. 1993. Detection of point mutations in the p53 gene: Comparison of single-strand conformation polymorphism, constant denaturant gel electrophoresis, and hydroxylamine and osmium tetroxide techniques. *Hum. Mutat.* **2**: 58–66.
 23. Beier, D.R. 1993. Single-strand conformation polymorphism (SSCP) analysis as a tool for genetic mapping. *Mamm. Genome* **4**: 627–631.
 24. Hunter, K.W., M.L. Watson, J. Rochelle, S. Ontiveros, D. Munroe, M.F. Seldin, and D.E. Housman. 1993. Single-strand conformational polymorphism (SSCP) mapping of the mouse genome: Integration of the SSCP, microsatellite, and gene maps of mouse chromosome 1. *Genomics* **18**: 510–519.
 25. Suzuki, Y., T. Sekiya, and K. Hayashi. 1991. Allele-specific polymerase chain reaction: A method for amplification and sequence determination of a single component among a mixture of sequence variants. *Anal. Biochem.* **192**: 82–84.
 26. Yap, E.P.H. and J.O'D. McGee. 1992. Nonisotopic SSCP and competitive PCR for DNA quantification: p53 in breast cancer cells. *Nucleic Acids Res.* **20**: 145.
 27. Lin, J.-C., B.K. De, and S.-C. Lin. 1993. Rapid and sensitive genotyping of Epstein-Barr virus using single-strand conformation polymorphism analysis of polymerase chain reaction products. *J. Virol. Methods* **43**: 233–246.
 28. Morohoshi, F., K. Hayashi, and N. Munakata. 1991. Molecular analysis of *Bacillus subtilis* *ada* mutants deficient in the adaptive response to simple alkylating agents. *J. Bacteriol.* **173**: 7834–7840.
 29. Fujita, K., J. Silver, and K. Peden. 1992. Changes in both gp120 and gp41 can account for increased growth potential and expanded host range of human immunodeficiency virus type 1. *J. Virol.* **66**: 4445–4451.
 30. Robertson, D.L. and G.F. Joyce. 1990. Selection *in vitro* of an RNA enzyme that specifically cleaves single-stranded DNA. *Nature* **344**: 467–468.
 31. Bartel, D.P., M.L. Zapp, M.R. Green, and J.W. Szostak. 1991. HIV-1 rev regulation involves recognition of non-Watson-Crick base pairs in viral RNA. *Cell* **67**: 529–536.

Single-strand conformational polymorphism.

K Fujita and J Silver

Genome Res. 1994 4: S137-S140

References

This article cites 31 articles, 2 of which can be accessed free at:
<http://genome.cshlp.org/content/4/3/S137.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

**Affordable, Accurate
Sequencing.**

To subscribe to *Genome Research* go to:
<https://genome.cshlp.org/subscriptions>