

# Single strand conformation polymorphism: principle and troubleshoots

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## Abstract

One of the common techniques used to identify unknown mutations is Single Strand Conformation Polymorphism (SSCP). SSCP is used frequently because the technique is simple, fast, sensitive and reproducible. It has been frequently reported that it is very easy to establish this technique with minimal infrastructure and expenditure. This short communication presents the basic principles of SSCP and possible troubleshoots of standardization of this technique in the lab.

**Keywords:** Single strand conformation polymorphism; PCR; Primer

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## Introduction

Mutations are permanent changes in DNA either in germ line or in somatic cells. Once in the germ cells, mutations result in conception of an individual defected from birth. Defects can be in the form of insertion, deletion or translocations [1]. Human genome project mapped the human genetic code and enabled us to understand the molecular genetic defects. Since then, molecular methods of detecting mutations have evolved from older time-consuming tedious methods to rapid and easy experiments that can be completed in basic laboratories. For identifying a disease, molecular analysis of the mutations can be a great tool. Mutation detection methods formally divided into 2 steps: change in DNA primary structure followed by visualization of changed products by labeling that screen the mutations. The other approach of mutational analysis involves methods based upon previous knowledge of genetic aberrations (known mutations) or screening of unknown mutations.

The usage of a technique for mutational screening depends upon a number of factors (1) the size of the gene of interest and (2) the available resources; sensitivity and specificity required. One of the common techniques used to identify unknown mutations is SSCP [2]. SSCP is used frequently because the technique is simple, fast, sensitive and reproducible. Moreover, it is very easy to establish this technique with minimal infrastructure and expenditure. It does not involve radioactivity thus making it easier. SSCP showed better resolution as compared to other mutation screening techniques like RFLP in distinguishing different mutations [3].

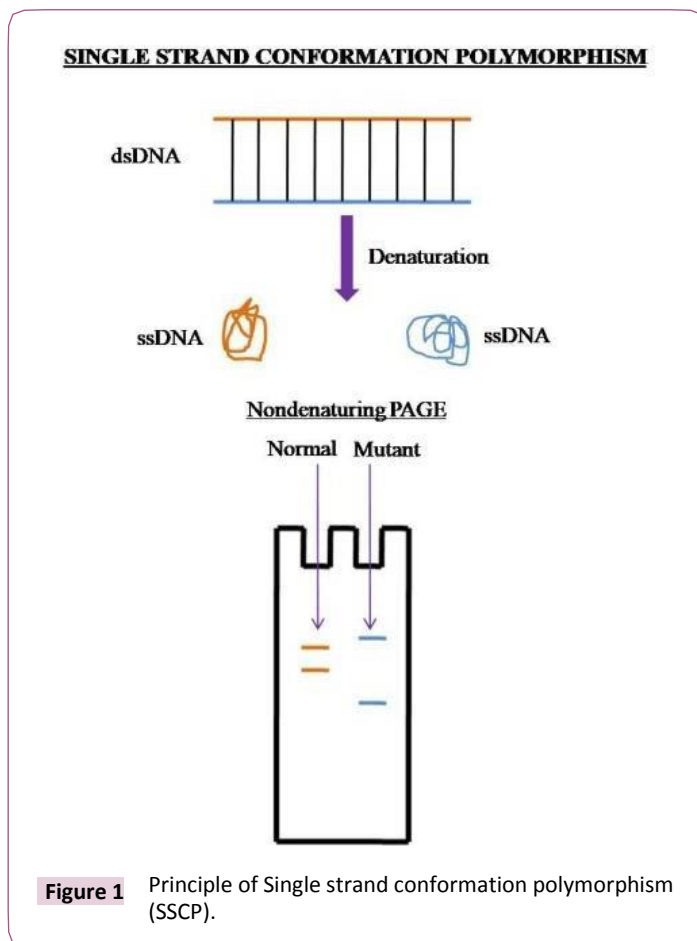
SSCP can identify deletion, insertion or rearrangements in amplified DNA. Identification of novel gene mutations improves our understanding of genetic disorders. The technique was first described by Orita and Hongyo and since then it has been used

extensively [4-9]. Although recommended as a reliable and sensitive technique, the above stated studies also confirmed their results of PCR-SSCP by sequencing. SSCP is an adequate and rapid screening technique useful to identify nucleotide changes as a first approach [8]. RFLP and SSCP were compared and shown that the latter was more reliable for identifying mutants. Although RFLP identified the mutants but SSCP provided higher resolutions for the identification of different mutations [3]. Several modifications have been done to effectively identify conformational changes and migration pattern of ssDNA. DNA fragments can be easily visualized by silver staining, ethidium bromide and fluorescent labelled primers [10-12].

## Principle and Troubleshoots

In PCR-amplified product, SSCP can detect even a single nucleotide change. It is based on the principle that changes in nucleic acids affect the migration of ssDNA fragment. This leads to change in visible mobility across a non-denaturing polyacrylamide gel [4] (Figure 1). The differential mobility of test and control sample may suggest possibility of mutations. The possible mutations can be screened further to determine the nucleotide change.

For conducting SSCP, DNA is isolated from blood or tissue followed by primer designing and PCR amplification. For optimal visualization of the amplified product, the PCR amplicon is run on non-denaturing polyacrylamide gel. After the primer band confirmation, the PCR amplified products are processed further for SSCP. The detailed steps can be reviewed from our previous publication [9].



SSCP is a simple and rapid method that is easily reproducible for detecting mutations (deletions, insertions and rearrangements) in PCR of amplified DNA [13]. For better results, fragment size should be between 150-300 bp [14]. Under non-denaturing conditions, ssDNA acquires a unique conformation and even if a single base alteration is present it can result in a conformational change [15]. This change can be detected by the altered mobility of these ssDNA. The conformation and movement of ssDNA on polyacrylamide gel can be affected by various factors like type of mutation, size of DNA fragment, GC content, gel matrix composition, temperature of the gel, concentration, pH of buffer and presence of denaturing agents in gel [4]. While standardizing SSCP, following troubleshootings may need optimizing the experiment conditions:

- (1) The denaturation of the PCR products is a crucial step. As given in the literature, formamide should be added in equal quantity as of the PCR amplicon. But, our previous study showed better results when formamide concentration used was more than the PCR product [9].
- (2) As the gel needs to be run at higher voltage, maintenance of low temperature is required. Therefore, the central cooling core of electrophoretic setup should be filled with water or coolant, or it is kept in 4°C/ cold room when it is to run overnight.
- (3) Setting up of electrophoretic unit is also crucial. Proper alignment of the glass plates is required to avoid leakage of the buffer as it may cause current drop many times. Continuous drop in current causes resistance in movement of bands and

thus bands produced may be of poor resolution. Any drop or fluctuation in the voltage should also be prevented.

Buffer quality is equally important. Buffer should not be used more than twice and should be loaded after cleaning of glass plates at each time when gel is run. Spilling of the sample outside the well should also be prevented in order to avoid ambiguous results.

## References

1. Al-Haggag M (2013) Evolving molecular methods for detection of mutations. *Gene Technology* 2:1-2.
2. Kakavas VK, Noulas A, Chalkias C, Hadjichristodoulou C, Georgiou L, et al (2006) Identification of the four most common  $\beta$ -globin gene mutations in greek  $\beta$ -thalassaemic patients and carriers by non-radioactive PCR-SSCP: Advantages and limitations of the method *JCLA* 20:1-7.
3. Zhou J, Deng K, Cheng Y, Zhong Z, Tian L, et al (2017) CRISPR-Cas9 based genome editing reveals new insights into microRNA function and regulation in rice 13:1-12.
4. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T, et al (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proceedings of the National Academy of Science* 86:2766-2770.
5. Kumar D, Gupta N, Ahlawat SPS, Campos M, Lavinha J, et al (2006) Single strand confirmation polymorphism (SSCP) detection in exon I of the  $\alpha$ -lactalbumin gene of Indian Jamunapari milk goats (*Capra hircus*). *Genetics and Molecular Biology* 29(2): 287-289.
6. Dubey PP, Sharma A, Gour DS (2008) Leptin gene polymorphism in Indian Sahiwal cattle by single strand conformation polymorphism (SSCP). *South African Journal of Animal Science* 38:131-136.
7. Rodríguez F, Cai D, Teng Y, Spooner D (2011). Asymmetric single-strand conformation polymorphism: an accurate and cost-effective method to amplify and sequence allelic variants. *Am J Bot* 98:1061-1067.
8. Barrera GP, Villamizar LF, Espinel C, Quintero EM, Belaich MN, et al (2017) Identification of *Diatraea* spp. (Lepidoptera:Crambidae) based on cytochrome oxidase II. *PLoS ONE* 12:e0184053.
9. Rishi I, Halder A, Sharma JB, Jain M, Sharma M, et al. (2020) Single Strand Conformation Polymorphism and sequencing to detect HS6T2 gene mutations in patients of idiopathic premature ovarian failure. *JCDR* 1-9.
10. Merril CR (1990) Silver staining of proteins and DNA. *Nature* 343:779-780.
11. Xie T, Ho SL, Ma OCK (1997) High resolution SSCP analysis using formamide and ethidium bromide staining. *Mol Pathol* 50:276-278.
12. Takahashi A, Ishino Y, Kato I, Fukumaki Y (1994) Rapid and practical detection of  $\beta$ -globin mutations causing  $\beta$ -thalassaemia by fluorescence-based PCR-single strand conformation polymorphism analysis. *Mol Cell Probes* 8:385-393.
13. Kakavas VK, Plageras P, Vlachos TA, Papaioannou A, Noulas VA, et al (2008) PCR-SSCP: a method for the molecular analysis of genetic diseases *Mol Biotechnol* 38:155-163.
14. Hayashi K, Yandell DW (1993) How sensitive is PCR-SSCP? *Hum Mutat* 2:338-346.
15. Kusakabe T, Maekawa K, Ichikawa A, Uesugi M, Sugiura Y, et al (1993) Conformation-selective DNA strand breaks by dynemicin: A molecular wedge into flexible regions of DNA. *Biochemistry* 32: 11669-11675.