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REVIEW

Sensitivity and applications of the PCR Single-Strand Conformation Polymorphism method

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Abstract

PCR Single-Strand Conformation Polymorphism is a method used to identify and detect mutations and is now well known for its many applications on living beings. This paper will discuss the experimental details, limitations and sensitivity of the PCR Single-Strand Conformation Polymorphism method in relation to all existing literature available to us until today. Genomic DNA extraction, PCR amplification and Single-Strand Conformation Polymorphism conditions (concentration of polyacrylamide slab gel electrophoresis, dissociation treatment of double- stranded DNA) and comparison with PCR Restriction Fragment Length Polymorphism are presented. Since its discovery in 1989, there have been many variations, innovations, and modifications of the method, which makes it very easy, safe, fast and for this reason widely applied in clinical diagnostic, forensic medicine, biochemical, veterinary, microbiological, food and environmental laboratories. One of the possible applications of the method is the diagnosis and identification of mutations in new strains of coronaviruses, because science needs more tools to tackle the problem of this pandemic. The PCR Single-Strand Conformation Polymorphism method can be applied in many cases provided that control samples are available and the required conditions of the method are achieved.

Keywords SSCP · Applications · Coronavirus

Abbreviations

dsDNA	Double-Stranded DNA
mtDNA	Mitochondrial DNA
PCR-SSCP	Polymerase Chain Reaction—Single-Strand Conformation Polymorphism
PCR–RFLP	Polymerase Chain Reaction—Restriction Fragment Length Polymorphism
SSCP	Single-Strand Conformation Polymorphism
ssDNA	Single-Stranded DNA
SNPs	Single Nucleotide Polymorphisms
SARS-CoV-2	Coronavirus

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SSCP analysis

The SSCP method follows the steps of DNA extraction, PCR amplification of the target fragment and finally denaturation of the double-stranded PCR product by heat and formamide and electrophoresis on a non-denaturing polyacrylamide gel. During electrophoresis, single-stranded DNAs are converted to stable conformations (there is none theory can predict the exact folded structure of ssDNA) depending on their major sequence. The SSCP method is based on the ability of single-stranded DNAs to fall into unique conformations depending on their primary sequence whose structures are stabilized by intramolecular interactions, under non-denaturing conditions. As a result, even a base change can lead to a modulation change, which can be detected by the altered mobility of the single-stranded DNA molecule in the SSCP method. Therefore, for each ssDNA fragment, the number of constant configurations that create bands of different mobility during SSCP electrophoresis must be determined experimentally under strictly controlled conditions. Several parameters have been found empirically to affect the sensitivity of SSCP analysis [1–5]. Among them is (i) a type of mutation (base substitutions, small insertions, deletions and rearrangements); (ii) DNA fragment size (no longer than

300 bp); (iii) G and C fragment; (iv) polyacrylamide (use of low percentage of crosslinker) or other gel matrix composition (addition of glycerol enhances the sensitivity); (v) gel size and potential; (vi) electrophoresis gel temperature (ssDNA have more compact conformation at lower temperatures); (vii) DNA concentration; (viii) buffer composition, including ionic strength and pH (low pH buffer can improve the sensitivity) and (ix) buffer additives. All these parameters are described in detail by Kakavas et al. [6].

SSCP vs RFLP

A detailed comparison of these methods is made in the work of Hashim et al. [7]. Both of them have advantages and disadvantages. On the one hand PCR-SSCP can detect unknown mutations and is rapid, inexpensive and convenient on the other hand PCR-RLFP is fast and can detect only known SNPs. We can't claim that one method is better than the other.

Medical diagnosis applications

Detection of somatic mutations in cancer, genes responsible for hereditary diseases, polymorphisms and other applications are described in detail at Kakavas et al. [8–10]. Recent researches in the last 4 years shows dispersion of SSCP method in the above applications: on cancer prognosis [11, 12], on asthma disease [13], on blood groups [14], on gilbert's syndrome [15], on diabetes disease [16], on respiratory distress syndrome [17], on infertile men with *varicocele* [18], on gastric mucosa [19], on traditional Chinese medicine [20], detection of bacterial DNA [21], identification of *trichomonas vaginalis* [22].

Other applications

Animals

PCR-SSCP has been used for several traits of animals such as wool characteristics [23], milk production traits [24], profile of *sheep* fatty acids [25], detection of *Escherichia coli* in the *black swan* [26], growth and carcass traits in *lambs* [27], reproductive traits in *cattle* [28], egg production traits in *hens* [29], body weight traits on *chicken* [30–32], gene study for fat-tailed and nonfat-tailed *sheep* [33], serotyping *dichelobacter nodosus* [34], mitochondrial diversity role in the productivity of *quails* [35], *miR-9* gene affects litter size in *goats* [36], mutations in *porcine* [37], *prolactin* gene and association with egg production in *ducks* [38], wool fibre diameter in *ewes* [39], *lactoferrin* gene affects milk content in *buffaloes* [40], milk traits in *yaks* [41], association between *MyoG* gene and egg quality [42].

Birds

Genes identification in *ostrich* populations [43] and mtDNA identification of *Alectoris rufa* populations [44].

Fishes

Identification of *shrimp* species [45], SNPs and white spot syndrome virus resistance in *black tiger shrimp* [46].

Amphibians

Caretta-caretta mtDNA polymorphisms detection [47].

Plants

PCR-SSCP could be used for the authentication of *snapper* species by SSCP [48], the population genetic structure of *zeugodacus tau* species complex [49], for the genetic diversity of *citrus* trees [50], identification of *phytophthora* genes polymorphism of *potato* [51], identification of genetic diversity of *lemon* and *mandarin* varieties [52], detection of the genetic variation of *alfalfa* gene [53], prediction of the *chloroplast* gene polymorphism in *barley* varieties [54] and bacteria identification in wood *tick* [55].

Microbial organisms

Molecular epidemiological studies on the exposure of farm children to bacteria in environmental dust [56], analysis of the fungal flora in environmental dust samples [57], development of methods to analyze bacterial species diversity in freshwater and soil ecosystems [58], impact of Fe oxides mineralogy on iron solubilization and associated microbial communities [59], difference in some biological properties of soils [60], gene comparison from culturable denitrifying bacteria [61], food waste composting and microbial community structure profiling [62].

Food

Microbial communities identification in cheese [63] and for food authenticity [64].

Applications in forensic medicine and biochemistry

Usually, a big number of biological samples used, are found in a crime because SSCP uses mtDNA for it´s different variations [65, 66].

Table 1 Applications of SSCP

Field of Applied Genetic polymorphism	References
Medical diagnosis applications	
β-globin gene mutations in Greek β-thalassemic patients and carriers with PCR-SSCP	[8]
Cystic fibrosis transmembrane regulator gene ΔF508 mutation with PCR-SSCP	[9]
Association of the CTCF gene with breast cancer progression	[11]
TCF 4 tumor suppressor: prognosis of sporadic colorectal cancer in humans	[12]
Haplotype in ABCC4 gene by PCR-SSCP technique in Iraqi asthmatic patients	[13]
O blood group in India: Peeping through the window of molecular biology	[14]
Gilbert's syndrome associated UDP-glucuronyl transferase on serum bilirubin level	[15]
Polymorphism in genes and female fecundity in diabetes type 2	[16]
Respiratory distress syndrome and SP-A1 gene polymorphism in Mongolian infants	[17]
Mitochondrial genetic variation in Iranian infertile men with varicocele	[18]
Effect of DNMT3A polymorphisms on CpG island hypermethylation in gastric mucosa	[19]
Methods in traditional Chinese medicine based on cytochrome b gene	[20]
Detection of bacterial DNA on neurostimulation systems in patients without overt infection	[21]
Genetic identification of trichomonas vaginalis by using the actin gene and molecular based methods	[22]
Animals	
Polymorphism of KRT83 and its association with selected wool traits in Merino-cross lambs	[23]
Association between genes polymorphisms with milk and the profile of fatty acids in sheeps	[24, 25]
First sporadic case of pathogenic *Escherichia coli* infection in black swan in china	[26]
Variation of the ovine uncoupling protein-1 gene and association with growth and carcass traits in New Zealand Romney lambs	[27]
Polymorphisms in synthetase gene with reproductive traits in indigenous and cross-bred cattle of Indian Origin	[28]
Genetic effects of the transcription factors on egg production-related traits in Chinese hens	[29]
Body weight, composition traits on chicken	[30–32]
Gene study for fat-tailed and nonfat-tailed sheep	[33]
Serotyping *Dichelobacter nodosus*	[34]
Mitochondrial diversity role in the productivity of quails	[35]
miR-9 gene affects litter size in goats	[36]
Mutations in porcine	[37]
Prolactin gene and association with egg production in ducks	[38]
Wool fibre diameter in new Zealand Romney ewes	[39]
Lactoferrin gene affects milk content in buffaloes	[40]
Variation in the fatty acid synthase gene and its association with milk traits in gannan yaks	[41]
Association between gene polymorphism and egg quality of egg quail	[42]
Birds	
Variations in genes are the potential adaptation of the immigrated African ostrich population	[43]
Mitochondrial DNA variability in populations of *Alectoris rufa*	[44]
Fishes	
Identification of shrimp species	[45]
SNPs and white spot syndrome virus resistance in black tiger shrimp	[46]
Amphibians	
Detection of polymorphisms of the mtDNA control region of *Caretta caretta*	[47]
Plants	
Authentication of snapper species by SSCP	[48]
Population genetic structure of *Zeugodacus tau* species complex in Thailand	[49]
Genetic diversity of citrus trees	[50]
Phytophthora genes polymorphism of potato	[51]
Identification of genetic diversity of lemon and mandarin varieties	[52]
Detection of the genetic variation of alfalfa gene	[53]
Prediction of the chloroplast gene polymorphism in barley varieties	[54]
Identification of bacteria in wood tick	[55]

Table 1 (continued)

Field of Applied Genetic polymorphism	References
Microbial organisms	
Epidemiological studies on the exposure of farm children to bacteria and fungal flora in dust	[56, 57]
Molecular biological methods to analyze bacterial species diversity in freshwater and soil ecosystems	[58]
Impact of Fe(III) oxides mineralogy on iron solubilization and associated microbial communities	[59]
Difference in some biological properties of soil under sugarcane cultivation	[60]
Community structures and comparison of nosZ and 16S rRNA genes from culturable denitrifying bacteria	[61]
Food waste composting and microbial community structure profiling	[62]
Food	
Culture-independent methods for identifying microbial communities in cheese	[63]
DNA Techniques to Verify Food Authenticity	[64]
Applications in forensic medicine and biochemistry	
Fluorescent SSCP of overlapping fragments: A highly sensitive method for the screening of mtDNA variation	[65]
The application of PCR-SSCP in forensic mtDNA typing	[66]
Future applications	
Biological importance of the mutations detected in the genome of feline coronaviruses from naturally infected cats	[67]

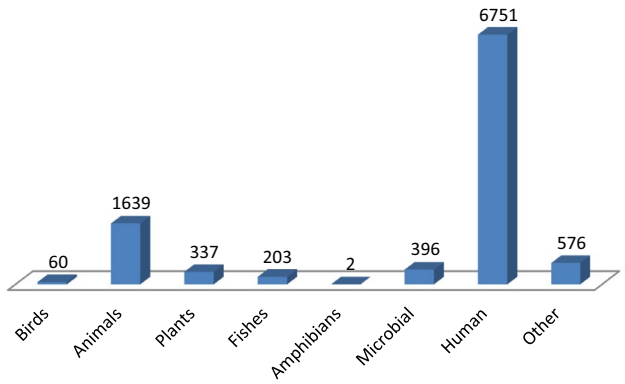

Fig. 1 Number of SSCP applications on deferent living beings

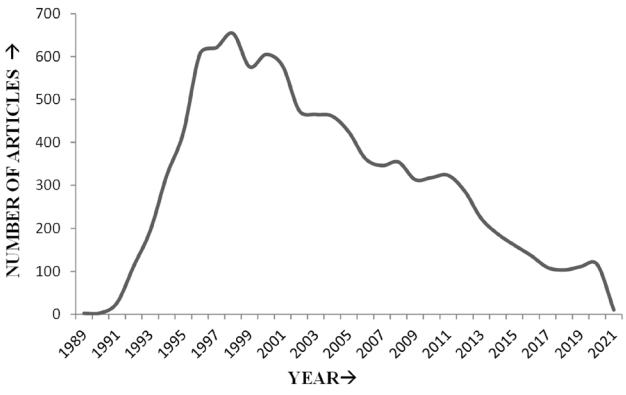

Fig. 2 Number of articles on the subject of SSCP over the past 32 years

An application with coronavirus

A very interesting research work concerned the cat infection with *coronaviruses* by Battilani et al. [67]. This study investigated the biological importance of the mutations detected in the genome of *feline coronaviruses* from naturally infected *cats*.

All the applications of SSCP analyzed above are shown in Table 1 and Fig. 1.

An exhaustive search in the literature determined that the SSCP method was first used in 1989, it reached its peak in the year 1999 and by 2021 demonstrated a downward trend with spread to applications of environmental, veterinary and forensic medicine interest. The total of the research works amounts to 9.944 in a depth of 32 years (Fig. 2). This may encourage us to use SSCP in routine analyses in clinical, environmental, veterinary, microbiological, and forensic medicine laboratories.

Conclusions

The PCR-SSCP method is widely used in the detection of mutations in both basic and applied biological and environmental science. The separation of mutated sequences could be further improved by using a gel matrix rather than a polyacrylamide one [68] or adding agents that can affect the folding of single-stranded DNA. Thus, PCR-SSCP is considered still modern as a method not only to screen sequence variations but also to identify new mutations. Among the various methods used for the detection of mutations, SSCP is one of the simplest and most sensitive methods, thus making it

attractive for use in the clinical diagnostic, veterinary, environmental, microbiological, food and forensic medicine laboratories. Everyone's expectation for the future application of SSCP technique in genotyping experiments is very high especially with the detection of different and new coronavirus (SARS-CoV-2) strains.

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Author contributions KK began his career with the establishment of the SSCP method during his doctoral dissertation in the Laboratory of Biochemistry, Medical school of the University of Thessaly. Now from his new position as a Lecturer in the Department of Environment at the same University, he is focusing on expanding this method in other applications. KK is the one who implemented all the experiments from 1999 until today and the inspirer of this manuscript.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The author declares that all the data are available from the author.

Declarations

Conflict of interest The author declares that he has no competing interests.

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