DETECTION OF SINGLE BASE CHANGES IN PCR-AMPLIFIED DNA USING DOUBLE AND SINGLE STRAND CONFORMATIONAL POLYMORPHISMS (SSCP AND DSCP)

F. Barros, M.S. Rodriguez-Calvo, I. Muñoz, C. Pestoni, M.V. Lareu and A. Carracedo

Institute of Legal Medicine, Faculty of Medicine, 15705 Santiago de Compostela. Spain.

Among the methods to detect single base changes (without sequencing) DSCP (Barros, 1991), also known as HA, is, together with SSCP (Orita, 1989), one of the more widely used.
The application of DSCP and SSCP in Forensic Genetics is investigated through the study of variation in coding DNA (HLA-Class II loci), the analysis of sequence variation in STRs and the analysis of mt-DNA

METHODS

PCR amplification:

System	Primer	PCR mixture
HLA-DQA1	5' GTGCTGCAGGTGTAAACTTGTACCAG 3'	A
	5' CACGGATCCGGTAGCAGCGGTAGAGTTG 3'	
HLA-DPB1	5' CAGGTACCCGCAGAGAATTAC 3'	A
	5' CCCTCACTCACCTCGGCG 3'	
HUMTH01	5' GTGGGCTGAAAAGCTCCCGATTAT 3'	B
	5' ATTCAAAGGGTATCTGGGTCTTGG 3'	
mtDNA	5' CACCATTAGCACCCAAAG 3'	C
	5' AGGGGGGGTTTGGTGGAAATTT 3'	

Mixture A: 1 - 10 ng DNA, 10 mM Tris-CLH (ph 8.3), 2.5 mM MgCl2, 50 mM Kcl, 0.01% gelatin, 200 µM of each dNTP, 0.2 µM of each primer and 2.5 units of AmpliTaq DNA Polymerase (Cetus, Emeryville, USA).

Mixture B: 5 - 25 ng DNA, 10 mM Tris-CLH (ph 8.3), 1.5 mM MgCl2, 50 mM Kcl, 0.01% gelatin, 200 µM of each dNTP, 0.25 µM of each primer and 1.25 units of AmpliTaq DNA Polymerase.

Mixture C: 5 - 25 ng DNA, 10 mM Tris-CLH (ph 8.3), 1.5 mM MgCl2, 50 mM Kcl, 0.01% gelatin, 200 µM of each dNTP, 0.2 µM of each primer and 5 units of AmpliTaq DNA Polymerase.

Amplification conditions:

Loci	Denaturation	Annealing	Extension	Cycles
HLA-DQA1	94°C, 1 min	60°C, 30 sec	72°C, 30 sec	32
HLA-DPB1	95°C, 1 min	55°C, 1 min	72°C, 90 sec	32
HUMTH01	94°C, 45 sec	54°C, 1 min	72°C, 1 min	30
mtDNA	95°C, 10 sec	55°C, 20 sec	72°C, 1 min	32

SSCP and DSCP analysis:

SSCP: denaturing the PCR reaction mixture after amplification at 95°C for 8 mins with 95 % formamide and 20 mM EDTA (1:1). The denatured product was immediately frozen on ice.
DSCP: heating the PCR product at 95°C for 8 mins. The product was immediately frozen on ice and finally heated at 40°C for 10 mins.
The mtDNA fragments were previously cut with the restriction enzyme Dde I.

PAGE and SDS-PAGE:

PAGE was carried out in PhastGels (Pharmacia Biotech, Uppsala, Sweden) Homogeneus T = 20 % and SDS-PAGE in PhastGels Gradient T = 8-25 %. Buffer systems, electrode strips and electrophoretic conditions used were as descrited in Barros 1994.

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RESULTS AND DISCUSSION

1. PCR conditions for DSCP and SSCP analysis

SSCP: Single strand DNA was made by denaturing the amplified DNA fragment at 95°C with formamide (Fig. 1.A).
DSCP: Heteroduplexes are created only after a denaturation / renaturation cycle. The optimal conditions for creating heteroduplexes are first to form single-strand DNA without formamide, to freeze the sample on ice, and then to heat the sample at a temperature below the melting domain for heteroduplexes (Fig. 1.B).
Conditions can be designed to obtain homoduplexes, heteroduplexes and single-strand DNA in the same gel (Fig 1.C)

2. Electrophoretic conditions

SSCP and DSCP patterns were analyzed in miniaturized polyacrilamide gels. The best type of gels using PhastGels are:

For SSCP T = 20 % (homogeneus)
For DSCP T = 8 - 25 % (gradient)

3. Analysis of mutations in coding DNA

Single base mutations can be distinguished in the two HLA-Class II loci studied (HLA-DQA1 and HLA-DPB) (Fig 1) using both SSCP and DSCP. SSCP has advantages over DSCP in typing systems with high number of alleles, because it is not necessary to have a diagram of all possible combinations of the alleles (Barros, 1994).

4. Analysis of mutations in mt-DNA

Most mtDNA variation is within the non-coding region which contains the origen of replication for H strand, both origins of transcription and the D-loop region (Anderson, 1981). A 902 bp fragment was amplified and subsequently cut with Dde I. So three fragments are obtained, two of them available for SSCP and DSCP analysis (Fig. 2).

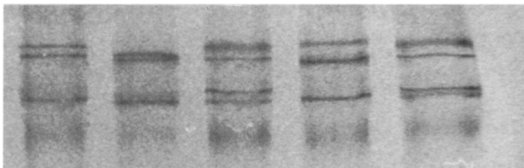

Figure 1A. SSCP patterns of HLA-DPB1 alleles in PhastGel T = 20 %. From left to right: lane (1) *0201-0402*, (2) *0401-0402*, (3) *0201-0401*, (4) *0201-0901*, (5) *0202-0401*.

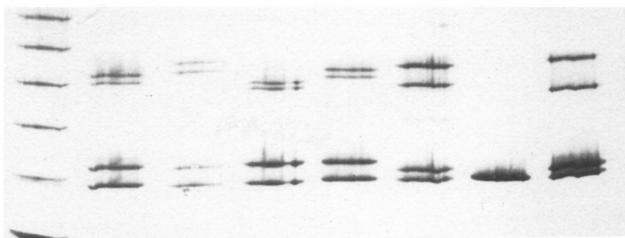

Figure 1B. DSCP patterns of HLA-DQA1 alleles in PhastGel T = 8-25 %. From left to right: 123 bp ladder marker, lane (1) 1.2-4, (2) 1.1-2, (3) 1.2-4, (4) 1.1-4, (5) 2-3, (6) 4-4, (7) 3-4.

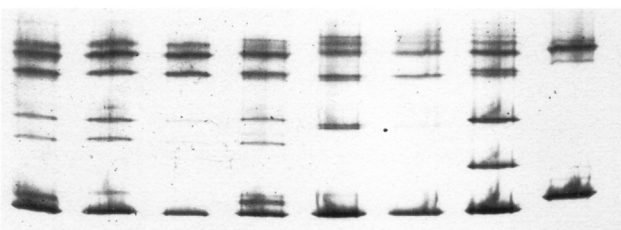

Figure 1C. SSCP and DSCP patterns of HLA-DQA1 alleles. From left to right: lane (1) 3-4, (2) 1.1-4, (3) 2-2, (4) 2-3, (5) 1.1-2, (6) 2-2, (7) 2-4, (8) 3-3.

The SSCP patterns of seven samples, with three variants, are shown in Figure 3. In the used conditions SSCP seems to be advantageous for detecting mutations and the analysis and lecture of the patterns is considerably easier.

5. Analysis of sequence variations in STRs

Single base substitutions or one base deletions in the repeating unit of STRs can be investigated through DSCP and SSCP analysis. One example is shown in the Figure 4 , where HUMTH01 9.3 - 10 heterozygote is detected in miniaturized gels using DSCP. HUMTH01 10 is a rare allele and can be confused with the 9.3 allele, since both fragments differ in a single base. DSCP offers, in this case, a fast and useful method to detect hidden variation in STRs.

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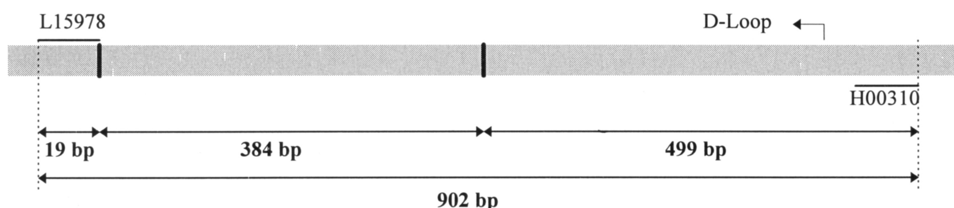

Figure 2. Diagram of the non-coding mtDNA fragment amplified with the Dde I restriction sites.

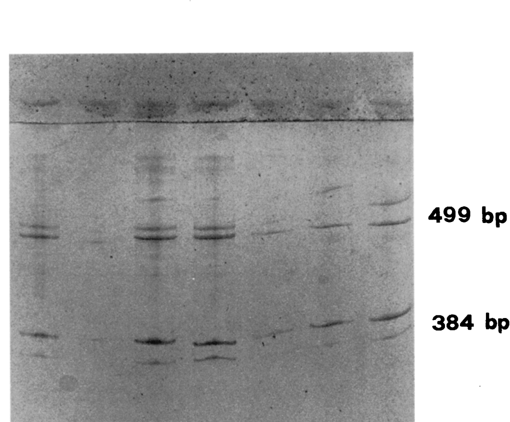

Figure 3. SSCP patterns of 384 bp and 499 bp mtDNA fragments in PhastGel T = 20 %.

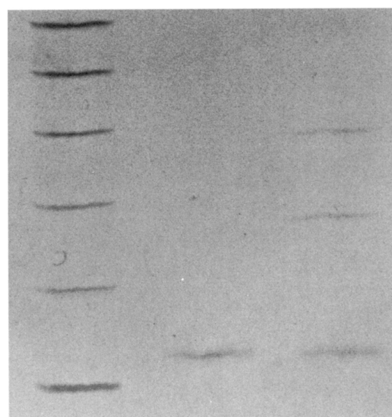

Figure 4. DSCP patterns of HUMTH01 alleles in PhastGel T = 8-25 %. From left to right: 123 bp ladder marker, lane (1) 9.3-9.3, (2) 9.3-10.

References

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