APPLICATION OF HLA-DR TYPING BY PCR-SSP TO FORENSIC SAMPLES

M. Ota, Y. Katsuyama, Y. Hama, N. Harashima, CY. Liu and H. Fukushima

Department of Legal Medicine, Shinshu University, Matsumoto, Japan

INTRODUCTION

An anlysis of the HLA complex by molecular methods has revealed an extensive degree of polymorphism. These genes are one of useful human genetic markers in forensic investigations. Class II HLA polymorphisms have been defined at the DNA level, due to the availability of allelic nucleotide sequences in this region as well as the successful application of molecular typing techniques. Various PCR based HLA class II DNA typing methods have been developed and applied. To date, one commercial kit for HLA-DQA1 typing has been validated for forensic investigations, and is used routinely in many laboratories. Among HLA class II genes, the DRB1 gene is one of the most useful in forensic genetics. In this study we have investigated the applicability of HLA-DR "low-resolution" typing by PCR-SSP (sequence specific primers) to forensic practice.

MATERIALS and METHODS

To define HLA-DR1 to DR10 by PCR-SSP, we designed 9 forward primers and 9 reverse primers (Table 1), which were created with a little modification in previous publications (1, 2).

DNA for investigation

This study uses twenty randomly selected healthy individuals who have been serologically DR typed. DNA was prepared by phenol/chloroform extraction. DNA from homozygous cells of 10th International Histocompatibility Workshop was used to verify primer specificities. The cell lines represented the following DRB1 alleles: 0101, 0102, 1501, 1502, 1601, 1602, 0301, 0401, 0402, 0404, 0405, 1101, 1102, 1103, 1201, 1301, 1302, 1401, 0701, 0801, 0802, 0803, 0901.

Amplification conditions

The PCR reactions were carried out in 25 μl volumes, which contained genomic DNA template, 10 pmol of each sequence-specific primer, 200 μM of each dNTP, 2 units of Taq DNA polymerase, 1.5 mM $MgCl_2$ 10 mM Tris-HCl, 50 mM KCl, and 0.1% Triton-X. Samples were amplified after

initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of 94 °C denaturation for 60 seconds, 60 °C annealing for 90 seconds, 72 °C for extension for 60 seconds and a final 72 °C extension for 5 minutes, using a thermal sequence TSR-300 (Iwaki Glass Co., Japan)

Nested PCR

For typing from extremely small amounts of DNA, we applied the nested PCR method to increase amplification sensitivity. Generic primers were generated from outside sequences to the nested primers used for PCR-SSP(table 1). After performing the first round of PCR using generic primers, the amplification mixture was purified by Centri-Sep column (Princeton Separations, Inc. NJ USA) to remove excess primers and dNTPs. An appropriate volume (usually 1μl) of purified DNA was subjected to a second round of PCR. The second PCR was performed under the following conditions: 1μl of the first amplification product was used as the template in 100μl of reaction mixture. Constituents and concentrations of the reaction buffer and reaction thermocycles were the same as in the first round of PCR, except that the number of cycles was reduced to 20.

We applied this method to determine the HLA-DR type of a DNA sample extracted from hair and left at 4 °C for 3 years.

RESULTS and DISCUSSION

The PCR-SSP method could type DR1 to DR10 specifically (DR1, DR2, DR3, DR4, DR11, DR12, DR13, DR14, DR7, DR8, DR9, DR10) for the DNA samples obtained from cells from randomly selected healthy individuals and from 10th International Histocompatibility Workshop. Furthermore, when combined with the PCR-RFLP method(2), this method is able to determine the sub-allelic types of DR2, DR4 and DR8, which have relatively high gene frequencies in Japanese (0.182, 0.228 and 0.133).

We could determine DR types with as little as 10ng DNA. Moreover, when we used the nested PCR method, it was possible to determine the DR type using only 10pg of DNA. This nested PCR method made it possible to conduct typing using only 0.2ng of DNA extracted from hair (Figure 1). This system yielded a 0.90 power of discrimination and a 0.71 chance of exclusion. This method promises to be a useful tool for forensic investigations.

REFERENCES

1) O. Oleraup and H, Zetterquist, Tissue Antigens, 1992:39:225-235.
2) M. Ota et al., Tissue Antigens, 1992: 39:187-202.

Table 1. Primer pairs for identification of DR1-DR10 specificities by PCR-SSP technique

G.P.	5' primer	3' primer
	DRBF:5'-CCGGATCCTTCGTGTCCCCACAGCACG	DRBR:5'-CCGCTGCACTGTGAAGCTCT
SSP		
DR1	5'R1: 5'-GGTTGCTGGAAAGATGCATCT	DRBR: 5'-CCGCTGCACTGTGAAGCTCT
DR2	5'R2: 5'-TTCCTGTGGCAGCCTAAGAGG	DRBR: 5'-CCGCTGCACTGTGAAGCTCT
DR3	5'R3: 5'-TACTTCCATAACCAGGAGGAGA	3'R3: 5'-AGTAGTTGTCCACCCGGC
DR4	5'R4: 5'-GTTTCTTGGAGCAGGTTAAAC	DRBR: 5'-CCGCTGCACTGTGAAGCTCT
DR11	5'R3568:5'-ACGTTTCTTGGAGTACTCTACG	3'R11: 5'-CTGGCTGTTCCAGTACTCCT
DR12	5'R12: 5'-AGTACTCTACGGGTGAGTGTT	3'R12: 5'-CACTGTGAAGCTCTCCACAG
DR13.1	5'R3568:5'-ACGTTTCTTGGAGTACTCTACG	3'R13-1:5'-CCCGCTCGTCTTCCAGGAT
DR13.2	5'R3568:5'-ACGTTTCTTGGAGTACTCTACG	3'R13-2:5'-TGTTCCAGTACTCGGCGCT
DR14.1	5'R3568:5'-ACGTTTCTTGGAGTACTCTACG	3'R14-1:5'-TCTGCAATAGGTGTCCACCT
DR14.2	5'R3568:5'-ACGTTTCTTGGAGTACTCTACG	3'R14-2:5'-TCCACCGCGGCCCGCCT
DR7	5'R7: 5'-AGTTCCTGGAAAGACTCTTCT	DRBR: 5'-CCGCTGCACTGTGAAGCTCT
DR8	5'R3568:5'-ACGTTTCTTGGAGTACTCTACG	3'R8: 5'-CTGCAGTAGGTGTCCACCAG
DR9	5'R9: 5'-GAAGCAGGATAAGTTTGAGTG	DRBR: 5'-CCGCTGCACTGTGAAGCTCT
DR10	5'R10: 5'-GGTTGCTGGAAAGACGCGTCC	DRBR: 5'-CCGCTGCACTGTGAAGCTCT

G.P. Generic Primers, SSP: Sequence Specific Primers

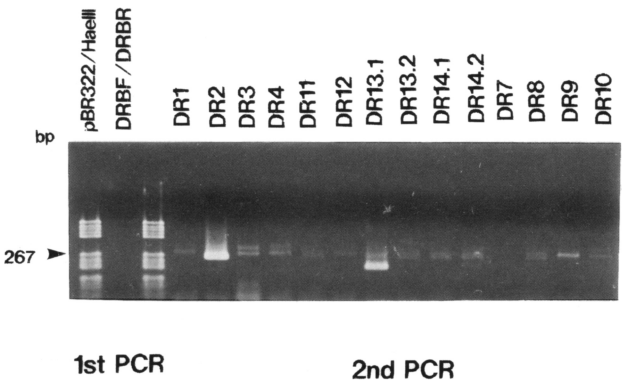

Figure 1.
HLA-DR"low-resolution"PCR-SSP typing of a DNA sample extracted from hair (3 cm length with a root) and left at 4 °C for 3 years. First PCR with primers DRBF and DRBR. Second PCR was perfomed by using 1 μl of first PCR product and each sequence specific primers(SSP). By second PCR using SSP typing method the sample could be typed DR2 and DR13.2 heterozygotes.