# Typing by polymerase chain reaction mediated amplification of DNA fragments in the HLA class I region and direct DNA sequencing

K.Schönborn-Sobolewski, J. Holtz, J.-P. Faber, M. Bormann

Dr.Dr. J. Holtz, Institut für Rechtsmedizin, Stiftsplatz 12, 5300 Bonn 1, FRG

Until now, exploiting the polymorphism of the HLA class I genes for forensic purposes is limited to the surface (protein) antigens. The DNA polymorphism detected by restriction fragment length polymorphisms (RFLP) revealed a large number of bands varying between 15 and 25 (Mayr 1986). The DNA used for RFLP analysis has to be intact, a condition seldom met in forensic tasks. In addition, for RFLP analysis a certain microgram amount of DNA is necessary.

As assessed by Southern blotting, the HLA class I gene family contains 15-20 members, many more than can be accounted for the HLA-A, -B and -C genes (Geraghty et al., 1987). Until now, there are reports about the DNA amplification in the HLA class II region comprising a relatively low number of alleles (Higuchi et al. 1988).

Materials and Methods:
Genomic DNA was prepared from peripheral blood cells according to standard procedures. The primer sequences were chosen to span the variable alpha 1-domain, corresponding the exon 2, alpha 2-domain, corresponding exon 3, and the alpha 3-region, corresponding exon 4 (Fig. 1).

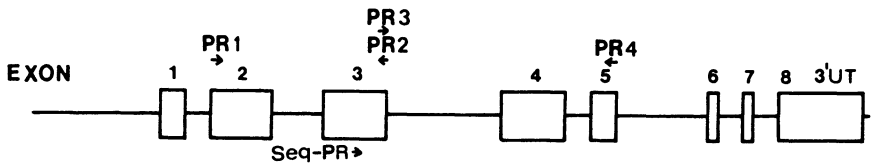



Figure 1 Amplification and sequencing strategy. The regions chosen for amplification and direct sequencing are shown along with the oligodeoxynucleotide amplification primers (PR) and the sequencing primer (Seq-PR). Arrows indicate the 5'→3' orientation of the primers.

PR 1 : 5'GCTCCCACTCCATGAGGTATT 3'
PR 2 : 3'CTATGGACCTCTTGCCCTTCC 5'
PR 3 : 5'AGATACCTGGAGAACGGGAAG 3'
PR 4 : 3'ACACCTCCTCCTTCTCGAGT 5'
Seq-PR: 5'GCGGGTACCGGCAGGACGCCTACGACGGCA 3'

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr

DNA amplification was performed according to Saiki et al.(1988) with 1 µg of genomic DNA, 300 ng of each primer and 1,5 mM $MgCl_2$ in 100 µl. Samples were heated to 94 C for 45 sec. (to denature the DNA), cooled to 60 C for 30 sec. (to anneal the primers) and heated to 72 C for 60 sec. (to extend the annealed primers). After 15 cycles the extension time was extended by 5 sec. a cycle for another 15 cycles, followed by a final extension step of 5 minutes.

PCR products were digested with restriction endonuclease TaqI (10 units/ug DNA) after precipitation with ethanol. Both digested and undigested samples were analyzed by agarose gel electrophoresis (3% NuSieve/1% SeaKem in TAE) and staining with ethidiumbromide (Fig. 2).

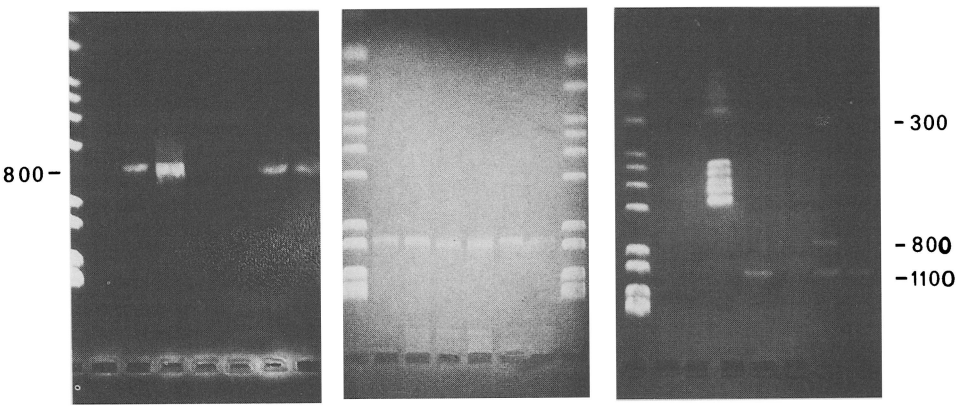


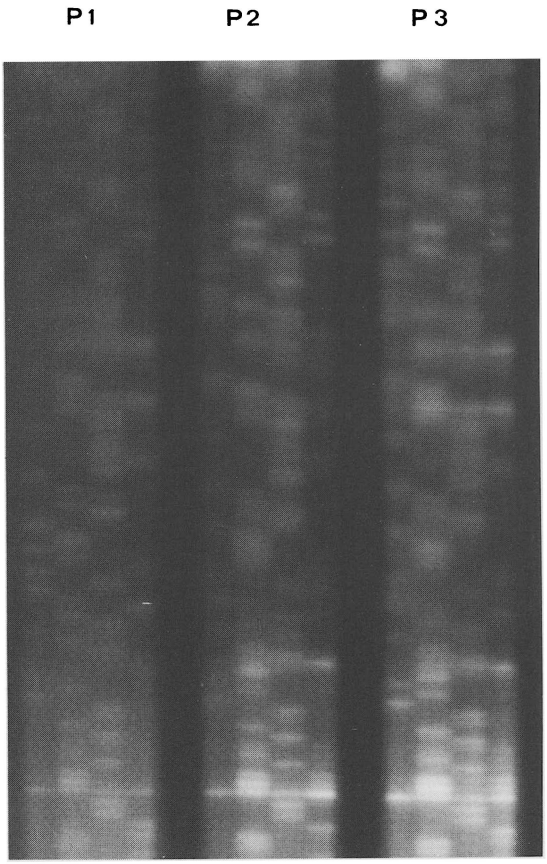

Figure 2 PCR-products with PR 1 and 2 (left), PR 3 and 4 (middle) and PR 3 and 4 digested with TaqI restriction enzyme (right). A panel of following serotypes was used: A28,w19 B5,35; A1,3 B35,57 Cw4; A2 B35 Cw4; A1,2 B16,44 Cw5; A1,24 B17,39 Cw6,7; A2,3 B15,35 Cw1,4; A2 B35 Cw4; A2,11 B17,44 Cw6,7.

Prior to DNA sequencing the PCR products were purified by Centricon 30 microconcentrators. Sequencing was carried out by the dideoxynucleotide chain-termination reaction (Sanger et al., 1977) using Sequenase kit from USB. The primer was radiolabeled using polynucleotide kinase and ($\gamma$-$^{32}$P)ATP. Samples were electrophoresed in a polyacrylamide/urea sequencing gel (Fig. 3).

Results:
The segments of genomic DNA chosen for amplification are shown in Fig. 1. Since the PCR primers are chosen to anneal to the HLA coding DNA regions with little or no variation , we succeeded in visualizing only one band in the ethidiumbromide stained agarose gels. With the primers PR 1 and 2 - annealing to known sequences of the serotypes A2, 3, w24, 11, B7 and 13 - the amplified DNA

fragment possessed a length of about 800 bp. The haplotypes defined by serologic HLA-ABC typing are genetically heterogenous. Irrespective of the serotype all PCR products had the same length. Differences of some nucleotides could not be detected.

The amplification products with primers PR 3 and PR 4 resulted in a uniform band of about 1100 bp, the expected length calculated with the help of the known sequences (including the known sequences of A2, 3 and w24).




```
A3    GCGCTCTTGGACCGCGGCGGACACGGCGGCTCAGATCACCAAGCGCAAGTGGGAGGCG
A2    ----------------------------A-------C--------A-------------
B7    ------C-----------------C---T--G---------C-----------------
Cw1   ------C--------C-------CC---------------C-----------------
P1    ----------------------------A------------------------------
P2    ------C--------C------?-----A-G---
P3    ---------------C--------?--A------
```

Figure 3 DNA sequences in exon 3 (nucleotide 120-178) of HLA A3 (Strachan et al. 1984), A2 (Koller et al. 1985), B7 (Davidson et al. 1985), Cw1 (Güssow et al. 1987) and the sequences we found. P1: A1,w24 B17,39 Cw6,7; P2: A2 B35 Cw4; P3: A1,3 B17,35 Cw4

In order to specify the PCR products we looked for TaqI digestibility. The 1100 bp fragment could indeed be digested by TaqI into fragments of 300 and 800 bp. The fragment of 1100 bp was not completely digested by TaqI; about half of the amplification product possessed no TaqI digestion site. It seems that this band at 1100 bp represents a mixture of DNA sequences despite the specific PCR primers.

An unequivocal characterization of the PCR product was done by direct DNA sequencing of the 800 bp fragments using an internal start primer (Seq-PR) in the middle of exon 3, a variable region. The amplification with this primer and primer PR 2 resulted in the expected PCR product of 210 bp length. The sequence ladder can be read 29 bp apart from the 3'end of the start primer. Compared to the known sequence of A3 the homology was 98% (1 nt of a total of 58 nt) (Fig. 3, P1).

This should help to improve individual typing even by sequencing a short piece of 50 nucleotides in the variable HLA sequence.

Literature:

Davidson WF, Kress M, Khoury G, Jay G (1985) Comparison of HLA class I gene sequences. J.Biol.Chem. 260: 13414-13423

Geraghty DE, Koller BH, Orr HT (1987) A human major histocompatibility complex class I gene that encodes a protein with a shortened cytoplasmic segment. Proc.Natl.Acad.Sci.USA 84: 9145-9194

Güssow D, Rein RS, Meijer I, de Hoog W, Seemann GHA, Hochstenbach FM, Ploegh HL (1987) Isolation, Expression and Primary Structure of HLA-Cw1 and HLA-Cw2 Genes: Evolutionary Aspects. Immunogenetics 25: 313-322

Higuchi R, von Beroldingen CH, Sensabaugh GF, Erlich HA (1988) DNA typing from single hairs. Nature 332: 543-546

Koller BH, Orr HT (1985) Cloning and complete sequence of an HLA-A2 gene: Analysis of two HLA-A alleles at the nucleotide level. J.Immunology 134: 2727-2732

Mayr WR (1986) The use of DNA polymorphisms demonstrated by means of the HLA system. Vox Sang. 50: 193-197

Saiki RK, Gelfand DH, Stoffel S, Scharf SH, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487-491

Sanger F, Nicklen S, Coulson AR (1977) Proc.Natl.Acad.Sci.USA 74: 5463-5467

Strachan T, Sodoyer R, Dramotte M, Jordan BR (1984) Complete nucleotide sequence of a functional class I HLA gene, HLA-A3: implications for the evolution of HLA genes. EMBO 3: 887-894