Application of HLA-class II Genotyping by the Modified PCR-RFLP Method to the Forensic Science

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INTRODUCTION

The HLA class II antigens(HLA-DR, -DQ, -DP), located in the short arm of chromosome 6, show a great deal of polymorphisms. These antigens have been usually defined by serological procedures (for DR and DQ) using alloantisera or monoclonal antibody and cellular assay procedure (for Dw and DP). Definition of HLA class II antigens became possible at the DNA level using Southern blot hybridization technique with class II antigen cDNA probes. More recently, detection of nucleotide sequence polymorphisms in the HLA class II region has become feasible with the advent of polymerase chain reaction (PCR) technique. The PCR method permits precise and direct analysis of allelic variations with as little as 1 ng of genomic DNA. We earlier reported the modified PCR-RFLP methods (1-3), which were legible and easy to use in the accurate definition of HLA class II (DQA1, DQB1, DRB1 and DPB1) alleles. This method allows discrimination of 36, 91, 946 and 190 combinations, including homozygotes and heterozygotes of the DQA1(8 alleles), DQB1(13 alleles), DRB1(43 alleles) and DPB1(18 alleles) respectively. In this study, we examined the possibility of HLA-class II genotyping by the modified PCR-RFLP method for DNA samples extracted from hairs, a small volume of whole blood, fresh or old dental pulp tissues.

MATERIALS AND METHODS

1) DNA extraction and amplification: Genomic DNAs were extracted from blood, a single plucked hair with a length of 3 cm and fresh or old dental pulp tissues (300 mg) by a rapid DNA extraction technique (Fig. 1) using Centricon 30 dialysis/concentration tube (Amicon Co.).
2) Amplification: DQA1, DQB1, DPB1 and DRB1 genes were amplified by using PCR primers (1,2,3). After amplification, aliquots (10 µl) of the reaction mixture were digested with each objective restriction endonuclease (1,2,3).
3) Acrylamide gel electrophoresis: Samples of the amplified DNAs cleaved with restriction enzyme were subjected to electrophoresis in 12% polyacrylamide gel in a minigel apparatus (Mupid, Cosmo Bio Co.). Cleavage or non-cleavage of amplified fragments was detected by staining with ethidium bromide.

RESULTS

1) DQA1 and DQB1 genotyping from blood.
DQA1 and DQB1 genes could be amplified with DNAs extracted

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from 250 µl of stored blood in a 1.5 ml eppendorf tube for 2 years at room temperature (Fig. 2). Their genotypes could be determined by the modified PCR-RFLP method (I: DQA1*0103/0301, DQB1*0601/0401; II: DQA1*0101=2/0301, DQB1*0501/0303).

2) DRB1 genotyping from hair.

DRB1 gene could be amplified with DNAs extracted from a single plucked hair with 3 cm length using group-specific primers. This genotype was determined by the modified PCR-RFLP method (Fig. 3 : DRB1*0803/0803).

3) DQA1 and DPB1 genotyping from dental pulp tissues.

The DQA1 genes from both fresh and old (over one year) dental pulp tissues could be amplified, but the DPB1 genes could be amplified only from fresh samples (Fig. 4. A; DQA1*0103/0301: DPB1*0402/0402, B; DQA1*0103/0301: DPB1*0202/1801, C; DQA1*0103/0301: DPB1*0501/0501, D; DQA1*0102/0103, E; DQA1*0102/0102).

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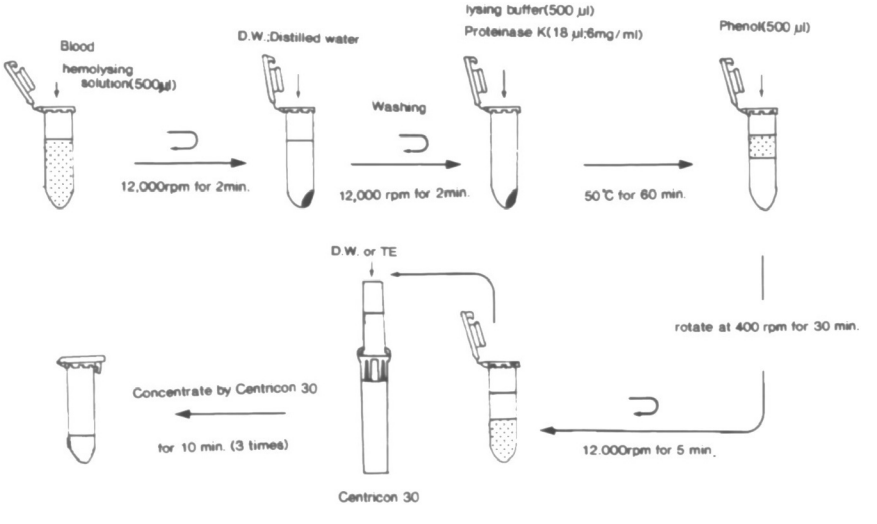
Fig.1 A rapid DNA extraction technique using Centricon 30 dialysis/concentration tube



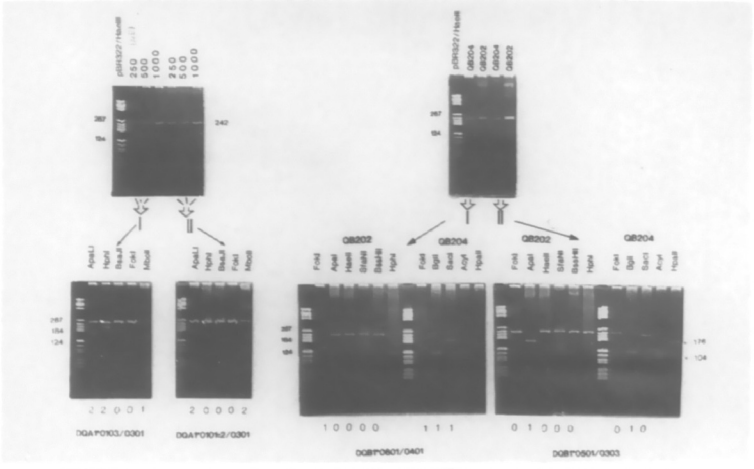


Fig. 2 Modified PCR-RFLP typing for the DQA1 and DQB1 genes using DNAs extracted from stored blood for 2 years

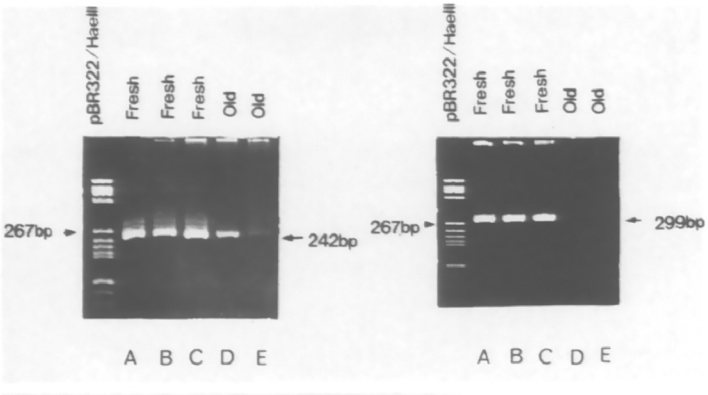


Fig.4 PCR-RFLP typing for the DQA1 and DPB1 genes using DNAs extracted from dental pulp tissues

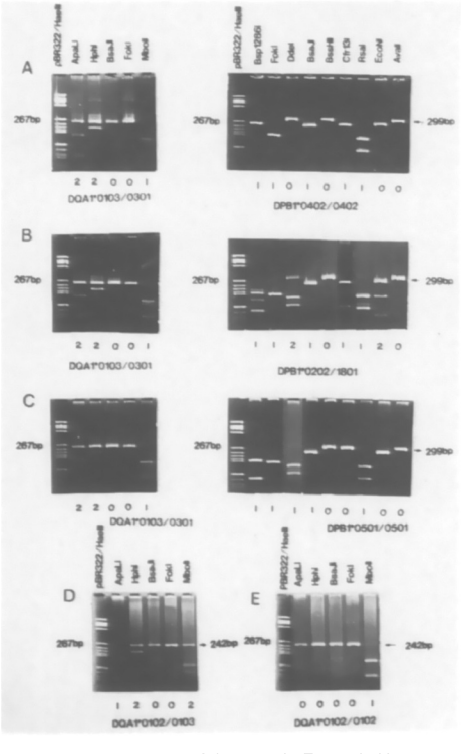


Fig.3 Modified PCR-RFLP typing for the DRB1 genes amplified by group specific primers using DNAs extracted from a single hair