

Species Identification by Analysis of the Genes for ABO Blood Group.

Yamada M¹, Ushiyama I², Sato M³, Ueyama H³, Ohkubo I³, Nishimura A² and Nishi K²

¹) Department of Human Life Science, Seibo Jogakuin Women's College, Fukakusa, Fushimi-ku, Kyoto 612, Japan, and Departments of ²) Legal Medicine and ³) Medical Biochemistry, Shiga University of Medical Science, Seta-Tsukinowacho, Otsu 520-21, Japan.

INTRODUCTION

The species identification is of a great importance in the forensic field. The immunological techniques have been utilized to distinguish human from other animal samples, such as blood stain, saliva or seminal fluid, however a keen method for identification of the origin of a sample has not been established. We have previously reported that ABH(0) blood group substances are detected in various species and that the lectin- and immuno-histochemistry can be available for species identification from tissue particles (Nishi 1992). It is considered that some animals possess the genes homologous to those for human ABO blood group. We have also reported the determination of ABO genotypes with DNAs extracted from fresh blood or formalin-fixed, paraffin-embedded tissues by PCR amplification and restriction enzymes digestion (Yamada 1994), depending on the polymorphism of the gene encoding blood group A-transferase. Here we describe that the DNA analysis of the genes for ABO blood group might be a tool of species identification.

MATERIALS AND METHODS

DNAs were prepared (Sambrook 1989) from the blood of human, Japanese monkeys, pigs, cats, rabbits, Chinese hamsters, rats (Wister and Fischer), mastomys and mice and from the liver of goldfishes.

Amplification with Taq DNA polymerase (Sambrook 1989) was performed with oligonucleotide primers 1) and 2), 3) and 4) (Lee 1992) and primers 5) and 6), those were designated as PCR-1+2, PCR-3+4 and PCR-5+6, respectively. Primers 1) and 2) were put across nucleotide 258 (a single base deletion site of the O allele) and primers 3) and 4) across nucleotide 700 (a single base substitution site in the A and B alleles) of cDNA at the human ABO gene locus (Yamamoto 1990). Primers 5) and 6) amplify the region includes three single base substitution sites, nucleotides 700, 793 and 800, in the human A and B alleles. Sequences of the primers used are as follows; 1) CACCGTGAAGGATGTCCT C, 2) AATGTCCACAGTCACTCGCC, 3) TGGAGATCCTGACTCCGCTG, 4) GTAGAAATCGCCCTCGTCT, 5) CTGGTGTGCGTGGAC and 6) ACCTCTGCACCGACCC.

Amplified DNAs were electrophoresed on 6% polyacrylamide gels and the bands were visualized by ethidium bromide staining (Sambrook 1989).

The PCR products from human DNA, labelled with digoxigenin-dUTP by random primed, were used as probes. After electrophoresis, the DNAs were transferred on to Nylon membranes (Hybond N+, Amersham) followed by hybridization with the

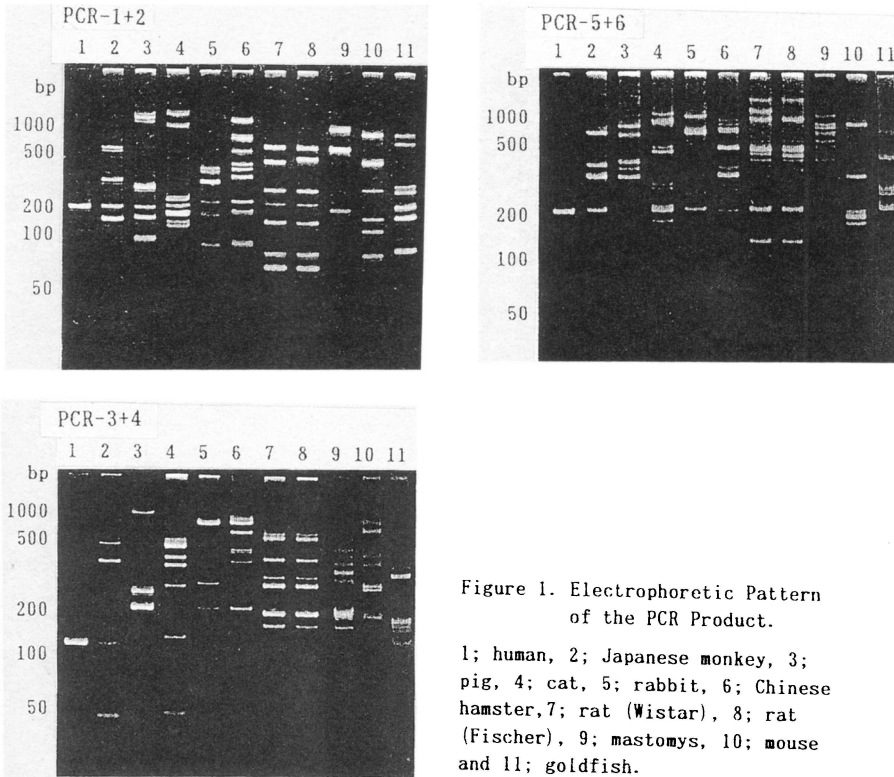


Figure 1. Electrophoretic Pattern of the PCR Product.

1; human, 2; Japanese monkey, 3; pig, 4; cat, 5; rabbit, 6; Chinese hamster, 7; rat (Wistar), 8; rat (Fischer), 9; mastomys, 10; mouse and 11; goldfish.

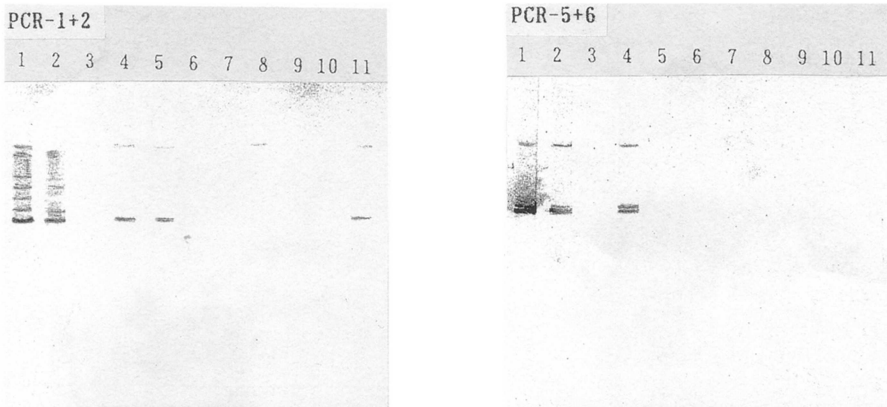


Figure 2. Hybridization Analysis.

Filters were washed in 0.1x SSC-0.1% SDS at 68 °C.

See the legend for Fig. 1.

corresponding probes. The filters were washed (Sambrook 1989) in 0.1x SSC-0.1% SDS at 68 °C or 2x SSC-0.1% SDS at 42 °C and the hybrids were detected by enzyme immunoassay (DNA Labelling & Detection Kit, Boehringer).

RESULTS AND DISCUSSION

Electrophoretic Analysis of The PCR Products (Fig. 1)

Every pair of primers presented a single band from human DNA and several bands from the other animals electrophoretically. The band patterns obtained from each species in PCR-1+2, PCR-3+4 and PCR-5+6 were distinct and no individual difference was observed (data not shown). Two strains of rat had identical electrophoretic patterns.

Hybridization Analysis (Fig. 2)

In Japanese monkey, cat, rabbit and goldfish, the amplified fragments in PCR-1+2 with the length as same as the human product hybridized with the probe, the human product, when washing at high stringency. After washed at lower stringency, the hybrids were observed in all the animals examined (data not shown). The PCR-3+4 fragments from mastomys and goldfish did not hybridize with the corresponding probe at both high and low washing stringencies (data not shown) and the PCR-5+6 fragments from Japanese monkey and cat showed hybridization.

Combination of the electrophoretic and hybridization analyses of the PCR products might be utilized for species identification. It is considered that the species identification followed by ABO typing might be desired when analyzing the stains of body fluids, such as saliva, semen and urine. Moreover, the results might explain the ABO substances detected in animals.

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