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Genetic analysis using fingernail DNA

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For the genetic analysis with the polymerase chain reaction (PCR), template DNA is generally prepared from blood cells. However, in some instances, when blood samples are not available, DNA from hair can be used for the analysis (1, 2). Both mitochondrial DNA (mtDNA) and nuclear DNA can be amplified using DNA extracted from one hair. While hair sampling is easy, there are still some problems, e.g., hair plucking is accompanied by slight pain, and sampling itself is impossible from hairless animals. Therefore, we have focused on the fingernail, which is available from most species of animals, as another source of DNA, and we have attempted to extract and amplify fingernail DNA.

DNA was extracted from 20–30 mg of fingernail clippings. The specimen was cut into small pieces, i.e., approximately 1 mm×2 mm, then rinsed once with 1.0 N NaOH, and twice with distilled water in an 1.5 ml Eppendorf tube. After adding 500 μl of extraction buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl, 0.45% NP-40, 0.45% Tween 20 and 0.01% gelatin), 7.5 μl of proteinase K solution (10 mg/ml) and 25 μl of 10% SDS solution, the sample was incubated at 37°C for 16 hr. Nucleic acids were extracted with phenol/chloroform. The aqueous phase was mixed with 4 μl of RNase A solution (10 mg/ml), incubated at 37°C for 30 min., then extracted once with phenol/chloroform, and once with chloroform/isoamylalcohol. DNA was precipitated with ethanol and sodium acetate. After centrifugation, the pellet was washed in 70% ethanol, air-dried and dissolved in distilled water. The DNA concentration was calculated from the optical density at 260 nm. The mean recovery of purified DNA in 10 individuals was 0.60 μg (SD = 0.53)/10 mg fingernail. DNA was also prepared from blood cells of the same individuals in a similar way.

Enzymatic amplification was carried out on three polymorphic loci, i.e., the D-loop region (3) in mtDNA, the second exon of

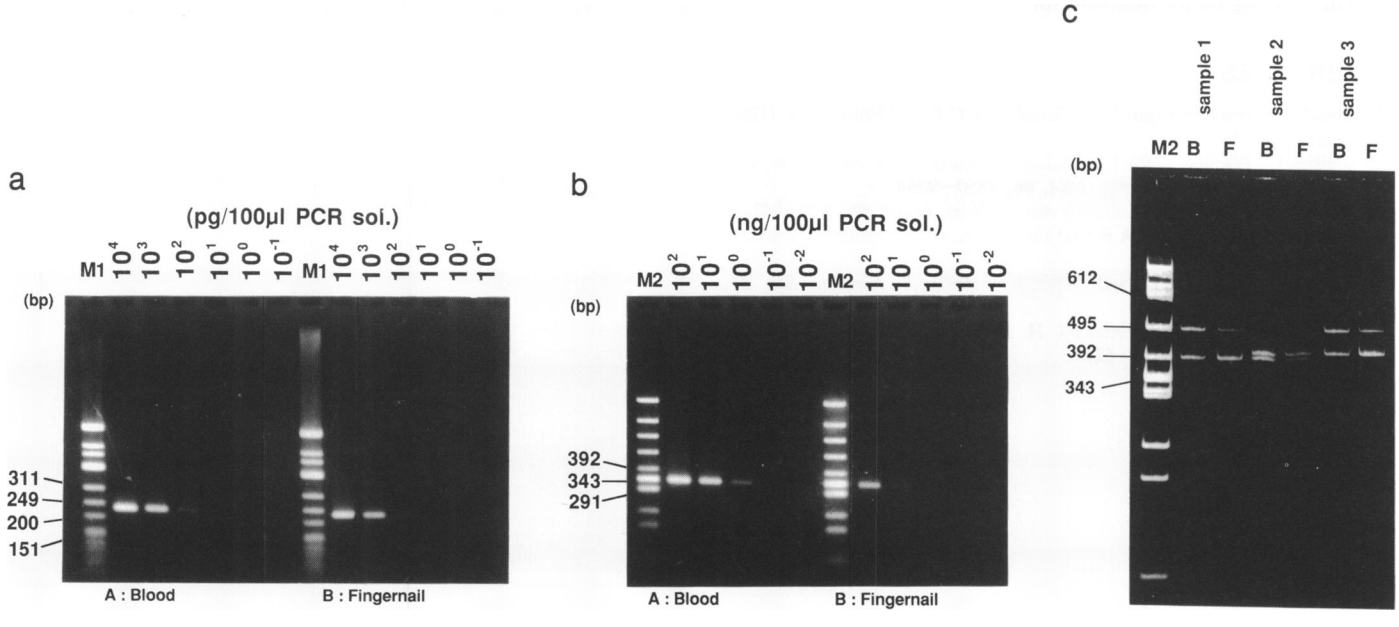

Figure 1. Gel electrophoresis of PCR product. Size markers are ΦX174 RF/HinfI (M1) and ϕX174 RF/HincII (M2). The PCR condition was programmed in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus) for a) and b) or Thermal Sequencer TSR-300 (IWAKI Glass Co., Ltd) for c). a) D-loop region of mtDNA (233 bp). Primers were 5′-CCCCATGCTTACAAGCAAG-3′ (forward) and 5′-ATTGATTTCACGGAGGATGG-3′ (reverse). Denaturation: 96°C·10 sec/annealing 45°C·10 sec/extention: 72°C·15 sec. 30 cycles. b) HLA–DPB1 gene (338 bp). Primers were 5′-CGGATCCGGCCCAAAGCCCTCACTC-3′ (forward) and 5′-GCTGC-AGGAGAGTGGCGCCTCCGCTCAT-3′ (reverse). Denaturation: 95°C·30 sec/annealing 67°C·10 sec/extention: 72°C·15 sec. 35 cycles. c) MCT118 locus. Primers were 5′-GAAACTGGCCTCCAAACACTGCCCGCCG-3′ (forward) and 5′-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3′ (reverse). Denaturation: 96°C·1 min/annealing 65°C·2 min/extention: 73°C·15 sec. 28 cycles for blood cell DNA (B) and 36 cycles for fingernail DNA (F).

HLA−DPB1 gene (4) and the MCT118 locus (5) in nuclear DNA. The PCR product was analyzed in either 3% agarose gels (D-loop and HLA-DPB1) or 4% polyacrylamide gels (MCT118), and visualized by ethidium bromide staining (Figure 1). All the DNA samples extracted from the fingernails, as well as from the blood cells, were effectively amplified at the three loci. However, the efficiency of the amplification was considerably higher with fingernail DNA. The minimum amount of DNA from fingernail samples required for amplification was less than one tenth of that from the blood samples with the mtDNA locus, and one-tenth to one-hundredth with the nuclear DNA locus (Figure 1). Also, on the electrophoresis and following the ethidium bromide staining, the fingernail DNA had smaller fragments than the DNA from blood samples. To confirm the genotype matching, DNA typing was carried out on the second exon of the HLA−DPB1 gene by reverse dot-blot hybridization (6). The fingernail genotype matched completely of the blood genotype in all 10 individuals (unpublished data). In the MCT118 locus, which contains a variable number of tandem repeats, two fragments amplified from two out of three samples had the same size (Figure 1), which indicated that the number of the repeats was the same in the two samples.

This study has demonstrated the usefulness of fingernail DNA for genetic analysis. The collection of a fingernail specimen takes only a short time, and is sometimes easier and safer than that of a hair specimen. DNA can be extracted easily from fingernail clippings by a conventional DNA extraction method. Its quality is sufficient for enzymatic amplification and genotyping or individual identification.

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