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SHORT COMMUNICATION

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Determination of the HUMTH01 alleles by the APLP method

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Abstract We present a simple and rapid technique for determination of alleles at the HUMTH01 locus. The amplified product length polymorphism (APLP) method using two primers different in length, permits the differentiation between allele 9.3 and other alleles. The primers were designed to have an allele-specific nucleotide at the 3′ terminal and 11 non-complementary nucleotides were added to the 5′ terminal of one of the primers for the allele 9.3. The amplified fragment sizes for the alleles 9.3 and 10 were 80 bp and 70 bp, respectively. This method has proved to be very useful for forensic applications.

Key words HUMTH01 · STR polymorphism · Allele specific primer · APLP method

Introduction

HUMTH01 is a highly polymorphic STR located on chromosome 11p15.5 [1–3] and many reports on allele frequencies in various populations have been published [4–7]. In the present paper, we describe an advanced technique for determination of the HUMTH01 alleles using the amplified product length polymorphism (APLP) method [8].

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Materials and methods

Oligonucleotide primers used for APLP [8] were designed according to the genomic information of the tyrosine hydroxylase gene [1] as showed in Fig. 1. The primer sequences were as follows:

TH01F; 5′-CTTATTTCCCTCATTgATTCATgCATTCA-3′
TH93F; 5′-agacgatagagCTTATTTCCCTCATTtATTCATaCATCAT-3′
TH01R; 5′-GAACACAGACTCCATGGTGA-3′

The non-complementary nucleotides are written in small letters. PCR was performed in a total reaction volume of 10 µl containing 5–10 ng genomic DNA, 10 mM Tris-HCl buffer (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 250 µM each dNTP, 4 pmol each primer and 0.5 U Taq polymerase (Takara, Ohtsu, Japan). Amplification was carried out using 30 cycles in a DNA Thermal Cycler PJ2000 (Perkin-Elmer, Foster City, Calif.) at 94 °C for 30 s, 57 °C for 30 s and 72 °C for 30 s.

Separation was carried out with 3 µl of amplicon in native polyacrylamide gels (12%T, 5%C, size 8 cm × 7 cm × 1 mm) with 0.375 M Tris-HCl buffer (pH 8.9) and 0.025 M Tris-glycine buffer (pH 8.3) as tank buffer for 60 min at 300 V. Bands were visualized by SYBER Green I (Molecular Probes, USA).

Results and discussion

We applied the APLP method to determine the genotypes of the HUMTH01. Figure 2 shows that each allele of

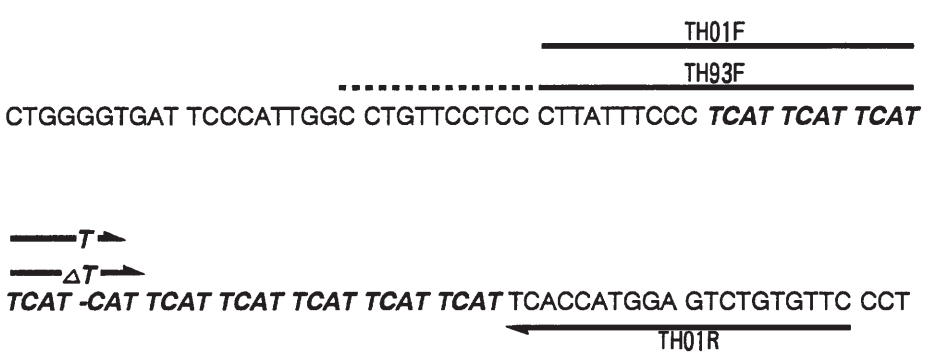

Fig. 1 Sequence of the allele 9.3 and annealing regions for primer. The sequences in bold type correspond to the repeat region, and the dotted line indicates the non-complimentary sequence region

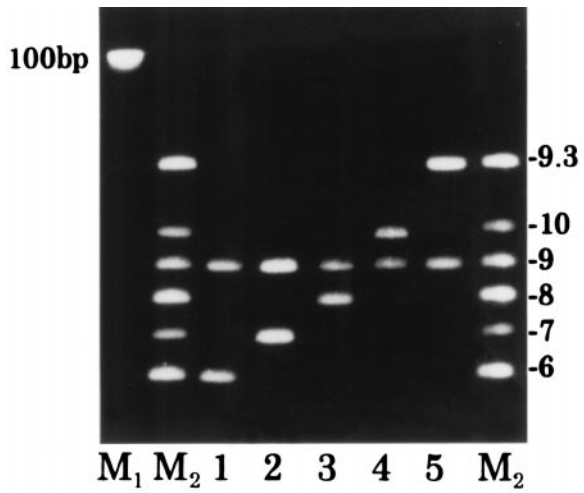

Fig. 2 Banding patterns of PCR products using the primer set (TH01F, TH93F and TH01R). *Lane 1*: 6–9, *lane 2*: 7–9, *lane 3*: 8–9, *lane 4*: 9–10, *lane 5*: 9–9.3, *lane* M_1: DNA size marker, *lane* M_2: allelic ladder marker

HUMTH01 can be clearly and unambiguously distinguished by comparison with the corresponding band in the allelic ladder. The allele 9.3 was amplified only with the primer TH93F and other alleles were amplified only with the primer TH01F. Since the allele-specific primer for the allele 9.3 (TH93F) has 11 non-complementary nucleotides, the PCR product for the allele 9.3 was 10 bp longer than that for the allele 10. This method is very simple easy and rapid, because it can be performed on short native polyacrylamide gels requiring only a short running time. HUMTH01 genotypes obtained by the APLP method were identical to those obtained by the previous method [9]. The alleles 8.3, 10.3 [6] and 13.3 [10] were not encountered in the present study. However, the allele 8.3 and 13.3 can be amplified only with the primer TH93F and the allele 10.3 can be amplified only with the primer TH01F. Consequently, all alleles of the HUMTH01 can be discerned by the present method.

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