



A method to help the detection of false homozygous samples at the D17S5 locus

S. Pelotti, G. Ferri, C. Colalongo, A. Abbondanza,
M. Falconi, G. Pappalardo *

*Department of Medicine and Public Health, Section of Legal Medicine, University of Bologna,
via Irnerio 49, Bologna 40126, Italy*

Abstract

Here we describe a method to check single-banded samples for the correct evaluation of this polymorphic region. Forward primers annealing within the D17S5 repeat sequences were designed and utilised in PCR with Horn's reverse primer. The method allows false homozygous states to be revealed and may be suitable to avoid mistyping in population studies and in forensic casework. © 2003 Elsevier Science B.V. All rights reserved.

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1. Introduction

PCR-assisted typing at the D17S5 locus (YNZ22) allows discrimination of alleles differing by the repetition of a 70-bp core sequence. Because of the large number of alleles and its high heterozygosity of over 80% in Caucasians, YNZ22 amplification has been reported as being suitable for forensic identification [1,2,3]. Some authors [4,5] analysing this polymorphism, referred that when two closed spaced large alleles were present the amplification fragments were equal, but when the alleles size differed significantly in a heterozygous sample, the small allele amplified more efficiently and the band corresponding to the large allele was weak or absent. This condition can lead to mistyping of a heterozygous individual as homozygous for the small allele. Batanian et al. [4] improved the amplification of the larger allele by reducing the amount of DNA from 1 µg to 20–50 ng. Deka et al. [5] proposed a rigorous standardization procedure utilizing suitable controls of large and small

* Corresponding author. Tel.: +39-51-243250; fax: +39-51-248896.
E-mail address: labemat@medleg.unibo.it (G. Pappalardo).

alleles and a modification of amplification extension step associated to 200 ng of template DNA. PCR conditions were varied by Ivey et al. [6] without overcoming the problem of allelic drop-out so they do not recommend the system for routine forensic analysis. Here we describe a method to check the single-banded samples that may be helpful for the evaluation of this polymorphic region. Forward primers “wandering” along the D17S5 repeat sequences were designed and utilised for PCR with Horn’s reverse primer.

2. Materials and methods

DNA from blood donors was extracted by the salting out method [7] and amplified with primers described by Horn et al. [1] at the following conditions: 150 ng of genomic DNA was amplified in a total volume of 25 μ l reaction mixture containing 50 mM Tris–HCl, 0.5 mM MgCl₂, 100 μ M each dNTP, 0.8 μ M each primer, 1.25 U Taq DNA polymerase (PE Applied Biosystem, USA). Initial denaturation was at 95 °C for 3 min followed by 30 cycles of 95 °C (1 min), 55 °C (1 min), 72 °C (4 min) with a final 72 °C extension for 7 min. Among these samples we then amplified five heterozygotes consisting of large and small alleles (2–12; 2–10; 1–9; 1–10; 1–12), five heterozygotes consisting of small and close alleles (2–3; 3–4) and 10 single-banded samples. PCR conditions were as above substituting the forward Horn’s primer with the “wandering” primer 5’ CCTTACA-GAAGCAATGAG 3’. Another internal “wandering” primer was designed as follows: 5’ acaCCTTACAGAAGCAATGAG 3’ (lower case: bases non-complementary to template strand) and utilized at the same PCR conditions. All the PCR products were separated by electrophoresis on 2% agarose gels containing ethidium bromide. A size marker 123-bp ladder (Gibco BRL) was used.

3. Results and discussion

The amplification of heterozygous samples with the first “wandering” primer produced a ladder-like set of fragments whose number was linked to the number of repeats of the largest allele. The amplicons size started from 94 bp for the first allele increasing by 70 bp for each subsequent repeat. The anodic fragments of the higher alleles very close to each other on agarose gels were often overlapped by a smear, unaffected by nuclease S1 treatment (not shown). Besides, as seen especially with the small alleles of heterozygous samples, one or two bands of the repeat size, albeit weak, sometimes appeared over the specific fragments. These observations suggested the occurrence of a misannealing of the amplicons, which may act as self-primers, particularly in the last cycles of PCR. Indeed surplus bands, never seen in true homozygous 1 samples, weakened or disappeared reducing the cycle number but typing may be impaired. We found that the addition of a ACA tail at the 5’ end of the wandering primer can prevent the extension of misannealed amplicons. Primer tailing works by incorporating a non-homologous sequence at the 3’ end of the forward primer strand. The result was a correct number of bands of the ladder-like pattern without troubling smear. Assuming a drop out, there is unlikely to be a failure of all the fragments of the largest allele impairing the heterozygous state determination.

In this way among the analysed single banded samples, seven showed a number of fragments corresponding to the repeats whereas three with small alleles (1, 2) showed the ladder profile with a number of PCR fragments higher than the expected revealing a false homozygous state.

A similar pattern was obtained in a parentage testing for mother and child samples which, previously typed as homozygous 2 and 4, respectively, would have been incompatible only for this locus.

In conclusion, the method may help to discriminate false homozygotes and to avoid mistyping in forensic and anthropological evaluations.

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