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International Congress Series 1288 (2006) 654–656

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Development of a new multiplex assay for STR typing of telogen hair roots

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Abstract. We have developed a new strategy in which 10 STR systems plus amelogenin were simultaneously amplified from telogen hair roots with maximal fragment sizes smaller than 270 base pairs. The multiplex includes six STR loci from the European standard set of loci (ESS) for DNA databases (D3S1358, D8S1179, D21S11, THO1, FGA and VWA) as well as four additional STR systems selected for their robustness and short amplicon sizes (D2S1338, D12S391, TPOX and D5S818). Due to the biotinylation of the reverse primers from five STRs systems, these PCR products can be separated from the other six amplicons after PCR amplification. The two sub-multiplexes were then analyzed in two different runs on a capillary gel electrophoresis instrument. © 2005 Elsevier B.V. All rights reserved.

Keywords: DNA degradation; Short amplicon PCR; MiniSTRs; Forensic DNA typing

1. Introduction

Short Tandem Repeats are used for routine forensic identification purposes for many years. In telogen hair roots, DNA is shortened during the hair development by an apoptotic process which results in fragmentation of the DNA to pieces smaller than 200 bp [1]. Therefore, the length of the amplifiable PCR product depends on the degradation rate of the DNA in use and is restricted to that size. Due the primer length and the number of tetrameric repeat units of the corresponding STR system, the length of the maximal PCR products is fixed. The main problem for the establishment of large multiplex systems for short STR systems is this length restriction. With the commonly used fluorescent dyes 6-FAM, JOE and NED, only five to six STR systems can be amplified in the same multiplex reaction. Therefore, we have combined two short STR multiplex reactions to a large multiplex [2]. The reverse primers of one of the sub-multiplexes were biotinylated so that these PCR fragments can be separated from the unbiotinylated fragments using streptavidin-coated Sepharose beads.

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doi:10.1016/j.ics.2005.12.030

To verify the specificity of the new STR multiplex undegraded human DNA samples from blood were amplified at least twice, separated and analysed by capillary gel electrophoresis. The results were compared to the typings with the SGM Plus™ (Applied Biosystems) or PowerPlex® 16 (Promega) kits and the single amplification of the D12S391 STR system. Furthermore, DNA samples from artificially degraded DNA and from real casework were analyzed.

2. Material and methods

2.1. DNA extraction

Human genomic DNA was extracted from blood and forensic telogen hair samples. Blood samples were extracted with the E.Z.N.A. Blood DNA Kit II (peqlab Biotechnologie GmbH, Germany). Hair samples were utilized from previous casework examinations. DNA from telogen hairs was extracted as described earlier by Hellmann et al. [3].

2.2. DNA amplification of autosomal STRs

The 25 μl PCR reaction mixture consisted of 12.5 μl reaction mix from the Qiagen® multiplex PCR Kit (Qiagen®, Germany), 10 μM of forward and reverse amplification primer and 11 μl of DNA. PCR was carried out with Perkin Elmer® 2400/2700 thermoyclers for 34 cycles using as follows: initial denaturation at 95 °C for 15 min, denaturation at 94 °C for 30 s, annealing at 57 °C for 90 s, extension at 60 °C for 90 s, followed by a final extension step at 60 °C for 30 min.

2.3. Multiplex separation and capillary gel electrophoresis

Three microliters of Sepharose beads (Streptavidin Sepharose™ HP, Amersham Biosciences Ltd.) was mixed with 20 μl PCR products, binding buffer and water to a final volume of 80 μl. The mix was incubated for 15 min at RT with continuous mixing. After immobilization, the beads were spun down, the supernatant was further purified with the MSB Spin PCRapace Kit (Invitek GmbH, Germany) according to the manufacturer's instructions and the PCR products were finally collected in 20 μl elution buffer. PCR products immobilized on the Sepharose beads were washed twice, first with 150 μl washing buffer and than with the same volume of 70% ethanol. Finally, the beads were resuspended in 20 μl elution buffer from the MSB Spin PCRapace Kit. Five microliters of each purified PCR fraction were analysed by capillary electrophoresis (using POP-6 polymer) in an ABI PRISM 310 Analyzer (Applied Biosystems, Foster City, CA, USA).

3. Results and discussion

To verify the specificity and reproducibility of the new STR multiplex, 66 pg of undegraded human DNA from 15 blood samples was amplified, separated and analysed by capillary gel electrophoresis. All typings were in accordance with the results from the SGM Plus™ (Applied Biosystems) or PowerPlex® 16 (Promega) kits and the single amplification of the D12S391 STR system. Less than 1% drop-out-alleles and no extra alleles among all analysed samples were observed. Allele dropout occurred randomly and was never observed twice for the same allele of a given sample. After separation and purification of the two multiplexes, a cross contamination between the two small multiplexes was never observed.

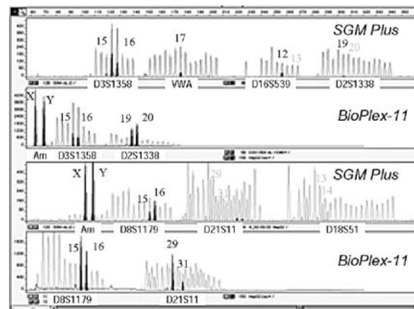

Fig. 1. Amplification of degraded DNA with the SGM Plus kit and the BioPlex-11.

To validate the assay sensitivity, DNA samples were typed from diluted samples from blood and cell lines down to 6.25 pg of DNA. Using more than 100 pg resulted in N+1 fragments typically found in cases of over amplification [4]. With 50 pg DNA, the first allelic drop-outs were observed. With lower DNA amount, the risk of drop-outs increased significantly. "Drop in" alleles were not observed.

Dilution studies cannot mimic the typing of degraded DNA properly, since the quality of the DNA is not considered. While the dilutions consist of high quality DNA from an unlimited source, the degraded DNA is a mixture of molecules of different fragment sizes from a limited source. Allele drop-out is a more random stochastic process and depends on the amount of available targets in the sample. To receive a more realistic impression of the typing capacity of our kit, we typed artificially degraded DNA [5] with the SGM Plus kit from ABI and the BioPlex-11 and compared the results (Fig. 1). With the SGM Plus kit, one or both alleles from the longer STR systems dropped out. Sometimes we also observed false alleles, as shown here for D21S11. In contrast, the BioPlex-11 amplified all alleles correctly due to the small size of the resulting fragments.

The kit contains six of the seven loci for ESS database STR systems and eight CODIS loci. The combination of 10 autosomal markers results in a high value for the power of discrimination (PD) in identity cases and is calculated for all ten loci to 1 in 4 billion, and for the 6 ESS database marker alone to 1 in 50 million. From all telogen hair samples which we have analysed until now 24% gave a full DNA profile, 58% a partial DNA profile and 18% showed no results. Therefore, we think that the BioPlex-11 seems to be a good tool for the typing of telogen hair roots, and can also be used for other problematic samples like bone or decomposed bodies.

References

[1] H. Matsuda, et al., Technical investigation of DNA extraction from single hair shaft, Rep. Nat. Res. Inst. Police Sci. 50 (1997) 23–28.

[2] K. Bender, P.M. Schneider, Validation and casework testing of the BioPlex-11 for STR typing of telogen hair roots. Forensic Sci. Int. 24 (in press).

[3] A. Hellmann, et al., STR typing of human telogen hairs—a new approach, Int. J. Leg. Med. 114 (2001) 269–273.

[4] R. Sparkes, et al., The validation of a 7-locus multiplex STR test for use in forensic casework: II. Artefacts, casework studies and success rates, Int. J. Leg. Med. 109 (1996) 195–204.

[5] K. Bender, M.J. Farfan, P.M. Schneider, Preparation of degraded human DNA under controlled conditions, Forensic Sci. Int. 139 (2004) 135–140.