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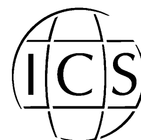
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Multiplex typing with 5 Y-chromosomal SNPs

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Abstract. Many different methods have been established for SNP detection and especially minisequencing has often been used. For our study we have selected 5 Y-chromosomal SNPs (M9, M17, M45, M170, M173) based on the degree of polymorphism. PCR primers were designed with the aim to get amplicon lengths of <200 bp. The Y-SNPs were optimized in singleplex reactions and combined to multiplex approaches by sequential optimization. Finally, we were able to analyse these 5 Y-chromosomal SNPs in one PCR/SNaPshot reaction. To validate this method for forensic stain analysis we investigated bones, bloodstains, cigarette butts and epithelial cells. Additionally, we typed 50 unrelated Caucasian individuals to obtain our regional haplotype frequencies. © 2005 Published by Elsevier B.V.

Keywords: Y-SNPs; Short amplicon; SnaPshot; Caucasian

1. Introduction

Within the last years PCR based SNP (*s*ingle *n*ucleotide *p*olymorphism) typing has become more and more important in forensic DNA analysis [1,2]. Minisequencing by using the SNaPshot Kit (Applied Biosystems, Darmstadt, Germany) is the most frequent method in forensic SNP analysis and was also used for our multiplex approach. For our study we selected 5 Y-chromosomal SNPs (M9, M17, M45, M170, M173) [3] based on the degree of polymorphism to be most informative for stain analysis. The Y-SNPs were optimized in singleplex reactions and then combined to multiplex approaches. Finally, we were able to analyse these 5 Y-chromosomal SNPs in one highly sensitive PCR/SNaPshot reaction.

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2. Material and methods

DNA from blood and from buccal swabs was extracted using the Chelex method described elsewhere. DNA from other stains was extracted with the First-DNA all-tissue DNA kit (GEN-IAL® GmbH, Troisdorf) according to manufacture's protocol. Apart from that bones were handled in a different way. The protocol for DNA extraction with the First-DNA all-tissue DNA kit was modified by using silica beads (glass milk). In addition DNA extraction from bones was performed with the Charge Switch® Forensic DNA Purification Kit (Invitrogen Life Technologies, Karlsruhe). Cell line DNA was used as standard DNA (9948, male DNA/9947A female DNA, Promega, Mannheim). The five Y-chromosomal SNPs M9, M17, M45, M170 and M173 were analysed using the minisequencing technique. PCR products were amplified with primers taken from the literature. PCR amplification was performed in 30 cycles following the profile: 95 °C for 45 s, 60 °C for 45 s, 65 °C for 45 s. PCR products were purified applying the two restriction enzymes SAP and ExoI. For the minisequencing assay the reaction mix from the SNaPshot® kit (Applied Biosystems, Weiterstadt) was used. Minisequencing primer were designed using PrimerSelect (DNA STAR). Minisequencing was performed as described in the manufacture's protocol. After purification minisequencing reactions were analysed on an ABI 310.

3. Results and discussion

If mixed stains consisting of male and female DNA have to be analysed the nonrecombining region of the Y-chromosome is especially relevant for forensic stain analysis. We validated a set of five Y-chromosomal SNPs for forensic stain analysis by optimizing the singleplex typing up to a five system multiplex (Fig. 1). Additionally, we analysed crime scene stains (Fig. 2). To validate this method for forensic stain analysis we investigated vaginal/sperm cell mixtures, bones, bloodstains, cigarette butts and epithelial cells (data not shown). Additionally, we typed 50 unrelated Caucasian individuals to obtain our regional SNP frequencies/haplotypes. The comparison of these frequencies with the data of Sanchez et al. [2] shows similar results (data not shown).

In conclusion, the use of minisequencing for Y-chromosomal SNP typing has the advantage that short PCR products can be analysed. Although STR typing lead to a much higher degree of information the SNaPshot method enables multiplex detection of biallelic

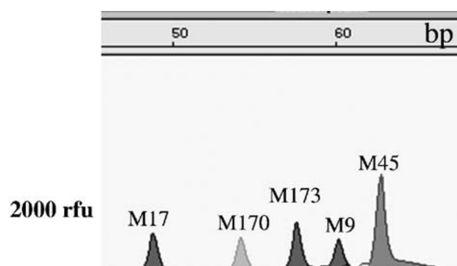

Fig. 1. Example of a five SNP multiplex after electrophoretic separation on ABI310.

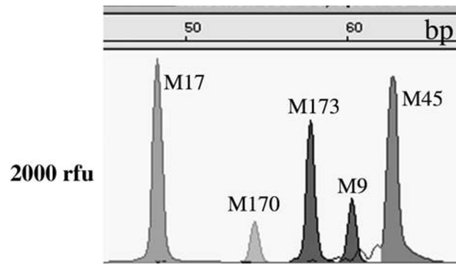

Fig. 2. Y-SNP result of degraded bone DNA from a male skeleton which was found in a wood; STR analysis was only successful for shorter amplicons.

markers for the investigation of highly degraded biological stains and a very sensitive detection of the male component in mixed stains.

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