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Multiplex genotyping of 22 autosomal SNPs and its application in the forensic field

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Abstract. This study reports the selection of 22 autosomal SNPs and the setting of PCR and minisequencing multiplexes suitable for forensic purposes. © 2005 Published by Elsevier B.V.

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1. Introduction

Due to their high abundance in the genome [1], SNPs have caused great interest in several fields of medical genetics. At the moment, public and private databases are being set up, collecting information on SNPs as concerns mapping data, flanking regions, allele frequency, etc. Various SNP typing technologies have also been developed [2].

These developments have all led to the possibility of considering SNPs as a new class of forensic genetic markers. Several aspects render SNPs as valuable markers for forensic investigations: their mutation rate, the applicability of high throughput genotyping technologies and the possibility of analyzing highly degraded DNA samples.

On the other hand this new class of markers has considerable limitations with respect to commonly used STRs: wide STR databases already exist; it is still unclear what the most appropriate typing method would be for SNPs; SNPs are more or less useless in mixture interpretation [3]. Moreover, due to their limited polymorphic content, a large

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number of SNPs are required to achieve a similar discrimination power to that of STRs [4,5].

An evaluation of an SNP-panel for forensic applications is reported here.

2. Materials and methods

22 SNPs, one for each autosomal chromosome, were selected from the “The SNP Consortium” database. We chose SNPs located within non-coding DNA regions and with an allele frequency of around 0.5 as estimated from at least two different population studies West Eurasians.

DNA was extracted from the whole blood of 50 healthy subjects by phenol chloroform and quantified by 1% agarose gel electrophoresis.

Multiplex PCR primer sequences were designed by Primer Express™ 1.0 (AB). Primer pair design and correct annealing were checked by singleplex amplification and direct sequencing of singleplex PCR products. 1 ng of genomic DNA was submitted to multiplex amplification in a final volume of 25 μl.

Polymorphic sites were detected by minisequencing reactions using the ABI PRISM® SNaPshot™ Multiplex Kit (AB). Primer extension products were separated by capillary electrophoresis on an ABI PRISM™ 310 Genetic Analyzer.

Multiplexes were also tested on low molecular weight DNA after fragmenting in a sonicator device. Sonicated DNA was amplified with the AmpF*l*STR Identifiler PCR Amplification kit (AB) and analyzed on an ABI 310 (AB).

3. Results and discussion

DNA regions containing 22 polymorphisms were amplified in 3 multiplex PCR reactions each consisting of 7 or 8 amplicons. Primers were designed so that lengths of the amplified fragments ranged between 53 and 151 bp. The extension primers were designed to be well-spaced from one another by adding poly-T or poly-GACT tails, with final primer lengths of between 20 and 70 nucleotides; identification of the extension products was always easy and certain. Fig. 1 shows the electropherograms of the 3 multiplexes.

To test the sensitivity of PCR for forensic applications, we performed experiments with decreasing amounts of DNA template. These experiments showed that the 3 multiplex reactions worked well in the range from 100 pg to 1 ng of DNA. To explore the suitability of the multiplexes with fragmented DNA, experiments were performed by amplifying sonicated DNA, with fragments sized less than 150 bp. Positive amplifications were obtained for all 3 SNP multiplexes, while the

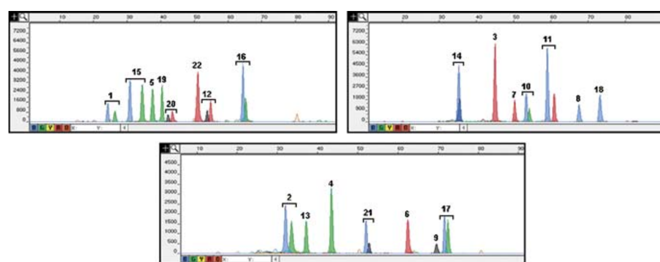

Fig. 1. Electropherograms of the 3 multiplexes, each consisting of 7 or 8 SNPs.

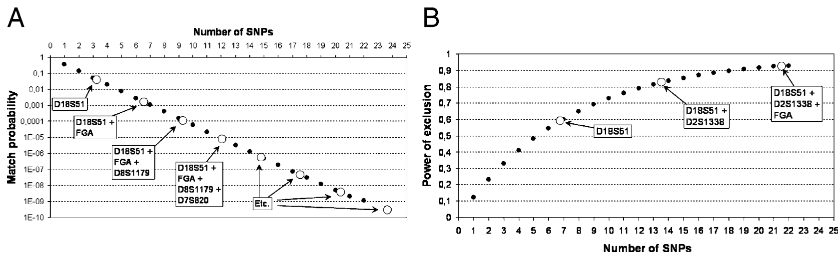

Fig. 2. (A) Comparing SNPs and STRs for the probability that unrelated subjects share the same multilocus genotype; (B) comparing SNPs and STRs for the probability that unrelated subjects are excluded as parent–child pairs.

STR multiplex displayed high background noise and only questionable peaks were obtained for STRs sized below 150 nucleotides.

In order to check the reproducibility of the multiplexes and evaluate their utility in forensic applications, 50 samples from central Italy were analyzed. No sample shared the same genotype. We were interested in comparing the efficiency of SNPs and STRs in a forensic context. How many SNPs are required to reach the same probability levels attained with standard STRs in casework studies? We addressed this question by computing the match probability (MP) and the exclusion probability (PE). We compared the SNPs characterized in this work with the loci included in the Profiler™ set.

Fig. 2A shows the cumulative match probability obtained by using an increasing number of SNPs (dots) compared with that obtained using an increasing number of STRs; both SNPs and STRs are ordered by decreasing values of heterozygosity. It can be seen that there is a rough equivalence 1 STR = 3 SNPs. Fig. 2B shows the comparison of cumulative PE. In this case, the equivalence is about 1 STR = 7 SNPs.

In conclusion, the technique used for our work is simple, efficient and applicable in any forensic laboratory. Amplifications can be performed successfully on small amounts of DNA template and on fragmented DNA. Moreover, our analyses show that autosomal SNPs can effectively replace autosomal STRs in some situations that are encountered in forensic genetics. However, for the time being, STRs remain the preferred markers.

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