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SNPs: tools for individual identification

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Abstract. SNPs, DNA sequence alterations at a single nucleotide position, present useful advantages over other markers for forensic purposes. The most important advantage is the analysed DNA sequence size, which is much shorter than the one used for STR research. Therefore, SNP profiles could have an application in filiations and individual identification, especially in anthropology where samples are old and often degraded. The aim of this study is the selection of a panel of informative SNPs and the confirmation of their transmission on unrelated French paternity cases. SNPs being biallelic, the analysis of about 50 of them is necessary to obtain the discrimination power of the existing STR multiplexes. We selected a set of informative SNPs and optimised their amplification through multiplex (6–10 fragments) reactions. Pools of two to three loci are analysed simultaneously using MALDI-TOF mass spectrometry. We consider automatic analysis and profile-sample attribution through collaboration with Bruker Daltonique (France). Besides applying these markers to paternity cases, we also evaluate selected SNPs on different populations in order to evaluate population specific frequency data.

Keywords: Forensic genetics; Single nucleotide polymorphism (SNP); Paternity; Individual identification; MALDI-TOF MS

1. Introduction

Despite the fact they are biallelic and therefore of limited discriminatory value, SNPs offer attractive features as genetic markers. For forensic and anthropology applications, the particular advantage of SNPs over other genetic markers is in the length of the studied DNA region. The needed DNA sequence being much shorter, SNP profiles could have an application in studies where only small or degraded samples are available analysis. Furthermore, it has been demonstrated that relatively small arrays of approximately 50 loci are comparable to existing STR multiplexes provided that their alleles range in proportion between 0.2 and 0.8 [1].

The aim of the present study is the selection and validation of a panel of informative biallelic markers, i.e. phenotypically not expressed but conserved and represented at high frequencies, their analysis by matrix assisted laser desorption ionisation time of flight mass

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Table 1
Masses of fragments expected after MALDI-TOF MS analysis

Product	Annotation in Fig. 1	Product mass
Primer AB1T	1	4772 Da
Primer AB2I	2	4858 Da
AB1T-allele A	1a	5085 Da
AB2I-allele A	2a	5171 Da
AB1T-allele G	1b	5406 Da
Primer WI 1732	3	5588 Da
WI 1732-allele A	3a	5901 Da
AB2I-allele G	2b	6159 Da
WI 1732-allele G	3b	6230 Da

In this example, the PEX medium requires dGTP, ddATP and ddTTP to detect the three concerned polymorphisms AB1T [3], AB2I [4] and WI 1732 [5].

spectrometry (MALDI-TOF MS) in order to establish individual specific profiles and evaluate the potential discrimination power.

2. Materials and methods

The SNPs were chosen from published results or the SNP consortium LTD database according to the criteria described in the introduction. Seventy-six individuals belonging to 21 unrelated STR-confirmed and 3 exclusionary paternity cases were genotyped for the 39 selected markers. DNA was extracted from blood samples. Six to 10 SNP containing fragments were co-amplified in multiplex PCR reactions. SNP detection was achieved

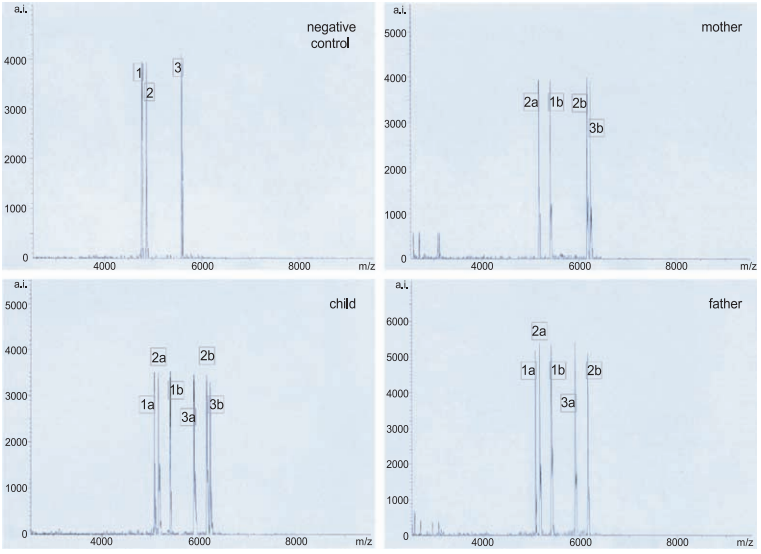

Fig. 1. Spectra obtained by analysing a paternity trio on three SNP loci in a multiplexed PEX reaction. Here, the absolute intensity of the signal is shown as function of the *m*/*z* ratio (laser shooting inducing monoprotonation, we virtually observe *m*). The sizes of the possible products are resumed in Table 1. Primers can only be observed in the negative control spectrum and not in the other ones as they have been consumed by the PEX reaction.

Table 2
Summary of genotypes observed in the example illustrated in Fig. 1 and the thereout calculated paternity indexes for each of the three loci

SNP	Mother	Child	Father	PI
AB1T	G/G	G/A	G/A	2.0243
AB2I	G/A	G/A	G/A	1
WI1732	G/G	G/A	A/A	2

through multiplexed (two to three SNPs) primer extension reactions (PEX) in order to have a minimal 80 Da size difference between the different products. These reactions were designed according to primer compatibility, nucleotides necessary to the PEX and masses of the possible products. An example of fragment masses expected after MALDI-TOF MS analysis are summarised in Table 1. Purification steps were accomplished using Genopure™ technology (Bruker Daltonics). Raw data obtained thanks to an Ultraflex™ MALDI-TOF mass spectrometer (Bruker Daltonics) were interpreted manually. A project of automatic analysis and sample-profile attribution is underway.

3. Results and discussion

An example of the obtained results can be observed in Fig. 1. The MALDI-TOF spectra are easily interpreted by the assessment of the presence or absence of a peak at distinct time-of flight points (corresponding to the mass of the fragment). The genotypes of the paternity trio on these three loci are summarized in Table 2 along with the paternity indexes, which can be calculated thanks to the formulae routinely used in STR analysis [2] and the reported allelic frequencies.

4. Interpretation and discussion

When five loci genotypes inconsistent with paternity were observed, exclusion was concluded, as the probability of the five inconsistencies being linked to mutation is minimal. The obtained results allowed to calculate partial probabilities of paternity by the formula $W = \mathrm{PI}/(1 + \mathrm{PI})$ estimating the probability prior to considering genetic evidence to 50%. These partial W ranged between 99.98% and 99.9999%, which confirms the utility of SNPs as markers for individual identification. Despite the bias due to the presence of persons sharing their genetic material (parents and children), the frequencies we observed on these 76 individuals neighbour the ones reported. This indicates the chosen SNPs are suitable for this application. Furthermore, an SNP profile was obtained from difficult forensic samples such as hairs that did not allow a complete STR profile. This method could allow the achievement of individual specific genetic profiles in short time and at low costs.

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