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SNP-genotyping on human Y-chromosome for forensic purposes: comparison of two different methods

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Abstract. Y-chromosomal DNA polymorphisms and especially the Y-STRs are well established for forensic routine casework. Concern of our study was to use Y-SNPs for forensic purposes, which are mainly investigated in molecular anthropology for evolutionary studies. We compared two minisequencing methods with different detection principles.

Keywords: Y-chromosomal SNP; Minisequencing; SNaPshot; MALDI-TOF MS

1. Introduction

Whereas short tandem repeats on the human Y-chromosome (Y-STRs) are well established in forensic routine casework, Y-chromosomal single nucleotide polymorphisms (Y-SNPs) are mostly used in molecular anthropology for evolutionary research up to now. In recent studies, several Y-SNPs have been shown to be potentially useful for forensic purposes, as well. In the field of forensic routine, methods for genotyping Y-SNPs have to comply with strong quality requirements. In particular, high efficiency in the analysis of minimal DNA-amounts, extremely high reliability, automation and high-throughput capability as well as cost-efficient tests are important in forensic work. According to an international study by Kayser et al. (personal communication), eight Y-chromosomal SNPs were chosen and combined in a hierarchical system to two multiplex reactions: M9mut (M9, M74, M173, M46, M17) and M9anc (M170, M172, M35), respectively. The hierarchical genotyping strategy allows a correlation of samples to different European haplogroups. In a proof-of-principle study, the samples from four different European populations were analysed with both methods.

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2. Materials and methods

Samples from routine casework were analysed. DNA extraction was performed using the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). To amplify the targeted sequences of DNA, the set of Y-SNPs chosen by M. Kayser (personal communication) was used. The sequences of the primers following M9: Kayser et al. (2000), M74: Kayser (pers. comm.), M46: Zerjal et al. (1997), M17, M172: Cordeaux (pers. comm.) and M173, M170, M35: unpublished. Allele identification was achieved by PCR amplification of 10–50 ng of genomic DNA.

2.1. SNaPshot minisequencing

The amplified products were purified from dNTPs and primers by shrimp alkaline phosphatase (SAP) and exonuclease I (EXO I) treatment to avoid participation in the subsequent primer-extension reaction. For the minisequencing reaction, the commercial kit SNaPshot Multiplex of ABI was used. After the extension and labelling reaction, the unincorporated ddNTPs were removed by enzymatic treatment using 0.2 U of SAP to avoid comigration of [F] ddNTPs with the fragment of interest. The sequences of the SNaPshot primers are of all markers are self-designed for a length of the product between 19 and 61 bp (Fig. 1).

2.2. Electrophoresis and detection

The products were analyzed on the ABI Prism™ 310/ABI Prism™ 3100 Avant Genetic Analyzers.

For MALDI-TOF MS-based Y SNP genotyping, the PCR-products containing the polymorphic site were treated with SAP and EXO I. In the following primer extension reaction, special primers with a biotin label at the 5′ end and a photocleavable building block at position 8 or 9 from the 3′ end were used. The extension primers hybridized directly adjacent to the SNP and were extended allelespecific by one nucleotide. Subsequently, the primer extension products were purified using the Bruker genostrep™ purification system. There by, the primer extension products were bound to streptavidin coated microtiter plates, salts and other undesirable components were removed by washing

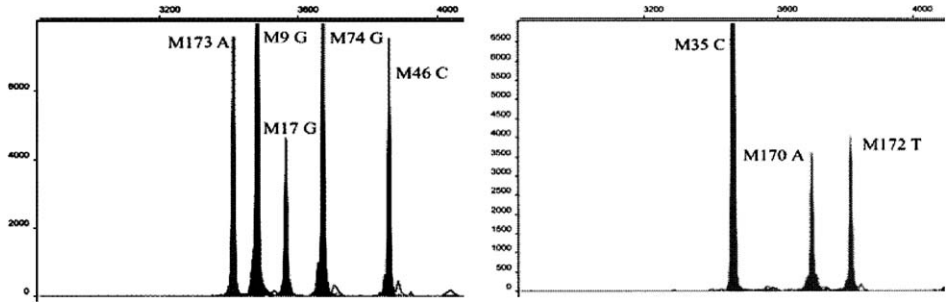

Fig. 1. Left: result of a pentaplex-reaction. Right: result of a triplex-reaction.

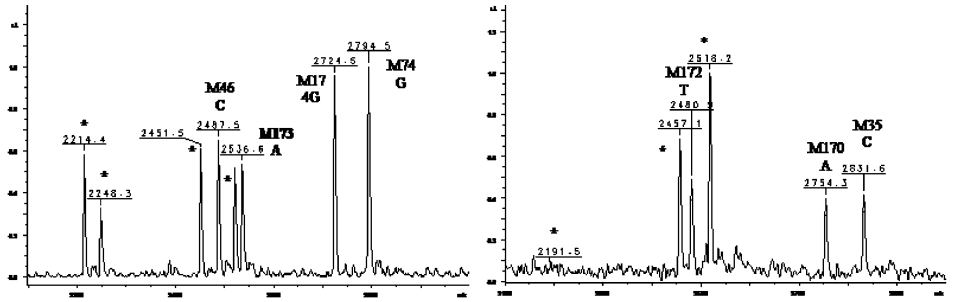

Fig. 2. Left: result of a quadruplex-reaction. Right: result of a triplex-reaction. The peaks marked with (*) show the remaining primers and the peaks labelled with the respective SNP and the genotype represent the primer extension products in all mass spectra.

and finally the purification plate was exposed to UV-light (366 nm, UV unit CL-366™, Bruker). Via UV-cleavage the 3′ part of the primer extension products containing the genotyping information was released to the elution buffer and could be transferred to the MALDI-TOF MS sample carrier. The shortened primer extension products were measured subsequently in a Bruker autoflex™ mass spectrometer in linear mode using 3-hydroxypicolinic acid (3-HPA) as matrix (Fig. 2).

3. Results

Fig. 1 shows results of the minisequencing with SNaPshot-reaction. An example of the pentaplex (left) shows the samples belong to the haplogroup Eu13/14 following the definition of Semino et al. (2000). In contrast, a result of the triplex for a sample of the haplogroup Eu4 is shown on the right.

Alternatively, Fig. 2 shows results of MALDI-TOF MS Y-SNP genotyping of the same samples. On the left the quadruplex-reaction (M74, M173, M46 and M17) detecting a sample representing the haplogroup Eu13/14 and on the right a result of the triplex-reaction (M35, M170, M172) of a sample representing the haplogroup Eu4.

4. Discussion

The results of our study show, that both fluorescent-based minisequencing using the ABI Prism SNaPshot multiplex kit and the MALDI-TOF MS-based genotyping in combination with the Bruker genoSNIP assay are well suited for genotyping Y-SNP markers. The European haplogroups could be accurately genotyped with the presented multiplex assays using both methods. The sample preparation procedure of both systems comprising PCR, SAP/*Exo*I-digest and single base extension reaction are very similar. To apply the SNaPshot minisequencing multiplex kit, the same equipment can be used as for genotyping STRs. The analysis costs for genotyping Y-SNPs are in the same range as for STR-analysis. In comparison to SNaPshot minisequencing, genotyping of Y-SNPs using the genoSNIP assay based on MALDI-TOF MS is less time consuming. Up to 384 samples can be prepared in parallel automatically using a dedicated pipetting robot (Bruker puredisk™) and measured with high accuracy and speed in the mass spectrometer.