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MtDNA coding region SNPs for rapid screening and haplogroup identification of forensic samples

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Abstract. Analysis of single nucleotide polymorphisms (SNPs) is a promising application in forensic human identification. We selected 16 SNPs from the coding region of the human mitochondrial DNA in order to ascribe samples to 1 of the 9 major West European Caucasian mitochondrial haplogroups. The selected SNPs are targeted in two multiplex-systems, via the application of the SNaPshot™ kit, a multiplex method based on the dideoxy single-base extension of unlabeled oligonucleotide primers. By screening these SNPs prior to sequencing analysis of the hypervariable regions in the control region, we would be able to rapidly differentiate between stains or hairs in high volume case work or to eliminate multiple suspects from an inquiry.

Keywords: West European Caucasian mtDNA haplogroups; Mitochondrial SNPs; Multiplex SNP typing; Primer extension method; Forensic profiling

1. Introduction

Every European can trace his or her evolutionary history back to nine ancestral matriarchal groups, referred to as mitochondrial haplogroups, from which all Europeans appear to be descended. The assignment to these haplogroups relies on the identification of a series of phylogenetic informative single nucleotide polymorphisms (SNPs). The set of selected markers in this study had to meet certain conditions: to discriminate between major European mtDNA lineages, to have a substantial power of exclusion with a minimal set of SNPs that may be targeted in two multiplex reactions and to be sensitive for degraded DNA or for low amounts of intact DNA.

Snapshot™ (AB) is a mini-sequencing method that relies upon the single-base extension of a primer immediately adjacent to the SNP using fluorescently labelled ddNTPs. The fluorescently labeled extension products can then be separated and visualized by electrophoresis and fluorescence detection.

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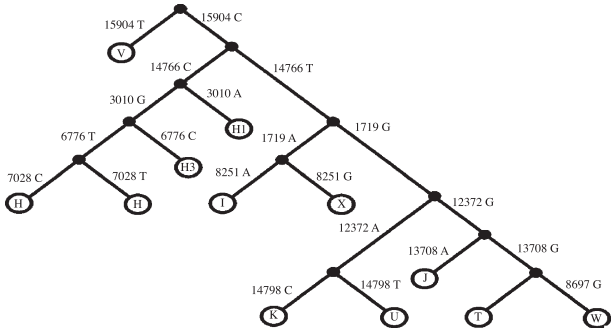

Fig. 1. Bifurcating decision tree for haplogroup assignment.

2. Materials and methods

For amplification, 19 μl of the PCR master mix (1.0 unit of Advantage 2 (Clontech), 1.0 unit of reaction buffer (Clontech), 200 μM each dNTP (AB); amplification primers and their concentrations are specified in Ref. [1]) was mixed with 1 μl DNA extract (containing 0.5–25 pg of genomic DNA). The amplification reaction was conducted in two eightplex PCR-reactions. Multiplex I comprised the SNP target sites G709A, G1719A, G3010A, C7028T, A11251G, G12372A, T14798C and C15904T. Multiplex II comprised the SNP target sites A1811G, T6365C, T6776C, G8251A, G8697A, G9055A, G13708A and C14766T. After an initial denaturation at 95 °C for 2 min, the reactions were put through 28–32 cycles of denaturation at 95 °C for 30 s, annealing at 66 °C for 30 s and extension at 72 °C for 30 s. PCR primers and unincorporated dNTPs were removed by adding ExoSAP-IT (USB). The reactions comprised 2.5 μl of the SNaPshot™ Multiplex Ready Reaction Mix, 2.0 μl of PCR product, 1.0 μl of pooled extension primers (0.2 μM final concentration for each primer) and water up to 10 μl. Thermal cycling and post-extension treatment were conducted following the manufacturers protocol. Unincorporated ddNTPs were removed

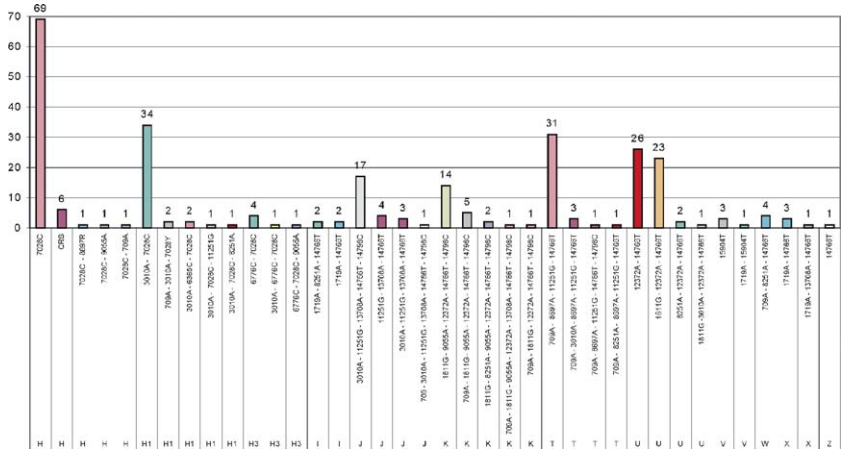

Fig. 2. Distribution mtDNA haplotypes in 277 unrelated Austrian Caucasians.

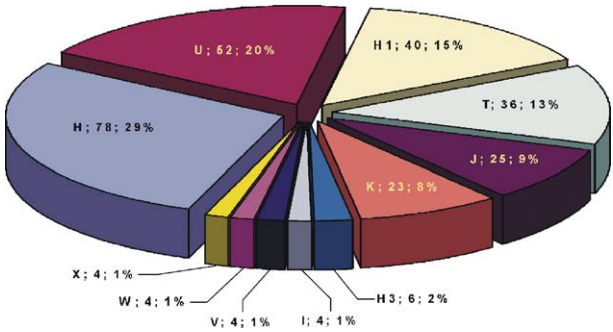

Fig. 3. Distribution of West European Caucasian haplogroups in 277 unrelated Austrian Caucasians.

with SAP (USB). The assignment to West European Caucasian haplogroups and was performed by following the branches of a bifurcating tree (Fig. 1).

3. Results

All 277 samples were successfully typed with the two multiplexes. The haplotypes found in this study are summarized in Fig. 2. All nine major West European Caucasian haplogroups (and two sub-haplogroups) were observed in our sample (Fig. 3). The most common haplotype found was a profile matching the rCRS, thus assigned to haplogroup H (24.5%). Overall, 37 different lineages were found in 277 individuals and 16 of them appeared only once in the dataset. The probability of a random match between two unrelated individuals was calculated as 11.4%.

The screening procedure presented here proved to be practical for unambiguously ascribing haplogroup origin to unknown DNA samples, and the method itself was shown to be very sensitive and suitable to type samples containing at least 1 pg genomic DNA. The entire procedure may be completed in 1 day and integrates easily with existing laboratory equipment. In addition, the assay is well suited for use on high degraded samples, as the amplicon lengths do not exceed 100 bp. Apart from the ability to eliminate non-probative DNA samples from further investigations, the assignment of a sample to a certain haplogroup gives the investigator the possibility to target specific control region fragments, which show a higher variability for certain haplogroups. This novel, multiplex PCR amplification and typing system for 16 mtDNA coding region SNPs promises to be a convenient and informative new DNA profiling system in the forensic field.

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Reference

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