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Dissection of mitochondrial haplogroup H using coding region SNPs

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Abstract. Analysis of single nucleotide polymorphisms (SNPs) is a promising application in forensic human identification. We selected 45 SNPs from the coding region of the human mitochondrial DNA in order to ascribe samples belonging to mitochondrial haplogroup H (hg-H) to one of the previously described sub-lineages of hg-H. SNP selection was carried out using the available literature on population and forensic genetics and extended by means of phylogenetic analysis of complete genomes (>400) and control region profiles. The selected SNPs are amplified in two PCR-multiplex reactions and subsequently targeted in three multiplex systems via the application of the SNaPshot kit. Samples belonging to haplogroup H (approximately 40% of West Eurasians), in most cases, cannot be distinguished from each other based on control region polymorphisms. By screening the selected coding region SNPs after sequencing of the control region, however, we would be able to rapidly differentiate between stains or hairs in high volume case work or to eliminate multiple suspects from an inquiry. The presented hg-H screening strategy was conceived as a high-throughput method and the distribution of the selected SNPs and targeted haplogroups was inferred from a huge population sample.

Keywords: West Eurasian mtDNA haplogroup; Mitochondrial SNP; Multiplex SNP typing; Primer extension method; Forensic DNA profiling

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1. Introduction

Analysis of single nucleotide polymorphisms (SNPs) is a promising application in forensic human identification [1]. The latest developments focus on rapid screening procedures that provide information on the mitochondrial DNA haplogroup affiliation of samples [2,3], thus enabling a quick elimination of exclusionary samples from further investigations.

West Eurasian mtDNA profiles can be classified into 10 major haplogroups (H, J, K, N1, T, U4, U5, V, X and W; [2,4–7]), with superhaplogroup H being predominant (~40–50% of samples). To increase the power of discrimination inside hg-H without leaving the level of screening applications, a rapid SNP screening method for a detailed dissection of hg-H was developed applying the SNaPshot (AB, Applied Biosystems, Foster City, CA, USA) minisequencing technology.

2. Materials and methods

Blood samples from 2214 unrelated Austrians were Chelex extracted [8] and pre-screened for hg-H by typing the markers 2706 and 7028 in a real-time PCR assay (H. Niederstätter, personal communication). In total, 859 samples (38.8%) turned out to belong to hg-H. The haplogroup diagnostic sites were selected from the literature [3–6,9–11]. Primers for amplification and minisequencing were designed using the program VisualOMP (DNA Software Inc., Ann Arbor, MI, USA). SNP sites, primer sequences and PCR conditions are going to be published soon (manuscript in preparation).

3. Results and discussion

A total of 35 different haplotypes were found in 859 samples belonging to hg-H. The majority of samples (67.1%) were resolved into smaller sub-haplogroups of hg-H (Table 1). Haplogroup H1 encompassed 25.3% of the hg-H samples, and a further subdivision could be established by the presence of polymorphisms T6365C (H1a1; 0.3%), A3796G (H1b; 2.8%), A9150G (H1c1; 1.9%), C12858T (H1c2; 0.7%), A9066G (H1f; 0.2%), T8602C (H1-8602; 0.2%) and T8473C (H1-8473; 0.2%). The remaining 33 haplogroups occurred at frequencies lower than 5% (Table 1).

The SNP markers selected for this study provide a very fine resolution within hg-H. Two major groups (H* and H1) still remain to be resolved by the application of appropriate sites. The presented hg-H dissecting multiplex system serves the forensic investigator in two important ways: as a screening tool, it provides quick information for the elimination of exclusionary samples; in addition, the strategy supplies further discrimination of samples that cannot be discriminated with control

Table 1
Frequencies of the different haplogroup H sub-haplogroups

Hg	%	Hg	%	Hg	%	Hg	%	Hg	%	Hg	%
H*	32.9	H1-8602	0.2	H3*	2.2	H4a1	0.2	H6a2	0.1	H13a1	2.1
H1*	19.0	H1-8473	0.2	H3a	4.6	H4a1a	0.5	H7*	2.3	H14	0.8
H1a1	0.3	H1f	0.2	H3b	0.9	H5a	2.9	H8*	0.1	H15	2.0
H1b	2.8	H2*	1.5	H3c	0.1	H5a1	2.0	H10	3.6	H16	2.0
H1c1	1.9	H2a	2.6	H4*	0.9	H6a	0.5	H11	1.4	H-709	2.0
H1c2	0.7	H2b	2.4	H4a	0.7	H6a1	2.4	H13a	1.0		

region sequencing. Thus, the presented hg-H dissecting multiplex system poses a new and innovative tool for forensic casework, which becomes very effective in investigations involving West Eurasian populations.

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