Int J Legal Med (2008) 122:517–523
DOI 10.1007/s00414-008-0267-x

TECHNICAL NOTE

A rapid mtDNA assay of 22 SNPs in one multiplex reaction increases the power of forensic testing in European Caucasians

S. Köhnemann · U. Sibbing · H. Pfeiffer · C. Hohoff

Received: 2 April 2008 / Accepted: 20 June 2008 / Published online: 19 August 2008

Abstract We have developed a multiplex mitochondrial (mtDNA) assay of 21 coding region single nucleotide polymorphisms (SNPs) and one control region SNP outside hypervariable region 1 (HVR1) and hypervariable region 2 (HVR2) that can be amplified in a single reverse touchdown polymerase chain reaction. Single base extension using the SNaPshot technique is also carried out as one multiplex. Besides the nine major European haplogroups (i.e. H, I, J, K, T, U, V, W, and X), 16 additional subclades (i.e. N1, X2, X2b, U2′-4/7′-9′, J/T, J1, J1c, HV, H1, H1a1, H1c, H3, H4, H6a, H7a H10) can be detected and classified into a phylogenetic mtDNA tree. By analyzing 130 Caucasoid samples from Germany, 36 different haplotypes were found resulting in a power of discrimination of 93.2%. Although 49% of all samples belonged to superhaplogroup H, the most common haplotype, i.e., haplogroup-specific SNPs plus haplogroup unspecific SNPs, had a frequency of only 18%. This assay is applicable for high-throughput mtDNA analysis and forensic mass screening. It will give additional information to the common control region sequencing of HVR1 and HVR2.

Keywords Multiplex mtDNA forensic assay · Phylogenetic tree · SNP · Power of discrimination · SNaPshot · haplogroup H

Electronic supplementary material The online version of this article (doi:10.1007/s00414-008-0267-x) contains supplementary material, which is available to authorized users.

S. Köhnemann · U. Sibbing · H. Pfeiffer · C. Hohoff (✉)
Institut für Rechtsmedizin, Universitätsklinikum Münster,
Röntgenstr. 23,
48149 Münster, Germany
e-mail: hohoff@uni-muenster.de

C. Hohoff
Forensische Genetik, Prof. B. Brinkmann,
Röntgenstr. 23,
48149 Münster, Germany

Introduction

Single nucleotide polymorphisms (SNPs) are distributed over the entire mitochondrial (mt) genome. It is assumed that regional varieties in the mtDNA coding region result from specific mtDNA replacement mutations [1]. Furthermore, mtDNA coding region SNPs are involved in a broad spectrum of diseases [2]. Hence, mtDNA haplogroup discrimination is interesting not only for phylogenetic and clinical but also for forensic studies. In forensic studies, knowledge about one suspect's or victim's haplogroup may help in crime scene investigations [3]. MtDNA typing is also important for degraded samples from hard tissues, especially bones [4] but even hairs [6], because there are 100–1,000 copies of mtDNA per cell, whereas there is only one copy of nuclear DNA (nDNA) per cell. Single base extension using SNaPshot (Applied Biosystems, Foster City, CA, USA) has crucial advantages than the routinely used hypervariable region (HVR) sequencing. For example, the amplicon size is typically smaller than 150 nucleotides (nt), whereas the size of HVR sequences is longer than 300 nt. Unfortunately, a maternal inheritance that is due to a lack of recombination and a size of 16,569 nt only causes a limiting power of discrimination compared to nDNA.

Simple haplogroup discrimination only will not lead to sufficient results because 39% [5] up to 50% [7] of all European Caucasians belong to superhaplogroup H. Thus, a subtyping of superhaplogroup H with further discriminating SNPs is inevitably necessary. This can be accomplished by a careful selection of SNPs that characterize different subclades [8] and seem to have more restricted geographic distributions [9]. A selection of haplogroup unspecific SNPs like hot-spot SNPs [10] that mutate in different haplogroup backgrounds is possible, too. In summary, it seems to be best to have both, an mtDNA forensic assay with hot-spot SNPs and SNPs discriminating for subclades of superhaplogroup H.

A fast and economic mtDNA SNP assay will save time and money and is suitable for mass screenings [11]. Therefore, all SNPs should be analyzed in one multiplex reaction.

Materials and methods

Selected mtDNA SNP sites

The SNPs (Table ESM 1) were chosen to discriminate between the main haplogroups present in Europe and their subclades. Furthermore, some SNPs were chosen because they are prone to mutate on any haplogroup background (hot-spot SNPs, i.e., 709, 1719, 3010, 8251, 13708; [10]). Site position numbering refers to the revised Cambridge Reference Sequence (rCRS; GenBank acc. no.: AC_000021).

Synthesized oligonucleotides

The polymerase chain reaction (PCR) and extension primers were synthesized by Biomers (Ulm, Germany). Lyophilized PCR primers were diluted to a stock solution of 100 pmol/μl and then diluted to working solutions of 25 and 2.5 pmol/μl. Extension primers were diluted to working solution of 10 and 1 pmol/μl. Both stock solutions and working solutions were stored at −20°C.

Primer design

PCR primers (Table ESM 2) were designed with the web-based software Primer3 (http://primer3.sourceforge.net). A test for hairpin structure and primer dimerization was carried out according to [12]. Extension primers for SNaPshot reactions, except the tags for electrophoretic separation, were taken as described elsewhere [8, 13–16]. The tag was selected according to [17]. The extension primers (Table ESM 3) were also screened for hairpin structure and primer dimerization.

Samples

DNA from buccal swabs, teeth, and hair shafts of voluntary donors was extracted as described elsewhere [18–20].

Multiplex PCR conditions

Multiplex PCR amplification was carried out in a total volume of 12.5 μl. Final primer pair concentrations for PCR are given in Table ESM 1. Final concentrations for standard PCR reagents were 1.25 μl $MgCl_2$ (5 mM), 1.25 μl Ampli Taq Gold PCR buffer (×10), 0.2 μl Ampli Taq Gold DNA polymerase (5 U/μl; all Applied Biosystems), 1.25 μl deoxynucleotide triphosphates (dNTPs; 250 μM; GE Healthcare, München, Germany), and 0.2 μl bovine serum albumin (20 mg/ml; MoBiTec, Göttingen, Germany).

Thermal cycling for PCR was carried out by reverse touchdown in a modified method according to [16] in a 2700 GeneAmp (Applied Biosystems) as follows: initial denaturation for 10 min at 95°C, three cycles of 95°C for 30 s, 55°C for 45 s, 72°C for 30 s, then 19 cycles 95°C for 30 s, 55°C for 45 s and 0.2°C per cycle, 72°C for 30 s, 11 cycles of 95°C for 30 s, 60°C for 45 s, 72°C for 30 s, final extension at 72°C for 7 min and storage at 4°C.

Multiplex extension primer conditions

Multiplex primer extension was carried out in a total volume of 10 μl. Final extension primer concentrations are given in Table ESM 1. Final concentrations for SNaPshot single base extension reagents were 2.5 μl of ABI Prism SNaPshot multiplex kit mix, 0.5 μl of ×10 Ampli Taq Gold PCR buffer and 1 μl of purified multiplex PCR product and 6 μl of balanced extension primer stock solution (see Table ESM 1).

Thermal cycling was carried out according to the manufacturer's recommendations (Applied Biosystems): 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s.

Purification

After PCR thermal cycling, 2.5 μl of shrimp alkaline phosphatase (SAP; Roche, Mannheim, Germany) and 0.05 μl of Exonuclease I (New England Biolabs, Frankfurt am Main, Germany) were added to each PCR reaction tube. Incubation was carried out in a 2700 GeneAmp thermal cycler (Applied Biosystems) at 37°C for 75 min, denaturation followed at 75°C for 15 min.

After SNaPshot reaction, 1 μl of SAP only was added. Incubation on a 2700 GeneAmp was carried out at 37°C for 45 min, denaturation followed at 75°C for 15 min.

Amplicon analysis

Of the purified extension primer product, 1 μl was mixed with 9.75 μl Hi-Di formamid and 0.25 μl LIZ-120 internal sizing standard (both Applied Biosystems). Amplicon analysis was carried out on an ABI Prism 3100 Avant Genetic Analyzer (Applied Biosystems), using 36 cm capillary arrays and default instrument settings as recommended by the manufacturer.

Data analysis and haplogroup assignment

The raw data were analyzed using GeneScan (version 3.7) and Genotyper software (version 2.5, Applied Biosystems). The main analysis, especially the labeling of the peaks, was

accomplished with a self-written macro in Genotyper software. Resulting information was exported to and analyzed by Microsoft Excel software (version 5.1; Microsoft, Redmond, WA, USA).

We chose the common phylogenetic network [21] of the mt genome and designed an assay specific haplogroup decision tree as can be seen in Fig. 1.

Random matching probability

According to Brandstätter et al. [13], the probability of a chance match $P(M)$ between two unrelated individuals is calculated as:

$$P(M) = n^{-2} \sum_{i=1}^{m} x_i^2$$

where n is the number of individuals in the database, m is the number of haplotypes in the database, and x_i is the number of times the ith haplotype was seen in the database.

Results and discussion

SNP sites

We chose SNP sites similar to [13, 16], i.e., 1719, 1811, 7028, 8251, 11251, 12372, 14470, 14766 and 15904, to detect the nine major European haplogroups H, I, J, K, T, U, V, W, and X. Ten sites were chosen to detect subclades, i.e., 1719, 3010, 3915, 5004, 6365, 6776, 8251, 9055, 13708, and 14798. Furthermore, two SNPs were chosen to provide additional power of forensic testing, i.e., 709 and 16519 [10, 16]. We also had included 4793, but it was hardly detectable in degraded DNA samples and remained in one allelic state, i.e., 4793G, in 130 samples. Increased primer concentration for 4793 in PCR and SNaPshot reaction led to artifacts in amplicon analysis (data not shown). In the end site, 4793 was excluded from this assay. Of the remaining 22 SNP sites, 21 were biallelic, and one remained in one allelic state (i.e., 12858C). Although there may be three variants for site 14470 (i.e. 14470T, 14470A, and 14470C), we did not find the 14470A transversion, which is indicative for haplogroup H10, but the 14470C transition, which is indicative for haplogroup X, exclusively. All SNP sites found are transitions in our study (Table ESM 1). Results of our SNaPshot assay were confirmed by retyping samples from the 2006 European DNA Profiling Group (EDNAP) collaborative exercise [23].

PCR primers

In this assay, the size of PCR amplicons was kept below 200 nt to increase the chance of successful amplification in degraded samples. Amplicon sizes range from 55 to 195 bp, on average 109 bp. In similar SNaPshot assays with forensic background, PCR amplicons ranged from 80 to 224 bp with an average of 143 bp [15] or from 122 to 186 bp with an average of 138 bp [16]. There was no hairpin structure as indicated by the program AutoDimer [12]. Primers were tested for cross-reactivity with AutoDimer, too. The highest score value for primer–dimer interaction was calculated as 7 for the self-compatibility of forward (FWD) primer 12372. Interaction was found at the 5′-end having an estimated melting temperature of only 38.5°C, leading to the conclusion that multiplex PCR primers would work well [22].

Every primer pair was confirmed in a singleplex reaction via polyacrylamide gel electrophoresis (data not shown). The

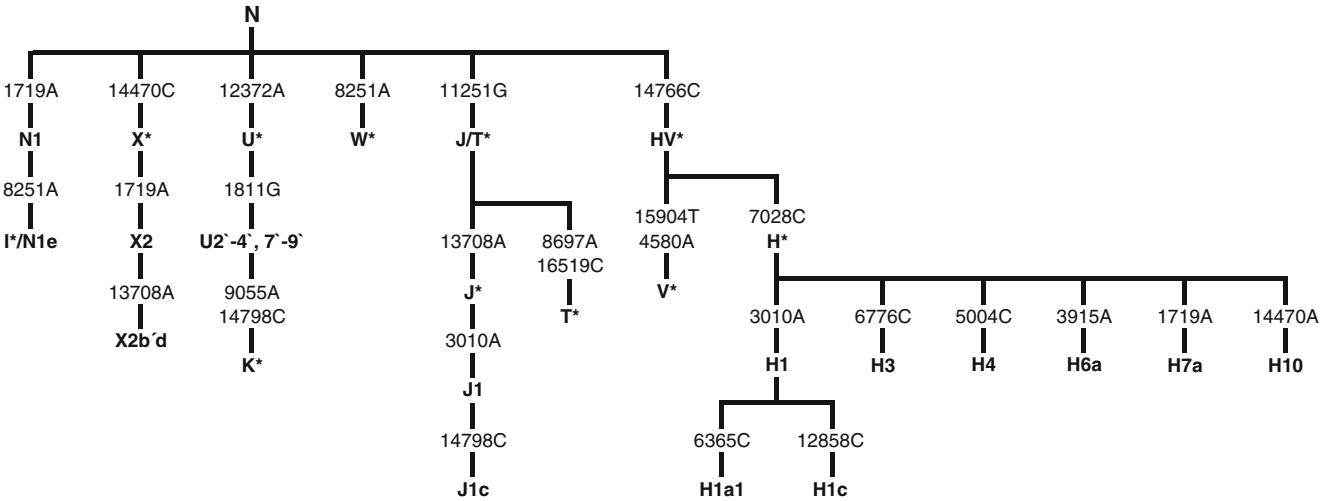

Fig. 1 Decision tree for haplogroup assignment. The *asterisks* indicates that this haplogroup may be discriminated into subclades

estimated size was consistent with the expected size for all primer pairs.

PCR thermal cycling conditions

The reverse touchdown PCR was carried out according to Vallone et al. (16) using modified settings. Electrophoretic separation of PCR products using 9.3% polyacrylamide gels at 30 mA and 1,000 V for 22 min exhibited that there are less unspecific PCR amplicons if the reverse touchdown PCR was carried out at 55°C instead of 50°C annealing temperature (data not shown). An annealing time of 45 s was sufficient. This higher annealing temperature and shorter annealing time could help decrease the amplification of additional unspecific PCR products.

Extension primers

No primer–dimer and hairpin structures were found for 22 extension primers.

Every extension primer was confirmed in a singleplex reaction, with its corresponding PCR amplicon only and within all PCR amplicons. The singleplex results corresponded to the multiplex results. Nevertheless, there were discrepancies from expected size and estimated size (for nomenclature see [12]) in a range of −0.2 nt (i.e., 14798C) to +7.3 nt (i.e., 709A). The following general observations were made:

1.) There was a tendency that extension primers with an expected size below 60 nt have a higher deviation in their estimated size than extension primers with an expected size above 60 nt. For example, the average Δnt (estimated size–expected size) for an incorporated G is 1.4 nt if the extension primer has a size below 60 nt, whereas it is only 0.5 nt for a primer above 60 nt. The same trend was shown for incorporated A, C, and T.
2.) There was a tendency that Δnt for G<Δnt for A<Δnt for C<Δnt for T. For example, the average values of Δnt for extension primers of 23–84 nt expected size

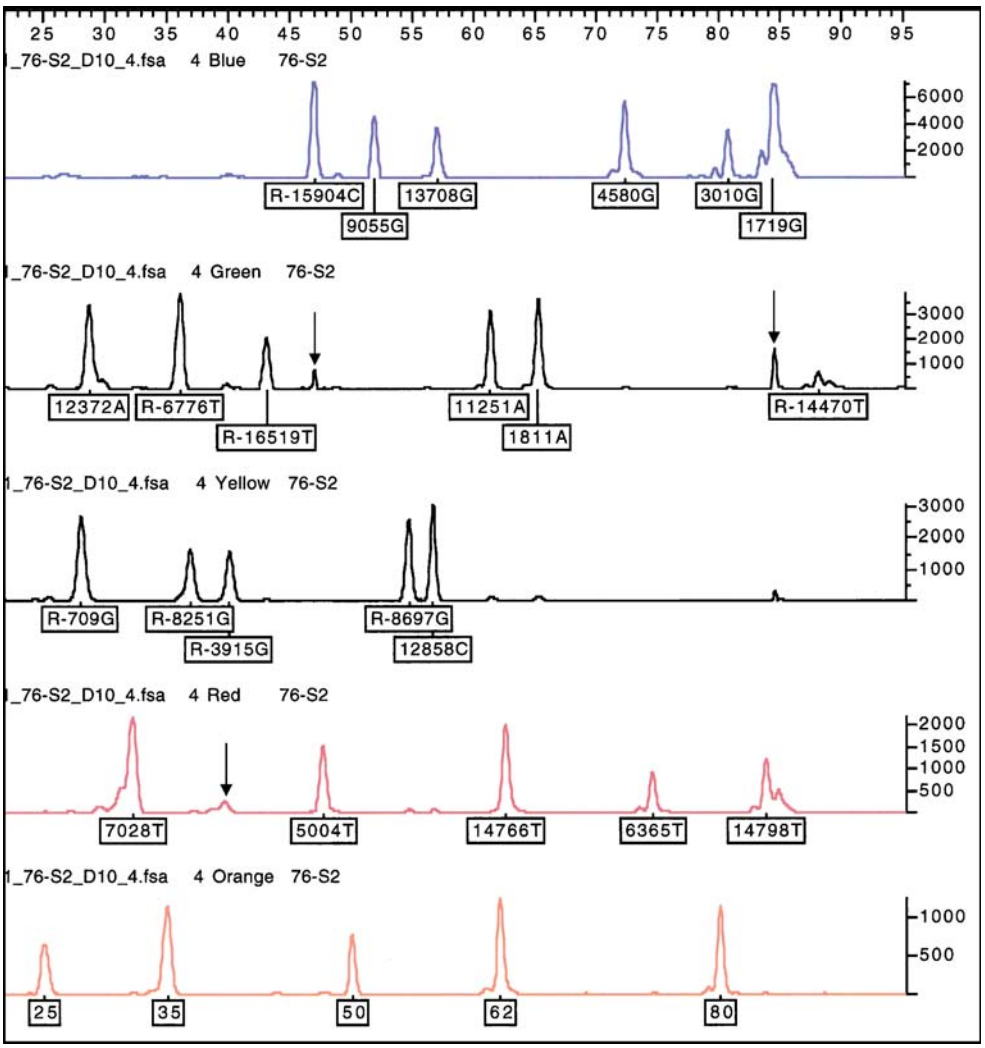

Fig. 2 Results for amplicon analysis of an EDNAP telogen hair sample (no. 2). "R" denounces that the extension primer attaches to the mtDNA H-strand. Numbers indicate the SNP site. The *last letter* indicates the polymorphism. Pull-ups are indicated by *arrows*

are: 1.4 for G<2.3 for A<B2.6 for C<3.4 for T. The same trend was true for extension primers of 61–84 nt expected size.

SNP genotyping

All 130 samples were typed successfully. A typical electropherogram of mtDNA extracted from telogen hair shafts of the EDNAP exercise 2006 can be seen in Fig. 2. The raw data of all samples were evaluated via macros in Genotyper software and in Microsoft Excel software exhibiting automated allele calling. It was obvious that the peak of site 14470 is the weakest compared to the other ones, although it had the highest concentration of PCR primer pairs and extension primers. This was described by [16] as well. Nevertheless, it was possible to balance most of the peaks to similar sizes. Similar to [16], we observed varying signal intensity based on the incorporated fluorescent-labeled dideoxynucleotide triphosphate (ddNTP). In our experiments, the signal intensity of fluorescent-labeled ddG>ddA>ddC>ddT. We confirm that this phenomenon is important for evaluating heteroplasmy detection, which was found in some of our samples. In addition, we have found that two possible products of one extension primer should have different electrophoretic mobilities in amplicon analysis of at least 0.5 nt, or otherwise a pull up might be misinterpreted as heteroplasmy.

In a mother–daughter–son trio, we observed stable and nearly identical profile peak ratios for each sample in two different analyses, if all conditions mentioned for this assay were kept stable. The same was true for retyped samples. In samples with poor quality of DNA, we observed more artifacts than in samples with high quality. The amount of DNA did not challenge our assay too much if the quality was good.

Haplogroup assignment

Out of 29 detectable haplogroups in 126 out of 130 samples from Germany, 24 were assigned to a particular haplogroup; the other four samples were assigned as “other” and belonged to two different haplotypes.

A total of 36 different haplotypes were found; 49% of all samples belonged to superhaplogroup H; nevertheless, subtyping reduced the most common haplotype (i.e., haplogroup H1) within this assay to 18% only. The other haplotypes ranged from 11% down to 1%. Fourteen haplotypes were observed only once, leading to a random matching probability of 6.8% for 130 samples from Germany. This is equivalent to a power of discrimination of 93.2%. For haplogroup H, the different haplotypes found can be seen in Fig. 3 (see also Tables ESM 4, and ESM 5).

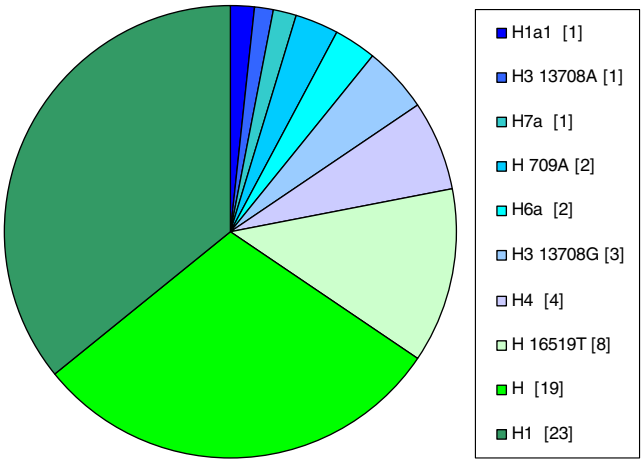

Fig. 3 Subtyping of haplogroup H, the legend illustrates the haplogroup and a haplotype specific SNP, respectively; numbers in brackets indicate the amount of samples

There were three hot-spot SNPs, namely 709, 13708, and 16519, with a relative frequency of 0.15, 0.08, and 0.69, respectively. A relative frequency between 0.20 and 0.80 is the best for individualizing SNPs; thus, site 16519 seems to be very good for forensic mtDNA typing in German Caucasoids.

There are three more SNPs having a frequency between 0.20 and 0.80 (i.e., 3010, 7028, 14766), but they are differentiating for haplogroup HV and H (i.e., 14766 and 7028) or for subclades of haplogroup H and J (i.e., 3010).

We then typed 16 European (i.e., five Italians, three Lithuanians, one Croatians, one Dutch, one Luxembourger, one Bulgarian, one Romanian, one English, and one French) and eight non-European (three Turkish, two Syrians, two Japanese, one Korean) samples and found that the distribution of haplogroup H had a relative frequency of 0.44 in the European sample, which is similar to that of the German sample. One more haplogroup that was not found in the German sample before could be identified, i.e., haplogroup preV. In the non-European samples, haplogroup H was not found. In these 24 non-German samples, nine more haplotypes were found, and the random matching probability declined to 6.1%. Although it was not possible to assign four of eight non-European samples to a haplogroup, all four samples were unique because of different haplotypes.

SNPs 1719, 3010, 8251, and 13708 were mentioned to be prone to mutate in different haplogroup backgrounds [10]. This was true for our non-German samples.

There were 56 samples sequenced for HV1. In 35 samples, the haplogroup discrimination of HV1 sequencing and SNP typing was identical. In eight samples, the SNP typing was more accurate than the HV1 sequencing. In 11 samples, the HV1 sequencing was more accurate than SNP typing, because

Table 1 HV1 sequencing and haplogroup typing, columns indicate the sample name, the predicted haplogroup after HV1 sequencing and the analyzed haplogroup after SNP typing

Sample	HV1 sequencing	SNP typing	Sample	HV1 sequencing	SNP typing
i.H. 15	D	other	i.H. 26	I	I
i.H. 47	H	H	i.H. 54	I	I
i.H. 12	H	H	i.H. 55	J	J/T
i.H. 25	H	H	i.H. 27	J	J1c
i.H. 48	H	H	i.H. 30	J	J1c
i.H. 04	H	H	i.H. 02	J	J1c
i.H. 07	H	H	i.H. 34	K	K
i.H. 13	H5	H	i.H. 40	K	K
i.H. 19	H	H	i.H. 33	K	K
i.H. 50	H	H	i.H. 41	K	K
i.H. 03	H	H'n	i.H. 08	K	K
i.H. 53	H	H'n	i.H. 09	K	K
i.H. 42	H	H'n	i.H. 39	N1	N1
i.H. 32	H5	H'n	i.H. 51	T	T
i.H. 37	H	H'n	i.H. 16	T	T
i.H. 56	H5	H'n	i.H. 20	T	T
i.H. 35	H	H1	i.H. 22	T	T
i.H. 36	H1a1	H1	i.H. 18	U5	U
i.H. 24	H1a1	H1	i.H. 10	U5	U
i.H. 06	H1*	H1	i.H. 11	U5	U
i.H. 38	H1*	H1	i.H. 31	U5	U
i.H. 21	H1*	H1	i.H. 49	U4	U'n
i.H. 29	H	H1	i.H. 43	V	V
i.H. 01	H?	H1	i.H. 28	V	V
i.H. 46	H	H1	i.H. 52	V	V
i.H. 17	H	H3	i.H. 14	W	W
i.H. 44	H	HV	i.H. 05	W	W
i.H. 45	HV	HV	i.H. 23	X	X

An n indicates that this sample has a C at position 16519.

SNP sites to detect these haplogroups are not included in our assay. In two samples, there was a discrepancy between SNP typing and HV1 sequencing, i.e., sample i.H. 15 and i.H. 44 (Tables 1, ESM 4, and ESM 5). For i.H. 15, there is a lack of further SNP sites in our assay, too, but for i.H. 44, there was a discrepancy in haplogroup discrimination by SNP typing and HV1 sequencing, respectively.

Challenging DNA samples

We typed hair shafts of the EDNAP collaborative exercise 2006 and found the same results as was required plus additional information of SNPs not included in the EDNAP assay. Furthermore, we typed five teeth from voluntary donors. Although the pulp was removed by a trephine, there were good results for all 22 SNPs. We also typed DNA extracts of 2-year-old telogen hair shafts and found the same results as were found in samples from buccal swabs of the same donor.

Conclusion

We have designed a forensic mtDNA assay of 22 SNPs. Compared to similar assays, this new multiplex assay has an increased discriminatory power in European Caucasians, because it combines SNPs of former assays [13, 16] to a power of discrimination of 93.2% in 130 Caucasian German samples. This assay is time and money saving because all 22 SNPs can be amplified in one multiplex PCR and one multiplex SNaPshot reaction. Nevertheless, there are still some samples with an identical haplotype after 22 SNPs typing. It is obvious that it will be impossible to discriminate any individual by mtDNA due to maternal inheritance and lack of recombination, yet mtDNA analysis is a powerful tool in forensics that benefits from small amplicons. This has been achieved for HV1 sequencing by using Mitominis, too [25]. A combination of fast and economic SNP typing that is followed by HV1 sequencing seems to be very efficient for forensic mass screenings. In the first step, it is possible to reduce the amount of samples to a frequency that equals the most common SNP haplotype, at least. In our study, this would have been 82% of all samples that could have been excluded from HV1 sequencing after SNP typing.

We will expand this assay to an even larger multiplex to distinguish between all major branches of the mtDNA phylogeny [21], and we will perform a forensic validation performing sensitivity studies, bone/teeth studies, and species specificity studies. We will also test if this assay is able to detect mtDNA point-heteroplasmy [24] or mixed stains.

References

1. Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, Wallace DC (2004) Effects of purifying and adaptive selection on regional variation in human mtDNA. Science 303:223–226
2. Fernandez-Moreno MA, Bornstein B, Petit N, Garesse R (2000) The pathophysiology of mitochondrial biogenesis: towards four decades of mitochondrial DNA research. Mol Genet Metab 71:481–495
3. Tolk HV, Barac L, Pericic M et al (2001) The evidence of mtDNA haplogroup F in a European population and its ethnohistoric implications. Eur J Hum Genet 9:717–723
4. Holland MM, Cave CA, Holland CA et al (2003) Development of a quality, high throughput DNA analysis procedure for skeletal samples to assist with the identification of victims from the World Trade Center attacks. Croat Med J 44:264–272
5. Turchi C, Buscemi L, Previderè C, Grignani P, Brandstätter A, Achilli A, Parson W, Tagliabracci A (2008) Italian mitochondrial DNA database: results of a collaborative exercise and proficiency testing. Int J Legal Med 122:199–204
6. Gilbert MT, Djurhuus D, Melchior L et al (2007) mtDNA from hair and nail clarifies the genetic relationship of the 15th century Qilakitsoq Inuit mummies. Am J Phys Anthropol 133(2):847–853
7. Loogväli EL, Roostalu U, Malyarchuk BA et al (2004) Disuniting uniformity: a pied cladistic canvas of mtDNA haplogroup H in Eurasia. Mol Biol Evol 21:2012–2021

8. Brandstätter A, Salas A, Niederstätter H, Gassner C, Carracedo A, Parson W (2006) Dissection of mitochondrial superhaplogroup H using coding region SNPs. Electrophoresis 27: 2541–2550
9. Grignani P, Peloso G, Achilli A et al (2006) Subtyping mtDNA haplogroup H by SNaPshot minisequencing and its application in forensic individual identification. Int J Legal Med 120:151–156
10. Salas A, Bandelt HJ, Macaulay V, Richards MB (2007) Phylogeographic investigations: the role of trees in forensic genetics. Forensic Sci Int 168:1–13
11. Szibor R, Plate I, Schmitter H, Wittig H, Krause D (2006) Forensic mass screening using mtDNA. Int J Legal Med 120:372–376
12. Vallone PM, Butler JM (2004) Auto Dimer: a screening tool for primer–dimer and hairpin structures. Biotechniques 37:226–231
13. Brandstätter A, Parsons TJ, Parson W (2003) Rapid screening of mtDNA coding region SNPs for the identification of west European Caucasian haplogroups. Int J Legal Med 117:291–298
14. Coble MD, Just RS, O'Callaghan JE et al (2004) Single nucleotide polymorphisms over the entire mtDNA genome that increase the power of forensic testing in Caucasians. Int J Legal Med 118:137–146
15. Quintans B, Álvarez-Iglesias V, Salas A, Phillips C, Lareu MV, Carracedo A (2004) Typing of mitochondrial DNA coding region SNPs of forensic and anthropological interest using SNaPshot minisequenzing. Forensic Sci Int 140:251–257
16. Vallone PM, Just RS, Coble MD, Butler JM, Parsons TJ (2004) A multiplex allele specific primer extension assay for forensically informative SNPs distributed throughout the mitochondrial genome. Int J Legal Med 118:147–157
17. Sanchez JJ, Børsting C, Morling N (2005) Typing of Y chromosome SNPs with multiplex PCR methods. Methods Mol Biol 297:209–228
18. Singer-Sam J, Tanguay RL, Riggs AD (1989) Use of chelex to improve the PCR signal from a small number of cells. Amplifications: A Forum for PCR Users. 3:11
19. Pfeiffer H, Steighner R, Fisher R, Mörnstad H, Yoon CL, Holland MM (1998) Mitochondrial DNA extraction and typing from isolated dentin-experimental evaluation in a Korean population. Int J Legal Med 111:309–313
20. Hellmann A, Rohleder U, Schmitter H, Wittig M (2001) STR typing of human telogen hairs—a new approach. Int J Legal Med 114:269–273
21. Torroni A, Achilli A, Macaulay V, Richards M, Bandelt HJ (2006) Harvesting the fruit of the human mtDNA tree. Trends Genet 22:339–345
22. Butler JM, Ruitberg CM, Vallone PM (2001) Capillary electrophoresis as a tool for optimization of multiplex PCR reactions. J Anal Chem 369:200–205
23. Parson W, Fendt L, Ballard D et al (2008) Identification of West Eurasian mitochondrial haplogroups by mtDNA SNP screening: Results of the 2006–2007 EDNAP collaborative exercise. Forensic Sci Int 2:61–68
24. Lutz-Bonengel S, Sänger T, Parson W, Müller H, Ellwart JW, Follo M, Bonengel B, Niederstätter H, Heinrich M, Schmidt U (2007) Single lymphocytes from two healthy individuals with mitochondrial point heteroplasmy are mainly homoplasmic. Int J Legal Med 122:189–197
25. Eichmann C, Parson W (2008) 'Mitominis': multiplex PCR analysis of reduced size amplicons for compound sequence analysis of the entire mtDNA control region in highly degraded samples. Int J Legal Med doi:10.1007/s00414-008-0227-5