Application of a Panel of 14 D-loop mtDNA SNPs for the Screening of Highly Degraded Specimens in Missing Persons Cases

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Abstract. In order to validate the application of a previously published panel of 14 highly discriminatory control region mtDNA SNPs typed using SNaPShot[TM] (Life Technologies), the assay was applied to identify maternal relationships in ten missing person cases. Bone samples from unidentified skeletal remains present in mortuaries were cleaned and ground in a cryogenic mill. DNA was extracted using the DNA IQ® (Promega) and Prepfiler[TM] Automated Forensic Extraction kit in the Automate Express[TM] Forensic DNA Extraction System (Applied Biosystems). Buccal swab samples from putative family members of the missing persons were extracted with Chelex® (Bio-Rad). mtDNA HVS-1 and HVS-2 segments were amplified in a single duplex PCR and SNPs were typed in a single multiplex SNaPShot reaction. When SNP profiles were compared, matches were only observed between the remains and their respective maternal relatives. One of the remains didn't match any of the references. We did not get results from one of the bone samples. All results were further confirmed by autosomal STR analysis. Our results suggest that the method is straightforward and can be used for exclusionary purposes in the screening of casework samples, missing persons and mass disaster identifications, saving time and laboratory resources when mtDNA analysis is necessary.

Keywords: Single nucleotide polymorphism; Mitochondrial DNA; Haplotype diversity; Brazil; Forensic DNA.

1. Introduction

Mitochondrial DNA (mtDNA) analysis is usually a last resort in routine forensic DNA casework. However, it has become a powerful tool for the analysis of highly degraded samples or samples containing too little or no nuclear DNA, such as old

bones and hair shafts[1,2,3]. Moreover, it can be of great value for the identification of missing persons when comparisons between maternal relatives are necessary. The golden standard methodology still constitutes the direct sequencing of PCR products or cloned amplicons from the HVS-1 and HVS-2 control region[4]. When a mtDNA sequence from an evidence sample and one from a reference sample cannot be excluded as originating from the same source, the haplotype´s frequency is obtained from population data databases.

We have developed an assay containing a panel of 14 highly discriminatory control region mtDNA SNPs to be typed using SNaPShot[TM] (Applied Biosystems)[5] for the screening of highly decomposed human remains in the forensic casework when mtDNA analysis is needed. The main goal of the assay is to apply a less labor intensive and less expensive screening method for mtDNA analysis, in order to aid in the exclusion of non-matching samples and as a presumptive test prior to final confirmatory DNA sequencing. The assay was validated by typing more than a hundred HVS-1/HVS-2 sequenced samples. No differences were observed between the SNP typing and DNA sequencing when results were compared, with the exception of null alleles observed in a few haplotypes. Haplotype diversity for a Brazilian population using 160 mtDNA sequences was of 0.9794.

In this work, we present the results obtained when the assay was applied to identify maternal relationships in ten missing persons cases.

2. Methods

2.1. DNA Extraction

Bone samples from ten unidentified skeletal remains present in mortuaries were cleaned and ground in a cryogenic mill. DNA was extracted from 100 mg of the bone powder using the DNA IQ® (Promega) and Prepfiler[TM] Automated Forensic Extraction kit in the Automate Express[TM] Forensic DNA Extraction System (Applied Biosystems). Buccal swab samples from twenty putative family members of the missing persons were extracted with Chelex® (Bio-Rad). After DNA extraction, samples autosomal DNA was quantified by real time PCR using the Quantifiler® Human kit (Life Technologies).

2.2 mtDNA Amplification

mtDNA HVS-1 (16024-16365) and HVS-2 (72-340) segments were amplified in a single duplex Polymerase Chain Reaction (PCR) using primers L15997, H16391, L48 and H408[6] as previously described[5].

2.3 mtDNA SNPs Typing

We have selected 14 highly discriminatory SNPs according to Salas & Amigo[7] to be typed using SNaPShot[TM] (Applied Biosystems) in a single assay (Figure 1). Following HVS-1 and HVS-2 amplification, SNPs were typed using SNaPShotTM[5].After the single base extension assay, samples were treated with SAP and then separated by capillary electrophoresis in an ABI 3130 Genetic Analyzer.
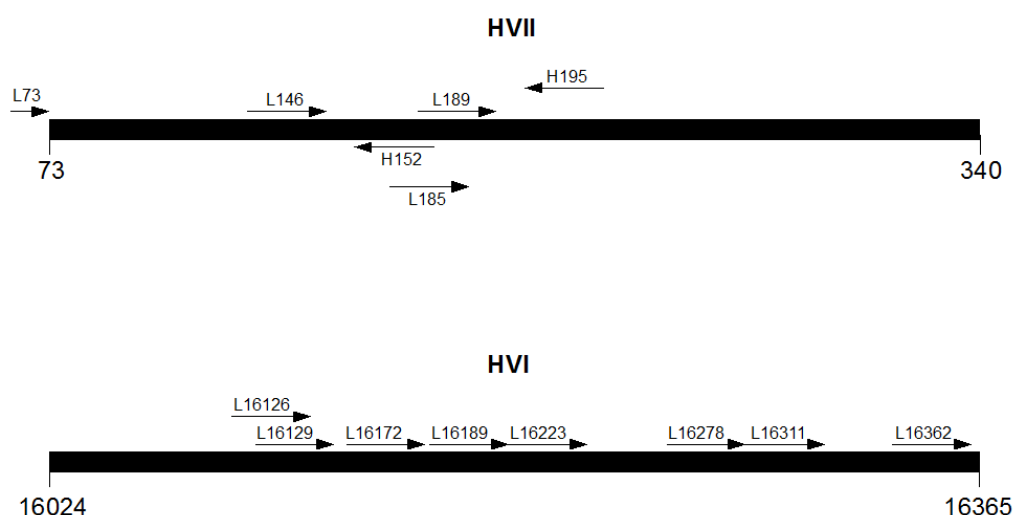

Figure 1. Set of primers used in the SNP assay. Primers were named according to the SNP position being typed.

3.   Results and Discussion

SNP profiles were obtained for all samples, with the exception of bone sample 1 (Figure 2), probably due to extensive DNA degradation. The autosomal short tandem repeat profile was also not obtained for this sample. Dropouts were observed for bone sample 10 at the SNP position L16311 and for samples 5 and 6 at positions L189 and H195, respectively. The same dropouts were observed for their respective maternal relatives. No differences in resolution were observed between bone and reference samples.

SNP profiles from the bone samples were then compared to those of relatives (Table 1). Matches were only observed between the remains and their

respective maternal relatives. In only one case (bone sample 9), besides the match with the mother (B-mother), a relative from another family (F-father) was not excluded. One of the remains didn´t match any of the references (bone sample 2). All relationships were further confirmed by autosomal STR analysis (Data not shown).
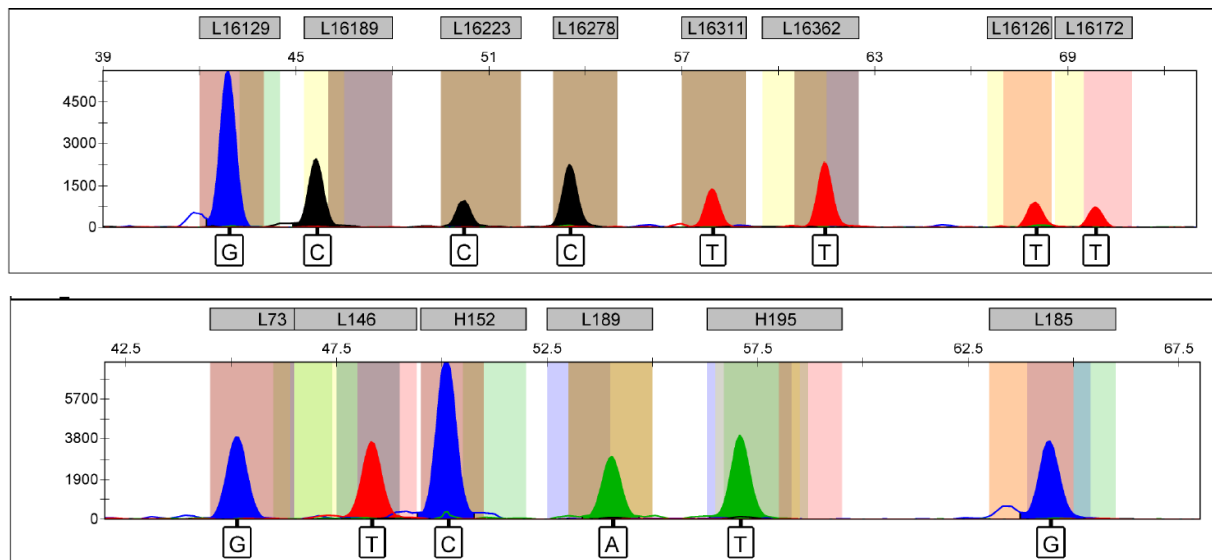

Figure 2. mtDNA SNP typing. Typing of sample 9 as an example of the SNPs panel of the assay. SNP positions and nucleotide genotyped are shown. Forward primers are labeled with an L before the SNP position and reverse primers with an H. Nucleotides genotyped with reverse primers were converted to their complementary strand

Our results suggest that the assay is straightforward and can be used for exclusionary purposes in the screening of casework samples, missing persons and mass disaster identifications, saving time and laboratory resources when mtDNA analysis is necessary.

Selected SNPs showed a high discriminatory power for exclusionary purposes, as theoretically predicted by Salas & Amigo[7]. Our validation study showed that the assay is very robust and sensitive. Assays were validated with highly degraded samples, mostly highly decomposed human remains. As the methodology is simple, fast and cheaper than DNA sequencing, it can be easily applied to solve maternal relationships in degraded samples as an exclusionary tool. Interpretation of results can be easily done using the software GeneMapper ID 3.2 (Applied Biosystems), which is available in most Forensic DNA laboratories.

Samples	S2	S3	S4	S5	S6	S7	S8	S9	S10	A-Mother	B-Father	C-Wife	C-Son	C-Brother	D-Father	E-Mother	F-Father	G-Father	H-Father	B-Mother	E-Father	H-Mother	C-Mother	I-Sister	J-Mother	F-Mother	G-Mother	D-Mother	I-Mother
S2	0	5	1	6	2	4	2	3	6	2	2	7	7	1	1	5	3	6	8	3	2	4	1	6	2	1	6	6	6
S3		0	6	5	6	3	5	4	5	6	5	4	4	6	4	0	4	1	9	4	5	3	6	5	5	6	5	1	5
S4			0	6	1	5	3	4	6	1	3	7	7	2	2	6	4	7	7	4	3	5	2	6	3	0	6	7	6
S5				0	7	6	8	5	1	7	6	1	1	7	5	5	5	5	5	6	6	7	1	8	6	0	5	5	1
S6					0	5	4	5	7	0	4	7	7	3	3	6	5	7	8	5	4	5	3	7	4	1	7	7	7
S7						0	6	3	6	5	6	5	5	5	3	3	3	4	8	3	4	0	5	6	6	5	6	4	6
S8							0	5	8	4	2	9	9	1	3	5	5	6	10	5	4	6	1	8	0	3	8	6	8
S9								0	5	5	5	6	6	4	2	4	0	5	9	0	1	3	4	5	5	4	5	5	5
S10									0	7	7	2	2	7	5	5	5	4	6	5	6	6	7	0	8	6	1	4	0
A-Mother										0	4	7	7	3	3	6	5	7	8	5	4	5	3	7	4	1	7	7	7
B-Father											0	7	7	3	3	5	5	5	8	5	4	6	3	7	2	3	6	5	7
C-Wife												0	0	8	6	4	6	4	6	6	7	5	8	2	9	7	1	4	2
C-Son													0	8	6	4	6	4	6	6	7	5	8	2	9	7	1	4	2
C-Brother														0	2	6	4	7	9	4	3	5	0	7	1	2	7	7	7
D-Father															0	4	2	5	7	2	1	3	2	5	3	2	5	5	5
E-Mother																0	4	1	9	4	5	3	6	5	5	6	5	1	5
F-Father																	0	5	9	0	1	3	4	5	5	4	5	5	5
G-Father																		0	9	5	6	4	7	4	6	7	5	0	4
H-Father																			0	9	8	8	9	6	10	7	5	9	6
B-Mother																				0	1	3	4	5	5	4	5	5	5
E-Father																					0	4	3	6	4	3	6	6	6
H-Mother																						0	5	6	6	5	6	4	6
C-Mother																							0	7	1	2	7	7	7
I-Sister																								0	8	6	1	4	0
J-Mother																									0	3	8	6	8
F-Mother																										0	6	7	6
G-Mother																											0	5	1
D-Mother																												0	4
I-Mother																													0

Table 1. Pairwise comparison of the mtDNA SNP typing results. Number of SNP differences observed between profiles are shown in the cells. S: bone sample; A-H: family relatives followed by relationship to the missing person. Green Boxes: Maternal relationships confirmed by autosomal STRs. Orange boxes: non-related but not excluded by mtDNA SNPs.

Acknowledgements

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