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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We have developed an assay containing a panel of 14 highly discriminatory control region mtDNA SNPs to be typed using SNaPShot™ (Applied Biosystems) (Chemale et al. Forensic Sci Int Genet. 2013 7(3):353-8) 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 0.9794. In order to validate the assay, it was applied to identify maternal relationships in ten missing persons 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™ Automated Forensic Extraction kit in the Automate Express™ 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.

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