DEVELOPMENT AND VALIDATION OF A D-LOOP MTDNA SNPs ASSAY FOR THE SCREENING OF SPECIMENS IN FORENSIC CASEWORK

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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. The golden standard methodology still constitutes the direct sequencing of PCR products or cloned amplicons from the HV1 and HV2 control region segments. Identifications using mtDNA are time-consuming, expensive and can be very complex, depending on the amount and nature of the material being tested.

The main goal of this work is to develop 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.

We have selected 14 highly discriminatory SNPs according to Salas & Amigo (2010) to be typed using SNaPShot™ (Applied Biosystems). The assay was validated by typing more than a hundred HV1/HV2 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 simulations were performed using 160 mtDNA sequences representative of the Brazilian population and a score of 0.9794 was obtained when the 14 SNPs were used. As the main goal of the work is to develop a screening assay to skip the sequencing of all samples in a particular case, a pairwise comparison of the sequences was done using the selected SNPs. When both HV1/HV2 SNPs were used for simulations, at least 2 differences were observed in 93.2% of the comparisons performed. The assay was validated with casework samples. Results show that the method is straightforward and can be used for exclusionary purposes, saving time and laboratory resources. Allelic diversity of the selected SNPs, primer interactions, power of discrimination conferred by the assay and perspectives of the work are discussed throughout the poster.

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