BEST PRACTICES FOR REPORTING mtDNA HETEROPLASMY IN FORENSIC CASEWORK WHEN USING A MASSIVELY PARALLEL SEQUENCING APPROACH AND CONSIDERING RATES, DNA DAMAGE & DRIFT

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Current practice provides for reliable reporting of mitochondrial (mt) DNA haplotypes. However, Sanger-type sequencing (STS) does not effectively identify heteroplasmic variants, especially low-level variants (<10%). Even when heteroplasmy is observed at high levels, the information is typically not used during a forensic investigation. The ability to resolve and report heteroplasmy will significantly enhance the value of mtDNA analysis in forensic casework (Ivanov et al., Nat. Genet., 1996;12:417-20). A massively parallel sequencing (MPS) approach will allow the forensic community to achieve this goal, while maintaining the integrity of producing reliable haplotypes (Holland et al., Croatia Med. J., 2011;52:299-313, McElhoe et al., Forensic Sci. Int.: Genet., 2014;13:20-29, Just et al., Forensic Sci. Int.: Genet., 2015;18:131-9). In order to effectively report heteroplasmy, weight estimates will be needed that reflect measured rates of heteroplasmy. Rates were determined in the mtDNA control region (CR) on a per individual and nucleotide basis, in a large European population group (>550), and across different age groups. MPS data was generated on a MiSeq from Illumina, Inc., and secondary data analysis was completed using NextGENe®, as well as a newly developed software package GeneMarker® HTS, from SoftGenetics, Inc. Sites of heteroplasmy were asymmetrically distributed, with a relatively small number of positions accounting for a high percentage of the total heteroplasmy observed, while the vast majority of nucleotide positions exhibited no heteroplasmy. Instrument and system noise were evaluated and had little impact on reporting thresholds. However, DNA damage associated with sample types most often encountered in forensic cases (for example, hair shafts and older skeletal remains) had a measurable effect on the reporting of sequence variants. In addition, drift observed both at the germline and somatic cell levels further illustrated the challenges faced when comparing evidence profiles to reference information, or comparison between items of evidence from the same individual. The body of knowledge gained from these studies has allowed for the development of *early-stage* best practices for the reporting of heteroplasmy in forensic casework when using an MPS approach and when considering rates, DNA damage, and variant drift.