IMPROVED MITOCHONDRIAL CONTROL REGION ANALYSIS OF DEGRADED BONE AND HAIR SAMPLES UTILIZING A NEW SMALL OVERLAPPING AMPLICON LIBRARY PREPARATION METHOD FOR THE MiSeq FGx®

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Forensic human identification (HID) by DNA analysis of low quality and quantity samples remains a significant challenge in today's laboratories. Emerging applications with new technologies and approaches continue to increase the success rate but short tandem repeat (STR) genotyping, the gold standard, is imperfect. In particular, highly degraded bones or remains, and hair often prove difficult for standard STR workflows. Mitochondrial genotyping has proven its utility as a powerful tool to supplement or replace STR analysis with these challenging samples. Traditionally, Sanger sequencing of the mitochondrial control region has been employed but its labor-intensive process with relatively high error rates limits the technique to just a few laboratories. Massively parallel sequencing (MPS) has changed this paradigm and democratized the capability of mitochondrial sequencing by simplifying workflows, increasing the confidence of the sequencing through depth of coverage, and simplifying the result interpretation process. In this study, we evaluated a new assay, the ForenSeq™ mtDNA Control Region Kit for the MiSeq FGx®, to genotype a variety of challenging hair and bone samples. The assay is designed with multiple overlapping amplicon primer sets that cover the mitochondrial control region specifically targeting degraded or damaged samples with small amplicons (all <150 bp, 118 bp average). Operationally, the assay in interesting to our lab as it can be scaled for small or large sample sets, depending on the needs of the user. The workflow was familiar to our laboratory staff as it was almost identical to the existing ForenSeq workflow which reduced implementation errors. Additionally, the integrated ForenSeq™ Universal Analysis Software performs the data analysis, simplifies data review for the casual user, and maintains the ability to export data files for further analysis with third party software at the discretion of the investigator. Furthermore, we compared our results and workflow with parallel analysis of the same challenging samples analyzed with traditional sanger sequencing. We will present results of the ForenSeq™ mtDNA Control Region Kit outperforming our Sanger workflow in sample preparation, reliability, and sensitivity with the capability of generating full HV1 and HV2 haplotypes from as little as 1 pg of DNA from challenging samples.