

## **COMPARISON OF DNA EXTRACTION METHODS FOR HAIR SHAFTS AND EVALUATION OF THE 10-PLEX PowerSeq KIT FOR MPS ANALYSIS OF LOW COPY NUMBER SAMPLES**

Jamie Gallimore, Jennifer McElhoe, PhD & Mitchell Holland, PhD, Pennsylvania State University, Forensic Science Program

DNA extraction from hair shafts is a common practice in forensic casework. However, the amount of nuclear (n) DNA recovered from hair shafts is usually below what is needed for STR analysis, leading forensic scientists to rely on mitochondrial (mt) DNA analysis due to its high copy number characteristics. Three DNA extraction methods for hair shafts were compared and evaluated: hair lysis and size-exclusion filtration (LF), hair grinding, lysis, PCIA purification and size-exclusion filtration (GLPF), and lysis and magnetic bead purification (LMB). Although extraction methods have been compared before, these three protocols have not been evaluated side-by-side using an mtDNA-based qPCR method (mtqPCR) as the assessment tool, followed by a massively parallel sequencing (MPS) approach to mtDNA analysis. Each method was used to extract four hairs from five donors; two of 0.5cm in length and two of 2.0cm in length. Extractions were performed in separate sets of 10 samples; for example, 0.5 and 2.0 cm hair shafts from the five donors using the LF protocol. Therefore, duplication of the data reflected true replicate analysis. The extracts were quantified in duplicate using a custom mtqPCR method; two target amplicons of 69 and 283 base pairs. As expected, the data exhibited considerable variability between donors, the extracts from the 2.0cm hair shafts had more copies of mtDNA than the 0.5cm hair shafts, and DNA degradation was observed. The LMB extraction method yielded the most copies of mtDNA per donor, with the LF and GLPF protocols producing similar, but lower yields. The performance of Promega's 10-Plex PowerSeq Prototype library preparation protocol for MPS analysis was evaluated on the 60 hair shaft extracts. The library process involves a post-amplification fluorometric quantification. Interestingly the quant values between the samples were similar, regardless of the extraction method. The subsequent MPS analysis was performed on an Illumina MiSeq. A previous study in our laboratory compared the polymerase supplied by the PowerSeq kit to the proofreading TaKaRa Hot Start ExTaq enzyme. The non-proofreading polymerase provided with the kit produced considerable sequencing errors, while the ExTaq produced no errors; at a reporting threshold of 1% minor sequence variants. Therefore, ExTaq was used for the MPS study of the hair shaft extracts. As an additional assessment, the 2.0cm hair shafts extracted with the LMB protocol (10 samples total) were also run through the MPS process using the kit polymerase. A complete evaluation of the findings will be presented.