ASSESSING CRUDE EXTRACTS FROM REFERENCE SAMPLES IN MASSIVELY PARALLEL SEQUENCING WORKFLOWS

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Massively parallel sequencing (MPS) is a tool which enables forensic scientists to obtain a greater level of discriminatory information from a forensic sample than ever before. In addition to providing length-based allele information for short tandem repeats (STRs), MPS reveals the sequence data for each allele, stutter, and artifact read obtained from the sample. For mitochondrial DNA (mtDNA) testing, MPS generates deep sequencing data, revealing previously unobservable low level heteroplasmy. The recent availability of kit-based MPS commercial products has advanced the viability for applying this technology in crime laboratories. In this study, three MPS products were evaluated for use with direct amplification of crude extracts from reference samples: a prototype version of the PowerSeq™ Auto/Y System, the ForenSeq™ kit with DNA Primer Mix A (DPMA), and the PowerSeq™ CRM Nested System, Custom. The resultant data demonstrate that both the PowerSeq™ Auto/Y and ForenSeq™ kits produce genotypes concordant with results from validated capillary electrophoresis (CE) based testing. Furthermore, the two kits yielded concordant sequence data for thirty reference samples typed with each kit. The PowerSeq™ CRM Nested kit generated mitochondrial haplotypes concordant with results generated at the Armed Forces DNA Identification Laboratory (AFDIL) using crude extracts from identical samples. These findings demonstrate the successful performance and capability for each of the three MPS kits with crude reference sample extracts for direct amplification. Compatibility with direct amplification establishes compelling evidence for the implementation of routine MPS-based testing in forensic laboratories.