ASSESSMENT OF ENRICHMENT METHODS FOR MASSIVELY PARALLEL SEQUENCING (MPS) OF HUMAN MITOCHONDRIAL DNA FROM COMPROMISED SAMPLES

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In forensic casework, short tandem repeat (STR) typing is often the preferred method of DNA analysis due to its high power of discrimination. However, many evidentiary samples contain limited or degraded DNA that is not suitable for STR typing. Under these circumstances, mitochondrial DNA (mtDNA) sequence analysis is typically performed. Massively parallel sequencing (MPS) has proven to be well suited for recovery of a considerable amount of data from forensic evidence, even enabling routine evaluation of the whole mitochondrial genome (mtGenome). However, limitations still preclude analysis of some samples, though enrichment may facilitate sequencing when conventional methods are not successful. Here, we investigate the utility of several enrichment strategies for MPS of Illumina® mtDNA libraries derived from challenging samples.

For this study, DNA was extracted from a set of samples including ancient hairs, maggot crops, and cremated remains. Extracts were prepared for MPS using five enrichment methods including the xGen® Lockdown® panel from IDT, Agilent Technologies SureSelect^{XT} target enrichment system, Sygnis TruePrime™ single cell whole genome amplification (WGA), a multiplex PCR assay developed in our laboratory, and WGA combined with multiplex PCR. Enriched libraries were independently sequenced on the Illumina® MiSeq®. Resulting reads were mapped to the revised Cambridge Reference Sequence (rCRS) and variant calling was performed with CLC Genomics Workbench. Data outputs were compared to determine which approach yielded the highest quality data.

The xGen® Lockdown® and TruePrime™ WGA methods did not work well in our hands. SureSelect^{XT} and multiplex PCR were successful for a myriad of sample types, each yielding analyzable data for 78.6% of the 14 samples tested. Average coverage across the mtGenome was comparable for both methods however, consistency was superior in SureSelect^{XT} data. Haplogroup classifications were concordant between libraries originating from the same donor.

Neither of the two successful enrichment methods significantly outperformed the other. While data obtained with SureSelect^{XT} was slightly higher in quality overall due mainly to higher coverage attained versus multiplex PCR, the cost and labor associated with the kit are likely prohibitive for many laboratories. Our multiplex PCR assay was sufficient for whole mtGenome analysis of most of the samples we assessed. However, in cases where samples are extremely limited or degraded, hybridization enrichment may be a more effective approach.