TYPING HIGHLY DEGRADED DNA USING CIRCULARIZED MOLECULES AND TARGET ENRICHMENT

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Forensic genetic typing of highly compromised DNA samples is one of the primary challenges faced by forensic analysts. These degraded or damaged biological samples are difficult to profile genetically using traditional methods employing short tandem repeat (STR) typing, due to the highly fragmented nature of the target molecules. Molecular biology approaches have been developed that may be applied to severely compromised samples to increase the capability of DNA profiling. Once DNA degrades to a certain point, most STR markers fail to amplify by the PCR. Our traditional typing techniques do not possess the capacity to overcome the molecular limitations of low quality and poor quality DNA; therefore, it is essential to develop methods that can analyze these challenged biological samples. There are two approaches that can be considered to improve upon current methods for analyzing highly degraded samples. First, single nucleotide polymorphisms (SNPs) can be targeted as their amplicon size can be constructed to be substantially smaller than those for STRs, making these markers a viable alternative for typing degraded DNA samples. Second, whole genome amplification (WGA), which amplifies all genomic DNA present in a sample. Rolling circle amplification (RCA) is a WGA technique that uses circular templates. Amplification of a circular molecule essentially creates an infinitely long template. RCA generates linear tandem copies of the circular template sequence by incorporating random oligonucleotide primers that bind to any template region. However, nuclear DNA is not circular and thus RCA cannot be exploited to its full potential. CircLigase™ II is an enzyme that circularizes single-stranded DNA through intrastrand ligation. Massively parallel sequencing (MPS) can sequence the enriched targets in a shotgun approach.

Three candidate human identity SNPs were targeted. Synthetic oligonucleotide sequences of varying lengths were generated with the candidate SNPs of interest within the sequence. Circularization of short synthetic DNA fragments, down to approximately 50 nucleotides (nts) in length, was achieved and amplified via RCA. Sequence data support that circularization was successful in that tandem copies of the target sequence were generated during amplification and confirmed in sequencing. In a controlled manner, the inherent error of the circularization and amplification process was assessed. Understanding the impact of sequence error in the process will allow for better interpretation of results obtained from forensic samples.