TYPING HIGHLY DEGRADED DNA USING CIRCULARIZED MOLECULES AND TARGET ENRICHMENT

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Forensic genetic typing of severely compromised biological samples is one of the primary challenges faced by forensic analysts. For over 30 years, forensic DNA analysis has focused its efforts on the advancement of developing assays with high sensitivity and power of discrimination. However, the types of samples encountered today are far different than those for which these techniques were first established. Currently, a substantial demand for analysis has been placed on challenged biological samples such as touch evidence and highly degraded or damaged human remains (i.e., those pertaining to missing persons cases and mass disasters). Our traditional methods do not possess the capacity to overcome the restrictions of low quantity and poor quality DNA; therefore, there is a considerable need to develop systems that can analyze severely challenged biological samples.

Whole genome amplification (WGA) shows promise in that it is a technique that has proven to increase viable template in low or trace level DNA samples. WGA essentially amplifies all genomic DNA present in a sample and, in theory, without bias and with high fidelity. Rolling circle amplification (RCA) is a WGA technique in which replication of circular templates generate linear tandem copies by incorporating random short oligonucleotide primers that bind to any template region. CircLigase™ II is an enzyme that circularizes single-stranded DNA through intrastrand ligation, essentially creating an infinite template since there is no end point in a circular molecule. This approach of circularization to facilitate amplifying target sites is well-suited for single nucleotide polymorphisms (SNPs) as the target site is relatively short in length. Massively parallel sequencing (MPS) techniques such as capture-enrichment of the circular products will enrich selected genomic regions containing the SNPs of interest. MPS essentially can sequence enriched targets in a shotgun approach.

Initial results, targeting a subset of three human identity SNPs, were evaluated for their efficiency to achieve circularization and capture-enrichment for downstream amplification. The potential development of a target enrichment and genomic amplification methodology will introduce a means to recover genetic information from evidentiary sources that were once believed to be impossible to analyze. With this approach, samples that once were thought to be untypable may become viable sources of genetic information for investigative leads or associations.