B86 Typing Highly Degraded DNA Using Circularized Molecules and Target Enrichment

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Learning Overview: After attending this presentation, attendees will understand the limitations often encountered with current forensic genetic typing in the processing of challenged biological samples and the potential for a novel circular molecule directed method to overcome the molecular restrictions of highly degraded and damaged DNA.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing potential methods that could make human identification testing possible for samples that were previously untypable. Attendees will benefit through the presentation of alternative methods to employ that (if successful) will enhance the capabilities for forensic scientists to analyze the most severely compromised DNA samples.

Compromised biological samples often present considerable challenges for forensic genetic typing due to containing highly fragmented DNA. Current forensic typing methods do not possess the capacity to overcome the molecular limitations of low quantity and poor-quality DNA. Forensic DNA profiling employs short tandem repeat (STR) typing. However, once DNA degrades to a certain point, most STR markers fail to amplify by the Polymerase Chain Reaction (PCR). There is a considerable need to develop systems that can analyze severely challenged biological samples. First, the typing of single nucleotide polymorphisms (SNPs) can be targeted as a marker of interest as they possess an increased capacity for the typing of shorter DNA fragments due to their small amplicon size, a target size substantially smaller than that required for STR typing. Second, complete genomic amplification can be achieved within a sample through whole genome amplification (WGA). Expanding on these molecular approaches, there are two circular molecule directed techniques that are considered as a potential means to improve upon current methods for the analysis of severely compromised biological samples. Rolling circle amplification (RCA), a WGA technique, generates linear tandem copies of the circular template sequence by employing random oligonucleotide primers that bind to any template region. Amplification of a circular template essentially creates an infinitely long template. However, nuclear DNA is not found in a circular conformation and thus RCA cannot be exploited to its full potential without modification of the DNA template into a circular molecule. CircLigase™ II (Lucigen®; Middelton, WI) is an enzyme that circularizes single-stranded DNA through intrastrand ligation by the formation of a phosphodiester bond between the 5′-phosphate and the 3′- hydroxyl group. Additionally, molecular inversion probes (MIPs) are an alternative circular molecule-based enrichment approach. A MIP is a single-stranded oligonucleotide that contains two terminalized complementary regions flanking a SNP of interest (or any target sequence) and a set of internal universal PCR primer binding sites for controlled downstream amplification. The two complementary regions hybridize to the template DNA, the gap is filled via a polymerase resulting in the complementary state of the SNP of interest. The MIP will then dissociate from the target and the target site is amplified by PCR via to the internally incorporated primer binding sites. Massively parallel sequencing (MPS) can sequence the enriched circular molecules via a shotgun approach; thus, a specific target sequence is not required to obtain sequencing results.

Three candidate human identity SNPs were targeted for both circular molecule approaches. Oligonucleotide sequences of various lengths (50-mer, 100-mer, and 200-mer) were designed and synthetically generated with the candidate SNPs of interest contained within the sequence. Circularization of short synthetic DNA fragments, down to approximately 50 nucleotides (nts) in length, was achieved and amplified via RCA using the REPLI-g Mini Kit (Qiagen; Valencia, CA). Sequence data depict tandem copies of the target sequence supporting successful circularization and subsequent amplification by RCA of the circular template by the CircLigase approach. Preliminary design for the MIP approach is underway.

Forensic DNA, Target Enrichment, SNPs