EVALUATION OF CIRCULAR DNA SUBSTRATES FOR WHOLE GENOME AMPLIFICATION PRIOR TO FORENSIC ANALYSIS

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Forensic biological evidence often contains low quantities (LCN) of DNA or may be substantially degraded making the sample refractory to forensic genetic analysis. One approach to overcome the limited quantity of DNA is the use of whole genome amplification (WGA). In principle, WGA is an unbiased amplification of all DNA contained in a sample, yielding increased amounts of template that can be subsequently analyzed using standard forensic technologies. One WGA technique, termed rolling circle amplification (RCA), involves the amplification of circular DNA fragments. This report describes the application of a single-stranded (ss) DNA ligase enzyme to generate circular DNA templates for RCA prior to downstream forensic analysis.

Our study focused on optimizing ssDNA ligase reaction conditions and efficiency of ligation utilizing several sizes of ss- and double-stranded (ds) DNA templates. The optimum conditions for circularization were determined, and a multi-step process was designed to utilize ssDNA ligase prior to WGA and short tandem repeat (STR) analysis. Simulated LCN and fragmented DNA were then tested using this method. However, the use of ssDNA ligase provided no apparent improvement for STR analysis following WGA. The multi-step process required to optimize samples for ssDNA ligase treatment prior to WGA likely results in the loss of template DNA and negative STR genotyping results. In contrast, input of linear genomic DNA template directly into WGA prior to STR analysis improved STR genotyping results compared to non-WGA samples.