B169 Degenerate Oligonucleotide-Primed PCR: 'Proofreading' a Method for Forensic DNA Analysis

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The goal of this research project is to provide the forensic DNA community with a Whole Genome Amplification (WGA) tool – Degenerate Oligonucleotide-Primed PCR (DOP-PCR) – that can readily increase the success rate of analysis of degraded, aged, or otherwise compromised biological evidence samples using existing conventional lab technologies and standard procedures for data analysis. This presentation will aim to build upon the data that was previously presented by showing improved amplification success using a dual-enzyme approach.

This presentation will impact the forensic community and/or humanity by allowing for an increased success rate for cases where a very low amount or unusable DNA was obtained from a crime scene, either through lack of evidence altogether or through severe degradation of samples. These samples can be analyzed by technicians without additional training or equipment, and with minimal additional costs to the labs.

Whole genome amplification techniques have been utilized in a number of molecular diagnostic areas, including embryonics, cancer biology, histopathology, and in a variety of studies on molecular diseases and genetic linkage. Degenerate Oligonucleotide-Primed PCR (DOPPCR) is one such whole genome amplification technique which allows for low copy number DNA samples to be preamplified such that high quality, high yield DNA samples can be available for downstream forensic STR amplification and analysis. Thus far, standard DOP-PCR techniques have shown to sufficiently generate enough high yield, high molecular weight DNA for STR analysis by capillary electrophoresis. However, results show some preferential amplification and allele/locus drop out in these subsequent multiplex STR amplifications. By adding a proofreading enzyme (i.e., *Pyrococcus furiosus)* in a small ratio to the *Thermus aquaticus* enzyme currently used with DOP-PCR and increasing extension times during thermalcycling, longer-sized products should be achieved. This will greatly reduce the preferential amplification and allele/locus drop out seen in multiplex STR amplifications. Initially, input DNA amounts ranging from 0.25 nanograms to 7.5 picograms were tested in four DOP-PCR setups using either *Taq* enzyme with *Taq* buffer, *Pfu* enzyme with *Pfu* buffer, 16:1 *Taq*/*Pfu* enzyme combination in *Taq* buffer, or 16:1 *Taq/Pfu* enzyme with *Pfu* buffer in either 50ul or 100ul reaction volumes. All resulting DNA was visualized by agarose gel electrophoresis and human DNA was quantitated by the traditional Quantiblot method. Preliminary results indicate that while the standard DOP-PCR reaction (using *Taq* enzyme in *Taq* buffer) produced products with a size range of approximately 250bp to 2,000bp, the *Taq/Pfu* enzyme combination produced products with a size range of approximately 500bp to over 5,000bp. Additionally, preliminary data from the *Taq/Pfu* enzyme combination experiments indicate that input DNA amounts as low as 7.5 picograms (~2 cells) yielded enough DNA to be visualized on a 1% agarose gel, and DNA yields increased by several thousand-fold for samples with as low as 62 picograms input DNA. Further research will evaluate the ability of DOPPCR products produced with the enzyme combination approach to generate a correct profile with distinct and balanced peaks at all multiplexed STR loci with minimal stochastic variation. In addition, other DOP-PCR enzyme combinations and WGA techniques will also be evaluated in a similar manner.

These research findings will impact the forensic DNA community by allowing for an increased success rate for cases where a very low amount or unusable DNA was obtained from a crime scene, either through lack of evidence altogether or through severe degradation of samples. These samples can be analyzed by technicians without additional training or equipment, and with minimal additional costs to the labs.

Whole Genome Amplification, DNA, STR Analysis

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