B42 Taq/Proofreading Enzyme Combinations: A Method to Enhance Degenerate Oligonucleotide Primed- PCR Results in Forensic DNA Analysis

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After attending this presentation, attendees will have information regarding Degenerate Oligonucleotide-Primed PCR (DOP-PCR), one WGA technique, in which a degenerate primer is used to amplify different regions across the entire genome to provide overlapping DNA fragments for theoretical full genome coverage.

This presentation will impact the forensic community and/or humanity by providing a tool that utilizes current laboratory personnel, equipment, and procedures with minimal additional costs and modifica- tions in order to achieve successful multiplex STR amplification of degraded, aged, or otherwise compromised biological evidence.

It is expected that by adding a proofreading enzyme (*i.e.*, Pyrococcus furiosus, Thermococcus gorgonarius) in a small ratio to the Thermus aquaticus enzyme currently used with Degenerate Oligonucleotide-Primed PCR (DOP-PCR), the efficiency of the amplification reaction will be increased due to the ability of the proofreading enzyme to correct base pair mismatches and maintain elongation through minor errors that normally occur when using Taq alone.

Whole genome amplification (WGA) is being actively researched in the forensic DNA community in order to achieve successful multiplex STR profiles from low quality/low copy number samples that were not ampli- fiable using standard STR amplification techniques. Currently, WGA is commonly being used in various non-forensic fields in order to amplify a single locus of a low copy yield sample, or multiple loci of higher yield samples. In Degenerate Oligonucleotide-Primed PCR (DOP-PCR), one WGA technique, a degenerate primer is used to amplify different regions across the entire genome to provide overlapping DNA fragments for theo- retical full genome coverage. Previous reports indicate that after DOP- PCR, the resulting DNA yield is increased significantly compared to the original sample. However, results thus far have shown some preferential amplification and allele/locus drop out in the subsequent multiplex STR amplifications. It is expected that by adding a proofreading enzyme (*i.e.*, *Pyrococcus furiosus, Thermococcus gorgonarius)* in a small ratio to the *Thermus aquaticus* enzyme currently used with DOP-PCR, the efficiency of the amplification reaction will be increased due to the ability of the proofreading enzyme to correct base pair mismatches and maintain elon- gation through minor errors that normally occur when using *Taq* alone. It is necessary to continue use of *Taq* and not convert to a reaction that solely uses a proofreading enzyme because *Taq* provides the increased rate nec- essary for full elongation and the poly-A addition quality that proofreading enzymes typically lack – all of which is required for the standard STR analyses that is conducted in most forensic laboratories. It is believed that these modifications will greatly reduce the preferential amplification and allele/locus drop out currently seen in multiplex STR amplifications that use DNA from the standard (*Taq*- only) DOP-PCR reaction.

In this study, the initial approach previously described (amplification by traditional DOP-PCR methods, concentration of post-DOP-PCR products, followed by inputting this high yield, high molecular weight DNA into the multiplex STR amplification) was used with several modifi- cations. First, real-time PCR using ABI's Quantifiler® kit was used to achieve more accurate quantitation of pre- and post-DOP-PCR products. Following the initial quantitation, input DNA amounts ranging from 0.25 nanograms to 7.5 picograms were tested in DOP-PCR setups using enzyme combinations including *Pfu* and *Tgo* in a 16:1 ratio with *Taq*. All post- DOP-PCR DNA was visualized by agarose gel electrophoresis and human DNA was quantitated using Quantifiler®. Preliminary results indicate an increase in both size range and DNA yield, up to several thousand-fold for low input DNA samples. Following the analysis of DNA yields and size, STR analysis was performed using an ABI 3100-*Avant* for DNA separation and detection. The STR results from the enzyme combination experiments showed that an increased number of loci can be recovered in the samples with an initial DNA input of 0.25ng and 0.125ng when utilizing a *Taq*/proofreading enzyme combination. However, the number of loci recovered decreases with decreasing DOP-PCR DNA input amounts.

Further research will evaluate the ability of DOP-PCR products pro- duced with the enzyme combination from even lower input DNA amounts to generate a correct profile with distinct and balanced peaks at *all* multi- plexed STR loci with minimal stochastic variation. In addition, other WGA techniques such as MDA and LCN techniques (increased cycle number for thermalcycling) will also be evaluated in a similar manner in order to make a comparison to the optimized *Taq*/proofreading enzyme DOP-PCR method.

DNA, STR, WGA

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