A87 dcDOP-PCR for the Analysis of Compromised Mock and Non - Probative Casework Samples

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The goal of this presentation is to evaluate a newly described low copy number technique ("dcDOP-PCR") using mock and non - probative casework samples like those frequently encountered in the forensic laboratory. Because the recommended input range for commercially available STR multiplex amplification kits is between 0.5 - 2.5 ng of template DNA, there is a need for techniques which can be used to analyze samples that fall below this range and/or samples whose DNA is severely degraded. Previously, this method has been evaluated using serially diluted DNA samples, however, to fully understand the potential of this method for forensic utility, it is important to evaluate mock/non - probative casework samples similar to those frequently encountered in the forensic laboratory.

This presentation will impact the forensic community by proving samples that were pre - amplified using the dcDOP - PCR method had a significantly increased STR allele success rate compared to those amplified with traditional STR procedures (no WGA), producing strong partial or full profiles in many cases where little to no STR data was obtained from traditional STR analysis. Further, STR data quality from samples pre - amplified with dcDOP - PCR was generally equivalent to or superior to traditional STR analysis. This method could have a significant impact on the forensic community by providing a relatively easy, inexpensive alternative for analyzing compromised and/or low copy number DNA evidence.

The goal of this research project was to evaluate a newly described low copy number technique ("dcDOP-PCR") using mock and non- probative casework samples like those frequently encountered in the forensic laboratory. Although the genetic analysis of DNA has proven to be an invaluable tool in forensic science, it can be problematic when DNA samples are of either low quantity or low quality. Because the recommended input range for commercially available STR multiplex amplification kits is between 0.5-2.5 ng of template DNA, there is a need for techniques which can be used to analyze samples that fall below this range and/or samples whose DNA is severely degraded. One such potential technique, whole genome amplification (WGA) is a method which theoretically preamplifies the whole genome using random or degenerate primers. Studies that have used this approach report that high quality/high yield samples can be obtained from low quantity/low quality samples, increasing the success of downstream applications. It is unknown whether any WGA technique will be beneficial for downstream forensic multiplex STR analysis. Previous reports have described the optimization of one WGA technique, degenerate oligonucleotide primed PCR (DOP-PCR), for use with low copy number, serial-diluted DNA samples. However, in order to fully understand the potential of this method for forensic utility, it is important to evaluate mock/non-probative casework samples similar to those frequently encountered in the forensic laboratory.

Samples evaluated in this study included aged bloodstains exposed to various environmental conditions, cigarette butts, bone, teeth, dermal fingerprints, hair roots, hair shafts, and fired cartridge cases. With the exception of the hairs and cartridge cases, DNA from all samples was initially extracted using the organic extraction method; however, these were later re-extracted using the Qiagen QIAamp® DNA Mini Kit method. Hair roots and hair shafts were extracted using the Qiagen QIAamp® DNA Micro Kit. Cartridge cases were extracted using the Promega DNA IQ™ System. Following extraction, the samples were quantified using the Quantifiler® Human DNA Quantification Kit with the ABI 7500 Real-Time PCR instrument. The samples were then amplified using the dcDOP-PCR method which features a 10N degenerate primer (22-mer, 5'-OH CTCGAGNNNNNNNNNN OH-3', N=A, T, G, C), 12 non-specific DOP-PCR cycles, and *Platinum® Taq*, a *Taq*:proofreading enzyme combination of *Taq:DeepVent*.After Microcon® concentration, the samples were amplified using the AmpfℓSTR® Profiler Plus™ PCR Amplification Kit and then post-PCR purified using the Qiagen MinElute® Post-PCR Purification Kit. Finally, the STR fragments were separated on an ABI 3100*Avant* Genetic Analyzer with results analyzed using ABI GeneMapper® ID Software v.3.2 with a threshold of 75 RFUs. Samples that were pre-amplified using the dcDOP-PCR method had a significantly increased STR allele success rate compared to those amplified with traditional STR procedures (no WGA), producing strong partial or full profiles in many cases where little to no STR data was obtained from traditional STR analysis. Further, STR data quality from samples pre-amplified with dcDOP-PCR was generally equivalent to or superior to traditional STR analysis. Unfortunately, samples extracted organically, particularly those that were environmentally challenged, displayed significant CE artifacts. Thus, it is recommended that this method be used selectively with non-organically extracted DNA samples. This method could have a significant impact on the forensic community by providing a relatively easy, inexpensive alternative for analyzing compromised and/or low copy number DNA evidence.

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Low Copy Number, Whole Genome Amplification, Degenerate Oligonucleotide Primed PCR

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