B61 Primer Modifications for the Improvement of Degenerate Oligonucleotide Primed- PCR-Based Whole Genome Amplification From Limited Quantities of DNA

Michelle D. Bonnette, BS, 3412 Spendthrift Drive, #610, Richmond, VA 23294; Denise N. Rodier, MS, 315 West Broad Street, #301, Richmond, VA 23220; Kelly L. Brown, BS, Virginia Commonwealth University, 1000 West Cary Street, Room 311, Richmond, VA 23284; Michelle B. Trevino, BS, 1900 East Cary Street, Apartment 210, Richmond, VA 23223; Jarrod R. Champagne, MSFS, 7003 Flagstaff Lane, #103, Richmond, VA 23228; and Tracey Dawson Cruz, PhD*, Virginia Commonwealth University, 1000 West Cary Street, PO Box 842012, Richmond, VA 23284*

After attending this presentation, attendees will learn an alternative form of WGA analysis for low-copy number forensic samples will be introduced to the attendees. In addition, results from optimization techniques will be discussed.

This presentation will impact the forensic science community by expanding on what has previously been reported on WGA methods for forensic analysis. These results may impact a laboratories decision to include low-copy analysis as a testing option.

Genetic analysis of compromised biological evidence is a frequent problem faced by forensic DNA analysts. Unfortunately, a complete and reliable STR (short tandem repeat) profile is difficult to obtain when using limited input quantities of DNA, whether a sample is limited in total quantity or limited in quality due to degradation. Currently there are several commercial STR multiplex kits available that allow simultaneous amplification of the core loci used in the Combined DNA Index System, or CODIS. However, the recommended input range is 0.5-2.5ng template DNA, a quantity that is not always available for testing from forensic casework samples. Therefore, researchers are attempting to formulate methodologies that can accommodate low-copy number samples (″100pg – equivalent to ~15 diploid cells or less) for accurate STR profiling. Whole genome amplification (WGA) is one technique that has been developed to pre-amplify the complete genome with minimal amplification bias prior to downstream applications such as locus specific STR multiplex amplification. This study sought to modify the Degenerate Oligonucleotide Primed (DOP)-PCR Whole Genome Amplification (WGA) method for improvement of downstream STR analysis of low copy number forensic DNA samples. Experiments involved increasing the degeneracy of the standard DOP primer which contains a six nucleotide degenerate region in its interior sequence, flanked by specific sequences on the 3' and 5' ends of the primer. These specific sequences in the standard DOP primer may prevent ample annealing across the genome limiting the ability to attain true whole genome coverage. By decreasing the specificity of the primer, or increasing its degeneracy, these modifications will likely increase the number of potential binding sites during the low annealing temperature cycles of the DOP-PCR reaction. These new primers may therefore aid in a more thorough amplification of the regions of the human genome containing STR loci and thus reduce allele drop out. Input DNA quantities of 0.125ng and 0.062ng were examined for each of three degenerate primers (6, 10, and 16 degenerate nucleotides) using standard DOP-PCR thermalcycling parameters. The total DNA recovered was quantified and electrophoresed to determine the yield and size of the resulting DNA fragments. All DOP-PCR products were then amplified using the AmpF*l*STR® Profiler Plus® STR kit followed by separation and detection by capillary electrophoresis (ABI 3100*Avant)*. While DOP-PCR products using the 10N and 16N primer were not able to be visualized or quantified using the standard conditions, use of the 10N primer significantly increased STR amplification success, producing as high as 78% of the expected alleles from as little as 0.062ng input DNA with a lower rate of drop-in or stochastic allele occurrence when compared to the 6N primer results. Further, both 10N and 16N primers averaged >50% heterozygote peak balance ratio for most input quantities examined. These results show that modifications of the traditional DOP-PCR reaction to include the use of a more degenerate primer (10N) allows for the generation of longer DNA fragments and more complete, balanced chromosome representation from limited and/or compromised clinical and forensic biological samples. Additionally, the DOP-PCR technique described herein utilizes equipment and technical skills already in place in most existing forensic and clinical DNA laboratories. Thus, if successful, the knowledge gained from this study may help to provide the forensic community with a procedure that would be highly beneficial and easy to implement.

Forensic DNA Analysis, Low Copy Number PCR, WGA

 ** Presenting Author*