B134 Degenerate Oligonucleotide-Primed PCR: A Whole Genome Amplification Approach to Forensic DNA

Kristin M. Meyer, BS, George Washington University, Washington, DC 20052; Kristen E. Lewis, BS, Virginia Commonwealth University, 1000 West Cary Street, Richmond, VA 23284; Moses S. Schanfield, PhD, George Washington University, Washington, DC 20052; Tracey C. Dawson, PhD, Virginia Commonwealth University, 1000 West Cary Street, Box 842012, Richmond, VA 23284*

These research findings will provide a complete set of validated protocols for the final optimized whole genome amplification experiments. All procedures will be developed for instrumentation and equipment that currently exists in most forensic DNA laboratories. These will impact the forensic DNA community by allowing for increased success rate for cases which contain compromised biological samples without a significant increase in costs or the need for specialized training.

The goal of this research project is to provide the Forensic DNA community with a tool – Whole Genome Amplification – that than can readily increase the success rate of the analysis of degraded, aged or otherwise compromised biological evidence samples using existing conventional lab technologies and standard procedures for data analysis. This presentation is aimed to familiarize the audience with one particular method, DOP-PCR, for nuclear STR amplification and analysis.

Whole genome amplification techniques such as primer extension preamplification (PEP), multiple displacement amplification (MDA), and degenerate oligonucleotide-primed PCR (DOP-PCR) have been utilized in a variety of scientific areas, including embryonics, cancer genetics, histopathology, and genetic disease diagnosis and linkage studies. Recently, DOP-PCR has been successfully used as a method to generate larger amounts of DNA that are necessary to perform SNP and microsatellite genotyping. DOP-PCR involves the use of a 16-mer degenerate primer which theoretically allows the statistical and representative amplification of the entire genome and has been shown to accomplish this from as little starting material as that contained within a single cell. This study focuses on using DOP-PCR to analyze forensic samples where the yields of high quality DNA are too low for genotyping using conventional nuclear DNA methods. A sensitivity experiment was performed using genomic DNA to evaluate the technique's lower limits of amplification. Initially, input DNA amounts ranging from 1 nanogram to 15 picograms were tested using the recommended 100ul amplification volume. In addition, mixed stain sperm fraction DNA samples from known male and female contributors (representing "compromised case" samples) that had previously failed to generate a profile were tested in order to approximate DOP-PCR's ability to generate an accurate genotype profile. Preliminary results indicate that DOP-PCR is capable of increasing the amount of total DNA, particularly for lower input DNA amounts. The total yield on average increased by several hundred-fold at the lower dilutions using the DOP-PCR method. However, the fragment sizes obtained after DOP-PCR indicate that larger fragments are not amplified as successfully as smaller fragments. The majority of DNA products resulting from DOP-PCR fell within a 500 bp to 1 kb range, which is slightly shorter than anticipated from published data reporting fragment sizes up to 3 kb. Additionally, the results for the "compromised case" samples suggest that the DOP-PCR products can successfully produce a correct profile with distinct and balanced peaks at STR loci. On average, Profiler Plus amplification of these samples produced balanced peaks for 7 out of the 10 loci, with unbalanced or failed amplification resulting mostly in the loci with larger expected fragment sizes. Further research will concentrate on the sensitivity aspect of this technique and the capability of obtaining larger amplicons from limiting or highly degraded forensic samples for both nuclear and mitochondrial genome applications.

DNA, STR, Whole Genome Amplification

 ** Presenting Author*