B42 Optimization of Degenerate Oligonucleotide Primed-Polymerase Chain Reaction for Forensic DNA Analysis Using Taq/Proofreading Enzyme Combinations

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The goal of this presentation is to discuss the research project to complete the optimization of a whole genome amplification (WGA) technique, Degenerate Oligonucleotide Primed – PCR (DOP-PCR). The optimized DOP-PCR should be able to overcome stochastic effects such as allelic drop out, stutter and peak imbalance that often arise when analyzing low copy number DNA evidence that is either degraded or of low quantity.

This presentation will impact the forensic community by providing a technique that may more efficiently analyze low copy number DNA evidence by increasing allelic success and profile acquisition. This technique is advantageous because it can be utilized with little additional cost to the forensic laboratories because it is designed to use equipment, kits, and techniques that are currently available.

Trace or latent DNA evidence including fingerprints, hairs, saliva, minute drops of blood and sweat are sometimes the only evidence available from crime scenes. This type of evidence usually contains less than 100 pg of DNA (~15 diploid cells or less) and is referred to as low copy number DNA evidence. Because of the limited quantity of DNA available, these types of samples can become difficult to analyze and interpret with traditional STR (short tandem repeats) analysis, preventing profile acquisition. Most analysis problems are due to stochastic effects that become prevalent with low copy number DNA samples. This includes allelic drop out, peak imbalance and high stutter. To overcome these limitations, techniques such as whole genome amplification (WGA) have been explored for forensic utility. Degenerate Oligonucleotide Primed-PCR (DOP-PCR) is a WGA technique that pre-amplifies large sections of the genome to produce enough DNA for downstream STR analysis. During non-specific cycling, a partially degenerate primer is utilized that binds at a low annealing temperature (30 ºC) at many random sites throughout the genome. The fragments produced are large, with good coverage and an overall higher yield. During the specific cycling, the temperature is increased to 62 ºC, increasing the stringency of the degenerate primers 5'end annealing to the previously amplified fragments. The final product size after DOP-PCR can be as much as 7 kb in length. It is believed that combining proofreading enzymes such as Pyrococcus furiosus (Pfu), Thermococcus gorgonarius (Tgo), or Pyrococcus species GB-D (Deep Vent) with Taq (*Thermus aquaticus*), may result in an increase in fidelity and final product size as well as a decrease in error rate. This is important because if longer products can be obtained, then a larger representative portion of the genome can be amplified, decreasing allelic drop out and increasing the probability of obtaining a complete STR profiles.

Five different DOP-PCR enzyme combinations were tested with DNA input values ranging from 0.25 nanograms to 7.8 picograms. The enzyme combinations included Taq:Pfu, Taq:Deep Vent, Taq:Tgo, Platinum Pfx, and ABI GeneAmp High Fidelity which consists of TaqGold and an unknown proprietary enzyme. All samples were amplified using optimized DOP-PCR technique (dcDOP-PCR). Samples were visualized on a 1% agarose gel to determine the DNA sample fragment size. All DNA sample fragments were then quantified to obtain the total yield using the ABI QuantiBlot® Human DNA Quantitation kit. dcDOP-PCR product DNA was then concentrated and amplified using AmpF® Profiler Plus® PCR Amplification Kit. The products from this STR multiplex amplification were separated and analyzed via capillary electrophoresis using the ABI 3100*Avant* Genetic Analyzer. The success was measured by percentage of alleles present, heterozygous peak balance and occurrence of other stochastic effects. Fragment length, yield and STR analysis data will be presented and discussed.

These research findings will impact the forensic community by providing a technique that may more efficiently analyze low copy number DNA evidence by increasing allelic success and profile acquisition. This technique is advantageous because it can be utilized with little additional cost to the forensic laboratories because it is designed to use equipment, kits and techniques that are currently available.

DOP-PCR, Low Copy Number DNA, Proofreading Enzymes

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