A48 Strategies for the Enrichment of Low Copy Number DNA Templates

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After attending this presentation, attendees will understand a novel genome amplification protocol, useful with forensic-type samples. They will be presented with the steps taken that were necessary to optimize the reaction, minimizing typical low coy number artifacts.

This presentation will impact the forensic science community by introducing a novel technique useful in the analysis of limited DNA samples and describing the experimental steps taken for its optimization.

In recent years, whole genome amplification (WGA) techniques have been used to enrich the genomic material in a sample so that low-level DNA can be successfully amplified. Using a WGA protocol, the entire genome is pre-amplified using multiple random start points for the molecular xeroxing process, providing an enriched full-genomic template for a second polymerase chain reaction in which the specific DNA fragments of interest can be amplified. Therefore, as it has been applied in human forensics, WGA is essentially the first step in a genome-wide nested PCR protocol. Due to the particulars of the forensic sample, (e.g., the use of loci containing repetitive DNA, the possibility of heterozygote imbalance, stutter and other PCR artifacts) no currently available WGA protocol completely addresses the needs of the forensic scientist.

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The premise for an improved WGA technique is simple. Standard WGA unnecessarily complicates a sample by enriching for total DNA when, in fact, there are a limited number of well-defined loci of interest for forensic profiling. A more judicious strategy would be to target only the regions of interest for pre-amplification, providing them as an enriched template for the follow-on profiling of the specific loci of interest. This modified WGA has been termed Targeted Genome Amplification (TGA).

Briefly, TGA primers are designed to contain 5' and 3' invariable tails that are noncomplementary to, and therefore do not bind, any site in the human genome. Rather than enriching the sample for total DNA, only the regions containing the loci of interest, i.e., regions about 400 base pairs in length surrounding each STR locus are pre-amplified. In the same TGA reaction, primers complementary to these invariable TGA primer tails are included. They bind the tails and amplify the intervening sequences. The goal of this step is a balanced amplification of all loci maintaining the relative ratios of mixtures.

The technique has been optimized as a 3µl TGA reaction requiring no sample transfer between tubes. A fast PCR protocol is employed for the TGA. The reaction can be completed in less than 30 minutes. Subsequently, the STR multiplex PCR mix is added directly to the completed TGA reaction. Using this technique, PCR artifacts commonly observed with low copy number profiling such as peak height imbalance and stutter have been reduced to permit correct genotyping. This study successfully generated six-locus STR profiles from as little as 25pg DNA and three locus profiles from as little as 15pg DNA. The technique has been applied with some success to forensic-type samples. Results of the experiments will be presented and the course of future studies will
be discussed.

Whole Genome Amplification, DNA Damage, DNA Repair

 ** Presenting Author*