B66 Low Copy Number Methodologies: A Comparison Study of Low Copy Number PCR and Multiple Displacement Amplification

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After attending this presentation, attendees will understand the benefits and drawbacks of multiple displacement amplification and low copy number PCR as related to forensic DNA analysis of low quantity/low quality biological evidence.

This presentation will impact the forensic community and/or humanity by providing insight for further research avenues towards attaining a dependable method for analyzing degraded, aged, or otherwise limited biological evidence samples.

The use of DNA amplification kits has become commonplace in forensic labs for DNA analysis of short tandem repeat (STR) profiles. However, these kits require strict adherence to the suggested input quantities of DNA (1-2.5 ng) to yield accurate and reliable results. Oftentimes, the evidence available for a given forensic case falls below this recommended input range. Therefore, researchers have begun to investigate various methods of whole genome amplification (WGA) as a means of increasing the initial quantity of DNA available, to levels adequate for the downstream application of STR profiling using the polymerase chain reaction (PCR). This study compares one such method of WGA, multiple displacement amplification (MDA), to low copy number PCR (LCN) to determine their effectiveness when working with low copy number DNA samples. DNA was extracted from buccal swabs and diluted to 0.25 ng, 0.125 ng, 0.062 ng, 0.031ng, 0.016 ng, and 0.0075 ng concentrations for use in this comparison study. MDA was performed according to the GenomiPhi™ DNA Amplification kit guidelines. The total DNA recovered was then quantified using the ABI Quantifiler™ kit with the ABI Prism® 7000 for real-time PCR analysis. In addition, all post-MDA DNA was visualized by agarose gel electrophoresis to determine the size of the DNA fragments obtained. The samples were then subjected to an STR amplification reaction with the AmpF*l*STR Profiler Plus™ kit. The LCN STR amplification was performed using the ABI Profiler Plus™ kit but with an increased cycle number (from the traditional 28 cycles to 34 cycles). STR products were separated and detected by capillary electrophoresis (CE) using the ABI Prism® 3100-*Avant* Genetic Analyzer, and analyzed with either ABI Prism® GeneScan® Analysis Software v 3.7.1 and Genotyper® Software v 3.7 or Genemapper™ ID Software v 3.1. STR success rate and quality was compared between the two methods.

DNA yields after MDA were variable, showing no obvious trend and generating between 7.5 and 6734-fold increases in total DNA. Post- MDA fragments were consistently >40kb in size for all samples tested. It should also be noted that negative controls also consistently showed DNA fragments when visualized on agarose gels. As for the STR success rate of samples after MDA, there was a trend of decreasing locus success with decreasing input DNA quantities (from 88.3% success at
0.25 ng inputs to 24% at 0.016 ng inputs); however, high amounts of interlocus and intralocus peak imbalance were observed. Additionally, extra alleles were seen in the MDA products after STR amplification, with a greater number of alleles per locus in the higher input DNA samples (with averages of up to four alleles per locus for 0.25 ng inputs). Alleles were also seen in the negative control MDA samples but were not consistent with the positive control profile or the profile of the laboratory analyst. STR locus success for the LCN PCR samples was significantly improved to that of the MDA samples, showing an 80% success rate when >0.031 ng input DNA was used. Unfortunately, heterozygote peak balance greater than 50% was only seen with the 0.25 ng and 0.125 ng input samples subjected to LCN PCR, but peak imbalance was somewhat improved when injection time on the CE was decreased from five seconds to two seconds. Additional alleles were also seen in STR profiles when the LCN PCR technique was used. Again more typed alleles were noted in the higher input samples, although decreasing injection time to two seconds reduced the number of extraneous alleles present in the STR profiles. This study shows that although both MDA and LCN PCR show some improvement for analyzing low copy number DNA samples both would require extensive optimization before they could be readily accepted practices for reliably obtaining STR profiles in the forensic community. Furthermore, while the LCN PCR approach shows a greater success rate for obtaining the expected alleles in a profile, the occurrence of extraneous alleles and stochastic effects will likely hinder its immediate implementation as a low copy number analysis method for most forensic labs. These findings will impact the forensic community by providing insight for further research avenues towards attaining a dependable method for analyzing degraded, aged, or otherwise limited biological evidence samples.

Forensic DNA Analysis, Low Copy Number PCR, Multiple Displacement Amplification

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