A47 Analysis of N-4 STR Repeat Slippage with Amplification Enhancer on Low-Quantity DNA Samples

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After attending this presentation, attendees will better understand how the amplification enhancer affects allele and stutter peak height of low-quantity DNA samples. Attendees will also learn that the enhancer causes the stutter peaks to increase proportionately the peak heights. This is evident in some mixture DNA profiles.

This presentation will impact the forensic science community by introducing a possible new enhancer that could be used to enhance low- quantity DNA samples in criminal cases.

Forensic DNA profiles are based on small repetitive nucleotide sequences called short tandem repeats (STR) that vary by size in the population. Due to the stochastic effects, low copy number (LCN) DNA samples (~<100pg) produce more random fluctuations in allelic peak heights and more reproducible anomalies or artifacts than larger quantities of DNA. Artifacts in the electropherograms can interfere with the interpretation of the true DNA peaks. One artifact, called repeat slippage or stutter, is due to the strand slippage during the PCR process. Based on sequence analysis of tetranucleotide STRs, stutter products are generally one repeat unit or n-4bp smaller than the true allele. Stutter percentages, or the ratio of the stutter height to the corresponding allele height, have been found to be ~<15% for tetranucleotide STR's. Stutter can complicate interpretation of profiles, especially in mixtures. Thus, minimizing amplification of stutter products is important when analyzing low-quantity DNA samples.

A new PCR enhancer, STRboost (SB), has been used to enhance amplification from low-quantity samples. SB has been reported to enhance alleles by 5-fold and is based on the natural biological mechanism called androhydrobiosis or life without water. The purpose of this study is to evaluate the percentage of stutter formation with various volumes of SB. The hypothesis is that amplification of low-quantity DNA samples will result in increased sensitivity (and higher peak heights) with no significant change in stutter percentage. Controlled human male DNA was diluted to 0.5ng/µl, 0.25ng/µl, and 0.125ng/µl. Extracted male and female DNA samples were quantified using real time quantitative PCR or QPCR and diluted to a 0.5ng/µl mixture of a 9:1 female to male ratio. The DNA samples were amplified in triplicates using the AmpFISTR Identifiler STR Multiplexing kit (Applied BioSystems) using SB at three volumes: 2.5µl, 5.0µl, and 9.0µl. The 0.5ng/ul mixed sample was tested at only 9.0ul of SB. The amplicons were separated by capillary electrophoresis using the ABI 310 Genetic Analyzer. A genetic profile containing sixteen core loci and amelogenin was generated using the software GeneMapper ID. From the profiles, the analyses of allele and stutter peaks were performed. The stutter percentages in the triplicate runs at each concentration were compared using the Single-Factor ANOVA and the Independent Two-Sample t-test with alpha being 0.05. Results at the single-source DNA samples and the 0.5ng/µl mixture show that the allele and stutter heights increased with no significant change in stutter percentage in most of the loci. Stochastic effects greatly increased at lower concentrations of DNA causing the p- values to approach alpha. The Two-Sample t-test was used for the mixture sample and for loci that showed reproducible stutter peaks at two volumes of SB. Highest peak heights and sensitivity were obtained using between 5-9µl of the enhancer in most of the loci. Preliminary results of the 0.5ng/µl mixture show higher stutter percentages than in the 0.5ng/µl single-source sample. The proportional enhancement of stutter and the corresponding true allele supports the hypothesis. This may be explained

by the fact that the stutter sequence only differs from the true alleles by one repeat (4bp).

Stutter, STR Boost, Low-Quantity DNA

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