B96 Reducing Stutter Artifacts in Forensic DNA Analysis Using Polyhydric Compounds

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Attendees will learn improvements in the analysis of short tandem repeats in DNA typing.

This presentation will impact the forensic community and/or humanity by improve methods in analysis of DNA typing.

Current methods for PCR amplification of short tandem repeat (STR) loci result in an artifact named "Stutter." These stutter products are shorter than the target allele by multiples of the repeat unit. Due to the presence of the stutter artifact, multipeak patterns are obtained with STRs which results in complication in the interpretation of results specifically in mixture analysis in forensic samples and measuring microsatellite instability in cancer diagnosis.

Polyhydric compounds and specifically polyols have been shown to increase the thermal stability of proteins under stressful conditions. Additionally polyols have been shown to interact with the polynucleotide solvation sites replacing water surrounding the double helix. Due to the combination of the functions of polyols mentioned above, the processivity of the enzyme may be enhanced by the presence of the polyols during the amplification of short tandem repeats. A screening method has been developed to measure the effect of polyhydric compounds on reducing the stutter artifact. The screening method involves the use of a PCR fluorescent multiplex system specifically used for detecting DNA in Forensics. The multiplex system (Profiler Plus ID) is combined with the use of various polyhydric compounds. Following PCR using the fluorescent multiplex the products are analyzed on a capillary electrophoresis system (ABI Prism 310) and detected using the Gene Mapper ID software system. The results are determined based on stutter percentage ratios. The stutter percentage ratio is defined as the ratio of the stutter allele to the main allele peak. Among the polyhydric compounds tested, there was a 2-20% decrease in stutter as compared to the control in all loci. Arabinose, sorbitol, glucose and sucrose contributed to the maximum decrease in stutter reduction. Further studies include varying the concentration of the polyhydric compounds and testing osmolytes for further reduction in the stutter artifact. This will improve genotyping and allele assignment of microsatellites by eliminating errors in genotyping thereby improving the sensitivity of the technique. In the forensic field specifically samples containing mixtures of DNA samples will be easier to decipher simplifying analysis.

STRs, Stutter, Polyols

 ** Presenting Author*