B50   Separating Complex DNA Mixtures Containing Related Individuals Using TrueAllele® Mixture Interpretation Software

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After attending this presentation, attendees will better understand an analysis method that reliably separates (deconvolutes) familial DNA mixtures of two to five individuals using TrueAllele® technology. Furthermore, this presentation demonstrates the difficulty of deconvolution of mixtures with high levels of allele sharing at both optimal and low levels of DNA.

This presentation will impact the forensic science community by demonstrating the effectiveness of a reliable tool that can be used for the interpretation of complex familial DNA mixtures, as well as the benefits of automated DNA analysis.

According to the preliminary 2015 report from the Federal Bureau of Investigation (FBI), violent crimes have increased 1.7% from the reported 1,165,383 violent crimes in 2014.[1,2] Major devastating events such as the Boston Marathon bombing and incest sexual assaults are only some of the recent occurrences that have bombarded laboratories with biological evidence. In the eyes of forensic scientists, many of these crimes have two matters in common: related individuals and complex DNA mixtures. DNA mixtures can pose multiple analysis issues; some of these issues stem from degraded DNA, small amounts of DNA (Low-Template (LT-DNA)), and the number of contributors in the mixture.[3,4] Using manual interpretation, the complexity of DNA mixtures often leads to inconclusive reports.[5]

The use of computer technology in the past ten years has successfully simplified DNA mixture interpretation. Innovative forensic Short Tandem Repeat (STR) commercial software available today is primarily used to separate contributors in a mixture. Recent studies are being conducted to validate these systems, especially for deconvoluting mixtures of more than two contributors; however, complex mixtures limit this new technology, especially when contributors share genetic material as a result of being from the same biological family.[3,4] There has been limited published research on how familial DNA mixtures affect automated interpretation.

This research focuses on the analysis of DNA mixtures containing related individuals and the prospects of using the continuous probabilistic STR analysis software TrueAllele® for statistical calculations in complex mixtures containing up to five individuals. It is proposed that TrueAllele® technology will resolve complex DNA mixtures containing three to five related individuals, generate Likelihood Ratio (LR) match statistics suitable for court, and reproduce those match results to show reliability in automated analysis.

Buccal swabs were collected from individuals in three separate families to create single-source DNA profiles. These samples were extracted using the DNA IQ™ System and quantified using Real-Time Polymerase Chain Reaction (RT-PCR) with Applied Biosystems® Quantifiler® assay. Based on the family pedigrees and the quantification results, 25 mixtures of two to five contributors were created using related individuals from those families. These mixtures were then replicated and diluted to provide mock LT-DNA samples. After amplification using the PowerPlex® Fusion STR kit and genotyping on Applied Biosystems® 3130 genetic analyzer, the mixtures were analyzed using TrueAllele®. The statistics produced across all mixtures were compared to determine if TrueAllele® could separate

mixture contributors, regardless of family origin. Reproducibility among triplicate runs was assessed within each mixture set to ensure the software was correctly separating the contributors and producing similar match statistics. Finally, the degree of shared alleles in a mixture (in relation to the produced match statistics) was compared.

Analysis of familial DNA mixtures provides an insight to the complexity of interpretation in a situation in which allele sharing is at its highest. Obtaining accurate and unbiased results with the help of automated analysis could lead to further advancements in other methods, such as kinship analysis and paternity testing. Conclusions from this study may not only provide a reliable tool for forensic scientists to analyze complex familial mixtures, but may also standardize the analysis process and decrease the number of inconclusive reports.

Reference(s):

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