B98 The Validation of a Statistical Tool for the Analysis of DNA Mixtures

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After attending this presentation, attendees will understand a method for the validation of a statistical tool for the probabilistic genotyping of the results of analysis of forensic DNA mixtures.

This presentation will impact the forensic science community by serving as a guideline to describe how laboratories can incorporate probabilistic genotyping into protocols for DNA interpretation of mixtures.

Improvements in time of analysis, sensitivity, and cost result in it not being uncommon to encounter touch DNA samples in the crime lab. The DNA profiles obtained from touch samples are often low-level complex mixtures that are not easily or consistently interpretable using traditional mixture interpretation approaches and statistics. New methods are increasingly available that are better suited for such data.

The Harris County Institute of Forensic Sciences (HCIFS) chose the R-based statistical software package Forensim and validated its use on complex mixtures. Forensim was appealing as it is open source and available at no cost. The Forensim module of interest to HCIFS is LRmix which follows the method developed by Gill.[1] It enables the calculation of likelihood ratios for genotypes derived from complex Short Tandem Repeat (STR) profiles. The system considers allele drop-in, drop-out, and multiple contributors utilizing a semi-continuous approach that does not include peak height.

Initial validation work focused on the calculation of the probability of drop-out and drop-in, values entered manually into the LRmix module before use. The probability of drop-out value of 0.14 was determined using the counting method, the tailed method, and logistic regression for validation samples of varying concentration. The probability of drop-in was determined to be <0.01 by reviewing hundreds of casework negative controls in search of spurious allele peaks below analytical threshold but above instrument baseline. The value was set to 0.01 as that is lowest probability of drop-in LRmix will accept. LRmix module also includes a Monte Carlo probability of drop-out simulator that is run after a profile is evaluated and an assumption of the number of contributors is made. This simulation was run throughout the validation work. The calculated probability of drop-out was found to be within the simulated range reinforcing the appropriateness of the calculated probability. These simulations will also be included in casework done with LRmix.

Forty two-person and three-person complex mixtures were created from known contributors and evaluated using LRmix to demonstrate sensitivity, reproducibility, and precision. All true contributors to the mixtures were correctly associated. The likelihood ratios obtained from these samples ranged from $1x10^5$ to $1x10^7$. Over 100 single-source profiles were created from randomly chosen alleles and all were correctly excluded when evaluated against the mixtures. LRmix is also capable of automating this step using the performance check module. The likelihood ratios obtained from these samples ranged from $1x10^{-55}$ to $1x10^{-7}$. Random profiles are created based on the population database used and compared to the mixture profile. The user chooses how many profiles to compare.

Overall, the validation of LRmix demonstrates it is an acceptable method for the calculation of the likelihood ratios for complex two- and three-person mixtures and it is an economical tool that can be incorporated by a laboratory.

Reference:

1. Gill P, Kirkham A, Curran J. LoComatioN: A software tool for the analysis of low copy number DNA profiles. *Forensic Sci Int* 2007:166(2-3):128-138.

Probabilistic Genotyping, LRmix, Validation