ANALYSIS OF ALLELIC DROP-OUT USING THE IDENTIFILER STR MULTIPLEX

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Many biological samples recovered from crime scenes contain a limited amount of DNA. Because such samples may contain relatively few copies of each locus, the random sampling of DNA molecules during the typing process may result in allelic drop-out, or the failure to observe some alleles that are actually present. In other words, the final DNA profile may not accurately represent the original sample. The possibility of allelic drop-out can severely complicate the interpretation of forensic DNA profiles—even those obtained using a standard number of PCR cycles. However, one statistical approach used to assess the strength of DNA evidence, the likelihood ratio (LR), has been extended to allow for allelic drop-out *(1)*. A freely available computer program enables one to calculate LRs for profiles containing components in the stochastic range, where allelic drop-out is possible *(2)*. One challenge to using this program is that it requires the user to specify a range of drop-out probabilities to consider for the calculation. However, few empirical studies to determine allelic drop-out probabilities over a variety of conditions have been performed to date *(3)*.

This study examined allelic drop-out in 60 single-source DNA profiles generated using the IdentiFiler system by Dr. John Butler's group at NIST *(4)*. This dataset contains profiles obtained by amplifying 100 pg, 30 pg, and 10 pg of DNA. Allelic drop-out is dependent on the concentration of DNA analyzed and, by definition, will vary from sample to sample. We used the average peak height (in RFUs) over all loci as an indicator of the relative amount of DNA present in the profile. We also tabulated the fraction of alleles that dropped out of each profile. Two instances of drop-out were observed for profiles with an average peak height >200 RFUs. For profiles with average peak heights between 100-200 RFUs, 0-80% of the alleles dropped out (median=10%). For profiles with an average peak height <100 RFUs, 17-100% (median=90%) of the peaks dropped out. Using a limit of detection/limit of quantitation (LOD/LOQ) approach to set the allelic detection threshold, rather than using a default threshold of 50 RFUs, resulted in fewer reported instances of allelic drop out (a median of 70% of alleles dropped out of profiles that had an average peak height of <100 RFUs). We next examined the fraction of alleles that dropped out for each locus. A correlation exists between the fraction of alleles that dropped out and length of the alleles genotyped at that locus. Longer alleles tended to drop-out more frequently than shorter alleles. Finally, we used logistic regression (following ref. 3) to model the fraction of alleles that have dropped out as a function of the average heights of the remaining peaks. The equation from the logistic regression model allows one to estimate the expected drop-out probability for a crime scene sample based on the average peak height of the profile or component of interest. These equations and instructions for use will be presented. In conclusion, use of a LR framework, incorporating an appropriate allelic detection threshold and estimated allelic drop-out probabilities, provides a means for extracting more useful information from low-template forensic DNA profiles, obviating the need to increase injection times or the number of PCR cycles.

References:
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