Characterization of the sources of peak height uncertainty resulting from ordinary alterations during forensic DNA processing: Examining validation schemes for the calibration of NOC

Kayleigh E. Rowan, Genevieve Wellner, Desmond S. Lun, Muriel Medard, and Catherine M. Grgicak

Boston University School of Medicine
Program in Biomedical Forensic Sciences
72 E. Concord Street, Boston, MA 02118

Introduction

Forensic DNA mixtures may be complex and be comprised of any number of contributors combined in any proportion. In these cases, classical DNA interpretation schemes may allow for comparison between evidence and standard. In response to the issues associated with complex, low-template DNA analysis, a number of methods and/or recommendations to determine the likelihood ratio, which requires an assumption regarding the number of contributors (NOC), have been published. Classical approaches to determine the NOC have relied on counting methods. However, as the complexity of the DNA profile increases, the minimum NOC may not be equivalent to the actual number. To accurately assess the probability that a certain NOC gives rise to an evidentiary item, the probability of drop-out (Pr(DO)), baseline noise, and stutter proportion must be considered. The authors have previously characterized the stutter proportion in a template DNA samples.

Methods

Results

Figure 1. Electropherograms for locus FGA at 0.25 ng for a) one sample preparation injected 4 different capillary lists, b) one sample preparation injected 4 times on one capillary, c) one sample preparation amplified 3 separate times with one kit list, and d) one sample preparation amplified using 4 different kit lots, demonstrating the variation caused by the parameters considered in this study.

Figure 2. A comparison of sample A(a), B(b), and C(c) of the average PHs are similar between samples.

Figure 3. Boxplot of normalized peak heights for injection list (a) versus injection list (b), showing visually there does not seem to be a difference between the two parameters.

Figure 4. Boxplot of normalized peak heights for injection list (a) versus amplification (b), showing amplification changes result in a greater variation than do injection changes.

Figure 5. Boxplot of normalized peak heights for injection list (a) versus kit lot (c), showing kit list and amplification changes result in similar levels of variation.

Figure 6. Cumulative histogram of concordance values for observed peak heights of 0.25, 0.0131, and 0.0071 ng for different injections ( ), capillary lots ( ), and kit lots ( ), showing that the average PHs are similar between samples.

Figure 7. Histogram of noise peak heights by channel for different injections ( ), capillary lots ( ), and kit lots ( ), showing that, within a dye color, there is no significant difference in the distribution of noise peak heights for the parameters examined.

Conclusions

Data indicate that variation in RFU signal was heavily affected by the uncertainty associated with the amplification, followed by capillary lot, then kit lot and injection. For example, the signal originating from a 0.25 ng target resulted in concordance ratios of 0.97 ± 0.02, 0.89 ± 0.07, 0.72 ± 0.15, and 0.71 ± 0.19 for injection, capillary amplification, kit lot changes respectively. Similar results were observed for all six targets tested. As a result, when calibrating NOC0, or when attempting to elucidate stochastic thresholds and/or rates of drop-out, the validation dataset should include, at a minimum, amplifications of multiple different samples such that several capillary lots are incorporated into the calibration scheme.

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