

Amplification Reproducibility of Profiles Generated Using Identifiler® and MiniFiler® PCR
Amplification Kits: Effects on Mixture Interpretation

Elisse Ruiz, M.S., Catherine M. Grgicak, Ph.D. and Robin W. Cotton, Ph.D.

Boston University, School of Medicine. Department of Anatomy and Neurobiology,
Biomedical Forensic Sciences. 715 Albany Street, R806. Boston MA, 02118.

Many biological samples deposited and collected from crime scenes contain mixtures from two or more individuals. Determining the total number of contributors, the relative ratio of DNA from each contributor and whether a known individual is included or excluded continues to be one of the most difficult areas of DNA testing.

Although courses for DNA Analyst training are offered and a number of publications with information regarding mixture interpretation exist¹⁻⁴, many of these analyses are constructed on a foundation of assumptions that presuppose amplification reproducibility. Another option available to the forensic DNA analyst is the implementation of expert systems into forensic DNA laboratories. These systems promise to be an important and indispensable means for accurate mixture interpretation, but have not yet been widely employed.

In this study, mixtures produced using various ratios of component DNAs were evaluated and findings related to results observed in single-source samples. More specifically this project focused on verifying and validating various assumptions used by analysts when interpreting mixture samples;

- 1) Peak heights and peak height ratios do not significantly differ between loci or amplifications using a fixed amount of DNA.
- 2) The peak heights and peak height ratios do not significantly differ between amplifications even when amplifying a variety of target amounts.
- 3) Lastly, is a generalized peak height ratio, derived from validation studies using single source samples at a particular target, an appropriate value when evaluating DNA mixture profiles? That is, does the DNA amplify independently during amplification when more than one contributor is present?

In this study the aforementioned assumptions were tested by amplifying single source samples (male and female) using various target amounts (4ng-0.0625ng). Samples were amplified in quadruplicate using Identifiler® and MiniFiler® Amplification Kits (Applied

Biosystems). Analysis of these results focused on examining the reproducibility of the peak heights between amplifications, loci and target amounts by utilizing the *paired t-test* to establish differences between these values and whether the differences are statistically significant.

The mixtures contained a series of 2-person mixtures (originating from the same individuals as the single source analysis) of human DNA. The DNAs were mixed at known ratios and amplified with Identifiler® and MiniFiler® with varying amounts of input DNA. The amplified products were prepared for injection onto a 3130 Genetic Analyzer (Applied Biosystems) using various injection times. The ~300 profiles were then analyzed using *GeneMapper ID* (Applied Biosystems) and the peak heights and peak height ratios were compared back to the single source samples to assess the viability of assumption 3. Simple statistical analysis such as the *paired t-test* and *ANOVA* were employed to establish the significance of observed differences.

References

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