A126 Validation Studies of Allele Drop-Out and Heterozygote Peak Imbalance of Single and Mixture Profiles Generated With AmpFlSTR® Identifiler® PCR Amplification Kit and Analyzed With Gene- Mapper IDX

Jackson Jeong, MS, Boston University School of Medicine, 72 East Concord Street, Room 806, Boston, MA 02118; Robin W. Cotton, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 East Concord Street, L1004, Boston, MA 02118; and Catherine M. Grgicak, PhD, Boston University, School of Medicine, Biomedical Forensic Sciences, 72 East Concord Street, Room R806D, Boston, MA 02118*

After attending this presentation, attendees will better understand various critical features of allele drop-out and heterozygote peak imbalance in single source DNA samples and samples containing mixtures.

This presentation will impact the forensic science community by providing validation data and practical guidance to support understanding of stochastic effects.

Reduction in the amount of DNA template below optimal levels results in loss of genetic information observed in the corresponding DNA profile. The information loss begins as heterozygous peak height imbalance and ultimately results in allele drop-out. This process is generally attributed to stochastic effects occurring during the primer binding steps in the early cycles of the polymerase chain reaction (PCR). Stochastic effects are generally defined as intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles from low quantity DNA template.[1] The effects may be locus and/or kit specific and the exact extent to which an evidence profile is effected cannot be known.

Data loss is commonly addressed through the use of a single stochastic threshold which defines the relative fluorescence units (rfu) value above which a single peak at a locus may be assumed to be homozygous. However, papers by Gill et.al. 2009 Forensic Science International and T. Tvedebrink et al. 2009 Forensic Science International provide methods which can be used to describe the distribution of rfu values where drop-out is unlikely to be observed to values where drop-out is likely to be observed.[2,3] The probability of allele drop-out can be characterized and used to develop laboratory interpretation guidelines which do not rely on the definition of a single stochastic threshold (in rfu) which is unlikely to be sufficiently predictive. This study investigates the characteristics of drop-out in samples having low template DNA in single source and multiple source samples. This is accomplished experimentally by steadily decreasing the amount of DNA template added to the PCR followed by careful characterization of both heterozygous peak imbalance and allele drop-out. Equivalent observations are made in profiles from single source samples and sample mixtures of known proportions.[4]

In this study, six different amounts of template (2, 1, 0.5, 0.25, 0.125 and 0.06ng) for four single source samples A, B, C, and D were amplified in quadruplicate using the AmpFℓSTR® Identifiler® PCR Amplification Kit. Additionally, combinations of A+B and C+D at nine different contributor ratios (1:19, 1:9, 1:4, 1:2, 1:1, 2:1, 4:1, 9:1, and 19:1) and the same six amounts of template were amplified using the AmpFℓSTR® Identifiler® PCR Amplification Kit. Procedures were used to ensure that each of the quadruplicate amplifications would have the minimum possible variation. Each sample was at injected for 2, 5, and 10 seconds.[5] Various features of allele drop-out and peak height ratio differences from all the above profiles were analyzed and interpreted using the GeneMapper® ID-X program from Applied Biosystems. Specifically, characterization of the change in heterozygote peak imbalance vs. the amount of DNA template as well as comparison of drop-out in the two person mixtures to drop-out in the single source samples of the same template mass was analyzed. Furthermore, the recovery of alleles falling below the analytical threshold when injection time was increased was also considered.

This data lays a foundation for better understanding the properties of stochastic effects which occur in the PCR. The data illustrate how careful characterization of a process can provide information which allows for improved analysis and interpretation procedures.

References:

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2. P. Gill, R. Puch-Solis and J. Curran. (2009) Forensic Science International: Genetics 3; 104-111.
3. T. Tvedebrink, P., S., Eriksen, H., S., Mogensen and N., Morling (2009) Forensic Science International 3;222-226
4. P. Gill, C.H. Brenner, J. S. Buckleton, A. Carracedo, M. Krawczak, W. R. Mayr et al.

 ** Presenting Author*

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 ** Presenting Author*