B139 Examining the Contribution of Sampling to Peak Height Imbalance in Low Template DNA Samples Using a Single-Tube Extraction Protocol

Thutrang Nguyen, BA, 221 Massachusetts, #708, Boston, MA 02115; and Robin W. Cotton, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, R 806, Boston, MA 02118*

After attending this presentation, attendees will understand that both pre-Polymerase Chain Reaction (PCR) sampling and PCR chemistry contribute to peak height imbalance as shown experimentally using a single-tube extraction and direct amplification protocol.

This presentation will impact the forensic science community by experimentally showing the contribution of pre-PCR sampling to peak height imbalance.

The developments of the PCR and the Short Tandem Repeat (STR) multiplex kits increased the ease and lowered the time and sample quantity required for Deoxyribonucleic Acid (DNA) typing compared to previous methods; however, the amplification of such a low mass of DNA can lead to increased stochastic effects, such as Allele Drop-Out (ADO) and heterozygous Peak Height (PH) imbalance, which make it difficult to determine the true donor profile. These stochastic effects are believed to be due to: (1) pre-PCR sampling from pipetting and sample transferal of dilute samples prior to amplification resulting in imbalanced heterozygous allele templates in the amplification reaction; and, (2) the kinetics of the PCR process in which there may be uneven amplification of heterozygous alleles during early PCR cycles when few target templates are initially available.

This study examines the contribution of PCR chemistry and pre-PCR sampling errors on stochastic effects by utilizing a single-tube DNA extraction and direct amplification method. Cells were collected into tubes using the McCrone Associates, Inc. cell transfer method, which allowed for approximation of DNA mass without quantification. The forensicGEM® saliva kit was used to lyse the cells and inactivate nucleases without inhibiting downstream amplification. The samples were then directly amplified with the AmpFℓSTR® Identifiler® Plus PCR Amplification kit. These samples should only show the effects of PCR chemistry since pipetting and tube transferal steps prior to amplification were removed with the expectation that equal numbers of heterozygous alleles are present in the sample pre-amplification. Comparisons of PH imbalance were made to samples extracted with forensicGEM® but had one or more pipetting and tube transferal steps prior to amplification; thus, these samples would exhibit the effects of both pre-PCR sampling and PCR chemistry errors and inefficiencies. Results indicate the imperfections observed in PH ratios are a combination of sampling and PCR-generated stochastic effects.

Peak Height Ratio, Low Template DNA, Single-Tube Extraction

Copyright 2016 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS.

*Presenting Author