B126 Pull-Up Problems: A Method to Better Identify and Characterize Pull-Up Peaks

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Learning Overview: After attending this presentation, attendees will understand how to develop and implement a standardized procedure to evaluate pull-up artifacts, allowing them to determine interpretation guidelines to reduce the complexity of electropherogram analysis.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a framework to develop interpretation guidelines regarding pull-up artifacts in electropherograms.

One of the key steps in DNA analysis is the evaluation of the peaks present in the electropherogram to determine if they are alleles or artifacts. For most of the peaks, alleles will be easily distinguished from artifacts. However, in complex DNA results such as mixtures, this exercise can prove to be more difficult and can be critical for the evaluation of the number of contributors, a crucial step for probabilistic genotyping interpretation. One type of artifact that can cause interpretation issues is the pull-up artifact, which appears at the capillary electrophoresis step, and can be labeled as alleles by the genotyping software. In mixtures, such peaks can be confused as a minor contributor's allele and could create situations of false exclusions.

The goal of this study was to develop a method of evaluation of pull-up artifacts and develop guidelines for the detection of these peaks. To achieve this goal, a plate of 22 pooled Polymerase Chain Reaction (PCR) products from known samples was created, with both single source profiles and mixtures. Capillary electrophoresis injection plates were set up from that PCR plate and the pull-up artifacts were compiled. The PCR product was sourced from positive controls amplified with Identifiler™ Plus, the capillary electrophoresis done on an Applied Biosystems™ 3500xL Genetic Analyzer, data was collected with the Applied Biosystems™ 3500 Series Data Collection Software 3 v3.0, and the analysis done using GeneMapper® ID-X v1.5. A total 36 injections were done over the course of five months, for a total of 792 samples analyzed and evaluated for the presence of pull-up artifacts. The size, height, and dye channel were compiled for the 5,092 pull-up peaks observed and also the alleles causing each of these artifacts. The relative height and the distance of the pull-up to its source were calculated and graphed out, as well as a few other parameters. The data collected was used to determine pull-up patterns from which interpretation guidelines were established. Although these results cannot be directly applied in other laboratories, pull-up artifacts are observed with all PCR-amplified DNA separated on a capillary electrophoresis instrument. Therefore, the presented method of characterization of pull-up artifacts could be implemented in any laboratory, using the amplification kit of choice and specific capillary electrophoresis injection parameters. Until these artifacts are successfully managed or eliminated by software or hardware, the study of pull-up can prove useful to analyze DNA results with increased confidence.

Pull-Up, Capillary Electrophoresis, DNA Analysis

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