B110 Quantitation and Characterization of Spectral Overlap Observed in Data Obtained From the 310 and 3100 Genetic Analyzers and the Use of These Data in Formulating Interpretation Guidelines

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The goal of this presentation is to provide a methodology by which one can apply specific diagnostic criteria to differentiate pull-up peaks from true alleles and/or other artifacts that commonly appear in data analyzed on the 310 and 3100 Genetic Analyzers (ABI).

Virtually all DNA analysis of forensic casework is performed using fluorescent detection. Typically, STR primers are labeled with fluorescent dyes allowing the detection of one strand of the denatured PCR product. For the AmpFISTR Profiler Plus® and COfiler® PCR Amplification Kits (ABI), the FAM, JOE, NED, and ROX fluorescent labels emit at wavelengths that correspond to the blue, green, yellow, and red regions of the spectrum. Spectral overlap between adjacent dyes can lead to the detection of extraneous peaks within a sample. To correct for this overlap, matrix standards are run to calculate the degree of spectral overlap between the dyes. The matrix file then subtracts this overlap resulting in analyzed data that does not contain extraneous peaks caused by spectral overlap. When the matrix fails to remove fluorescence detected from adjacent dyes, "pull-up" or "bleed through" peaks are observed on the electropherogram. One characteristic of pull-up peaks is their close correspondence in base pair size to the size of the causal peak, which customarily has a significantly higher peak height and usually originates from a spectrally adjacent color(s).

Data analysis would benefit from guidelines that indicate when and where a pull-up peak would most likely occur, particularly when the nature of these peaks varies between instruments (377, 310, 3100). This information can be critical in forensic casework due to the extensive overlap between loci labeled with different dyes. This is particularly important when a pull-up peak is labeled with an allele designation that corresponds to ladder allele. Interpretation errors can result in the inclusion of pull-up peaks or the exclusion of small, but true peaks from a profile.

For this study, data has been compiled from samples run on the 310 and 3100 Genetic Analyzers to establish an RFU (relative fluorescent unit) value below which pull-up is unlikely to occur. The 310 data reflects peak heights obtained from samples run on five different instruments, each with a unique matrix file. The 3100 data were obtained from a single instrument. When the data between the two instruments were compared, it was observed that pull-up occurs at relatively lower peak heights then on the 310. The data further characterizes pull-up in terms of the amount of "drift" between the base pair size of the pull-up peak vs. that of the causal peak. When both instrument platforms were compared, the amount of drift observed between the pull-up peak and its corresponding true allele was not only greater on the 3100 but was also directional in nature.

The quantitative information provided in this study will provide the analyst an additional tool with which to analyze fluorescent-based forensic casework data. It is anticipated that this information could further lead to the development of a diagnostic hierarchy for differentiating true alleles, pull-up peaks and spikes.

STR Analysis, Bleed Through, Interpretation

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