EVALUATION OF POWERPLEX FUSION (5C) DATA INTERPRETATION CHALLENGES BETWEEN THE 29 AND 30 CYCLE AMPLIFICATION METHODS

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Following Promega's recommended run conditions for the PowerPlex® Fusion (5C) 24-plex (30 cycles of PCR followed by a 5-second injection on a 3130xL), Sorenson Forensics' internal validation results mimicked those published in the developmental validation. However, conventional data evaluation for determining stochastic and peak height ratio (PHR) thresholds did not provide the confidence sufficient for analysts to correctly interpret mixed samples. Both Type I and Type II errors were observed from all analysts on mock test sets. It was estimated that if implemented, one in every two mixed Fusion profiles would include an allele call error and that proficiency test failure was likely.

At fault was data variability. The reproducibility study revealed a significant difference in peak heights both within and between amplification plates. PHR variability caused sporadic intra-locus imbalance events so extreme (lowest observed ratio was 16%) that they interfered with accurate stutter interpretation. In an effort to improve variability, a new series of sensitivity data was generated from a reduced cycle number amplification (29 cycles). Comparison of allele heights and peak height ratios showed a marked improvement in variability in the 29 cycle data over the 30 cycle data. The spread of possible peak heights obtained from any given DNA input was tighter. The PHR of lower DNA inputs also showed a tightening of range, but more importantly, the minimum PHR observed at lower DNA inputs was higher in the 29 cycle data (48% for 0.0625ng DNA input) than in the 30 cycle data (31% for 0.0625ng DNA input) thus making proper stutter interpretation possible. In fact, charting of elevated stutter events alongside imbalanced PHR events showed minimum overlap, only occurring below the analytical threshold.

The challenges presented by the PowerPlex® Fusion (5C) kit validation have shown that internal validation of a 29 cycle amplification method is superior to a 30 cycle setting for purposes of forensic casework. Therefore further evaluation studies on amplification cycle number are necessary prior to implementation into the laboratory.