A101 Measurement of a Stochastic Threshold and Development of a Reduced Volume Reaction for PowerPlex® 16 HS and Identifiler™ Plus

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After attending this presentation, attendees will learn how establishing a stochastic threshold can affect the interpretation of DNA profiles for lower template quantity samples and the resulting statistical analysis. In addition, attendees will learn how stochastic thresholds compare between PowerPlex® 16 HS, Identifiler™ Plus, and PowerPlex® 16.

This presentation will impact the forensic science community by allowing analysts to be more confident in their interpretations of DNA profiles, thus differing stochastic thresholds for commonly used STR multiplex kits can affect both the useful data obtained for forensic samples and the statistical analysis performed. The information gleaned from this study may allow other forensic laboratories in the midst of developing stochastic thresholds to improve their DNA typing result quality.

Validating a stochastic threshold has been deemed necessary by SWGDAM for interpreting STR typing results. The stochastic threshold is the threshold at which the analyst can be confident that if one peak for a heterozygote is above this threshold, then its sister allele will be present and should be at least above the analytical threshold. This increases confidence in homozygous calls made for single source samples and alleles attributed to each contributor in mixture analysis. Promega and Applied Biosystems have released two STR amplification kits (PowerPlex® 16 HS and Identifiler™ Plus, respectively), which claim to improve upon the DNA typing results obtained with their previous kits. Specifically, these kits allege better heterozygous locus balance, which could mean a lower stochastic threshold. To test the validity of these claims, the stochastic threshold was defined for both kits and compared to the PowerPlex® 16 threshold defined previously by the Virginia Department of Forensic Science. The amplification parameters of half-volume reactions (12.5mL) for PowerPlex® 16 HS and Identifiler™ Plus were first modified to closely mimic full-volume (25.0mL) reactions since typically, reducing the PCR reaction volume greatly enhances sensitivity. A half-volume (12.5ml) reaction with one cycle removed (31 cycles) provided sensitivity with PowerPlex® 16 HS that was comparable to its full-volume reaction. A half-volume reaction with the standard cycle number (28 cycles) provided sensitivity with Identifiler™ Plus multiplex that was similar to its full volume reaction, which was surprising. Stochastic thresholds were determined using half-volume reaction conditions under three injection times for each kit: two, five, and ten seconds for PowerPlex® 16 HS and five, ten, and twenty seconds for Identifiler™ Plus. The stochastic thresholds established using PowerPlex® 16 HS were 180, 320, and 370 rfu for a two, five, and ten second injection, respectively. The stochastic thresholds established using Identifiler™ Plus were 200, 300 and 380 rfu for a five, ten and twenty second injection, respectively. All of the thresholds established were equal to or lower than those for the corresponding injection time using PowerPlex® 16. The average peak height ratios for all three kits were statistically similar. The stochastic threshold was applied to single-source and mixture mock case samples typed using each of the kits and the useful data obtained, both for interpretation and statistical
analysis, compared.

PowerPlex® 16 HS, Identifiler™ Plus, Stochastic Threshold

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