IDENTIFILER PLUS VALIDATION FOR FORENSIC CASEWORK AND IMPLICATIONS FOR QUANTIFICATION OF INHIBITED SAMPLES

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Identifiler Plus offers several advantages over the Profiler Plus/COfiler and Identifiler Amplification kits. Chief among these are improvements in the buffer composition to overcome PCR inhibition and improvements in peak height ratio between sister alleles at a locus as compared to the original ABI Identifiler DNA typing kit. Another advantage is decreased amplification time due to combining the annealing and extension cycles making the PCR process two steps (denaturation and annealing/extension) rather than three.

The Harris County Institute of Forensic Sciences Forensic Biology Lab performed an internal validation of the Identifiler Plus Amplification Kit. Sensitivity and stochastic studies, accuracy, precision, reproducibility, mixed samples, inhibition, stutter, and contamination were assessed and compared to the currently validated casework systems, Profiler Plus and Cofiler. All studies were performed using 28-cycle amplification.

The most interesting attribute of Identifiler Plus is its ability to amplify a full DNA profile from samples containing a PCR inhibitor, humic acid; Profiler Plus, COfiler, Minifiler, and PowerPlex 16 HS were unable to amplify in the presence of the same amount of the inhibitor. These samples also showed undetermined sample and IPC Ct values during quantification with Quantifiler Duo. While this is an impressive feat, it creates difficulty for the forensic casework community. Now that an amplification kit is available that is more robust than commonly utilized qPCR systems, laboratories must use alternative approaches to estimate the amount of extracted DNA recovered in cases of extreme inhibition until a more robust quantification kit can be developed. Strategies for determining the amount of DNA to amplify from inhibited samples will be discussed during the presentation.

Identifiler Plus was slightly more sensitive than Profiler Plus/COfiler with a 0.75 ng optimal input amount of DNA for amplification, lower than the 1.0 ng target DNA amount for Profiler Plus/COfiler. Stochastic effects were observed from 50 to 145 relative fluorescent units (RFU), nearly the same as Profiler Plus/COfiler at 125 RFU. In addition, the minimum amount of DNA that enabled more than 90% of alleles to be detected above the laboratory's peak amplitude threshold of 50 RFU was 0.06 ng, almost identical to that of Profiler Plus/COfiler at 0.05 ng. In determining heterozygous peak balance, the peak height ratio (PHR) for samples where both sister alleles are above the stochastic region was 58%, while the PHR for samples within the stochastic region was 46% (average + 3SD). This is comparable to what is observed with Profiler Plus/Cofiler samples.

Identifiler Plus produced accurate and reliable results from repetitively tested single source blood, saliva, and bone samples. The base pair sizing precision of was within 0.5bp with a standard deviation below 0.15 bp. Stutter behavior was studied for each locus. The maximum observed stutter at D21S11, D7S820, and D5S818 slightly exceeded that reported by the manufacturer. Reciprocal male / female mixture studies demonstrated that Identifiler Plus recovered more alleles from minor mixture contributors than Profiler Plus/COfiler. Peak height ratios for Identifiler Plus and Profiler Plus/COfiler were similar.