B3 Validation of the AmpF*l*STR Identifiler™, Profiler Plus™, and COfiler™ STR Kits on the 3100 Capillary Electrophoresis Detection Platform

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The goal of this presentation is to validate the Identifiler™, Profiler Plus ™, and COfiler™ STR kits on the Applied Biosystems/Hitachi 3100 Capillary Electrophoresis platform for use in typing of convicted offender blood samples for the Connecticut Sex Offender DNA Database.

The Identifiler™ STR kit amplifies 15 STR loci (the core CODIS 13 STRs + D2S1338 + D19S433) and Amelogenin in a single reaction. The primary benefit of a single amplification system is the increased throughput achieved for processing specimens such as convicted offender database samples. In addition, there is a reduction in paperwork and tube labeling which minimizes the opportunity to mislabel or switch a sample during the processing procedure. A double amplification system (Profiler Plus and COfiler), while providing a sample check by having overlapping loci between the two systems, results in twice the amplification preparation and run time per sample when compared to the Identifiler kit. The greater throughput volume of the ABI 3100 instrument makes it an ideal detection platform for databasing. The Profiler Plus and COfiler systems were also validated on the ABI 3100 detection platform for known sample processing to introduce additional flexibility in the laboratory.

In assessing the Identifiler kit and comparing to Profiler Plus and COfiler results, 1 nanogram of input DNA was amplified in a 25 µl reaction volume. A final extension step of 90 minutes at 60°C was added to the thermal cycling parameters. Within each run, capillary-tocapillary precision for each of the locus in the Identifiler kit was in the standard deviation range of 0.02-0.10 nucleotides and the Profiler Plus and COfiler loci were comparable to the Identifiler kit. Capillary precision between runs was calculated for each locus and the values for the ABI 3100 instrument were well below the 0.15 nucleotide standard deviation specification set by Applied Biosystems, Inc.

However, on occasion, a standard deviation value of 0.19 nucleotide was observed for the FGA locus. To correct for this, it is important to follow ABI recommendations for injecting at least 1 ladder per run on the instrument. The average stutter values (Identifiler) were calculated for each locus and were as follows: D8S1179 (6.3%), D21S11 (9.1%), D7S820 (5.4%), CSF1PO (6.3%), D3S1358 (6.3%), THO1 (3.2%), D13S317 (8.1%), D16S539 (7.2%), D2S1338 (10.0%), D19S433 (5.0%), vWA (7.2%), TPOX (6.3%), D18S51 (6.8%), D5S818 (7.2%), and FGA (5.4%). The stutter values from Profiler Plus and COfiler systems were comparable to the Identifiler on the ABI 3100 instrument. For all three amplification systems, full STR profiles were obtained from 1 nanogram of DNA template; however, the sensitivity of the ABI 3100 instrument allowed the obtaining of profiles from as low as 100 picograms of input DNA template. In addition, the Profiler Plus and COfiler profiles generated on an ABI 377 instrument were concordant with those profiles generated on the ABI 3100 CE instrument.

In summary, this validation study demonstrates that the results of the Profiler Plus and COfiler amplification kits are comparable on the ABI 3100 instrument to the ABI 377 instrument. Identifiler, as a single megaplex amplification system in combination with the use of the ABI 3100 instrument, does improve the handling and abbreviates the preparation time resulting in increased throughput processing of convicted offender samples. Moreover, the profiles generated by the Profiler Plus and COfiler kits were in agreement with the profiles generated by the Identifiler kit.

Identifier, ABI 3100, Concordance

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