VALIDATION OF APPLIED BIOSYSTEMS 3730 GENETIC ANALYZER FOR HIGH-THROUGHPUT OFFENDER TESTING USING IDENTIFILER

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Background: The Applied Biosystems (AB) 3130XL genetic analyzer is a capillary electrophoresis-based instrument utilized in the human identification testing community for fragment analysis. With the release of the next-generation 3730 platform, higher throughput processing is achievable (up to four times greater than the 3130XL) while maintaining low failure rates. This study's objective was to validate the 3730 using the Identifiler multiplex (AB) for human identification testing applications in a high-throughput offender testing laboratory.

Methods: Genomic DNA used in this validation were isolated from whole blood samples using Qiagen 's DNA Blood Kit and the BioSprint96 robot or using Qiagen's DNA Investigator kit and the Qiagen BioRobot EZ1. The samples were quantitated using a modified PicoGreen assay and amplified using the Identifiler PCR kit on GeneAmp 9700 thermocyclers. Amplified products were processed on a 3730 48-capillary array and the resulting genotypic data was analyzed with GeneMapper (version 3.2.1), using CODIS version 1.1 panels and bin sets. Data quality parameters examined in this study included crosstalk, concordance, precision and sensitivity.

Results: Crosstalk was assessed with a "checkerboard " plate configuration whereby signal was examined in blank wells and was detected only when input DNA was greater than 5ng. Data concordance was examined by analyzing reference samples previously examined using the 3130XL (AB). No instances of non-concordance were observed. Run-to-run and capillary-to-capillary precision was evaluated by examining migration of a series of allelic ladders. For all allele peaks, the detected size ranges were within 1.0 base pair. Sensitivity studies involved input DNA quantities of 10.0ng, 5.0ng, 2.5ng, 1.25ng, 0.62ng, 0.31ng, 0.16ng and 0.05ng. With addition of less than 0.62ng, stochastic effects became evident.

Conclusion: This study demonstrates that the 3730 yields reliable and consistent results within the context of high-throughput fragment analysis for human identification testing laboratories.