



### **B41 Validation of the AB 3730xl DNA Analyzer for Paternity and Forensic Use**

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The goal of this presentation is to discuss the validation study of the AB 3730xl DNA Analyzer with a 48-capillary array with the AmpFISTR® Identifiler® PCR amplification kit for paternity and forensic testing applications.

This presentation will impact the forensic community by providing public forensic laboratories with a validated means for high throughput DNA testing with robust results.

The Applied Biosystems (AB) 3130 Genetic Analyzers and 3730 DNA Analyzers are capillary electrophoresis-based instruments that generate genotypic information by separating amplified DNA fragments based upon their sizes and detecting fluorophores covalently-bound to incorporated primers. The 3130 Genetic Analyzers are increasing in popularity in labs performing forensic STR fragment analysis. The 3730 DNA Analyzers are considered medium-to-high throughput instruments that are not currently used in forensic laboratories. Sorenson Forensics primarily uses a 3130xl Genetic Analyzer for fragment analysis. The purpose of this study was to validate the AB 3730xl DNA Analyzer with a 48-capillary array with the AmpFISTR® Identifiler® PCR amplification kit for paternity and forensic testing applications.

This validation was performed for both Sorenson Forensics and Sorenson Genomics (paternity) laboratories. The following studies were performed in accordance with SWGDAM (Scientific Working Group on DNA Analysis Methods) guidelines and DAB (DNA Advisory Board) standards: Crosstalk, Concordance, Reproducibility and Precision, Sensitivity, Mixture, Match Criteria, Contamination, Stutter, and Peak Height Ratio Analysis. DNA samples used for this study included various sample types of simulated casework samples, such as buccal swabs, hairs, cigarette butts, blood on fabrics, and touched items, as well as NIST SRM 2391b samples. Sample quantities were determined either by the Quantifiler™ Human DNA Quantification Kit or a PicoGreen®-based assay. All samples were amplified using the AmpFISTR® Identifiler® PCR amplification kit and analyzed on a 3730xl DNA Analyzer. The default GeneMapper36\_POP7 run module for the 3730xl and reduced cross talk (G5-RCT) dye set were used. The data were analyzed using either GeneMapper® ID software v3.2 or GeneMapper® software v4.0.

The AB 3730xl DNA Analyzer has proven to reliably and robustly analyze DNA fragments from the AmpFISTR® Identifiler® PCR amplification kit. Experimental results obtained from a 3730xl DNA Analyzer were concordant with results obtained from a 3130xl and/or an ABI Prism® 3700 Analyzer. Observed crosstalk was below 75RFU which did not interfere with interpretation. Concordant and reproducible results were obtained from the known samples, NIST SRM 2391b samples, 9947A positive amplification controls, and allelic ladders. Precision of the instrument was demonstrated by analyzing multiple Identifiler® ladders, simulated casework samples, and GeneScan™ 500 or 600 LIZ® size standard. The results of the sensitivity study indicated that the optimal range of input DNA for amplification is between 1.5ng - 3ng. Mixture analyses studies provided insight as to what mixture ratios could be confidently interpreted from casework samples. Stutter percentages fell within manufacturer's published guidelines. These results demonstrate the usefulness and applicability of the 3730xl for high throughput commercial DNA testing.

The results of these studies provide support for the use of the AmpFISTR® Identifiler® PCR amplification kit in conjunction with a 3730xl DNA Analyzer with a 48-capillary array for forensic and paternity testing in our laboratory. This instrument allows for a higher throughput and may decrease cost per sample through labor savings. Further studies could be performed to optimize the 3730xl for forensic applications. An injection time study would benefit forensic casework samples so that multiple injection times could be used. Further investigation into the merits of GeneScan™ 600 LIZ® size standard could also be assessed. Other commercially available multiplexes should also be validated on this instrument. This study comprises an initial validation of the Identifiler® multiplex on the AB 3730xl DNA Analyzer with a 48-capillary array for forensic and relationship testing applications.

**Validation, 3730xl, Identifiler®**