A45 Internal Validation of the AmpfℓSTR® MiniFiler™ Amplification Kit for Use in Forensic Casework

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The goal of this presentation is to discuss the valdiation study of the AmpfℓSTR® MiniFilerTM PCR amplification kit with an ABI 310 Genetic Analyzer for use in a forensic casework laboratory.

This presentation will impact the forensic community by providing the basis of the five validation studies conducted according to SWGDAM (Scientific Working Group on DNA Analysis Methods) and DAB (DNA Advisory Board) standards: Sensitivity, Concordance, Mixtures, Peak Height Ratio Analysis, and Stutter.

The AmpfℓSTR® MiniFiler™ PCR amplification kits utilize a five- dye fluorescent system for fragment analysis and have been optimized for the analysis of degraded and/or inhibited DNA samples. The MiniFiler™ kit was designed with miniSTRs to amplify eight of the largest sized loci in the AmpfℓSTR® Identifiler® PCR Amplification Kit (D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, FGA). These loci along with the gender-identification locus, Amelogenin, enables simultaneous amplification of the loci that often fail detection during the amplification of degraded DNA. An internal validation of the MiniFiler™ amplification kit was completed for the Mesa Police Department Forensic Services Section for use in casework analysis.

DNA samples used for these studies included buccal swabs, hairs, cigarette butts, NIST-traceable bloodstain samples, semen samples, and touch samples. Samples were quantitated using Applied Biosystems Quantifiler™ Human DNA Quantification Kit. All samples were amplified with both the AmpfℓSTR® Identifiler® and MiniFiler™ amplification kits and electrophoresed on a 310 Genetic Analyzer. The data was analyzed using GeneMapper® ID v.3.2 analysis software.

The MiniFiler™ amplification kit yields reliable and robust DNA profiles when the input DNA amount is between 0.2-0.6 ng, with the optimal template amount being approximately 0.4 ng. However, DNA results were obtained with a DNA input amount of 12.5 pg. A significant increase in pull-up and off-ladder alleles was seen at concentrations greater than 0.625ng. Experimental results obtained from the MiniFiler™ amplification kit were concordant with the results obtained from the Identifiler® amplification kit. In the mixture study, full profiles were detected for the minor contributor at 1:2 and 1:4 ratios with a threshold set at 50 rfu. More dilute mixtures (1:8 and above) gave some results for the minor contributor, but not consistently. The peak height ratios between the two heterozygous peaks were above the recommended values from ABI and averaged approximately 83%. Heterozygote peak imbalance as low as 41% was observed within one loci of a given sample. Based on the empirical data obtained from the samples, the average stutter percentages fell below the manufacturer's published guidelines.

Based on the results of these studies, the use of GeneMapper® ID analysis software in conjuction with the MiniFiler™ amplification kit will yield results consistent with those currently found using GeneMapper® ID with Identifiler® amplification technology. It will also yield more complete profiles when used in combination with the Identifiler® amplification kit. This validation study supports the use of the MiniFiler™ amplification kit on forensic casework samples that are degraded, contain PCR inhibitors, and/or have low template DNA amounts.

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MiniFiler™, Validation, Degraded DNA

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