INTERNAL VALIDATION OF THE AMPFℓSTR® IDENTIFILER® PCR AMPLIFICATION KIT

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Internal validation of the AmpFℓSTR® Identifiler® PCR Amplification Kit was performed on ABI PRISM 3130xl Genetic Analyzers for use on automated platforms in forensic casework. Various parameters were examined including precision, sensitivity, reproducibility, stutter peak heights, DNA mixtures and forensically relevant samples were tested. The manufacturer's suggested conditions for PCR and electrophoresis were followed with the exception of the primer annealing temperature which on our PCR equipment, the GeneAmp® PCR System 9700, was optimal at 58 ºC instead of 59 ºC. The validation included the use of two different assays of 10 µl and 15 µl final reaction volumes to target low and high DNA concentrations respectively. Our results show that it is possible to consistently obtain full profiles with as little as 60 pg of DNA in 10 µl reaction volumes and with 125 pg in 15 µl. In mixture analysis, the minor contributors were clearly detected down to a 1:10 ratio in 10 µl reaction volumes. Allele frequencies and stutter values for each locus were determined from a population study of 280 caucasian individuals from the Montreal and Lac St-Jean areas of Québec, Canada. Forensic samples were tested to ascertain the system's ability to produce significant results with DNA extracts from various biological sources and substrates.