THE FORENSIC VALIDATION STUDIES OF PROFILER PLUS™ AND ALLELE FREQUENCIES OF PROFILER LOCI IN A POLISH POPULATION

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Short tandem repeat (STR) loci are widely used in forensic genetics for identification and paternity testing. Introduction of the multiplex PCR technique gave the possibility of simultaneous, quick and robust amplification of several DNA loci from minute biological stains or fresh blood. One of the commercially available multiplex kits is Profiler Plus™ (Perkin-Elmer) consisting of nine polymorphic loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820) and amelogenin. Fluorescent detection technology gives the possibility of simultaneous automatic analysis of all ten amplified loci. The main aim of our work was multiloci analysis of DNA samples from Northern Poland population using Perkin-Elmer Profiler Plus™ kit (P+) and ABI™ 310 automatic sequencer. Moreover we would like to analyze the influence of different factors on Profiler Plus™ amplification fidelity, sensitivity and reproducibility based mainly on TWGDAM guidelines on validation.

DNA was isolated from 202 blood samples from unrelated individuals living in Poland. The extraction and quantitation was carried out using phenol-chloroform method and QuantiBlot® kit (PE, USA) detection respectively. DNA samples were amplified in a multiplex reaction with subsequent automatic detection using capillary electrophoresis (ABI™ Prism® 310 DNA sequencer). Some statistical parameters characterizing system and compliance with the Hardy-Weinberg equation were calculated.

Validation studies included effects of DNA concentration and degradation, extraction methods and different inhibitors on PCR amplification. We also analyzed the influence of different factors on stutter bands formation and analysis precision. We found the P+ very sensitive. The lowest DNA concentration needed to obtain a complete profile was 60pg (RFU cut off level 150). Moreover, according to TWGDAM guidelines, we performed other studies like mixed stains, stains on different substrata, non-human DNA, aging studies etc. The system was shown to be robust and reproducible.

Of the nine analyzed loci the most discriminating were D18S51 (PD=0.963), FGA (PD=0.961) and D21S11 (PD=0.950). The calculated probability of identity for P+ loci is $2{,}26 \times 10^{-11}$ giving on average 1 in 44 billion probability of identity in our population sample. All loci met Hardy-Weinberg equilibrium. During population studies rare allele in the FGA locus was observed. Sequencing analysis identified it as allele 16.

An attempt was made to improve precision by applying a different internal standard in the form of the fluorescent ladder (CXR) 60-400bp Promega (FL-CXR). It was observed that substitution of GS500 for FL-CXR drastically improves the precision of analysis. High SD values for GS500 were confirmed, ranging from 0.18 to 0.75bp. For FL-CXR all the SD values were located within the range 0.06–0.16bp. Such high precision with the application of FL-CXR, allows not only for the differentiation of alleles differing by 4 or 2 nucleotides but also of those differing by 1 nucleotide (±3SD).