VALIDATION OF POWERPLEX®16 SYSTEM FOR STR ANALYSIS ON ABI 310 GENETIC ANALYZER

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The aim of the study is to evaluate the forensic usefulness of the PowerPlex® 16 system for STR analysis using PE ABI PRISM® 310 Genetic Analyser. The evaluation was performed on biological evidence, which included some known paternity samples, NIST standards, previous proficiency samples and some other known samples. Fifteen polymorphic STR loci, Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Penta D, CSF1PO, D16S539, D7S820, D13S17, D5S818, and amelogenin were detected using PowerPlex®16 system (Promega Corp. WI). In order to determine the sensitivity of the machine for sample concentration: 2 ng, 1.5 ng 1 ng, 0.5 ng, 0.25 ng, 0.125 ng, 0.063 ng and 0.031 ng of standard DNA 9947A were amplified and tested. To determine the consistency of the system, two known samples DNA #1 and DNA #2 were re-injected five times. In order to determine the reproducibility of the analysis, DNA extracted from bloodstain, saliva, and nasal secretions from the same person were amplified and tested.

To evaluate the suitability of the system for mixture analysis, two DNA samples (DNA A and DNA B) of equal concentrations were mixed in ratios 9+1, 8+2, 7+3, 6+4, 5+5, 4+6, 3+7, 2+8, 1+9 and the mixtures were amplified and tested. In addition, known DNA samples #1, 2, & 3 DNA standard 9947A, NIST standard #1, previous CAP proficiency samples (DNA Data Base Set-2 1998, Set 1&2 2000, Set-1 2001 and FID 2001) and samples from five known paternity cases were also amplified and tested.

All stained samples except for the paternity case samples were extracted by organic extraction method and amplified by PowerPlex®16 System according to the manufacturer protocol using PE 9600 thermocycler. Samples from the five paternity cases (blood stains on FTA® cards taken as routine paternity samples in the lab) were amplified directly from FTA® cardpunches after cleaning with FTA® purification reagent and reducing the number of PCR cycles to 18. The amplified products were analysed as per the manufacturer protocol using GeneScan® software v 3.1. Genotype analysis was performed using PowerTyper™ 16 software (Promega Corp. WI).

The accuracy of DNA profiling by PowerPlex®16 was established by testing approximately 50 specimens. The identity of all the samples was compared and verified by using the Profiler Plus™ and COfiler™ systems. Genotypes of 13 STR loci so obtained from PowerTyper™ 16 agree with the results obtained from the Profiler Plus™ and COfiler™ systems.

The sensitivity testing shows that the range between 0.25 to 1 ng of DNA template is ideal for accurate results. Mixture analysis shows that the mixture sensitivity of the PowerPlex®16 system equals that of Profiler Plus™ and COfiler™ system. Also the results obtained from the PowerPlex® system are consistent and reproducible. Direct amplification from the FTA® cardpunches by reducing the PCR cycles to 18 produced accurate sizes and balanced alleles. Therefore the validation studies show that the Promega PowerPlex®16 system is an accurate, sensitive and reliable method of multiplexing STR typing for forensic casework.