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Internal validation of the AmpF*l*STR® Identifiler PCR™ amplification kit on the ABI Prism® 3100 genetic analyzer for use in forensic casework at the Department of Chemistry, Malaysia

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Abstract. Seven elements which comprised sensitivity, precision, reproducibility, non-probative casework, stutter, heterozygous peak height ratio and mixture interpretations were studied by the Forensic DNA Laboratory at the Department of Chemistry, Malaysia to validate the use of the AmpF*l*STR® Identifiler PCR™ Amplification Kit on the ABI Prism® 3100 Genetic Analyzer. The AmpF*l*STR® Identifiler PCR™ multiplex co-amplifies fifteen STRs loci, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and the Amelogenin, the gender marker. Based on the results obtained, guidelines for the interpretation of DNA STR profiles are drawn up for use in forensic casework at the Department of Chemistry, Malaysia. © 2005 Published by Elsevier B.V.

Keywords: Deoxyribonucleic acid (DNA); Internal validation; Short tandem repeats (STRs); Polymerase chain reaction (PCR); AmpFlSTR® Identifiler PCRTM; ABI Prism® 3100 Genetic Analyzer

1. Introduction

According to the guidelines of quality assurance standards for forensic DNA testing laboratories [1,2], prior to implementing a new/existing DNA analysis procedure, the forensic laboratory must first demonstrate reliability of the procedure by carrying out an internal validation [3,4]. Recent advances in forensic DNA analysis, particularly STR multiplexing, have focused on improving the robustness and efficiency of the system, such

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Table 1
PCR amplification parameters are as follows

Instrument system	Tube type	Initial incubation step	Denature	Anneal	Extend	Final extension	Final step
		HOLD	CYCLE (28 cycles)			HOLD	HOLD
GeneAmp PCR System 9600/9700	Thin-walled reaction tube with cap	95 °C 11 min	94 °C 1 min	59 °C 1 min	72 °C 1 min	60 °C 60 min	10 °C (forever)

as minimizing sample consumption and maximizing reproducible informative data output. The ABI Prism® 3100 Genetic Analyzer represent a high-throughput and sensitive automated capillary electrophoresis system that separate, detect and analyze up to 16 samples of fluorescently labeled DNA fragments in a single run [5].

2. Materials and methods

DNA was mainly extracted using the chelex extraction protocol, unless stated otherwise. Population database samples were purified and amplified directly using punched blood discs from FTA® cards. PCR amplification parameters as in Table 1.

Total PCR reaction volume, 20 μl = 12 μl of Master Mix + 8 μl of Template DNA.

Concentration of template DNA used is 0.15 ng/μl (= 1.2 ng), unless stated otherwise.

(i) Sensitivity: DNA from human cell line 9947A and two known DNA samples (from reference blood specimens) were amplified in duplicate at the following concentrations: 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, and 0.0125 ng/μl. Total number of observations = 42. **(ii) Precision:** (a) 16 samples of AmpF*l*STR® Identifiler Allelic Ladders were analyzed in duplicate on three different days. Total number of observations = 96. (b) At least four most common alleles of each genetic locus were selected and a minimum of 50 data points for each allele were obtained from population database samples. **(iii) Reproducibility:** DNA extracts from 4 different specimens (bloodstains, seminal stains, buccal swabs and hair roots) from two different individuals were amplified in duplicate at three different times and their PCR products were analysed on three different days. Total number of observations = 48. **(iv) Non-probative casework:** (a) Two bloodstains on fabric and plastic substrate; (b) Four liquid blood specimens preserved in EDTA; (c) Two muscle tissue specimens from phenol/chloroform extraction; (d) Four seminal stains (cotton swab and fabric substrate) from differential extraction; (e) One saliva stain from cigarette butt; 9f) One bone specimen from phenol/chloroform extraction after decalcification.

Blood specimens in (b) were from the next-of-kin of two families which were used for the identification of the two muscle tissue specimens in (c) from two severely burnt bodies. These 14 DNA extracts were amplified in duplicate and they were previously analyzed using the AmpF*l*STR® Profiler Plus/COfiler PCR™ Amplification Kits. Total number of observations = 28. **(v) Stutter:** Percent stutter were evaluated for each locus using peak height. A minimum of 100 data points from population database samples were used comprising homozygous alleles and heterozygous alleles which were greater than 4 bp apart to ensure that there is no overlapping peaks. **(vi) Heterozygous peak height ratio:** Differences in peak height of heterozygous alleles were evaluated for each locus. A

minimum of 55 data points from the population database samples were used. Data points used comprised heterozygous alleles which were greater than 4 bp apart to ensure that there is no contribution by stutter peaks. **(vii) Mixtures:** (a) DNA extracts from two pairs of individuals (liquid blood specimens) were mixed in ratios: 1:0, 0:1, 1:1, 1:2, 2:1, 1:5, 5:1, 1:10, 10:1, 1:20 and 20:1; b) Liquid blood from two pairs of individuals were mixed in the ratios: 1:0, 0:1, 1:1, 1:2, 2:1, 1:5, 5:1, 1:10, 10:1, 1:20 and 20:1.

These DNA extracts were amplified in duplicate using template DNA concentrations of 0.15 and 0.30 ng/μl. Total number of observations = 176.

3. Results and discussion

(i) Sensitivity: To obtain maximum homozygous allele peak height <4000 RFU and the minimum heterozygous allele peak height >150 RFU, the suitable range of template DNA concentration is 0.10–0.20 ng/μl (i.e. 0.8–1.6 ng DNA). **(ii) Precision:** The standard deviation of Allelic Ladders and population database samples were 0.20 bp and 0.22 bp, respectively, i.e. within the recommended ±0.5 bp window for genotyping. **(iii) Reproducibility:** Identical STR profiles were obtained from the 4 different specimens taken from the two individuals on all 3 occasions. **(iv) Non-probative casework:** Concordance between the Identifiler® and Profiler/COfiler™ systems was observed for all the 14 DNA extracts. **(v) Stutter:** The mean percent stutter varied from a low of 2.94 (SD 1.44) for TPOX and to a high of 8.97 (SD 3.06) for D18S51. At locus TPOX, at least 95% of the observations fell within the 6% stutter, followed by D7S820, CSF1PO, TH01–8%, D8S1179, D21S11, D13S317, D16S539, D5S818–9%, vWA–10%, D2S1338, D19S443–11%, D3S1358–12%, FGA–13% and D18S51–15%. **(vi) Heterozygous peak height ratio:** The mean percent heterozygous peak height ratio varied from a low of 84.52 (SD 9.61) at D18S51 to a high of 93.30 (SD 4.83) at TH01. At least 90% of the observations have peak height ratios of more than 70%, including the gender marker, Amelogenin. **(vii) Mixtures:** (a) Major and minor components in a two samples mixtures are rather significant from their peak height ratios when the minor component is 15–30%; (b) The detection limit of the minor component is about 10% when the threshold is set at 100 RFU; (c) It is recommended that mixed samples should be amplified using higher template DNA concentration, 0.25–0.30 ng/μl (i.e. 2.0–2.4 ng DNA).

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