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Further study on suitability of Profiler Plus in personal identification

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Abstract

This work reports a validation study on the AmpFlSTR Profiler Plus™ kit (PE/AB) on capillary electrophoresis (CE).

Keywords: STR; Profiler Plus; Capillary electrophoresis; Validation study

1. Introduction

In order to evaluate the performance of AmpFlSTR Profiler Plus™ kit for forensic applications, sensitivity tests were carried out to establish the optimal amount of DNA required for interpretable and reliable profiles on capillary electrophoresis (CE). The next stage of the study verified the suitability and efficiency of multiloci analysis in pratical forensic applications.

2. Materials and methods

Serial dilutions of the GM9947A cell line were performed, covering the whole range between 4 and 0 ng. The most significant casework samples reaching our laboratory over the last 2 years were selected; bloodstains aged from 2 to 16 years were also included (Table 1). DNA was quantified using the slot blot hybridization; the Microcon-30 procedure was used to purify DNA samples giving negative or partial profiles. Amplifi-

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Table 1
Forensically significant samples from examples of casework collected in our laboratory (125 samples)

Sample	Extraction method [reference]	Number tested	Number with complete typing profile	Number with partial typing profile
Bloodstains—100% cotton (aged from 2 to 16 years at room temperature)	Ch, Ph	10	10	–
Bloodstains (carpet, fabric, fingernail, glasses, leather, marble, paper, plastic, tape, terry cloth, wood)	Ch, Ph	48	43	2
Buccal cell swab	Ch	18	18	–
Chewing gum	Ch	2	2	–
Cigarette butt	Ch [1]	3	1	1
Condom—outside surface	Ch	1	1	–
Fingernail	Ch	2	1	1
Fingernail—bitten off	Ch	1	Mixed profile	
Glass	Ch	3	2	1
Hair (root)	Ch	4	3	–
Liquid blood	Ch	12	12	
Skeletal remains				
bone shaft (femur)	[2]	3	3	–
soft tissue	QI	1	1	–
dental pulp	QI	1	1	–
Teaspoon	Ch	1	1	–
Tin	Ch	3	3	–
Tissue (post-mortem):muscle, liver, kidney	QI	4	3	1
Tissue embedded in paraffin blocks—liver	[3]	1	1	–
Tissue sections—gastric biopsy	QI	2	1	1
Urine	Ch	2	1	1
Vaginal swab	Ch	3	3	–

Ch = chelex; Ph = phenol-chloroform; QI = QI Amp-Quiagen.

cation was carried out following the manufacturer's recommendations and PCR products were electrophoresed on an ABI Prism 310 Genetic Analyzer (PE/AB).

3. Results and discussion

Results of sensitivity studies are listed in Table 2. As expected, the smallest and most homozygous loci appeared more sensitive to low amounts of DNA. Considering an arbitrary cut-off peak height threshold of 25 RFU, complete typing profiles were obtained from as little as 0.063 ng of DNA. Locus drop-out phenomenon was noted for DNA concentrations below this value, with the loss of the longer loci. Over 0.25 ng, all the loci were clearly detectable above the background, with peak heights >150 RFU, a conventional threshold limit accepted for scoring alleles. Unequal amplification of heterozygous

Table 2
Results of template DNA titration experiments on ABI PRISM 310 genetic analyzer (L = light allele; H = heavy allele)

DNA (ng)	D3S1358 (L)	D3S1358 (H)	VWA (L)	VWA (H)	FGA (L)	FGA (H)	X	D8S1179	D21S11	D18S51 (L)	D18S51 (H)	D5S818	D13S317	D7S820 (L)	D7S820 (H)
Peak heights (relative fluorescence units)															
4	1291	1181	1119	907	701	678	2619	2221	2081	798	601	1789	1162	591	459
2	1005	927	773	693	593	538	1608	1624	1309	633	519	1368	1013	486	390
1	655	695	664	435	499	441	1131	906	1000	597	575	1110	1004	340	407
0.5	385	341	225	212	229	218	738	446	440	197	226	484	431	173	155
0.25	132	169	127	101	104	106	343	262	177	114	66	244	204	63	77
0.125	78	107	75	68	56	59	180	136	122	55	39	112	108	41	45
0.063	51	42	45	47	37	38	95	71	61	38	31	62	58	28	31
0.031	47	41	–	–	–	–	40	29	47	28	26	25	35	–	–
0.015	–	–	–	–	–	–	25	–	28	–	–	–	–	–	–
0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Genotype of female cell line 9947A: D3S1358 14–15; vWA 17–18; FGA 23–24; D8S1179 13; D21S11 30; D18S51 15–19; D5S818 11; D13S317 11; D7S820 10–11.

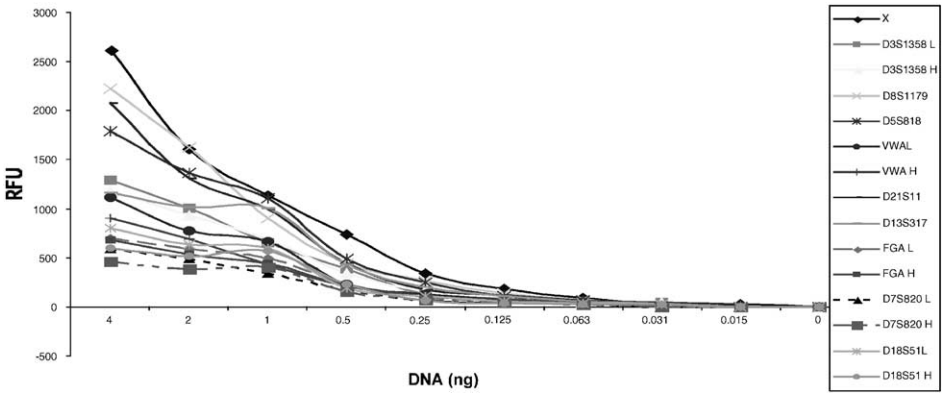

Fig. 1. Concentration–fluorescence linearity on ABI PRISM CE 310 Genetic Analyzer, using AmpFlSTR Profiler Plus™ kit. Legend show alleles of female cell line 9947A, from lightest (L) to heaviest (H).

profiles resulting in appreciable imbalance between peak heights was observed for 0.25 and 0.5 ng, due to a stochastic effect. Serial diluitions of the GM9947A cell line were also tested to examine the concentration–fluorescence linearity for each locus of the multiplex system (Fig. 1). With decreasing quantities of DNA, a linear trend appeared, except for the longer alleles of systems D7S820 and D18S51, where increased fluorescence was observed in the range 1–2 ng. It was interesting to note that, in the same range, most of the longer fragment loci (from D18S51 to vWa) of the multiplex system showed nearly comparable peak heights, suggesting that within this range, amplification of single loci has the same quality performance as the PCR reaction resulting in a balanced signals, in the range 500–1500 RFU. This indicates that a DNA amount of 1–2 ng is the optimum, thus avoiding differential amplification phenomena. In fact, with greater DNA amounts, an abundance of amplified products was observed for the homozygous loci with fluorescence >1500 RFU.

Unambiguous profiles were obtained for casework analysis when amplification was reached in the range 1–2 ng and quantities of DNA template higher than these did not produce valid results (locus drop-out phenomenon or negative results). The success rate in typing forensic casework samples was 90%. This percentage included positive results (15%) obtained after the easy additional step of purification by Microcon-30 in DNA extracts that failed to amplify.

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