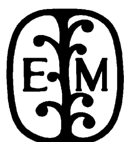

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Evaluation of the STR typing kit PowerPlex™ 16 with respect to technical performance and population genetics: a multicenter study

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1. Introduction

The recently distributed STR typing kit PowerPlex™ 16 (Promega, Madison, USA) was evaluated in 17 different European laboratories. The kit amplifies the loci D3S1358,

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TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, Amelogenin, vWA, D8S1179, TPOX and FGA. This broad study focused on technical aspects including: the impact of the amount of target DNA, balance of loci, peak height differences in heterozygotes, comparison of genotyping results obtained with different primers as well as the analysis of population data and mutation rates.

2. Methods

Each laboratory analysed unrelated individuals from its area with exception of the Teddington laboratory, which investigated individuals from immigration cases which could be assigned to either an Asian group (India, Pakistan and Bangladesh) or African Blacks. DNA was prepared from buccal swabs and blood by various well-established methods partially followed by quantification. PCR reaction volumes other than the recommended 25 μl varied from 10 to 50 μl. The analyses were performed on either an ABI 310 or ABI 377 analyses. Allele designation was performed by comparison with the allelic ladder provided with the kit. The allele frequencies were calculated from the number of each genotype of the sample set. Unbiased estimates of expected heterozygosity were computed as described by Edwards et al [1]. Possible deviation from Hardy–Weinberg–Equilibrium (HWE) was tested by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies [2–4] and the exact test [5] based on 2000 shuffling experiments. The F_{ST} values were determined as described by Weir and Cockerham [6].

3. Results and discussion

Each laboratory analysed 98–575 persons which added up to a total number of 3698 individuals. Between 1876 (vWA) and 695 (D16S539), samples were tested with at least one different primer set (loci CSF1PO, D13S317, D16S539, D18S51, D21S11, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX and vWA). Results (19,555) were compared. At locus D8S1179, PowerPlex™ 16 revealed heterozygotes in two parent/child pairs while amplification with AmpFlSTR® SGM Plus (Applied Biosystems, USA) showed only one allele. The parents were from either Vietnamese or Philippine decent. At locus TH01, one heterozygous sample (6/9.3) (PowerPlex™ 16) showed only the allele 9.3 with AmpFlSTR® SGM Plus (ABI). At locus vWA, one person showed the phenotype 16/17 with PowerPlex™ 16 while AmpFlSTR® SGM Plus and AmpFlSTR® Profiler 1 (ABI) amplified allele *16 only. Weak amplifications with PowerPlex™ 16 were observed at loci FGA (*24) and TPOX (*9) in parent /child pairs while balanced peak heights were obtained with AmpFlSTR® Profiler 1 (ABI). At locus D5S818, weak amplifications of allele * 10 were observed with PowerPlex™ 16 in two individuals. Locus CSF1PO showed a weak allele *8 with another sample while amplifications with Profiler 1 showed normal peaks.

The balance of the amplicons was measured by comparing the mean peak heights from heterozygous loci. The inter loci balance for the 'blue' systems is shown in Fig. 1. The

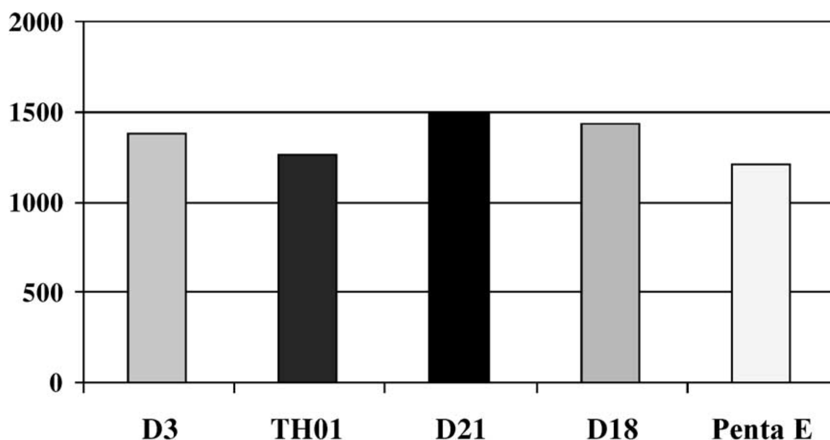

Fig. 1. Interloci balance. Mean peak heights (RFU) of heterozygous loci D3 ($n = 132$), TH01 ($n = 128$), D21 ($n = 134$), D18 ($n = 133$) and Penta E ($n = 131$).

peak height differences (%) in sister alleles of 140–147 heterozygotes was calculated for the loci D3, TH01, D21, D18 and Penta E with allele 1 as the smaller allele. Most peak height differences were observed in the range from −10% to 30%: D3 (96.6%), TH01 (94.3%), D21 (95.9%), D18 (98.6%) and Penta E (83.5%) (Fig. 2). Peak height differences > 60% can be caused by either technical or genetic reasons. Control amplification with purified DNA can rule out technical reasons while transmission of the weaker allele gives a strong indication of incomplete primer matching in these loci.

The allele frequencies of the various European populations were very similar while the Asian group and to a greater extent the group of African Blacks showed considerable differences. The genetic distance between the European populations varies between 0.96

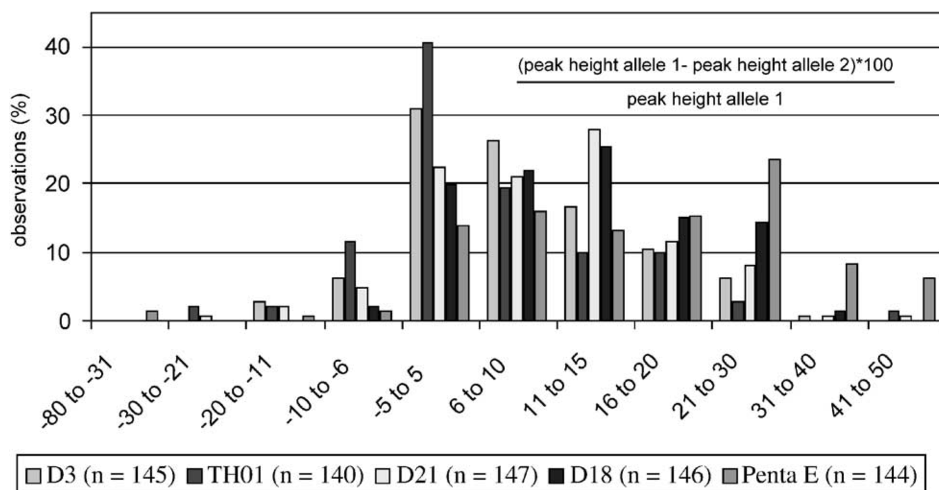

Fig. 2. Peak height differences (%) of the sister alleles in heterozygotes. Allele 1 is the smaller allele.

Table 1
Allele frequenqies (%) at the locus Penta E, laboratory: Amsterdam (AMS), BERN, Hamburg (HAM), Innsbruck (INN), Koeln (KLN), Ljubljana (LJU), Madrid (MAD), Mainz (MZ), Parma (PAR), Prague (PRA), Rome (ROM), Split (SPL), Genève (SWI), Tallin (TAL), Tuebingen (TUE), Asian Group (TED_A), African Black (TED_B)

Number of individuals tested	Alleles																
	AMS	BERN	HAM	INN	KLN	LJU	MAD	MZ	PAR	PRA	ROM	SPL	SWI	TAL	TUE	TED_A	TED_B
	106	107	575	270	418	190	197	104	98	220	233	104	120	151	300	232	190
5	4.72	7.48	8.61	9.81	6.58	8.16	3.05	3.85	7.65	5.91	6.65	6.73	5.83	6.29	6.67	4.31	3.16
6	0	0	0	0	0.24	0	0	0	0.51	0	0	0	0	0	0.5	0	0
7	12.74	13.08	13.3	15.56	16.75	13.42	13.96	16.35	14.29	17.73	16.09	14.9	15.42	18.87	16.5	9.05	7.37
8	2.36	0.47	1.13	2.04	0.36	0.79	2.28	0.96	1.02	0.68	1.93	1.92	2.92	0.33	1.5	0.65	14.74
9	2.83	1.87	1.65	1.3	1.56	1.58	2.03	0.96	1.53	2.95	1.07	0.96	0.42	1.32	2.17	2.59	4.47
10	10.38	11.21	11.74	9.26	10.05	12.89	12.94	12.02	8.16	14.09	9.23	15.38	12.08	10.93	10.33	4.53	4.21
11	9.43	10.75	11.48	10.37	10.29	8.68	12.69	12.02	18.88	9.09	10.94	5.77	10.83	9.27	13.17	14.44	8.42
12	18.87	21.96	15.74	16.3	17.34	18.42	19.54	17.79	14.29	16.14	21.03	15.38	19.58	13.91	17.67	10.56	11.84
13	7.55	9.81	10.7	10.37	9.45	10.26	11.68	8.65	9.18	7.73	12.23	12.02	12.08	6.95	10.33	9.91	6.32
14	3.77	4.21	6.96	4.26	4.55	6.32	3.3	6.25	6.12	4.09	6.87	7.21	3.33	9.6	4	7.54	8.95
15	7.08	5.14	4.61	4.26	4.67	5.26	4.57	6.25	4.59	6.14	4.08	3.85	4.58	4.97	4.17	7.54	13.16
16	6.6	3.74	4.78	5.37	5.5	4.21	4.57	5.29	3.57	5.23	1.93	6.25	4.17	6.29	4.67	9.48	5.26
16.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.22	0
17	6.6	3.27	3.22	3.7	5.74	5.53	4.57	5.29	6.12	2.05	3.22	6.73	3.33	6.62	4.17	6.03	5.26
17.4	0	0	0	0.19	0	0	0	0	0	0	0	0	0	0	0	0	0
18	2.36	3.27	3.04	3.33	3.23	2.11	1.27	3.37	2.04	5	2.36	1.92	3.33	1.99	1.67	6.03	4.47
19	1.89	1.4	1.3	2.59	2.51	1.58	1.78	0.48	1.53	2.27	1.07	0	0.42	1.32	1	2.59	1.05
20	1.42	1.4	1.3	1.3	1.08	0.53	1.02	0.48	0.51	0.91	0.86	0	0.83	0.66	0.67	3.23	1.32
21	0.47	0.93	0.26	0	0.12	0.26	0.51	0	0	0	0.43	0.96	0.83	0	0.67	0.86	0
22	0	0	0.09	0	0	0	0.25	0	0	0	0	0	0	0.66	0.17	0.22	0
23	0.94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.22	0
26	0	0	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2
Allele frequenqies (%) at locus Penta D, laboratory: Amsterdam (AMS), BERN, Hamburg (HAM), Innsbruck (INN), Koeln (KLN), Ljubljana (LJU), Madrid (MAD), Mainz (MZ), Parma (PAR), Prague (PRA), Rome (ROM), Split (SPL), Genève (SWI), Tallin (TAL), Tuebingen (TUE), Asian Group (TED_A), African Black (TED_B)

Number of individuals tested	Alleles																
	AMS	BERN	HAM	INN	KLN	LJU	MAD	MZ	PAR	PRA	ROM	SPL	SWI	TAL	TUE	TED_A	TED_B
	106	107	575	270	418	190	197	104	98	220	233	104	120	151	300	232	190
2.2	0	0	0.52	0	0	0	0.76	0	1.02	0	0.21	0	0.42	0	0.17	0	15
3.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.05
5	0.47	0	0.09	0	0.12	0	0	0	0	0	0	0	0.42	0	0	0	3.42
5.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.26
6	0	0	0.09	0	0.12	0	0	0	0	0.23	0.21	0	0	0	0.17	0.22	0.26
7	0.47	0.47	0.7	0.19	0.72	0	0.76	0	0	1.14	0.43	0	0.42	1.32	0.67	1.08	3.95
8	1.42	2.34	1.57	2.22	1.08	0.53	1.52	1.44	1.02	0.45	1.07	0	2.92	0.66	2.33	1.29	10.00
9	20.75	21.03	22	22.59	20.1	24.47	20.56	20.19	19.9	22.73	20.17	28.37	18.75	29.14	20.33	19.4	12.11
10	13.68	8.88	11.83	10	10.41	12.37	10.66	9.62	11.73	12.05	10.73	10.1	12.92	14.57	11.83	21.55	17.89
11	11.32	12.15	15.74	13.7	15.19	15.26	16.24	17.31	15.31	16.14	18.03	15.87	15.42	11.59	19	22.63	14.47
12	21.7	20.09	20.09	18.7	22.49	16.84	18.02	21.63	15.82	19.32	17.81	22.6	22.08	22.85	18.83	14.01	11.84
12.1	0	0	0	0	0	0.26	0	0	0	0	0	0	0	0	0	0	0
13	24.06	25.7	19.57	21.3	21.41	21.05	19.04	18.75	23.98	19.55	21.67	10.1	17.08	13.91	17.17	10.78	6.84
13.4	0	0	0	0.37	0	0	0	0	0	0	0	0	0	0	0	0	0
14	4.25	8.41	5.57	8.15	6.1	6.84	8.88	7.69	6.63	6.14	5.79	10.58	8.33	4.3	6.5	6.9	2.63
15	1.89	0.47	1.65	2.41	1.91	2.11	2.79	2.88	4.08	2.05	3	0.96	1.25	0.99	2.33	1.29	0.26
16	0	0.47	0.52	0.37	0.24	0.26	0.76	0.48	0.51	0.23	0.86	0	0	0.66	0.67	0.22	0
17	0	0	0.09	0	0.12	0	0	0	0	0	0	1.44	0	0	0	0.65	0

Table 3
Meioses and genetic inconsistencies at loci Penta D and Penta E

	GEC	Maternal meioses	Paternal meioses	Maternal inconsistencies	Paternal inconsistencies
Penta D	0.66	864	829	–	1 (0.12%)
Penta E	0.80	924	894	1 (0.11%)	1 (0,12%)

and 0.99. Variants were observed at most loci. The frequencies for the loci Penta D and Penta E revealed a high level of polymorphism (Tables 1 and 2). In spite of the high general exclusion rates of Penta D and Penta E, their mutation rates were low (Table 3). All mutations observed at loci other than the Pentas were confirmed by using alternative primer sets.

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