USE OF POWERPLEX® 16 IN COMPLEX MIXTURE ANALYSIS OF FORENSIC SAMPLES

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ABSTRACT
We are currently testing the PowerPlex® 16 System in our laboratory in order to use it routinely and change our current management procedure for casework samples, that is to say, the use of two amplifications (Profiler™ Plus and Profiler™) for one sample. So far, our conclusions are that the PowerPlex® 16 System seems to be a good solution for reference samples (database samples or victims/suspects) and samples whose DNA quantity is critical. We are more cautious concerning the mixture samples: we still have to test different conditions to minimize the risk of pull-ups and thus, the risk of misinterpretation (true allele or pull-up?).

INTRODUCTION
After successfully testing the PowerPlex® 16, prototype from Promega, some questions remained to be answered before using the commercial version PowerPlex® 16 with casework samples (mainly possible mixtures) in our laboratory. Among these, we were mostly interested in the possible effects of a higher number of amplification cycles (32 in place of 28), the sensitivity, the stutter bands and the heterozygote peak ratios.

MATERIALS AND METHODS
Samples were categorized in three groups: reference (n=77), casework (n=19) and experimental mixtures (n=2). For the latter, two reference samples were mixed in different ratios (1:1, 6:4, 7:3, 8:2, 9:1, 20:1 and vice versa). Five such sets were prepared, but only two of these have been analysed.

DNA was extracted from the reference samples either with QIAamp (3) or classic Phenol/Chloroform (4). This method was also used for all casework samples.

Amplification was done using the ABI 9600 thermocycler. Comparisons made with PowerPlex® 16.2 (reference samples) showed little difference between 0.5ng and 1ng amplified DNA. We thus chose to amplify 0.5ng for all reference and experimental mixtures. For casework samples, 2ng of DNA was amplified (same quantity as with Profiler™ (5)/Profiler™ Plus (6)).
All samples were amplified according to the PowerPlex® 16 System Technical Manual. Casework samples were amplified twice: once using 32 cycles (10+22), once with 28 cycles (10+18).

All amplified samples were analyzed using ABI 377 sequencer with Genescan® 3.1. Cut-off was set at 50 rfu for all dyes.

RESULTS AND DISCUSSION

Before analyzing either experimental mixtures or casework samples (some of which had been identified as mixtures using Profiler™/Profiler™ Plus), it was important to determine the heterozygous balance and the stutter ratio for the different loci amplified with PowerPlex® 16.

To do so, we analyzed 77 reference samples and calculated stutter and heterozygous ratios for each locus. We could detect no significant differences between peak height and peak area and decided to use peak height for our study.

Results show a high level of stutter for several loci (Table 1). For some systems (D3S1358, D5S818, vWA, D7S820 & D8S1179), this can be easily explained as each of these loci presents nearly identical allele sizes. Excessively high peak heights (> 2000 rfu) could also be an explanation for some samples. As expected, both pentanucleotides presented very low stutter level (less than 4%).

When compared with Profiler™/Profiler™ Plus though, PowerPlex® 16 shows higher stutter level for all common loci (Figure 1). No discrepancies were observed between PowerPlex® 16 and Profiler™/Profiler™ results.

Regarding heterozygous balance, results are satisfying and are similar to those obtained with Profiler™ and/or Profiler™ Plus (Figure 1).

When comparing peak heights between loci for one sample, D5S818 showed consistently lower values than other loci. On the other hand, peak heights for both pentanucleotides were higher, with, in some samples, a 10 to 1 difference between Penta D and other loci (data not shown)!

One last note about the tested reference samples is that, even with very high peaks (> 3000 rfu), results were always sharp and could be analyzed without problem.

After reference samples, casework samples were analyzed with PowerPlex® 16. Two series of amplifications were made, one using 32 (10+22) cycles, the other 28 (10+18) cycles.
The main reason for those tests was to assess the risks of artefactual peaks with 32 cycles amplification, but results obtained with both amplifications were also compared with Profiler™ Plus profiles (Table 2).

As expected, the 32 cycle amplification produced higher peaks, and thus, more artefacts in the form of stutters and pull-up alleles than the 28 cycles amplification (either PowerPlex® 16 or Profiler™ Plus).

Pull-up problems were observed with six loci (D3S1358, D5S818, vWA and D21S11, D7S820, D8S1179) which form two groups showing similar allele sizes for different dyes (around 130-140 nucleotides for the first one, 220-236 for the second one).
For samples with a high number of pull-up and/or stutters, interpretation was a lot more difficult in the case of complex mixtures.

When comparing results between PowerPlex® 16 and Profiler™ Plus, interesting trends appeared. If, as should be expected, the 32 cycle amplification of PowerPlex® 16 gave higher peaks than the 28 cycle amplification of Profiler™ Plus, 28 cycle amplification of PowerPlex® 16 was lower than its Profiler™ Plus counterpart, save for three loci: D21S11, D18S1179 and D7S820.

Among the other loci, FGA showed the weakest results with PowerPlex® 16, especially with 28 cycle amplification. In that case, 20 alleles detected with Profiler™ Plus where either absent or under the cut-off of 50 in PowerPlex® 16!

On the other hand, PowerPlex® 16 showed, for the D7S820 and D13S317 loci, alleles not detected in Profiler™ Plus but present in one of the reference sample found in the mixed profile of the analyzed casework sample.

For the experimental mixtures amplified, preliminary results show that ratios between samples and dilutions are conserved. Mixture can still be detected for 20:1 and 1:20 ratios for some loci, even if some alleles were not detected for 3:7 ratio (D18S518 locus).
The cut-off used for casework mixtures can sometimes be as low as 30 rfu (depending on background noise), some alleles could still be detected that way.

CONCLUSIONS

PowerPlex ® 16 seems to be a very powerful, cheap and interesting tool, especially for very small and reference samples (DNA databases and victim/suspect in casework). Regarding casework samples, particularly possible mixtures, some work must still be done before using it routinely, the most important being to determine the to be amplified DNA amount. The first tests we made, with the amount used for Profiler plus ™ and Profiler ™ amplifications (2ng), showed a lot of artefactual peaks (stutter and pull-up) caused mainly by very high peak heights (more than 2000 rfu) and overlapping of alleles of systems with different dyes.

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Tables and Figures

	Summary								
	Stutter Maximum		Hz			% Homoz.	Number		
	Maximum	Moyenne	Minimum	Maximum	Mean		Samples	Total Hz	Stutter
D3S1358	0.241	0.08893	0.511	1.59	0.91629	40.26%		46	54
TH01	0.075	0.04311	0.68	1.44	0.99138	45.97%		57	32
D21S11	0.185	0.07927	0.555	1.89	0.94868	20.78%		61	32
D18S51	0.198	0.07715	0.33	1.87	0.96894	10.39%		53	50
Penta E	0.062	0.03701	0.63	2.13	0.97755	14.29%		66	44
D5S818	0.302	0.10663	0.617	1.47	0.89924	31.17%		53	23
D13S317	0.174	0.07511	0.61	1.53	0.96376	31.17%		53	31
D7S820	0.081	0.06135	0.46	2.02	0.98622	15.58%	77	65	20
D16S836	0.13	0.06865	0.61	1.57	0.90386	20.78%		61	49
CSF1PO	0.141	0.06997	0.507	1.7	0.95372	31.17%		53	35
Penta D	0.041	0.02947	0.459	2.01	0.96321	25.97%		57	7
Amelo	0	0	0.72	2.03	1.20079	44.16%		43	0
vWA	0.15	0.07792	0.47	1.575	0.90305	14.29%		66	56
D8S1179	0.105	0.07493	0.556	1.99	0.99315	19.48%		62	32
TPOX	0.127	0.03966	0.583	1.54	1.0236	46.75%		41	13
FGA	0.13	0.07397	0.56	1.79	0.95371	12.99%		67	58

Table 1: Reference samples analysis, heterozygous balance and stutter level for Powerplex®16 loci

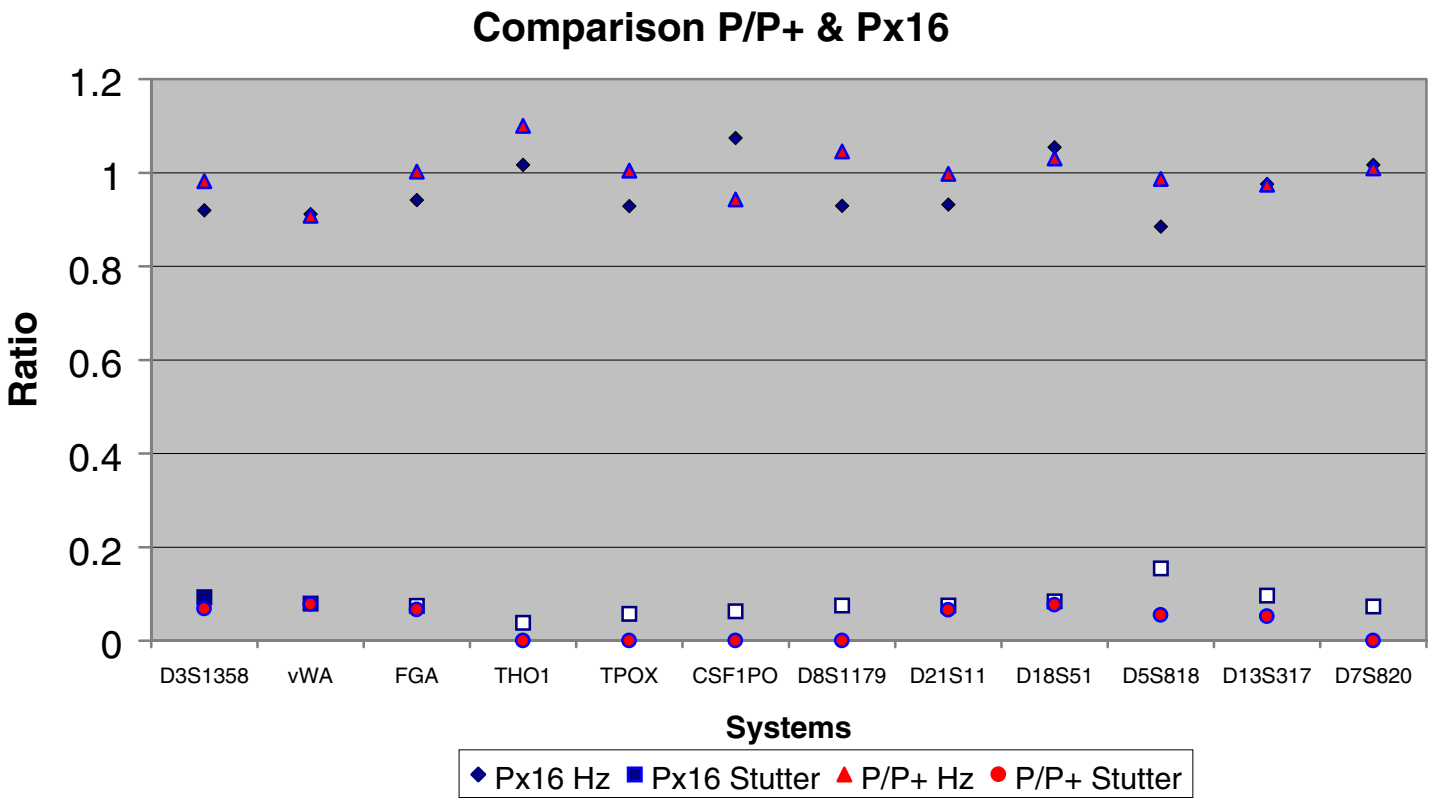

Figure 1: Reference samples analysis, comparison of heterozygous balance and stutter level between Profiler™/Profiler™ Plus and PowerPlex®16

	P+/Px16 32	P+/Px16 28	Alleles seen in[1]				Stutters			Range	
			P+ not Px16 28	Px16 28 not P+[2]	P+ not Px16 32	Px16 32 not P+[2]	P+	Px16 32	Px16 28	P+	Px16
D3S1358	0.495429	1.6775692	3	-	-	5 (3)	11	21	8	114-143	114-146
vWA	0.4731828	2.6128852	9	2 (2)	-	11 (4)	6	17	2	157-197	123-171
FGA	0.6989232	3.6798551	20	1	3	8	6	17	1	220-268	321-444
D8S1179	0.4121148	1.8648084	7	1 (1)	-	8 (2)	6	19	4	127-168	203-247
D21S11	0.2650663	0.8783192	-	6	-	13 (6)	5	15	8	189-244	202-258
D18S51	0.2503498	0.8276382	-	3	-	9	6	23	8	274-342	283-359
D5S818	0.5384045	1.7694345	3	3 (3)	-	17 (13)	4	9	4	134-171	118-154
D13S317	0.3833117	1.5496508	3	6	-	8	4	18	2	207-235	175-207
D7S820	0.1960443	0.8308495	1	2 (1)	-	11 (6)	1	17	4	262-294	215-247

Table 2: Casework samples analysis, PowerPlex® 16 (28 and 32), Profiler™ Plus (28)

[1] stutters excluded

[2] number between parentheses indicate the number of alleles present at potential pull-up positions

2 ng sample DNA in Px16: 25 µl mix 32 cycles amplification 28 cycles amplification
P+: 50 µl mix 28 cycles amplification
Analyzed with Genescan 3.1, cut-off set at 50 rfu

Clearly more stutters with Px16 amplification, for all P+/P common systems.
Main reason is higher peaks observed with Px16 (sometimes more than 10 times the peak height of P+ amplified allele).
Stutter bands are then above the set cut-off (50) and reported during analysis.
Other reason (and real problem for Px16) are pull-ups. They can exagerate the peak height of a stutter and make it appear bigger than it really is.
Pull-ups are a real problems for the three smallest systems (D3S1358, D5S818 and vWA) as some alleles have identical amplified length.