



How Low Can You Go? – A comparative sensitivity study

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Background:

Forensic casework samples come in a variety of shapes and sizes. Along with this variety of evidentiary samples comes a wide range of DNA concentrations that are encountered. It is important that the chemistry used to amplify these samples is capable of handling such variety, especially when dealing with low copy number DNA.

This study examined the performance of six different amplification kits. Four of the kits were autosomal STR kits, one manufactured by Promega, PowerPlex16 (PP16) and three manufactured by Applied Biosystems, Profiler Plus (Pro+), Cofiler (Co), and Identifier (ID). The remaining two kits were Y-STR kits, one manufactured by Promega, PowerPlexY (PPY) and one produced by Applied Biosystems, Yfiler (Yf). Sensitivity was examined to determine the saturation point of the kits as well as the minimum detection limits.

Methods:

Sample and instrument preparation:

All normalized DNA samples were prepared at the same time. These normalized samples were used for the entire study to maintain consistency and reduce pipetting variability.

The same analyst/technician prepared and loaded all of the amplification plates for all the kits, again to help reduce person to person variation. Three GeneAmp® PCR System 9700 thermocyclers were used at random. Samples were run according to recommended manufacturers' protocols.

All samples were run with 1ul of amplification product on the same ABI 3130xl Genetic Analyzer. The default run modules were used, with the exception of using a 5 second injection rather than a 10 second, as this is our validated standard injection time. Samples were then analyzed with GeneMapperID v3.2. Seventy-five RFU was used as the threshold for calling an allele for all studies conducted, as this is our validated parameter.

Sensitivity Study:

For the autosomal study, three different genomic samples, female 1 (F1), male 2 (M2), and male 4 (M4) were used. A range of input DNA from each of these samples (4ng, 2ng, 1ng, 0.5ng, 0.25ng, 0.125ng, 0.0625ng, and 0.0312ng) were amplified and analyzed in triplicate with each kit. The heterozygote peak balance, average peak height, and locus to locus balance were calculated for each sample. The heterozygote peak balance was calculated by dividing the smaller of the two peak heights by the larger. The average peak height was calculated by taking the average of the two heterozygote peak heights, or half of the homozygote peak height. The locus to locus balance was calculated by dividing the smaller of the two average peak heights by the larger. Locus to locus balance was calculated within each dye set. The total number of alleles called was also recorded for each profile.

For the Y-STR study three different genomic samples were used, male 1 (M1), male 2 (M2), and male 4 (M4). A range of input DNA from each of these samples (1ng, 0.5ng, 0.25ng, 0.125ng, 0.0625ng, 0.0312ng) were amplified and analyzed in triplicate. Heterozygote peak balance and locus to locus balance were calculated for each sample. The heterozygote peak balance was calculated for DYS395 in the same fashion as described above. The locus to locus balance was calculated by dividing the smaller of the two peak heights by the larger. For the DYS395 locus, the peak height used was the average of the peak heights of the two alleles present. Locus to locus balance was calculated within each dye set. The total number of alleles was also recorded for each profile.

Results:

STR Kits

Profiler Plus produced a full profile for at least one of the replicate down to 0.125ng for female 1 and male 2, and down to 0.25ng for male 4. A full profile was obtained in at least on replicate down to 0.125ng for female 1 and male 2, and 0.25ng for male 4 for Cofiler. Identifier was successful in producing a full profile in at least one replicate at 0.25ng for female 1 and male 2, and 0.50ng for male 4. Powerplex16 was able to obtain a full profile down to 0.0625ng for female 1 and male 2, while obtaining a full profile for male 4 at 0.125ng. Profiler Plus obtained a full profile for all replicates at 0.25ng for female 1, male 2, and male 4 (Tables 1,2,3 respectively). All replicates of 0.25ng for female 1 and male 4, and 0.125ng for male 2 obtained a full profile with the Cofiler multiplex (Tables 1, 3, and 2 respectively). Identifier obtained a full profile for all replicates for female 1, male 2, and male 4 at 0.5ng (Tables 1, 2, and 3 respectively). PowerPlex16 obtained full profiles in all replicates for female 1 at 0.0625ng, and male 2 and 4 at 0.125ng (Tables 1,2, and 3 respectively). The average peak height at 1ng (typical targeted amount of DNA) for Profiler Plus was 1080 RFU, Cofiler averaged 1357 RFU, Identifier averaged 478, while PowerPlex16 topped the charts with 4104 RFU (Table 4). PP16 had the highest peak height at all DNA concentrations. All multiplexes for each concentration had heterozygote peak height ratios greater than 60% (Table 5).

Profiler Plus had 5 out of the possible 19 alleles called for male 2 at an input amount of 0.0625ng of DNA with a 5 second injection (Figure 1), while Cofiler had 11 out of the possible 13 alleles called (Figure 2). Identifier had only 2 of the possible 28 alleles called (Figure 3), while PowerPlex16 was able to call 30 out of the possible 30 alleles called (Figure 4).

Y-STR kits:

Yfiler was able to obtain a full profile for at least on of the replicates down to 0.0625 ng for male 1 and male 2, and down to 0.125ng for male 4. PowerPlexY successfully produced a full profile down to 0.0312ng in at least one of the replicates for male 1, male 2, and male 4. Yfiler obtained a full profile in all replicates for male 1, male 2 and male 4 at 0.125ng (Tables 6, 7, and 8 respectively). PowerPlexY obtained a full profile in all replicates at 0.125ng for male 1, and 0.0625ng for male 2 and male 4 (Tables 6, 7, and 8 respectively). The average peak height at 1ng of input DNA was 2447 RFU for Yfiler, and was 6336 RFU for PowerPlexY (Table 9).

Yfiler was able to get 9 out of the possible 17 alleles called for male 1 at an input amount of 0.0312ng with a 5 second injection (Figure 5), while PowerPlexY was able to get 12 out of the possible 12 alleles called (Figure 6).

STR- Results

F1	Average Percentage of the Total Number of Alleles Called								
	Multiplex	4ng	2ng	1ng	0.5ng	0.25ng	0.125ng	0.0625ng	0.0312ng
Profiler Plus	100	100	100	100	100	90	48.8	0	
Cofiler	100	100	100	100	100	94.2	83.3	5.8	
Identifier	100	100	100	100	95.1	27	19.6	1.1	
PowerPlex16	100	100	100	100	100	100	100	78.8	

Table 1: The average percentage of the total number of alleles called for each multiplex at each of the DNA concentrations of female 1 DNA.

M2	Average Percentage of the Total Number of Alleles Called								
	Multiplex	4ng	2ng	1ng	0.5ng	0.25ng	0.125ng	0.0625ng	0.0312ng
Profiler Plus	100	100	100	100	100	66.8	24.7	22.6	
Cofiler	100	100	100	100	100	100	71.5	10	
Identifier	100	100	100	100	79	33.3	2.3	0	
PowerPlex16	100	100	100	100	100	100	97.7	82.3	

Table 2: The average percentage of the total number of alleles called for each multiplex at each of the DNA concentrations of male 2 DNA.

M4	Average Percentage of the Total Number of Alleles Called								
	Multiplex	4ng	2ng	1ng	0.5ng	0.25ng	0.125ng	0.0625ng	0.0312ng
Profiler Plus	100	100	100	100	100	37	11.7	5.9	
Cofiler	100	100	100	100	100	47.5	2.5	2.5	
Identifier	100	100	100	100	72.5	14.3	0	0	
PowerPlex16	100	100	100	100	100	100	89.2	70.3	

Table 3: The average percentage of the total number of alleles called for each multiplex at each of the DNA concentrations of male 4 DNA.

F1,M2,M4	Average RFU of Alleles Called								
	Multiplex	4ng	2ng	1ng	0.5ng	0.25ng	0.125ng	0.0625ng	0.0312ng
Profiler Plus	3,142.8	1,799.8	1,080.0	503.3	247.3	104.7	54.8	53.3	
Cofiler	3,294.3	1,759.1	1,357.2	685.3	276.3	175.0	77.3	50.5	
Identifier	1,558.5	896.8	477.9	250.3	102.6	53.6	46.6	41.0	
PowerPlex16	5,347.8	5,395.4	4,104.4	2,947.4	1,397.5	737.1	400.8	194.9	

Table 4: The average peak height, RFU, for all of the samples for each multiplex at each concentration of DNA.

Overall	Average Heterozygote Peak Balance								
	Multiplex	4ng	2ng	1ng	0.5ng	0.25ng	0.125ng	0.0625ng	0.0312ng
Profiler Plus	0.924	0.901	0.882	0.851	0.783	0.840	0.711	0.683	
Cofiler	0.921	0.907	0.885	0.800	0.770	0.762	0.694	-	
Identifier	0.920	0.918	0.871	0.842	0.819	0.858	-	-	
PowerPlex16	0.913	0.906	0.867	0.850	0.792	0.713	0.648	0.660	

Table 5: The average heterozygote peak balance for all of the DNA samples for each multiplex at each DNA concentration.

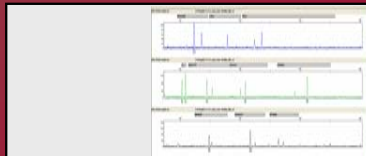


Figure 1: Profiler Plus 0.0625ng amplification of male 2 for 5 second injection.

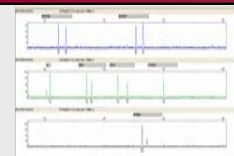


Figure 2: Cofiler 0.0625ng amplification of male 2 for 5 second injection.

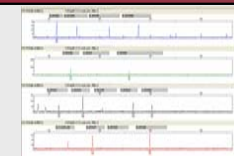


Figure 3: Identifier 0.0625ng amplification of male 2 for 5 second injection.

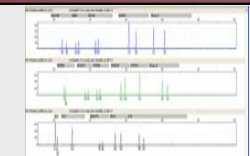


Figure 4: PowerPlex16 0.0625ng amplification of male 2 for 5 second injection.

Y-STR Results:

M1	Average Percentage of the Total Number of Alleles Called						
	Multiplex	1ng	0.5ng	0.25ng	0.125ng	0.0625ng	0.0312ng
Yfiler	100	100	100	100	82.4	41.2	
PowerPlexY	100	100	100	100	97.5	81.7	

Table 6: The average percentage of total number of alleles called for each Y-STR multiplex at each DNA concentration of male 1.

M2	Average Percentage of the Total Number of Alleles Called						
	Multiplex	1ng	0.5ng	0.25ng	0.125ng	0.0625ng	0.0312ng
Yfiler	100	100	100	100	92.3	64.7	
PowerPlexY	100	100	100	100	100	97.5	

Table 7: The average percentage of the total number of alleles called for each Y-STR multiplex at each DNA concentration of male 2.

M4	Average Percentage of the Total Number of Alleles Called						
	Multiplex	1ng	0.5ng	0.25ng	0.125ng	0.0625ng	0.0312ng
Yfiler	100	100	100	100	58.8	31.5	
PowerPlexY	100	100	100	100	100	90.9	

Table 8: The average percentage of the total number of alleles called for each Y-STR multiplex for each DNA concentration of male 4.

Overall	Average RFU of Alleles Called						
	Multiplex	1ng	0.5ng	0.25ng	0.125ng	0.0625ng	0.0312ng
Yfiler	2447.4	1081.4	566.7	279.3	149.9	109.4	
PowerPlexY	6936.4	5077.9	3590.5	1434.6	747.0	438.3	

Table 9: The average peak height, RFU, for each Y-STR multiplex for each DNA concentration of all samples.

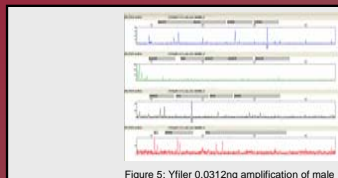


Figure 5: Yfiler 0.0312ng amplification of male 1.



Figure 6: PowerPlexY 0.0312ng amplification of male 1.

Conclusions: PowerPlex16 was on average twice the sensitivity as Profiler Plus and Cofiler, and four times as sensitive as Identifier. The average peak height showed that Profiler Plus and Cofiler were below our stochastic threshold at 0.125ng and Identifier was below our threshold at 0.25ng, while PowerPlex16 did not drop below the threshold until 0.0312ng. PowerPlexY was on average twice as sensitive as the Yfiler kit. The peak heights were also on average 4 times higher than those of Yfiler.