



The Evaluation of Eight Commercially Available STR Kits

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Abstract:

The National Forensic Science Technology Center (NFSTC) conducted a study to evaluate the performance of eight STR amplification kits: Applied Biosystems' AmpfSTR® Profiler Plus® ID kit, Cofiler® kit, Identifiler® kit, MiniFiler™ kit and the Yfiler® kit and Promega's PowerPlex® 16 system, PowerPlex® Y system and the PowerPlex® S5 system.

The performance of a single source serial dilution was assessed with each STR amplification kit based on a defined set of criteria:

- Sensitivity
- Heterozygosity
- Baseline noise
- Reproducible amplification artifacts

Heterozygosity and sensitivity from two person mixture series were compared to results obtained from single source samples.

Introduction:

There are various commercial STR multiplex kits available to the forensic science DNA community that are designed to address the ever-changing needs of crime laboratories. The primary goal of this study is to provide an overview of key performance measures of the eight STR kits tested.

Materials and Method:

Materials:

- Standards from 2 male donors
- Phenol:chloroform:isoamyl alcohol (25:24:1)
- TE Buffer, DTT and Proteinase K (10ng/ul)
- Applied Biosystems' Human DNA Quantifiler® Kit
- Applied Biosystems' AmpfSTR® Profiler Plus® kit, Cofiler® kit, Identifiler® kit, MiniFiler™ kit and the Yfiler®
- Promega's PowerPlex® 16 system, PowerPlex® Y system and the PowerPlex® S5 system
- Running Buffer, 10X
- 16 capillary array, 36cm
- POP-4™ polymer for 3130x/
- Matrix standards
- Internal Lane Size Standards
- Hi-D™ Formamide
- 96-Well GeneAmp® PCR System 9700
- 7500 Real-Time PCR System
- 3130x/ Genetic Analyzer

Method:

- Two separate known human male DNA standards were prepared utilizing a standard organic extraction method in conjunction with the Millipore Microcon® 100 centrifugal filter device.
- The samples were serially diluted to yield the following target concentrations: 1.0, 0.5, 0.25, 0.125, 0.03125, 0.015625, and 0.0078 ng.
- The two standards were systematically combined to create the following mixture ratios: 1:20, 1:15, 1:12, 1:10, 1:8, and 1:5. The targeted concentrations varied per kit and were based on the performance of the dilution series. (Profiler Plus®, Cofiler®, Identifiler® targeted 1 ng. PowerPlex® 16, PowerPlex® S5, PowerPlex® Y and Yfiler® targeted 0.5ng. MiniFiler™ target was 0.25ng.)
- The samples were quantitated using the Applied Biosystems Quantifiler® Human Quantification Kit on an Applied Biosystems 7500 Real-Time PCR System. The results were normalized with NIST quantitation standards.
- Samples were amplified on an Applied Biosystems GeneAmp® PCR 9700 thermal cycler following manufacturer's specifications. Applied Biosystems' AmpfSTR® Profiler Plus® kit, Cofiler® kit, Identifiler® kit, MiniFiler™ kit,

and the Yfiler® kit and Promega's PowerPlex® 16 system, PowerPlex® Y system, and the PowerPlex® S5 system.

- The samples were then separated and detected using an Applied Biosystems 3130x/ Genetic Analyzer using manufacturer's recommended conditions:
 - Applied Biosystems kits: 3kv, 10 sec injections, 8.7 µl Formamide, 0.3 µl GS 500, 1 µl of sample.
 - Promega kits: 3kv, 10 sec injections, 9.5 µl Formamide, 0.5 µl ILS 600, 1 µl of sample

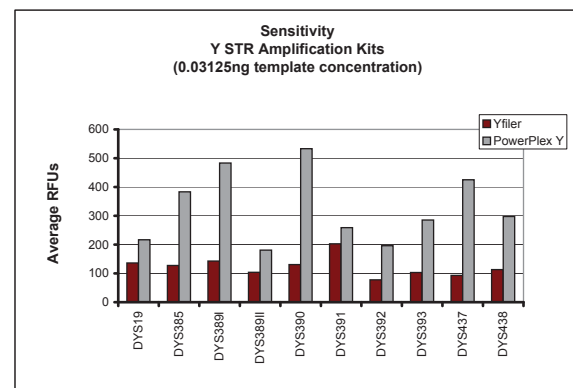
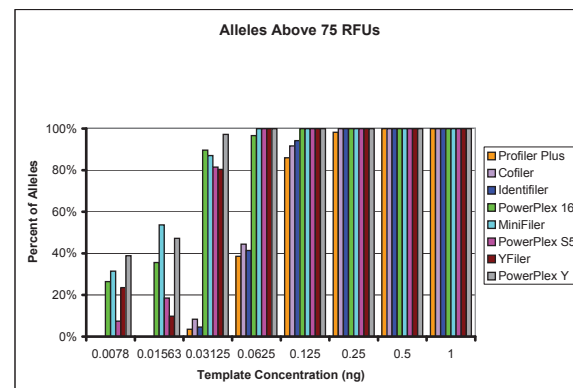
— Promega kits: 3kv, 10 sec injections, 9.5 µl Formamide, 0.5 µl ILS 600, 1 µl of sample

- Data was analyzed using GeneMapper® ID Software v3.2.

Results and Discussion:

Sensitivity:

- Concentrations at which alleles fell below 75 RFUs for each kit are depicted in table 1.
- MiniFiler™ and PowerPlex® 16 produced the highest RFUs for each concentration.
- Identifiler®, Profiler Plus®, and Cofiler® exhibited comparable peak heights to each other for each concentration.
- Profiler Plus® displayed an RFU value less than 75 at 0.25 ng concentration at D7S820 in one injection of the triplicate data set.
 - Note: Profiler Plus® and Cofiler® amplifications were performed with manufacturer's recommended 50 µl reaction.
- The RFU range was higher for PowerPlex® Y than Yfiler® at each concentration.



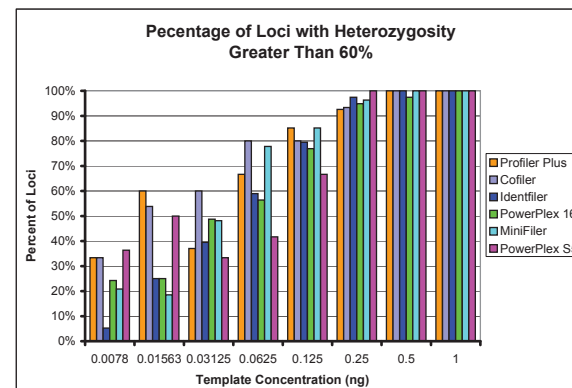
Heterozygosity:

- As sample concentration decreases, the number of loci with heterozygosity greater than 60% also decreases.
- At concentrations of 0.5 ng and below, PowerPlex® 16 did not maintain a minimum heterozygosity of 60% at D8S1179

— 0.5 ng corresponds with an average RFU of 2200

- Note: this occurred once in the triplicate injection

- At concentrations of 0.25 ng and lower, Profiler Plus®, Cofiler®, Identifiler®, MiniFiler™ did not maintain minimum of 60% heterozygosity.
 - 0.25 ng concentration corresponds with the following peak height ranges:
 - Profiler Plus® - 138 to 273 RFUs
 - Cofiler® - 210 to 350 RFUs
 - Identifiler® - 255 to 445 RFUs
 - MiniFiler™ - 1870 to 3250 RFUs
- At concentrations of 0.125 ng and lower, PowerPlex® S5 displays loss of 60% heterozygosity.
 - 0.125 ng corresponds with the RFU range 228 to 753



Amplification Artifacts:

- Several dye blobs were present in the MiniFiler™, Yfiler®, Identifiler®, PowerPlex® 16, PowerPlex® Y, Profiler Plus® kits.
- Some instances of elevated stutter were observed for Profiler Plus®, Identifiler®, PowerPlex® 16.
- No elevated stutter was observed with Cofiler®, MiniFiler™, PowerPlex® S5.
- Indications of plus stutter were observed in several kits and in some instances were greater than 75 RFUs.
- Minus A was observed in several kits, most notably in PowerPlex® S5.

Baseline Noise:

- All amplification kits displayed low background noise with the limit of detection (LOD) ranging from 10 to 15 RFUs.

$$LOD = \bar{X}_{blank} + 3\sigma_{blank}$$

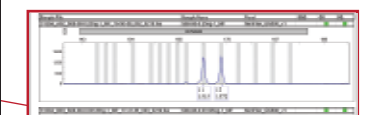
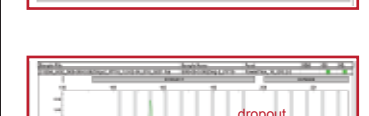
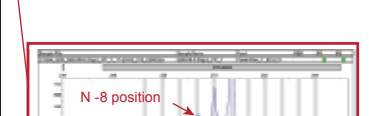
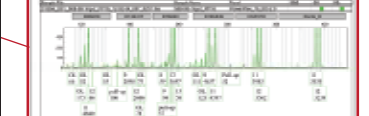
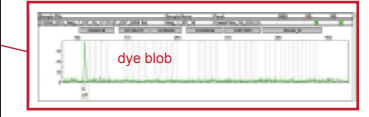
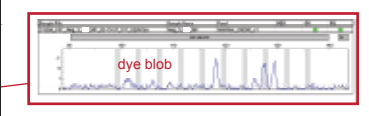
- The limit of quantitation (LOQ) ranging from 24 to 36 RFUs for all kits.

$$LOQ = \bar{X}_{blank} + 10\sigma_{blank}$$

- In general, PowerPlex® 16 displayed a higher level of noise than the other kits tested.

TABLE 1

	Profiler Plus®	Cofiler®	Identifiler®	PowerPlex®16	PowerPlex®S5	MiniFiler™	Yfiler®	PowerPlex® Y
Manufacturer's suggested reaction volume	50µl	50µl	25µl	25µl	25µl	25µl	25µl	25µl
Artifacts	109 bp and 221 bp (blue), 113 bp and 213 bp (green), 162 bp (yellow), 198 bp (red)	135 bp (Green)	119 bp (blue), 113 bp (green), 162 bp (yellow), 198 bp (red)	116 bp (green), 247 bp (yellow)	not observed	117 bp and doublet at 1271/129 bp (blue), 104 bp and 118-120 bp (green), 77 and 106 bp (yellow)	95 bp (yellow), 200 and 213 bp (red)	114 bp (green), 156 bp (yellow)
Optimal range	0.5 ng - 1.0 ng	0.5 ng - 1.0 ng	0.5 ng - 1.0 ng	0.125ng to 0.5 ng	0.125ng to 0.5 ng	0.125ng to 0.5 ng	0.125ng to 0.5 ng	0.125ng to 0.5 ng
Elevated Stutter (N-4)	not observed	not observed	vWA	Excess noise, offscale/overloaded	not observed	Excess noise, offscale/overloaded	Excess noise, offscale/overloaded	Excess noise, offscale/overloaded
(N-4) - Below 75 RFUs	FGA, D18	not observed	D18	Excess noise, offscale/overloaded	D18, FGA	Excess noise, offscale/overloaded	Excess noise, offscale/overloaded	Excess noise, offscale/overloaded
(N-4) - Above 75 RFUs	not observed	not observed	not observed	Excess noise, offscale/overloaded	FGA	Excess noise, offscale/overloaded	DYS456, DYS392 (n=3)	Excess noise, offscale/overloaded
Minus (-) A	not observed	slight -A observed @ Amel, not callable	not observed	Excess noise, offscale/overloaded	(-) A present, TH01, FGA, Filtered out	not observed	not observed	Excess noise, offscale/overloaded
Percentage of loci that maintain 60% heterozygosity	100%	100%	100%	100%	100%	100%	N/A	N/A
RFU range	629-2195	729-2172	743-2855	2030 - offscale	3371-6640	5192-offscale	2886-offscale	4762-offscale
Percentage of alleles above 75 RFUs	100%	100%	100%	100%	100%	100%	100%	100%
Elevated Stutter (N-4)	not observed	not observed	vWA	vWA	not observed	not observed	DYS456 (N-8) present at DYS389 II, Filtered out	not observed
(N-4) - Below 75 RFUs	D18	not observed	D18	Excess noise	D8, FGA	Excess noise	DYS456, DYS389II, DYS393	DYS390, DYS393, DYS389II, DYS392
(N-4) - Above 75 RFUs	not observed	not observed	not observed	not observed	D18	D18	DYS456, DYS392 (n=3)	DYS390, DYS393, DYS389II, DYS392
Minus (-) A	not observed	slight -A observed @ Amel, not callable	slight -A observed @ Amel, not callable	slight -A observed @ Amel, not callable	(-) A present, TH01, FGA, Filtered out	not observed	not observed	not observed
Percentage of loci that maintain 60% heterozygosity	100%	100%	100%	100%	100%	100%	N/A	N/A
RFU range	306-1014	333-1017	295-1503	1441-5778	1314-3120	1696-3390	1314-1468	2431-1792
Percentage of alleles above 75 RFUs	100%	100%	100%	100%	100%	100%	100%	100%
Elevated Stutter (N-4)	vWA (below 75 RFUs)	not observed	D19 (below 75 RFUs)	vWA	not observed	not observed	DYS456 (N-8) present at DYS389 II, Filtered out	not observed
(N-4) - Below 75 RFUs	not observed	not observed	not observed	not observed	D7, D18, CSF, D16, D18, D8, FGA	D18, D21	DYS456, DYS389II, DYS393	DYS390, DYS393, DYS389II, DYS392
(N-4) - Above 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	DYS456	DYS390, DYS393, DYS389II, DYS392
Minus (-) A	not observed	slight -A observed @ Amel, not callable	slight -A observed @ Amel, not callable	slight -A observed @ TH01, not callable	(-) A present, TH01, FGA, Filtered out	not observed	not observed	not observed
Percentage of loci that maintain 60% heterozygosity	93%	95%	88%	87%	100%	99%	N/A	N/A
RFU range	154-641	153-617	122-767	122-3602	663-3685	1287-3391	642-3817	1156-5619
Percentage of alleles above 75 RFUs	98%	100%	100%	100%	100%	100%	100%	100%
Elevated Stutter (N-4)	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
(N-4) - Below 75 RFUs	not observed	not observed	not observed	not observed	D18, FGA	not observed	DYS392 (n=3)	DYS392 (n=3), DYS439
(N-4) - Above 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
Minus (-) A	not observed	slight -A observed @ Amel, not callable	slight -A observed @ Amel, not callable	slight -A observed @ TH01, not callable	(-) A present, TH01, FGA, Filtered out	not observed	not observed	not observed
Percentage of loci that maintain 60% heterozygosity	85%	80%	79%	77%	87%	85%	N/A	N/A
RFU range	52-205	76-339	51-306	116-2773	262-763	395-1348	239-1363	451-2183
Percentage of alleles above 75 RFUs	88%	92%	94%	100%	100%	100%	100%	100%
Elevated Stutter (N-4)	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
(N-4) - Below 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
(N-4) - Above 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
Minus (-) A	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
Percentage of loci that maintain 60% heterozygosity	67%	80%	59%	86%	42%	76%	N/A	N/A
RFU range	110-195	80-192	50-208	62-1499	103-559	178-1241	89-355	1367-1157
Percentage of alleles above 75 RFUs	39%	44%	41%	97%	100%	100%	100%	100%
Elevated Stutter (N-4)	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
(N-4) - Below 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
(N-4) - Above 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
Minus (-) A	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
Percentage of loci that maintain 60% heterozygosity	37%	60%	39%	49%	33%	48%	N/A	N/A
RFU range	0-104	0-124	0-106	0-871	29-276	0-583	49-364	0-867
Percentage of alleles above 75 RFUs	4%	8%	5%	80%	97%	81%	97%	47%
Elevated Stutter (N-4)	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
(N-4) - Below 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
(N-4) - Above 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
Minus (-) A	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
Percentage of loci that maintain 60% heterozygosity	60%	54%	25%	25%	50%	19%	N/A	N/A
RFU range	0-43	0-67	0-61	0-356	0-160	0-361	0-203	0-633
Percentage of alleles above 75 RFUs	0%	0%	0%	3%	19%	54%	10%	47%
Elevated Stutter (N-4)	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
(N-4) - Below 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
(N-4) - Above 75 RFUs	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
Minus (-) A	not observed	not observed	not observed	not observed	not observed	not observed	not observed	not observed
Percentage of loci that maintain 60% heterozygosity	33%	33%	6%	24%	36%	21%	N/A	N/A
RFU range	0-24	0-30	0-39	0-312	0-171	0-179	0-213	0-292
Percentage of alleles above 75 RFUs	0%	0%	0%	26%	7%	31%	24%	39%
Note:	Percentage of loci that maintain 60% heterozygosity.		Calculated using the # of loci above 60% total # of loci with callable heterozygotes. Includes alleles below 75 RFUs. The total # of loci is adjusted for the lower concentration and the loci with complete dropout are not included in the total count in the triplicate data sets.					
	1st concentration to fall below 75 RFUs				1st concentration to fall below 60% heterozygosity			

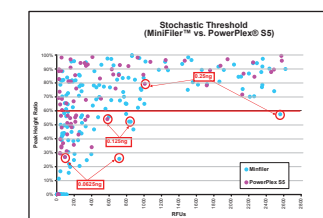
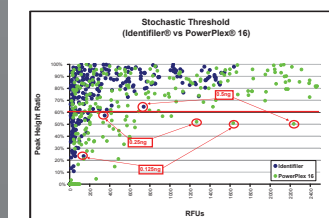


Mixtures:

Mixture ratio	Percentage of Minor Contributor Alleles Above 75 RFUs							
	Profiler Plus®	Cofiler®	Identifiler®	PowerPlex® 16	PowerPlex® S5	MiniFiler™	Yfiler®	PowerPlex® Y
1:5	100%	100%	100%	100%	100%	100%	100%	83%
1:8	100%	100%	100%	88%	100%	75%	87%	77%
1:10	82%	100%	85%	77%	100%	73%	83%	67%
1:12	59%	92%	74%	79%	94%	73%	87%	56%
1:15	54%	42%	68%	73%	94%	46%	78%	38%
1:20	19%	78%	58%	80%	81%	45%	69%	44%

Conclusions:

- In general, the concentration at which heterozygosity fell below 60% was always higher than the concentration for which peak heights fell below 75 RFUs.
- PowerPlex® 16 appeared to be more sensitive than Identifiler®, Profiler Plus® and Cofiler®, however it displayed stochastic effects at higher RFU values/concentrations when compared to these kits.
- MiniFiler™ appeared to be more sensitive than PowerPlex® S5 however it displayed stochastic effects at higher RFU values/concentrations.
- Dropout (where a heterozygote appears as a homozygote) was observed with MiniFiler™ and PowerPlex® 16 with approximate peak heights at 200 and 500 RFUs, respectively.
- Yfiler® and PowerPlex® Y perform similarly. Each kit had alleles that were below or less than 75 RFUs at 0.03125 ng.
- Laboratories should perform appropriate validation studies in order to establish interpretation guidelines which should include assessment of LOD, LOQ and stochastic threshold for each amplification kit and instrument.
- The mixture series performed as expected when compared to single source samples at comparable concentrations.



References:

Internal Validation of STR Systems Reference Manual. www.promega.com part # GE053, revised 9/06.

The most current user and technical manuals for each kit were referenced in this study. Manuals procured from www.appliedbiosystems.com (published 1998-2006) and www.promega.com/bs/ (published 2008.)

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