

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.

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, 0.0625, 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 0.5 ng. MiniFiler[™] target was 0.25 ng.)
- 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' AmpflSTR[®] 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 samples were then separated and detected using an Applied Biosystems 3130 xI Genetic Analyzer using manufacture'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.
- Data was analyzed using GeneMapper[®] ID Software v3.2.

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Results and Discussion:

Sensitivity:

- Concentrations at which alleles fell below 75 RFUs for each kit are depicted in Fig. 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 manufacture's recommended 50 µl reaction.
- The RFU range was higher for PowerPlex® Y than Yfiler® at each concentration. (Fig. 2)

Fig. 1

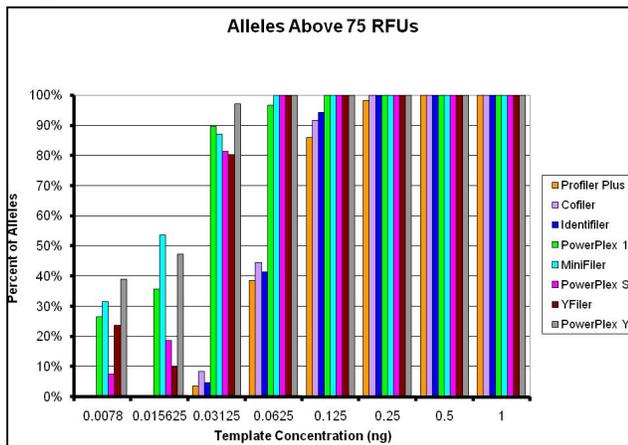
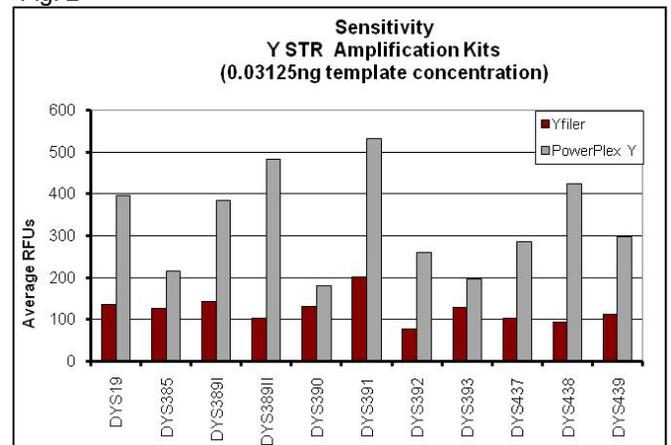


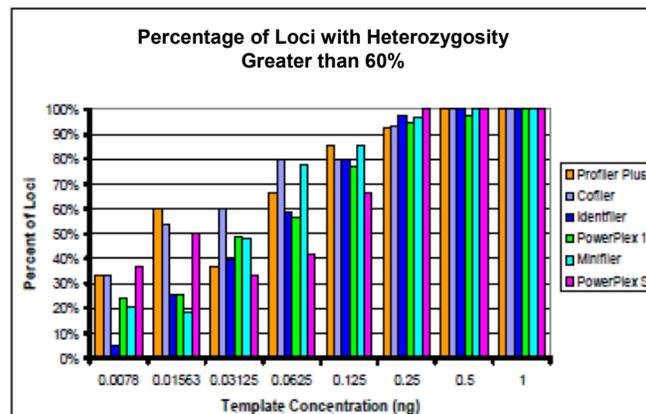
Fig. 2

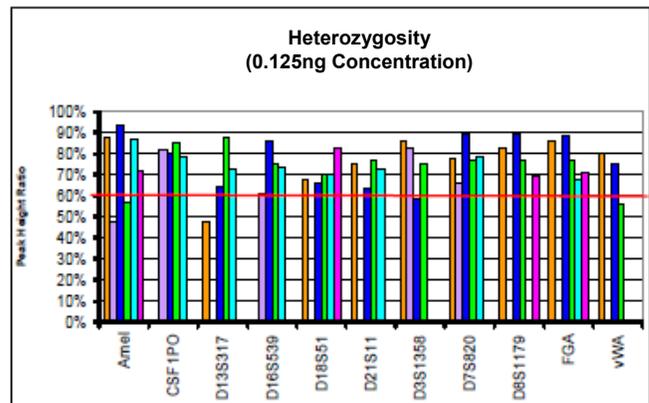
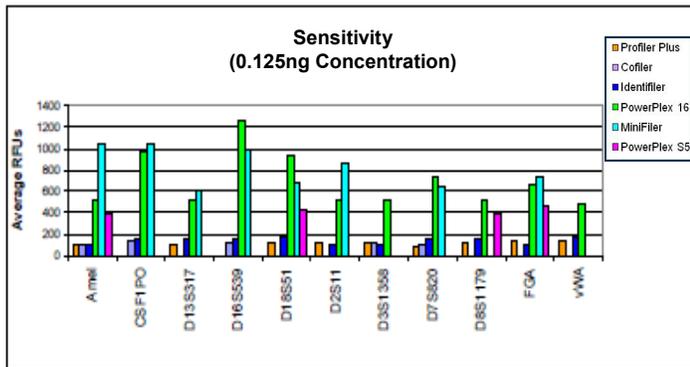


Heterozygosity:

- 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® and MiniFiler™ did not maintain a minimum heterozygosity of 60% .
 - 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®, Identifier®, PowerPlex® 16, PowerPlex® Y and Profiler Plus® kits. (Fig 3)
- Some instances of elevated stutter were observed for Profiler Plus®, Identifier® and PowerPlex® 16.
- No elevated stutter was observed with Cofiler®, MiniFiler™ or 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.
- Complete dropout occurred in PowerPlex® 16 and MiniFiler™ at 500 RFUs and 200 RFUs, respectively. (Fig 4)

Fig. 3

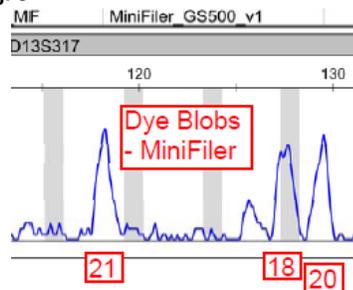
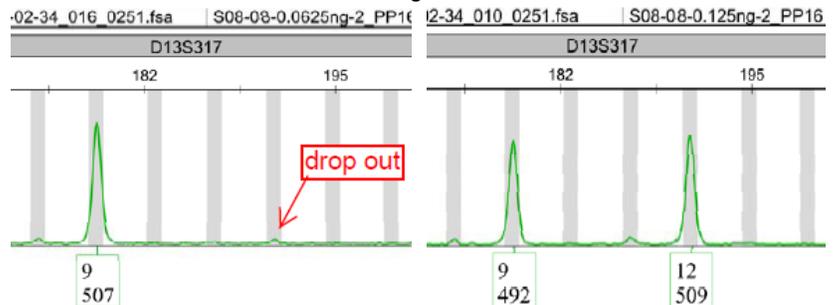


Fig. 4



Baseline noise:

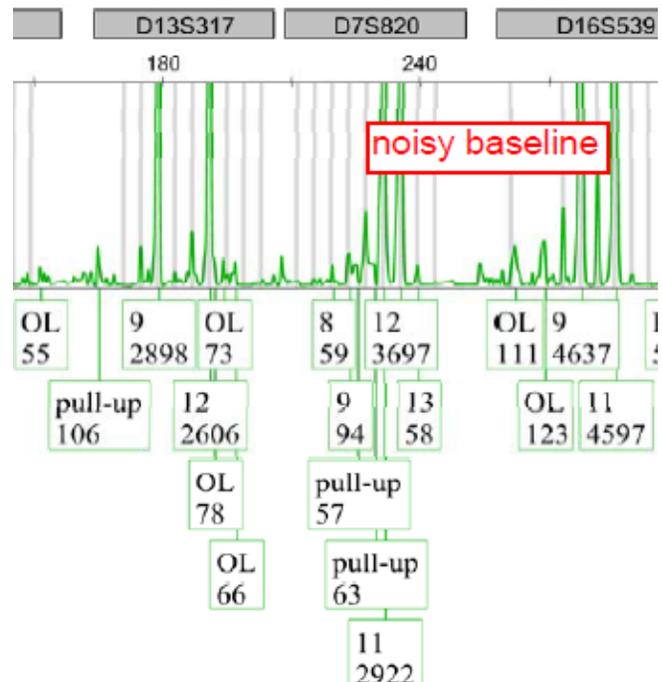
- All amplification kits displayed low background noise with the limit of detection (LOD) ranging from 10 to 15 RFUs.

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

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

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

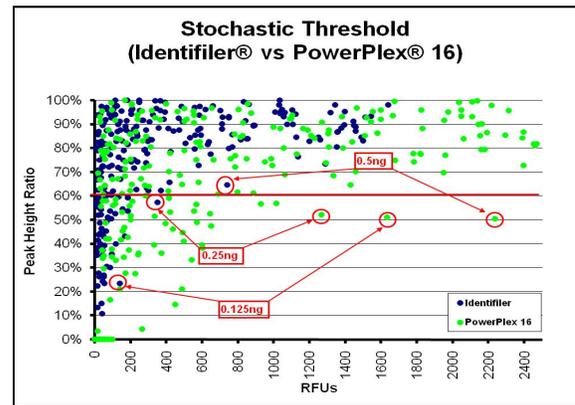
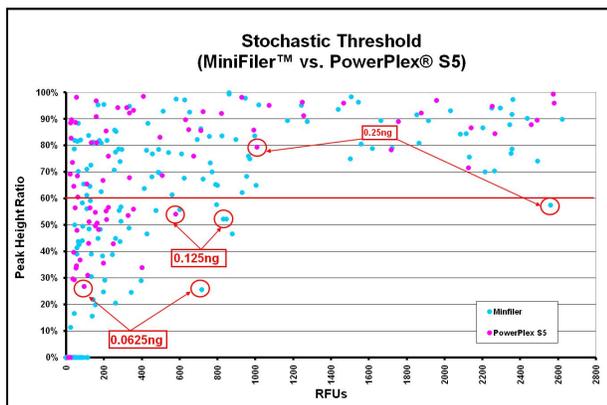
- In general PowerPlex® 16 displayed a higher level of noise than the other kits tested.



Mixtures:

	Profiler Plus®	Cofiler®	Identifiler®	PowerPlex® 16	PowerPlex® S5	MiniFiler™	Yfiler®	PowerPlex® Y
	Percentage of Minor Contributor Alleles Above 75 RFUs							
Mixture ratio								
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%	93%	67%
1:12	59%	92%	74%	79%	94%	73%	87%	56%
1:15	54%	42%	68%	73%	94%	48%	78%	38%
1:20	18%	75%	55%	50%	81%	45%	69%	44%

Stochastic Threshold:



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 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/tbs/ (published 2008.)