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Forensic Technology Testing & Evaluation Report Form

Project Information

Title: The Evaluation of Eight Commercially Available STR Kits
Evaluation Type: STR Amplification Kits
Stakeholder(s): NFSTC
Start Date: 09/15/08
End Date: 01/30/09
Report Date: 02/04/09

Evaluation Overview

A number of commercially available short tandem repeat (STR) amplification kits are available for use in forensic DNA laboratories. While it is the responsibility of each laboratory system to evaluate and choose the analytical methods that best suit its needs, it is important that forensic DNA analysts have a general understanding of the performance of commonly used STR amplification kits. In an effort to assist in this process, the National Forensic Science Technology Center (NFSTC) conducted a study to evaluate the performance of eight STR amplification kits: Applied Biosystems' AmpfℓSTR® Profiler Plus ID® kit, Cofiler® kit, Identifiler® kit, MiniFiler™ kit and Yfiler® kit, and Promega's PowerPlex® 16 system, PowerPlex® Y system and PowerPlex® S5 system.

The performance of each STR amplification kit was assessed based on a defined set of criteria: sensitivity, peak ratios at heterozygous loci, baseline noise, stutter ratio and amplification artifacts. These criteria were determined through the analysis of single-source human DNA samples. A mixture series was prepared and analyzed to assess the percent contribution necessary to detect a minor contributor in a two donor mixture for each STR amplification kit.

Two separate known human DNA standards were prepared utilizing a standard organic extraction method in conjunction with the Millipore Microcon® 100 centrifugal filter device. The samples were quantitated using the Applied Biosystems Quantifiler® Human and Y Human Male DNA Quantification Kits on an Applied Biosystems 7500 Real-Time PCR System. To minimize variation, a large volume (1000 µl) of each sample was prepared and used for the dilution series and the mixture series. Samples were amplified on an Applied Biosystems GeneAmp® PCR 9700 thermal cycler following manufacturer's specifications. The samples were then separated and detected using an Applied Biosystems 3130*xl* Genetic Analyzer and the data was analyzed using GeneMapper® ID Software v3.2 using a threshold of 75 RFU.

A serial dilution was performed on a known human DNA sample to yield target concentrations of 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.0078 ng. The data obtained from each of these samples was used to assess the sensitivity and peak ratios at heterozygous loci for each of the eight STR amplification kits. In addition, the observation of any reproducible amplification artifact(s) in these data was noted. The baseline noise was assessed by evaluating the data from ten injections of amplification negative controls for each of the eight STR amplification kits.

A two-donor mixture experiment was performed to evaluate the percent contribution necessary to detect a minor contributor for all eight STR multiplexes. Two separate known human DNA standards were systematically combined to create the following mixture ratios: 1:20, 1:15, 1:12, 1:10, 1:8 and 1:5. The DNA profile from the minor contributor was evaluated at a 75 RFU threshold and the peak ratios at heterozygous loci was calculated and noted at all loci where the alleles were 75 RFU or higher.

There are various commercially available STR multiplex kits available to the forensic science DNA community that are designed to address the ever changing needs of crime laboratories. A primary goal of this study is to provide an overview of key performance measures of these eight commercially available STR kits. The demand on forensic DNA laboratories to employ methods that meet their system's needs is a continual challenge and is compelling DNA technical leaders and laboratory management to acquire relevant information that will aid in making these crucial decisions.

Evaluation Team

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Product Specifications

Brief Description: STR amplification kits

Product Uses: HID

Storage Conditions: Refrigerator/Freezer

Number of Reactions:

- Applied Biosystems AmpfℓSTR® Profiler Plus ID® kit – 200 reactions
- Applied Biosystems AmpfℓSTR® Cofiler® kit – 200 reactions
- Applied Biosystems AmpfℓSTR® Identifiler® kit – 200 reactions
- Applied Biosystems AmpfℓSTR® MiniFiler™ kit – 100 reactions
- Applied Biosystems AmpfℓSTR® Yfiler® kit – 100 reactions
- Promega PowerPlex® 16 system –100 or 400 reactions
- Promega PowerPlex® Y system –50 or 200 reactions
- Promega PowerPlex® S5 system –100 or 400 reactions

Detection System: Applied Biosystems 3130*xl* Genetic Analyzer

Analysis Software: GeneMapper ID ver. 3.2

Evaluation

Standards, Controls and Samples Used in Evaluation

- NIST Quantitation standards

- Dilution series of male DNA
- Mixture series of two male donors

Synopsis of Experiment(s)

To evaluate the performance of eight commercially available kits using 3130*xl* to evaluate different performance measures.

Reagents and Consumables:

- Buccal swabs from 2 donors
- Phenol:chloroform:Isoamyl alcohol (25:24:1)
- Proteinase K (10 ng/ul)
- NIST Human quantitation standards
- Applied Biosystems Human DNA Quantifiler Kit
- Applied Biosystems Human Male Y DNA Quantifiler Kit
- TE Buffer
- 16 capillary array
- POP4
- Applied Biosystems' AmpfℓSTR® Profiler PlusID® kit, Cofiler® kit, Identifiler® kit, MiniFiler™ kit and Yfiler® kit
- Promega's PowerPlex® 16 system, PowerPlex® Y system and PowerPlex® S5 system
- 10x Genetic Analyzer Buffer
- Dye Set G5 Spectral/ matrix standards
- Dye Set F Spectral/ matrix standards
- PowerPlex® matrix standards
- Internal lane size standards
- HiDi™ formamide

Equipment:

- AB 7500 RT PCR
- AB 9700 Thermal Cylcer
- AB 3130*xl* Genetic Analyzer

Experimental Design:

1. Collected standards from two male individuals.

2. Performed organic extraction in conjunction with Millipore Microcon 100 centrifugal filter device.

3. Quantified using Quantifiler Human and Quantifiler Human Y Kits on the AB 7500 RT PCR instrument and normalized the results with NIST quantitation standards.

4. Combined and diluted samples to generate a large volume of at least 1 ml to obtain the following target concentrations for amplification.

    - Single source dilutions
        - 1.0 ng
        - 0.50 ng
        - 0.25 ng
        - 0.125 ng
        - 0.0625 ng
        - 0.03125 ng
        - 0.015625 ng
        - 0.0078125 ng

    - Mixture of two males to include the following ratios:
        - 1:20
        - 1:15
        - 1:12
        - 1:10
        - 1:8
        - 1:5

5. Quantified after dilutions using Quantifiler Human Kit on the AB 7500 RT PCR instrument and normalized the results with the NIST quantitation standards.

6. Amplified the appropriate amount of each sample with each of the following kits: Applied Biosystems' AmpfℓSTR® Profiler Plus ID® kit, Cofiler® kit, Identifiler® kit, MiniFiler™ kit and Yfiler® kit, and Promega's PowerPlex® 16 system, PowerPlex® Y system, and the PowerPlex® S5 system using manufacturers' recommended amplification conditions.

    - Profiler/ Cofiler (50 µl reaction) cycling parameters: 95ºC hold (11 min); 28 cycles 94ºC (1 min), 59ºC (1 min) and 72ºC (1 min); and a 60ºC (45 min) final extension.

    - Identifiler (25 µl reaction) cycling parameters: 95ºC hold (11 min); 28 cycles 94ºC (1 min), 59ºC (1 min) and 72ºC (1 min); and a 60ºC (60 min) final extension.

    - MiniFiler (25 µl reaction) cycling parameters: 95ºC hold (11 min); 30 cycles 94ºC (20 sec), 59ºC (2 min) and 72ºC (1 min); and a 60ºC (45 min) final extension

    - YFiler (25 µl reaction) cycling parameters: 95ºC hold (11 min); 30 cycles 94ºC (1 min), 61ºC (12 min) and 72ºC (1 min); and a 60ºC (80 min) final extension.

- PowerPlex 16 (25 µl reaction) cycling parameters: 95ºC hold (11 min); 96ºC hold (1 min); 10 cycles ramp 100% to 94ºC (30 sec), ramp 29% to 60ºC (30 sec) and ramp 23% to 70ºC (45 sec); 22 cycles ramp 100% to 90ºC (30 sec), ramp 29% to 60ºC (30 sec) and ramp 23% to 70ºC (45 sec); and a 60ºC (30 min) final extension.

- PowerPlex Y (25 µl reaction) cycling parameters: 95ºC hold (11 min); 96ºC hold (1 min); 10 cycles ramp 100% to 94ºC (30 sec), ramp 29% to 60ºC (30 sec) and ramp 23% to 70ºC (45 sec); 22 cycles ramp 100% to 90ºC (30 sec), ramp 29% to 58ºC (30 sec) and ramp 23% to 70ºC (45 sec); and a 60ºC (30 min) final extension.

- PowerPlex S5 (25 µl reaction) cycling parameters: 96ºC hold (2 min); 30 cycles 94ºC (30 sec), 60ºC (2 min) and 72ºC (90 sec); and a 60ºC (45 min) final extension.

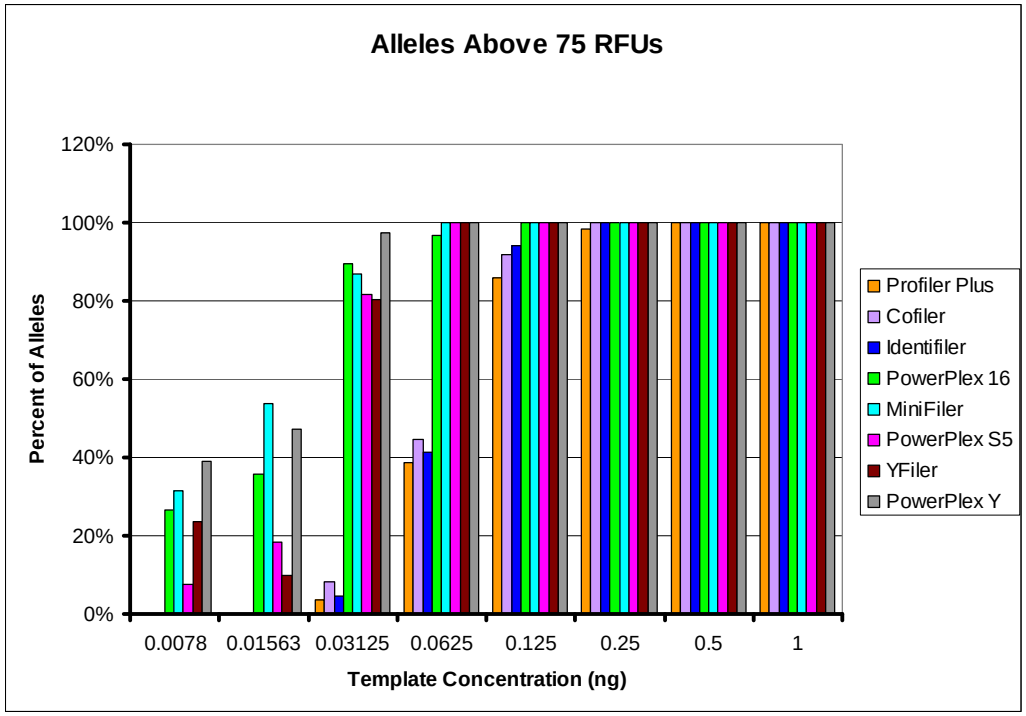
7. Prepared and ran samples on 3130*xl* using manufacturer's recommended running conditions.
    - Applied Biosystems kits: 3 kv, 10 sec injections, 8.7 µl Form, 0.3 µl GS 500, 1 µl sample
    - Promega kits: 3 kv, 10 sec injections, 9.5 µl Form, 0.5 µl ILS 600, 1 µl sample
8. Analyzed data using Genemapper ID v 3.2. Evaluate and tabulate data for a defined set of criteria, to include: sensitivity, peak height ratios at heterozygous loci, baseline noise, increased stutter and amplification artifacts

Findings

Sensitivity :

- Concentrations at which alleles fell below 75 RFUs for each kit are depicted in Graph 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 0.5 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.
        - Each kit had alleles that were below or less than 75RFUs at 0.03125 ng.

Graph 1



Heterozygosity:

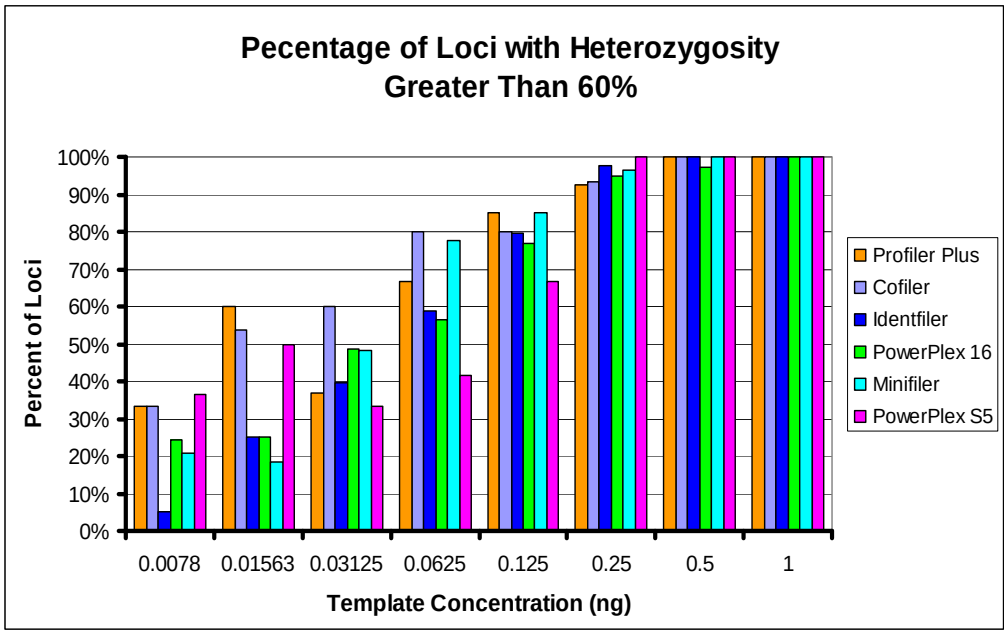
- As sample concentration decreases, the number of loci with heterozygosity greater than 60% also decreases. (Graph 2)
- At concentrations of 0.5 ng and below, PowerPlex® 16 did not maintain a minimum heterozygosity of 60% at D8S1179
    - 0.5 ng corresponds with an average RFU of 2200
        - Note: this occurred once in the triplicate injection

- At concentrations of 0.25 ng and lower, Profiler Plus®, Cofiler®, Identifiler® and MiniFiler™ did not maintain 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

Graph 2



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 was 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).

Baseline Noise:

- All amplification kits displayed low background noise with the limit of detection (LOD) ranged from 10 to 15 RFUs.
- The limit of quantitation (LOQ) ranged from 24 to 36 RFUs for all kits.
- In general PowerPlex® 16 displayed a higher level of noise than the other kits tested.

Mixtures:

- The mixture series performed as expected, when compared to single source samples at comparable concentrations.
- The mixture with a 1:5 ratio yielded a full profile for the minor donor for all amplification kits except PowerPlex® Y.
- Cofiler® and PowerPlex® S5 are the only two kits that displayed a full profile for the minor donor down to 1:8 ratio.
- A full profile for the minor donor was expected but not obtained for most kits at the 1:10 ratio.

Stochastic Threshold:

- A different stochastic threshold should be evaluated for each amplification kit.
- Cofiler®, Identifiler® and PowerPlex® S5 displayed less stochastic effects when compared to kits of similar size.
- As sample concentration decreases, the number of loci with heterozygosity greater than 60% also decreases.
- At the concentration of 0.25 ng, most kits displayed data points below 60% for heterozygosity.
- PowerPlex® 16 and MiniFiler® displayed stochastic effects at higher RFUs than the other amplification kits.

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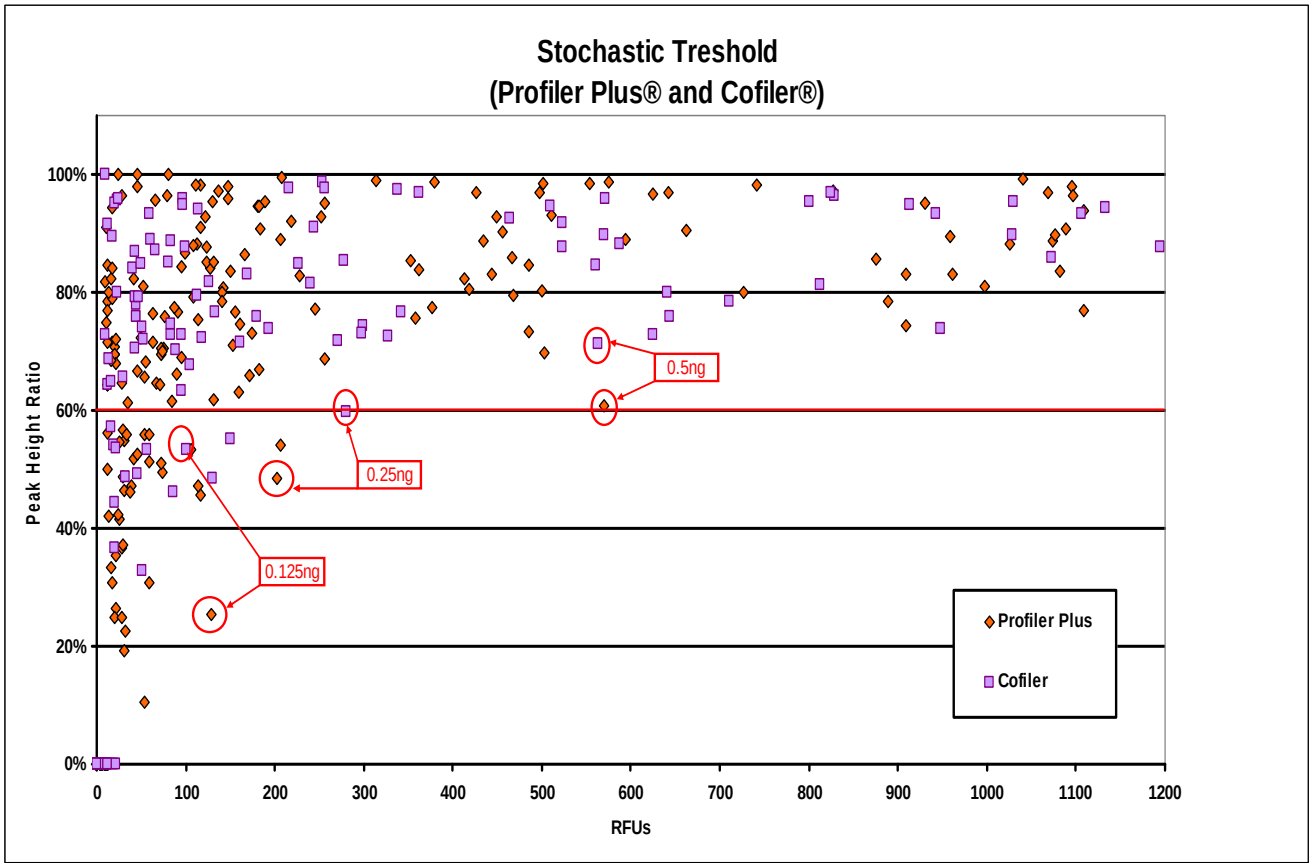
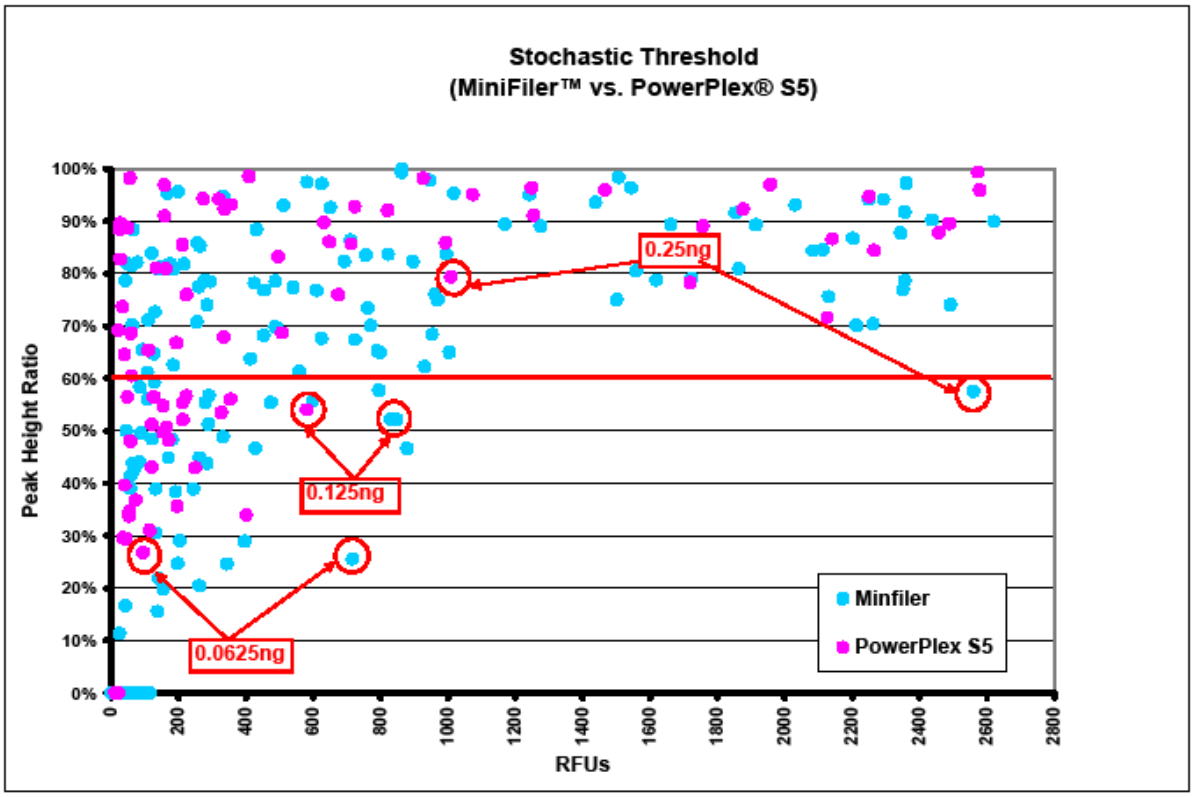
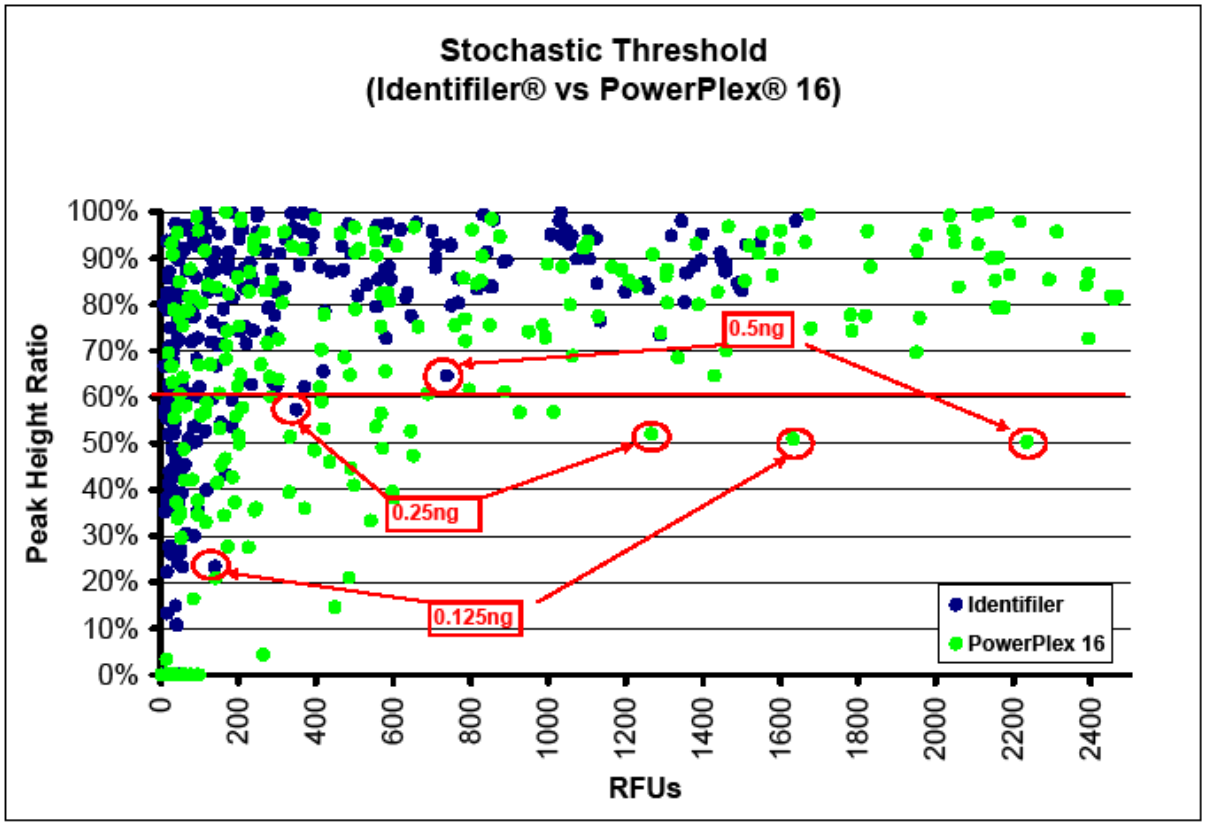



Stochastic Threshold
(MiniFiler™ vs. PowerPlex® S5)
Peak Height Ratio
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
RFUs
0.25ng
0.125ng
0.0625ng
Minfiler
PowerPlex S5



Strengths:

- PowerPlex® 16 exhibited greater sensitivity than Identifiler®, Profiler Plus® and Cofiler®.
- MiniFiler™ exhibited greater sensitivity than PowerPlex® S5.
- Identifiler®, Profiler Plus®, and Cofiler® demonstrated comparable peak heights to each other for each concentration.
- All of the amplification kits, with the exception of PowerPlex® 16, maintain a 60% heterozygosity ratio for 1 ng and 0.5 ng concentrations.
- Identifiler® maintained heterozygosity ratios of 60% more frequently than PowerPlex® 16 for concentrations from 0.0625 ng to 1 ng.

- Profiler Plus® and Cofiler® maintained heterozygosity ratios more frequently than Identifiler®, for the lower concentrations in the dilution series (0.078 ng to 0.125 ng).
- PowerPlex® Y displayed higher RFU range than Yfiler® at each concentration.
- Cofiler®, Identifiler® and PowerPlex® S5 displayed less stochastic effects when compared to kits of similar size.
- The mixture sample with a 1:5 ratio yielded a full profile for the minor donor for all amplification kits except PowerPlex® Y.
- Cofiler® and PowerPlex® S5 are the only two kits that displayed a full profile for the minor donor down to 1:8 ratio.

Areas of Improvement:

- For all amplification kits, heterozygosity of 60% is not maintained at the concentrations that sensitivity is maintained.
- PowerPlex® 16 displayed more stochastic effects at higher RFU values/concentrations when compared to Identifiler®, Profiler Plus® and Cofiler®.
- PowerPlex® 16 displayed a higher baseline noise than all other amplification kits.
- PowerPlex® 16 displayed a large range in RFU values across all loci within a sample.
- MiniFiler™ displayed more stochastic effects at higher RFU values/concentrations when compared to PowerPlex® S5.
- Amplification artifacts that are unique to each kit are discussed above in the Findings and may have an effect on interpretation.
- For the mixture series, a full profile for the minor donor was expected but not obtained for most kits at the 1:10 ratio.

Limitations:

- The limitations vary per kit as discussed in the above text and are based on the proprietary chemistry of the kits.
- 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.

Training:

- No additional training necessary for a trained DNA analyst to use these products.
- Additional training may be necessary to interpret data and apply statistics for Y-STR amplification kits.

Health and Safety Issues:

- Normal laboratory practices are required.

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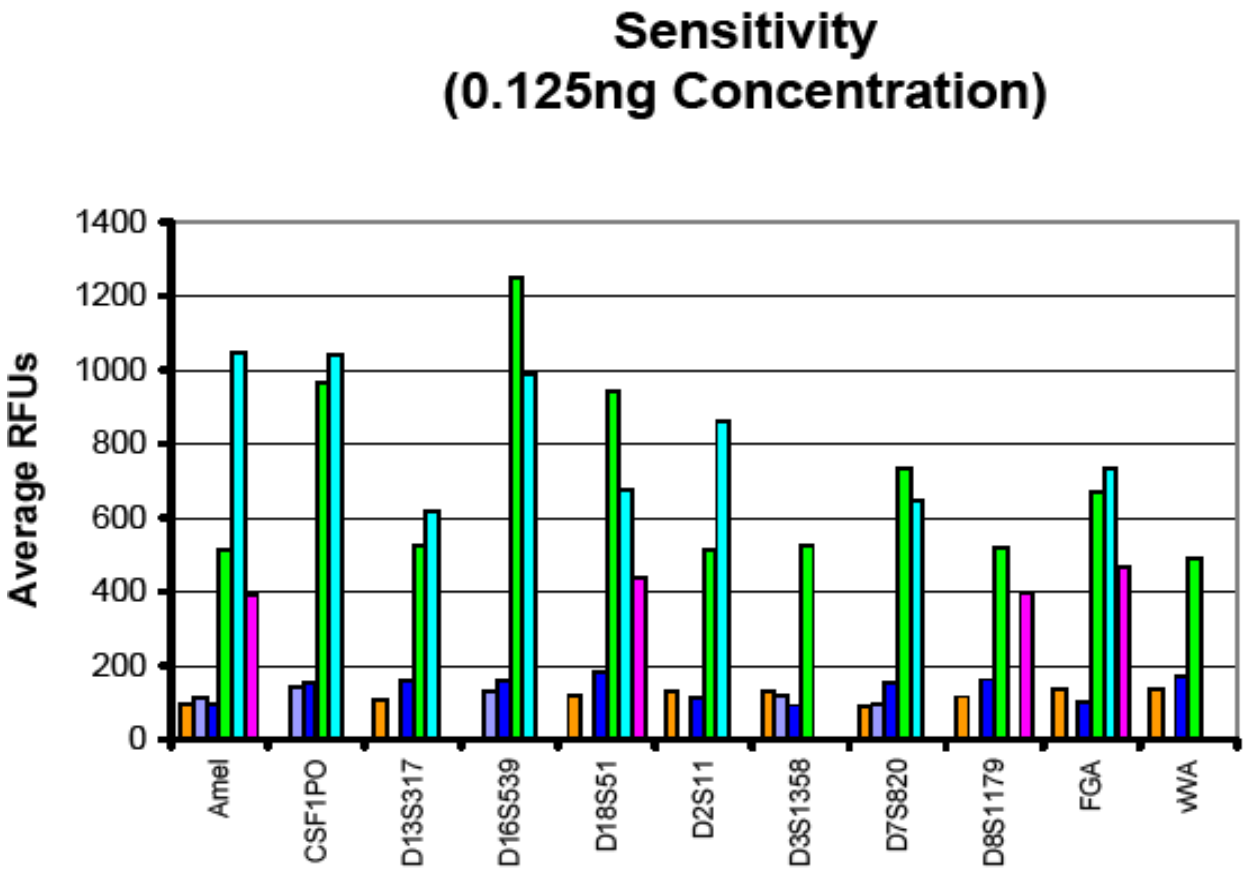
Additional Images:



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