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Evaluation of Applied Biosystems' AmpFℓSTR® Identifiler® Plus Amplification Kit

Project Information

Title: Evaluation of Applied Biosystems' AmpFℓSTR® Identifiler® Plus Amplification Kit

Evaluation Type: Kit comparison study

Stakeholder: Forensic Science Community

Start Date: 04/01/2010 End Date: 06/01/2010

Lot Number(s): 1001002

Manufacturer Information

Manufacturer: Applied Biosystems Inc.

Phone Number: (800) 248-0281

Internet address:
http://www.appliedbiosystems.com/absite/us/en/home.html

Stakeholder Information

Contact Person: N/A

Phone Number: N/A

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Evaluation Team

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Evaluation Summary

The demand for forensic DNA laboratories to employ new methods to meet their system's needs is a continual challenge to DNA technical leaders and laboratory management and creates a growing need for relevant information to aid in making these crucial decisions. The objective of this evaluation is to provide relevant performance information to the forensic science community through an overview of key performance measures of Applied Biosystems' Identifiler® Plus amplification kit compared to Promega's PowerPlex® 16 HS, Applied Biosystems' Identifiler® and Applied Biosystems' MiniFiler™ kits.

There are a number of commercially available short tandem repeat (STR) amplification kits for use in forensic DNA laboratories. While the responsibility lies with 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.

To assist forensic laboratories in understanding the general performance of commercially available STR amplification kits, the National Forensic Science Technology Center (NFSTC) previously evaluated kits: Applied Biosystems' AmpFℓSTR® Profiler Plus ID®, Cofiler®, Identifiler®, MiniFiler™ and Yfiler® kits; and Promega's PowerPlex® 16 system, PowerPlex® Y system, PowerPlex® S5 system, and PowerPlex® 16 HS. The kits were assessed for: sensitivity, peak height ratios at heterozygous loci, baseline noise, stutter ratio and amplification artifacts. These criteria were evaluated by analysis of single-source human DNA samples and a mixture series.

The current study evaluated a new amplification kit, Applied Biosystems' AmpFℓSTR® Identifiler® Plus using the same sample set and performance standards as the previous amplification kit study.

Product Specifications

Product Name(s): Applied Biosystems' Identifiler® Plus kit

Cost: $3,500 per 200 reactions

Power Requirements: N/A

Weight: N/A

Storage Conditions: -15°C to -25°C prior to initial use; 2°C to 8°C after initial use

Operational Conditions: N/A

Associated Costs: $4,000 (miscellaneous supplies charged partially to this evaluation)

Photos

Instrument Setup Comments

N/A

Level of Operator Knowledge (Set per Manufacturer)

☐ Non-Scientist ☐ Technician ☒ Scientist

Procedure

1) Collect standards from two male individuals.
2) Perform organic extraction in conjunction with Millipore Microcon 100 centrifugal filter device.
3) Quantify using Quantifiler Human and Quantifiler Human Y Kits on the AB 7500 RT PCR instrument and normalized the results with NIST quantitation standards.
4) Combine and dilute 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
    - Quantify after dilutions are prepared using Applied Biosystems' Quantifiler Human Kit on the Applied Biosystems' 7500 Real-Time PCR System and normalize the results with the NIST SRM 2372 quantitation standards.

5) Amplify the appropriate amount of each sample using manufacturers' recommended amplification conditions.
    - Identifiler® Plus (28 cycles) cycling parameters: 95°C hold (11 min); 28 cycles of 94 °C (20 sec) and 59 °C (3 min) and a 60°C (10 min) final extension.
    - Identifiler® Plus (29 cycles) cycling parameters: 95°C hold (11 min); 28 cycles of 94 °C (20 sec) and 59 °C (3 min) and a 60°C (10 min) final extension.
    - PowerPlex 16 HS (30 cycles) cycling parameters: 95°C hold (11 min); 96°C hold (1 min); 10 cycles ramp 100% to 94°C (30 sec), ramp 29% to 60°C (30 sec) and ramp 23% to 70°C (45 sec); 22 cycles ramp 100% to 90°C (30 sec), ramp 29% to 60°C (30 sec) and ramp 23% to 70°C (45 sec); and a 60°C (30 min) final extension.

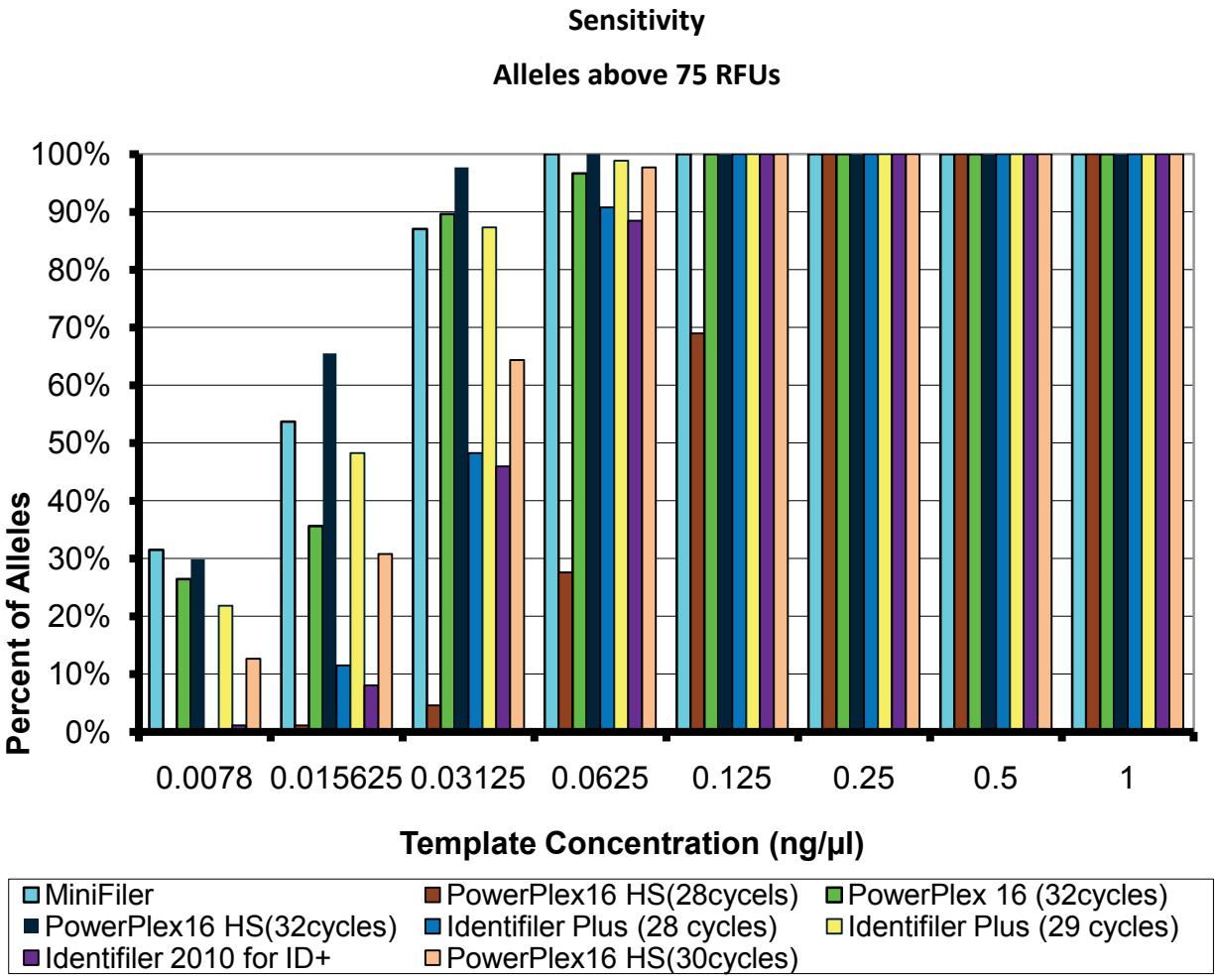
6) Prepare and run samples on the 3130*xl* Genetic Analyzer using manufacturer's recommended running conditions.
    - Applied Biosystems' kits: 3 kv, 10 sec injections, 8.7 µl Form, 0.3 µl GS 500, 1 µl sample
    - Promega kits: 3 kv, 10 sec injections, 9.5 µl Form, 0.5 µl ILS 600, 1 µl sample

7) Analyze data using Applied Biosystems' 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.

Standards, Controls and Samples Interrogated During Evaluation

- Standards from two male donors
- NIST SRM 2372 quantitation standards
- Amplification positive control DNA 9947A
- Amplification negative control
- Identifiler® Plus allelic ladder
- GS 500 – LIZ
- PowerPlex® standards

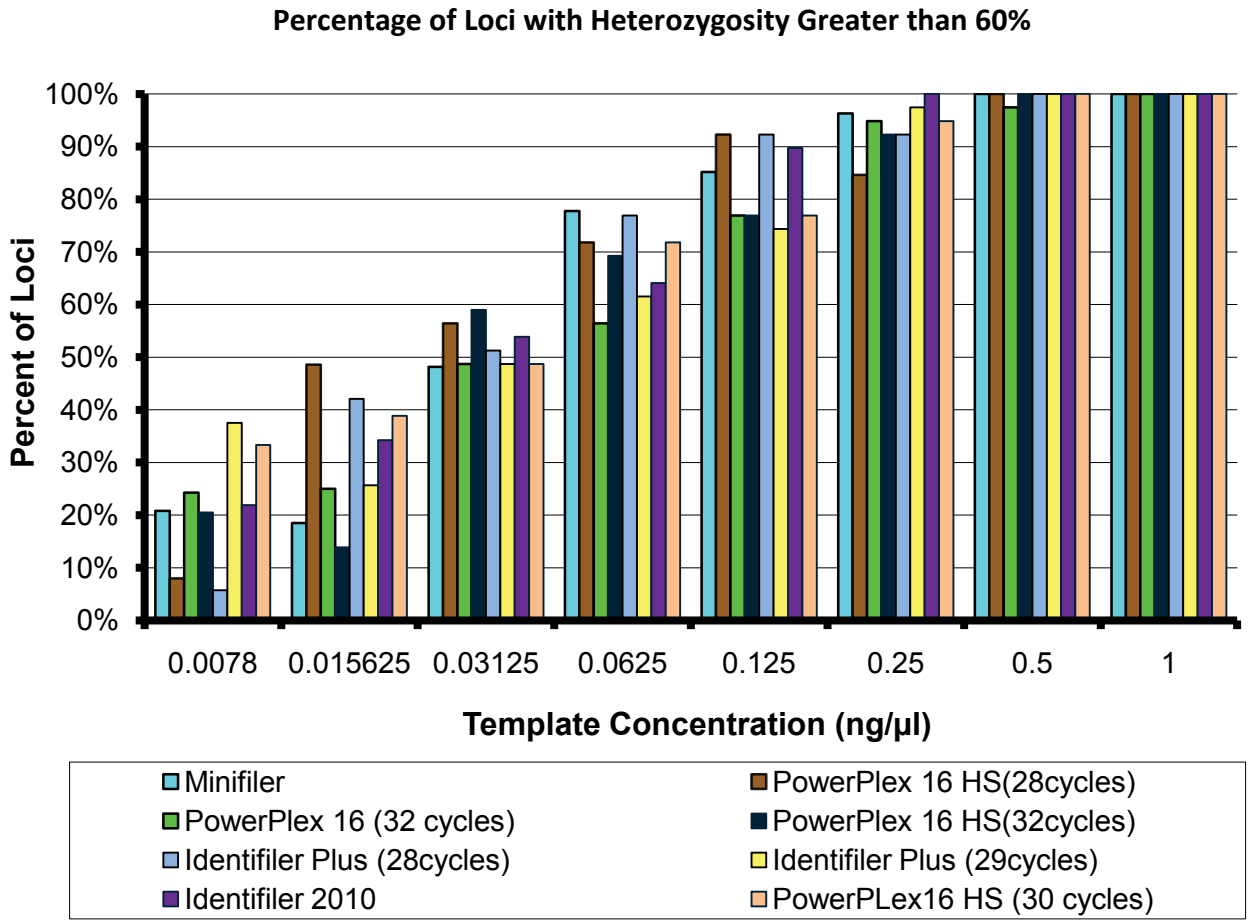
Findings



Sensitivity

- Concentrations at which alleles fell below 75 RFUs for comparable kits are depicted in the table above.
  - 0.03125 ng/µl
    - PowerPlex® 16 HS (32 cycles)
    - MiniFiler ™
  - 0.0625 ng/µl
    - PowerPlex® 16 (32 cycles)
    - PowerPlex® 16 HS (30 cycles)
    - Identifiler® Plus (28 cycles)
    - Identifiler® Plus (29 cycles)

    - 0.125 ng/µl
        - PowerPlex® 16 HS (28 cycles)
- Sensitivity Summary:
    - PowerPlex® 16 HS (32 cycles) produced the highest RFUs for each of the concentrations in the dilution series.
    - PowerPlex 16 (32 cycles) and MiniFiler™ RFU values were comparable for all concentrations.
    - Identifiler® Plus (29 cycles) displayed higher average peak heights than Identifiler® Plus (28 cycles); however, both kits had alleles fall below 75 RFUs at the same concentration of 0.0625 ng/µl.
    - Identifiler® Plus (28 cycles), in general, displayed higher average peak heights than Identifiler® at all concentrations.
    - PowerPlex® 16 HS (30 cycles), in general, displayed slightly higher average peak heights than Identifiler ® Plus (28 cycles). Both kits had alleles fall below 75 RFUs at the same concentration of 0.0625 ng/µl.



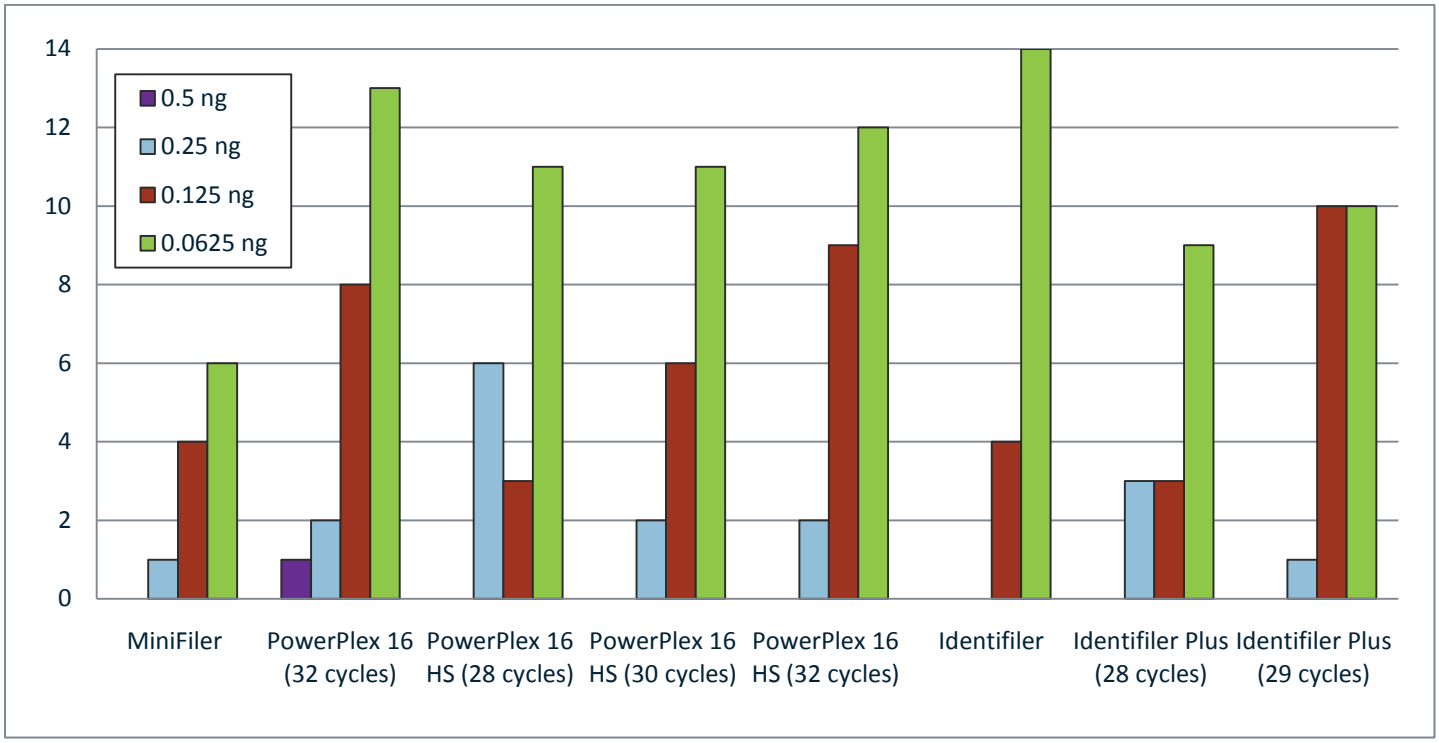
Heterozygosity

- First concentration where heterozygosity falls below 60% with corresponding ratios and peak heights:
  - 0.5 ng/µl: PowerPlex® 16 (32 cycles) – one occurrence: D8 at 50% (2974,1497 RFUs)
  - 0.25 ng/µl:
    - MiniFiler™ – one occurrence: D21 at 58% (3250,1870 RFUs)
    - PowerPlex® 16 HS(28 cycles) – six occurrences:
      - CSF at 58% (142,243 RFUs)
      - D18 at 59% (309,182 RFUs)
      - D18 at 52% (160,306 RFUs)
      - D3 at 55% (169,306 RFUs)
      - Penta D at 41% (434,178 RFUs)
      - Penta E at 44% (299,132 RFUs)

- PowerPlex® 16 HS(32 cycles) – two occurences
  - D16 at 51% (1338,2612 RFUs)
  - D3 at 43% (2344,5454 RFUs)
- PowerPlex® 16 HS(30 cycles) – two occurences:
  - D3 at 56% (536,963 RFUs)
  - D7 at 54% (981,533 RFUs)
- Identifiler® Plus (28 cycles) – three occurences:
  - D16 at 59% (902,529 RFUs)
  - D18 at 52% (754,396 RFUs)
  - D19 at 54% (657,355 RFUs)
- Identifiler® Plus (29 cycles) – one occurrence: D16 at 55% (2011,1104 RFUs)

- 0.125 ng/µl:
  - Identifiler® – four occurrences:
    - Amelogenin at 56% (146,263 RFUs)
    - D13 at 56% (273,486)
    - D21 at 42% (77,185)
    - D2 at 50% (482,242)

Number of Loci in the Triplicate Data Set with Heterozygosity below 60%



Amplification Artifacts

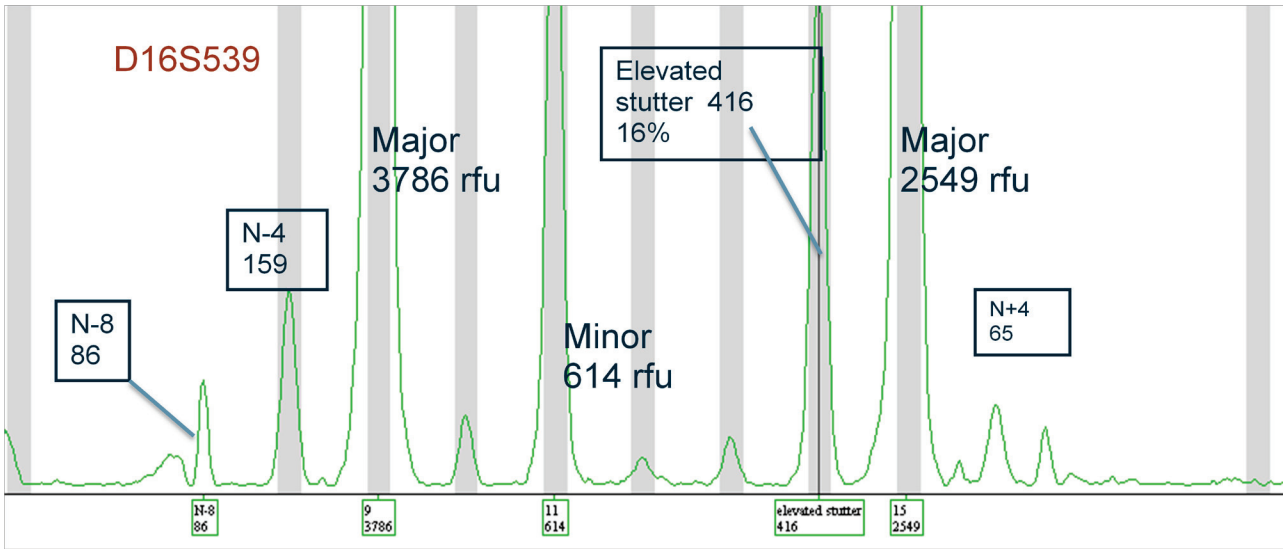
- Dye blobs – every kit displayed at least one or more dye artifacts
  - Identifiler®: 119 bp (blue), 113 bp (green), 162 bp (yellow), 198 bp (red).
  - MiniFiler™: 117 bp and doublet at 127/129 bp (blue); 104 bp and 118-120 bp (green), 77 and 105 bp(yellow).
  - PowerPlex® 16: 116 bp (green), 247 bp (yellow).
  - PowerPlex® 16 HS: 131 bp (blue), 151 bp (yellow).
  - Identifiler® Plus: 119 bp (green).
- Minus A
  - Minus A was generally not observed for the PowerPlex® 16 HS system.
  - PowerPlex® 16 HS and Identifiler® Plus showed minimal minus A, unless the sample was overloaded due to increased cycle number.
  - Identifiler® and PowerPlex® 16 showed more minus A than the enhanced kits.
  - No minus A was observed with overloaded samples with MiniFiler™.

- N-4
    - Elevated stutter was observed:
        - Identifiler® Plus (28 and 29 cycles) – shows several occurrences (D19 and vWA) of elevated, callable N-4 at 0.25 ng/µl and 0.125 ng/µl, respectively.
        - PowerPlex®16 HS (28, 30 and 32 cycles) – shows elevated, callable N-4 at the vWA locus for 0.5 ng/µl, 0.25 ng/µl and 0.03125 ng/µl, respectively.

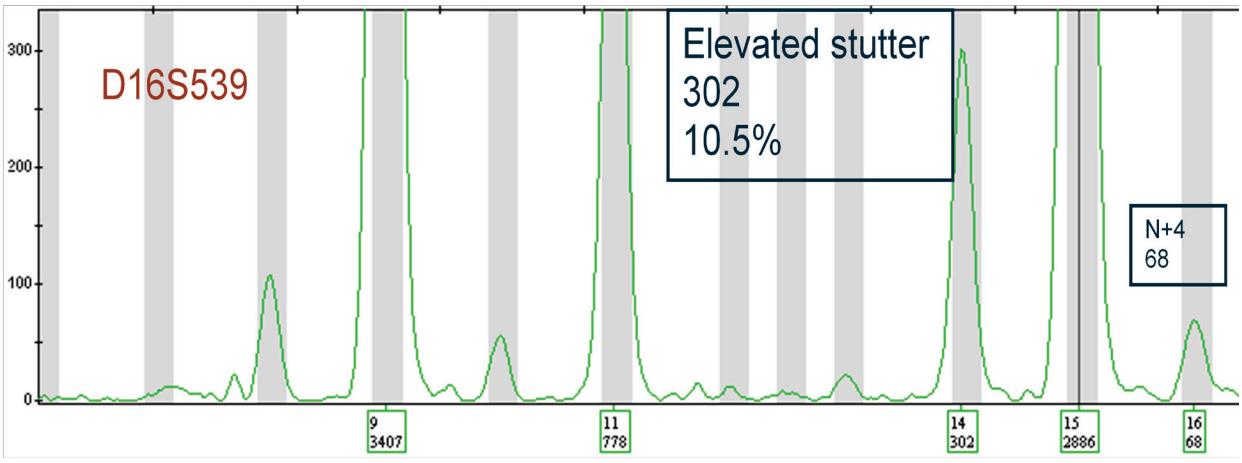
          NOTE: N-4 at vWA occurs in all Identifiler® and PowerPlex® 16 kits. It is not observed in Profiler® or Cofiler® kits.
- N+4
    - Indications of plus stutter were observed as noted:
        - PowerPlex® 16 and Identifiler – no callable plus stutter observed in dilution series.
        - PowerPlex® 16 HS (28 cycles) – no callable plus stutter observed. Below 75 RFUs plus stutter was observed down to 0.5ng/µl.
        - Identifiler® Plus (28 cycles) – no callable plus stutter observed. Below 75 RFUs plus stutter was observed down to 0.125ng/µl.
        - PowerPlex® 16 HS (30 cycles) – callable plus was stutter observed at 1ng/µl. Below 75 RFUs plus stutter was observed down to 0.25 ng/µl.
        - Identifiler® Plus (29 cycles) – callable plus was stutter observed at 1 ng. Below 75 RFUs plus stutter was observed down to 0.03125 ng.
        - PowerPle®x16 HS (32 cycles) – callable plus was stutter observed at 0.25 ng/µl. Below 75 RFUs plus stutter was observed down to 0.03125 ng/µl.
        - MiniFiler™ – callable plus was stutter observed at 0.5ng/µl. Below 75 RFUs plus stutter was observed down to 0.25 ng/µl.

- Artifacts displayed in Mixture Series:

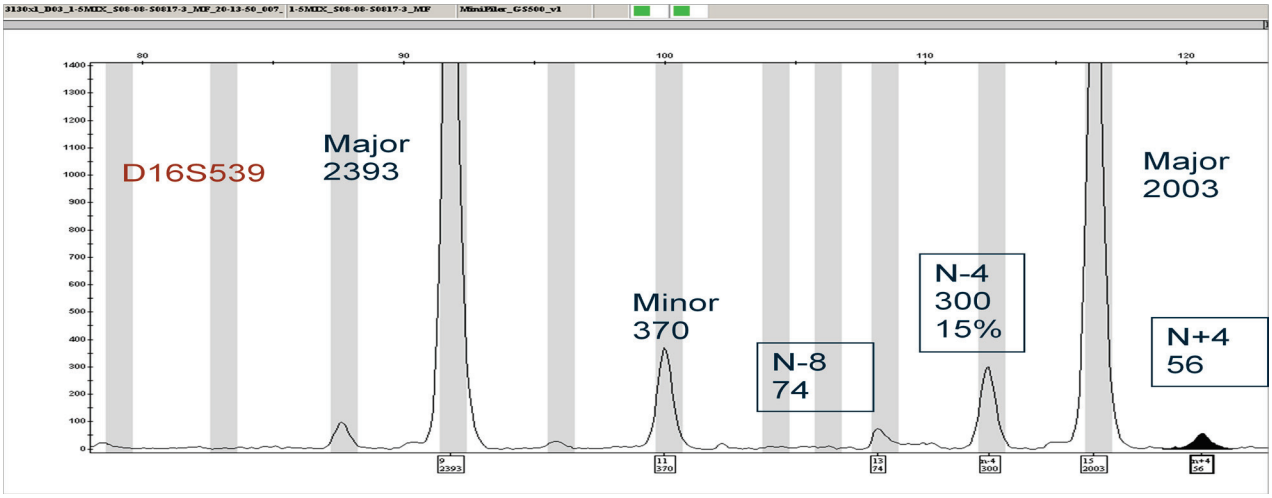
1:5 Mixture
PowerPlex® 16 HS
30 cycles (1 ng/µl)



1:5 mixture
Identifiler® Plus
29 cycles (0.5 ng/µl)



1:5 Mixture
MiniFiler™
(0.25 ng/µl)



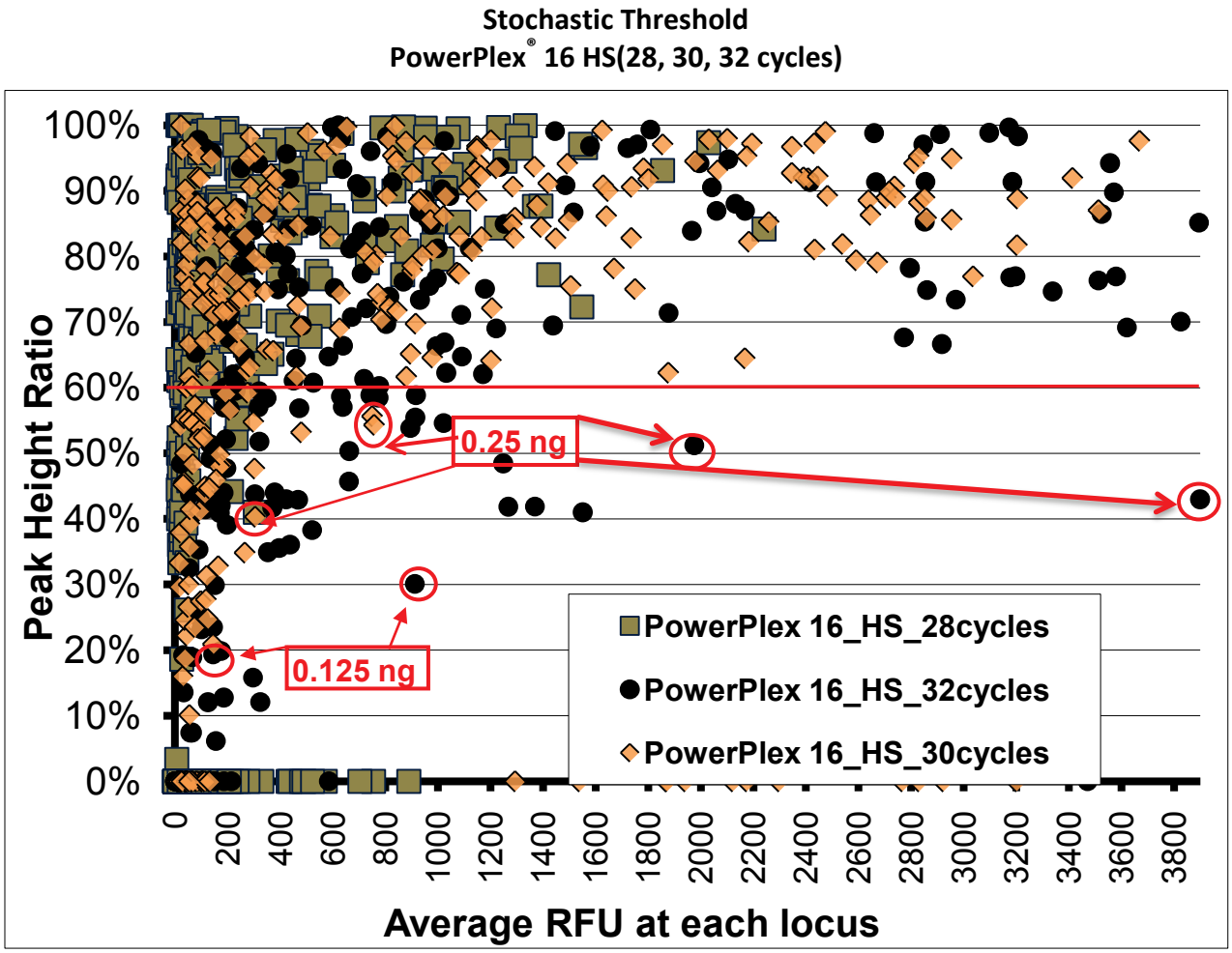
Baseline Noise

		Profiler®	Cofiler®	MiniFiler™	Identifiler®	PP16	PP16 HS 32 cycles	ID+ 28cycles	ID+ 29cycles
LOD	Ave + 3X std dev	15.7	15.5	14.3	14.2	13.6	14.5	13.5	14.1
LOQ	Ave +10X std dev	29.6	28.7	25.9	25.5	24.4	26.7	23.9	25.5

- Using 8 RFU minimum:
  - All amplification kits displayed background noise with the limit of detection (LOD) between 13 and 15 RFUs.
  - The limit of quantitation (LOQ) is between 24 and 29 RFUs.

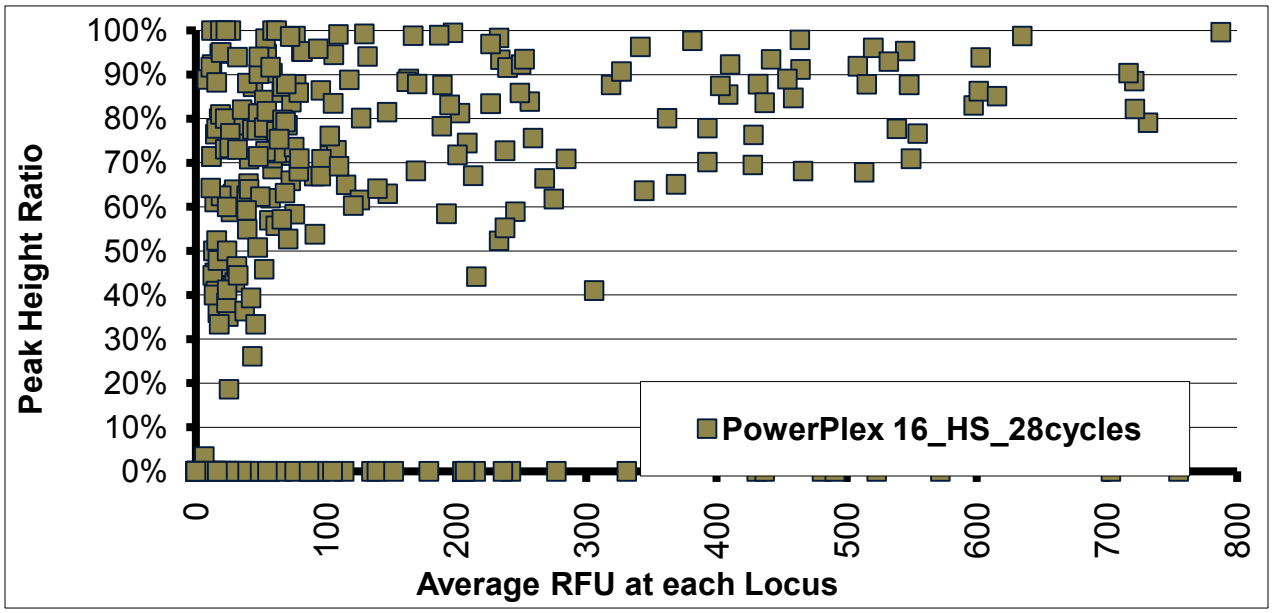
Stochastic Threshold

- The greater the cycle number the more stochastic effects at higher RFUs are observed.

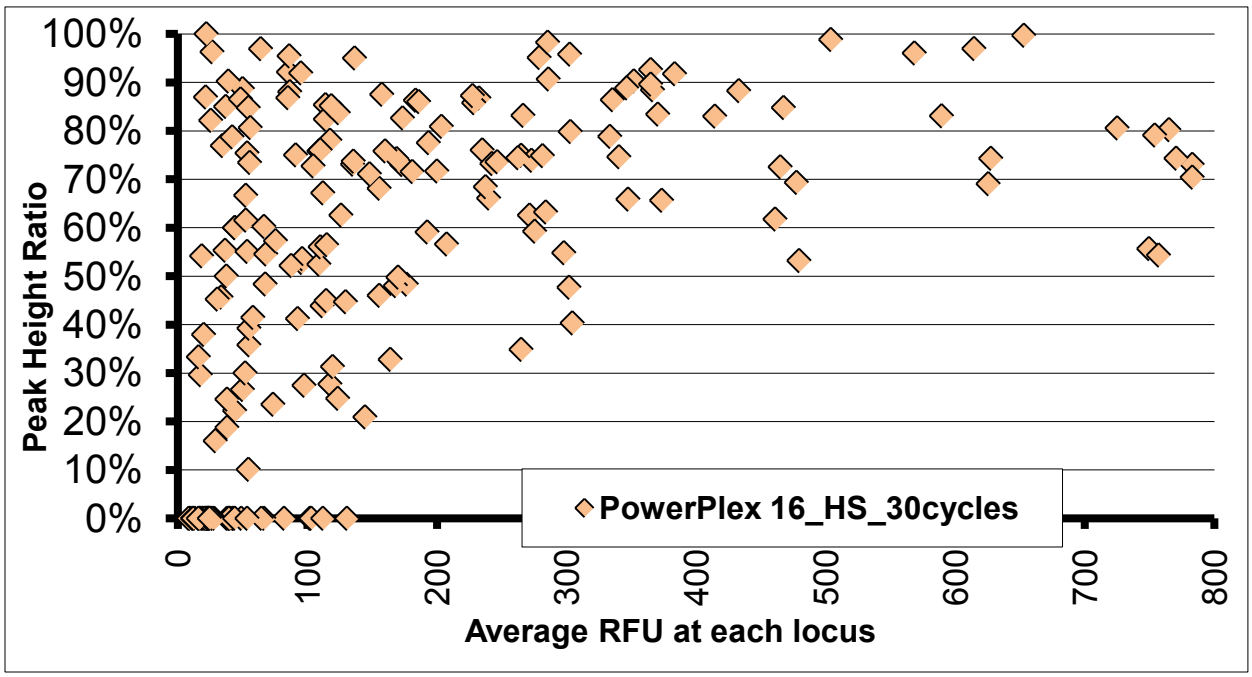


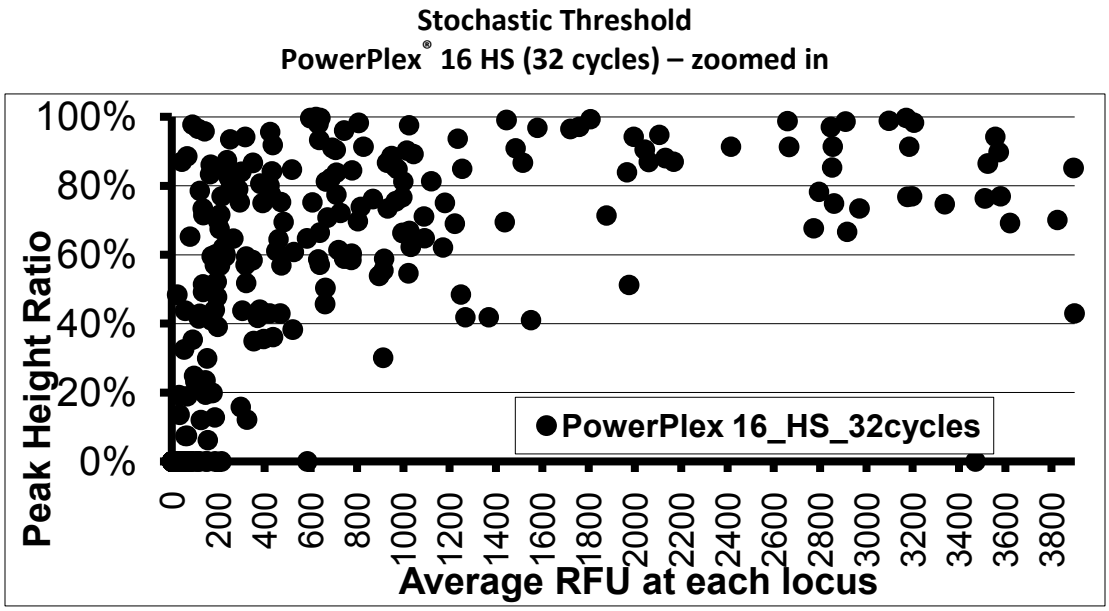
- Select Outliers (occurrence with highest RFUs that falls below 60% heterozygosity) depicted in the chart above:
  - PowerPlex® 16 HS (32 cycles): 3899 RFUs, 43% heterozygosity, 0.25 ng/µl
  - PowerPlex® 16 HS (30 cycles): 757 RFUs, 54% heterozygosity, 0.25 ng /µl
  - PowerPlex® 16 HS (28 cycles): 306 RFUs, 40% heterozygosity, 0.25 ng /µl
  - PowerPlex® 16 (32 cycles): 2235 RFUs, 50% heterozygosity, 0.5 ng/µl (not shown in this graph)

Stochastic Threshold
PowerPlex® 16 HS (28 cycles) – zoomed in

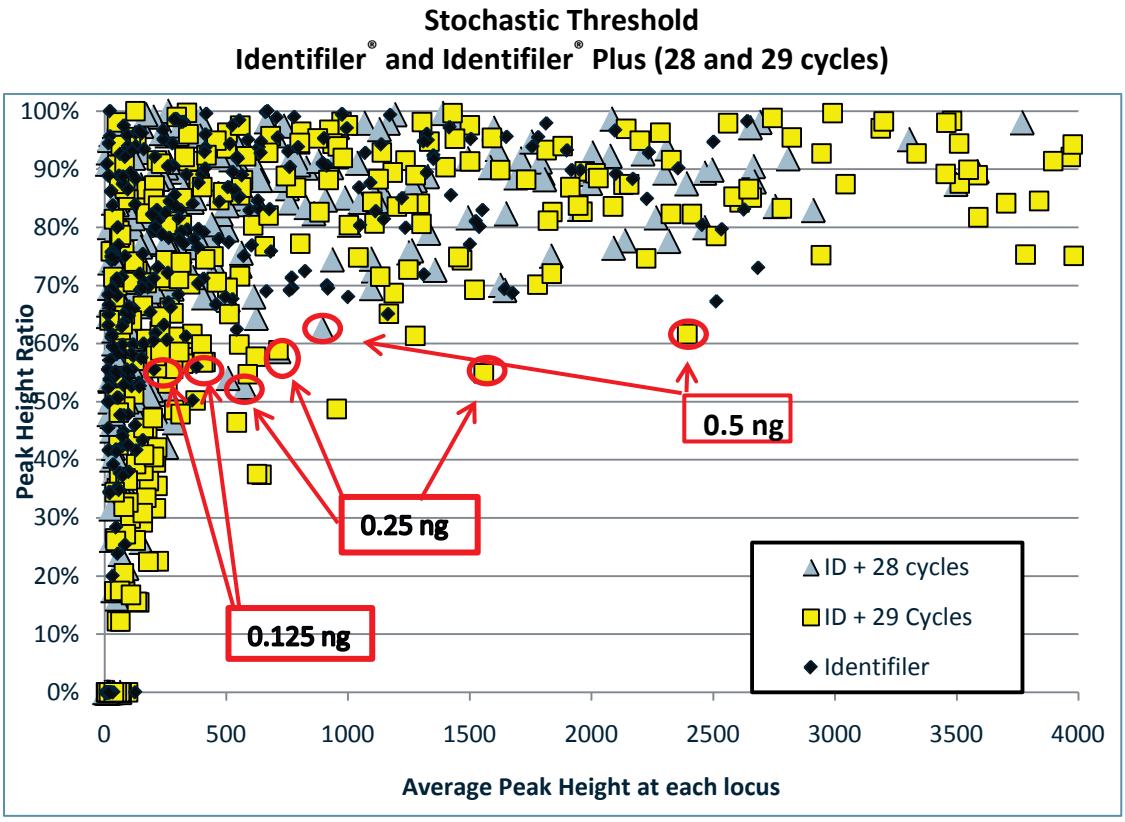


Stochastic Threshold
PowerPlex® 16 HS (30 cycles) – zoomed in

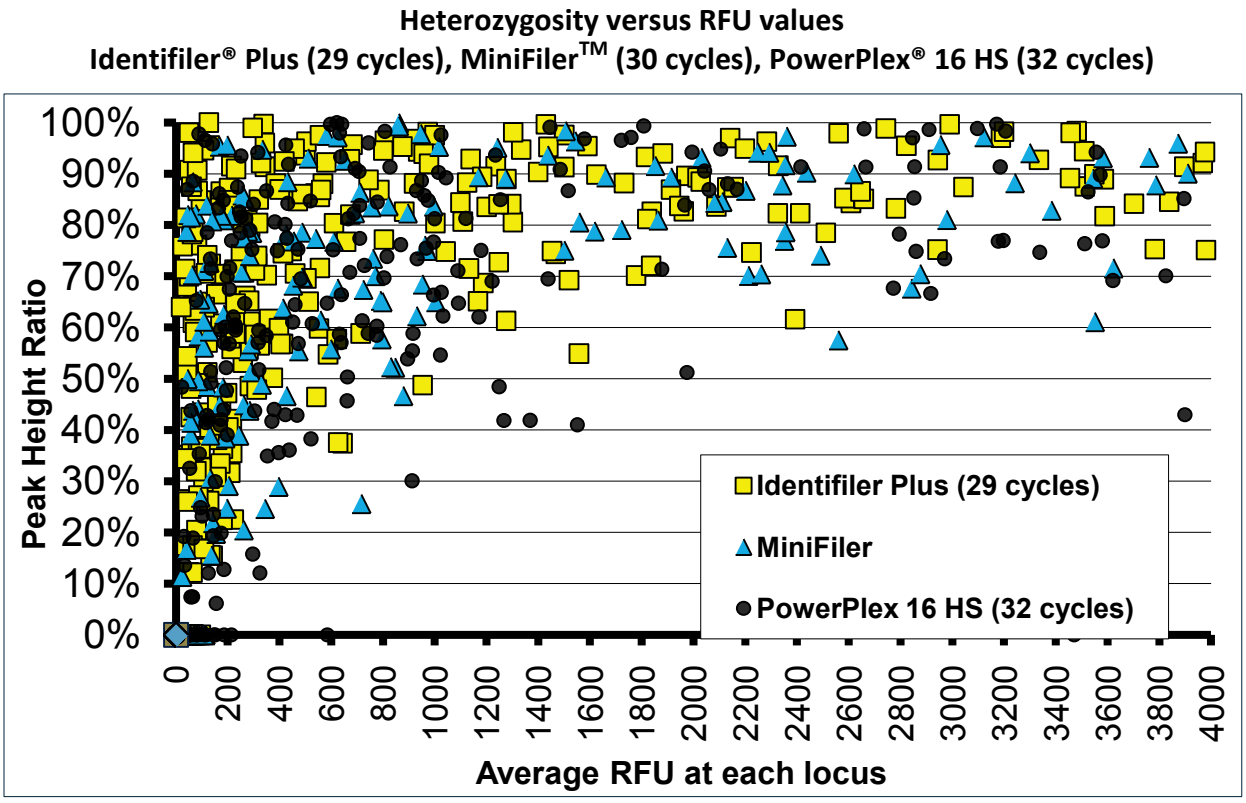




- Each of the graphs above demonstrate the need for different stochastic thresholds for different cycling parameters for the same amplification kit

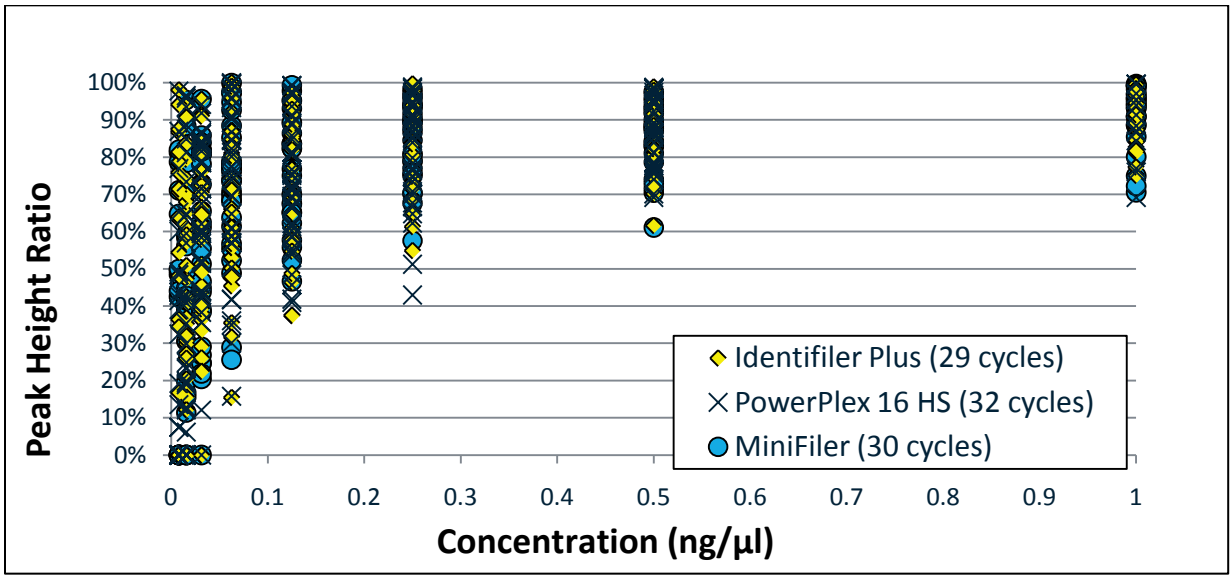


- Select Outliers (occurrence with highest average RFUs that falls below 60% heterozygosity) depicted in the chart above:
  - Identifiler® Plus (29 cycles): 1557 RFUs, 55% heterozygosity, 0.25 ng/µl
  - Identifiler® Plus (28 cycles): 715 RFUs, 59% heterozygosity, 0.25 ng /µl
  - Identifiler® : 350 RFUs, 57% heterozygosity, 0.125 ng/µl
  - MiniFiler™: 2500 RFUS, 57% heterozygosity, 0.5 ng/µl (not shown in this graph)
- This graph supports that increased cycle number increases stochastic effects.



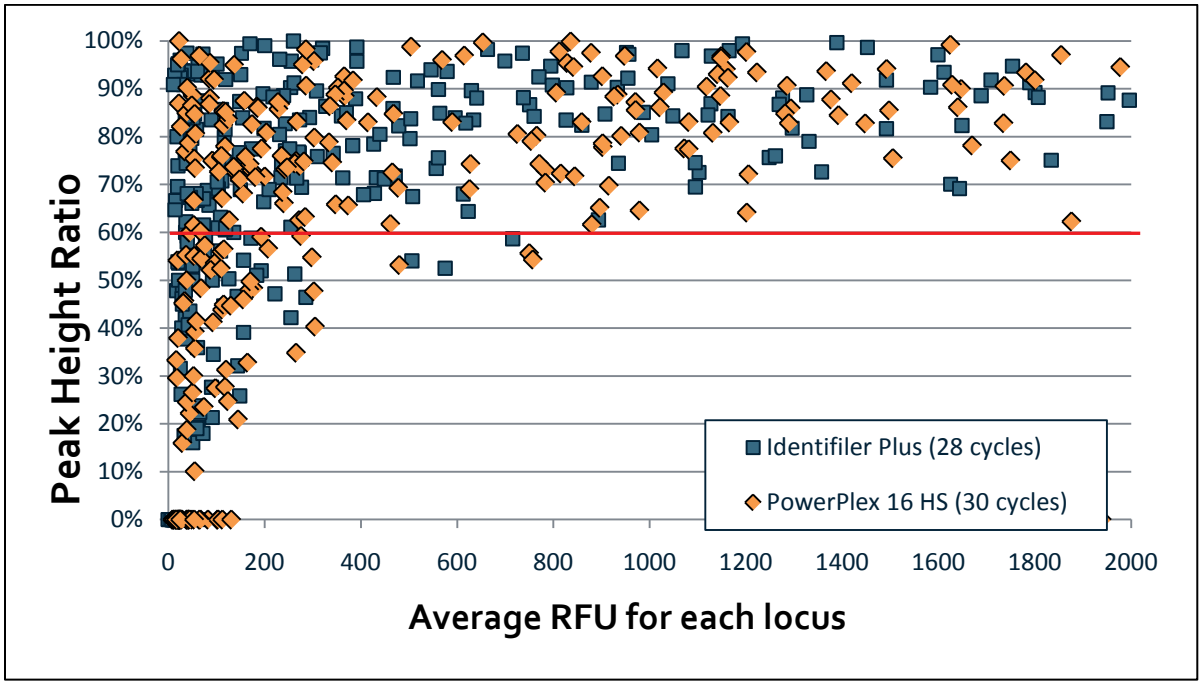
- PowerPlex® 16 HS (32 cycles) shows more occurrences of stochastic effects at greater RFU values than MiniFiler™ and Identifiler® Plus (29 cycles).
    - PowerPlex® 16 HS (32 cycles) has 17 occurrences of heterozygosity less than 60% at RFU values greater than or equal to 600 RFUs.
    - MiniFiler™ has seven occurrences of heterozygosity less than 60% at RFU values greater than or equal to 600 RFUs.
    - Identifiler® Plus (29 cycles) has six occurrences of heterozygosity less than 60% at RFU values greater than or equal to 600 RFUs.

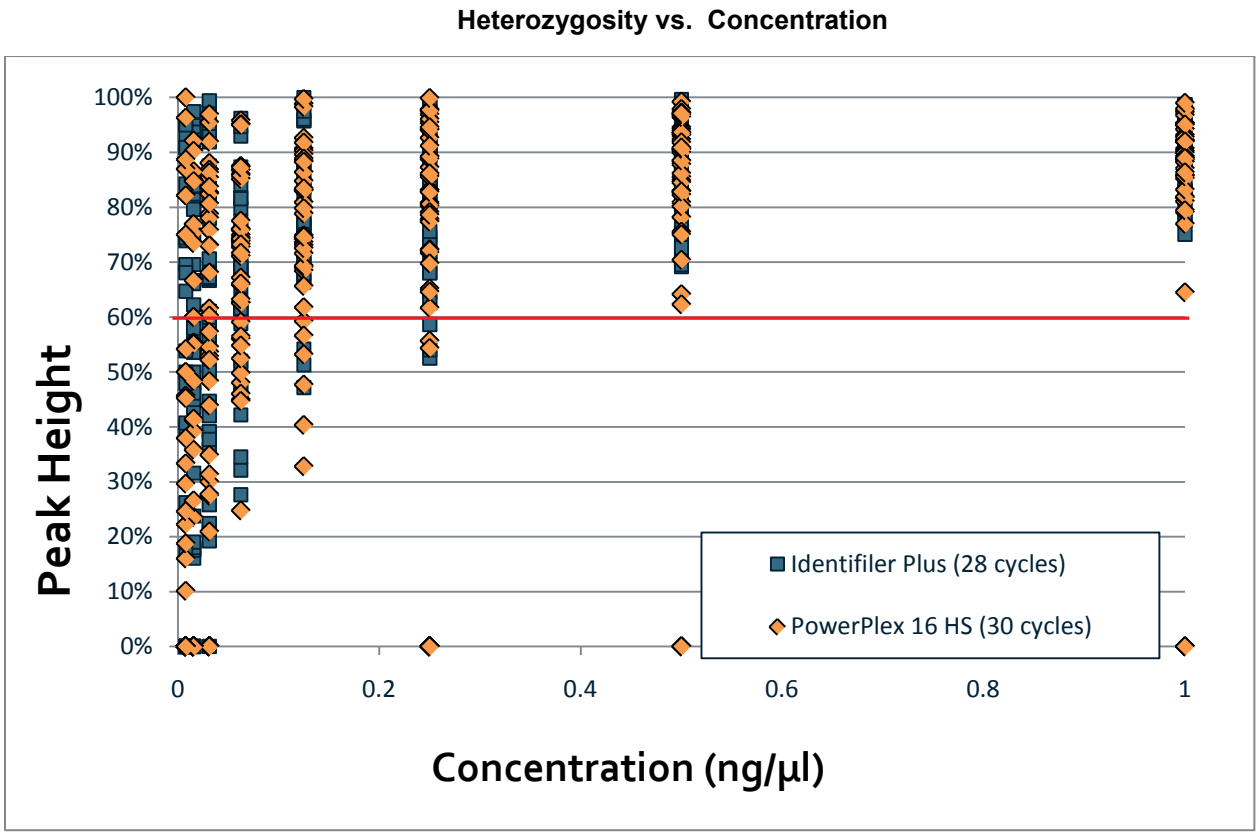
Heterozygosity vs. Concentration
Identifiler® Plus (29 cycles), MiniFiler™ (30 cycles), PowerPlex® 16 HS (32 cycles)



- All three of these kits show that heterozygosity below 60% occurs at 0.25 ng/µl.

Heterozygosity vs. RFU values
Identifiler® Plus (28 cycles), PowerPlex® 16 HS (30 cycles)

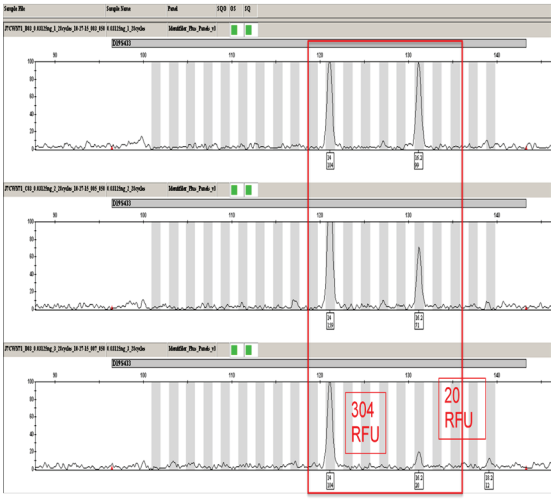




- These graphs support that Identifiler® Plus (28 cycles) is similar to PowerPlex® 16 HS (30 cycles).
  - Both show similar stochasm with equal number of occurrences of data falling below 60% heterozygosity with RFU values at 600 or above.
  - Both kits have data points that fall below 60% heterozygosity at 0.25 ng/µl.

- Examples of stochastic effects:

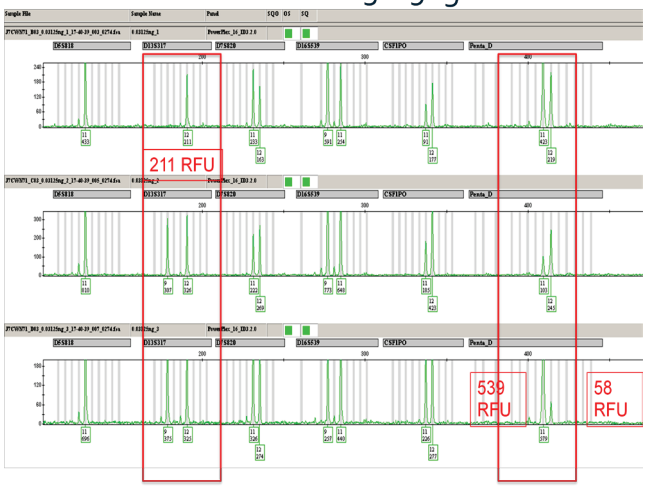
Identifiler Plus (28 cycles)
0.03125ng



Identifiler Plus (29 cycles)
0.03125ng

PowerPlex 16 (32cycles)
(0.0625ng)

PowerPlex 16 HS (32 cycles)
0.03125ng



Mixtures

	MiniFiler	Identifiler	PP16	PP16HS 28 cycles	PP16 HS 30 cycles	ID+ 28 cycles	ID+ 29 cycles
target (ng)	0.25 ng	1 ng	0.5 ng	1 ng	1 ng	1 ng	0.5 ng
1:5 mix	100%	100%	100%	100%	100%	100%	100%
1:8 mix	75%	94%	88%	67%	89%	91%	96%
1:10 mix	73%	85%	77%	32%	84%	89%	87%
1:12 mix	73%	76%	79%	32%	75%	85%	81%
1:15 mix	48%	63%	73%	12%	49%	67%	72%

Note: percent of minor donor alleles 75 RFUs and greater (excludes peaks that fall in stutter position that are filtered out by GeneMapper®)

- In general, heterozygosity for major holds for all ratios tested
- Point at which heterozygosity for minor falls below 60%:
  - 1:5 mix: MiniFiler™, PowerPlex® 16 (32 cycles), Identifiler® Plus (29 cycles)
  - 1:8 ratio: PowerPlex® 16HS (28 cycles), Identifiler® Plus (28 cycles)
  - 1:10 mix: Identifiler®, PowerPlex® 16 HS(30 cycles)

Summary of Select Kits

Approx Target (ng)	0.125 - 0.5	0.5 - 1.0	0.5 - 1.0	0.25 - 0.5	0.125 - 0.5	0.5 - 1.0	0.25 - 0.5	0.06 - 0.125
Full profile (RFU range)	0.0625 ng (178-1141 RFU)	0.125 ng (77-756 RFU)	0.125 ng (100-1127 RFU)	0.125 ng (243-2068 RFU)	0.125 ng (115-2773 RFU)	0.25 ng (91-819 RFU)	0.125 ng (81 -1165)	0.0625 ng (81-2603 RFU)
Loss of 60% heterozygosity (RFU ave for locus of occurrence with highest RFU)	0.25 ng (2560 RFU)	0.125 ng (379 RFU)	0.25 ng (575 RFU)	0.25 ng (1557RFU)	0.5 ng (2235 RFU)	0.25 ng (307 RFU)	0.25 ng (757 RFU)	0.25 ng (3899 RFU)
Allelic Dropout (heterozygote allele height)	0.03125 ng (216 RFU)	0.03125 ng (126 RFU)	0.03125 ng (59 RFU)	0.03125 ng (192 RFU)	0.0625 ng (507 RFU)	0.0625 ng (51 RFU)	0.03125 ng (107 RFU)	0.03125 ng (211 RFU)
Minor Donor alleles below 75 RFUs (click on stutter)	1:10	1:10	1:12	1:10	1:8	1:8	1:8	N/A
Loss of 60% heterozygosity for minor donor	1:5	1:10	1:8	1:5	1:5	1:8	1:10	N/A

Conclusions

- Comparing Identifiler® and Identifiler® Plus:
  - Identifiler® and Identifiler® Plus (28 cycles) similar peak heights.
  - Identifiler® Plus (29 cycles) yielded higher peak heights than Identifiler® and Identifiler® Plus (28 cycles).
  - Identifiler® and Identifiler® Plus (28 cycles) are less susceptible to stochastic effects than Identifiler ®Plus (29 cycles).
  - Identifiler® Plus (28 cycles) demonstrated fewer loci falling below 60% heterozygosity than Identifiler® and Identifiler® Plus (29 cycles) at 0.125 ng/µl and 0.0625 ng/ µl.
  - Identifiler® demonstrated fewer loci falling below 60% heterozygosity than Identifiler® Plus (28 and 29 cycles) at 0.25 ng/µl.
- Comparing PowerPlex® 16 and PowerPlex® 16 HS (28, 30, and 32 cycles):
  - PowerPlex® 16 HS (32 cycles) showed fewer stochastic effects than PowerPlex® 16.
  - Peak heights for PowerPlex® 16 HS (32 cycles) are greater than PowerPlex® 16.
  - PowerPlex® 16 HS (28 cycles) is less susceptible to stochastic effects than PowerPlex® 16 and PowerPlex® 16 HS (30 and 32 cycles).
  - PowerPlex® 16 HS (30 cycles) demonstrated better heterozygosity for the minor donor in 1:10 mixture series than the PowerPlex® 16 and PowerPlex® 16 HS (28 and 32 cycles).
  - PowerPlex® 16 HS (30 cycles) demonstrated fewer loci falling below 60% heterozygosity than Identifiler® Plus (28 cycles) at 0.25 ng/µl.
  - Identifiler® Plus (28 cycles) demonstrated fewer loci falling below 60% heterozygosity than PowerPlex® 16 HS (30 cycles) at 0.125 ng/µl and 0.0625 ng/ µl.
- Comparing PowerPlex® 16 HS (30 cycles) to Identifiler Plus (28 cycles):
  - PowerPlex 16 HS (30) displayed slightly higher average peak heights; however it maintained comparable peak height ranges when compared to the Identifiler® Plus (28 cycles).
  - Both kits displayed partial profiles at 0.125 ng/µl and a loss of heterozygosity at 0.25 ng/µl.
  - PowerPlex® 16 HS (30), maintained heterozygosity for minor donor 1:10 mixture ratio and full profile for minor for 1:8. Identifiler® Plus (28 cycles) maintained heterozygosity for minor for the 1:8 mixture ratio and full profile for minor for 1:12.
- Comparing Identifiler® Plus (29 cycles) and PowerPlex® 16 HS (32 cycles) to MiniFiler™:
  - Identifiler® Plus (29 cycles):
    - Identifiler® Plus (29 cycles) is slightly less sensitive than MiniFiler™.
    - Identifiler® Plus (29 cycles) and MiniFiler™ performed similarly as far as detecting the minor donor in the mixture series.
    - Identifiler® Plus (29 cycles) appears to be less susceptible to stochastic effects than MiniFiler™.

- PowerPlex® 16 HS(32 cycles):
  - PowerPlex® 16 HS (32 cycles) and MiniFiler™ showed alleles below 75 RFUs at the same concentration of 0.03125 ng/µl; however, PowerPlex® 16 HS (32 cycles) generated higher RFU values for all concentration in the dilution series.
  - PowerPlex® 16 HS (32 cycles) is more susceptible to stochastic effects than MiniFiler™; however, they both show stochasm at higher RFU values than expected.

Areas for Improvement

- Improved heterozygosity for greater number of cycles.
- All kits display artifacts that, if removed, would be helpful for data interpretation

Limitations

- Quantitation system is an estimate and can be inaccurate. This can effect concentration of template added to the amplification reaction which, can in turn, yield greater stochastic effects.
- The limitations vary with each 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 Requirements

- 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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