

Additional Study¹: Evaluation of an Additional Amplification Kit—the PowerPlex® 16 HS System

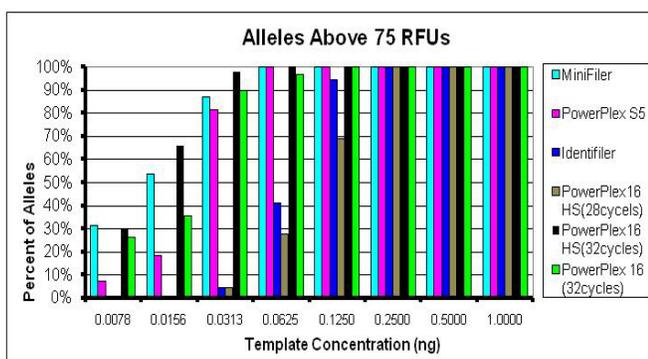
Introduction:

As a continuation to “The Evaluation of Eight Commercially Available Amplification STR Kits” conducted by the NFTSC, a newly available PowerPlex® 16 HS system was evaluated using the same experimental design and performance criteria. The only variation is the PowerPlex® 16 HS system only was evaluated using 28 and 32 cycles for the dilutions series, while the mixtures series was evaluated using 28 cycles.

Additionally, a set of four challenged samples prepared by Promega were evaluated using the Identifier®, MiniFiler™ and PowerPlex® 16 HS system (28 cycles). The amplification target suggested for these samples is 2 µl of the 0.25 ng/µl sample. This was performed in triplicate.

Results and Discussion:

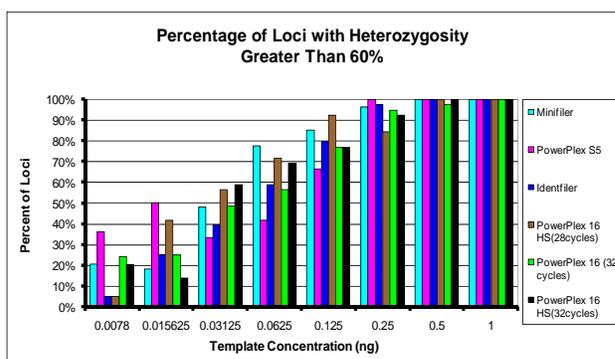
Sensitivity:



Concentrations at which alleles fell below 75 RFUs for comparable kits are depicted in the table above.

- PowerPlex® 16 HS (32cycles) produced the highest RFUs for each concentration.
- PowerPlex® 16 HS (28cycles) had alleles fall below 75 RFUs at 0.125 ng.
- PowerPlex® 16 HS (32cycles) had alleles fall below 75 RFUs at 0.03125 ng.

Heterozygosity:



At concentrations of 0.25 ng and lower, PowerPlex® 16 HS (28 and 32 cycles) did not maintain heterozygosity of 60%.

- 0.25 ng concentration corresponds with the following peak height ranges:
 - PowerPlex® 16 HS (28 cycles) - 91 to 889RFUs
 - PowerPlex® 16 HS (32cycles) - 556 to 7586 RFUs

Amplification Artifacts:

- Two dye blobs were present in the PowerPlex® 16 HS system.
- Elevated stutter was observed at vWA at concentrations as low as 0.03125ng for PowerPlex® 16 HS (32 cycles).
- Elevated stutter was observed at vWA for the 0.5ng and 1.0ng concentrations with PowerPlex® 16 HS (28 cycles).
- Indications of plus stutter (below 75 RFUs) were observed for concentrations as low as 0.03125 ng for PowerPlex® 16 HS (32 cycles) and were greater than 75 RFUs at concentrations of 0.25 ng and higher.
- Indications of plus stutter (below 75 RFUs) were observed at 0.5 ng and higher for PowerPlex® 16 HS (28 cycles).
- Minus A was generally not observed for the PowerPlex® 16 HS system.

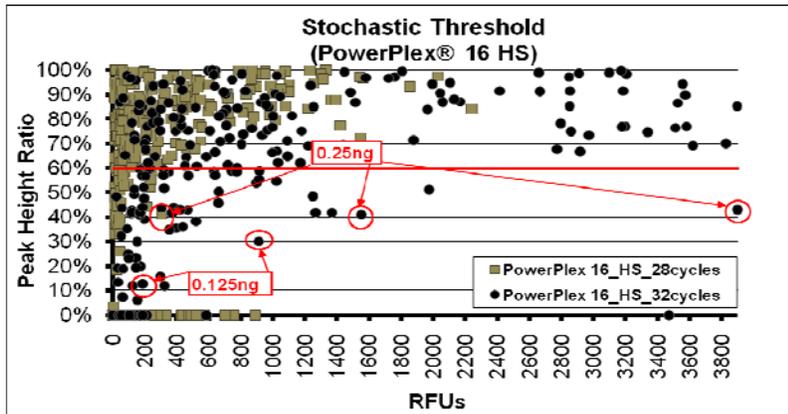
¹Original Study: *The Evaluation of Eight Commercially Available Amplification STR Kits*, 2008

Authors: Sutherland, Carrie B, BS¹; O'Brien, Robert I, BS¹; Figarelli, Debra A, BS¹; Ring, Joan G, MS¹, and Grates, Kirk M, BA¹

Baseline Noise:

- PowerPlex® 16 HS (32 cycles) amplification kit displayed background noise with the limit of detection (LOD) at approximately 15 RFUs.
- The limit of quantitation (LOQ) is approximately 27 RFUs.

Stochastic Threshold:



Mixtures:

PowerPlex® 16 HS (28 cycles) performed similarly to the previously evaluated mixture series for PowerPlex® 16 (32 cycles). Mixture ratios and heterozygosity of the major donor held throughout the series.

Percentage of Minor Alleles 75 RFUs and above (excludes minor donor alleles that fall in stutter position and filtered out by GeneMapper)					
1:5 mix	1:8 mix	1:10 mix	1:12 mix	1:15 mix	1:20 mix
100%	67%	32%	32%	12%	7%

Challenged samples:

Percentage of alleles called above 50 RFUs			
	Identifiler®	MiniFiler™	PowerPlex® 16 HS (28 cycles)
DNA #1: 9948 and hematin	0%	4%	100%
DNA #2: 9948 and tannic acid	0%	33%	100%
DNA #3: 9948 and humic acid	99%	100%	100%
DNA #4: 9948 control	100%	100%	100%

Conclusions:

- PowerPlex® 16 HS (32 cycles) amplification kit yielded higher RFUs for the dilutions series than the previously tested PowerPlex® 16 (32 cycles) amplification kit.
- PowerPlex® 16 HS (28 cycles) performed similar to Identifiler® with regards to the minimum RFU value in the range at most concentrations, heterozygosity loss at 0.25 ng, and alleles below 75 RFUs at 0.125 ng and 0.0625 ng, respectively.
- Identifiler® displayed higher maximum RFU value in the range at each concentration than PowerPlex® 16 HS (28 cycles).
- PowerPlex® 16 HS (28 cycles) out performed MiniFiler™ and Identifiler® amplification kits for the challenged samples.
- Dropout was observed at a lower concentration (0.03125 ng) for the PowerPlex® 16 HS (32 cycles) opposed to 0.0625 ng for the previously tested PowerPlex® 16 (32 cycles) amplification kit.

