Forensic Technology Testing & Evaluation Report

Forensic Technology Testing & Evaluation Project

Project Title:	Projected Start Date:
Evaluation of PowerPlex® 16 HS Amplification Kit	July 1, 2009
Evaluation Type: (Instrument, kit, procedure, product-to-product comparison study, etc.)	Projected End Date:
Kit	August31, 2009
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Manufacturer Information for product(s) being evaluated:

Manufacturer	Address	Contact Person	Phone
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This project was supported by Award No. 2008-MU-MU-K003 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.

Evaluation Overview

Evaluation Summary:
Overview of this project, including background information, objectives and forensic applications.

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 a previous effort to assist in this process, the National Forensic Science Technology Center (NFSTC) conducted a study and evaluated 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. A new amplification kit, Promega's PowerPlex® 16 HS, will be evaluated using the same sample set and performance standards as the previous study. The only variation is that the PowerPlex® 16 HS system was evaluated using both 28 and 32 cycles for the dilutions series, while the mixtures series was only evaluated using 28 cycles.

The performance of Promega's PowerPlex® 16 HS amplification kit will be assessed based on the same previously defined set of criteria: sensitivity, peak ratios at heterozygous loci, baseline noise, stutter ratio and amplification artifacts. These criteria will be evaluated through the analysis of single-source human DNA samples as well as a mixture series.

Additionally, a set of four challenged samples prepared by Promega were evaluated using the Identifiler®, 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.

A primary goal of this study is to provide an overview of key performance measures of Promega's PowerPlex® 16 HS compared to the eight commercially available STR kits that were previously studied by the NFSTC. 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.

Experimental Design:
This evaluation followed the procedure outlined below:

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 normalize 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
5. Quantify after dilutions using Quantifiler Human Kit on the AB 7500 RT PCR instrument and normalize the results with the NIST quantitation standards.
6. Amplify the appropriate amount of each sample with each of the following kits: Promega's PowerPlex® 16 HS system using manufacturers' recommended amplification conditions.
    - PowerPlex 16 HS (32 cycles) 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 16 HS (28 cycles) 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); 18 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.
7. Prepare and run samples on the 3130*xl* Genetic Analyzer using manufacturer's recommended running conditions.
    - Promega kits: 3 kv, 10 sec injections, 9.5 µl Form, 0.5 µl ILS 600, 1 µl sample
8. Analyze 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.

Standards, Controls and Samples Interrogated During Evaluation:

- Standards from 2 male donors
- NIST SRM 2372 Quantitation Standards
- Amplification positive (9947A)
- Amplification Negative
- PowerPlex 16 HS Allelic Ladder

- GS 500 - LIZ
- PowerPlex® Standards

Product(s) Specifications:

Brief description of Product(s)/Technology/Procedure being evaluated:

Product Name(s)	**Model Number:**	**Serial/Lot Number:**	**Dimensions:**
PowerPlex 16 HS			
	Cost:	**Weight:**	**Power Req.:**
	2195/100rxns		
Storage Conditions:	**Frozen**		
Operational Conditions:	**N/A**		
Associated costs: *(consumables, maintenance, etc.)*	**N/A**		

Evaluation

Instrument Setup Performed by*:*

____ Manufacturer
____ Manufacturer and Evaluator(s)
____ Evaluator(s) Only

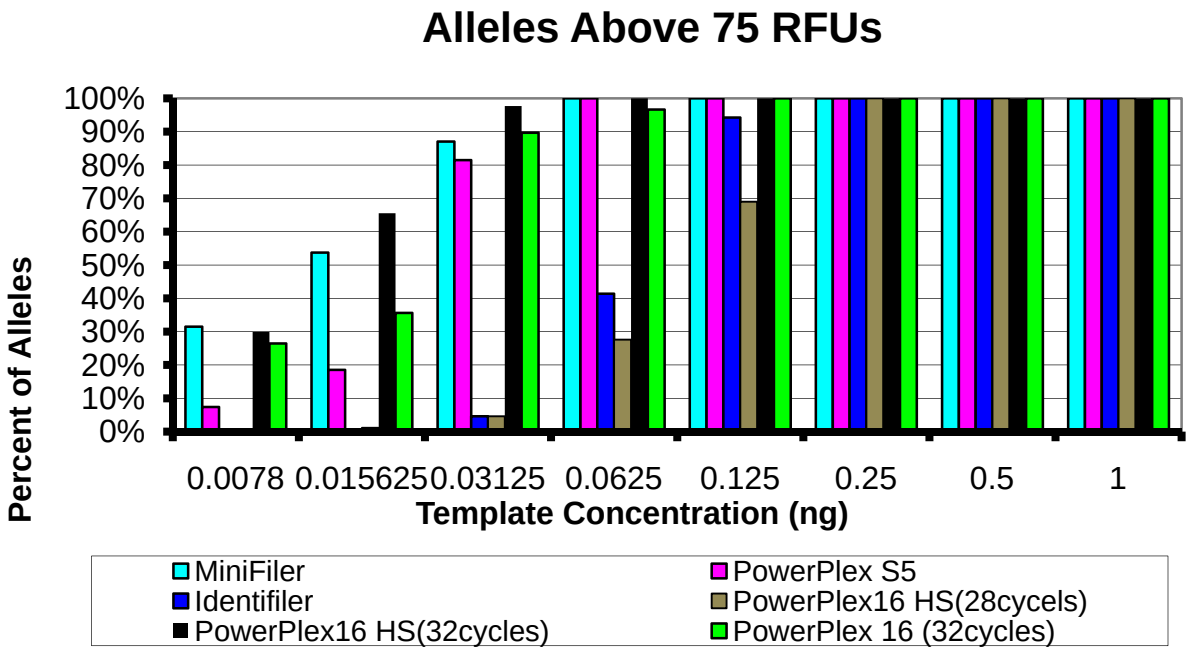
Instrument Setup Comments:

N/A

Level of Operator Knowledge as Set by Manufacturer:

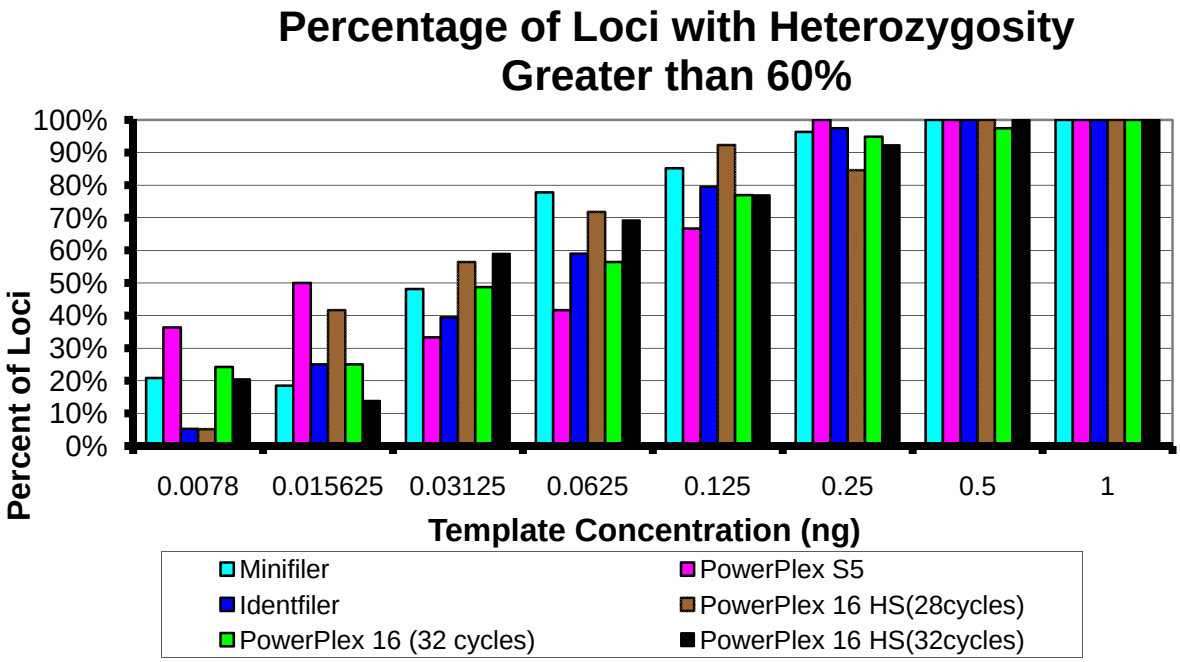
____ Non-Scientist
____ Technician
____ Scientist

Results of Evaluation (Tables, Graphs)



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 minimum of 60% heterozygosity.
    - 0.25 ng concentration corresponds with the following peak height ranges:
        - PowerPlex® 16 HS (28 cycles): 40 to 333 RFUs
        - PowerPlex® 16 HS (32cycles): 341 to 4065 RFUs

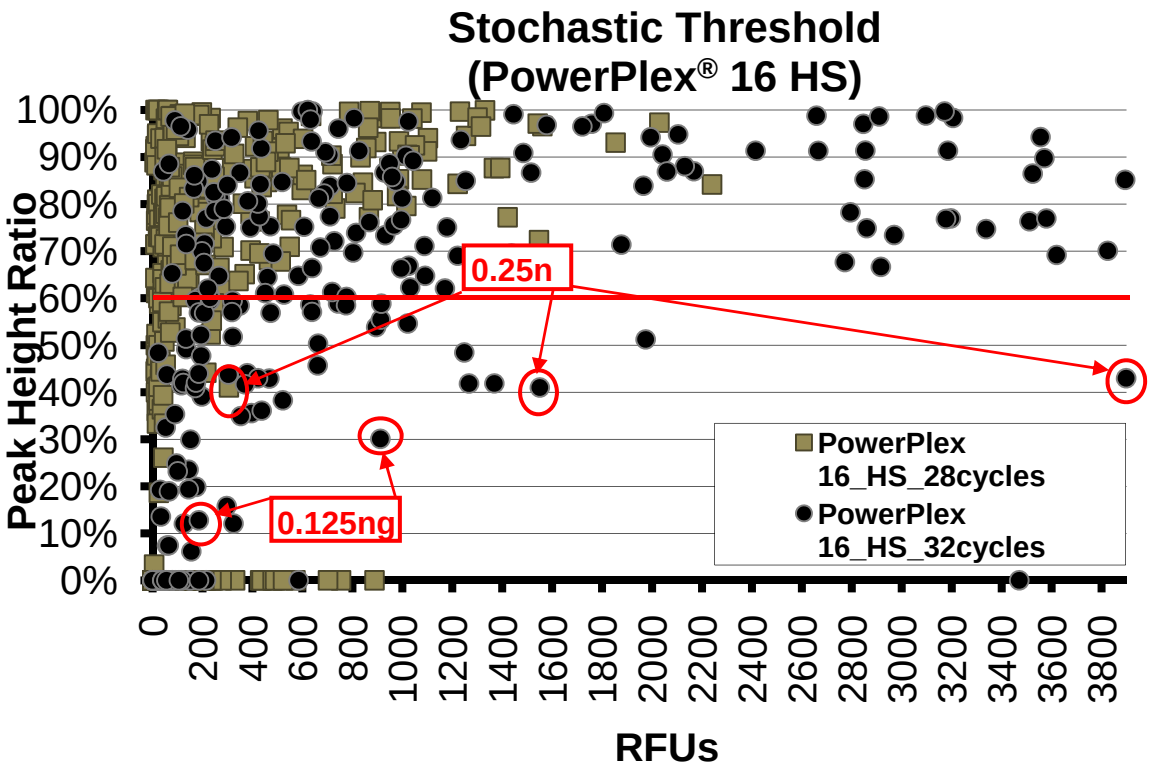
Amplification Artifacts:

- Two dye blobs were present in the PowerPlex® 16 HS system.
- Elevated stutter was observed at vWA for concentrations as low as 0.03125 ng for PowerPlex® 16 HS (32 cycles).
- Elevated stutter was observed at vWA with PowerPlex® 16 HS (28 cycles).
- Indications of plus stutter were observed at lower concentrations for PowerPlex® 16 HS (32 cycles) and were greater than 75 RFUs at concentrations of 0.25 ng and higher.
- Indications of plus stutter 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.

Baseline Noise:

- PowerPlex® 16 HS (32 cycles) amplification kit displayed background noise with the limit of detection (LOD) at approximately 17 RFUs.
- The limit of quantitation (LOQ) is approximately 35 RFUs.

Stochastic Threshold:



Mixtures:

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

Percentage of Alleles 75 RFUs and above					
1:5 mix	1:8 mix	1:10 mix	1:12 mix	1:15 mix	1:20 mix
100%	90.48%	77.55%	76.19%	68.03%	66.67%

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%

Post-Evaluation Findings

Strengths/Results:

- PowerPlex® 16 HS (32 cycles) yielded the highest RFU values for all the amplification kits.
- PowerPlex® 16 HS (28 cycles) outperformed MiniFiler™ and Identifiler® amplification kit for the challenged samples.
- PowerPlex® 16 HS (28 cycles) performed similar to Identifiler® with regards to peak heights at each concentration, heterozygosity loss at the concentration of 0.25 ng, and alleles below 75 RFUs at 0.125 ngs.

Areas for Improvement:

- PowerPlex® 16 HS displayed a higher baseline noise than all other amplification kits.
- Heterozygosity for PowerPlex® 16 HS with 32 cycle yielded more occurrences with less than 60% balance than PowerPlex® 16 HS (28 cycles) as well as other amplification kits.

Limitations of Technology:

- 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 Requirements:

- No additional training is 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 health and safety practices are required.