

Validation of PowerPlex® 18D



FORENSIC SCIENCE

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Abstract

This study sought to validate the performance of the PowerPlex® 18D direct amplification kit (PP18D) for use with single-source FTA® samples.

PP18D was determined to yield full profiles from samples with concentrations as low as 0.4ng/μL. The kit was shown to be precise at all 18 loci. Known samples were collected from LVMPD employees, and all samples were concordant. Seven samples that were amplified and run on different instruments and different days to test reproducibility were also concordant. Other common sample substrates (cotton swabs, Omni® swabs, extracted DNA) were also tested and a full profile was obtained from all samples.

Introduction

The purpose of this study was to ensure that the PowerPlex® 18D kit, when used for direct amplification of FTA® card samples, would produce DNA profiles of acceptable quality to be entered into CODIS.

This study will impact the forensic science community by showing the reliability of a new technology that can potentially save a significant amount of time and money during the production of forensic DNA profiles. It will also provide other laboratories considering adopting direct amplification with a general internal validation scheme.

Materials and Methods

- Samples collected using Whatman® EasiCollect™ devices

- Punches of the FTA® cards taken using the Harris 1.2mm Manual Punch

- Half reaction amplification (7.5μL water, 2.5μL 5X Master Mix, 2.5μL 5X Primer Pair Mix per sample)

- 27 cycle amplification on Applied Biosystems GeneAmp® PCR System 9700 thermal cyclers

- Capillary electrophoresis on Applied Biosystems 3130xl Genetic Analyzers

- Applied Biosystems GeneMapper® ID-X v1.1.1 used for data analysis

Sensitivity and Stochastic

Calvin	Rio	Luxor
5 ng	5 ng	5 ng
4 ng	4 ng	4 ng
3 ng	3 ng	3 ng
2 ng	2 ng	2 ng
1 ng	1 ng	1 ng
0.8 ng	0.8 ng	0.8 ng
0.6 ng	0.6 ng	0.6 ng
0.4 ng	0.4 ng	0.4 ng
0.2 ng	0.2 ng	0.2 ng
0.1 ng	0.1 ng	0.1 ng

- 3kV/5sec for Calvin

- 3kV/3sec for Rio and Luxor

* Due to instrument maintenance, a stochastic threshold is currently being established by LVMPD

Offscale peaks
Good profile
Dropout

Relative sensitivity of three 3130xl genetic analyzers

Database-Type Samples

- Extracts from cotton-tipped swabs using DNA IQ™ with Biomek® NX

$$\frac{\text{Average quant value (0.152ng/}\mu\text{L)}}{\text{Volume added to amp (7.5}\mu\text{L)}} = \text{Average target (1.14ng)}$$

- Full profiles below 5ng recommended input

Known/Non-Probative and Reproducibility

- 65 samples compared to LVMPD staff database

- 7 samples processed on

- 3 separate days
- Different thermal cyclers
- Different genetic analyzers

Concordance

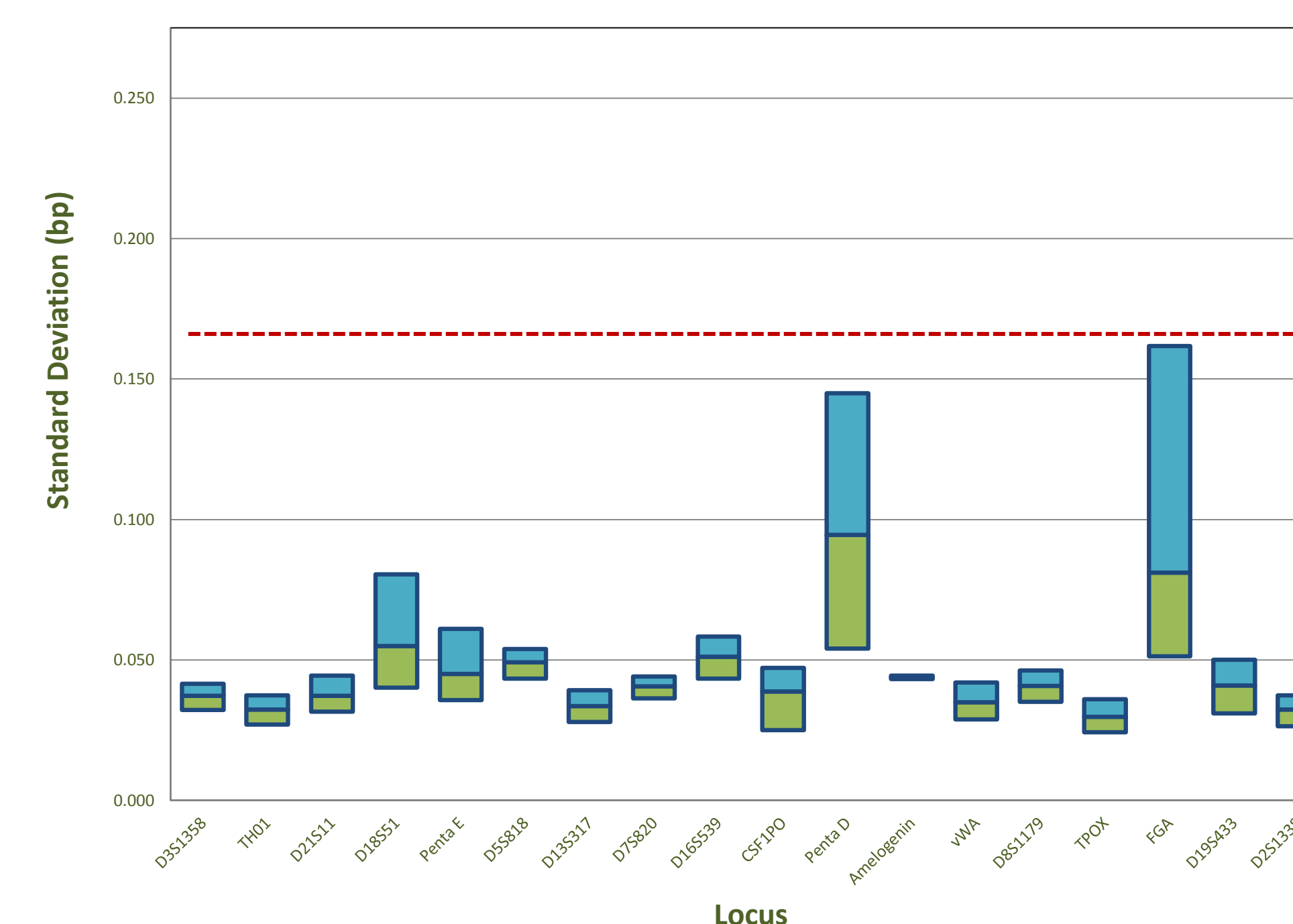
- All profiles obtained in this validation matched a known profile from the same donor

- NIST samples yielded expected profiles

- No null alleles or primer binding site mutations detected

Precision

Overall Basepair (bp) Sizing Precision



Casework-Type Samples

- Manual extraction of buccal swabs
 - PrepFiler™
 - Organic
 - Qiagen
 - Chelex®

- 5ng amplification target

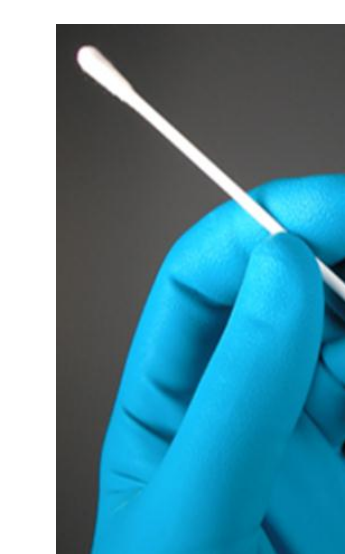
- Full profiles

Sample Type

- Full profile from manual FTA® cards at 27 cycles

- Partial profile from cotton and Omni® swabs at 27 cycles

- Full profile from cotton and Omni® swabs at 28 cycles **without lysis buffer**



<http://www.evidentimescene.com/cata/blood/swabster2.jpg>



http://www.fishersci.com/ecomm/servelet/productdetail_10652_1631910_29104_1_0

Contamination

- All negative controls and reagent blanks contained no evidence of contamination

- One mixed sample – minor peaks found to match neighboring sample; attributed to human error

Conclusions

- PowerPlex® 18D is a robust kit that consistently produced accurate and reproducible results.

- PowerPlex® 18D will be adopted by the LVMPD Biology/DNA Detail for use with database samples.

- Further studies will be done on non-FTA® card samples once the lysis buffer becomes commercially available.

References

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Acknowledgements

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