PowerPlex® Fusion

Overview & Developmental Validation Preliminary Summary

Ann MacPhetridge, Marketing Manager

October 2012

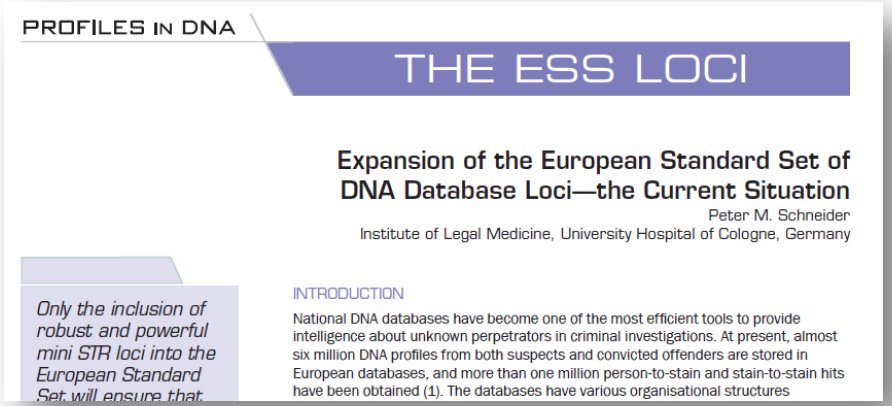
Promega

PowerPlex® Fusion System
Webinar outline

- Expansion of CODIS
- Overview of the PowerPlex® Fusion System
- PowerPlex® Fusion Developmental Validation Data – a summary of work performed to-date by Promega

Expansion of Databases… Expansion of Multiplexes

- ✓ Need for common loci is recognized
- ✓ European Standard Set has expanded
- ✓ CODIS will expand in the coming years
- ✓ Goals:
  - More data sharing
  - Fewer adventitious matches



PROFILES IN DNA

THE ESS LOCI

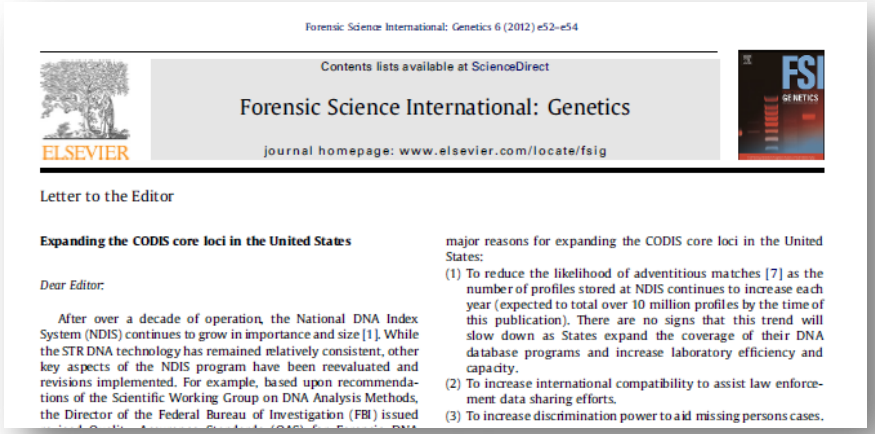
Expansion of the European Standard Set of DNA Database Loci—the Current Situation

Peter M. Schneider
Institute of Legal Medicine, University Hospital of Cologne, Germany

Only the inclusion of robust and powerful mini STR loci into the European Standard Set will ensure that

INTRODUCTION

National DNA databases have become one of the most efficient tools to provide intelligence about unknown perpetrators in criminal investigations. At present, almost six million DNA profiles from both suspects and convicted offenders are stored in European databases, and more than one million person-to-stain and stain-to-stain hits have been obtained (1). The databases have various organisational structures



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Letter to the Editor

Expanding the CODIS core loci in the United States

Dear Editor:

After over a decade of operation, the National DNA Index System (NDIS) continues to grow in importance and size [1]. While the STR DNA technology has remained relatively consistent, other key aspects of the NDIS program have been reevaluated and revisions implemented. For example, based upon recommendations of the Scientific Working Group on DNA Analysis Methods, the Director of the Federal Bureau of Investigation (FBI) issued

major reasons for expanding the CODIS core loci in the United States:

(1) To reduce the likelihood of adventitious matches [7] as the number of profiles stored at NDIS continues to increase each year (expected to total over 10 million profiles by the time of this publication). There are no signs that this trend will slow down as States expand the coverage of their DNA database programs and increase laboratory efficiency and capacity.
(2) To increase international compatibility to assist law enforcement data sharing efforts.
(3) To increase discrimination power to aid missing persons cases.

Supporting Expansion of Core Loci
Product Requirements

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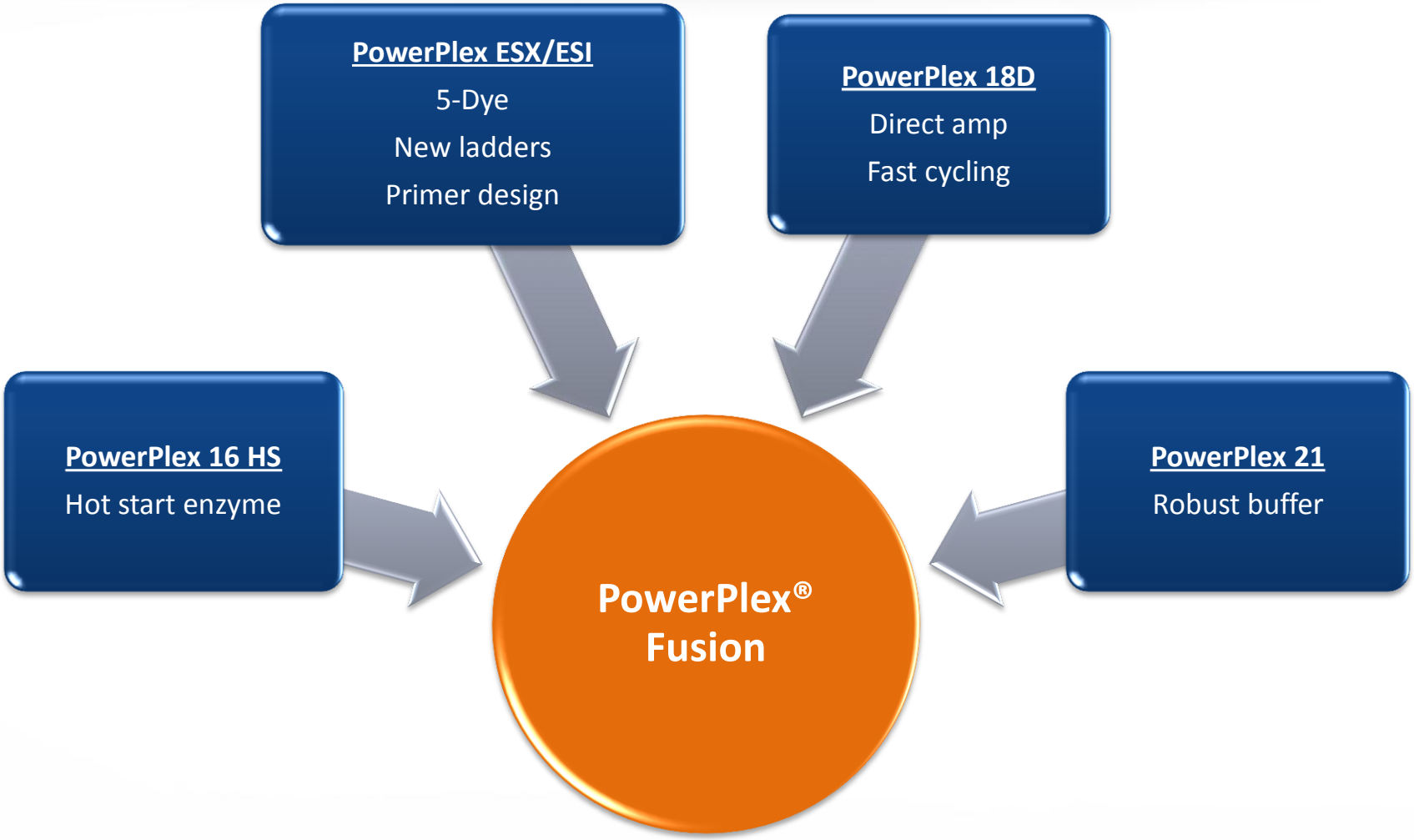
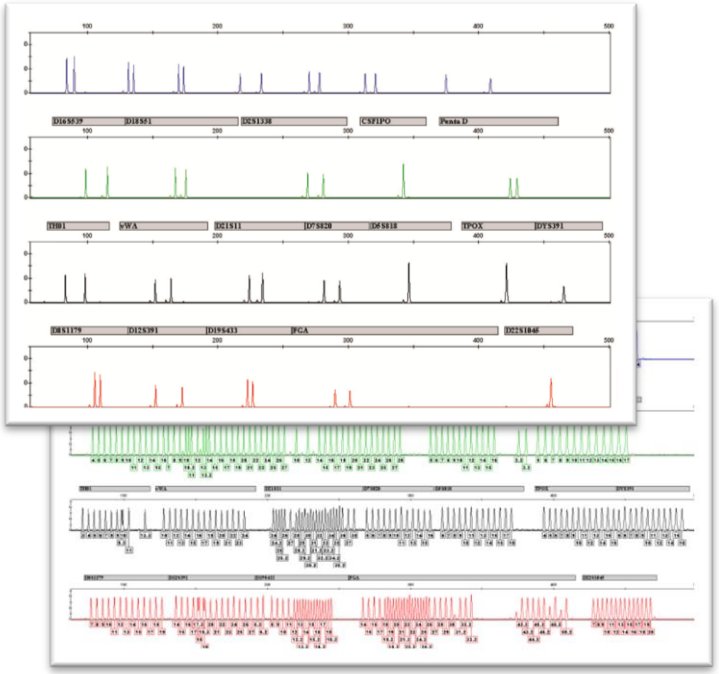
1. Include all current CODIS and ESS loci, plus Amelogenin
   - US SWGDAM: include DYS391
2. Support current CE instruments and software
   - 5-dye chemistry proven on the 31xx and 3500 Genetic Analyzer
3. Enable fast cycling
4. Create one kit for all labs to simplify validation, purchasing, etc.
   - Provide database (i.e. direct amp) and casework protocols

Development Approach
Building on Proven Technology

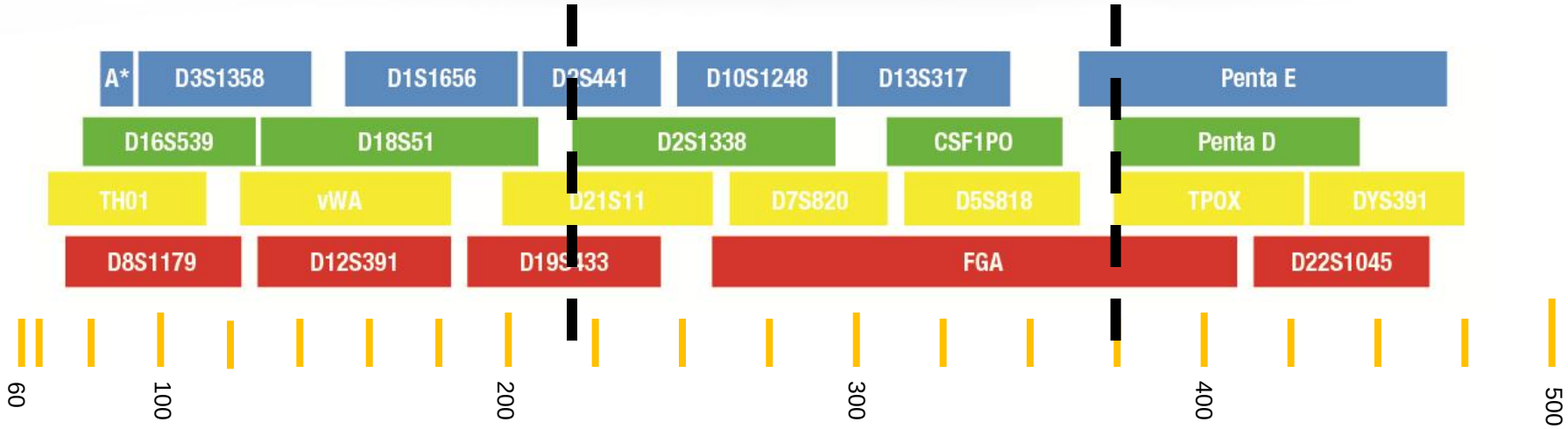


What is the PowerPlex® Fusion System?

- Analyzes 24 loci in a single reaction
  - CODIS + ESS + Pentas + D2S1338 + D19 +DYS391 + Amelogenin
  - Highest discrimination
- Uses rapid PCR technology (~85 min)
- Offers casework *and* database protocols ***in one kit***
  - One kit to validate, purchase & QC
- Designed to work with 31xx CE
  - No 6-dye upgrade required



PowerPlex® Fusion System
Locus placement



Size (bp) * Amelogenin

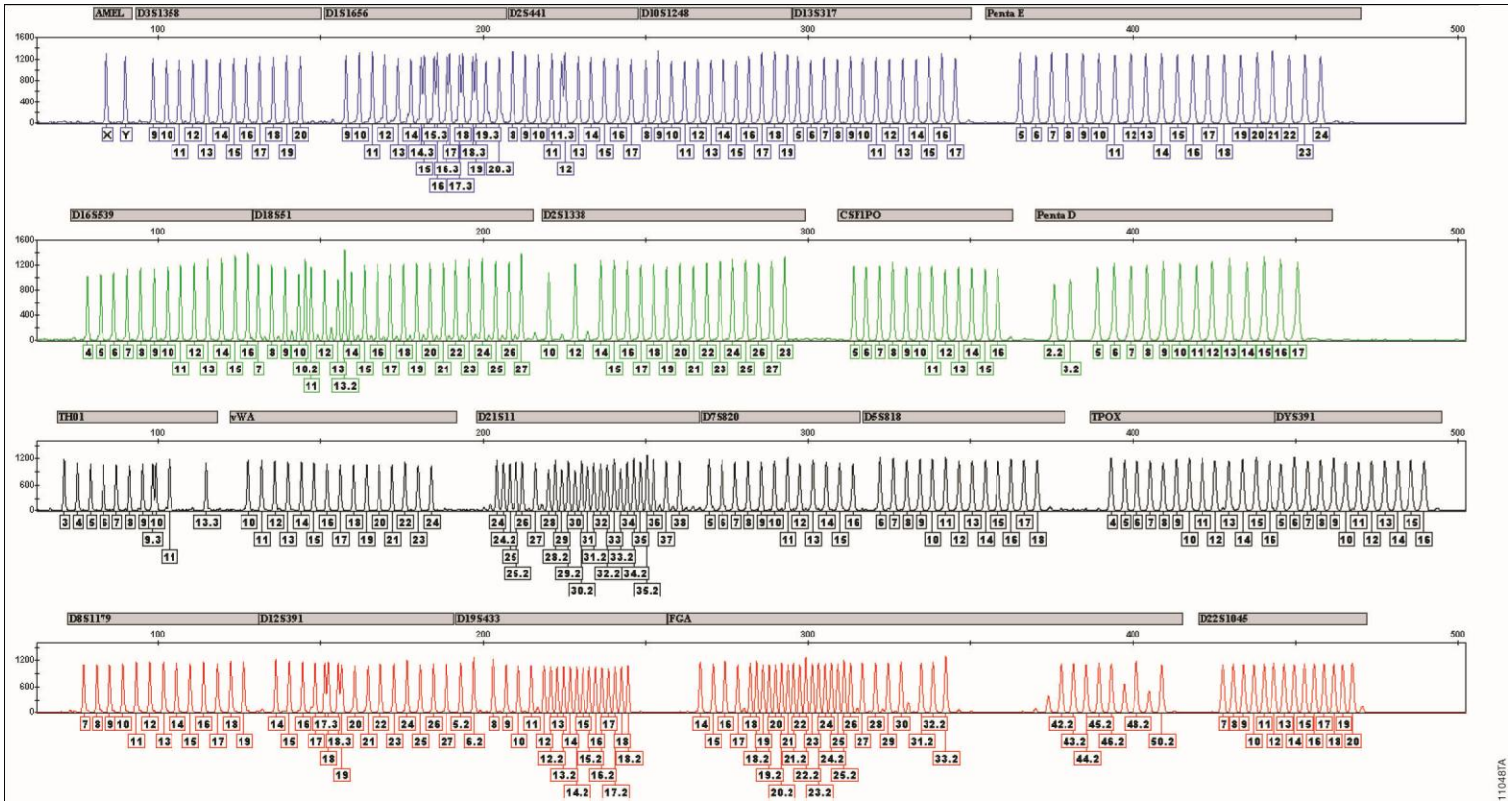
Most essential loci located below ~400bp; nine under 220bp

- Excluding “extended” FGA alleles, the most informative required loci are <375bp
- All required “new CODIS loci” (“Panel A”)
- 2 of 3 “recommended” loci, TPOX and D22 (“Panel B”) -- no SE33
- Pentas

Locus Selection
Inclusion of Pentas

- Straightforward inclusion in multiplexes because of “size range” (allele range)
- Commercially-available for more than a decade
- Easy mixture interpretation due to low stutter
- High discrimination added by two highly polymorphic loci
- Used routinely in Asia, Europe, North and South America
  - >1M samples in US NDIS contain Pentas

Enhanced allelic ladder



- 36 new alleles compared to PowerPlex® 16
- New virtual bins to increase genotyping efficiency
- Fewer inconclusive calls

Configuration and protocol

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Product Overview—Configuration

Kit sizes

- 200 reactions (DC2402)
- 800 reactions (DC2408)

Components

- PRE-AMP: 5X Master Mix (w/ hot start *Taq*), 5X Primer Mix, 2800M Control DNA, Amplification Grade Water
- POST-AMP: Size standard (ILS 500), Allelic Ladder

Related Products

- 5-dye Matrix for AB 31xx & 3500 (DG4700)
- GeneMapper ID & ID-X panel/bin/stutter file downloads (free)

Supported Instruments

Thermal Cycler:

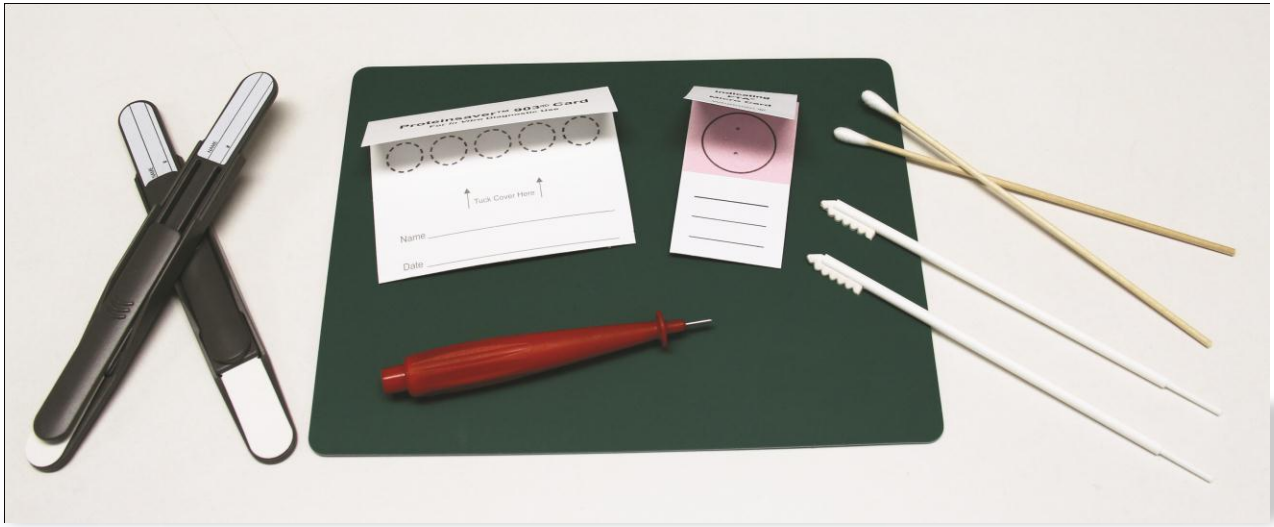
- GeneAmp 9700

Capillary Electrophoresis:

- 31xx Genetic Analyzer
- 3500 Genetic Analyzer

Usable Sample Types

- Extracted DNA
- Direct amplification from solid support materials
  - FTA® and Indicating FTA® cards
  - Swabs – Cotton, Omni Swab [treated with SwabSolution™]
  - Bode Buccal DNA Collector™ [treated with PunchSolution™]
  - S&S 903 Specimen Collection paper [treated with PunchSolution™]



PowerPlex® Fusion Protocol

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PowerPlex® Fusion System

Extracted DNA
OR punch
OR swab extract

Reaction Assembly
- 5X Master Mix
- 5X Primer Mix
- 15µl volume available

Amplification: <1.5 hours

1 cycle	96°C for 1 min
30	94°C for 10 seconds
cycles:	59°C for 1:00min
	72°C for 30 seconds
	60°C for 10 minutes
	4°C soak

Detection and Analysis
- 5 Dye Chemistry (G5)
- 3130/3130xl or 3500/3500xl

Protocols

Extracted DNA, FTA® punches, NonFTA punches, Swabs

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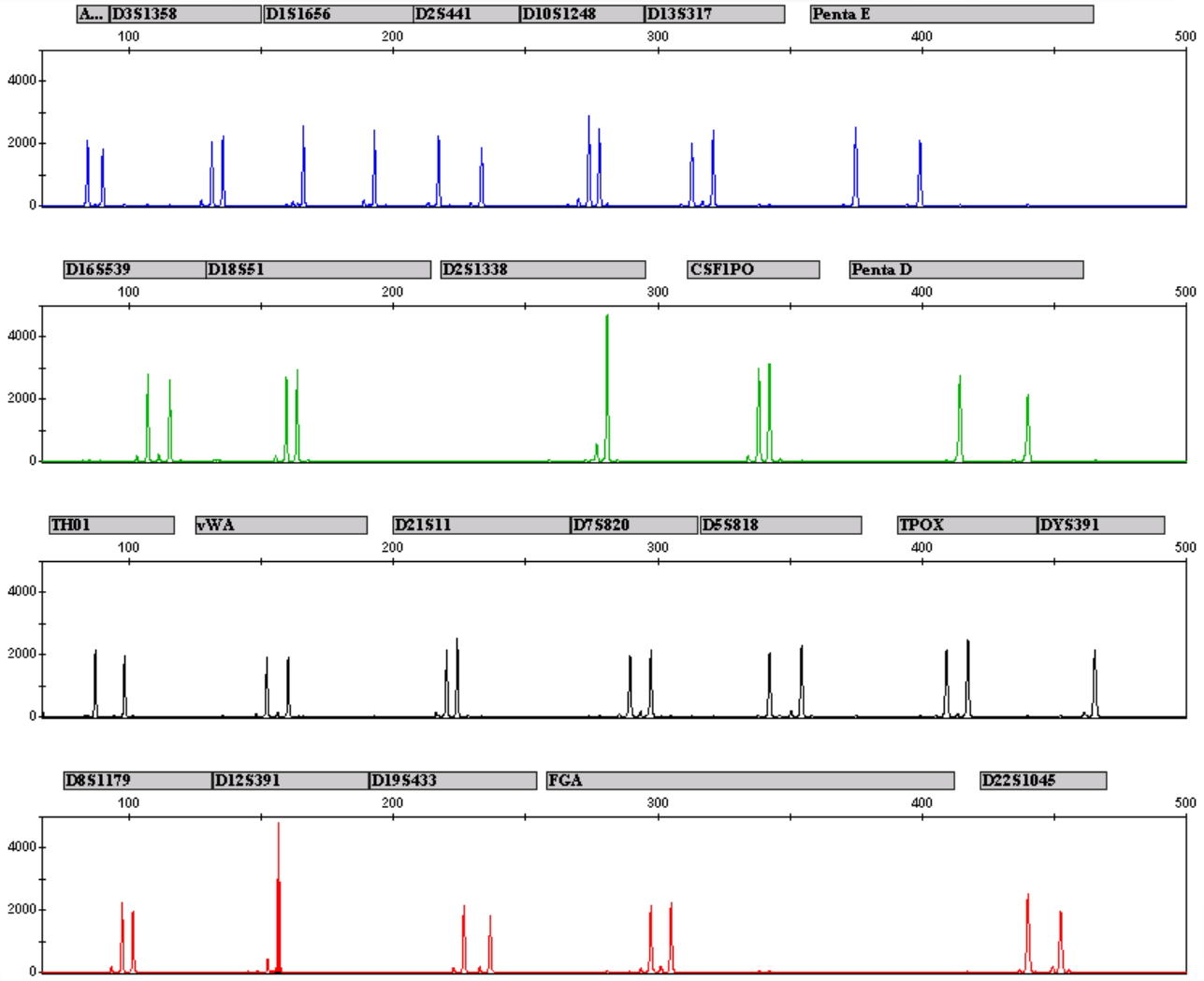
Extracted DNA	FTA punches	NonFTA Punches	Swabs
Extract DNA from sample	Punch FTA® into plate	Punch card into plate, treat with PunchSolution™	SwabSolution™ incubation of swab
Add up to 15µl of sample into reaction	Add reaction mix	Add reaction mix to dried punch	Add 2µl of extract into reaction mix

Cycling time ~85 minutes for all applications

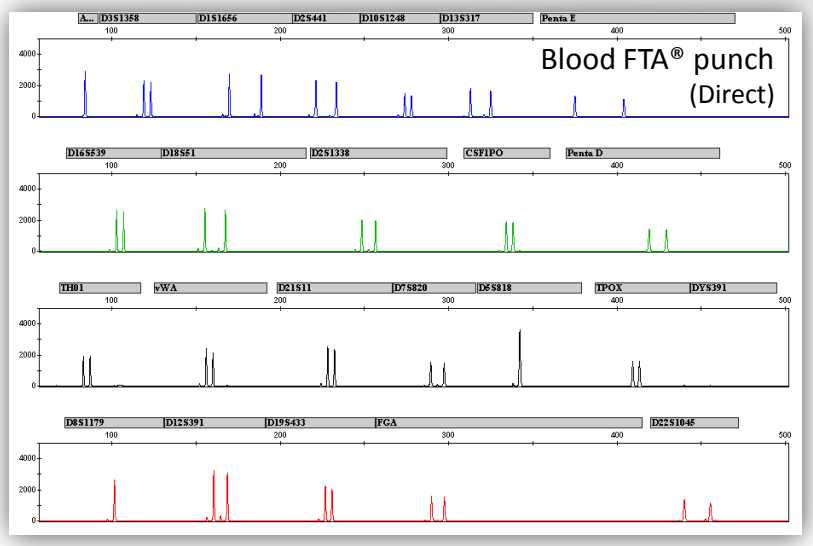
Extracted DNA
0.5ng

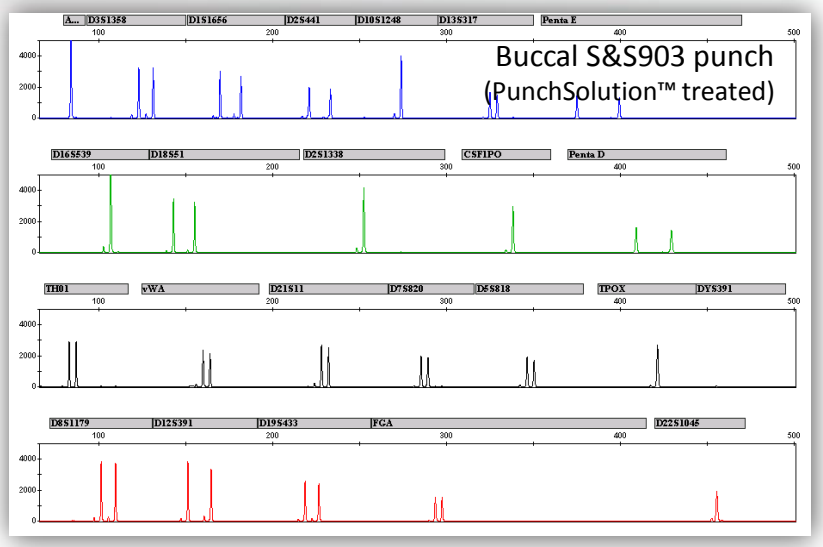
3130xl

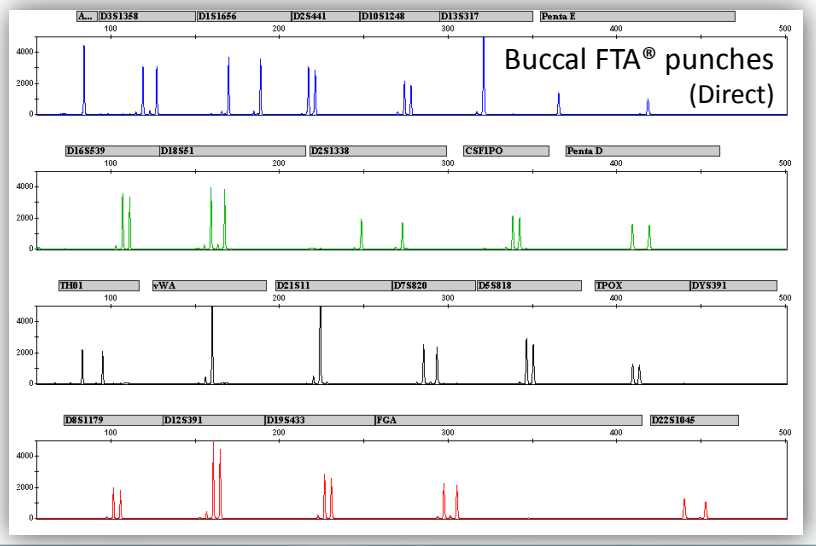


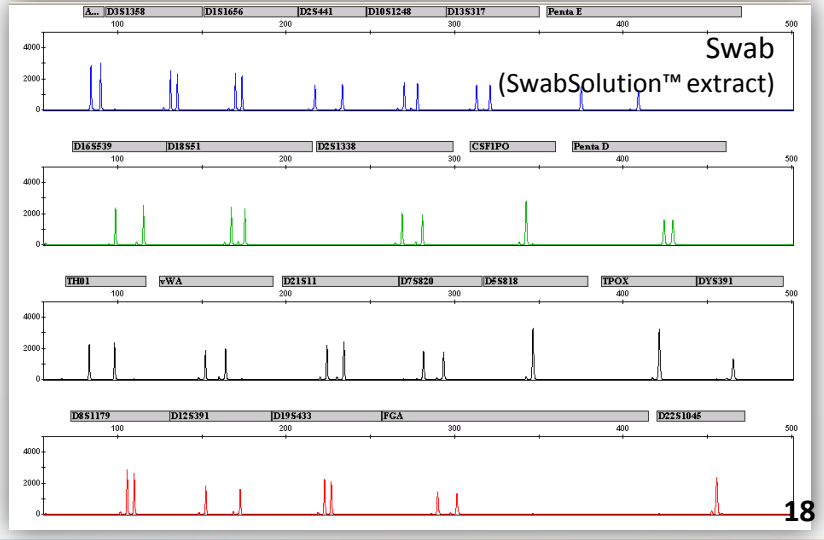
3kv 5s

Example "Direct Amp" Data

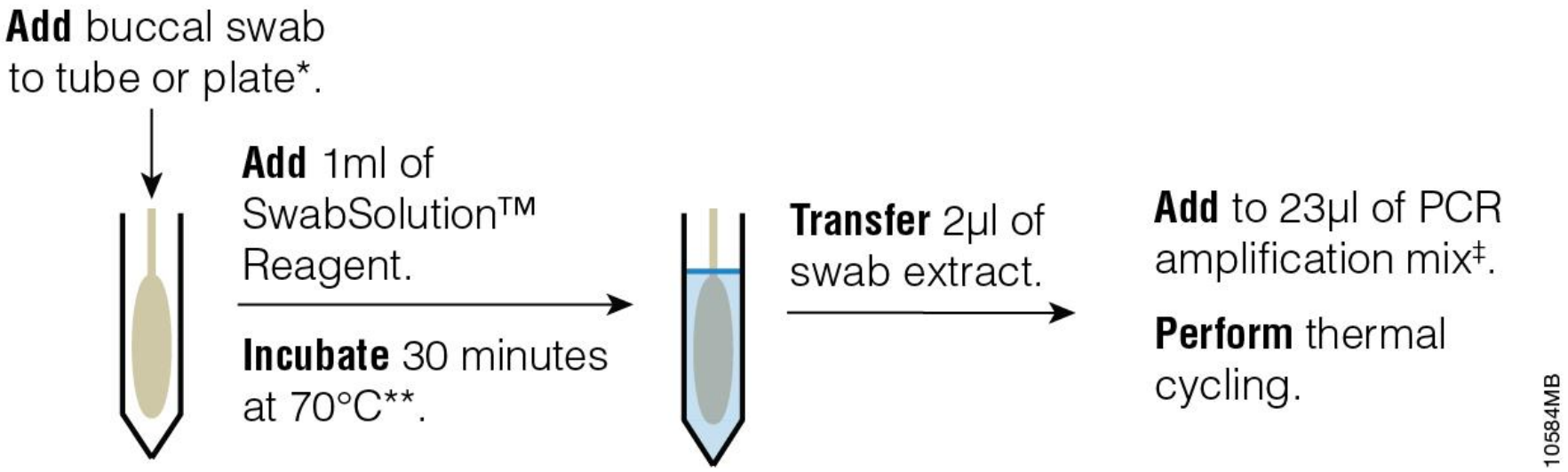








Buccal Swab Workflow



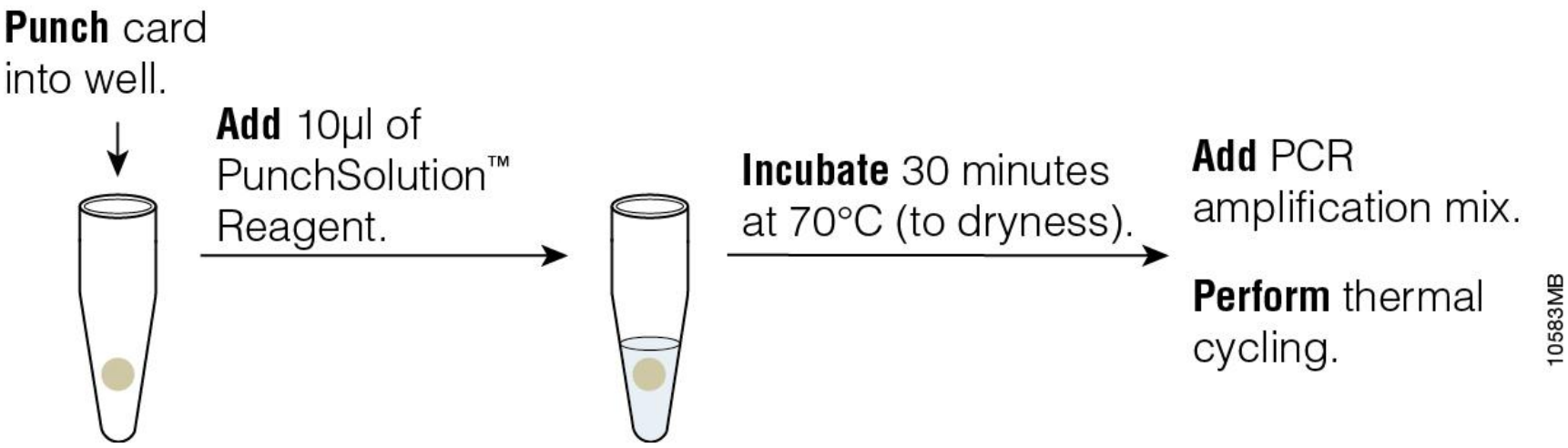
Simple, rapid process for all swabs

* *For plate format, use 2.2mL deep well plate (V6781)*

** *Use new heat transfer block (A2661)*

‡ *For reactions other than PowerPlex® 18D and PowerPlex® 21, add AmpSolution™ Reagent*

NonFTA® punch workflow
S&S903, Bode Buccal DNA Collector™ Device



Rapid, same-plate processing of non-FTA® punches

Performance

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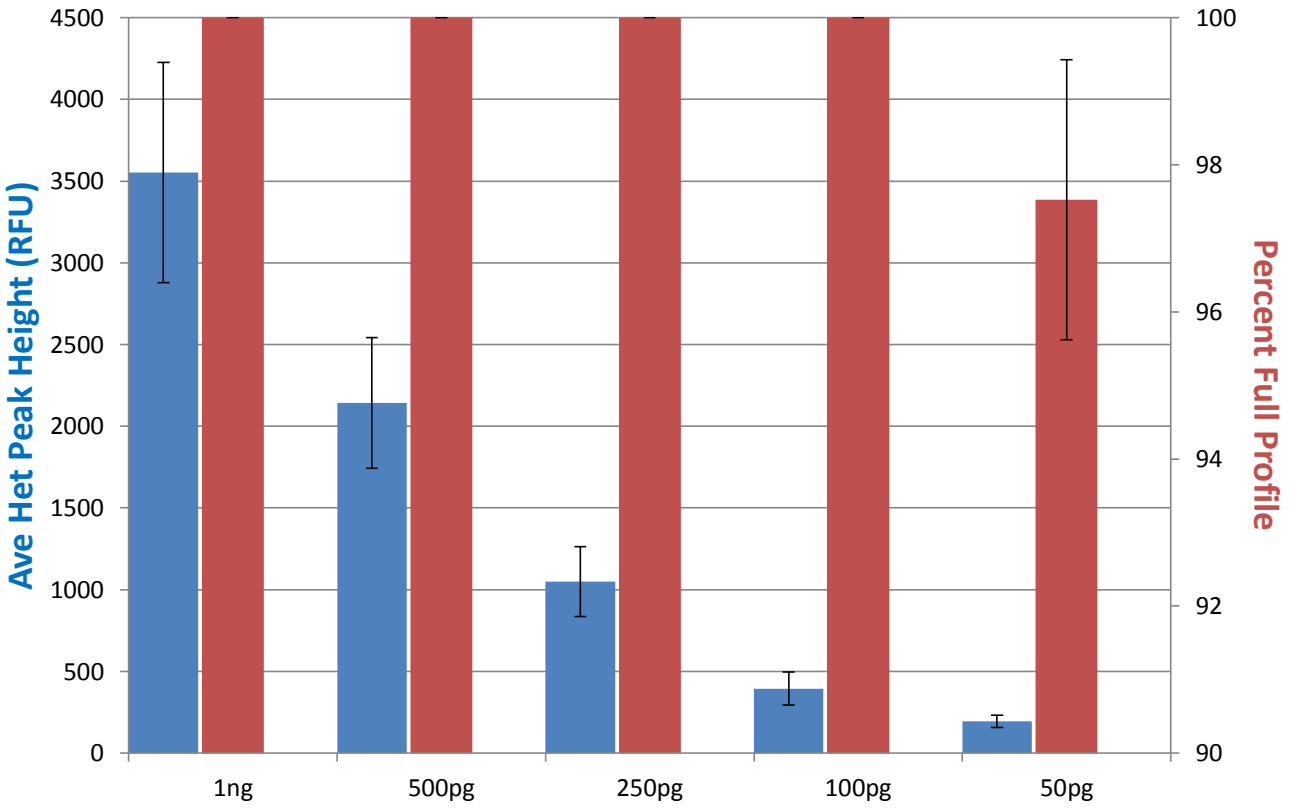
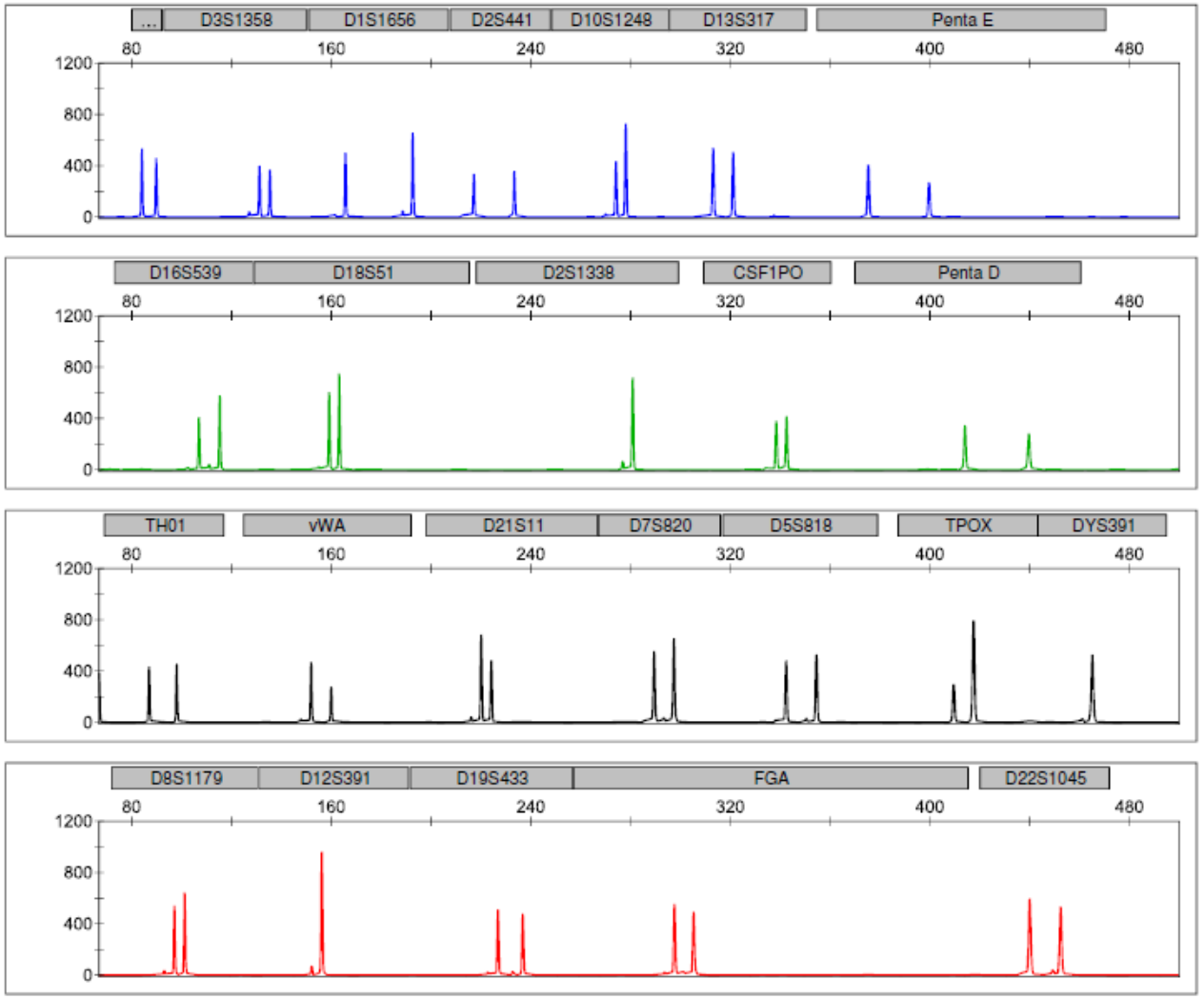
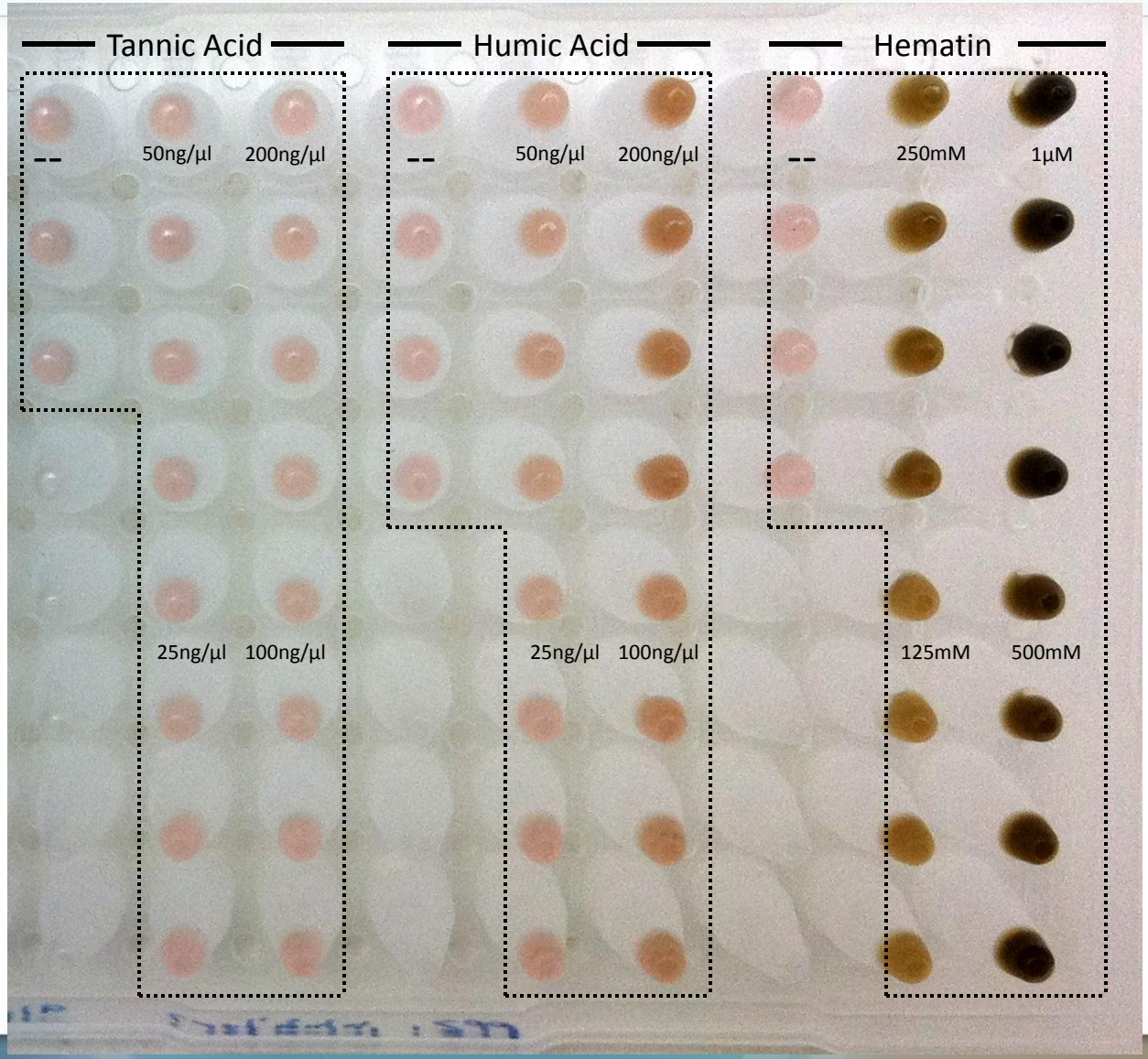
Sensitivity
Titration of Purified DNAs



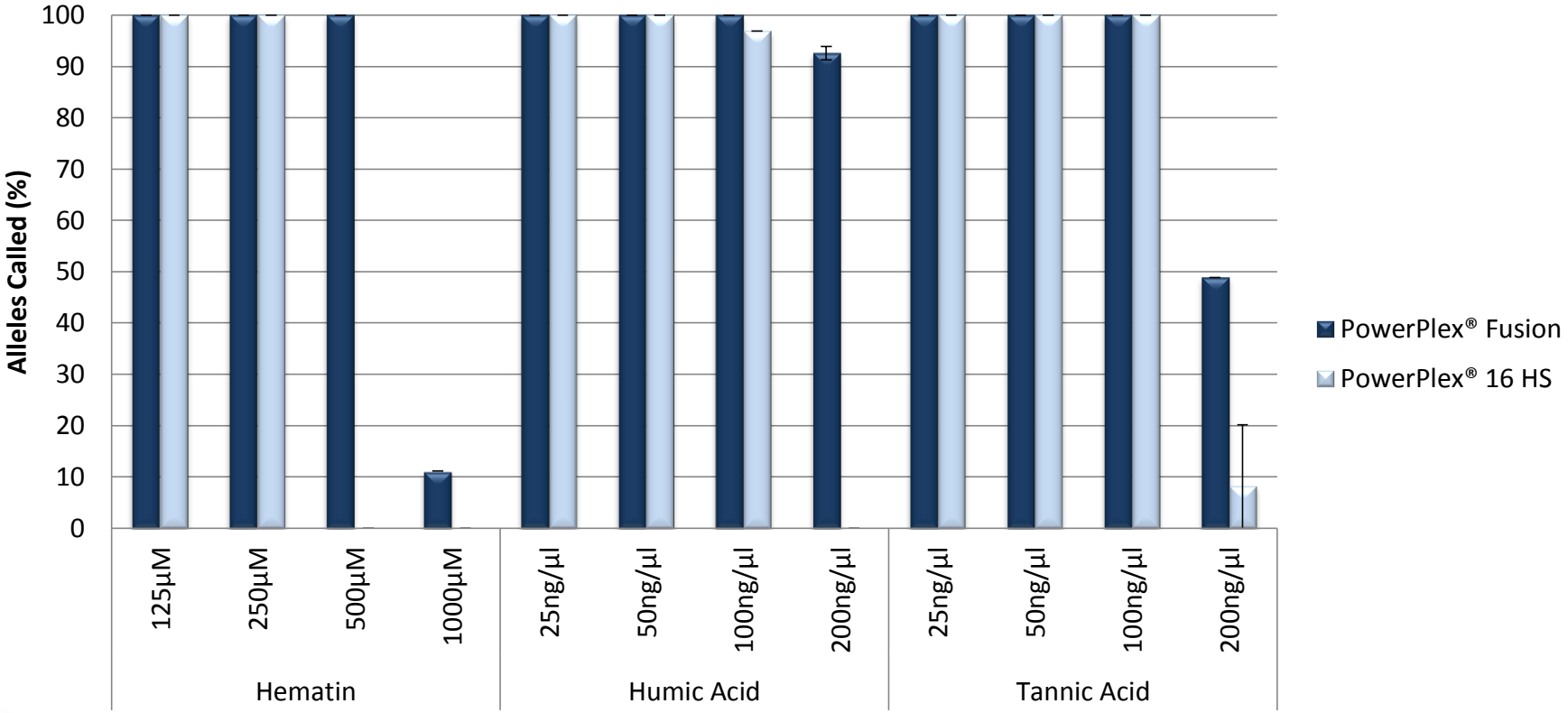
Sensitivity
100pg Amplification



PowerPlex® Fusion Inhibitor Titration



Inhibitor Tolerance
Titration of Common Forensic PCR Inhibitors



500pg of DNA (n=3) was amplified with each kit in the presence of the listed inhibitors.

PowerPlex® Fusion Developmental Validation Data

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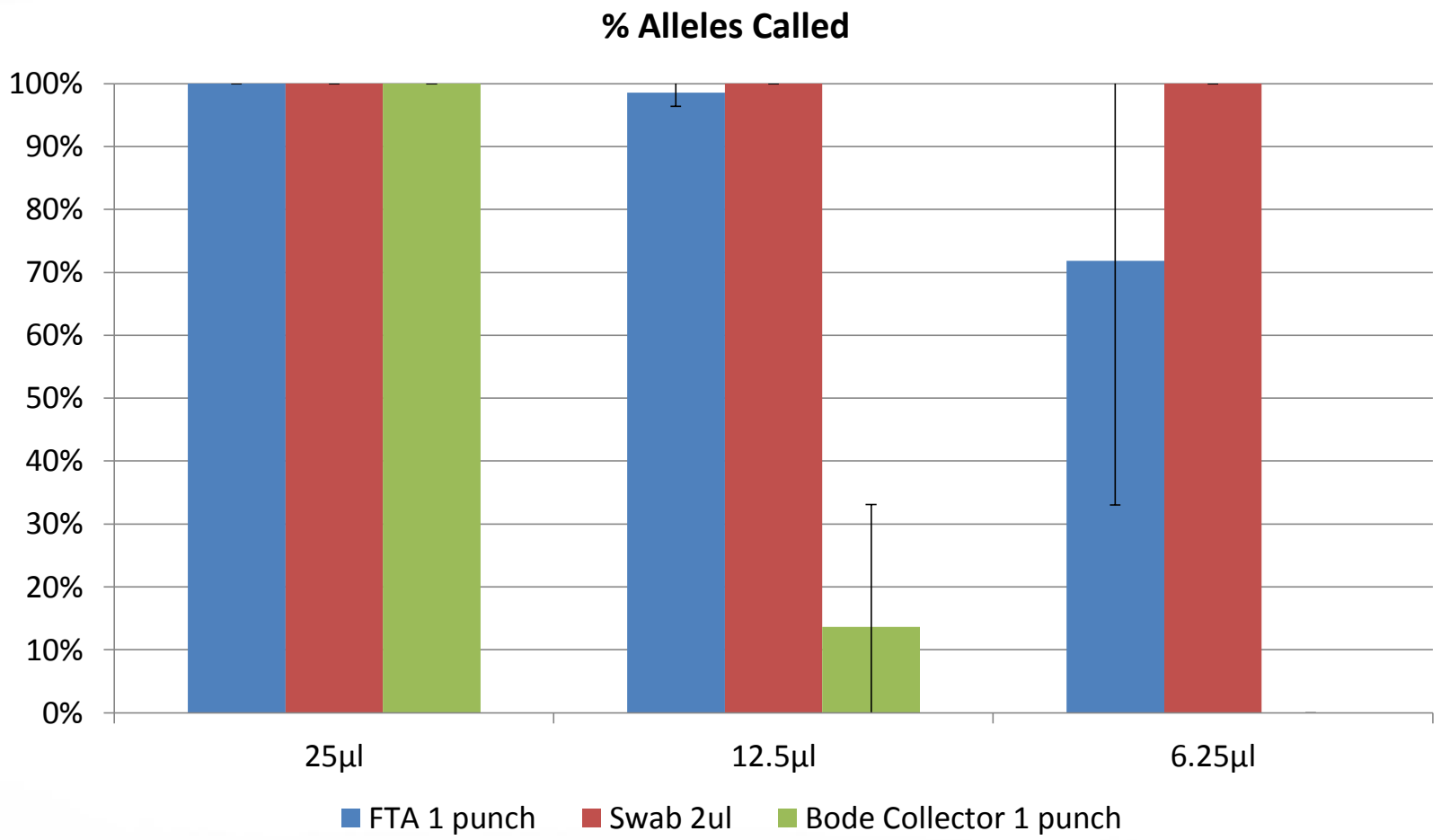
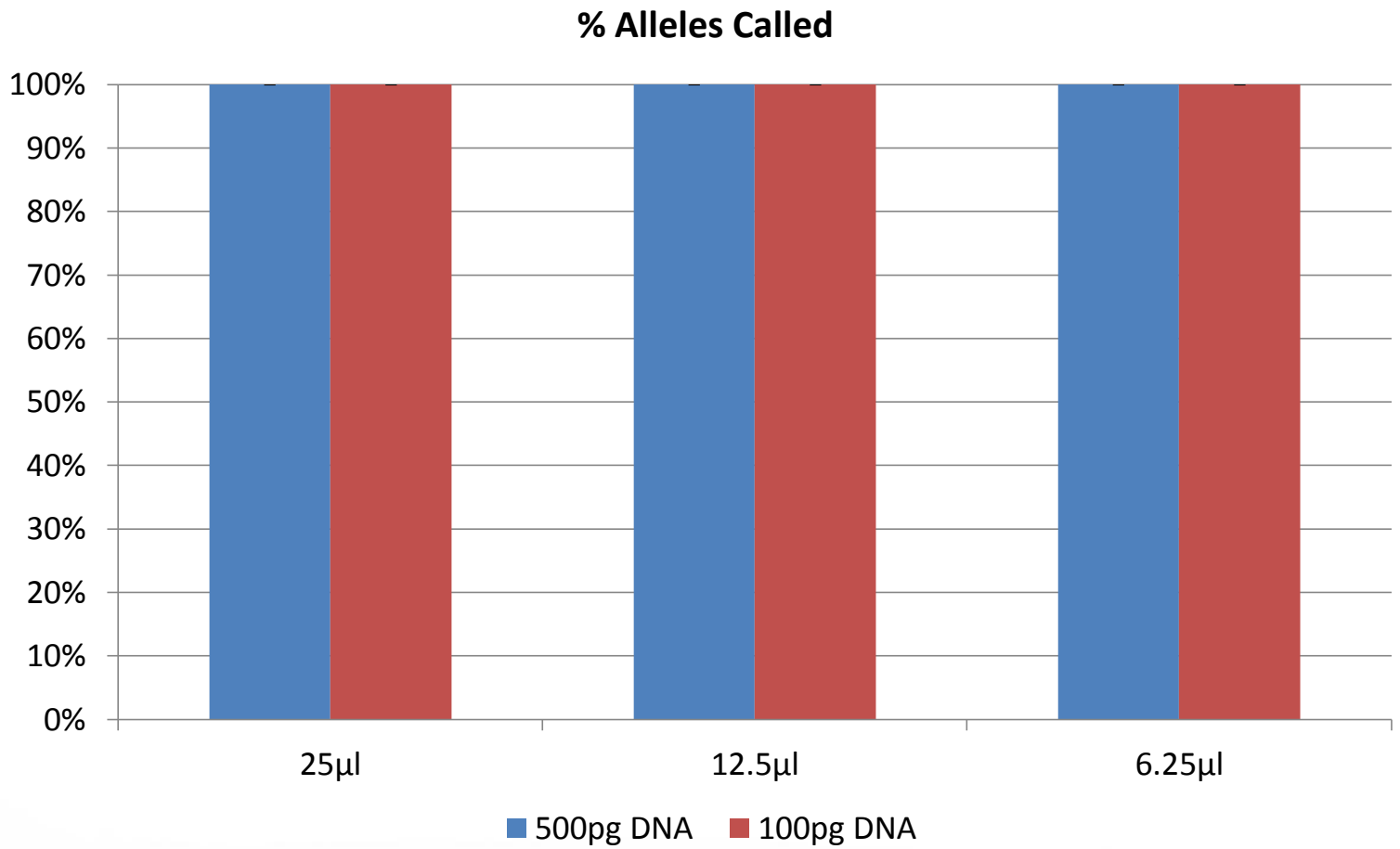
Developmental Validation – Preliminary data
Reaction Volume – Solid Support Samples



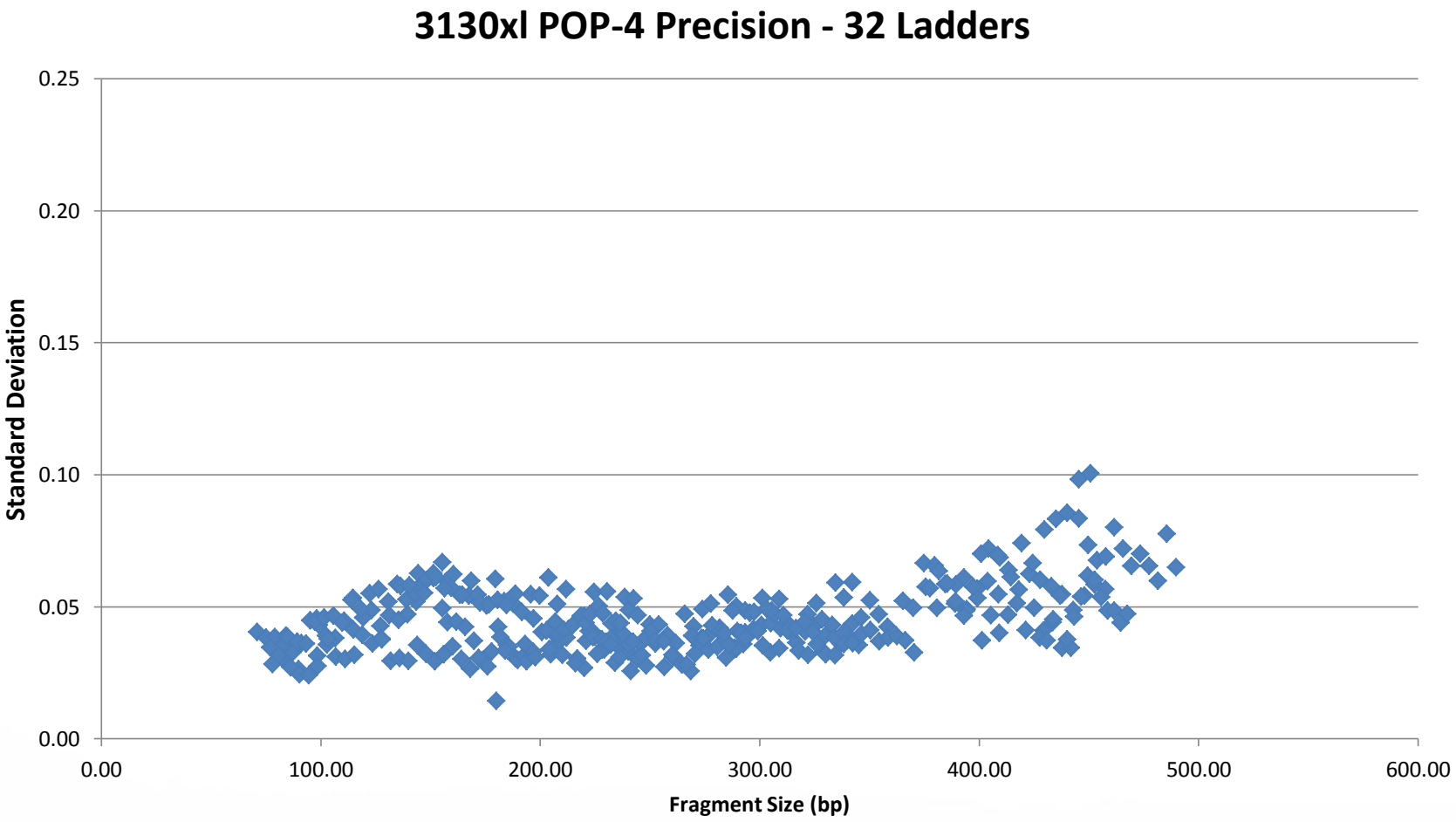
N = 3

Developmental Validation – Preliminary data
Reaction Volume – Extracted DNA

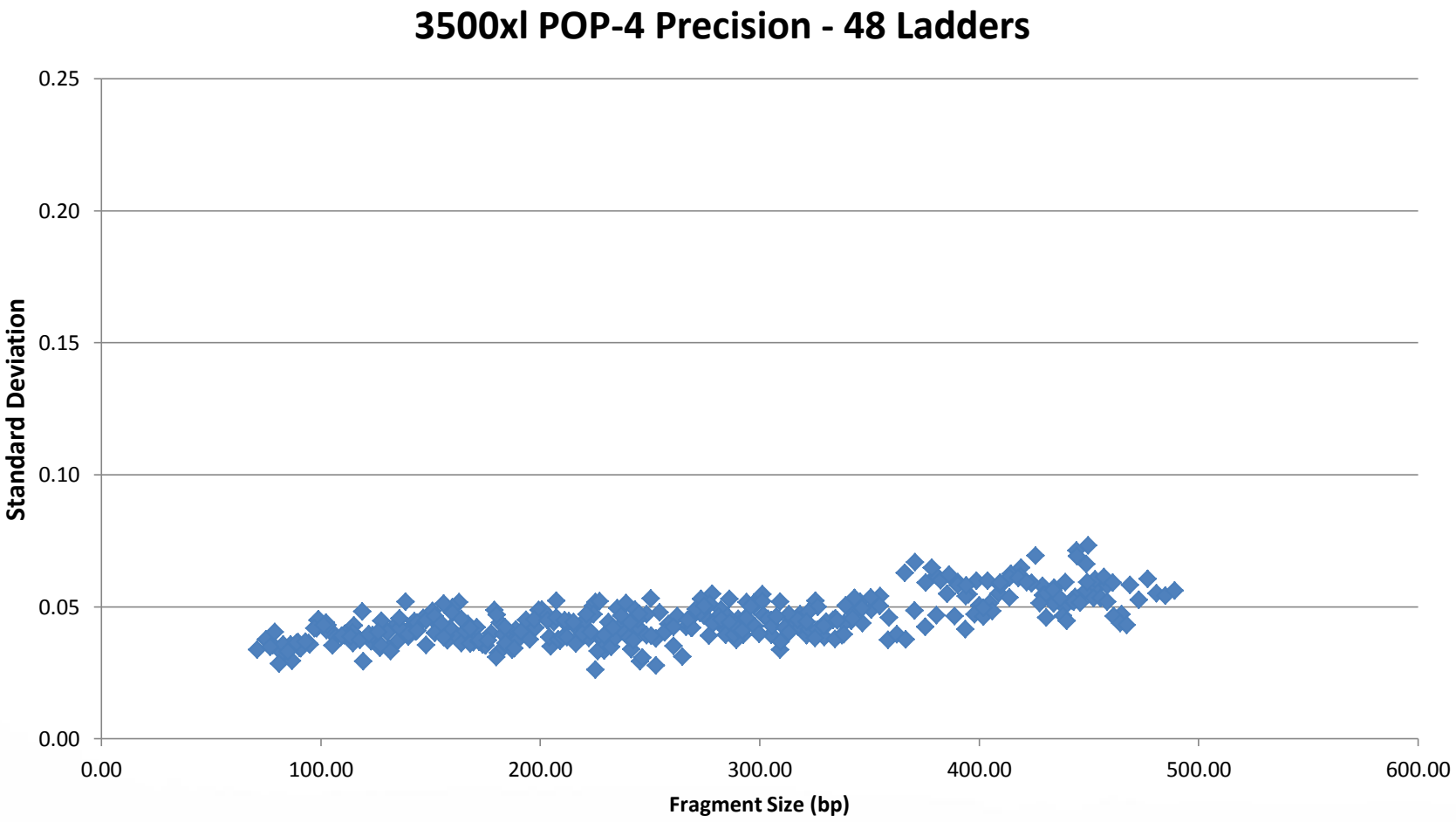


N = 3

Developmental Validation – Preliminary data
Precision Studies AB 3130xl



Developmental Validation – Preliminary data
Precision Studies AB 3500xl



Developmental Validation
Population Study - NIST Concordance Testing

- PowerPlex® Fusion results compared to all other kits tested including: Sinofiler/NGM/Identifiler/Yfiler/IDplex/ESSplex/PP16/PP21 kits with **652 unrelated individuals** (NIST U.S. population set)
- Fusion is **fully concordant with NIST SRMs 2391b&c** certified values
- No PP Fusion **null alleles**
- No PP Fusion **discordance** with other PowerPlex kits, discordance with ABI or Qiagen kits is on their end and are previously documented

Developmental Validation
Population Study - Probability of Identity Values

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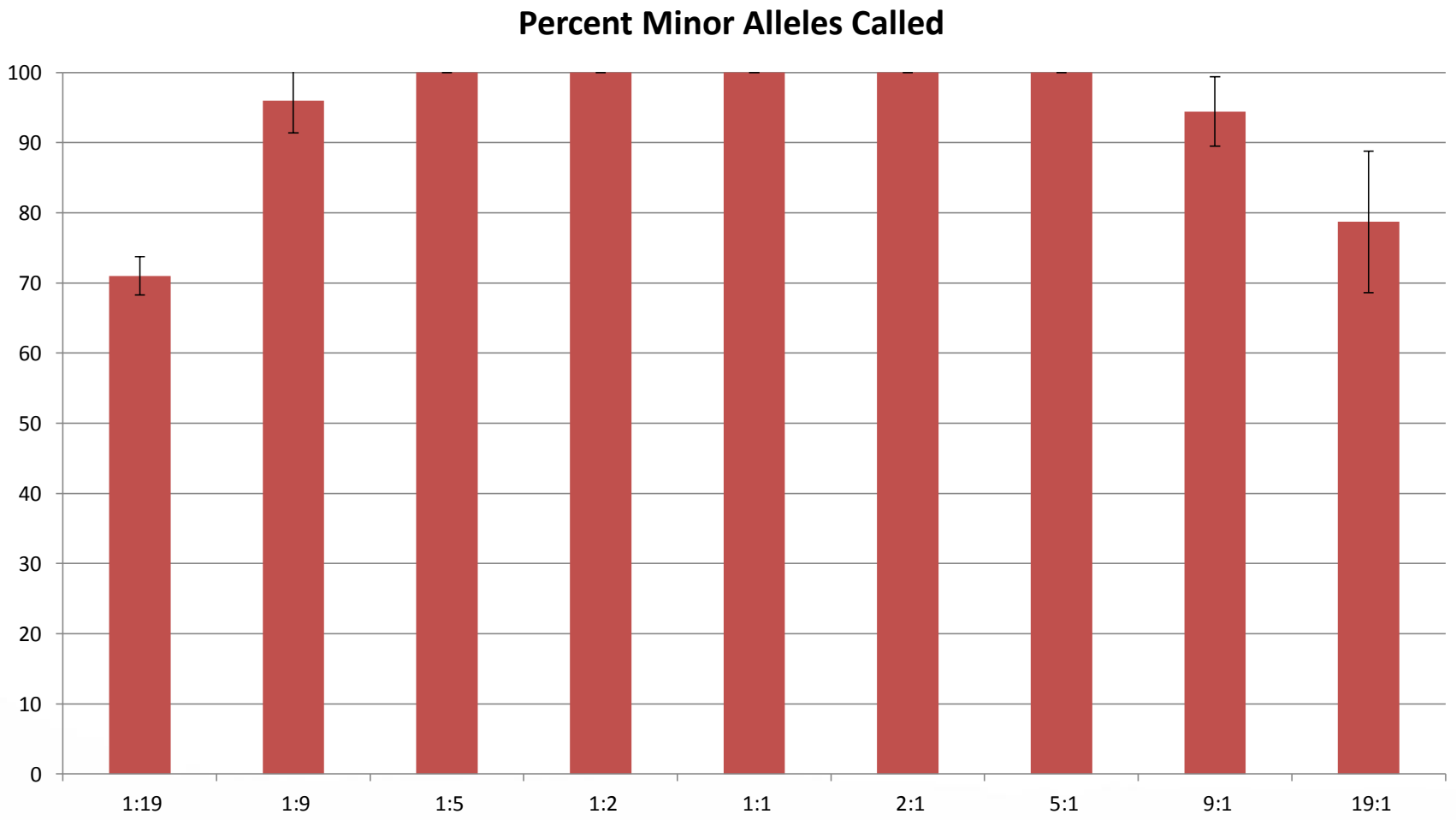
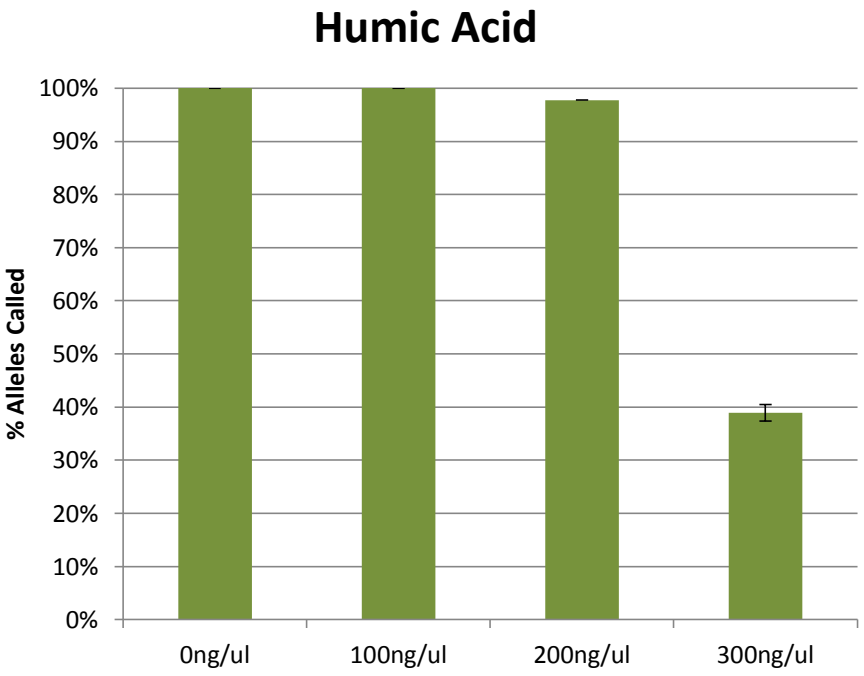
STR Typing Kits (Locus Combinations)	**Total (N=1036)**	African Americans (N=342)	U.S. Caucasians (N=359)	U.S. Hispanics (N=238)	U.S. Asians (N=97)
CODIS 13	**5.02E-16**	**1.13E-15**	**3.01E-15**	**1.39E-15**	**1.71E-14**
Identifiler	6.17E-19	1.03E-18	6.94E-18	2.76E-18	5.30E-17
PowerPlex 16	**2.82E-19**	**6.08E-19**	**4.22E-18**	**1.29E-18**	**2.54E-17**
PowerPlex 18D	3.47E-22	5.55E-22	9.76E-21	2.58E-21	7.87E-20
ESS 12	2.99E-16	9.14E-16	9.68E-16	2.64E-15	3.41E-14
ESI 16 / ESX 16 / NGM	2.74E-20	6.02E-20	2.21E-19	4.03E-19	9.80E-18
ESI 17 / ESX 17 / NGM SElect	**1.81E-22**	**6.44E-22**	**1.74E-21**	**3.99E-21**	**1.86E-19**
PowerPlex 21	6.71E-27	2.08E-26	2.51E-25	7.96E-26	5.80E-24
CODIS 19 (-DYS391)	1.95E-23	1.08E-22	1.66E-22	1.93E-22	6.01E-21
GlobalFiler (-DYS391)	1.60E-27	5.63E-27	2.95E-26	5.10E-26	2.57E-24
PowerPlex FUSION (-DYS391)	**1.36E-28**	**2.83E-28**	**5.25E-27**	**4.81E-27**	**2.01E-25**

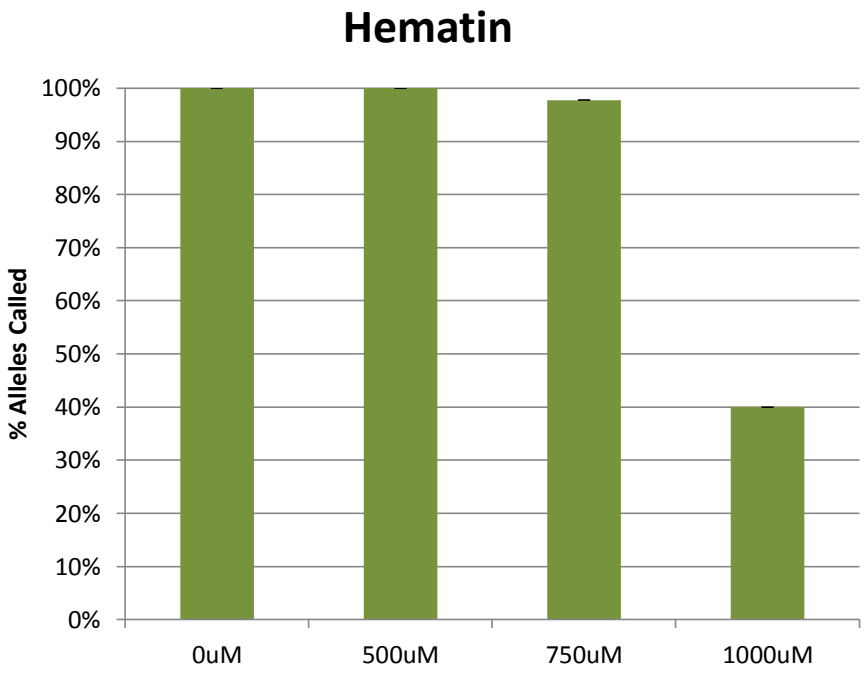
Slide kindly provided by John Butler (NIST) using data collected by Becky Hill & analysis tools developed by Dave Duewer

Developmental Validation – Preliminary data
Mixture Study



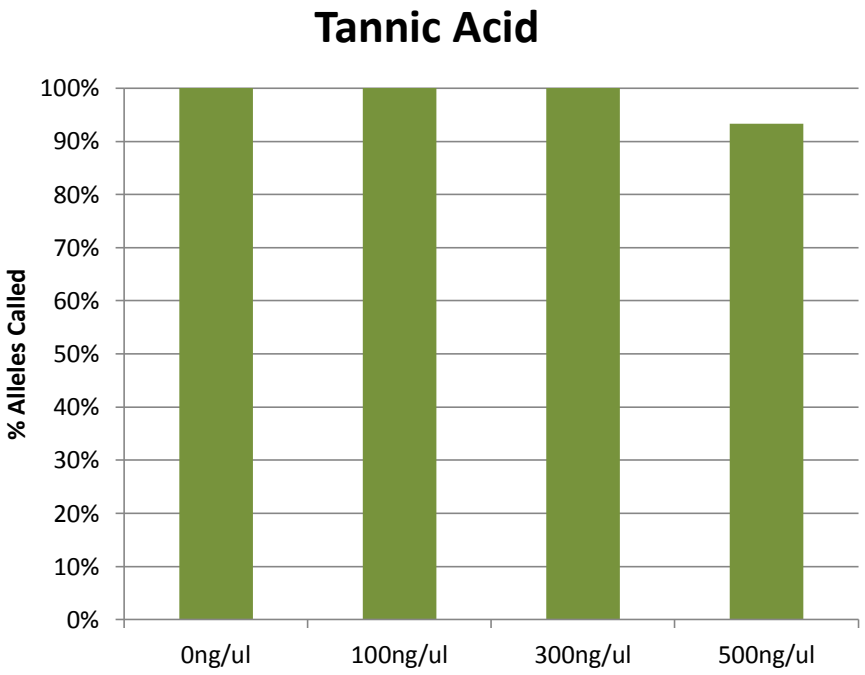
Developmental Validation – Preliminary data
Inhibitors Study

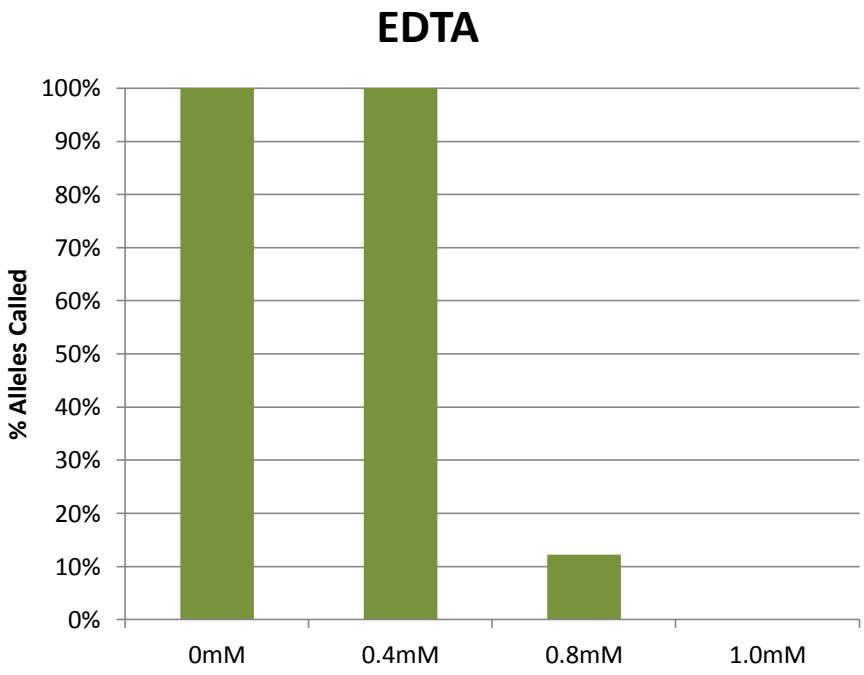




N = 2

Developmental Validation – Preliminary data
Inhibitors Study





N = 2

PowerPlex® Fusion System

Promega

- Provides a rapid thermal cycling protocol
- Sensitive and robust for casework
- Allows for direct amplification for swabs and punches in databasing
- Is fully compatible with current 31xx & 3500 series Genetic Analyzers
- Includes all core CODIS and ESS loci as well as a Pentas and DYS391
  - Most discrimination available
- Will allow searching of *all* autosomal STRs historically entered in NDIS

Questions?

Promega