

# ***PowerPlex<sup>®</sup> Y23 Developmental Validation***

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# ***PowerPlex® Y23 System***

## ***Presentation outline***



- Benefits of Y-STR Analysis
- Background information on PowerPlex® Y23
- Developmental requirements of SWGDAM
- PowerPlex® Y23 Developmental Validation Data
- Update on Y-STR Statistical Databases
  - US Y-STR Database
  - YHRD Database

## *The Benefits of Y-STR Analysis*

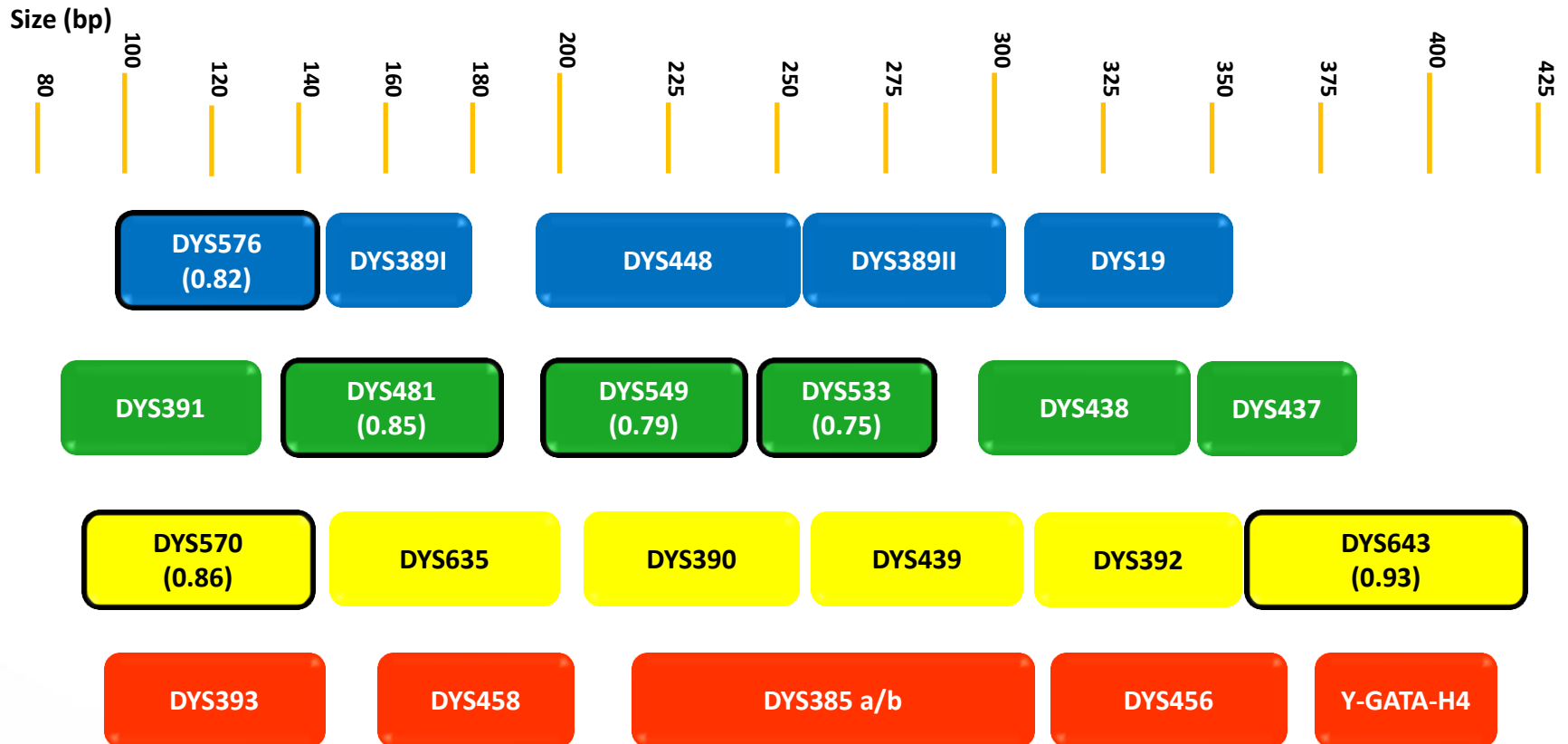
- Forensic casework on sexual assault evidence
  - Relatively high amount of female DNA
  - Mixtures with DNA from multiple male donors
- Paternity testing
- Familial searching
- Missing persons investigations
- Migration and evolutionary studies
- Historical and genealogical research

## ***Overview of the PowerPlex® Y23 System***

- Includes all of the loci from PowerPlex® Y as well as AmpF/STR® Yfiler®
  - Includes six Y-STR loci that are not in any other commercially available kit
- One system capable of extracted and direct amplification applications
- Amplify male genomic DNA in an excess of female genomic DNA
  - No amplification products for female genomic DNA
- Rapid thermal cycling
- Compatible with the Applied Biosystems' 3130 and 3500 Genetic Analyzers

# PowerPlex® Y23 System

Six new loci with high gene diversity



# Gene Diversity of STR Loci in Y-STR Systems



Promega

Locus	Gene Diversity	PowerPlex® Y	AmpFISTR® Yfiler™	PowerPlex® Y 23
DYS643	0.92			√
DYS570	0.85			√
DYS481	0.85			√
DYS385 a/b	0.83	√	√	√
DYS576	0.82			√
DYS549	0.78			√
DYS458	0.78		√	√
DYS390	0.75	√	√	√
DYS533	0.75			√
DYS635	0.71		√	√
DYS456	0.70		√	√
DYS448	0.69		√	√
DYS438	0.68	√	√	√
DYS439	0.67	√	√	√
DYS392	0.64	√	√	√
DYS19	0.64	√	√	√
DYS437	0.62	√	√	√
DYS389II	0.62	√	√	√
Y GATA H4	0.60		√	√
DYS389I	0.56	√	√	√
DYS391	0.53	√	√	√
DYS393	0.46	√	√	√

## *Value of Additional Loci*

- NIST concordance study: 12 sets of two individuals matched at all 17 AmpF/STR® Yfiler® loci:
  - With the addition of the new six loci, 8 of 12 sets were distinguished from each other (only 4 sets of two individuals still match)
  - Remaining sets appear to be related individuals based on mitochondrial DNA and autosomal testing
  - NIST manuscript submitted
- UNT concordance study
  - Yfiler had 708 unique profiles
  - Additional 6 loci alone had 557 unique profiles
  - All 23 loci together had 758 unique profiles

C. Davis, et. Al. Prototype PowerPlex®Y23 System: A concordance study. Forensic Sci. Int. Genet. (2012). <http://dx.doi.org/10.1016/j.fsigen.2012.06.005>

# PowerPlex® Y23 System

## Thermal Cycling



PowerPlex® Y23 System	
1 cycle	96°C for 2 min
30 or 26 cycles	94°C for 10 sec 61°C for 1 min 72°C for 30 sec
1 cycle	60°C for 20 min

- Extracted DNA
  - 30 cycles
  - 1 h 40 min total time
- Punches and swab extract
  - 26 cycles
  - 1 h 30 min total time

AmpF/STR® Y-Filer®	
1 cycle	95°C for 11 min
27 or 30 cycles	94°C for 1 min 61°C for 1 min 72°C for 1 min
1 cycle	60°C for 80 min

- Extracted DNA
  - 30 cycles
  - >3 h total time
- Washed FTA
  - 27 cycles



# PowerPlex® Y23 System

## Kit components



Optimized for up to 17.5µl extracted DNA sample in 25µl reaction volume

Component	200 reactions (DC2320)	50 reactions (DC2305)
<b>Pre-amplification</b>		
PowerPlex® Y23 5X Master Mix	4 x 250µl	1 x 250µl
PowerPlex® Y23 10X Primer Pair Mix	4 x 125µl	1 x 125µl
2800M Control DNA, 10ng/µl	25µl	25µl
Water, Amplification Grade	5 x 1,250µl	5 x 1,250µl
<b>Post-amplification</b>		
PowerPlex® Y23 Allelic Ladder Mix	4 x 25µl	1 x 25µl
CC5 Internal Lane Standard 500 Y23*	2 x 300µl	1 x 300µl

\* Optimized for PowerPlex® Y23 only



***PowerPlex® Y23 Developmental  
Validation Data***



# ***SWGDM Guidelines for Developmental Validation***



- 2.1 – Inheritance, mapping, detection, polymorphism
- 2.2 – Species Specificity
- 2.3 – Sensitivity studies
- 2.4 – Stability
- 2.5 – Reproducibility
- 2.6 – Case-type samples/non-probative samples
- 2.7 – Population studies
- 2.8 – Mixture studies
- 2.9 – Precision and accuracy
- 2.10 – Specificity and robustness, balance and stochastic, known artifacts, positive and negative controls

# ***PowerPlex® Y23 System***

## ***Developmental Validation Collaborators***



- Four US laboratories have been identified to participate in the developmental validation
  - Illinois State Police -sponsor for NDIS approval
  - Sorenson Forensics
  - Texas Department of Safety – Casework DNA Section and CODIS Section
  - Promega

## 2.1

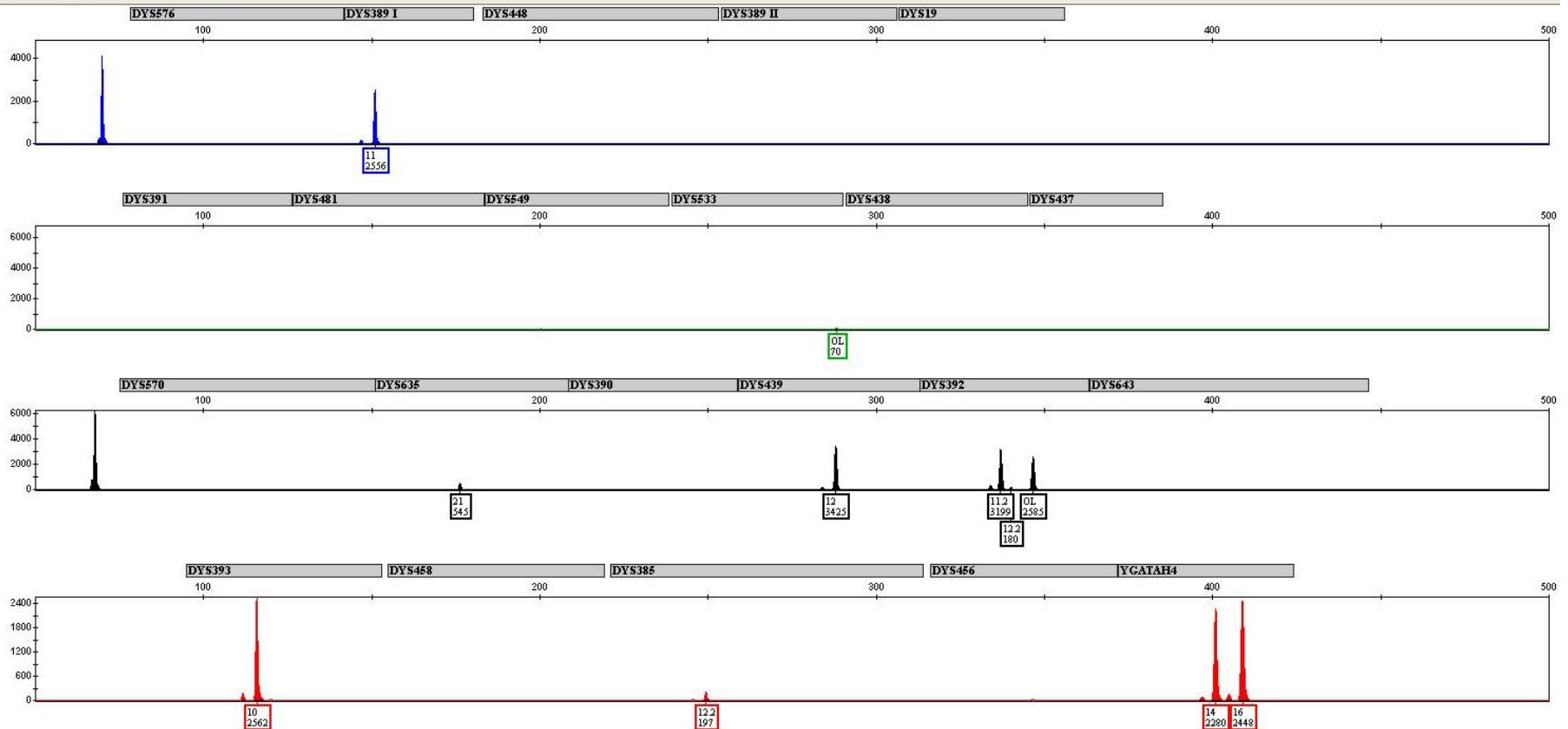
- Inheritance:
  - Vermeulen, M. *et al.* (2009) Improving global and regional resolution of male lineage differentiation by simple single-copy Y-chromosomal short tandem repeat polymorphisms. *For. Sci. Int. Genet.* 3, 205-213
  - DYS576 and DYS570 have mutation rates of >1%, considered rapidly mutating
  - NIST studies for father son data in progress
- Mapping
  - Hanson, E.K and J. Ballantyne. Comprehensive annotated STR physical map of human Y chromosome: Forensic implications. *Legal Medicine* 8 (2006) 110-120.
  - Article describes the six new loci included in PowerPlex® Y23

## 2.2 Species specificity

- Promega tested on both the 3130 and 3500
  - Three higher primates: Chimpanzee, Gorilla, Orangutan
  - Nine domestic animals: pig, horse, deer, cat, rabbit, chicken, dog, cow, mouse
  - Seven microorganisms: *E.coli*, *E.faecalis*, *S.servisiae*, *F. nucleatum*, *M. luteus*, *S. salivarius*, *S. mitis*
  - Results showed no artifacts with 10ng of domestic animal or microorganism DNA
  - Partial profiles obtained from 500pg DNA from higher primates, however profile pattern was not consistent with human male profile
- ISP tested
  - 4 primates: Gibbon, Macaque, DeBrazza's Monkey, Lemur
  - 4 mammals: Cat, Dog, Fox, Mouse
  - 2 microorganisms: *C. perfringens* and *B.subtilis*
  - One allele was seen in the Macaque sample
  - No other artifacts were seen from the domestic animals or microorganisms

# 2.2 Species Specificity

## 500pg Chimpanzee DNA



# ***Developmental Validation***

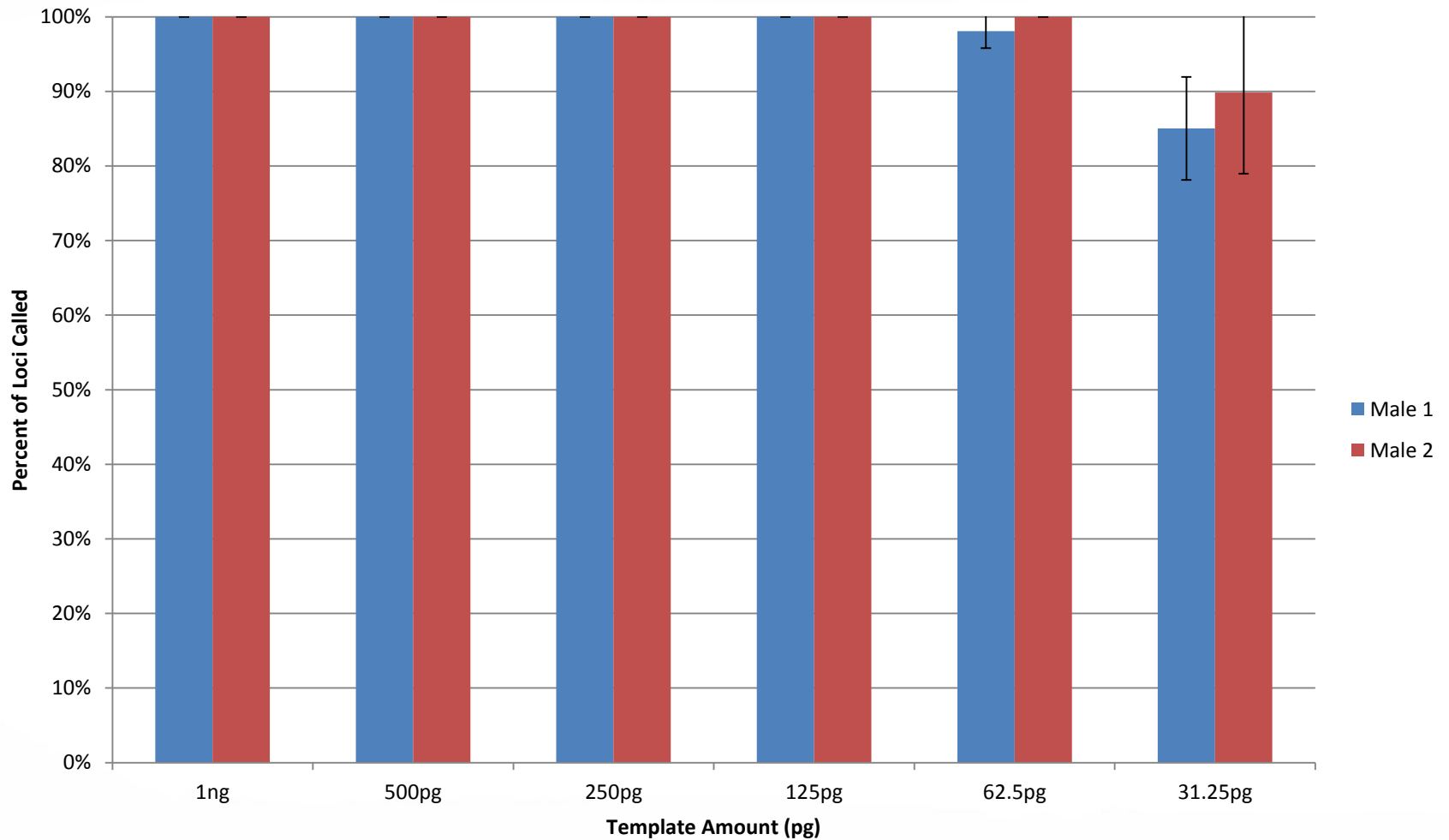
## ***2.3 Sensitivity studies***



- **Extracted DNA**
  - 2 male DNA samples were tested by 3 laboratories
  - All samples were tested in triplicate
  - 1ng, 500pg, 250pg, 125pg, 62.5pg, 31.25pg
- **Direct Blood on FTA**
  - Blood samples from 5 males were tested by 1 laboratory
  - All samples tested in triplicate
  - 1, 2, or 3 – 1.2mm punches
- **Direct Buccal on FTA**
  - Buccal samples from 5 males were tested by 1 laboratory
  - All samples tested in triplicate
  - 1, 2, or 3 – 1.2mm punches

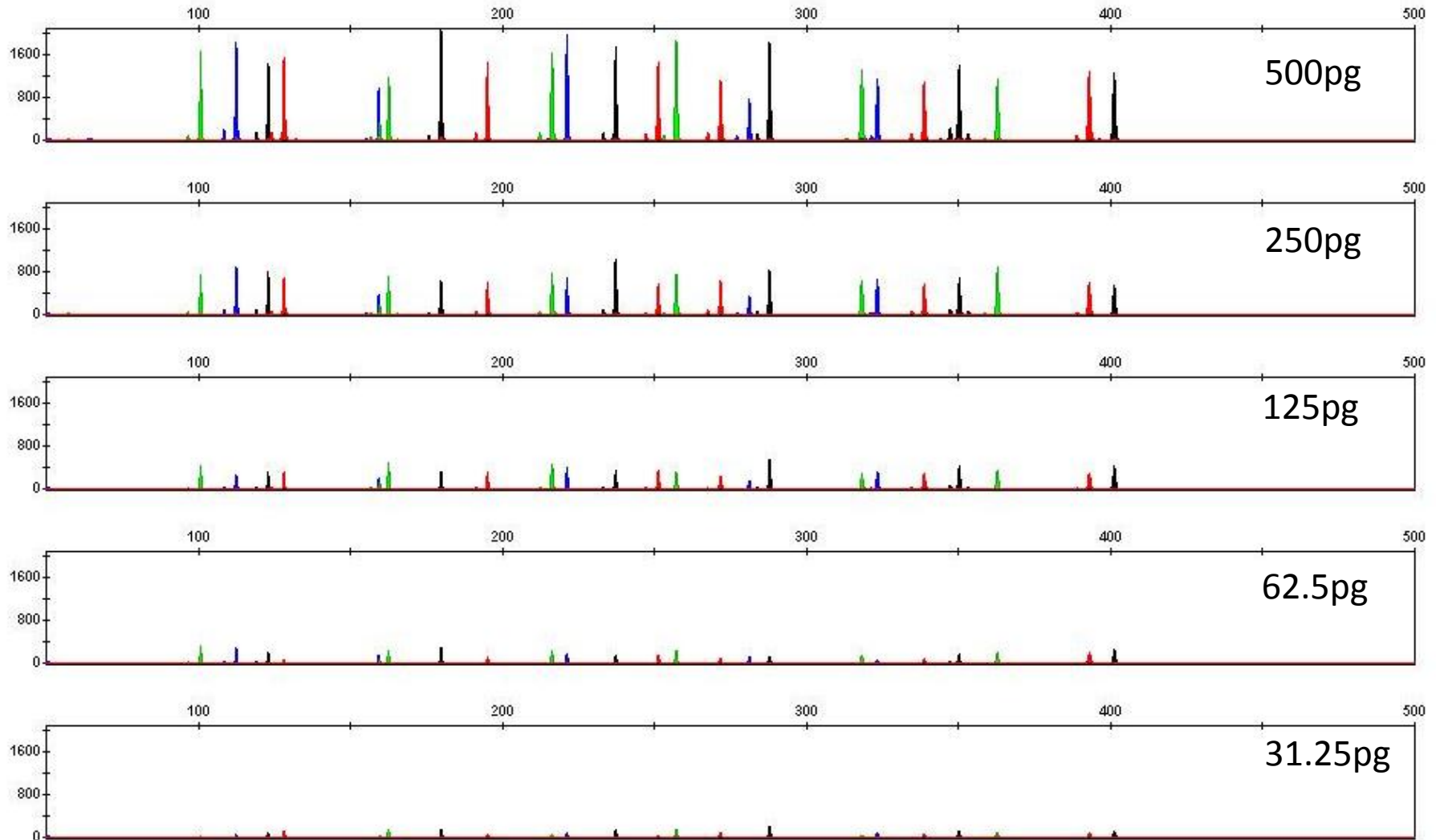


# Sensitivity of Extracted DNA



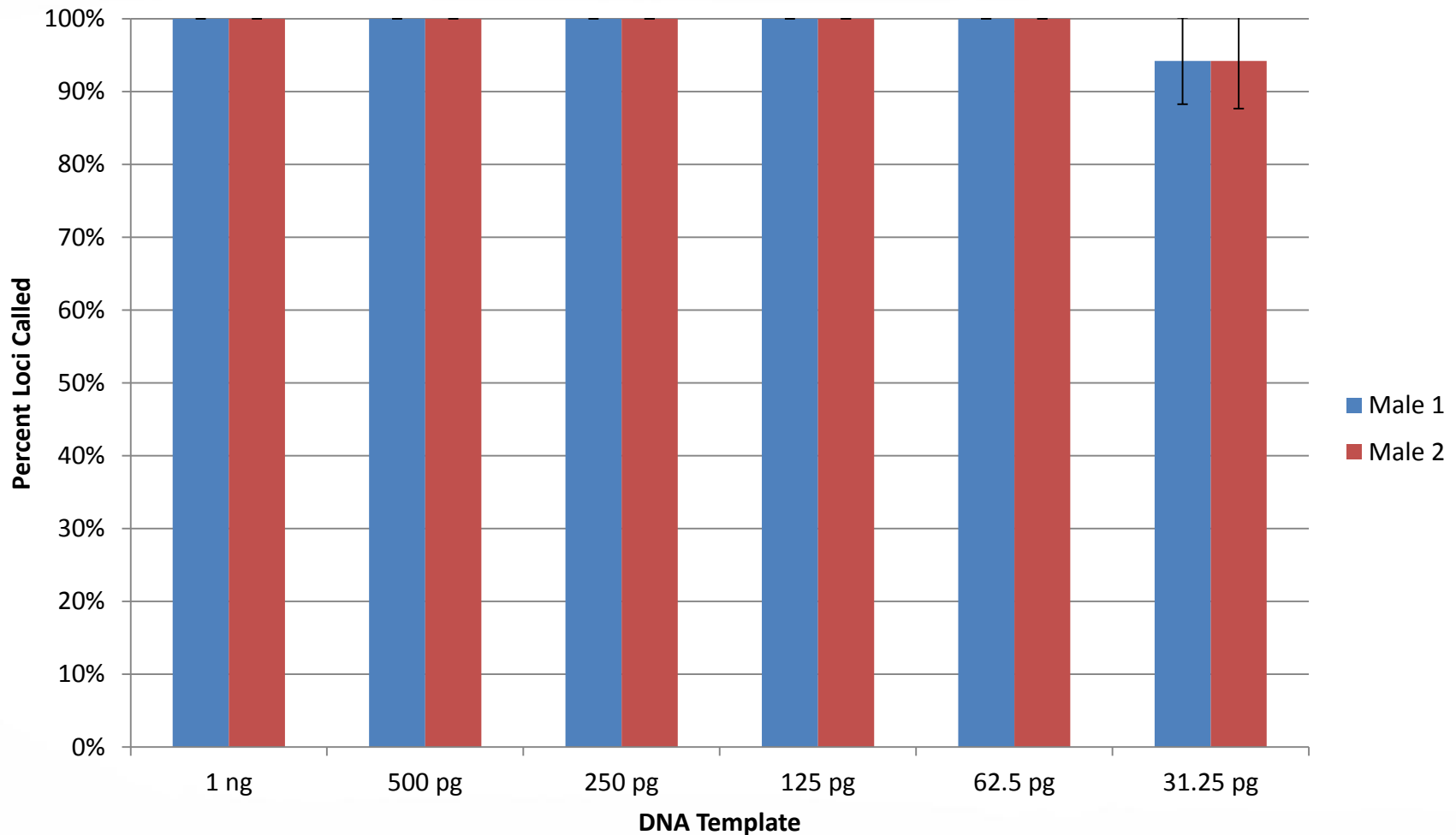
## 2.3 Sensitivity Studies

*Extracted DNA – full profiles consistently down to 62.5pg*



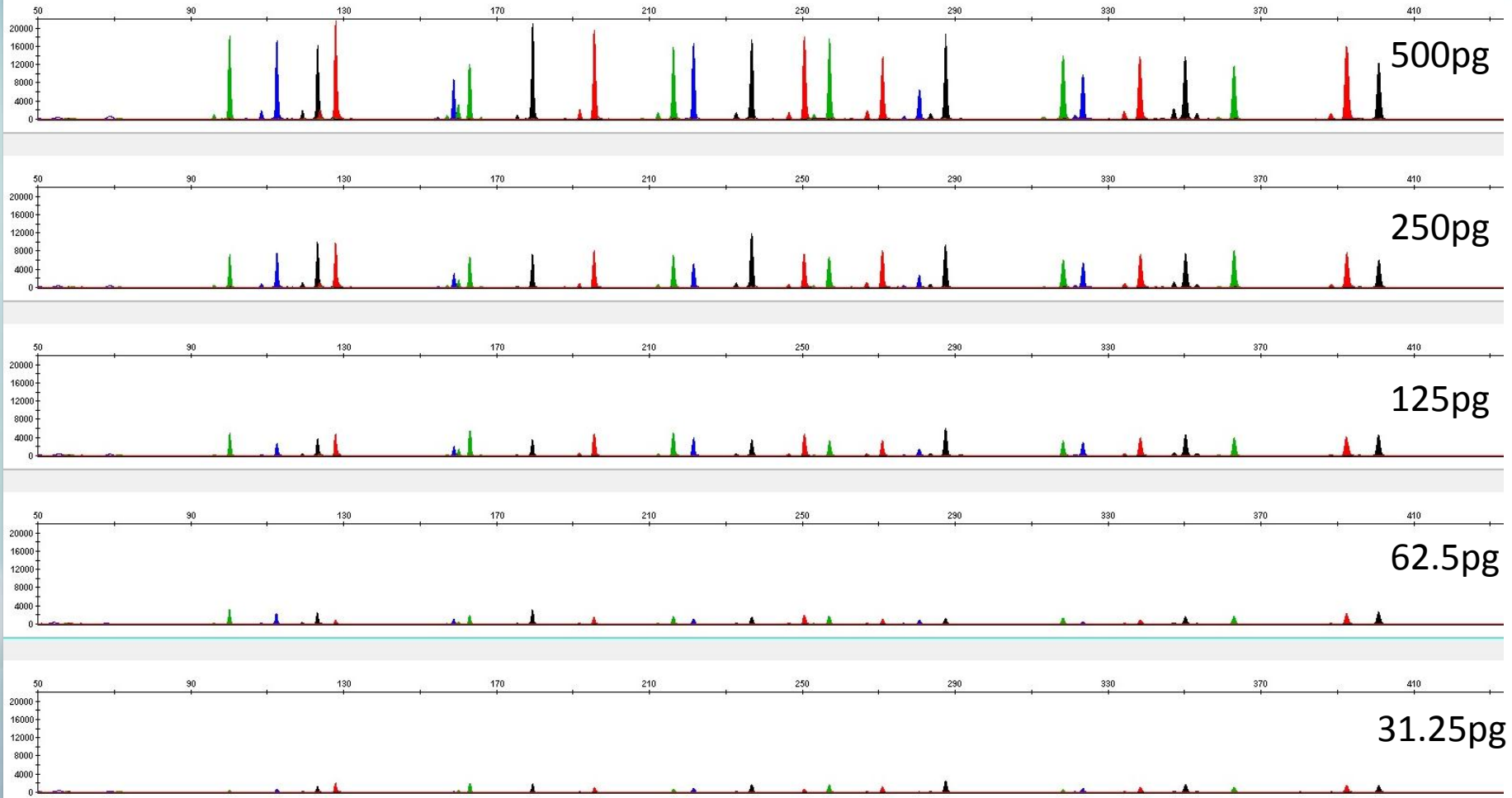
## 2.3 Sensitivity Studies

### Extracted DNA on 3500 Genetic Analyzer



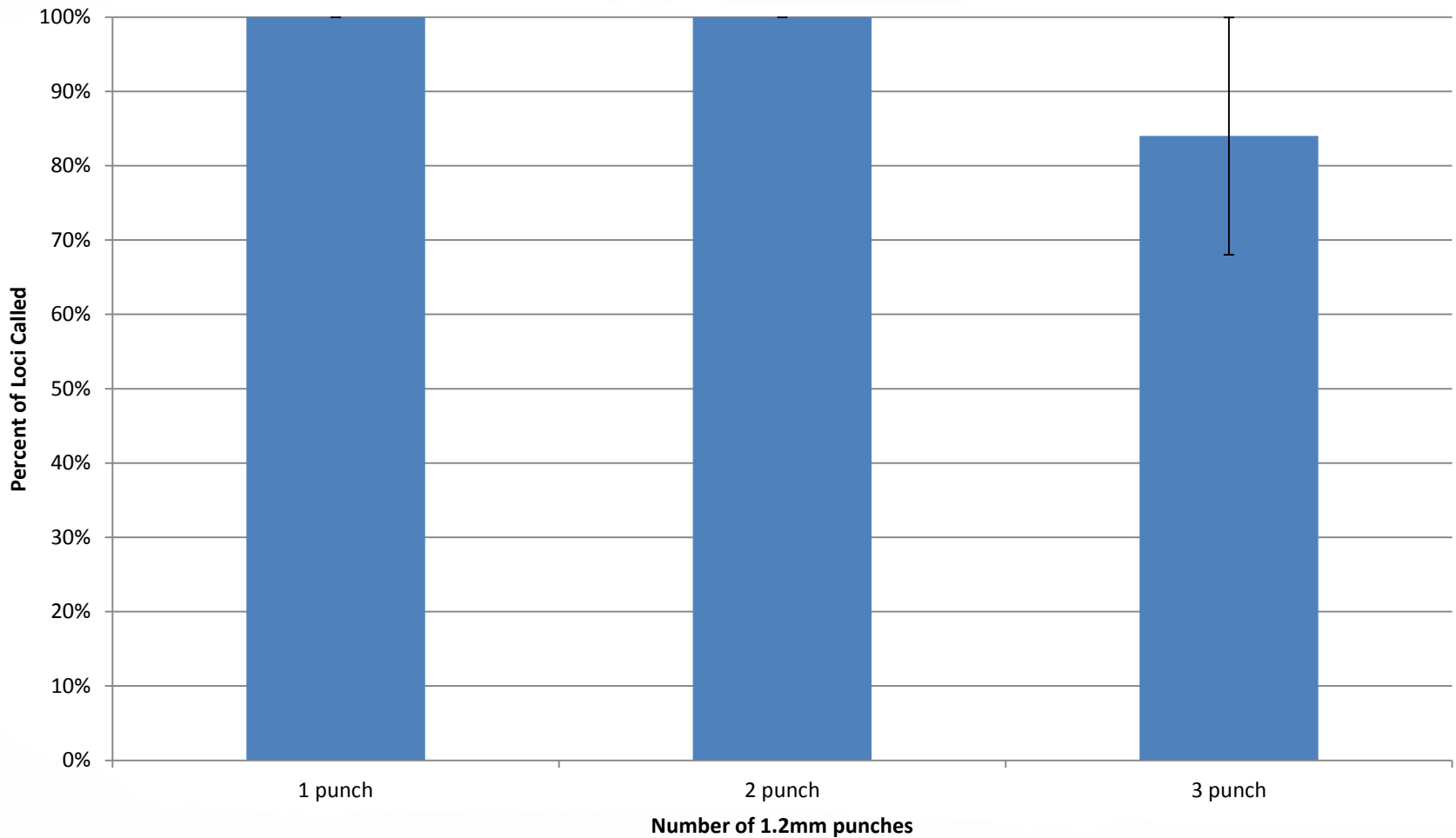
## 2.3 Sensitivity Studies

### Extracted DNA on 3500 Genetic Analyzer



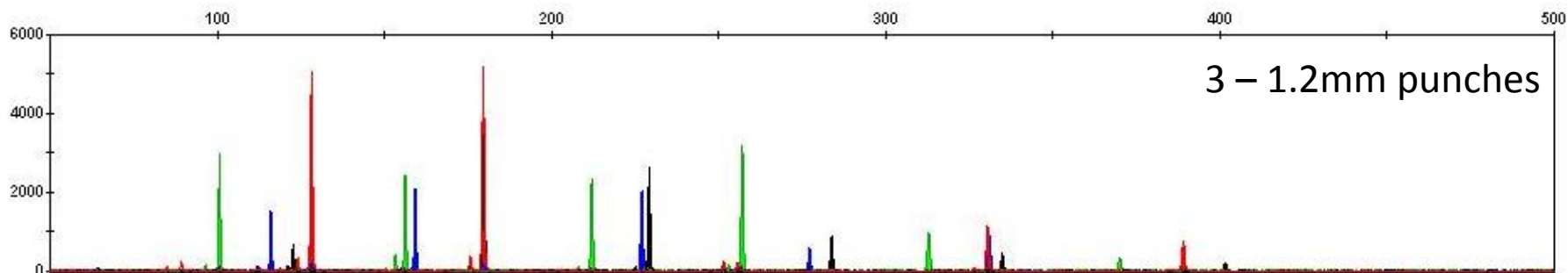
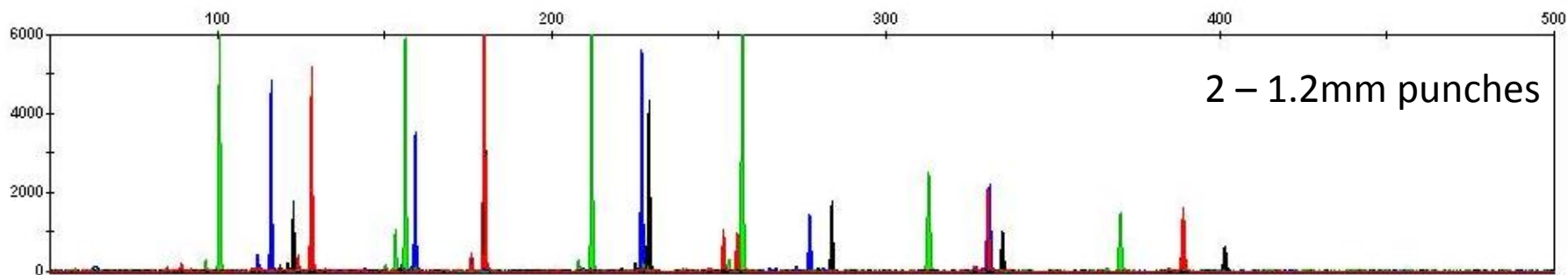
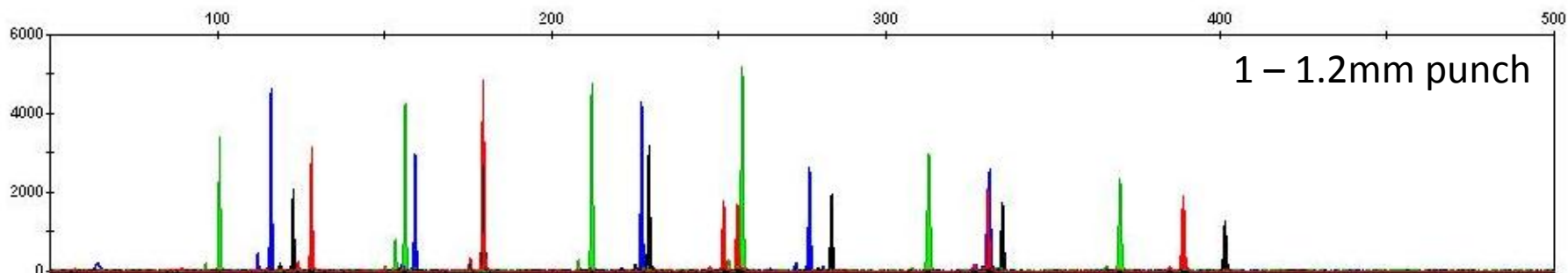
## 2.3 Sensitivity Studies

### *Direct amplification of blood on FTA*



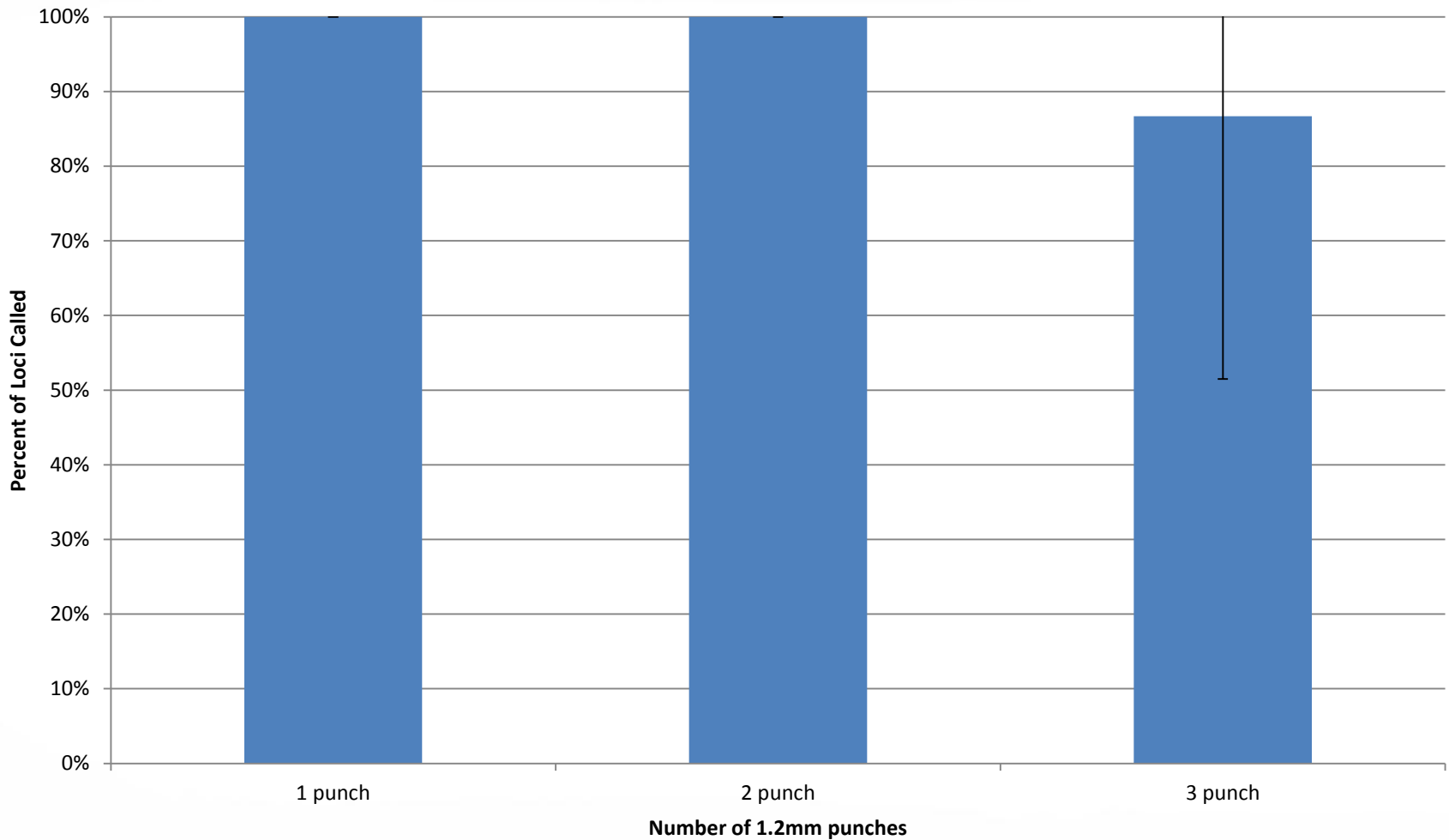
## 2.3 Sensitivity Studies

### Direct amplification of blood on FTA



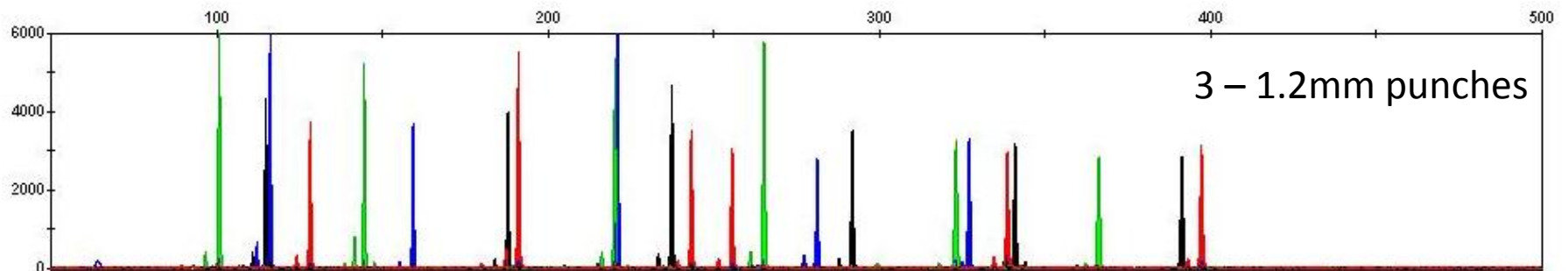
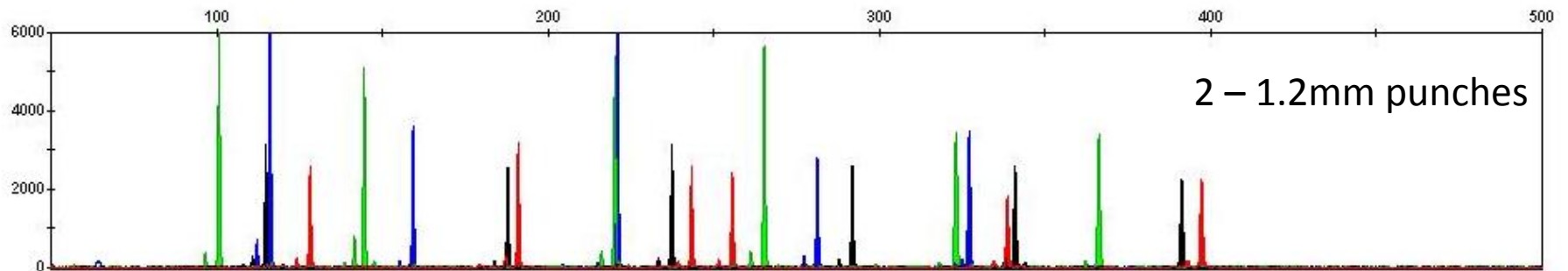
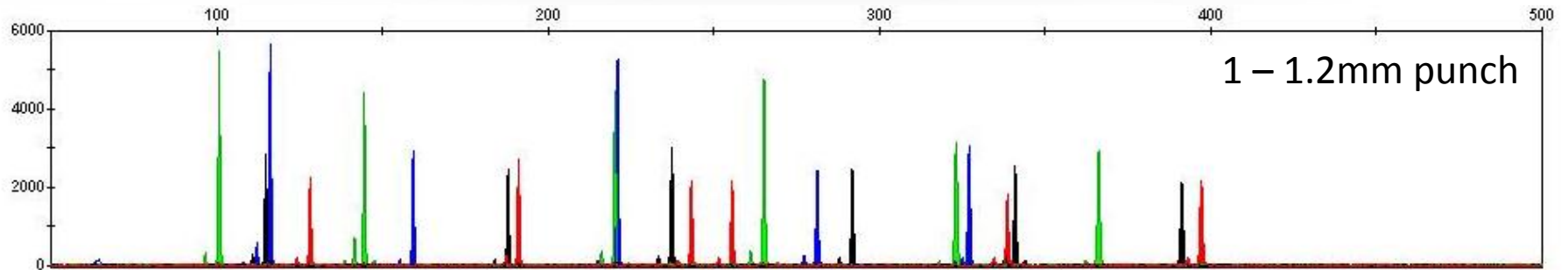
## 2.3 Sensitivity Studies

### *Direct amplification of buccal on FTA*



## 2.3 Sensitivity Studies

### Direct amplification of buccal on FTA





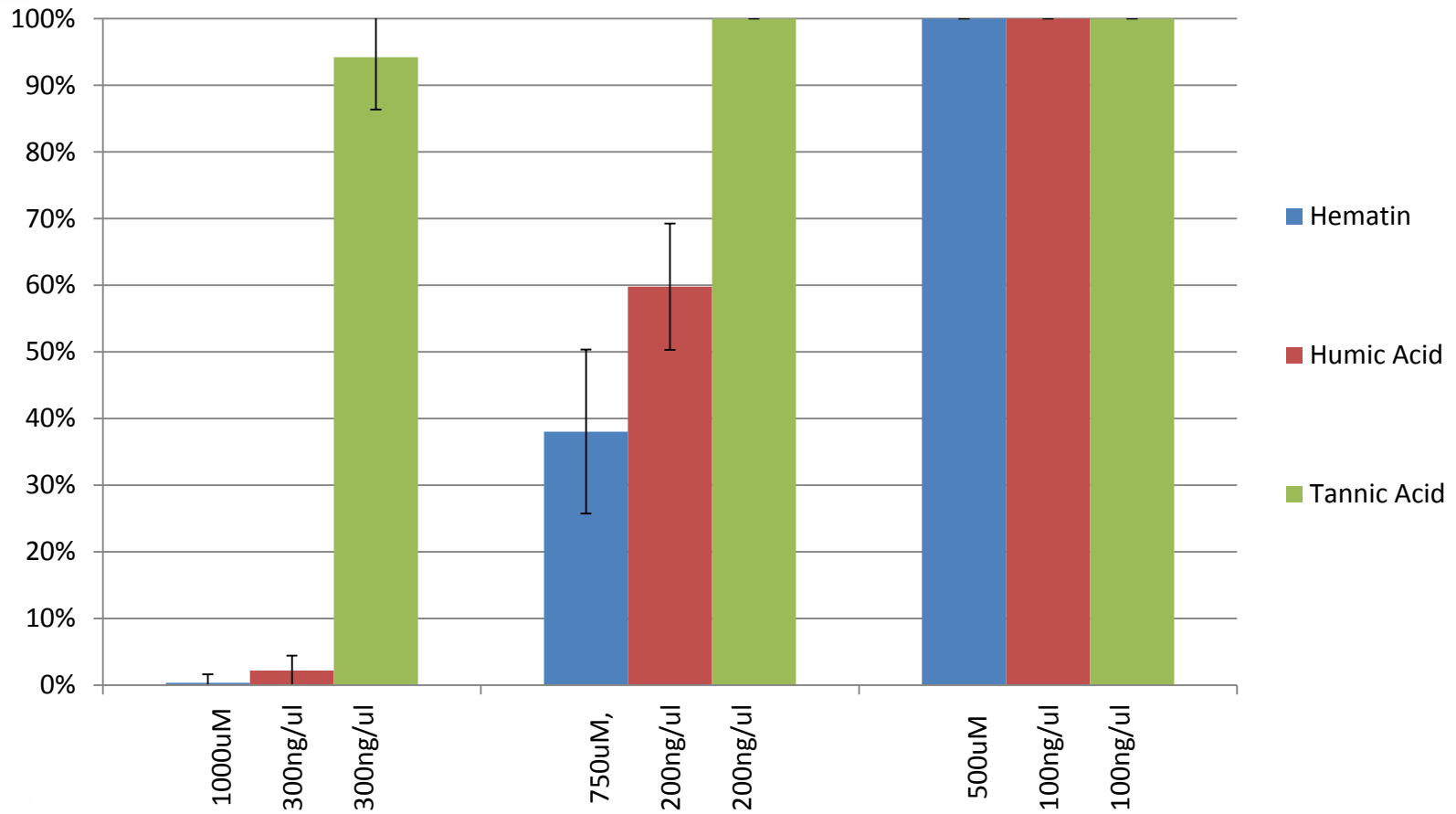
# ***Developmental Validation***

## ***2.4 Stability studies – inhibitors***



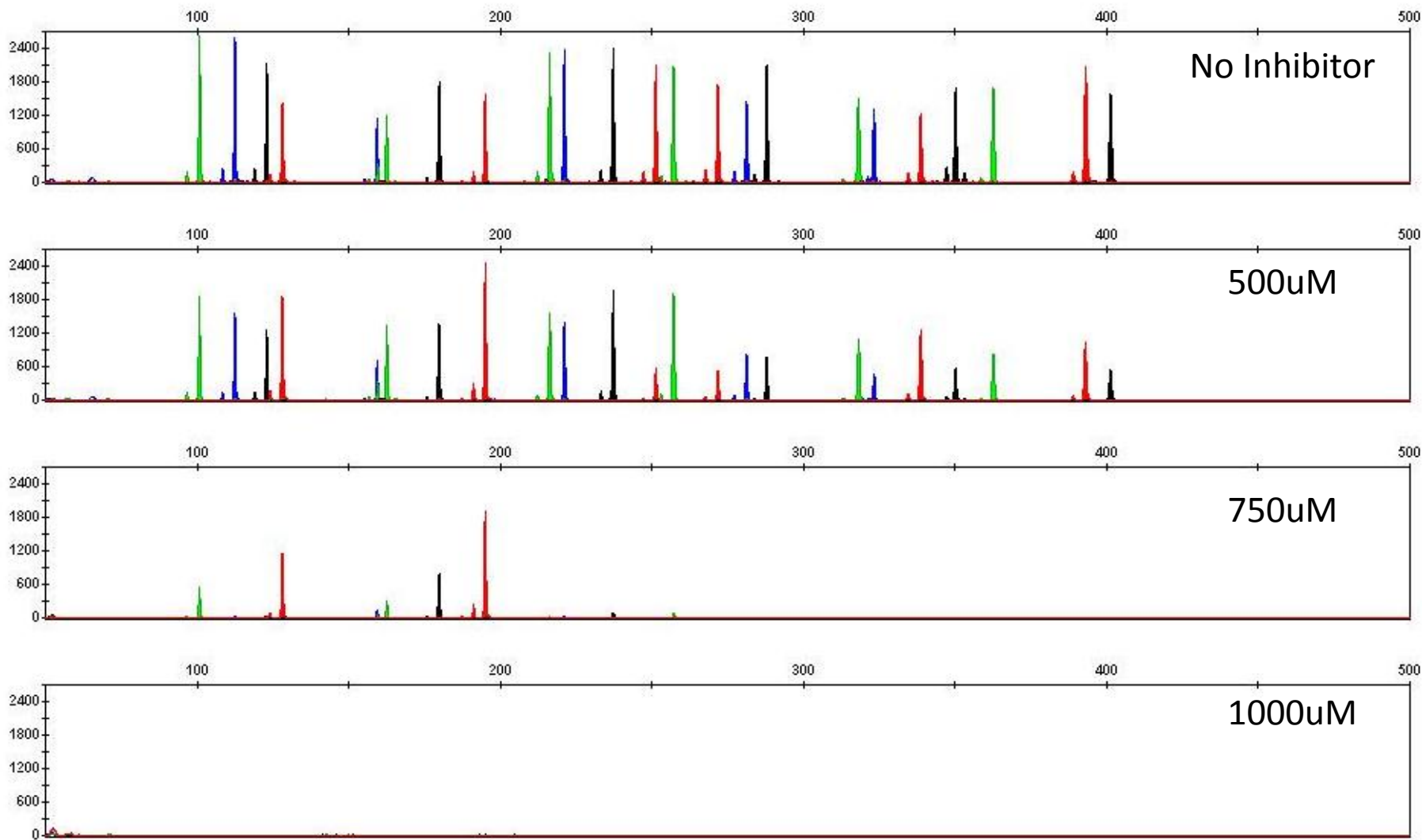
- Three inhibitors
  - Hematin: 500, 750, and 1000 $\mu$ M
  - Humic acid: 100, 200, and 300ng/ $\mu$ l
  - Tannic acid: 100, 200, and 300ng/ $\mu$ l
- 500ng extracted DNA from 2 males were tested in triplicate amplifications for each sample type
- Testing performed at 2 laboratories

## 2.4 Stability Studies – Inhibitors



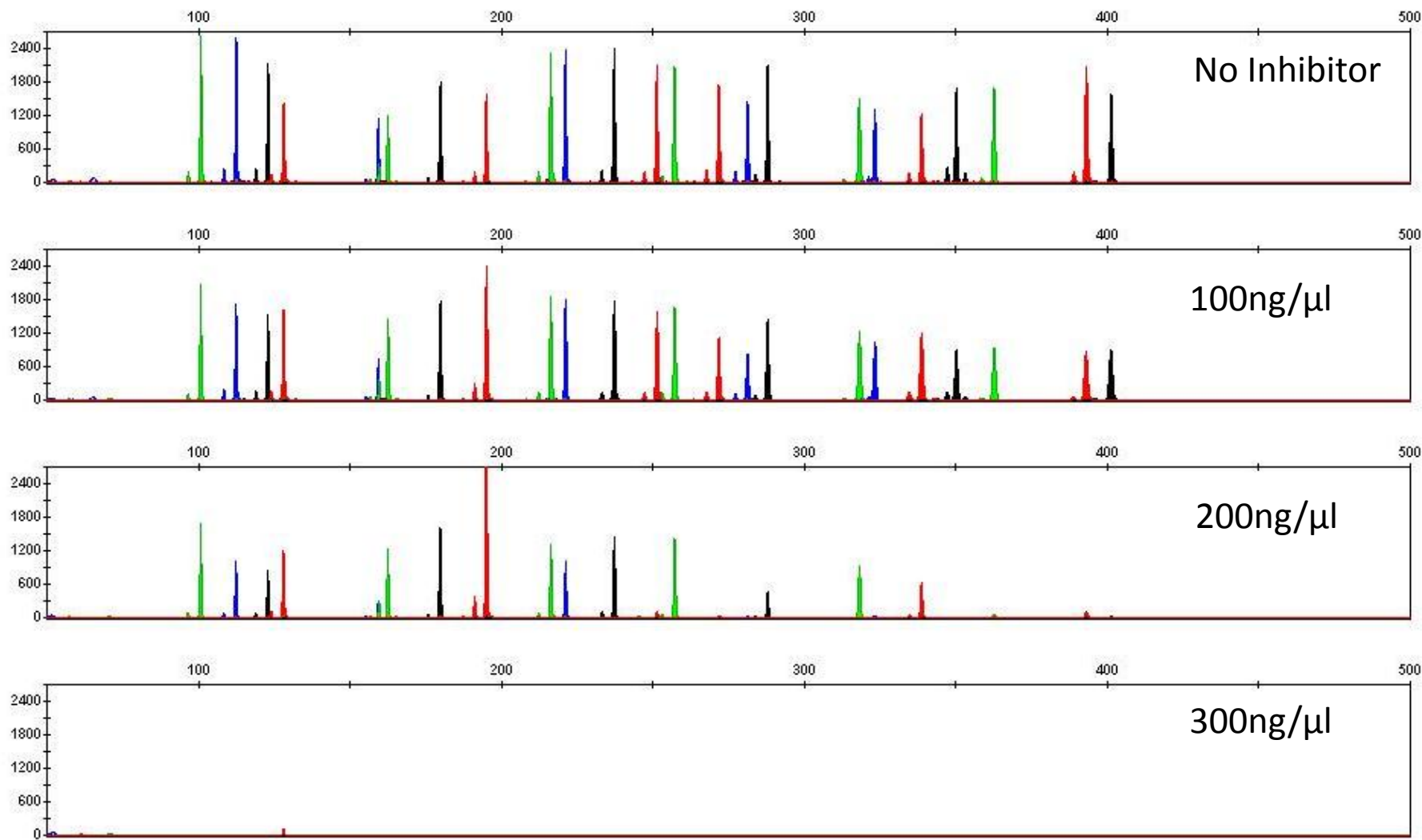
## 2.4 Stability Studies – Inhibitors

### Hematin



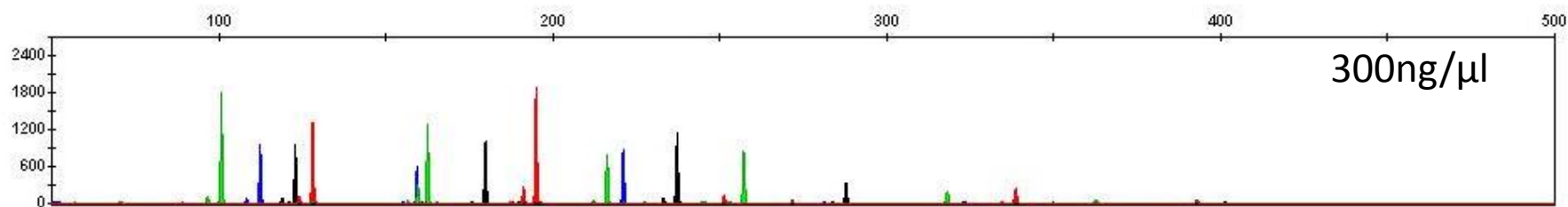
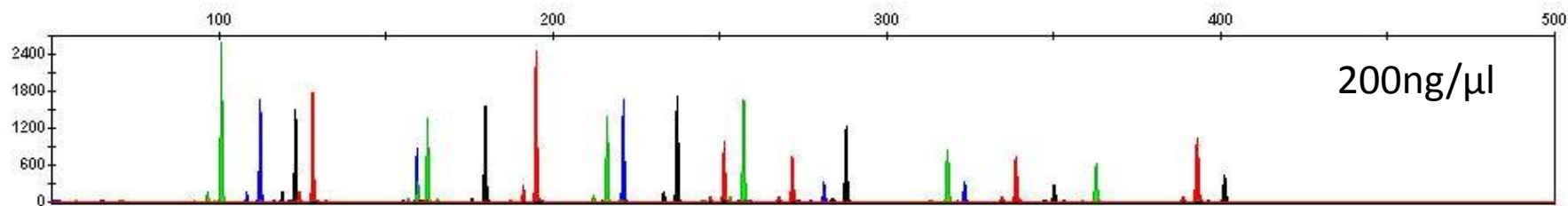
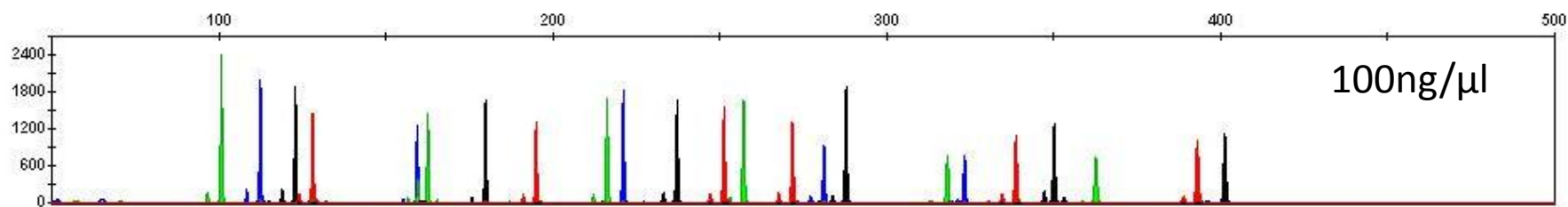
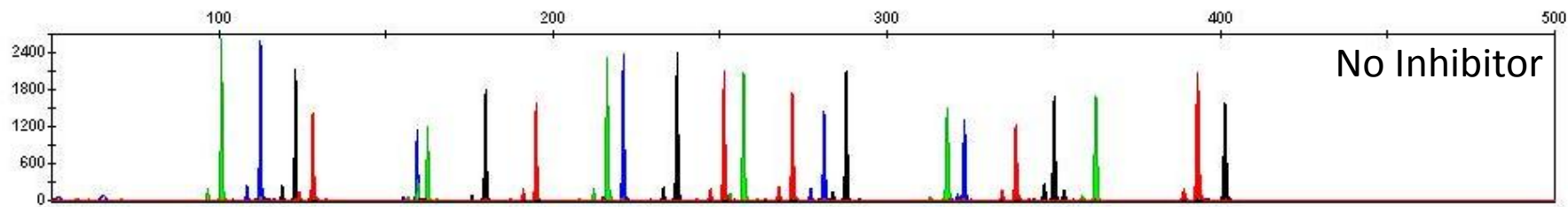
## 2.4 Stability Studies – Inhibitors

### Humic acid



## 2.4 Stability Studies – Inhibitors

### Tannic acid



# ***Developmental Validation***

## ***2.5 Reproducibility***



- Extracted DNA
  - NIST 2395
  - Tested by 3 laboratories
- Blood on FTA – 1 – 1.2mm punch
  - 2 males
  - Tested by 3 laboratories
- Buccal on FTA – 2 – 1.2mm punch
  - 2 males
  - Tested by 2 laboratories

# 2.5 Reproducibility

## Extracted DNA: NIST 2395



	NIST A	NIST B	NIST C	NIST D	NIST E
DYS576	18	16	17	18	17
DYS389 I	13	13	14	12	14
DYS448	19	21	21	21	20
DYS389 II	29	28	32	28	31
DYS19	14	14	16	15	17
DYS391	11	11	12	10	10
DYS481	22	23	28	23	28
DYS549	14	12	11	13	13
DYS533	12	12	10	9	12
DYS438	12	9	11	11	10
DYS437	15	14	14	16	14
DYS570	17	18	18	17	18
DYS635	23	21	23	21	21
DYS390	25	23	21	22	24
DYS439	12	12	11	11	11
DYS392	13	11	11	11	12
DYS643	10	10	15	11	12
DYS393	13	12	13	14	14
DYS458	16	15	17	16	16
DYS385	12,15	14,17	17,20	14,15	13,15
DYS456	15	15	15	15	15
YGATAH4	12	12	12	12	11

Note, samples were run on both the 3130 and 3500. All samples were concordant with certificate from NIST for all loci available, and were consistent across all amplifications and platforms for the ones not available.

## 2.5 Reproducibility

### Blood and buccal on FTA



	Promega	Sorenson Forensics	Texas DPS
Sample Type	Blood	Blood	Blood
# Reps Male 1	3	3	3
# Reps Male 2	3	3	3
Concordant?	YES	YES	YES
Sample Type	Buccal	Buccal	
# Reps Male 1	3	3	
# Reps Male 2	3	3	
Concordant?	YES	YES	

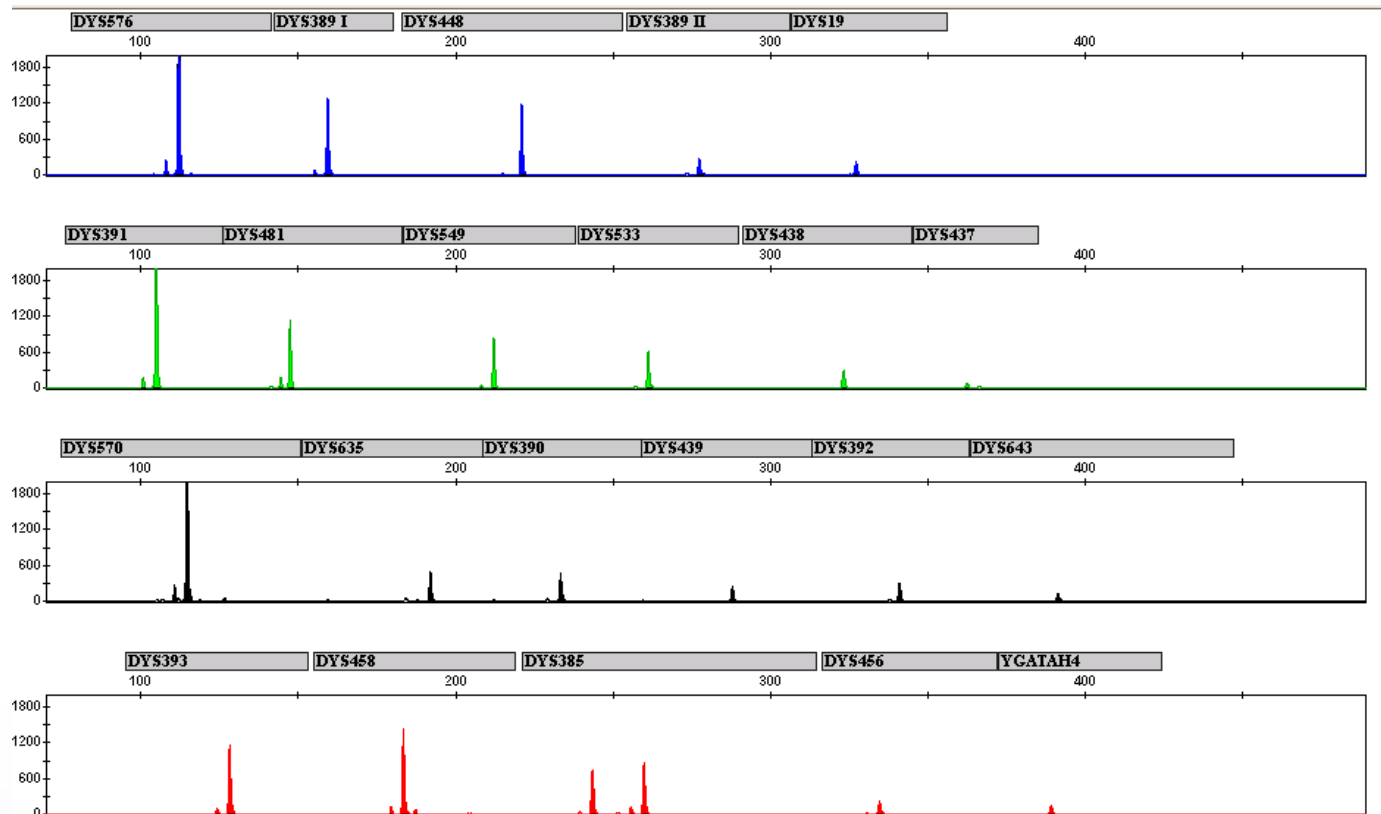


## ***2.6 Case-type samples/non-probative samples***

- Case samples tested
  - Sperm and epithelial fractions from:
    - Underwear
    - Bedding
    - Coat
    - Shirt
  - Epithelial fractions from:
    - Underwear
    - Blue jeans
  - Bloodstain:
    - Sheet

## 2.6 Case-type samples/non-probative samples

This sample is actually an “unstained area” adjacent to an area where semen was identified. Autosomal STR results identified a complete female source profile associated with the person wearing the garment (underwear) and 4 minor alleles plus Amelogenin Y.



## 2.7 – Population Study -Concordance

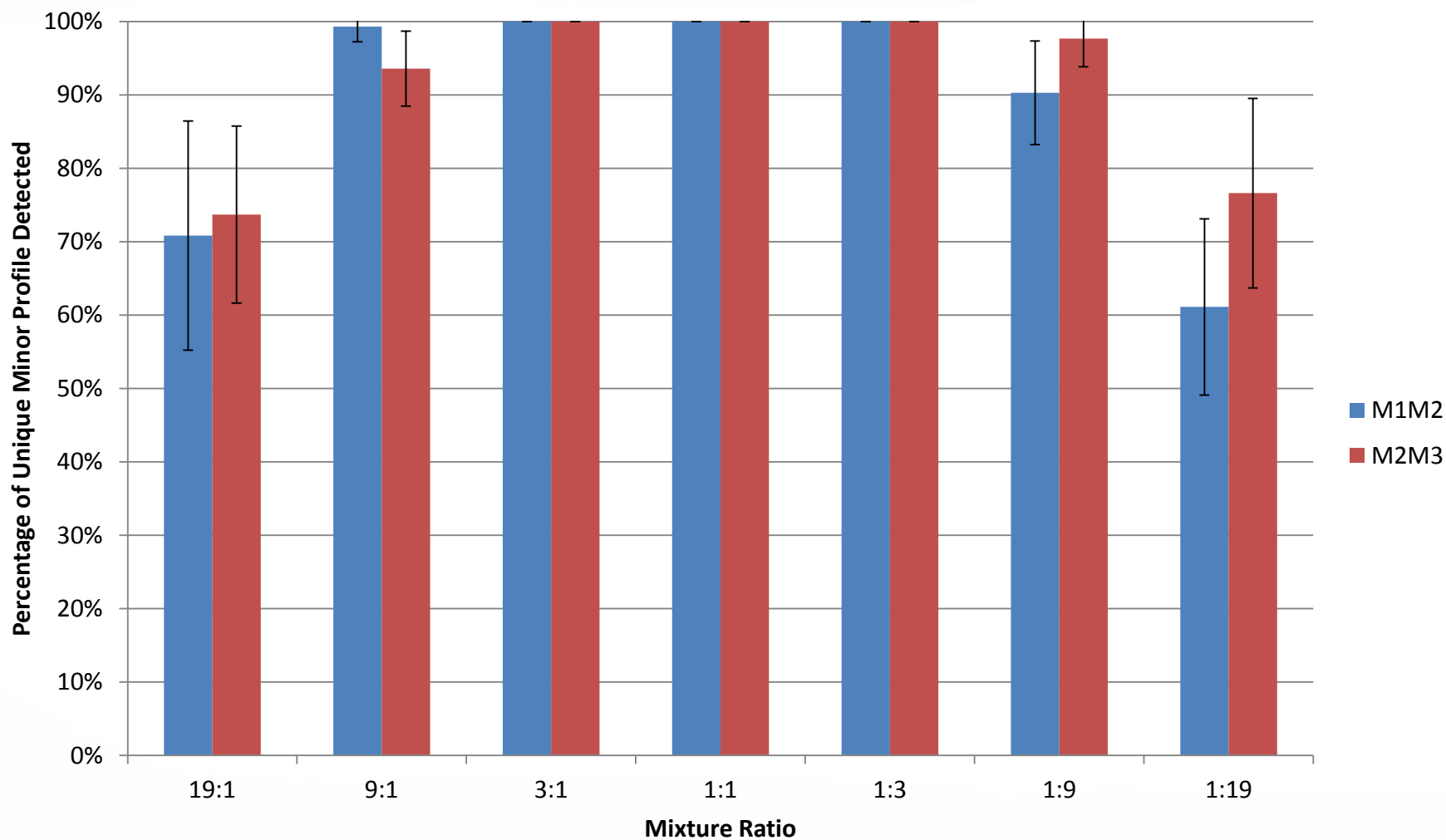
- University of North Texas Health Science Center
  - > 950 US population samples tested
    - C. Davis, et. Al. Prototype PowerPlex®Y23 System: A concordance study. Forensic Sci. Int. Genet. (2012). <http://dx.doi.org/10.1016/j.fsigen.2012.06.005>
  - Concordance in 17 common loci with AmpF/STR® Yfiler® kit
  - Null allele in DYS448 with duplication in DYS576 due to a 42 base deletion.
    - This deletion is also observed with AmpF/STR® Yfiler® and has been published Budowle, et. al., (2008) Null allele sequence structure at the DYS448 locus and implications for profile interpretation, Int J Legal Med 122:421-427.
- National Institute of Standards and Technology
  - > 600 US population samples tested
  - Concordance in 17 common loci with AmpF/STR® Yfiler® kit and 4 new loci (NIST primer sequences)

## 2.8 Mixture studies

- Male/Male Mixtures
  - 2 male/male mixture sets sent to laboratories, 500pg total DNA
  - 3 laboratories completed studies
  - Samples amplified in triplicate
  - 19:1, 9:1, 3:1, 1:1, 1:3, 1:9, and 1:19
- Male/Female Mixtures
  - Male Varied/Female constant
    - 2 male/female mixture sets sent to each laboratory
    - 3 laboratories completed studies
    - Samples amplified in triplicate
    - Female held constant at 400ng; male at 500pg, 250pg, 125pg, 62.5pg, 31.25pg
  - Male Constant/Female Varied
    - 1 male mixture set used
    - 1 laboratory completed study
    - Samples amplified in triplicate
    - Male held constant at 125pg or 500pg; female at 500ng, 1000ng, 2000ng, or 3000ng

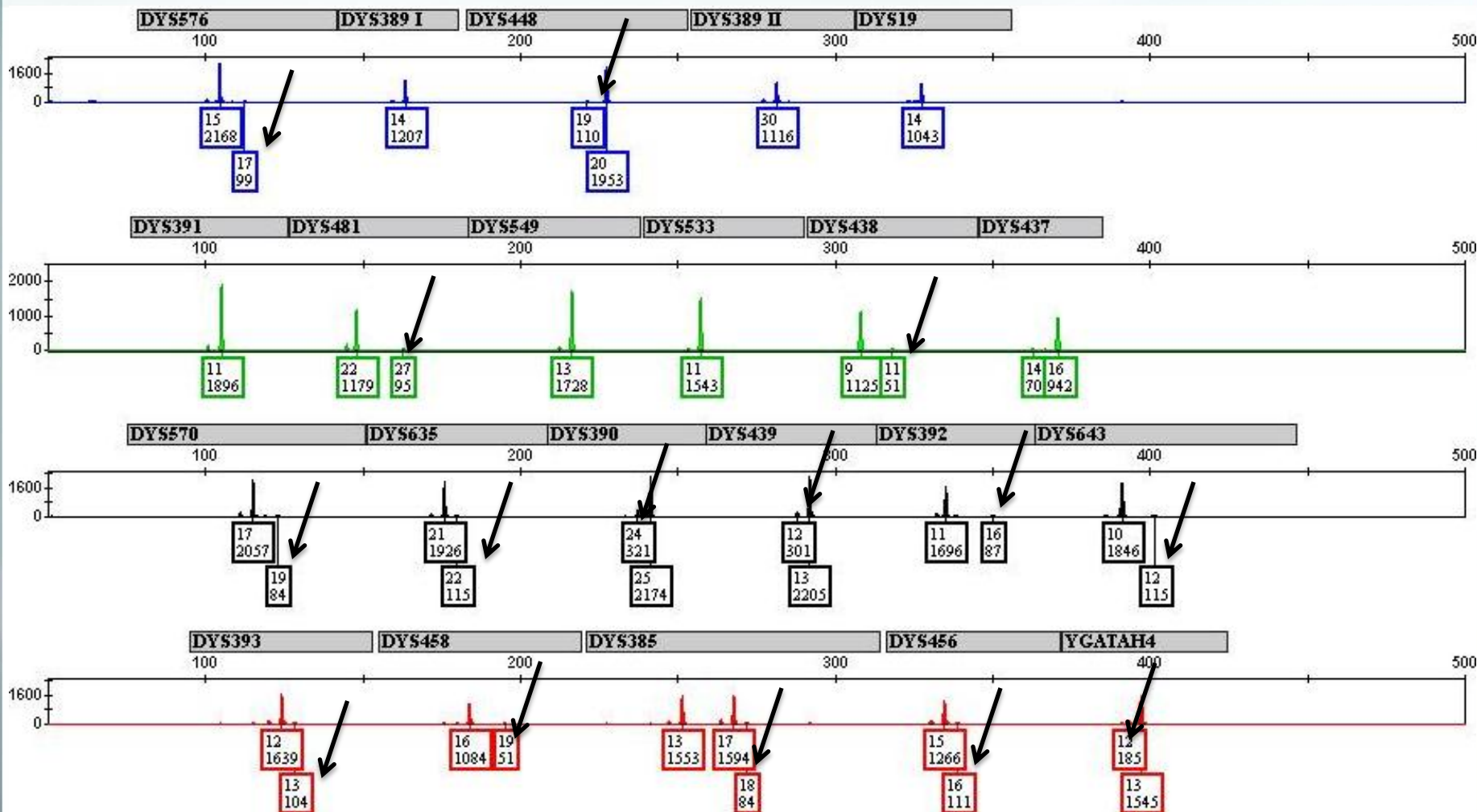
## 2.8 Mixture Studies

### Male/Male Mixtures



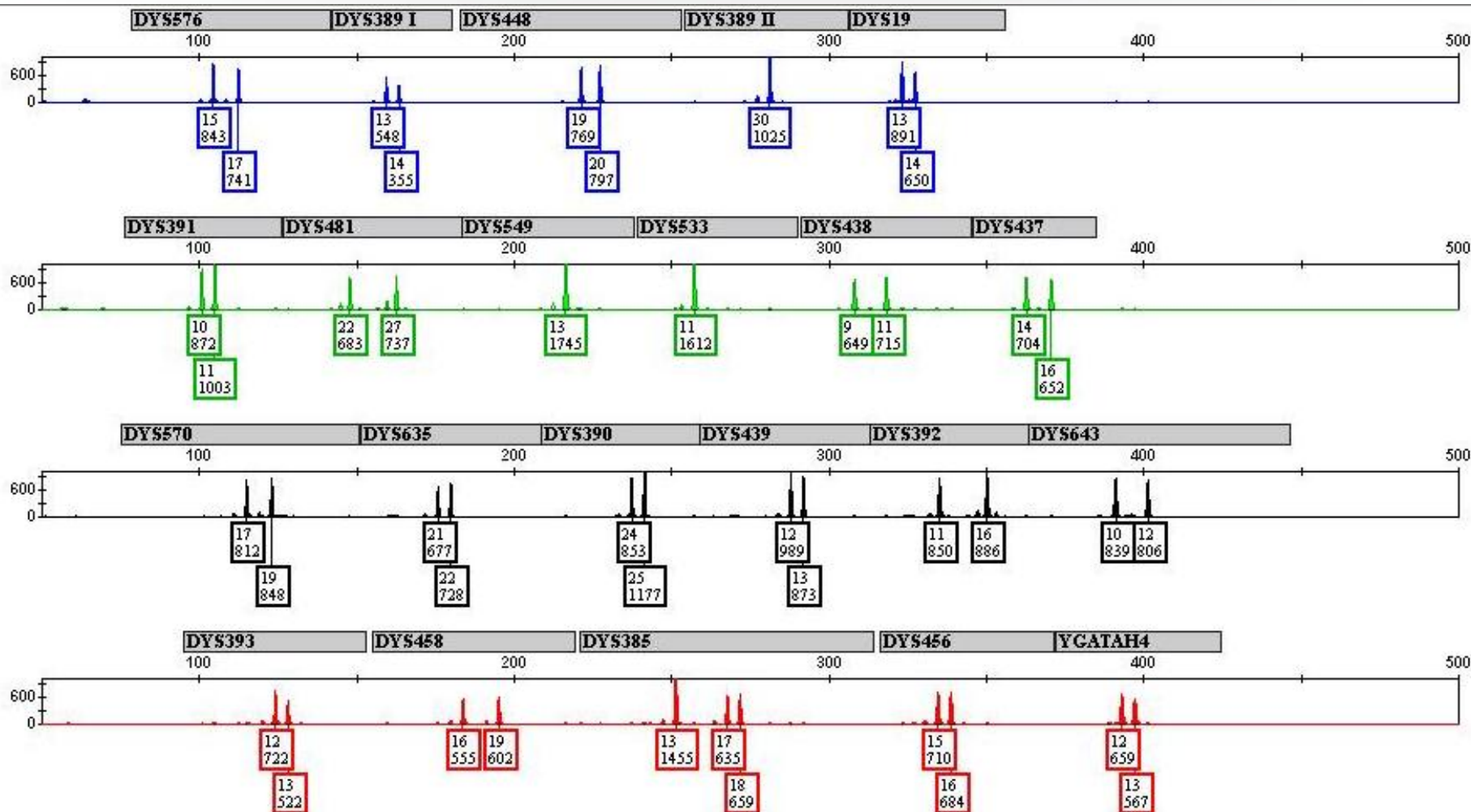
## 2.8 Mixture Studies

### Male/Male Mixtures, 19 to 1 ratio



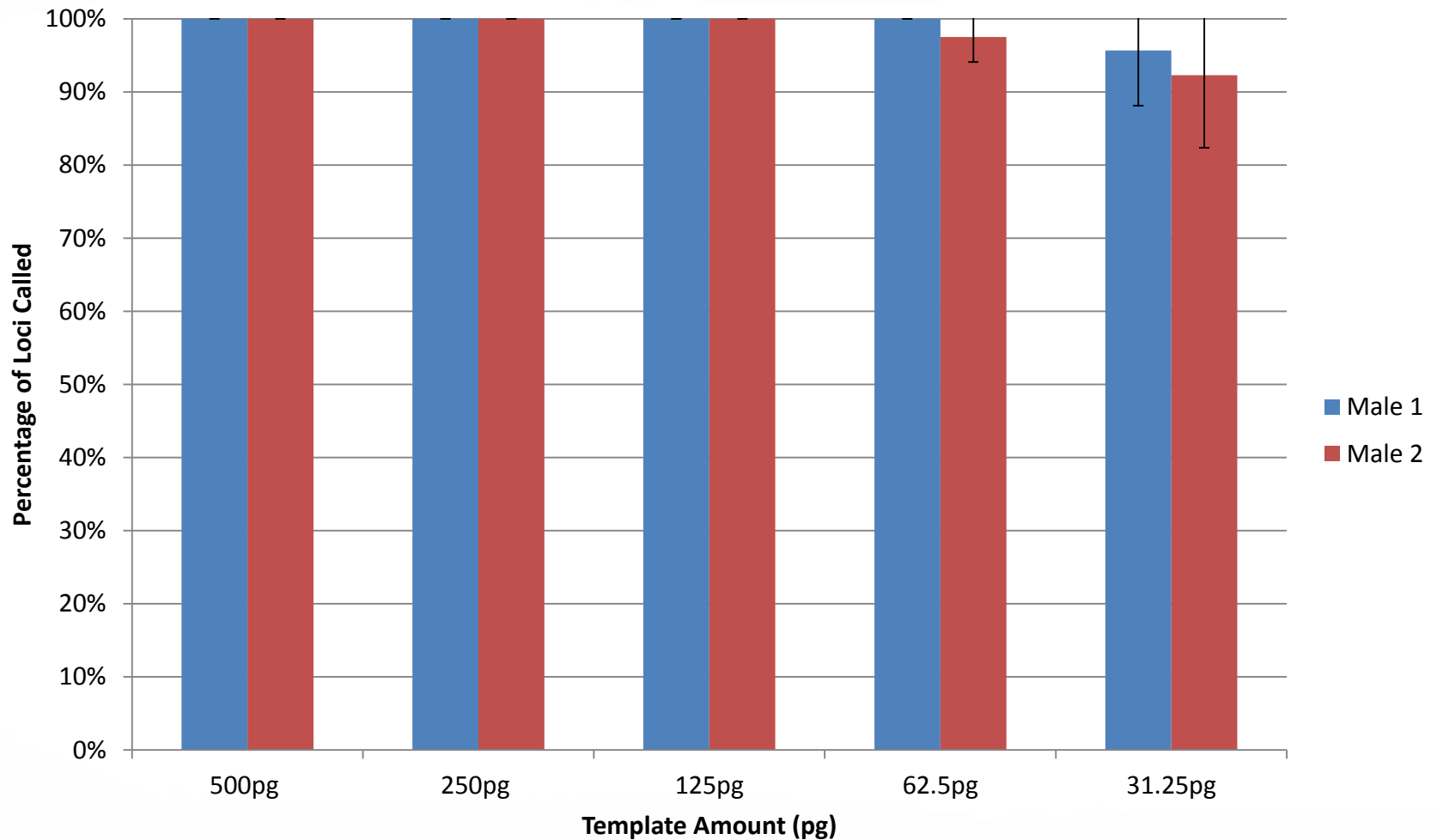
## 2.8 Mixture Studies

### Male/Male Mixtures, 1 to 1 ratio



## 2.8 Mixture Studies

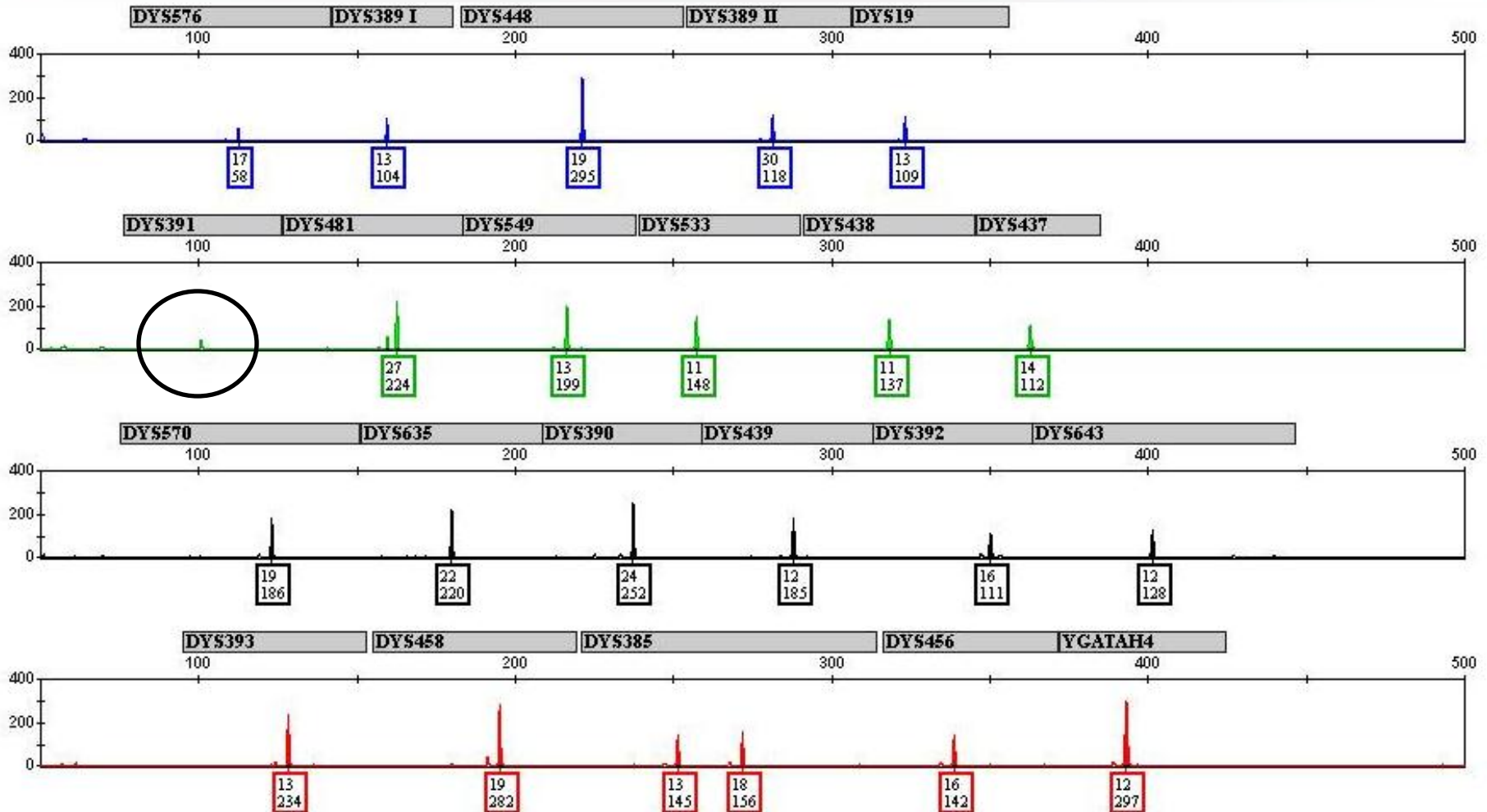
Male/female Mixtures – decreasing male DNA with 400ng female DNA





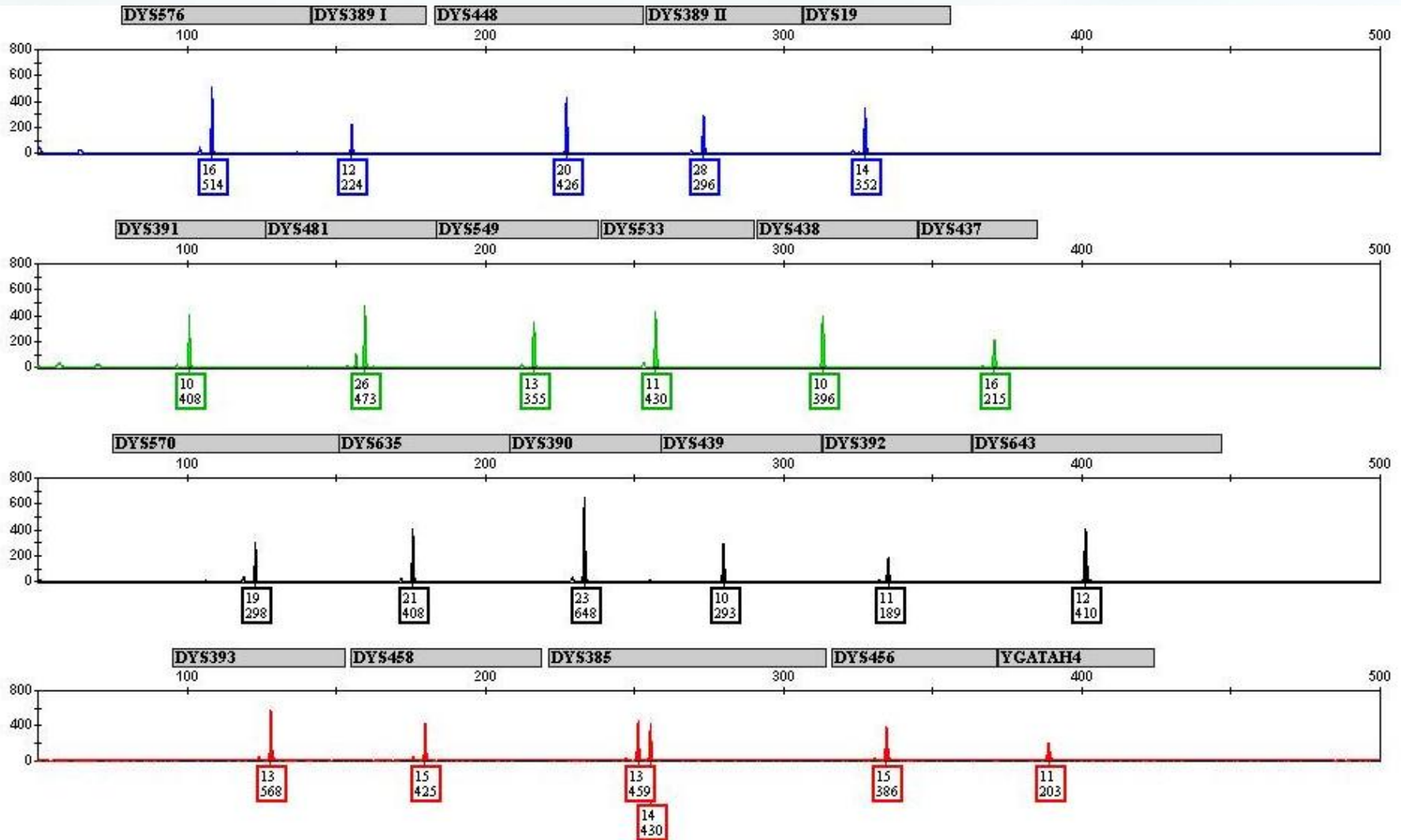
## 2.8 Mixture Studies

Male Varied/Female Constant Mixtures – 62.5pg of male DNA with 400ng female DNA (6400X)



## 2.8 Mixture Studies

Male Constant/Female Varied Mixtures – 125pg male DNA with 3000ng of female DNA (24,000X)



## ***2.9 Precision study***

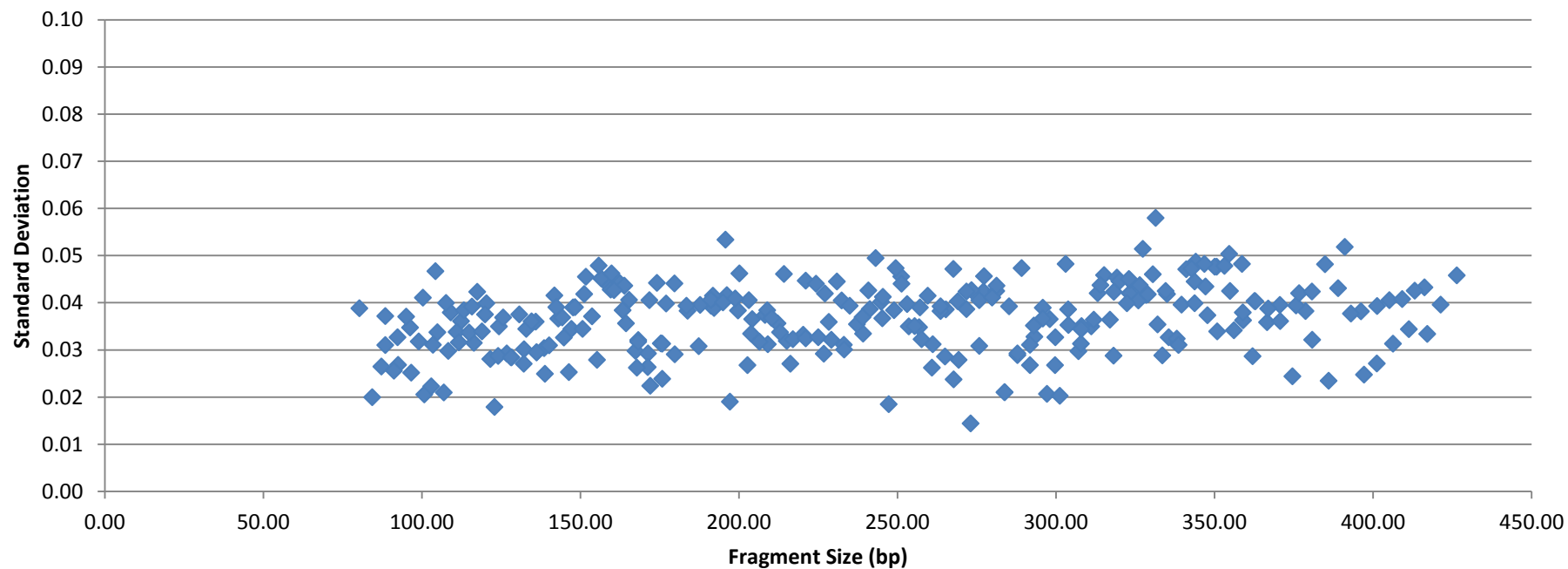
- Precision was performed at each laboratory
- Both 3130 and 3500 series of Genetic analyzers were tested

## 2.9 Precision Studies

### AB 3130



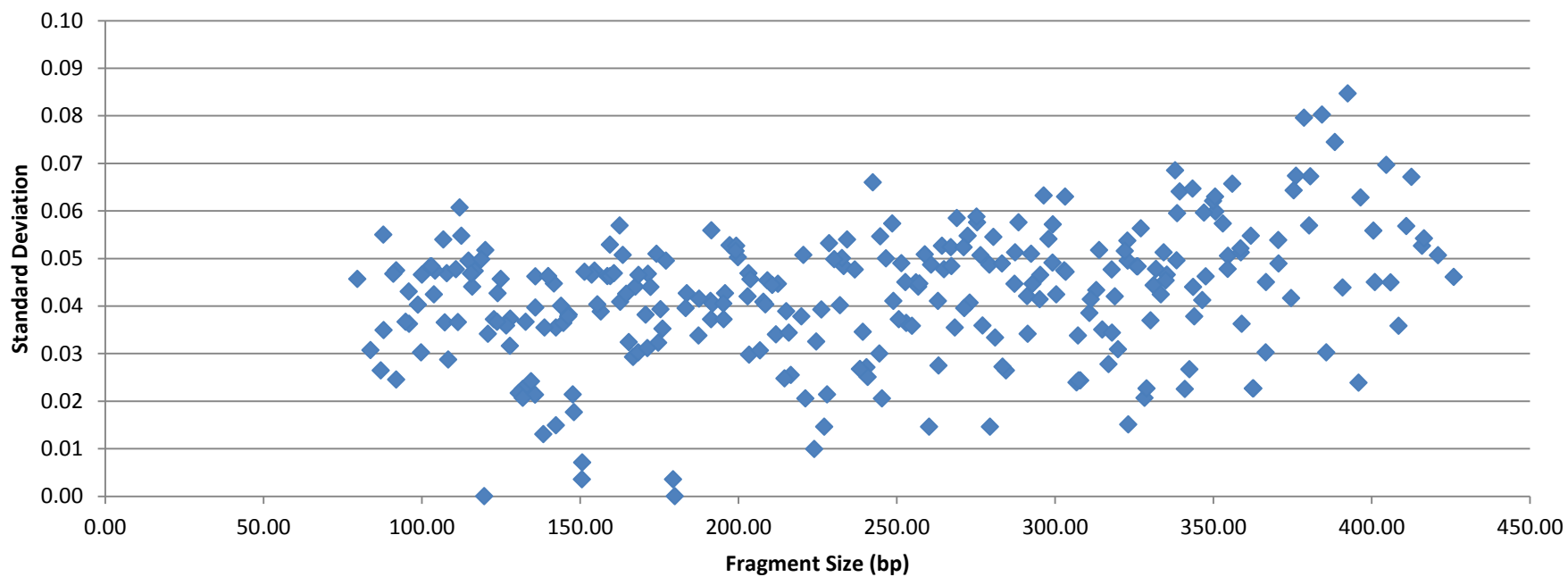
Precision of 14 Ladders on 3130 with POP-4®



## 2.9 Precision Studies AB 3500xl



Precision of 8 Ladders on 3500xl with POP-4®



## 2.10.1 Specificity and robustness

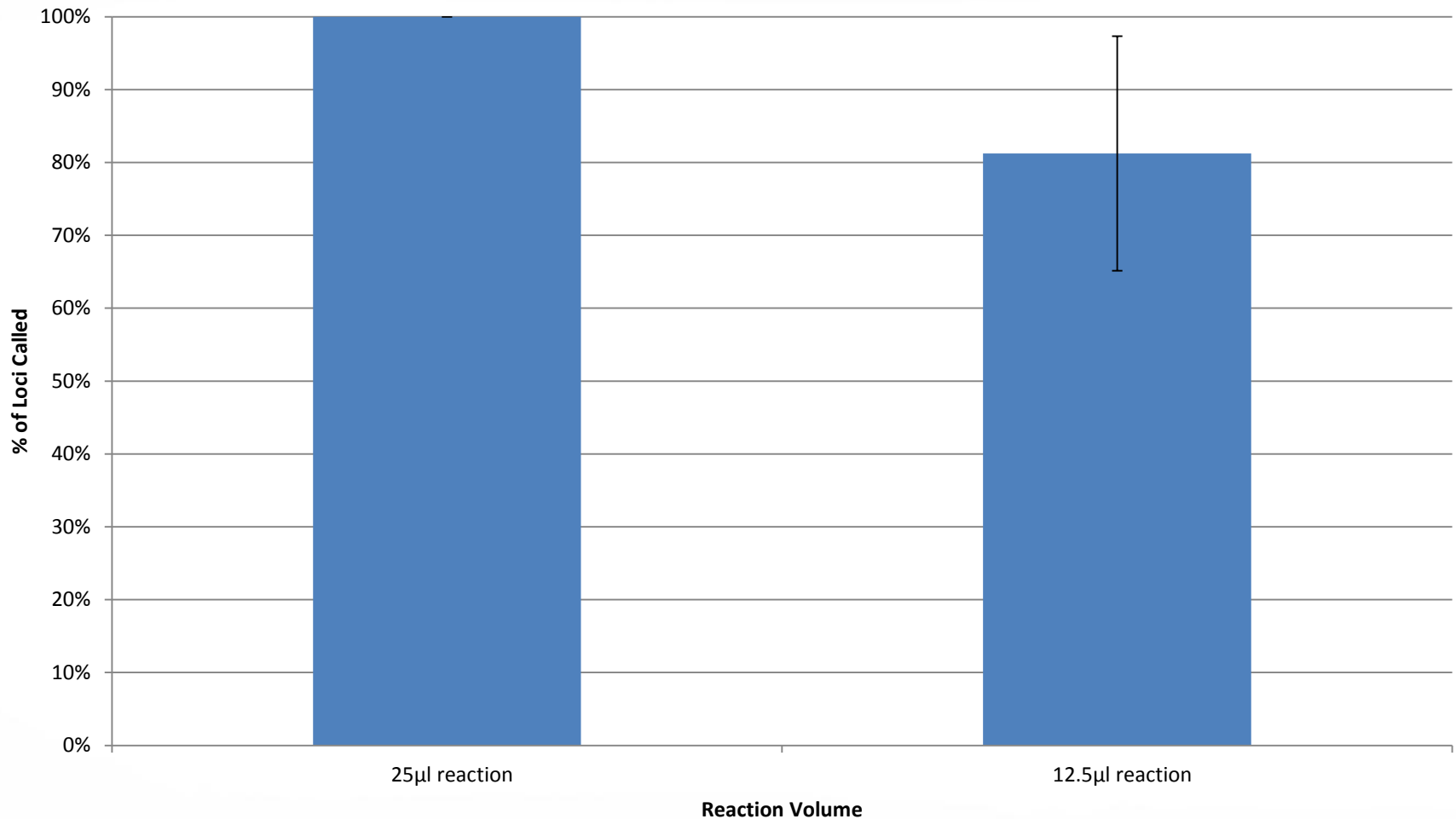
- Reaction Volume - 25 $\mu$ l vs 12.5 $\mu$ l
  - Extracted DNA - data not yet available
  - Blood on FTA – 1 – 1.2mm punch
  - Buccal on FTA – 1 or 2 – 1.2mm punch
- Cycle Number
  - Extracted DNA – 28, 30, and 32 cycles
  - Blood on FTA, 1 – 1.2mm punch – 25, 26, and 27 cycles
  - Buccal on FTA, 2 – 1.2mm punch – 26, 27, and 28 cycles
  - Bode Buccal Collector™, 1 – 1.2mm punch, with PunchSolution™ - 25, 26, and 27 cycles
  - Blood on S&S 903, 1 – 1.2mm punch with PunchSolution™ - 25, 26, and 27 cycles
  - Omni swabs with SwabSolution™ , 1 entire swab head- 25, 26, and 27 cycles
  - Cotton swabs with SwabSolution™, 1 entire swab head - 25, 26, and 27 cycles

## ***2.10.1 Specificity and robustness***

- Annealing Temperature - 59°C, 61°C, 63°C,
  - Extracted DNA – 500pg male DNA
  - Blood on FTA – 1 – 1.2mm punch
  - Buccal on FTA – 2 – 1.2mm punch
- PCR Reaction Components, magnesium and EDTA titration
  - Extracted DNA – 500pg male DNA
- Primer Concentration – 0.75X, 1X, 1.25X
  - Extracted DNA – 500pg male DNA – data not yet available
  - Blood on FTA – 1 – 1.2mm punch
  - Buccal on FTA – 2 -1.2mm punch

## 2.10.1 Specificity and robustness

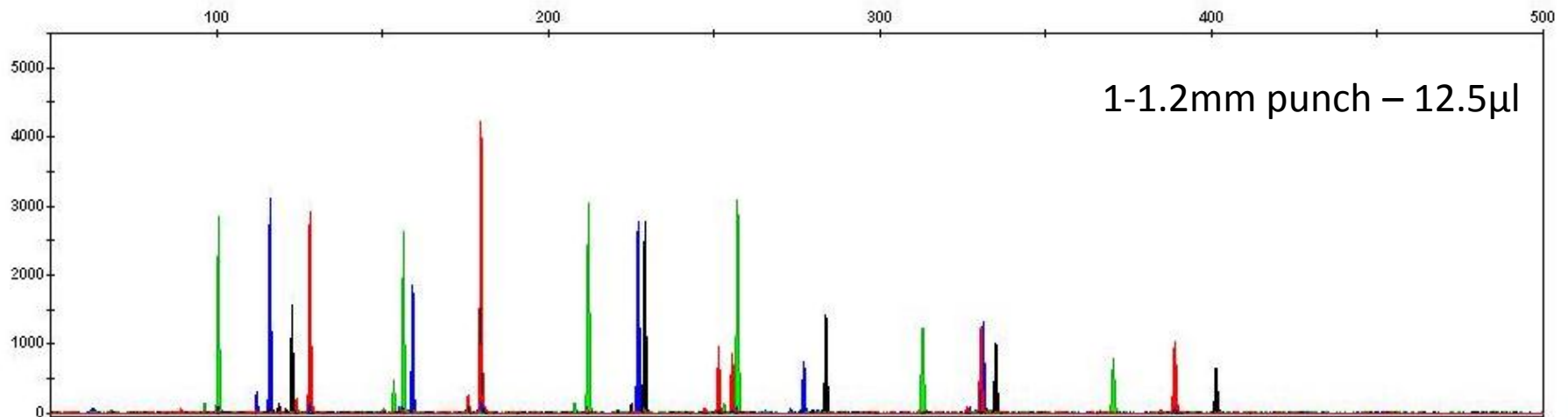
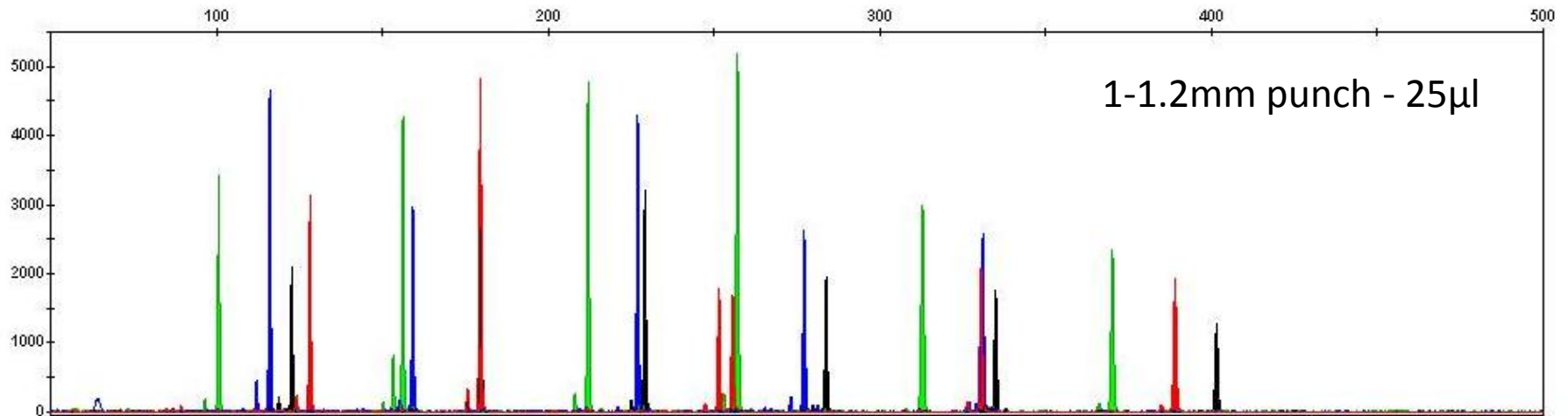
### Reaction Volume – Blood on FTA





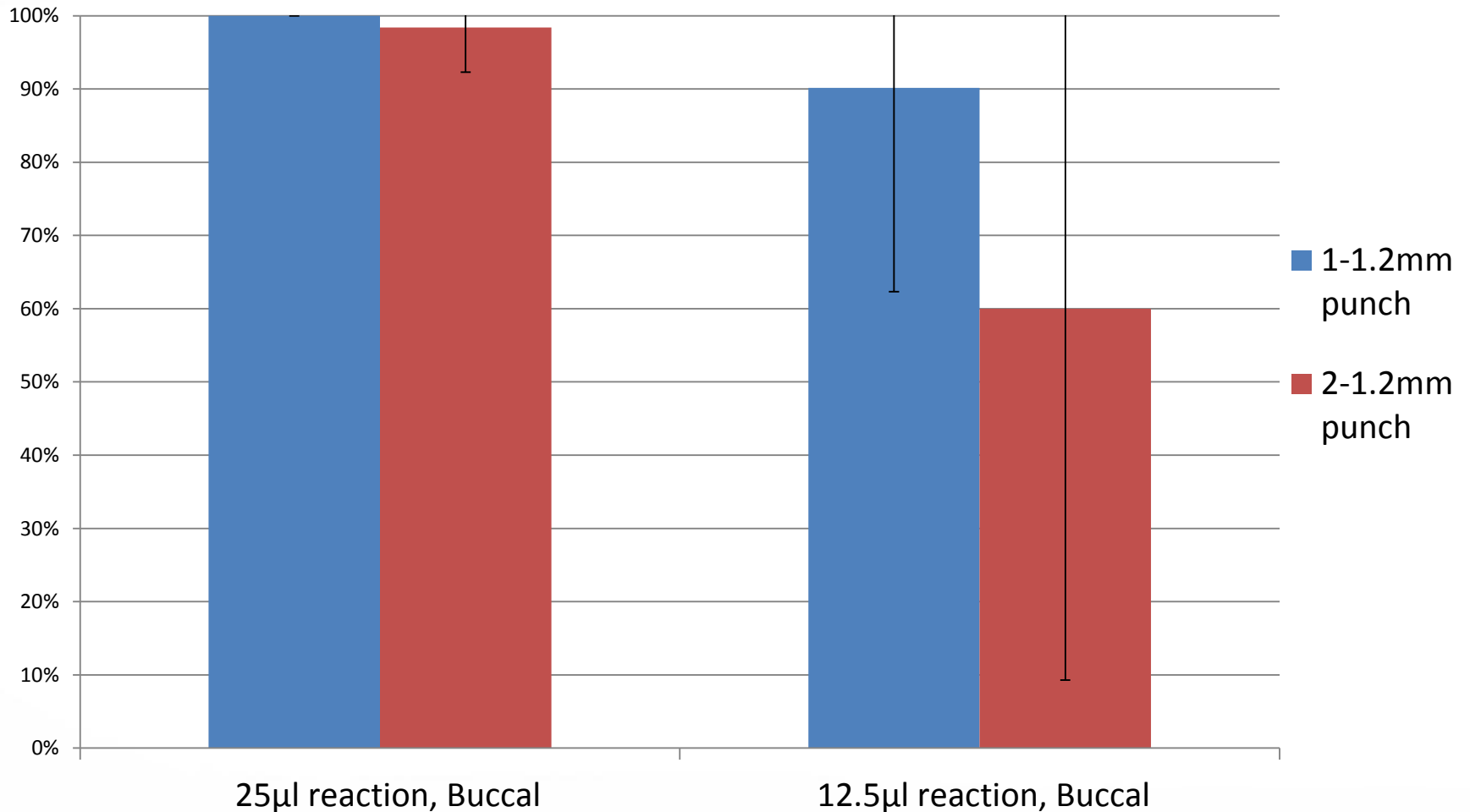
## 2.10.1 Specificity and robustness

### Reaction Volume – Blood on FTA



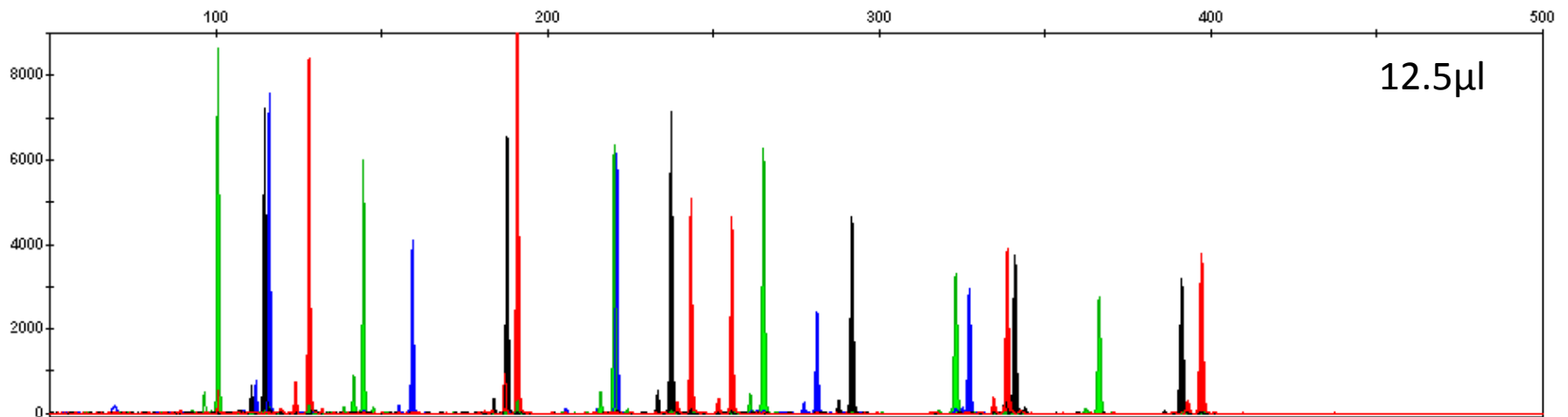
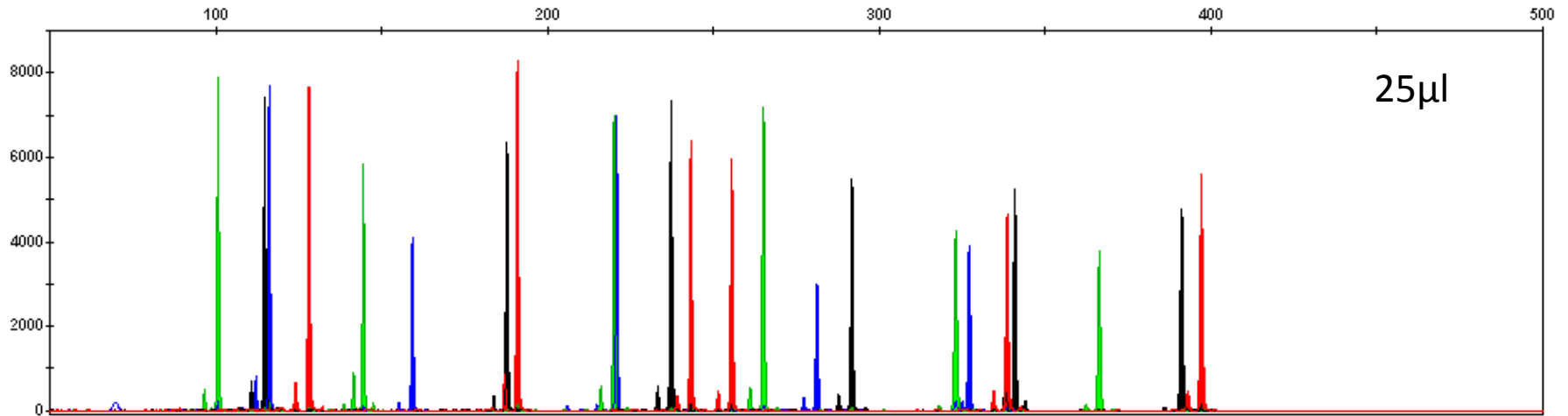
## 2.10.1 Specificity and robustness

### Reaction Volume – Buccal on FTA

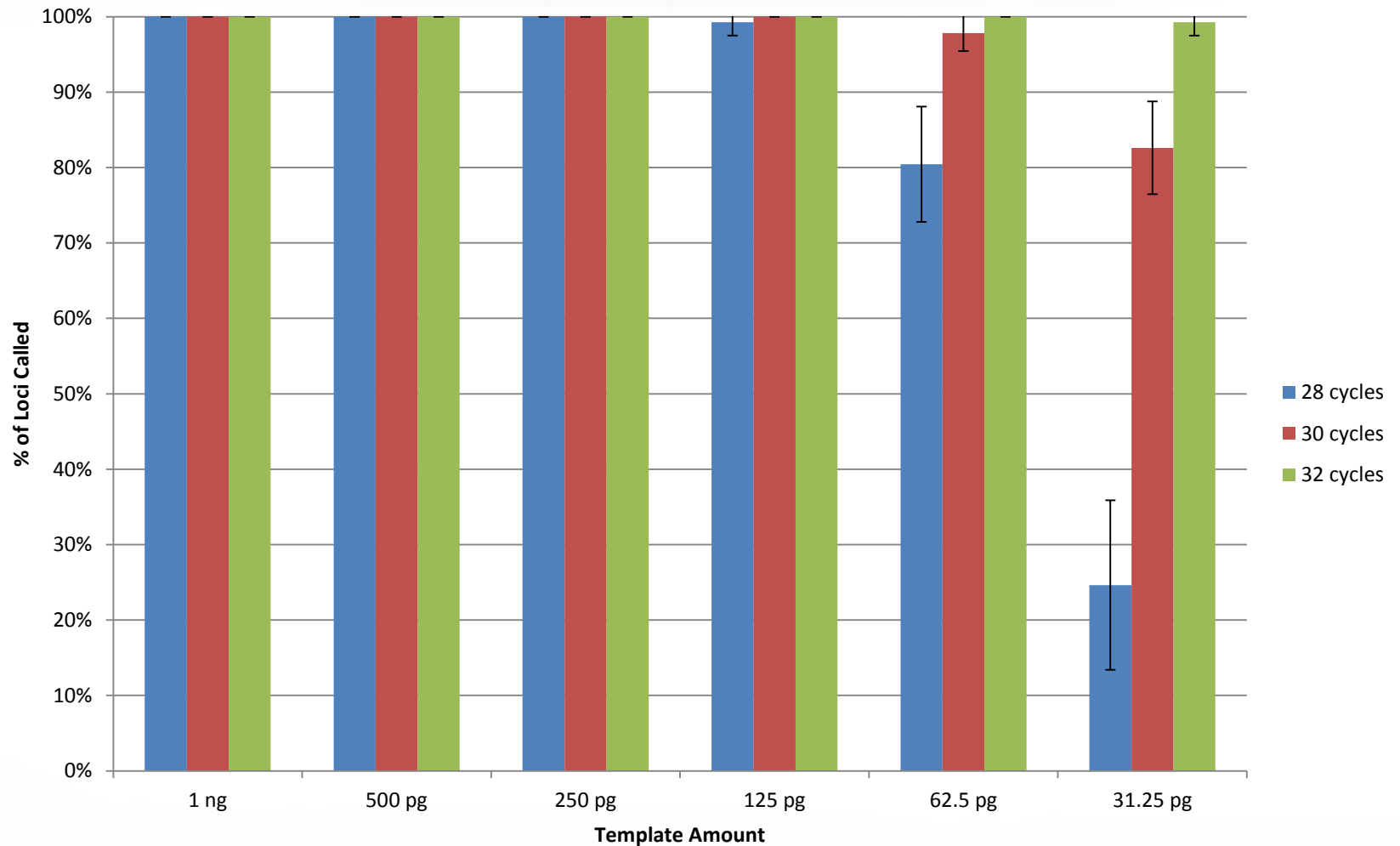


## 2.10.1 Specificity and robustness

Reaction Volume – Buccal on FTA, 1 – 1.2mm punch

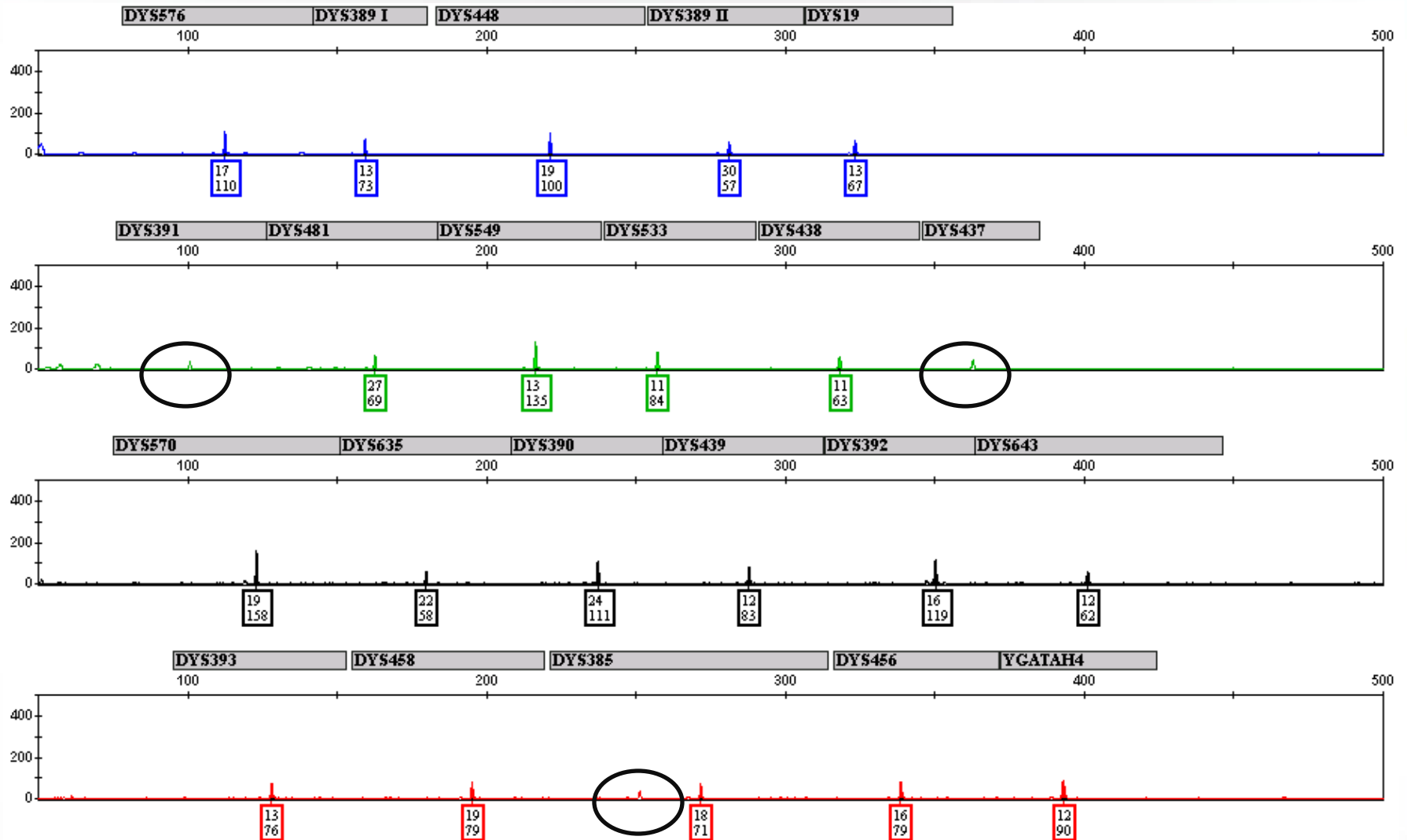


## 2.10.1 Specificity and robustness Cycle Number – Extracted DNA



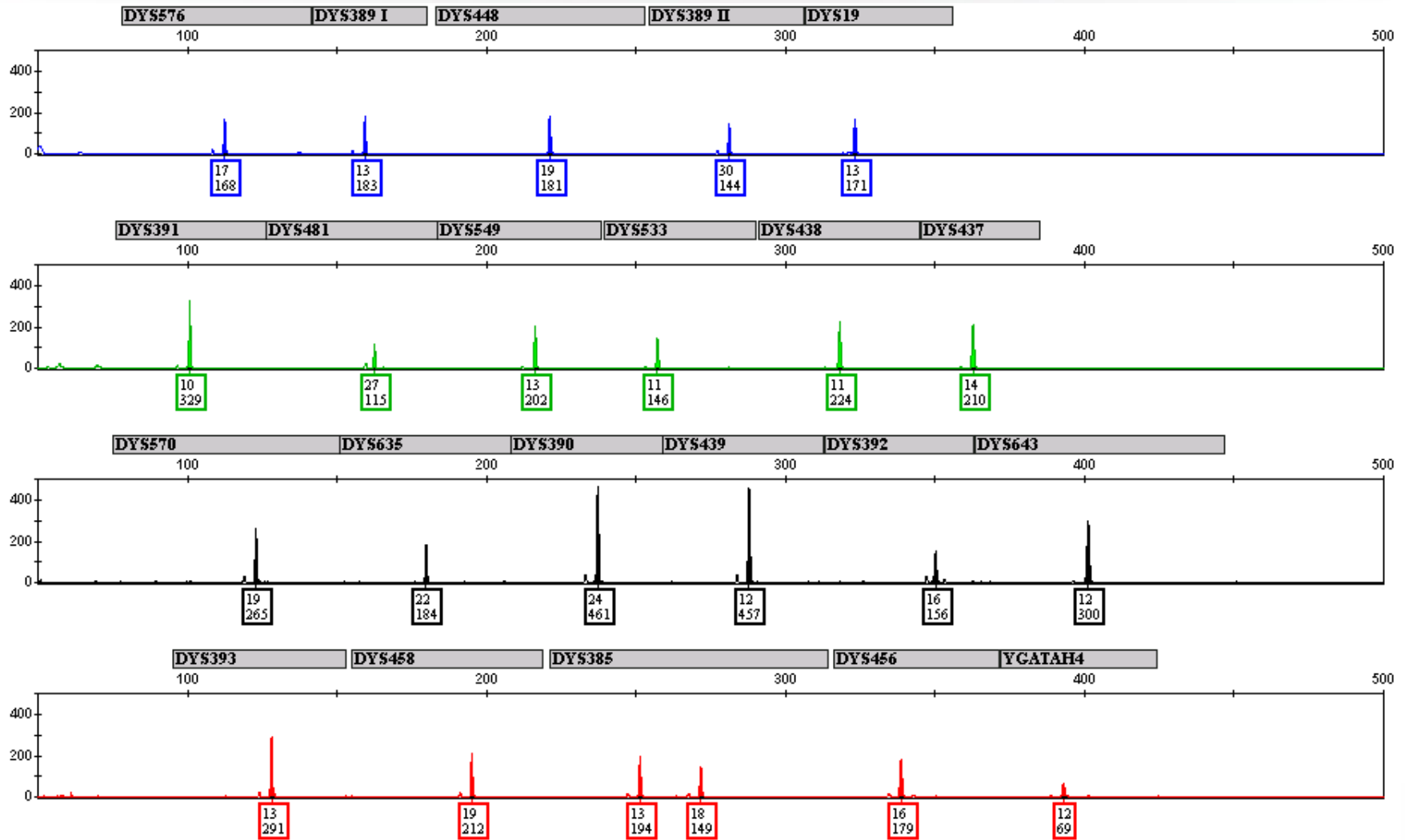
## 2.10.1 Specificity and robustness

Cycle Number – 62.5pg, 28 cycles



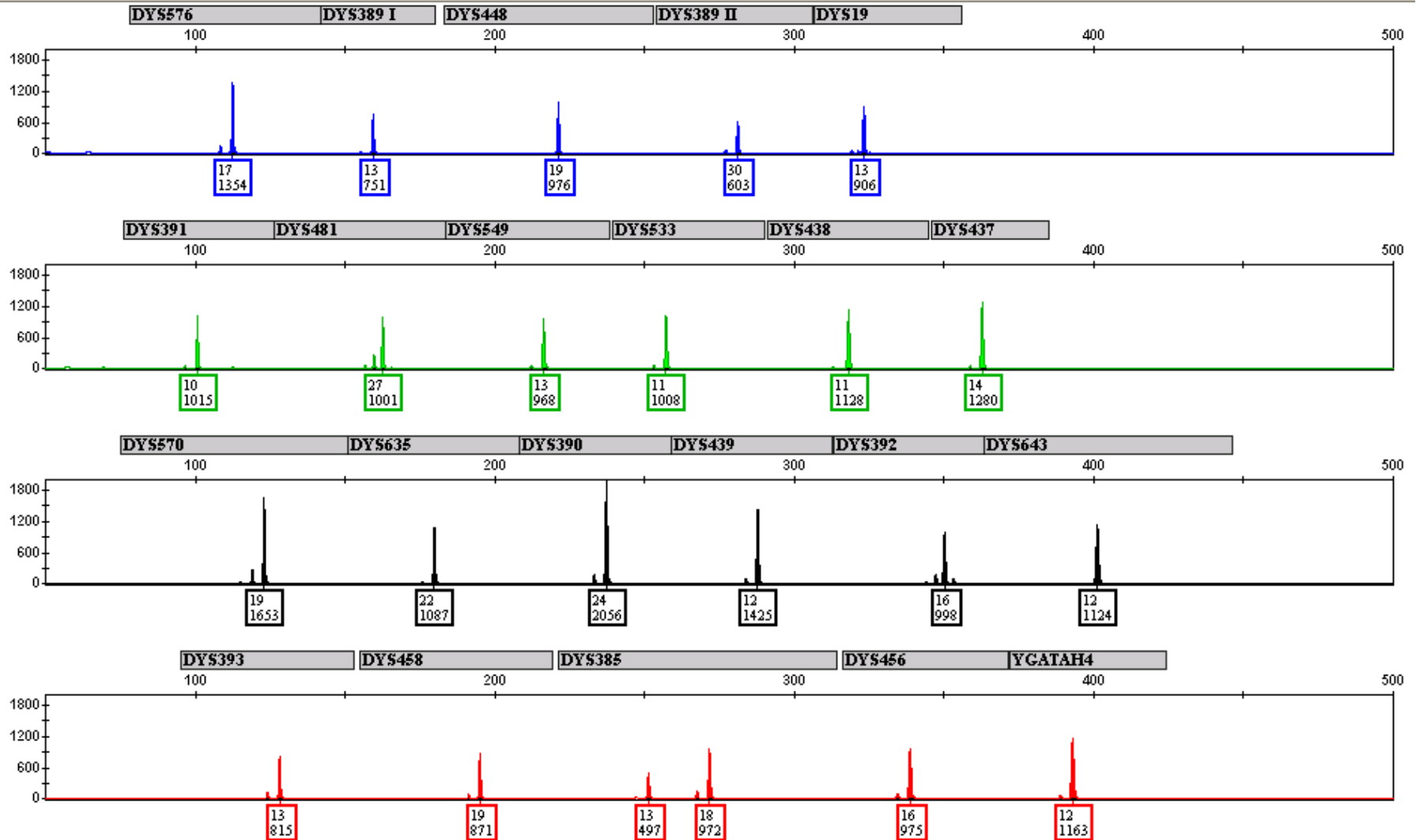
# 2.10.1 Specificity and robustness

Cycle Number – 62.5pg, 30 cycles



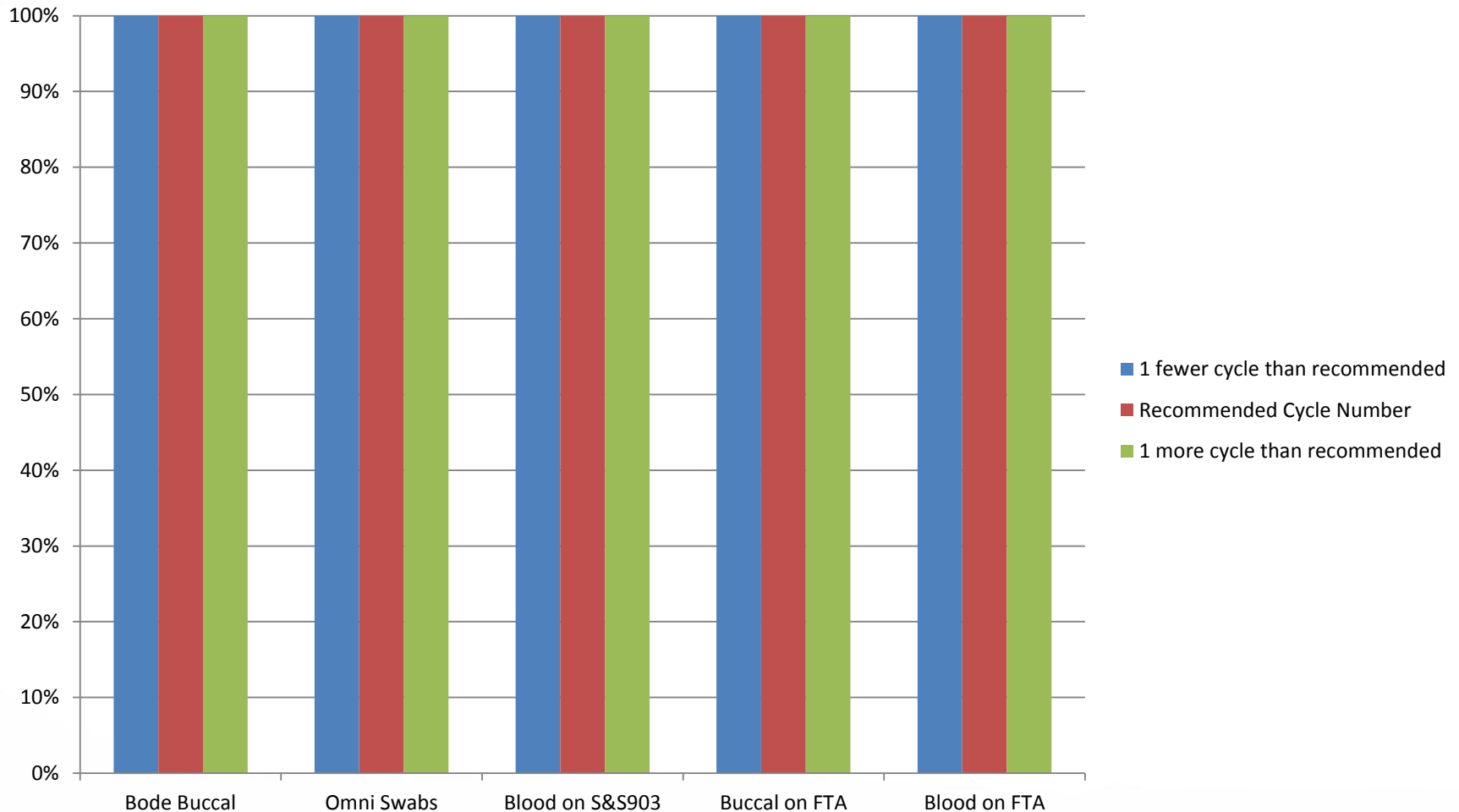
# 2.10.1 Specificity and robustness

Cycle Number – 62.5pg, 32 cycles



## 2.10.1 Specificity and robustness

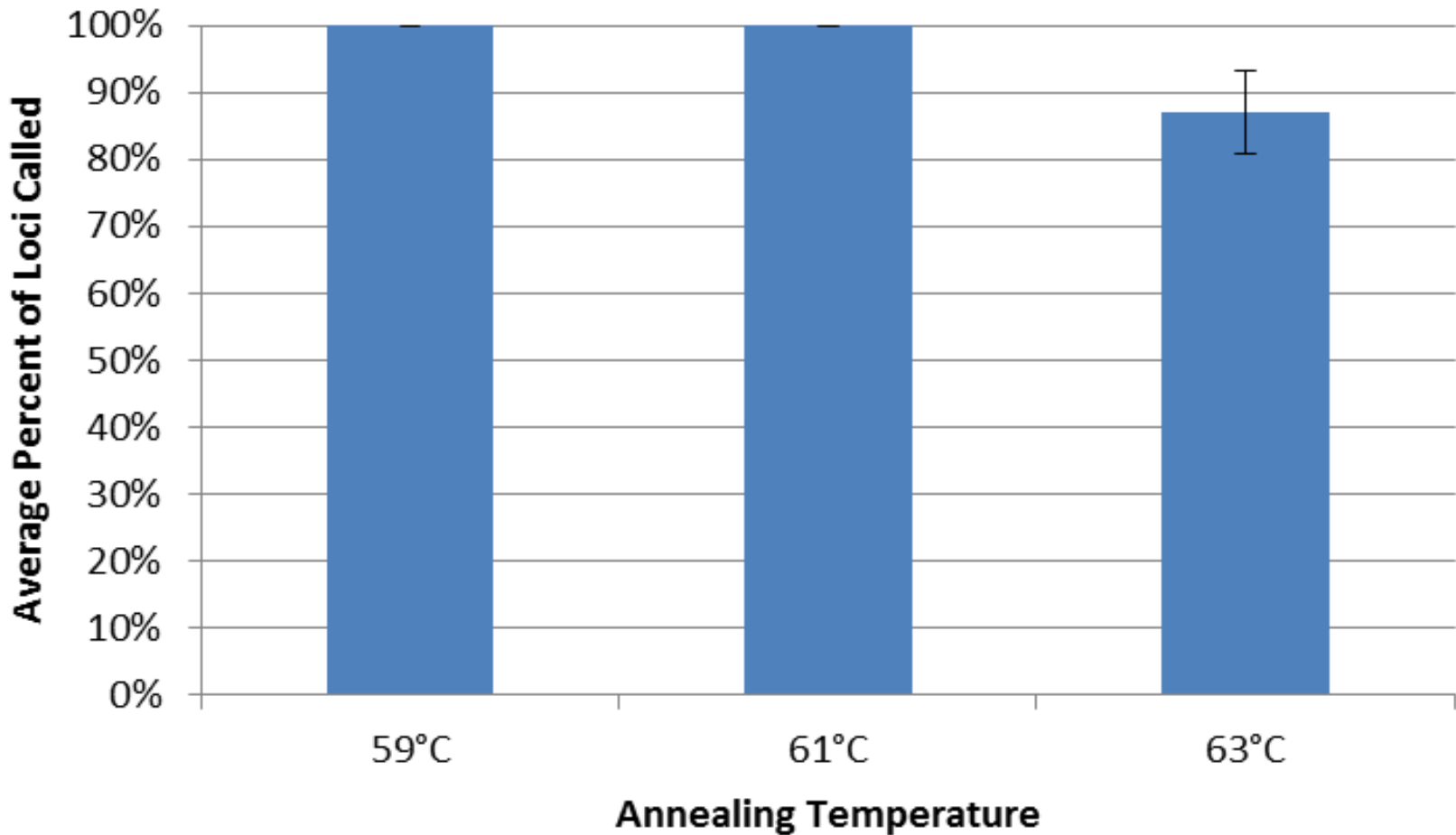
### Cycle Number – Direct Amplifications





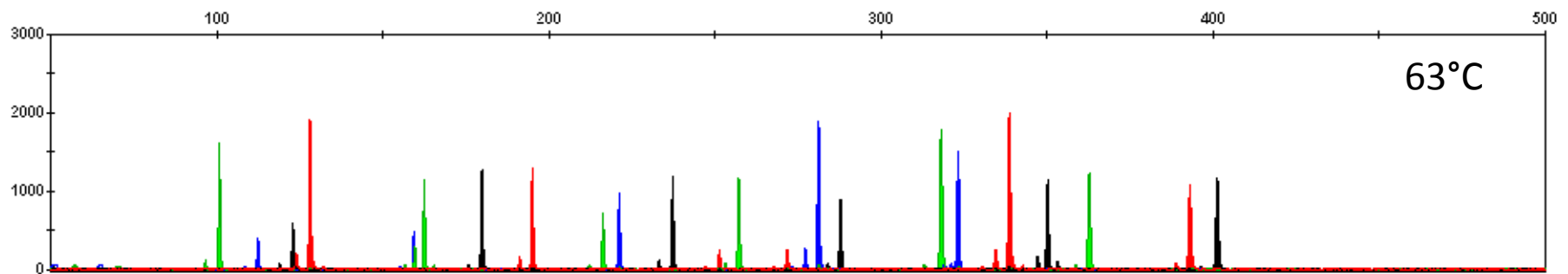
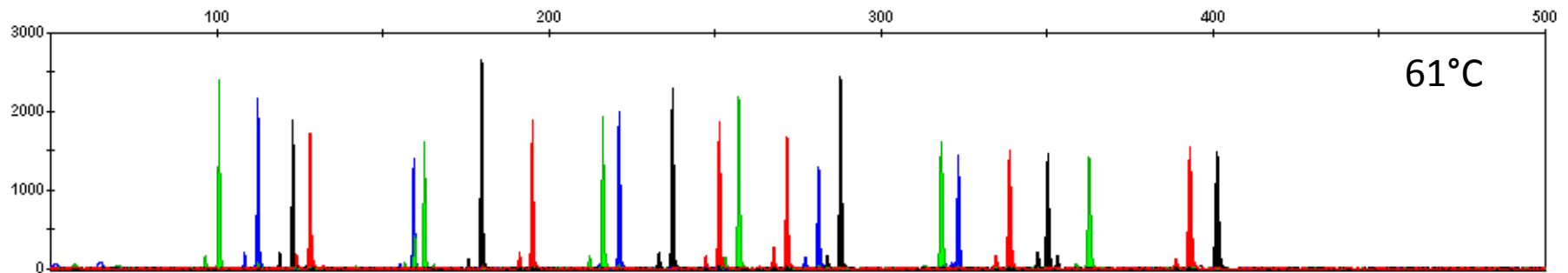
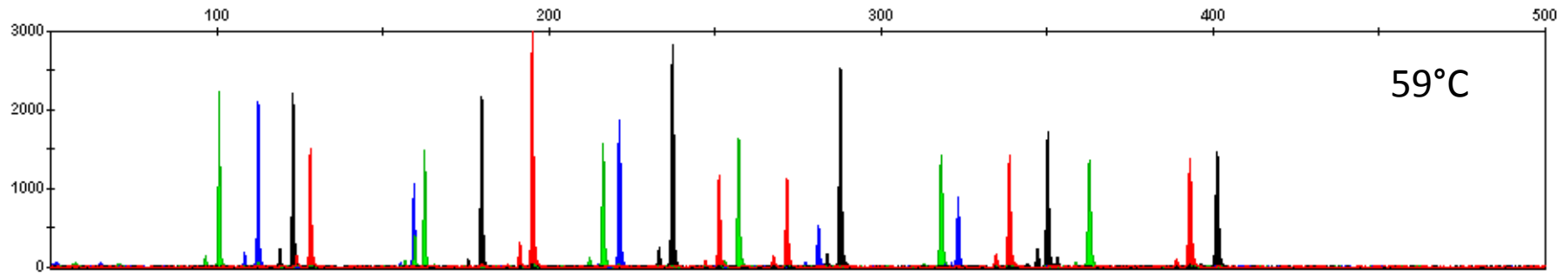
## 2.10.1 Specificity and robustness

Annealing Temperature – Extracted DNA, 100pg data



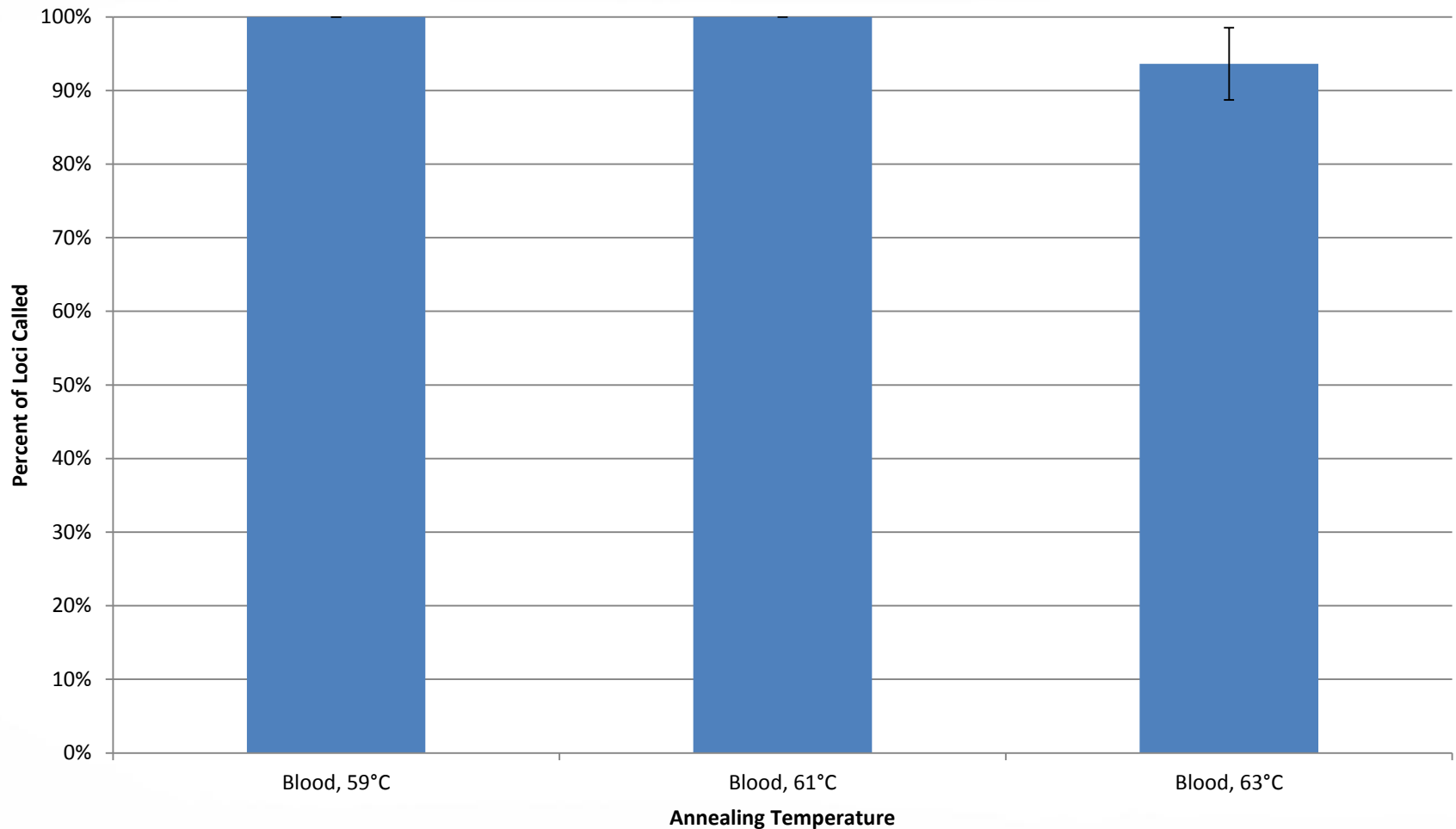
## 2.10.1 Specificity and robustness

### Annealing Temperature – Extracted DNA



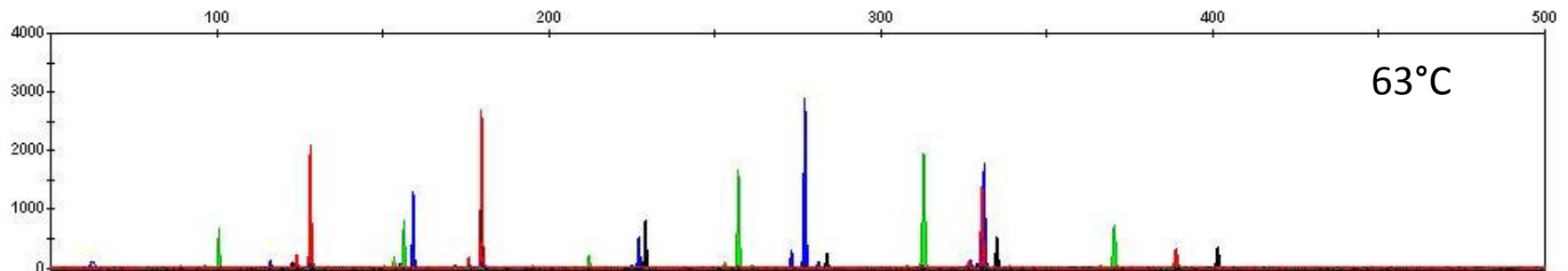
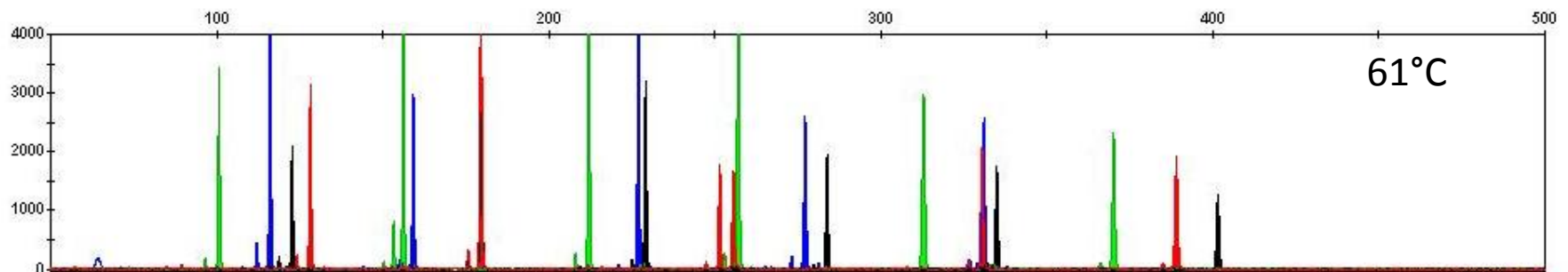
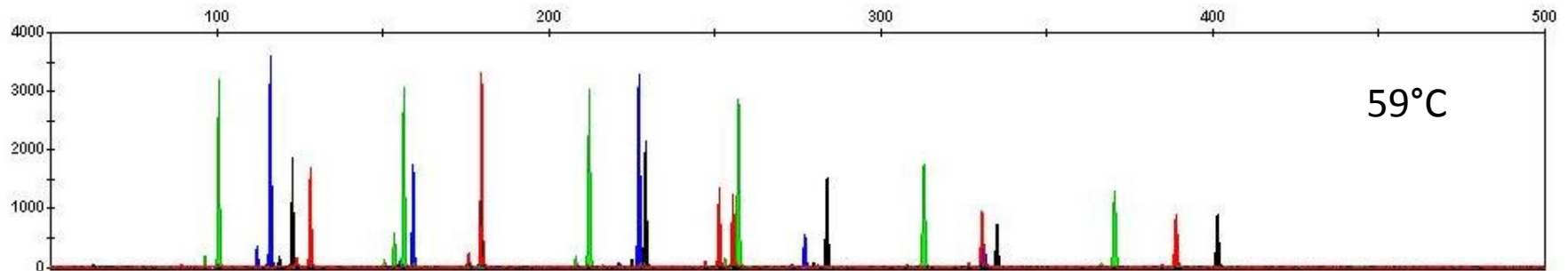
## 2.10.1 Specificity and robustness

### Annealing Temperature – Blood on FTA



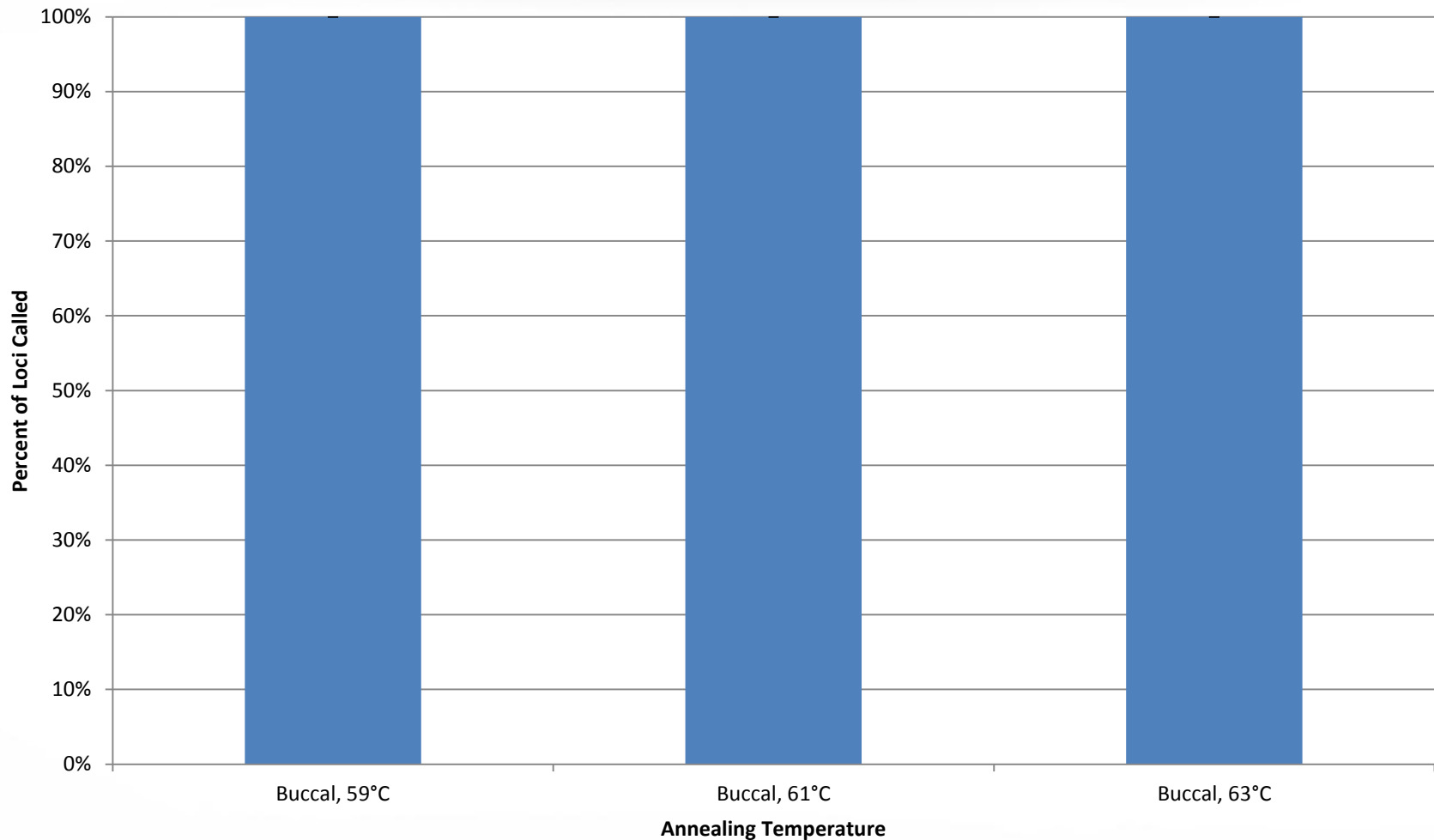
## 2.10.1 Specificity and robustness

### Annealing Temperature – Blood on FTA



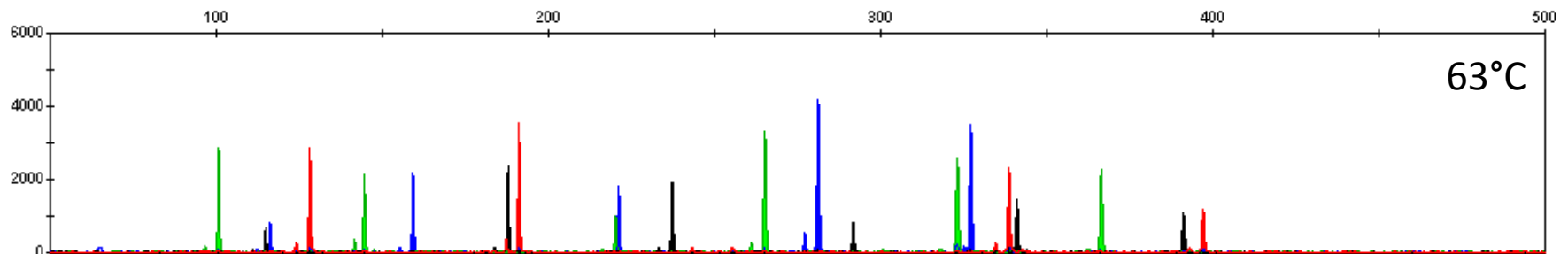
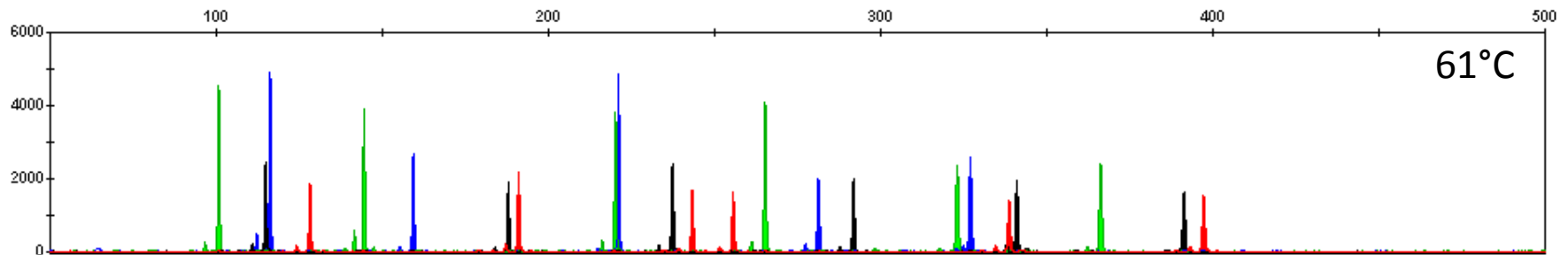
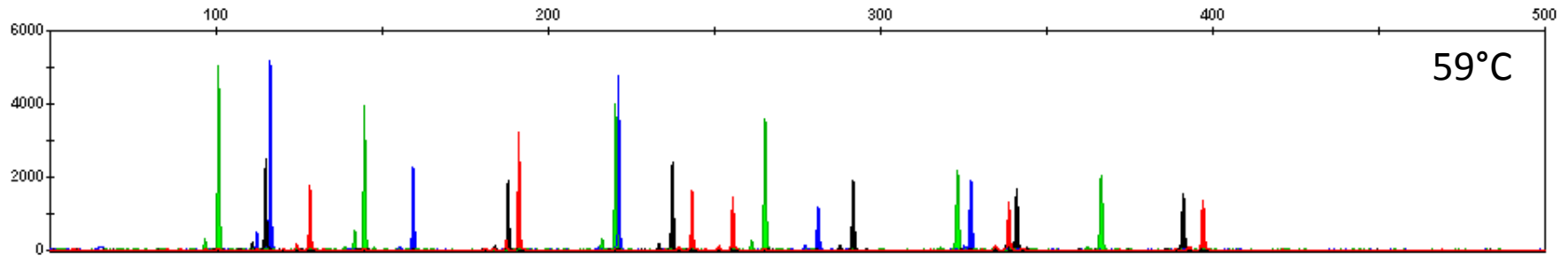
## 2.10.1 Specificity and robustness

### Annealing Temperature – Buccal on FTA



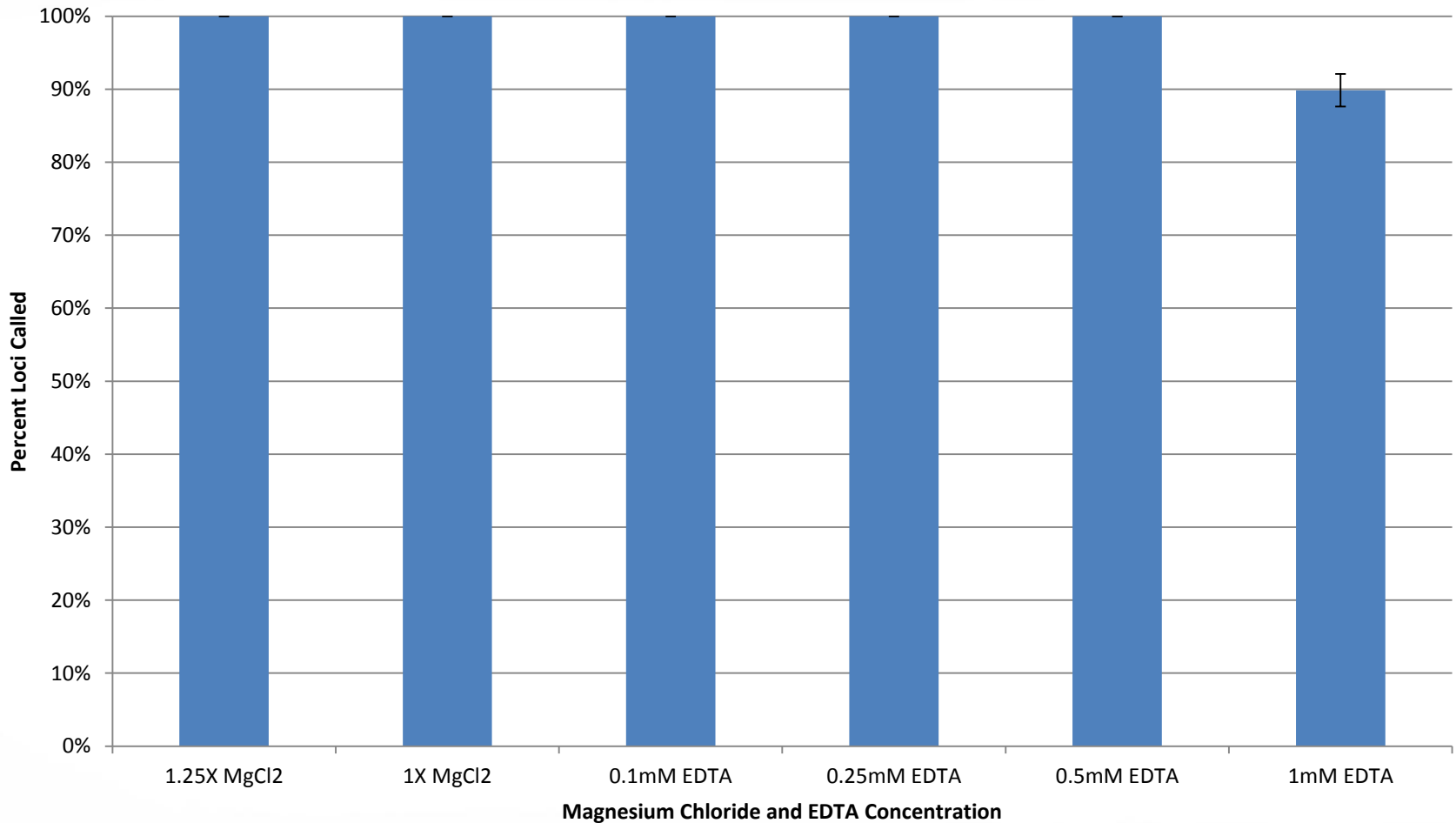
## 2.10.1 Specificity and robustness

### Annealing Temperature – Buccal on FTA



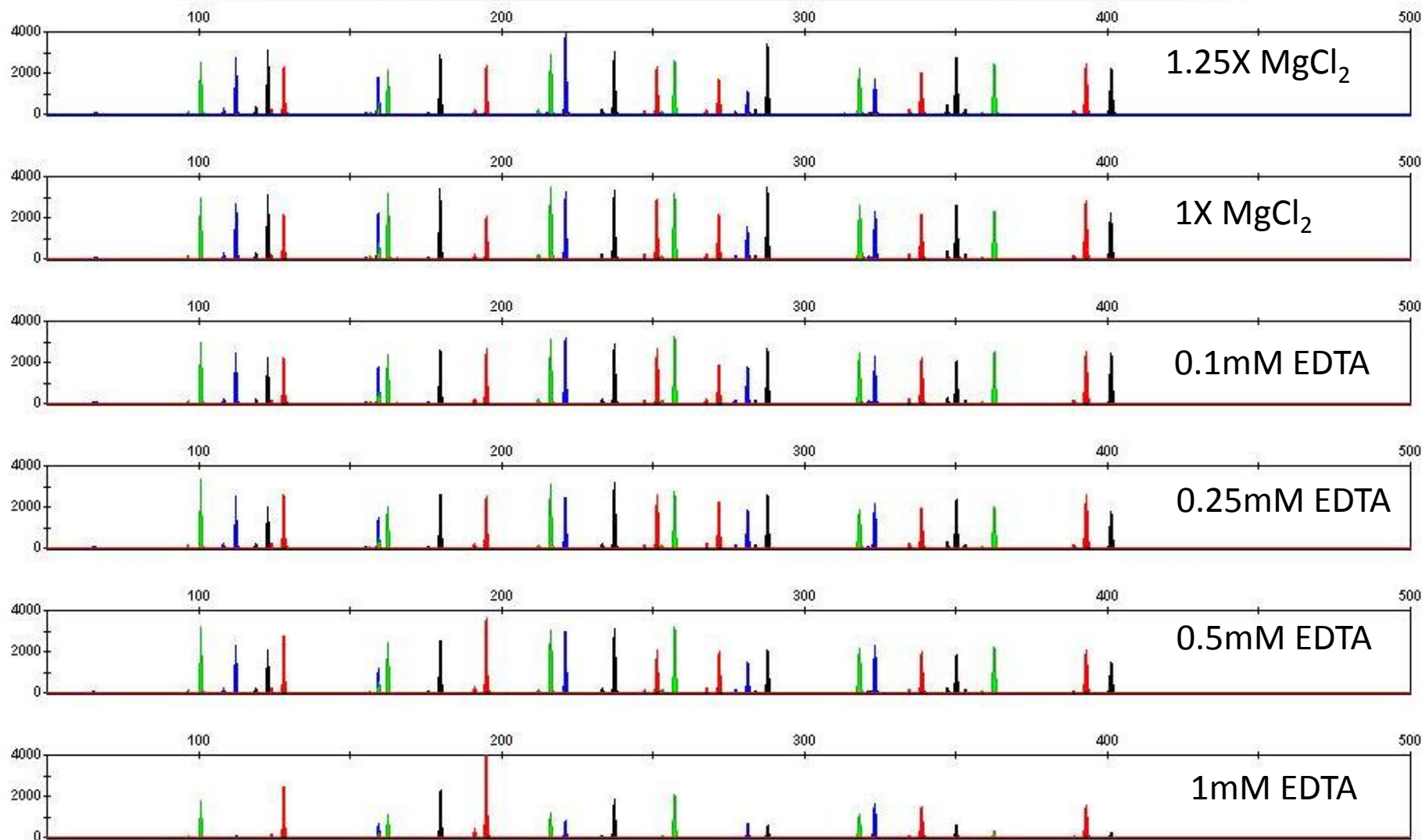
## 2.10.1 Specificity and robustness

### PCR reaction components – Extracted DNA



## 2.10.1 Specificity and robustness

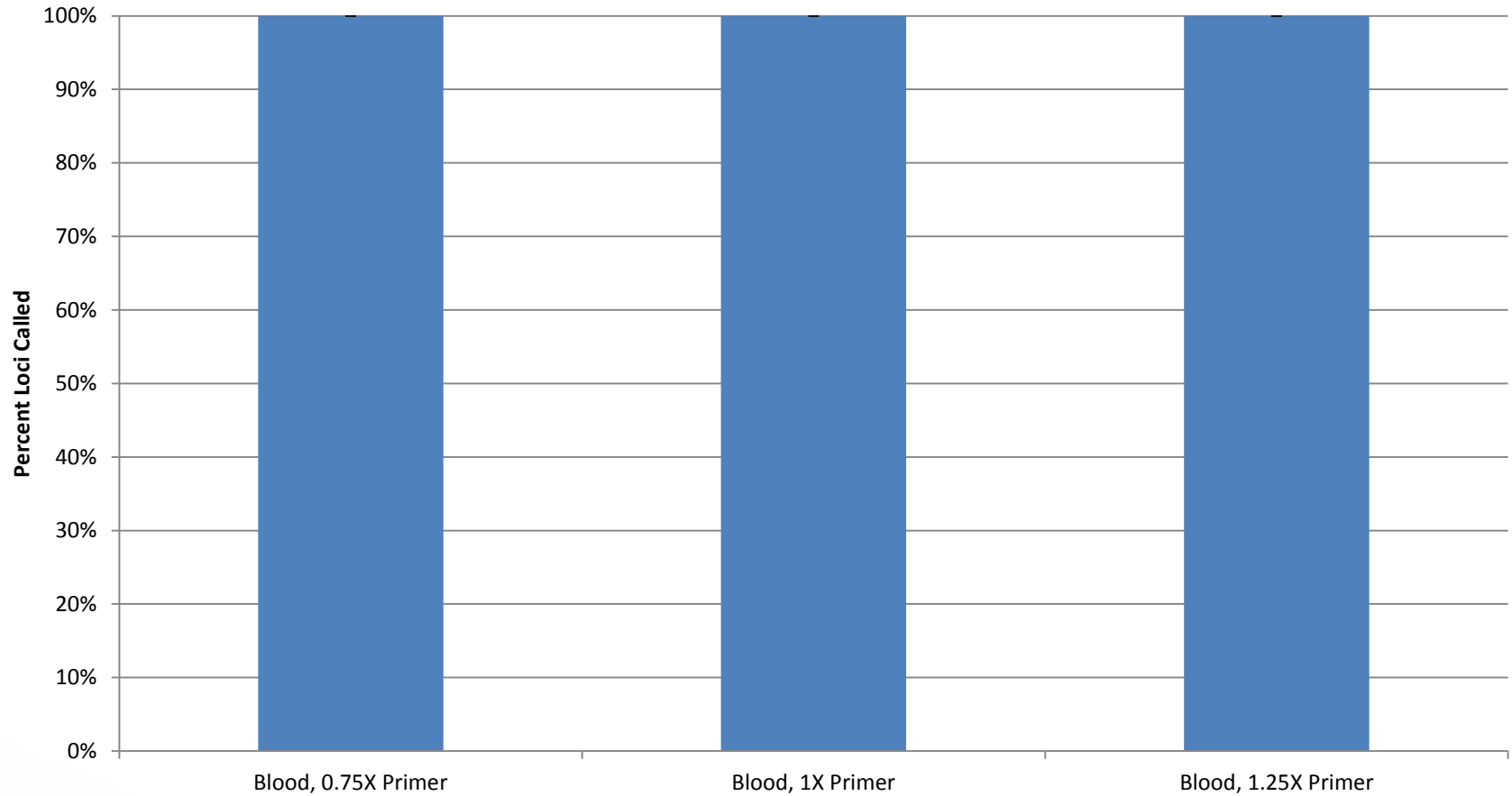
### PCR reaction components – Extracted DNA





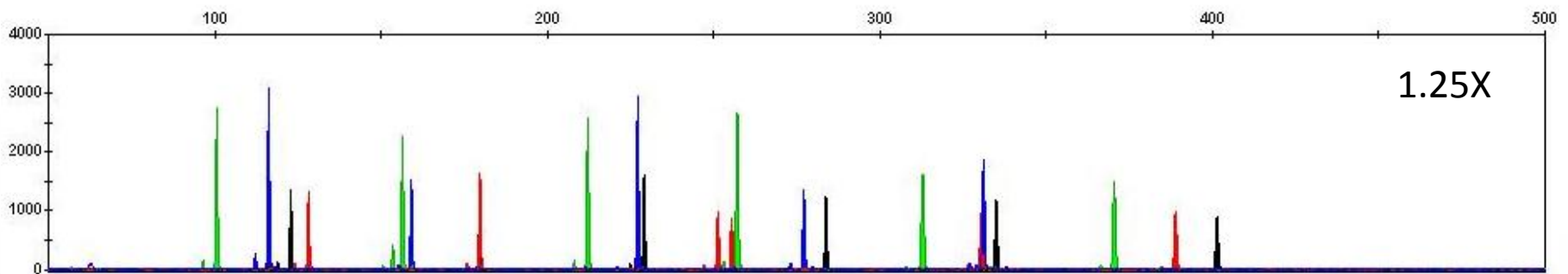
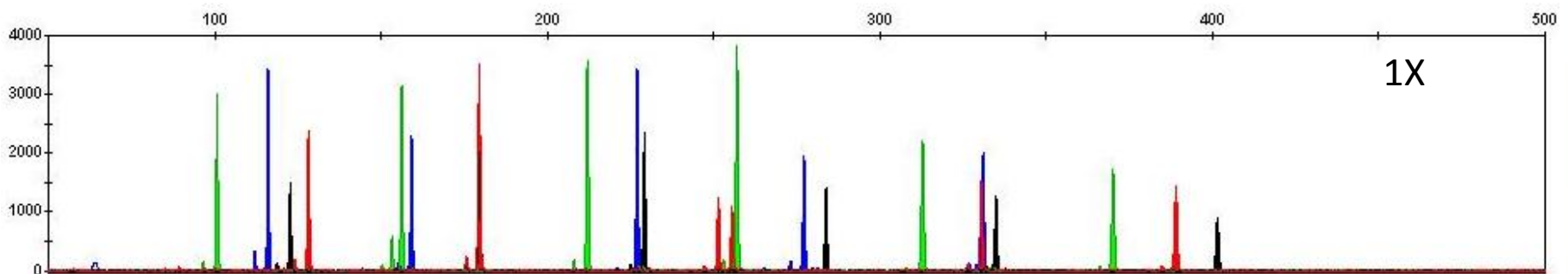
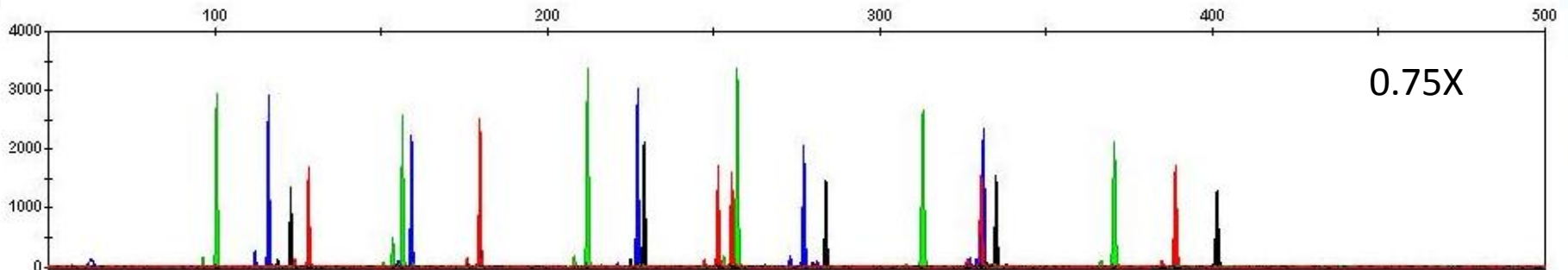
## 2.10.1 Specificity and robustness

### Primer Concentration – Blood on FTA



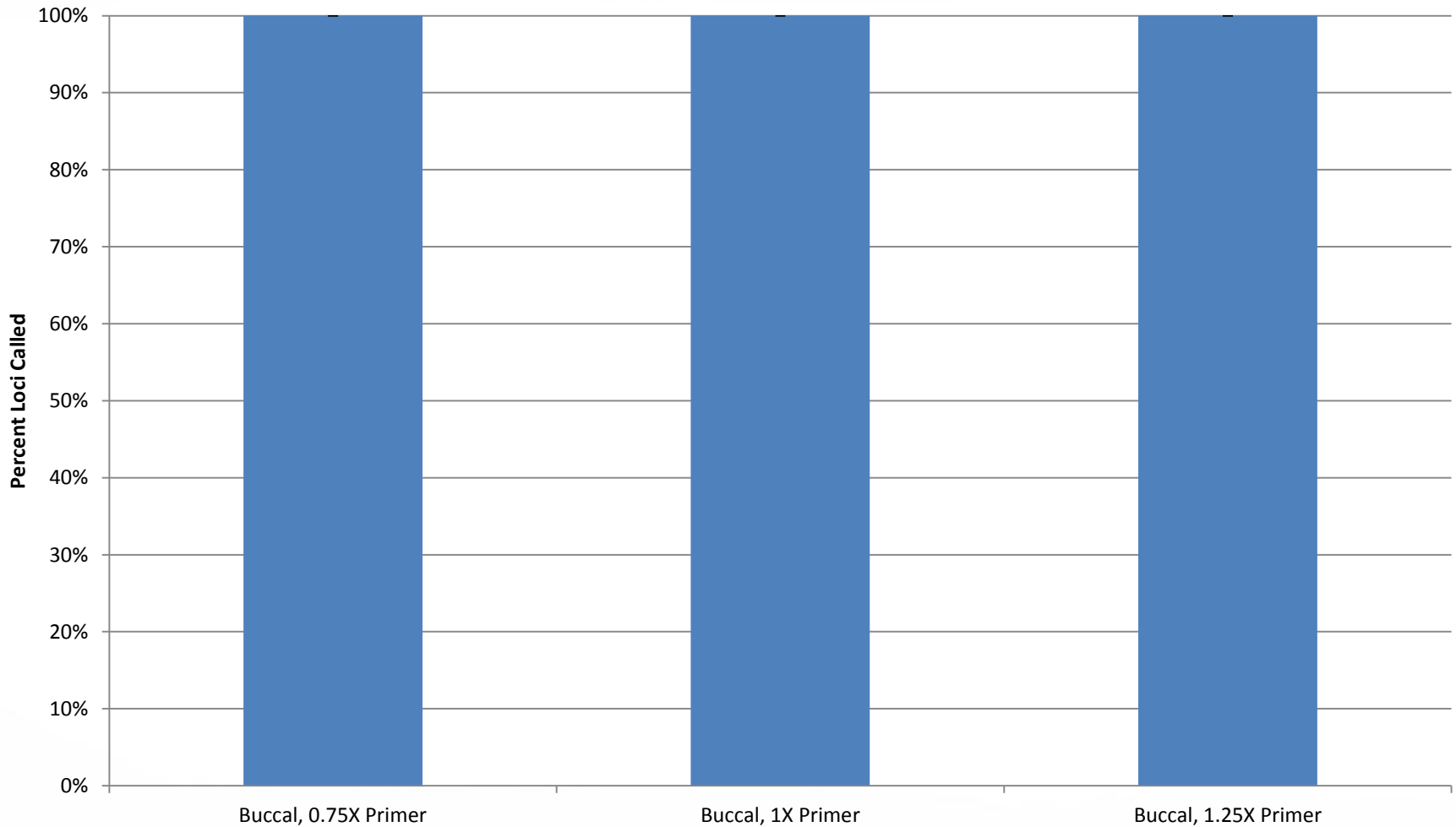
## 2.10.1 Specificity and robustness

### Primer Concentration – Blood on FTA



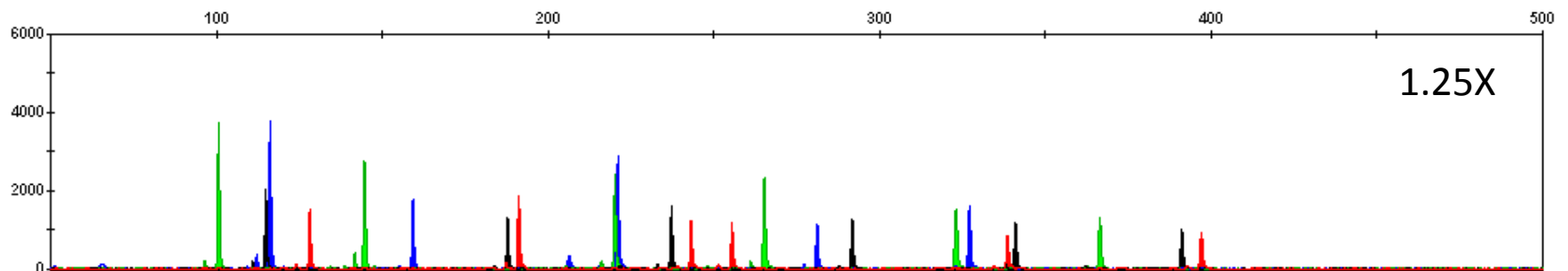
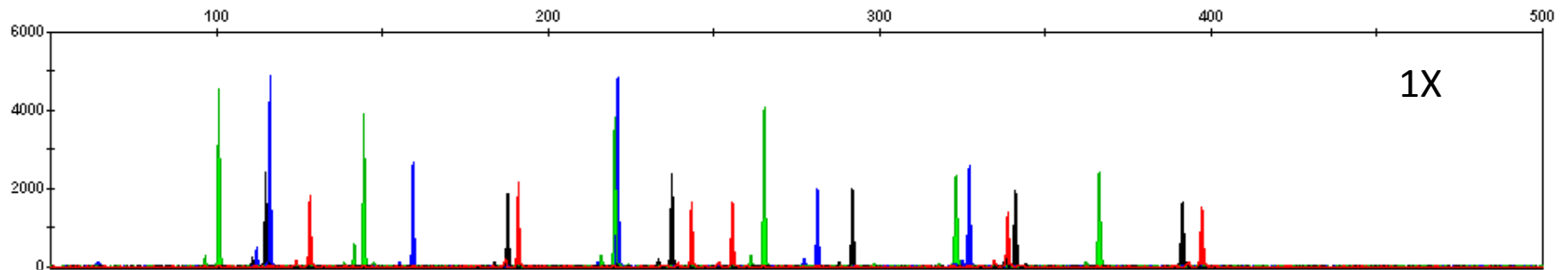
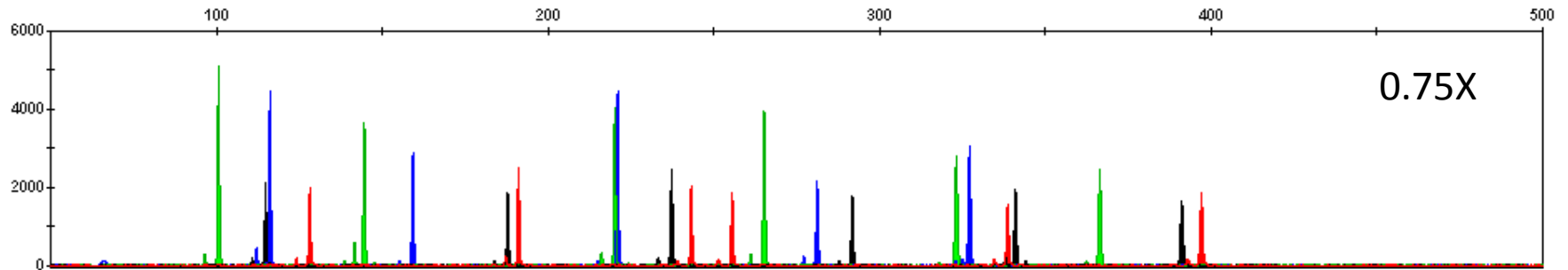
## 2.10.1 Specificity and robustness

### Primer Concentration – Buccal on FTA

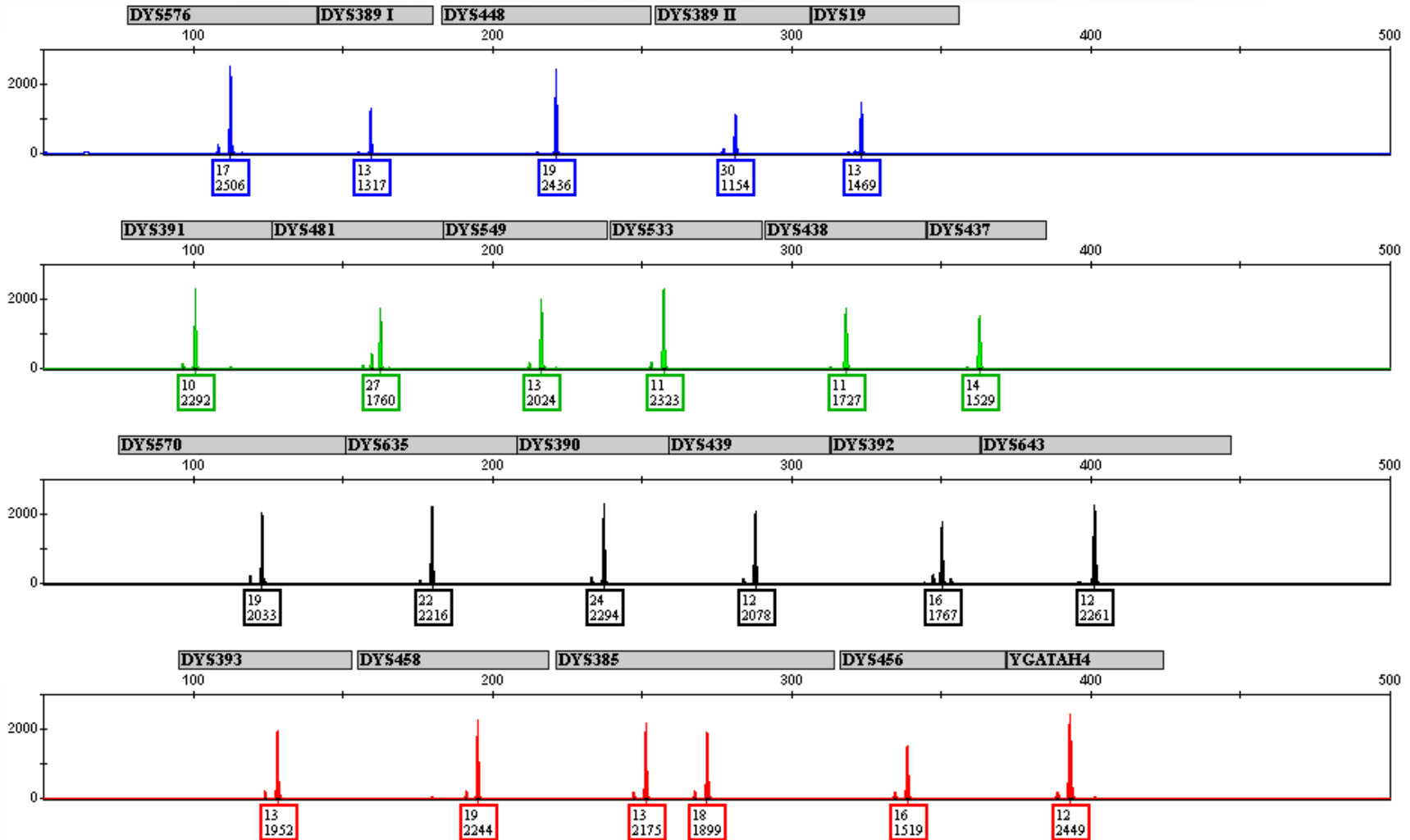


## 2.10.1 Specificity and robustness

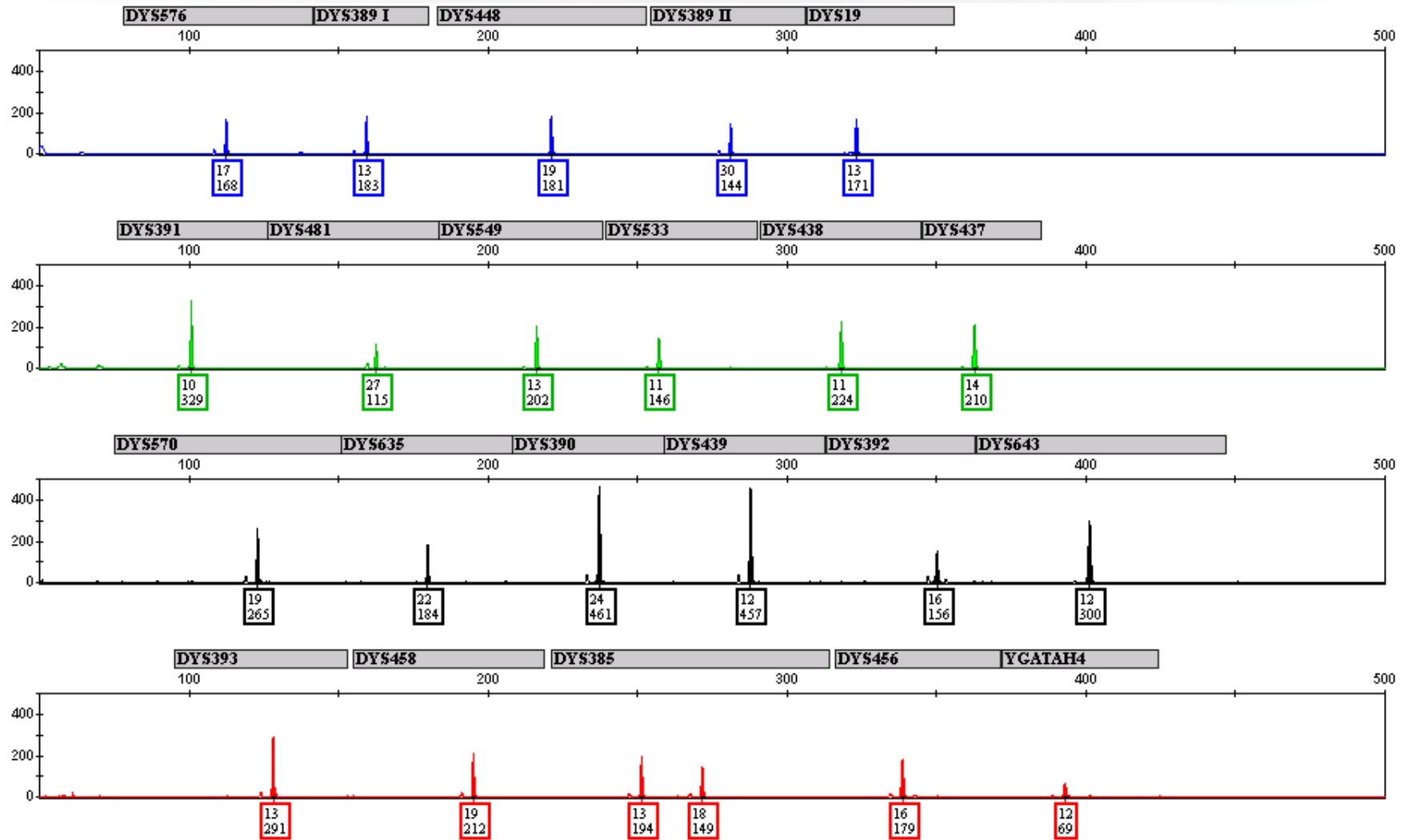
### Primer Concentration – Buccal on FTA



## 2.10.2 Balance and stochastic 500pg Male DNA



## 2.10.2 Balance and stochastic 62.5pg Male DNA



## 2.10.3 Known Artifacts

### Artifacts Listed in the Technical Manual



Table 4. DNA-Dependent Artifacts.

Locus	Artifact Size
DYS19	n-2; n+2 <sup>1</sup>
DYS448	n-9 to n-15 <sup>2,3</sup>

<sup>1</sup>Two bases below and above the true allele peak, respectively.

<sup>2</sup>These variably sized peaks on the Applied Biosystems® 3130 and 3500 Genetic Analyzers may represent double-stranded DNA derived from the DYS448 amplicon. (Double-stranded DNA is known to migrate faster than single-stranded DNA on capillary electrophoresis [CE] instruments.)

<sup>3</sup>The low-level, DNA-dependent artifact is noticeable only with high input template amounts and allele peak heights.

Table 5. DNA-Independent Artifacts.

Dye Label	Instrument	Artifact Size
Fluorescein	Applied Biosystems® 3130 Genetic Analyzers with POP-4™ polymer	68-71 bases <sup>1</sup>
	Applied Biosystems® 3500 Genetic Analyzers with POP-4™ polymer	66-69 bases <sup>1</sup>
JOE	Applied Biosystems® 3130 Genetic Analyzers with POP-4™ polymer	60-62 bases <sup>1</sup>
	Applied Biosystems® 3500 Genetic Analyzers with POP-4™ polymer	58-60 bases <sup>1</sup>

<sup>1</sup>The signal strength of these artifacts increases with storage of the amplification plate at 4°C, sometimes in as short a time period as overnight but more commonly when plates are left at 4°C for a few days. We recommend storing amplification products at -20°C.

## 2.10.3 Known Artifacts

### Artifacts Listed in the Technical Manual – POP7



- The PowerPlex®Y23 System is optimized for POP-4™ polymer. We recognize that some laboratories use POP-7™ polymer and therefore have included a protocol in this manual.
- Some DNA-independent artifacts migrate in the panel range with the POP-7™ polymer .
- Global filters used for database analysis will generally filter these artifact peaks. However, these peaks may be labeled with casework samples.
- Internal validation should be performed and interpretation guidelines created that describe the artifacts and their impact on data analysis.

Table 9. DNA-Independent Artifacts.

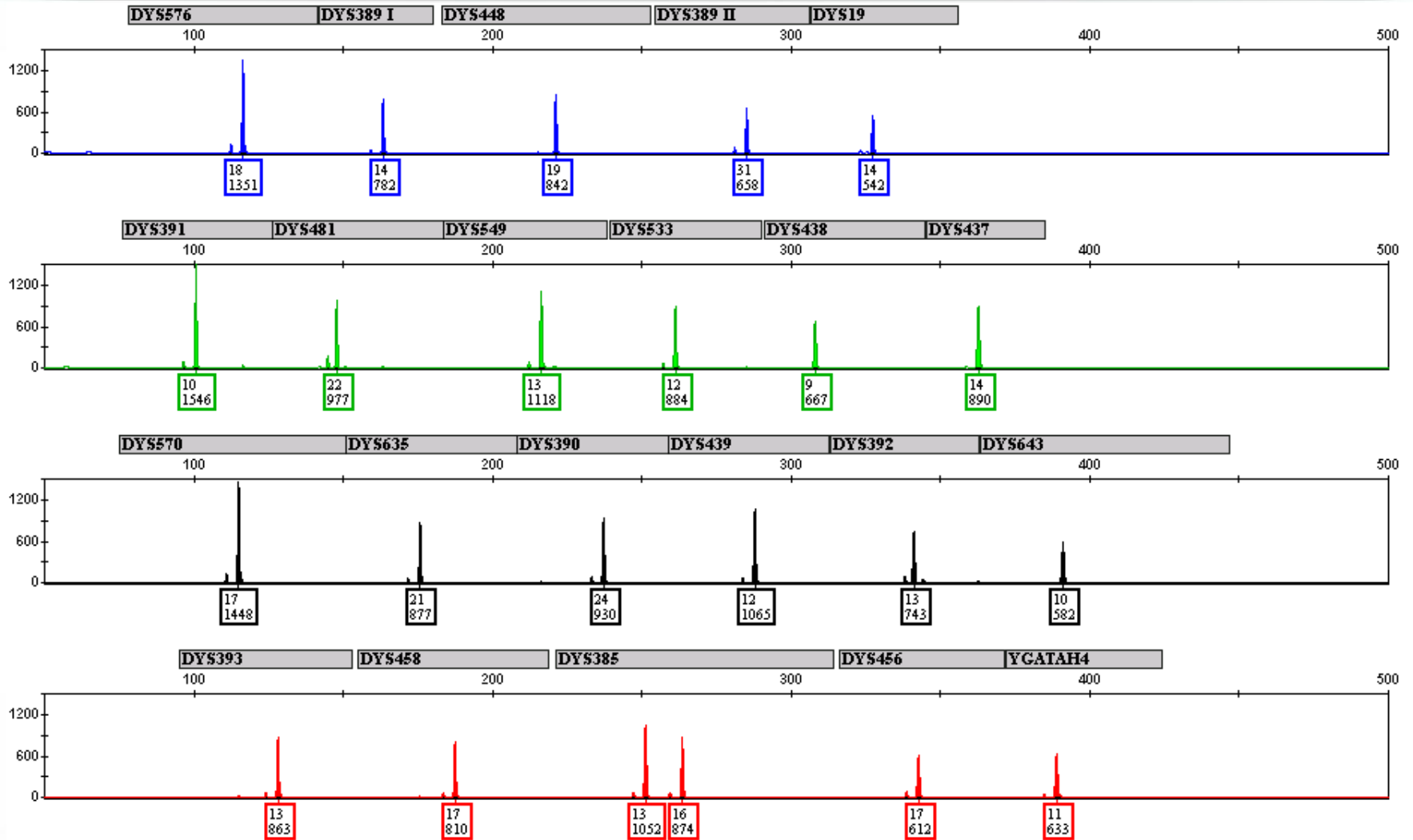
Dye Label	Instrument	Artifact Size
Fluorescein	Applied Biosystems® 3130 Genetic Analyzers with POP-7™ polymer	65–68 bases
		73–75 bases
		85–87 bases
		100–104 bases <sup>1</sup>
JOE	Applied Biosystems® 3130 Genetic Analyzers with POP-7™ polymer	66–69 bases
		88–91 bases <sup>1</sup>

<sup>1</sup>The signal strength of these artifacts increases with storage of the amplification plate at 4°C, sometimes in as short a time period as overnight but more commonly when plates are left at 4°C for a few days. We recommend storing amplification products at –20°C.

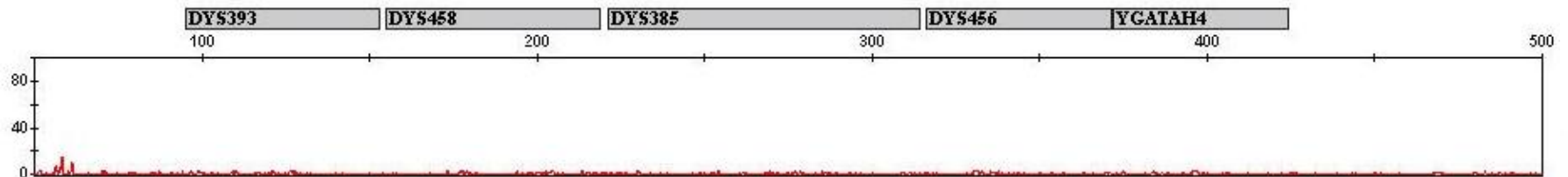
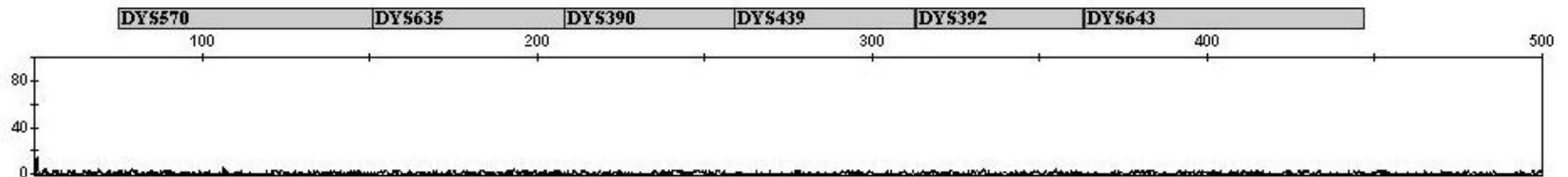
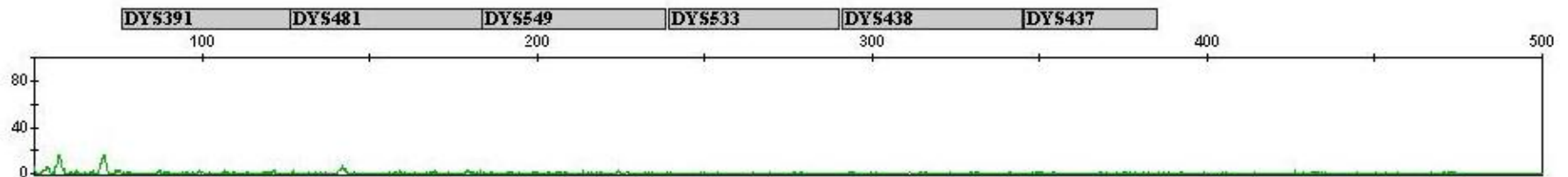
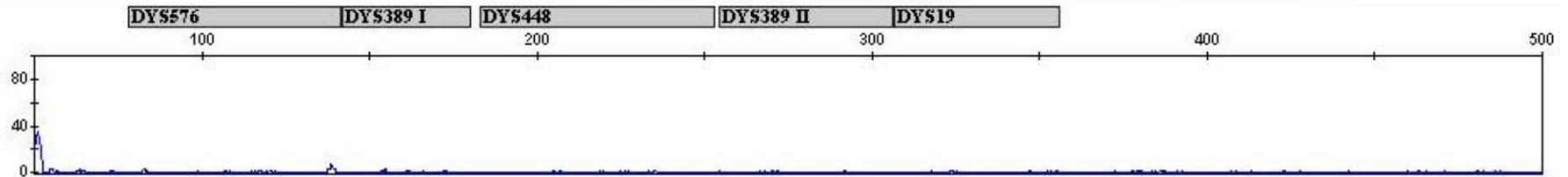


# 2.10.4 Positive Control

## 500pg 2800M



## 2.10.4 Negative Control



# ***Stutter Samples***



# PowerPlex® Y23 System

## Stutter Values



3500 minus stutter						
n=105	Average	St.Dev	Average + 3SD	Max	Count	Occurrence %
DYS576	0.108	0.013	0.147	0.150	94	89.52%
DYS389I	0.052	0.010	0.081	0.076	85	80.95%
DYS448	0.026	0.005	0.040	0.037	83	79.05%
DYS389II	0.114	0.012	0.152	0.153	93	88.57%
DYS19	0.068	0.013	0.106	0.102	88	83.81%
DYS391	0.074	0.016	0.123	0.184	93	88.57%
DYS481	0.207	0.031	0.298	0.280	94	89.52%
DYS549	0.077	0.012	0.114	0.147	92	87.62%
DYS533	0.068	0.013	0.107	0.131	92	87.62%
DYS438	0.031	0.006	0.050	0.062	76	72.38%
DYS437	0.050	0.012	0.084	0.116	83	79.05%
DYS570	0.109	0.017	0.159	0.172	94	89.52%
DYS635	0.068	0.016	0.115	0.112	91	86.67%
DYS390	0.086	0.016	0.134	0.125	93	88.57%
DYS439	0.069	0.013	0.107	0.121	93	88.57%
DYS392	0.102	0.023	0.171	0.188	93	88.57%
DYS643	0.024	0.005	0.039	0.037	66	62.86%
DYS393	0.102	0.016	0.151	0.194	95	90.48%
DYS458	0.097	0.016	0.147	0.185	95	90.48%
DYS385	0.092	0.019	0.149	0.150	93	88.57%
DYS456	0.111	0.013	0.150	0.156	94	89.52%
Y-GATA	0.074	0.013	0.111	0.116	93	88.57%

3500 plus stutter						
n=105	Average	St.Dev	Average + 3SD	Max	Count	Occurrence %
DYS576	0.017	0.010	0.048	0.050	25	23.81%
DYS389I					0	0.00%
DYS448					0	0.00%
DYS389II	0.010			0.010	1	0.95%
DYS19	0.017	0.017	0.067	0.036	3	2.86%
DYS391	0.012	0.003	0.022	0.016	7	6.67%
DYS481	0.027	0.009	0.055	0.094	80	76.19%
DYS549	0.018	0.010	0.048	0.047	25	23.81%
DYS533	0.014	0.006	0.031	0.023	12	11.43%
DYS438					0	0.00%
DYS437	0.008			0.008	1	0.95%
DYS570	0.013	0.008	0.036	0.047	30	28.57%
DYS635	0.012	0.004	0.024	0.017	4	3.81%
DYS390	0.012	0.003	0.020	0.016	5	4.76%
DYS439	0.011	0.005	0.027	0.028	19	18.10%
DYS392	0.051	0.018	0.105	0.115	88	83.81%
DYS643					0	0.00%
DYS393	0.015	0.004	0.027	0.026	72	68.57%
DYS458	0.010	0.004	0.022	0.021	12	11.43%
DYS385	0.015	0.014	0.056	0.031	3	2.86%
DYS456	0.021	0.006	0.038	0.035	56	53.33%
Y-GATA	0.012	0.005	0.026	0.019	13	12.38%

3500 DYS19 N-2						
N=105	Average	St.Dev	Average + 3 STD	Max	Count	Occurrence %
N-2	0.081	0.007	0.102	0.099	90	85.71%

3500 DYS19 N+2						
N=105	Average	St.Dev	Average + 3 STD	Max	Count	Occurrence %
N+2	0.025	0.004	0.038	0.036	44	41.90%

# PowerPlex® Y23 System

## Stutter Filters for GeneMapper ID-X



Marker Name	DYS576			
	0.147	3.25	4.75	Minus
Marker Name	DYS389 I			
	0.081	3.25	4.75	Minus
Marker Name	DYS448			
	0.04	5.25	6.75	Minus
Marker Name	DYS389 II			
	0.152	3.25	4.75	Minus
Marker Name	<b>DYS19</b>			
	0.107	3.25	4.75	Minus
	<b>0.102</b>	<b>1.25</b>	<b>2.75</b>	<b>Minus</b>
	<b>0.038</b>	<b>1.25</b>	<b>2.75</b>	<b>Plus</b>
Marker Name	DYS391			
	0.124	3.25	4.75	Minus
Marker Name	<b>DYS481</b>			
	0.298	2.25	3.75	Minus
	<b>0.055</b>	<b>2.25</b>	<b>3.75</b>	<b>Plus</b>
Marker Name	DYS549			
	0.114	3.25	4.75	Minus
Marker Name	DYS533			
	0.107	3.25	4.75	Minus
Marker Name	DYS438			
	0.05	3.25	4.75	Minus
Marker Name	DYS437			
	0.084	3.25	4.75	Minus

Marker Name	DYS570			
	0.159	3.25	4.75	Minus
Marker Name	DYS635			
	0.115	3.25	4.75	Minus
Marker Name	DYS390			
	0.134	3.25	4.75	Minus
Marker Name	DYS439			
	0.107	3.25	4.75	Minus
Marker Name	<b>DYS392</b>			
	0.171	2.25	3.75	Minus
	<b>0.105</b>	<b>2.25</b>	<b>3.75</b>	<b>Plus</b>
Marker Name	DYS643			
	0.039	4.25	5.75	Minus
Marker Name	DYS393			
	0.151	3.25	4.75	Minus
Marker Name	DYS458			
	0.147	3.25	4.75	Minus
Marker Name	DYS385			
	0.149	3.25	4.75	Minus
Marker Name	DYS456			
	0.151	3.25	4.75	Minus
Marker Name	YGATAH4			
	0.111	3.25	4.75	Minus

# PowerPlex® Y23 System Stutter Filters for GeneMapper® ID



**Analysis Method Editor - HID**

General | Allele | Peak Detector | Peak Quality | Quality Flags

Bin Set: PowerPlexY23\_Bins\_v1.0

Use marker-specific stutter ratio if available

Marker Repeat Type :	Tri	Tetra	Penta	Hexa
Cut-off Value	0.0	0.0	0.0	0.0
MinusA Ratio	0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0
	To	0.0	0.0	0.0
Minus Stutter Ratio	0.0	0.0	0.0	0.0
Minus Stutter Distance	From	2.25	3.25	4.25
	To	3.75	4.75	5.75
Plus Stutter Ratio	0.06	0.0	0.0	0.0
Plus Stutter Distance	From	2.25	0.0	0.0
	To	3.75	0.0	0.0

Amelogenin Cutoff: 0.0

Range Filter... Factory Defaults

OK Cancel

The value of 0.06 will filter the plus stutter for DYS481 (5.5%) but not for DYS392 (10.5%).  
This is noted in the Technical Manual

#GeneMapper ID v3.2  
Version GM v 3.0  
Kit type: MICROSATELLITE  
Chemistry PowerPlex null  
Panel PowerPlex null

DYS576	blue	78	141.5	18	4	0.147
DYS389 I	blue	142	180	14	4	0.081
DYS448	blue	183	253	19	6	0.04
DYS389 II	blue	254	306	31	4	0.152
DYS19	blue	306.5	356	14	4	0.107
DYS391	green	76	126.1	10	4	0.124
DYS481	green	126.4	183.3	22	3	0.298
DYS549	green	183.5	238	13	4	0.114
DYS533	green	239	290	12	4	0.107
DYS438	green	291	345	9	5	0.05
DYS437	green	345.5	385	14	4	0.084
DYS570	yellow	75	150.9	17	4	0.159
DYS635	yellow	151	208.3	21	4	0.115
DYS390	yellow	208.4	258.7	24	4	0.134
DYS439	yellow	258.8	312.8	12	4	0.107
DYS392	yellow	313	363.1	13	3	0.171
DYS643	yellow	363.5	446.5	10	5	0.039
DYS393	red	95	153	13	4	0.151
DYS458	red	155	219	17	4	0.147
DYS385	red	221	314	13, 16	4	0.149
DYS456	red	316	371.5	17	4	0.151
YGATAH4	red	372	424	11	4	0.111

Minus stutter is included in the panels file



# ***Y-STR Statistical Databases***



# US Y-STR Database



[Introduction](#) | [User Directions](#) | [Database Descriptive](#)

## US Y-STR Database

Release: 3.0 | Last Updated: 07/29/2012

**Select Alleles** | [Input Haplotype\(s\) From Your File](#) | [Mixture Analysis Tools](#)

### Common Markers

DYS19	DYS385	DYS389I	DYS389II
* <input type="text"/>	* <input type="text"/>	* <input type="text"/>	* <input type="text"/>
DYS390	DYS391	DYS392	DYS393
* <input type="text"/>	* <input type="text"/>	* <input type="text"/>	* <input type="text"/>
DYS437	DYS438	DYS439	DYS448
* <input type="text"/>	* <input type="text"/>	* <input type="text"/>	* <input type="text"/>
DYS456	DYS458	DYS635 (YGATAC4)	YGATAH4
* <input type="text"/>	* <input type="text"/>	* <input type="text"/>	* <input type="text"/>
DYS481	DYS533	DYS549	DYS570
* <input type="text"/>	* <input type="text"/>	* <input type="text"/>	* <input type="text"/>
DYS576	DYS643		
* <input type="text"/>	* <input type="text"/>		

### Search By Ancestry

All

African American

Asian

Caucasian



# US Y-STR Database



## Overall Database Summary:

The selected haplotype is found in 0 of 1375 total individuals within the database with a frequency of 0. Applying the 95% upper confidence interval results in a frequency of 0.002176, which is equivalent to approximately 1 in every 460 individuals.

The selected haplotype is found in 0 of 362 African American individuals within the database, with a frequency of 0.000000. Applying the 95% upper confidence interval results in a frequency of 0.008241, which is equivalent to approximately 1 in every 121 individuals.

The selected haplotype is found in 0 of 0 Asian individuals within the database, with a frequency of 0.

The selected haplotype is found in 0 of 295 Caucasian individuals within the database, with a frequency of 0.000000. Applying the 95% upper confidence interval results in a frequency of 0.010103, which is equivalent to approximately 1 in every 99 individuals.

The selected haplotype is found in 0 of 277 Hispanic individuals within the database, with a frequency of 0.000000. Applying the 95% upper confidence interval results in a frequency of 0.010756, which is equivalent to approximately 1 in every 93 individuals.

The selected haplotype is found in 0 of 441 Native American individuals within the database, with a frequency of 0.000000. Applying the 95% upper confidence interval results in a frequency of 0.00677, which is equivalent to approximately 1 in every 148 individuals.

**Queries Performed:** 95254

As of 8/9/12, 1375 samples contained all 23 loci. This number is anticipated to increase with submissions in the upcoming months.

# Updated Y-HRD Database Interface



YHRD.ORG 3.0



R40: 104174 haplotypes

Search

Haplotypes

SNPs

Populations

Contributors

Contributions

[→ Sign in](#) [→ Register](#) [→ Home](#)

Download Manual

Analyse

Research

Contribute

Meet

YHRD Standard | Promega PowerPlex Y | Applied Biosystems AmpFISTR Yfiler | Promega PowerPlex Y23

DYS576	DYS389I	DYS448	DYS389II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
DYS570	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385	DYS456	YGATAH4
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

National database | Metapopulations | SNP

Search

Reset

Whole database

**Please note:** The database size will vary based on the loci you have entered.

- 7 loci haplotype (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393): **104174 haplotypes**
- Minimal Haplotype (+ DYS385a/b): **102377 haplotypes**
- SWGDAM haplotype (+ DYS438, DYS439): **75290 haplotypes**
- Promega PowerPlex Y (+ DYS437): **55747 haplotypes**
- Applied Biosystems AmpFISTR Yfiler (+ DYS448, DYS456, DYS458, DYS635, YGATAH4): **44469 haplotypes**
- Promega PowerPlex Y23 (+ DYS576, DYS481, DYS549, DYS533, DYS570, DYS643): **951 haplotypes**

# *Summary*



## *Performance Summary*

- PowerPlex® Y23 System is a very flexible system that is capable of working for multiple applications including casework (extracted) samples as well as multiple substrates for direct amplification
- Full profiles obtained consistently at 62.5pg of male DNA
- Ability to overcome excessive amounts of female with full profiles of 125 pg in 3000 ng of female DNA
- Rapid cycling of 1 hour 40 minutes for casework samples and 1 hour 30 minutes for direct amplification samples
- Robust against many of the commonly encountered inhibitors; humic acid, hematin, and tannic acid

## ***Supported instruments & software summary***

### Thermal Cycler:

- GeneAmp® PCR System 9700 (Applied Biosystems)

### Capillary Electrophoresis Instrument:

- 3500 & 3500xL Genetic Analyzer (Applied Biosystems)
- 3130 & 3130xl Genetic Analyzer (Applied Biosystems)
- ABI PRISM® 3100 & 3100-Avant Genetic Analyzer (Applied Biosystems)



### Data Analysis Software:

- GeneMapper® ID-X Software , version 1.2 (Applied Biosystems)
  - Includes plus stutter filters for DYS481 and DYS392 (trinucleotide repeat loci)
  - Includes N-2 and N+2 filters for DYS19
- GeneMapper® ID Software, version 3.2 (Applied Biosystems)

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***Questions?***

