ISHI Workshop on New Loci and Kits
October 10, 2013 (Atlanta, GA)
New Autosomal and Y-STR Loci and Kits: Making Data Driven Decisions

Experience with PowerPlex Fusion

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Michigan State Police

Michigan State Police
STR Kit Chemistry History

- Transitioned from RFLP directly to STRs
- Initial casework kits included AMPFℓSTR Blue, Green I and II
  - Provided 9 STR Loci
- Casework moved to Profiler Plus/Cofiler
  - Provided 13 STR Loci
- Database group utilized PowerPlex 16
  - Provided 13 STR Loci plus Penta E and Penta D
- Casework moved to PowerPlex 16 HS in 2011
  - Interest in capturing Penta E and Penta D markers
  - Detailed comparison of Identifiler Plus and PowerPlex 16 HS

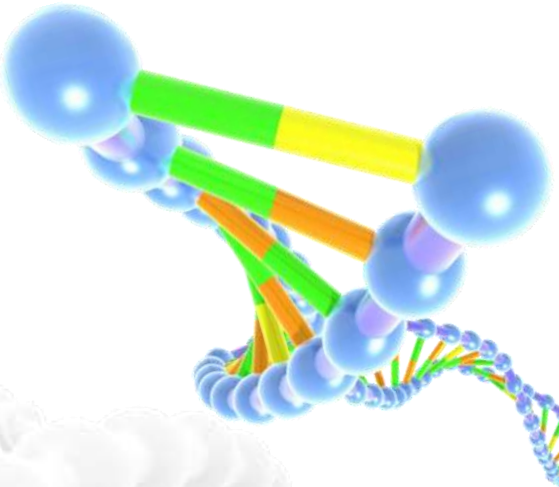
PowerPlex Fusion

- Interest in looking at extended STR Loci panels
- Currently use 3130 and 3130xl Genetic Analyzer platforms
  - Contracts to purchase 3500s being established
- Offender database of 300,000+ include Penta loci

•INTERNAL VALIDATION

- Casework and Database units

- Studies completed include:
  - Precision
  - Sensitivity
  - Baseline noise evaluation
  - Contamination Assessment
  - Mock Casework
  - Mixtures
  - Concordance
  - NIST SRM



Validation at One Laboratory (Recently Completed)

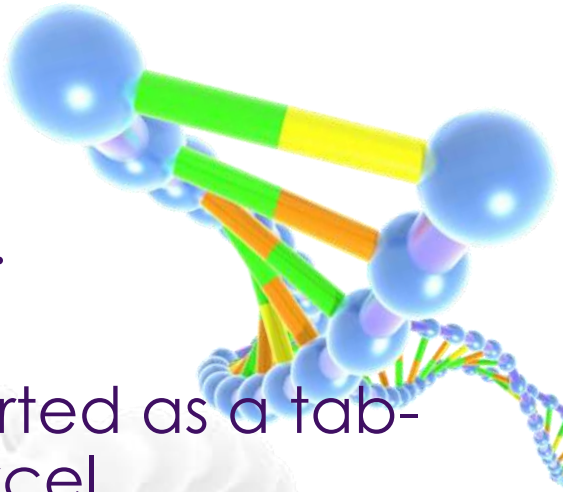
Performance Verification Studies at Two Casework Laboratories and Our Databasing Laboratory

Training, Competency Testing and Planned Implementation System-wide.

Internal Validation

Precision

Ladders were run multiple times over a few days. All capillaries were tested.



A table in GMID was created and exported as a tab-delimited file for import into Microsoft Excel

The average bp size and standard deviation were calculated for each allele of each locus

Internal Validation

Precision

In general:

Smaller loci are averaging differences approximately 0.03 to 0.05bp

Larger loci (FGA, PentaE, PentaD) are averaging 0.08-0.09bp

Well below 0.5bp window

Internal Validation

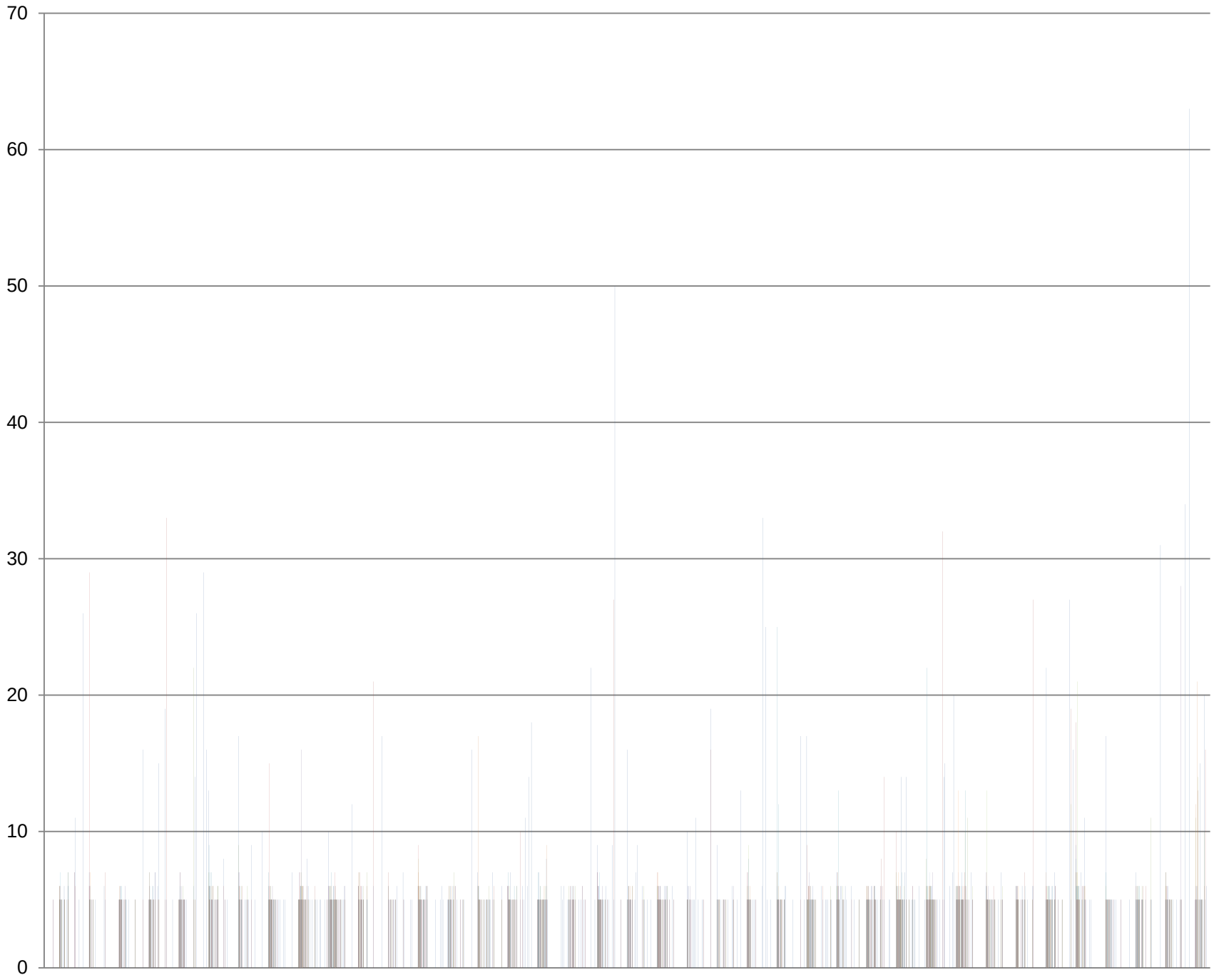
Contamination Assessment and Baseline Noise Evaluation

40 Reagent Blanks were amplified
- Stain Extraction Blanks
- Maxwell Extraction Blanks
- Epithelial Extraction Blanks
- Sperm Fraction Blanks

- Run on 3130
- Analyzed at 5 rfu
- GMID table created and exported to Excel

- Average Peak height was 5.8rfu +/- 3.2 rfu

- A few peaks were labeled > 25 rfu


70
60
50
40
30
20
10
0

Internal Validation

Sensitivity

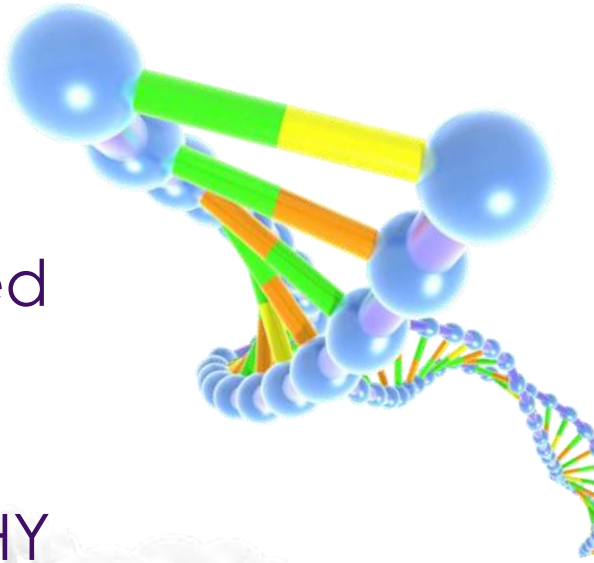
3 samples of extracted DNA were used
(Many heterozygous loci)

Samples were quantified using Plexor HY

Serial dilutions were made

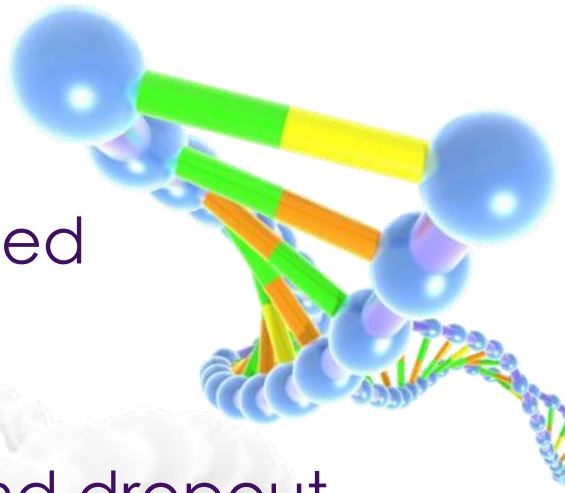
Each dilution was quantified in triplicate

Replicates were amplified. (Range of >1ng to ~ 8 pg)



Internal Validation

Sensitivity



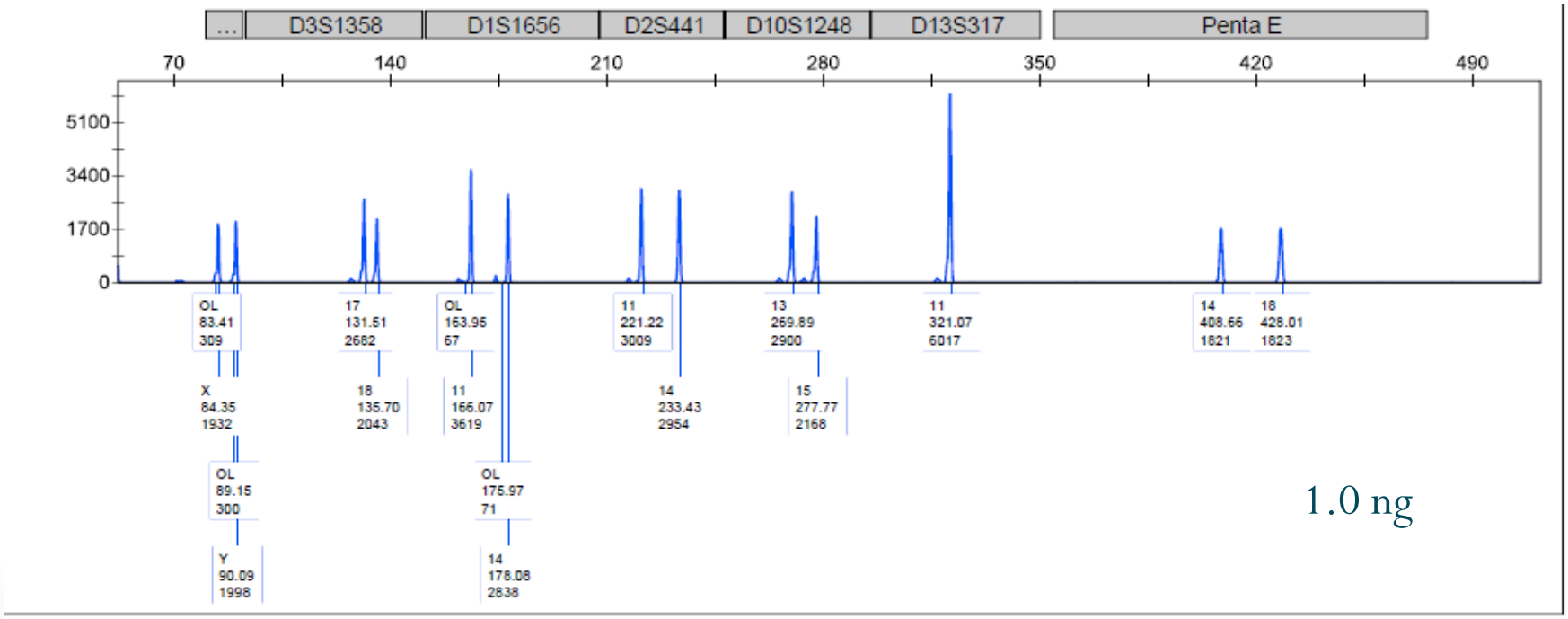
Samples less than 0.5 ng were amplified 12 times each

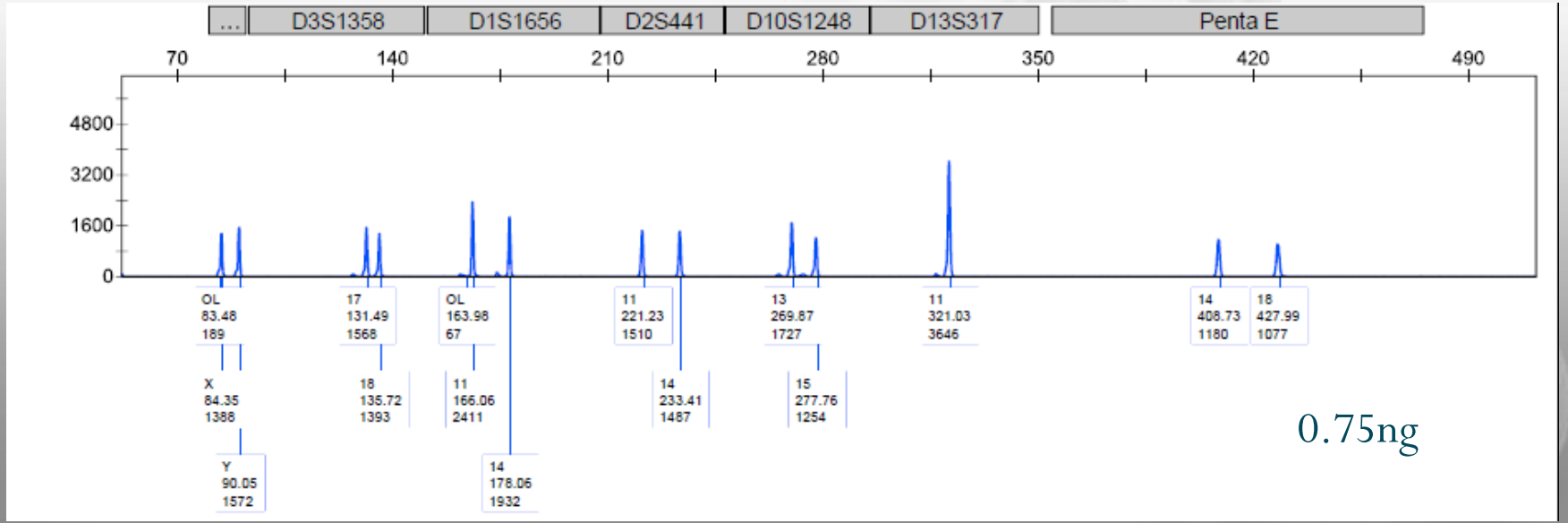
Data was reviewed for imbalance and dropout

Full profiles consistently down to 125pg

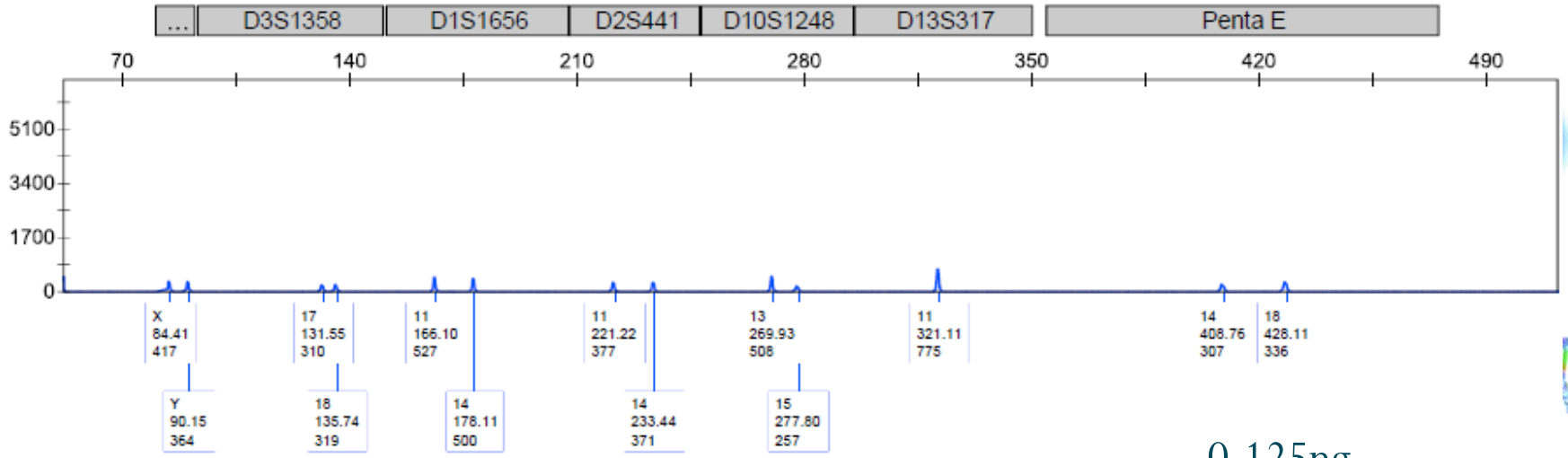
Increase in concentration = better PHR

Internal Validation





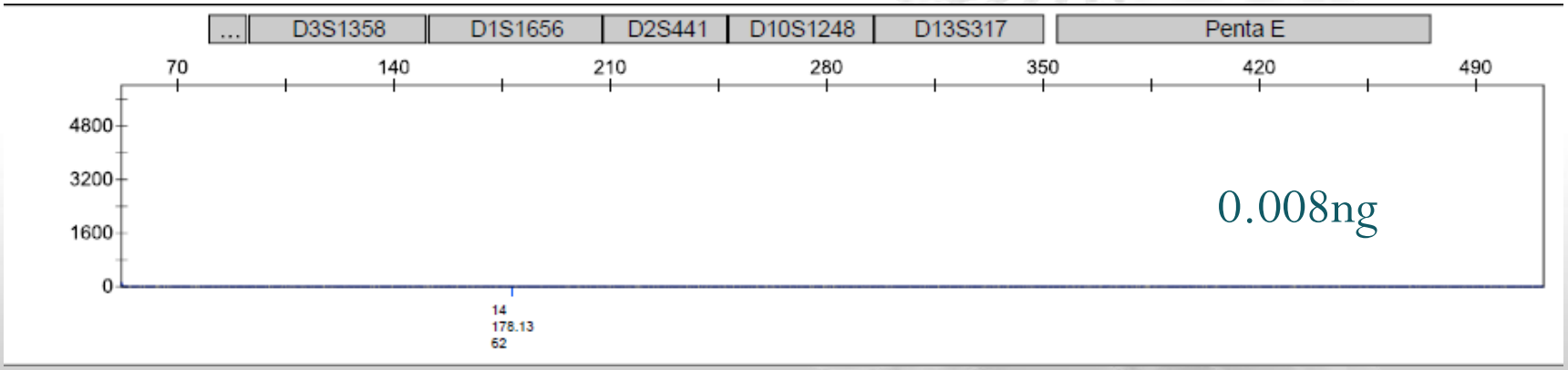
Internal Validation



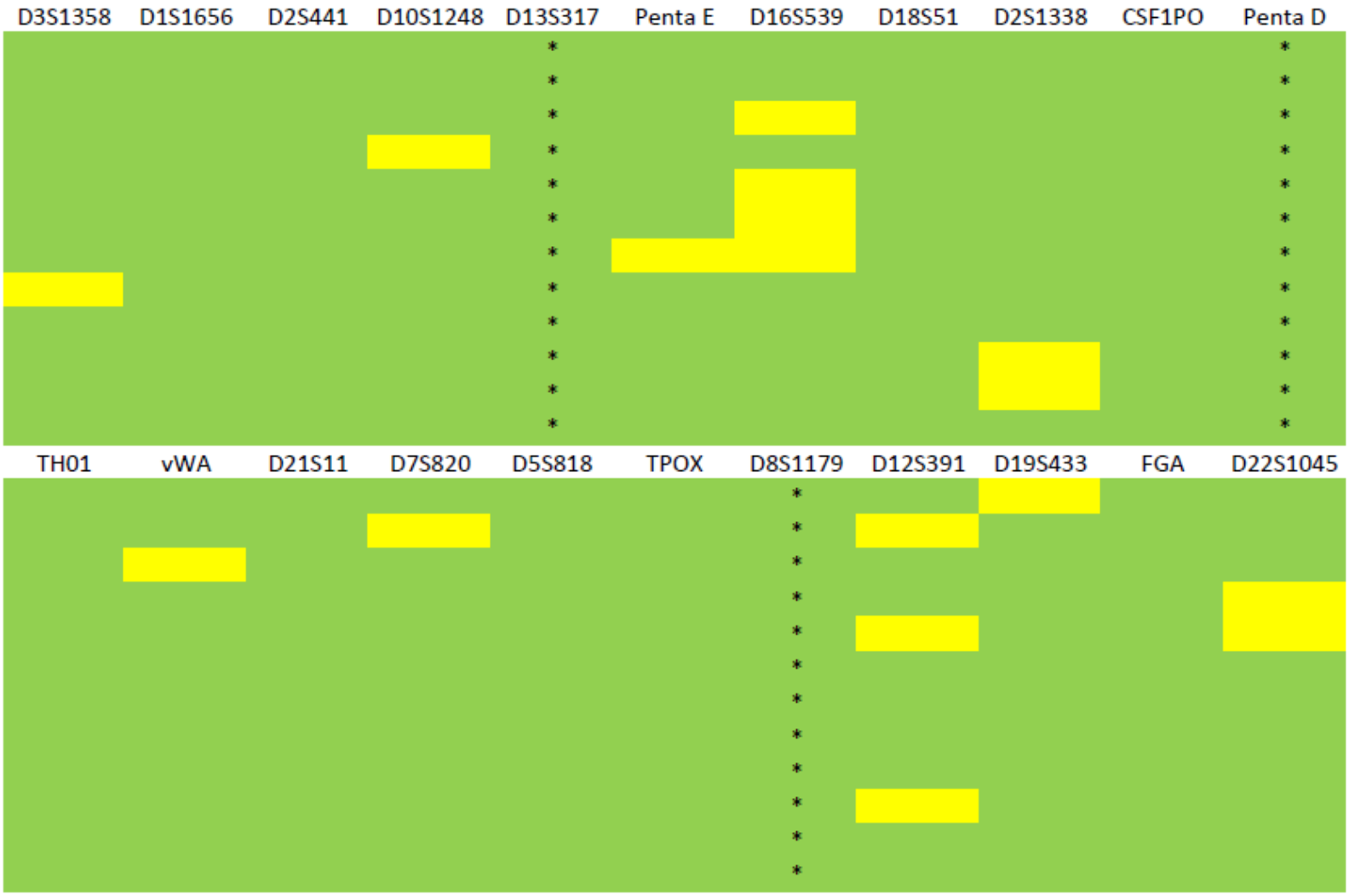
0.125ng

0.06ng

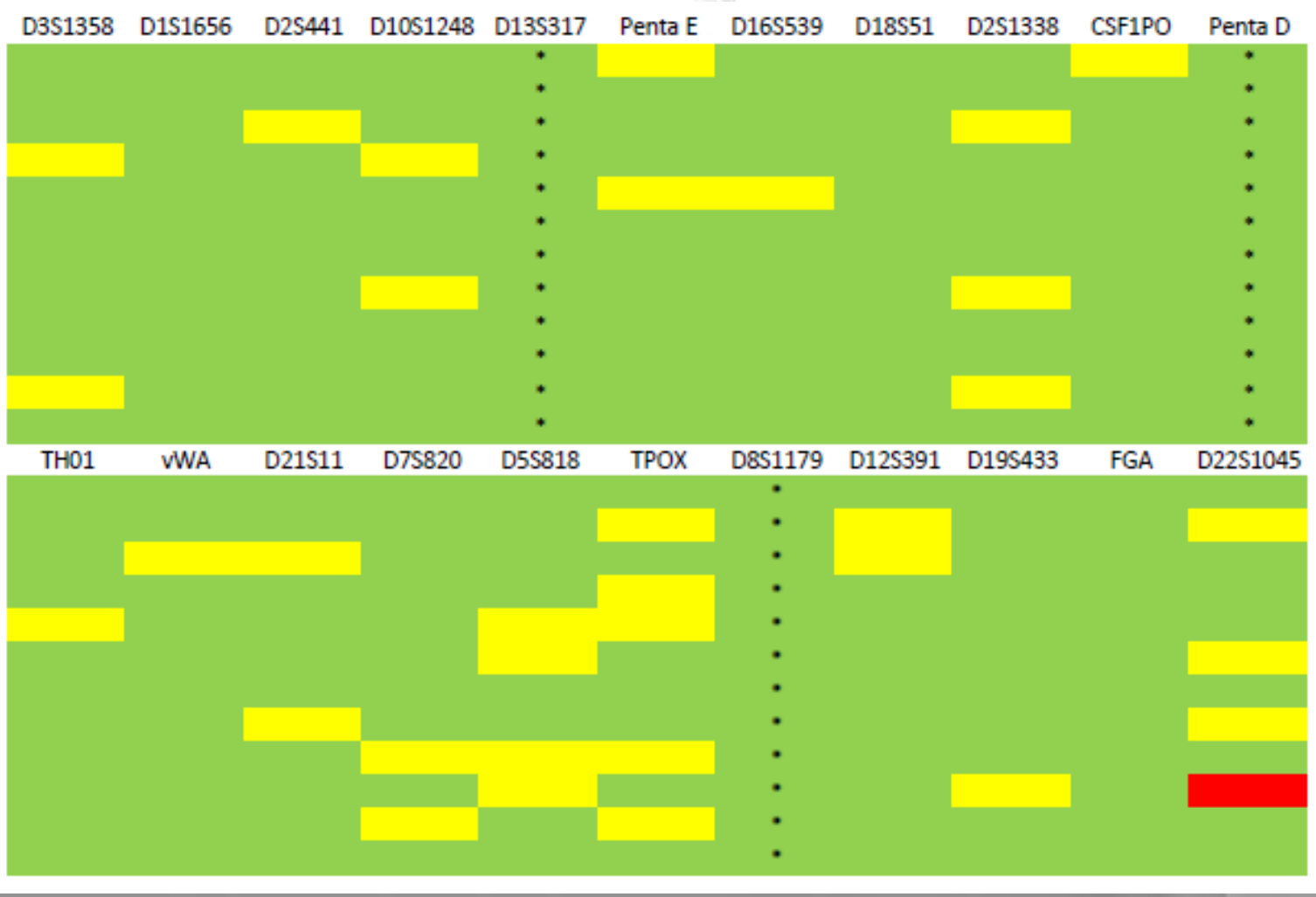
Internal Validation



250 pg, 50% PHR, AT 75, ST 300

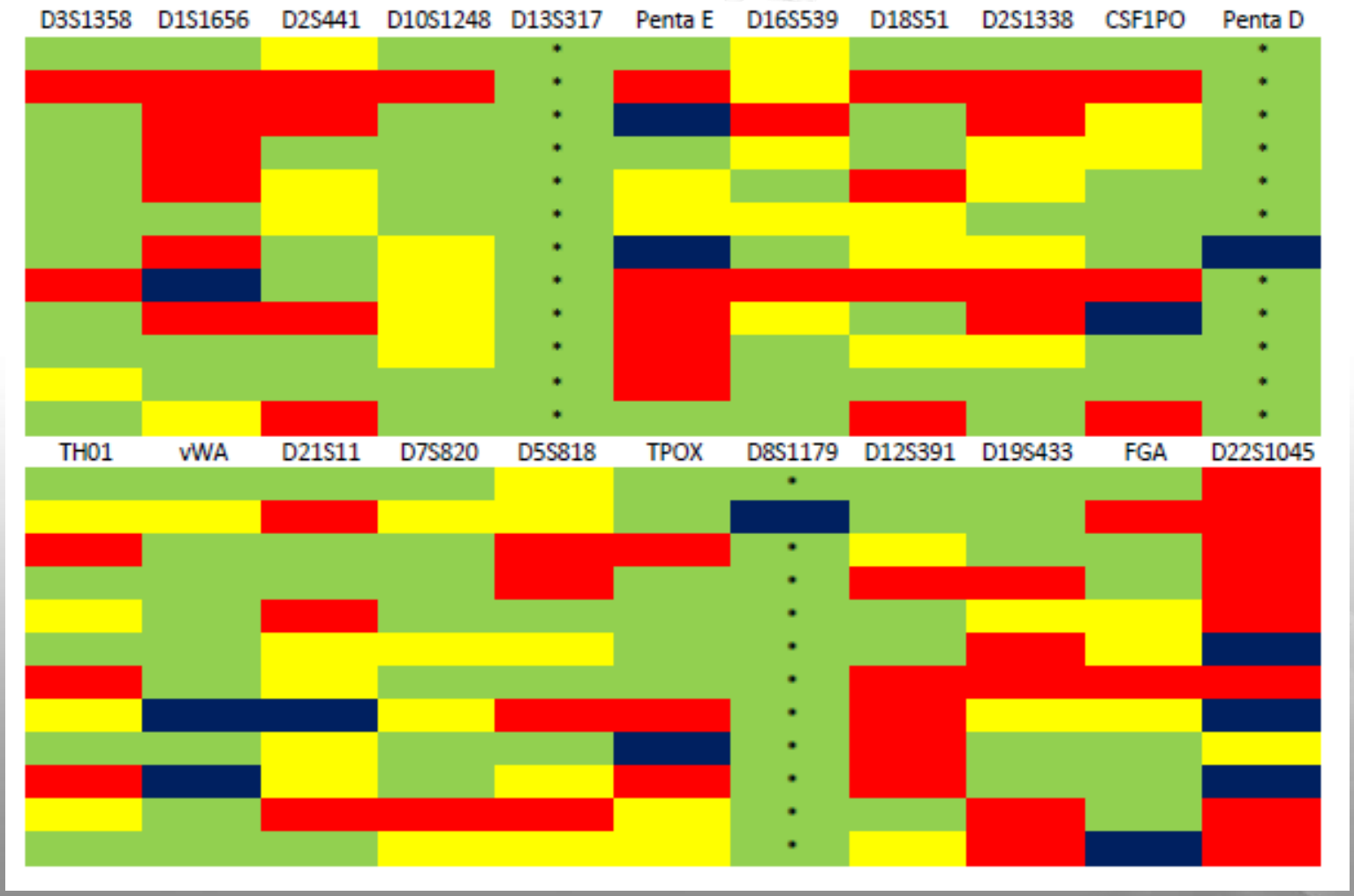


125 pg, 50% PHR, AT 75, ST 300



62 pg, 50% PHR, AT 75, ST 300

31 pg, 50% PHR, AT 75, ST 300



Internal Validation

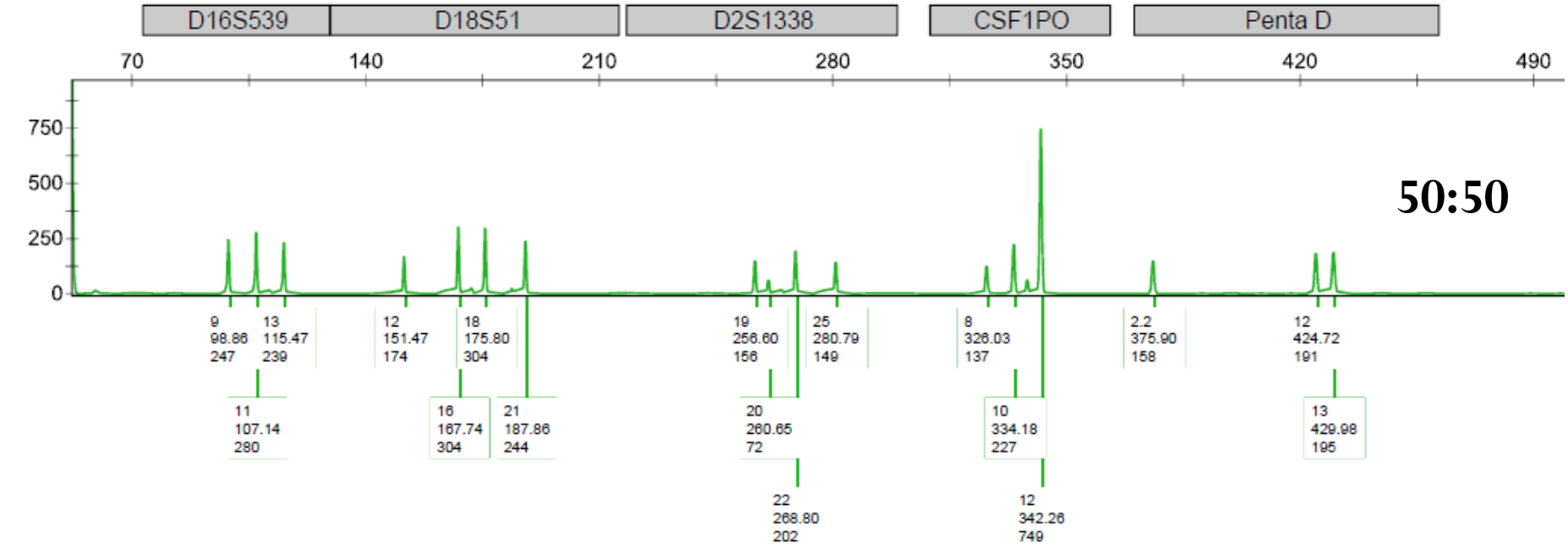
Mixtures

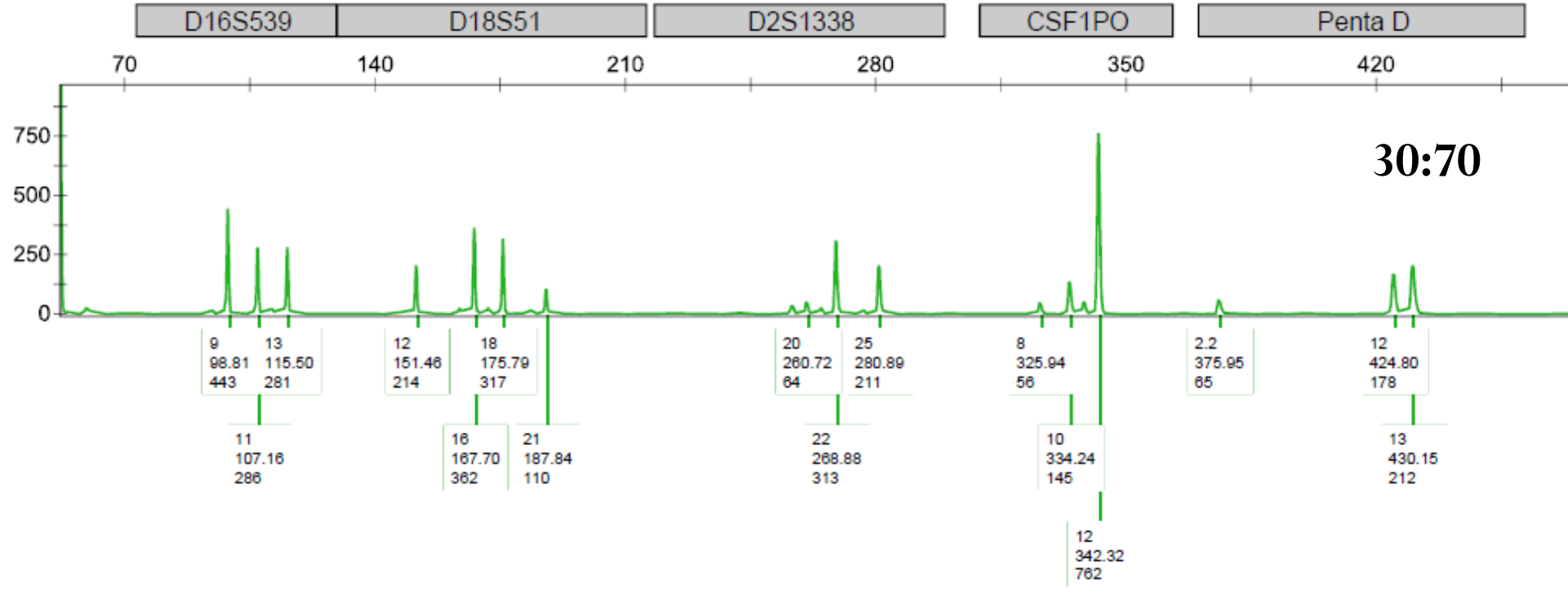
Mixtures prepared from extracted reference sample DNA

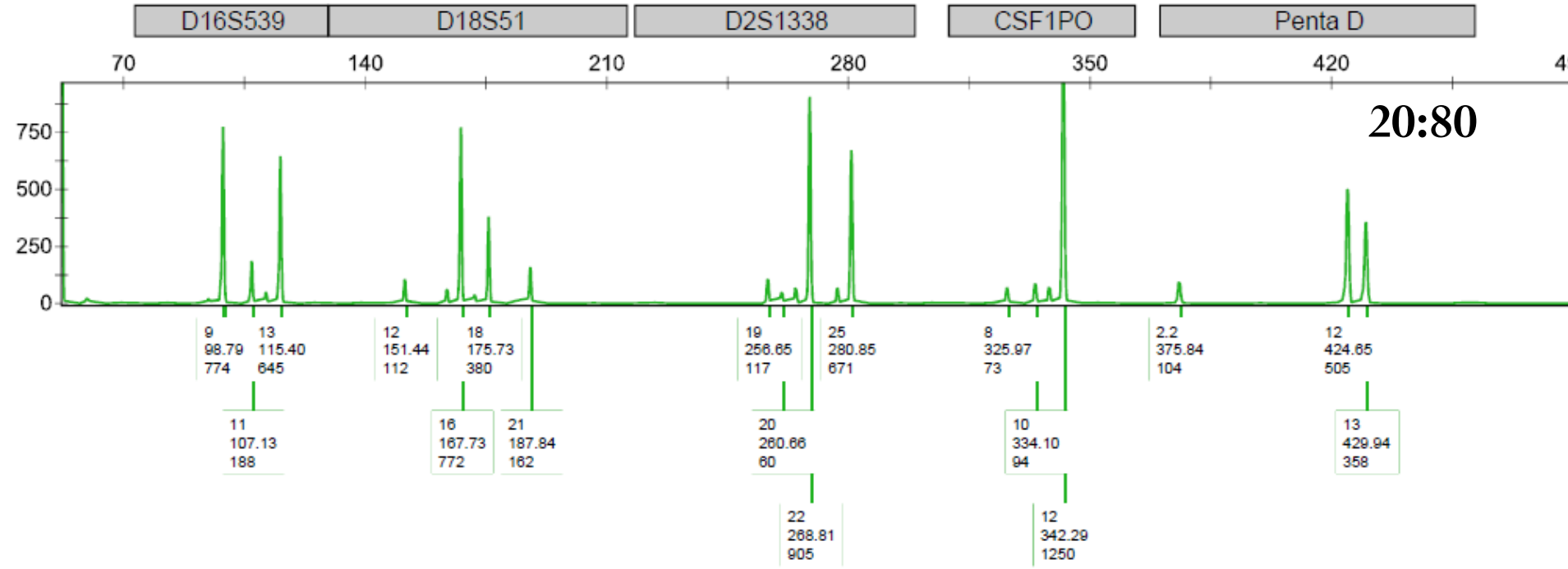
Ratios from 100:0 to 0:100 were prepared with an input template amount of 0.75ng

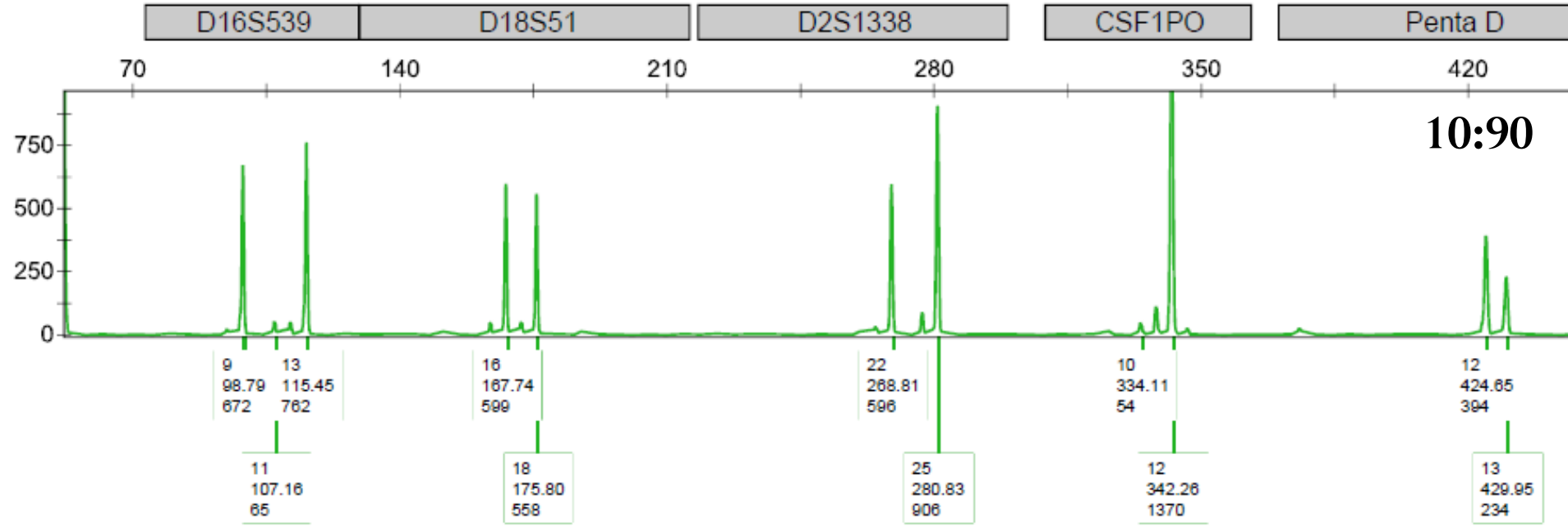
Amplified product was ran on a 3130 with a standard 5 second injection time

Minor donor detected at all mixture ratios


D16S539
D18S51
D2S1338
CSF1PO
Penta D
70
140
210
280
350
420
490
750
500
250
0
50:50
9 98.86 247
13 115.47 239
11 107.14 280
12 151.47 174
18 175.80 304
16 167.74 304
21 187.86 244
19 256.60 156
25 280.79 149
20 260.65 72
22 268.80 202
8 326.03 137
10 334.18 227
12 342.26 749
2.2 375.90 158
12 424.72 191
13 429.98 195

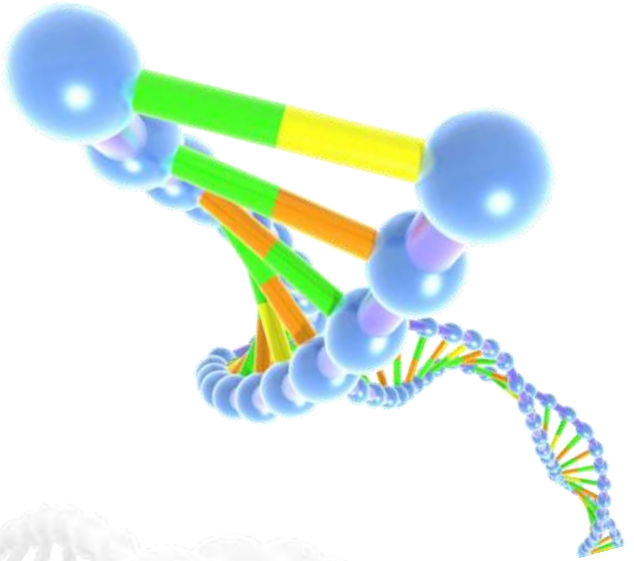

D16S539
D18S51
D2S1338
CSF1PO
Penta D
70
140
210
280
350
420
750
500
250
0
30:70
9 98.81 443
13 115.50 281
11 107.16 286
12 151.46 214
18 175.79 317
16 167.70 362
21 187.84 110
20 260.72 64
25 280.89 211
22 268.88 313
8 325.94 56
10 334.24 145
12 342.32 762
2.2 375.95 65
12 424.80 178
13 430.15 212


D16S539
D18S51
D2S1338
CSF1PO
Penta D
70
140
210
280
350
420
750
500
250
0
20:80
9 98.79 774
13 115.40 645
12 151.44 112
18 175.73 380
19 256.65 117
25 280.85 671
8 325.97 73
2.2 375.84 104
12 424.65 505
11 107.13 188
16 167.73 772
21 187.84 162
20 260.66 60
10 334.10 94
13 429.94 358
22 268.81 905
12 342.29 1250


D16S539
D18S51
D2S1338
CSF1PO
Penta D
70
140
210
280
350
420
750
500
250
0
10:90
9 98.79 672
13 115.45 762
16 167.74 599
22 268.81 596
10 334.11 54
12 424.65 394
11 107.16 65
18 175.80 558
25 280.83 906
12 342.26 1370
13 429.95 234

Internal Validation

Casework



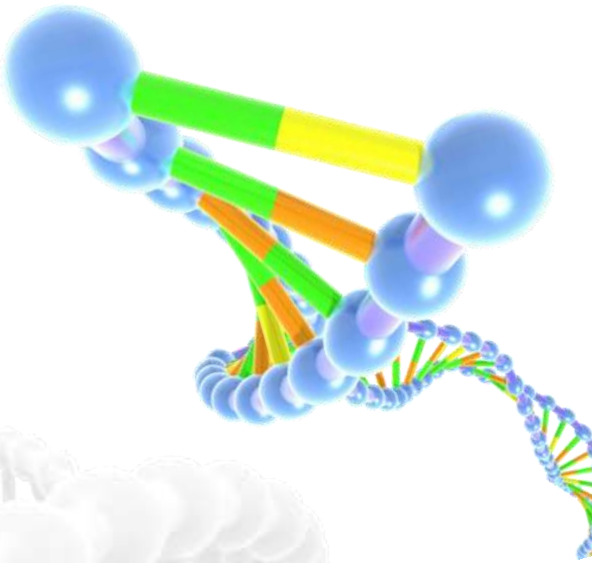
Fortunate to be able to use actual casework samples

More than 40 cases have been analyzed with Fusion- only able to show a small portion here

Concordant data overall

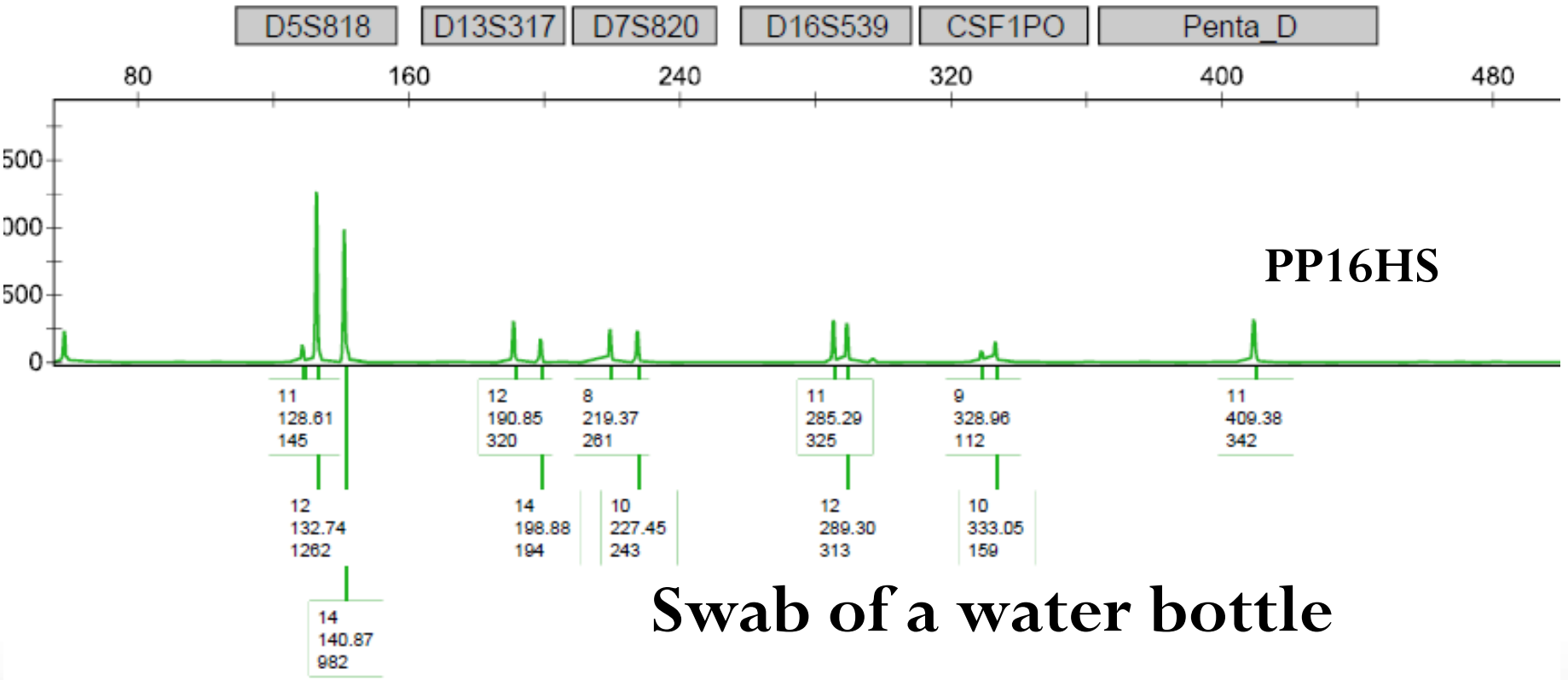
Description	Quantification Value
Left Hand Fingernail Scrapings	0.0304
Right Hand Fingernail Scrapings	0.1650
swabbings from gray hat	0.0074
swab of piece of glove	0.0702
swab of water bottle	0.3330
swab of Miller Lite bottle	0.0118
swab of hat	1.0500
swab of Gatorade bottle	0.0077
swab of Stanley tool	0.0071
swab of glove (under TV)	0.5710
swab of glove (parking lot)	1.8300
Cigarette Butt	0.1210
Cigarette Butt	0.0916
Leather Apron - Lower Left	0.3520
Leather Apron - Lower Left Corner	0.1330
Leather Apron - Upper Center	0.0064
swab from inside stairs	1.1900
cigarette	2.4200
blood from jewelry box	0.5510
blood from kitchen table	0.2680
blood from glass	0.1160
cigarette butt (8A)	0.5890
cigarette butt (CPeak-1)	4.4800
blood from air bag	0.1400
blood on gift card sleeve	0.1150

Description	Quantification Value
swab of cords	0.1730
swab of bottle	0.5870
swab of sweatshirt	0.5490
swab of mouth of water bottle	3.2800
blood from camp fuel can	0.7290
envelope cutting	0.3710
blood from glass	0.5560
suspected flesh	5.1500
cigarette butt	2.9100
blood from POE	0.4110
blood from lottery slip	0.1550
blood from broken window	0.6800
blood from computer bag	0.1840
blood from broken window	0.3430
blood from floor	4.0500



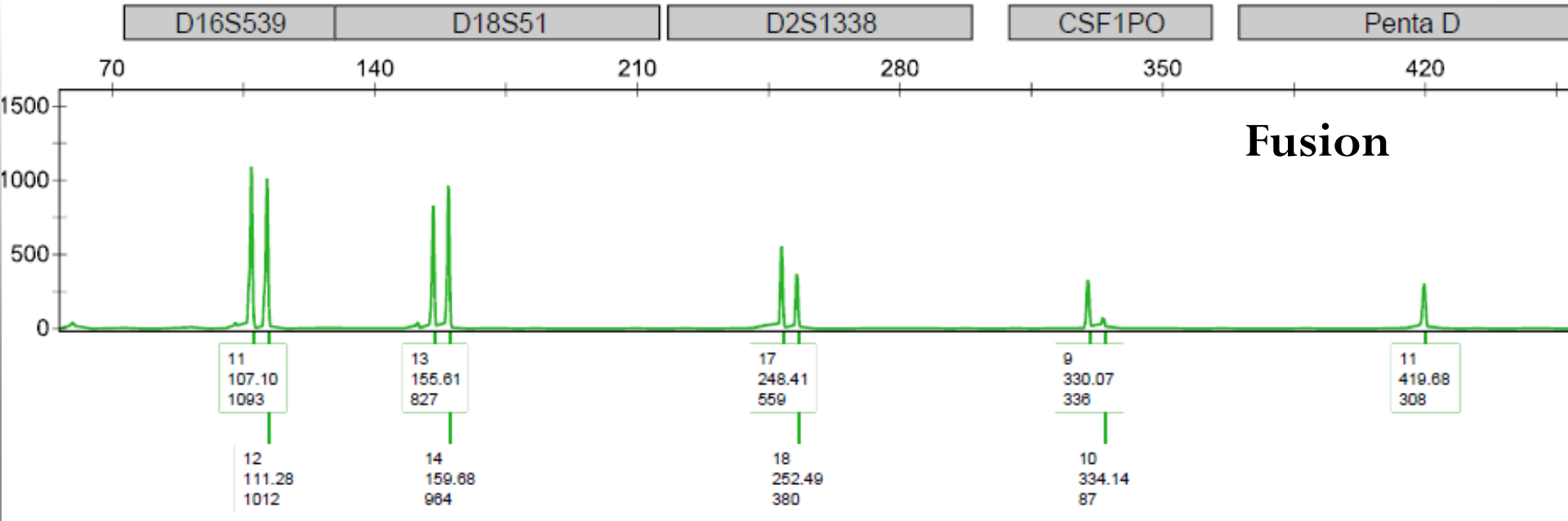
Description	Quantification Value	Y Quant
Vaginal / Cervical - EF	1.1200	0.0075
Anal / Rectal - EF	1.3400	0.0250
Vaginal / Cervical - EF	1.1900	N/A
Vaginal - EF	0.6990	0.0940
Vaginal - EF	1.0900	N/A
Vaginal / Cervical - SF	2.3900	5.0700
Anal / Rectal - SF	0.1570	0.3490
Vaginal / Cervical - SF	10.3000	0.0080
Vaginal - SF	9.3700	9.3800
Vaginal - SF	6.8100	0.0346


D3S1358
TH01
D21S11
D18S51
Penta_E
80
160
240
320
400
480
3600
2400
1200
0
15
122.60
796
17
130.91
756
7
164.42
863
9.3
175.28
1031
29
218.86
833
30
222.89
741
15
311.19
3678
7
385.73
1457
17
436.58
1163
Reference PP16HS
...
D3S1358
D1S1656
D2S441
D10S1248
D13S317
Penta E
70
140
210
280
350
420
2400
1600
800
0
OL
83.30
145
X
84.23
834
OL
89.11
148
Y
89.99
15
123.21
1008
17
131.55
993
15
181.95
2138
11
221.12
1239
14
233.35
1260
13
269.95
902
17
285.65
924
8
308.94
1115
12
325.00
1076
7
374.70
693
17
423.03
684
Reference Fusion

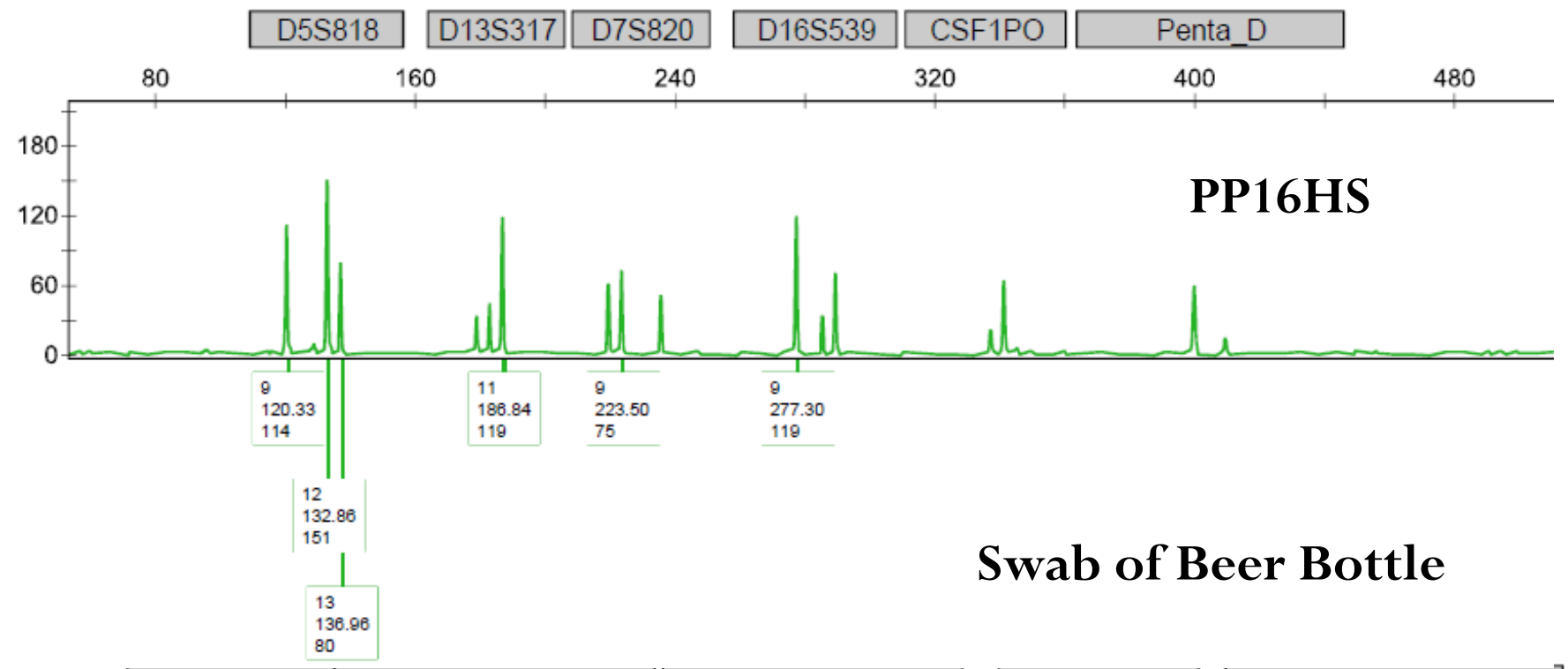


PP16HS

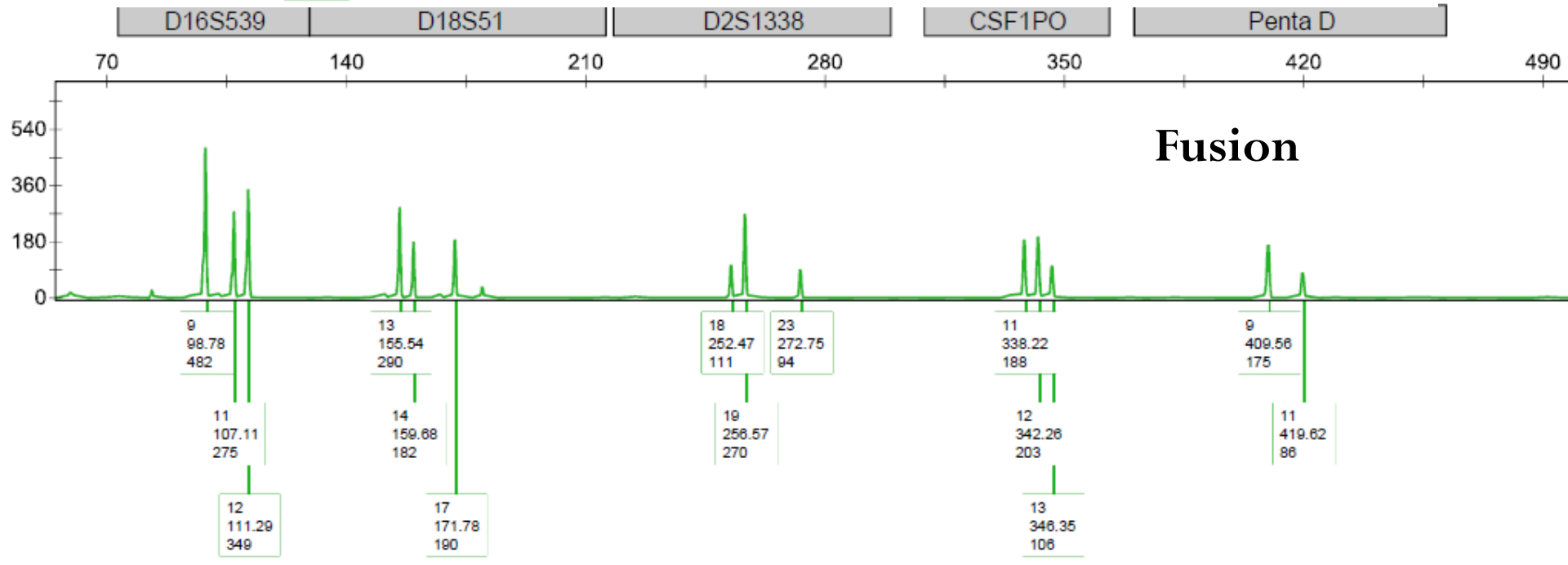
Swab of a water bottle

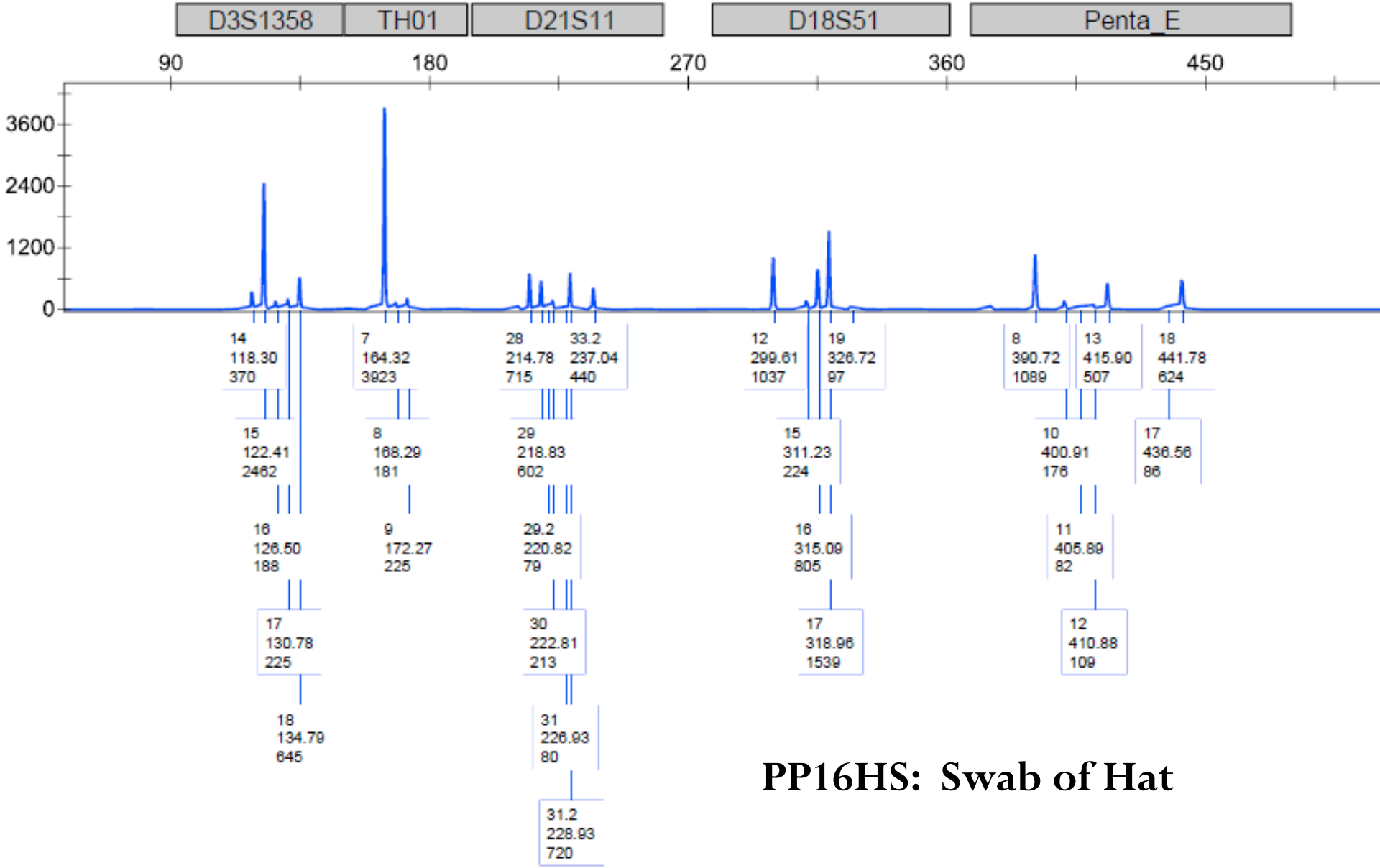


Fusion

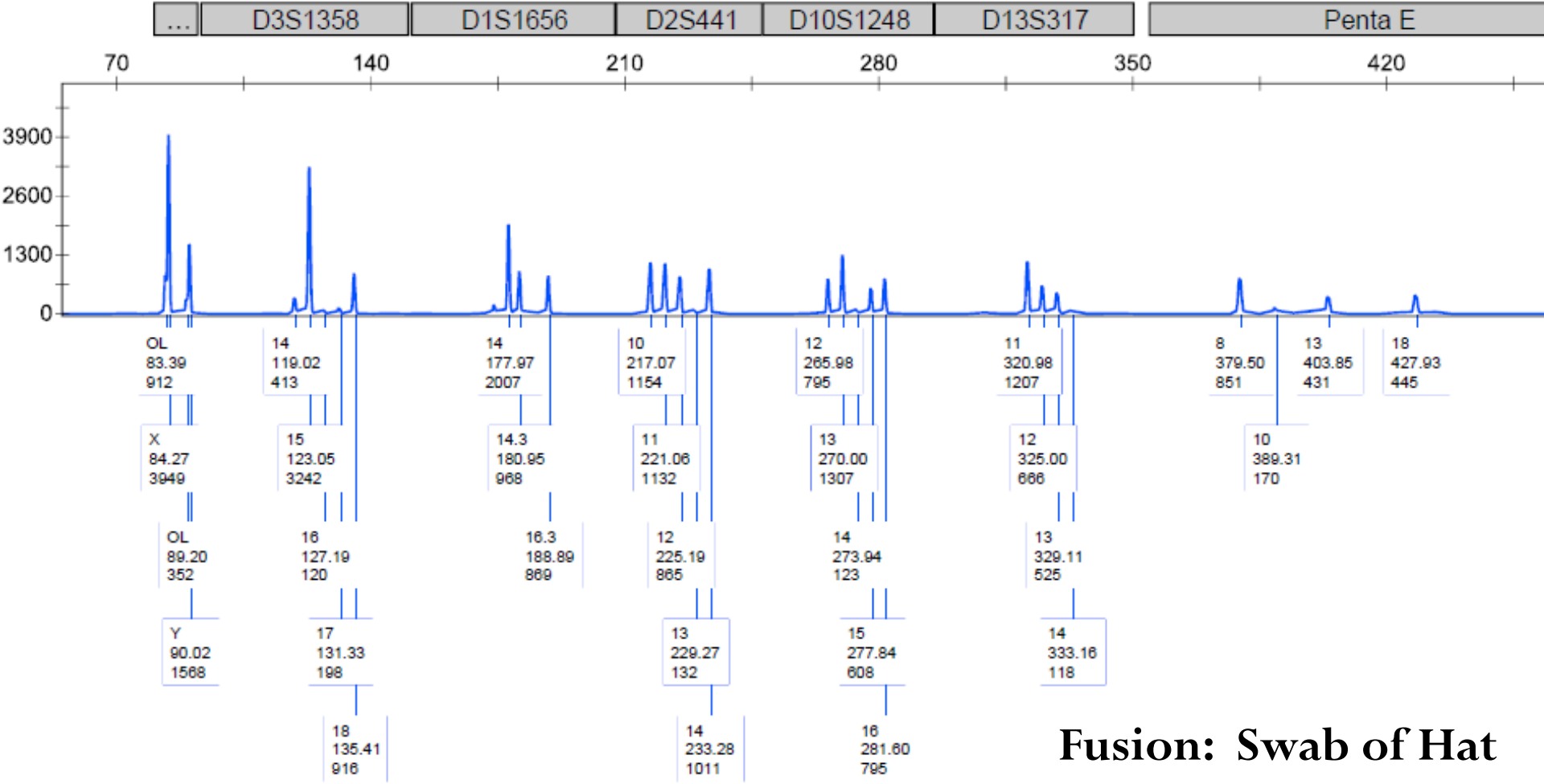


Swab of Beer Bottle



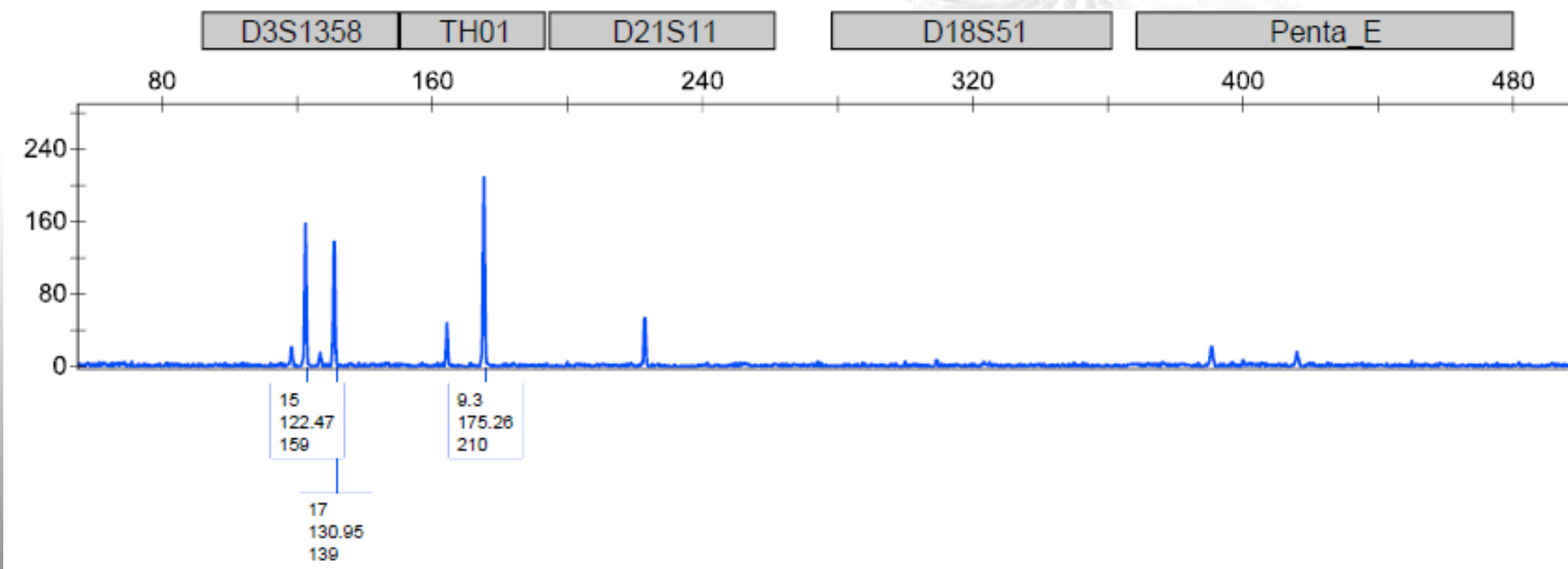


PP16HS: Swab of Hat

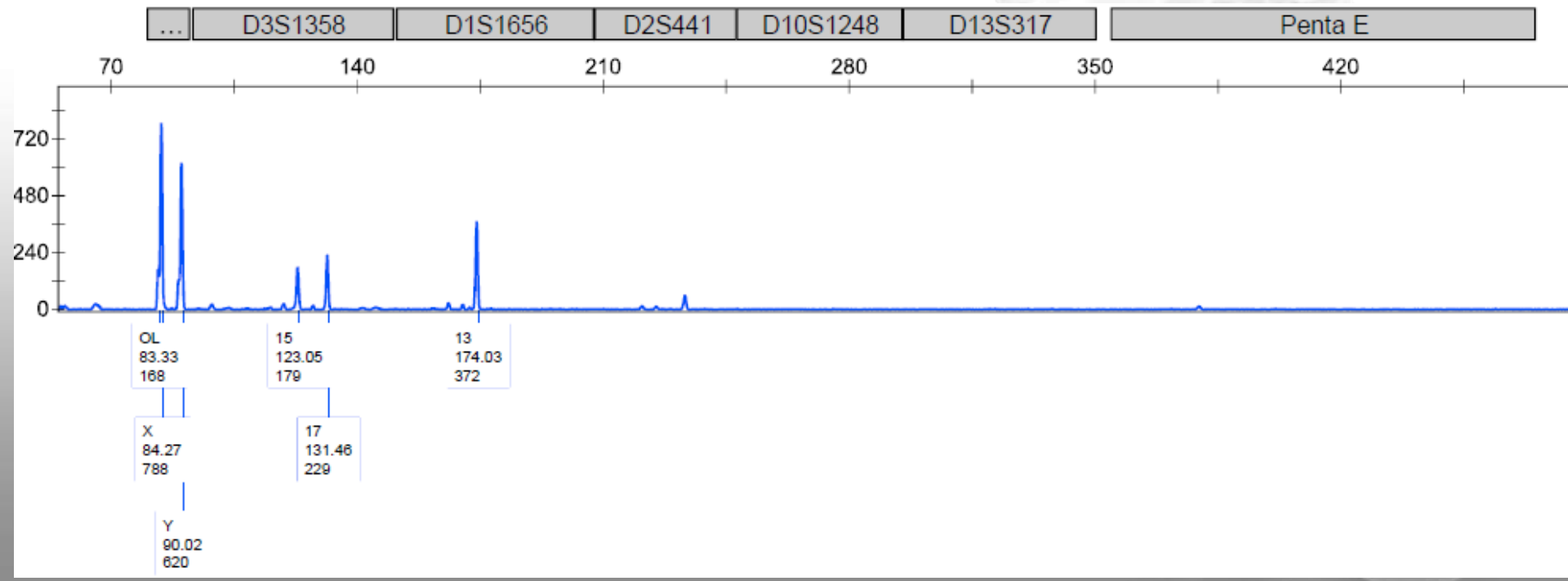


Fusion: Swab of Hat

PP16HS: Fingernail Scrapings
5 total markers interpretable

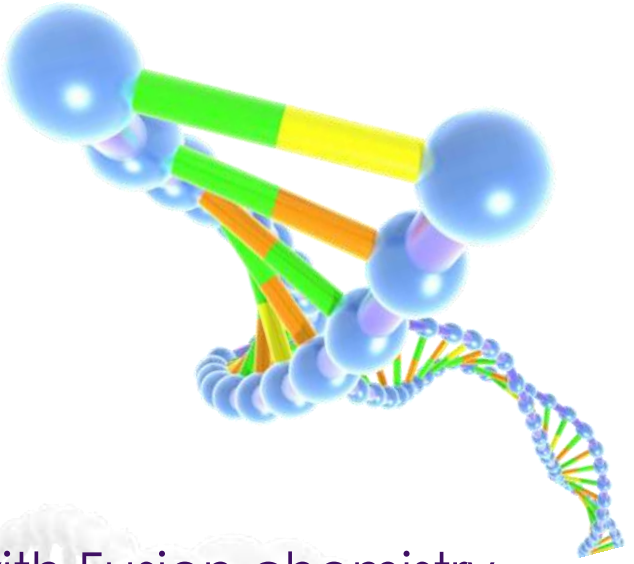


Fusion: Fingernail Scrapings
10 total markers interpretable



•INTERNAL VALIDATION

- NIST Standards Concordance



- NIST sample set 2931b was amplified with Fusion chemistry.
- All profiles obtained were concordant with the published profiles for the various components provided in the kit.

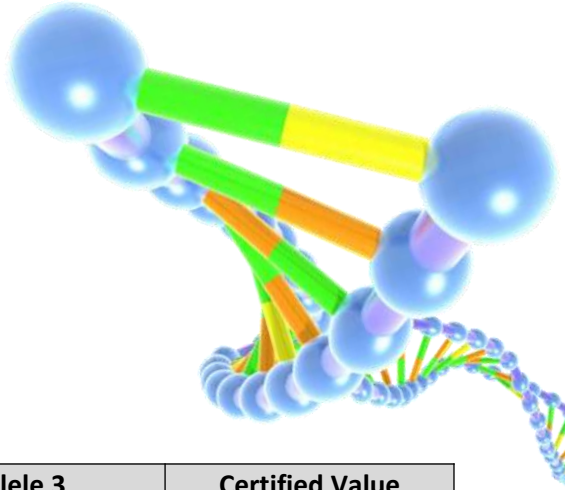
•INTERNAL VALIDATION

- NIST Standards Concordance
  - 2800M

Sample Name	Marker	Allele 1	Allele 2	Certified Value
2800M071713 [3 sec]	CSF1PO	12		12,12
2800M071713 [3 sec]	D3S1358	17	18	17,18
2800M071713 [3 sec]	D5S818	12		12,12
2800M071713 [3 sec]	D7S820	8	11	8,11
2800M071713 [3 sec]	D8S1179	14	15	14,15
2800M071713 [3 sec]	D13S317	9	11	9,11
2800M071713 [3 sec]	D16S539	9	13	9,13
2800M071713 [3 sec]	D18S51	16	18	16,18
2800M071713 [3 sec]	D21S11	29	31.2	29,31.2
2800M071713 [3 sec]	FGA	20	23	20,23
2800M071713 [3 sec]	TH01	6	9.3	6,9.3
2800M071713 [3 sec]	TPOX	11		11,11
2800M071713 [3 sec]	vWA	16	19	16,19
2800M071713 [3 sec]	AMEL	X	Y	X,Y
2800M071713 [3 sec]	Penta D	12	13	12,13
2800M071713 [3 sec]	Penta E	7	14	7,14
2800M071713 [3 sec]	D2S1338	22	25	22,25
2800M071713 [3 sec]	D19S433	13	14	13,14
2800M071713 [3 sec]	D10S1248	13	15	13,15
2800M071713 [3 sec]	D2S441	10	14	10,14
2800M071713 [3 sec]	D22S1045	16		16,16
2800M071713 [3 sec]	D12S391	18	23	18,23
2800M071713 [3 sec]	D1S1656	12	13	12,13
2800M071713 [3 sec]	DYS391	10		10,10

•INTERNAL VALIDATION

- NIST Standards Concordance
  - Genomic DNA 3



Sample Name	Marker	Allele 1	Allele 2	Allele 3	Certified Value
303 - Genomic DNA 3 [3 sec]	CSF1PO	11	12		11,12
303 - Genomic DNA 3 [3 sec]	D3S1358	9 (95 RFU = 2.7%)	15		15,15
303 - Genomic DNA 3 [3 sec]	D5S818	11			11,11
303 - Genomic DNA 3 [3 sec]	D7S820	12	13		12,13
303 - Genomic DNA 3 [3 sec]	D8S1179	14	15 (287 RFU = 12.1%)	16	14,16
303 - Genomic DNA 3 [3 sec]	D13S317	11	12		11,12
303 - Genomic DNA 3 [3 sec]	D16S539	11	12		11,12
303 - Genomic DNA 3 [3 sec]	D18S51	16	20		16,20
303 - Genomic DNA 3 [3 sec]	D21S11	28	31.2		28,31.2
303 - Genomic DNA 3 [3 sec]	FGA	23	25		23,25
303 - Genomic DNA 3 [3 sec]	TH01	9.3			9.3,9.3
303 - Genomic DNA 3 [3 sec]	TPOX	8	11		8,11
303 - Genomic DNA 3 [3 sec]	vWA	18	19		18,19
303 - Genomic DNA 3 [3 sec]	AMEL	X	Y		X,Y
303 - Genomic DNA 3 [3 sec]	Penta D	11	12		11,12
303 - Genomic DNA 3 [3 sec]	Penta E	13	14		13,14
303 - Genomic DNA 3 [3 sec]	D2S1338	20	24		20,24
303 - Genomic DNA 3 [3 sec]	D19S433	12	14		12,14
303 - Genomic DNA 3 [3 sec]	D10S1248	13	16		13,16
303 - Genomic DNA 3 [3 sec]	D2S441	10	14		10,14
303 - Genomic DNA 3 [3 sec]	D22S1045	15	16		15,16
303 - Genomic DNA 3 [3 sec]	D12S391	15	21		N/A
303 - Genomic DNA 3 [3 sec]	D1S1656	14	15		N/A
303 - Genomic DNA 3 [3 sec]	DYS391	10			N/A

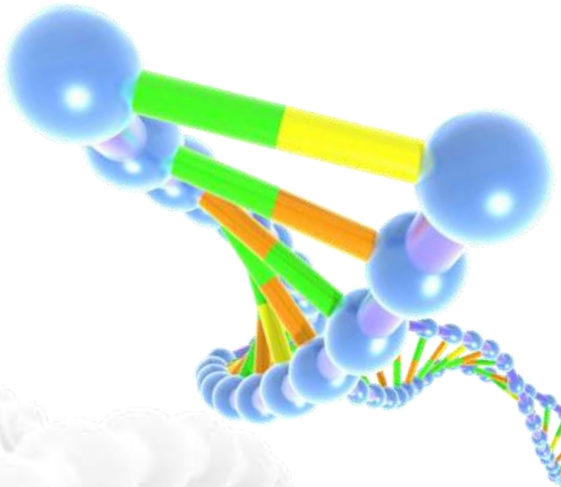
•INTERNAL VALIDATION

- NIST Standards Concordance
  - Genomic DNA 10 (GM09948)

Sample Name	Marker	Allele 1	Allele 2	Allele 3	Certified Value
310 - Genomic DNA 10 [3 sec]	CSF1PO	10	11	12	10,11,12
310 - Genomic DNA 10 [3 sec]	D3S1358	15	17		15,17
310 - Genomic DNA 10 [3 sec]	D5S818	11	13		11,13
310 - Genomic DNA 10 [3 sec]	D7S820	11			11,11
310 - Genomic DNA 10 [3 sec]	D8S1179	12	13		12,13
310 - Genomic DNA 10 [3 sec]	D13S317	11			11,11
310 - Genomic DNA 10 [3 sec]	D16S539	11			11,11
310 - Genomic DNA 10 [3 sec]	D18S51	15	18		15,18
310 - Genomic DNA 10 [3 sec]	D21S11	29	30		29,30
310 - Genomic DNA 10 [3 sec]	FGA	24	26		24,26
310 - Genomic DNA 10 [3 sec]	TH01	6	9.3		6,9.3
310 - Genomic DNA 10 [3 sec]	TPOX	8	9		8,9
310 - Genomic DNA 10 [3 sec]	vWA	17			17,17
310 - Genomic DNA 10 [3 sec]	AMEL	X	Y		X,Y
310 - Genomic DNA 10 [3 sec]	Penta D	8	12		8,12
310 - Genomic DNA 10 [3 sec]	Penta E	11			11,11
310 - Genomic DNA 10 [3 sec]	D2S1338	23			23,23
310 - Genomic DNA 10 [3 sec]	D19S433	13	14		13,14
310 - Genomic DNA 10 [3 sec]	D10S1248	12	15		12,15
310 - Genomic DNA 10 [3 sec]	D2S441	11	12		11,12
310 - Genomic DNA 10 [3 sec]	D22S1045	16	17 (225 RFU = 16.9%)	18	16,18
310 - Genomic DNA 10 [3 sec]	D12S391	18	24	24.3 (112 RFU = 4.5%)	N/A
310 - Genomic DNA 10 [3 sec]	D1S1656	14	17		N/A
310 - Genomic DNA 10 [3 sec]	DYS391	10			N/A

•INTERNAL VALIDATION

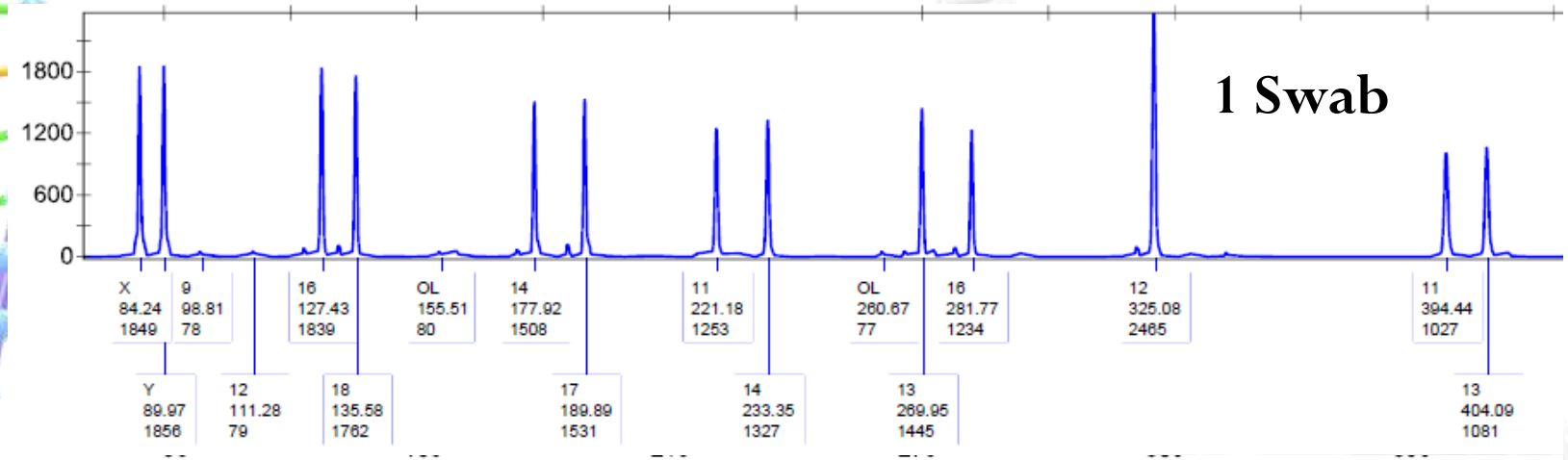
Direct Amplification of Knowns

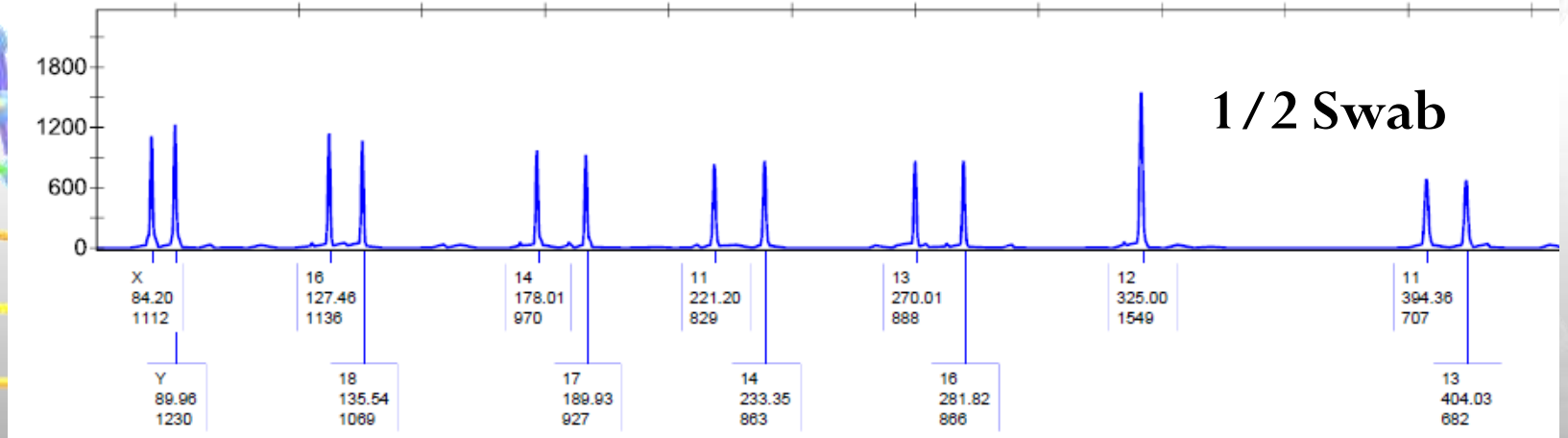


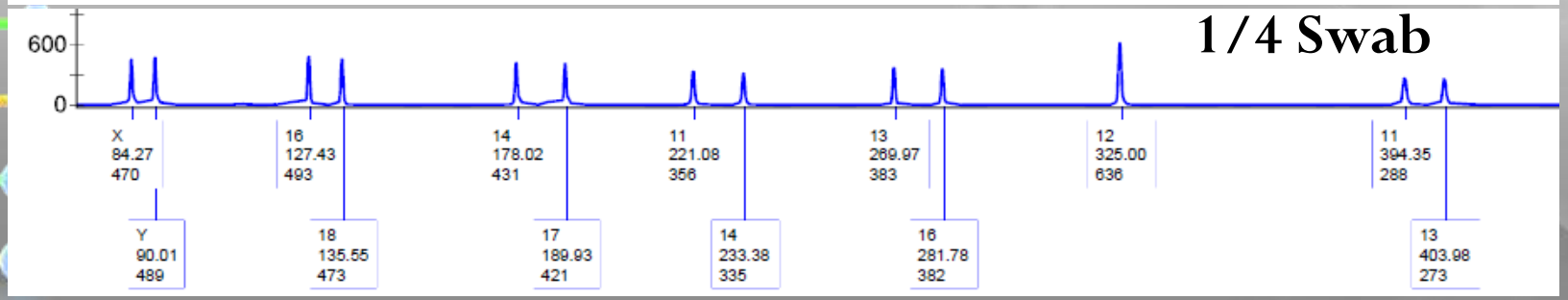
- Vary amount of swab
  - Cotton Swab – 1 swab, ½ swab, ¼ swab
  - Omni Swab – 2 teeth, 1 tooth, ½ tooth
- Cycle Number Optimization
  - 30 Cycles / 27 Cycles / 25 Cycles
- Procedure:
  - Add 1 mL of swab solution to swab amounts listed above.
  - Place samples in 70 C hot plate for 30 minutes.
  - Use 1 uL of solution for amp 1 and 2 uL solution for amp 2.
  - Amp samples at same cycle / extension as evidence cycle parameters. (30 cycles)
  - Additional Amps performed at 27, 26, and 25 Cycles

•INTERNAL VALIDATION

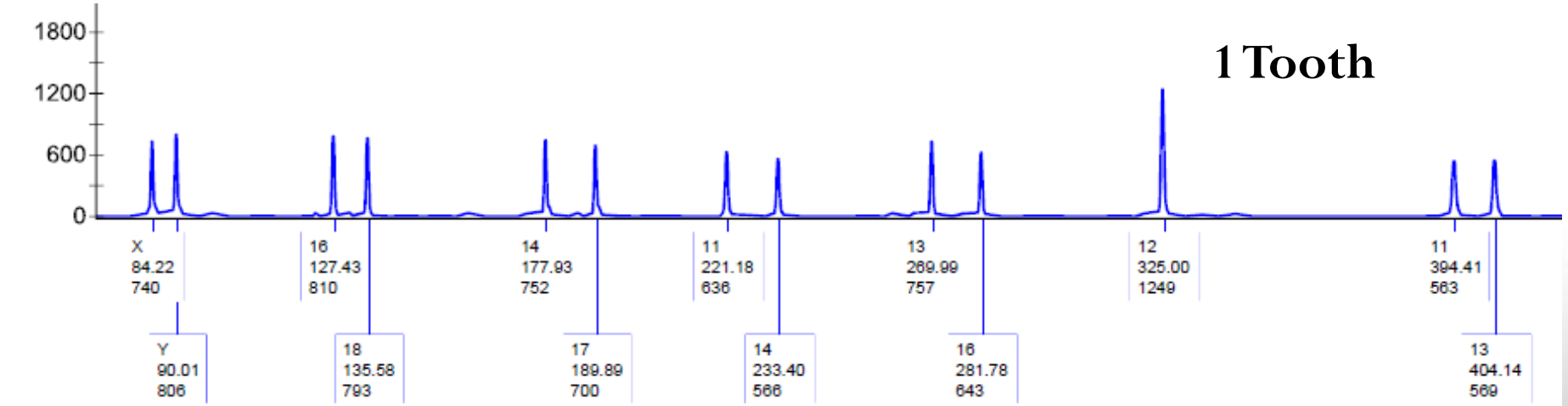
Direct Amplification of Knowns








1800
1200
600
0
2 Teeth
X
84.31
756
16
127.45
885
14
177.96
771
11
221.18
649
13
269.95
713
12
325.00
1297
11
394.33
598
Y
90.01
808
18
135.59
815
17
189.86
722
14
233.38
616
16
281.77
776
13
404.02
617


1800
1200
600
0
1 Tooth
X
84.22
740
16
127.43
810
14
177.93
752
11
221.18
636
13
269.99
757
12
325.00
1249
11
394.41
563
Y
90.01
806
18
135.58
793
17
189.89
700
14
233.40
566
16
281.78
643
13
404.14
569


1800
1200
600
0
1/2 Tooth
X
84.21
739
16
127.46
884
14
177.94
822
11
221.15
778
13
269.96
744
12
325.00
1419
11
394.40
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Y
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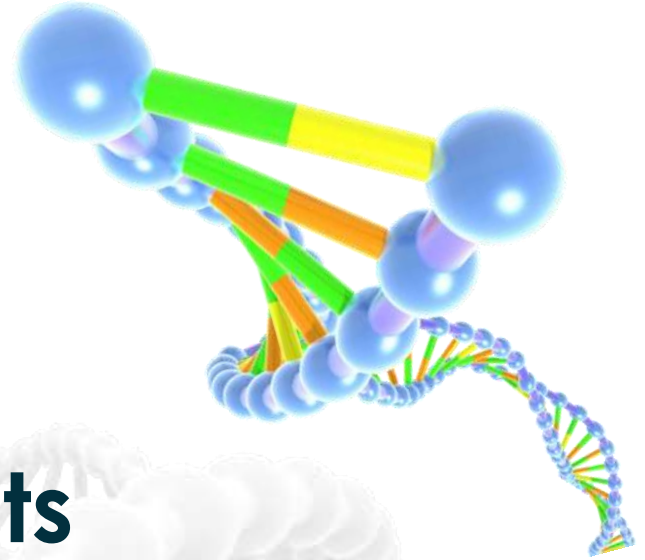
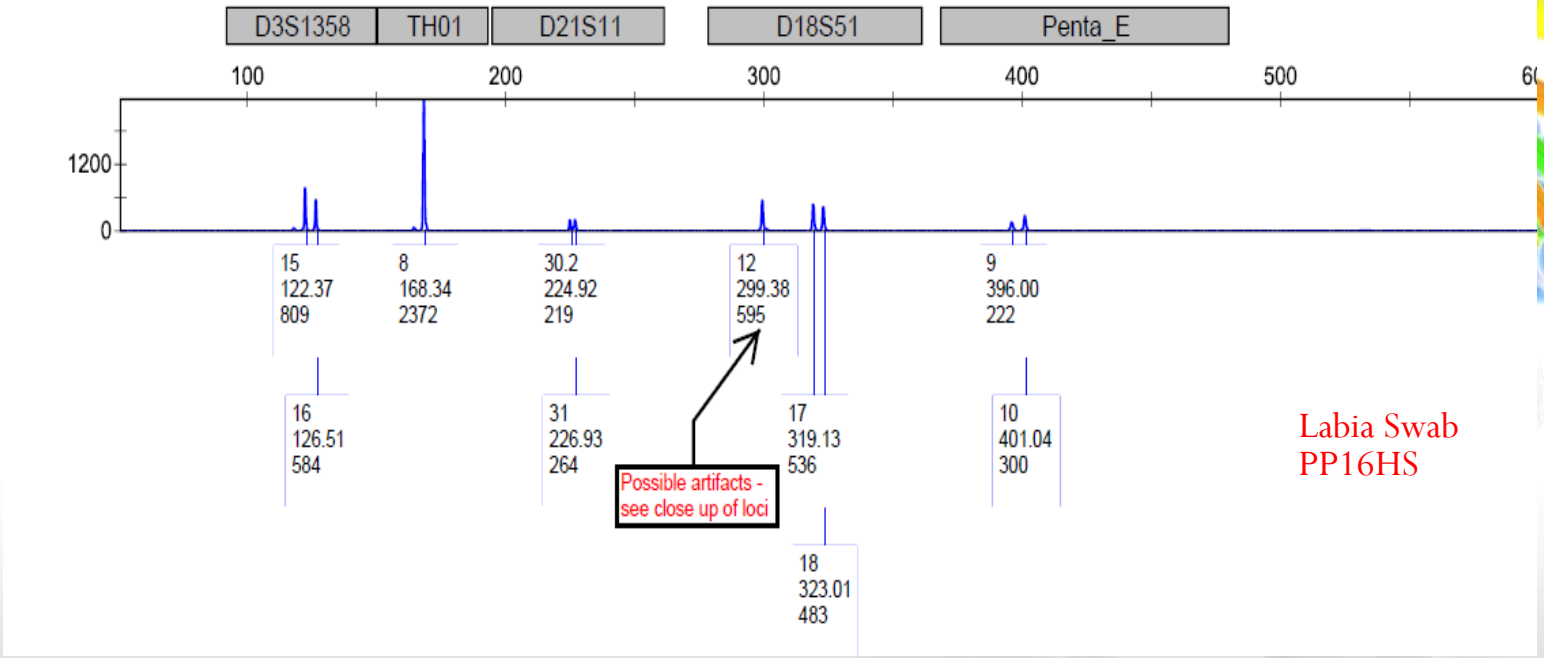
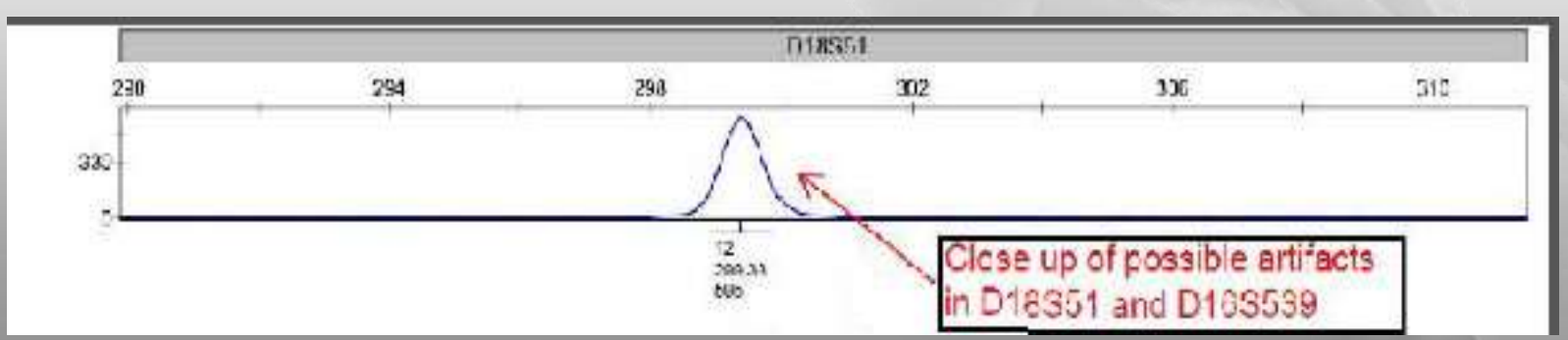


Artifact Assessments

•Additional Studies

- Comparison of artifacts

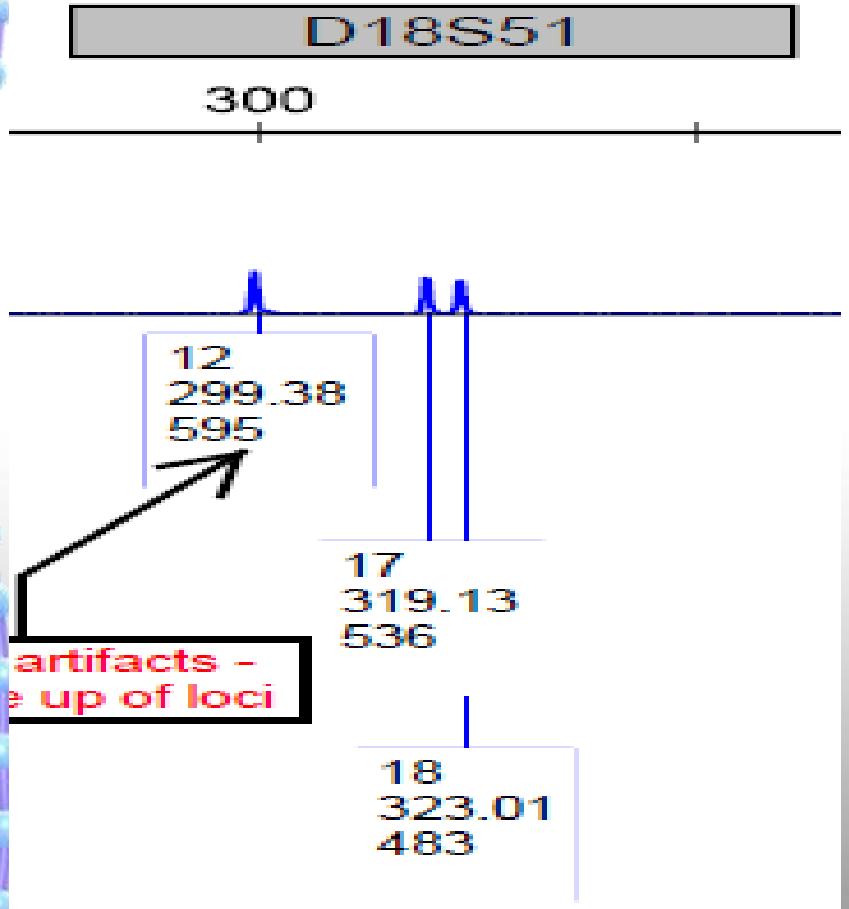




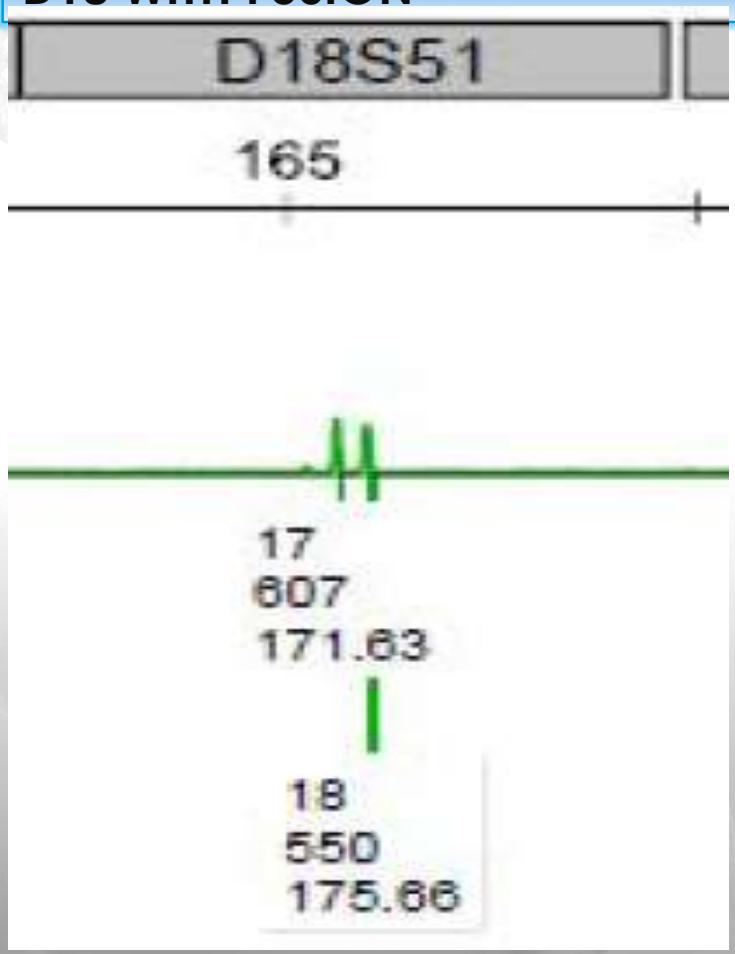
Additional Studies

Comparison of artifacts

D18 WITH PP16HS

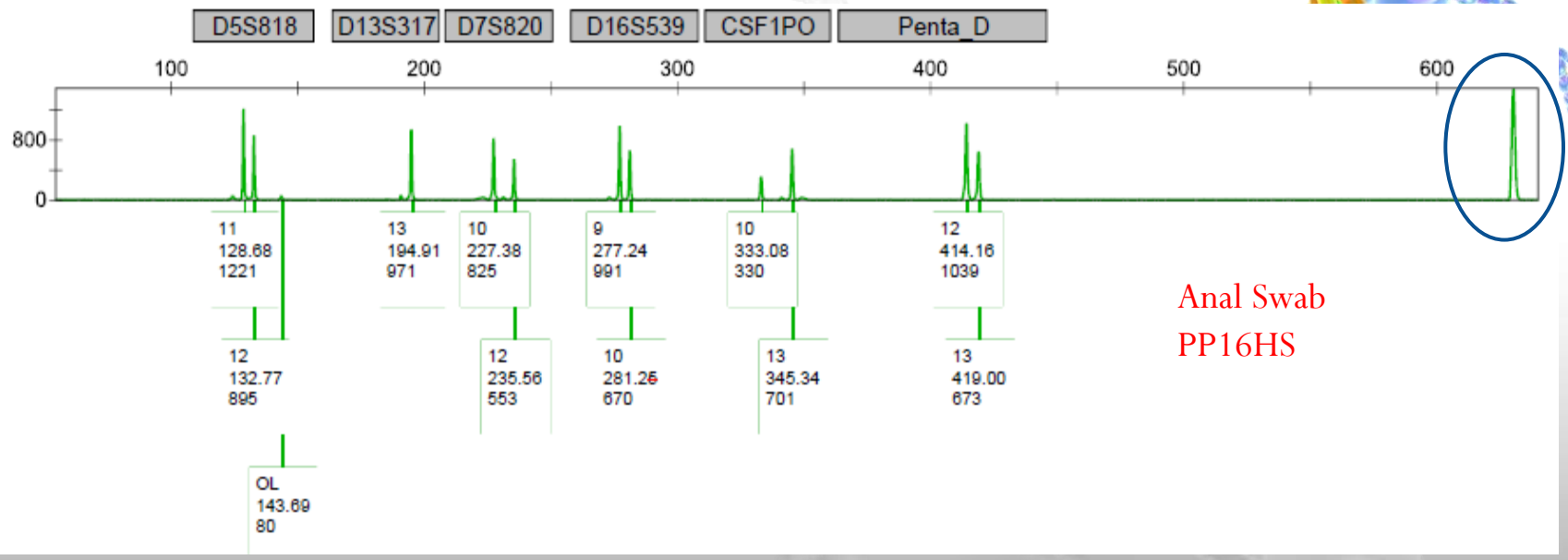


D18 WITH FUSION



•Additional Studies

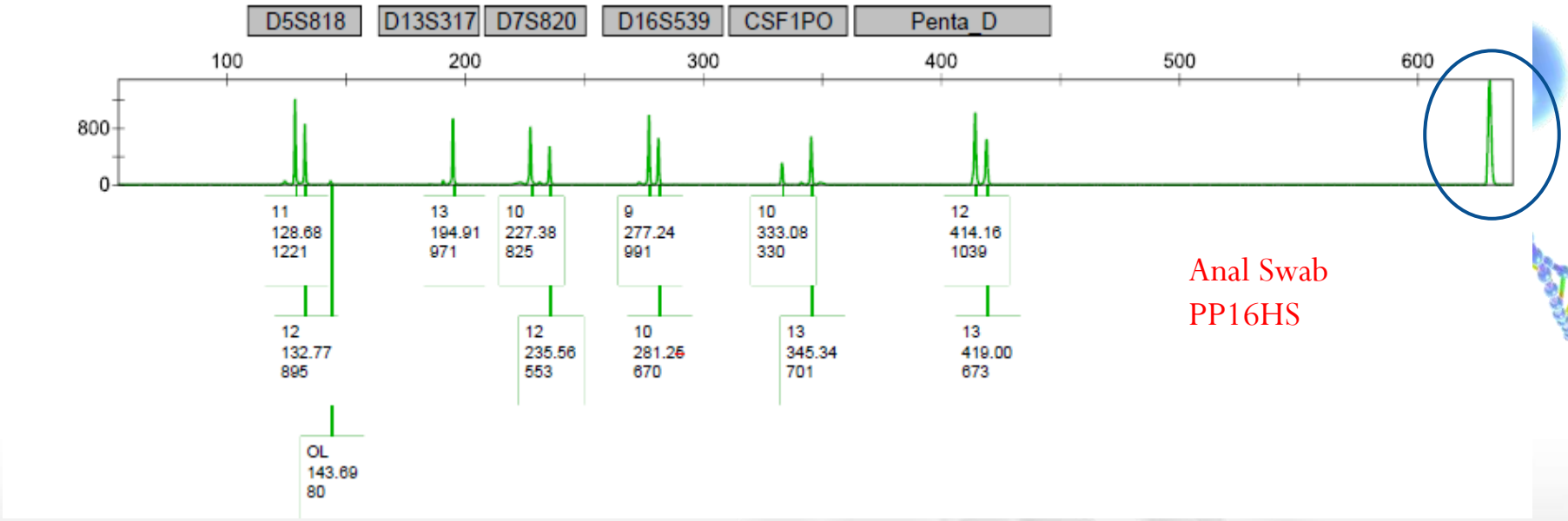
- Comparison of artifacts



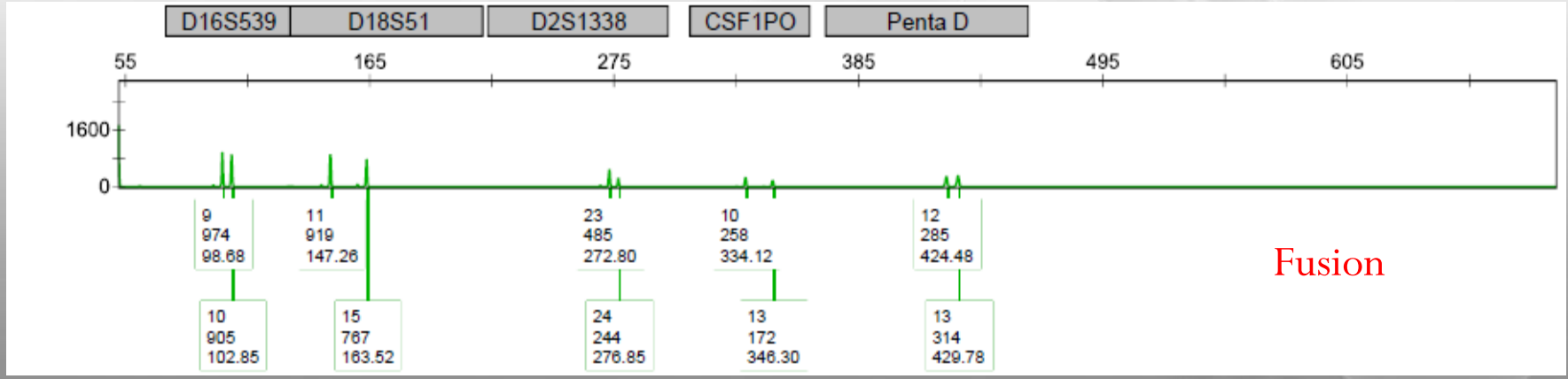
Anal Swab
PP16HS

•Additional Studies

- Comparison of artifacts



Anal Swab
PP16HS



Fusion

Considerations for Selecting a Kit

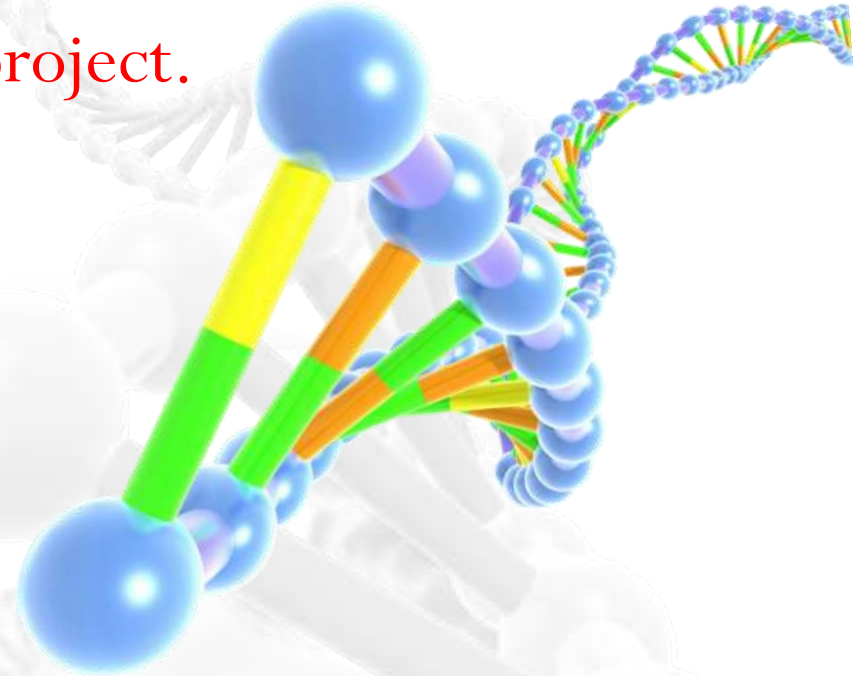
- One kit vs. two kits
- 5 dye vs. 6 dye
- Familiarity

- Software changes
- Ease of Direct Amplification
- Support

- Use of Pentas
- Cost
- Required CODIS loci (No changes yet)

ACKNOWLEDGEMENTS

Thank you to Promega Corporation for asking us to participate and all of the other collaborators on the project.



A special thanks to Kirk DeLeeuw, Josh Strong, DonaldYet and Kristin Schelling for all of the effort in conducting the internal validation.