Validation and Implementation of the Promega PowerPlex® Y23 STR System

WSP CLD Forensic DNA Continuing Education Day – June 18, 2015

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Choosing a Kit

- Started Validation in Mid-2012
- Something we could drop into available workflow (5 dye chemistry, 3130*xl*)
  - AmpFLSTR® Yfiler® Amplification Kit
  - PowerPlex® Y23 STR System

Yfiler® vs Y23

- **Dynamic range and sensitivity**
  - Take a previously extracted and quantitated male DNA sample
  - Amplify five times each at the following targets, using the male DNA quantitation result for input calculations
    - 2ng, 1ng, 0.5ng, 0.25ng, 0.1ng, 0.05ng, and 0.02ng
  - Compare results between the two kits with respect to limits of detection, sensitivity, inter-dye balance, detection of artifacts

Yfiler® vs Y23

Yfiler® vs Y23

- **Dynamic range and sensitivity**
  - Both kits appeared to have roughly equal sensitivity and dye balance. 0.5ng appeared to be ideal target template for single source samples.
  - Both kits started exhibiting dropout at approximately 0.05ng of input DNA
    - Y23 had a slight edge regarding number of loci with respect to allelic drop out

Yfiler® vs Y23

- **Male/female mixture studies**
  - Does the presence of female DNA in a sample have any effect on the Y-STR amplification results when the male DNA is held at a given target?

Yfiler® vs Y23

- **Male/female mixture studies**
  - Constant male, increasing female
    - Create the following ratios of male:female DNA and amp in duplicate

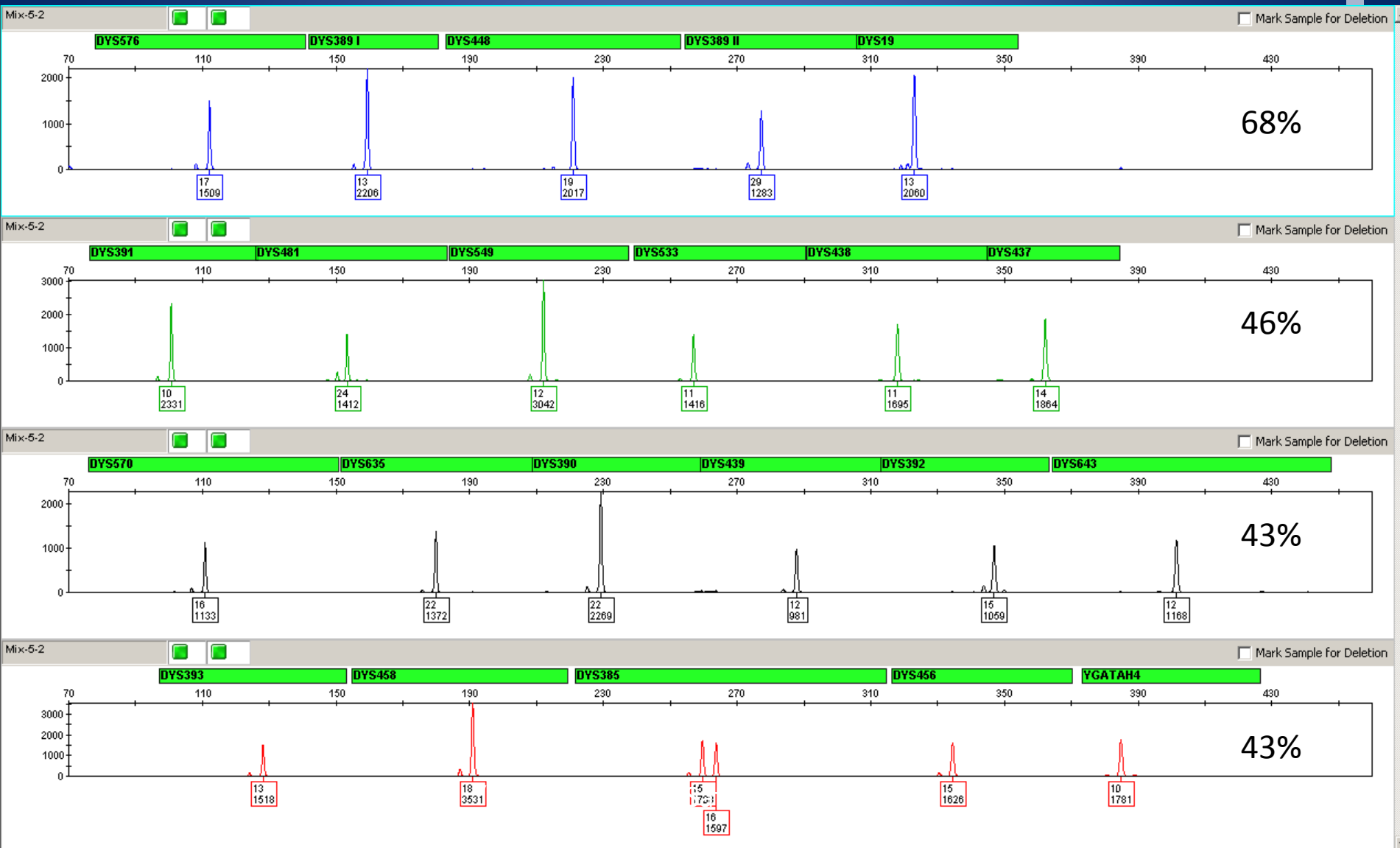
Amp Target: ng male/ng female	male:female ratio
0.5/0.5	1:1
0.5/5	1:10
0.5/50	1:100
0.5/240	1:480
0.5/1000	1:2000

Yfiler ®

0.5ng male input (1:2000)

Y23

0.5ng male input (1:2000)



Selected the Y23 Kit for Further Validation

- Greater discrimination power from extra alleles
- Resistance to inhibitory effects of excess female DNA

Y23 Validation

Using Male Quantitation Results

- ***Practical determination of H:M ratio as a decision point for casework***
  - Can a cut-off be determined with respect to the ratio of male to total human DNA in a sample, at which analysis with Y-STRs should be attempted over analysis with Identifiler Plus?

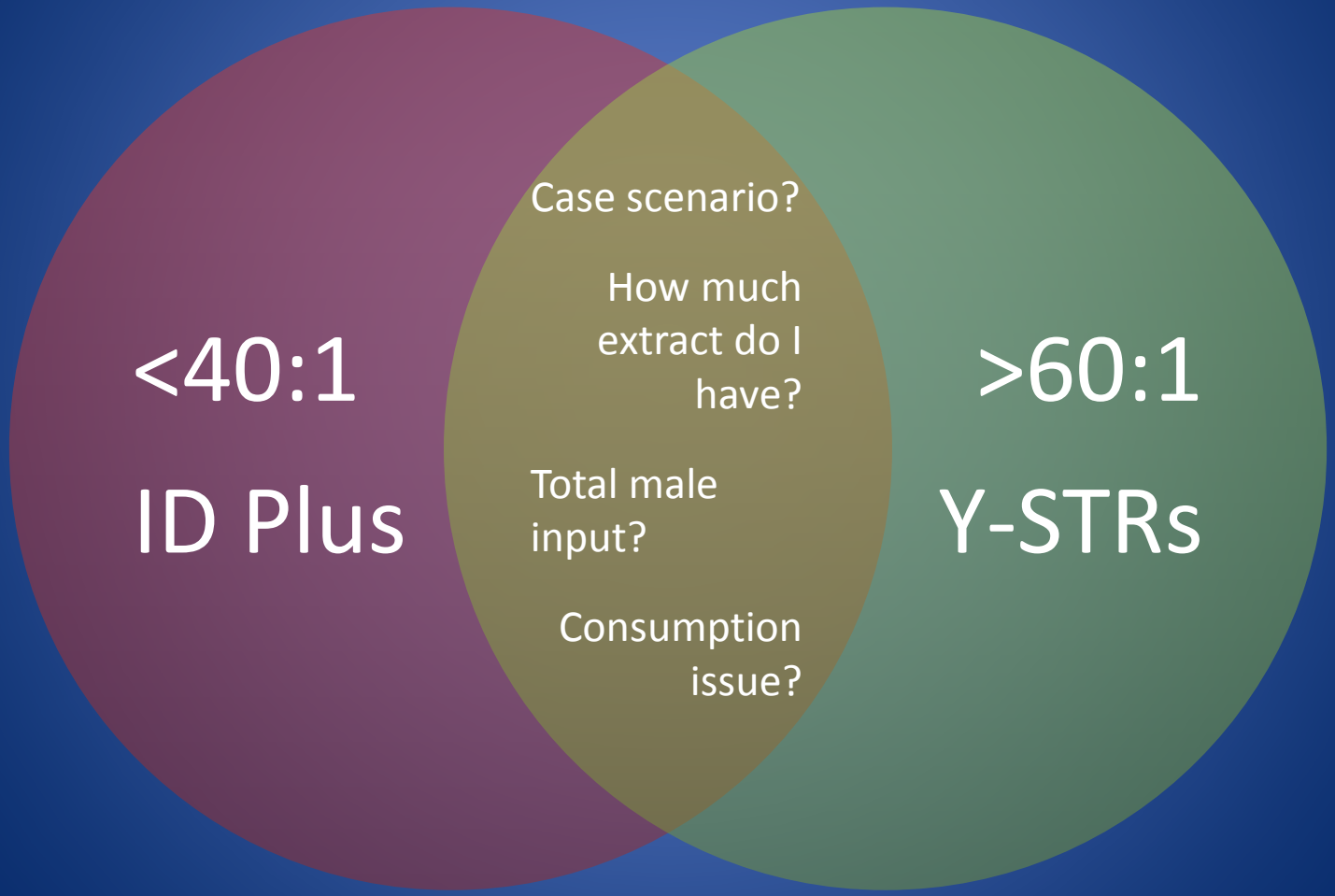
Y23 Validation

Using Male Quantitation Results

- Create DNA extracts in the following total human:male DNA ratios: 10:1, 20:1, 30:1, 40:1, 50:1, 60:1
  - Quant each simulated in sample in triplicate—average results to determine "true" H:M ratio
  - Amplify each dilution in triplicate using the Identifiler Plus kit targeting 1ng of human DNA
  - Compare results with respect to minor profile obtained with ID Plus vs completeness of profile obtained with Y-STRs

Y23 Validation

Using Male Quantitation Results



Y23 Validation

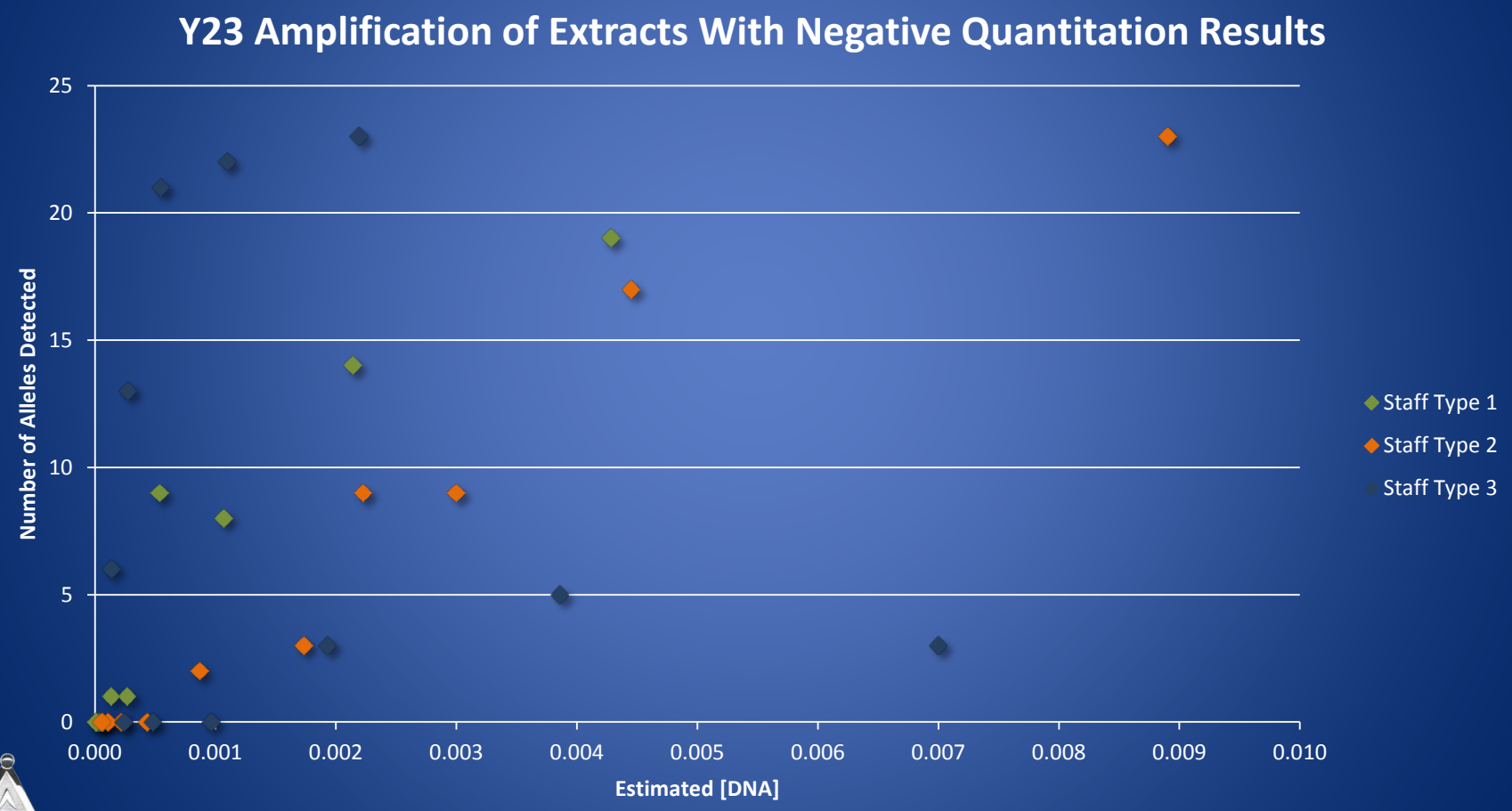
Negative Male Quants

- Three male staff member extracts were serially diluted for a total of 16 dilutions. The dilution was 1:1 each time, resulting in the DNA concentration being cut in half with each dilution.

- The 48 extracts were each quantitated
  - 10ul of each extract with no quant was amplified using Y23
  - Simulates what would occur when a 50ul extract was concentrated to 11ul and quanted with 10ul of extract remaining for amplification

Y23 Validation

Negative Male Quants



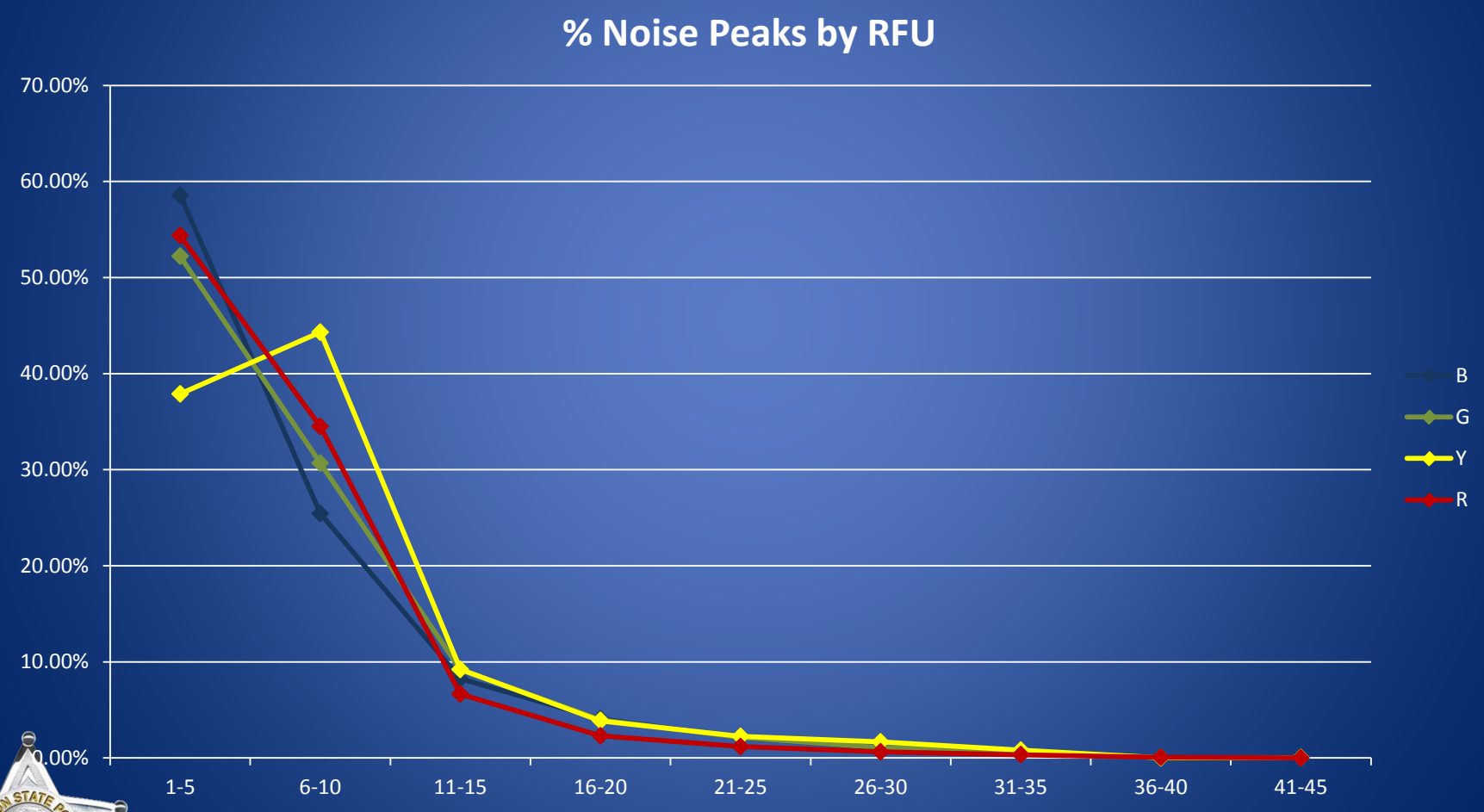
Y23 Validation

Analytical Threshold

- Used all available genotyping data from previous experiments
- Analyzed in GMID-X using a 10RFU detection threshold
- Cleaned up data to remove allelic peaks, pull up, non-filtered stutter, etc.

Y23 Validation

Analytical Threshold



Y23 Validation

Analytical Threshold

- No stark differences noted between dye channels
- Set detection threshold to 45 RFU

Y23 Validation

Stutter

- When do we use stutter?
  - Determination of minimum number of contributors
  - Mixture deconvolution

Y23 Validation

Stutter

- When doing a major/minor deconvolution:
  - Subtract the maximum expected stutter from the candidate minor allele
    - For this allele to be called, it has to be greater than the GMID-X stutter threshold
  - If after this subtraction the allele is greater than the detection threshold, the peak may be assigned as an obligate minor allele
  - If after this subtraction the allele falls below the detection threshold, the peak is considered to be indistinguishable from stutter and is not assigned as a minor allele

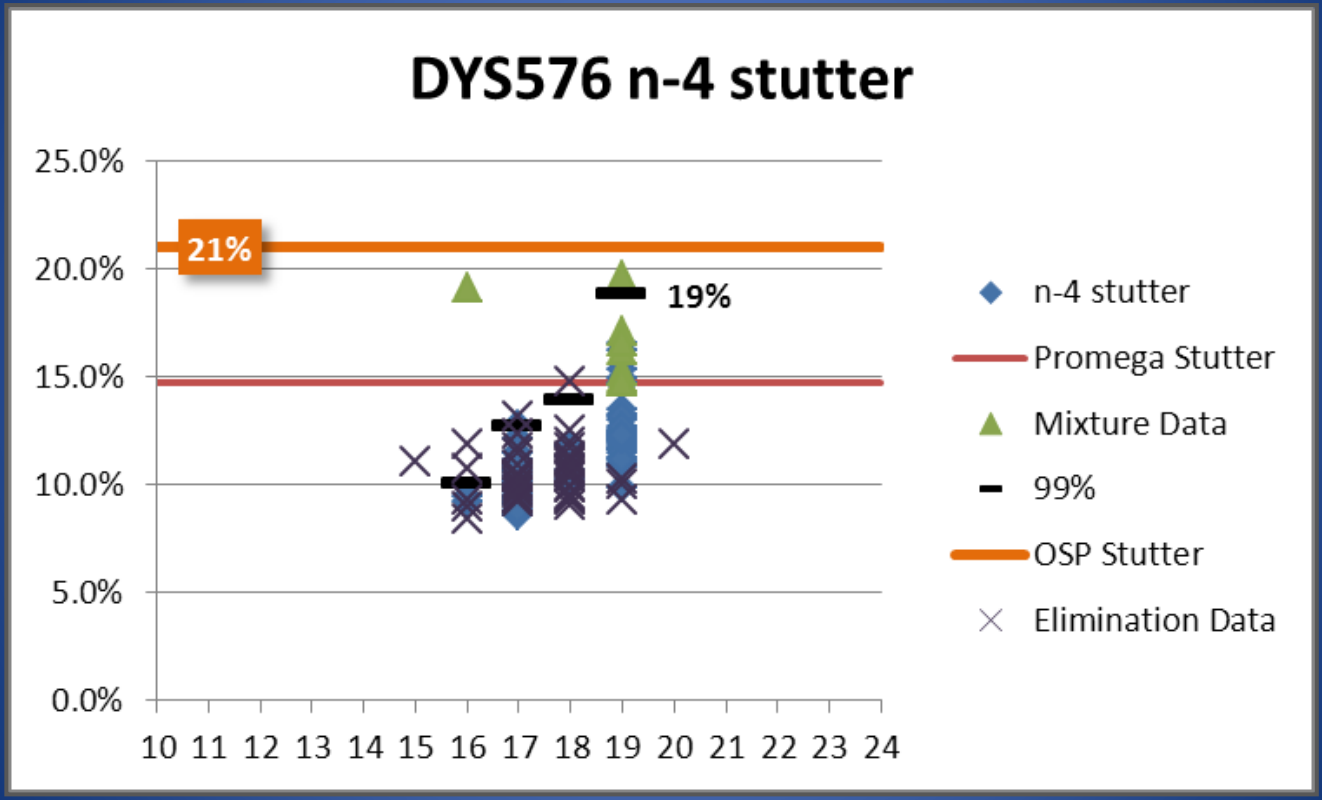
Y23 Validation

Stutter

- Genotyping data generated as part of validation experiments thus far
- Genotyping data obtained from OSP Forensic Services Division elimination samples
- Analyzed with no stutter filters, 10RFU detection

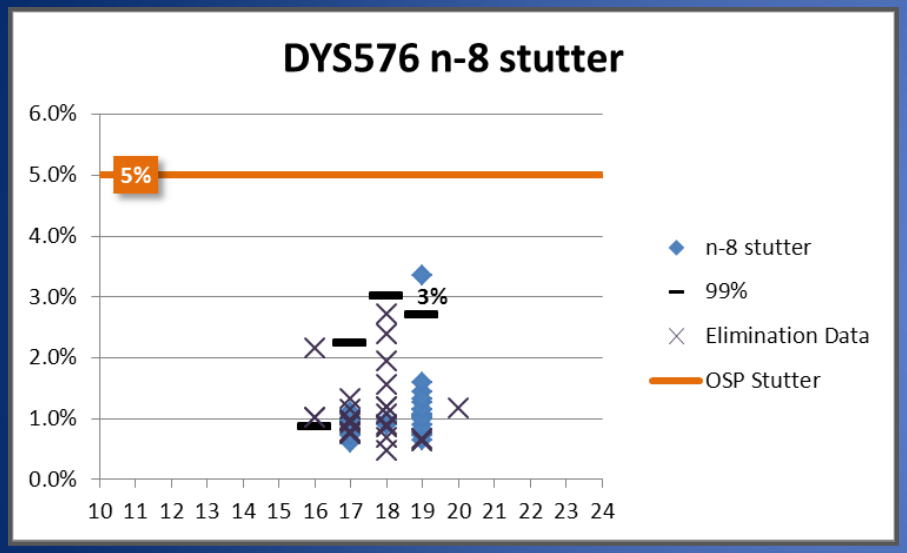
Y23 Validation

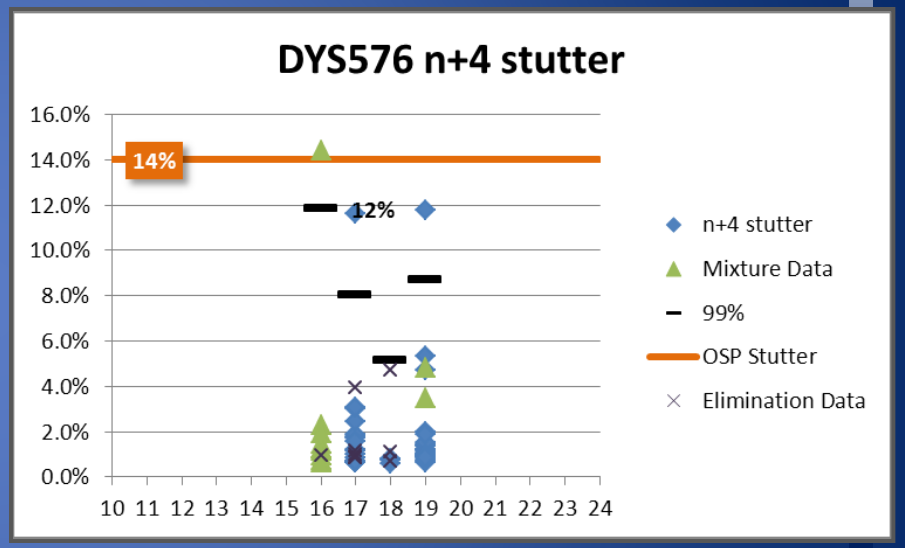
Stutter



Y23 Validation

Stutter





Locus	Repeat Size	-2 Repeats	-1 Repeat		+1 Repeat	
DYS576	4	5%	21%		14%	
DYS389 I	4		10%		4%	
DYS448	6		6%		2%	
DYS389 II	4	5%	18%		7%	
DYS19	4		-4bp: 13%	-2bp: 16%	+2bp: 8%	+4bp: 4%
DYS391	4	4%	13%		2%	
DYS481	3	9%	33%		9%	
DYS549	4	3%	15%		5%	
DYS533	4	3%	17%		5%	
DYS438	5		7%		5%	
DYS437	4		12%		7%	
DYS570	4	4%	16%		5%	
DYS635	4	4%	16%		8%	
DYS390	4	5%	14%		5%	
DYS439	4	4%	12%		8%	
DYS392	3	5%	20%		14%	
DYS643	5		7%		4%	
DYS393	4	4%	15%		5%	
DYS458	4	4%	18%		5%	
DYS385 a/b	4	4%	17%		6%	
DYS456	4	4%	18%		6%	
Y-GATA-H4	4	3%	13%		5%	

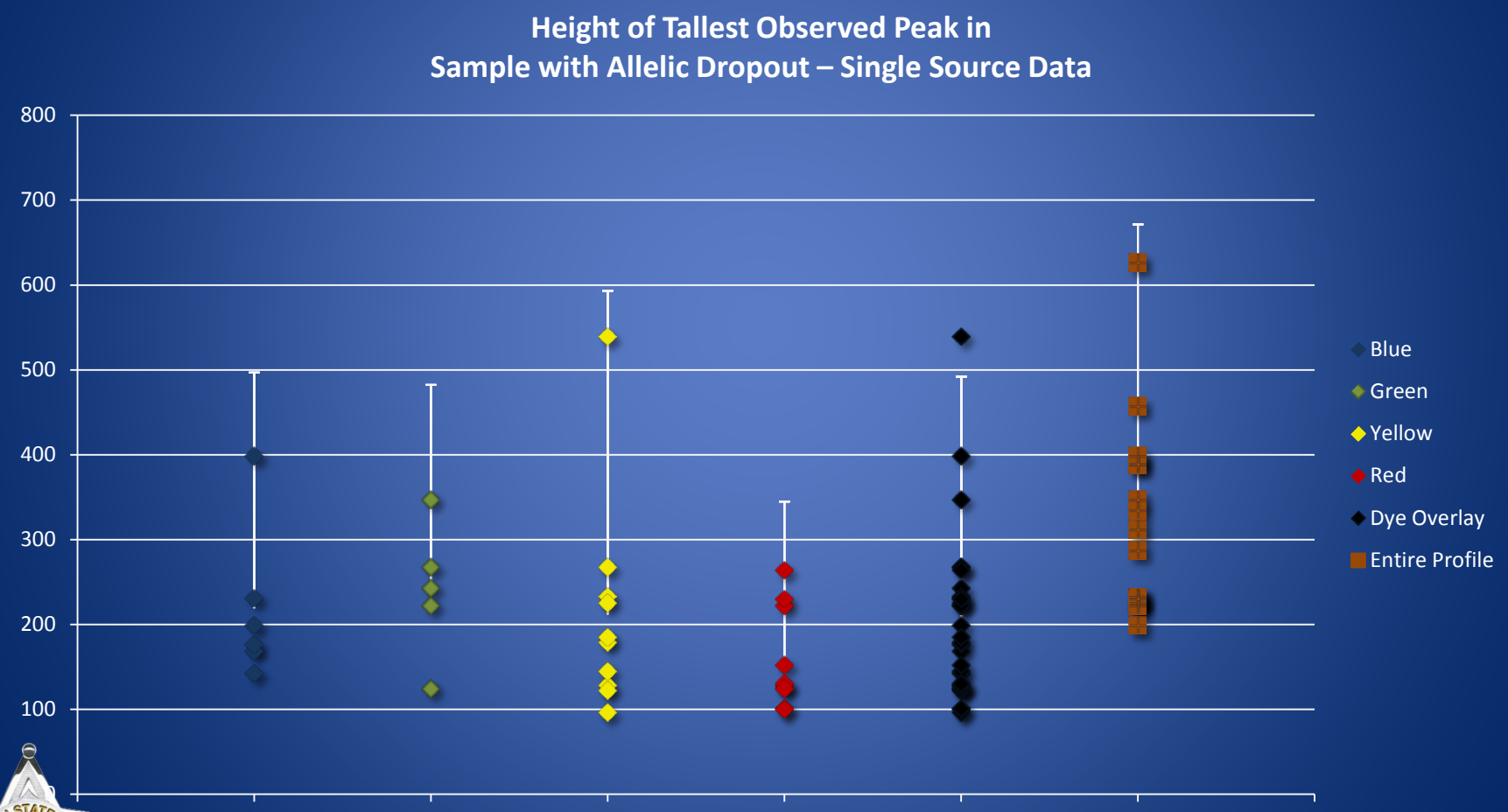
Y23 Validation

Dropout Threshold

- At what point can we assume a contributor has not dropped out?
  - Look at single source data to determine if an RFU based threshold can be determined
  - Test this threshold on the mixture data

Y23 Validation

Dropout Threshold



Y23 Validation

Mixture Studies

- Approach to mixture studies
  - Mixtures of two males
  - Mixtures of three males
  - Can I use PHR to deconvolute?
    - Examine data for peak height inversions
  - If I have only a single peak, can I assign to major contributor?
    - Examine data for inverted dropout

Y23 Validation

Mixture Studies

- Two contributors
  - Create male/male mixtures in the following ratios: 1:1, 1:2, 1:3, 1:4, 1:8, 1:16, 1:32
  - Amplify in triplicate targeting 0.5ng, 0.25ng, 0.1ng, 0.05ng, and 0.02ng

Y23 Validation

Mixture Studies

- Two contributors
  - Didn't assess their individual haplotypes before setting up the mixtures
  - 11 loci (not including DYS385 a/b) where the genotypes differ between the two contributors

Y23 Validation

Mixture Studies

- Three contributors
  - Create male/male/male mixtures in the following ratios: 70-20-10, 50-25-25, 45-45-10, 35-35-30
  - Amplify in triplicate targeting 0.5ng, 0.25ng, 0.1ng, 0.05ng, and 0.02ng

Y23 Validation

Mixture Studies

- Three contributors

Locus	Contributor #1	Contributor #2	Contributor #3
DYS576	19	19	16
DYS389 I	13	14	13
DYS448	19	19	19
DYS389 II	29	30	29
DYS19	14	15	14
DYS391	11	11	10
DYS481	22	22	22
DYS549	14	13	13
DYS533	12	12	12
DYS438	12	12	12
DYS437	15	15	15
DYS570	16	18	17
DYS635	23	23	23
DYS390	23	23	24
DYS439	12	12	12
DYS392	13	13	14
DYS643	10	9	10
DYS393	13	13	13
DYS458	18	18	17
DYS385 a/b	11,14	11,14	11,15
DYS456	15	16	16
Y-GATA-H4	12	11	12

Notice anything?

Locus	Contributor #1	Contributor #2	Contributor #3
DYS576	19	19	16
DYS389 I	13	14	13
DYS448	19	19	19
DYS389 II	29	30	29
DYS19	14	15	14
DYS391	11	11	10
DYS481	22	22	22
DYS549	14	13	13
DYS533	12	12	12
DYS438	12	12	12
DYS437	15	15	15
DYS570	16	18	17
DYS635	23	23	23
DYS390	23	23	24
DYS439	12	12	12
DYS392	13	13	14
DYS643	10	9	10
DYS393	13	13	13
DYS458	18	18	17
DYS385 a/b	11,14	11,14	11,15
DYS456	15	16	16
Y-GATA-H4	12	11	12

Notice anything?

Y23 Validation

Mixture Studies

- Had *lots* of data on two allele loci
- Still needed a good representation of three allele loci
- Went back and chose three more haplotypes from the elimination database

Y23 Validation

Mixture Studies - Dropout

- Review mixture data from mixtures of two males – test the dropout threshold
  - Height of tallest detected peak within a dye channel where dropout occurred at another locus

Y23 Validation

Mixture Studies - Dropout

	BLUE	GREEN*	YELLOW	RED	ALL
MAX	445		444	596	596
AVE	168		169	138	160
99%	492		486	523	499

**In the known contributor mixture, DYS570 was the only locus that could be used to discriminate major and minor contributors. The two staff members had the same haplotype at the remaining green loci. No minor peak detected at DYS570 meant total dropout in the dye channel.*

Y23 Validation

Mixture Studies - Dropout

	BLUE	GREEN*	YELLOW	RED	ALL
MAX	445		444	596	596
AVE	168		169	138	160
99%	492		486	523	499

Dropout threshold set to 600 RFU

If all assigned minor haplotype peaks are >600 RFU, then it can be assumed that the minor has not dropped out at other loci and major contributor peaks can be "split" between the major and the minor.

This is supported by an assessment of the single source data.

Y23 Validation

Mixture Studies - Dropout

	BLUE	GREEN*	YELLOW	RED	ALL
MAX	445		444	596	596
AVE	168		169	138	160
99%	492		486	523	499

Dropout threshold set to 600 RFU

Dropout threshold also applies to indistinguishable mixtures of two males. If a balanced two-male mixture is detected, and one male can be assumed, the peaks at the single allele loci can be assigned to deduced contributors provided no peaks less than 600RFU are assigned to the deduced contributor.

Y23 Validation

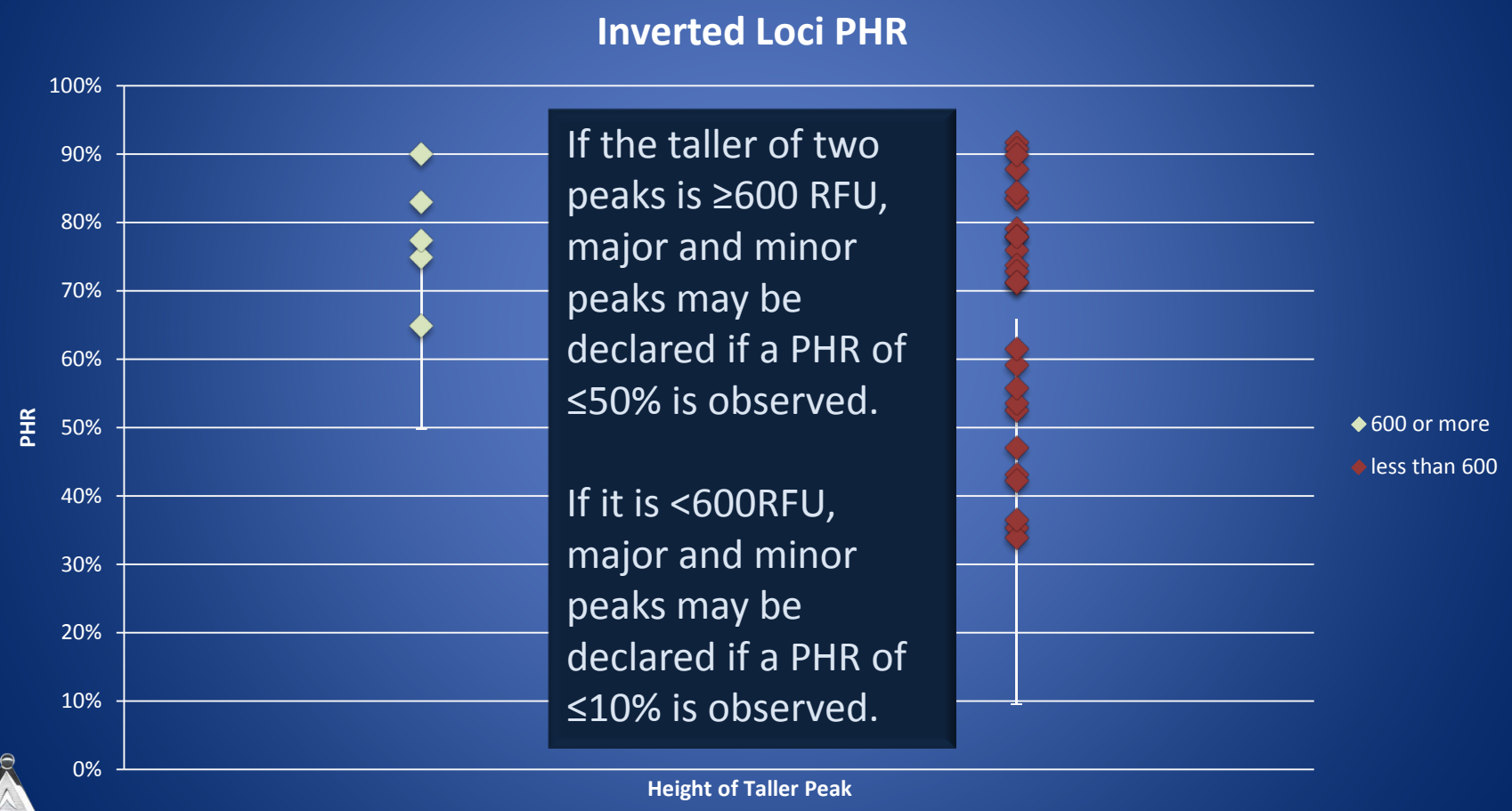
Mixture Studies – Two males

11 loci x 5 ratios x 5 input targets x 3 replicates = 825 loci to assess behavior of major and minor contributor alleles for each ratio

Ratio	PHR Inversion
1:2	12/825
1:3	11/825
1:4	6/825
1:8	1/825
1:16	1/825

Y23 Validation

Mixture Studies



If the taller of two peaks is ≥600 RFU, major and minor peaks may be declared if a PHR of ≤50% is observed.

If it is <600RFU, major and minor peaks may be declared if a PHR of ≤10% is observed.

Y23 Validation

Mixture Studies

- Inverted dropout

Ratio	PHR Inversion	Inverted Dropout
1:2	12/165	4
1:3	11/165	1
1:4	6/165	3
1:8	1/165	2
1:16	1/165	1

- *If only a single peak is present, can it be automatically assigned to the major contributor?*

Y23 Validation

Mixture Studies

- *If only a single peak is present, can it be automatically assigned to the major contributor?*
  - 11 instances of inverted dropout observed. RFU: 50, 52, 53, 58 (x2), 63 (x2), 66, 96, 132, 212
  - Set threshold for inverted dropout to 250 RFU (average + 3STD = 231)

Y23 Validation

Mixture Studies

- **Two primary questions for three contributor mixtures**
  - **When can I confidently assign a single major contributor haplotype?**
  - **If I can't assign a major, can I identify obligate minor alleles?**
    - Data not shown/discussed today, we find it has limited application in actual casework.

Y23 Validation

Mixture Studies

- **Two primary questions for three contributor mixtures**
  - **When can I confidently assign a single major contributor haplotype?**
    - Used the 600RFU and 250RFU thresholds from the two person mixture data to guide data evaluation
      - First data set, each sample had the potential to exhibit 14 mixed loci = 840 loci to assess
      - Second data set, each sample had the potential to exhibit 21 mixed loci = 1470 loci to assess

Y23 Validation

Mixture Studies

- **Two primary questions for three contributor mixtures**
  - **When can I confidently assign a single major contributor haplotype?**
    - Examined all mixed loci for peak height inversions and inverted dropout

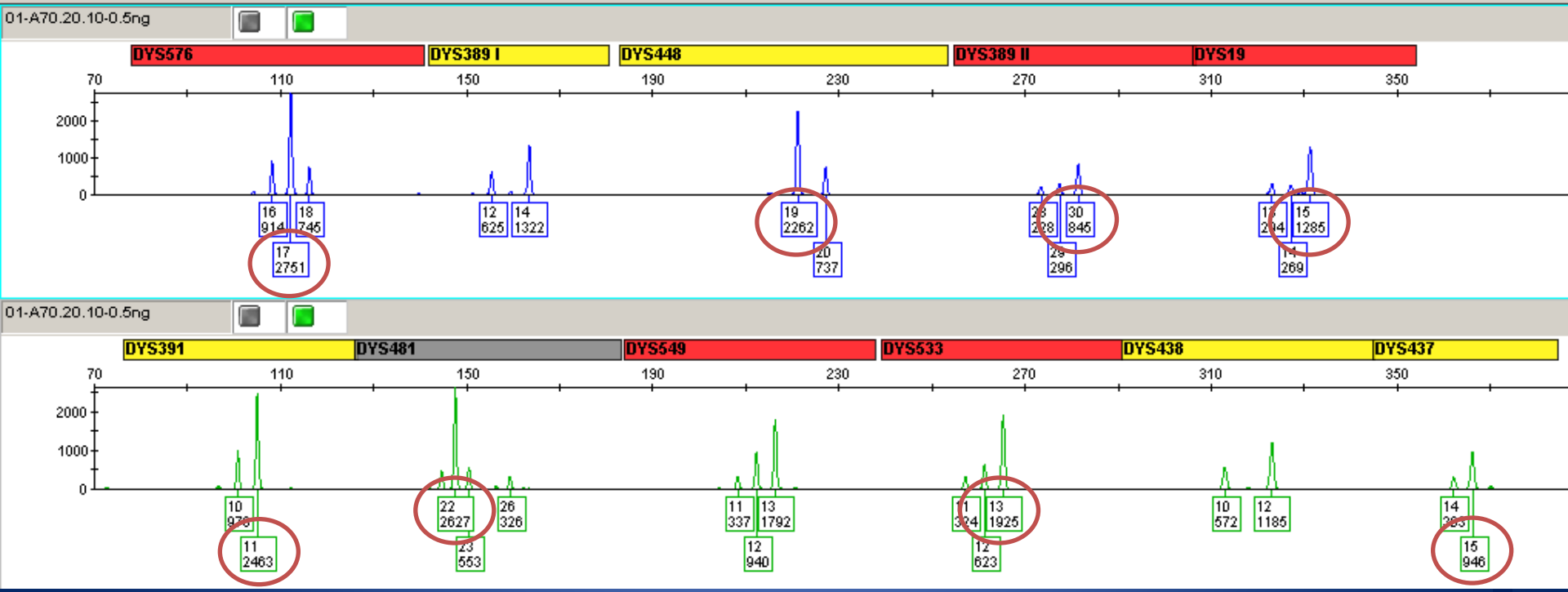
Y23 Validation

Mixture Studies

- A total of 159 peak height inversions were observed
  - Grouped into samples in which the tallest peak was ≥600RFU and those in which the tallest peak was <600RFU
    - ≥600RFU, declare a major when PHR is ≤35% with respect to the next tallest peak
    - <600RFU, declare a major when PHR is ≤10% with respect to the next tallest peak

Y23 Validation

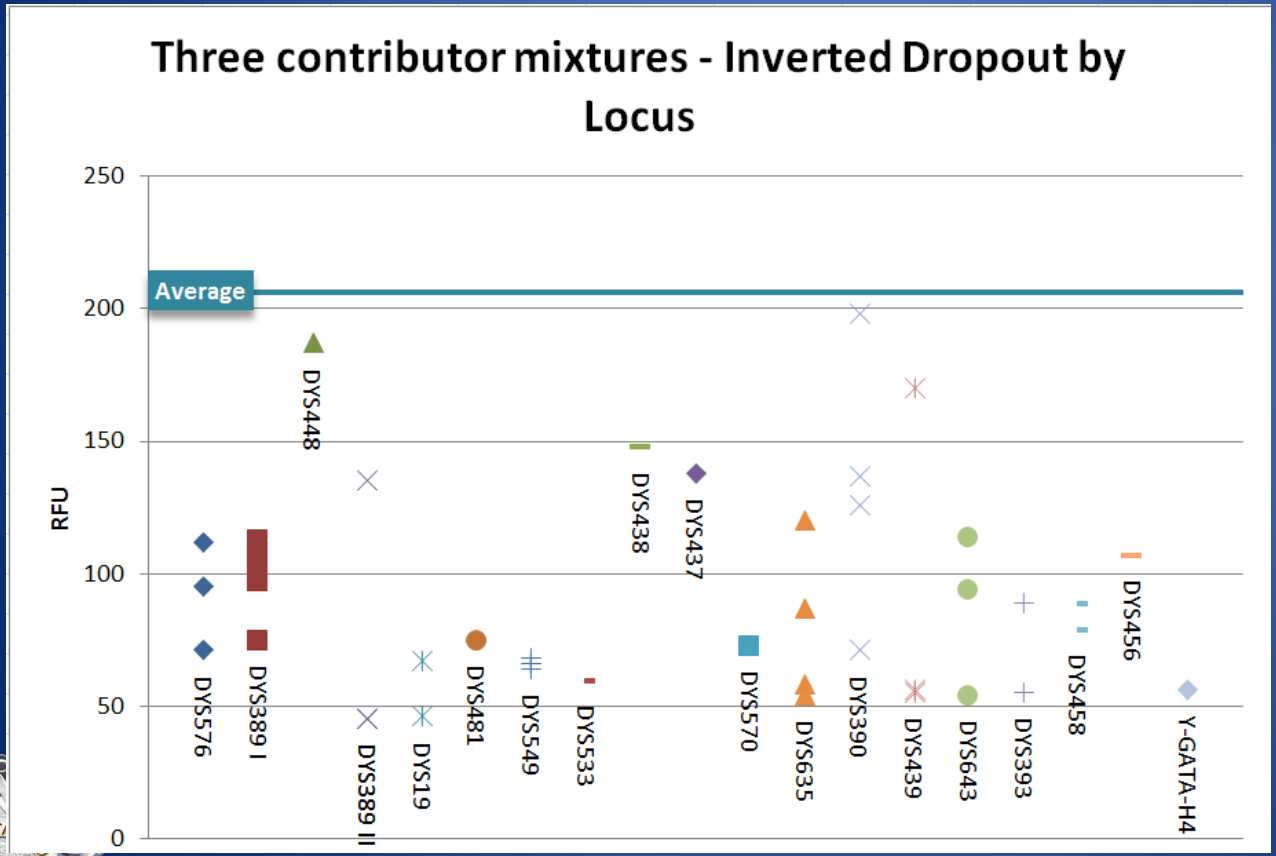
Mixture Studies



Y23 Validation

Mixture Studies

- A total of 43 observations of inverted dropout



250 RFU threshold developed from 2 male data held for mixtures of 3 males

Y23 Validation

Mixture Studies

- One last note on mixture interpretation…
  - Just because you *can*, doesn't mean you *should*
    - The PHR and thresholds developed *only* make sense if you can visually assess the mixture and make an assumption about the (minimum) number of contributors and about the relative contributor ratios

… be smarter than the math…

Y23 Statistics

- US Y-STR database web interface doesn't allow for
  - Mixtures
  - Population samples with varying loci
    - SWGDAM Y-STR interpretation guidelines 10.1.1
      - Essentially says you can use all of the concordant loci in the database provided all of the non-concordant loci are removed.
        - » If it matches at all SWGDAM 11, then use (count) it
        - » If it matches at SWGDAM 11 but doesn't match at one of 17 Y-filer, then it doesn't get counted.

Y23 Statistics

- Validated Steven Myers' CA DOJ Y-Mix Tool
- Simulated 6-locus, 17-locus, and 23-locus profiles
  - Profile probabilities calculated with US Y-STR database and with Y-Mix Tool
  - Looking for concordance
- Simulated 2 and 3 person mixtures
  - Searched mixture against Y-Mix tool
  - Searched all possible combinations against US Y-STR database
  - Looking for concordance

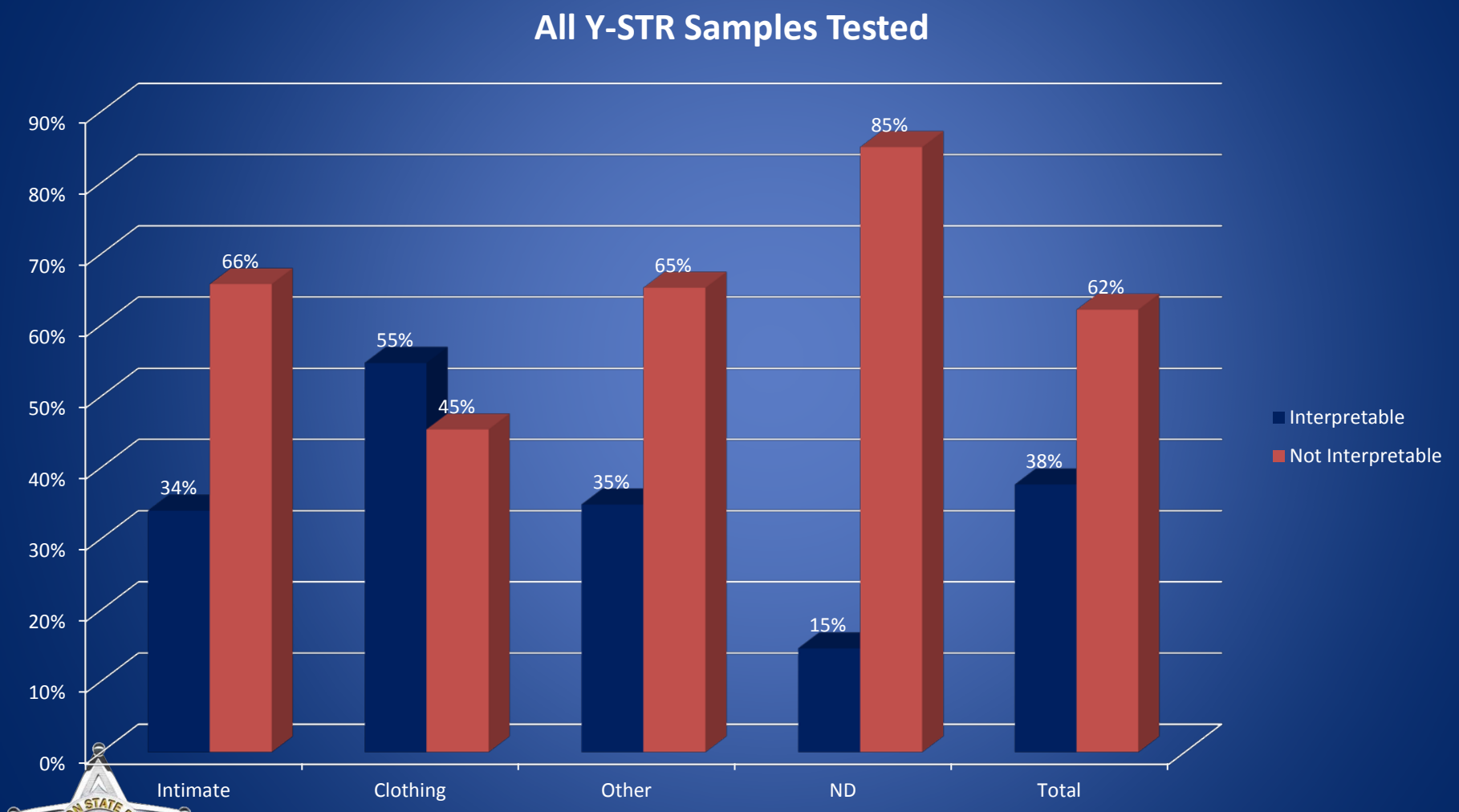
Y23 Implementation

- Policies
- Procedures
- Analyst training
- LEA training
- Additional Excel Tools

Y23 Implementation

- OSP Implemented April 19, 2013
- ~175 cases completed
- 10-15 in the Y-STR backlog

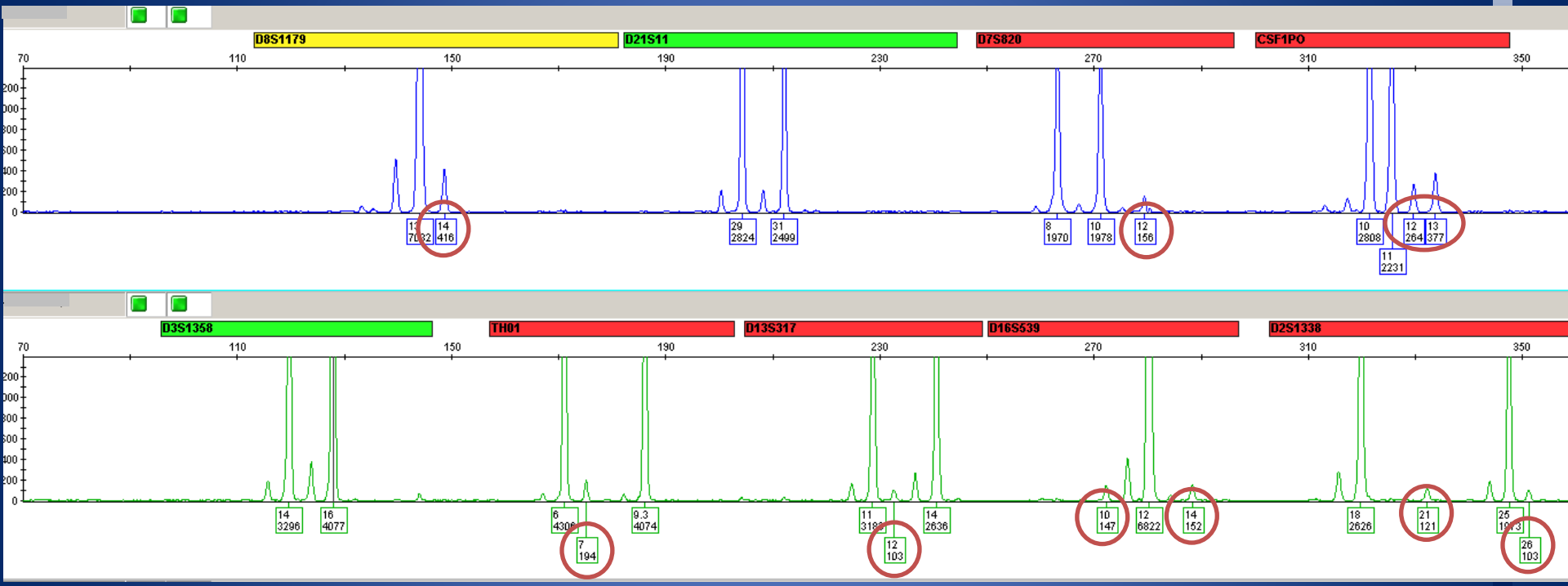
Y-STR Program



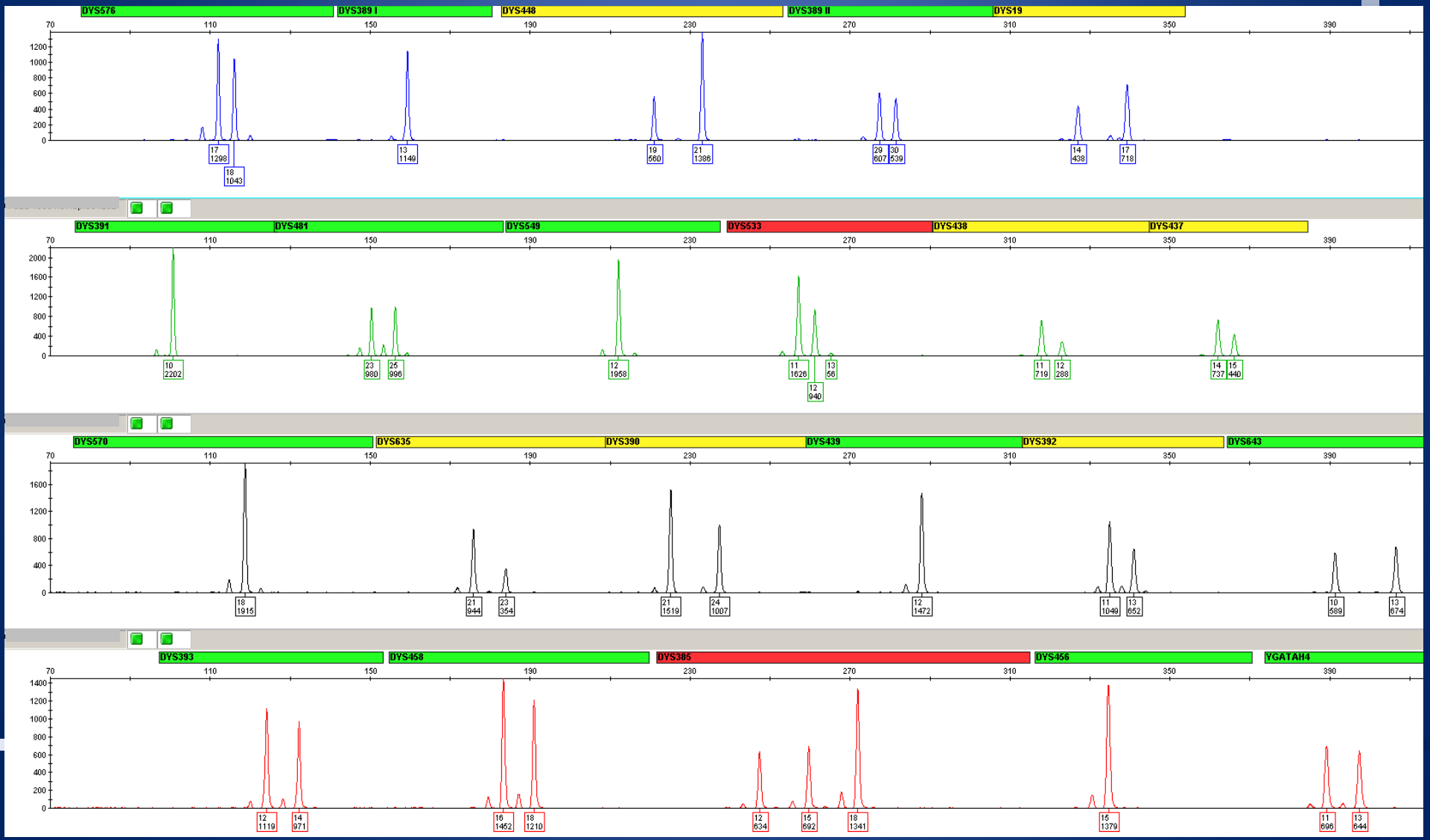
Casework

- 2002 Homicide
- Vaginal swab sperm cell fraction, extracted in June 2002
- Mixture consistent with two contributors
  - Major victim
  - Minor male into CODIS

Casework


D8S1179
D21S11
D7S820
CSF1PO
D3S1358
TH01
D13S317
D16S539
D2S1338

Casework


DYS576
DYS389 I
DYS448
DYS389 II
DYS19
17 1298
18 1043
13 1149
19 560
21 1386
29 607
30 539
14 438
17 718
DYS391
DYS481
DYS549
DYS533
DYS438
DYS437
10 2202
23 980
25 996
12 1958
11 1626
13 56
12 940
11 719
12 288
14 737
15 440
DYS570
DYS635
DYS390
DYS439
DYS392
DYS643
18 1915
21 944
23 354
21 1519
24 1007
12 1472
11 1049
13 652
10 588
13 674
DYS393
DYS458
DYS385
DYS456
YGATAH4
12 1119
14 971
16 1452
18 1210
12 634
15 692
18 1341
15 1379
11 696
13 644

Casework

- Removed original “unknown male” profile from CODIS
- Revised assumption of two contributors to the autosomal profile, issued supplemental report
  - Major contributor profile matches victim
  - Typing results from minor contributors are insufficient for comparison purposes

Casework

- Father and two sons typed; each son had a mutation that gave them a slightly different Y-STR haplotype

Exhibit #	x11 - Son #1	x12 - Father	x13 - Son #2
DYS576	16	16	16
DYS389I	12	12	12
DYS448	20	20	20
DYS389 II	28	28	28
DYS19	14	14	14
DYS391	10	10	10
DYS481	27	27	27
DYS549	12	12	12
DYS533	11	11	11
DYS438	10	10	10
DYS437	16	16	16
DYS570	19	19	18
DYS635	22	22	22
DYS390	22	22	22
DYS439	11	11	11
DYS392	11	11	11
DYS643	12	12	12
DYS393	13	13	13
DYS458	16	15	15
DYS385 a/b	13,16	13,16	13,16
DYS456	14	14	14
Y-GATA-H4	11	11	11

1.24%

0.64%

DYS570 mutation rate obtained from presentation: "Increased Efficiency of Forensic Y-STR Analysis with the New PowerPlex Y23 System" by Lutz Roewer,

DYS458 mutation rate obtained from data compiled by YHRD

Questions?

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Thank You!