2014 Rapid DNA Maturity Assessment Results

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CODIS State Administrator Meeting

May 12, 2015

Norman, OK

National Institute of Standards and Technology

U.S. Department of Commerce

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Purpose of Maturity Assessment

- To assess the current status of rapid DNA typing technology for the CODIS core loci
  - In support of lab and future external (non-lab-based) Rapid DNA implementation
- Integrated (swab in – allele detection) instruments capable of genotyping the core CODIS 13 STR markers were eligible for the study

Rapid DNA Instruments

ANDE (NetBio)

- One biochipset
  - Stored at RT
  - Shelf life ≈ 6 months

PowerPlex 16 loci
≈86 min runtime
(5 samples)

ANDE PP16

RapidHIT 200 (IntegenX)

- Kit = 4 components
  - Stored between RT-4ºC
  - Shelf life ≈ 5 months @ 4ºC

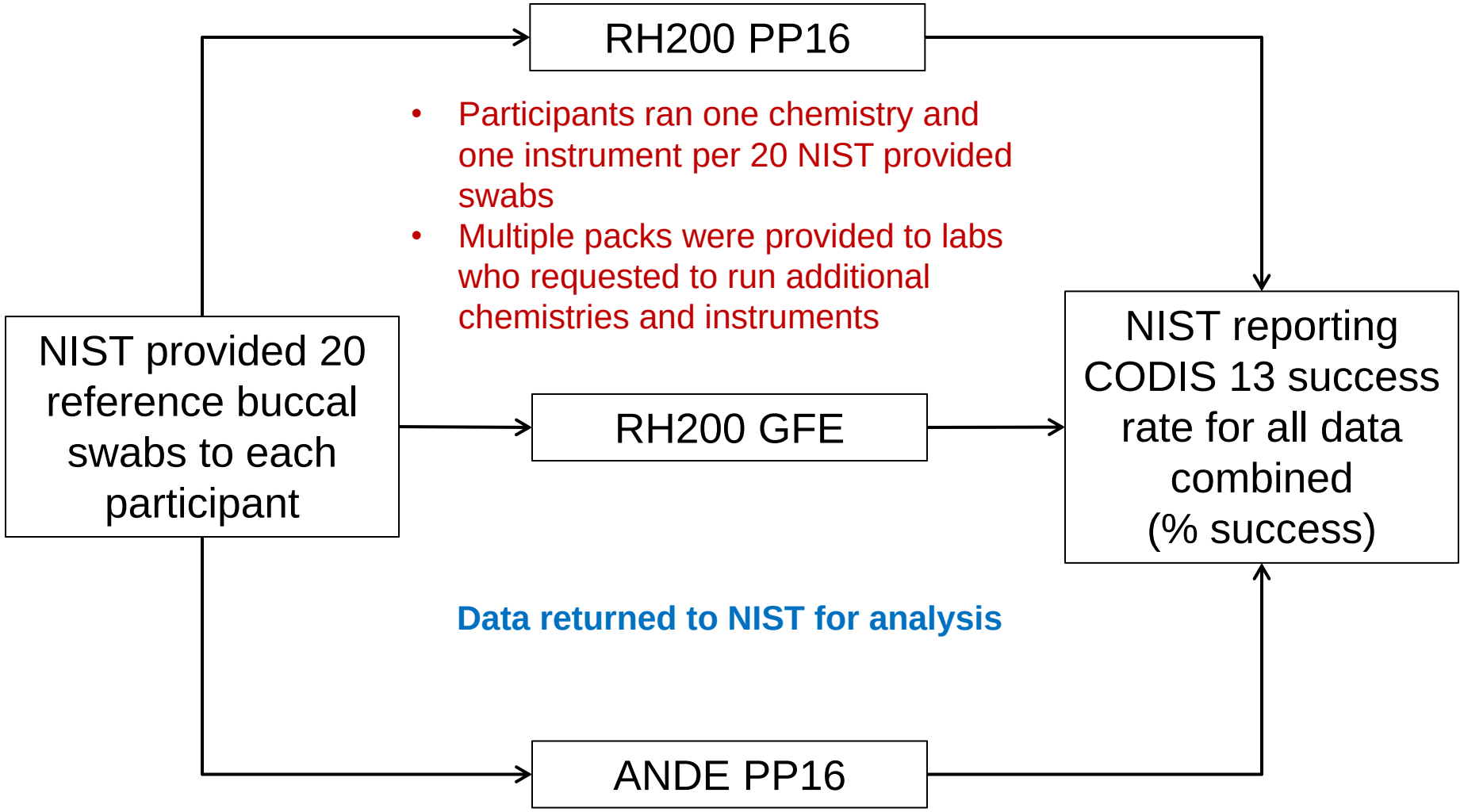
PowerPlex 16 loci
≈90 min runtime
(5 samples)

RH200 PP16

GlobalFiler Express loci
≈120 min runtime
(1-7 samples)

RH200 GFE

R-DNA Maturity Assessment



Timeline of Maturity Assessment

January 2014: Buccal samples collected at NIST and stored at RT

October 2014: Samples shipped to participating laboratories

October-December 2014: Data electronically returned to NIST

November 2014 – May 2015: Data analyzed at NIST

20 Swabs provided

Maturity Assessment

Participating Laboratories (7)	Instrument Platforms (2)	Independent Instruments (11)	Chemistry	Total Samples Tested (280)
Federal State	NetBio ANDE 	5	PowerPlex 16	100
Private	IntegenX RapidHIT 200 	6	PowerPlex 16	60
			GlobalFiler Express	120

NIST Analysis Parameters

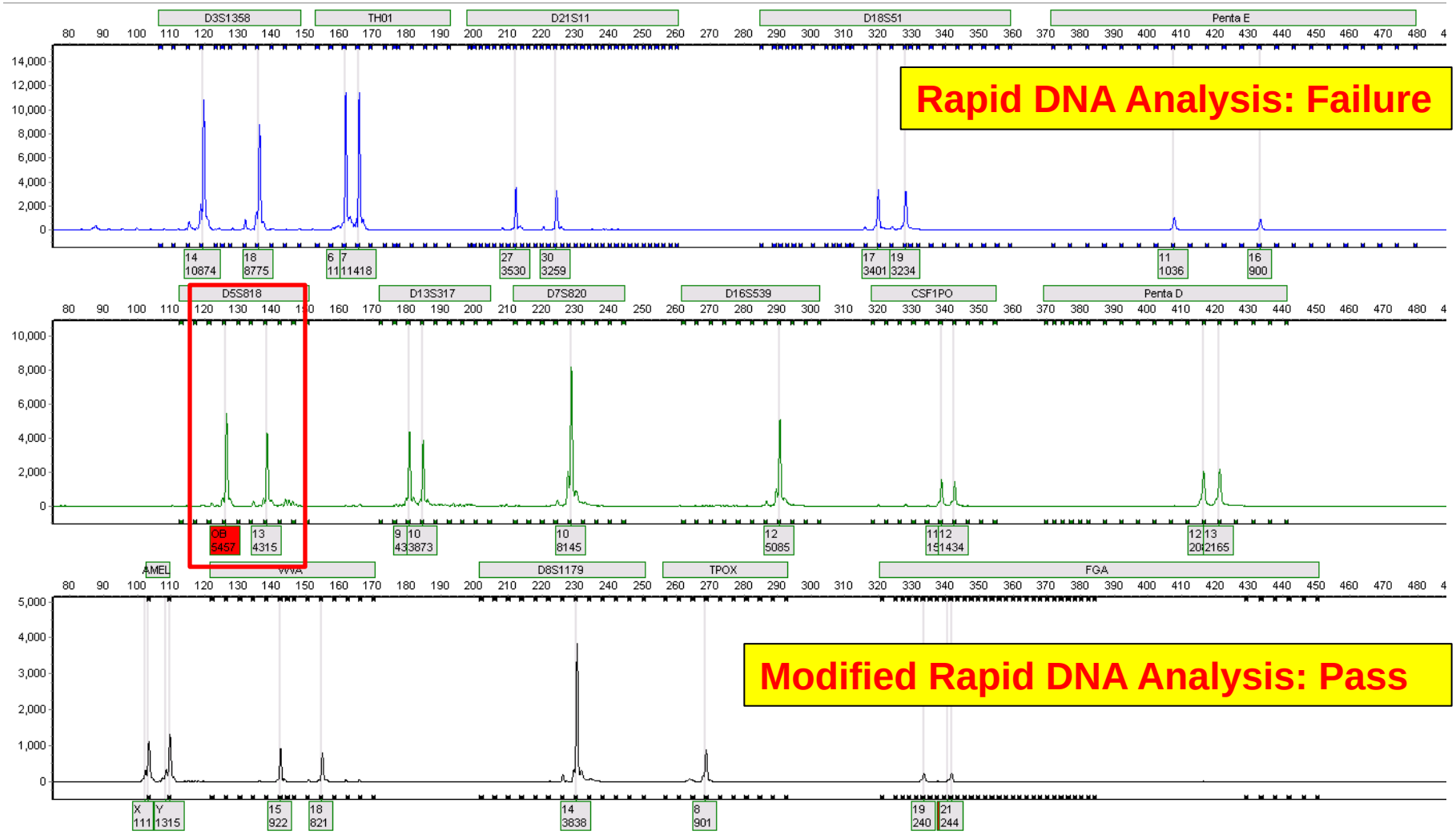
- **Rapid DNA Analysis**: Without human intervention

- **Modified Rapid DNA Analysis**: Expert interpretation and analysis of electropherogram

http://swgdam.org/docs.html

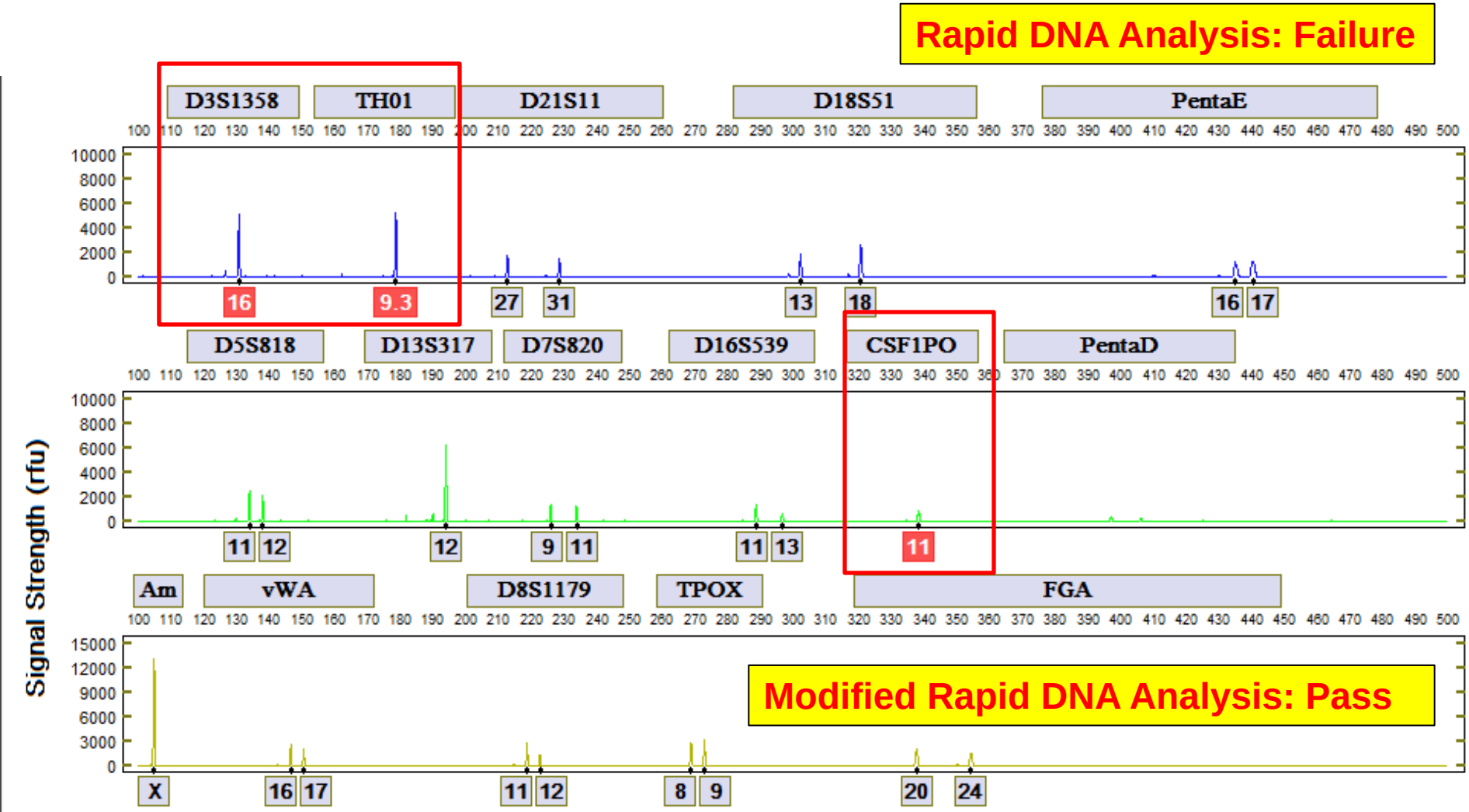
- Additional analysis (PHR, Stutter, etc.) of the data performed with GeneMapper IDX v 1.3

D3S1358	TH01	D21S11	D18S51	PentaE	D5S818	D13S317	D7S820	D16S539	CSF1PO	PentaD	AMEL	vWA	D8S1179	TPOX	FGA



D3S1358	TH01	D21S11	D18S51	PentaE	D5S818	D13S317	D7S820	D16S539	CSF1PO	PentaD	AMEL	vWA	D8S1179	TPOX	FGA

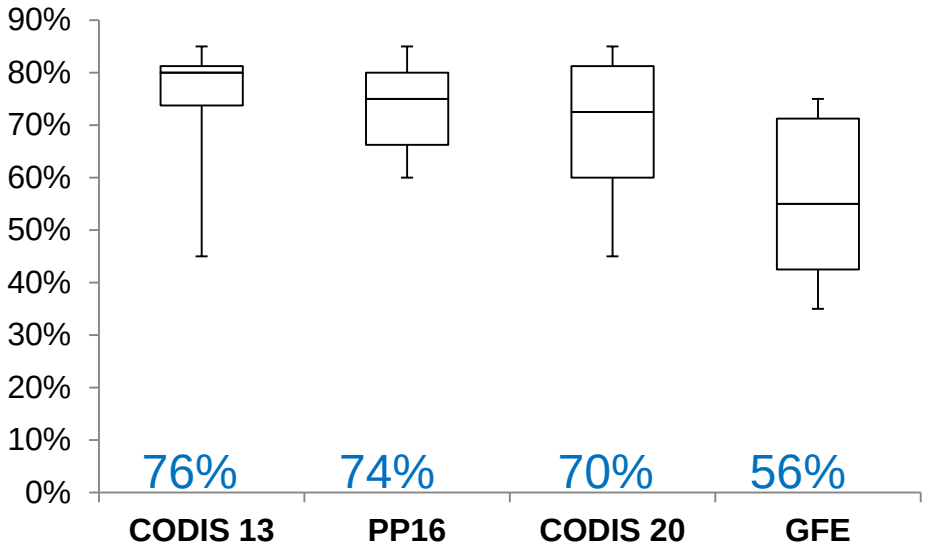
D3S1358	TH01	D21S11	D18S51	PentaE	D5S818	D13S317	D7S820	D16S539	CSF1PO	PentaD	AMEL	vWA	D8S1179	TPOX	FGA



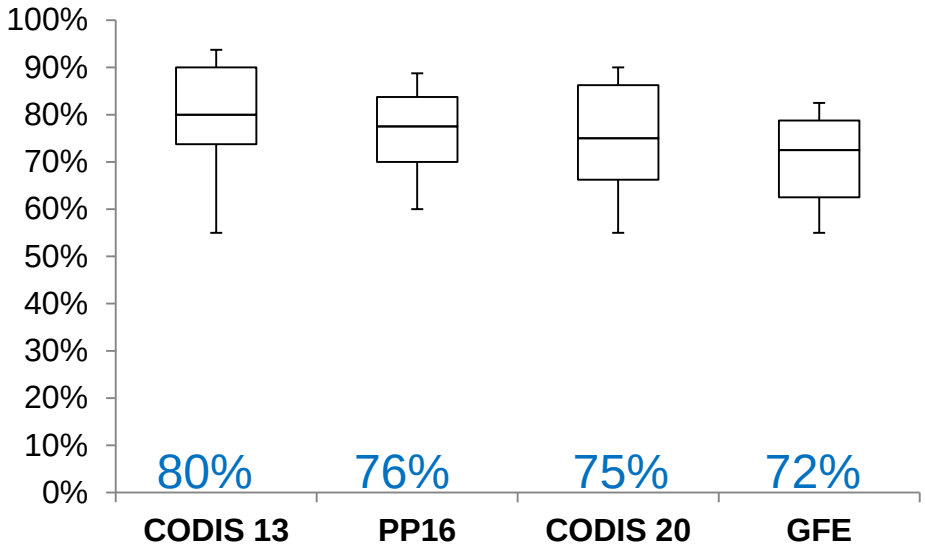
D3S1358	TH01	D21S11	D18S51	PentaE	D5S818	D13S317	D7S820	D16S539	CSF1PO	PentaD	AMEL	vWA	D8S1179	TPOX	FGA

Overall Success

Rapid DNA Analysis



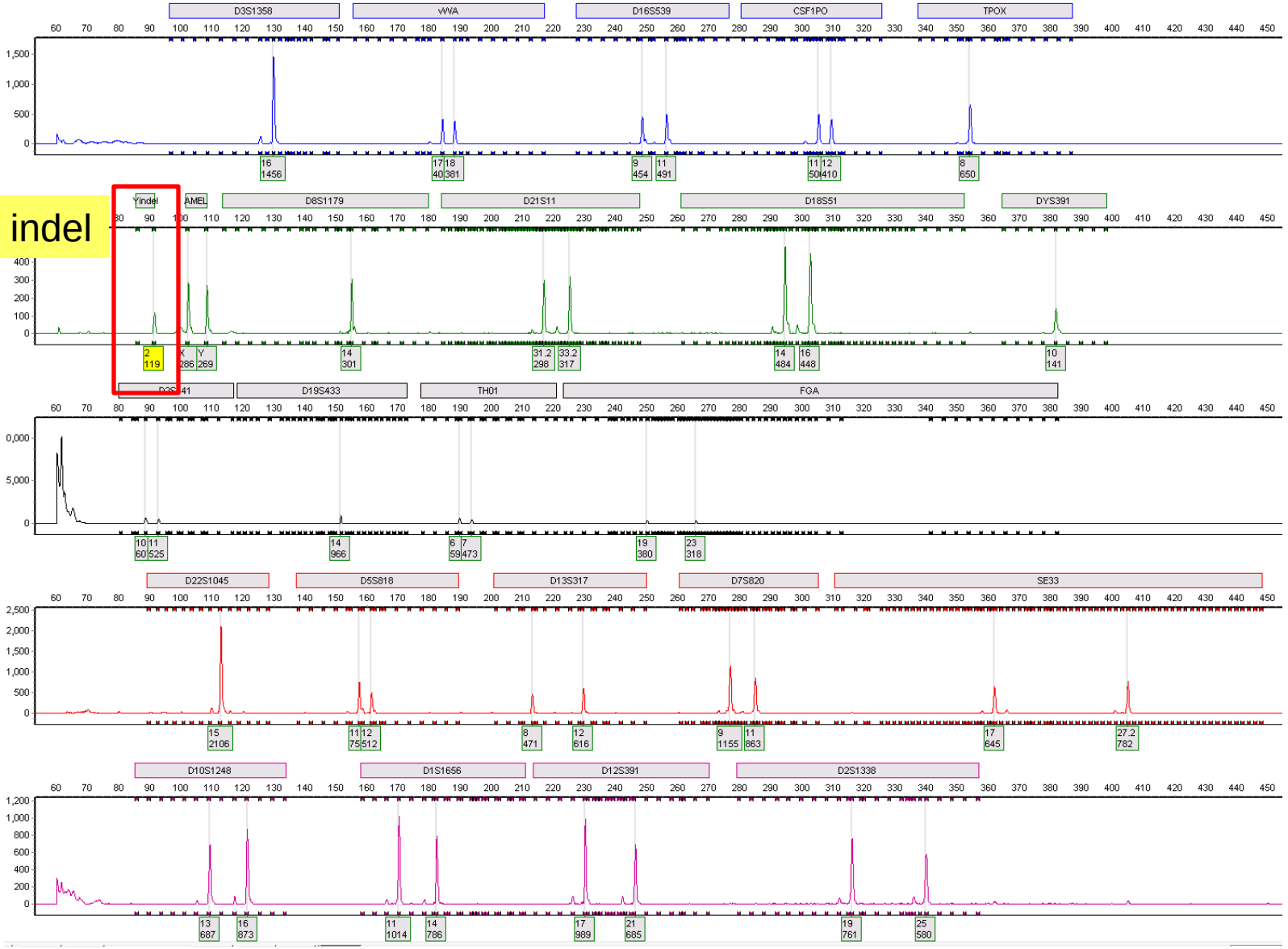
Modified Rapid DNA Analysis



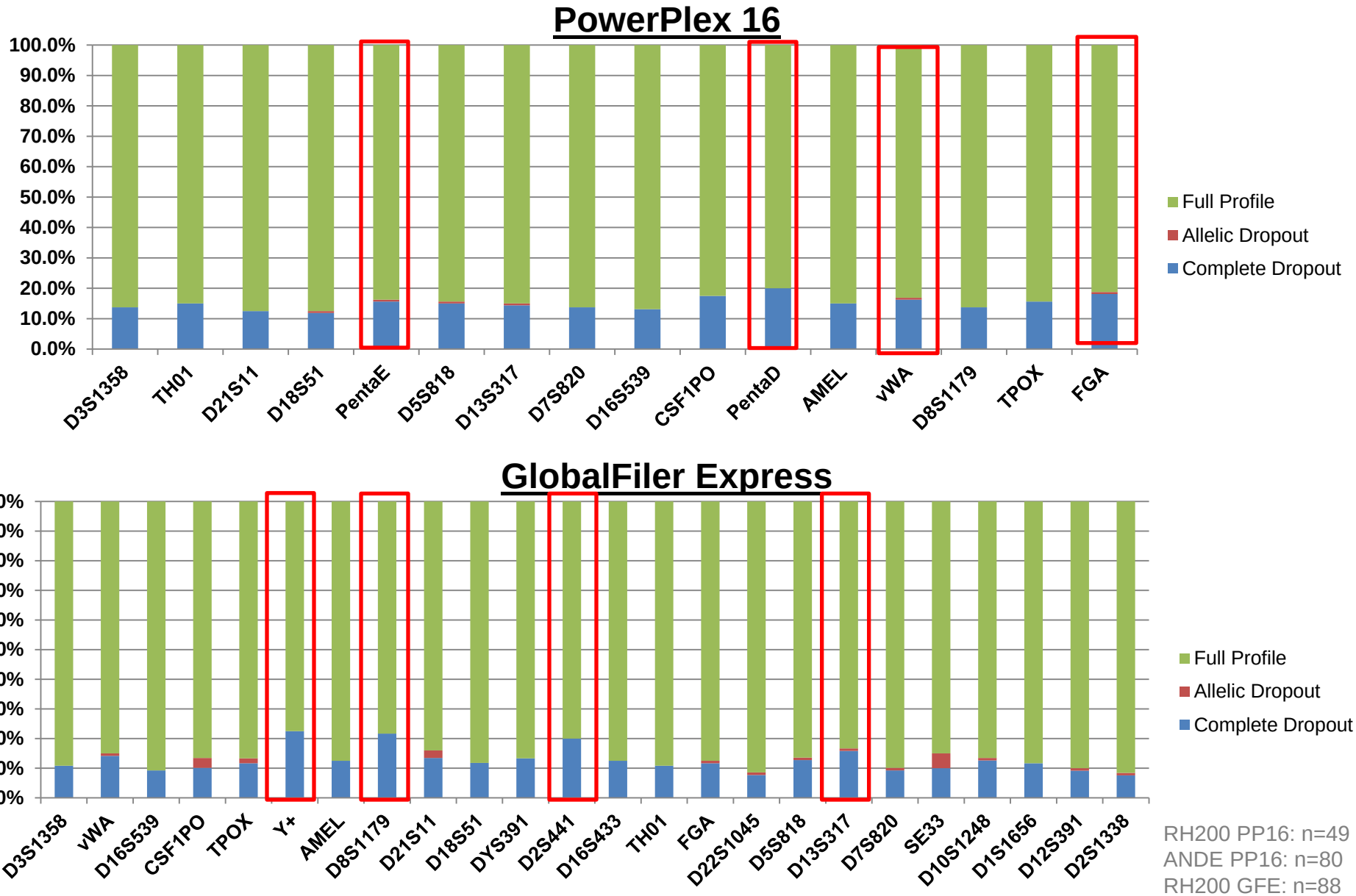
8 samples were passed (for CODIS 13) by performing modified rapid DNA analysis across all platforms (76 → 80%)

- 2 Samples within the RH200 PP16
- 3 Samples within the RH200 GFE
- 3 Samples within the ANDE PP16

Higher success with manual review of GFE

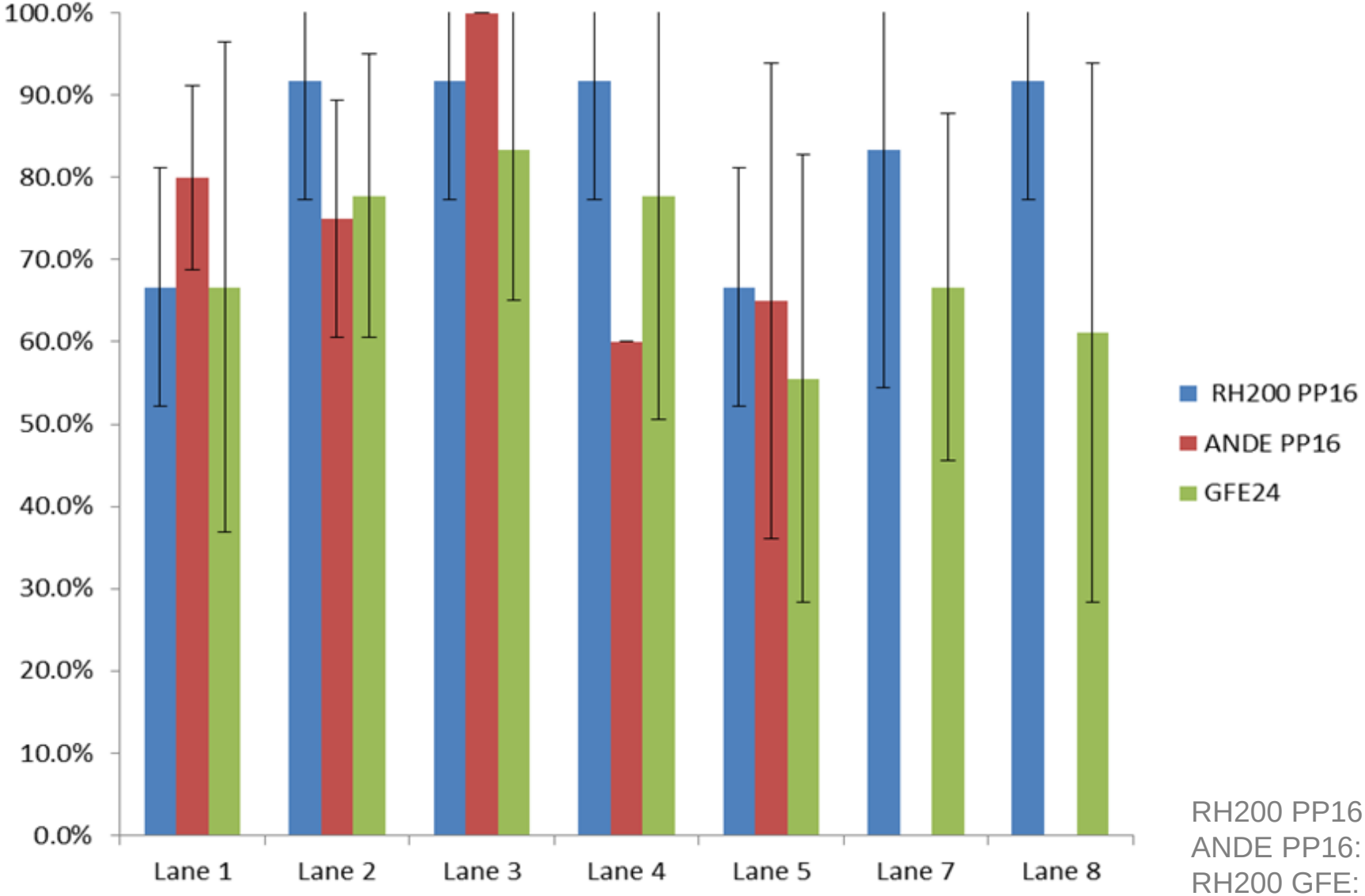


Locus Success (Rapid Analysis)



RH200 PP16: n=49
ANDE PP16: n=80
RH200 GFE: n=88

Lane Success (CODIS 13)

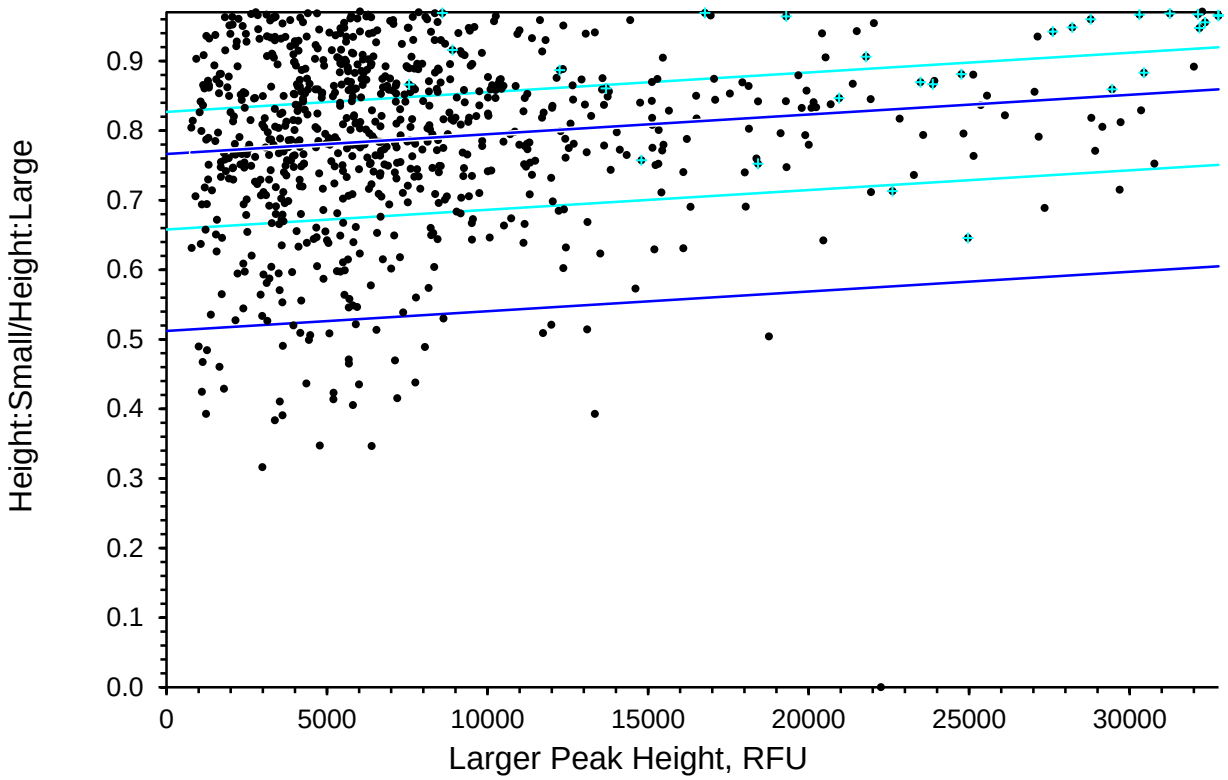


RH200 PP16: n=49
ANDE PP16: n=80
RH200 GFE: n=88

Peak Height Ratios

Median PHR for CODIS 13 loci

Locus	#Ratio	Median
D8S1179	55	0.813
D21S11	43	0.825
D18S51	62	0.827
FGA	67	0.829
TPOX	44	0.832
D16S539	52	0.835
vWA	65	0.844
D7S820	47	0.850
D3S1358	61	0.855
D5S818	36	0.863
CSF1PO	61	0.870
D13S317	44	0.871
TH01	28	0.939



Median peak height ratio greater than 75% for all loci

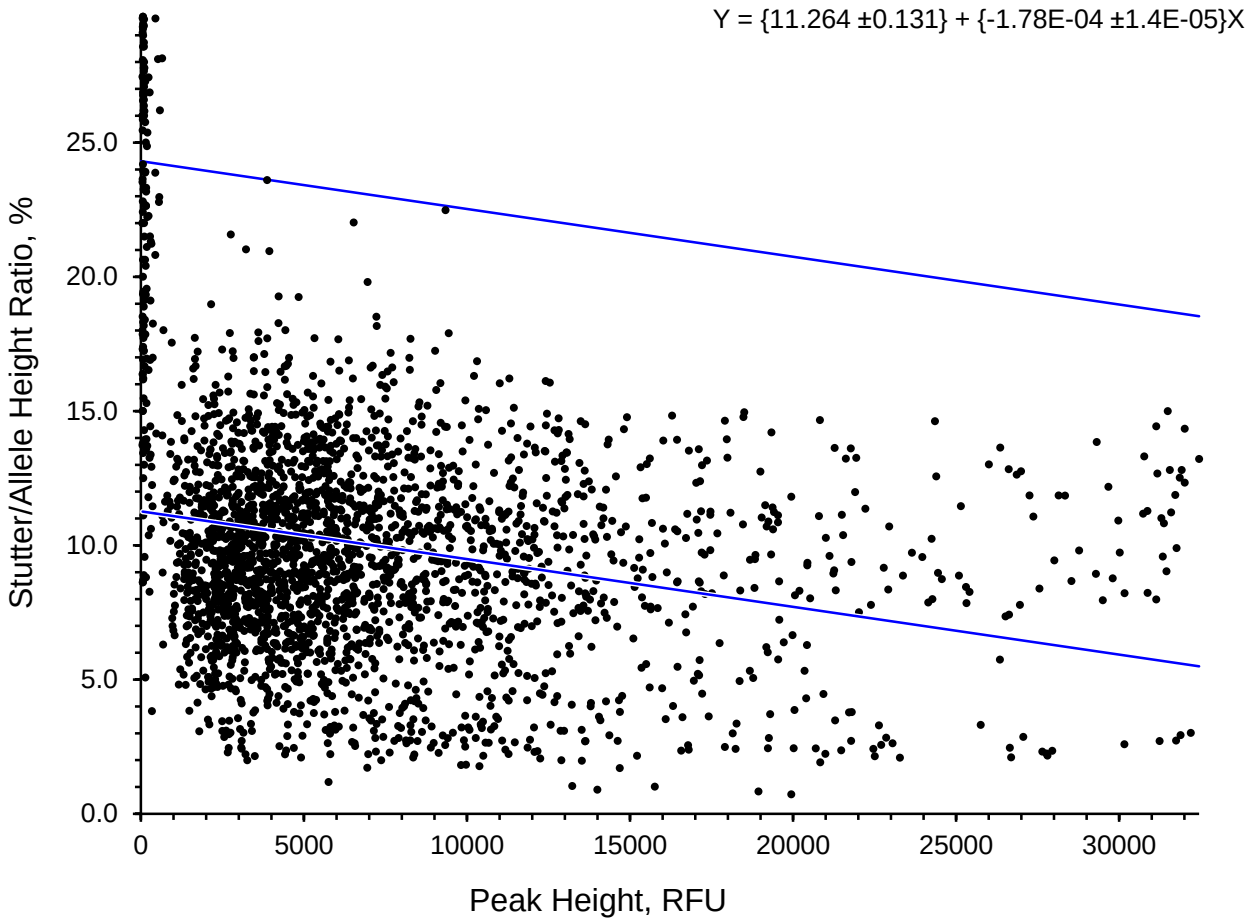
Full Profiles: n=252

Stutter Percentage

Mean stutter for CODIS 13 loci

Locus	Mean	SD
TH01	4.2	4.3
TPOX	5.7	4.4
D7S820	8.1	4.3
D8S1179	9.3	3.2
D18S51	9.7	3.9
D13S317	9.6	3.6
FGA	10.3	4.0
D5S818	10.2	2.4
D16S539	10.5	4.0
vWA	10.7	4.4
CSF1PO	11.3	3.5
D3S1358	13.0	3.1
D21S11	13.9	3.6

Median stutter from 4.2% to 13.9%



Full Profiles: n=252

Thresholds

As described by vendors and currently understood at NIST

- IntegenX employs dynamic thresholding
  - Each run has an independent analytical and stochastic threshold calculated via a programed algorithm
  - Locus specific dynamic thresholds set within software
- NetBio employs a static threshold applied to all data uniformly

Thanks to Sanae Lembirik

IntegenX Thresholds

Analysis Parameters

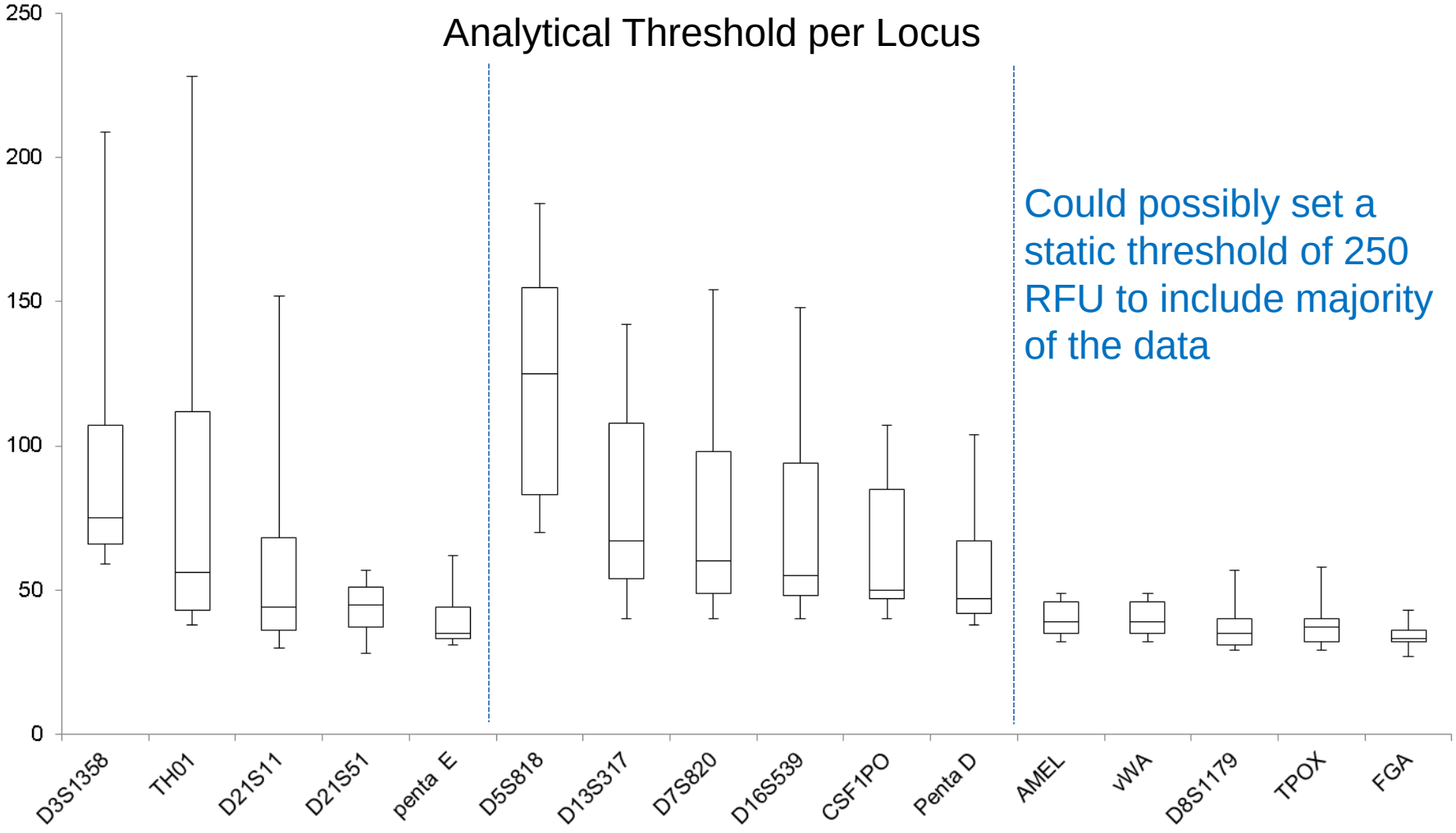
Locus	Stochastic Threshold	Analytical Threshold
D3S1358	112	56
vWA	49	25
D16S539	37	18
CSF1PO	36	18
TPOX	33	17
Yindel	221	111
AMEL	49	25
D8S1179	100	50
D21S11	75	37
D18S51	66	33
DYS391	77	38
D2S441	419	209
D19S433	224	112
TH01	111	55
FGA	77	39
D22S1045	198	99
D5S818	142	71
D13S317	133	66
D7S820	145	72
SE33	164	82
D10S1248	701	350
D1S1656	422	211
D12S391	270	135
D2S1338	169	84

- Can be located in the report file generated per sample
- Table format with PDF report
- Data has been transcribed into an Excel file for analysis

Thanks to Sanae Lembirik

One run's data omitted due to being a significant outlier

IntegenX PP16 Thresholds

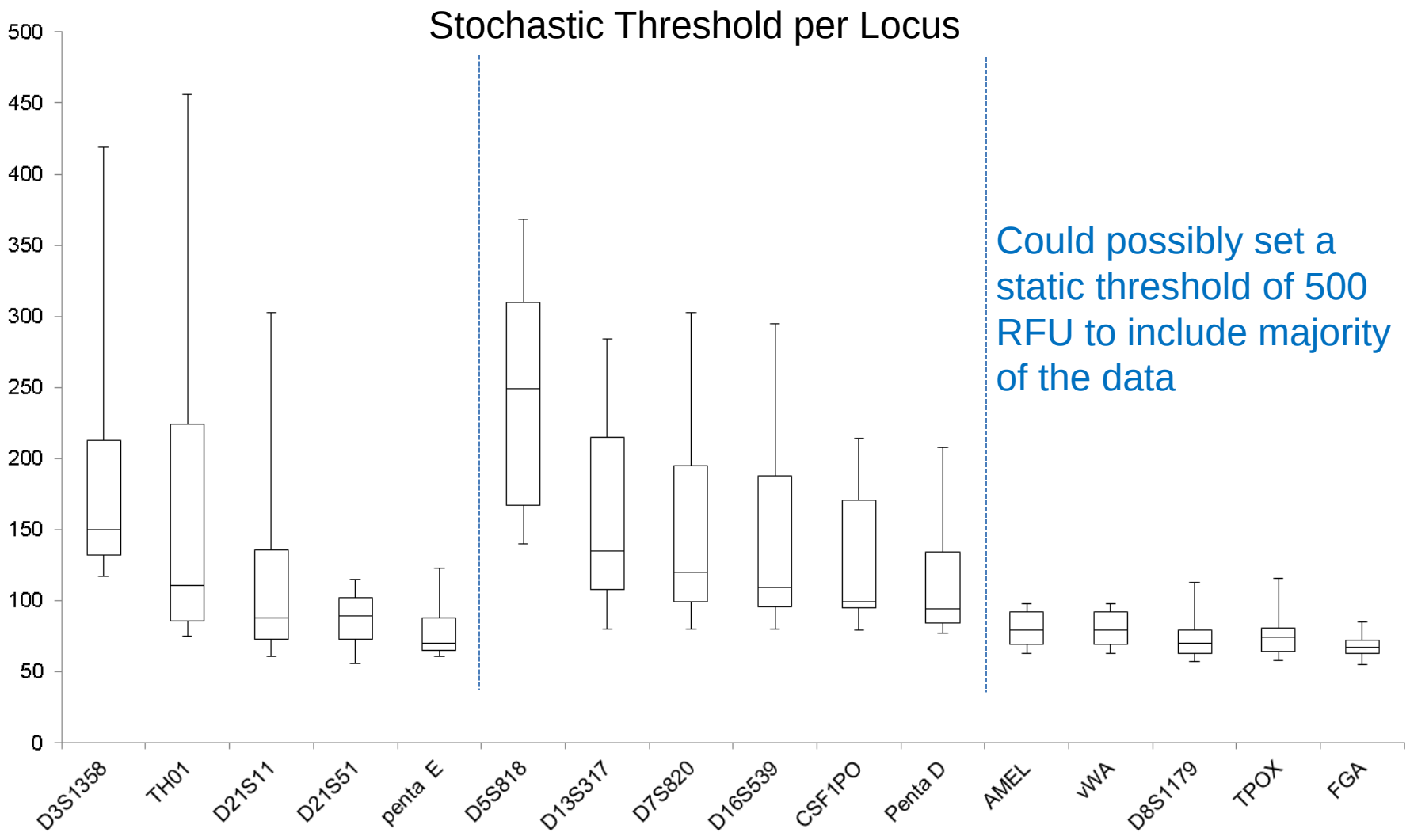


Could possibly set a static threshold of 250 RFU to include majority of the data

Thanks to Sanae Lembirik

One run's data omitted due to being a significant outlier

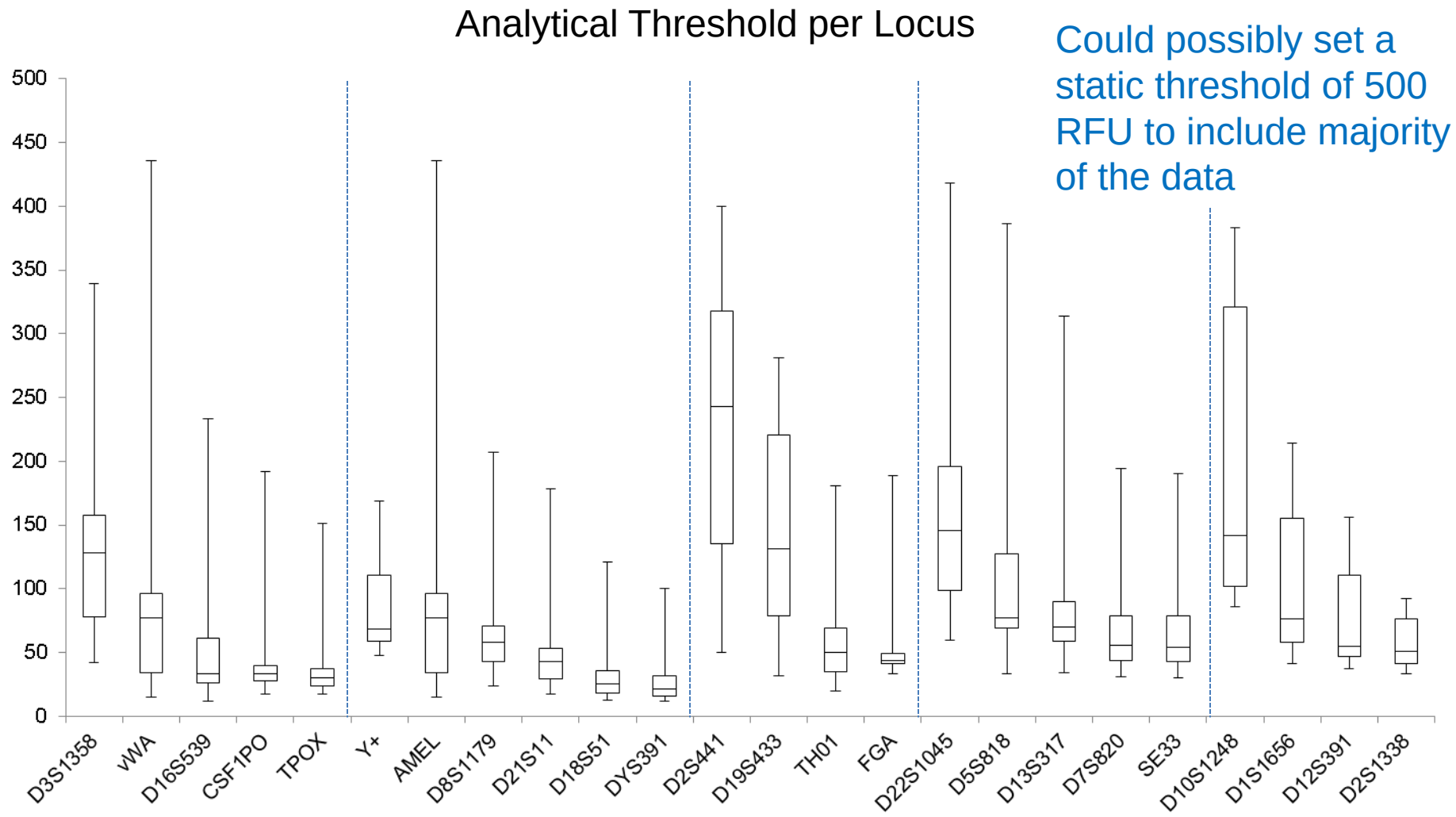
IntegenX PP16 Thresholds



Could possibly set a static threshold of 500 RFU to include majority of the data

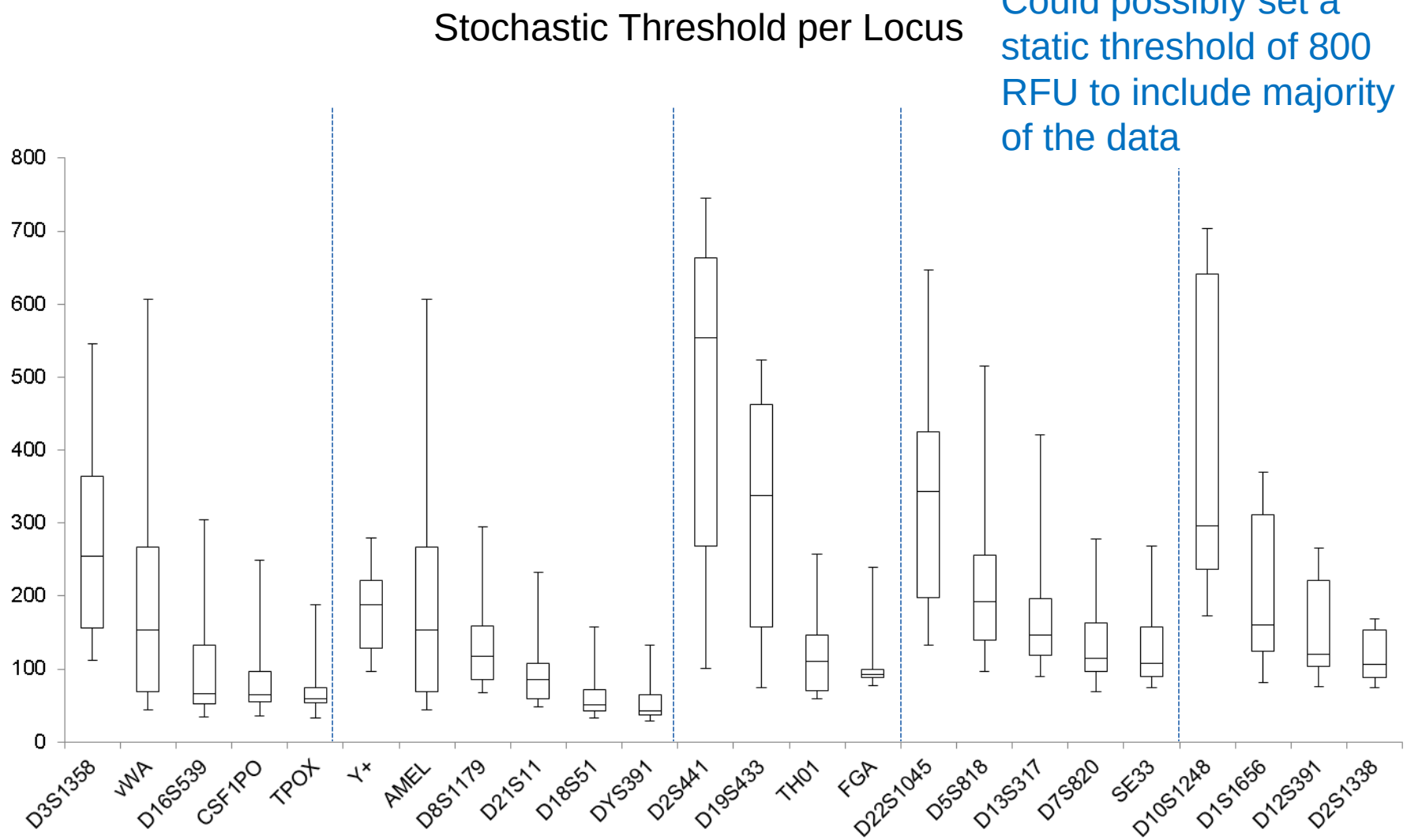
Thanks to Sanae Lembirik

IntegenX GFE Thresholds



Thanks to Sanae Lembirik

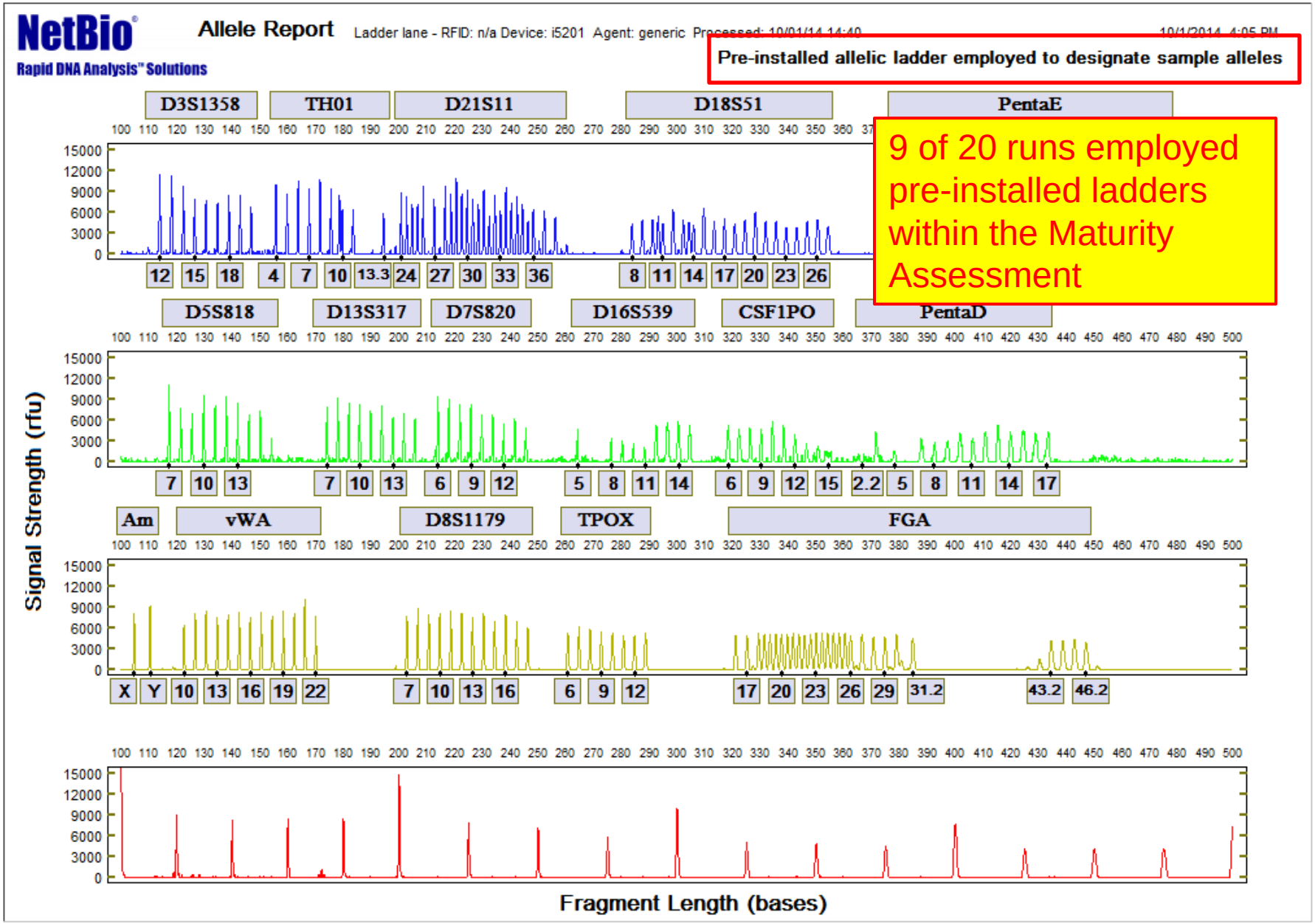
IntegenX GFE Thresholds



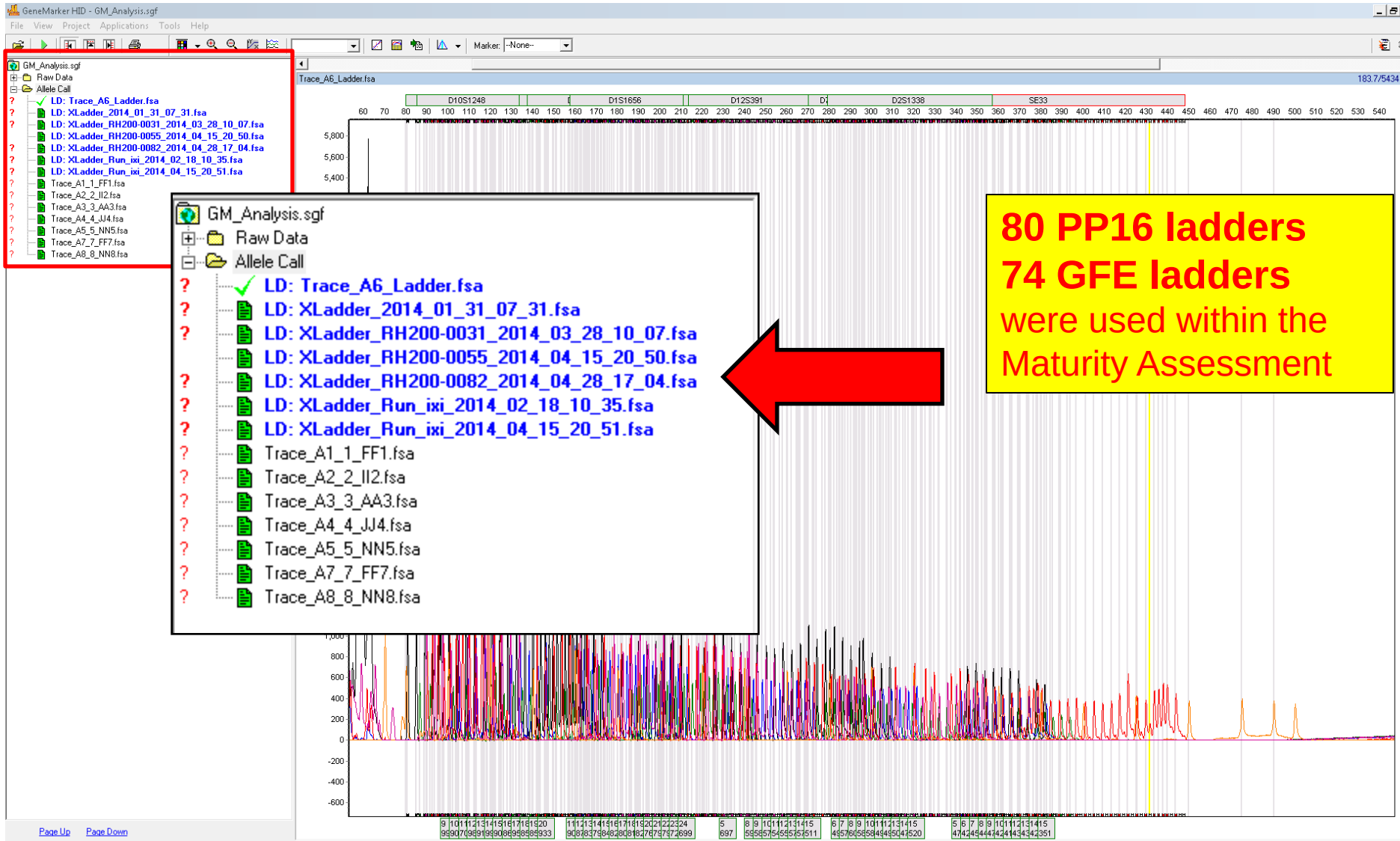
Ladders

As described by vendors and understood by NIST

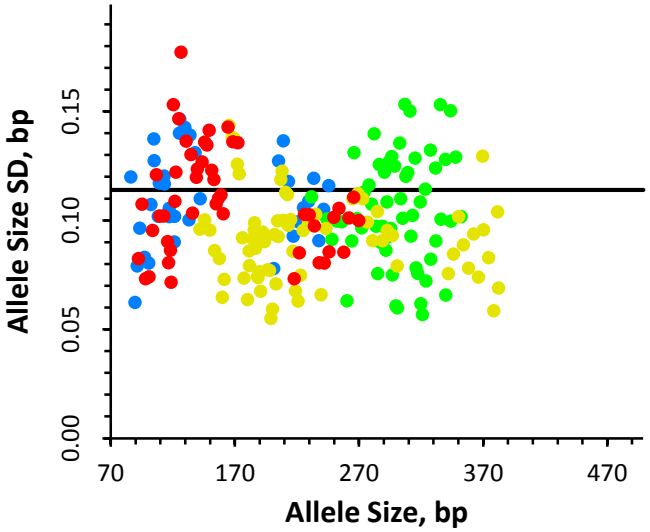
- Both instruments contain “onboard” ladders
  - For use if the ladder on the chip fails
- IntegenX
  - “Fit best Ladder” Algorithm
  - Database of ~200 ladders uploaded into each instrument used to find best ladder per sample
    - Uses size standard and algorithm fitting per sample
- ANDE
  - Onboard ladder file saved and applied to data when chip ladder fails


NetBio
Rapid DNA Analysis™ Solutions
Allele Report
Ladder lane - RFID: n/a Device: i5201 Agent: generic Processed: 10/01/14 14:40
10/1/2014 4:05 PM
Pre-installed allelic ladder employed to designate sample alleles
9 of 20 runs employed pre-installed ladders within the Maturity Assessment
D3S1358
TH01
D21S11
D18S51
PentaE
12 15 18 4 7 10 13.3 24 27 30 33 36
8 11 14 17 20 23 26
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
7 10 13 7 10 13 6 9 12 5 8 11 14 6 9 12 15 2.2 5 8 11 14 17
Am
vWA
D8S1179
TPOX
FGA
X Y 10 13 16 19 22 7 10 13 16 6 9 12 17 20 23 26 29 31.2 43.2 46.2
Signal Strength (rfu)
Fragment Length (bases)

IntegenX Ladders



IntegenX Ladder Precision

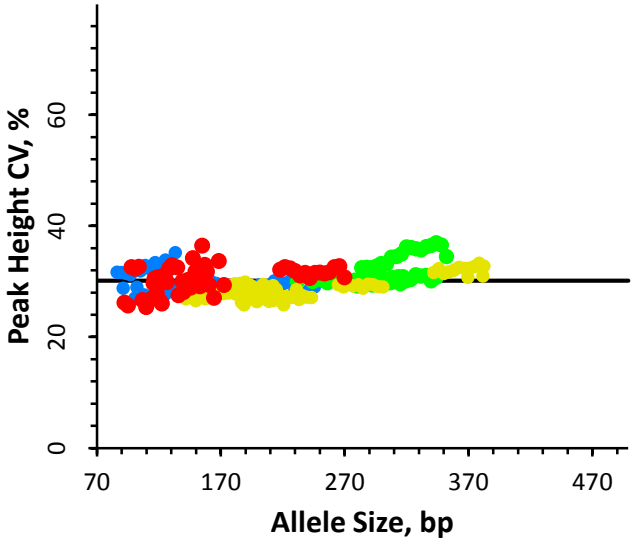


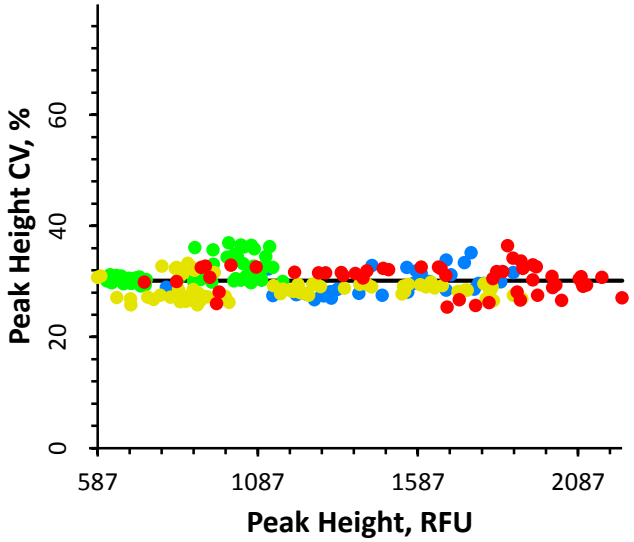
RapidHIT 200: GlobalFiler Express

Allele size SD: 0.114 bp

Peak Height CV: 30.1 %
Mean Peak Height: 1251 RFU

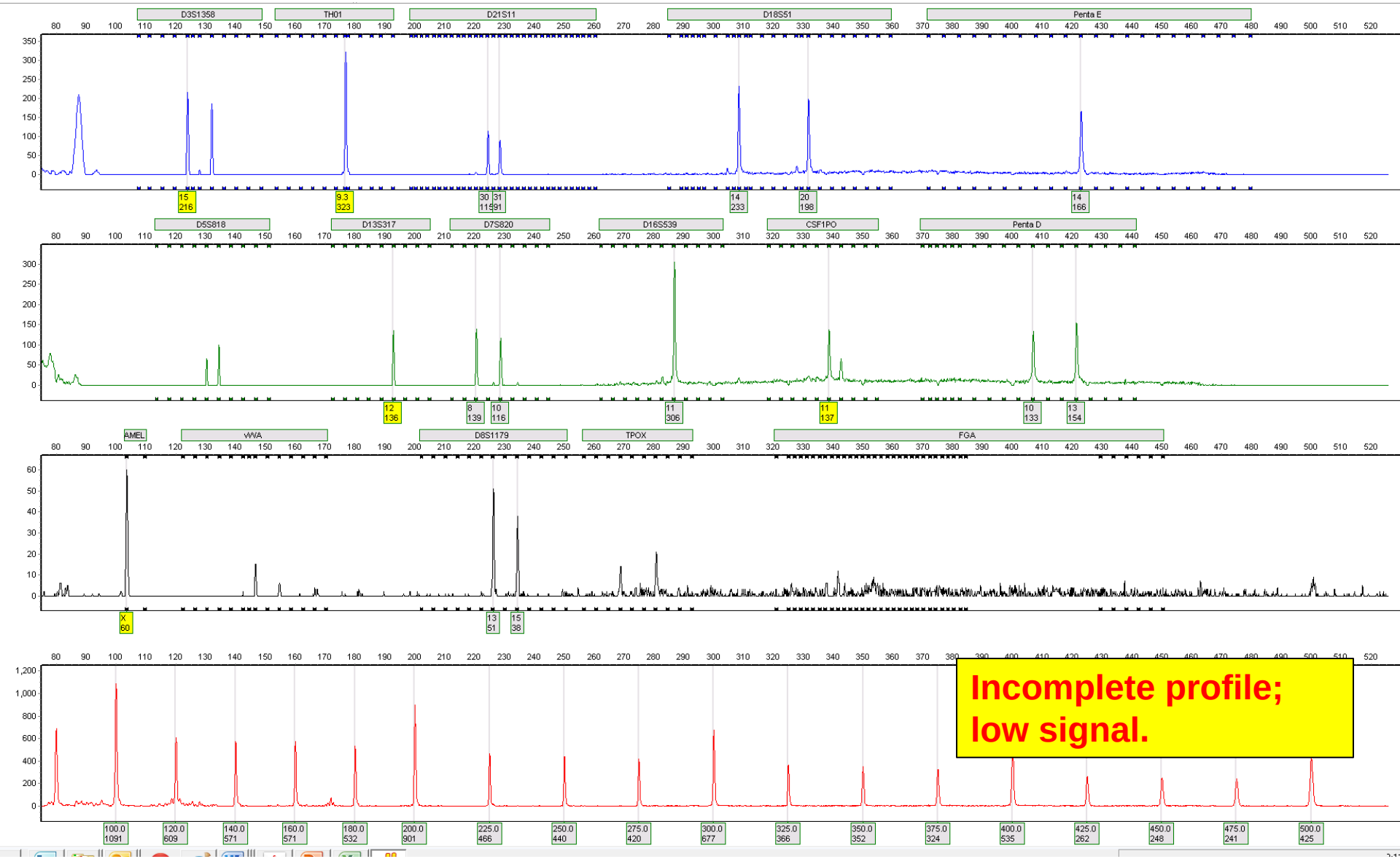
Mean Peak Area: 6909 RFU × bp
Peak Area CV: 30.1 %
Mean Peak Width: 5.4 bp
Slope {CV(Area),CV(Height)}: 0.998





Examination of the remaining ~20% samples that were not successful

Examples observed between at least 2 individual datasets within the Maturity Assessment Data

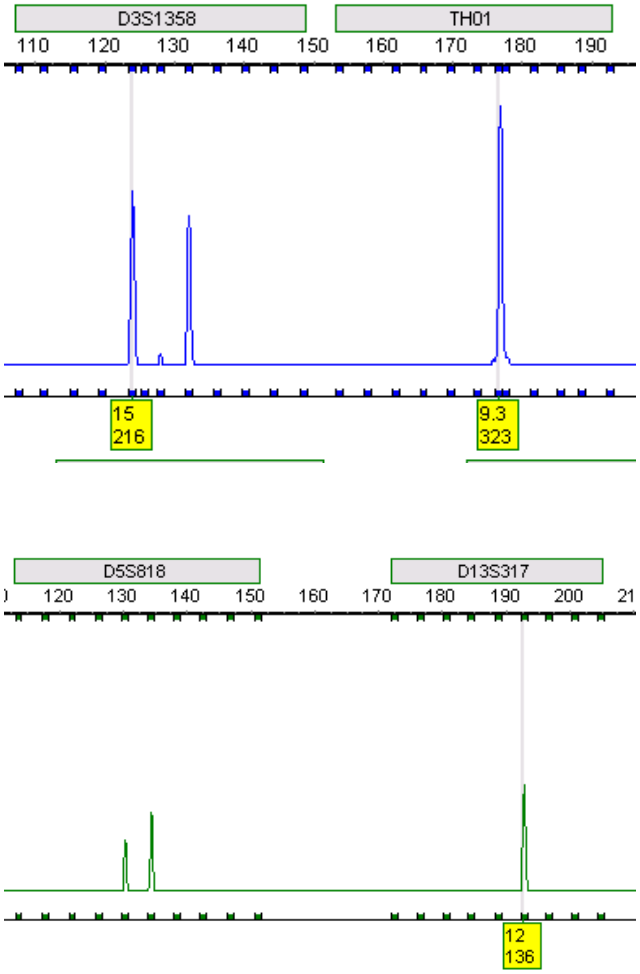


Incomplete profile; low signal.

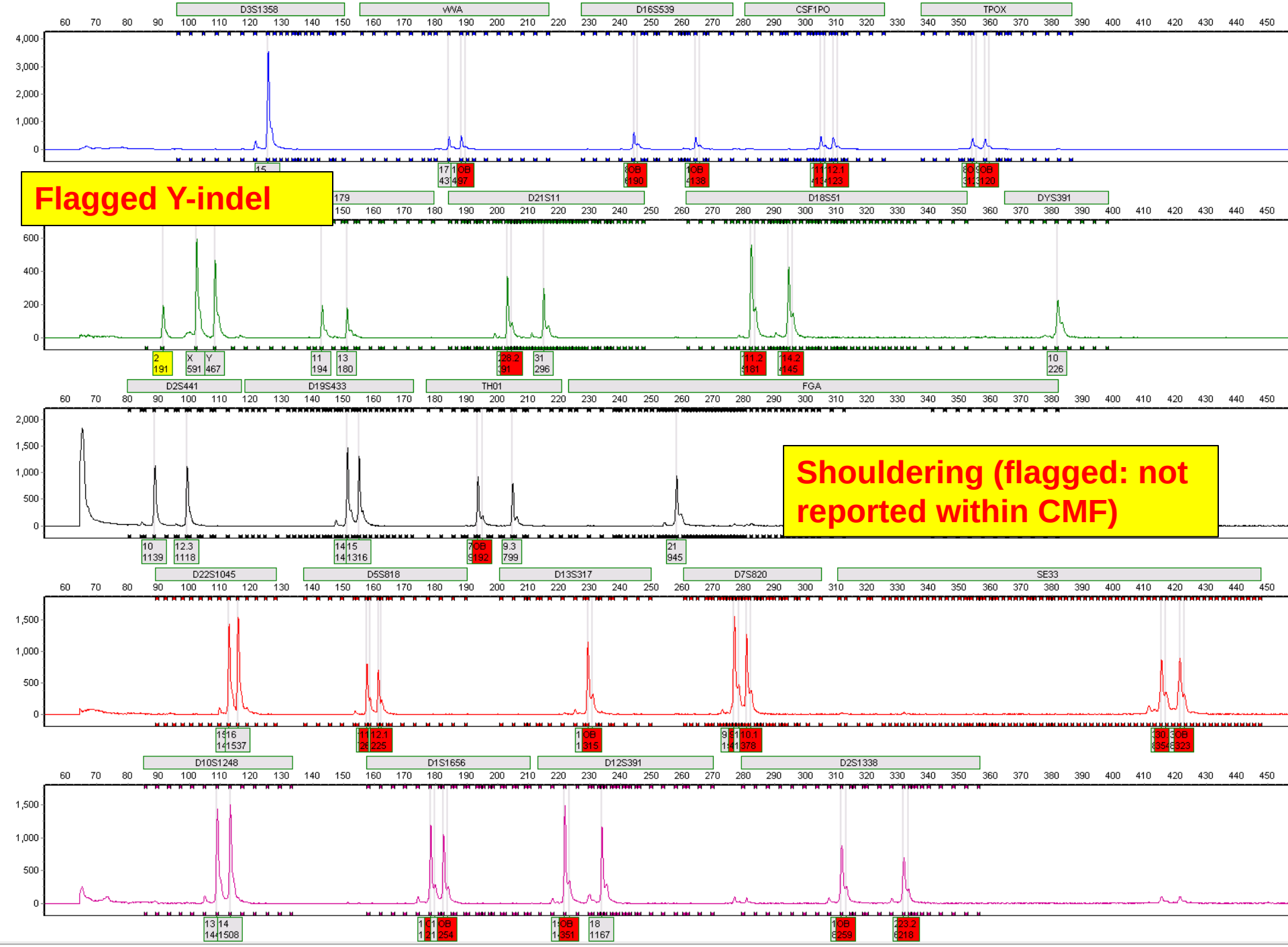
This is happening for PP16 and GFE Chemistries on the RH200

Report for Previous Sample

Loci	5. LabC_NN_5
D3S1358	15,
TH01	9.3,
D21S11	30, 31,
D18S51	14, 20,
Penta E	14,
D5S818	
D13S317	12,
D7S820	8, 10,
D16S539	11,
CSF1PO	11,
Penta D	10, 13,
AMEL	X,
vWA	
D8S1179	13, 15,
TPOX	
FGA	



Incomplete profiles reported (in xml file)


Flagged Y-indel
Shouldering (flagged: not reported within CMF)
D3S1358
vWA
D16S539
CSF1PO
TPOX
D21S11
D18S51
DYS391
D2S441
D19S433
TH01
FGA
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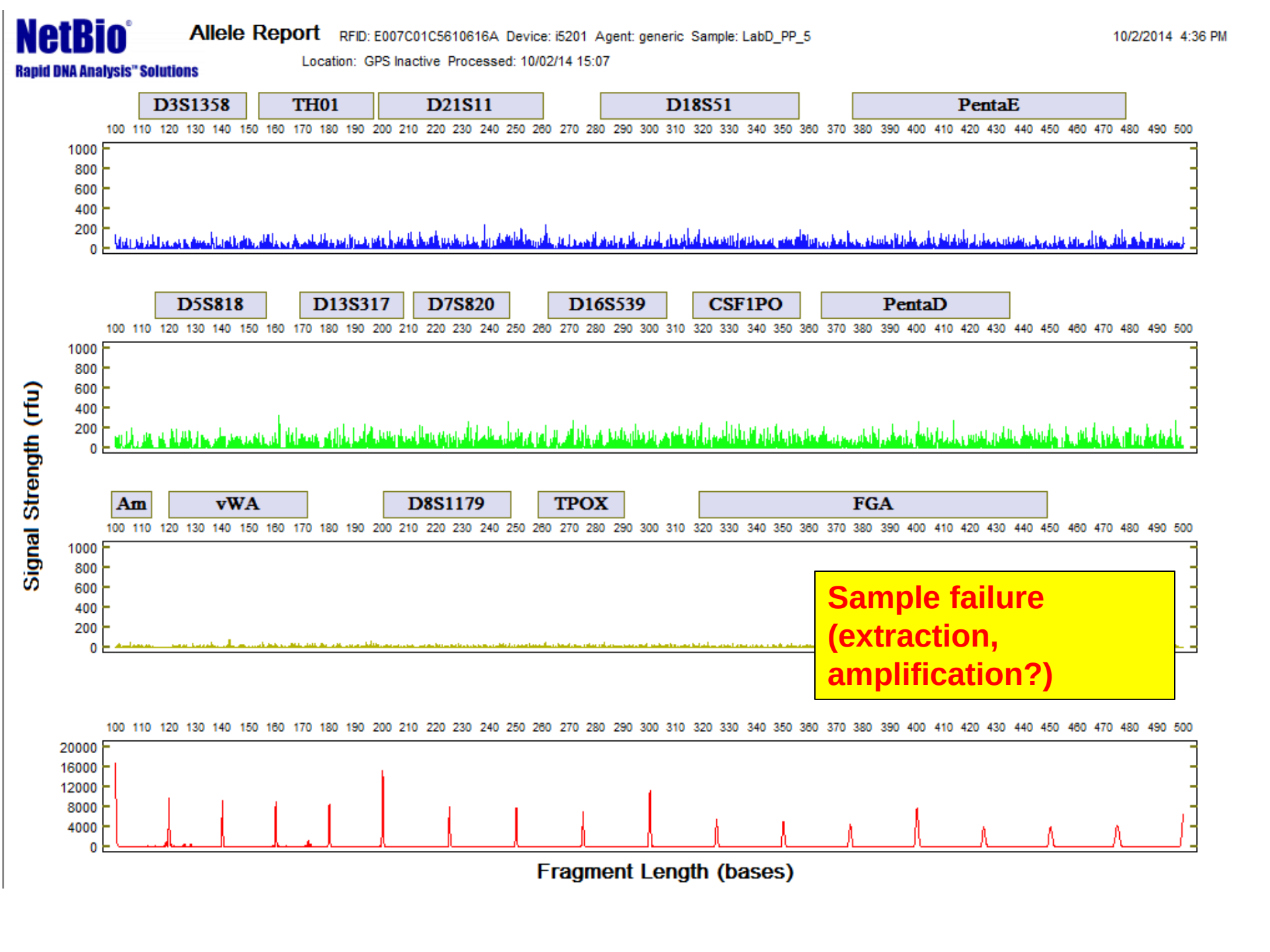
D5S818
D13S317
D7S820
SE33
D10S1248
D1S1656
D12S391
D2S1338


NetBio
Rapid DNA Analysis™ Solutions
Allele Report
RFID: E007C01C5610616A Device: i5201 Agent: generic Sample: LabD_PP_5
Location: GPS Inactive Processed: 10/02/14 15:07
10/2/2014 4:36 PM
D3S1358
TH01
D21S11
D18S51
PentaE
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
Am
vWA
D8S1179
TPOX
FGA
Signal Strength (rfu)
Sample failure (extraction, amplification?)
Fragment Length (bases)

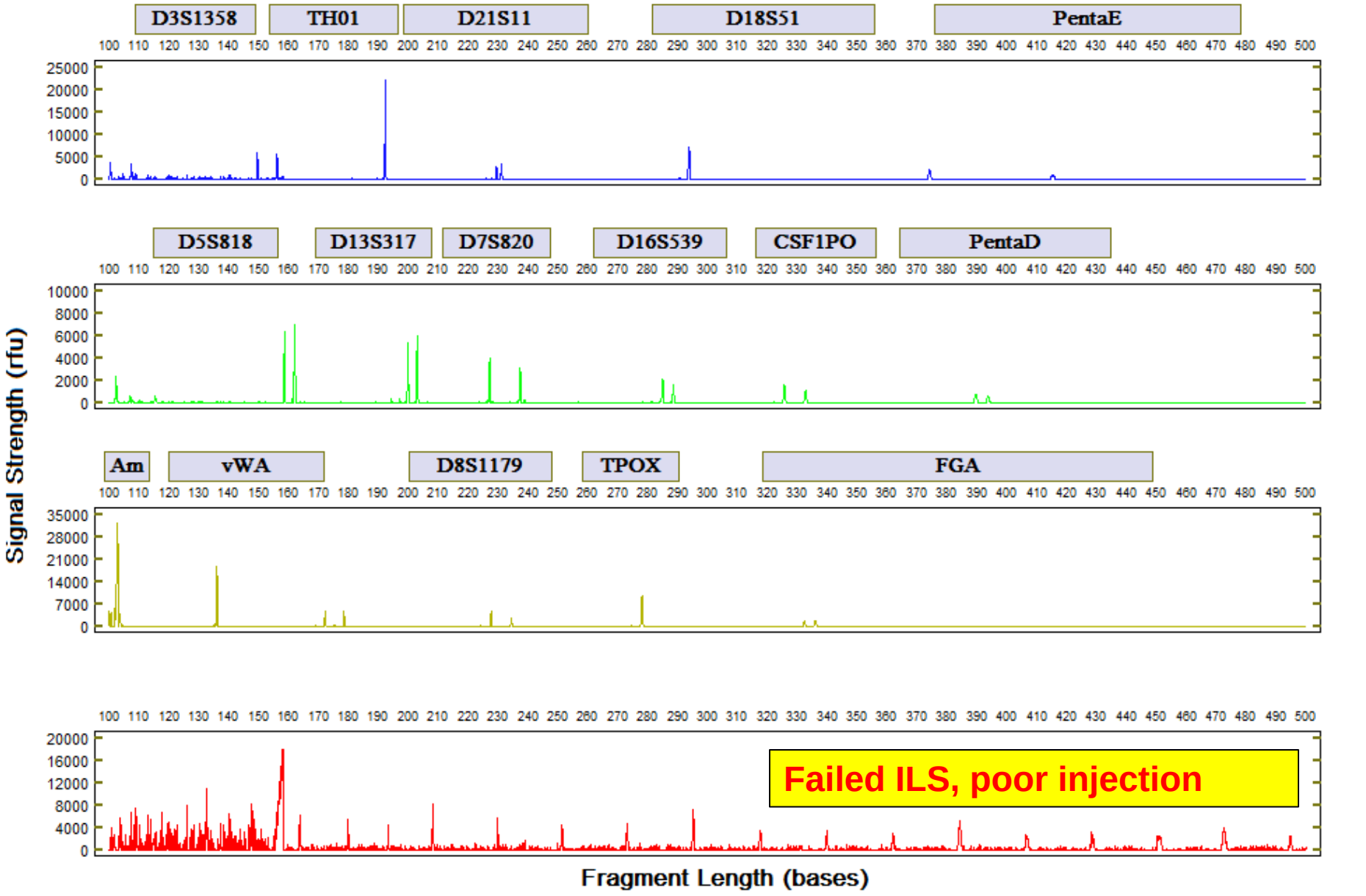
NetBio®
Rapid DNA Analysis™ Solutions

Allele Report

RFID: E007C01C56101D7D Device: i0901 Agent: SuperAdmin1 Sample: FF

11/24/2014 3:47 PM

Location: 3509.7212,N,07900.3309,W Processed: 11/24/14 14:17



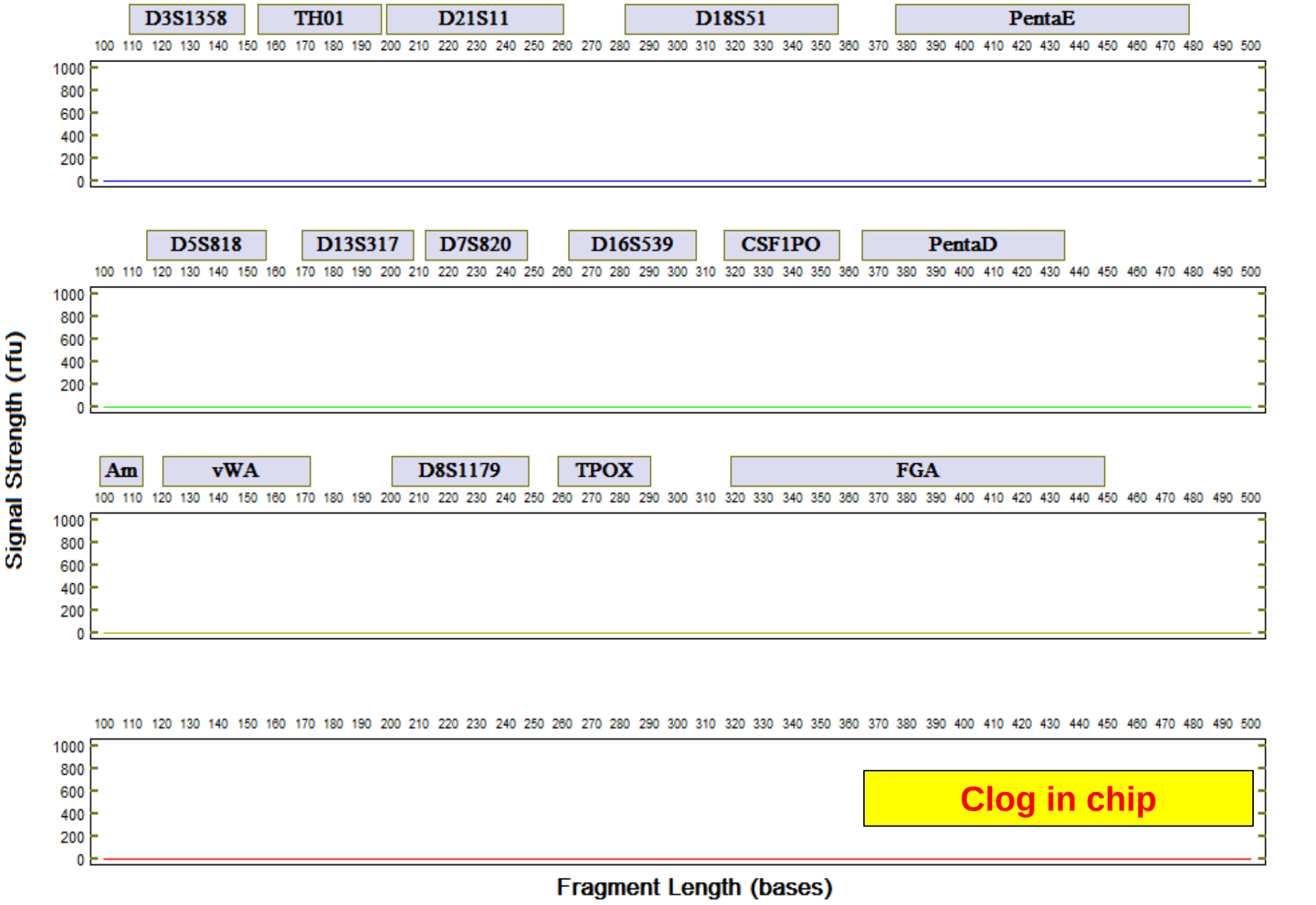
NetBio®
Rapid DNA Analysis™ Solutions

Allele Report

RFID: E007C01C5610202A Device: i0904 Agent: SuperAdmin Sample: LabC_AA_1

10/1/2014 12:48 PM

Location: GPS Inactive Processed: 10/01/14 11:17



Improvements Since R-DNA MA

- Problems with IXI GFE cartridge manufacturing Summer/Fall of 2014 (during this study)
  - Leaking buffer cartridges
  - Leaking PCR mastermix during injection of pogos
- Bad lot of polymer (exposed to extreme heat in AZ and began to break down)
- New RH200 software version (2.0)
  - Added kinship function (pair-wise comparisons)
  - Rapid DNA Process mode (complete expert mode)
    - GlobalFiler Express with GeneMarker HID 2.7

Chips were redesigned in January 2015

Summary

- 2014 R-DNA Maturity Assessment exhibited a 76% success rate for the CODIS 13 Core Loci using Rapid DNA Analysis
  - Success ranged from 45% to 85% across laboratories, chemistries, and instruments
- Precision is within 0.114 bp on average for RH200 GFE
- Continuing to run R-DNA platforms with newer chemistries and upgrades
  - GFE with the RapidHIT200
  - 27plex 6-dye chemistry with ANDE

Final Results

Rapid DNA Instrument Platforms	**Number of Participating Labs**	**Total Instruments**	**Samples Attempted**	**Core CODIS Success** (Rapid DNA Analysis)	**Core CODIS Success** (Modified Rapid DNA Analysis)
2	7	11	280	76.1%	80.0%

Overall success for the R-DNA maturity assessment will be reported:

http://www.nist.gov/mml/bmd/genetics/dna_biometrics.cfm

This data will be made available to the public.

Thank you for your attention!

Thanks to David Duewer and Sanae Lembirik for assistance with data analysis

Contact Info:
erica.romsos@nist.gov
301-975-5107

Funding

DHS – Rapid DNA Prototype and Kinship Performance Evaluation

FBI - the Evaluation of Forensic DNA Typing as a Biometric Tool