DNA Mixture Interpretation Workshop | *Brad Jenkins*

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Virginia and the New SWGDAM Guidelines

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NIJ Disclaimer

- **This project was supported by NIJ Award #2008-DN-BX-K073 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice."**

Virginia Procedures

- **DFS will be following the published guidelines**
- **Complete implementation is expected in Spring 2011**
- **Yes we ran a lot of gels for years**
- **Online with CE for 1 year (3130xl)**

Virginia's Lab System

- **4 Labs**
- **70 staff**
- **Data Bank Unit**
- **Casework Unit**
- **Y STR Unit**
- **Mito Unit**
- **Familial DNA Unit ??**
- **Research Unit**

Virginia DNA Testing

- **PowerPlex 16**
- **2 sec, 5 sec and 10 sec injections**
- **Stats**
  - **Random Match Probability (single source and major/minor)**
  - **Likelihood ratio (2 person mixtures)**
  - **CPI (all other mixtures)**

SWGDAM Guidelines 2010

- **Section 1 Preliminary evaluation of the data**
  - **Analytical Threshold**
  - **Controls**

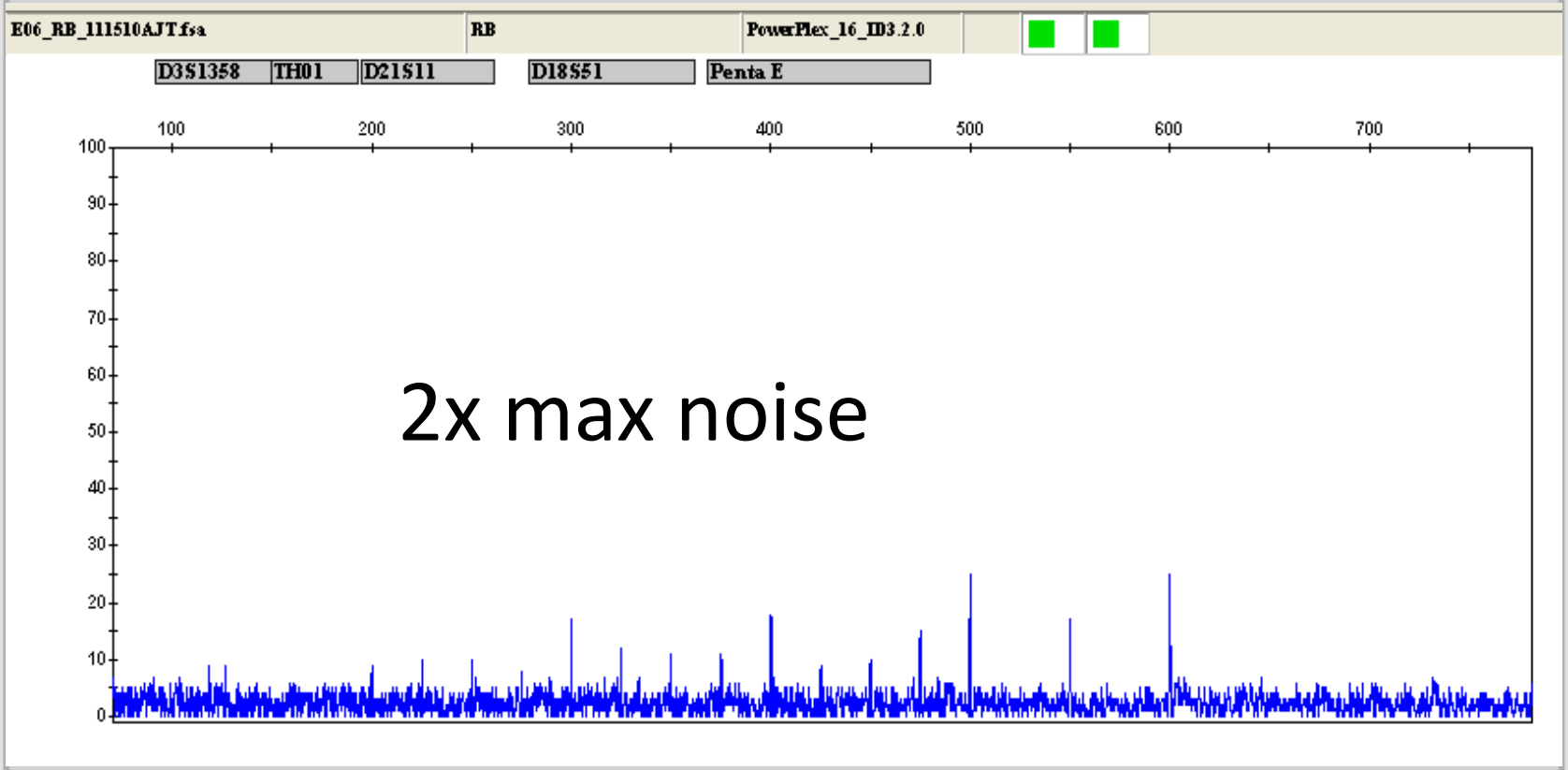
How do you apply your analytical threshold to repaired or new instruments

If your controls have issues will you interpret the data

Limit of Detection (LOD)

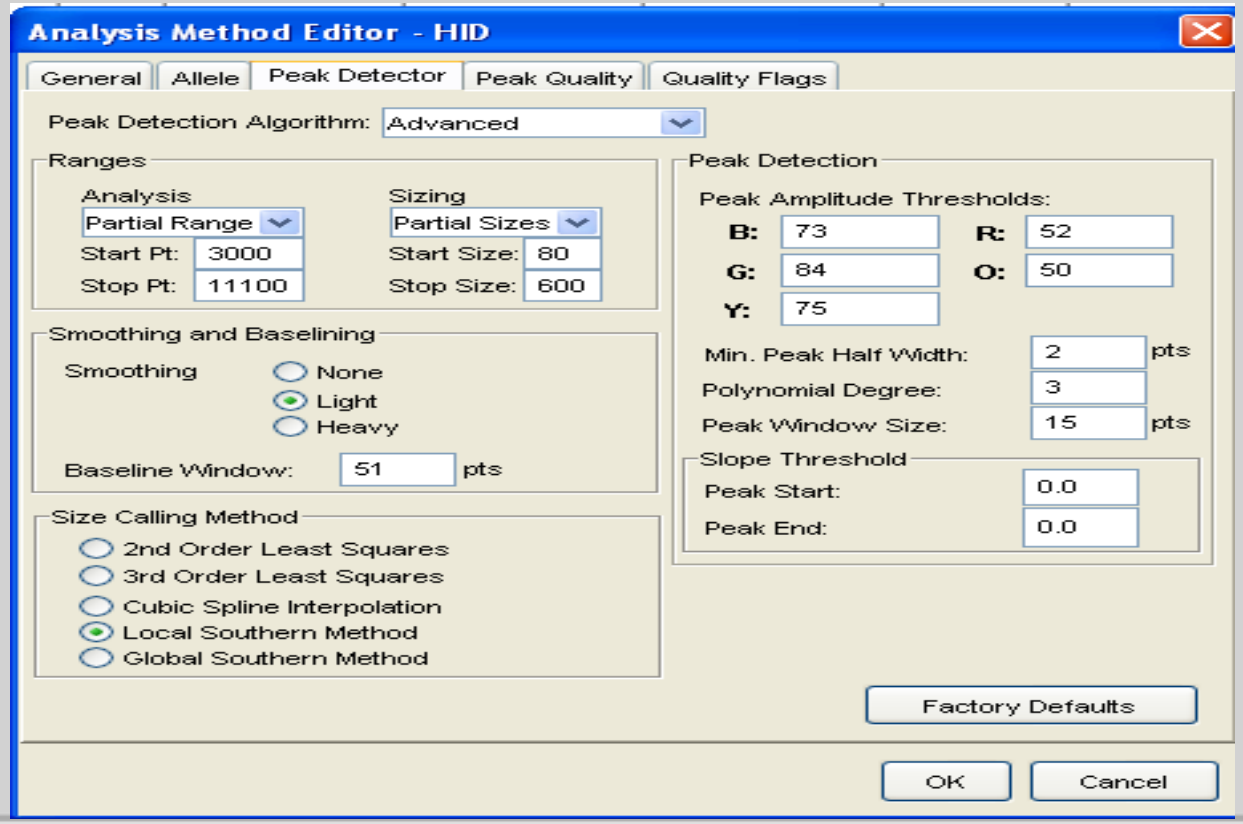
- **Measured the LOD from all types of samples**
- **3 Different Injection Times (3sec, 5sec and 10sec)**
  - **No sig change per injection time**
- **9 Instruments**
- **Statewide Average**

Limit of Detection



Section 1

- **Analytical Threshold**
  - **2x max noise per dye**



Evaluating The LOD Yearly

Once a year, when a new instrument is brought on line and/or following laser replacement (or similar repair) the LOD is calculated/re-calculated

- **20 samples from multiple runs are reviewed**
- **LOD calculated (2x max noise) per dye**
- **Statewide LOD is the average of all the instruments**
- **New or repaired instrument compared to the statewide average**

Current LOD Measurements

	Blue	Green	Yellow	Red
Average LOD	73	84	75	52
Standard Deviation	5.6	5.2	6.1	11.5

Jan 2011 LOD values

	Blue	Green	Yellow	Red
Central-1	98	106	48	48
Central-2	78	84	46	58
Central-3	58	64	66	58
Central-4	74	80	58	38
Northern-1	60	76	52	60
Northern-2	58	82	52	28
Eastern-1	28	26	20	26
Eastern-2	32	32	38	48
Western-1	58	82	62	62
Average	60.4	70.2	49.1	47.3

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Establishing A Cut Off For Low Level Samples

- **Validation suggested 30pg of DNA in amplification cocktail.**
- **Testing this with real casework samples**
- **Have seen that Plexor is reproducible at the low end of detection**

SWGDAM Guidelines 2010

Section 2

- **Allele Designations**

< or > the respective ladder allele

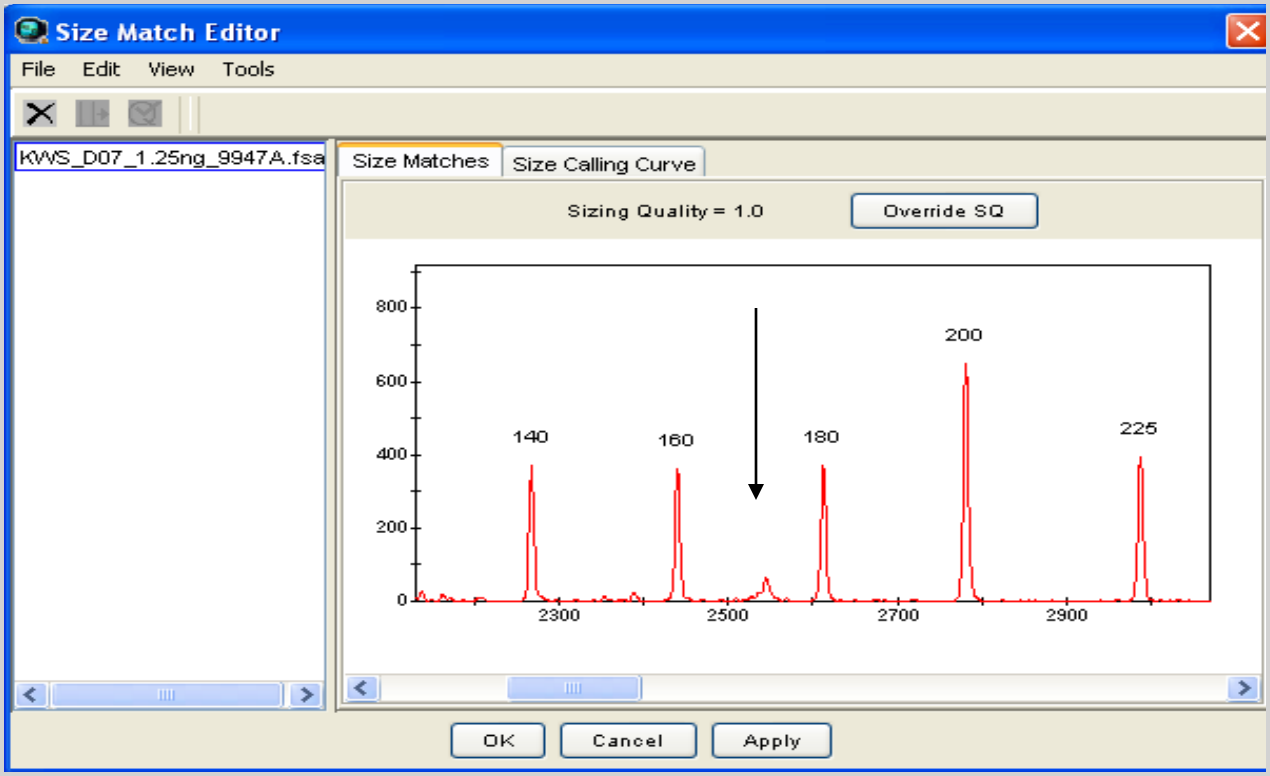
Incomplete repeats TH01 9.3

Extrapolation

SWGDAM Guidelines 2010

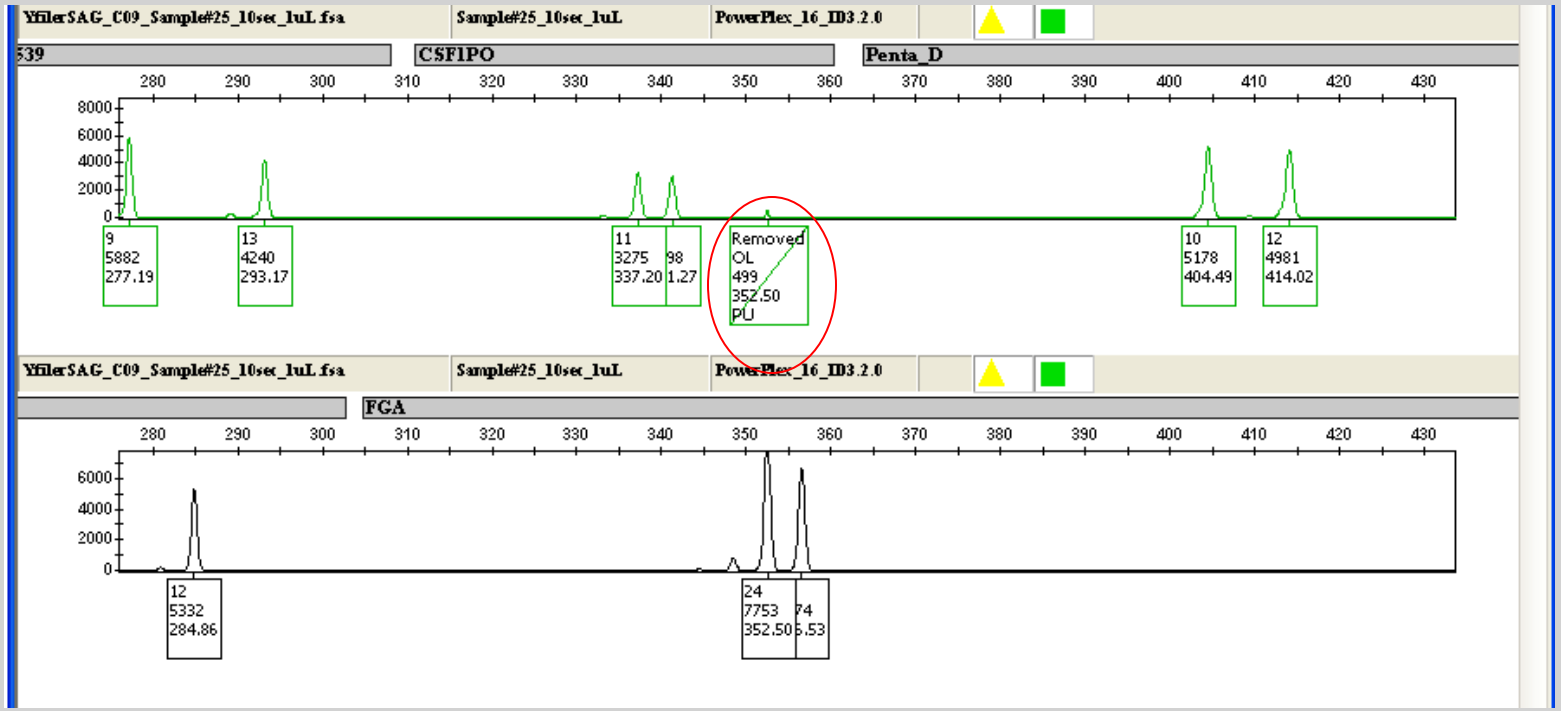
- **Section 3 Interpretation** **Gels were not so bad**
  - **Non-allele peaks**
    - **Spikes**
    - **Blobs**
    - **Known artifacts**
  - **Off scale data**
  - **Stochastic Threshold**
  - **Peak Height Ratios**
  - **Numbers of contributors**
  - **Mixture interpretation**

Non-allele peaks



Size Match Editor window showing the ILS 600 size standard. The arrow points to the artifact at approximately 172 bp.

Non-allele peaks



Pull-up from the yellow/black channel (TMR) into the green channel (JOE), indicated by the red circle and shown as an edited off-ladder (OL) peak that has been changed to indicate pull-up (PU). Editing allele calls is described in 4.2.7.6.

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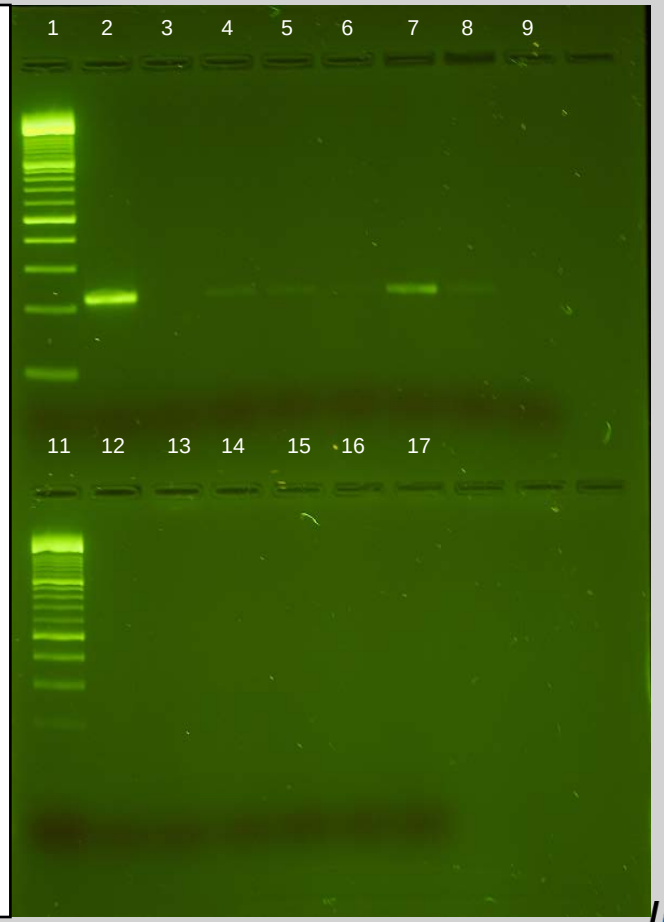
Off Scale

Top Origin
Lane 1: DNA MW Marker XIV
Lane 2: Positive (HL-60) -200pg
Lane 3: Negative
Lane 4: Dried 1ng – 1 Joule/Cm^2
Lane 5: Dried 2ng – 1 Joule/Cm^2
Lane 6: 0.1ng/ul liquid – 1 Joule/Cm^2
Lane 7: 0.2ng/ul liquid – 1 Joule/Cm^2
Lane 8: Dried 1ng – 2 Joule/Cm^2
Lane 9: Dried 2ng – 2 Joule/Cm^2
Bottom Origin
Lane 11: DNA MW Marker XIV (1:2)
Lane 12: 0.1ng/ul liquid – 2 Joule/Cm^2
Lane 13: 0.2ng/ul liquid – 2 Joule/Cm^2
Lane 14: Dried 1ng – 3 Joule/Cm^2
Lane 15: Dried 2ng – 3 Joule/Cm^2
Lane 16: 0.1ng/ul liquid – 3 Joule/Cm^2
Lane 17: 0.2ng/ul liquid – 3 Joule/Cm^2

*Product gel from 0.2ml PCR tube amplification results



Stochastic Threshold

- **2 samples hetero at most loci**
- **Quant and made dilutions to 10pg, 30pg, 100pg**
- **Re-quant dilutions and amplified 10 replicates of each sample in the same TC**
- **Injected each sample for 2, 5 and 10 sec**
- **Blister plots**

	D3S1358	TH01	D21S11	D18S51	Penta E	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta D	Amel	vWA	D8S1179	TPOX	FGA
	16,17	6,9.3	29,30	14,17	7,17	12,13	13	10,11	11,12	11,12	10,14	X.Y	17,18	13,14	8,11	20,25
10pg	146 126	- -	84 -	- 84	- -	176 112	-	102 -	197 -	- -	- -	- -	- -	- -	236 -*	- -
	- -	127 -*	93 214	101 103	83 -	152 -*	124	*- 205	- -	- -	119 -	94 -*	127 -*	- -	- -	*- 114
	- -	- -	98 -*	- -	- -	87 94	90	- -	- -	- -	- -	- -	103 -*	- -	- 171	- -
	107 - *	- -	*- 142	- -	- -	- -	-	- -	- -	- -	- -	- -	*- 106	- -	*- 83	- -
	*- 101	- -	*- 102	- -	- -	*- 110	199	- -	- -	92 -	- -	138 81	150 -*	84 -*	- -	- -
	*- 92	95 150	153 134	- -	- -	119 -	-	323 -*	- -	- -	- 96	78 242	113 -*	144 152	*- 172	99 -
	150 89	- -	88 -*	- -	- -	- -	170	*- 112	85 163	- -	185 -	- -	*- 147	- -	*- 128	- -
	150 84	305 205	80 89	122 -*	- -	- -	89	- -	186 134	- -	91 -	*- 255	113 -	*- 105	88 88	-
	- -	*- 107	81 228	- -	- -	138 -*	150	- -	201 201	- -	- 131	*- 159	- -	- -	146 78	88 -*
	- -	287 82	103 -*	- -	- -	*- 114	-	- 105	158 -	- -	*- 141	- -	77 -	174 -	87 82	- 86
30pg	176 244	345 360	253 243	81 291	176 -	*- 125	311	266 160	182 280	248 -	189 86	256 -*	352 115	*- 115	245 295	256 -
	260 219	250 235	86 118	141 -*	74 -	116 144	272	292 665	517 243	- -	*- 99	170 334	156 274	275 89	251 378	224 -*
	288 218	164 364	368 142	*- 89	157 388	210 264	398	109 281	207 144	- -	226 456	211 372	209 329	140 174	140 193	*- 174
	221 86	214 125	365 -*	- -	126 126	116 230	384	244 238	119 110	84 -*	- -	203 285	383 183	101 188	116 119	*- 184
	217 -*	123 87	202 191	148 -*	98 -*	145 281	175	*- 144	87 -*	- -	- 161	279 309	289 -*	104 131	95 -*	77 -*
	433 225	122 139	203 322	- -	236 376	138 358	360	441 350	386 89	155 -*	186 242	500 290	243 266	295 174	163 305	221 -*
	497 125	113 131	165 -*	392 155	308 96	280 161	420	200 191	272 112	244 180	420 114	374 494	293 499	125 135	305 153	423 189
	364 423	211 171	229 214	422 256	299 73	300 252	298	299 312	*- 174	- 214	- 611	516 190	248 278	315 212	186 -	113 315
	230 193	245 222	172 90	- -	- -	308 272	326	141 -*	203 232	229 268	- 270	116 234	229 374	164 158	138 -	319 100
	254 454	374 102	140 329	78 106	*- 205	266 119	361	505 236	493 222	485 242	239 207	220 365	351 245	267 -*	226 472	261 -*
100pg	370 537	708 438	314 455	186 488	716 384	350 417	852	340 95	556 268	*- 321	420 -	301 790	789 655	534 316	389 435	184 208
	653 587	473 717	484 275	245 567	451 452	496 414	823	436 559	259 607	433 534	511 422	844 897	702 640	289 361	470 363	212 185
	403 788	760 684	442 805	275 553	992 174	621 839	395	605 343	1051 496	341 237	481 365	721 840	797 912	163 82	1359 263	313 481
	934 270	926 777	535 697	180 486	134 485	394 447	783	747 432	216 346	301 360	135 235	590 430	638 417	316 314	357 371	290 546
	539 393	928 1152	668 486	164 94	512 202	489 758	693	493 497	468 157	218 181	403 554	663 1012	966 907	426 301	298 916	256 553
	470 407	316 429	484 363	366 240	273 -*	480 342	410	458 129	453 527	216 189	205 320	430 511	799 410	165 305	263 362	258 264
	268 745	637 283	398 207	370 378	228 314	243 469	827	655 391	192 285	245 361	283 505	430 526	679 298	209 398	542 486	210 125
	324 751	540 388	222 240	348 485	79 -*	470 476	711	377 392	438 303	324 282	303 411	796 888	850 534	531 170	400 378	523 285
	365 759	506 473	507 656	441 354	239 178	472 329	755	605 345	195 -*	383 335	328 365	908 1077	964 854	329 539	696 744	409 286
	665 880	168 407	646 379	*- 612	384 243	578 630	644	432 243	615 346	773 375	598 298	803 983	741 543	405 281	694 407	190 132

* Indicates the sister allele was observed, but was below the LOD.

Stochastic Threshold

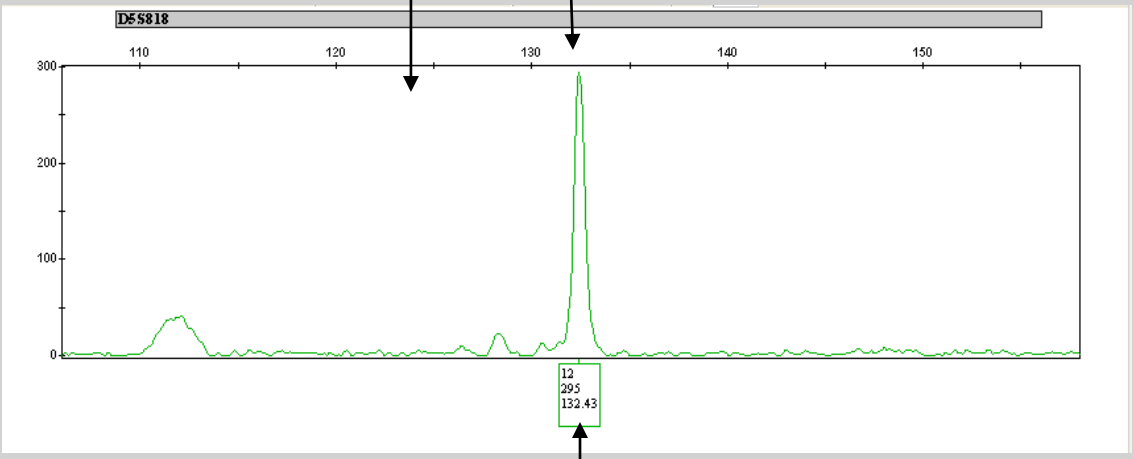
- **Averaged the RFU values of the surviving sister peak where the other sister peak fell below the LOD or was not visible per injection time**
- **Added standard deviation**
- **Apply this data to 2,3 and 4 person mixtures**

Stochastic Threshold

Injection Time	Average peak height	SD	Stochastic Threshold	N
2 seconds	121.5	42.45	160	159
5 seconds	152.85	80.67	230	205
10 seconds	202.93	128.49	330	168

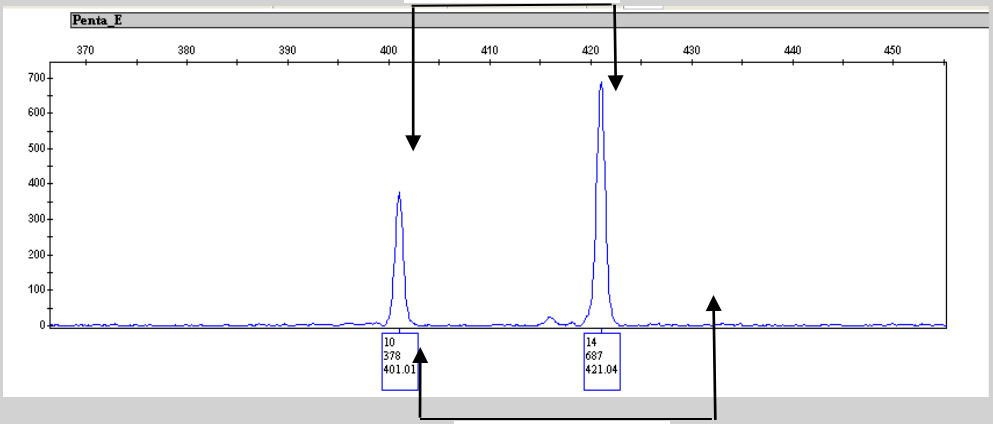
Stochastic threshold values generated using the average of the peak heights where allelic drop-out was observed plus one standard deviation.

Reference 5



Reference 4

Reference 7



Reference 4

Peak Height Ratios

Locus	Lower Limit	Locus	Lower Limit
Penta E	33%	VWA	60%
D18S51	61%	Amelogenin	64%
D21S11	72%	Penta D	53%
TH01	53%	CSF1PO	61%
D3S1358	69%	D16S539	51%
FGA	50%	D7S820	59%
TPOX	59%	D13S317	55%
D8S1179	78%	D5S818	75%

NOTE: Although average peak height ratios for a single source sample at a heterozygous locus may be above 0.6 when DNA quantity is above stochastic levels (250 pg and greater), minimum heterozygote balances have been observed below 0.6 for single source samples even at sufficient quantities of input DNA (250 pg and greater,).

Numbers of Contributors

- **Number of alleles**
- **Data below analytical threshold (LOD)**
- **Masking considerations**

Mixture Interpretation Major/minor

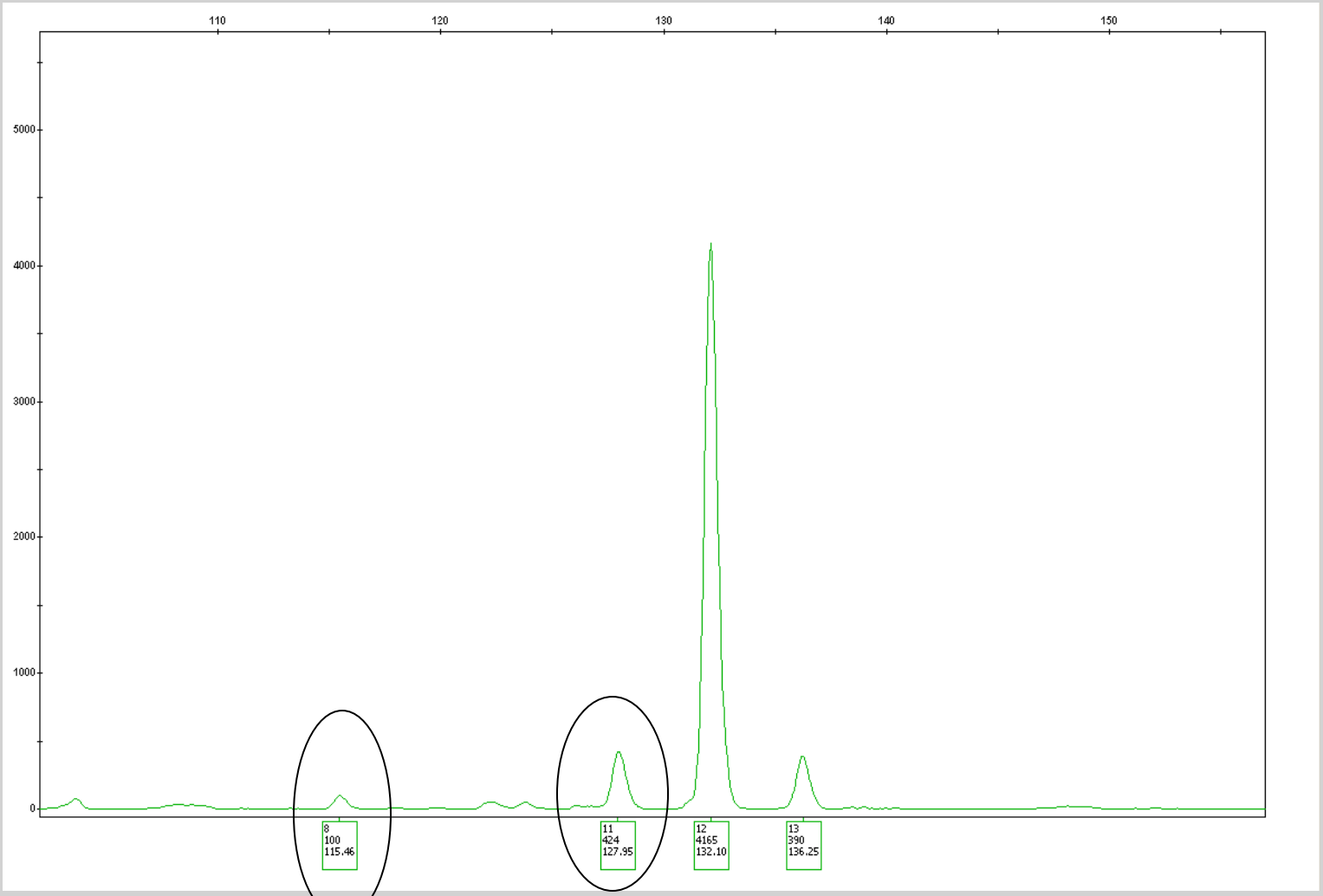
- **Try and limit to 2 person mixtures**
- **Multiple majors/minors**

A major profile will be called when the peak height of the minor source of DNA is 33.0% or less than the peak height of the major source of DNA. If one or both the minor and the major contributors are heterozygous, then the 33.0% value applies to the largest of the minor contributor peaks and the smallest of the major contributor peaks.

Consider Stutter

Take the largest stutter RFU value at a locus and compare to the RFU value of the smallest minor allele (not in a stutter position)

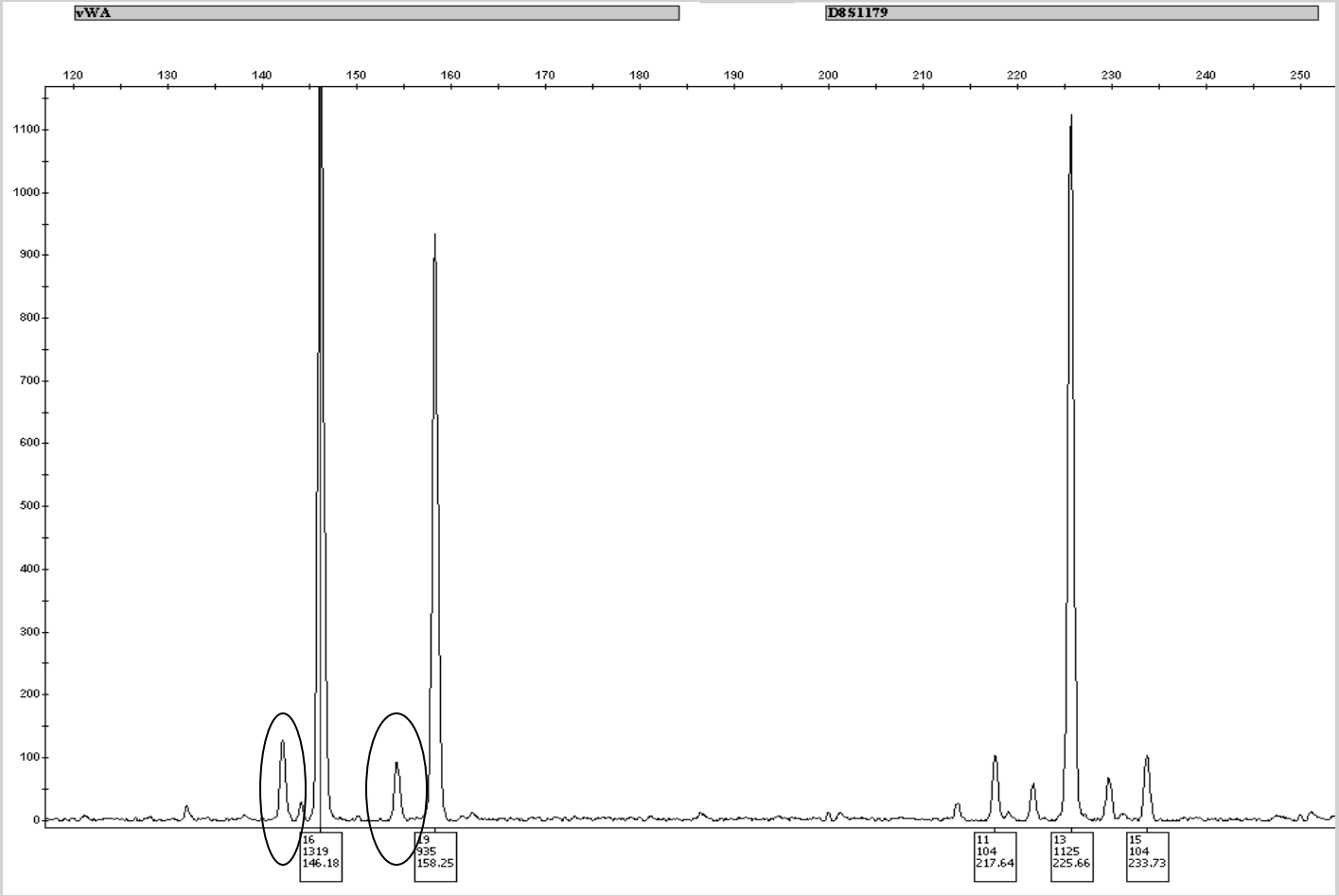
If the stutter RFU is greater than or equal to the minor allele RFU value use all the stutter alleles in the CPI calculation



Minor Allele

Stutter

Stutter Considerations



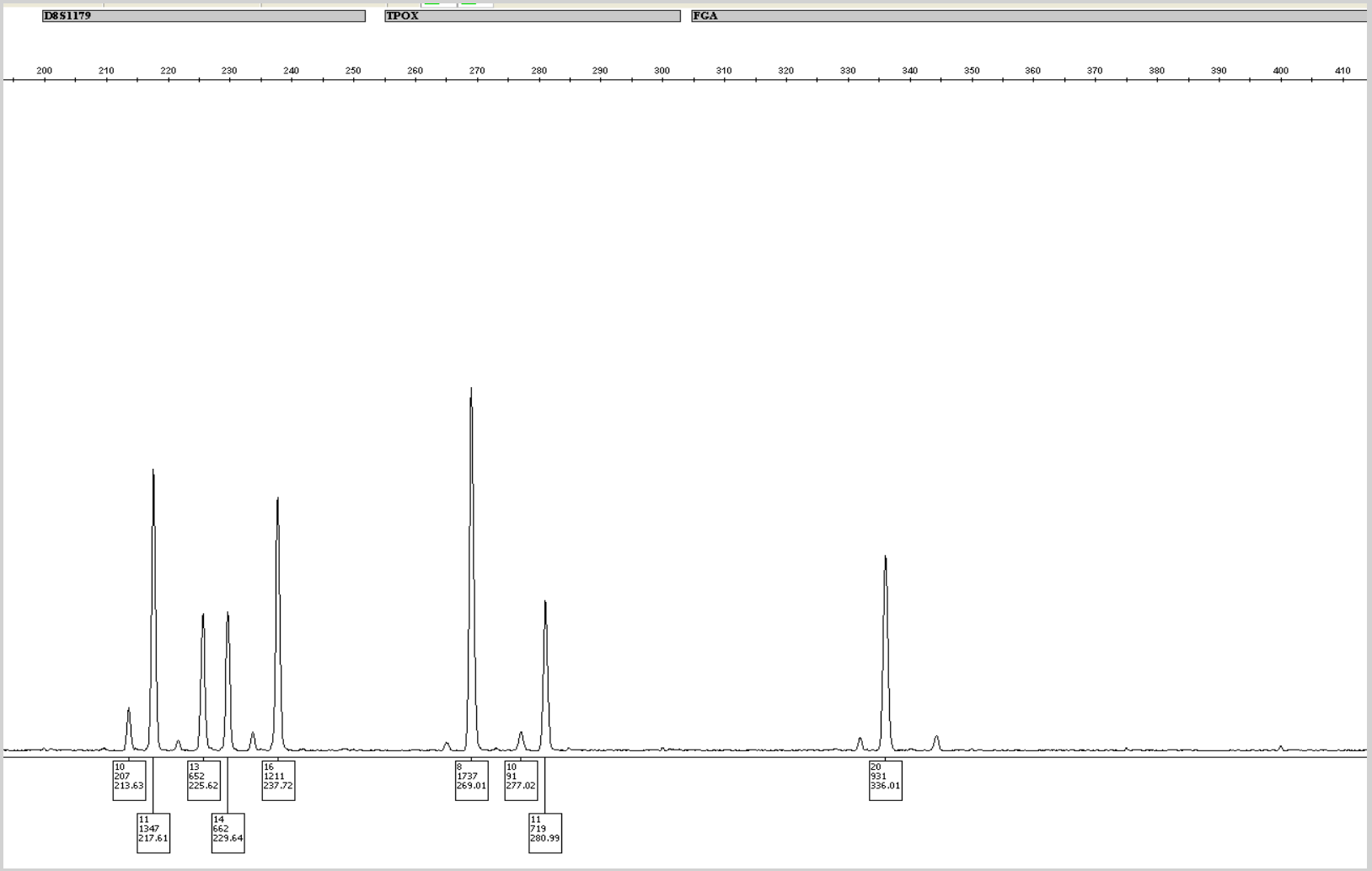
Evidence Prior to References

- **All evidentiary sample electropherogram data must be analyzed prior to the reference samples.**
- **A technical review of the data analysis will be conducted by a second qualified examiner/analyst using the GeneMapper® ID software. The reviewer will review the evidentiary samples prior to the reference samples.**
- **Expert system on casework reference samples?**

Putting Mixture Interpretation Into Practice

- **Validation to define your system**
- **Test your mixture interpretation on real forensic casework**
- **Electronic examples for your staff**
- **Get buy in from your staff**


TH01
15 1409 122.09
16 1481 126.19
17 2164 130.44
18 3279 134.47
6 2709 160.08
8 1091 168.00
9 3629 172.00
MOCK-2010A.1.ground.sp
PowerPlex_16_ID3.2.0
D5S818
D13S317
7 2416 111.39
11 5438 128.05
12 1744 132.08
8 1299 174.49
11 3307 186.49
12 2054 190.51
AMEL
vWA
X 4538 103.59
Y 2849 109.51
16 952 146.20
17 1287 150.21
18 2546 154.23
19 2662 158.25


D8S1179
TPOX
FGA
200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410
10
207
213.63
11
1347
217.61
13
652
225.62
14
662
229.64
16
1211
237.72
8
1737
269.01
10
91
277.02
11
719
280.99
20
931
336.01

Future

- **PHR and Stutter at the Low End**
- **% Contributor**
- **Quant cut off 30pg**
- **Mixture cut off 7 alleles at 2 or more loci**
- **Likelihood ratio with a drop-out factor**
- **Expert system**

Contact Information

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