DNA Mixture Analysis:

Principles and Practice of Mixture Interpretation and Statistical Analysis Using the SWGDAM STR Interpretation Guidelines

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Putting it all Together: A Case Example

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NIST

AAFS 2011 Workshop #17
Chicago, IL
February 22, 2011

Bureau of Alcohol, Tobacco, Firearms and Explosives
1972

CE User's Group (December 5, 2008)

- Bruce Heidebrecht organized
- Held at Maryland State Police Forensic Lab
- Presentations & discussion on 4 mixture cases
- ~60 people attended from 16 labs

- Bruce has developed several helpful tools for mixtures…

Steps in the Mixture Interpretation Process

[Adapted from Clayton *et al. (1998) Forensic Sci. Int. 91:55-70*]

Step 1. Identify the Presence of a Mixture

↓

Step 2. Designate Allele Peaks

↓

Step 3. Identify the Number of Potential Contributors

↓

Step 4. Estimate the Relative Ratio of the Individuals Contributing to the Mixture

↓

Step 5. Consider All Possible Genotype Combinations

↓

Step 6. Perform statistical analysis

↓

Step 7. Compare Reference/Casework Samples

Some Information

Victim’s Vaginal Swab

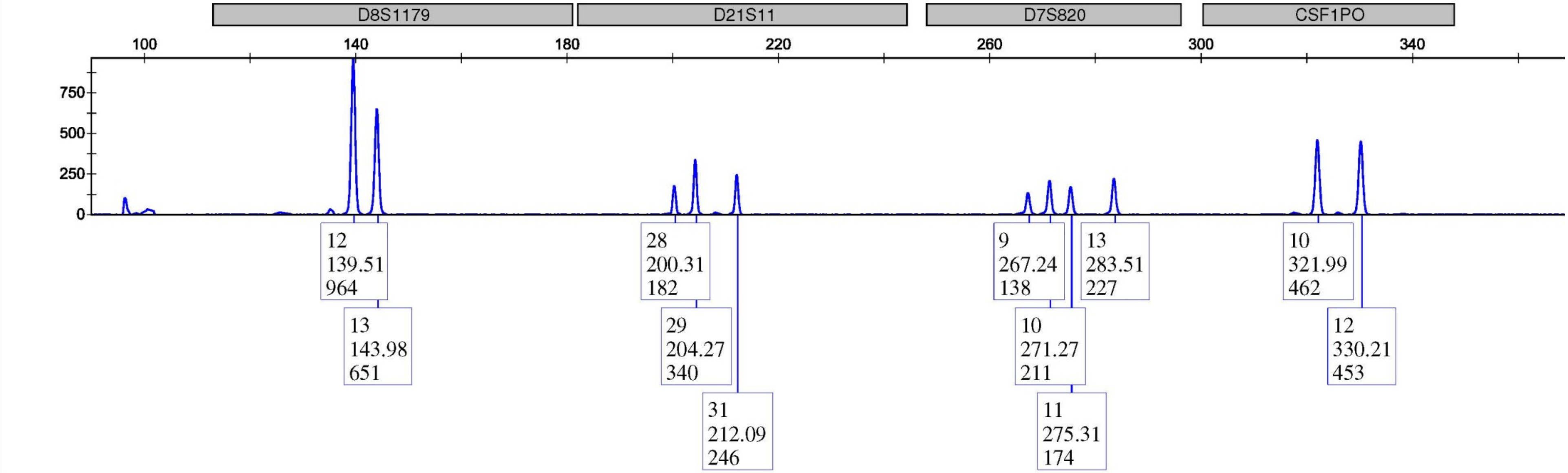
(sperm fraction)

ST = 150 RFUs

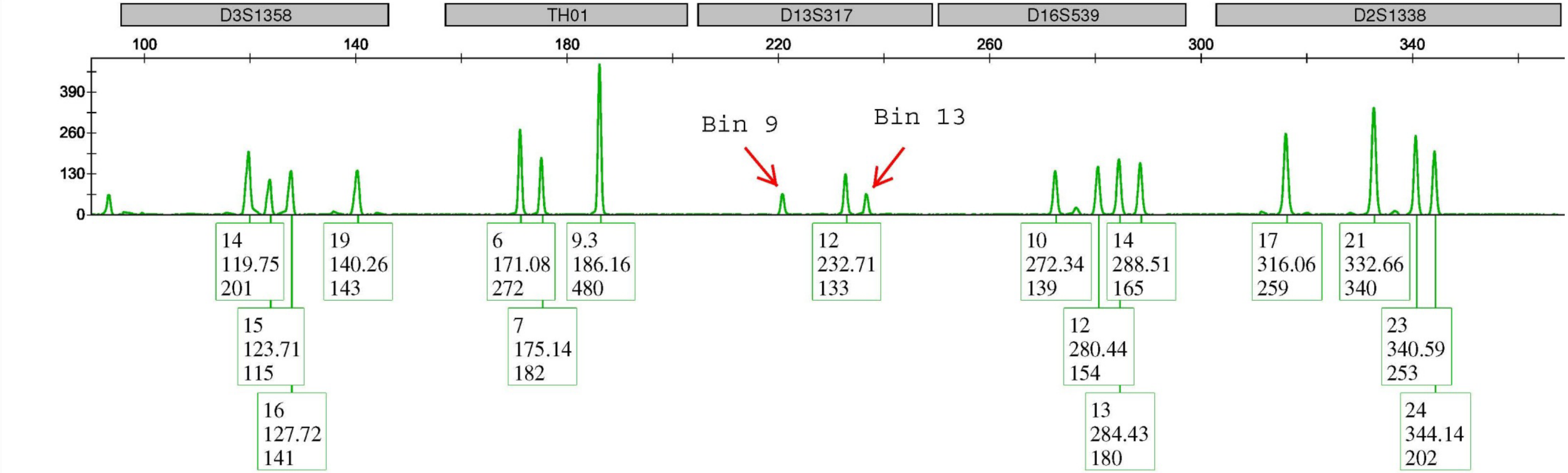
Biosystems
neMapper ID v3.2.1

[redacted]

Sample File	Sample Name	Panel
[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1



[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1



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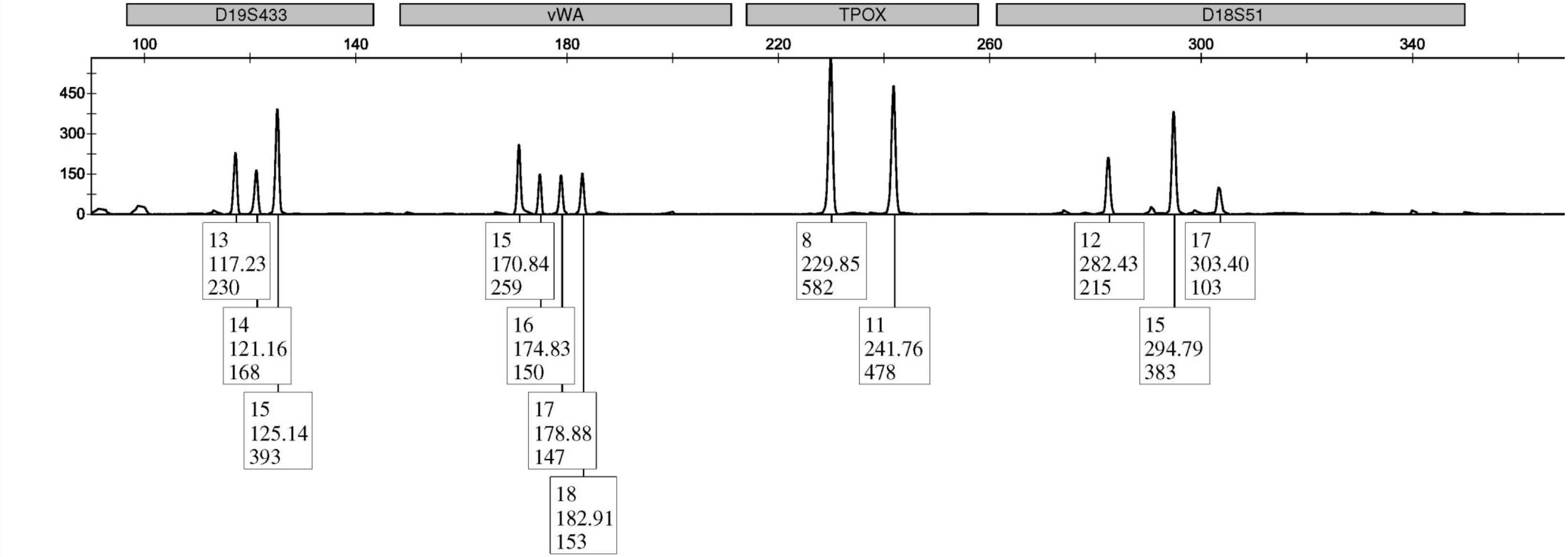

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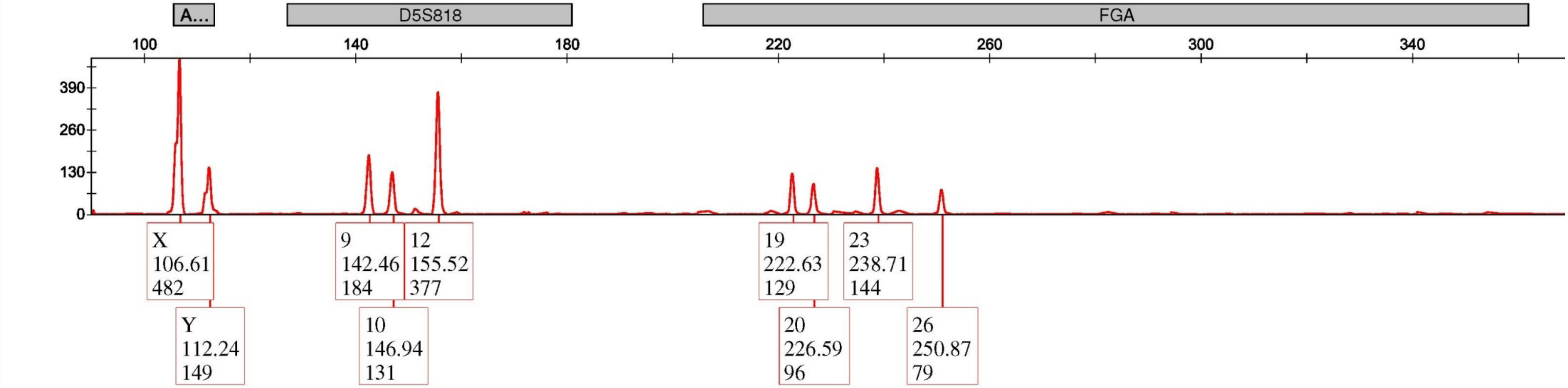

Determine number of contributors

2 person mixture

(no more than 4 alleles at any locus)

Applied Biosystems
GeneMapper ID v3.2.1

[redacted]

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[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1
[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1

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2 person mixture

(no more than 4 alleles at any locus)

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[redacted]

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[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1

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Determine if there is a distinct major contributor

No distinct major contributor

Applied Biosystems
GeneMapper ID v3.2.1

[redacted]

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Sample File	Sample Name	Panel
[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1

[redacted] 2007-06-01_E01_10-43-12.fsa [redacted] Identifiler_v1

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No distinct major contributor

Applied Biosystems
GeneMapper ID v3.2.1

[redacted]

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Sample File	Sample Name	Panel
[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1

[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1

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Determine if there are stochastic issues

Some loci have stochastic issues (obvious)

Applied Biosystems
GeneMapper ID v3.2.1

[redacted]

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Sample File	Sample Name	Panel
[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1
[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1

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Some loci have stochastic issues (less than 150rfu)

Applied Biosystems
GeneMapper ID v3.2.1

[redacted]

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[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1
[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1

Less than 150rfu, but 4 alleles present

Less than 150rfu, but 4 alleles present

XY

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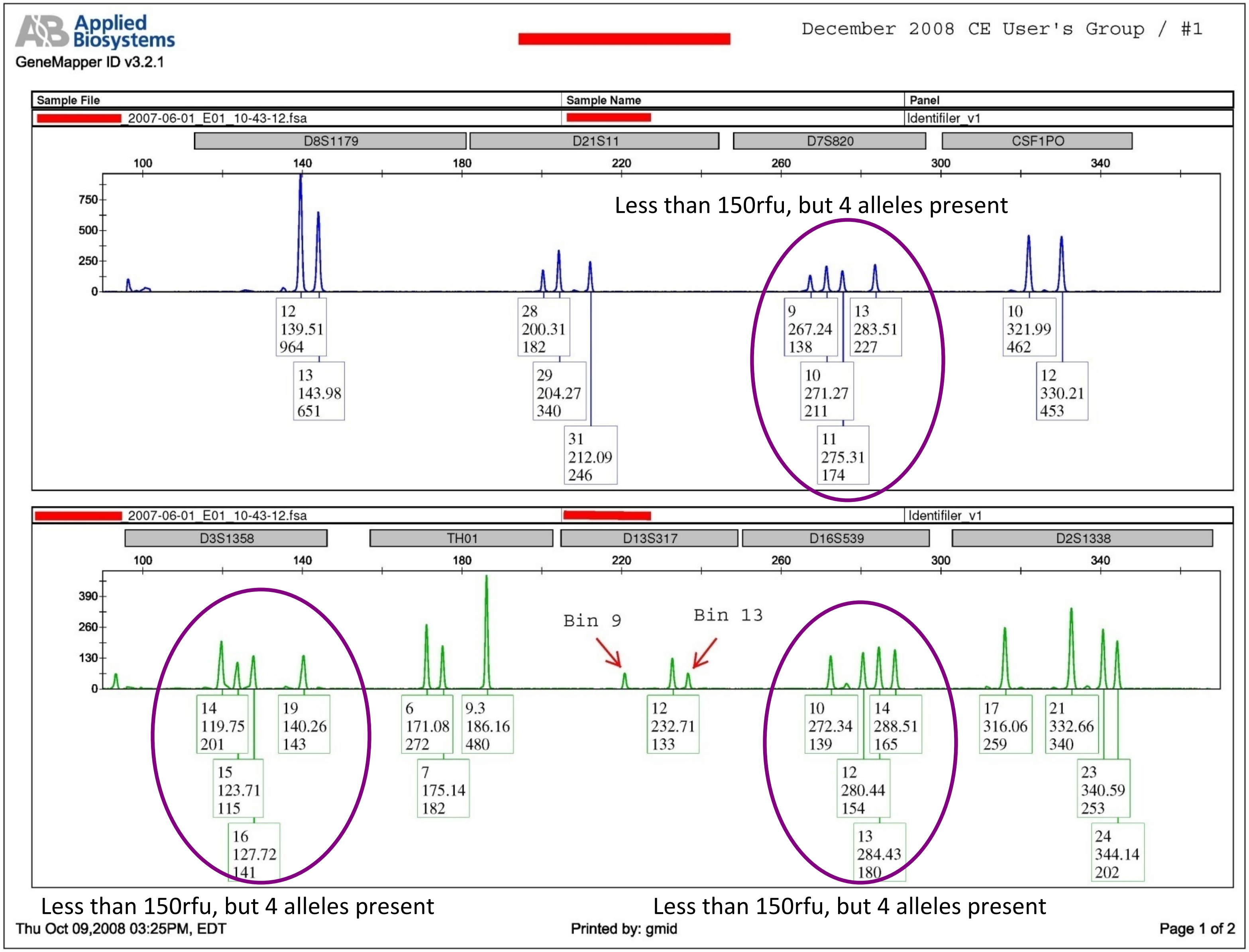
Re-examine D8-D2 for alleles less than 150rfu



Applied Biosystems
GeneMapper ID v3.2.1

[redacted]

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[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1
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Less than 150rfu, but 4 alleles present

Less than 150rfu, but 4 alleles present

Less than 150rfu, but 4 alleles present

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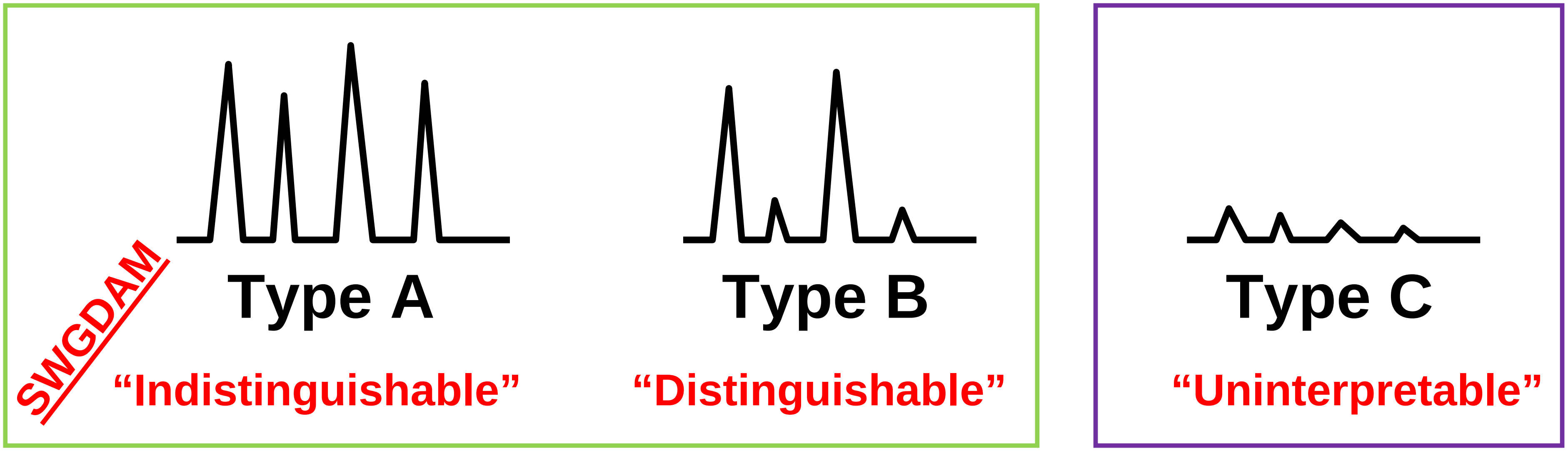
Define the mixture category

- 2 person mixture
- No distinct major contributor
- Some loci have stochastic level peaks

Schneider *et al.* (2009) and SWGDAM



SWGDAM

Type A

"Indistinguishable"

Type B

"Distinguishable"

Type C

"Uninterpretable"

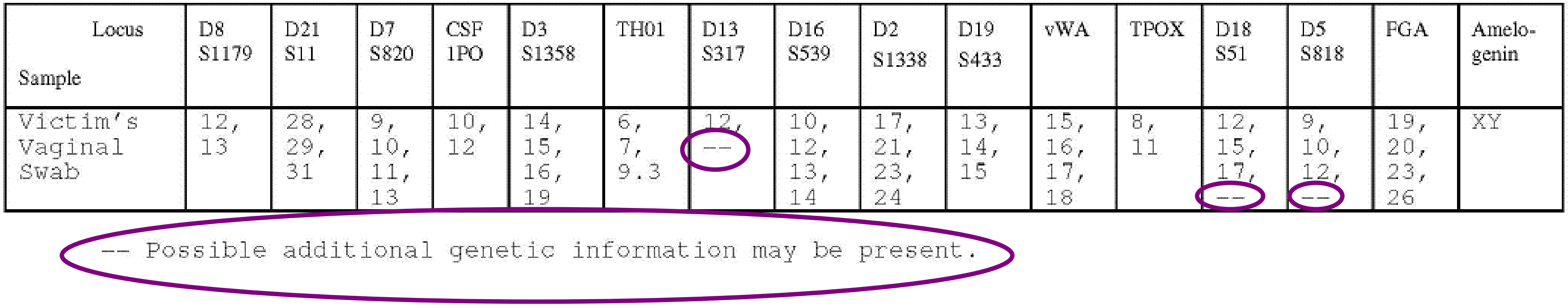
A statistical analysis must be performed

A statistical analysis should not be performed

Category A Mixture

(with some Category C loci)

Basic results chart (before looking at reference standards)



Locus Sample	D8 S1179	D21 S11	D7 S820	CSF 1PO	D3 S1358	TH01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Amelo-genin
Victim's Vaginal Swab	12, 13	28, 29, 31	9, 10, 11, 13	10, 12	14, 15, 16, 19	6, 7, 9.3	12, --	10, 12, 13, 14	17, 21, 23, 24	13, 14, 15	15, 16, 17, 18	8, 11	12, 15, 17, --	9, 10, 12, --	19, 20, 23, 26	XY

-- Possible additional genetic information may be present.

Due to the sample being Category A mixture

(with some Category C loci),

with one “known contributor”,

most likely to try

Mixture Deconvolution

Steps in the Mixture Interpretation Process

[Adapted from Clayton *et al. (1998) Forensic Sci. Int. 91:55-70*]

Step 1. Identify the Presence of a Mixture

Step 2. Designate Allele Peaks

Step 3. Identify the Number of Potential Contributors

Step 4. Estimate the Relative Ratio of the Individuals Contributing to the Mixture

Step 5. Consider All Possible Genotype Combinations

Step 6. Perform statistical analysis

Step 7. Compare Reference/Casework Samples

Overall profile appears to be 1:1 ratio

Applied Biosystems
GeneMapper ID v3.2.1

[redacted]

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[redacted] 2007-06-01_E01_10-43-12.fsa	[redacted]	Identifiler_v1

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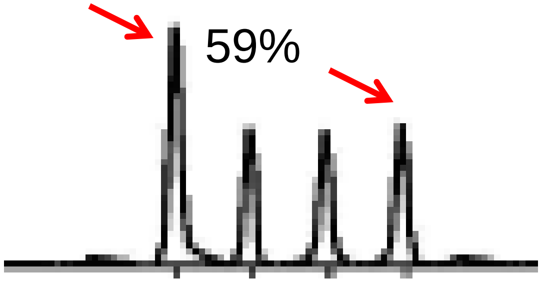
Imperfect PHR’s exist in Victim’s profile where 4 alleles are present in the mixture

- Since 4 alleles present in the mixture, and two match to the victim, the other two MUST be the “true attacker,” even if not perfectly balanced.
- vWA

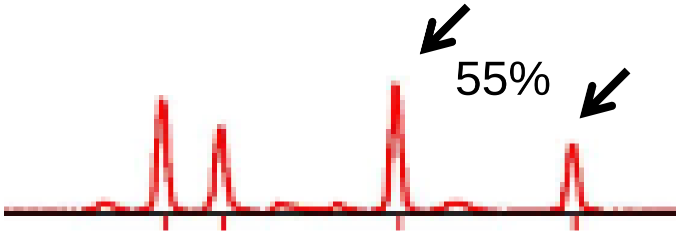


- Evidence = 15,16,17,18
- Victim = 15,18
- “true attacker” must be 16,17 even if

PHR for Victim is less than perfect (59%)

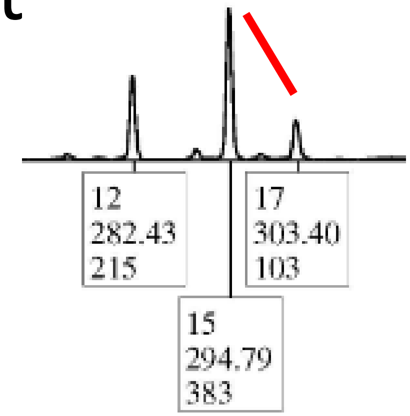
Imperfect PHR's exist in Victim's profile where 4 alleles are present in the mixture

- Since 4 alleles present in the mixture, and two match to the victim, the other two MUST be the "true attacker," even if not perfectly balanced.
- FGA
- Evidence = <u>19,20</u>,23,26
- Victim = 23,26
- "true attacker" must be <u>19,20</u> even if PHR for Victim is less than perfect (55%)

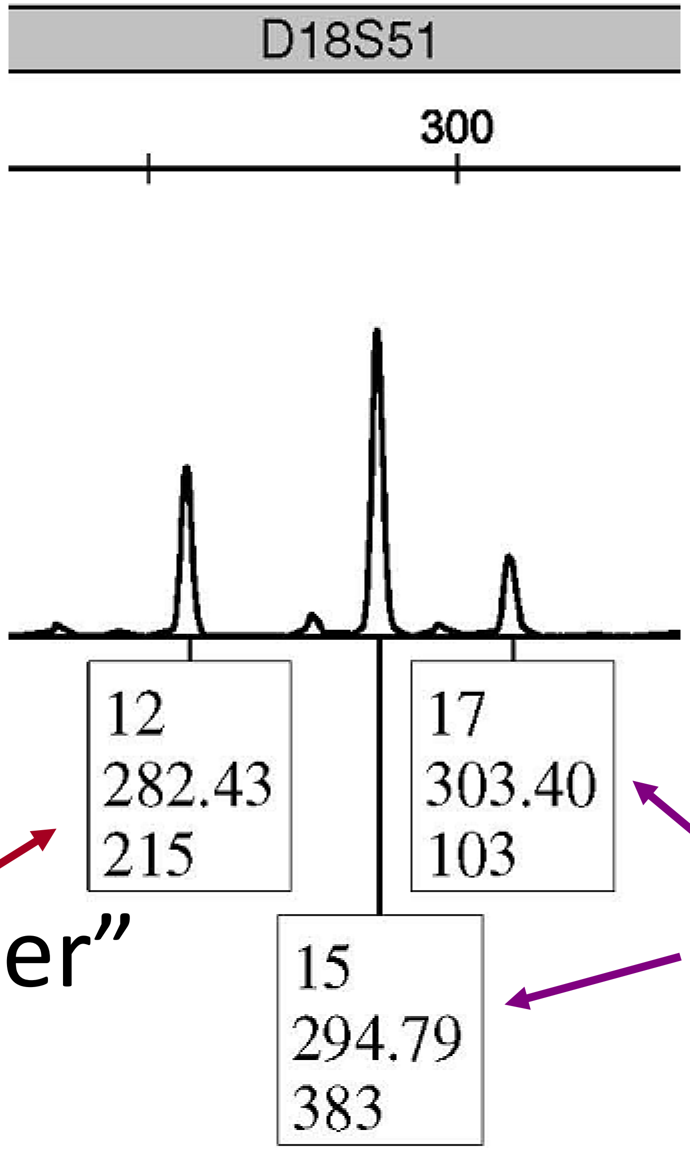


Imperfect PHR's exist in Victim's profile where 3 alleles are present in the mixture

- Since 3 alleles present in the mixture, and two match to the victim, the other one MUST belong to the "true attacker." This person may also share an allele with the Vict



- D18
- Evidence = 12,15,17,--
- Victim = 15,17
- "true attacker" must have allele 12
- "true attacker" may be 12,12 or 12,15 or 12,17 or 12,--



“True attacker”

Victim

Steps in the Mixture Interpretation Process

[Adapted from Clayton *et al. (1998) Forensic Sci. Int. 91:55-70*]

Step 1.	Identify the Presence of a Mixture
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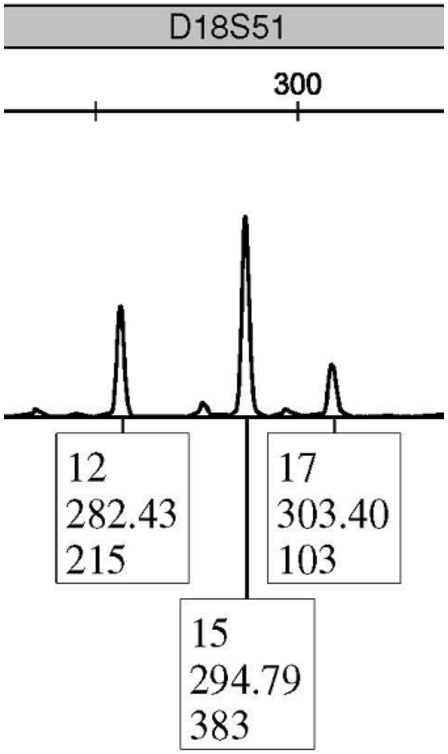
Step 3.	Identify the Number of Potential Contributors
Step 4.	Estimate the Relative Ratio of the Individuals Contributing to the Mixture
Step 5.	Consider All Possible Genotype Combinations
Step 6.	Perform statistical analysis
Step 7.	Compare Reference/Casework Samples

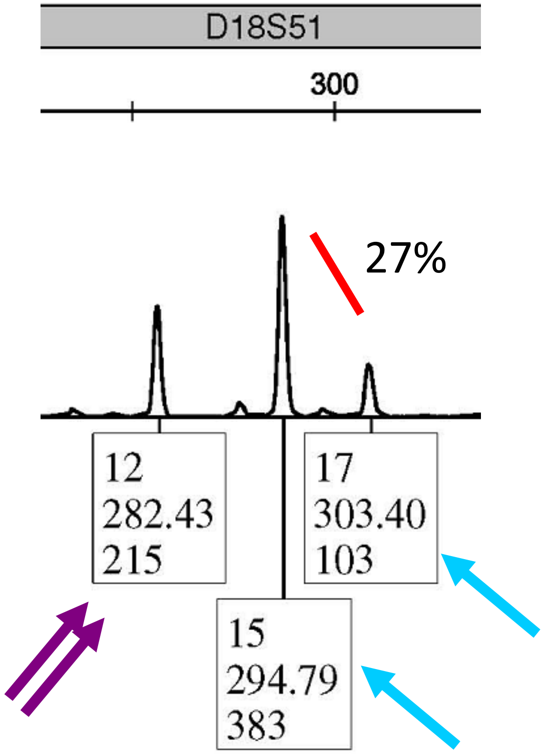
Consider all possible genotypes

- “true attacker” may be 12,12 or 12,15 or 12,17 or 12,--



Consider all possible genotypes

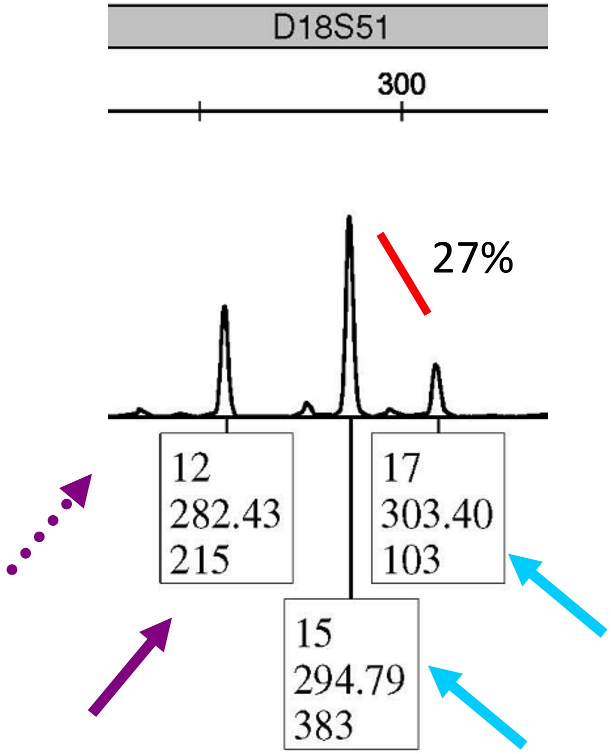
Victim
15, 17



If “true attacker” is <u>12,12</u> that leaves 15,17 entirely as the Victim with 27%PHR (103/383)

Consider all possible genotypes

Victim
15, 17

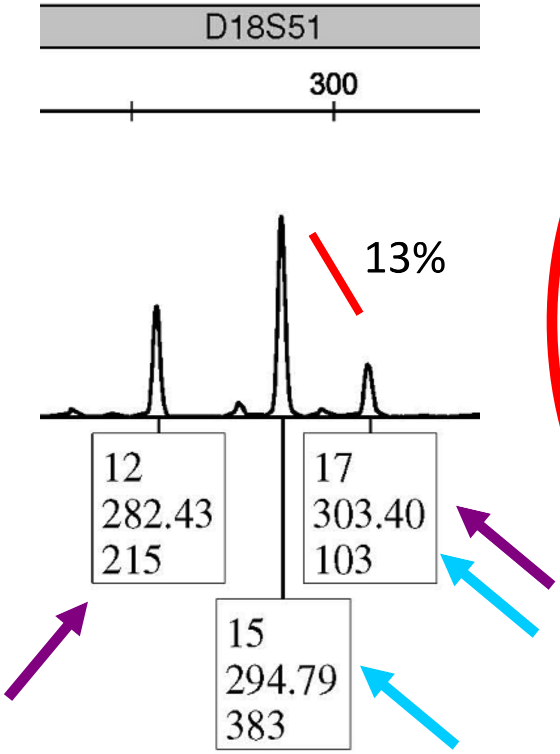


~~If "true attacker" is <u>12,--</u> that leaves 15,17 entirely as the Victim with 27%PHR (103/383)~~

~~And unreasonable to assume dropout associated with allele 12~~

Consider all possible genotypes

Victim
15, 17

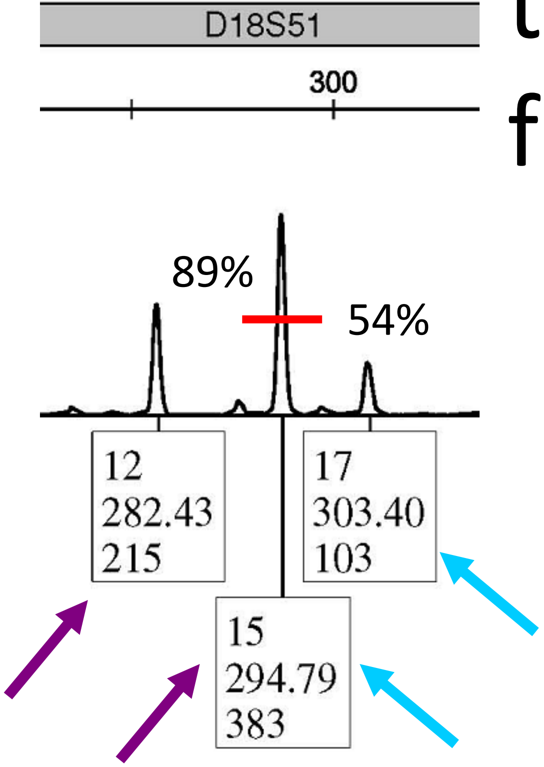


~~If "true attacker" is 12,17 then that splits the rfu value for allele 17, leaving the Victim with 13%PHR~~

~~assuming a 1:1 ratio of contributors = (103/2)/383~~

Consider all possible genotypes

Victim
15, 17

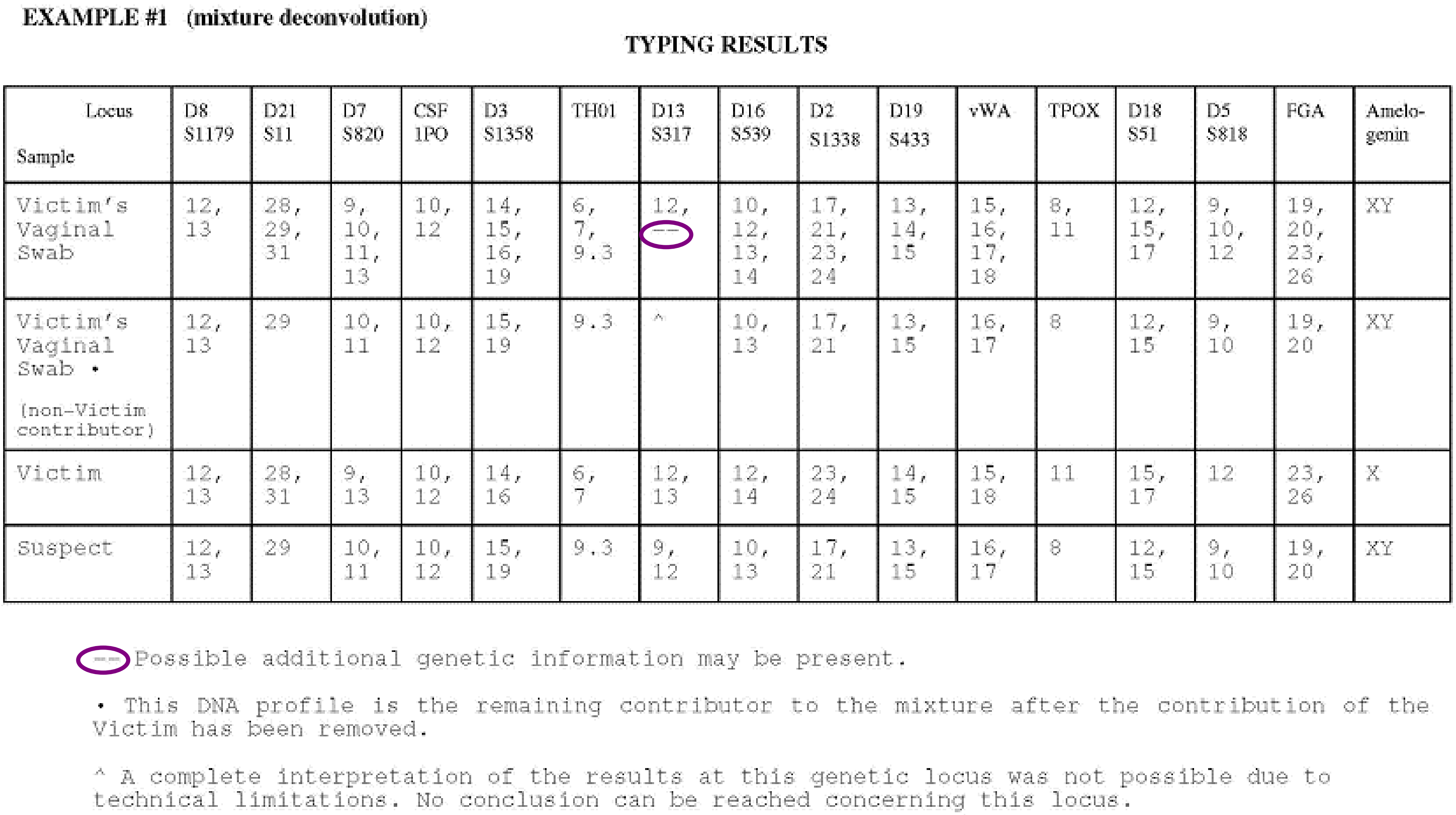


If “true attacker” is 12,15 then that splits the rfu value for allele 15,

leaving the Victim with 54%PHR {103/(383/2)}

and “true attacker” with 89%PHR {(383/2)/215}

Mixture Deconvolution results chart



EXAMPLE #1 (mixture deconvolution)

TYPING RESULTS

Locus Sample	D8 S1179	D21 S11	D7 S820	CSF 1PO	D3 S1358	TH01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Amelo-genin
Victim's Vaginal Swab	12, 13	28, 29, 31	9, 10, 11, 13	10, 12	14, 15, 16, 19	6, 7, 9.3	12, --	10, 12, 13, 14	17, 21, 23, 24	13, 14, 15	15, 16, 17, 18	8, 11	12, 15, 17	9, 10, 12	19, 20, 23, 26	XY
Victim's Vaginal Swab • (non-Victim contributor)	12, 13	29	10, 11	10, 12	15, 19	9.3	^	10, 13	17, 21	13, 15	16, 17	8	12, 15	9, 10	19, 20	XY
Victim	12, 13	28, 31	9, 13	10, 12	14, 16	6, 7	12, 13	12, 14	23, 24	14, 15	15, 18	11	15, 17	12	23, 26	X
Suspect	12, 13	29	10, 11	10, 12	15, 19	9.3	9, 12	10, 13	17, 21	13, 15	16, 17	8	12, 15	9, 10	19, 20	XY

-- Possible additional genetic information may be present.

• This DNA profile is the remaining contributor to the mixture after the contribution of the Victim has been removed.

^ A complete interpretation of the results at this genetic locus was not possible due to technical limitations. No conclusion can be reached concerning this locus.

Enter deconvoluted profile into
sole source calculation worksheet

Steps in the Mixture Interpretation Process

[Adapted from Clayton *et al. (1998) Forensic Sci. Int. 91:55-70*]

Step 1. Identify the Presence of a Mixture

↓

Step 2. Designate Allele Peaks

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Step 3. Identify the Number of Potential Contributors

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Step 4. Estimate the Relative Ratio of the Individuals Contributing to the Mixture

↓

Step 5. Consider All Possible Genotype Combinations

↓

Step 6. Perform Statistical Analysis

↓

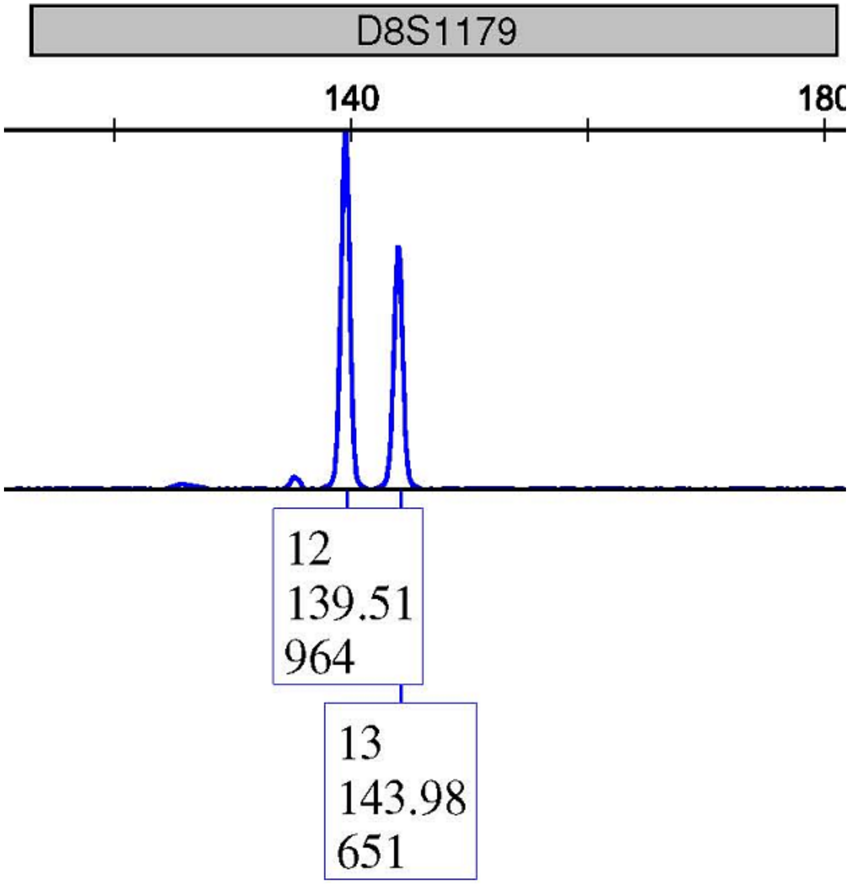
Step 7. Compare Reference/Casework Samples

Statistical Analysis

- The MDSP uses the “unrestricted likelihood ratio”

- Allele heights for shared alleles are NOT taken into consideration using this statistical method.

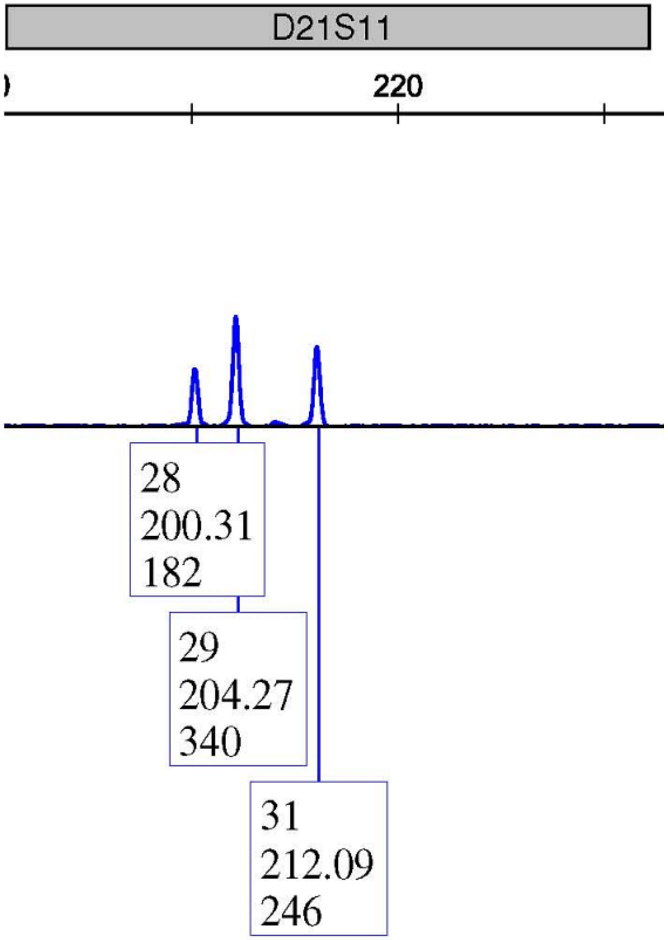
Determine alleles requisite to the attacker



Victim is 12,13

No alleles are requisite to the attacker

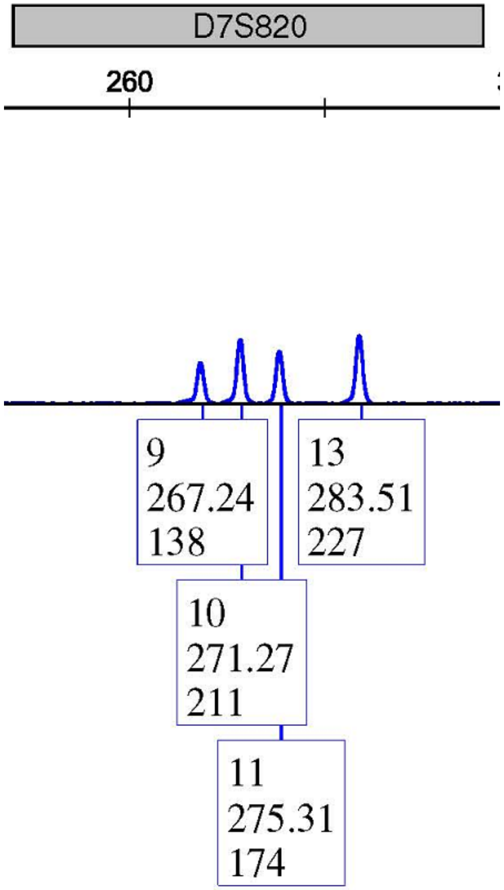
Determine alleles requisite to the attacker



Victim is 28,31

Allele 29 is requisite to the attacker

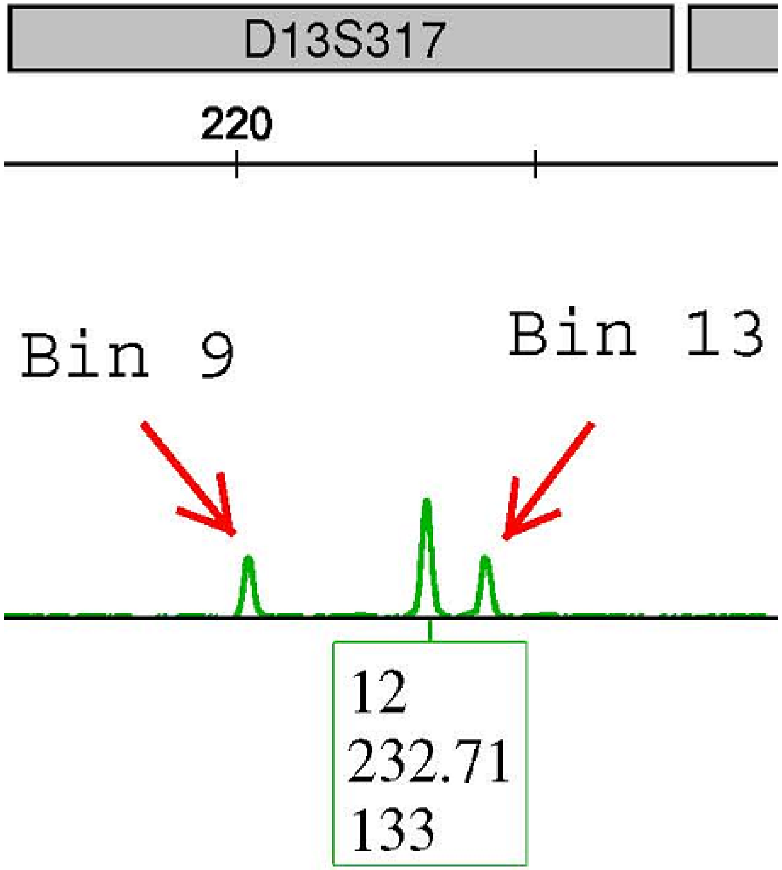
Determine alleles requisite to the attacker



Victim is 9,13

Alleles 10,11 are requisite to the attacker

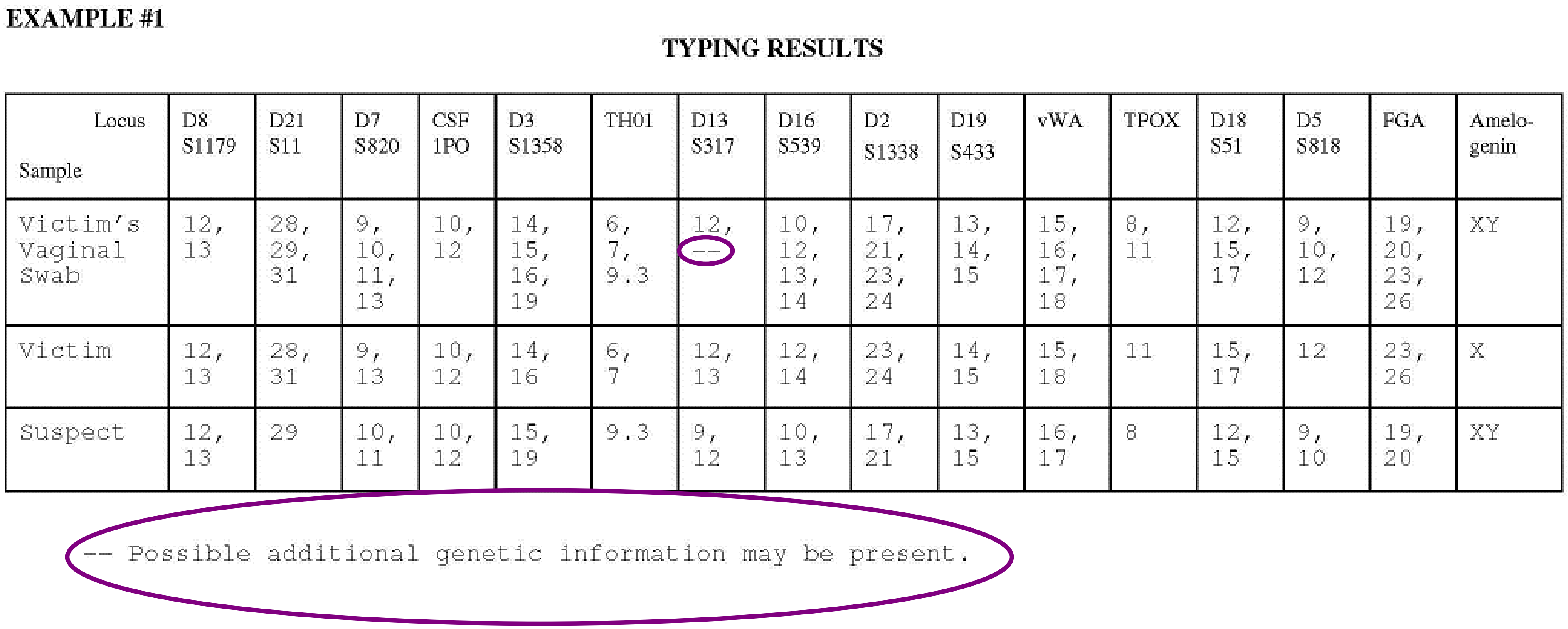
Determine alleles requisite to the attacker



Victim is 12,13
Since Victim is not fully represented, and
allele 12 is below stochastic,
DROP LOCUS

Report for
Likelihood Ratio

Likelihood Ratio results chart



EXAMPLE #1

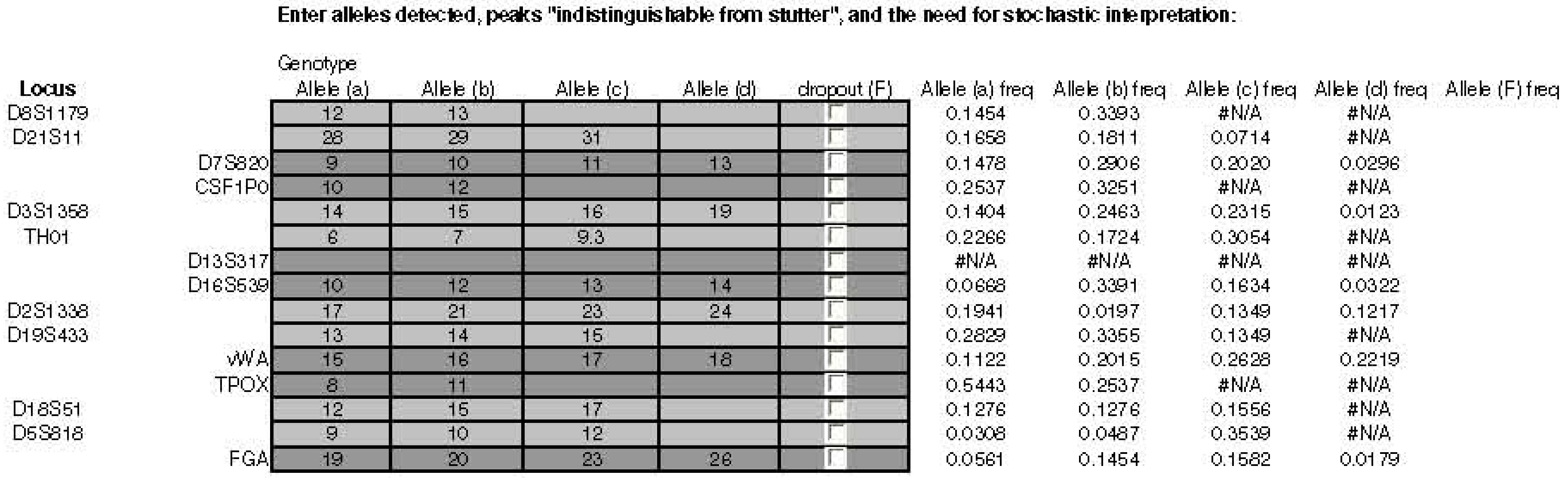
TYPING RESULTS

Locus Sample	D8 S1179	D21 S11	D7 S820	CSF 1PO	D3 S1358	TH01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Amelo-genin
Victim's Vaginal Swab	12, 13	28, 29, 31	9, 10, 11, 13	10, 12	14, 15, 16, 19	6, 7, 9.3	12, --	10, 12, 13, 14	17, 21, 23, 24	13, 14, 15	15, 16, 17, 18	8, 11	12, 15, 17	9, 10, 12	19, 20, 23, 26	XY
Victim	12, 13	28, 31	9, 13	10, 12	14, 16	6, 7	12, 13	12, 14	23, 24	14, 15	15, 18	11	15, 17	12	23, 26	X
Suspect	12, 13	29	10, 11	10, 12	15, 19	9.3	9, 12	10, 13	17, 21	13, 15	16, 17	8	12, 15	9, 10	19, 20	XY

-- Possible additional genetic information may be present.

Suspect cannot be excluded from the mixture, so proceed with LR stats.

Enter alleles of the mixture into ISFG LR worksheet

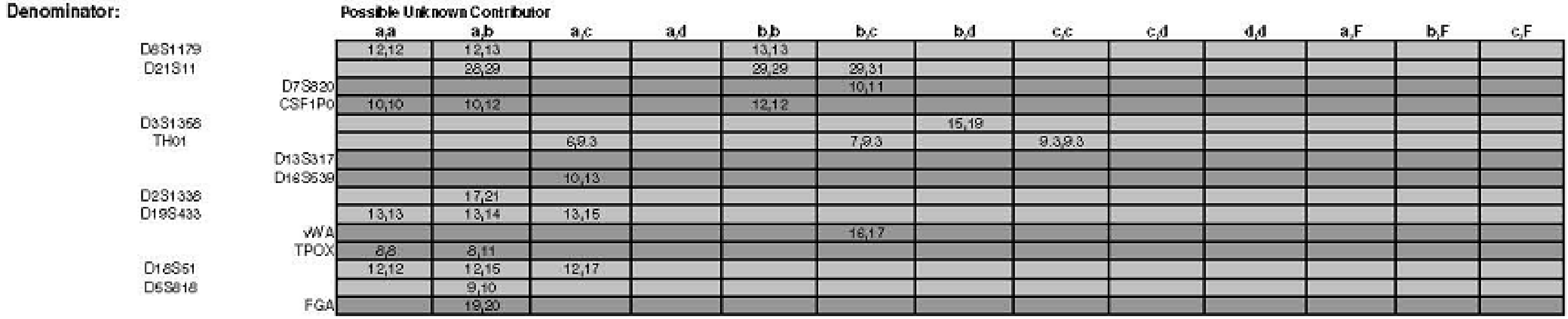


Enter alleles detected, peaks "indistinguishable from stutter", and the need for stochastic interpretation:

Locus	Genotype Allele (a)	Allele (b)	Allele (c)	Allele (d)	dropout (F)	Allele (a) freq	Allele (b) freq	Allele (c) freq	Allele (d) freq	Allele (F) freq
D8S1179	12	13			☐	0.1454	0.3393	#N/A	#N/A	
D21S11	28	29	31		☐	0.1658	0.1811	0.0714	#N/A	
D7S820	9	10	11	13	☐	0.1478	0.2906	0.2020	0.0296	
CSF1PO	10	12			☐	0.2537	0.3251	#N/A	#N/A	
D3S1358	14	15	16	19	☐	0.1404	0.2463	0.2315	0.0123	
TH01	6	7	9.3		☐	0.2266	0.1724	0.3054	#N/A	
D13S317					☐	#N/A	#N/A	#N/A	#N/A	
D16S539	10	12	13	14	☐	0.0668	0.3391	0.1634	0.0322	
D2S1338	17	21	23	24	☐	0.1941	0.0197	0.1349	0.1217	
D19S433	13	14	15		☐	0.2829	0.3355	0.1349	#N/A	
vWA	15	16	17	18	☐	0.1122	0.2015	0.2628	0.2219	
TPOX	8	11			☐	0.5443	0.2537	#N/A	#N/A	
D18S51	12	15	17		☐	0.1276	0.1276	0.1556	#N/A	
D5S818	9	10	12		☐	0.0308	0.0487	0.3539	#N/A	
FGA	19	20	23	26	☐	0.0561	0.1454	0.1582	0.0179	

Numerator has no alleles unaccounted for by the Prosecutor’s theory

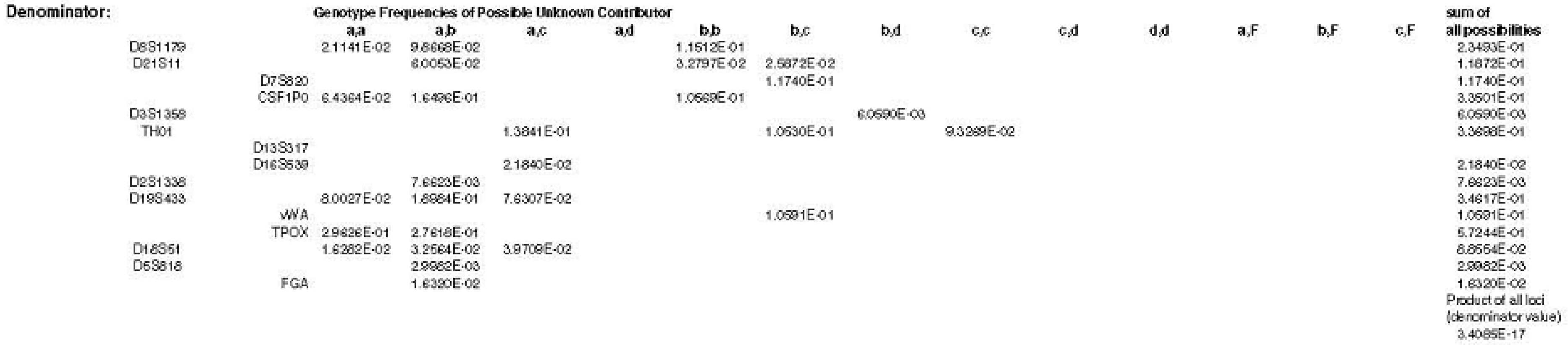
Denominator has these possibilities for the “true attacker”



Denominator:

Possible Unknown Contributor

	a,a	a,b	a,c	a,d	b,b	b,c	b,d	c,c	c,d	d,d	a,F	b,F	c,F
D8S1179	12,12	12,13			13,13								
D21S11		28,29			29,29	29,31							
D7S820						10,11							
CSF1PO	10,10	10,12			12,12								
D3S1358							15,19						
TH01			6,9.3			7,9.3		9.3,9.3					
D13S317													
D16S539			10,13										
D2S1338		17,21											
D19S433	13,13	13,14	13,15										
vWA						16,17							
TPOX	8,8	8,11											
D18S51	12,12	12,15	12,17										
D5S818		9,10											
FGA		19,20											



Denominator:

Genotype Frequencies of Possible Unknown Contributor

	a,a	a,b	a,c	a,d	b,b	b,c	b,d	c,c	c,d	d,d	a,F	b,F	c,F	sum of all possibilities
D8S1179	2.1141E-02	9.8668E-02			1.1512E-01									2.3493E-01
D21S11		6.0053E-02			3.2797E-02	2.5872E-02								1.1872E-01
D7S820						1.1740E-01								1.1740E-01
CSF1PO	6.4364E-02	1.6496E-01			1.0669E-01									3.3601E-01
D3S1358							6.0590E-03							6.0590E-03
TH01			1.3841E-01			1.0630E-01		9.3289E-02						3.3798E-01
D13S317														
D16S539			2.1840E-02											2.1840E-02
D2S1338		7.6823E-03												7.6823E-03
D19S433	8.0027E-02	1.8984E-01	7.6307E-02											3.4617E-01
vWA						1.0591E-01								1.0591E-01
TPOX	2.9626E-01	2.7618E-01												5.7244E-01
D18S51	1.6282E-02	3.2564E-02	3.9709E-02											8.8554E-02
D5S818		2.9962E-03												2.9962E-03
FGA		1.6320E-02												1.6320E-02
														Product of all loci (denominator value)
														3.4085E-17

Final LR calculation
numerator / denominator
29,338,605,880,333,700

29,000,000,000,000,000

Likelihood Ratio Conclusions

DNA from two individuals was obtained from the sperm fraction of the Victim's vaginal swab. The DNA profile present is consistent with the combined known profiles from the Victim and the Suspect.

The probability of the DNA profile at all genetic loci tested, except D13S317, is 29 Quadrillion times more likely if it originated from the Victim and the Suspect than from the Victim and an unknown individual in the Caucasian population.

The probability of the DNA profile at all genetic loci tested, except D13S317, is 330 Quadrillion times more likely if it originated from the Victim and the Suspect than from the Victim and an unknown individual in the African American population.

The genetic locus D13S317 is consistent with the Victim and the Suspect being contributors of the DNA profile obtained from this item. However, for technical considerations, this locus was not used in the above calculations.

If CPI/CPE stats used

Since statistic cannot adjust for the possibility of dropout, and does not take the number of contributors into account, any loci where alleles are below stochastic levels cannot be used in the CPI stat without modifications to the calculation.

If CPI/CPE stats used

Can use

- D8
- D21
- CSF
- TH01
- D2
- D19
- TPOX

Cannot use

- D7
- D13
- vWA
- D5
- D3
- D16
- D18
- FGA

If CPI/CPE stats used

- CPI statistics using POPSTATS

- 1 in 1,670 Caucasians included
- 99.940% Caucasians excluded

- 1 in 11,930 African Americans included
- 99.991% African Americans excluded

Acknowledgments

- Bruce Heidebrecht (Maryland State Police)

http://www.cstl.nist.gov/biotech/strbase/training.htm
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