




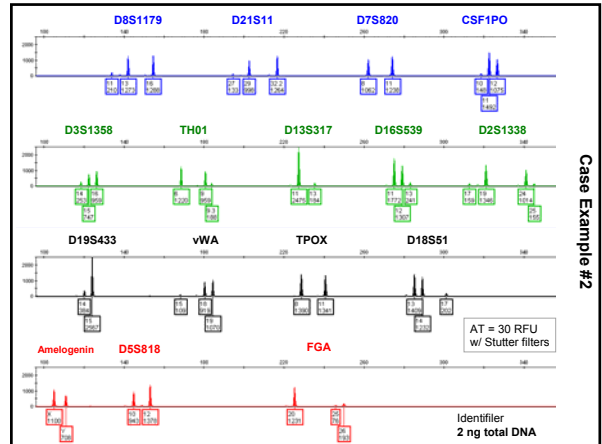
ISHI 2010 Mixture Interpretation Workshop:
Principles, Protocols, and Practice
October 11, 2010 – San Antonio, TX



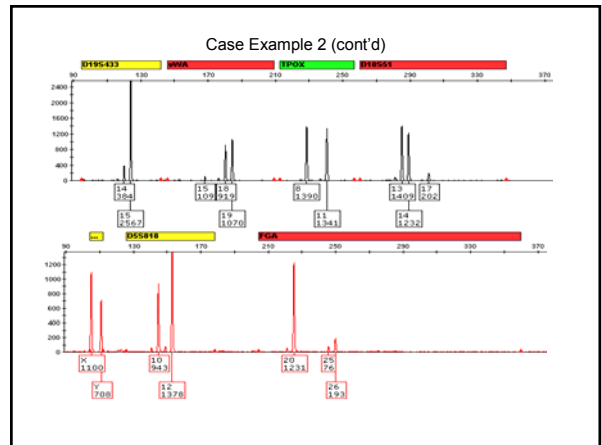
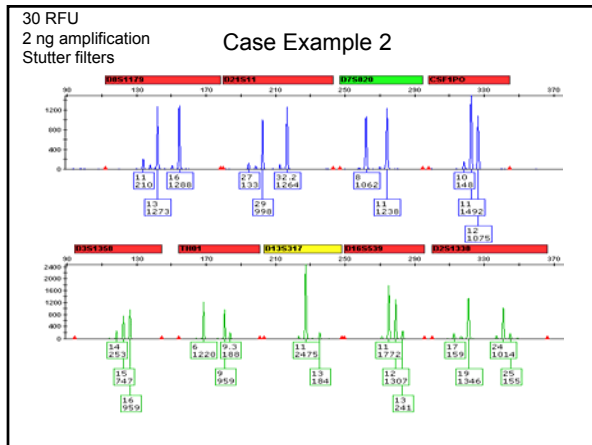
Case Example #2

Charlotte J. Word
Michael D. Coble



Case Example #2



This sample is...

1. Single-source DNA
2. Mixture of DNA from 2 people
3. Mixture of DNA from at least 2 people
4. Mixture of DNA from 3 or more people
5. None of the above

Option	Percentage
1	20%
2	20%
3	20%
4	20%
5	20%

This is a mixture of DNA from 2 people. This statement is a...

1. Fact
2. One assumption that can be used for the interpretation and reporting of the profile data
3. A falsehood

Option	Percentage
1	33%
2	33%
3	33%

Is there a distinguishable major contributor to this profile?

1. Yes
2. No
3. Not sure

Using Worksheet

- Record genotypes for **major contributor**
- Note any loci where there is a question regarding the alleles/genotype of the **major contributor**

Using Worksheet Major Contributor

PROFILE INTERPRETATION WORKSHEET IDENTIFIER

PROFILE NAME: Case Example 2 - Major

ANALYST: _____

DATE: _____

MIXTURE: yes no unsure

Analytical threshold: _____
 Stutter % used: _____
 Stochastic threshold: _____
 Peak height ratio: _____
 Comments: _____

ID LOCUS	Alleles called	Alleles above Stochastic Threshold	Stutter or other peaks to consider	Possible allele dropout? Y/N	Stochastic issues? (e.g., stutter, peak resistance, drop-in, etc.) Y/N	Degradation/Inhibition? Y/N	If mixture, restricted genotypes can be used? Y/N	Can this locus be interpreted? Y/N	Additional Comments
D8S1179	11,13,16						Y 13,16	Y	
D21S11	27,29,32,2						Y 29,32,2	Y	

The gender of the major contributor(s) is...

1. Male
2. Female
3. Not sure

Statistics for the major contributor would be best done using...

1. Random match probability (RMP)
2. Likelihood ratio (LR)
3. Combined Prob. of Exclusion (CPE)
4. RMP and/or LR
5. RMP and/or CPE
6. LR and/or CPE

RMP and LR for major contributor

Population	Genotype		Allele (P)	Allele (Q)	Allele (P) freq	Allele (Q) freq	Genotype Freq	Formula Used
Caucasian (FBI)	D8S1179	13 16	0.3393	0.0128	0.0128	0.0128	8.68E-03	2P*Q
	D21S11	29 32,2	0.1811	0.1122	0.1122	0.1122	4.069E-02	2P*Q
	D15S10	8 11	0.1626	0.2020	0.2020	0.2020	6.569E-02	2P*Q
	CSF1PO	11 12	0.3005	0.3251	0.3251	0.3251	1.953E-01	2P*Q
	D3S1358	15 16	0.2463	0.2315	0.2315	0.2315	1.144E-01	2P*Q
	TH01	6 9	0.2295	0.1650	0.1650	0.1650	7.477E-02	2P*Q
	D13S317	11 11	0.3189	0.3189	0.3189	0.3189	1.0387E-01	P*P+(1-P)*(0.01)
	D16S539	11 12	0.2723	0.3381	0.3381	0.3381	1.8487E-01	2P*Q
	D2S1338	19 24	0.1447	0.1217	0.1217	0.1217	3.523E-02	2P*Q
	D18S433	15 15	0.1349	0.1349	0.1349	0.1349	1.9357E-02	P*P+(1-P)*(0.01)
	vWA	18 19	0.2219	0.0842	0.0842	0.0842	3.739E-02	2P*Q
	TPOX	8 11	0.1443	0.2537	0.2537	0.2537	2.781E-01	2P*Q
D18S51	13 14	0.1225	0.1735	0.1735	0.1735	4.250E-02	2P*Q	
D5S818	10 12	0.0487	0.3539	0.3539	0.3539	3.444E-02	2P*Q	
FGA	20 20	0.1454	0.1454	0.1454	0.1454	2.234E-02	P*P+(1-P)*(0.01)	
Combined Freq							1.711E-19	Product of all loci used
Inverse							5.84E+18	
Random Match Probability					1 in		5.84E+18	Quintillion

LR = 1/RMP = 5.84 Quintillion

Spreadsheet from Bruce Heidebrecht

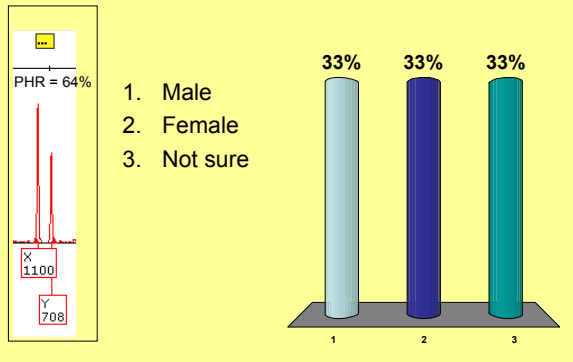
What do we know so far?

- Mixture of DNA from 2 or more contributors
- There is DNA from a major contributor and at least one minor contributor
- Major contributor is a male
- Single-source statistics for major contributor can be calculated (even without standard for comparison)

Minor Contributor(s)

- We know:
 - There is at least 1 minor contributor (but could be more)
- What can we determine:
 - Gender?
 - Obligate alleles?
 - Obligate genotypes?
 - Ratio of Major:Minor
 - Sufficient for comparison purposes?

The gender of the minor contributor(s) is...



Using Worksheet Minor Contributor(s)

- Determine which alleles are from minor contributor(s) (i.e., obligate alleles)
- Are there any labeled alleles that might be artifacts?
- Full profile or possibility of missing alleles?
- Stochastic issues possible?
- Degradation possible?
- Can any genotypes be determined?

Using Worksheet Minor Contributor(s)

- Can any genotypes be determined?

MUST consider results under two assumptions:

- 1) 2 total contributors → 1 major + 1 minor
- 2) ≥3 total contributors → 1 major + 2 or more minor contributors

Using Worksheet Minor Contributor(s)

PROFILE INTERPRETATION WORKSHEET
IDENTIFIER

PROFILE NAME: Case Example 2 - Minor

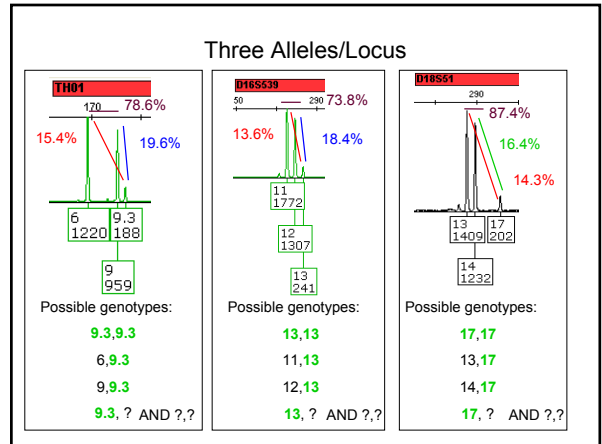
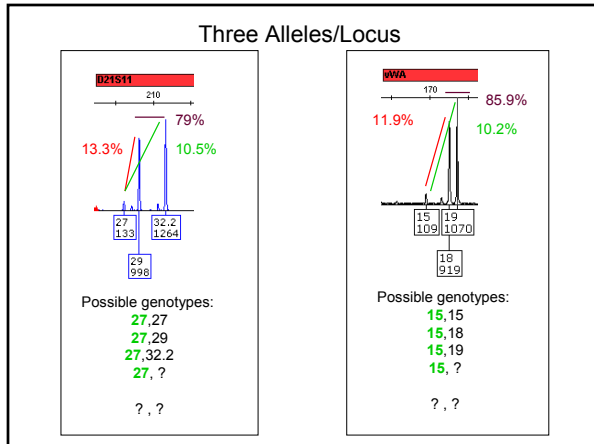
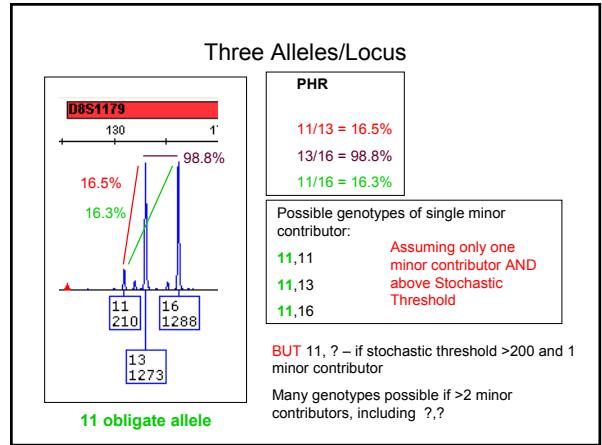
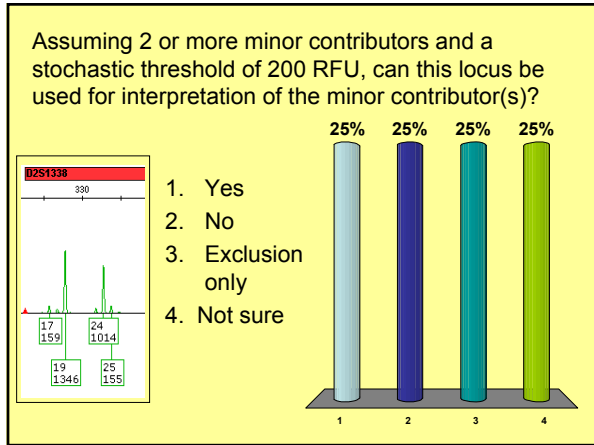
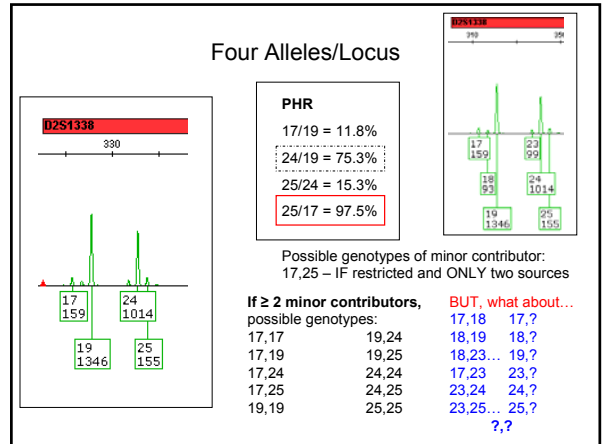
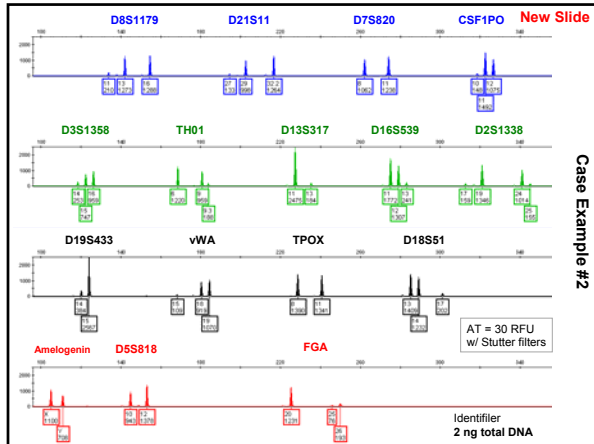
ANALYST: _____

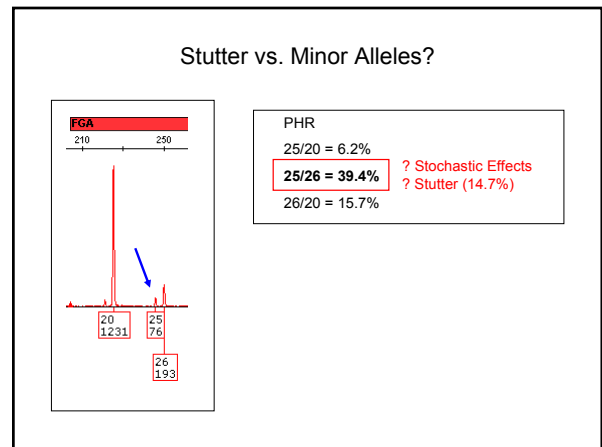
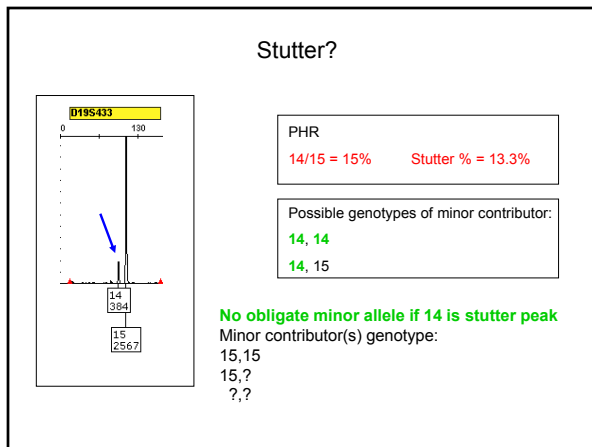
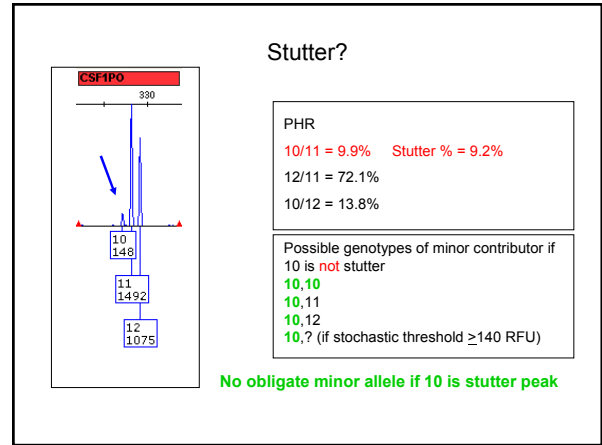
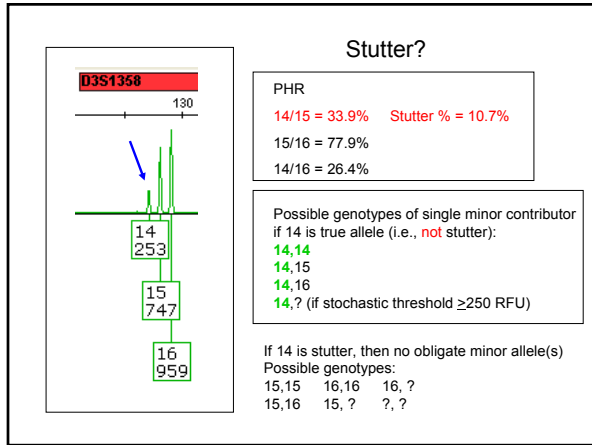
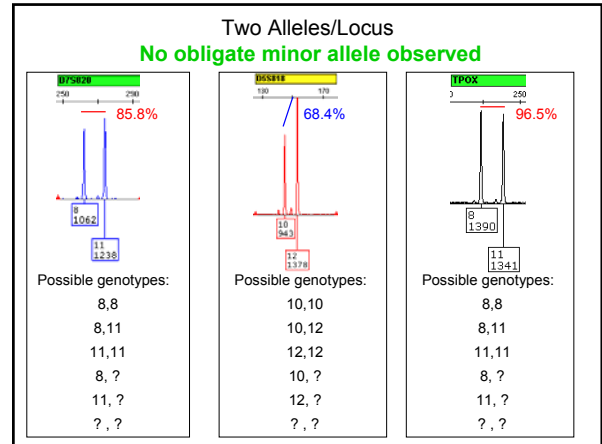
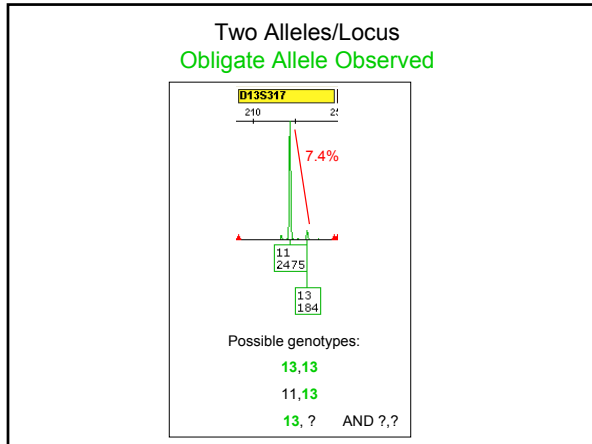
DATE: _____

MIXTURE: yes no unsure

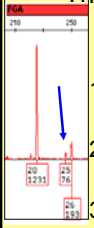
ID LOCUS	Alleles called	Alleles above Stochastic Threshold	Stutter or other peaks to consider	Possible allele dropout? Y/N	Stochastic issues? (e.g., mixed stutter, peak imbalance, drop-in, etc.) Y/N	Degradation/Inhibition? Y/N	If mixture, restricted genotypes can be used? Y/N	Can this locus be interpreted? Y/N	Additional Comments
D8S1179	11,13,16	11,13,16	12,15	?	?	N	N	?	
D21S11	27,29,32.2	29,32.2	28,31.2	Y	?	N	N	?	

Analytical threshold: _____
Stutter % used: _____
Stochastic threshold: _____
Peak height ratio: _____
Comments: _____

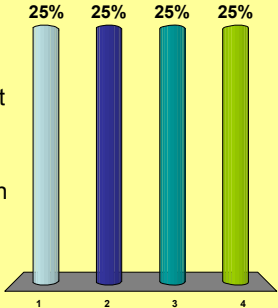




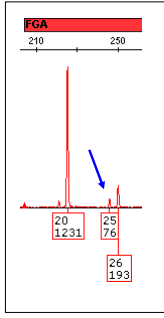
The 25 allele should be considered a ...



1. True allele (interpret)
2. Stutter peak (not interpret)
3. Not sure – help!
4. Will decide when I see the standard profile(s)



Stutter vs. Minor Alleles?



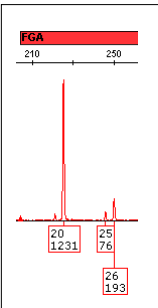
PHR

25/20 = 6.2%
25/26 = 39.4% ? Stochastic Effects
 ? Stutter (14.7%)
 26/20 = 15.7%

If assume 1 minor contributor and NO stutter, then restricted genotype is 25,26

If assume 25 is stutter, obligate allele is 26
 Possible genotypes are: 20,26 or 26,?
 depending on stochastic threshold if only one minor contributor

Stutter vs. Minor Alleles?



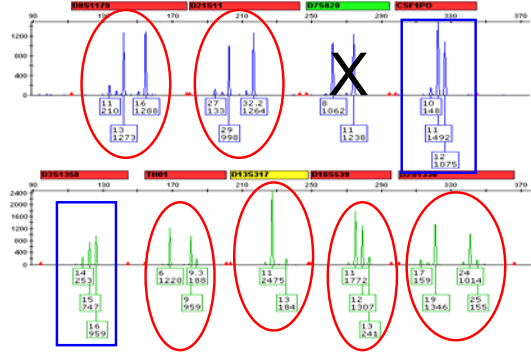
PHR

25/20 = 6.2%
25/26 = 39.4% ? Stochastic Effects
 ? Stutter (14.7%)
 26/20 = 15.7%

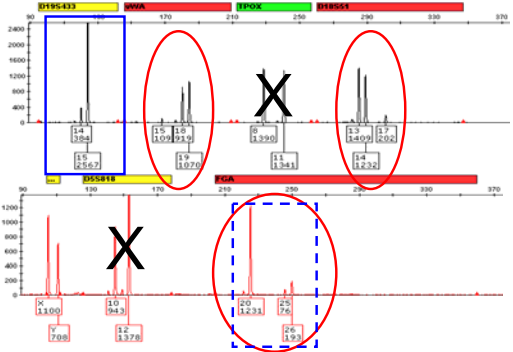
If more than 2 minor contributors:

20,20	26,26
20,25	20,?
20,26	25,?
25,25	26,?
25,26	?,?

>4:1 ratio



>4:1 ratio



Summary

- If multiple possibilities exist for the number of contributors, MUST consider data and determine possible genotypes under all reasonable possibilities (e.g., two contributors, three contributors, etc.)
- Genotype possibilities (and thus inconclusive loci) for the minor contributor increase as:
 - Peak heights approach stutter peak heights
 - Peak heights approach the stochastic threshold
 - Peak heights fall below the stochastic threshold
 - Major:minor ratio becomes more disparate
 - The number of minor contributors increases beyond 1
- Choice of stochastic threshold may have significant effect on the data that may be interpreted with these types of mixtures

Summary

- These decisions MUST be made prior to looking at any profiles from known contributors

Alleles above ST = 17, 19, 24, 25 ST = 150 RFU

Alleles below ST = none* PHR

CPI

17/18* = 58.5%
17/23* = 62.3%
18*/25 = 60.0%
23*/25 = 63.9%

$$CPI = (p + q + r + s + t + u)^2$$

$$CPI = (0.1941 + 0.0526 + 0.1447 + 0.1349 + 0.1217 + 0.0953)^2$$

$$CPI = 0.5525 \text{ or } 55.24\%$$

ST = 200 RFU

$$CPI = (p + q + r + s + t + u + v...)^2$$

$$CPI = 1$$

$$CPI = 1.0 \text{ or } 100\%$$

No assumption for the number of contributors

Alleles above ST = 17, 19, 24, 25 ST = 150 RFU

Alleles below ST = none* CPI

$$CPI = (p + q + r + s + t + u)^2$$

$$CPI = (0.1941 + 0.0526 + 0.1447 + 0.1349 + 0.1217 + 0.0953)^2$$

$$CPI = 0.5525 \text{ or } 55.24\%$$

RMP = 17, 25
RMP = 2pv
RMP = 2(0.1941)(0.0953)

$$RMP = 0.0369 \text{ or } 1 \text{ in } 27$$

Assume two contributors, 4 alleles present
no need to include stutter peaks

Alleles above ST = 17, 19, 24, 25 ST = 150 RFU

Alleles below ST = none* Restricted LR

$$\frac{P(E|H_1)}{P(E|H_2)} = \frac{V + S}{V + U}$$

$$= \frac{2(f_{19})(f_{24}) + 1}{2(f_{19})(f_{24}) + 2(f_{17})(f_{25})}$$

LR_{res} = 17, 25
LR_{res} = 1/2pv
LR_{res} = 1/2(0.1941)(0.0953)

$$LR_{res} = 27$$

Alleles above AT = 6, 9, 9.3 CPI or CPE

TH01 No assumption # of contributors

ST = 100 RFU YES
ST = 150 RFU YES
ST = 200 RFU NO
ST = 250 RFU NO

$$CPI = 6,6 + 6,9 + 6,9.3 + 9,9 + 9,9.3 + 9,3,9.3$$

$$CPI = (p + q + r)^2$$

$$CPI = (0.2266 + 0.1650 + 0.3054)^2$$

$$CPI = 1.0$$

Alleles above AT = 6, 9, 9.3 RMP

TH01 Assume 2 contributors

ST = 100 RFU YES

Genotypes = 6,9.3 + 9,9.3 + 9.3,9.3 (minor cont.)

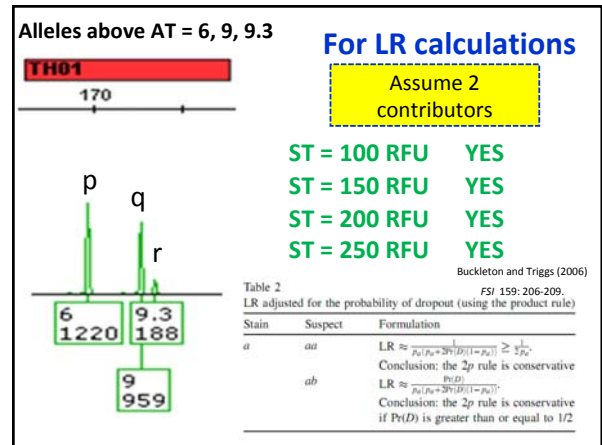
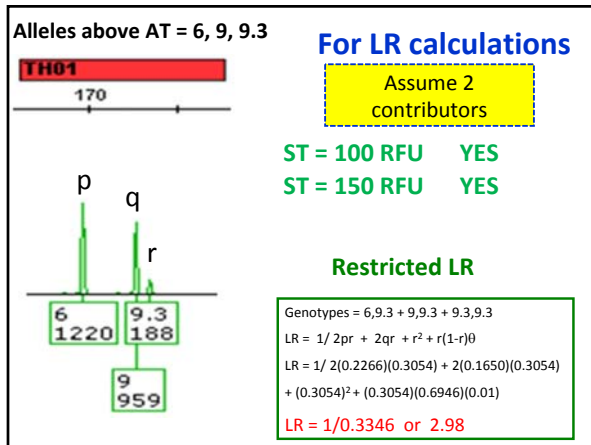
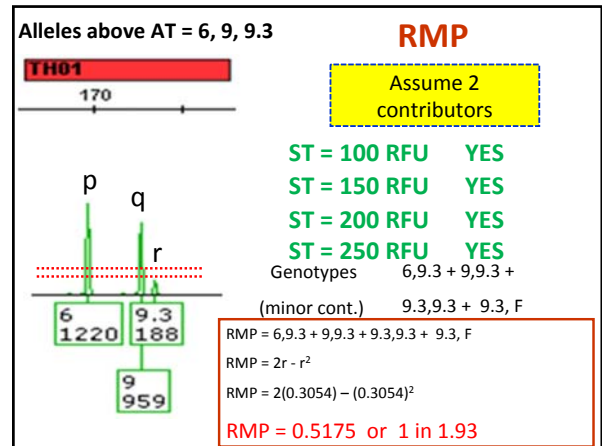
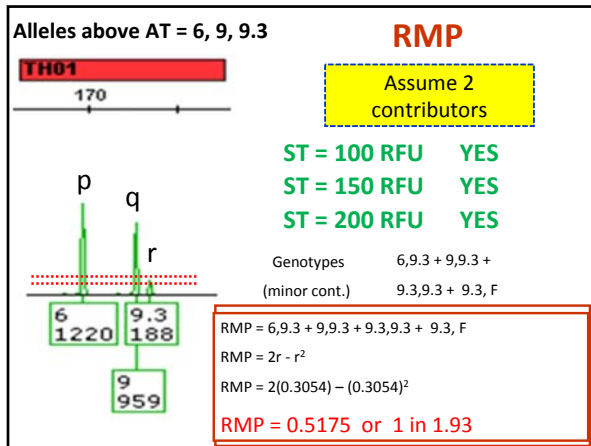
RMP = 2pr + 2qr + r² + r(1-r)θ

$$RMP = 6,9.3 + 9,9.3 + 9.3,9.3$$

$$RMP = 2pr + 2qr + r^2 + r(1-r)\theta$$

$$RMP = 2(0.2266)(0.3054) + 2(0.1650)(0.3054) + (0.3054)^2 + (0.3054)(0.6946)(0.01)$$

$$RMP = 0.3346 \text{ or } 1 \text{ in } 2.98$$



The major contributor profile matches...

- Person 1
- Person 2
- Person 3
- Person 4
- Person 5
- No one listed on the table

If we **assume** that there are ONLY 2 contributors to this mixture and therefore only 1 minor contributor, should we expect all of the minor peaks to belong to one person?

- Yes
- No
- Don't understand the question

If we assume that there is only 1 minor contributor to this mixture, Person 1 is...

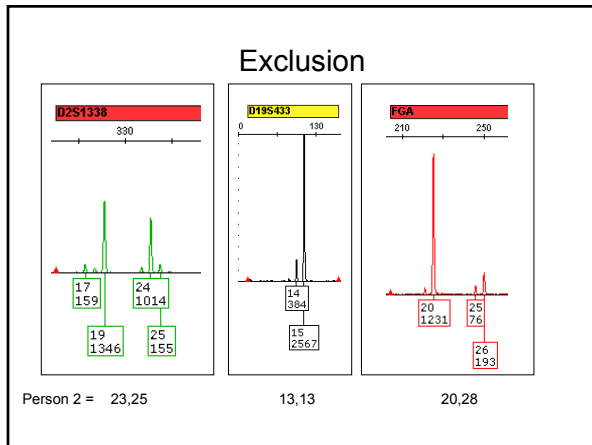
1. Excluded as a source
2. Cannot be excluded as a possible source
3. Included as a possible source
4. Inconclusive
5. Not sure

A bar chart with five bars, each labeled with a number from 1 to 5. Above each bar is the text '20%'. The bars are colored light blue, dark blue, teal, green, and grey from left to right.

If we assume that there is only 1 minor contributor to this mixture, Person 2 is...

1. Excluded as a source
2. Cannot be excluded as a possible source
3. Included as a possible source
4. Inconclusive
5. Not sure

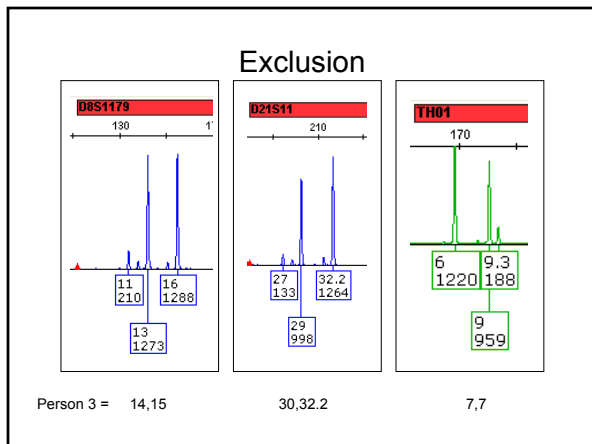
A bar chart with five bars, each labeled with a number from 1 to 5. Above each bar is the text '20%'. The bars are colored light blue, dark blue, teal, green, and grey from left to right.



If we assume that there is only 1 minor contributor to this mixture, Person 3 is...

1. Excluded as a source
2. Cannot be excluded as a possible source
3. Included as a possible source
4. Inconclusive
5. Not sure

A bar chart with five bars, each labeled with a number from 1 to 5. Above each bar is the text '20%'. The bars are colored light blue, dark blue, teal, green, and grey from left to right.



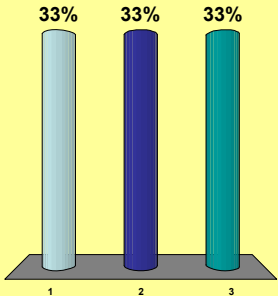
If we assume that there is only 1 minor contributor to this mixture, Person 4 is...

1. Excluded as a source
2. Cannot be excluded as a possible source
3. Included as a possible source
4. Inconclusive
5. Not sure

A bar chart with five bars, each labeled with a number from 1 to 5. Above each bar is the text '20%'. The bars are colored light blue, dark blue, teal, green, and grey from left to right.

If we assume that there are 3 or more contributors to this mixture, should we expect all of the minor peaks to belong to one contributor?

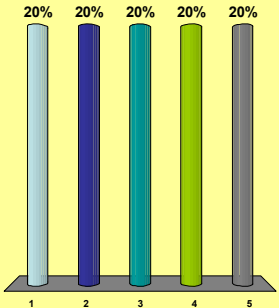
1. Yes
2. No
3. Don't understand the question



Category	Percentage
1	33%
2	33%
3	33%

If we assume that there are 3 or more contributors to this mixture, Person 1 is...

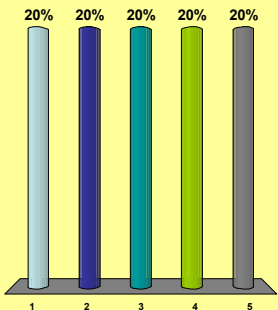
1. Excluded as a source
2. Cannot be excluded as a source
3. Included as a possible source
4. Inconclusive
5. Not sure



Category	Percentage
1	20%
2	20%
3	20%
4	20%
5	20%

If we assume that there are 3 or more contributors to this mixture, Person 2 is...

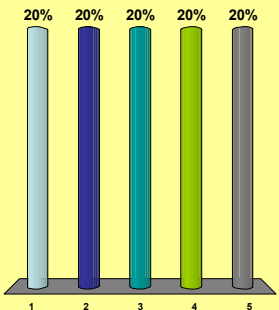
1. Excluded as a source
2. Cannot be excluded as a source
3. Included as a possible source
4. Inconclusive
5. Not sure



Category	Percentage
1	20%
2	20%
3	20%
4	20%
5	20%

If we assume that there are 3 or more contributors to this mixture, Person 3 is...

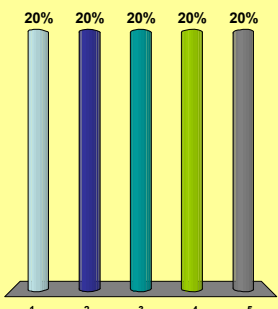
1. Excluded as a source
2. Cannot be excluded as a source
3. Included as a possible source
4. Inconclusive
5. Not sure



Category	Percentage
1	20%
2	20%
3	20%
4	20%
5	20%

If we assume that there are 3 or more contributors to this mixture, Person 4 is...

1. Excluded as a source
2. Cannot be excluded as a source
3. Included as a possible source
4. Inconclusive
5. Not sure



Category	Percentage
1	20%
2	20%
3	20%
4	20%
5	20%