Case Example #1

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Case Example 1: ID, 30 rfu, w/stutter filter
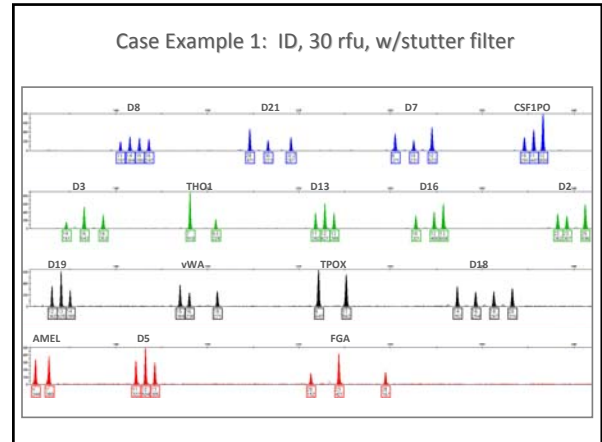
This sample is…?

1. Single-source
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. The evidence profile + my profile

20% 20% 20% 20% 20%

1 2 3 4 5

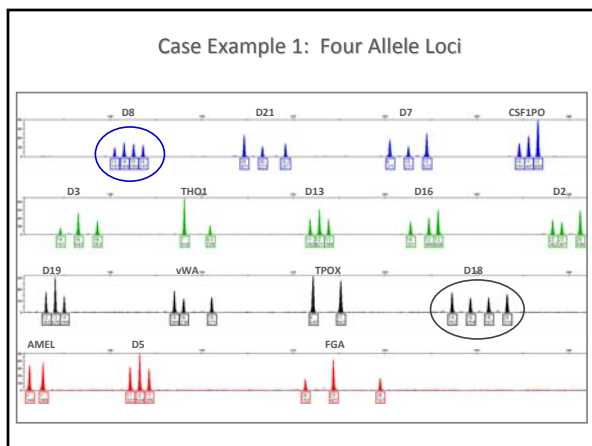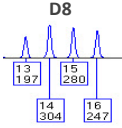## The statement "This is a mixture of DNA from 2 people" is….

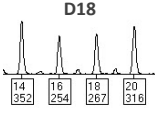1. True
2. A good starting point for the analysis
3. Only an assumption
4. Both 2 & 3
5. The answer the investigator is hoping for

20% 20% 20% 20% 20%

1 2 3 4 5

Case Example 1: Four Allele Loci

Beginning with the four allele loci and assuming two contributors can we estimate the ratio of the two contributors?

D8

| 13 | 15 |
| 197 | 280 |

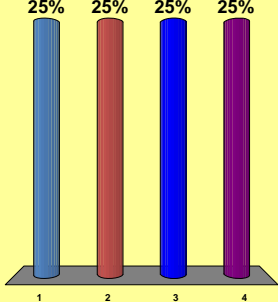| 14 | 16 |
| 304 | 247 |

D18

| 14 | 16 | 18 | 20 |
| 352 | 254 | 267 | 316 |

Possible genotype combinations →

13,14 + 15,16
13,15 + 14,16
13,16 + 14,15

&

14,16 + 18,20
14,18 + 16,20
14,20 + 16,18

Example: (rfu 13+ rfu 14) ÷ total rfu = proportion of contributor 1
(197 + 304) ÷ 1028 = 0.49

Proportion of Total rfu →

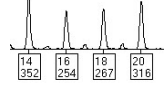0.49
0.46
or
0.43

≈

0.49
0.48
or
0.44

The average proportions of 2 contributors = 0.46 : 0.54. Is there a distinguishable major contributor to this profile?

1. Yes
2. No
3. Not sure
4. I need my computer

25%   25%   25%   25%

1   2   3   4

Consider peak height ratio at the 4 allele loci:

D18

| 14 352 | 16 254 | 18 267 | 20 316 |

possible pairs →
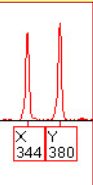
1) 14,16 + 18,20
2) 14,18 + 16,20
3) 14,20 + 16,18

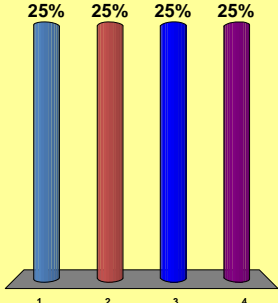Peak height ratios

1) 0.72 & 0.84
2) 0.76 & 0.80
3) 0.90 & 0.95

Based on the allowed peak height ratio, all three pairs of genotypes are possible.

Cannot restrict the possible combinations of genotypes

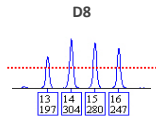**Assuming two contributors, the gender of the two contributors is likely...?**

| X 344 | Y 380 |

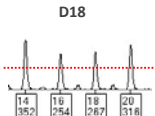1. Male + male
2. Male + Female
3. Probably M/M but cannot be certain
4. Can't know

25%   25%   25%   25%

1   2   3   4

Combined Probability of Exclusion

D8

ST = 150 RFU

D18

| 13 197 | 14 304 | 15 280 | 16 247 |

| 14 352 | 16 254 | 18 267 | 20 318 |

PI = (p + q + r + s)²
PI = (freq 13 + freq 14 + freq 15 + freq 16)
PI = (0.305 + 0.166 + 0.114 + 0.031)²
PI = (0.616)²
PI = 0.379
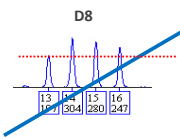
PI = (p + q + r + s)²
PI = (freq 14 + freq 16 + freq 18 + freq 20)
PI = (0.137 + 0.139 + 0.076 + 0.022)²
PI = (0.374)²
PI = 0.140

CPI = (0.379)(0.140) = 0.053
CPE = 1 − CPI = 0.947
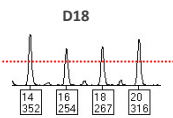
Combined Probability of Exclusion

D8

ST = 200 RFU

D18

| 13 197 | 14 304 | 15 280 | 16 247 |

| 14 352 | 16 254 | 18 267 | 20 316 |

PI = 1.0

PI = (p + q + r + s)²
PI = (freq 14 + freq 16 + freq 18 + freq 20)
PI = (0.137 + 0.139 + 0.076 + 0.022)²
PI = (0.374)²
PI = 0.140

CPI = (1.0)(0.140) = 0.140
CPE = 1 − CPI = 0.86

What do we know so far?

- Mixture of DNA from 2 or more contributors
- No evidence of degradation in the profile
- A few peaks are above analytical threshold, but below stochastic threshold
- There are no relatively small (minor) peaks except those in stutter positions
- No indication of a third contributor
  - (consider the points shown in grey above)

What do we know so far? Cont.
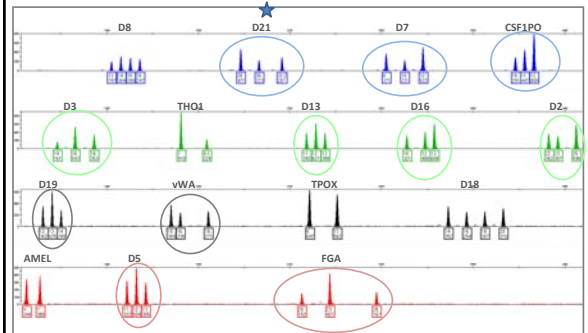
- If two contributors, the proportion of contributor 1 ≈ 0.46 and contributor 2 ≈ 0.54

- Using a stochastic threshold of:
  - 150 rfu, there are **no** peaks below the threshold
  - 200 rfu, there are **4** peaks below the threshold
  - 250 rfu, there are **8** peaks below the threshold

Case Example 1: Three Allele Loci

How will we analyze?

- <u>Assume two contributors</u> at a ratio of ≈ 1:1.
- List possible contributing genotypes.
- List possible pairs of contributing genotypes.
- Calculate the resulting peak height ratios.
- Use ½ rfu in this calculation when a peak would be shared between the two contributing genotypes.
  - (use ½ for ease of calculation today, could use range of proportions based on profile data)
  - For this exercise we are rounding the proportion of 0.46 and 0.54 to 0.5 and 0.5 ≈ 1:1.

**D21**

28	30	32.2
471	223	287

Possible Genotypes
a, a
a, b
a, c
b, b
b, c
c, c
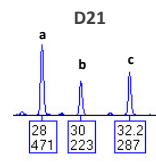
Possible Genotype Combinations

a, a + b, c	28, 28 + 30, 32.2
a, b + a, c	28, 30 + 28, 32.2
b, b + a, c	30, 30 + 28, 32.2
a, b + b, c	28, 30 + 30, 32.2
c, c + a, b	32.2, 32.2 + 28, 30
a, c + b, c	28, 32.2 + 30, 32.2

Can we rule out any of these combinations by looking at peak height ratio and incorporating the estimated ratio of contributors using the <u>two contributor assumption</u>?

D21

28	30	32.2
471	223	287

a   b   c
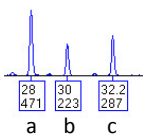
Peak Height Ratios ....

30, 32.2 = 0.78 & 28, 28
½(28), 30 = 0.95 & ½(28), 32.2 = 0.82

28, 32.2 = 0.61 & 30, 30
28, ½(30) = 0.47 & ½(30), 32.2 = 0.39

28, 30 = 0.47 & 3.2, 32.2
28, ½(32.2) = 0.31 & 30, ½(32.2) = 0.65

Possible Genotype Combinations

28, 28 + 30, 32.2 •
28, 30 + 28, 32.2 •

30, 30 + 28, 32.2 •
28, **30** + **30**, 32.2 •

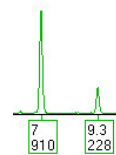32.2, 32.2 + 28, 30 •
28, **32.2** + 30, **32.2** •

28, 28 + **30, 32.2**
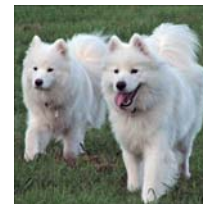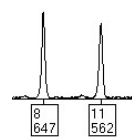
28, 30 + 28, 32.2

~~30, 30~~ + ~~28, 32.2~~

Possible Genotype Combinations

Two allele loci:

THO1

TPOX

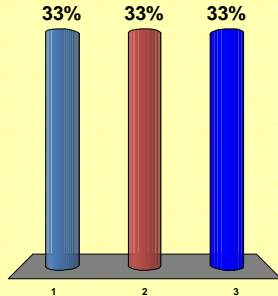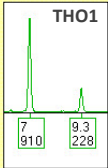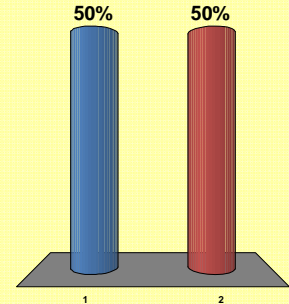| 7	9.3
910	228

8	11
647	562

Slide 1

Based on the information we have thus far, which possibility do you like most for the contributor genotypes?

1. 7, 7 + 9.3, 9.3
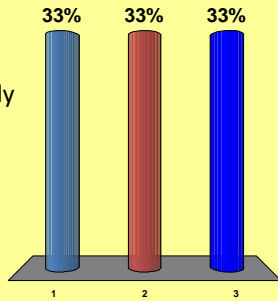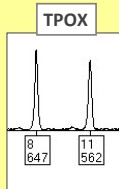2. 7, 9.3 + 7, 7
3. 7, 7 + 9.3, X

THO1

| 7 | 9.3 |
| 910 | 228 |

33%   **33%**   **33%**

1   2   3

Slide 2

Did you use the information about contributor ratio to help answer this question?

1. YES
2. NO

50%   **50%**

1   2

Slide 3

Which possibility do you like most for the contributor genotypes?

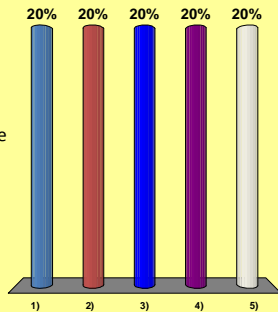1. 8, 11 + 8, 11
2. 8,8 + 11, 11
3. Both 1 & 2 are equally possible

TPOX

| 8 | 11 |
| 647 | 562 |

33%   **33%**   **33%**

1   2   3

Slide 4

Deducing a Second Contributor

Slide 5

If we want to deduce a second contributor, what do we need in order to do this?

1) A magician
2) An intimate sample from one contributor
3) A known profile from the same contributor
4) 2 and 3
5) None of the above

20%   **20%**   **20%**   **20%**   **20%**

1)   2)   3)   4)   5)

Slide 6

Assuming two contributors and one of them is known and assumed to be in the mixture:

- 4 alleles observed:
  - Known is heterozygous ⟶ deduce 2nd person

- 3 alleles observed:
  - Known is homozygous ⟶ deduce 2nd person
  - Known is heterozygous ⟶ ? Have obligate allele of 2nd person

- 2 alleles observed:
  - Known is homozygous ⟶ ? Have obligate allele of 2nd person
  - Known is heterozygous ⟶ May have no information

Slide 1

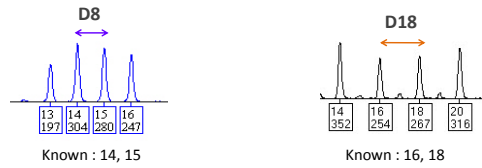**Four allele locus, assume two contributors:**

p   q   r   s
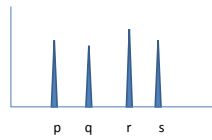
4 alleles are observed:

Known is heterozygous ⟶ deduce 2nd person
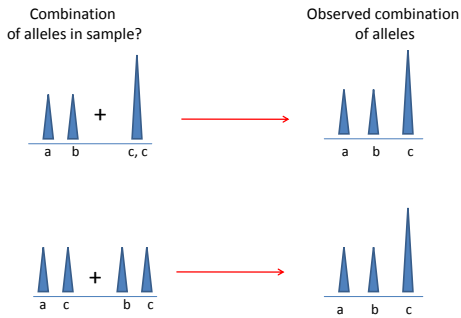
Known is: p, q ⟶ 2nd person is: r, s

Slide 2

Four Allele Loci

D8

| 13 197 | 14 304 | 15 280 | 16 247 |

Known : 14, 15

D18

| 14 352 | 16 254 | 18 267 | 20 316 |

Known : 16, 18

Can calculate the proportion of this contributor

Known Proportion = 0.57

Deduced 2nd contributor:
13, 16

Known proportion = 0.44

Deduced 2nd contributor:
14, 20

Slide 3

Three allele loci, assume 2 contributors and approximately 1:1 mixture

Combination of alleles in sample?

a   b   c, c

Observed combination of alleles

a   b   c

a   c   b   c

a   b   c

Slide 4

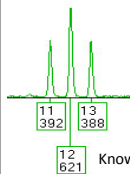**Three allele loci: known homozygous**

D5

| 11 322 | 12 504 | 13 305 |
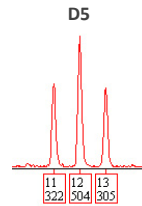
Deduced 2nd contributor = 11, 13

Peak height ratio (11, 13) = 0.94 &
Contributor ratio = 0.45

Contributor ratio ~ 1:1

Known : 12, 12

Easy!!

Slide 5

Three allele loci: Known heterozygous

D 13

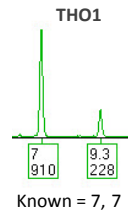| 11 392 | 13 388 |
| 12 621 |

Known : 11, 12

Contributor ratio ~ 1:1

Obligate allele of 2nd contributor = 13

13, 13

Second contributor = 11, 13

12, 13

known	2nd	PR (K)	PR(2nd)	Proportion K & 2nd
11, 12	13, 13	0.63	NA	0.72 &0.28
11, 12	11, 13	0.32	0.51	0.58 & 0.42
11, 12	12, 13	0.79	0.80	0.50 & 0.50

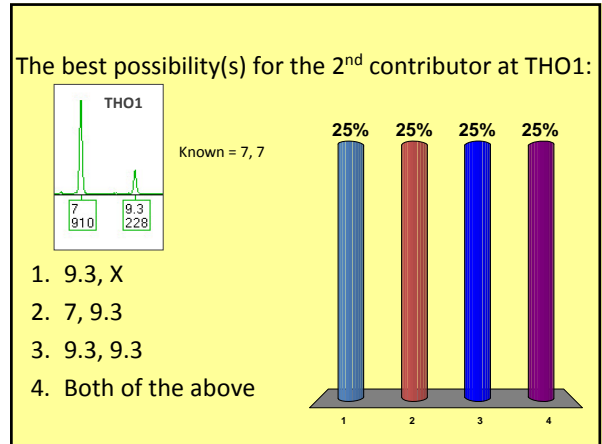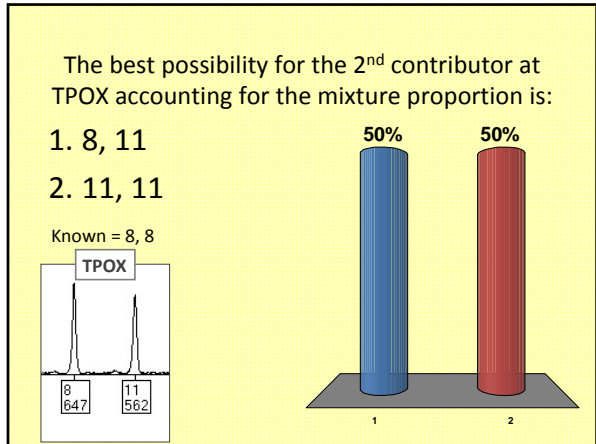Used ½(rfu) as estimate for shared allele rfu in this example.

Slide 6

Two allele loci:

THO1

| 7 910 | 9.3 228 |

Known = 7, 7

Second contributor = ?

TPOX

| 8 647 | 11 562 |

Known = 8, 8

The best possibility for the 2nd contributor at TPOX accounting for the mixture proportion is:

1. 8, 11
2. 11, 11

Known = 8, 8

TPOX

| 8 | 11 |
| 647 | 562 |

50%   50%

1   2

The best possibility(s) for the 2nd contributor at THO1:

THO1

Known = 7, 7

| 7 | 9.3 |
| 910 | 228 |

1. 9.3, X
2. 7, 9.3
3. 9.3, 9.3
4. Both of the above

25%   25%   25%   25%

1   2   3   4
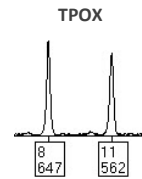
Deducing a Second Contributor

- Need a specified process that takes into account mixture proportion data from the profile and the range of allowable peak height ratio

- Computer processes are needed and available to test