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DNA Mixture Interpretation Webcast
April 12, 2013

http://www.nist.gov/oles/forensics/dna-analyst-training-on-mixture-interpretation.cfm

http://www.cstl.nist.gov/strbase/mixture.htm

FORENSICS@NIST

Worked Examples
&
Report Wording

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Process to mixture analysis

1) Look at overall e-gram to make assumptions of number of contributors, ratio of contributors, and if the mixture fits the lab's criteria for major/minor determinations.

2) Identify which alleles are below the stochastic threshold and therefore might have dropout at that locus.

3) For loci without unambiguous minor alleles, determine if minor contributor is reasonable to be considered masked by major, or might be dropping out completely.

4) Analyze mixture for peaks that are "indistinguishable from stutter." ("IFS")

Process to mixture analysis

- At this point, the analysis of the sample may be complete, dependent upon choice of statistics.

  - At this point, all loci should be identified as being useable for major/minor contributor(s) or CPE/CPI statistics.

  - All of this is done independently of the reference standards.

  - The application of which loci are useful for statistics utilizing assumptions (e.g. LR, RMP, and mixture deconvolution) may be influenced by the reference standard of the "known contributor."

Process to mixture analysis

5) Compare any reference standards that are to be considered "known" to the mixture (e.g. victim on own vaginal swab).

6) If doing stats involving a "known" contributor, re-evaluate non-known contribution to mixture for possible dropout and "indistinguishable from stutter".

7) NOTE: this re-evaluation is done without consideration of the probative reference standard.

Process to mixture analysis

8) Compare any reference standards that are to be considered probative to the mixture (e.g. suspect on victim's vaginal swab). If the probative reference standard is excluded from the mixture, declare an exclusion.

9) If the probative reference standard is not excluded from the mixture, determine the weight of that statement using statistics.

Process to mixture analysis

10) If statistics cannot be applied to support a statement of non-exclusion, then the probative reference standard can not be included, but *might* be able to be excluded, as a potential contributor to the mixture.

If can not exclude, but can not statistically support an inclusion, the association of the individual to the evidence is inconclusive.

2 person mixture, data below the stochastic threshold, reasonable to assume dropout

D21S11

30.2
423

31
446

31.2
79
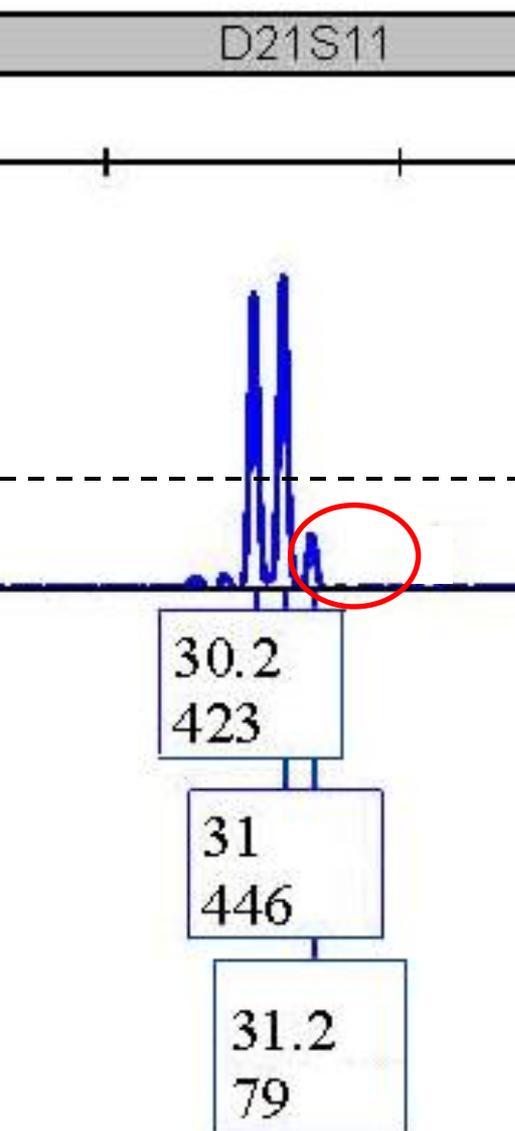
- Minor contributor has one detected allele (31.2) below stochastic threshold.
- Reasonable to assume sister allele to the 31.2 may be below the analytical threshold.
- Major alleles 30.2,31 are well balanced (95%PHR) … no indication that a sister allele to the 31.2 must be masked by major contributor.
- Include / exclude to the major based upon genotype 30.2,31 Easy, no need to discuss…
- Include / exclude to the minor based upon a requisite allele 31.2

2 person mixture, data below the stochastic threshold, reasonable to assume dropout

D21S11

30.2
423

31
446

31.2
79
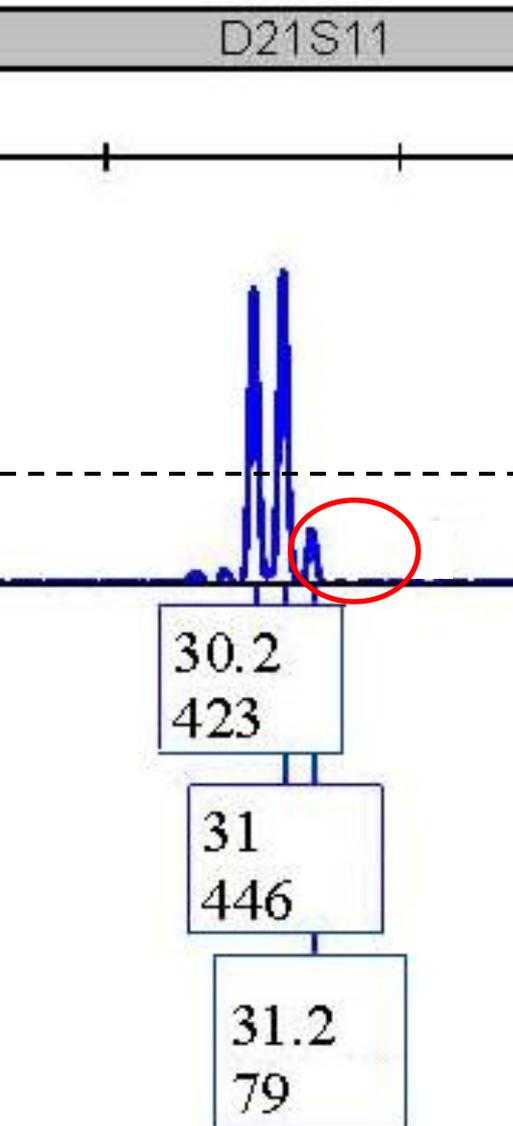
- RMP to probative minor contributor:

  $2P_{(31.2)}$

- Conclusion statements:

  DNA from two contributors was obtained from the evidence.

  John Q. Suspect cannot be excluded as the minor contributor of this mixture.

  The probability of selecting an unrelated individual at random who cannot be excluded as the minor contributor to the DNA profile obtained from this item is approximately: 1 in 5

2 person mixture, data below the stochastic threshold, reasonable to assume dropout
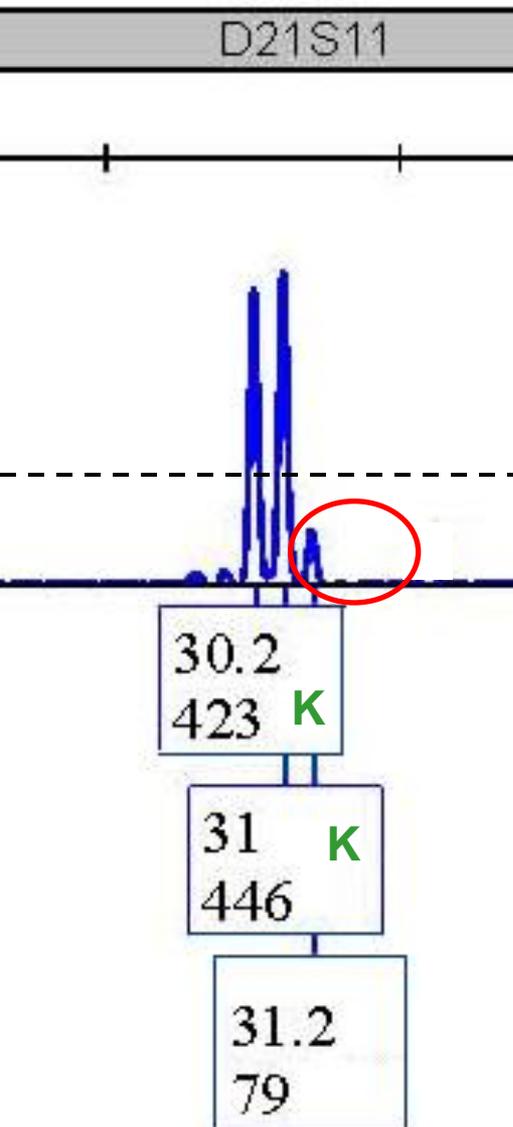
- "Known contributor" = major contributor.

- Likelihood Ratio for probative minor contributor:

$$1 / 2P_{(31.2)}$$

- Conclusion statements:

DNA from two individuals was obtained from the evidence.

Assuming the presence of Jane K. Victim, the DNA profile is approximately 5 times more likely to occur if it originated from Jane K. Victim and John Q. Suspect than from Jane K. Victim and an unknown individual in the Caucasian population.

2 person mixture, data below the stochastic threshold, reasonable to assume dropout
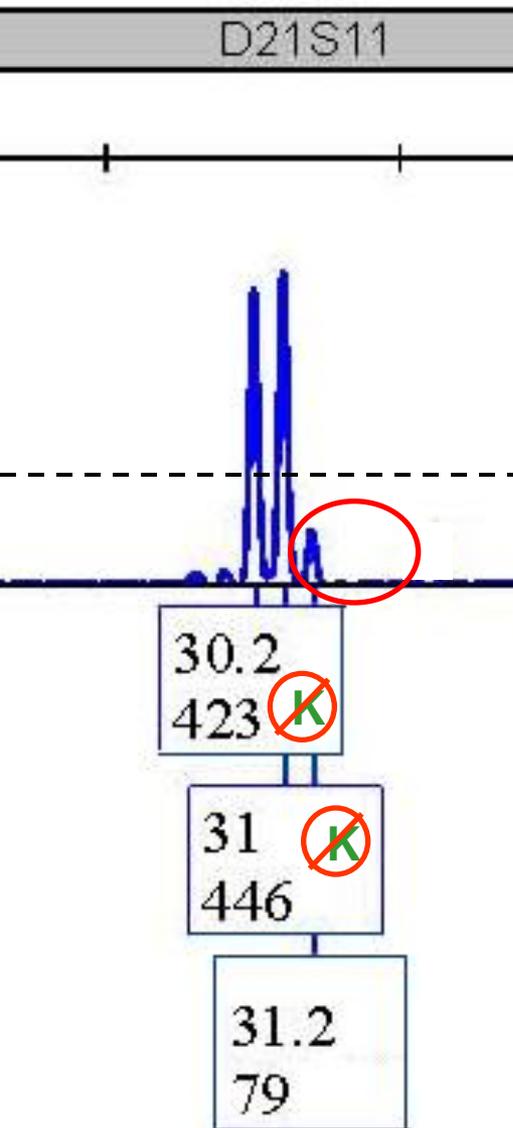
- CPI:

    Cannot perform CPI stats on the minor component due to data below the stochastic threshold.

- Conclusion statements:

    The minor component of the DNA profile obtained from this item does not satisfy the laboratory's inclusionary reporting criteria.

2 person mixture, data below the stochastic threshold, reasonable to assume dropout?

D21S11

30.2
423

31   K
446

31.2
79   K
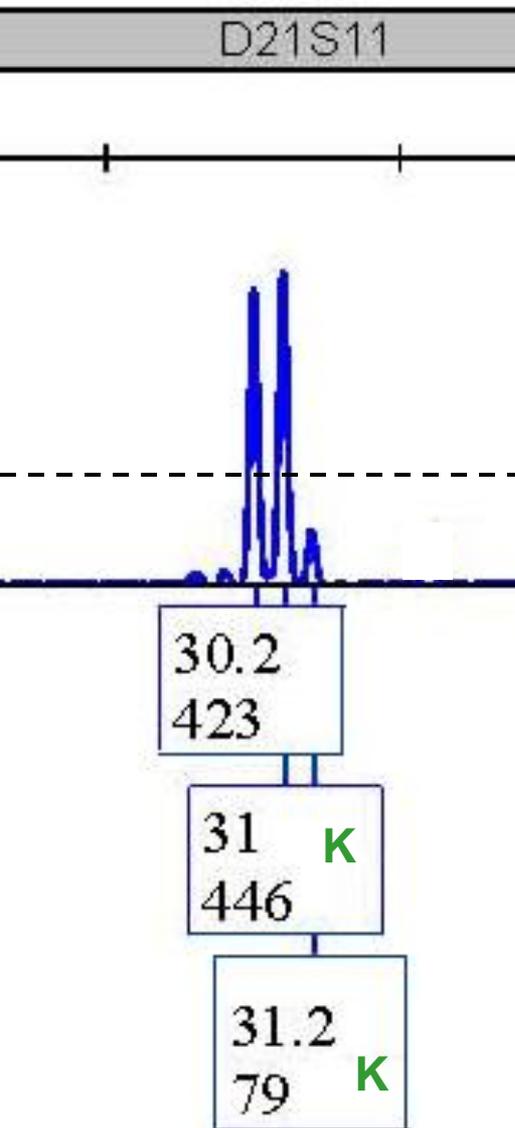
- What if minor contributor is "known"?
- Victim's fingernail scrapings. Victim's profile is 31,31.2.
- Dropout of the minor contributor is not happening. Victim's DNA is just at low levels.
- This decision is made based upon knowledge of the known contributor's profile in comparison to the mixture.
- This decision is not made based upon any probative reference profile.

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout
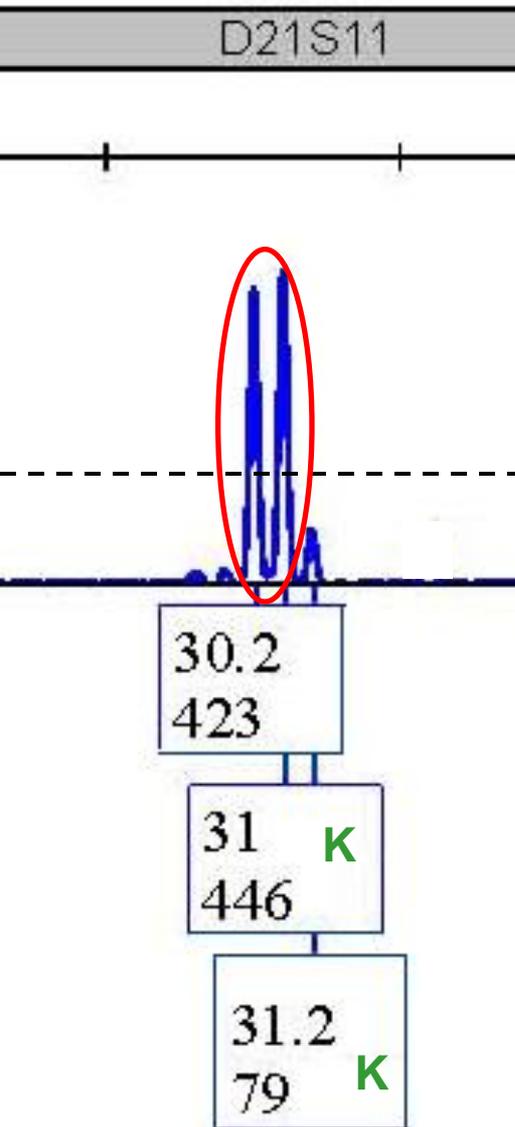
- RMP to probative major contributor:

  $$2P_{(30.2)}\, P_{(31)}$$

- Conclusion statements:

  DNA from two contributors was obtained from the evidence.

  John Q. Suspect cannot be excluded as the major contributor of this mixture.

  The probability of selecting an unrelated individual at random who cannot be excluded as the major contributor to the DNA profile obtained from this item is approximately: 1 in 180

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

D21S11

30.2
423

31   K
446

31.2
79   K
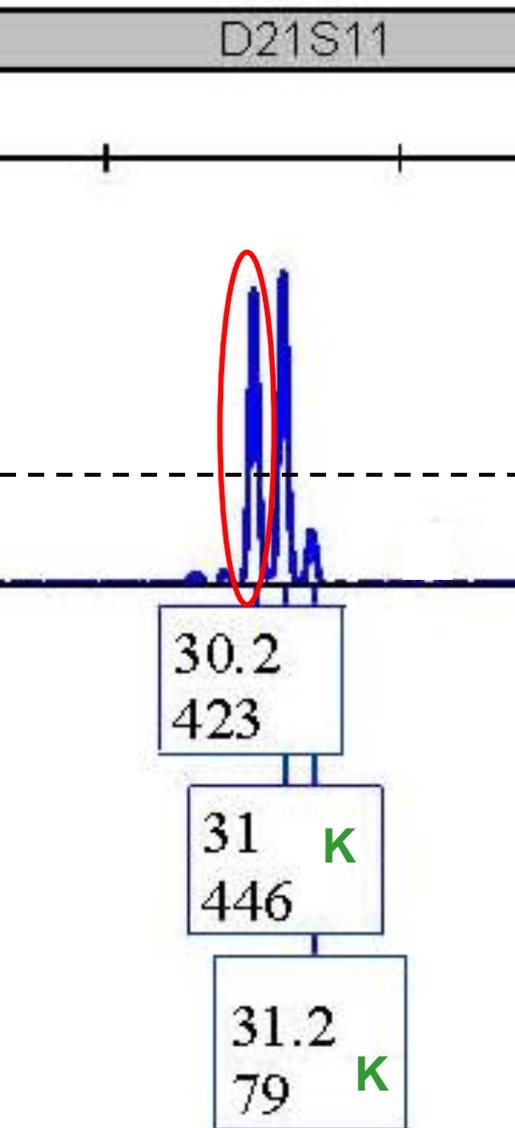
- Unrestricted Likelihood Ratio:

$$1 / \{ [2P_{(30.2)} P_{(31)}] + [2P_{(30.2)} P_{(31.2)}] + [P_{(30.2)}]^2 \}$$

- Conclusion statements:

DNA from two individuals was obtained from the evidence.

Assuming the presence of Jane K. Victim, the DNA profile is approximately 68 times more likely to occur if it originated from Jane K. Victim and John Q. Suspect than from Jane K. Victim and an unknown individual in the Caucasian population.

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

D21S11

30.2
423

31   K
446

31.2
79   K
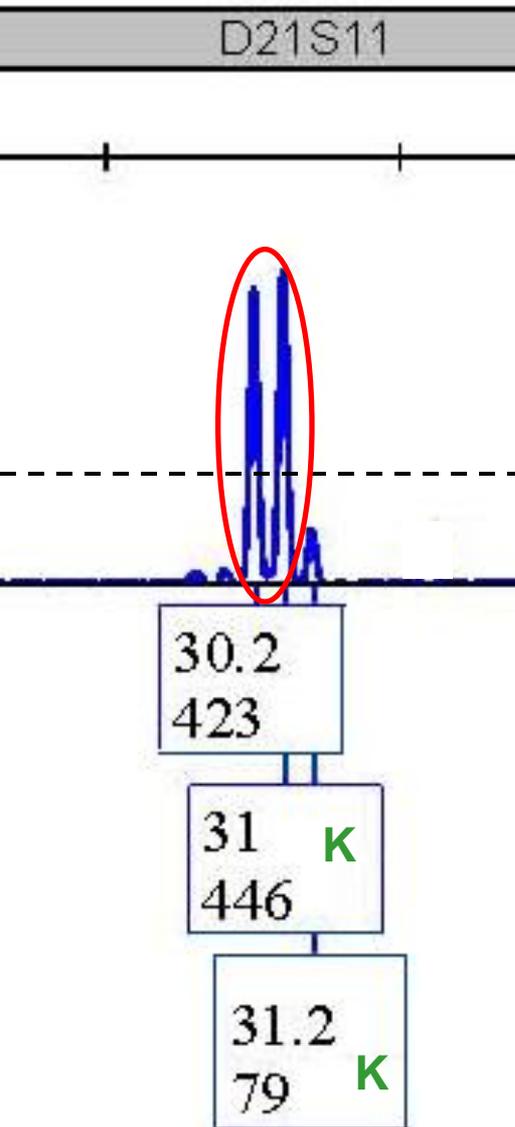
- Restricted Likelihood Ratio:

  $$1 / \{2P_{(30.2)}\, P_{(31)}\}$$

- Conclusion statements:

  DNA from two individuals was obtained from the evidence.

  Assuming the presence of Jane K. Victim, the DNA profile is approximately 180 times more likely to occur if it originated from Jane K. Victim and John Q. Suspect than from Jane K. Victim and an unknown individual in the Caucasian population.

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

D21S11

30.2
423

31   K
446

31.2 K
79

- CPI:
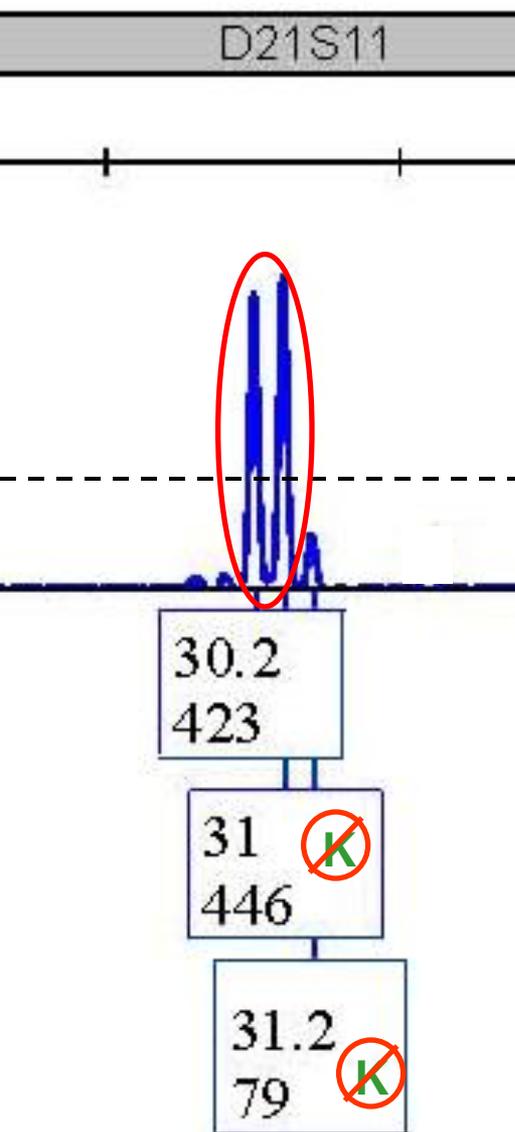
    Cannot perform on locus as a whole due to data below the stochastic threshold.

- Conclusion statements:

    The DNA profile obtained from this item does not satisfy the laboratory's inclusionary reporting criteria.

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout
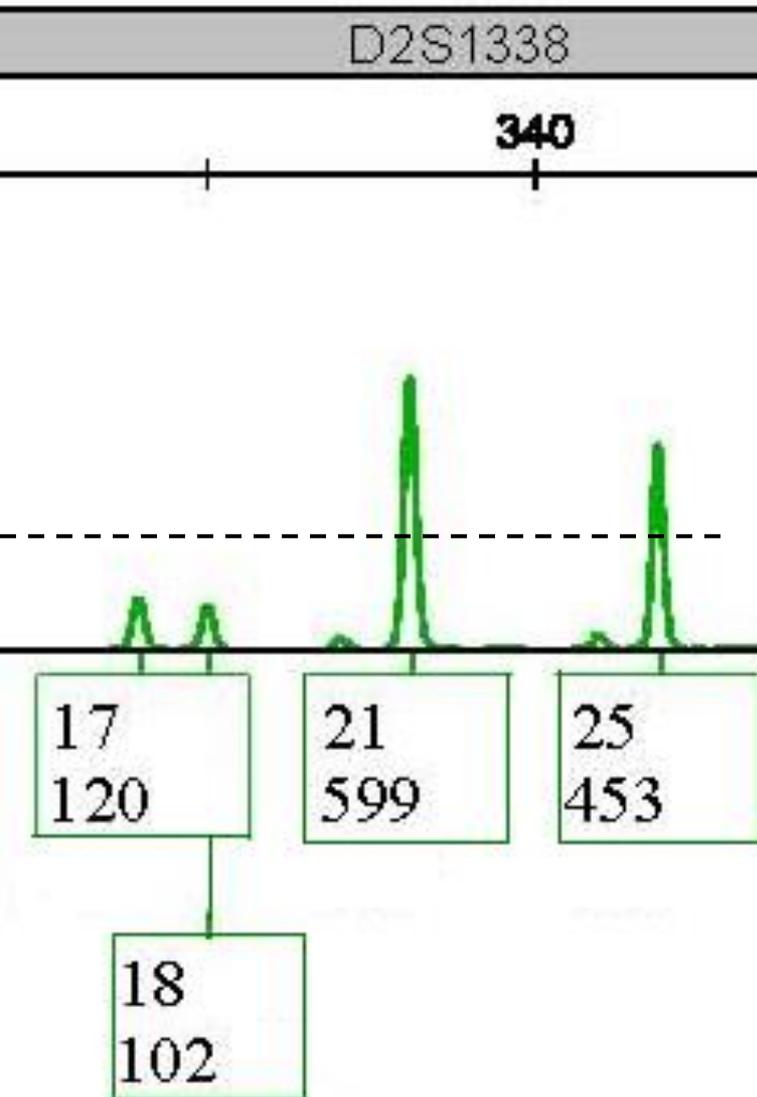
- Locus has two detected alleles (17,18) below stochastic threshold.
- Since four alleles detected in a mixture reasoned to be only two contributors, it is unreasonable to assume dropout is occurring.

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout
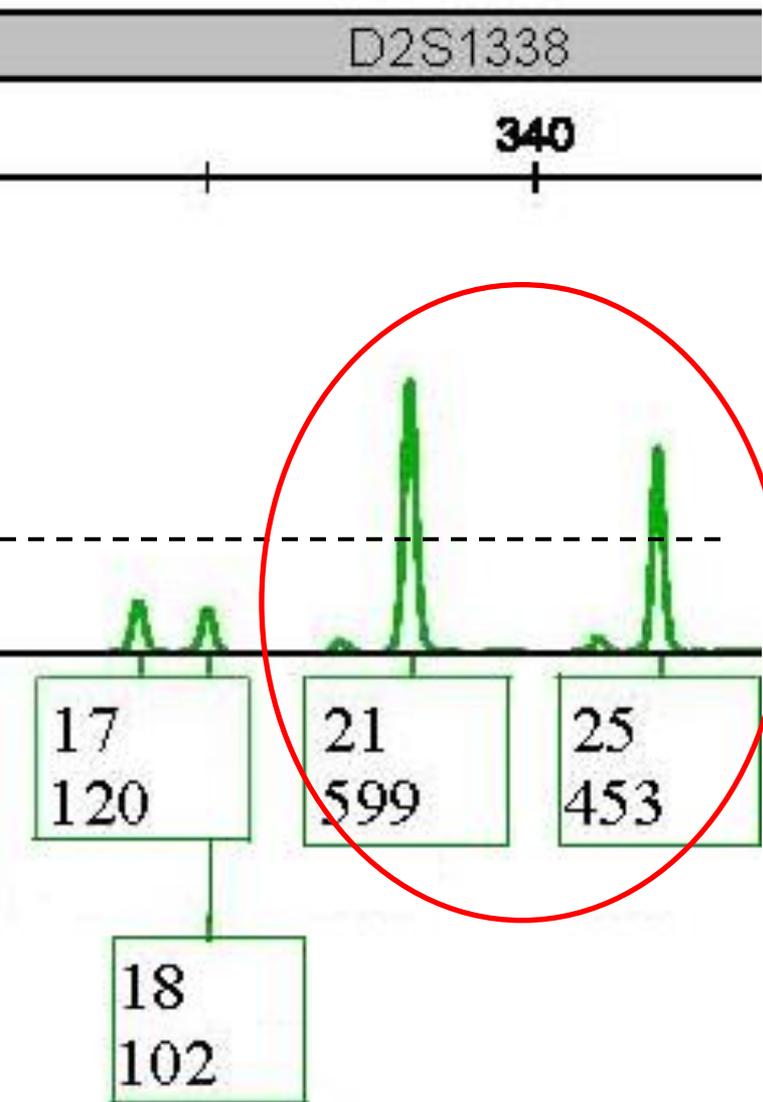
- RMP to probative major contributor:

$$2P_{(21)}P_{(25)}$$

- Conclusion statements:

DNA from two contributors was obtained from the evidence.

John Q. Suspect cannot be excluded as the major contributor of this mixture.

The probability of selecting an unrelated individual at random who cannot be excluded as the major contributor to the DNA profile obtained from this item is approximately: 1 in 260

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

- Unrestricted Likelihood Ratio for major contributor:

$$\frac{\{2P_{(17)}P_{(18)}\}}{\begin{array}{l}[\ \{2P_{(17)}P_{(18)}\} * \{2P_{(21)}P_{(25)}\}\ ] + \\ [\ \{2P_{(17)}P_{(21)}\} * \{2P_{(18)}P_{(25)}\}\ ] + \\ [\ \{2P_{(17)}P_{(25)}\} * \{2P_{(18)}P_{(21)}\}\ ] +\end{array}}$$

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

- Unrestricted Likelihood Ratio for major contributor:

- Conclusion statements:

DNA from two individuals was obtained from the evidence.

The DNA profile is approximately 44 times more likely to occur if it originated from John Q. Suspect and an unknown individual in the Caucasian population than from two unknown individuals in the Caucasian population.

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout
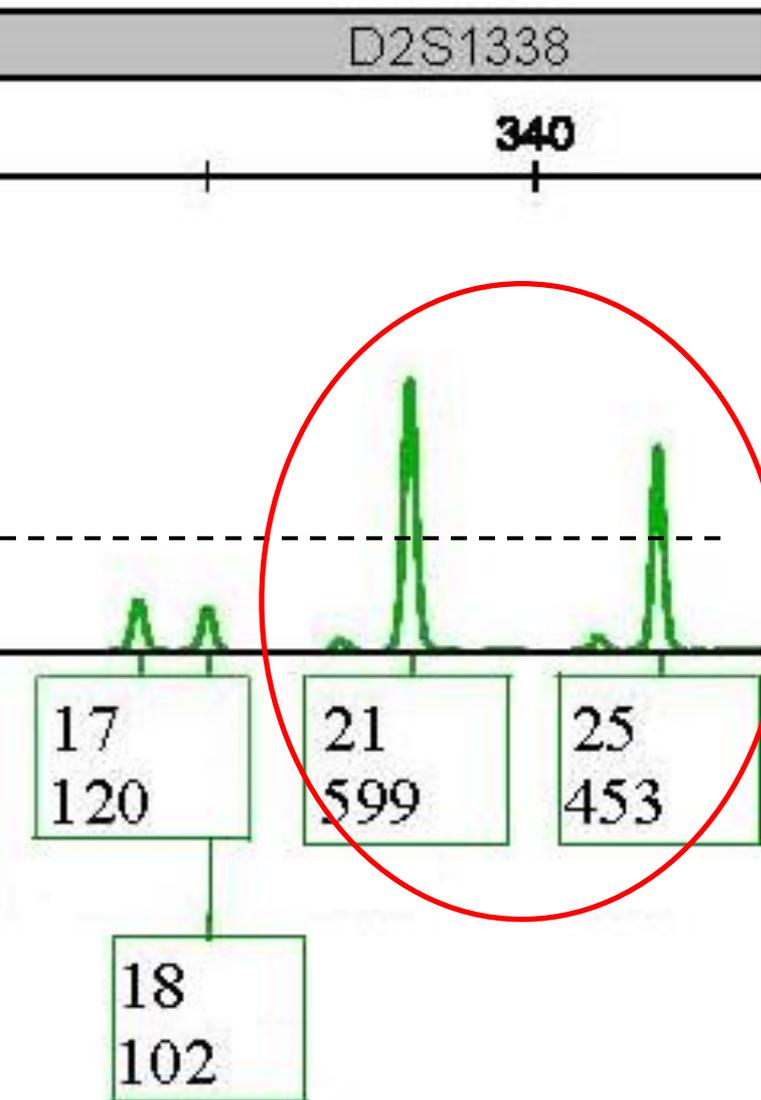
- Restricted Likelihood Ratio for major contributor:

$$\frac{\{2P_{(17)}P_{(18)}\}}{[\ \{2P_{(17)}P_{(18)}\}\ *\ \{2P_{(21)}P_{(25)}\}\ ]}$$

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

D2S1338

340

17
120

21
599

25
453

18
102
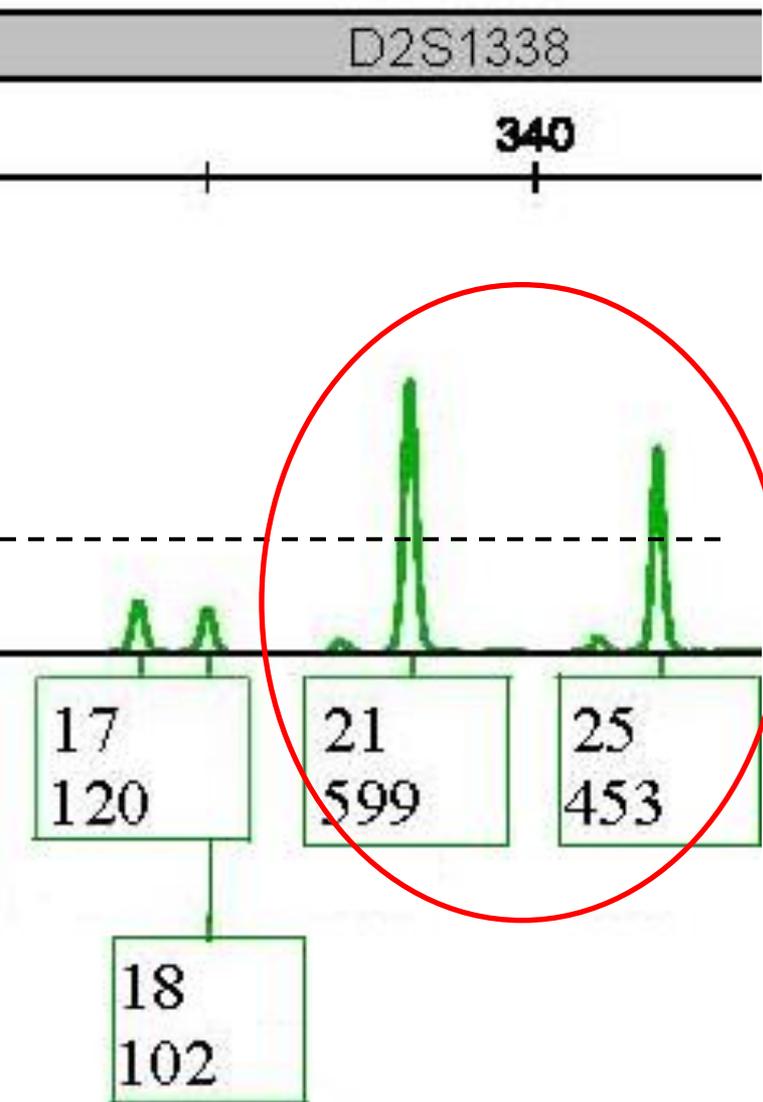
- Restricted Likelihood Ratio for major contributor:

- Conclusion statements:

DNA from two individuals was obtained from the evidence.

The DNA profile is approximately 260 times more likely to occur if it originated from John Q. Suspect and an unknown individual in the Caucasian population than from two unknown individuals in the Caucasian population.

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

D2S1338

340

17
120

21
599

25
453

18
102
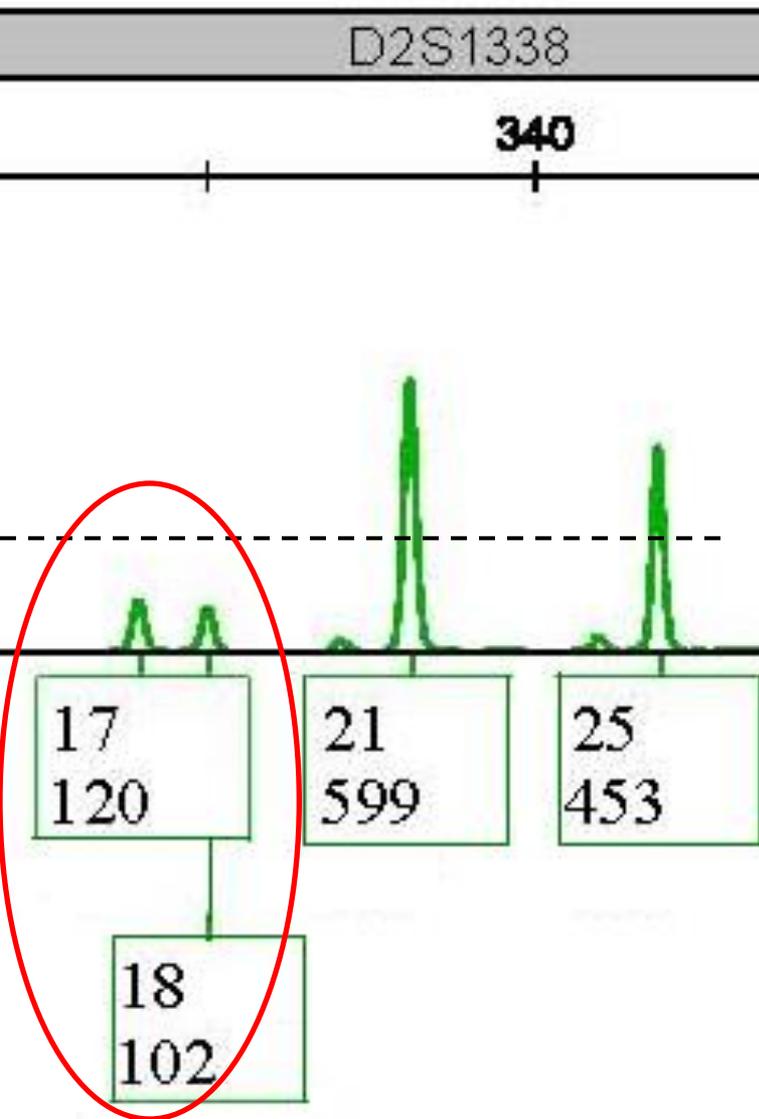
- RMP to probative minor contributor:
  $$2P_{(17)}P_{(18)}$$
- Conclusion statements:

DNA from two contributors was obtained from the evidence.

John Q. Suspect cannot be excluded as the minor contributor of this mixture.

The probability of selecting an unrelated individual at random who cannot be excluded as the minor contributor to the DNA profile obtained from this item is approximately: 1 in 48

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

- Unrestricted Likelihood Ratio for minor contributor:

$$\frac{\{2P_{(21)}P_{(25)}\}}{\begin{array}{l}[\,\{2P_{(17)}P_{(18)}\} * \{2P_{(21)}P_{(25)}\}\,] + \\ [\,\{2P_{(17)}P_{(21)}\} * \{2P_{(18)}P_{(25)}\}\,] + \\ [\,\{2P_{(17)}P_{(25)}\} * \{2P_{(18)}P_{(21)}\}\,] +\end{array}}$$

D2S1338

340

17
120

21
599

25
453

18
102

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

D2S1338

340

17
120

21
599

25
453

18
102

- Unrestricted Likelihood Ratio for minor contributor:

- Conclusion statements:

  DNA from two individuals was obtained from the evidence.

  The DNA profile is approximately 8 times more likely to occur if it originated from John Q. Suspect and an unknown individual in the Caucasian population than from two unknown individuals in the Caucasian population.

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

- Restricted Likelihood Ratio for minor contributor:

$$\frac{\{2P_{(21)}P_{(25)}\}}{[\,\{2P_{(17)}P_{(18)}\} * \{2P_{(21)}P_{(25)}\}\,]}$$
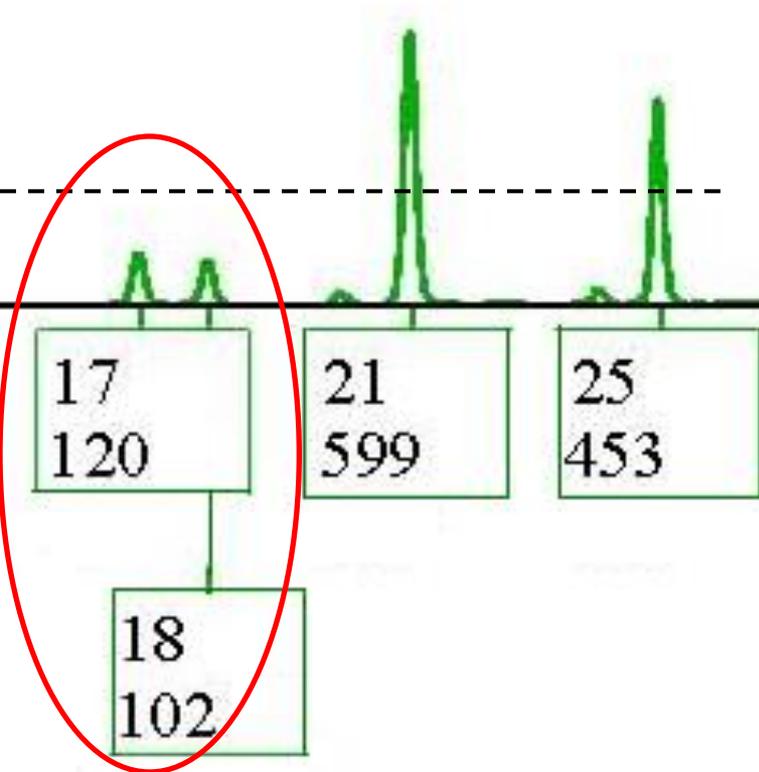
2 person mixture, data below the stochastic threshold, unreasonable to assume dropout
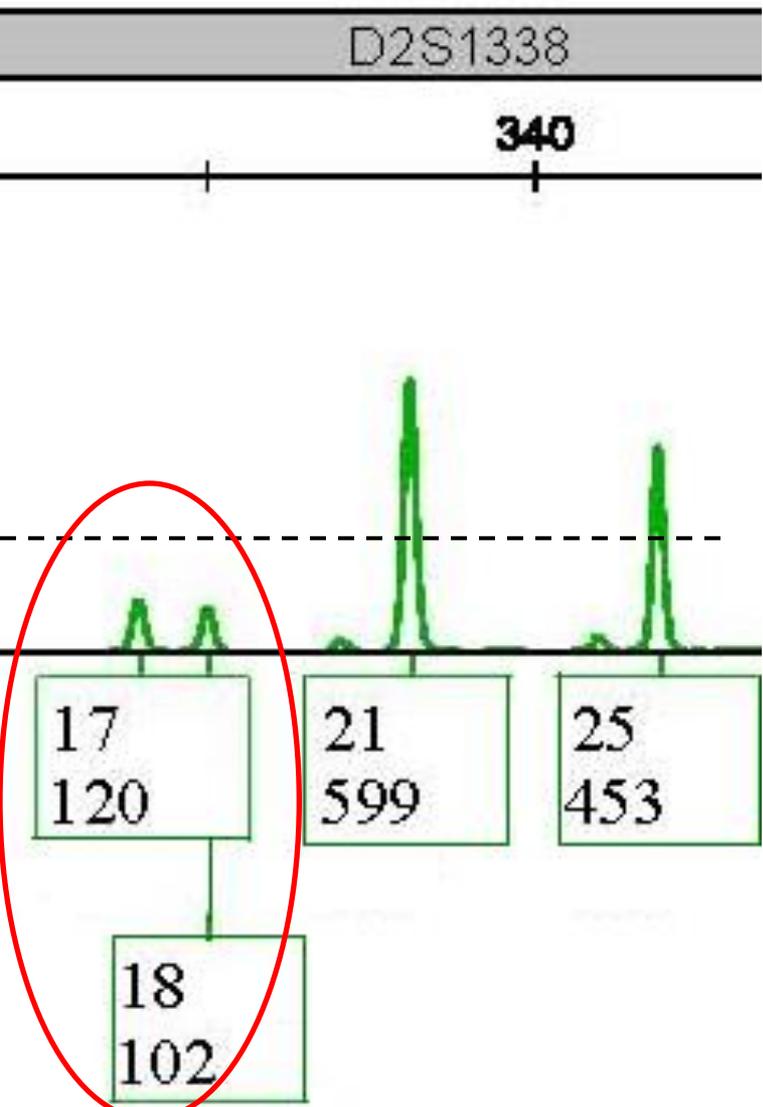
- Restricted Likelihood Ratio for minor contributor:

- Conclusion statements:

  DNA from two individuals was obtained from the evidence.

  The DNA profile is approximately 48 times more likely to occur if it originated from John Q. Suspect and an unknown individual in the Caucasian population than from two unknown individuals in the Caucasian population.

~~2 person~~ mixture, data below the stochastic threshold, ~~un~~reasonable to assume dropout

D2S1338

340

17
120

21
599

25
453

18
102

- CPI:

  Cannot perform on locus as a whole due to data below the stochastic threshold.

- Conclusion statements:

  The DNA profile obtained from this item does not satisfy the laboratory's inclusionary reporting criteria.

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

D3S1358
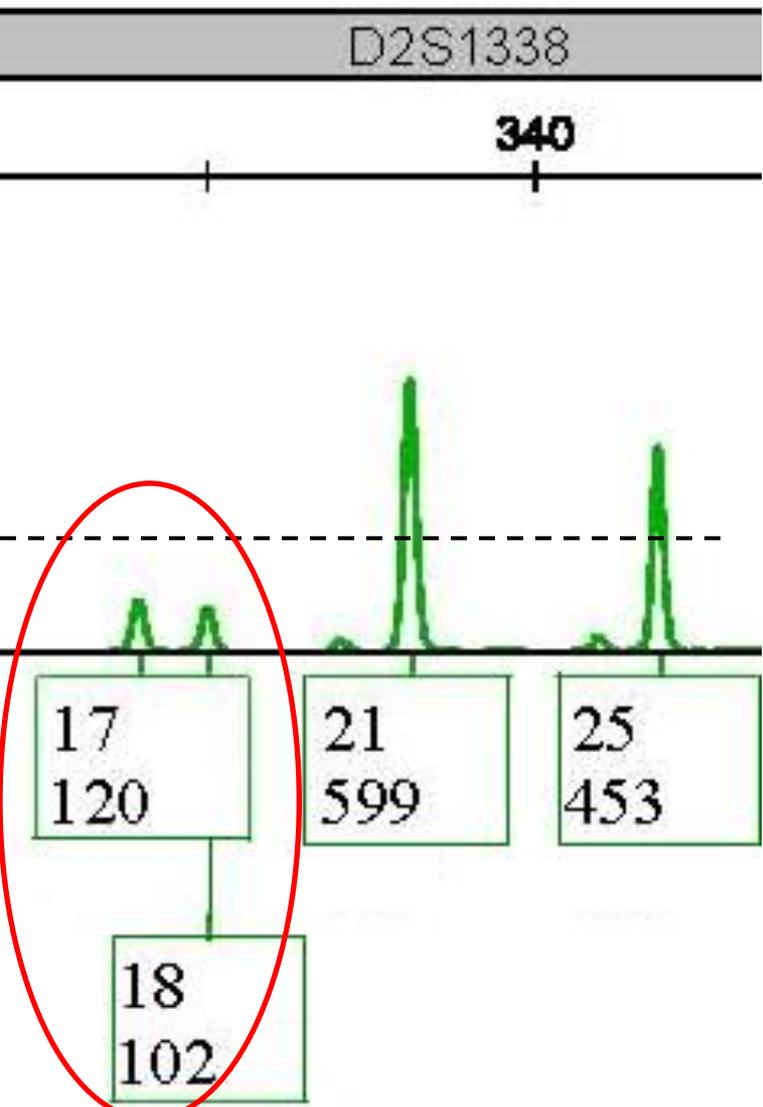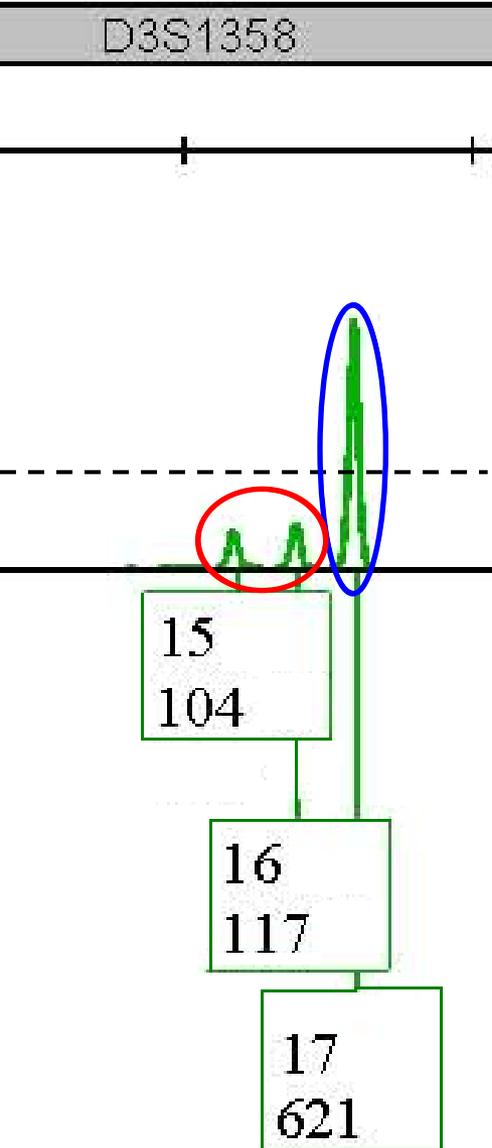
- Locus has two detected alleles (15,16) below stochastic threshold.

- Although less than four alleles detected in a mixture reasoned to be only two contributors, it is unreasonable to assume dropout is occurring based upon examination for potential genotypes.

15,F and 16,17 = unreasonable (19%PHR)

16,F and 15,17 = unreasonable (17%PHR)

15,16 and 17,F = unreasonable (allele 17 above stochastic threshold)

15,16 and 17,17 = reasonable (89%PHR)

15
104

16
117

17
621

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

D3S1358

15
104

16
117

17
621
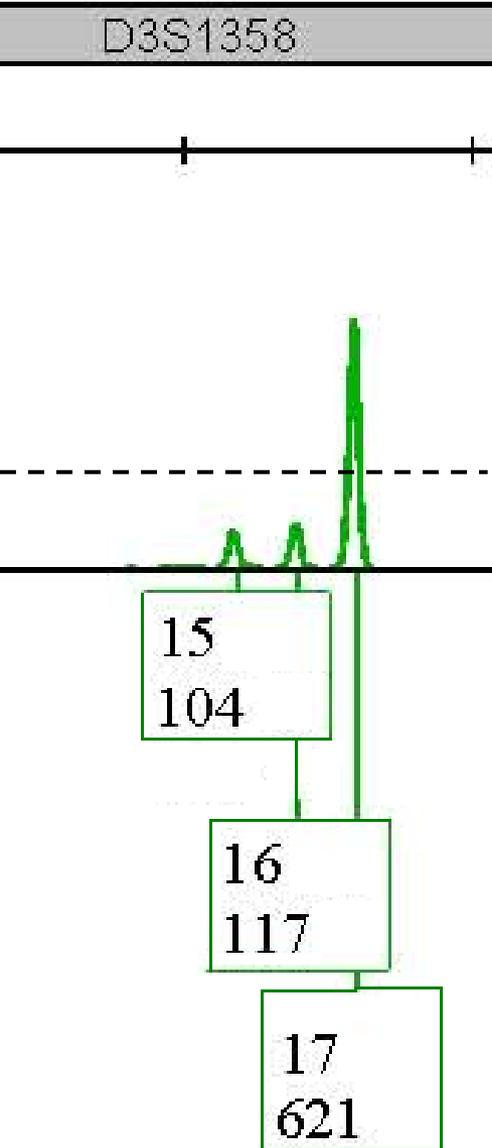
- Since locus has been reasoned to have no dropout and major/minor genotypes have been reasoned, can perform:
  - RMP for major
  - RMP for minor
  - ULR for major
  - ULR for minor
  - RLR for major
  - RLR for minor

2 person mixture, data below the stochastic threshold, unreasonable to assume dropout

D3S1358

- CPI:

  Cannot perform on locus as a whole due to data below the stochastic threshold.

- Conclusion statements:

  The DNA profile obtained from this item does not satisfy the laboratory's inclusionary reporting criteria.

15
104

16
117

17
621

2 person mixture, possible dropout?

D8S1179

13
587

14
555
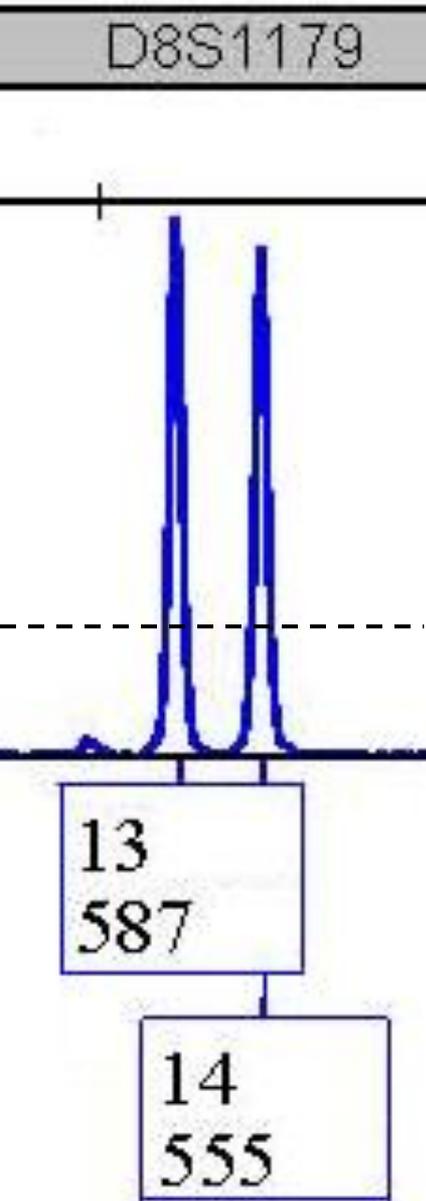
- Ratio of contributors v. stochastic threshold:
  - Is this <u>1:1</u>? Dropout is unreasonable
  - Is this <u>10:1</u>? Dropout is reasonable

- Overall height of minor contributor:
  - Minor contributor heterozygous alleles are <u>above</u> stochastic threshold? Dropout is unreasonable
  - Minor contributor heterozygous alleles are <u>below</u> stochastic threshold? Dropout is reasonable

- Molecular weight of locus:
  - Minor contributor alleles <u>are seen</u> in higher molecular weight loci? Dropout is *less* reasonable
  - Minor contributor alleles <u>are not seen</u> in higher molecular weight loci? Dropout is *more* reasonable

2 person mixture, reasonable to assume dropout at D8?

| D8S1179 | D21S11 |

13
587

14
555

30.2
423

31
446

31.2
79
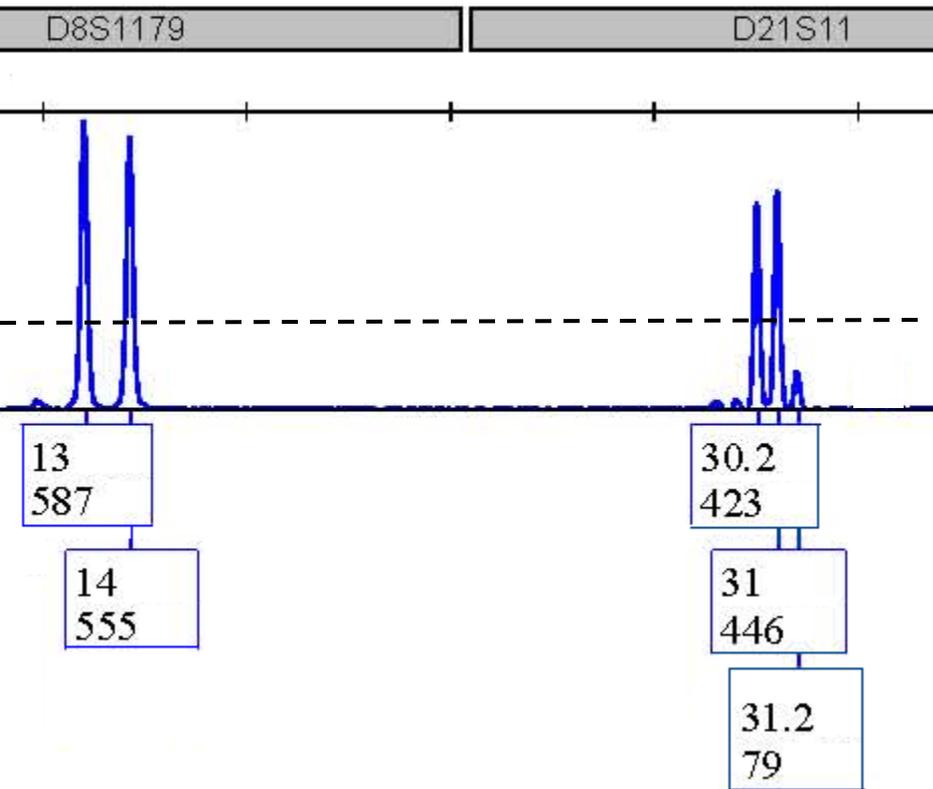
- Ratio of contributors:
  - D21 is ~ 5:1 or 10:1.
    Dropout at D8 is reasonable.

- Overall height of minor contributor:
  - Minor contributor allele at D21 is below stochastic threshold.
    Dropout at D8 is reasonable.

- Molecular weight of locus:
  - Minor contributor allele is seen in D21.
    Dropout is less reasonable.

2 person mixture, reasonable to assume dropout at D8?
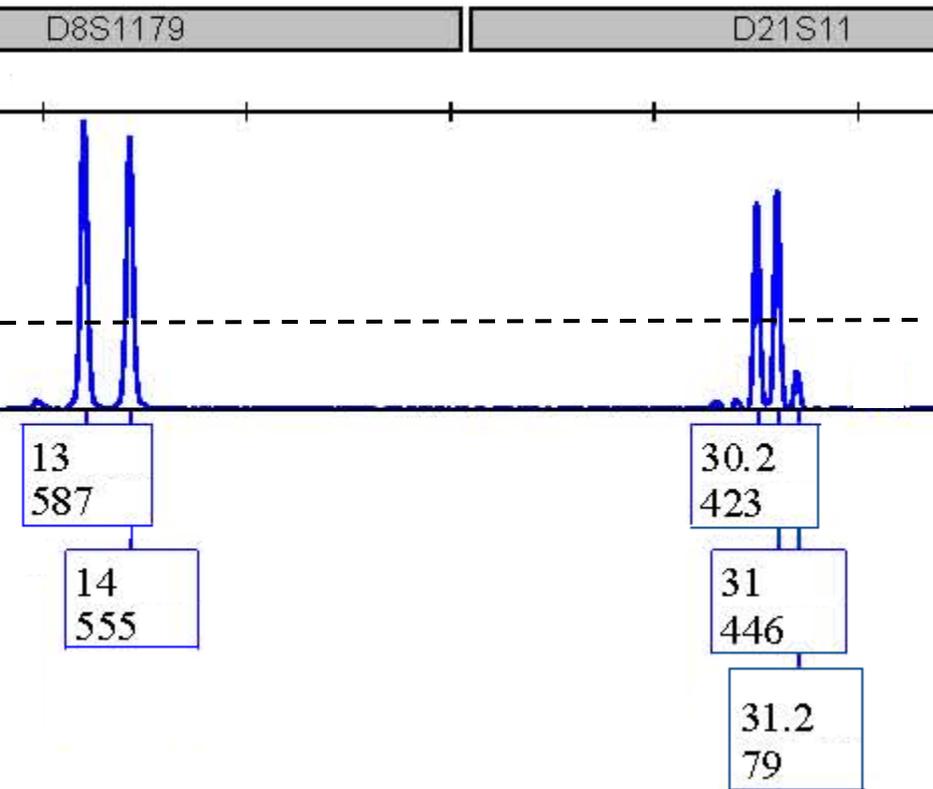
D8S1179	D21S11

| 13 | 30.2 |
| 587 | 423 |

| 14 | 31 |
| 555 | 446 |

| | 31.2 |
| | 79 |

- Make a decision <u>before</u> comparing the profile of the probative reference standard.

- If declaring <u>possible dropout</u> at D8, then the true minor contributor could be any profile.

- This renders the locus useless for statistics for the minor.

• Even if the probative reference standard is fully represented by the detected alleles!

2 person mixture, reasonable to assume dropout at D8?
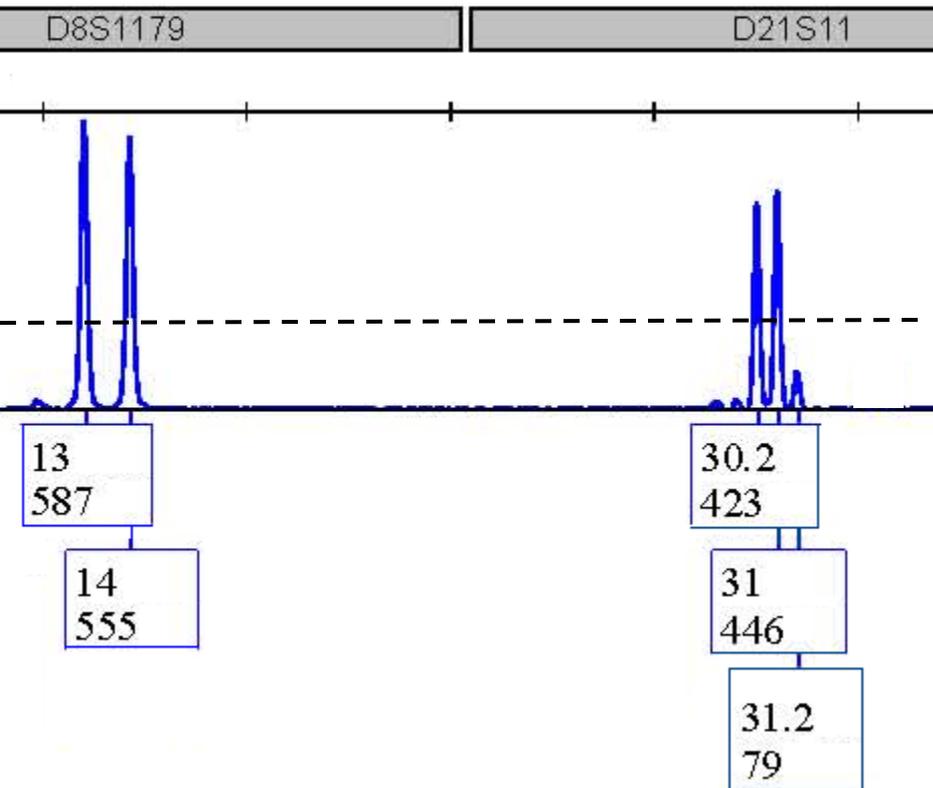
- Make a decision <u>before</u> comparing the profile of the probative reference standard.

- If declaring <u>dropout is unreasonable</u> at D8, then the true minor contributor must be masked.

- This renders the locus useful for statistics for the minor.

- However, if the probative reference standard is not masked by the detected alleles, then exclude!

Minor allele in stutter position (consider stutter percentage)
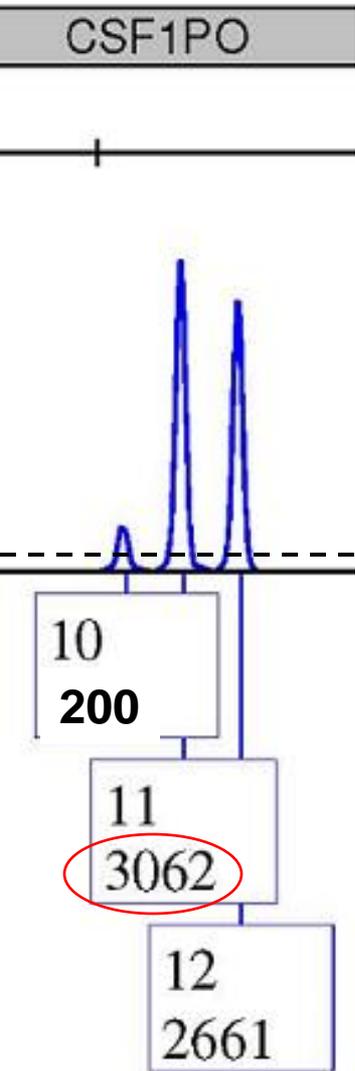
- Major alleles 11,12 are well balanced (87%PHR) … no indication that a sister allele to the 10 must be masked by major contributor.

- 200rfu stochastic threshold.

- 477-277 = 200.

- If 277rfu in bin 10 is stutter, the true value of allele 10 may be below stochastic threshold; sister allele to the 10 may be undetected.

- 277 / 3062 = 9%

- Is 9% stutter reasonable?

Minor allele in stutter position (consider stutter percentage)

CSF1PO

10
477

11
3062

12
2661
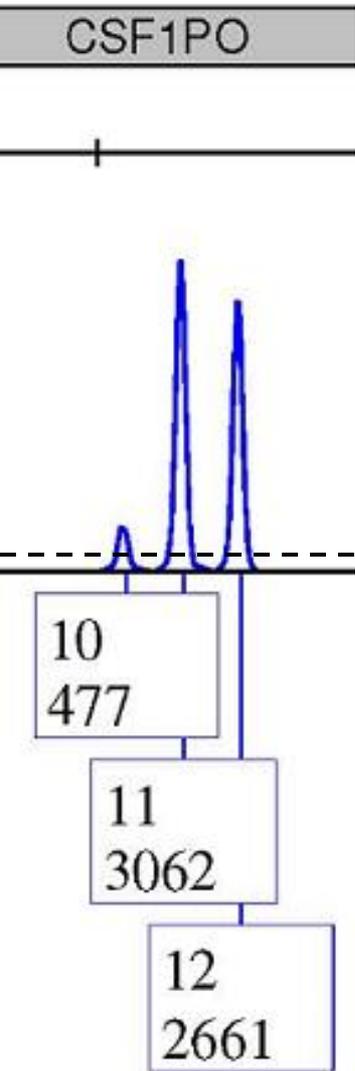
- Even though <u>all data is above stochastic threshold</u>, a thorough interpretation may show that dropout is still reasonable.

- Even if the probative reference standard is fully represented within the detected alleles, the true minor contributor may have an undetected allele.

- Stats for the minor contributor:

  RMP using a "2P" calculation

  ULR using a "2P" calculation

  RLR using a "2P" calculation

  CPI is not appropriate.

Minor allele indistinguishable from stutter

D8S1179

10
236

14
3310
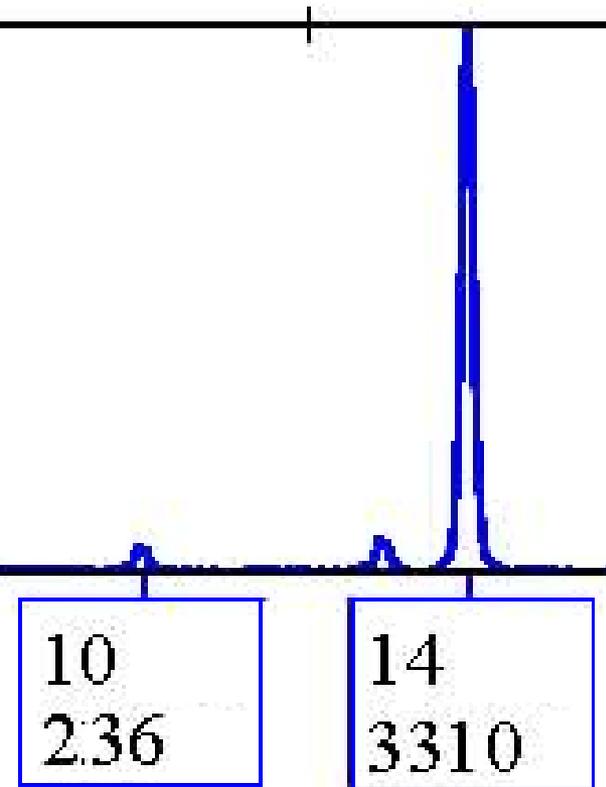
- Since unambiguous minor (10) is above stochastic threshold, and not in stutter position, unreasonable to assume dropout of a sister allele to the allele 10.

- However, the sister allele to the allele 10 may be in the stutter bin 13 and have been filtered out by the software.

- Reanalyze the mixture with stutter filters set to 0%.

Minor allele indistinguishable from stutter

D8S1179

10	13
236	271

14
3310
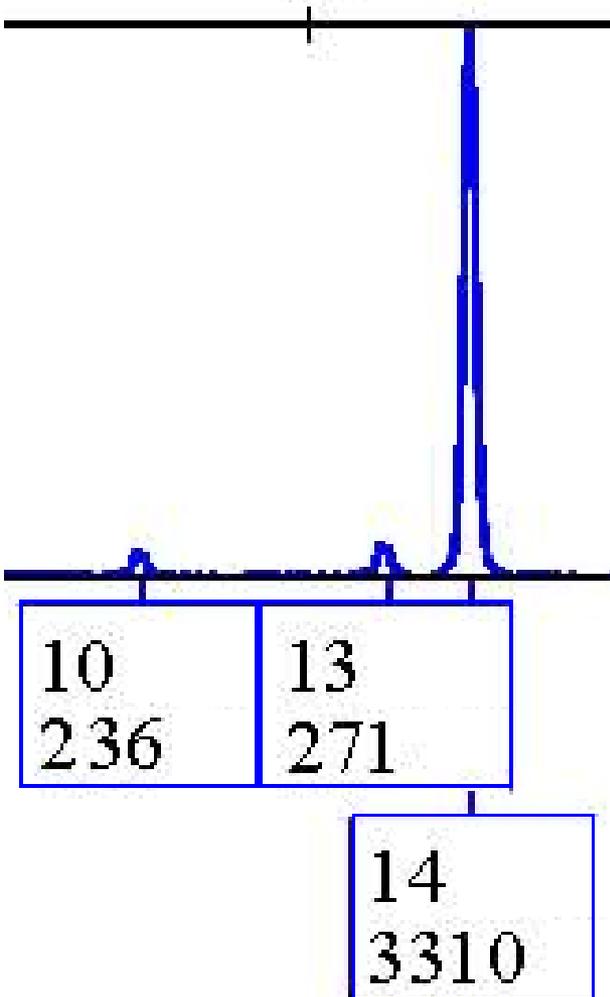
- Upon examining all data above analytical, without regards to stutter filters, compare peaks in stutter positions to unambiguous minor alleles.

- 271 / 236 = 115% PHR

- Even with some amount of stutter present in bin 13, this peak may contain a true sister allele to the allele 13.

- However, it could also be only stutter.

- As such, it is "indistinguishable from stutter" (IFS).

Minor allele indistinguishable from stutter
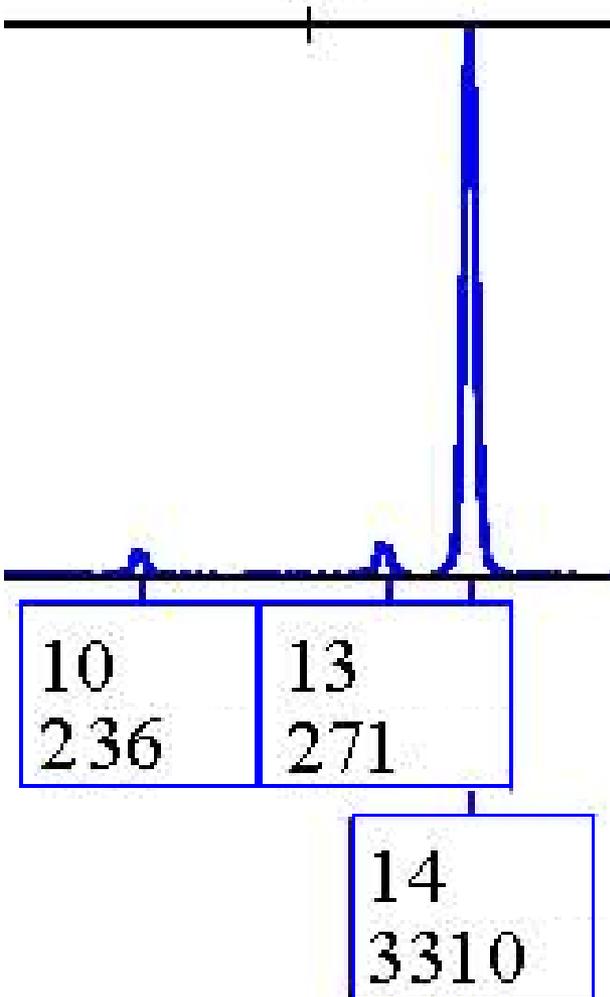
- Statistics for the minor contributor (or mixture as a whole) must incorporate both ideas of the peak being stutter and being a true minor allele.

- RMP for minor:

$$(P_{(10)}^2) + (2P_{(10)}P_{(14)}) + (2P_{(10)}P_{(13)})$$

The probability of selecting an unrelated individual at random who cannot be excluded as the minor contributor to the DNA profile obtained from this item is approximately: 1 in 8

Minor allele indistinguishable from stutter
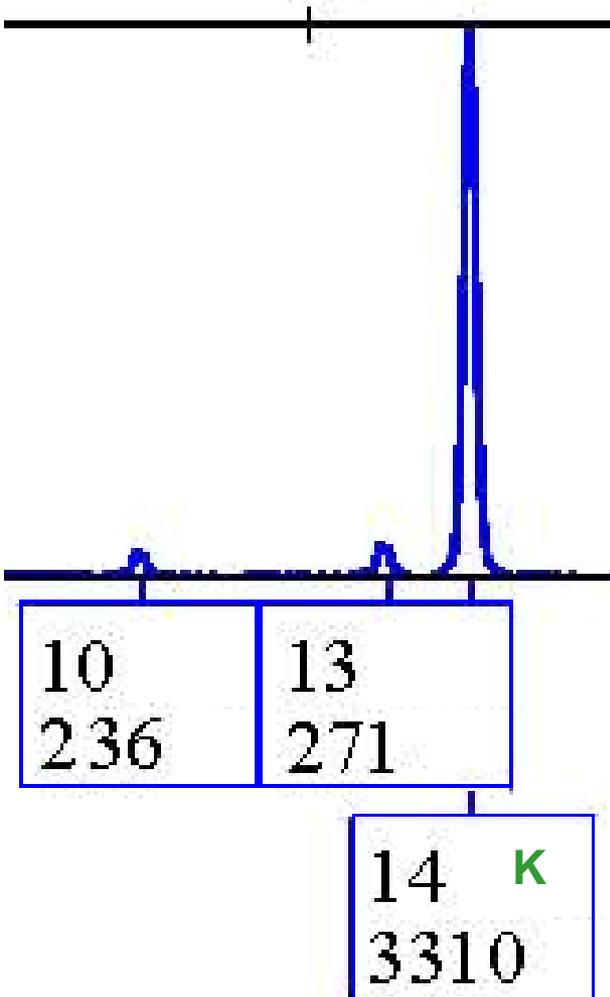
D8S1179

10
236

13
271

14 **K**
3310

- Statistics for the minor contributor (or mixture as a whole) must incorporate both ideas of the peak being stutter and being a true minor allele.

- ULR for minor (considering major is "known")

$$\frac{1}{P_{(10)}^2 + 2P_{(10)}P_{(14)} + 2P_{(10)}P_{(13)}}$$

The DNA profile is approximately 8 times more likely to occur if it originated from Jane K. Victim and John Q. Suspect than from Jane K. Victim and an unknown individual in the Caucasian population.

Minor allele indistinguishable from stutter

D8S1179

10
236

13
271

14 **K**
3310
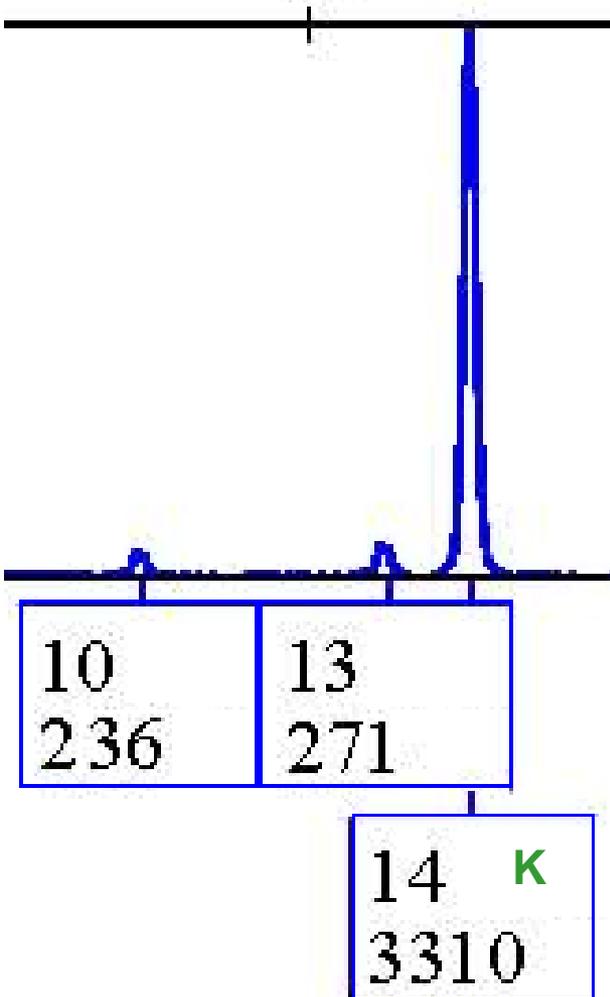
- Statistics for the minor contributor (or mixture as a whole) must incorporate both ideas of the peak being stutter and being a true minor allele.

- RLR for minor (considering major is "known")

$$\frac{1}{P_{(10)}^2 + 2P_{(10)}P_{(14)} + 2P_{(10)}P_{(13)}}$$

The DNA profile is approximately 8 times more likely to occur if it originated from Jane K. Victim and John Q. Suspect than from Jane K. Victim and an unknown individual in the Caucasian population.

Minor allele indistinguishable from stutter

D8S1179

10	13
2 36	271

14
3310
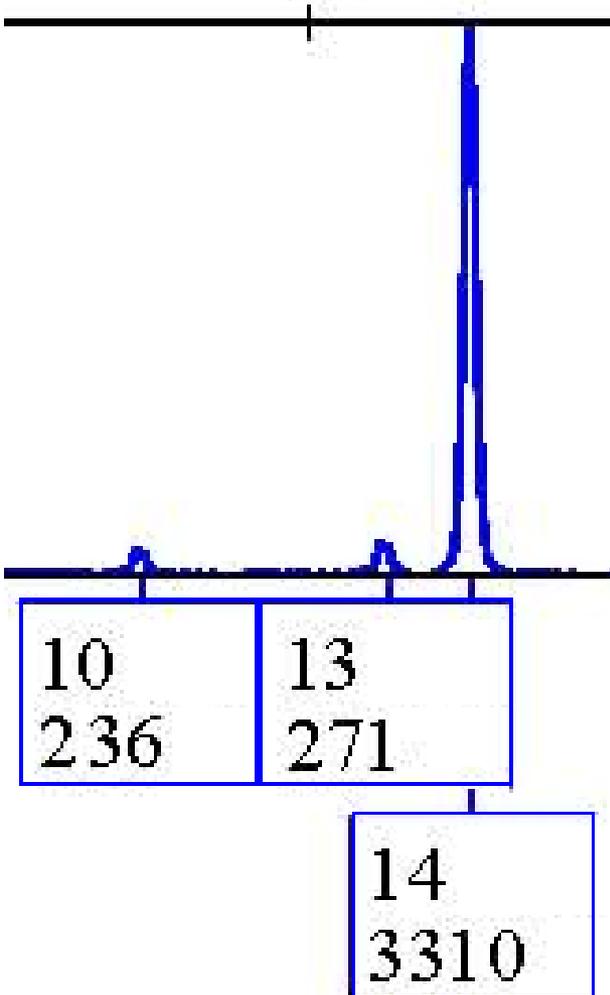
- Statistics for the minor contributor (or mixture as a whole) must incorporate both ideas of the peak being stutter and being a true minor allele.

- CPI for mixture as a whole:

$$\{P_{(10)} + P_{(13)} + P_{(14)}\}^2$$

The probabilities of selecting an unrelated individual at random who cannot be excluded as one of the possible sources of the DNA profile obtained from this item are approximately 1 in 2 in the Caucasian population.

Minor peak distinguishable as stutter

- Not every peak in every stutter bin is worthy of being designated as IFS.

- If the mixture has no distinction of major and minor, then there is no minor contributor at the rfu level of stutter peaks.

- If a locus has already been declared to have the possibility of dropout, the statistics that incorporate dropout account for IFS peaks. "Dropout trumps IFS."

- If the minor contributor already has a complete genotype defined by the unambiguous alleles.

- If the minor contributor is "known" and that genotype is already defined by the unambiguous alleles.

Documentation

- Documentation of the interpretation within the case folder is crucial:

  The technical reviewer can understand why the analyst made certain decisions.

  The analyst can refer to the case notes in court to recall the decisions.

  The analysis is open to the scrutiny of another expert.

Documentation

- Documentation of the interpretation within the case folder is crucial:

    Analytical and stochastic thresholds.

    Number of contributors hypothesized to be present.

    Presence of any "known" contributors.

    Reasons to discount dropout when data is present below the stochastic threshold.

    Reasons to include possible dropout when no data is visible below the stochastic threshold.

    Reasons to identify a peak as stutter or "indistinguishable from stutter".

Documentation

- Documentation of the assumptions (number of contributors, presence of "known" contributor, etc.) within the case report is crucial:

    Who may see only the report and never see the case notes?

    Law enforcement

    Prosecuting attorney

    Defense attorney

    Judge

    Jury