Complex Mixtures

The more you know the harder they get!

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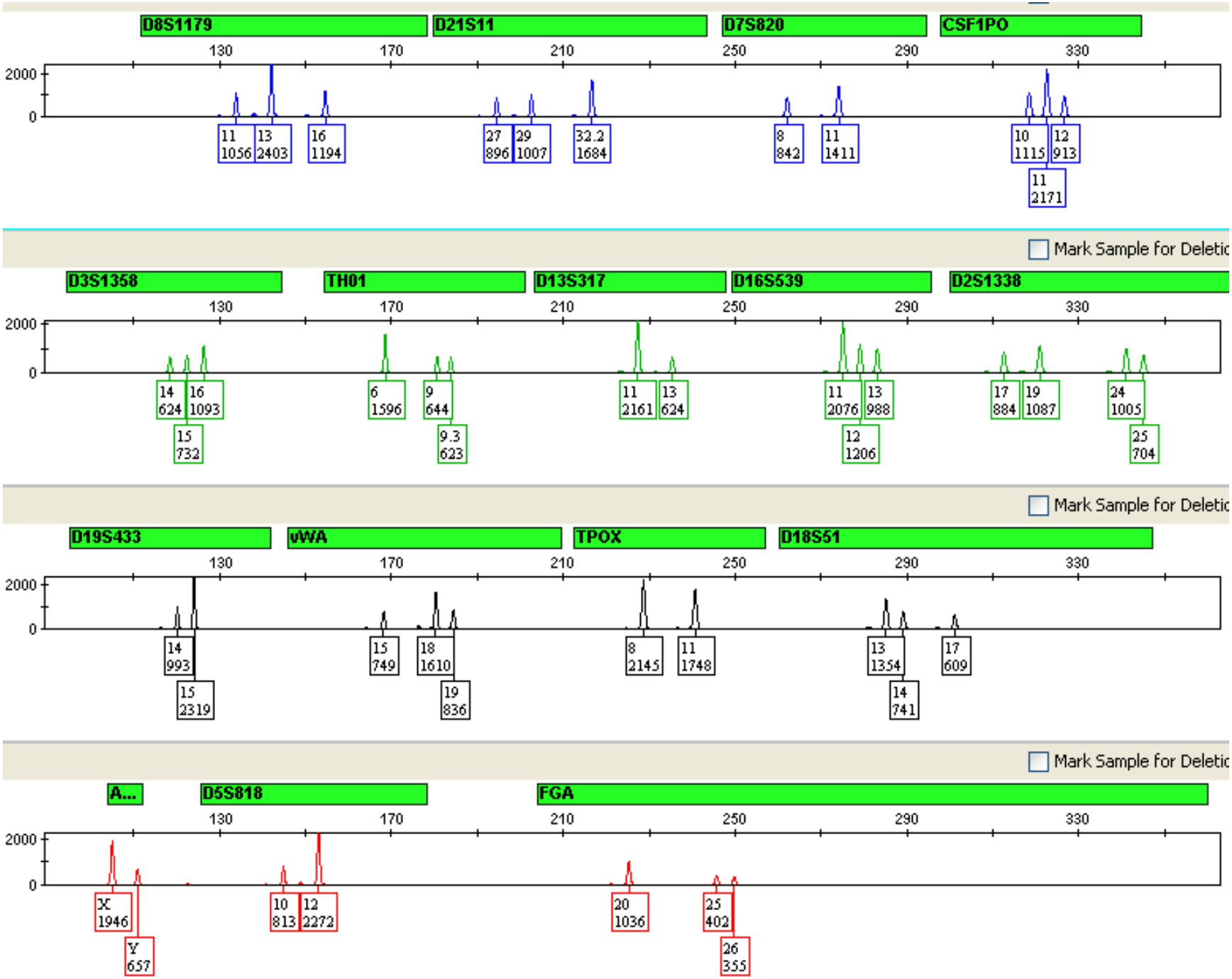
BOSTON UNIVERSITY

NIST

Two-Person Mixtures

- Lots of experience and familiarity with two-person mixtures, literature, validation studies, training samples
- Published guidelines for interpretation
- Well developed SOPs for interpretation
- Routine amount of input DNA in amplification generally leads to nice profiles


D8S1179
D21S11
D7S820
CSF1PO
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11 1056
13 2403
16 1194
27 896
29 1007
32.2 1684
8 842
11 1411
10 1115
12 913
11 2171
Mark Sample for Deletic
D3S1358
TH01
D13S317
D16S539
D2S1338
14 624
16 1093
15 732
6 1596
9 644
9.3 623
11 2161
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11 2076
13 988
12 1206
17 884
19 1087
24 1005
25 704
Mark Sample for Deletic
D19S433
vWA
TPOX
D18S51
14 993
15 2319
15 749
18 1610
19 836
8 2145
11 1748
13 1354
14 741
17 609
Mark Sample for Deletic
A...
D5S818
FGA
X 1946
Y 657
10 813
12 2272
20 1036
25 402
26 355

Two-Person Mixtures

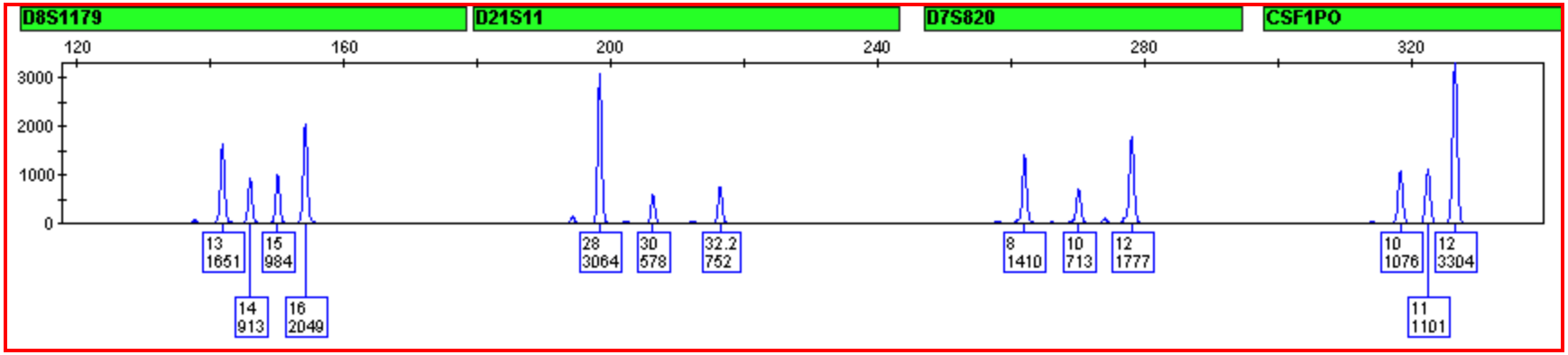
High Certainty Leads to High Confidence

- Only two contributors present
- Distinguishing stutter/artifacts from true alleles
- Use stochastic threshold to assess if all alleles are likely present vs. LT DNA with stochastic effects
- Assessing mixture ratio (distinguishable/ major:minor or indistinguishable mixture)
- Deducing second contributor if one contributor is known

Two-Person Mixtures

Assume number of contributors is two:

- Aids in allele association at each locus based on peak height ratios
- May aid in genotype association for full profile based on mixture ratio
- Statistics calculations often straight forward

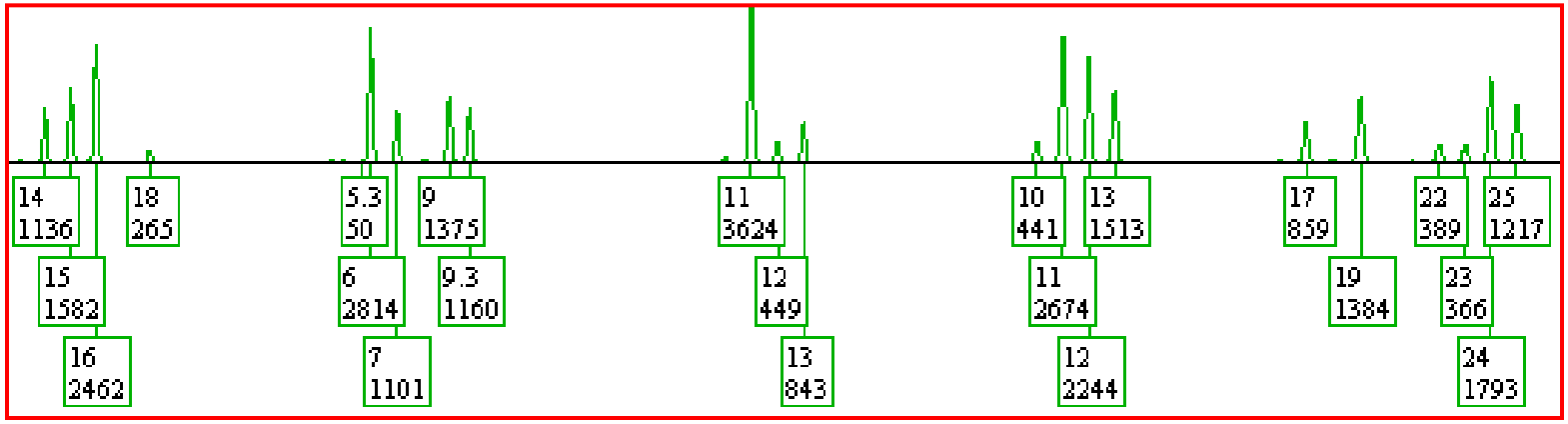


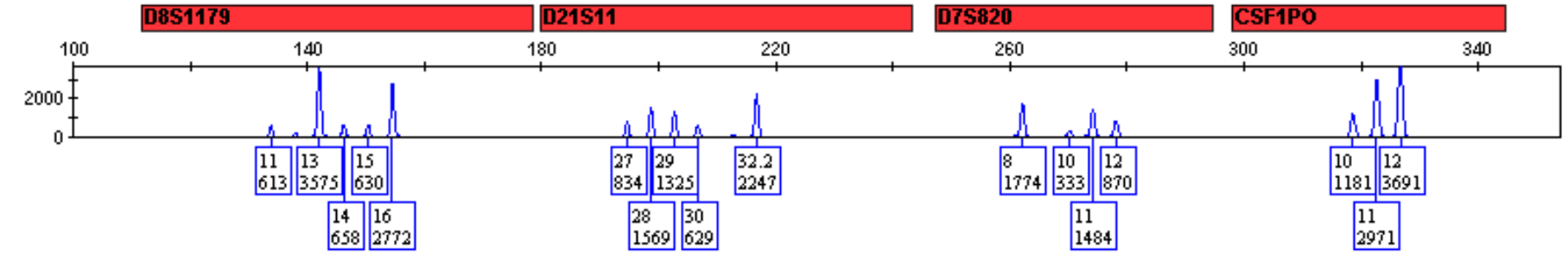
Complex Mixtures

- Multiple contributors
  - 3- & 4- person (or more!)
- Relatives in Mixtures

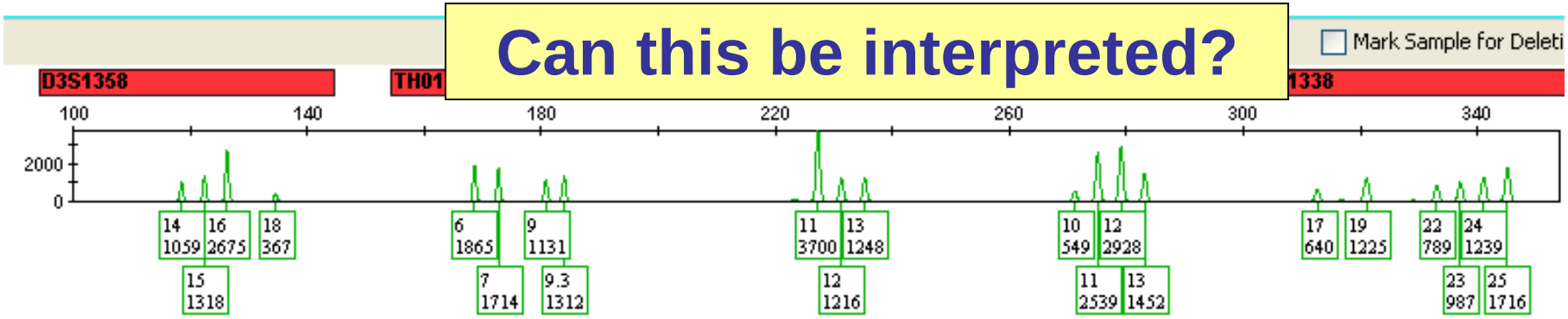
Complex Mixture Interpretation

Is hard because the parameters used to interpret two-person mixtures often may not be directly applicable to complex mixtures

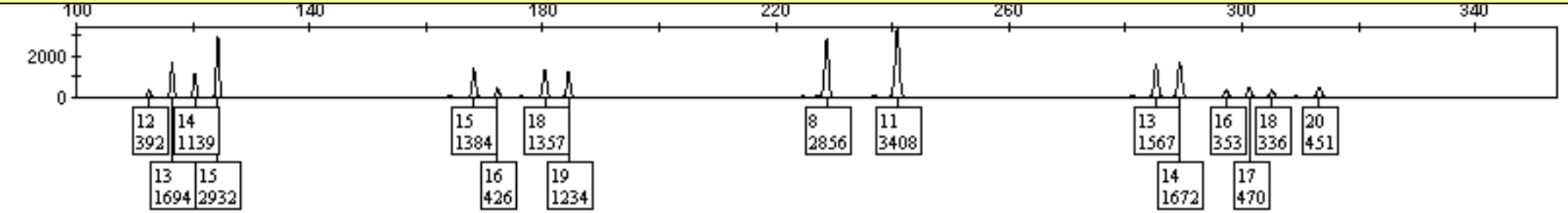




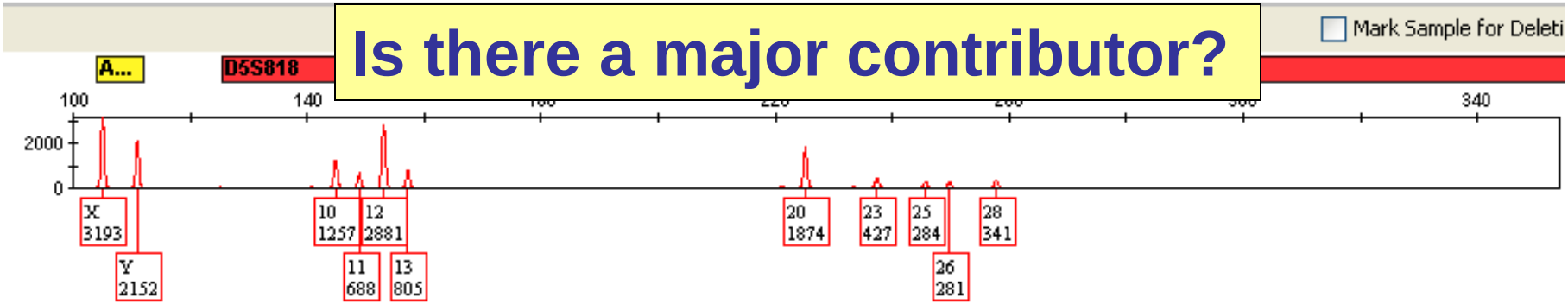
Can this be interpreted?



How many contributors assumed for interpretation?

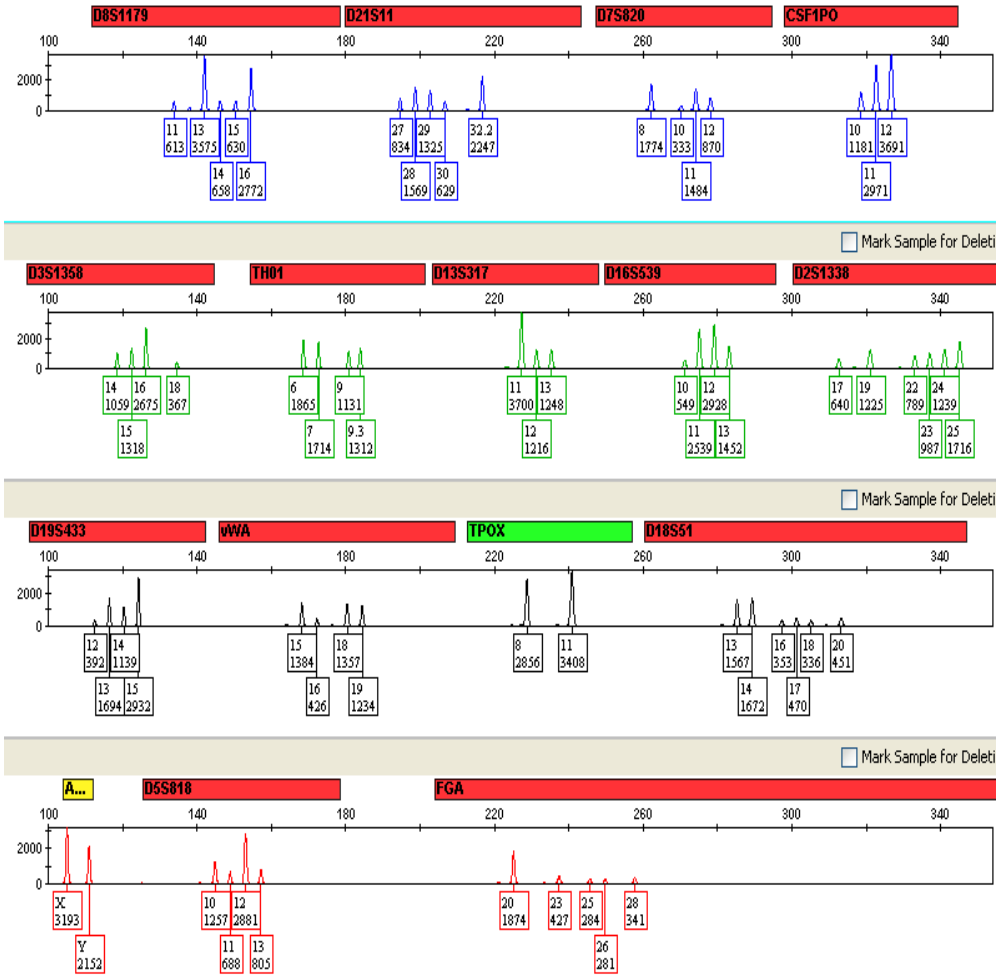


Is there a major contributor?



Complex Mixture – Allele Summary

- 6 alleles at 2 loci
- 5 alleles at 3 loci
- 4 alleles at 7 loci
- 3 alleles at 2 loci
- 2 alleles at 1 locus
- 1 allele at 0 loci
- 63 total alleles

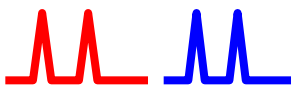


Two-Person Mixtures

Observed profile

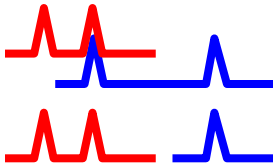
A **B**

14 total combinations



4 alleles

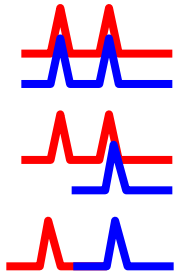
All heterozygotes and non-overlapping alleles



3 alleles

Heterozygote + heterozygote, one overlapping allele

Heterozygote + homozygote, no overlapping alleles

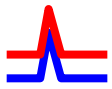


2 alleles

Heterozygote + heterozygote, two overlapping alleles

Heterozygote + homozygote, one overlapping allele

Homozygote + homozygote, no overlapping alleles



1 allele

Homozygote + homozygote, overlapping allele

3-Person Mixtures

Observed profile

150 total combinations

6 alleles

All heterozygotes and non-overlapping alleles

5 alleles

Two heterozygotes and one homozygote
Three heterozygotes, one overlapping allele

4 alleles

Six combinations of heterozygotes, homozygotes and overlapping alleles

3 alleles

Eight combinations of heterozygotes, homozygotes, and overlapping alleles

2 alleles

Five combinations of heterozygotes, homozygotes, and overlapping alleles

1 allele

All homozygotes, overlapping allele

4-Person Mixtures

Observed profile

8 alleles

All heterozygotes and non-overlapping alleles

MANY combinations

7 alleles

Several combinations of heterozygotes, homozygotes, and overlapping alleles

6 alleles

Many combinations

5 alleles

Many combinations

4 alleles

Many combinations

3 alleles

Many combinations

2 alleles

Many combinations

1 allele

All homozygotes, overlapping allele

Two-Person Simulated Mixtures – SGM+ Number of Alleles at each Locus

Table 1

The probability of observing a given number of alleles in a two-person mixtures for simulated profiles at the SGM^{+TM} loci

Loci	No. of alleles			
	1	2	3	4
D3	0.011	0.240	0.559	0.190
vWA	0.008	0.194	0.548	0.250
D16	0.016	0.287	0.533	0.164
D2	0.003	0.094	0.462	0.441
D8	0.011	0.194	0.521	0.274
D21	0.007	0.147	0.505	0.341
D18	0.003	0.095	0.472	0.430
D19	0.020	0.261	0.516	0.203
THO	0.016	0.271	0.547	0.166
FGA	0.003	0.116	0.500	0.381

Buckleton et al. Forensic Science International: Genetics 1 (2007) 20–28

Three-Person Simulated Mixtures – SGM+ Number of Alleles at each Locus

Table 2

The probability of observing a given number of alleles in a three-person mixtures for simulated profiles at the SGM+™ loci

Loci	No. of alleles showing					
	1	2	3	4	5	6
D3	0.000	0.053	0.366	0.463	0.115	0.002
vWA	0.000	0.037	0.285	0.468	0.194	0.016
D16	0.001	0.086	0.397	0.411	0.100	0.005
D2	0.000	0.008	0.104	0.385	0.393	0.110
D8	0.001	0.041	0.258	0.436	0.236	0.029
D21	0.000	0.023	0.192	0.428	0.302	0.055
D18	0.000	0.007	0.109	0.392	0.396	0.096
D19	0.003	0.078	0.352	0.401	0.152	0.014
THO	0.001	0.074	0.395	0.439	0.088	0.002
FGA	0.000	0.012	0.144	0.424	0.346	0.074

Buckleton et al. Forensic Science International: Genetics 1 (2007) 20–28

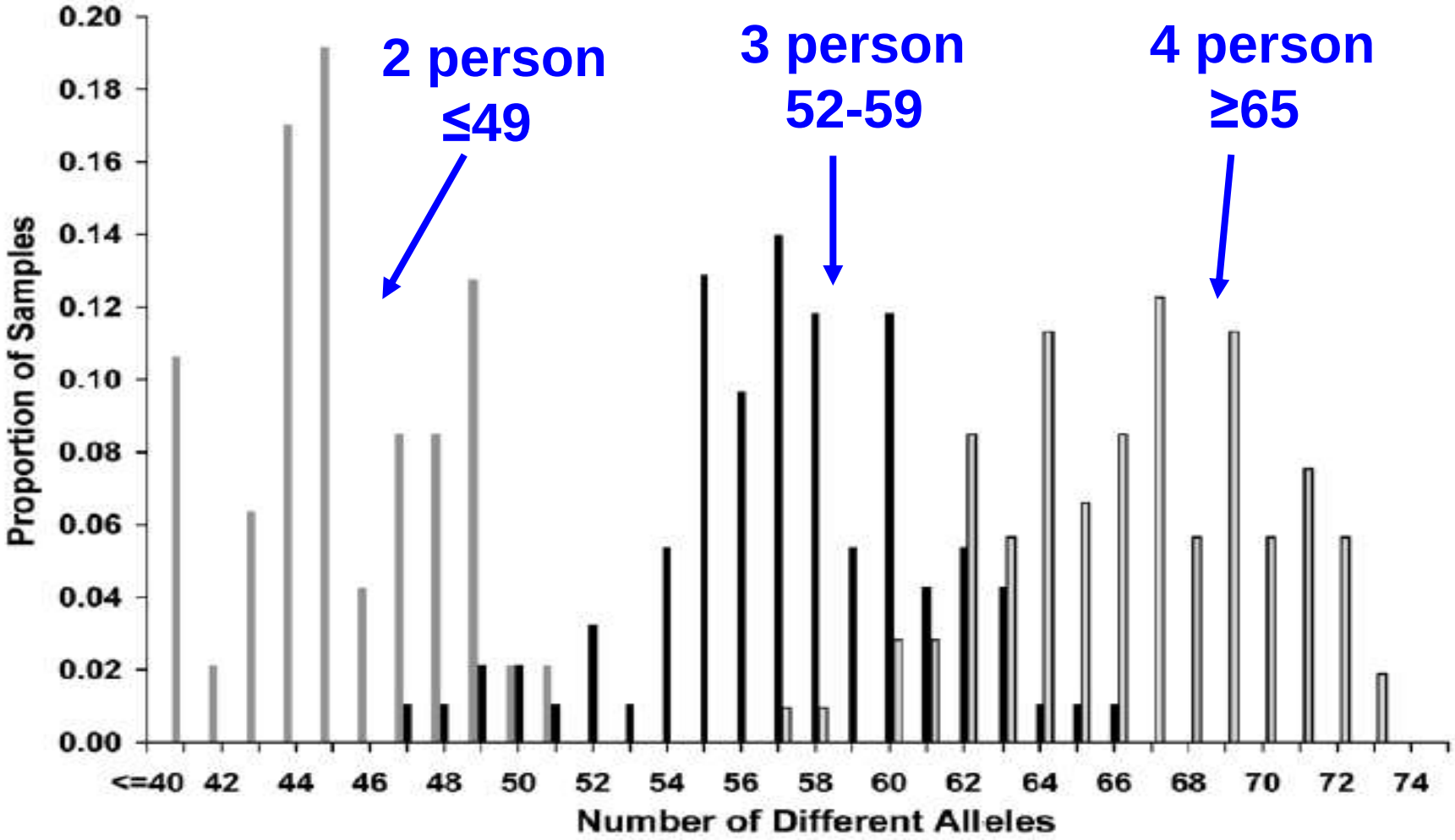


Figure 1. Expected # of different alleles from mixtures.

Estimating the number of contributors to two-, three-, and four-person mixtures containing DNA in high template and low template amounts
Perez et al., Croat Med J. 2011; 52:314-26

Two-Person Mixture Studies Summary

- **Always** recognized as a mixture – no risk of confusing as a single-source
  - Loci with 3 or 4 alleles
  - Peak height ratio imbalance at loci with 2 alleles
- Observe more loci with 2 or 3 alleles than 4 alleles – even when DNA from two heterozygous individuals were mixed
- 49 or fewer total alleles

Buckleton et al. Forensic Science International: Genetics 1 (2007) 20–28; Paoletti et al. J Forensic Sci, Nov. 2005, Vol. 50, No. 6; Haned et al. J Forensic Sci, January 2011, Vol. 56, No. 1; Perez et al., Croat Med J. 2011; 52:314-26

Three-Person Mixture Studies Summary

- No risk of confusing as a single-source
- Small risk of confusing with two-person mixture
  - Observe at least one locus with 5 or 6 alleles in ~97% of profiles (3% have ≤4 alleles)
  - 3% profiles look like 2-person mixture
  - Risk if LT-DNA, degradation, inhibition, primer mutation to look like 2-person mixture
- Most loci have 3 or 4 alleles
- 52-59 total alleles

Buckleton et al. Forensic Science International: Genetics 1 (2007) 20–28; Paoletti et al. J Forensic Sci, Nov. 2005, Vol. 50, No. 6; Haned et al. J Forensic Sci, January 2011, Vol. 56, No. 1; Perez et al., Croat Med J. 2011; 52:314-26

Four-Person Mixture Studies Summary

- No risk of confusing as a single-source
- Very small risk of confusing with two-person mixture
  - Likely to have peak height imbalance
- Very small number of loci with 8 alleles and very few with 7 alleles
  - High risk of confusing with three-person mixture
  - Risk if LT-DNA, degradation, inhibition, primer mutation
- ≥65 total alleles

Buckleton et al. Forensic Science International: Genetics 1 (2007) 20–28; Paoletti et al. J Forensic Sci, Nov. 2005, Vol. 50, No. 6; Haned et al. J Forensic Sci, January 2011, Vol. 56, No. 1; Perez et al., Croat Med J. 2011; 52:314-26

Four-Person Mixture Studies Summary

>70% of 4-person mixtures would NOT be recognized as 4-person mixtures based on allele count

Buckleton et al. Forensic Science International: Genetics 1 (2007) 20–28; Paoletti et al. J Forensic Sci, Nov. 2005, Vol. 50, No. 6; Haned et al. J Forensic Sci, January 2011, Vol. 56, No. 1; Perez et al., Croat Med J. 2011; 52:314-26

Five-, Six- Person Mixture Studies Summary

- >99% of 5 person mixtures would look like 4 person mixtures (~60%) or 3-person mixtures (~40%)
- Most 6 person mixtures would look like 5 person mixture (6%), 4-person mixtures (80%) or 3-person mixtures (14%)

Wang, T.W., Kalet, P., Pendleton, J., Gilbert, K., Lucas, L. and Birdwell, J.D. 2005 The probable number of contributors to a STR DNA mixture. http://www.promega.com/products/pm/genetic-identity/ishi-conference-proceedings/16th-ishi-poster-abstracts/; Haned et al. J Forensic Sci, January 2011, Vol. 56,(1), 23-28

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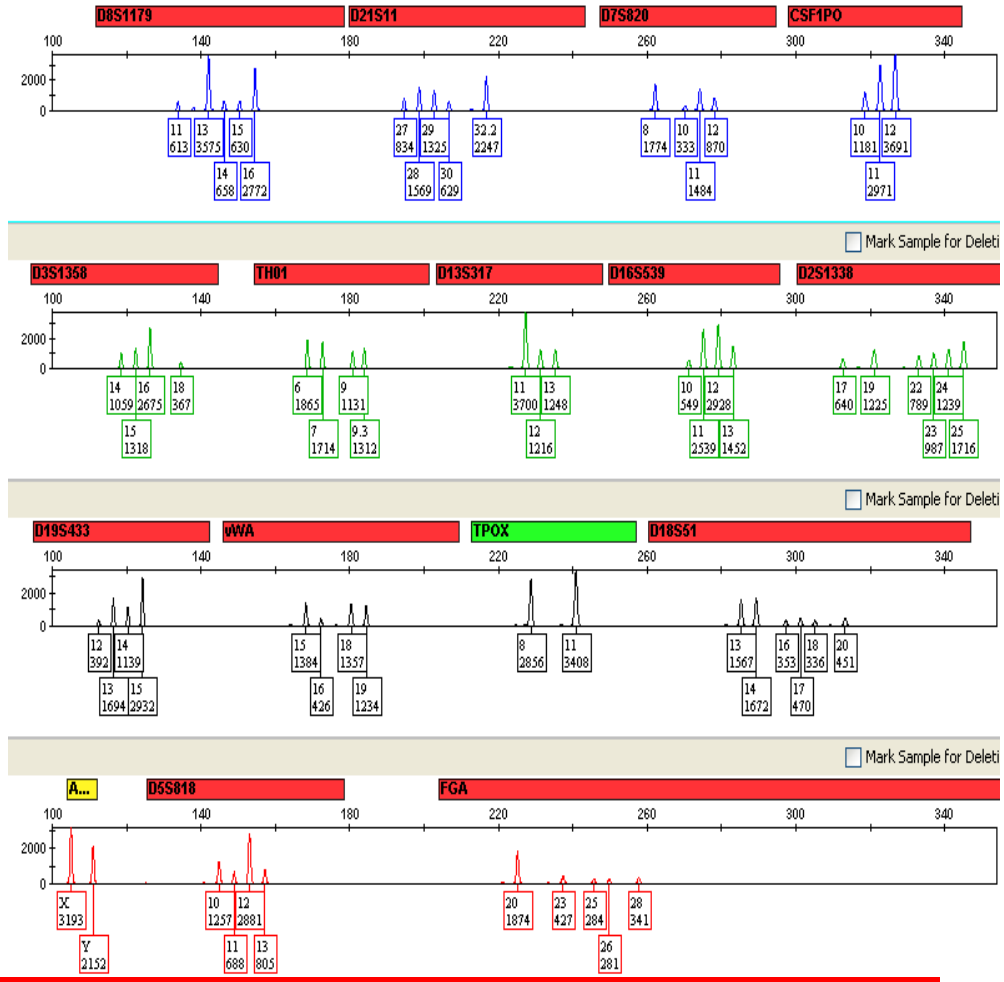
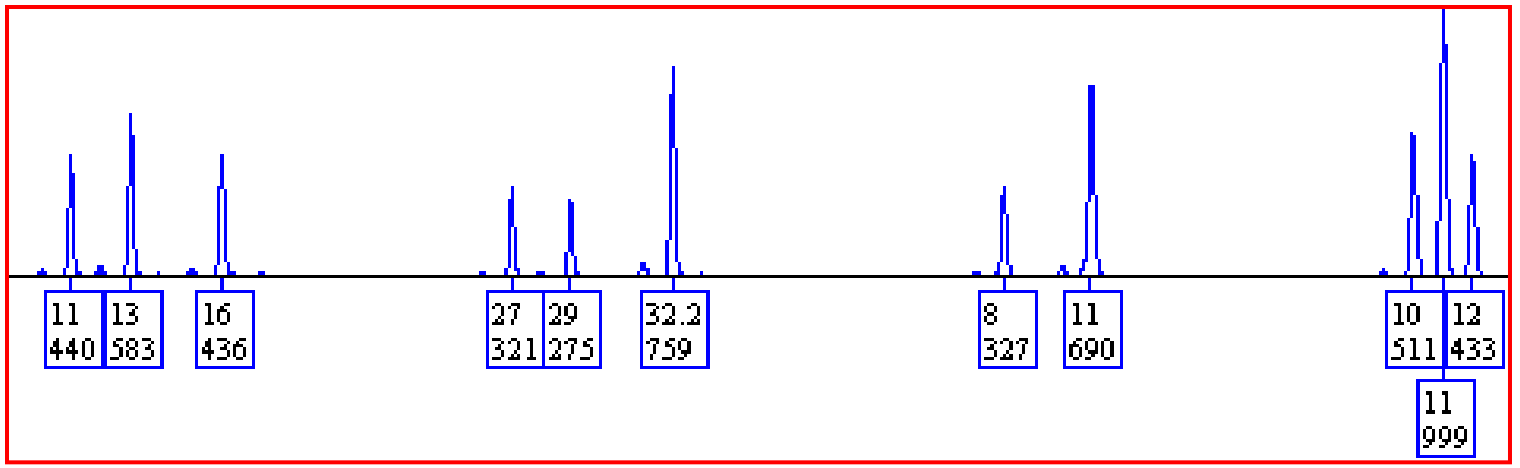


A 4-person mixture @ 1:1:1:2 ratio!!

Complex Mixtures

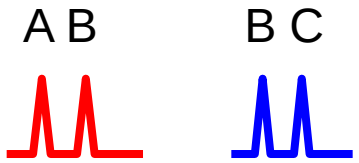
Mixtures with Relatives

Parent-Child
Sibling-Sibling



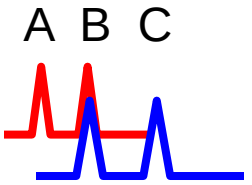
Parent + **Child**

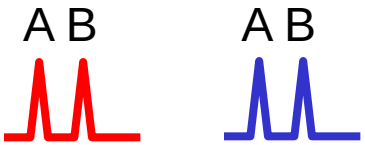
Mixture DNA Profile Pattern



Maximum: 3 alleles

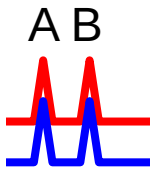
Both heterozygote, one shared allele

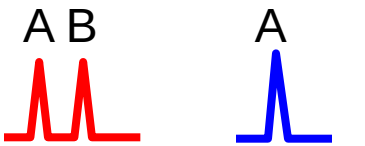




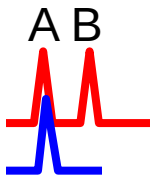
2 alleles

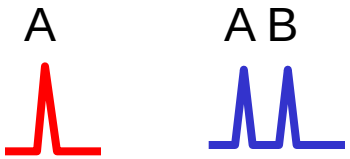
Heterozygote + heterozygote, two shared alleles



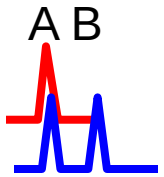


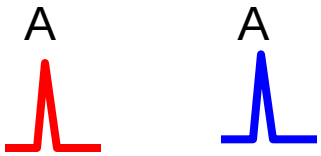
Heterozygote + homozygote, one shared allele





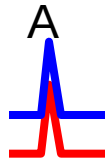
Homozygote + heterozygote, one shared allele



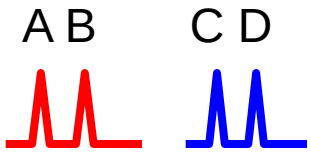
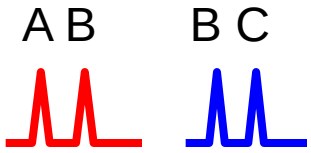
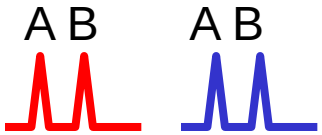
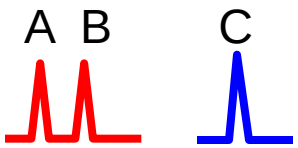
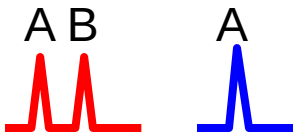
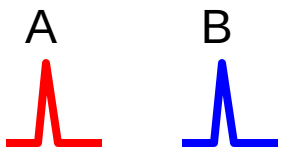
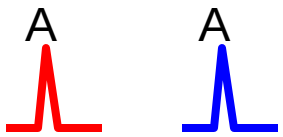


1 allele

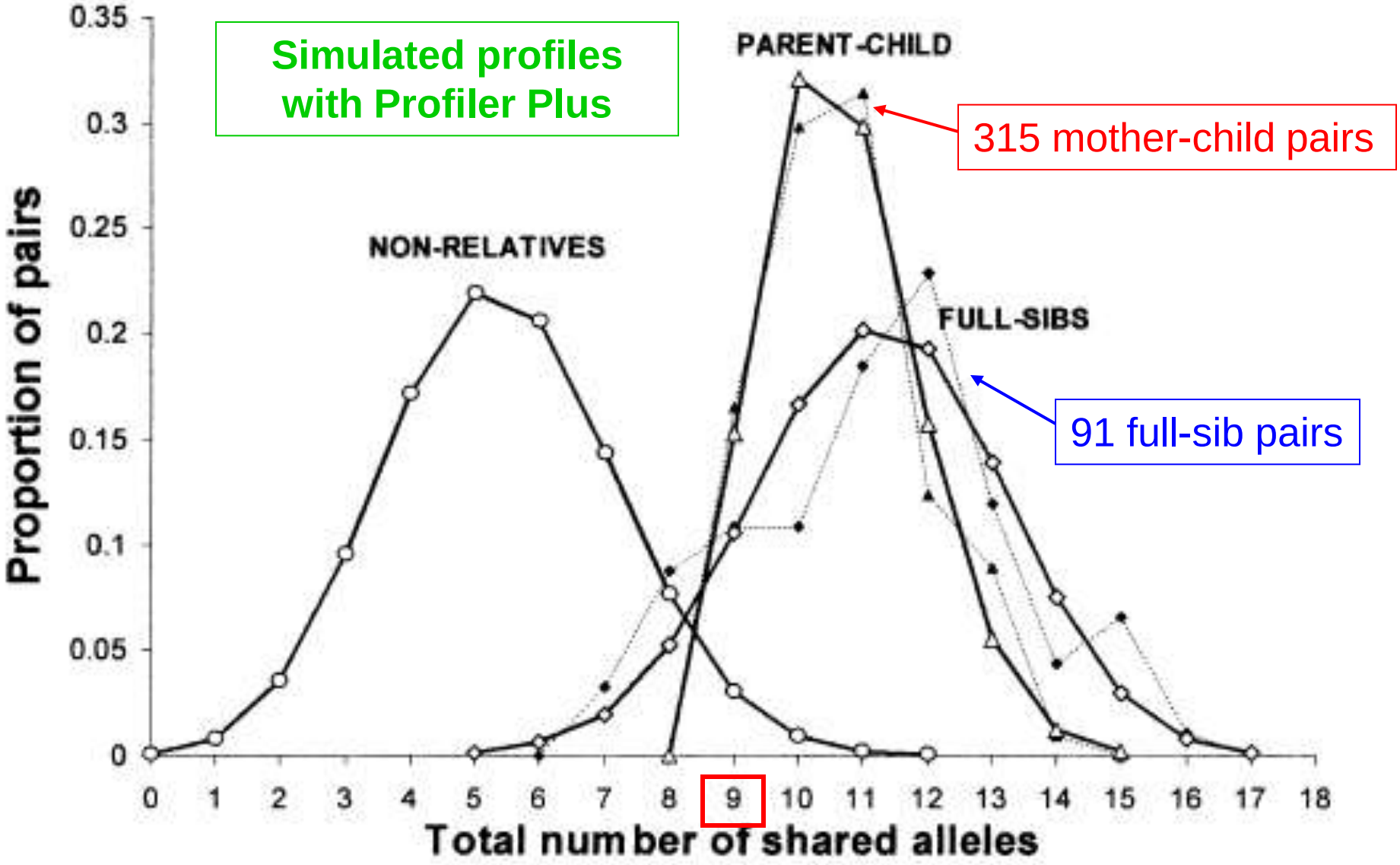
Homozygote + homozygote, one shared allele



ALLELE SHARE AT EACH LOCUS

P1 + P2	Genotypes of Children	% Sibling Allele Sharing
	AC or AD or BC or BD	0%, 50% or 100%
	AB or AC or BB or BC	0%, 50% or 100%
	AB/BA or AA or BB	0%, 50% or 100%
	AC or BC	50% or 100%
	AA or BA	50% or 100%
	AB	100%
	AA	100%

P1 = Parent 1; P2 = Parent 2



Forensic Science International 131 (2003) 85-89

Mixtures with Relatives – Summary

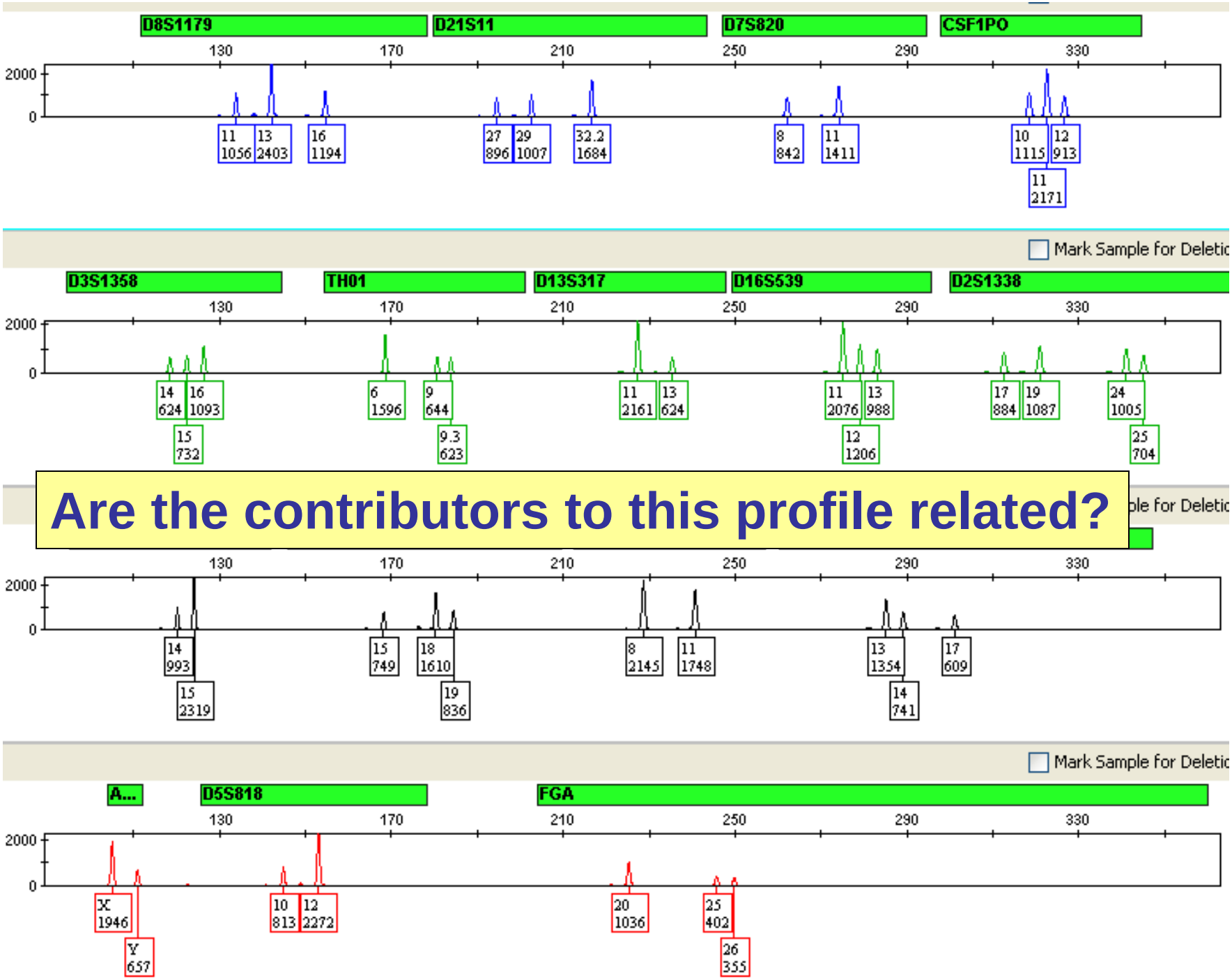
Parent-Child

- Expect at least 50% allele share
- Expect at least one shared allele at each locus
- Maximum 3 alleles per locus (in absence of mutation)
- If test X loci, expect >X allele shares (9-14 Profiler Plus; 13-20 CODIS)

Mixtures with Relatives – Summary

Sibling-Sibling

- Expect at least 50% allele share overall, but variable: 7-16 Profiler Plus; 12-22 CODIS (≥X-1)
- Expect 0, 50 or 100% allele share at each locus
- Expect at least one allele share at 9-13 loci (CODIS data)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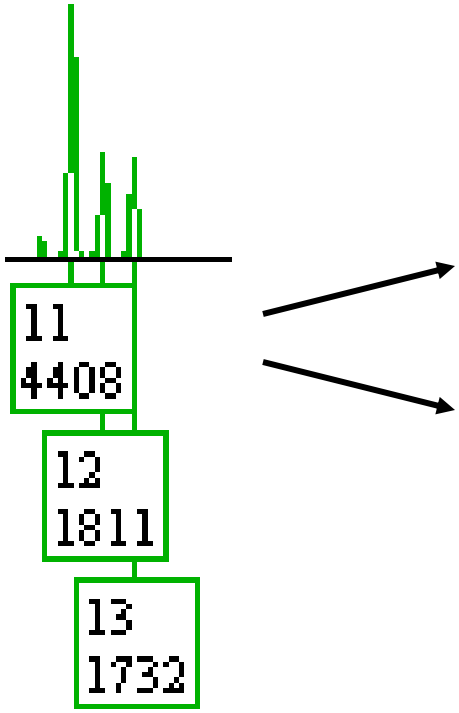
Are the contributors to this profile related?
ole for Deletic
14 993
15 2319
15 749
18 1610
19 836
8 2145
11 1748
13 1354
14 741
17 609
Mark Sample for Deletic
A...
D5S818
FGA
X 1946
Y 657
10 813
12 2272
20 1036
25 402
26 355

Mixtures with Relatives –
Working Backwards from Mixed DNA Profile

- With mixed DNA profile from unknowns, may not know if alleles are shared
- Data in the graphs are not helpful



11,12 + 11,13

or

11,11 + 12,13

Unrelated?

Relative?
Parent-Child?
Sibs?

Relative?
Sibs?

INCREASED COMPLEXITY

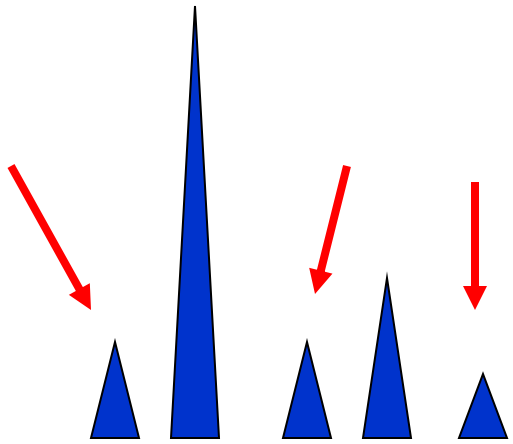
HIGH UNCERTAINTY

LACK OF CONFIDENCE

Complex Mixtures

More Uncertainty and Lack of Confidence

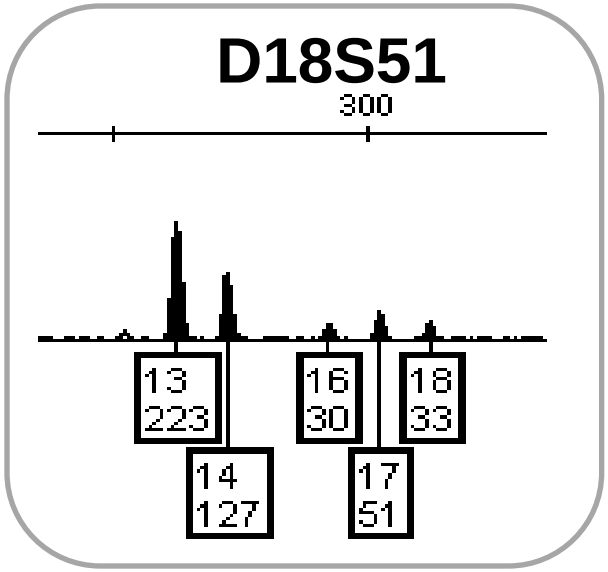
- Peak vs. Artifacts
  - Stutter?
  - Pull-up?
  - True Allelle?



Complex Mixtures

More Uncertainty and Lack of Confidence

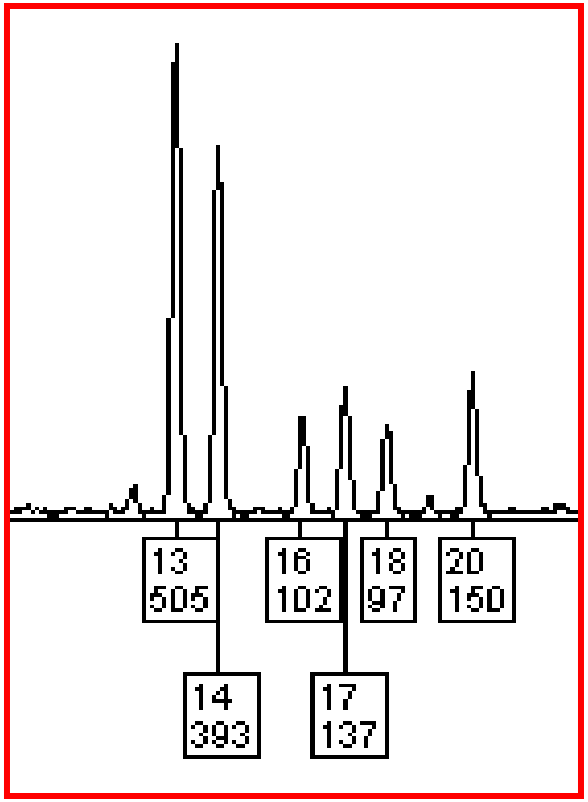
- High likelihood that DNA from one or more contributors is below optimal range
  - LT DNA = stochastic effects
  - Missing alleles? (allele drop out)
  - Elevated Stutter? True allele vs. Stutter?
  - Allele drop-in?



Complex Mixtures

More Uncertainty and Lack of Confidence

- Stochastic threshold
  - Only meaningful for the peaks below the value – may be missing sister allele
  - All alleles present?

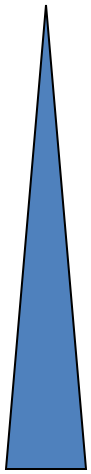


Complex Mixtures

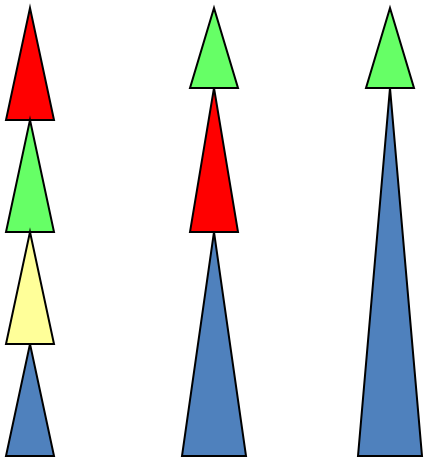
More Uncertainty and Lack of Confidence

- Stochastic threshold
  - NO meaning for peaks above the value –
    - Major contributor?
    - Shared alleles? How many shares? Relatives or unrelated

Major



vs.

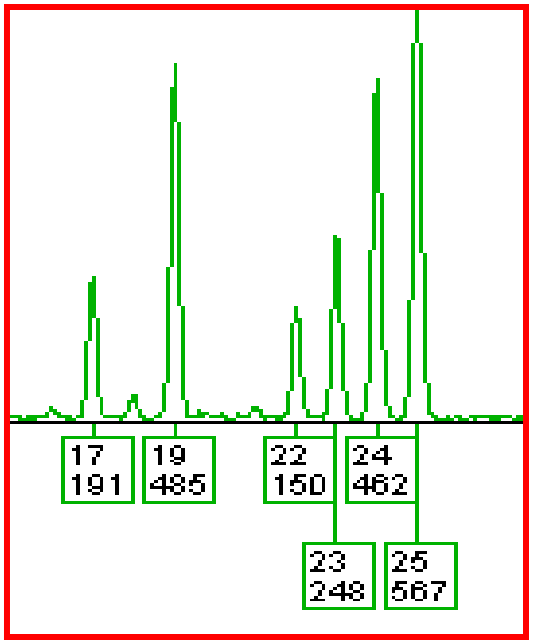


Shared alleles

Complex Mixtures

More Uncertainty and Lack of Confidence

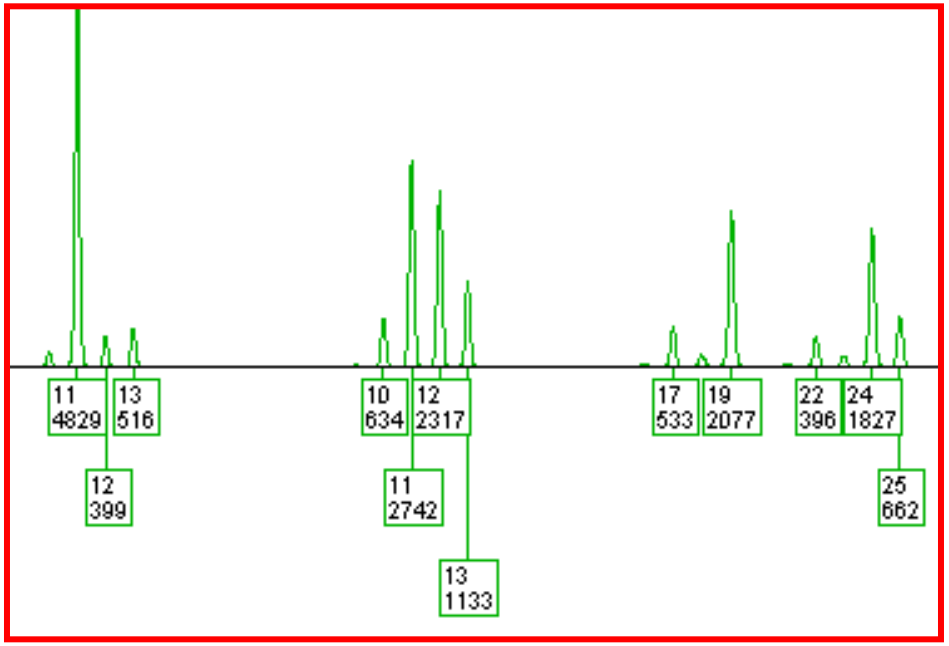
- Peak height ratios have no meaning at most or all loci
  - Cannot use to associate alleles into genotypes
  - Ability to deduce other contributors decreased even if know one contributor



Complex Mixtures

More Uncertainty and Lack of Confidence

- Mixture ratio cannot be calculated
  - Different amount from each contributor likely with no way to determine
  - Cannot use to associate genotypes into profiles



Complex Mixtures

More Uncertainty and Lack of Confidence

- Number of contributors – maximum allele/minimum number often an underestimate
  - What number to assume?
  - May need to interpret under multiple assumptions (especially if the conclusion changes)

Complex Mixtures

False Inclusions

- Increased risk as # of alleles increase
- Cannot assign meaningful statistical frequency

Exclusions less likely

- Can anyone be excluded if LT DNA present?
- Partial “inclusions”

Inconclusive reporting increased

Conclusions

- Criteria routinely used in crime laboratories for the interpretation of two-person mixtures may not apply for most complex mixtures

- LT-DNA, degradation, inhibition play more significant role

- Additional complex mixtures need to be generated and evaluated for establishment of interpretation guidelines

Thank you!

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