2012 Mixture Interpretation Workshop:

Mixtures **U**sing *SOUND* **S**tatistics, **I**nterpretation, & **C**onclusions

Complex Mixtures

Charlotte J. Word, Ph.D.

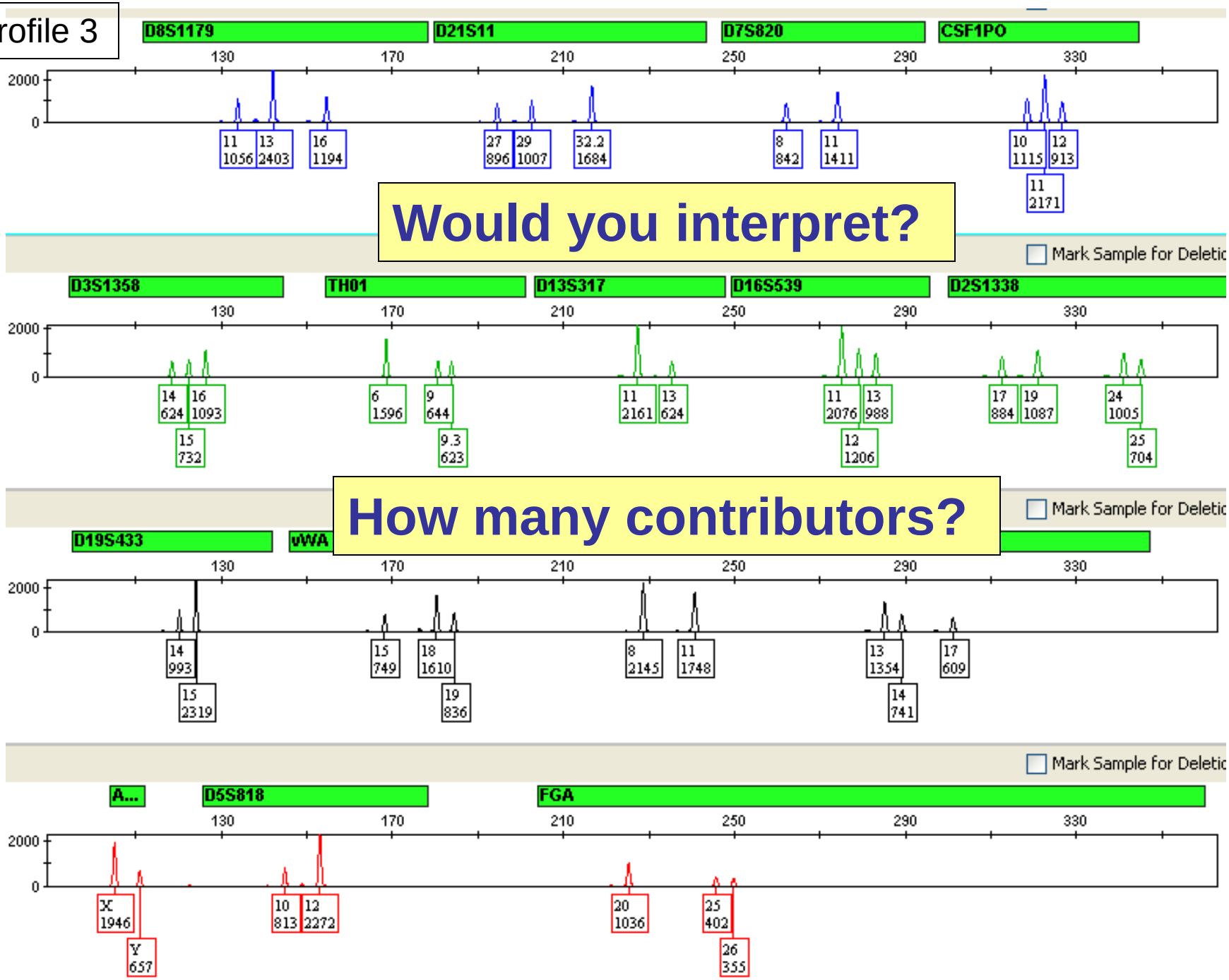
October 15, 2012

Nashville, TN

BOSTON UNIVERSITY

NIST

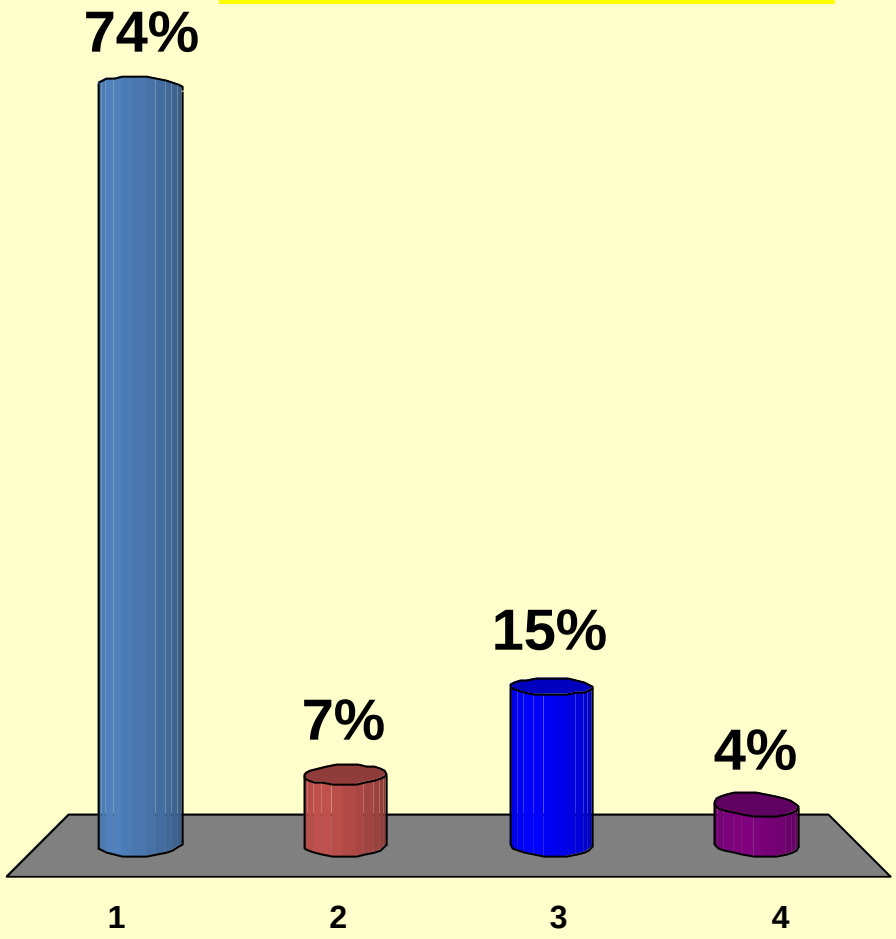
Profile 3


D8S1179
D21S11
D7S820
CSF1PO
130
170
210
250
290
330
2000
0
11 1056
13 2403
16 1194
27 896
29 1007
32.2 1684
8 842
11 1411
10 1115
12 913
11 2171
Would you interpret?
Mark Sample for Deletic
D3S1358
TH01
D13S317
D16S539
D2S1338
14 624
16 1093
15 732
6 1596
9 644
9.3 623
11 2161
13 624
11 2076
13 988
12 1206
17 884
19 1087
24 1005
25 704
How many contributors?
Mark Sample for Deletic
D19S433
vWA
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

Would you attempt to interpret the mixture in the previous slide?

1. Yes, definitely
2. No, definitely not
3. Only if I knew the profile for one contributor
4. Yes, but only with help from my technical reviewer

Data from 104 responses
ISHI Mixture Workshop (Oct 2012)



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

Two-Person Mixtures

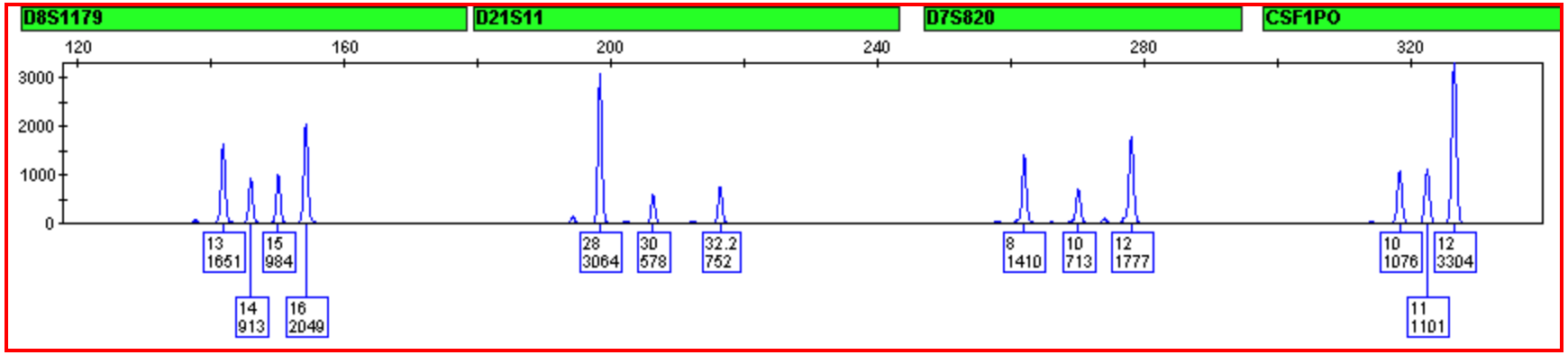
High Certainty Leads to High Confidence

- **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

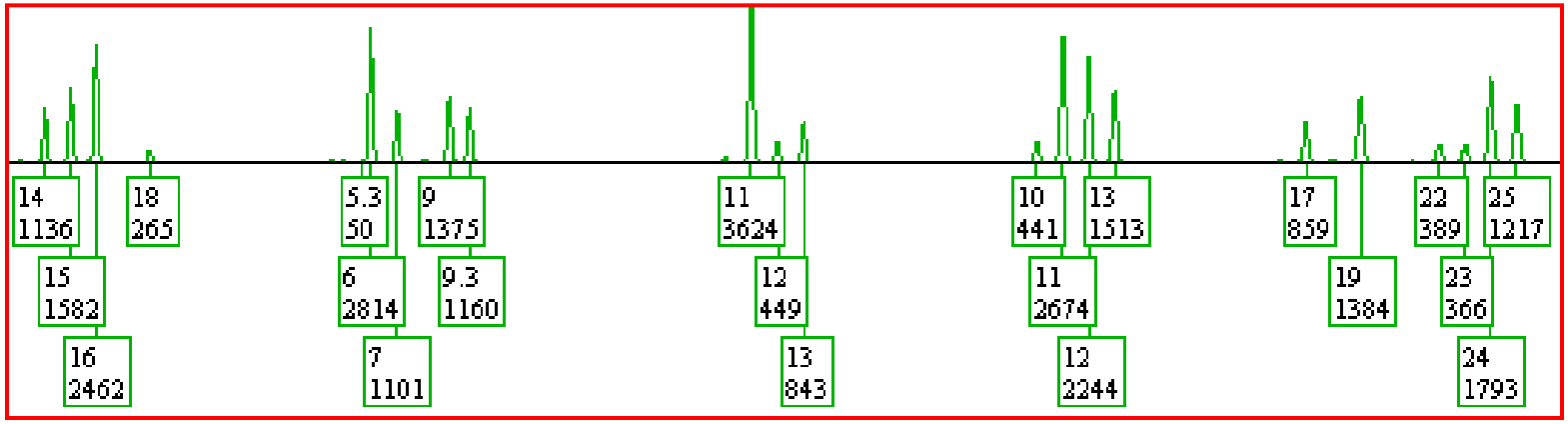


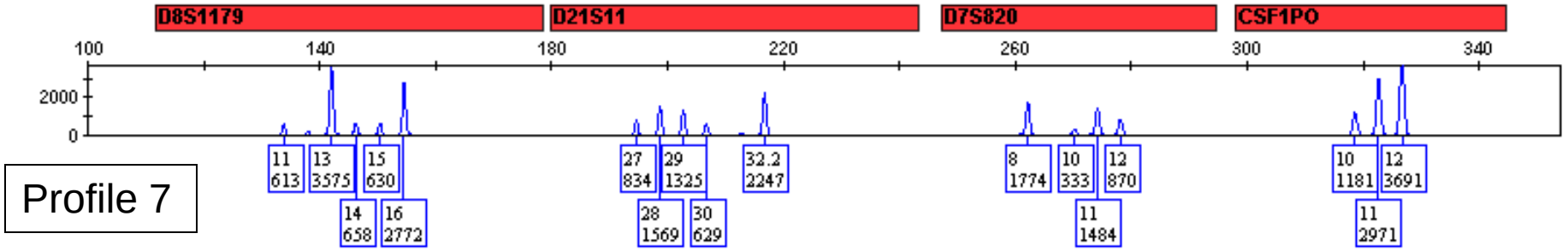
Complex Mixtures

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

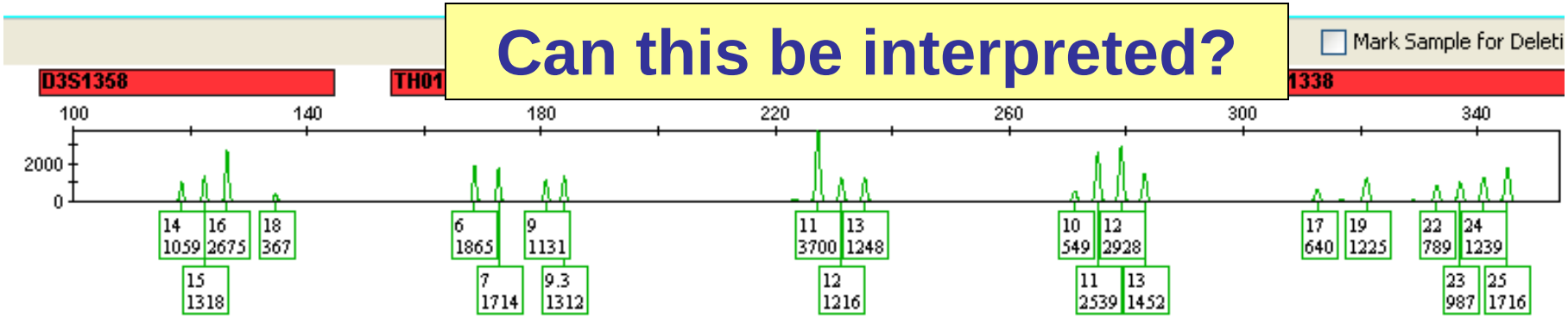
Complex Mixture Interpretation

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



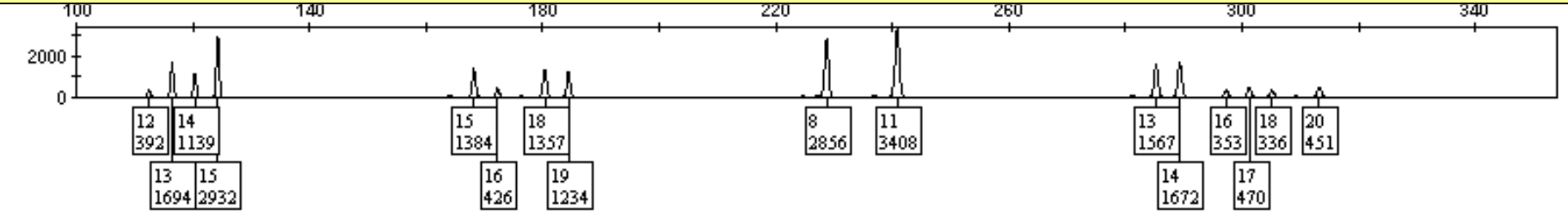


Profile 7

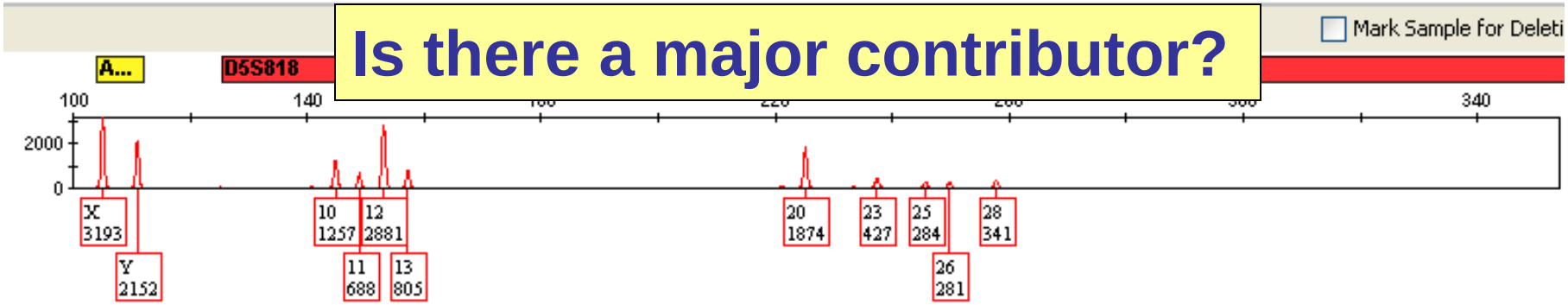


Can this be interpreted?

How many contributors assumed for interpretation?

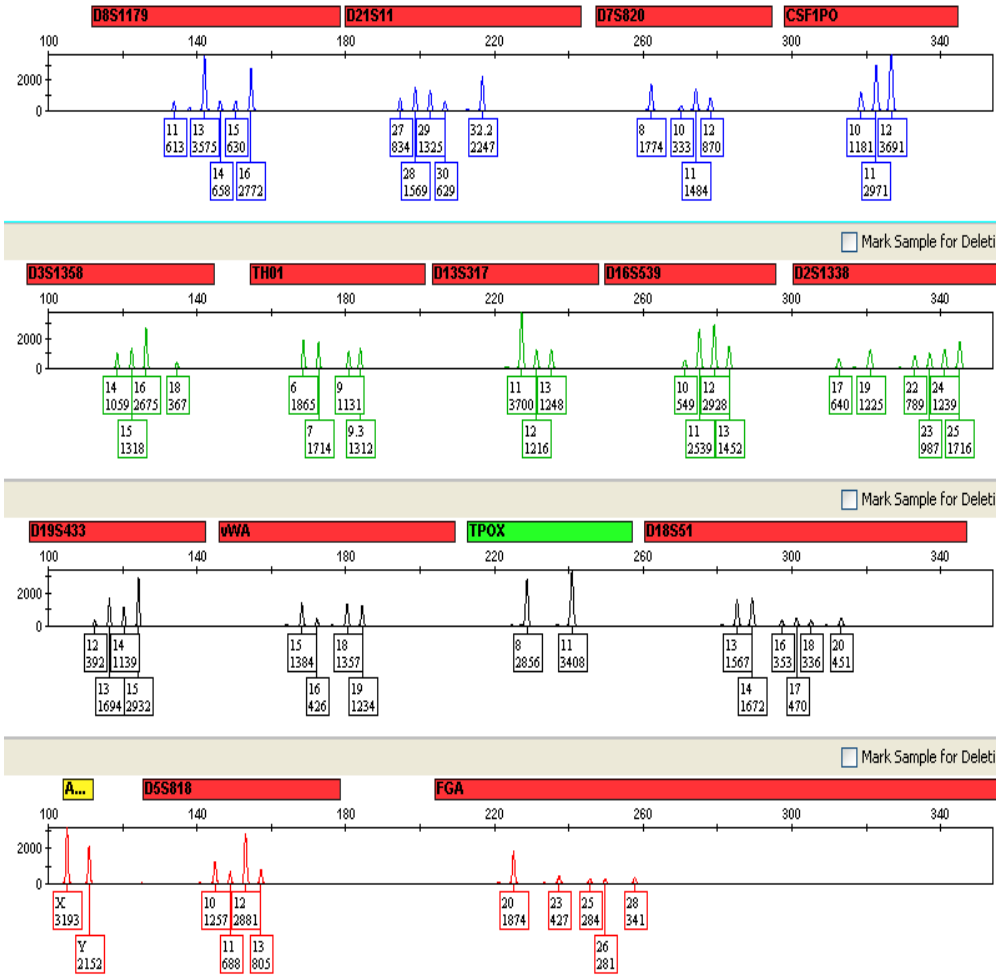


Is there a major contributor?



Complex Mixture – Allele Summary

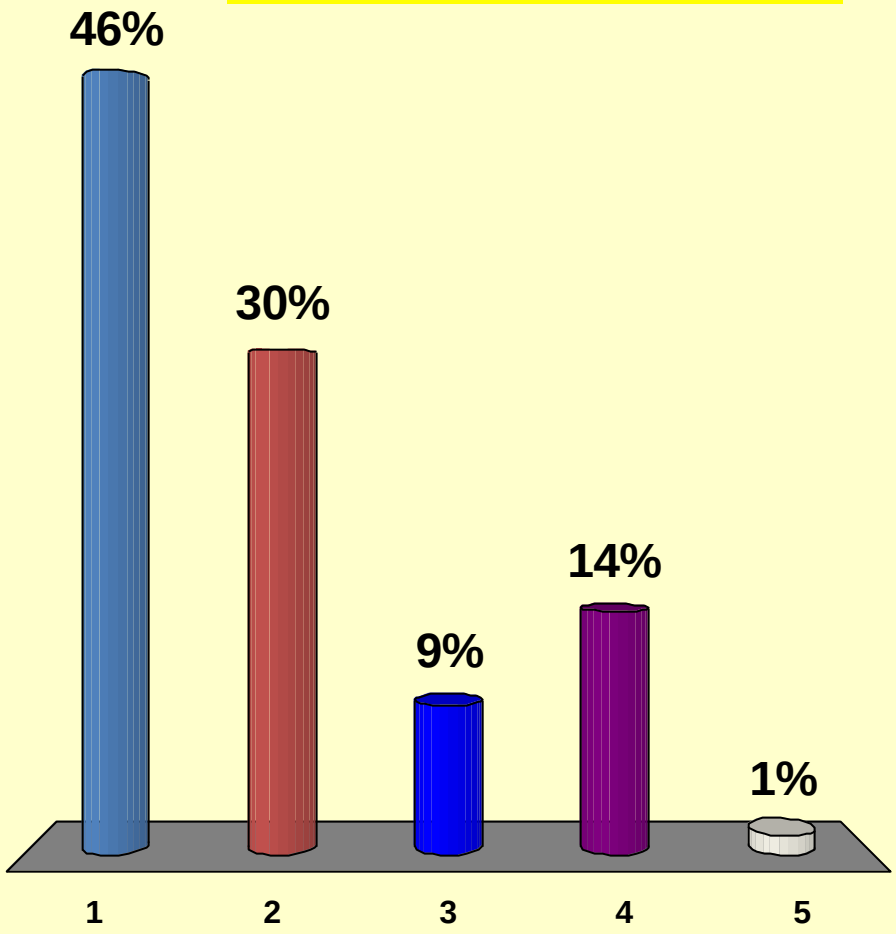
- 6 alleles at 2 loci
- 5 alleles at 3 loci
- 4 alleles at 7 loci
- 3 alleles at 2 loci
- 2 alleles at 1 locus
- 1 allele at 0 loci
- 63 total alleles



Would you attempt to interpret the mixture in the previous slide?

1. Yes, definitely
2. No, definitely not
3. Only if I knew the profile for one contributor
4. Yes, but only the major contributor
5. Yes, but only the minor contributor

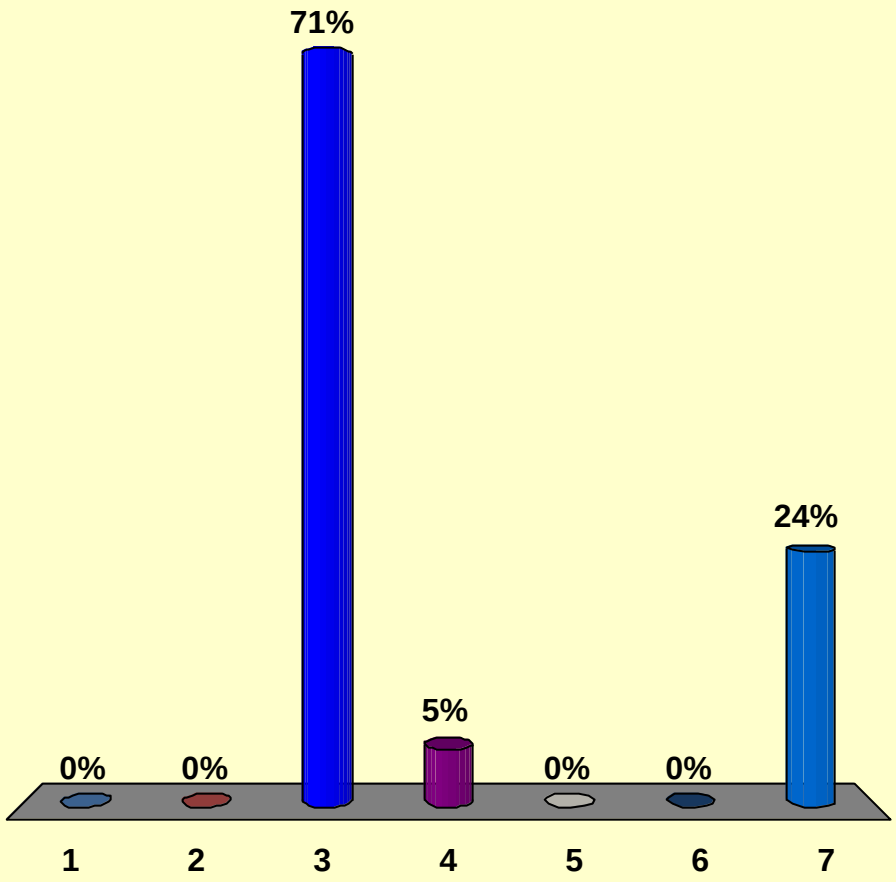
Data from 91 responses
ISHI Mixture Workshop (Oct 2012)



How many contributors should be assumed for interpretation?

1. 1
2. 2
3. 3
4. 4
5. 5
6. 6 or more
7. Use several assumptions

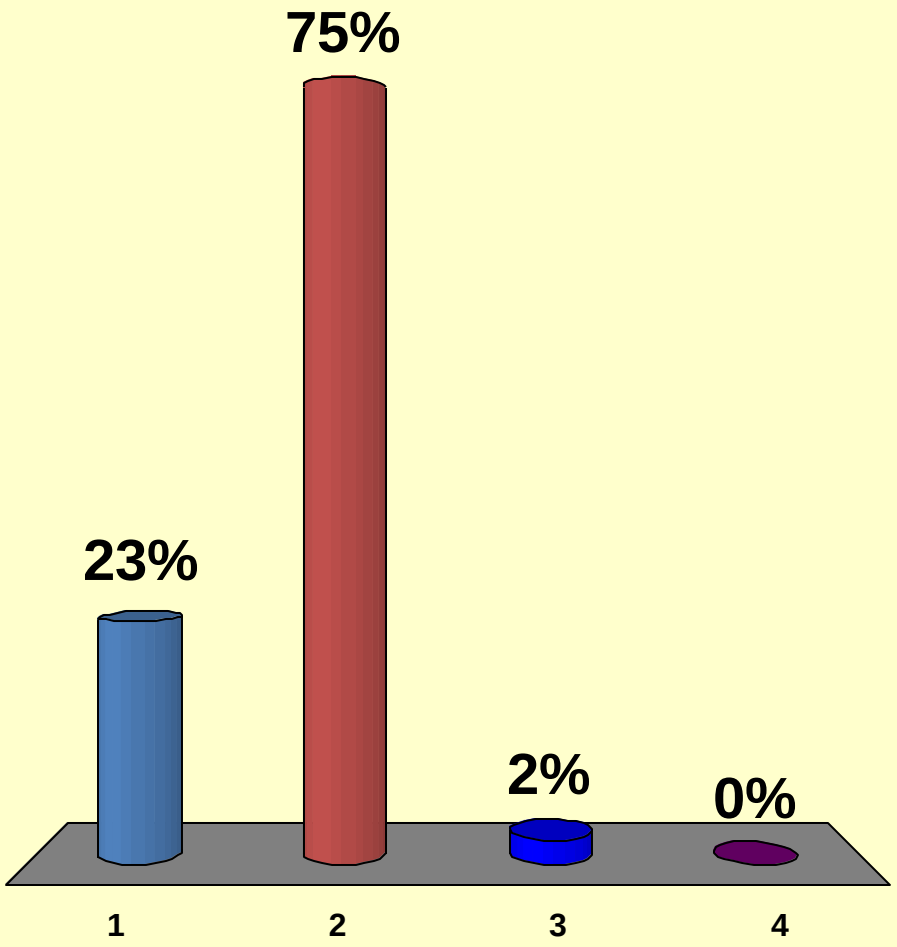
Data from 92 responses
ISHI Mixture Workshop (Oct 2012)



Can alleles for a major contributor be determined for this profile?

1. Yes, definitely
2. No, definitely not
3. Only if I knew the profile for one contributor
4. Only if the suspect is included

Data from 91 responses
ISHI Mixture Workshop (Oct 2012)

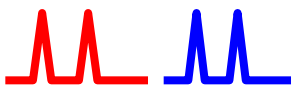


Two-Person Mixtures

Observed profile

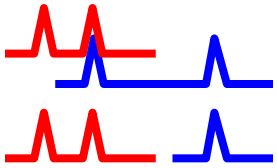
A **B**

14 total combinations



4 alleles

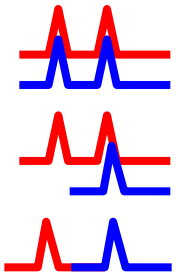
All heterozygotes and non-overlapping alleles



3 alleles

Heterozygote + heterozygote, one overlapping allele

Heterozygote + homozygote, no overlapping alleles

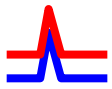


2 alleles

Heterozygote + heterozygote, two overlapping alleles

Heterozygote + homozygote, one overlapping allele

Homozygote + homozygote, no overlapping alleles



1 allele

Homozygote + homozygote, overlapping allele

Three-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

Four-Person Mixtures

Observed profile

MANY combinations

8 alleles

All heterozygotes and non-overlapping alleles

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

Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics 1 (2007) 20–28
FSI GENETICS
www.elsevier.com/locate/fsig

Towards understanding the effect of uncertainty in the number of contributors to DNA stains

John S. Buckleton [a], James M. Curran [b,*], Peter Gill [c]

[a] *The Institute of Environmental Science and Research Ltd., Private Bag 92021, Auckland, New Zealand*
[b] *Department of Statistics, University of Auckland, Private Bag 92019, Auckland, New Zealand*
[c] *The Forensic Science Service, Trident Court, Solihull Parkway, Birmingham Business Park, Solihull B37 7YN, UK*

Received 31 May 2006; received in revised form 12 September 2006; accepted 13 September 2006

Abstract

DNA evidence recovered from a scene or collected in relation to a case is generally declared as a mixture when more than two alleles are observed at several loci. However, in principle, all DNA profiles may be considered to be potentially mixtures, even those that show not more than two alleles at any locus. When using a likelihood ratio approach to the interpretation of mixed DNA profiles it is necessary to postulate the number of potential contributors. However, this number is never known with certainty. The possibility of a, say three-person mixture, presenting four or fewer peaks at each locus of the CODIS set was explored by Paoletti et al. [D.R. Paoletti, T.E. Doom, C.M. Krane, M.L. Raymer, D.E. Krane, Empirical analysis of the STR profiles resulting from conceptual mixtures, J. Forensic Sci. 50 (2005) 1361–1366]. In this work we extend this analysis to consider the profiler plus and SGM plus multiplices. We begin the assessment of the risk associated with current practice in the calculation of LR's. We open the discussion of possible ways to surmount this ambiguity.

Forensic Science International: Genetics 1 (2007) 20–28

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^{+TM} 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

2, 3, 4-Person Simulated Mixtures – CODIS Loci Number of Alleles at each Locus

J Forensic Sci, Nov. 2005, Vol. 50, No. 6
Paper ID JFS2004475
Available online at: www.astm.org

David R. Paoletti,[1] M.S.; Travis E. Doom,[1,2] Ph.D.; Carissa M. Krane,[3] Ph.D.; Michael L. Raymer,[1,2] Ph.D.; and Dan E. Krane,[4] Ph.D.

Empirical Analysis of the STR Profiles Resulting from Conceptual Mixtures

ABSTRACT: Samples containing DNA from two or more individuals can be difficult to interpret. Even ascertaining the number of contributors can be challenging and associated uncertainties can have dramatic effects on the interpretation of testing results. Using an FBI genotypes dataset, containing complete genotype information from the 13 Combined DNA Index System (CODIS) loci for 959 individuals, all possible mixtures of three individuals were exhaustively and empirically computed. Allele sharing between pairs of individuals in the original dataset, a randomized dataset and datasets of generated cousins and siblings was evaluated as were the number of loci that were necessary to reliably deduce the number of contributors present in simulated mixtures of four or less contributors. The relatively small number of alleles detectable at most CODIS loci and the fact that some alleles are likely to be shared between individuals within a population can make the maximum number of different alleles observed at any tested loci an unreliable indicator of the maximum number of contributors to a mixed DNA sample. This analysis does not use other data available from the electropherograms (such as peak height or peak area) to estimate the number of contributors to each mixture. As a result, the study represents a worst case analysis of mixture characterization. Within this dataset, approximately 3% of three-person mixtures would be mischaracterized as two-person mixtures and more than 70% of four-person mixtures would be mischaracterized as two- or three-person mixtures using only the maximum number of alleles observed at any tested locus.

Paoletti et al. J Forensic Sci, Nov. 2005, Vol. 50, No. 6

2- to 5-Person Simulated Mixtures – Identifiler Number of Alleles vs. Likelihood Estimator

J Forensic Sci, January 2011, Vol. 56, No. 1
doi: 10.1111/j.1556-4029.2010.01550.x
Available online at: interscience.wiley.com

PAPER

CRIMINALISTICS

Hinda Haned,[1] M.S.; Laurent Pène,[2] M.S.; Jean R. Lobry,[1] Ph.D.; Anne B. Dufour,[1] Ph.D.; and Dominique Pontier,[1] Ph.D.

Estimating the Number of Contributors to Forensic DNA Mixtures: Does Maximum Likelihood Perform Better Than Maximum Allele Count?

Haned et al. J Forensic Sci, January 2011, Vol. 56, No. 1

Number of Contributors – Total Number of Alleles

314 FORENSIC SCIENCE

doi: 10.3325/cmj.2011.52.314

CMJ

Estimating the number of contributors to two-, three-, and four-person mixtures containing DNA in high template and low template amounts

Jaheida Perez, Adele A. Mitchell, Nubia Ducasse, Jeannie Tamariz, Theresa Caragine

Office of Chief Medical Examiner of the City of New York, The Department of Forensic Biology, New York, NY, USA

Perez et al., Croat Med J. 2011; 52:314-26

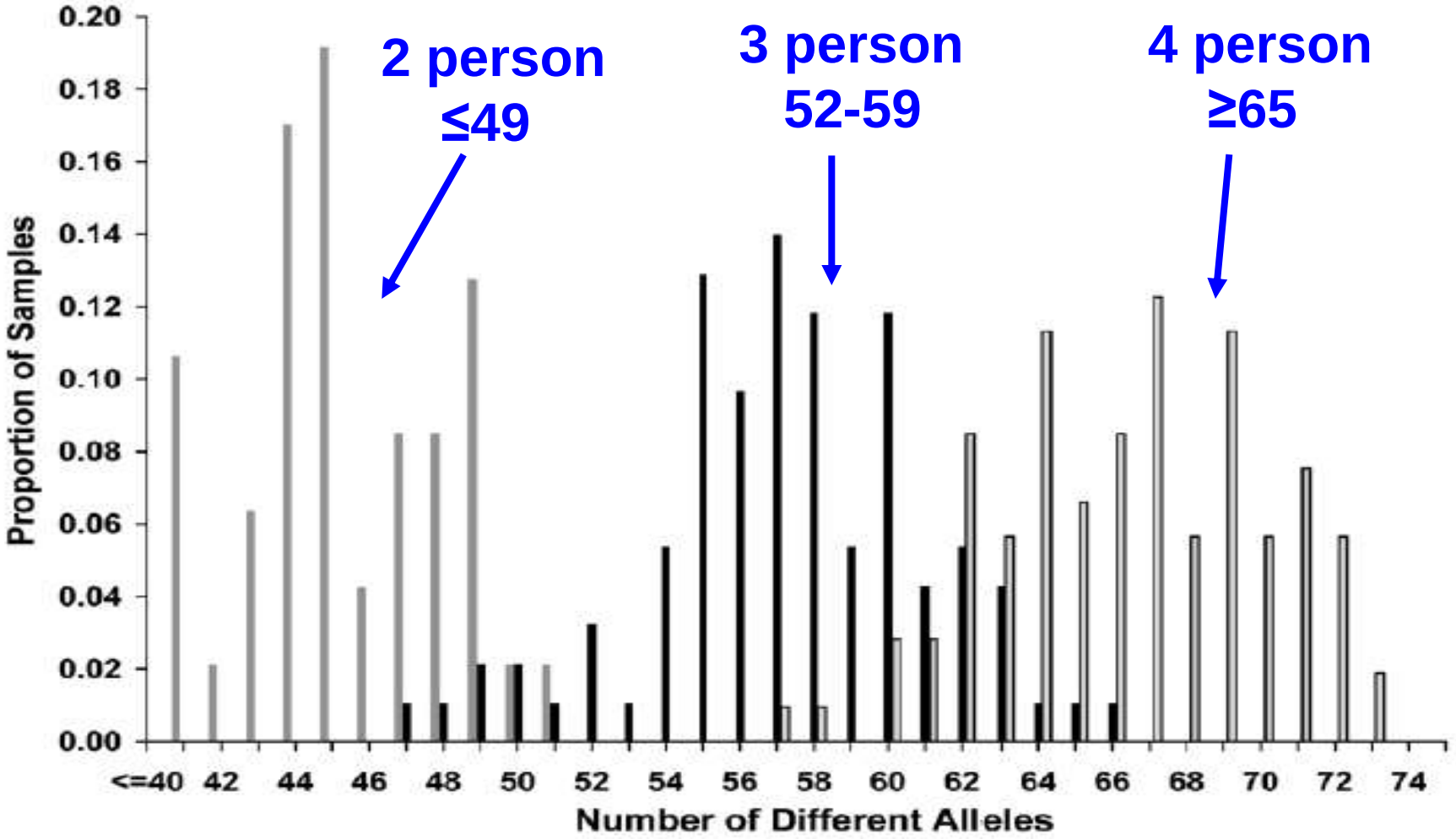


Figure 1. Expected # of different alleles from mixtures.

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

Based on Allele Counts Alone:

- **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)
  - Maximum allele count works most of time
  - 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 maximum number allele count at a locus

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

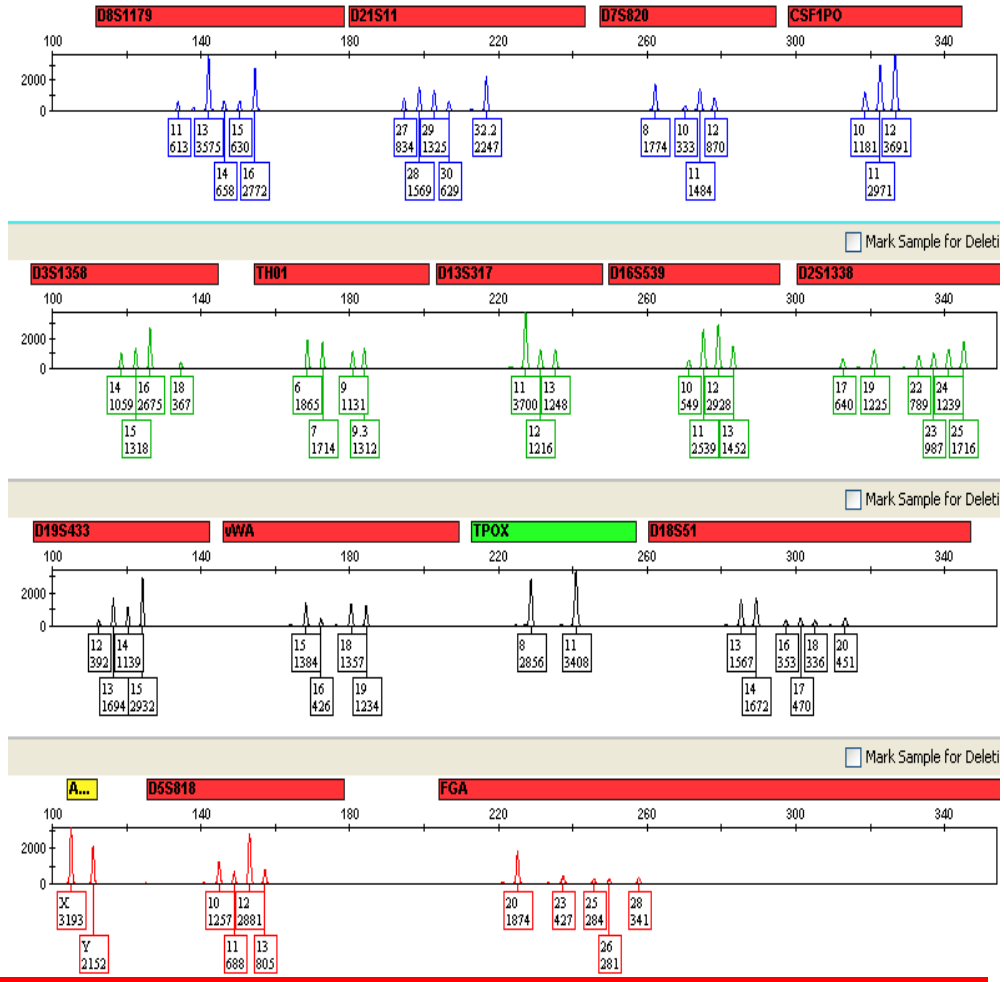
Five-, Six- Person Mixture Studies Summary

- >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

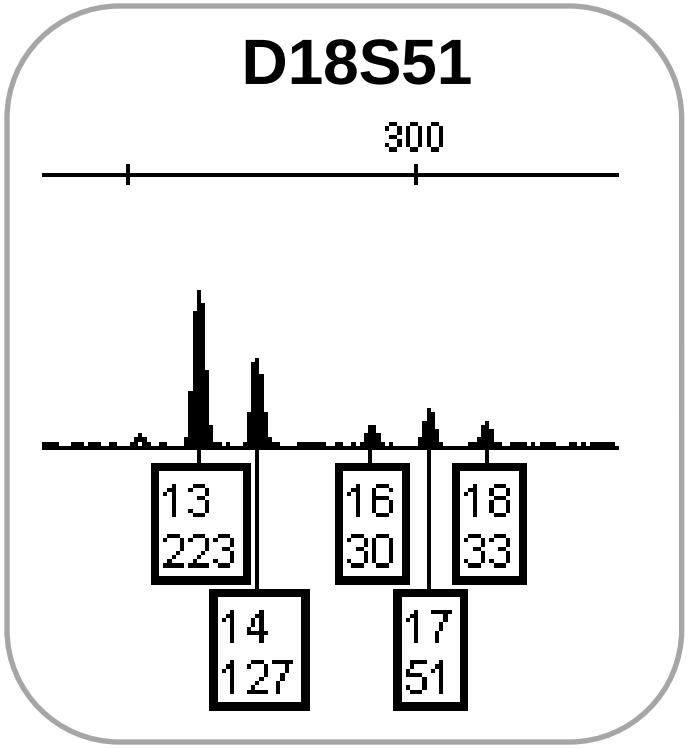
Complex Mixture – Allele Summary

- 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



A 4-person mixture @ 1.6:3:1:2 ratio!!

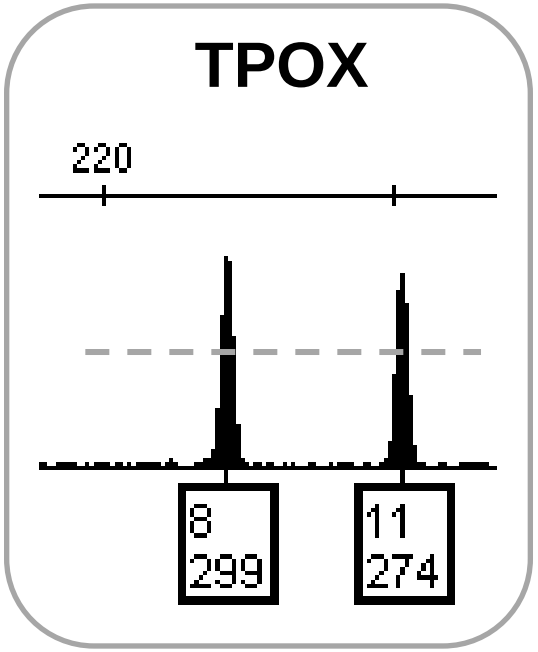
Uncertainty in the Potential Number of Contributors with this Result



5 alleles observed

- Several of the peaks are barely above the analytical threshold of 30 RFU
  - In fact, with an analytical threshold of 50 RFU or even 35 RFU, there would only be three detected alleles at D18S51
- Stochastic effects could result in a high degree of stutter off of the 17 allele making alleles 16 and 18 potential stutter products
- No other loci have >4 alleles detected

All Detected Alleles Are Above the Stochastic Threshold – **Or Are They?**



Stochastic threshold = 150 RFU

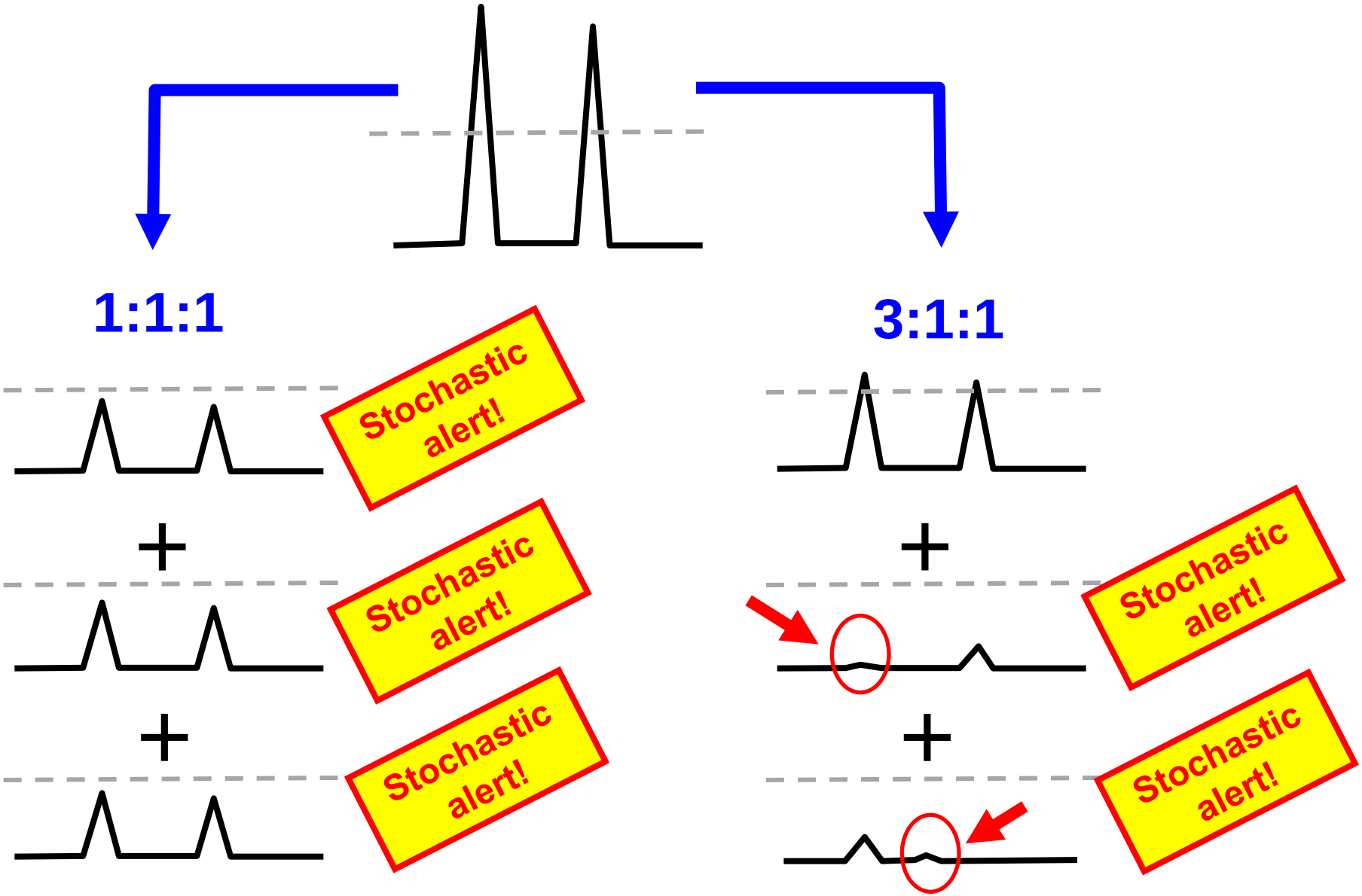
Does this result guarantee no allele drop-out?

We have assumed three contributors. If result is from an equal contribution of 3 individuals…

Then some alleles from individual contributors would be below the stochastic threshold and we could not assume that all alleles are being observed!

Assuming Three Contributors…

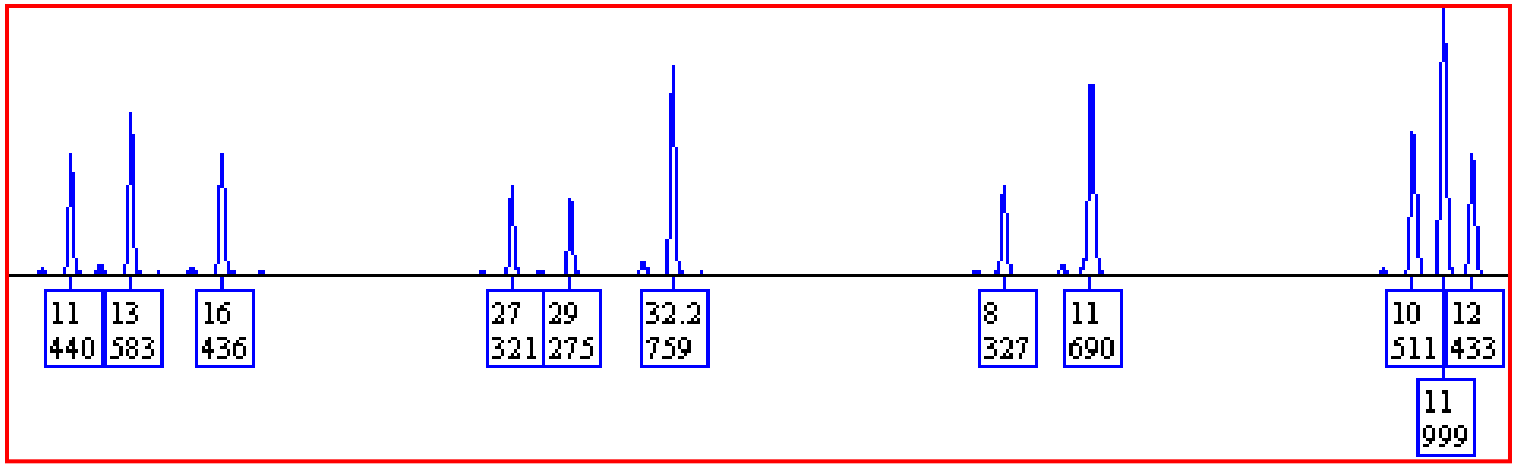
Some Possible Contributions to This Result



Complex Mixtures

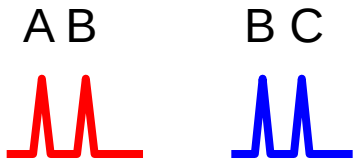
Mixtures with Relatives

Parent-Child
Sibling-Sibling



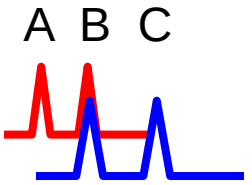
Parent + **Child**

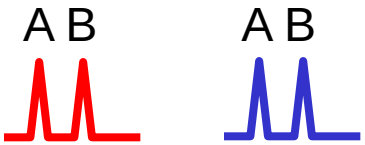
Mixture DNA Profile Pattern



Maximum: 3 alleles

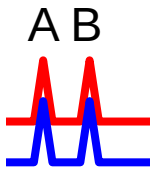
Both heterozygote, one shared allele

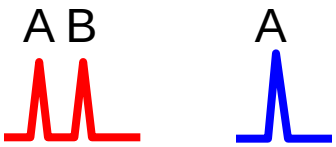




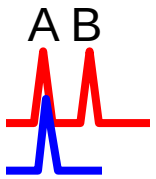
2 alleles

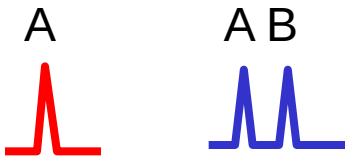
Heterozygote + heterozygote, two shared alleles



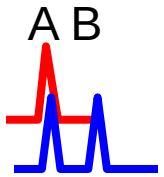


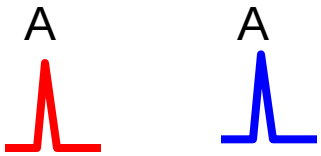
Heterozygote + homozygote, one shared allele





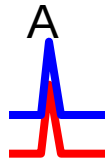
Homozygote + heterozygote, one shared allele



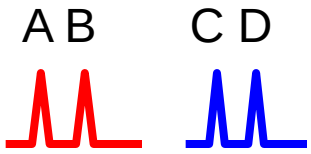
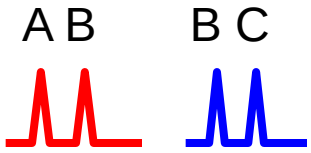
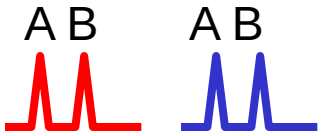
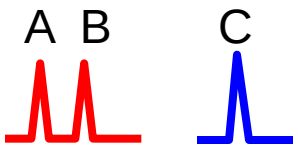
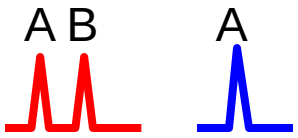
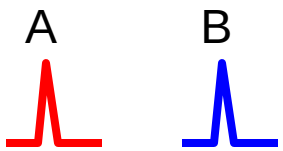
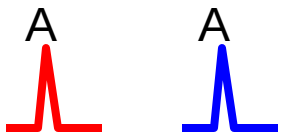


1 allele

Homozygote + homozygote, one shared allele



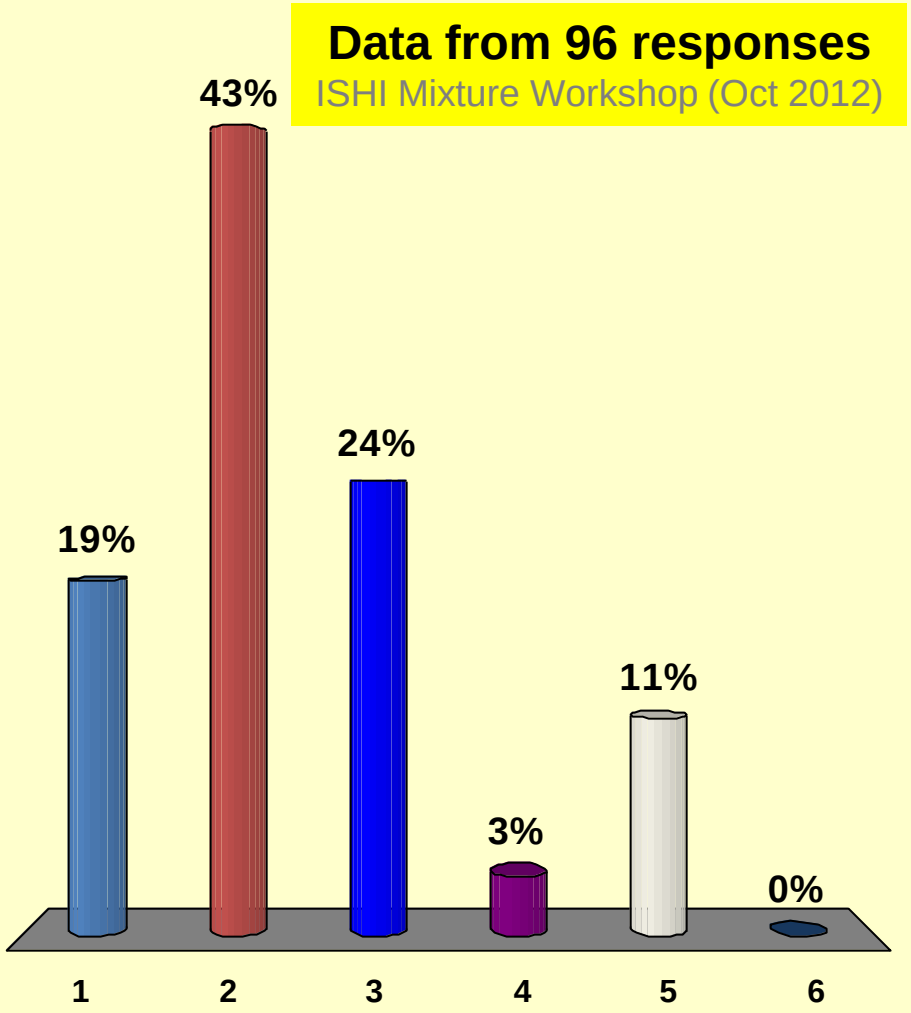
ALLELE SHARE AT EACH LOCUS

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

If I suspected the perpetrator in a case was related to one of the known contributors based on their DNA profiles, I would…

1. State it in a report
2. Tell the investigator
3. Tell the technical leader or lab director
4. Do nothing
5. Do Y STR testing (if males)
6. Do mtDNA testing



Allele Sharing in Relatives

ELSEVIER

Forensic Science International 131 (2003) 85–89

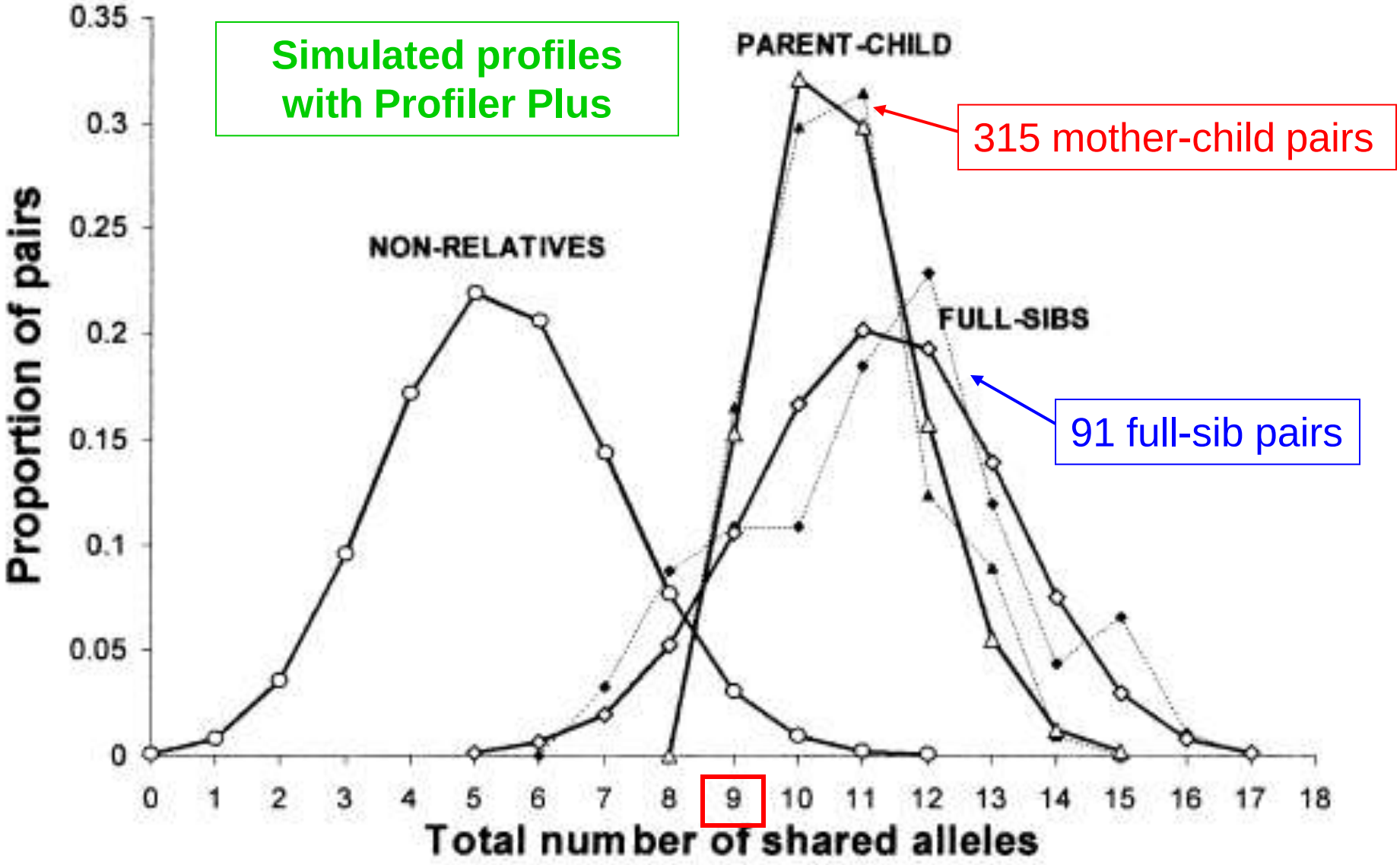
Forensic Science International

www.elsevier.com/locate/forsciint

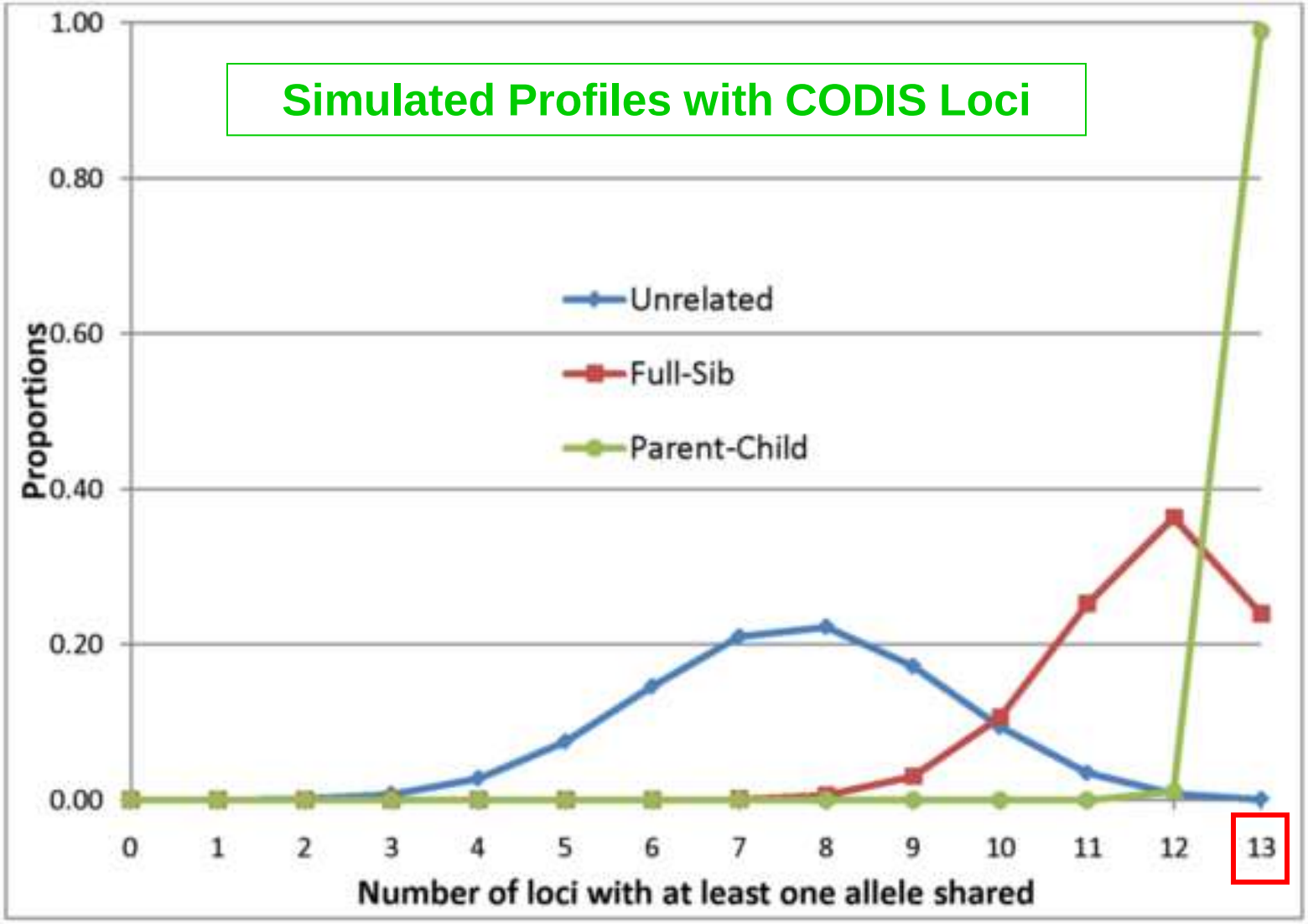
Allele sharing in first-degree and unrelated pairs of individuals in the Ge.F.I. AmpFlSTR® Profiler Plus™ database

Silvano Presciuttini[a,l,*], Francesca Ciampini[a], Milena Alù[b], Nicoletta Cerri[c], Marina Dobosz[d], Ranieri Domenici[e], Gabriella Peloso[f], Susi Pelotti[g], Andrea Piccinini[h], Elena Ponzano[i], Ugo Ricci[j], Adriano Tagliabracci[k], J.E. Baley-Wilson[l], Francesco De Stefano[m], Vincenzo Pascali[d,l]

Presciuttini et al. Forensic Science International 131 (2003) 85-89



Forensic Science International 131 (2003) 85-89



Ge et al. Comparisons of the familial DNA databases searching policies. J. Forensic Sci. 2011;56(6):1448-56.

Mixtures with Relatives – Summary

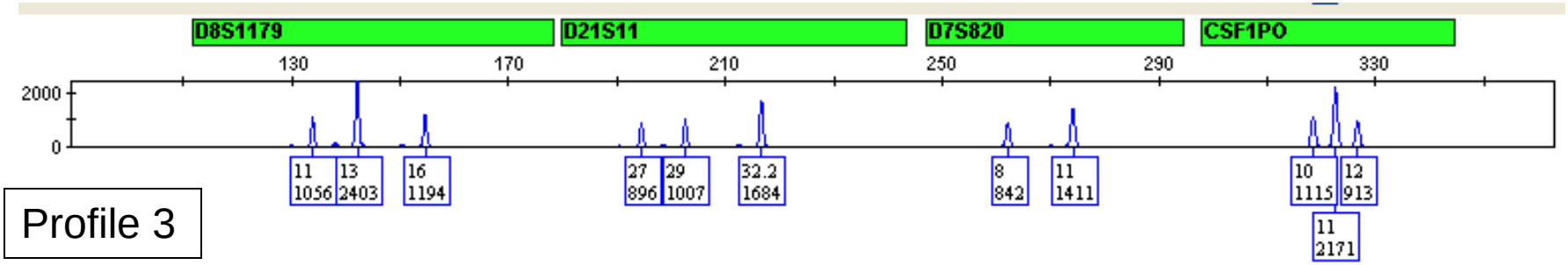
Parent-Child

- 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)

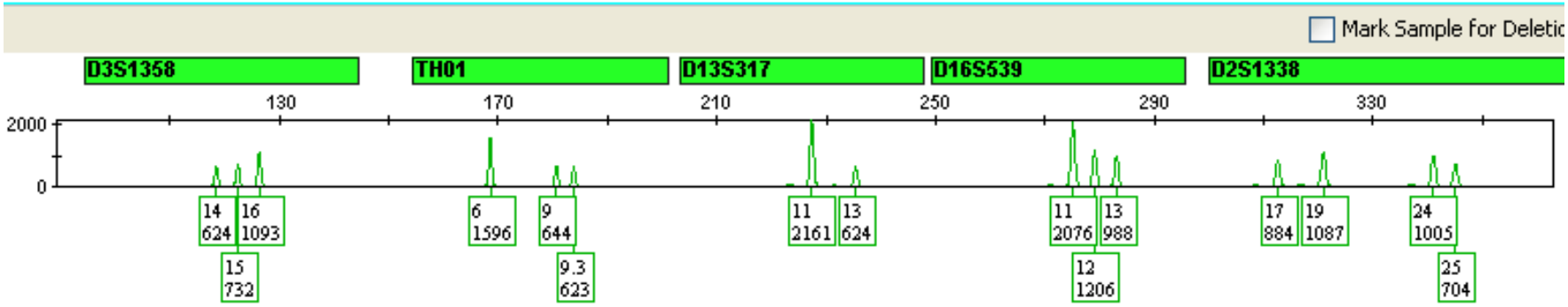
Mixtures with Relatives – Summary

Sibling-Sibling

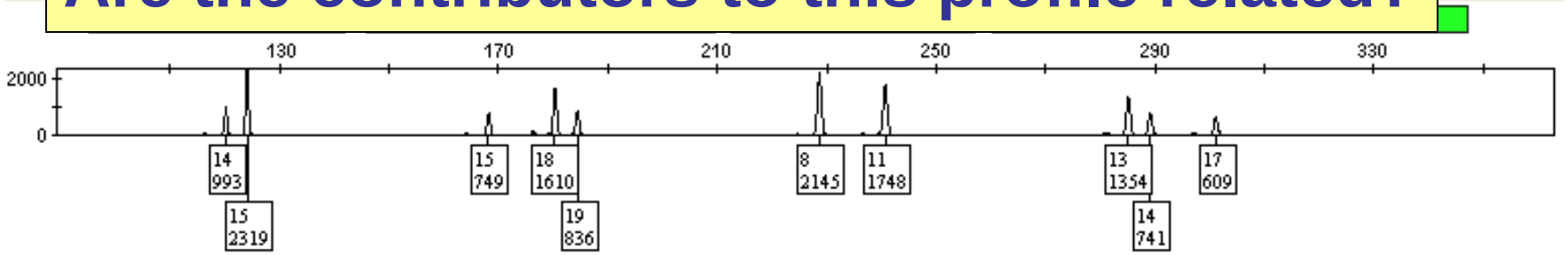
- Expect at least 50% allele share overall, but variable: 7-16 Profiler Plus; 12-22 CODIS (≥X-1)
- Expect 0, 50 or 100% allele share at each locus
- Expect at least one allele share at 9-13 loci (CODIS data)

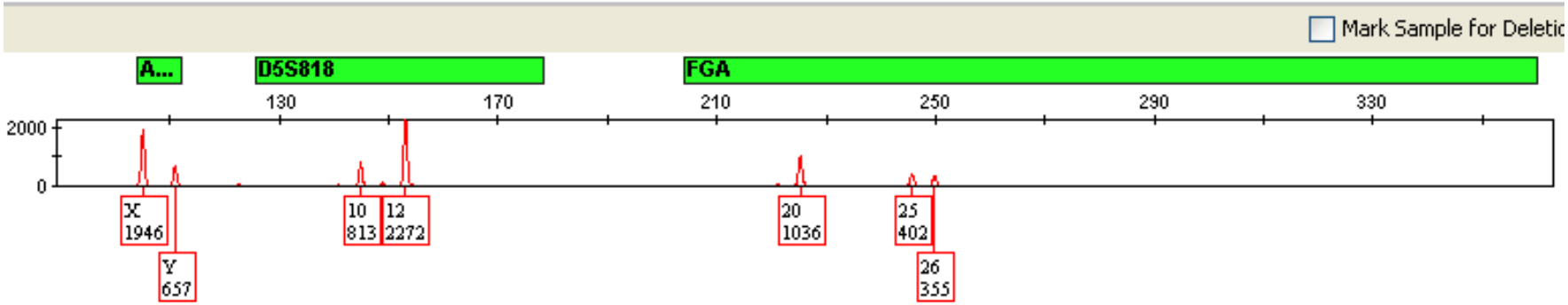


Profile 3



Are the contributors to this profile related?

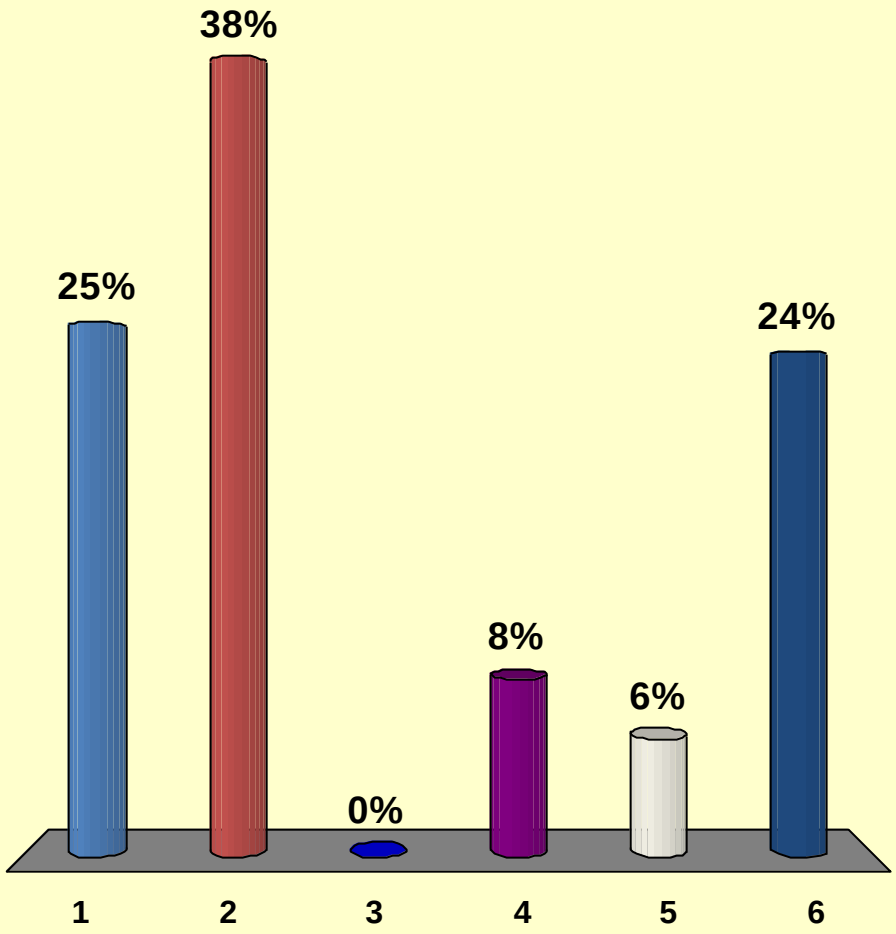




The contributors to the previous profile are most likely….

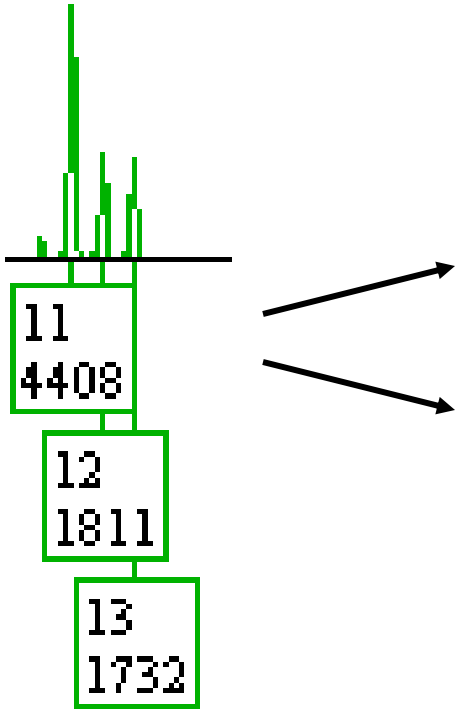
1. Related as parent-offspring
2. Related as siblings
3. Related as cousins or other non-first degree relatives
4. Related but mutations occurred
5. Unrelated
6. Insufficient information

Data from 72 responses
ISHI Mixture Workshop (Oct 2012)



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

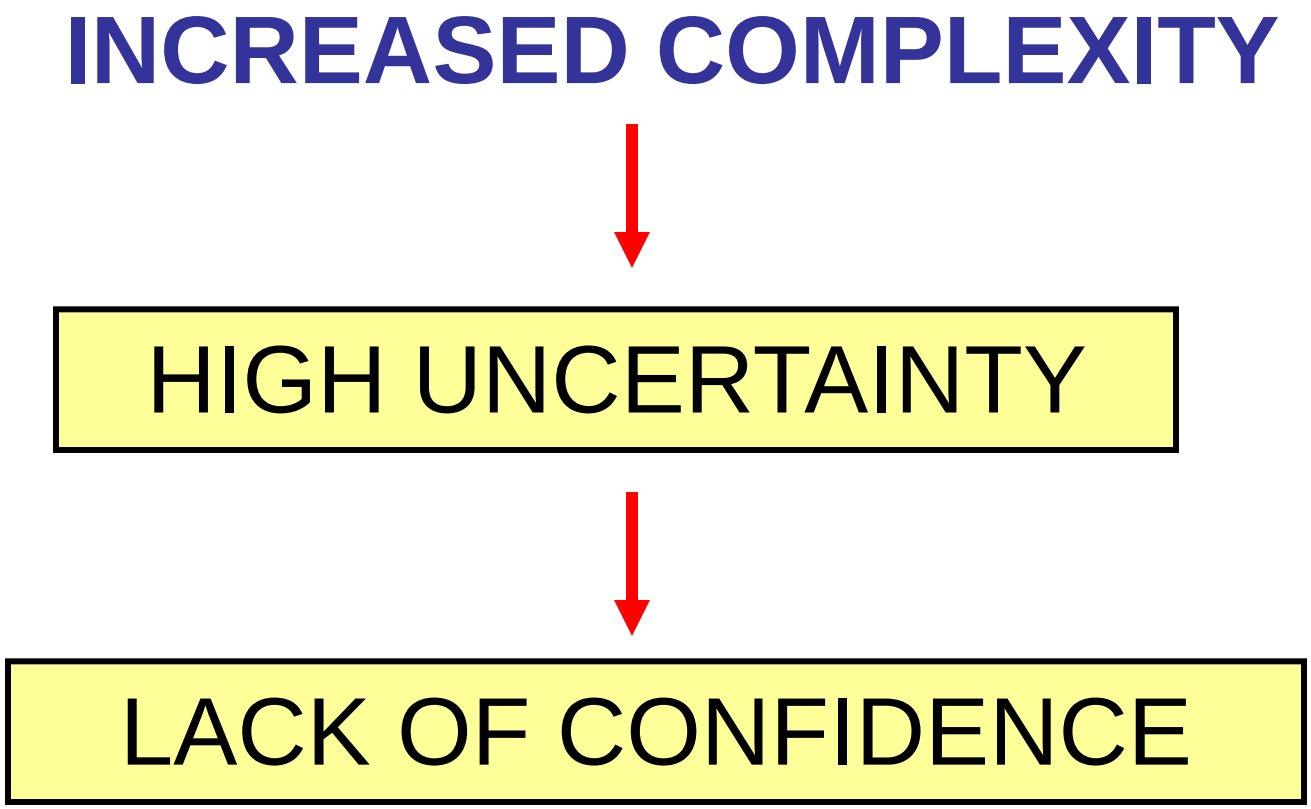
Unrelated?

Relative?
Parent-Child?
Sibs?

Relative?
Sibs?

True Known Contributors to Previous Profile

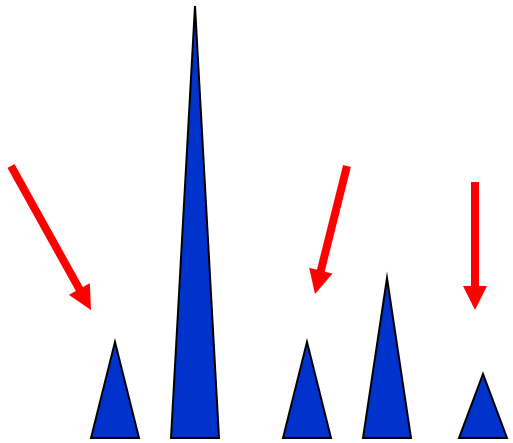
- Share 14 alleles over 15 Identifiler loci
  - 8 alleles at 9 Profiler Plus loci
  - 13 alleles at 13 CODIS loci
  - 15 alleles 17 loci (Identifiler + PowerPlex 16 HS)
- One allele in common at each locus, except D2, FGA and Penta E
- Likely not parent, unless mutations occurred
- Sibs?
  - Using known contributors' profiles : Inconclusive from allele #; Ge locus data suggests sibs
- **Provided as DNA from non-relatives**


INCREASED COMPLEXITY
HIGH UNCERTAINTY
LACK OF CONFIDENCE

Complex Mixtures

More Uncertainty and Lack of Confidence

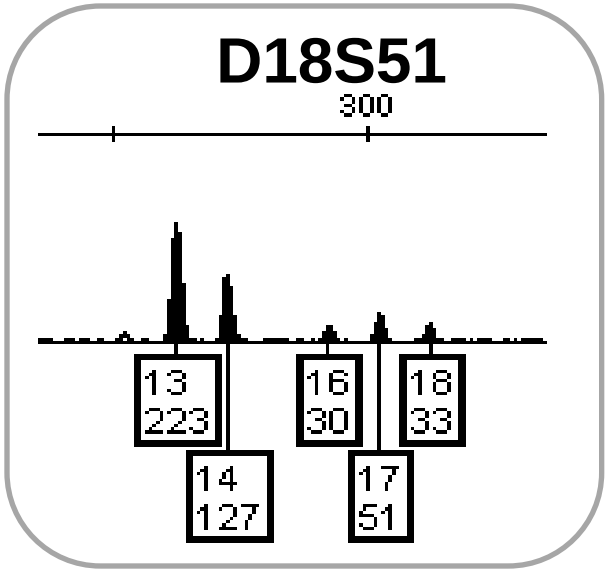
- **Peak vs. Artifacts**
  - **Stutter?**
  - **Pull-up?**
  - **True Allelle?**



Complex Mixtures

More Uncertainty and Lack of Confidence

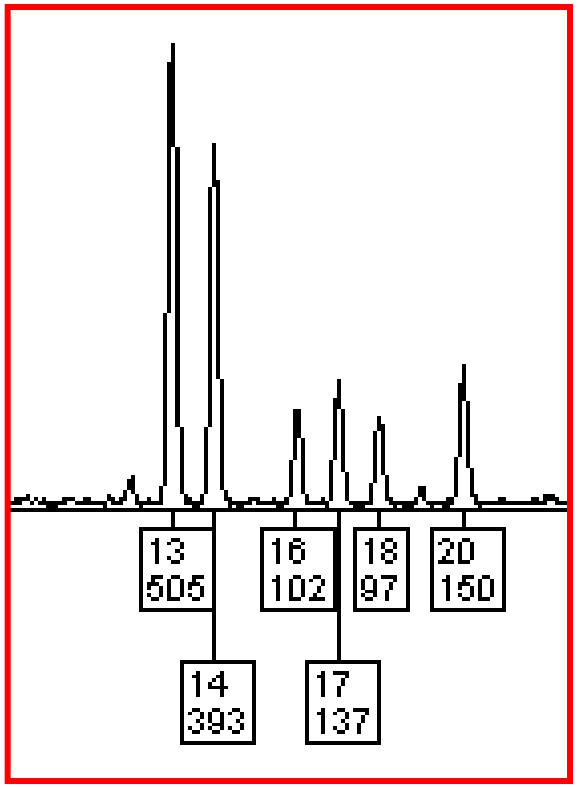
- High likelihood that DNA from one or more contributors is below optimal range
  - LT DNA = stochastic effects
  - Missing alleles? (allele drop out)
  - Elevated Stutter? True allele vs. Stutter?
  - Allele drop-in?



Complex Mixtures

More Uncertainty and Lack of Confidence

- **Stochastic threshold**
  - **Only meaningful for the peaks below the value – may be missing sister allele**
  - **Only helps with assessing if ALL alleles are likely present**

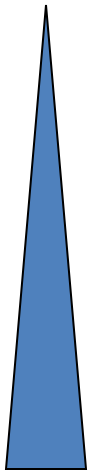


Complex Mixtures

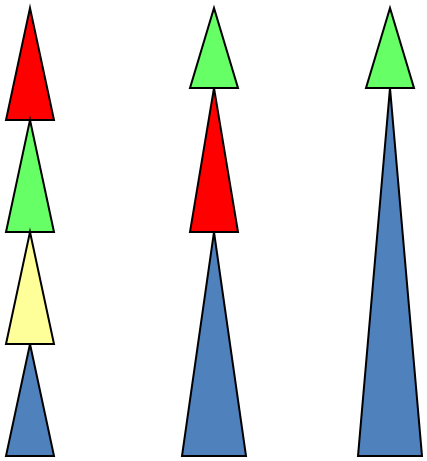
More Uncertainty and Lack of Confidence

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

Major



vs.

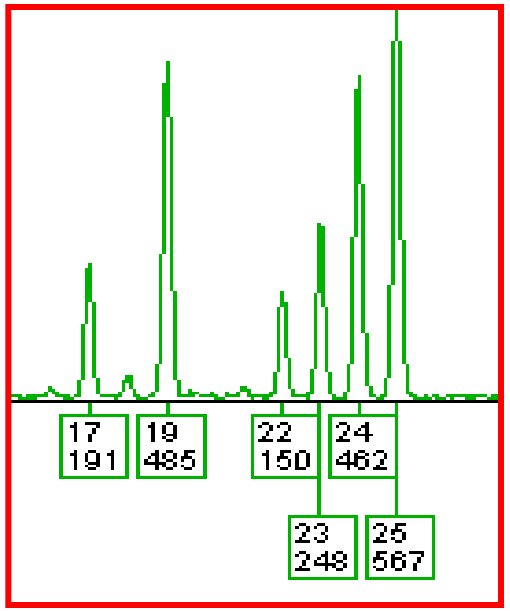


Shared alleles

Complex Mixtures

More Uncertainty and Lack of Confidence

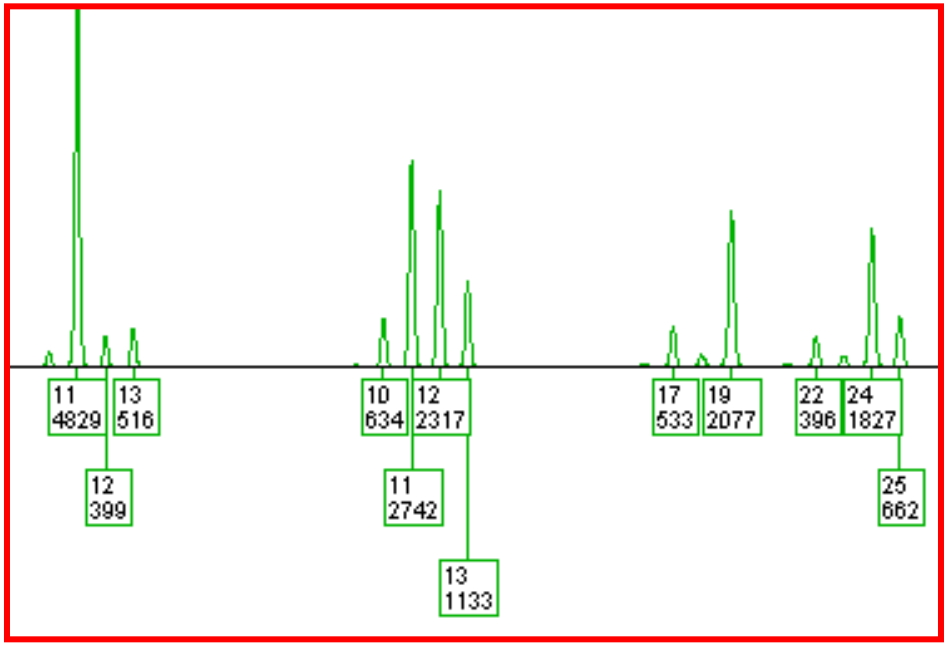
- Peak height ratios have no meaning at most or all loci
  - Cannot use to associate alleles into genotypes
  - Ability to deduce other contributors decreased even if you know one contributor



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 count/minimum number often an underestimate
  - What number to assume?
  - May need to interpret under multiple assumptions (especially if the conclusion changes)

Complex Mixtures

More Uncertainty and Lack of Confidence

- **"Inclusion" based on alleles NOT based on genotypes → may not be correct inclusion**
- **False Inclusions**
  - Increased risk as # of alleles increase
- **How calculate statistical frequency?**

Complex Mixtures

Exclusions less likely

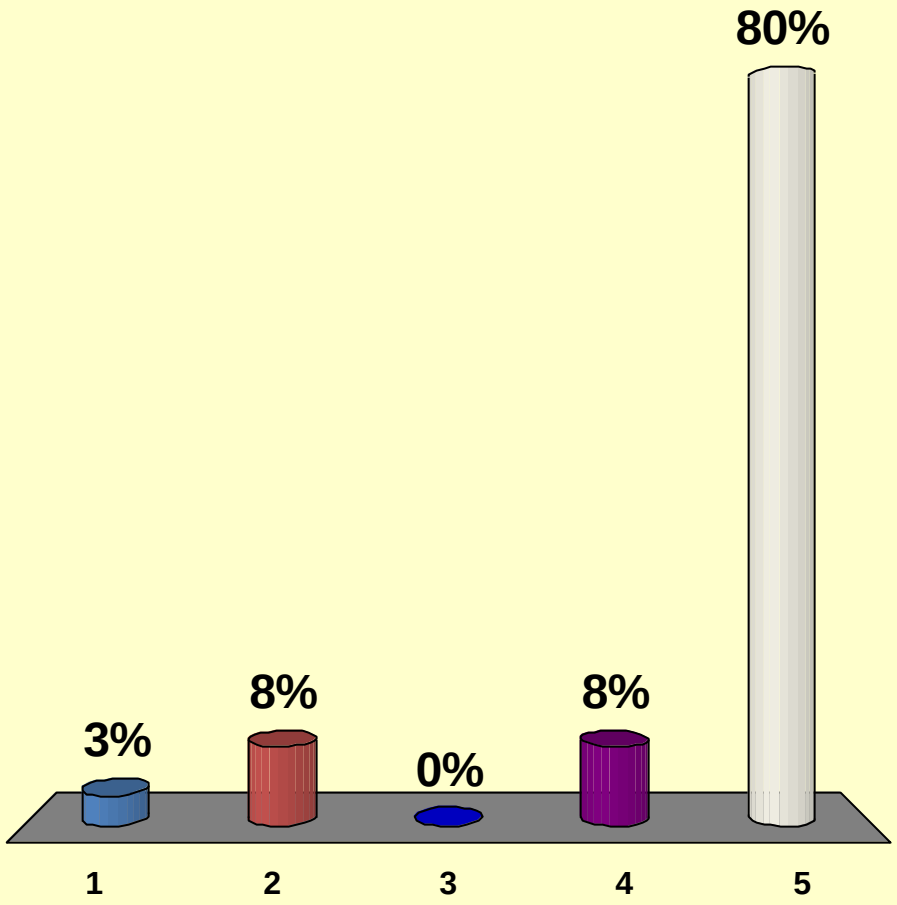
- Can anyone be excluded if LT DNA present?
- Partial “inclusions”

Inconclusive reporting increased

Should we be interpreting mixtures with 3 or more contributors?

1. Always
2. Never
3. Just in high profile cases
4. Only when one or more contributors are known
5. Maybe – depending on the profile

Data from 96 responses
ISHI Mixture Workshop (Oct 2012)



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 scientifically supported interpretation guidelines

Thank you!