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Criminal Justice Forensics
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Fordham University School of Law

MIX13: An interlaboratory study on the present state of DNA mixture interpretation in the U.S.

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National Institute of Standards and Technology

NIST and NIJ Disclaimer

Past and Present Funding: Interagency Agreement between the National Institute of Justice and NIST Office of Law Enforcement Standards

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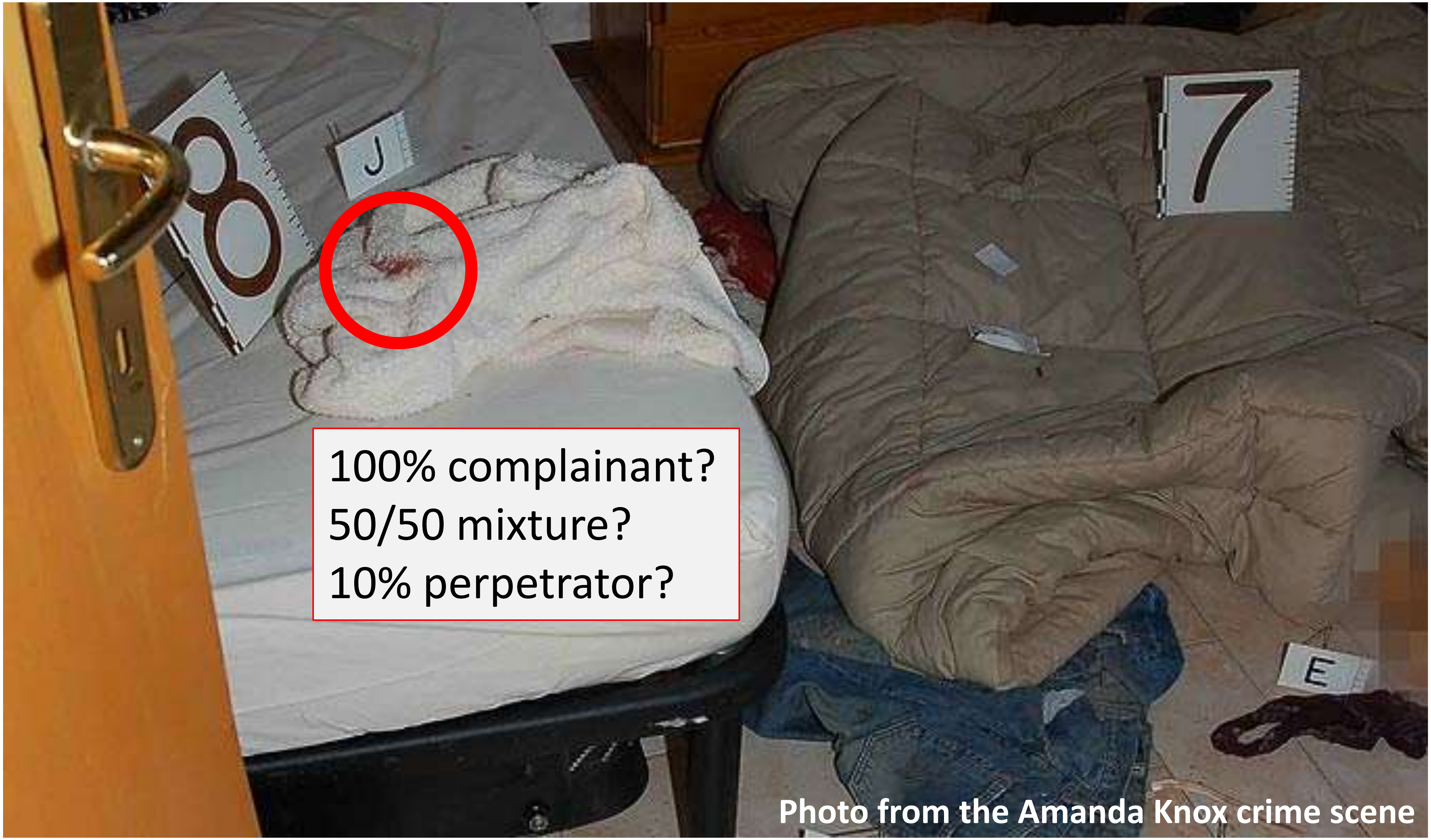
Why are Mixtures Difficult to Interpret?

1. We don't know *a priori* the relative contribution of each DNA component



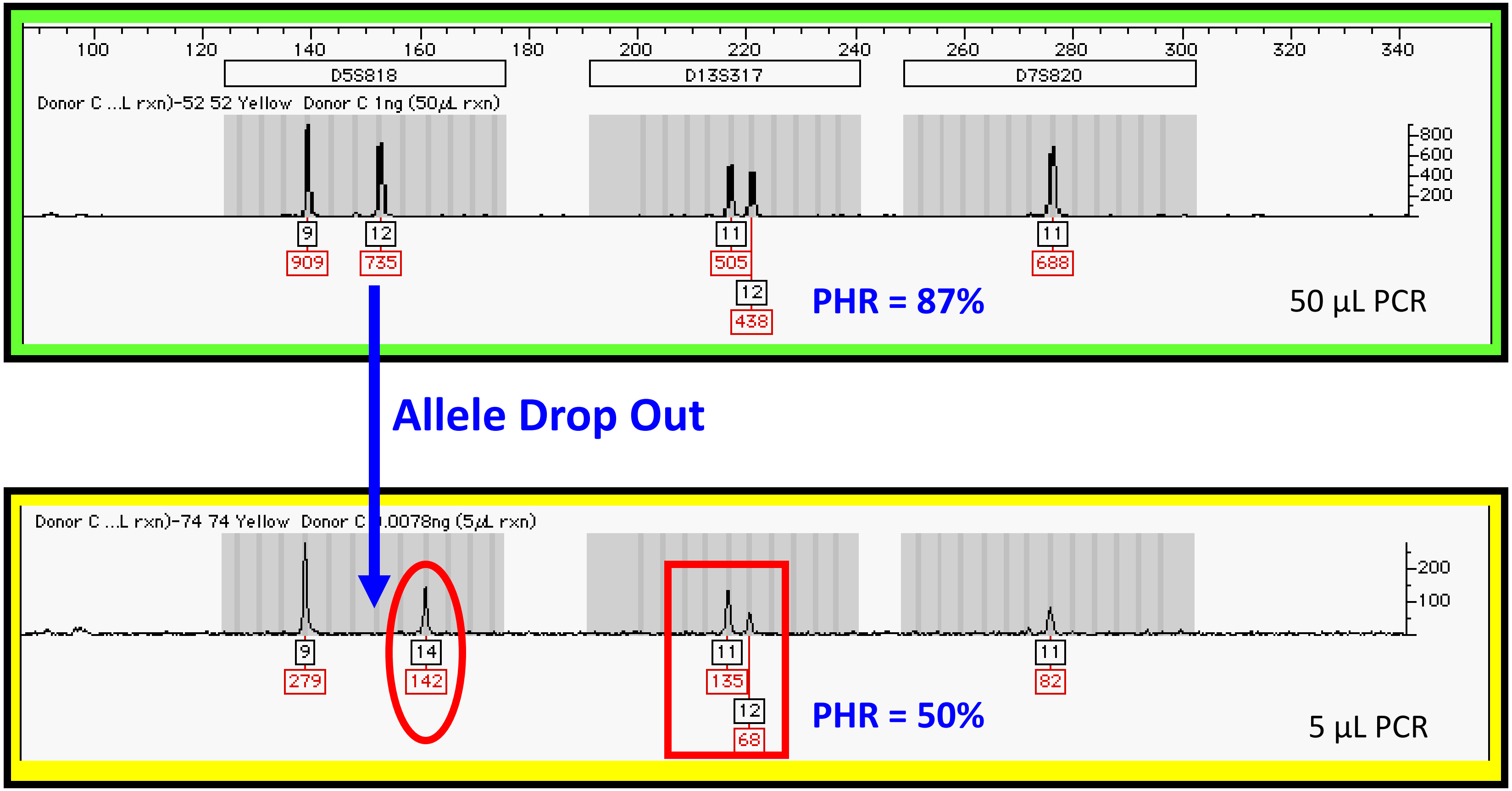
100% complainant?
50/50 mixture?
10% perpetrator?

Photo from the Amanda Knox crime scene

2. STR kits are like “Goldilocks”

Input DNA

Data from Debbie Hobson (FBI) – LCN Workshop AAFS 2003



1ng

PHR = 87%

50 µL PCR

Allele Drop Out

8pg

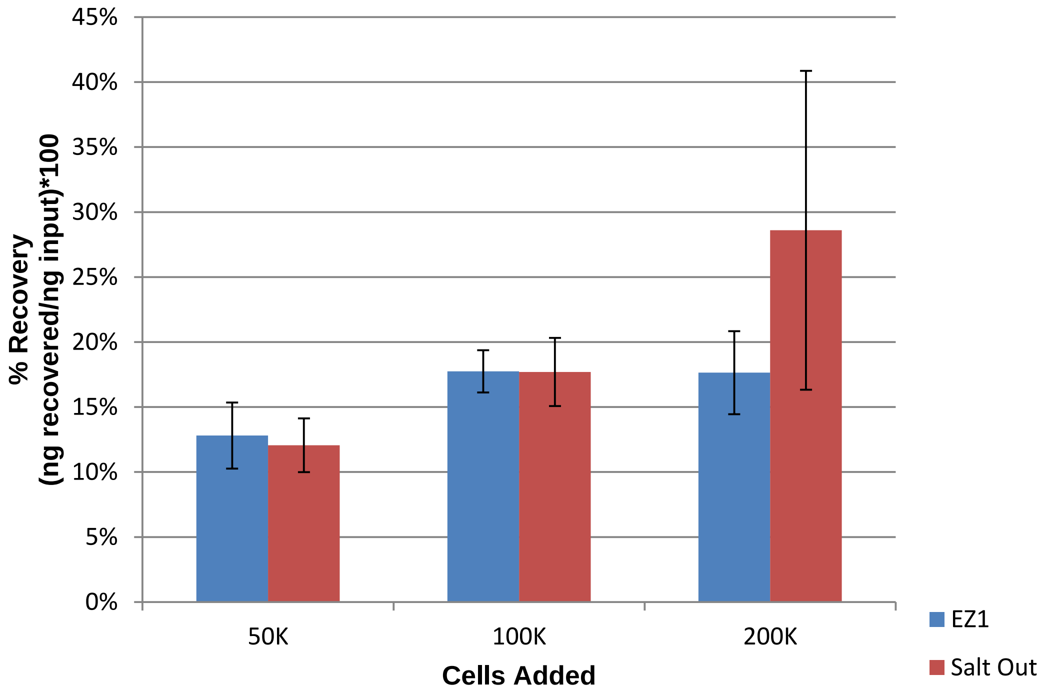
PHR = 50%

5 µL PCR

Allele Drop In

Heterozygote Allele Imbalance

3. DNA Extraction/Recovery is about 20-30%



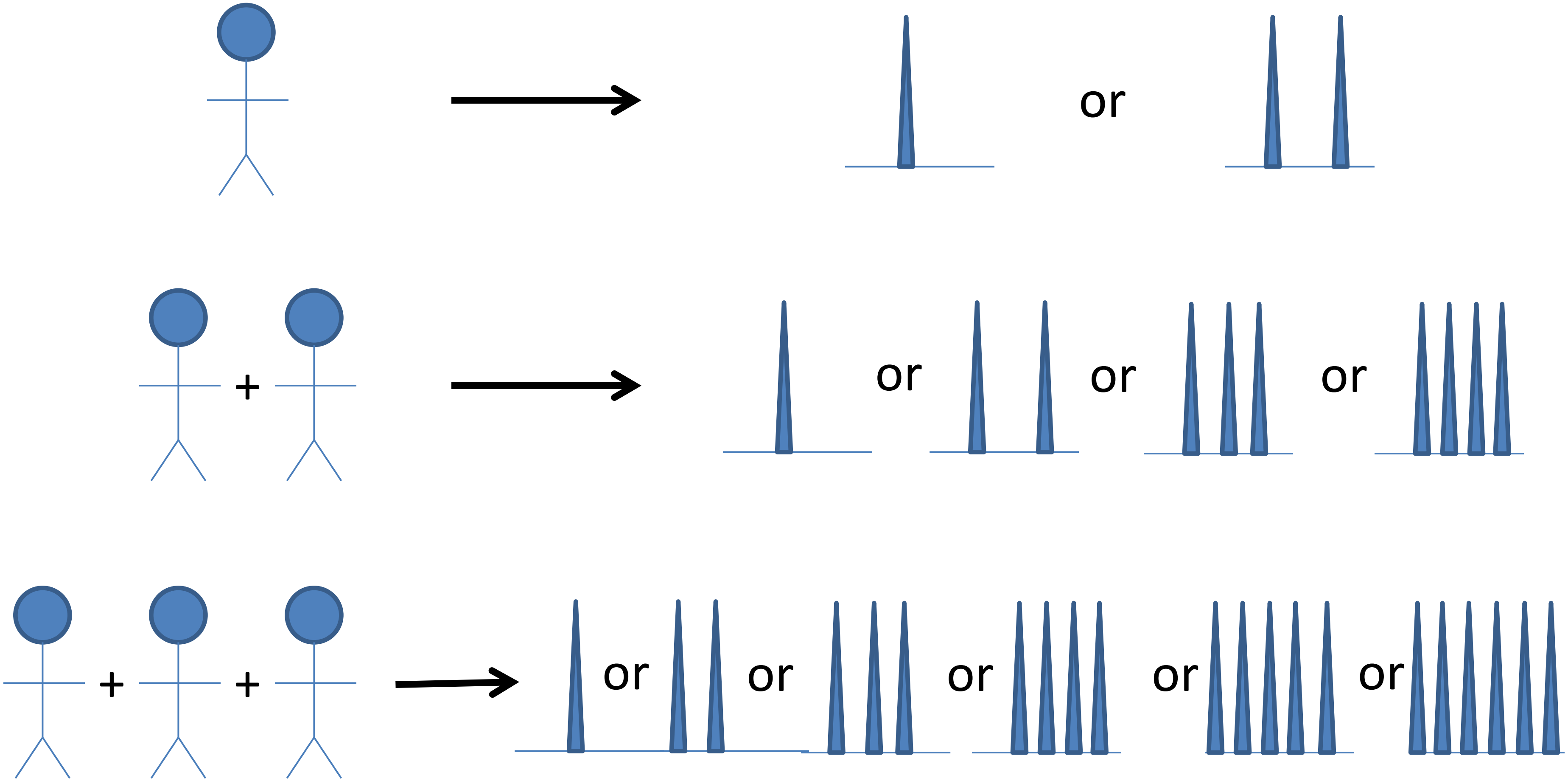
0.5 ng

Extraction process

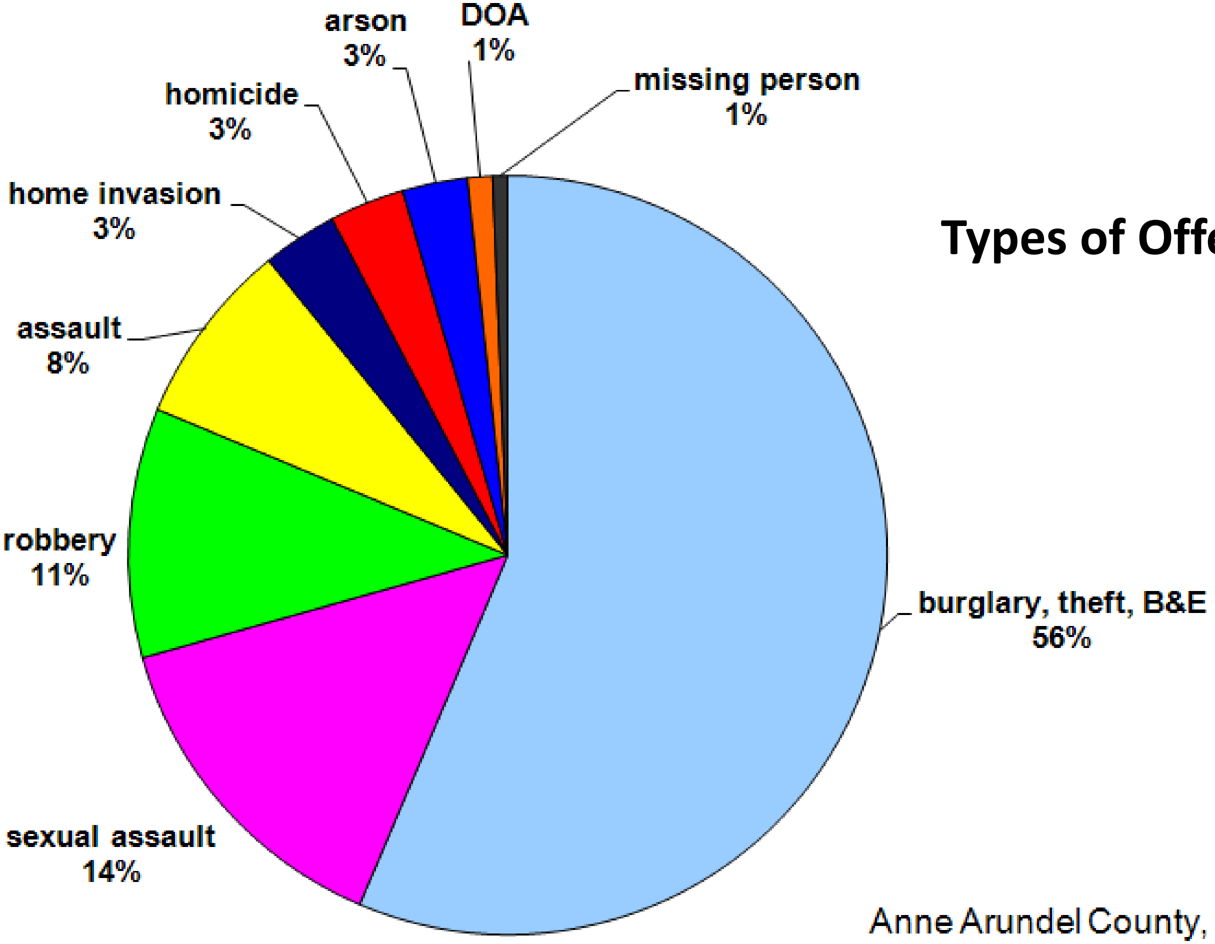
~ 70-80% sample loss

100 - 150 pg

4. It can be difficult to determine the number of contributors in the mixture



5. We can't say "NO"



Types of Offenses (2012)

Anne Arundel County, Maryland
470 total cases submitted

Slide courtesy of Sarah Chenoweth

Interlaboratory Studies

- The method by which multiple laboratories compare results and demonstrate that the methods used in one’s own laboratory are reproducible in another laboratory.

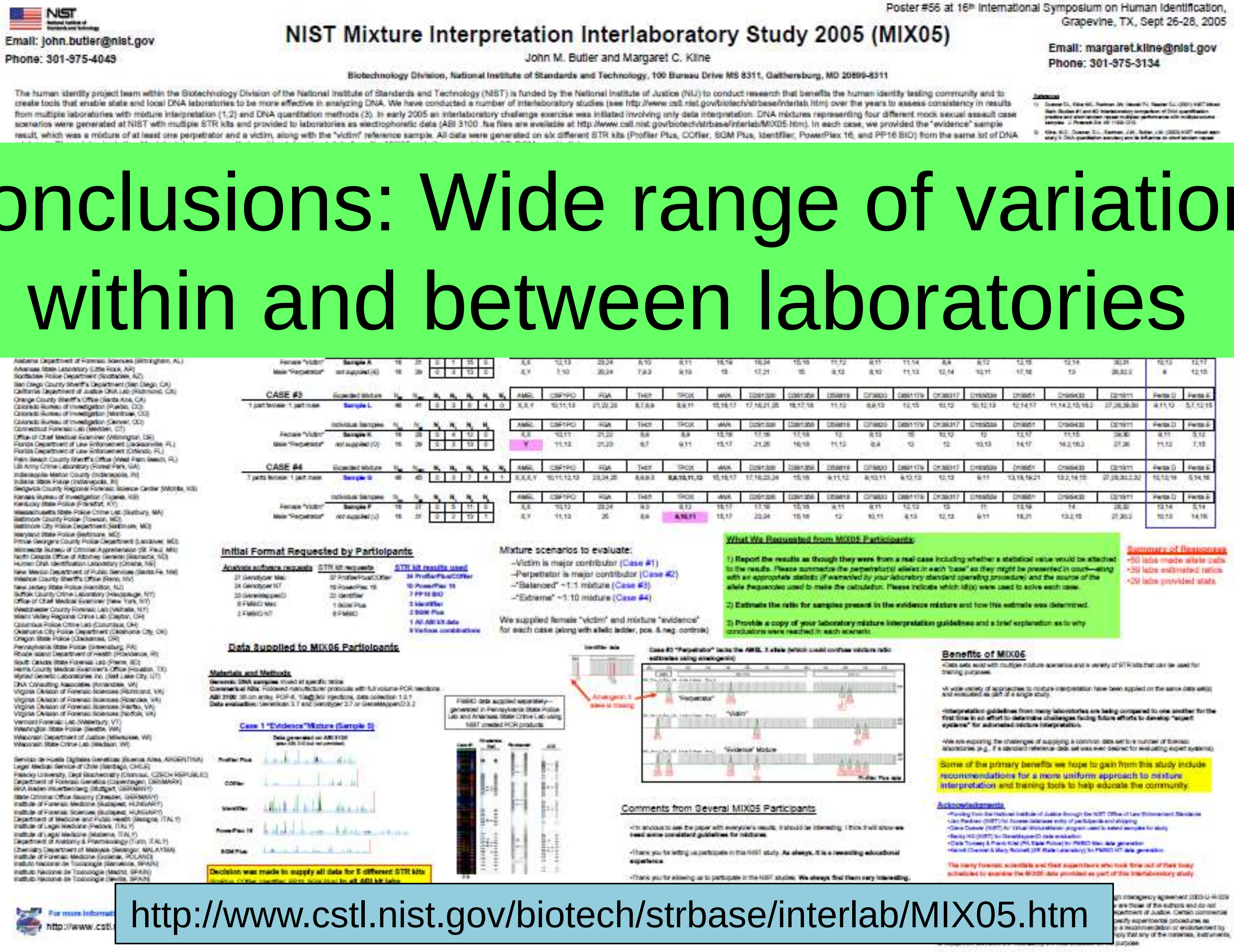
- These tests are essential to demonstrate consistency in results from multiple laboratories.

(J.M. Butler, *Forensic DNA Typing*, p. 216)

Previous Interlaboratory Studies

- MSS 1 (1997) – 22 labs participated
- MSS 2 (1999) – 45 labs participated
- MSS 3 (2000-2001) – 74 labs participated
- MIX05 (2005) – 69 labs participated

MIX05 Poster Presentation at ISHI



Poster #56 at 16th International Symposium on Human Identification, Grapevine, TX, Sept 26-28, 2005

NIST Mixture Interpretation Interlaboratory Study 2005 (MIX05)

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The human identity project team within the Biotechnology Division of the National Institute of Standards and Technology (NIST) is funded by the National Institute of Justice (NIJ) to conduct research that benefits the human identity testing community and to create tools that enable state and local DNA laboratories to be more effective in analyzing DNA. We have conducted a number of interlaboratory studies (see http://www.cstl.nist.gov/biotech/strbase/interlab.htm) over the years to assess consistency in results from multiple laboratories with mixture interpretation (1,2) and DNA quantitation methods (3). In early 2005 an interlaboratory challenge exercise was initiated involving only data interpretation. DNA mixtures representing four different mock sexual assault case scenarios were generated at NIST with multiple STR kits and provided to laboratories as electrophoretic data (ABI 3100 .fsa files are available at http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm). In each case, we provided the "evidence" sample result, which was a mixture of at least one perpetrator and a victim, along with the "victim" reference sample. All data were generated on six different STR kits (Profiler Plus, COfiler, SGM Plus, Identifiler, PowerPlex 16, and PP16 BIO) from the same lot of DNA

Conclusions: Wide range of variation within and between laboratories

CASE #3

CASE #4

Initial Format Requested by Participants

Mixture scenarios to evaluate:
- –Victim is major contributor (Case #1)
- –Perpetrator is major contributor (Case #2)
- –"Balanced" ~1:1 mixture (Case #3)
- –"Extreme" ~1:10 mixture (Case #4)

We supplied female "victim" and mixture "evidence" for each case (along with allelic ladder, pos. & neg. controls)

What We Requested from MIX05 Participants:

1) **Report the results as though they were from a real case including whether a statistical value would be attached to the results.** *Please summarize the perpetrator(s) alleles in each "case" as they might be presented in court—along with an appropriate statistic (if warranted by your laboratory standard operating procedure) and the source of the allele frequencies used to make the calculation. Please indicate which kit(s) were used to solve each case.*

2) **Estimate the ratio for samples present in the evidence mixture** and how this estimate was determined.

3) **Provide a copy of your laboratory mixture interpretation guidelines** and a brief explanation as to why conclusions were reached in each scenario

Summary of Responses
- •50 labs made allele calls
- •38 labs estimated ratios
- •29 labs provided stats

Data Supplied to MIX05 Participants

Benefits of MIX05

Comments from Several MIX05 Participants

http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm

How MIX13 differs from MIX05 study

	MIX13 (2013)	**MIX05 (2005)**
Response	107 labs	69 labs
Number of cases provided	5 cases	4 cases
Case types being mimicked	Sexual assault & touch evidence	Sexual assault evidence
Mixture complexity	2, 3, >3-person (potentially related, low-template, inclusion/exclusion)	all 2-person (all unrelated, male/female; various major/minor ratios)
Scenarios provided	Yes	No

MIX 13 – NIST Interlaboratory Study on Mixture Interpretation - **Purpose**

- MIX05 – conducted in 2005. Since then a great deal of effort has been focused on improvements in DNA mixture interpretation.

- 2010 SWGDAM Guidelines approved in January 2010 – many labs have changed their protocols recently.

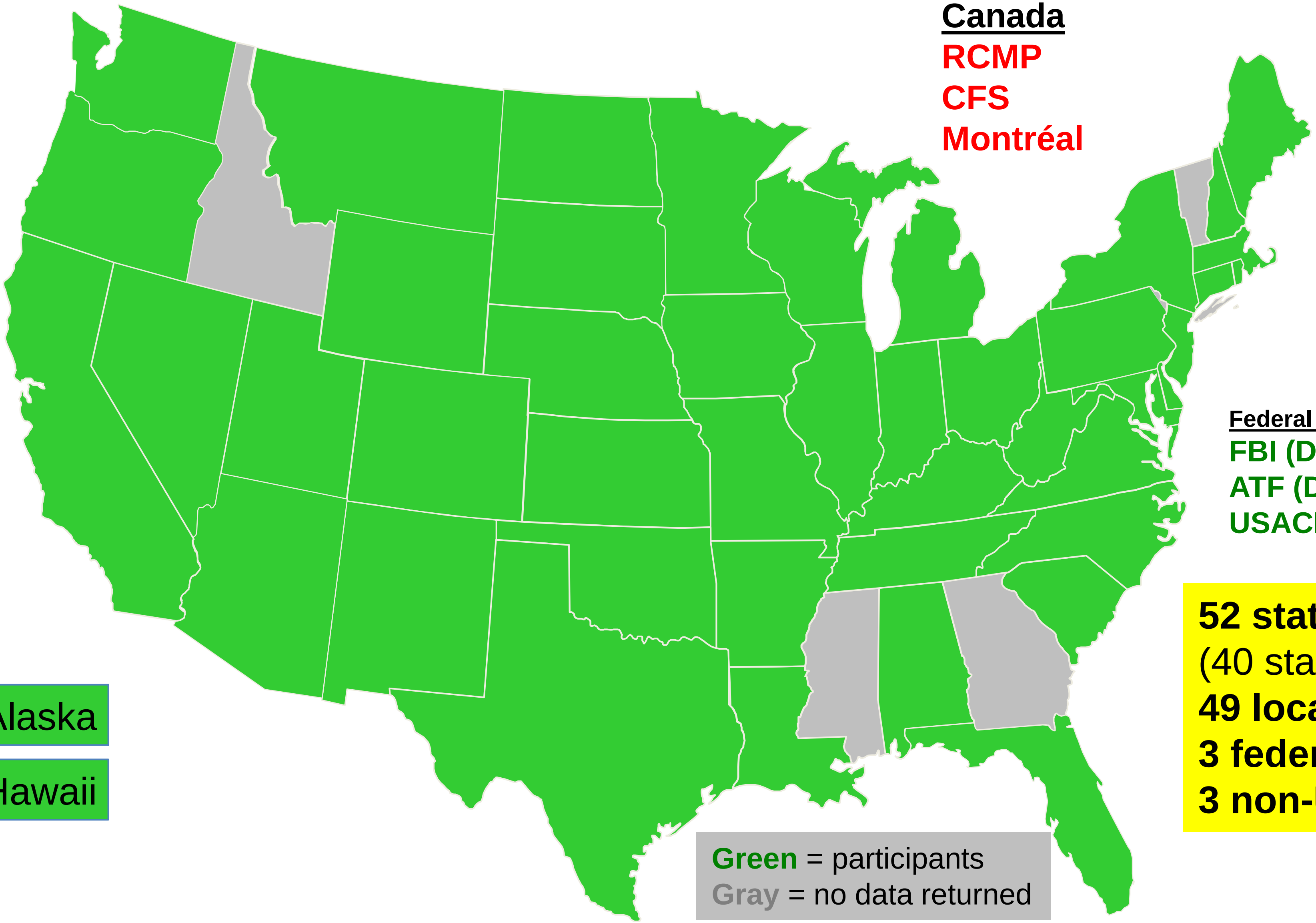
- MIX13 – Interpretation challenge – no samples to run.

MIX 13 – NIST Interlaboratory Study on Mixture Interpretation - **Goals**

- (1) To evaluate the current “lay of the land” regarding STR mixture interpretation across the community.
- (2) To measure consistency in mixture interpretation across the U.S. after the publication of the 2010 SWGDAM guidelines.
- (3) To learn where future training and research could help improve mixture interpretation and reporting.

MIX13 Participants from **107 Laboratories**

46 states had at least one lab participate



Canada

RCMP

CFS

Montréal

Federal Labs

FBI (DOJ)

ATF (DOJ)

USACIL (DOD)

52 state labs
(40 states)
49 local labs
3 federal
3 non-U.S.

Alaska

Hawaii

Green = participants
Gray = no data returned

Due to the number of laboratories responding and the federal, state, and local coverage obtained, this MIX13 interlaboratory study can be assumed to provide **a reasonable representation of current U.S. forensic DNA lab procedures across the community**

MIX13 was also used an intra-lab study

Comments from TL of a MIX13 Lab

- Thank you for the opportunity to participate in this exercise! Some of these were very challenging and provoked a lot of conversation.

- I had a majority of the analysts in our Forensic Biology Unit interpret these profiles ***independently in an effort to identify inconsistencies and areas where we need to improve.*** **It was very interesting how much the results varied!** I've included two spreadsheets that demonstrate this – "MIX13 summary of allele calls" and "MIX13 summary of stats and conclusions."

16 different analysts examined the data in this particular lab

Purpose of MIX13 Cases

	Challenge provided to study responses
Case 1	~1:1 mixture (2-person)
Case 2	**Low template** profile with potential dropout (3-person)
Case 3	Potential **relative** involved (3-person)
Case 4	Minor component (2-person)
Case 5	Complex mixture (>3-person) with # **of contributors**; inclusion/exclusion issues

According to German Stain Commission (2009) mixture types: 1 = A, 2 = C, 3 = ?, 4 = B, 5 = ?

Case 01 – Sexual Assault Evidence

~1:1 mixture (2-person)
“German Type A”

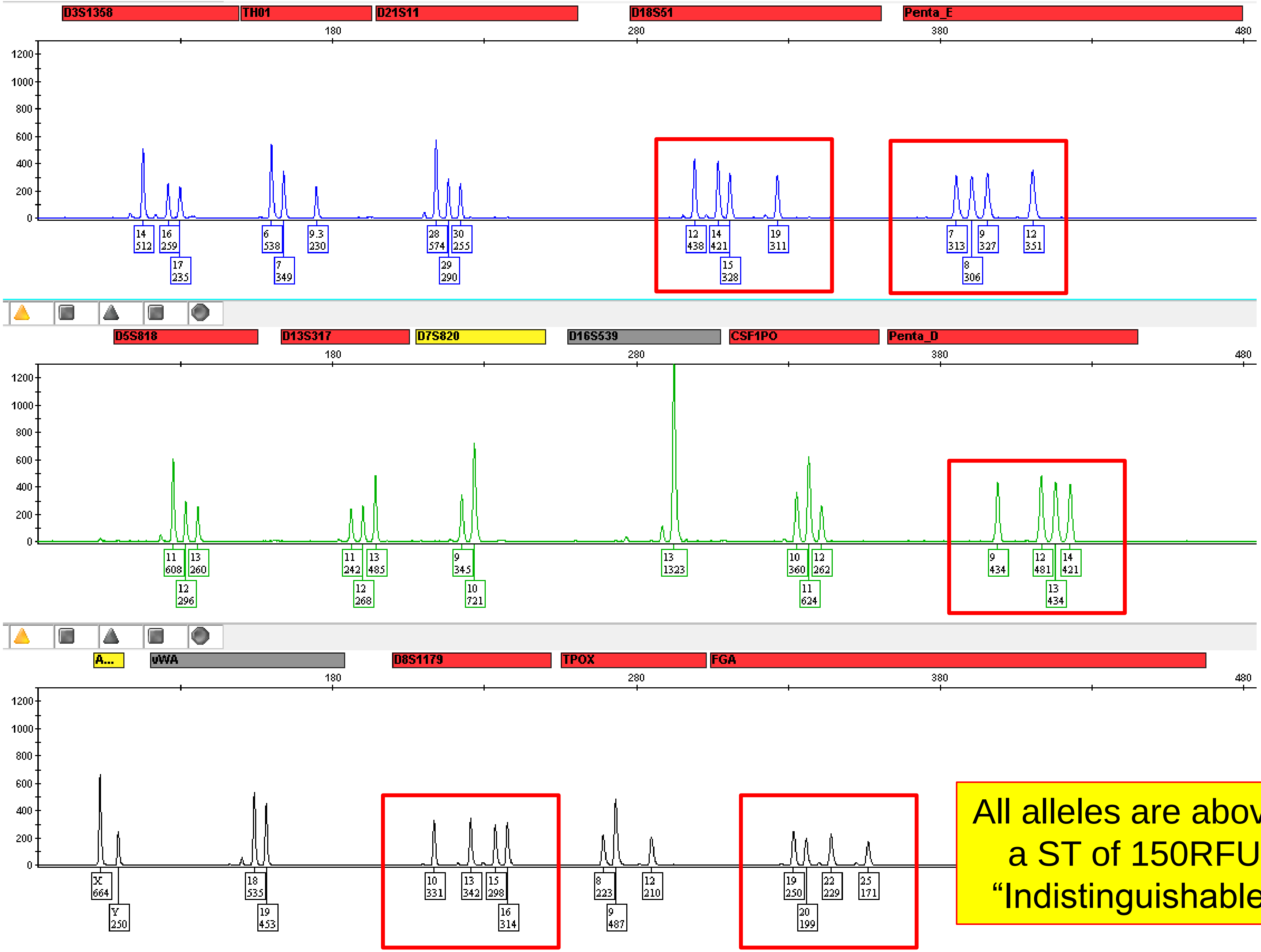
Scenario

- Evidence: sperm fraction from a vaginal swab.

- A female meets a male acquaintance at a bar after work and they return to her apartment for a nightcap. She recalls the drink tasting funny and then wakes up 14 hours later after a co-worker has her landlord to open her apartment. She is confident that she did not have consensual sex and was probably drugged. She reports the incident to the police and goes to the hospital for an examination.

- The accused male gives a buccal swab for comparison.

Case 01 – PP16HS

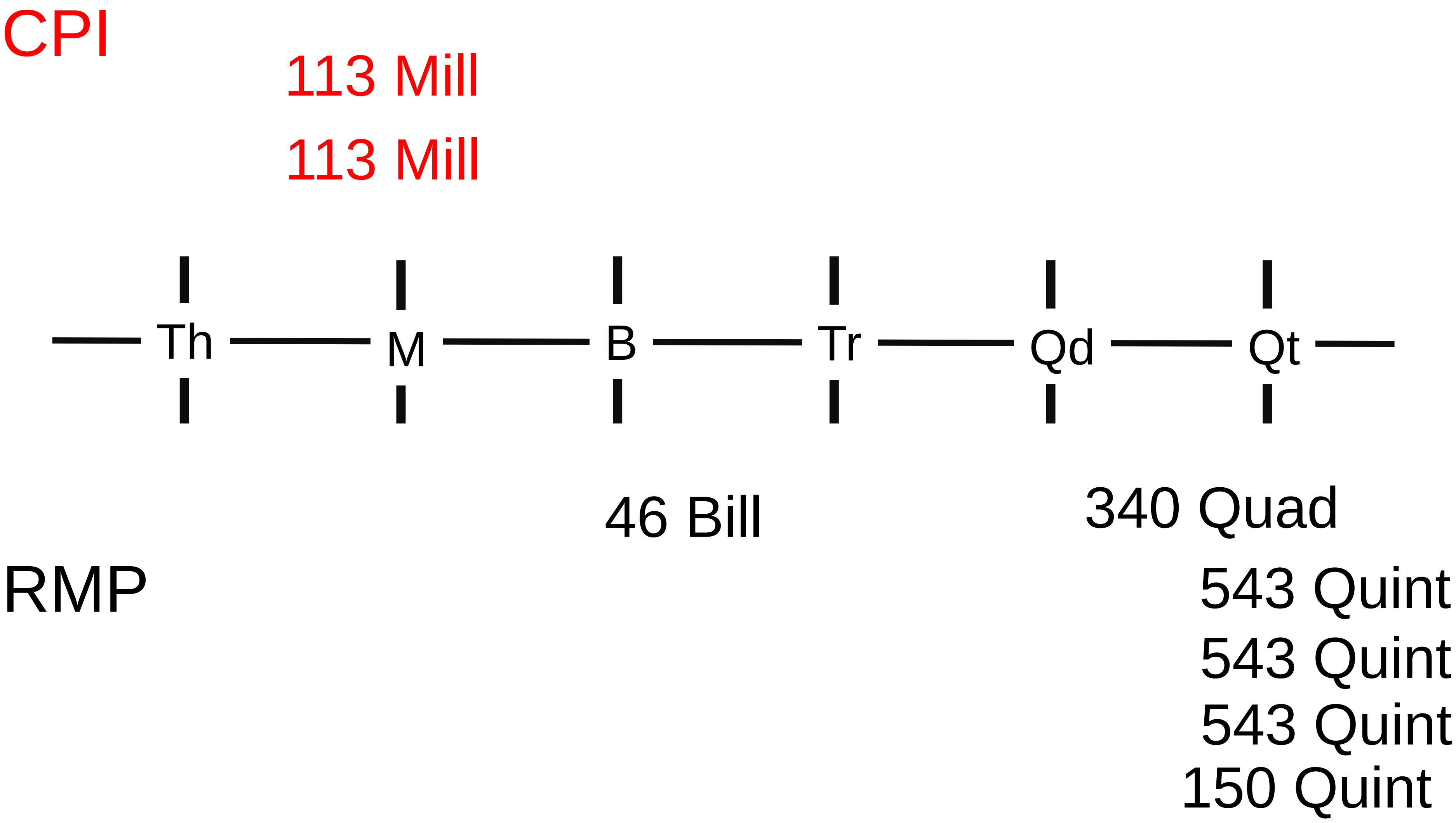


All alleles are above a ST of 150RFU “Indistinguishable”

Primary Goals

- Primary purpose – will labs attempt to subtract the victim from the evidence and generate a mRMP/LR stat **or** simply use CPI.
- So far, about 50/50 breakdown for CPI/mRMP – suspect is included in the mixture.

Intra-Laboratory Results (n = 8)



CPI

113 Mill

113 Mill

Th

M

B

Tr

Qd

Qt

46 Bill

340 Quad

RMP

543 Quint

543 Quint

543 Quint

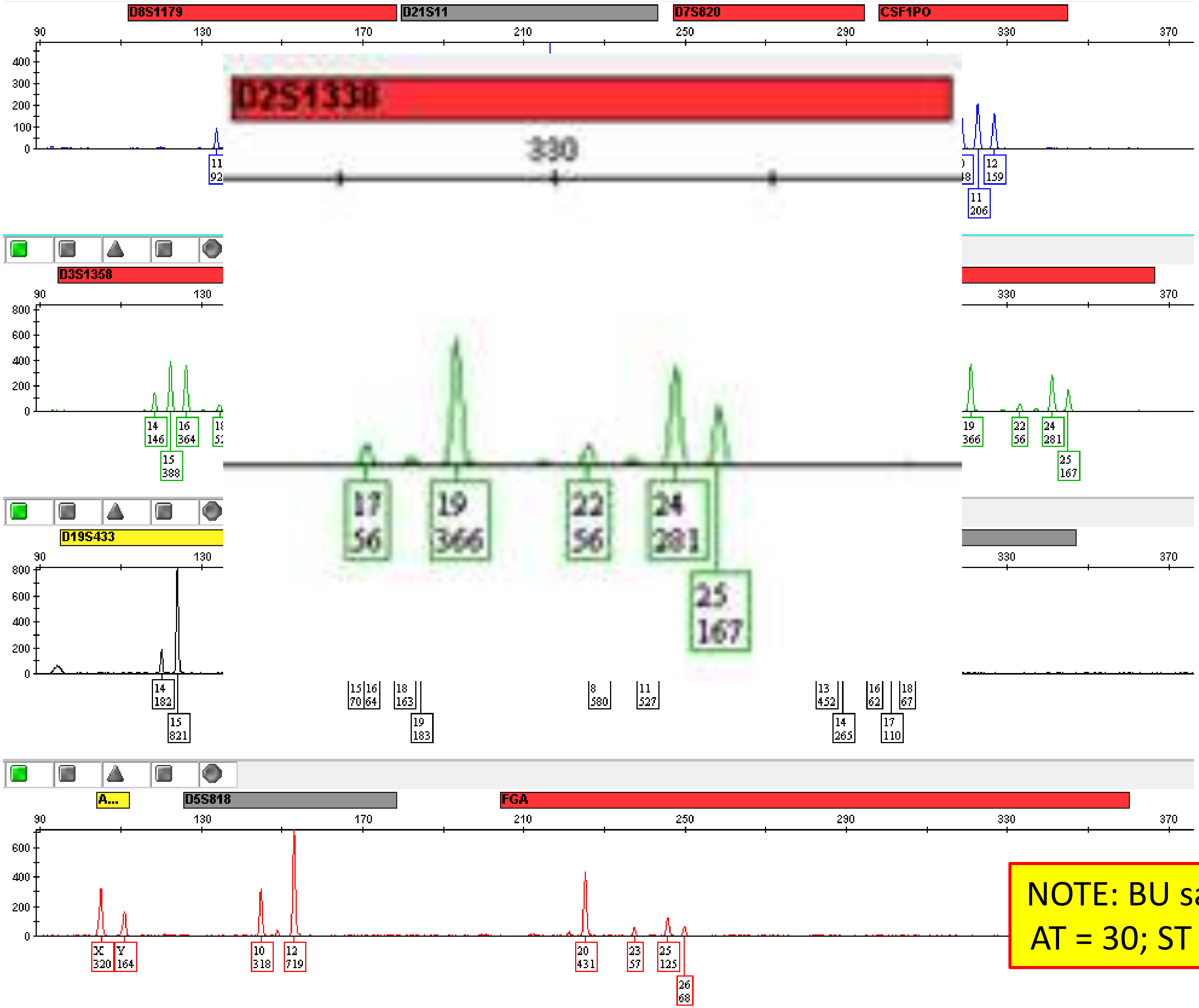
150 Quint

Case 02 – Handgun (Touch) Evidence

Low template profile with potential dropout (3-person)

“German Type C”

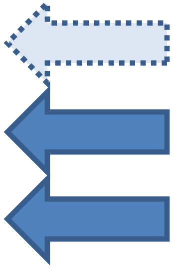
Case 02 – IDFiler



NOTE: BU sample
AT = 30; ST = 150

Case 02 – Four Suspects

	Individual	Included?	Ratio
212 pg	Suspect A	Yes	6
53 pg	Suspect B	Yes	1.5
35 pg	Suspect C	Yes	1
	Suspect D	No	--



Drop Out Is Possible

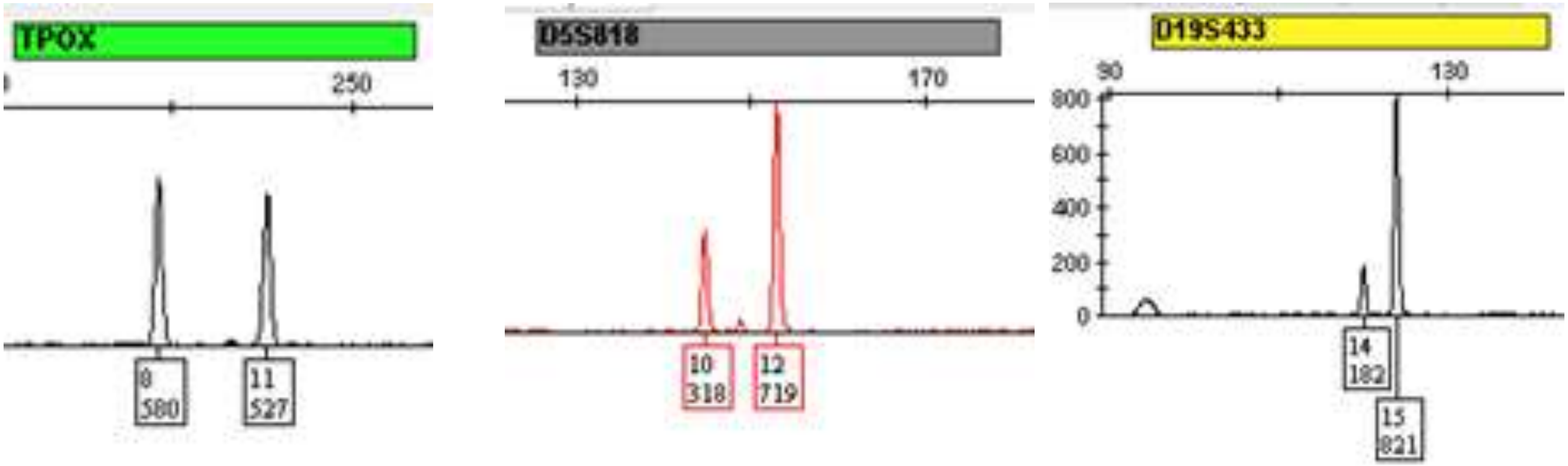
Total Input DNA = 300 pg

Primary Goals

- Primary purpose – is this mixture too complex for interpretation due to the potential of drop-out?

- Several labs – CPI for Suspects A, B and C using a limited number of loci.

- So far, no lab has included suspect D (has been reported as inconclusive).


TPOX
250
8
580
11
527
D5S818
130
170
10
318
12
719
D19S433
90
130
800
600
400
200
0
14
182
15
821

~ 1 in 35

Intra-Laboratory Results (n = 8)

Analyst	Suspect A	Suspect B	Suspect C	Suspect D
1	Inconclusive - A, B, C			Excluded
2	6.74 Quad	**23.6**	Excluded	Excluded
3	Inconclusive - A, B, C			Excluded
4	**9.4** for A, B, C			Excluded
5	4.1 Quint	**37**	Excluded	Excluded
6	**230** for A, B		Inconclusive	Excluded
7	**9.4** for A, B		Excluded	Excluded
8	**37.3** for A, B		Excluded	Excluded

Case 03 – Sexual Assault Evidence

Potential relative involved
(3-person)

Case 03 – Two Suspects

Individual	Inclusion?	Ratio
Victim	Included	7
Boyfriend (CP)	Included	2
Suspect 3A (Brother)	Included	1
Suspect 3B (Friend)	Excluded	--

Drop-out Possible

The Brothers

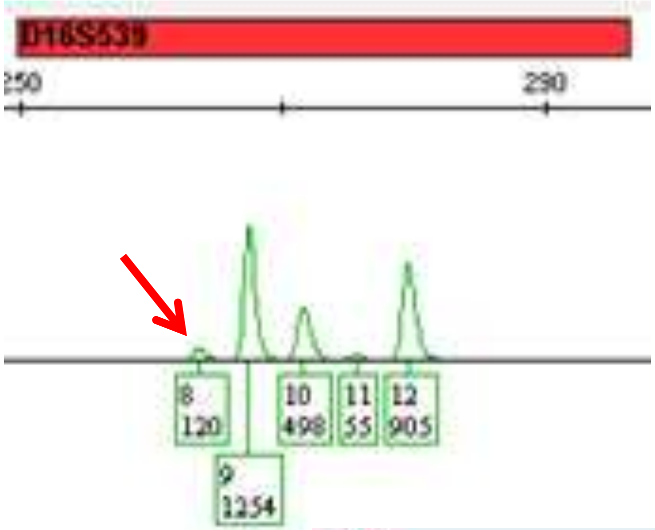
Markers	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	vWA	TPOX	D18S51	D5S818
Victim 03A	**12,15**	**31.2,31.2**	**10,10**	**10,11**	**14,14**	**9.3,9.3**	**11,12**	**15,15**	**9,11**	**12,13**	**11,12**
Cons Partner	**14,14**	**28,35**	**10,11**	**10,12**	**14,18**	**7,8**	**12,13**	**17,21**	**6,8**	**13,16**	**10,12**
Suspect 03A	**14,15**	**28,35**	**10,11**	**12,12**	**14,18**	**7,8**	**12,13**	**17,21**	**8,9**	**13,16**	**10,12**

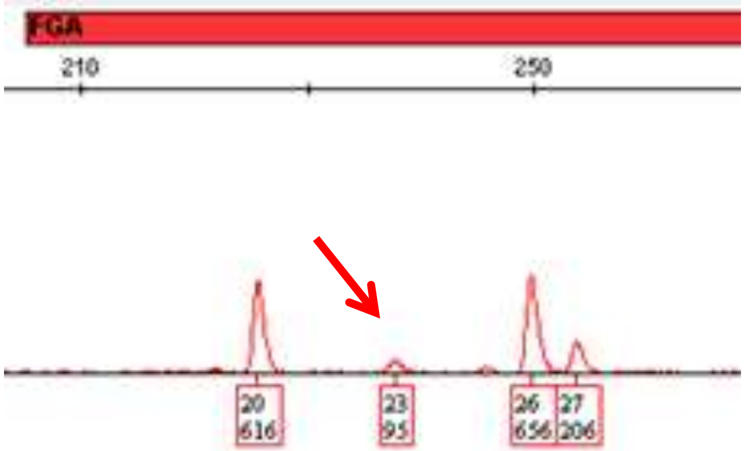
For 11 of the 13 CODIS loci – all of suspect 03A's alleles are masked by either his brother or the victim

The Brothers

For D16 and FGA – two alleles of the suspected brother are present in the epg

Markers	D16S539	FGA
Victim 03A	9,12	20,26
Cons Partner	10,10	26,27
Suspect 03A	8,9	23,27





The Brothers

The kit-specific markers give some additional information

Markers	D2S1338	D19S433
Victim 03A	**20,20**	**14,14**
Cons Partner	**16,20**	**14,14**
Suspect 03A	**16,17**	**14,14.2**

Primary Goals

- Primary purpose – is this mixture too complex for interpretation due to the potential of drop-out in the low level suspect?

- Most labs – including Suspect 3A and excluding Suspect 3B.

- Most labs are using ***CPI stats*** for this case…

An issue…

- Random Man Not Excluded (CPE/CPI) – The probability that a ***random person*** (unrelated individual) would be excluded as a contributor to the observed DNA mixture.

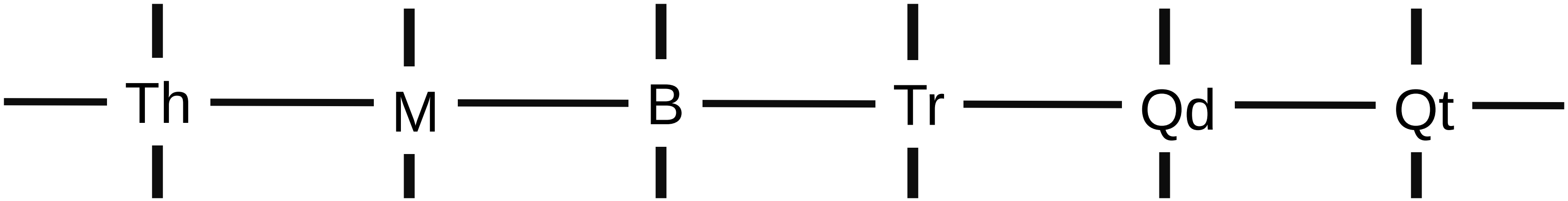
- Only a few labs have stated this – “Due to the relatedness of the exemplars submitted for comparison, a statistical analysis cannot be provided at this time.”

Intra-Laboratory Results (n = 8)

CPI

13.1 Mill

1.4 Mill



Th M B Tr Qd Qt

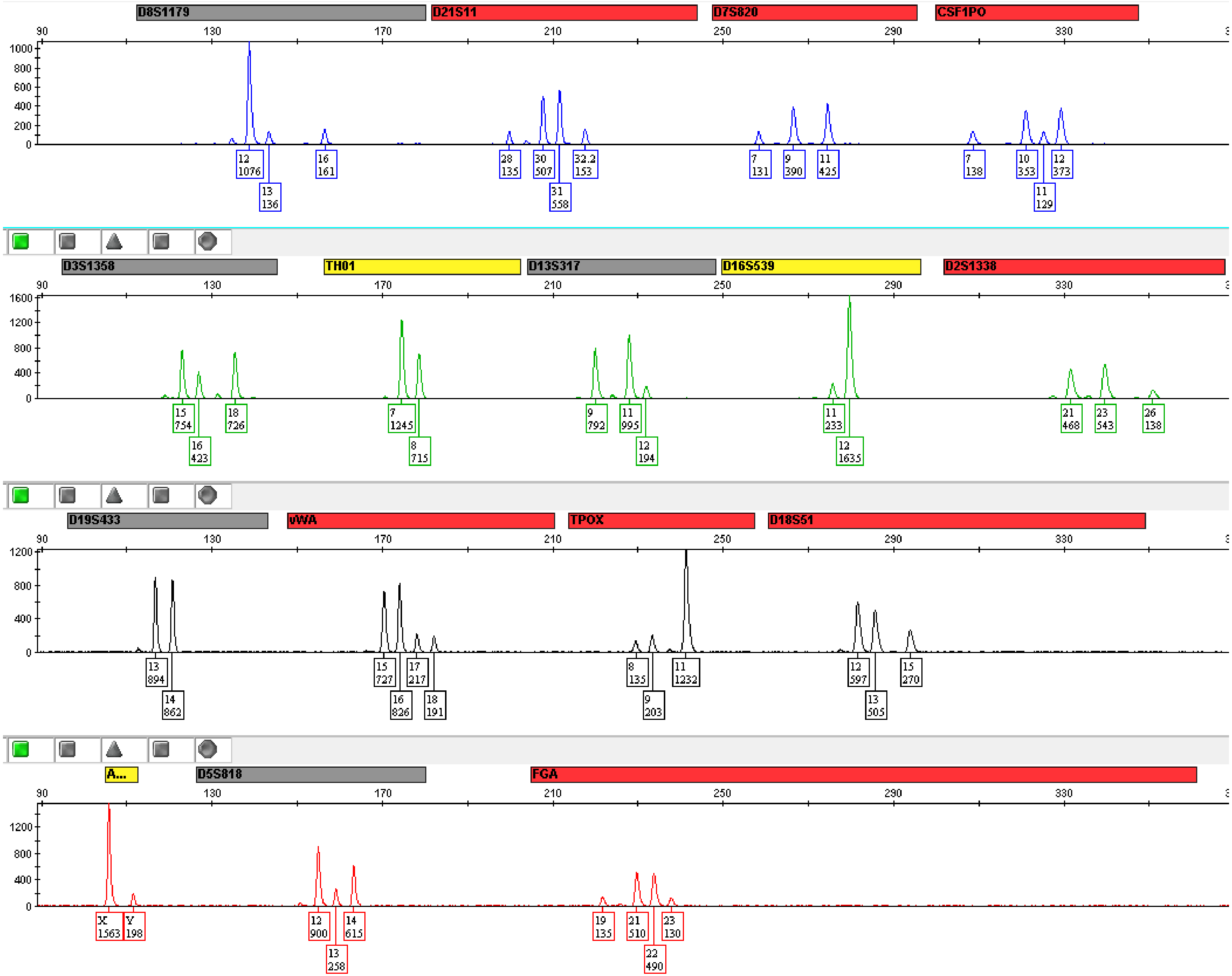
Inconclusive
Inconclusive
Inconclusive
Inconclusive

Excluded
Excluded

Case 04 – Bite Mark Evidence

Minor component (2-person)
“German Type B”

Case 04 – IDPlus



Case 04 – One Suspect

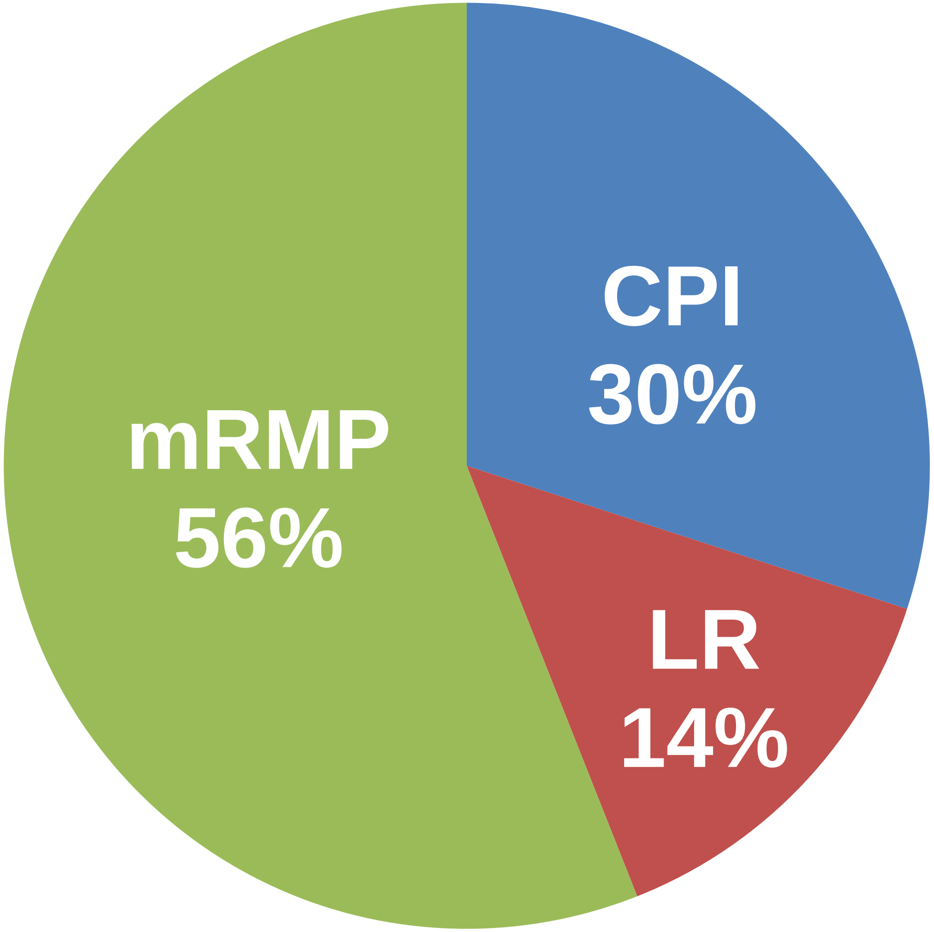
Individual	Inclusion?	Ratio
Victim	Included	~3.5
Suspect 4A	Included	1

Primary Goals

- Primary purpose – will labs choose to deconvolve this mixture since the mixture ratio is close to the limit of deconvolution for many labs?

- All labs have included the suspect in the mixture.

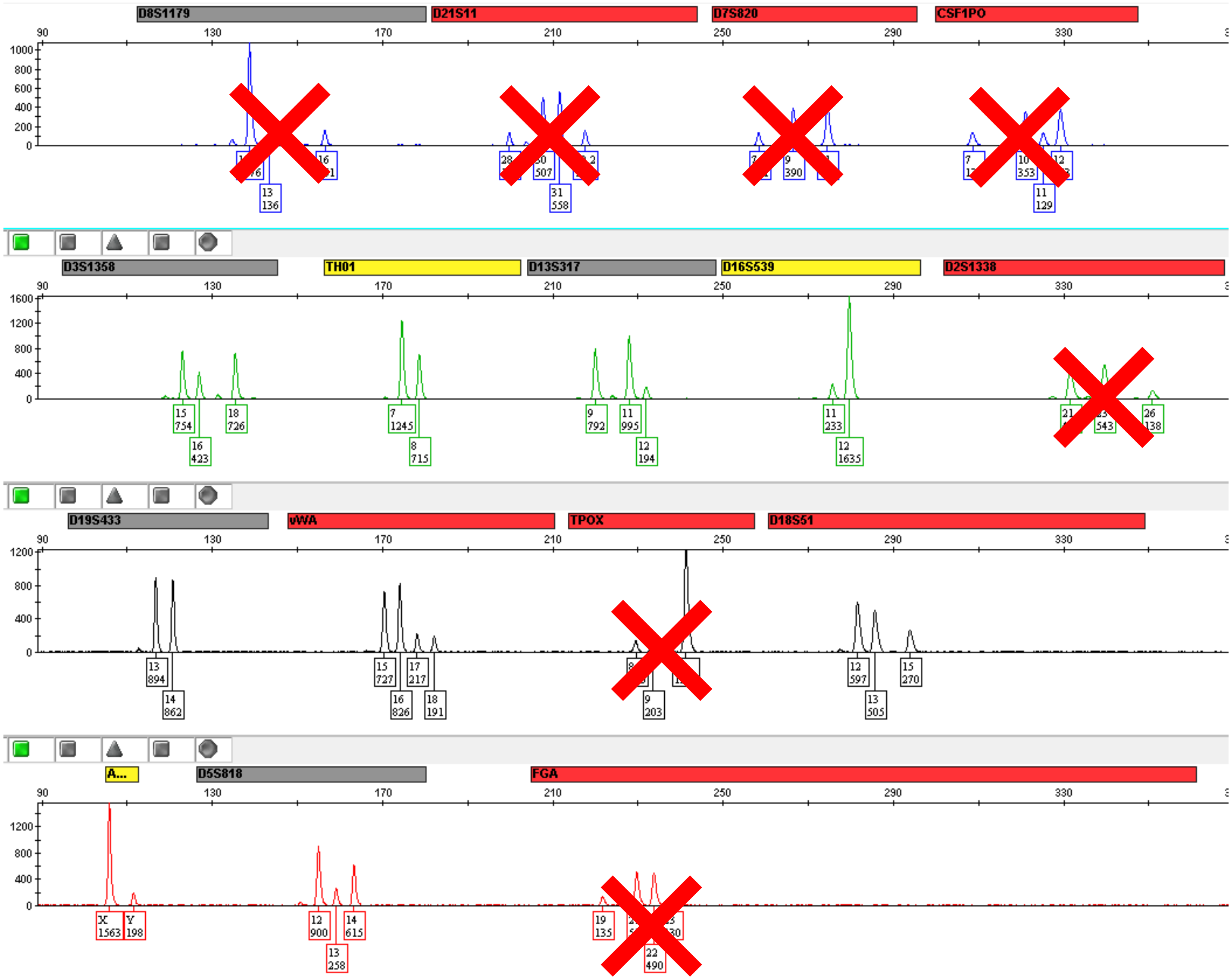
Statistical Interpretation



CPI Analysis

- Stats ranged from 1 in 3,070 to 1 in 862,000 with a median of 1 in 14,380.

Alleles below ST (ID+)



<u>7 Loci</u>

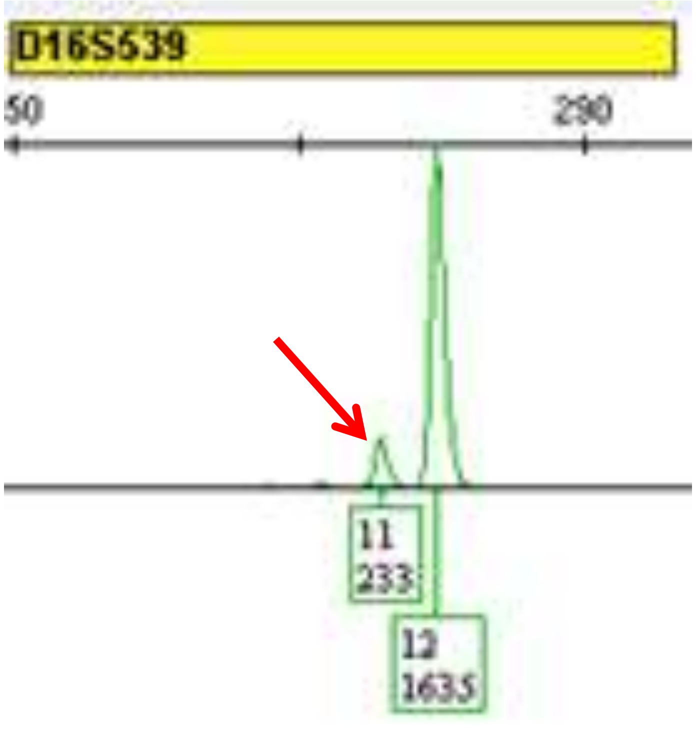
D8
D21
D7
CSF
D2
TPOX
FGA

1 of 12 ID+ labs included all but D7

mRMP/LR Analysis

- Stats ranged from 1 in 358,000 to 1 in 412 Quintillion with a median of 1 in 2.58 Quadrillion

Focus on Uncertainty with D16 (stutter)

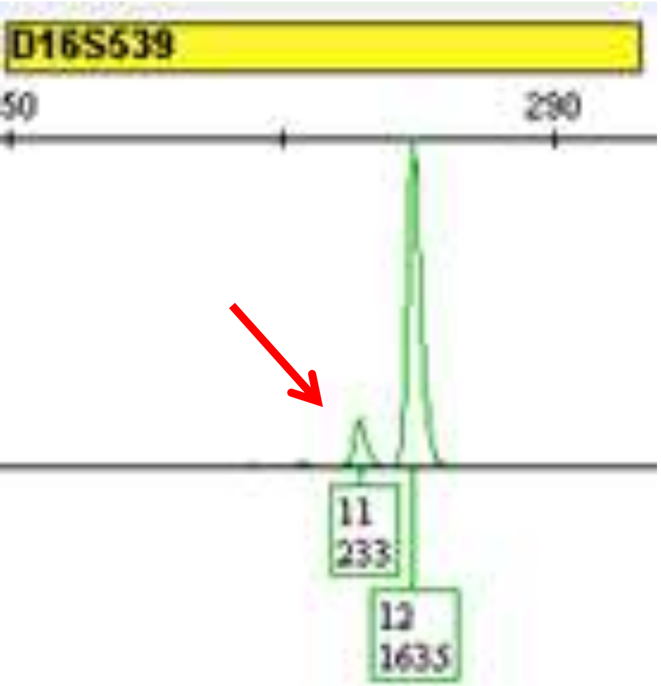


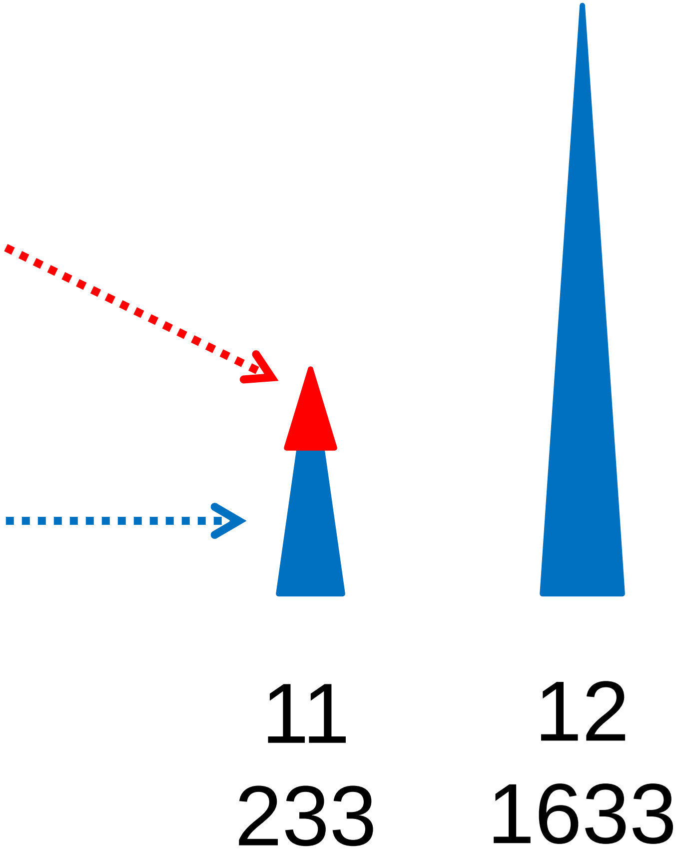
If 10% stutter from the 12 allele (163 RFU) is part of the 11 allele, then the remaining peak (70 RFU) is below the ST

No CPI labs excluded D16 from the stat

POI = 11, 12

Uncertainty with D16





70 RFU
(POI)

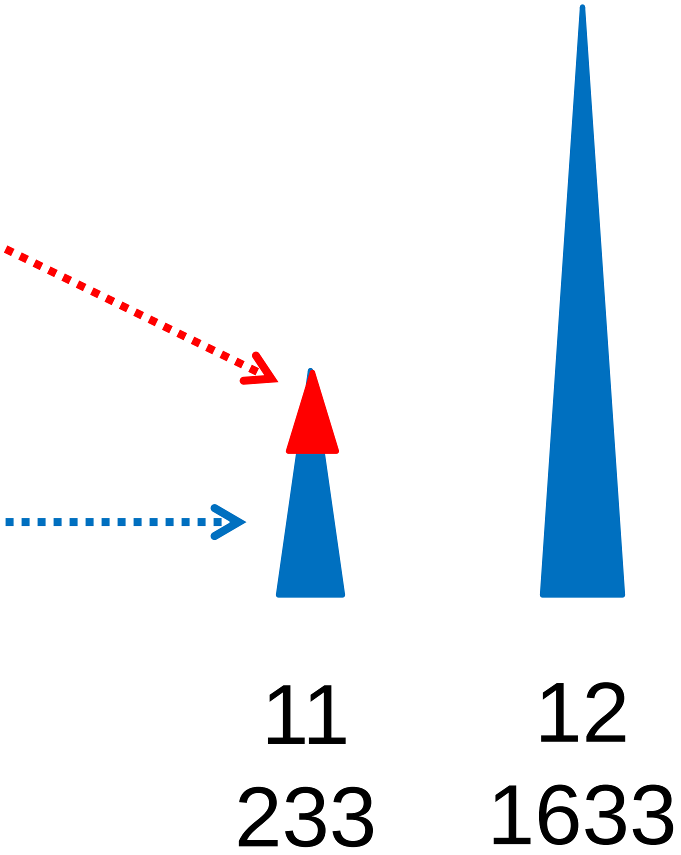
163 RFU
(Stutter)

11
233

12
1633

POI = 11, 12

Uncertainty with D16
Observed Approaches



70 RFU
(POI)

163 RFU
(Stutter)

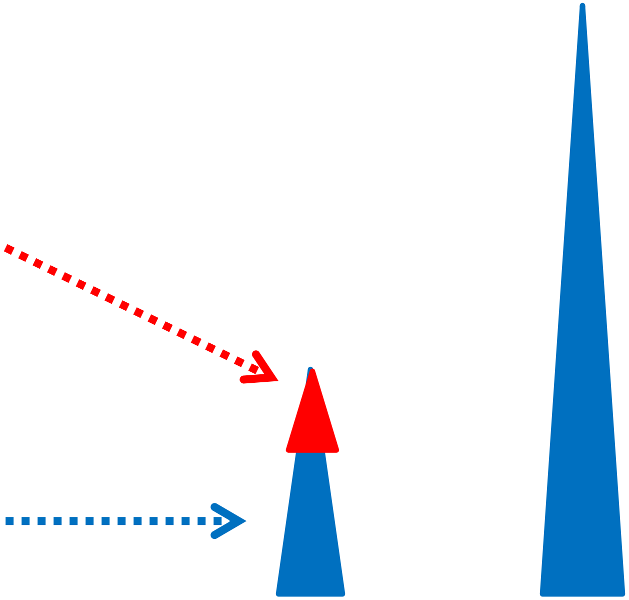
11
233

12
1633

Drop the locus
(Below ST)

POI = 11, 12

Uncertainty with D16
Observed Approaches



70 RFU
(POI)

163 RFU
(Stutter)

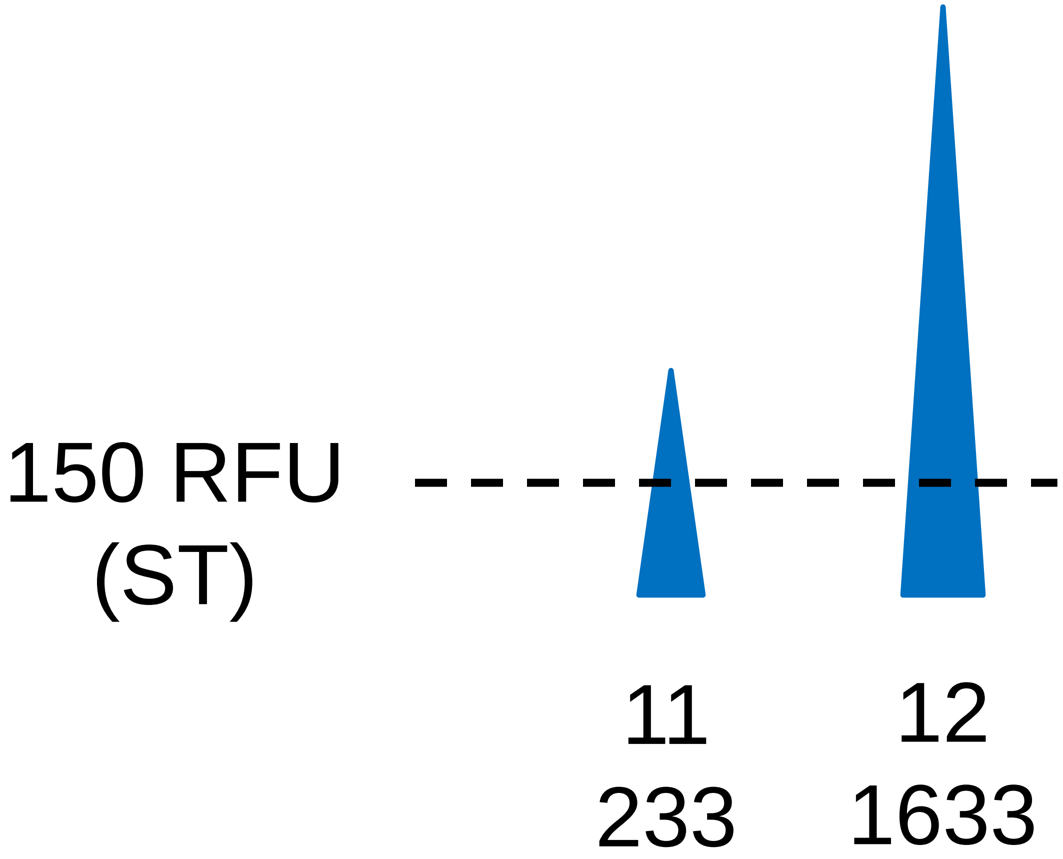
11
233

12
1633

Use 2p
(Below ST)

POI = 11, 12

Uncertainty with D16
Observed Approaches



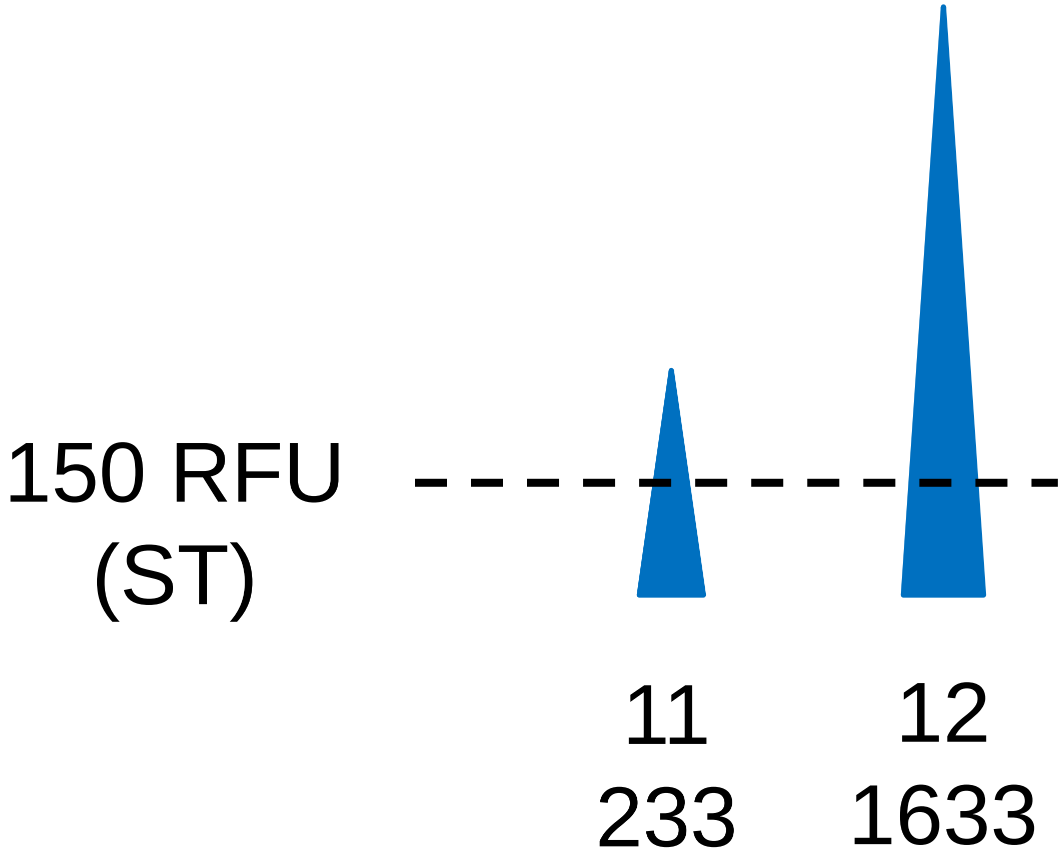
Use 2pq or p^2

(to infer genotypes)

11,12 or 11,11

POI = 11, 12

Uncertainty with D16
Observed Approaches



150 RFU
(ST)

11
233

12
1633

Use 2pq

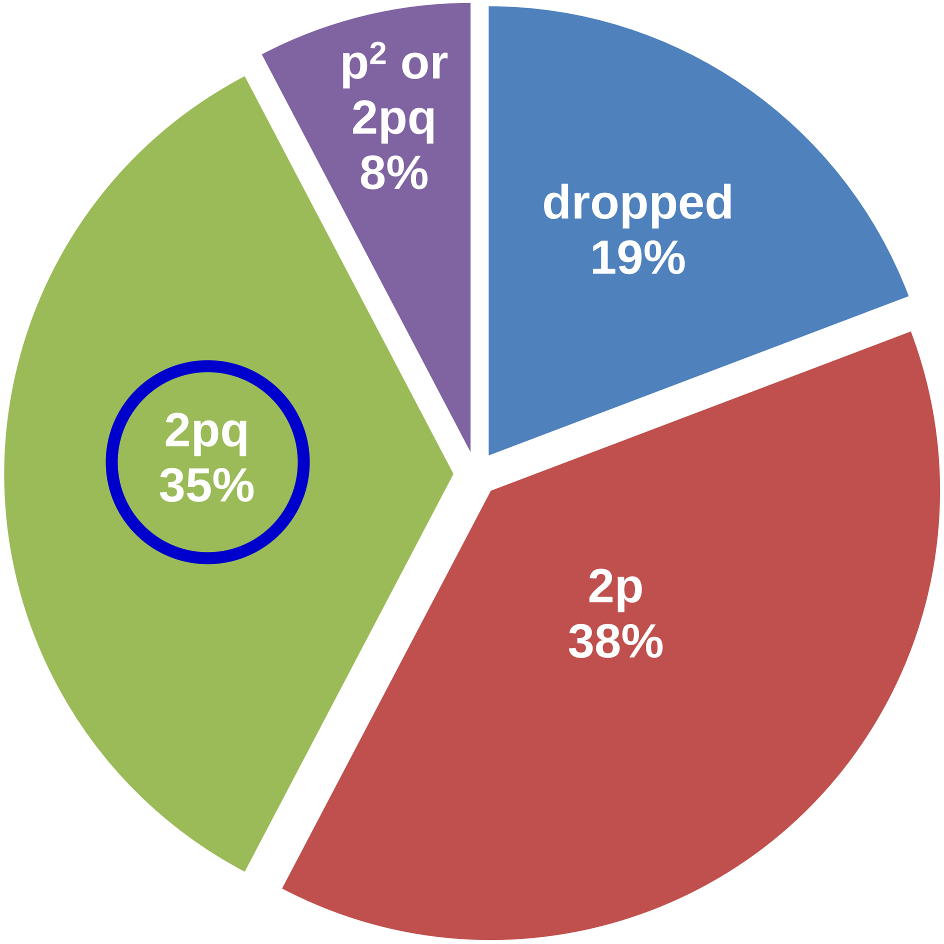
(to infer genotype)

11,12

(matches POI)
“Certainty”

POI = 11, 12

Statistics with D16 (mRMP/LR)



4.2. For calculating the CPE or RMP, any DNA typing results used for statistical analysis ***must be*** derived from **evidentiary items** and not known samples.

Probabilistic approaches to interpretation can be useful to reduce uncertainty and subjectivity

Summary

- Most labs have validated and implemented AT and STs since MIX05. However, there is still a great deal of variation in interpretation across the U.S.
- An Idea – if everyone uses the same AT/ST, then one would expect to see similar results.
- Reality – the results were all over the place, Some of this was to be expected since each lab’s protocol is different (e.g. dropping a locus vs, 2p).
- Probabilistic approaches will also deconvolve the mixture (without dropping loci), and can do so without bias.

Case 05 – Ski Mask (Robbery Evidence)

Complex mixture (>3-person) with # of contributors; inclusion/exclusion issues

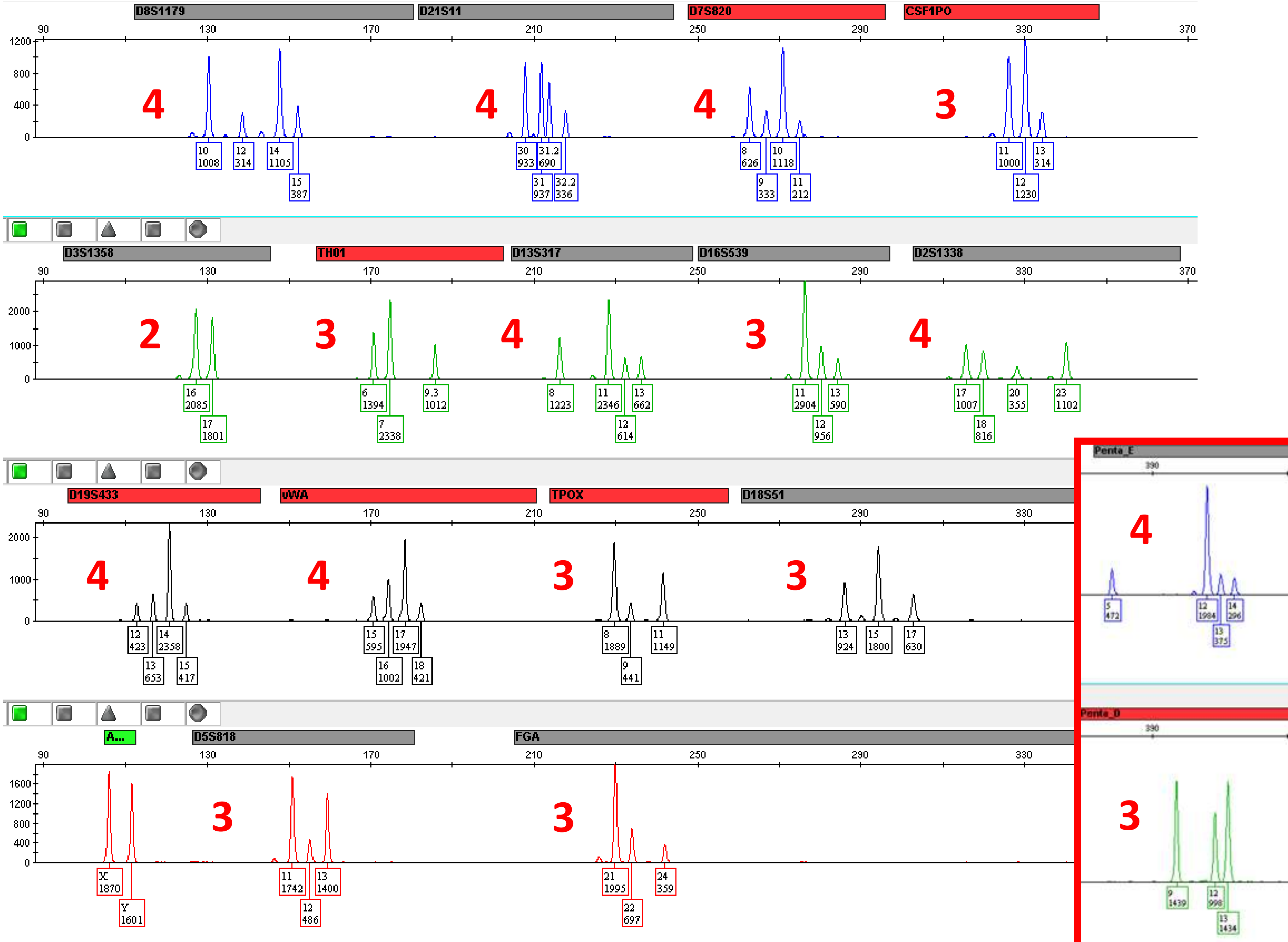
Scenario

- Evidence: Ski mask recovered at a bank robbery.

- A number of gang-related robberies have targeted several banks in the city. The robberies have typically involved 2-3 perpetrators. A ski mask was recovered in a trash can one block away from the latest bank robbery and is submitted for DNA testing.

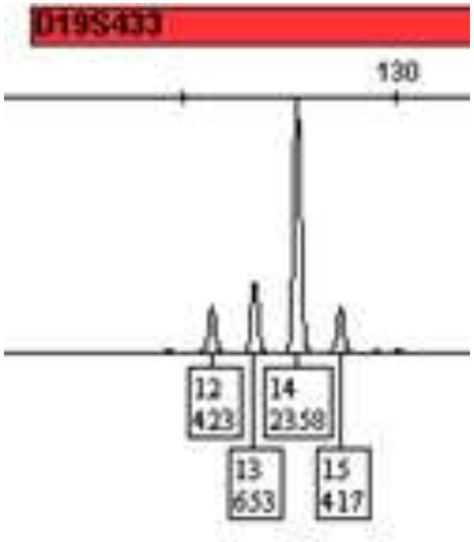
- A confidential informant has implicated two suspects in at least three of the armed robberies. Police have obtained buccal swab references from the two suspects identified from the CI, and another known accomplice of the suspects.

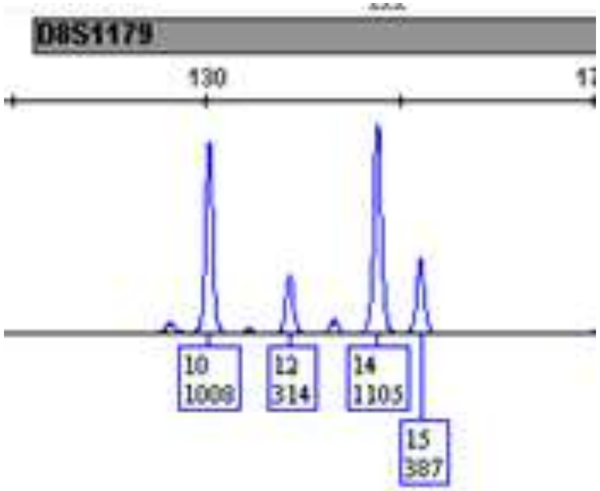
Case 05 – IDPlus

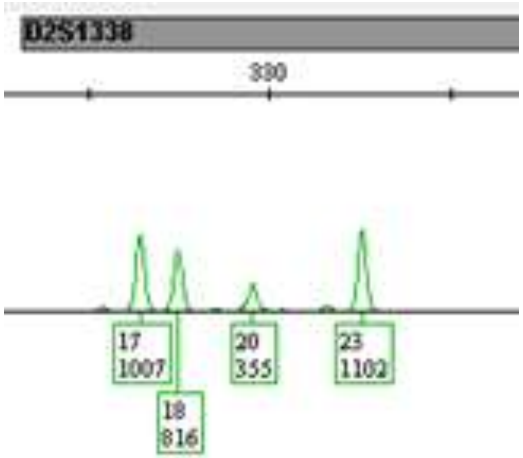


No more than 4 alleles at a locus

- Suggests a 2 person mixture







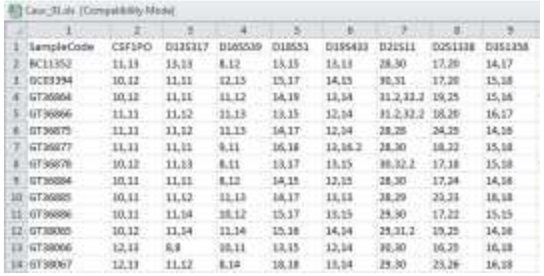
- Peak Height information does not agree

Case 05

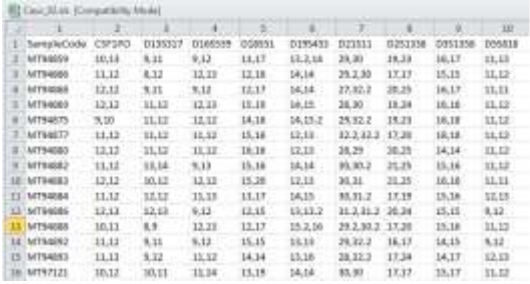
- Is actually a 4 person mixture with no more than 4 alleles at any locus.

- Created with Virtual Mixture Maker (David Duewer, NIST) using 259 Caucasian samples from the NIST population data.

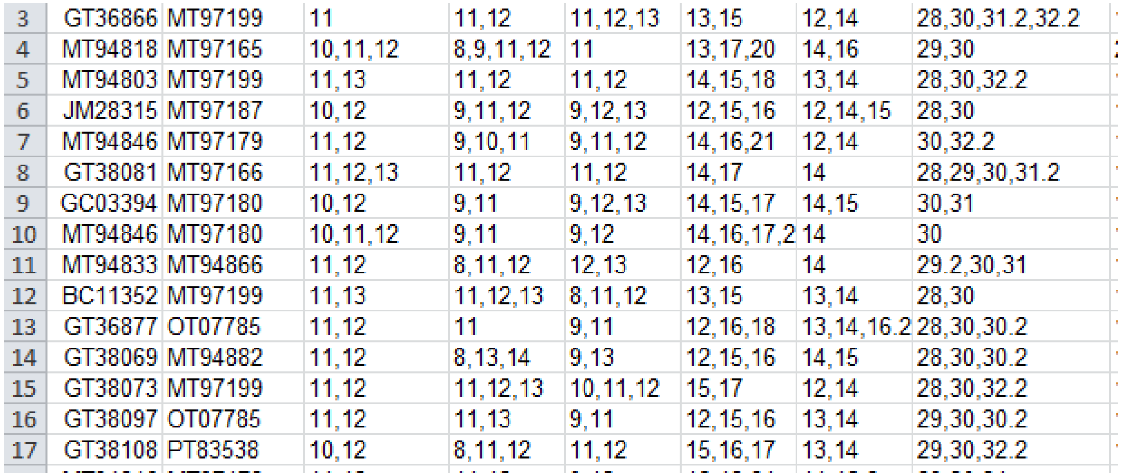
An overview of the mixture creation



65 samples
(ID and Pentas)



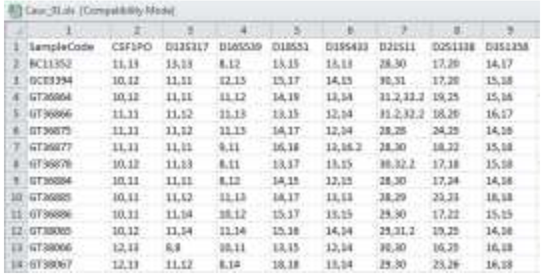
65 samples
(ID and Pentas)



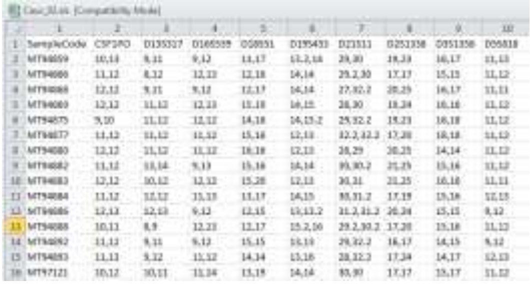
3	GT36866	MT97199	11	11,12	11,12,13	13,15	12,14	28,30,31.2,32.2
4	MT94818	MT97165	10,11,12	8,9,11,12	11	13,17,20	14,16	29,30
5	MT94803	MT97199	11,13	11,12	11,12	14,15,18	13,14	28,30,32.2
6	JM28315	MT97187	10,12	9,11,12	9,12,13	12,15,16	12,14,15	28,30
7	MT94846	MT97179	11,12	9,10,11	9,11,12	14,16,21	12,14	30,32.2
8	GT38081	MT97166	11,12,13	11,12	11,12	14,17	14	28,29,30,31.2
9	GC03394	MT97180	10,12	9,11	9,12,13	14,15,17	14,15	30,31
10	MT94846	MT97180	10,11,12	9,11	9,12	14,16,17,2	14	30
11	MT94833	MT94866	11,12	8,11,12	12,13	12,16	14	29.2,30,31
12	BC11352	MT97199	11,13	11,12,13	8,11,12	13,15	13,14	28,30
13	GT36877	OT07785	11,12	11	9,11	12,16,18	13,14,16.2	28,30,30.2
14	GT38069	MT94882	11,12	8,13,14	9,13	12,15,16	14,15	28,30,30.2
15	GT38073	MT97199	11,12	11,12,13	10,11,12	15,17	12,14	28,30,32.2
16	GT38097	OT07785	11,12	11,13	9,11	12,15,16	13,14	29,30,30.2
17	GT38108	PT83538	10,12	8,11,12	11,12	15,16,17	13,14	29,30,32.2

Groups 1 and 2

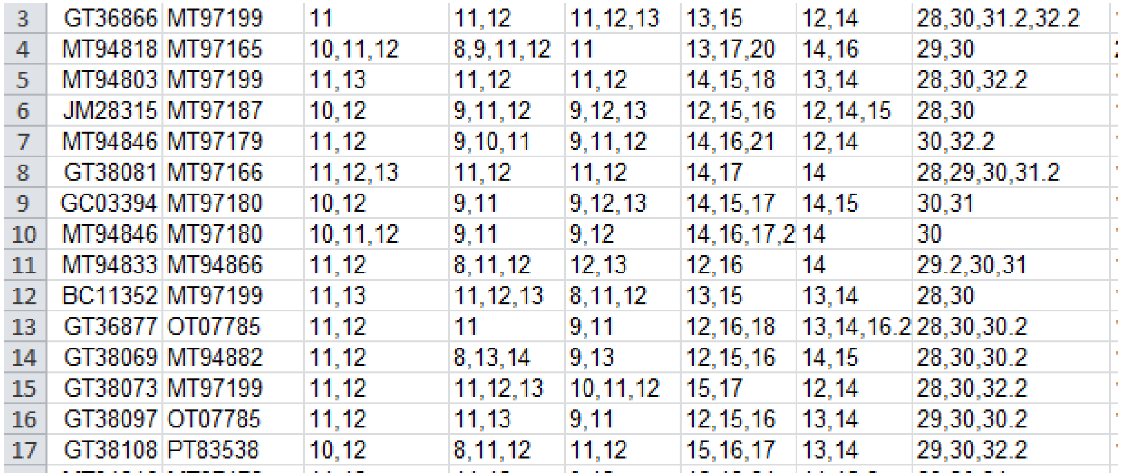
An overview of the mixture creation



65 samples
(ID and Pentas)

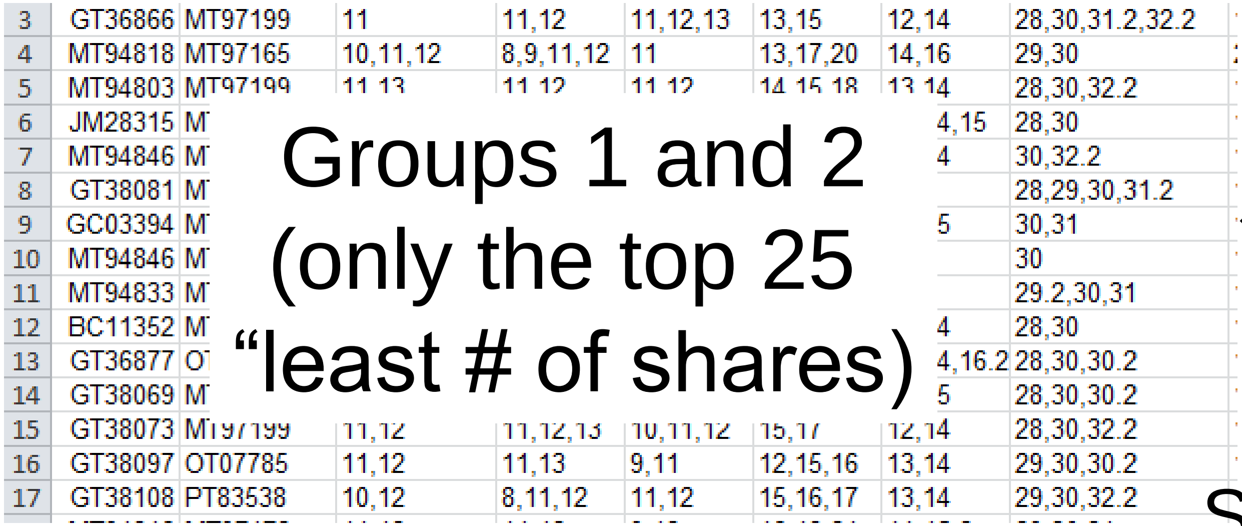


64 samples
(ID and Pentas)



3	GT36866	MT97199	11	11,12	11,12,13	13,15	12,14	28,30,31.2,32.2
4	MT94818	MT97165	10,11,12	8,9,11,12	11	13,17,20	14,16	29,30
5	MT94803	MT97199	11,13	11,12	11,12	14,15,18	13,14	28,30,32.2
6	JM28315	MT97187	10,12	9,11,12	9,12,13	12,15,16	12,14,15	28,30
7	MT94846	MT97179	11,12	9,10,11	9,11,12	14,16,21	12,14	30,32.2
8	GT38081	MT97166	11,12,13	11,12	11,12	14,17	14	28,29,30,31.2
9	GC03394	MT97180	10,12	9,11	9,12,13	14,15,17	14,15	30,31
10	MT94846	MT97180	10,11,12	9,11	9,12	14,16,17,2	14	30
11	MT94833	MT94866	11,12	8,11,12	12,13	12,16	14	29.2,30,31
12	BC11352	MT97199	11,13	11,12,13	8,11,12	13,15	13,14	28,30
13	GT36877	OT07785	11,12	11	9,11	12,16,18	13,14,16.2	28,30,30.2
14	GT38069	MT94882	11,12	8,13,14	9,13	12,15,16	14,15	28,30,30.2
15	GT38073	MT97199	11,12	11,12,13	10,11,12	15,17	12,14	28,30,32.2
16	GT38097	OT07785	11,12	11,13	9,11	12,15,16	13,14	29,30,30.2
17	GT38108	PT83538	10,12	8,11,12	11,12	15,16,17	13,14	29,30,32.2

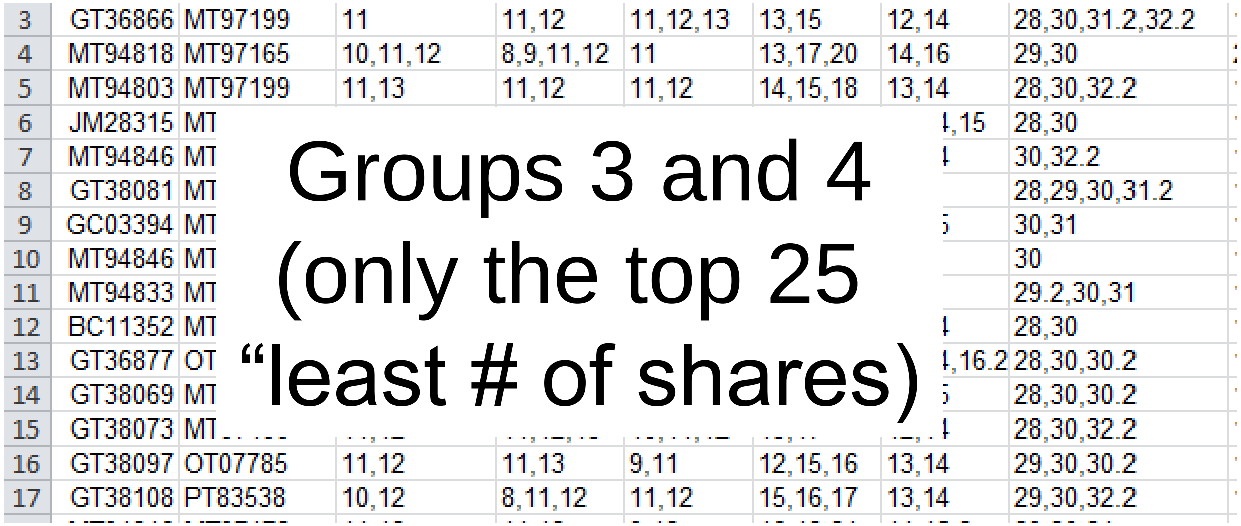
Groups 3 and 4



3	GT36866	MT97199	11	11,12	11,12,13	13,15	12,14	28,30,31.2,32.2
4	MT94818	MT97165	10,11,12	8,9,11,12	11	13,17,20	14,16	29,30
5	MT94803	MT97199	11,13	11,12	11,12	14,15,18	13,14	28,30,32.2
6	JM28315	MT					4,15	28,30
7	MT94846	MT					4	30,32.2
8	GT38081	MT						28,29,30,31.2
9	GC03394	MT					5	30,31
10	MT94846	MT						30
11	MT94833	MT						29.2,30,31
12	BC11352	MT					4	28,30
13	GT36877	OT					4,16.2	28,30,30.2
14	GT38069	MT					5	28,30,30.2
15	GT38073	MT97199	11,12	11,12,13	10,11,12	15,17	12,14	28,30,32.2
16	GT38097	OT07785	11,12	11,13	9,11	12,15,16	13,14	29,30,30.2
17	GT38108	PT83538	10,12	8,11,12	11,12	15,16,17	13,14	29,30,32.2

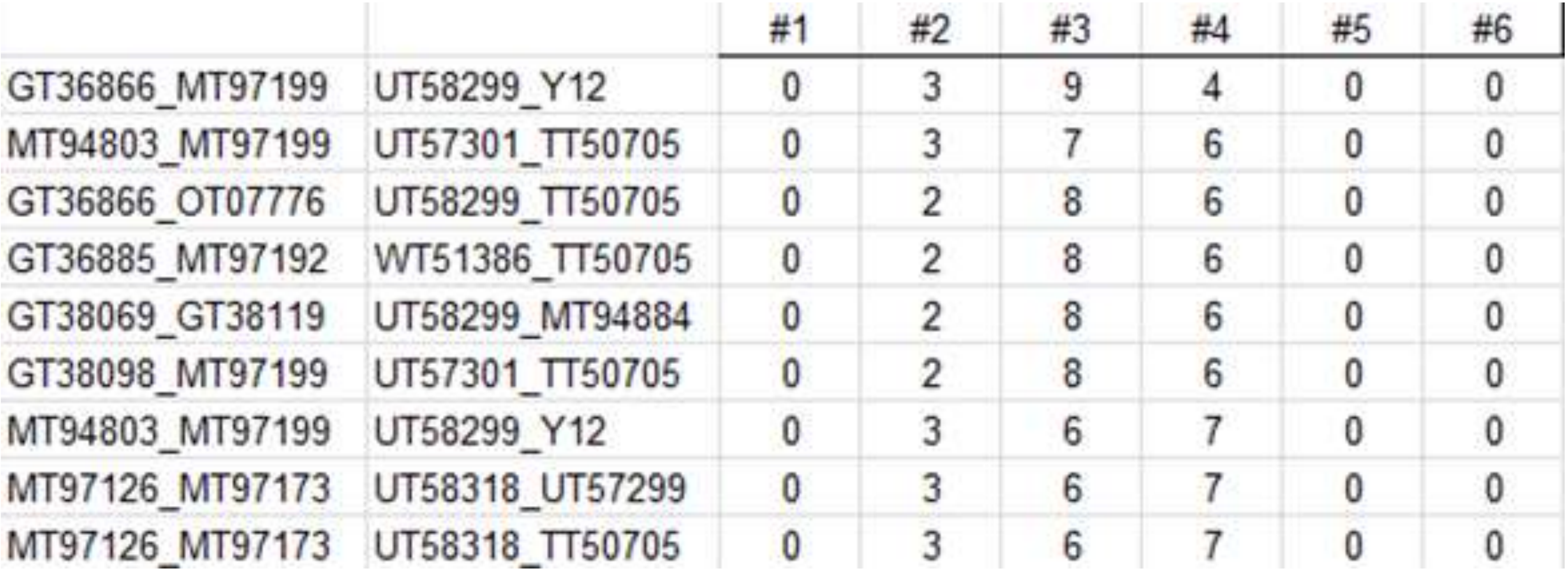
Groups 1 and 2
(only the top 25
"least # of shares)

Select combinations
with no more than
4 alleles at any locus



3	GT36866	MT97199	11	11,12	11,12,13	13,15	12,14	28,30,31.2,32.2
4	MT94818	MT97165	10,11,12	8,9,11,12	11	13,17,20	14,16	29,30
5	MT94803	MT97199	11,13	11,12	11,12	14,15,18	13,14	28,30,32.2
6	JM28315	MT					,15	28,30
7	MT94846	MT						30,32.2
8	GT38081	MT						28,29,30,31.2
9	GC03394	MT						30,31
10	MT94846	MT						30
11	MT94833	MT						29.2,30,31
12	BC11352	MT						28,30
13	GT36877	OT					,16.2	28,30,30.2
14	GT38069	MT						28,30,30.2
15	GT38073	MT						28,30,32.2
16	GT38097	OT07785	11,12	11,13	9,11	12,15,16	13,14	29,30,30.2
17	GT38108	PT83538	10,12	8,11,12	11,12	15,16,17	13,14	29,30,32.2

Groups 3 and 4
(only the top 25
"least # of shares)



		#1	#2	#3	#4	#5	#6
GT36866_MT97199	UT58299_Y12	0	3	9	4	0	0
MT94803_MT97199	UT57301_TT50705	0	3	7	6	0	0
GT36866_OT07776	UT58299_TT50705	0	2	8	6	0	0
GT36885_MT97192	WT51386_TT50705	0	2	8	6	0	0
GT38069_GT38119	UT58299_MT94884	0	2	8	6	0	0
GT38098_MT97199	UT57301_TT50705	0	2	8	6	0	0
MT94803_MT97199	UT58299_Y12	0	3	6	7	0	0
MT97126_MT97173	UT58318_UT57299	0	3	6	7	0	0
MT97126_MT97173	UT58318_TT50705	0	3	6	7	0	0

Note: All samples are unrelated
(relative testing, mtDNA, Y-STRs, X-STRs, etc…)

Case 05 – 3 Suspects

Individual	
Suspect 5A	Included
Suspect 5B	Included
Suspect 5C	Not in the mixture

D8S1179 Result with Case 5 Mixture

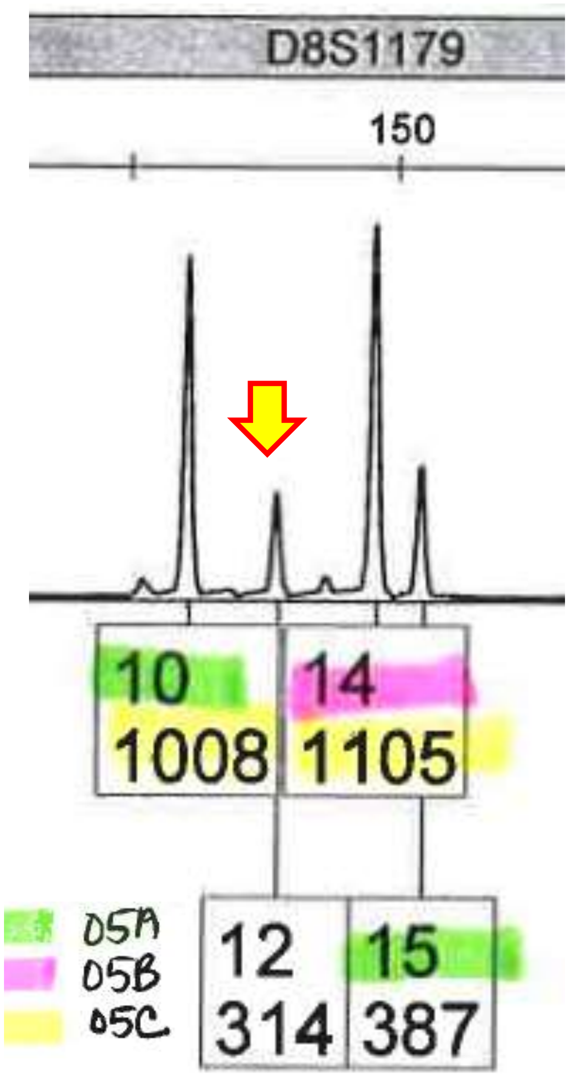
Provided Reference
Suspect Profiles
<u>at D8S1179</u>

5A : 10,15
5B : 14,14
5C : 10,14

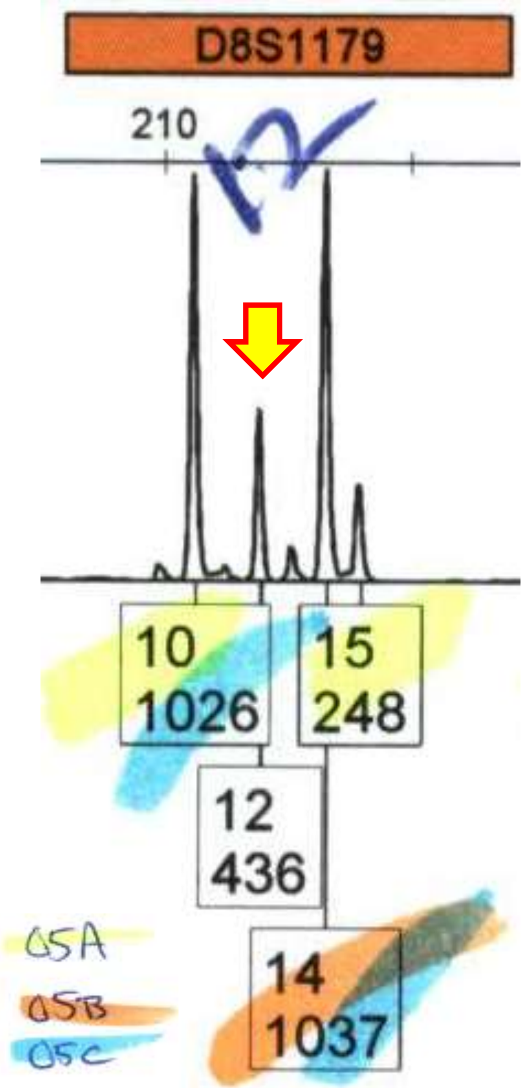
Allele 12 is in the evidence profiles but not in three provided reference samples

Is there another contributor not represented by the supplied suspects?

Identifiler Plus Result



PowerPlex 16HS Result



D19S433 Result with Case 5 Mixture

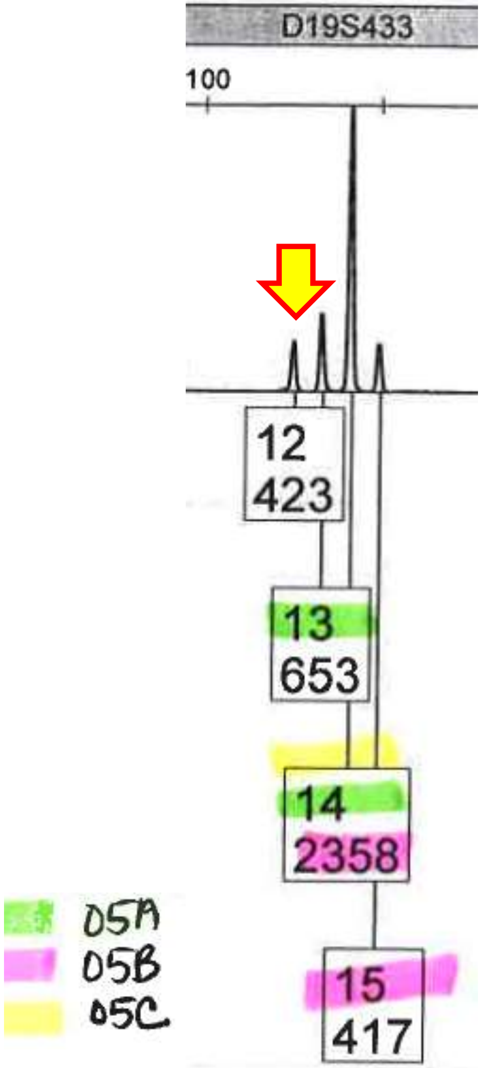
Provided Reference
Suspect Profiles
at xxxx

5A : 13,14
5B : 14,15
5C : 14,14

Allele 12 is in the evidence profiles but not in three provided reference samples

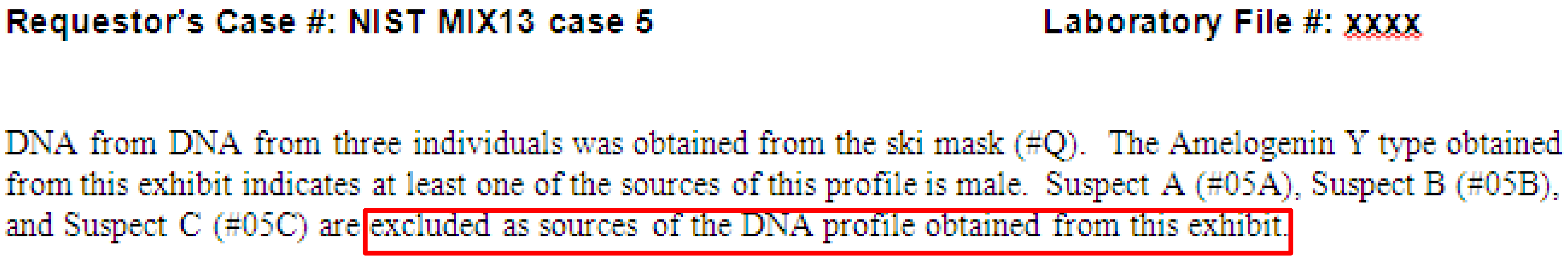
Is there another contributor not represented by the supplied suspects?

Identifiler Plus Result



The D19S433 peak height imbalance created concern among __ labs

Case 5: conclusion reached is **exclusion** of all three suspects

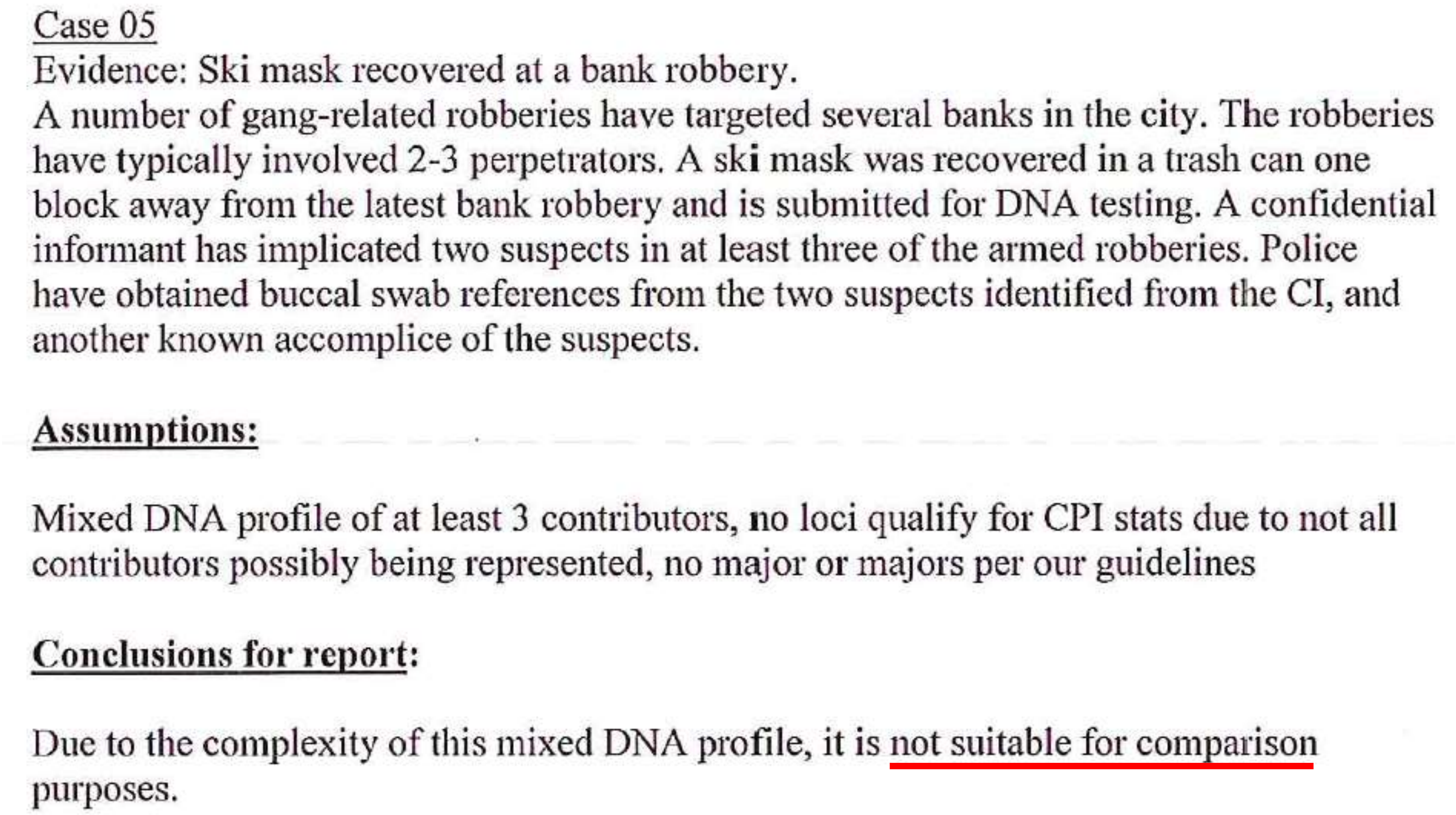


Requestor's Case #: NIST MIX13 case 5 **Laboratory File #: xxxx**

DNA from DNA from three individuals was obtained from the ski mask (#Q). The Amelogenin Y type obtained from this exhibit indicates at least one of the sources of this profile is male. Suspect A (#05A), Suspect B (#05B), and Suspect C (#05C) are excluded as sources of the DNA profile obtained from this exhibit.

Provides a 20-page detailed analysis and evaluation on a locus-by-locus basis

Case 5: conclusion of **inconclusive** (not suitable for comparison)



Case 05

Evidence: Ski mask recovered at a bank robbery.

A number of gang-related robberies have targeted several banks in the city. The robberies have typically involved 2-3 perpetrators. A ski mask was recovered in a trash can one block away from the latest bank robbery and is submitted for DNA testing. A confidential informant has implicated two suspects in at least three of the armed robberies. Police have obtained buccal swab references from the two suspects identified from the CI, and another known accomplice of the suspects.

Assumptions:

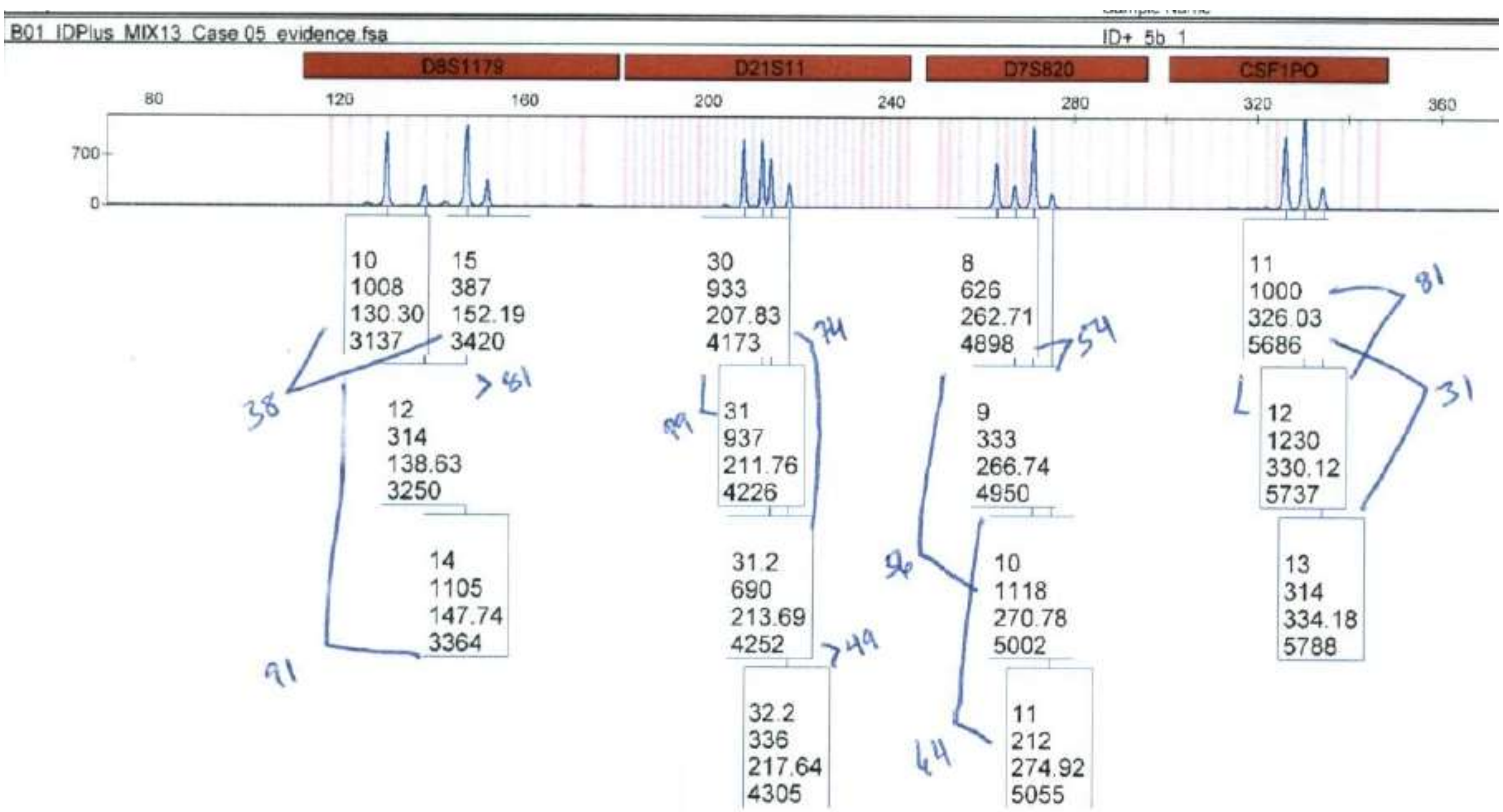
Mixed DNA profile of at least 3 contributors, no loci qualify for CPI stats due to not all contributors possibly being represented, no major or majors per our guidelines

Conclusions for report:

Due to the complexity of this mixed DNA profile, it is not suitable for comparison purposes.

Case 5: lab manually examines PHRs

no major, no conclusion – "not interpretable due to the complexity of the genetic information"

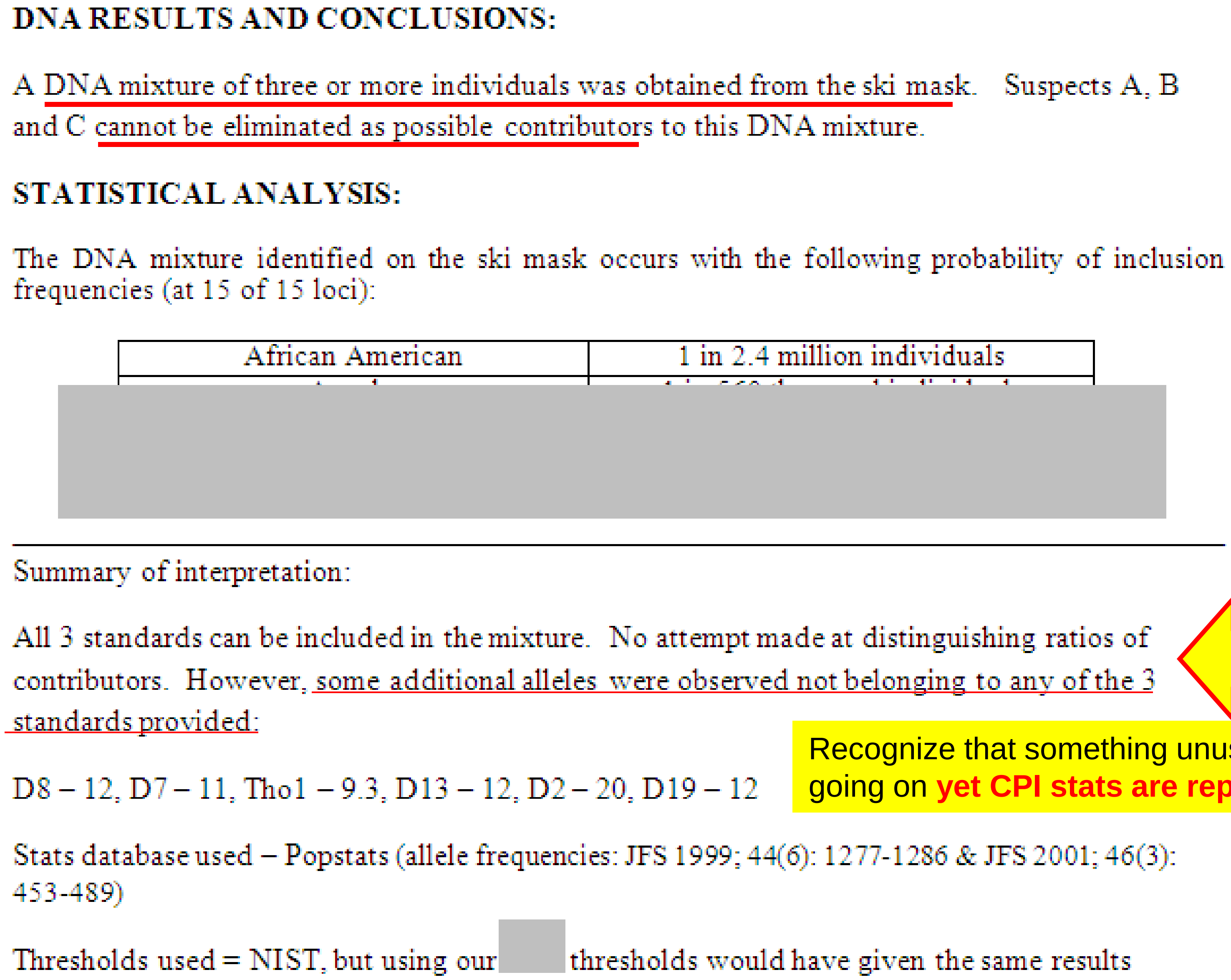


Case 05

A mixture of at least three individuals was detected Evidence Item 1 in which a major contributor could not be determined.

Evidence Item 1 is not interpretable due the complexity of the genetic information; therefore no conclusions will be drawn.

Case 5: all suspects **included** (cannot be eliminated as possible contributors)



DNA RESULTS AND CONCLUSIONS:

A DNA mixture of three or more individuals was obtained from the ski mask. Suspects A, B and C cannot be eliminated as possible contributors to this DNA mixture.

STATISTICAL ANALYSIS:

The DNA mixture identified on the ski mask occurs with the following probability of inclusion frequencies (at 15 of 15 loci):

African American	1 in 2.4 million individuals
[redacted]	[redacted]

Summary of interpretation:

All 3 standards can be included in the mixture. No attempt made at distinguishing ratios of contributors. However, some additional alleles were observed not belonging to any of the 3 standards provided:

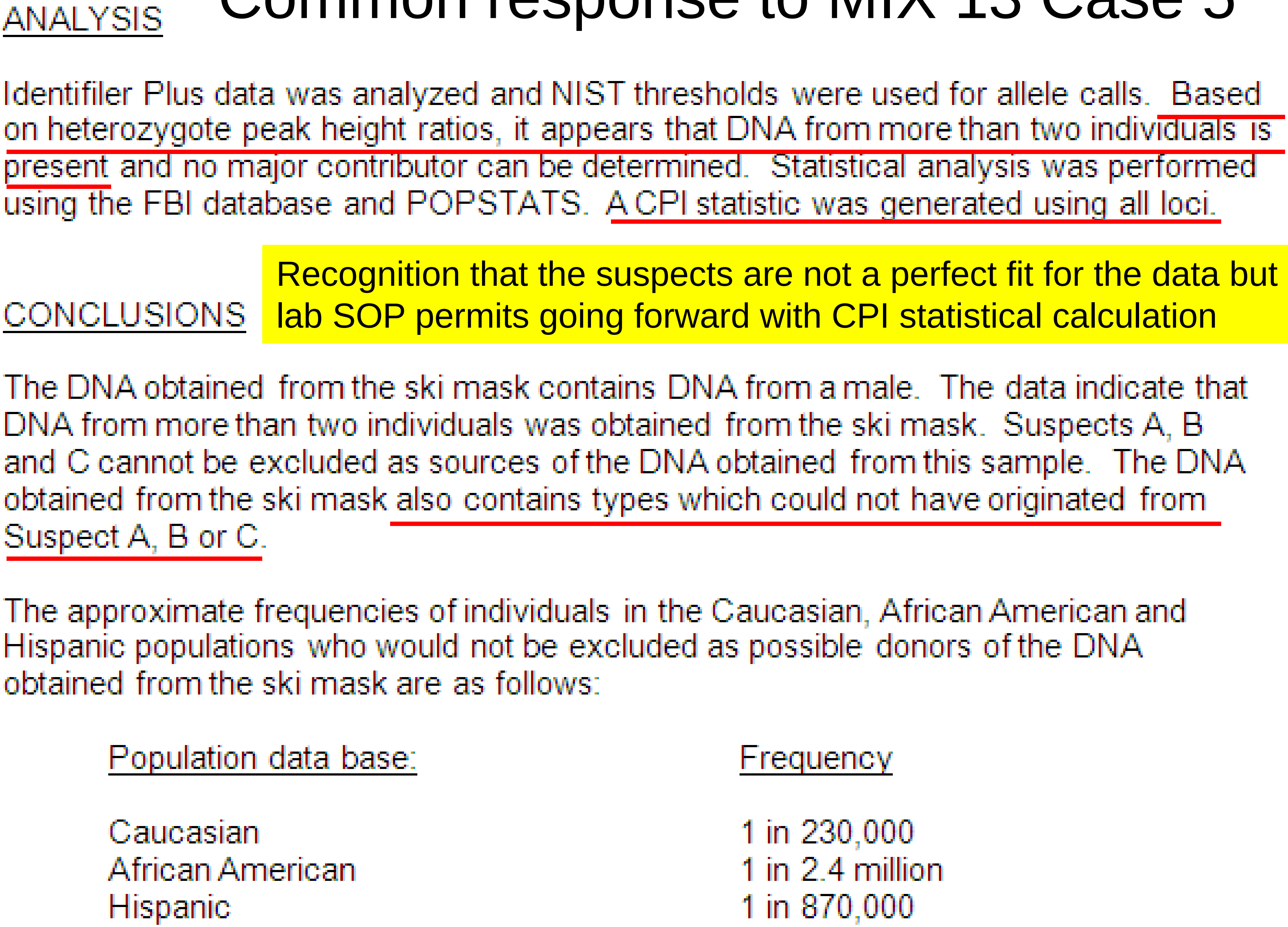
D8 – 12, D7 – 11, Tho1 – 9.3, D13 – 12, D2 – 20, D19 – 12

Stats database used – Popstats (allele frequencies: JFS 1999; 44(6): 1277-1286 & JFS 2001; 46(3): 453-489)

Thresholds used = NIST, but using our [redacted] thresholds would have given the same results

Recognize that something unusual is going on **yet CPI stats are reported**

Common response to MIX 13 Case 5



ANALYSIS

Identifiler Plus data was analyzed and NIST thresholds were used for allele calls. Based on heterozygote peak height ratios, it appears that DNA from more than two individuals is present and no major contributor can be determined. Statistical analysis was performed using the FBI database and POPSTATS. A CPI statistic was generated using all loci.

Recognition that the suspects are not a perfect fit for the data but lab SOP permits going forward with CPI statistical calculation

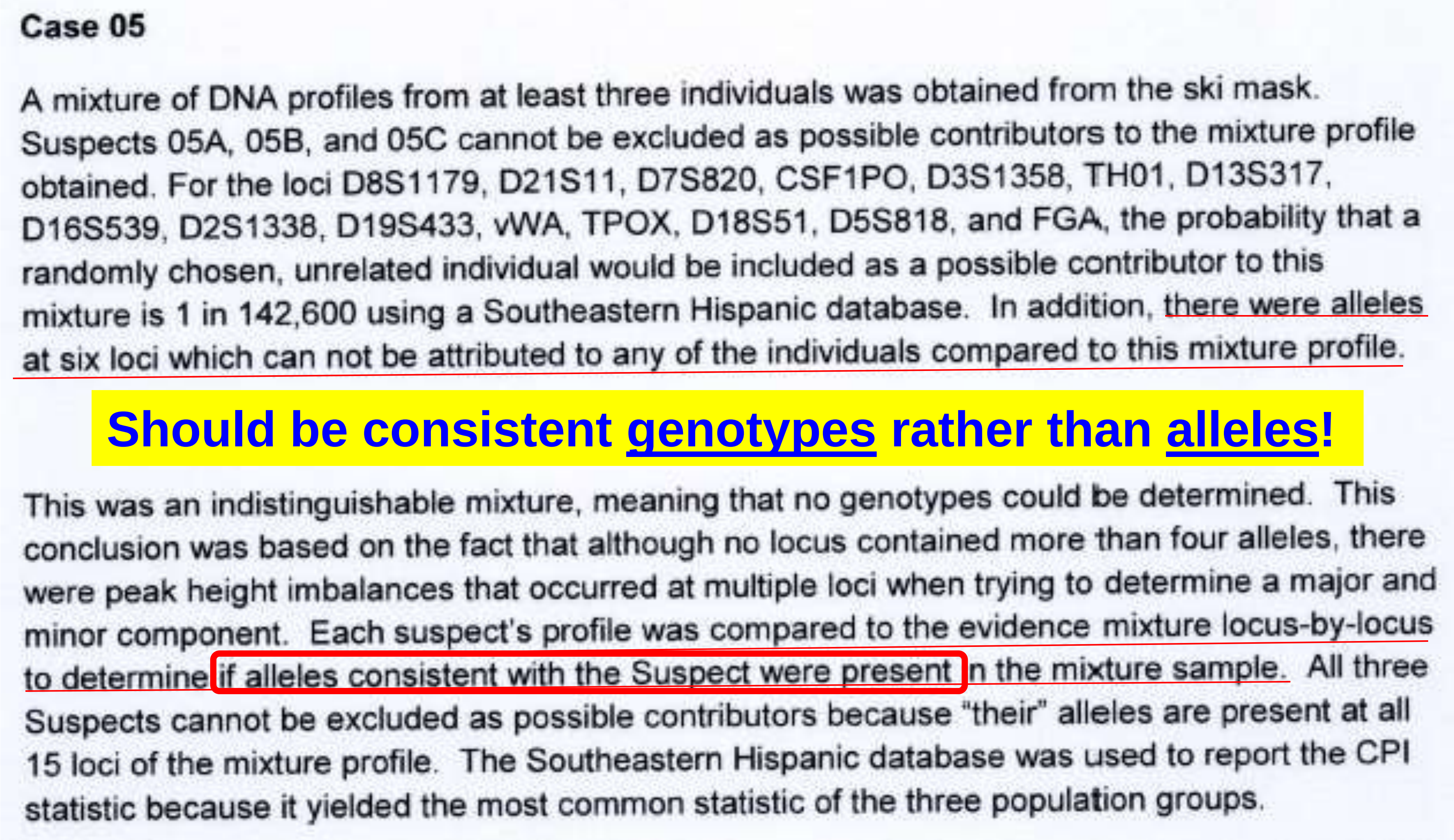
CONCLUSIONS

The DNA obtained from the ski mask contains DNA from a male. The data indicate that DNA from more than two individuals was obtained from the ski mask. Suspects A, B and C cannot be excluded as sources of the DNA obtained from this sample. The DNA obtained from the ski mask also contains types which could not have originated from Suspect A, B or C.

The approximate frequencies of individuals in the Caucasian, African American and Hispanic populations who would not be excluded as possible donors of the DNA obtained from the ski mask are as follows:

Population data base:	Frequency
Caucasian	1 in 230,000
African American	1 in 2.4 million
Hispanic	1 in 870,000

A fundamental misunderstanding due to overuse of CPI statistics



Case 05

A mixture of DNA profiles from at least three individuals was obtained from the ski mask. Suspects 05A, 05B, and 05C cannot be excluded as possible contributors to the mixture profile obtained. For the loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA, the probability that a randomly chosen, unrelated individual would be included as a possible contributor to this mixture is 1 in 142,600 using a Southeastern Hispanic database. In addition, there were alleles at six loci which can not be attributed to any of the individuals compared to this mixture profile.

Should be consistent genotypes rather than alleles!

This was an indistinguishable mixture, meaning that no genotypes could be determined. This conclusion was based on the fact that although no locus contained more than four alleles, there were peak height imbalances that occurred at multiple loci when trying to determine a major and minor component. Each suspect's profile was compared to the evidence mixture locus-by-locus to determine if alleles consistent with the Suspect were present in the mixture sample. All three Suspects cannot be excluded as possible contributors because "their" alleles are present at all 15 loci of the mixture profile. The Southeastern Hispanic database was used to report the CPI statistic because it yielded the most common statistic of the three population groups.

MIX13 Case 5 Outcomes with Suspect C

(whose genotypes were not present in the mixture)

# Labs	Report Conclusions	Reasons given
6	Exclude Suspect C	detailed genotype checks (ID+); TrueAllele negative LR (ID+); assumed major/minor and suspects did not fit (ID+); 3 labs noted Penta E missing allele 15 (PP16HS)
3	Inconclusive with C only (A & B included)	All these labs used PP16HS
21	Inconclusive for A, B, and C	
70	**Include & provide CPI statistics**	*All over the road…*

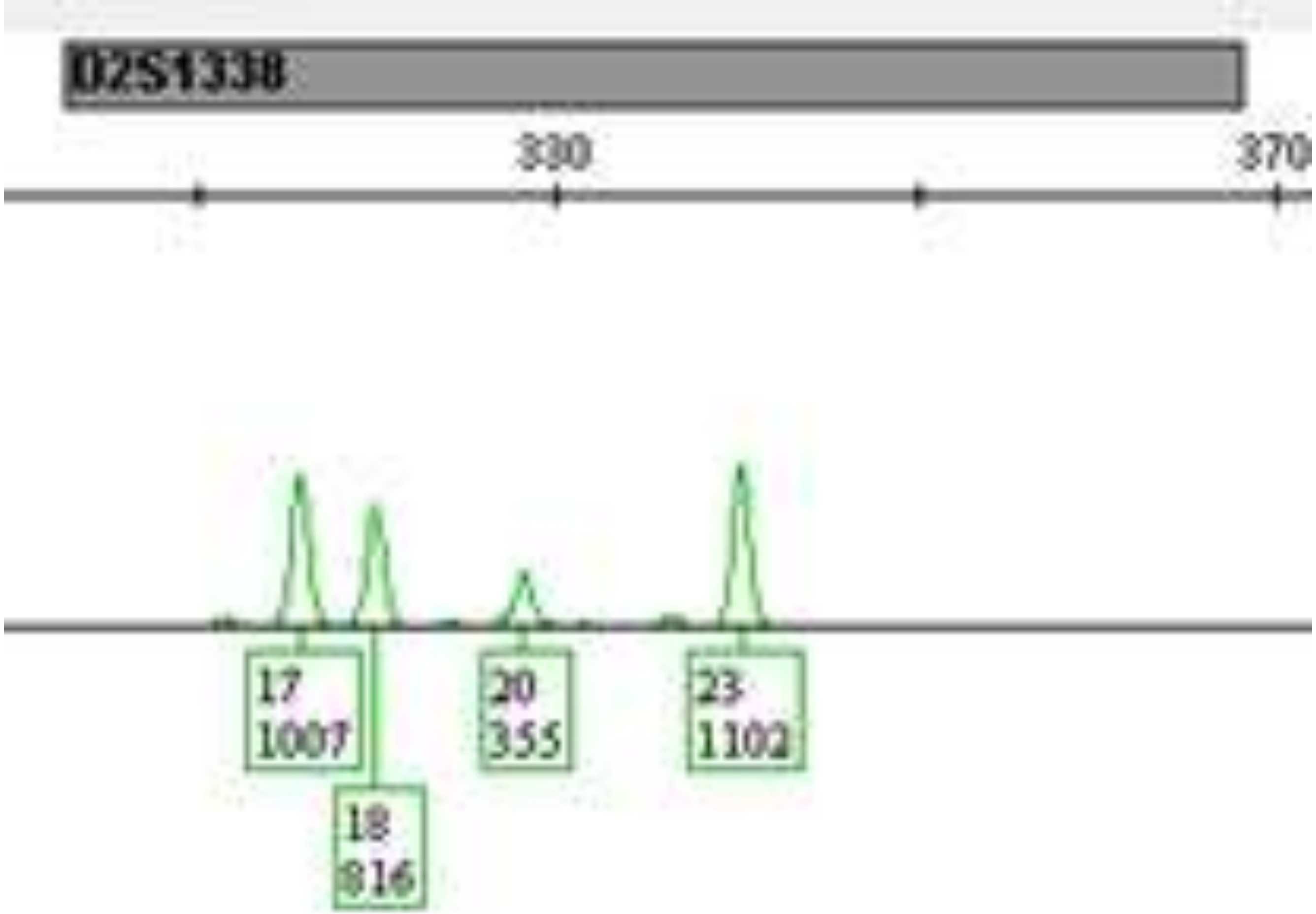
Range of CPI stats for Caucasian population:

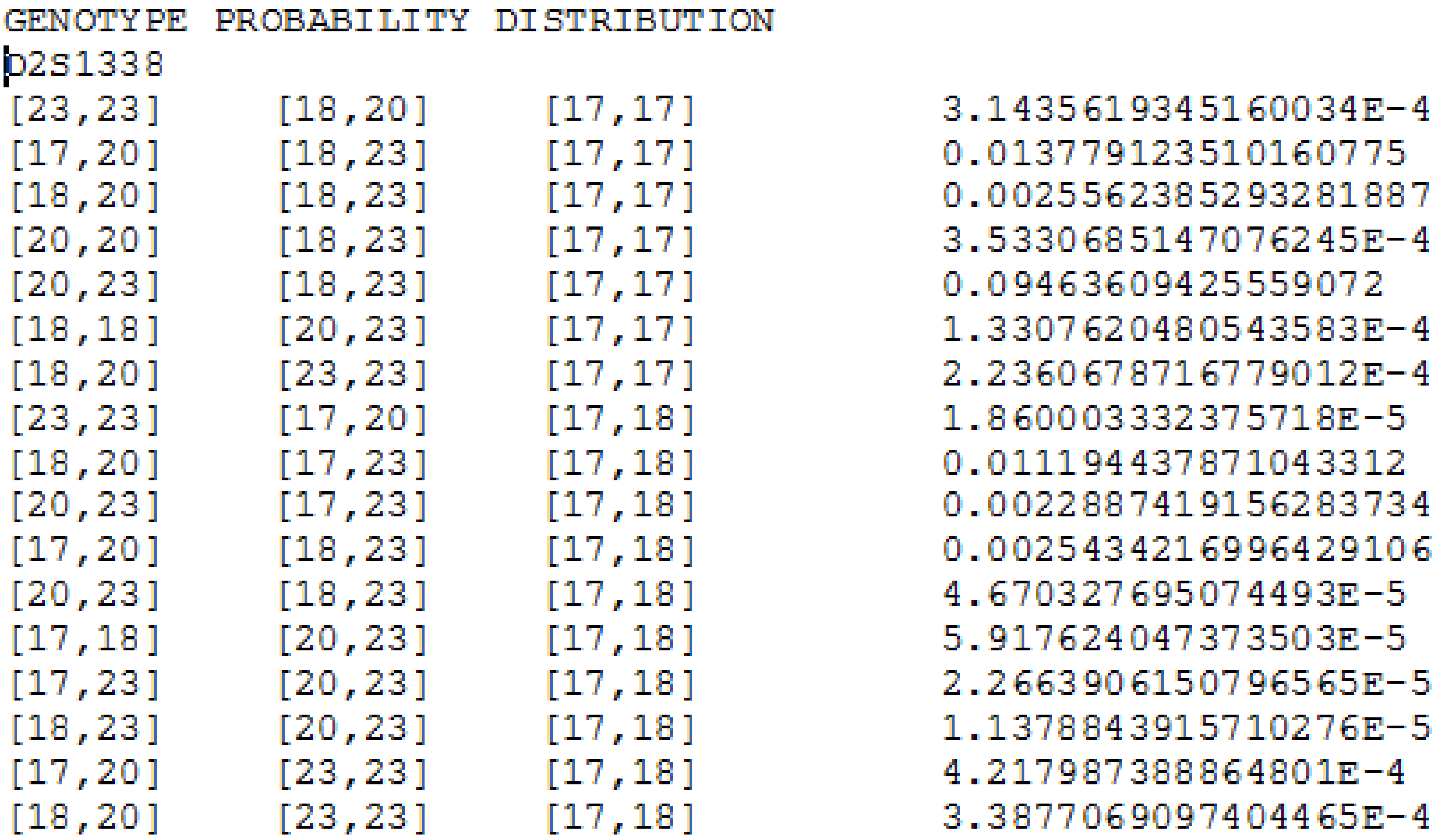
FBI allele frequencies: **1 in 9** (Labs 12 & 54) to **1 in 344,000** (Lab 107)

Case 05

“Couldn’t help but note the need for mix deconvolution software tools for case 05”

(a) Deconvolution as 3p mixture





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 [18,20]     [18,23]     [17,17]           0.0025562385293281887
 [20,20]     [18,23]     [17,17]           3.5330685147076245E-4
 [20,23]     [18,23]     [17,17]           0.09463609425559072
 [18,18]     [20,23]     [17,17]           1.3307620480543583E-4
 [18,20]     [23,23]     [17,17]           2.2360678716779012E-4
 [23,23]     [17,20]     [17,18]           1.860003332375718E-5
 [18,20]     [17,23]     [17,18]           0.011194437871043312
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 [17,20]     [18,23]     [17,18]           0.0025434216996429106
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 [17,18]     [20,23]     [17,18]           5.917624047373503E-5
 [17,23]     [20,23]     [17,18]           2.2663906150796565E-5
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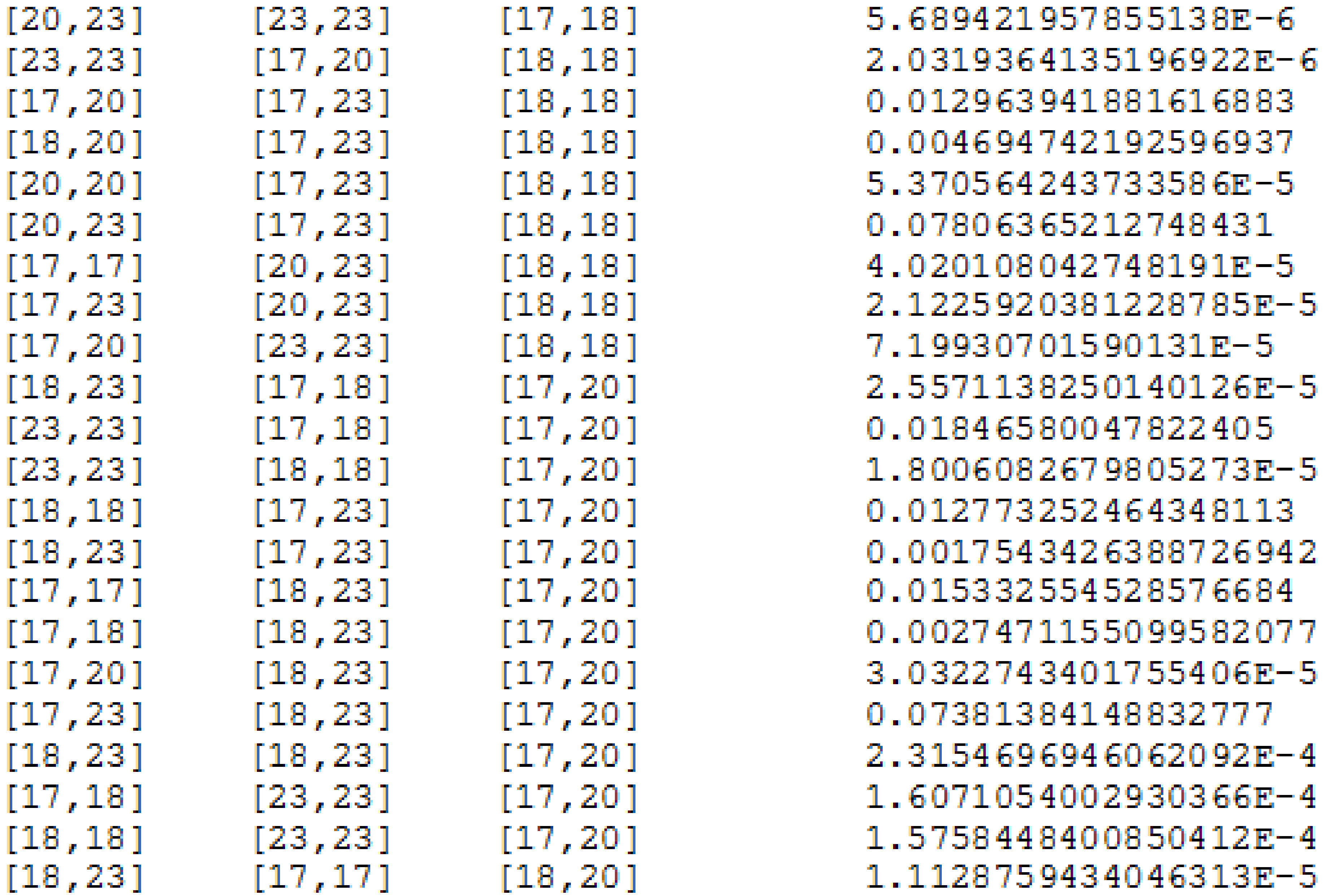

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[18,20]      [17,23]      [18,18]            0.004694742192596937
[20,20]      [17,23]      [18,18]            5.370564243733586E-5
[20,23]      [17,23]      [18,18]            0.0780636521274843‌1
[17,17]      [20,23]      [18,18]            4.020108042748191E-5
[17,23]      [20,23]      [18,18]            2.1225920381228785E-5
[17,20]      [23,23]      [18,18]            7.19930701590131E-5
[18,23]      [17,18]      [17,20]            2.5571138250140126E-5
[23,23]      [17,18]      [17,20]            0.018465800478224‌05
[23,23]      [18,18]      [17,20]            1.8006082679805273E-5
[18,18]      [17,23]      [17,20]            0.0127732524643481‌13
[18,23]      [17,23]      [17,20]            0.0017543426388726942
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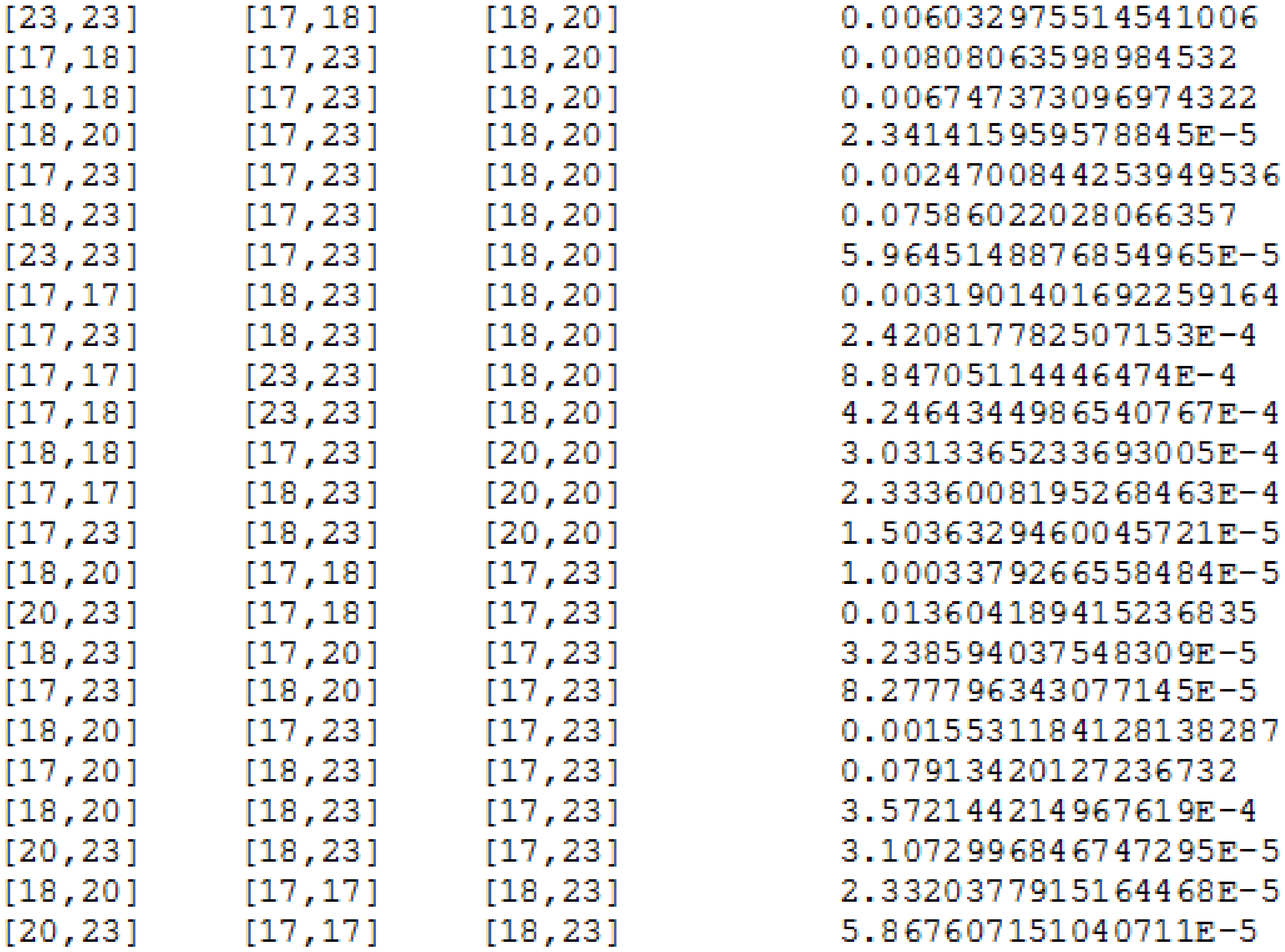

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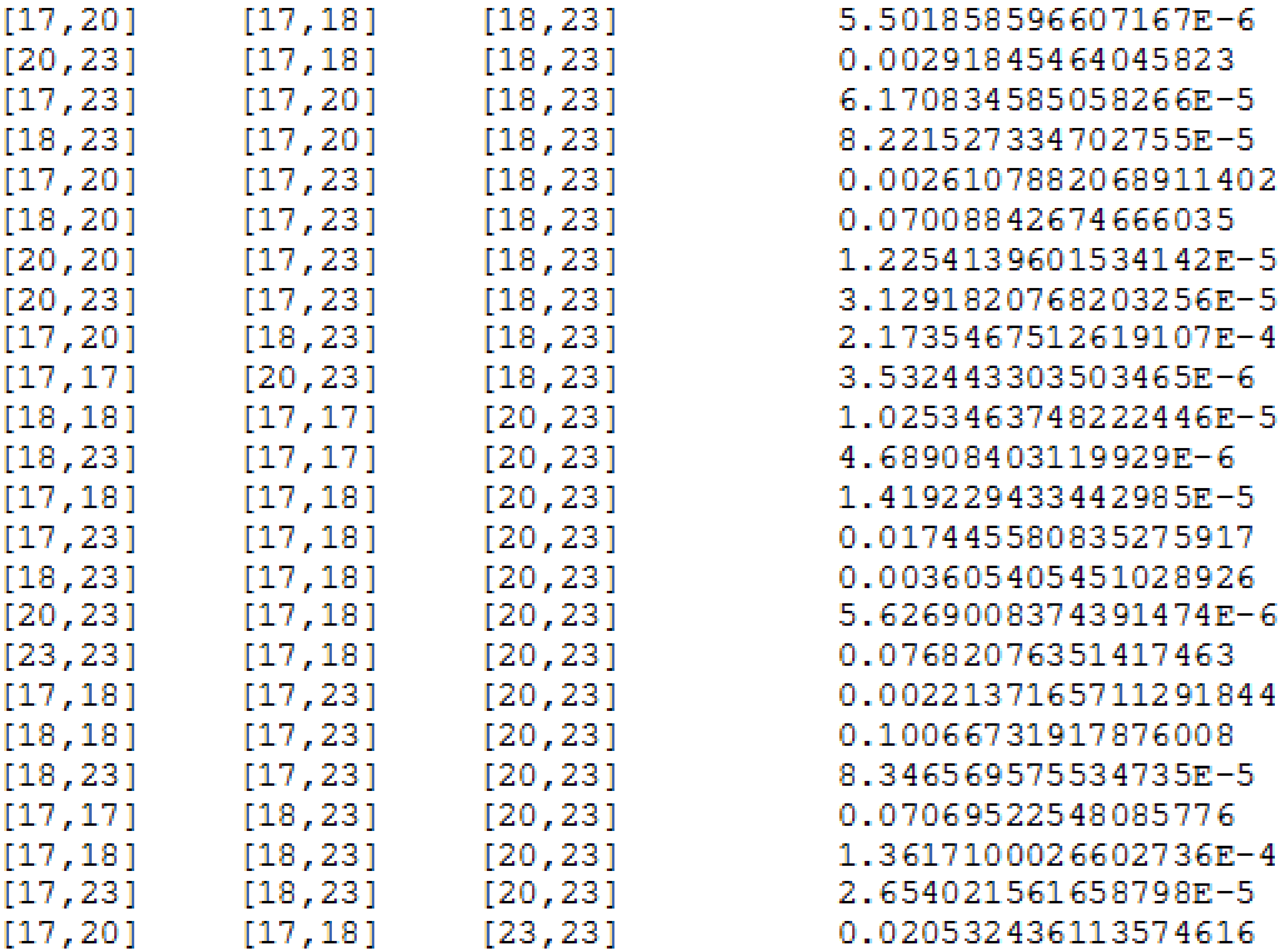
[18,18]     [17,23]     [20,20]          3.0313365233693005E-4
[17,17]     [18,23]     [20,20]          2.3336008195268463E-4
[17,23]     [18,23]     [20,20]          1.5036329460045721E-5
[18,20]     [17,18]     [17,23]          1.0003379266558484E-5
[20,23]     [17,18]     [17,23]          0.013604189415236835
[18,23]     [17,20]     [17,23]          3.238594037548309E-5
[17,23]     [18,20]     [17,23]          8.277796343077145E-5
[18,20]     [17,23]     [17,23]          0.0015531184128138287
[17,20]     [18,23]     [17,23]          0.07913420127236732
[18,20]     [18,23]     [17,23]          3.572144214967619E-4
[20,23]     [18,23]     [17,23]          3.1072996846747295E-5
[18,20]     [17,17]     [18,23]          2.3320377915164468E-5
[20,23]     [17,17]     [18,23]          5.867607151040711E-5
```



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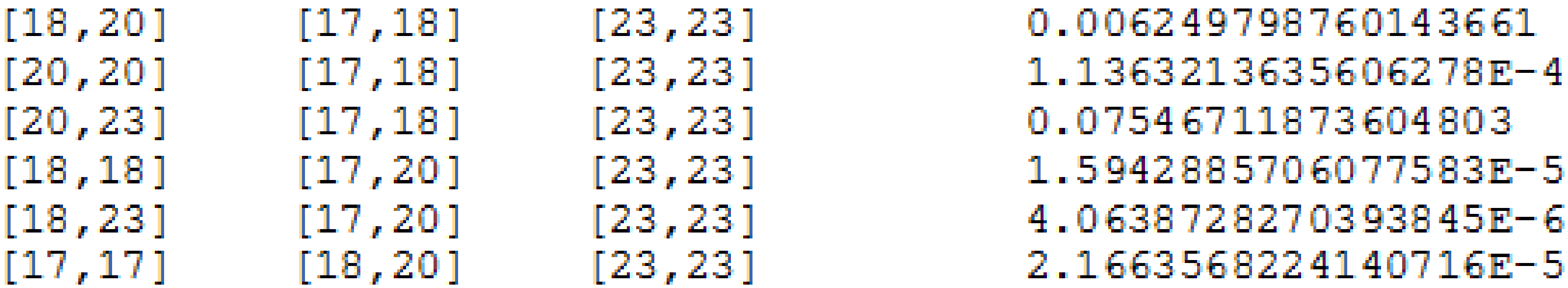
[17,20]      [17,18]      [18,23]            5.501858596607167E-6
[20,23]      [17,18]      [18,23]            0.00291845464045823
[17,23]      [17,20]      [18,23]            6.170834585058266E-5
[18,23]      [17,20]      [18,23]            8.221527334702755E-5
[17,20]      [17,23]      [18,23]            0.0026107882068911402
[18,20]      [17,23]      [18,23]            0.07008842674666035
[20,20]      [17,23]      [18,23]            1.2254139601534142E-5
[20,23]      [17,23]      [18,23]            3.1291820768203256E-5
[17,20]      [18,23]      [18,23]            2.1735467512619107E-4
[17,17]      [20,23]      [18,23]            3.532443303503465E-6
[18,18]      [17,17]      [20,23]            1.0253463748222446E-5
[18,23]      [17,17]      [20,23]            4.68908403119929E-6
[17,18]      [17,18]      [20,23]            1.419229433442985E-5
[17,23]      [17,18]      [20,23]            0.017445580835275917
[18,23]      [17,18]      [20,23]            0.003605405451028926
[20,23]      [17,18]      [20,23]            5.6269008374391474E-6
[23,23]      [17,18]      [20,23]            0.07682076351417463
[17,18]      [17,23]      [20,23]            0.0022137165711291844
[18,18]      [17,23]      [20,23]            0.10066731917876008
[18,23]      [17,23]      [20,23]            8.346569575534735E-5
[17,17]      [18,23]      [20,23]            0.07069522548085776
[17,18]      [18,23]      [20,23]            1.3617100026602736E-4
[17,23]      [18,23]      [20,23]            2.654021561658798E-5
[17,20]      [17,18]      [23,23]            0.020532436113574616
```



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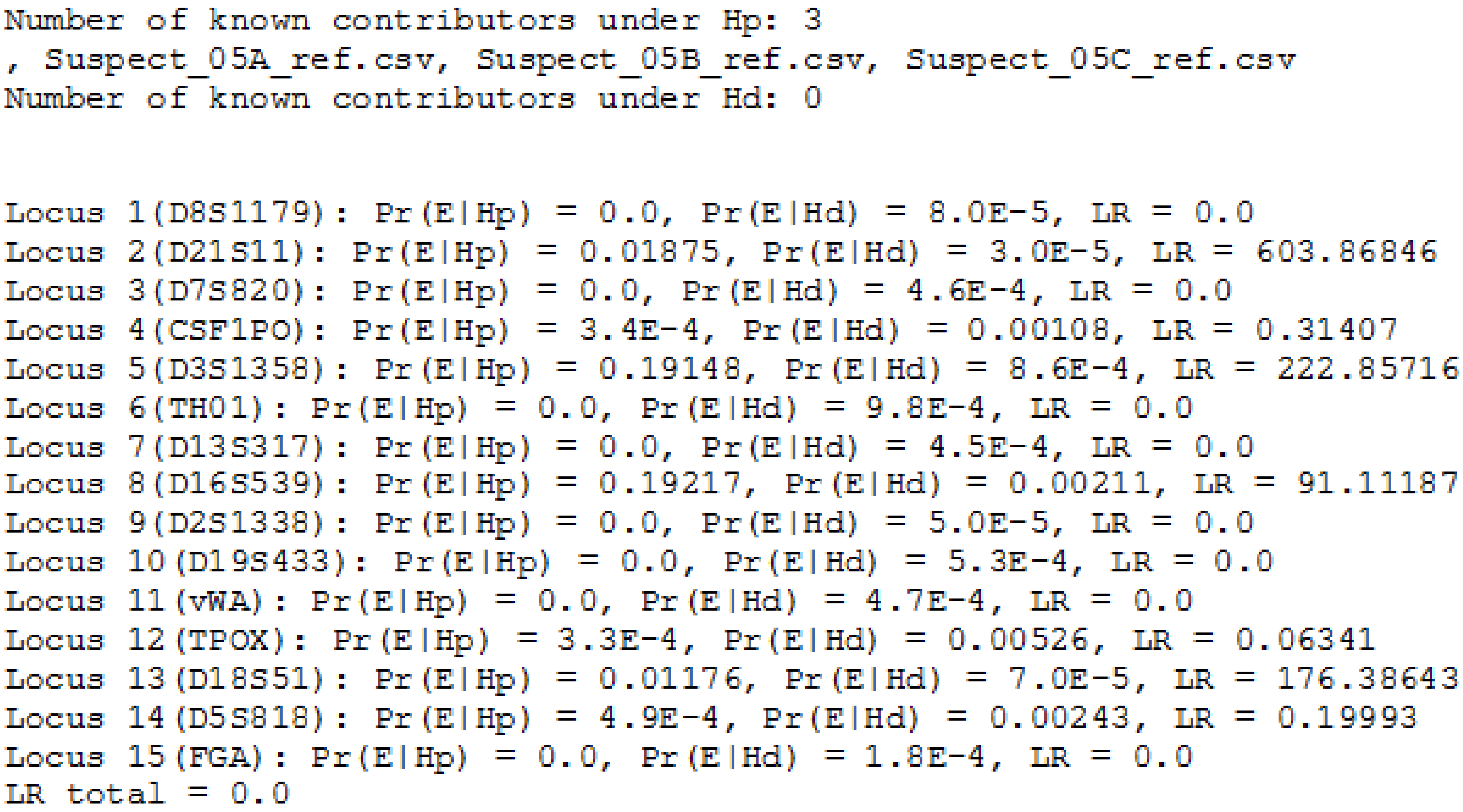
[18,20]     [17,18]      [23,23]            0.006249798760143661
[20,20]     [17,18]      [23,23]            1.136321363560627 8E-4
[20,23]     [17,18]      [23,23]            0.07546711873604803
[18,18]     [17,20]      [23,23]            1.5942885706077583E-5
[18,23]     [17,20]      [23,23]            4.063872827039384 5E-6
[17,17]     [18,20]      [23,23]            2.166356822414071 6E-5
```

93 !! Possible **Genotype** Combinations



```
Number of known contributors under Hp: 3
, Suspect_05A_ref.csv, Suspect_05B_ref.csv, Suspect_05C_ref.csv
Number of known contributors under Hd: 0


Locus 1(D8S1179): Pr(E|Hp) = 0.0, Pr(E|Hd) = 8.0E-5, LR = 0.0
Locus 2(D21S11): Pr(E|Hp) = 0.01875, Pr(E|Hd) = 3.0E-5, LR = 603.86846
Locus 3(D7S820): Pr(E|Hp) = 0.0, Pr(E|Hd) = 4.6E-4, LR = 0.0
Locus 4(CSF1PO): Pr(E|Hp) = 3.4E-4, Pr(E|Hd) = 0.00108, LR = 0.31407
Locus 5(D3S1358): Pr(E|Hp) = 0.19148, Pr(E|Hd) = 8.6E-4, LR = 222.85716
Locus 6(TH01): Pr(E|Hp) = 0.0, Pr(E|Hd) = 9.8E-4, LR = 0.0
Locus 7(D13S317): Pr(E|Hp) = 0.0, Pr(E|Hd) = 4.5E-4, LR = 0.0
Locus 8(D16S539): Pr(E|Hp) = 0.19217, Pr(E|Hd) = 0.00211, LR = 91.11187
Locus 9(D2S1338): Pr(E|Hp) = 0.0, Pr(E|Hd) = 5.0E-5, LR = 0.0
Locus 10(D19S433): Pr(E|Hp) = 0.0, Pr(E|Hd) = 5.3E-4, LR = 0.0
Locus 11(vWA): Pr(E|Hp) = 0.0, Pr(E|Hd) = 4.7E-4, LR = 0.0
Locus 12(TPOX): Pr(E|Hp) = 3.3E-4, Pr(E|Hd) = 0.00526, LR = 0.06341
Locus 13(D18S51): Pr(E|Hp) = 0.01176, Pr(E|Hd) = 7.0E-5, LR = 176.38643
Locus 14(D5S818): Pr(E|Hp) = 4.9E-4, Pr(E|Hd) = 0.00243, LR = 0.19993
Locus 15(FGA): Pr(E|Hp) = 0.0, Pr(E|Hd) = 1.8E-4, LR = 0.0
LR total = 0.0
```

LR Total = 0.0

Case 05

- Similar results when sample was analyzed as a 4 person mixture. True Allele results from one of the labs gave a negative logLR for Suspect C.

- Interpretation of this complex mixture (real or otherwise) requires an analysis of the genotypes, not the number of alleles above ST.

- CPI is not a substitute for interpretation with complex mixtures.

Eric Buel’s thoughts on this example (shared with John Butler)

“We should embrace this information and find a way to make the changes necessary to try to prevent this in the future. We must understand it is not about "us" but about **how to make sure we as a community provide the best possible services to those we serve - both victims and suspects.”**

Thank You!

John Butler
Robin Cotton
Charlotte Word

Margaret Kline
Becky Hill
Dave Duewer

Jill Spriggs

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