Topics and Techniques for Forensic DNA Analysis
Continuing Education Seminar

Mixture Interpretation

NYC OCME
Dept of Forensic Biology

New York City, NY
April 18, 2012

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National Institute of Standards and Technology

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SWGDAM Website and Resources Available

http://www.swgdam.org/resources.html

Link to http://www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm

Mixture Training Materials

Reviewed by SWGDAM Mixture Committee

http://www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm

Recent Training Workshops

- AAFS (February 22, 2011)
  - **Mixture Interpretation (with 6 other speakers)**
- ISFG (August 30, 2011)
  - **CE Fundamentals and Troubleshooting**
- Int. Symp. Human Ident. (October 3, 2011)
  - **Mixture Interpretation (with Boston University)**
- Int. Symp. Human Ident. (October 6, 2011)
  - **Troubleshooting Laboratory Systems**

Slide handouts available at
http://www.cstl.nist.gov/strbase/training.htm

Mixture Workshop (Promega ISHI 2010)

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

Regional workshops presented in FL, TX, MI, and AZ (April – June 2011)
Updated mixture workshop presented at ISHI 2011 (October 3, 2011)

Handout >200 pages
Literature list of >100 articles

13 Modules Pres
Introductio
SWG (John)
oids (Catherine)
astic effects (Robin)
eak height ratios (Charlotte)
Number of contributors (John)
Mixture ratios (John)
Mixture principles (Charlotte)
Statistics (Mike)

Case Example 1 (Robin)
Case Example 2 (Charlotte)
Case Example 3 (John)

Catherine Grgicak	Mike Coble	Robin Cotton	John Butler	Charlotte Word
Boston U.	NIST	Boston U.	NIST	Consultant

NIJ Grant to Boston University
funded ~150 state & local lab analysts to attend

Promega ISHI 2012 Mixture Workshop

- **John Butler**, Ph.D., NIST, Gaithersburg, MD
- **Michael Coble**, Ph.D., NIST, Gaithersburg, MD
- **Robin Cotton**, Ph.D., Boston University, Boston, MA
- **Catherine Grgicak**, Ph.D., Boston University, Boston, MA
- **Charlotte J. Word**, Ph.D., Gaithersburg, MD

This workshop is for analysts, technical reviewers and technical leaders performing and interpreting validation studies and/or interpreting and reviewing STR data, particularly more difficult mixtures. Various DNA profiles will be analyzed and interpreted using selected analytical thresholds and stochastic thresholds to demonstrate the impact of those values on the profiles amplified with low-template DNA vs. higher amounts of DNA. Different statistical approaches and conclusions suitable for the profiles will be presented.

Useful Articles on DNA Mixture Interpretation

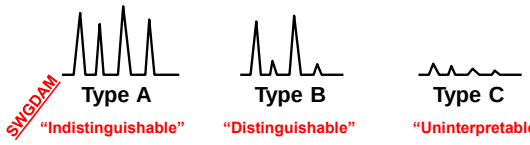
- Buckleton, J.S. and Curran, J.M. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.
- Budowle, B., *et al.* (2009) Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. *J. Forensic Sci.* 54: 810-821.
- Clayton, T.M., *et al.* (1998) Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Sci. Int.* 91: 55-70.
- Gill, P., *et al.* (2006) DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101.
- Gill, P., *et al.* (2008) National recommendations of the technical UK DNA working group on mixture interpretation for the NDNAD and for court going purposes. *FSI Genetics* 2(1): 76–82.
- Schneider, P.M., *et al.* (2009) The German Stain Commission: recommendations for the interpretation of mixed stains. *Int. J. Legal Med.* 123: 1-5.

German Mixture Classification Scheme

Schneider *et al*. (2009) *Int. J. Legal Med*. 123: 1-5

(German Stain Commission, 2006):

- **Type A**: no obvious major contributor, no evidence of stochastic effects
- **Type B**: clearly distinguishable major and minor contributors; consistent peak height ratios of ***approximately 4:1*** (major to minor component) for all heterozygous systems, no stochastic effects
- **Type C**: mixtures without major contributor(s), evidence for stochastic effects



Available for download from the ISFG Website:
http://www.isfg.org/Publication;Gill2006


DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

Our discussions have highlighted a significant need for continuing education and research into this area.

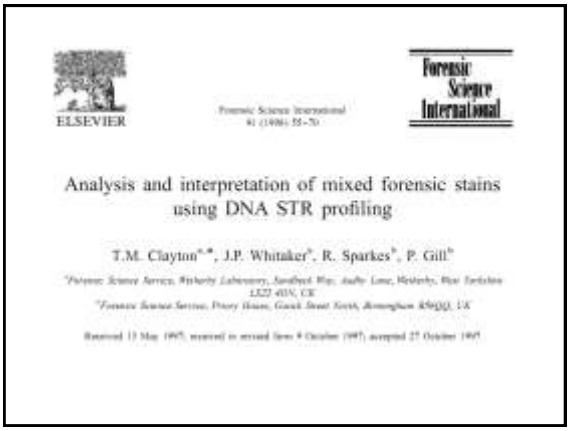
Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

ISFG Recommendations on Mixture Interpretation

http://www.isfg.org/Publication;Gill2006

1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE
2. Scientists should be trained in and use LRs
3. Methods to calculate LRs of mixtures are cited
4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
5. Prosecution determines H_p and defense determines H_d and multiple propositions may be evaluated
6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable
7. Allele dropout to explain evidence can only be used with low signal data
8. No statistical interpretation should be performed on alleles below threshold
9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101


Analysis and interpretation of mixed forensic stains using DNA STR profiling

T.M. Clayton, J.P. Whitaker, R. Sparkes, P. Gill

Steps in the interpretation of mixtures

(Clayton *et al.* *Forensic Sci. Int.* 1998; 91:55-70)

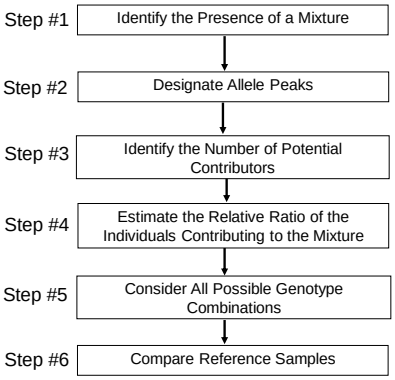
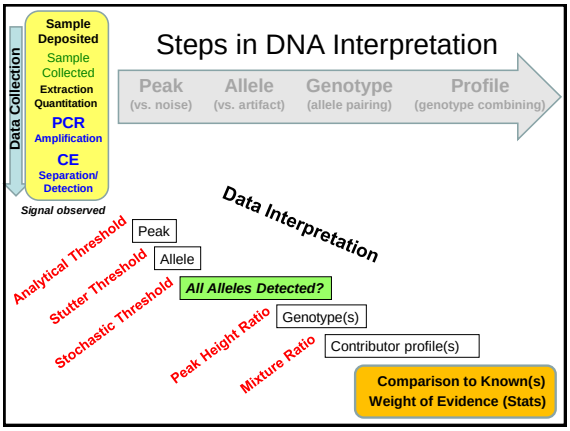
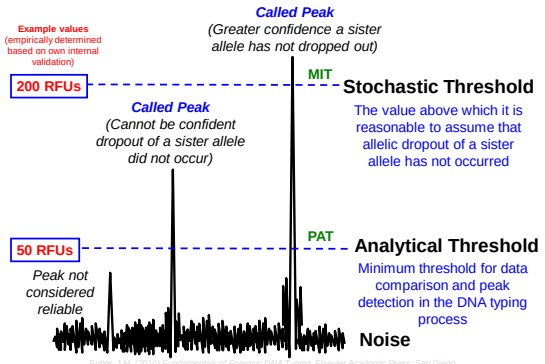


Figure 7.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

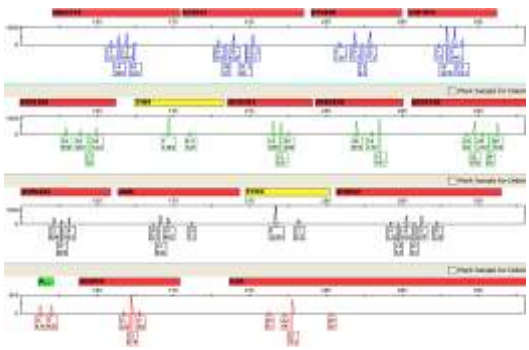
Steps in DNA Interpretation



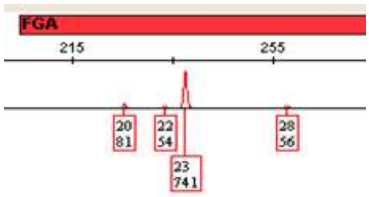
Overview of Two Thresholds



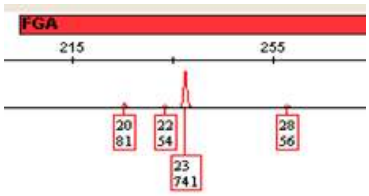
Profile 1 (stutter filter off)



Analytical Threshold (Peaks vs. Noise)

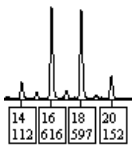


Stutter Threshold (Alelles vs. Artifacts)



Assumptions based upon # of contributors

Determination of Genotypes (PHR)



D18S51

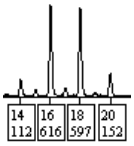
Possible Combinations

14, 16 (18%) and 18, 20 (25%)

14, 18 (19%) and 16, 20 (25%)

14, 20 (74%) and 16, 18 (97%)

Determination of Mixture Ratio



Major: 16,18
Minor: 14,20

D18S51

Total of all peak heights
= 112 + 616 + 597 + 152
= 1477 RFUs

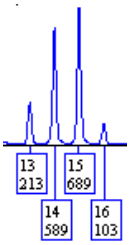
Minor component:
("14"+"20")/total = (112+152)/1477 = **0.179**

Major component:
("16"+"18")/ total = (616+597)/1477 = **0.821**

≈ 4.6 : 1

Four Peaks (4 allele loci)
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

Determination of Genotypes (PHR)



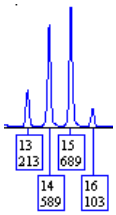
D8S1179

Possible Combinations

13, 14 (36%)	and	15, 16 (15%)
13, 15 (31%)	and	14, 16 (17%)
13, 16 (48%)	and	14, 15 (85%)

Includes "stutter" from the 14 allele

Determination of Mixture Ratio



Major: 14,15
Minor: 13,16

D8S1179

Total of all peak heights
= 213 + 589 + 689 + 103
= 1594 RFUs

Minor component:
("13"+"16")/total = (213+103)/1594 = **0.198**

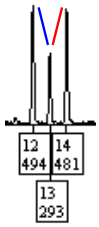
Major component:
("14"+"15")/ total = (589+689)/1594 = **0.802**

≈ 4 : 1

Four Peaks (4 allele loci)
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

Application of the Mixture Ratio

59% 61%



D19S433

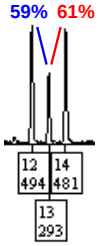
Using peak height ratio, all genotypes possible:

12,12	**12,13**
13,13	**12,14**
14,14	**13,14**

Is there a major:minor here?

Application of the Mixture Ratio

59% 61%



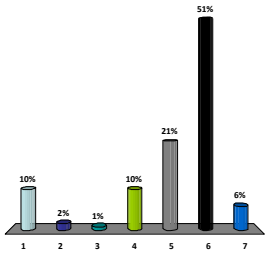
All possible genotype combinations:

12,12 + 13,14	**1:1.6**
13,13 + 12,14	**1:3.3**
14,14 + 12,13	**1:1.6**
12,13 + 12,14	**1:1.4**
~~**12,13 + 13,14**~~	~~**1:1**~~
12,14 + 13,14	**1:1.4**

Using MIXTURE RATIO calculations, can eliminate genotype pairs

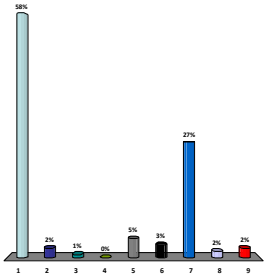
Does your lab use any software to help calculate mixture parameters (PHR, Mx, etc…)?

1. GMID-X
2. FSS I^3
3. GeneMarker HID
4. True Allele
5. In-house Excel program
6. On a calculator (painfully)
7. Other



Does your lab use any software to help calculate mixture stats?

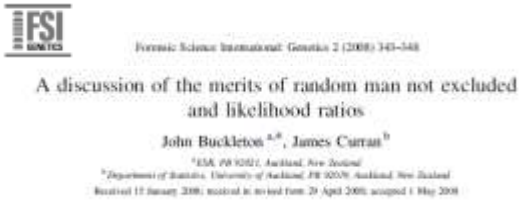
1. PopStats
2. GMID-X
3. FSS I^3
4. GeneMarker HID
5. True Allele
6. DNA·View
7. In-house Excel program
8. On a calculator (painfully)
9. Other



Statistical Approaches with Mixtures

See Ladd *et al.* (2001) *Croat Med J.* 42:244-246

"Exclusionary" Approach	"Inferred Genotype" Approach
Random Man Not Excluded (RMNE)	Random Match Probability (RMP)
Combined Prob. of Inclusion (CPI)	Likelihood Ratio (LR)
Combined Prob. of Exclusion (CPE)	



Forensic Science International: Genetics 2 (2008) 340–348

A discussion of the merits of random man not excluded and likelihood ratios

John Buckleton [a,*], James Curran [b]

[a] ESR, PB 92021, Auckland, New Zealand
[b] Department of Statistics, University of Auckland, PB 92019, Auckland, New Zealand

Received 15 January 2008; received in revised form 29 April 2008; accepted 1 May 2008

We conclude that the two matters that appear to have real force are:
(1) LRs are more difficult to present in court and
(2) the RMNE statistic wastes information that should be utilised.

If CPI/CPE Stats are Used

Since exclusionary statistics cannot adjust for the possibility of dropout, and does not take the number of contributors into account, any loci where alleles are below stochastic levels cannot be used in the CPI statistic.

If CPI/CPE Stats are Used

If CPI/CPE Stats are Used

Can use	Cannot use	
D21	D8	D2
CSF	D7	vWA
D3	TH01	D18
D19	D13	D5
TPOX	D16	FGA

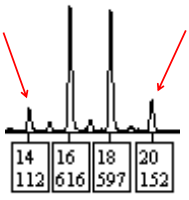
If CPI/CPE Stats are Used

- CPI statistics using FBI Caucasian Frequencies
- 1 in 71 Caucasians included
- 98.59% Caucasians excluded

If RMP/LR Stats are Used

- Since there is an assumption to the number of contributors, it is possible to use data that falls below the ST.

RMP - D18S51



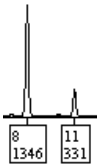
If Assume 2 Contributors….

Major	Minor
16,18	14,20

RMP_{minor} = 2pq
= 2 x f(14) x f(20)
= 2 x (0.1735) x (0.0255)
= 0.00884 or 1 in 113

(LR = 113)

RMP - TPOX



If Assume 2 Contributors….

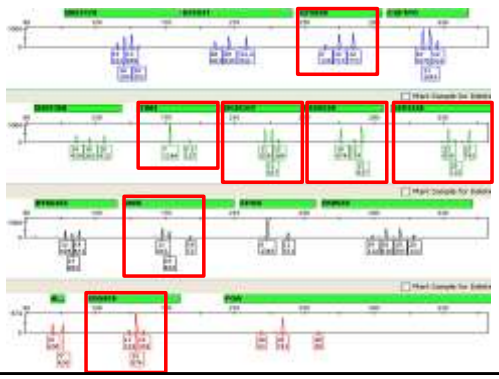
Major	Minor
8,8	11,8 *OR* 11,11

RMP = 8,11 + 11,11
RMP = 2pq + $(q^2 + q(1-q)\theta)$

RMP = 2(0.5443)(0.2537) +
$(0.2537)^2$ + (0.2537)(0.7463)(0.01)
= 0.3424 or 1 in 2.9

RMP/LR

Profile 1: ID_2_SCD_NG0.5_R4,1_A1_V1.2

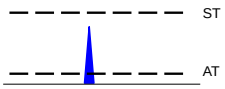


If RMP/LR Stats are Used

Can use	Loci with potential D-out	
D8	D7	D2
D21	TH01	vWA
D18	D13	D5
D3	D16	
D19		
TPOX		
FGA		
CSF		

The "2p" Rule

- The "2p" rule can be used to statistically account for zygosity ambiguity – i.e. is this single peak below the stochastic threshold the result of a homozygous genotype or the result of a heterozygous genotype with allele drop-out of the sister allele?



Resolved Question

To pee or not to pee? That is the question...?

"Drink sir, is a great provoker of three things…. nose painting, sleep and urine."

Macbeth: Act 2, Scene 3

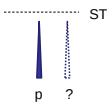
http://answers.yahoo.com/question/index?qid=20100419211523AA8pQEJ

2p – SWGDAM Guidelines

- 5.2.1.3.1. The formula 2p, as described in recommendation 4.1 of NRCII, may be applied to this result.
- 5.2.1.3.2. Instead of using 2p, the algebraically identical formulae **$2p - p^2$** and **$p^2 + 2p(1-p)$** may be used to address this situation without double-counting the proportion of homozygotes in the population.

$2p - p^2$ and $p^2 + 2p(1-p)$

Suppose 5 allele system – P, Q, R, S & T



The possible genotype could be anything…

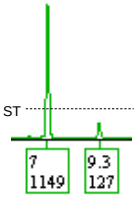
$= PP + PQ + PR + PS + PT$

$= p^2 + 2pq + 2pr + 2ps + 2pt$

$= p^2 + 2p\,(q + r + s + t) \longrightarrow = (1-p)$

$= \mathbf{p^2 + 2p\,(1-p)}$ $= p^2 + 2p - 2p^2 =$ **$2p - p^2$**

Profile 1 - TH01



Major – 7, 7

Possible Minor Contributors

7, 9.3 (2pq)

9.3, 9.3 p^2

9.3, ? 2p (or $p^2 + 2p(1 - p)$)

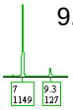
Profile 1 - TH01 (LR)

$$\frac{P(E|H_1)}{P(E|H_2)} = \frac{V \,\&\, S}{V \,\&\, U} = \frac{f_7^2 + f_7(1-f_7)\,\cancel{\theta}\;\&\;1}{\cancel{f_7^2 + f_7(1-f_7)\,\theta}\;\&} \qquad p^2 + 2p(1-p)$$

V = 7, 7

U = 7, 9.3
9.3, 9.3
9.3, ?

$$= \frac{1}{f_{9.3}^2 + 2f_{9.3}(1-f_{9.3})}$$



$f_{9.3} = 0.3054$

$= 1 / 0.5175 =$ **1.93**

Profile 1 - TH01 (LR)

$$\frac{P(E|H_1)}{P(E|H_2)} = \frac{V \& S}{V \& U} = \frac{1}{p^2 + p(1-p)\theta + 2pq}$$

V = 7, 7
U = 7, 9.3
9.3, 9.3

$$= \frac{1}{f_{9.3}^2 + f_{9.3}(1-f_{9.3})\theta + 2f_{9.3}f_7}$$

Let ST = 125 RFU

$f_{9.3}$ = 0.3054
f_7 = 0.1724

= 1 / 0.2007 = 4.98

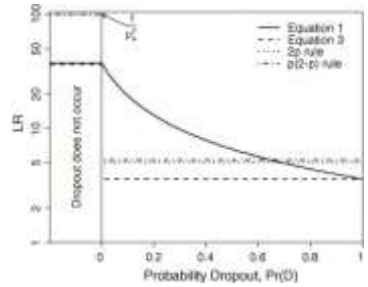
The "2p" Rule

- "This rule arose during the VNTR era. At that time many smaller alleles "ran off the end of the gel" and were not visualised."

- Buckleton and Triggs (2006)

"Is the 2*p* rule always conservative?"

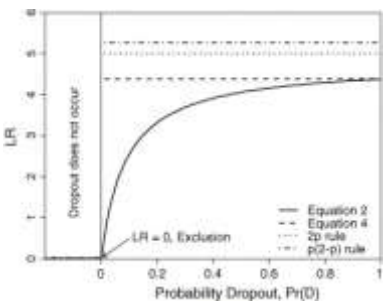
The "2p" Rule



Stain = aa

Suspect = aa

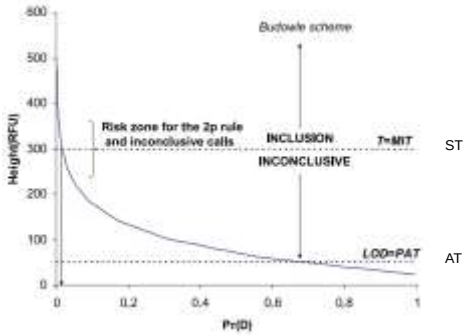
The "2p" Rule



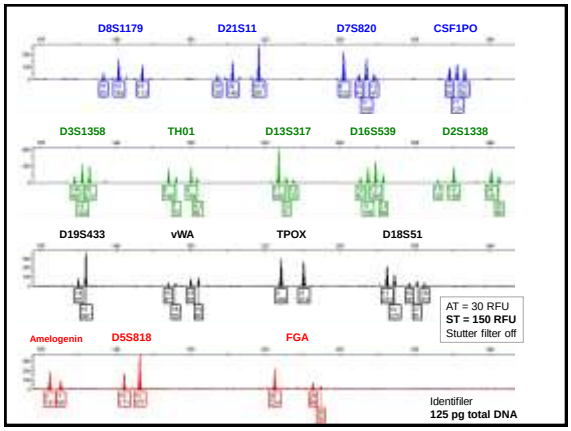
Stain = aa

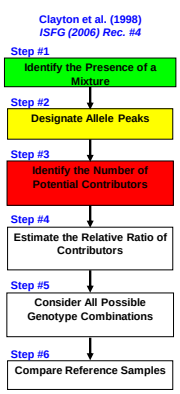
Suspect = ab

Gill and Buckleton (2010)



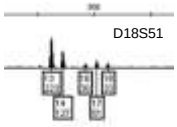
Challenges with low level, complex mixtures

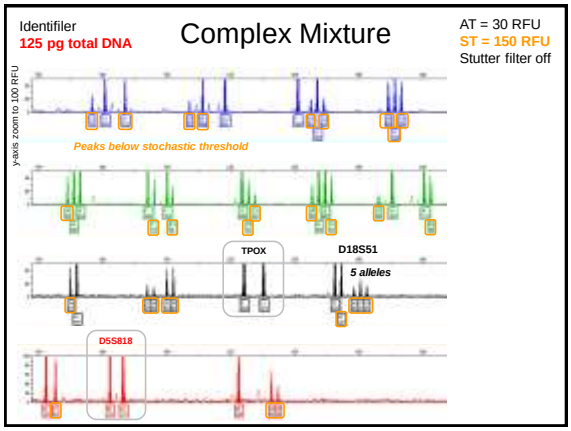




Impact of Results with Low Level DNA

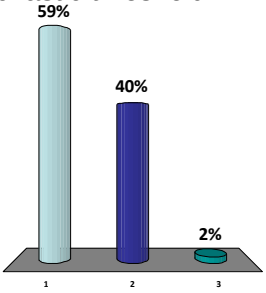
When amplifying low amounts of DNA (e.g., 125 pg), allele dropout is a likely possibility leading to **higher uncertainty** in the potential number of contributors and in the possible genotype combinations





Would you do a CPE/CPI statistic on TPOX and D5S818 because all alleles are above the stochastic threshold?

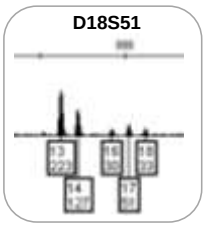
1. Yes
2. No
3. I don't work in a lab



What Can We Say about this Result?

- Low level DNA (only amplified 125 pg total DNA)
  - likely to exhibit stochastic effects and have allele dropout
- Mixture of at least 3 contributors
  - Based on detection of 5 alleles at D18S51
  - If at equal amounts, ~40 pg of each contributor (if not equal, then less for the minor contributors); **we expect allele dropout**
- At least one of the contributors is male
  - Based on presence of Y allele at amelogenin
- Statistics if using CPI/CPE
  - Would appear that we can only use TPOX and D5S818 results with a stochastic threshold of 150 RFU *(will explore this further)*
- **Due to potential of excessive allele dropout, we are unable to perform any meaningful Q-K comparisons**

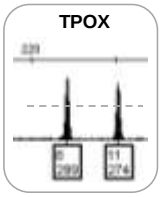
Uncertainty in the Potential Number of Contributors with this Result



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?**



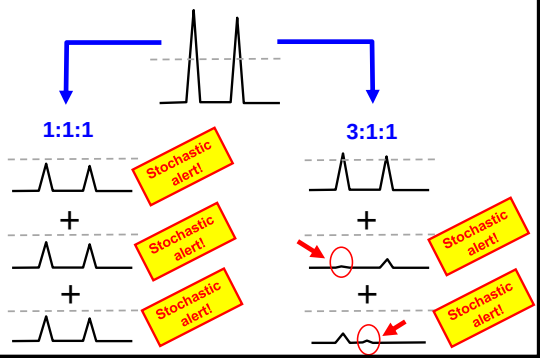
Stochastic threshold = 150 RFU

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!

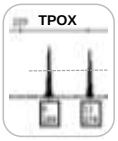
Assuming Three Contributors…
Some Possible Contributions to This Result



All Loci Are Not Created Equal
when it comes to mixture interpretation

- In the case of less polymorphic loci, such as TPOX, there are fewer alleles and these occur at higher frequency. Thus, there is a greater chance of allele sharing (peak height stacking) in mixtures.
- **Higher locus heterozygosity is advantageous for mixture interpretation** – we would expect to see more alleles (within and between contributors) and thus have a better chance of estimating the true number of contributors to the mixture

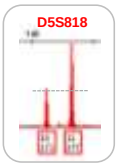
Even if you did attempt to calculate a CPI/CPE statistic using loci with all observed alleles above the stochastic threshold on this result…



TPOX Allele Frequencies (NIST Caucasian, Butler et al. 2003)
8 = 0.53
11 = 0.24
CPI = $(0.53 + 0.24)^2$ = 0.59 or **59%**

Combine loci = 0.59 x 0.18 = 0.11 or **11%**

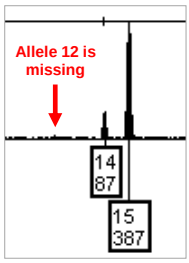
Approximately 1 in every 9 Caucasians could be included in this mixture



D5S818 Allele Frequencies (NIST Caucasian, Butler et al. 2003)
10 = 0.05
12 = 0.38
CPI = $(0.05 + 0.38)^2$ = 0.18 or **18%**

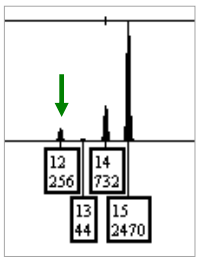
Impact of Amplifying More DNA

D19S433



125 pg total DNA amplified

D19S433



500 pg total DNA amplified

True Contributors
3 contributors with a 2:1:1 mixture

15,15 (2x)
14,15 (1x)
12,14 (1x)

How should you handle the suspect comparison(s) with this case result?

- **No suspect comparisons should be made** as **the mixture result has too much uncertainty** with stochastic effects that may not account for all alleles being detected
- **Declare the result "inconclusive"**

How not to handle this result

- "To heck with the analytical and stochastic thresholds", **I am just going to see if the suspect profile(s) can fit into the mixture allele pattern observed** – and then if an allele is not present in the evidentiary sample try to explain it with possible allele dropout due to stochastic effects
- This is what Bill Thompson calls "painting the target around the arrow (matching profile)…"

Thompson, W.C. (2009) Painting the target around the matching profile: the Texas sharpshooter fallacy in forensic DNA interpretation. *Law, Probability and Risk* 8: 257-276

What to do with low level DNA mixtures?

- **German Stain Commission "Category C"** (Schneider et al. 2006, 2009)
  - Cannot perform stats because stochastic effects make it uncertain that all alleles are accounted for
- **ISFG Recommendations #8 & #9** (Gill et al. 2006)
  - Stochastic effects limit usefulness
- *Fundamentals of Forensic DNA Typing* (2010) Butler 3rd edition (volume 1), chapter 18
  - Don't go "outside the box" without supporting validation

ISFG Recommendations on Mixture Interpretation

http://www.isfg.org/Publication;Gill2006

1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE
2. Scientists should be trained in and use LRs
3. Methods to calculate LRs of mixtures are cited
4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
5. Prosecution determines H_p and defense determines H_d and multiple propositions may be evaluated
6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable
7. Allele dropout to explain evidence can only be used with low signal data
8. No statistical interpretation should be performed on alleles below threshold
9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

A Complexity/Uncertainty Threshold

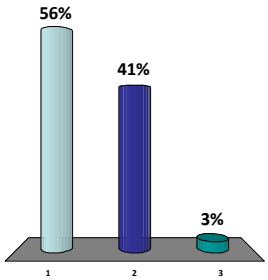
New Scientist article (August 2010)

- **How DNA evidence creates victims of chance**
  - 18 August 2010 by Linda Geddes
- From the last paragraph:
  - **In really complex cases, analysts need to be able to draw a line** and say "This is just too complex, I can't make the call on it," says Butler. "Part of the challenge now, is that every lab has that line set at a different place. But the honest thing to do as a scientist is to say: **I'm not going to try to get something that won't be reliable**."

http://www.newscientist.com/article/mg20727743.300-how-dna-evidence-creates-victims-of-chance.html

Has your laboratory implemented a "stop testing" approach with complex and/or low-level mixture?

1. Yes
2. No
3. I don't work in a lab



Is there a way forward?

"On the Threshold of a Dilemma"

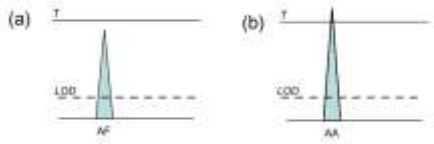
- Gill and Buckleton (2010)
- Although most labs use thresholds of some description, this philosophy has always been problematic because there is an inherent illogicality which we call the falling off the cliff effect.


FORENSIC SCIENCES

Commentary on: Buckwle B, Onorato AJ, Callaghan TF, Della Manna A, Gross AM, Guerrieri RA, Luttman JC, McClare DL. Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. J Forensic Sci 2009;54(4):810–21.

"Falling off the Cliff Effect"

- If T = an arbitrary level (e.g., 150 rfu), an allele of 149 rfu is subject to a different set of guidelines compared with one that is 150 rfu even though they differ by just 1 rfu (Fig. 1).

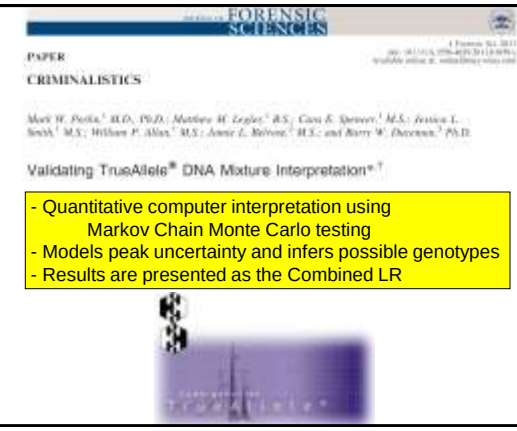


Gill and Buckleton *JFS* 55: 265-268 (2010)

Falling off the Cliff vs. Gradual Decline

Gill and Buckleton *JFS* **55:** 265-268 (2010)

- "The purpose of the ISFG DNA commission document was to provide a way forward to demonstrate the use of ***probabilistic models to circumvent the requirement for a threshold*** and to safeguard the legitimate interests of defendants."


FORENSIC SCIENCES

PAPER

CRIMINALISTICS

Validating TrueAllele® DNA Mixture Interpretation*,†

- Quantitative computer interpretation using Markov Chain Monte Carlo testing
- Models peak uncertainty and infers possible genotypes
- Results are presented as the Combined LR

True Allele Software (Cybergenetics)

- We purchased the software in September 2010.
- Three day training at Cybergenetics (Pittsburgh, PA) in October.
- Software runs on a Linux Server with a Mac interface.

True Allele Casework Workflow 5 Modules

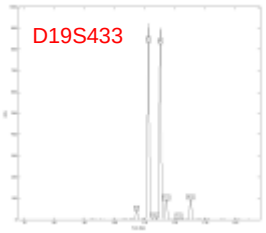
Analyze

.fsa files imported
Size Standard check
Allelic Ladder check
Alleles are called

True Allele Casework Workflow 5 Modules

Analyze → Data

Server



All Peaks above 10 RFU are considered

True Allele Casework Workflow 5 Modules

Analyze → Data → Request

Server

Computation

State Assumptions
2, 3, 4 unknowns
1 Unk with Victim?
Set Parameters
MCMC modeling
(e.g.50K)
Degradation?

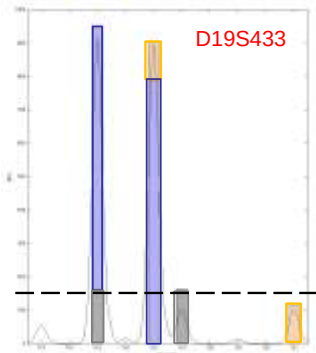
True Allele Casework Workflow 5 Modules

Analyze → Data → Request → Review

Server

Computation

Review of One Replicate (of 50K)

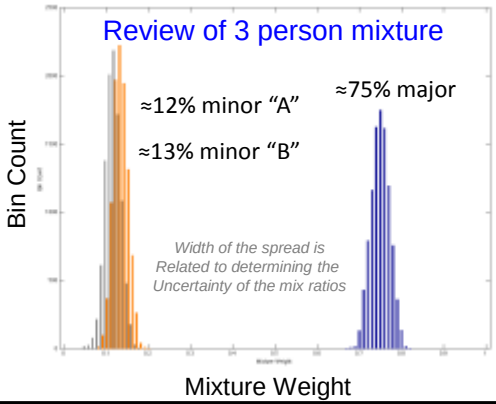


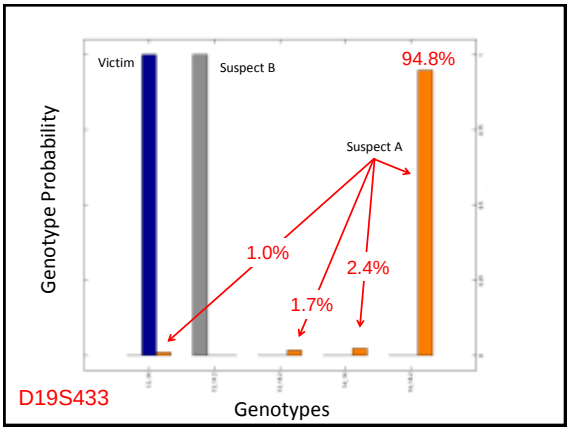
3P mixture,
2 Unknowns,

Conditioned on the Victim (major)

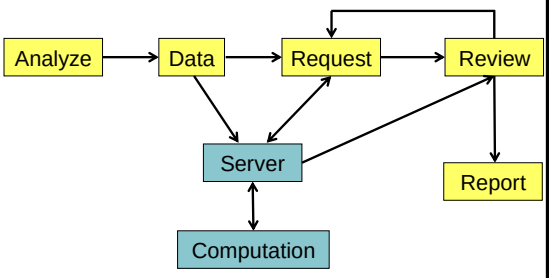
Good fit of the data to the model

Review of 3 person mixture





True Allele Casework Workflow 5 Modules



Determining the LR for D19S433

Suspect A = 14, 16.2 $H_P = 0.967$

Allele Pair	Probability Before Conditioning
14, 16.2	0.967
14, 14	0.003
13, 16.2	0.026
13, 14	0.001

$$LR = \frac{0.967}{}$$

Determining the LR for D19S433

Suspect A = 14, 16.2 $H_P = 0.967$

Allele Pair	Probability Before Conditioning	Genotype Frequency	Probability * Genotype Freq
14, 16.2	0.967	0.0120	0.01164
14, 14	0.003	0.0498	0.00013
13, 16.2	0.026	0.0131	0.00034
13, 14	0.001	0.1082	0.00009
		sum	0.0122

$$LR = \frac{0.967}{0.0122} = 79.26 \quad H_D$$

Combined LR = 5.6 Quintillion

			Genotype Probability Distribution			Weighted Likelihood	Likelihood Ratio		
	allele pair	Likelihood	Questioned	Reference	Suspect	Numerator	Denominator	LR	log(LR)
locus	x	l(x)	q(x)	r(x)	s(x)	l(x)*s(x)	l(x)*r(x)		
CSF1PO	11, 12	0.686	0.778	0.1448	1	0.68615	0.1292	5.31	0.725
D13S317	9, 12	1	1	0.0291	1	0.99952	0.02913	34.301	1.535
D16S539	9, 11	0.985	0.995	0.1238	1	0.98451	0.12188	8.036	0.905
D18S51	13, 17	0.999	1	0.0154	1	0.99915	0.01543	64.677	1.811
D19S433	14, 16.2	0.967	0.948	0.012	1	0.96715	0.01222	79.143	1.898
D21S11	28, 30	0.968	0.98	0.0872	1	0.96809	0.08648	11.194	1.049
D2S1338	23, 24	0.998	1	0.0179	1	0.99831	0.01787	55.866	1.747
D3S1358	15, 17	0.988	0.994	0.1224	1	0.98759	0.12084	8.14	0.911
D5S818	11, 11	0.451	0.394	0.0537	1	0.45103	0.07309	6.17	0.79
D7S820	11, 12	0.984	0.978	0.0356	1	0.98383	0.03617	27.198	1.435
D8S1179	13, 14	0.203	0.9	0.1293	1	0.20267	0.02993	6.771	0.831
FGA	21, 25	0.32	0.356	0.028	1	0.31986	0.01906	16.783	1.225
TH01	7, 7	0.887	0.985	0.1739	1	0.88661	0.15588	5.687	0.755
TPOX	8, 8	1	1	0.1375	1	1	0.13746	7.275	0.862
vWA	15, 20	0.998	0.996	0.0057	1	0.99808	0.00569	174.834	2.243

Results

- Results are expressed as logLR values

$$LR = 1{,}000{,}000 = 10^6$$

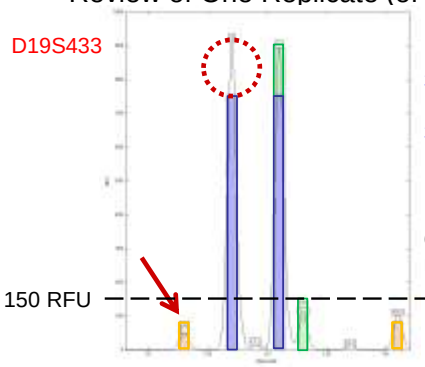
$$\log(LR) = \log 10^6$$

$$\log(LR) = 6 * \log 10\ (1)$$

$$\log(LR) = 6$$

Review of One Replicate (of 50K)

D19S433



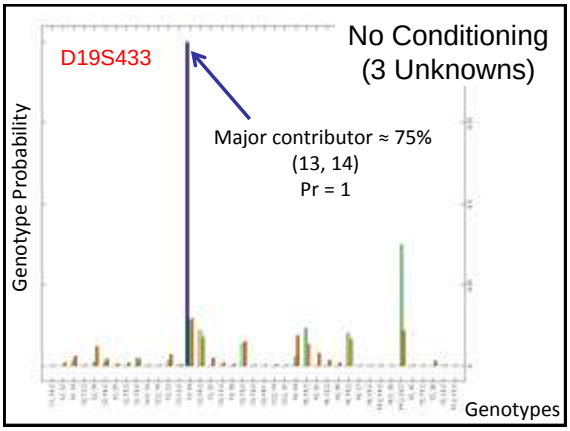
3P mixture,

3 Unknowns

Poor fit of the data to the model

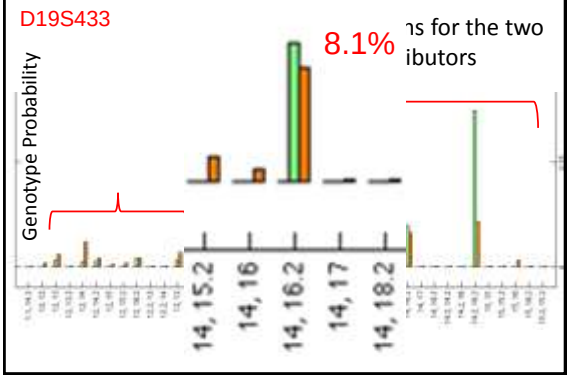
No Conditioning (3 Unknowns)

D19S433



No Conditioning (3 Unknowns)

D19S433



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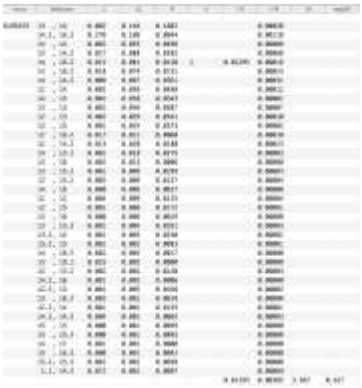


Suspect "A" Genotype

39 probable genotypes

D19S433

Suspect A = 14, 16.2 $H_P = 0.013$

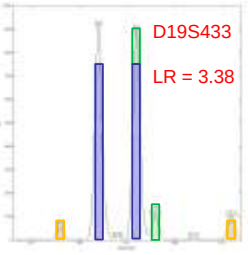
Allele Pair	Probability	Genotype Frequency	Prob * GenFreq
13,14	0.002	0.1082	0.00020
14.2, 16.2	0.270	0.0044	0.00118
14, 14	0.002	0.0498	0.00008
13, 14.2	0.017	0.0392	0.00068
14, 16.2	0.013	0.0120	0.00016
13, 16.2	0.018	0.0131	0.00023
etc…	etc…	etc…	etc…
		Sum	0.00385

$$LR = \frac{0.013}{0.00385} = 3.38$$ H_D

D19S433

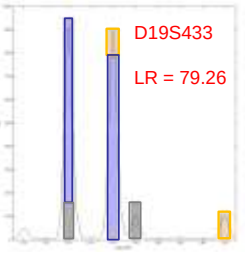
No Conditioning (3 Unknowns)

No Conditioning



Profile - Combined log(LR)
Suspect A log(LR) = 8.03
Suspect B log(LR) = 7.84

Conditioned on Victim



Profile - Combined log(LR)
Suspect A log(LR) = 18.72
Suspect B log(LR) = 19.45

Exploring the Capabilities

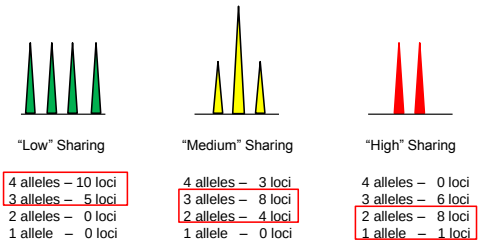
- **Degree of Allele Sharing**
- **Mixture Ratios**
- DNA Quantity

Mixture Data Set

- Mixtures of pristine male and female DNA amplified at a total concentration of 1.0 ng/μL using Identifiler (standard conditions).
- Mixture ratios ranged from 90:10, 80:20, 70:30 60:40, 50:50, 40:60, 30:70, 20:80, and 10:90
- Each sample was amplified twice.

Mixture Data Set

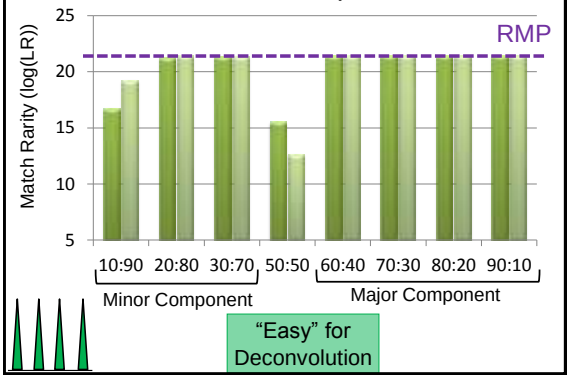
- Three different combinations:



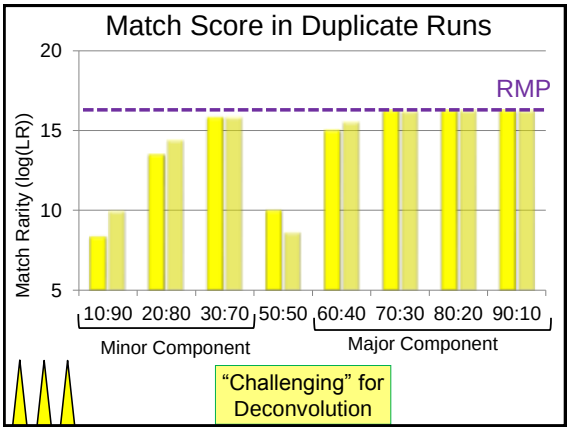
"Low" Sharing	"Medium" Sharing	"High" Sharing
4 alleles – 10 loci	4 alleles – 3 loci	4 alleles – 0 loci
3 alleles – 5 loci	3 alleles – 8 loci	3 alleles – 6 loci
2 alleles – 0 loci	2 alleles – 4 loci	2 alleles – 8 loci
1 allele – 0 loci	1 allele – 0 loci	1 allele – 1 loci

Virtual MixtureMaker - http://www.cstl.nist.gov/strbase/software.htm

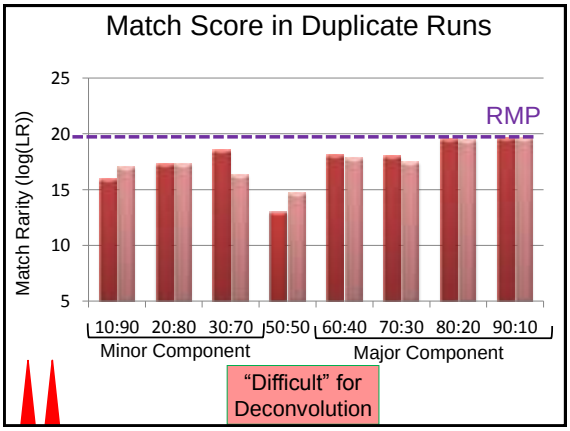
Match Score in Duplicate Runs

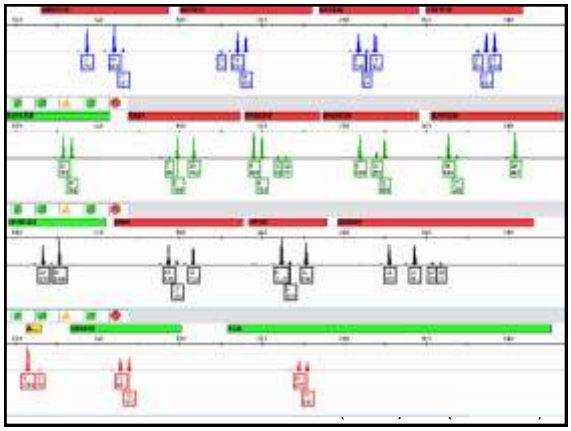


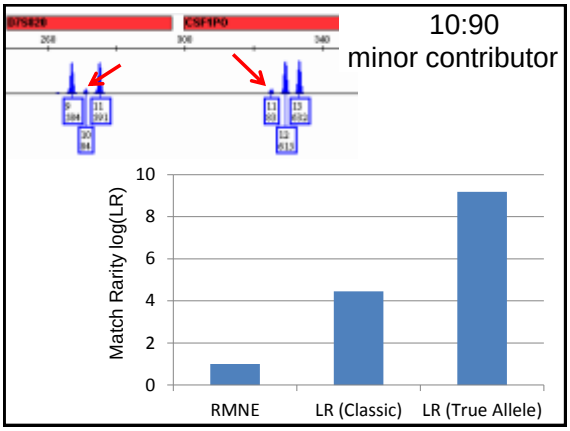
Match Score in Duplicate Runs



Match Score in Duplicate Runs

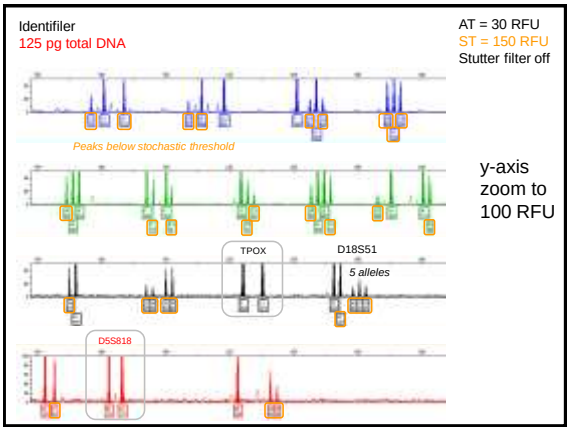


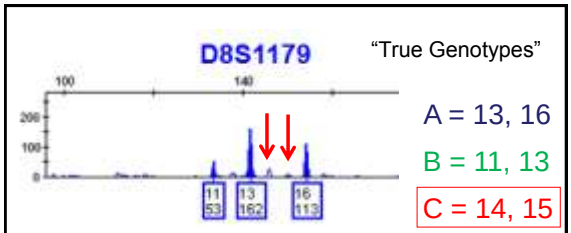




Exploring the Capabilities

- Degree of Allele Sharing
- Mixture Ratios
- **DNA Quantity**





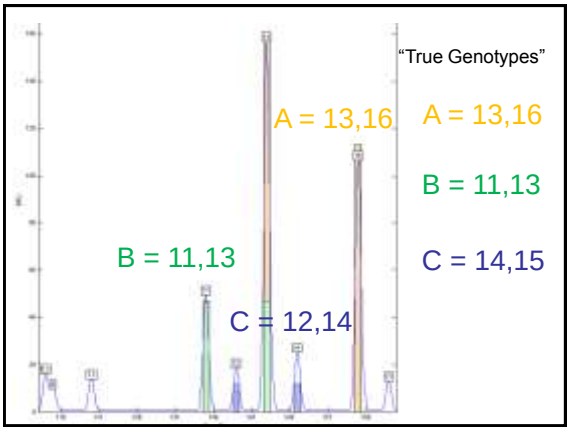
"True Genotypes"

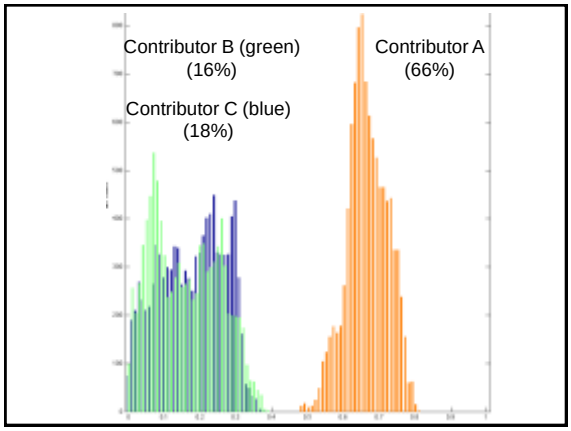
A = 13, 16

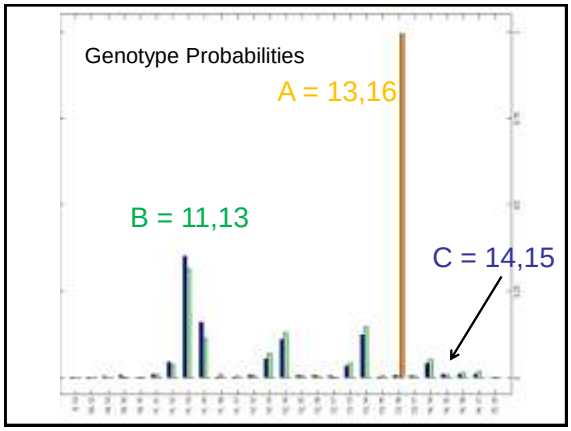
B = 11, 13

C = 14, 15

3 person Mixture – No Conditioning
Major Contributor ≈ 83 pg input DNA
2 Minor Contributors ≈ 21 pg input DNA







Results for Contributor A (male)

Locus	Allele Pair	Probability Likelihood	Genotype Frequency	Suspect	H_p Numerator	H_d Denominator	LR
CSF1PO	10, 11	0.572	0.1292			0.07395	
	11, 12	0.306	0.2133	1	0.30563	0.0652	
	10, 12	0.12	0.1547			0.01861	
					0.30563	0.15791	1.935
D13S317	11, 11	1	0.1149	1	1	0.11488	8.704
D8S1179	13, 16	0.998	0.0199	1	0.99786	0.0199	49.668

The match rarity between the evidence and suspect is 1.21 quintillion

Results for Contributor B (female)

Locus	Allele Pair	Probability Likelihood	Genotype Frequency	Suspect	H_p Numerator	H_d Denominator	LR
D8S1179	11, 13	0.073	0.0498	1	0.07338	0.00368	
	11, 14	0.034	0.0271			0.00092	
	13, 14	0.009	0.0996			0.00065	
	12, 14	0.011	0.0606			0.00068	
	12, 13	0.005	0.1115			0.0006	
	11, 12	0.018	0.0303			0.00054	
	14, 14	0.004	0.0271			0.00012	
	13, 13	0.003	0.0916			0.00031	
	14, 16	0.003	0.0108			0.00003	
	14, 15	0.001	0.0379			0.00003	

etc…

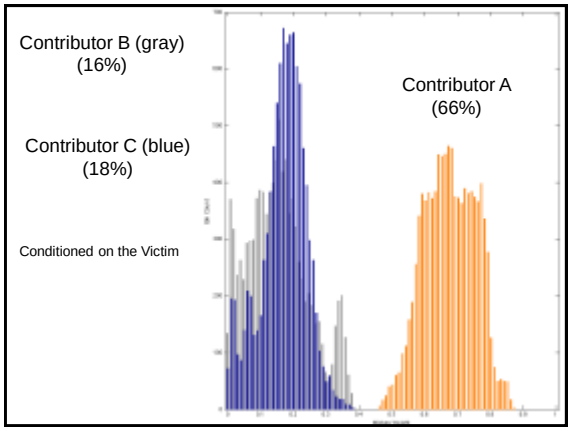
9.197

The match rarity between the evidence and suspect is 1.43 million

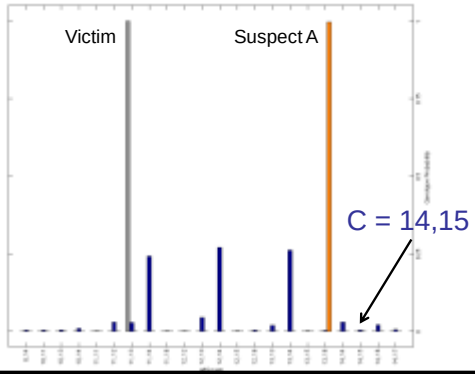
Results for Contributor C (male)

Locus	Allele Pair	Probability Likelihood	Genotype Frequency	Suspect	H_p Numerator	H_d Denominator	LR
D8S1179	11, 13	0.056	0.0498			0.00279	
	13, 14	0.007	0.0996			0.00066	
	12, 14	0.011	0.0606			0.00068	
	11, 14	0.021	0.0271			0.00056	
	12, 13	0.006	0.1115			0.00066	
	14, 14	0.005	0.0271			0.00013	
	etc…	etc…	etc…			etc…	
	14, 15	0.001	0.0379	1	0.00056	0.00002	
	12, 15	0.001	0.0424			0.00003	
	etc…	etc…	etc…			etc…	
	10, 15	0	0.0227			0.00001	
					0.00056	0.00665	**0.084**

The match rarity between the evidence and suspect is 9.16 thousand



The Power of Conditioning



The Power of Conditioning

	LR (no conditioning, 3unk)
Contributor A	1.21 Quintillion
Contributor B (victim)	1.43 Million
Contributor C	9.16 Thousand

	LR (conditioned on victim + 2unk)
Contributor A	1.32 Quintillion
Contributor B (victim)	2.19 Million
Contributor C	59.8 Thousand

Ranged from 1.13 to 800K

Summary

- True Allele utilizes probabilistic genotyping and makes better use of the data than the RMNE approach.
- However, the software is computer intensive. On our 4 processor system, it can take 12-16 hours to run up to four 3-person mixture samples.

Summary

- **Allele Sharing:** Stacking of alleles due to sharing creates more uncertainty.
- **Mixture Ratio:** With "distance" between the two contributors, there is greater certainty. Generally, True Allele performs better than RMNE and the classic LR with low level contributors.

Summary

- **DNA Quantity:** Generally, with high DNA signal, replicates runs on True Allele are very reproducible.
- However, with low DNA signal, higher levels of uncertainty are observed (as expected).
- There is a need to determine an appropriate threshold for an inclusion log(LR).

Thank You!

Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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Questions?

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