

California Association of Criminalists Fall Meeting 06 November 2012 – San Jose, California

Exploring the Capabilities of Mixture Interpretation using True Allele Software

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Which of the topics below would be your first choice for additional training?

- 1. Relevant literature
- 2. How to validate thresholds
- 3. How to develop relevant SOPs
- 4. Interpretation of low level mixtures
- 5. Statistics

2/3 want more information on these topics

From one of the regional mixture workshops (Apr – June 2011)



Stats Required for Inclusions

SWGDAM Interpretation Guideline 4.1:

"The laboratory must perform statistical analysis in support of any inclusion that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis."

Buckleton & Curran (2008): "There is a considerable aura to DNA evidence. Because of this aura it is vital that weak evidence is correctly represented as weak or not presented at all."

Buckleton, J. and Curran, J. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.

Statistics A Tragedy in 400 Quadrillion Acts



"Though this be madness, yet there is method in't."

— William Shakespeare, Hamlet

Statistical Approaches with Mixtures

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

"Exclusionary" Approach

Random Man Not Excluded (RMNE)

Combined Prob. of Inclusion (CPI)

Combined Prob. of Exclusion (CPE)

"Allele-centric"

"Inferred Genotype" Approach

Random Match Probability [modified] (mRMP)

> Likelihood Ratio (LR)

"Genotype-centric"



Forensic Science International: Genetics 2 (2008) 343-348

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

John Buckleton a,*, James Curran^b

^aESR, PB 92021, Auckland, New Zealand ^bDepartment 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.

Curran and Buckleton (2010)





J Forensic Sci, September 2010, Vol. 55, No. 5 doi: 10.1111/j.1556-4029.2010.01446.x Available online at: interscience.wiley.com

PAPER CRIMINALISTICS; GENERAL

James M. Curran,¹ M.Sc.(Hons.), Ph.D. and John Buckleton,² Ph.D.

Inclusion Probabilities and Dropout

Created 1000 Two-person Mixtures (Budowle et al. 1999 AfAm freq.).

Created 10,000 "third person" genotypes.

Compared "third person" to mixture data, calculated PI for included loci, ignored discordant alleles.

Curran and Buckleton (2010)



Review of Two Thresholds

Example values

(empirically determined

Called Peak

(Greater confidence a sister allele has not dropped out)



Butler, J.M. (2010) *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press: San Diego

2-person Mixture



2-Person Mixture



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

If CPI/CPE Stats are Used (ST = 200 RFU)







Shakespeare on Allelic Drop-Out



http://es.wikipedia.org/wiki/William_Shakespeare

"Hell is empty and all the devils are here."

— William Shakespeare, The Tempest





<u>Can use</u>	<u>Cannot use</u>		
D21	D8	D2	
CSF	D7	vWA	
D3	TH01	D18	
D19			
ΤΡΟΧ	D13	D5	
	D16	FGA	

Impact: discarding 2/3 of the data

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

If CPI/CPE Stats are Used (ST = 150 RFU)





The impact of changing thresholds

If mRMP/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.

mRMP - D18S51



(LR = 113)

If Assume 2 Contributors.... <u>Major</u> 16,18 <u>Minor</u> 14,20

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



Potential for Drop-out



If mRMP/LR Stats are Used

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

 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?



"2p" or not "2p"... That is the question.

Shakespeare on "2p"



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

— William Shakespeare, Macbeth

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 p² and p² + 2p(1-p) may be used to address this situation without double-counting the proportion of homozygotes in the population.

Macbeth/Duncan Profile - 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))		

Macbeth/Duncan Profile - TH01



Macbeth/Duncan Profile - TH01



Macbeth/Duncan Profile - TH01 $\frac{LR}{ST = 200 (2p \text{ is used}) \qquad 1.93}$ $ST = 125 (2pq \text{ is used}) \qquad 4.98$

2p is conservative...

 "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?"





 $f(a) = 0.10 \quad 1/2p = 5$

Three Questions

- According to William Shakespeare, what were the last words of Julius Caesar before he died?
- Et tu, Brute?
- What is the capital of Bangladesh?
- Dhaka

Three Questions

• How many people are in this mixture?



Mark Sample for Deleti



Mark Sample for Deleti






Do You Have Uncertainty in Your Data?

• If allele dropout is a possibility

(e.g., in a partial profile), then there is uncertainty in whether or not an allele is present in the sample...and therefore what genotype combinations Possible allele pairing with the 11

 If different allele combinations are possible in a mixture, then there is uncertainty in the genotype combinations that are possible...



Uncertainty and Probability

- "Contrary to what many people think, uncertainty is present throughout any scientific procedure."
 - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004)
 Statistics and the Evaluation of Evidence for Forensic
 Scientists, Second Edition
- "It is now recognized that the only tool for handling uncertainty is probability."
 - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004) Statistics and the Evaluation of Evidence for Forensic Scientists, Second Edition

Is there a way forward?

Next Issue of FSI-Genetics

Forensic Science International: Genetics xxx (2012) xxx-xxx



Editorial

Focus issue—Analysis and biostatistical interpretation of complex and low template DNA samples

Article in press...



DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

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Whatever way uncertainty is approached, probability is the *only* sound way to think about it.



-Dennis Lindley

Haned et al.

Forensic Science International: Genetics xxx (2012) xxx-xxx



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journal homepage: www.elsevier.com/locate/fsig



Exploratory data analysis for the interpretation of low template DNA mixtures

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Mitchell et al.

Forensic Science International: Genetics xxx (2012) xxx-xxx



Validation of a DNA mixture statistics tool incorporating allelic drop-out and drop-in

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PAPER

J Forensic Sci, 2011 doi: 10.1111/j.1556-4029.2011.01859.x Available online at: onlinelibrary.wiley.com

CRIMINALISTICS

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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



3 Person Mixture



Review of One Replicate (of 50K)



Alternative Explanations of the Data





Mixture Weight



Determining the LR for D19S433

Suspect A = 14, 16.2 $H_P = 0.967$

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



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



Combined LR = 5.6 Quintillion

			Genotype Probability Distribution			Weighted Likelihood		Likelihood Ratio	
	allele pair	Likelihood	Questioned	Reference	Suspect	Numerator	Denominator	LR	log(LR)
locus	Х	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
ТРОХ	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

Review of One Replicate (of 50K)







locus	allele pair	L	Q	R	S	L*S	L*R	LR	log(LR)
0105/33	13 14	0 002	0 146	0 1087			0 00020		
0193433	14 2 16 2	0.002	0.140	0.1002			0.00020		
	14.2, 10.2	0.270	0.105	0.0044			0.00110		
	14, 14	0.002	0.095	0.0490			0.00000		
	13, 14.2	0.017	0.000	0.0392	1	0 01205	0.00000		
	14 , 10.2	0.015	0.001	0.0120	1	0.01295	0.00010		
	13, 10.2	0.010	0.074	0.0151			0.00025		
	14 , 14.2	0.009	0.007	0.0408			0.00031		
	12 , 14	0.002	0.039	0.0498			0.00012		
	14, 15	0.001	0.034	0.0545			0.00002		
	13, 13	0.001	0.034	0.0587			0.00007		
	12 , 13	0.002	0.029	0.0541			0.00010		
	13, 15	0.001	0.024	0.0373			0.00002		
	12 , 16.2	0.017	0.021	0.0060			0.00010		
	12 , 14.2	0.013	0.020	0.0180			0.00023		
	14 , 15.2	0.001	0.018	0.0275			0.00003		
	15, 16	0.002	0.015	0.0006			0.00000		
	13 , 15.2	0.001	0.009	0.0299			0.00003		
	12 , 15.2	0.003	0.009	0.0137			0.00004		
	14 , 16	0.000	0.009	0.0017			0.00000		
	12 , 12	0.004	0.009	0.0125			0.00004		
	12 , 15	0.001	0.006	0.0172			0.00001		
	13 , 16	0.000	0.006	0.0019			0.00000		
	13 , 13.2	0.001	0.004	0.0261			0.00003		
	13.2, 14	0.001	0.003	0.0240			0.00002		
	13.2, 15	0.001	0.002	0.0083			0.00001		
	14 , 18.2	0.002	0.002	0.0017			0.00000		
	13 , 19.1	0.019	0.002	0.0000			0.00000		
	12 , 13.2	0.002	0.002	0.0120			0.00003		
	14.2, 16	0.001	0.002	0.0006			0.00000		
	12.2, 13	0.001	0.002	0.0168			0.00002		
	13 , 18.2	0.002	0.001	0.0019			0.00000		
	12.2, 14	0.001	0.001	0.0155			0.00001		
	14.2, 14.2	0.004	0.001	0.0065			0.00003		
	15 , 15	0.000	0.001	0.0059			0.00000		
	15 , 15.2	0.000	0.001	0.0095			0.00000		
	14 , 17	0.001	0.001	0.0000			0.00000		
	15 , 16.2	0.000	0.001	0.0042			0.00000		
	15.2, 15.2	0.001	0.001	0.0038			0.00000		
	1.1, 14.2	0.072	0.001	0.0097			0.00069		
						0.01295	0.00385	3.367	0.527

Suspect "A" Genotype

39 probable genotypes

D19S433

Susp	ext A = 1	4, 16.2	$H_{P} = 0$	0.013	8	
			Genotype		Prob *	
	Allele Pair	Probability	Frequency		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	
		0.01:	3	Sum	0.00385	H,
	LR =		- = 3.	38		1
		0.0038	35			122
No Conditioning (3 Unknowns)					0193	9433

No Conditioning

Conditioned on Victim



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

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



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

4 alleles – 10 loci 3 alleles – 5 loci 2 alleles – 0 loci 1 allele – 0 loci



"Medium" Sharing

"High" Sharing

4 alleles –	3 loci
3 alleles –	8 loci
2 alleles –	4 loci
1 allele –	0 loci

4 alleles –	0 loci
3 alleles –	6 loci
2 alleles –	8 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











Complex Mixture





Mark Sample for Deleti TPOX D19S433 υWA D18S51 2000 -16 18 20 353 336 451 12 14 392 1139 1384 2856 3408 1567 1672 1694 2932

🔄 🔄 Mark Sample for Deleti



True Allele Results – 3 person mixture





True Allele Results – 4 person mixture






Potential Suspects

- A, B, C and D are the four individuals in the mixture.
- John Butler is also a suspect (The Butler did it).
- "Omni man" is also a possible suspect.



"The Butler"







Omni Man



Strategies

- Conditioning will help...
- This may not be possible.
- Multiple replicates will be necessary.
- There is a need to determine an appropriate method for an inclusion log(LR).

Summary of the Issues

- We need to move away from the interpretation of mixtures from an "allele-centric" point of view.
- Methods to incorporate probability will be necessary as we make this transition and confront the issues of low-level profiles with drop-out.
- "Just as logic is reasoning applied to truth and falsity, probability is reasoning with uncertainty" -Dennis Lindley

Summary of the Issues

- The LR is the preferred method to evaluate lowlevel, complex mixture evidence with drop-out.
 ISFG recommendations are in press.
- This will require (obviously) software solutions... however, we need to better understand and be able to explain the statistics as a community.
- "But, for my own part, it was Greek to me"
 William Shakespeare, Julius Caesar
- "We know what we are, but know not what we may be." — William Shakespeare, Hamlet

Summary of the Issues

 Extensive training will be necessary – and a single 8 hour workshop will once a year will not suffice.

"Do, or do not. There is no try." — Yoda



Thank You

• "I can no other answer make but thanks, and thanks." - William Shakespeare, *Twelfth Night*



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http://es.wikipedia.org/wiki/William_Shakespeare