


## DNA Interpretation Workshop 2


# Probabilistic Genotyping

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<http://www.cstl.nist.gov/strbase/training.htm>



**ISFG Pre-Conference Workshop**  
Melbourne, Australia  
September 2-3, 2013



## NIST and NIJ Disclaimer


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

**Points of view are mine** and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

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**Our publications and presentations are made available at:**  
<http://www.cstl.nist.gov/strbase/NISTpub.htm>

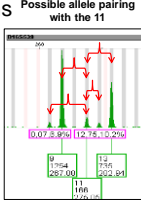
Whatever way uncertainty is approached, probability is the *only* sound way to think about it.



-Dennis Lindley

## 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 are possible
- **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*

## “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.

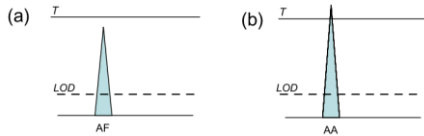
**JOURNAL OF FORENSIC SCIENCES**

Commentary on: Budowle B, Onorato AJ, Callaghan TF, Della Manna A, Gross AM, Guerrieri RA, Luttman JC, McClure 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.

*J Forensic Sci*, January 2010, Vol. 55, No. 1  
 doi: 10.1111/j.1556-4029.2009.01257.x  
 Available online at: [intrescience.wiley.com](http://intrescience.wiley.com)

### “Falling off the Cliff Effect”

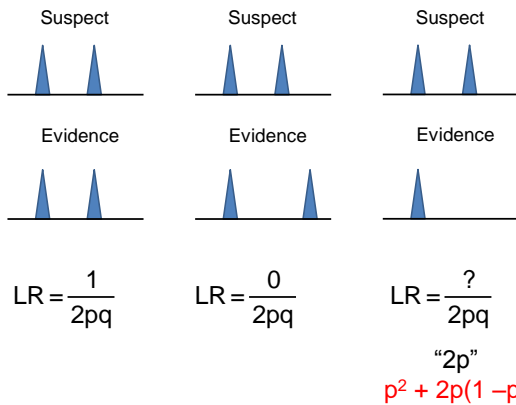
- If  $T$  = an arbitrary level (e.g., 150 rfu), an allele of 149 rfu is subject to a different set of guidelines compared with one that is 150 rfu even though they differ by just 1 rfu (Fig. 1).



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

### 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.”



### What should we do with discordant data?

- Continue to use RMNE (CPI, CPE) (not optimal)
- Use the Binary LR with  $2p$  (not optimal)
- Semi-continuous methods with a LR (Drop models)

### Some Drop Model Examples

- LR mix (Haned and Gill)
- Balding (likeLTD - R program)
- FST (NYOCME, Mitchell *et al.*)
- Kelly *et al.* (University of Auckland, ESR)
- Lab Retriever (Lohmueller, Rudin and Inman)

### Semi-continuous methods

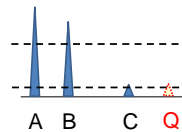
- Use a Pr(DO) and LR's
- Speed of analysis – “relatively fast”
- The methods do not make full use of data - only the alleles present.

### What should we do with discordant data?

- Continue to use RMNE (CPI, CPE) (*not optimal*)
- Use the Binary LR with 2p (*not optimal*)
- Semi-continuous methods with a LR (Drop models)
- Fully continuous methods with LR

### Continuous Models

- Mathematical modeling of “molecular biology” of the profile (mix ratio, PHR (Hb), stutter, etc...) to find optimal genotypes, giving **WEIGHT** to the results.



#### Probable Genotypes

- AC – 40%
- BC – 25%
- CC – 20%
- CQ – 15%

### Some Continuous Model Examples

- TrueAllele (Cybergenetics)
- STRmix (ESR [NZ] and Australian collaboration)
- Cowell et al. (FSI-G (2011) 5:202-209)

Weights are determined by performing simulations of the data (Markov Chain Monte Carlo - MCMC)

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

PAPER  
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 a Combined LR

### True Allele Software (Cybergenetics)

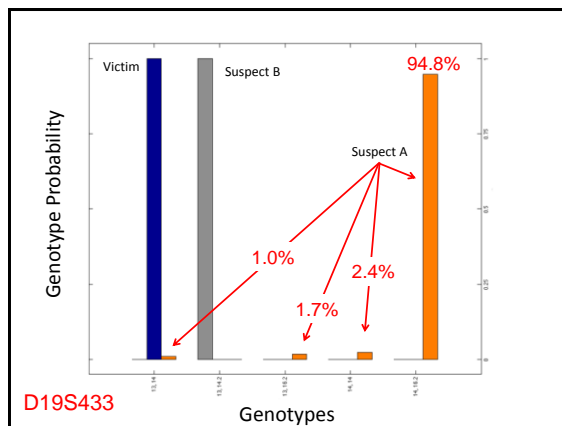
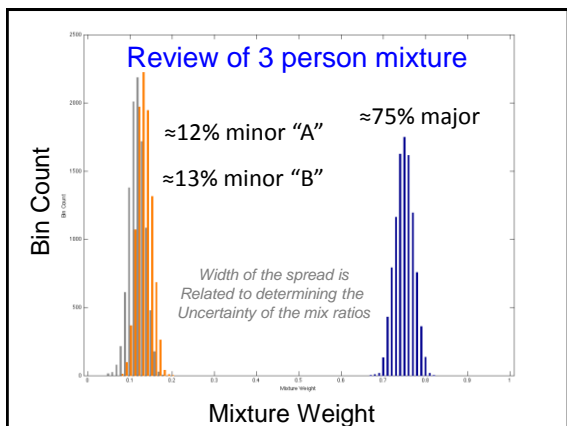
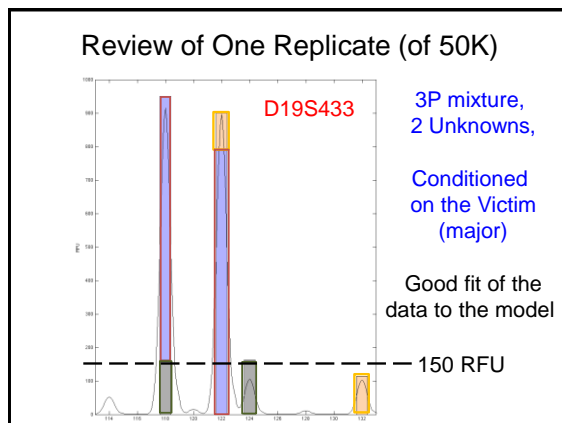
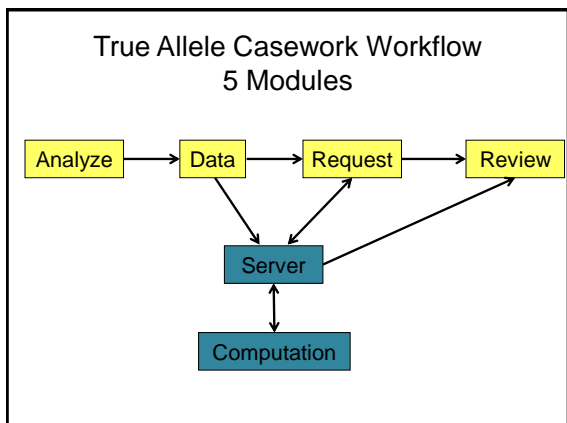
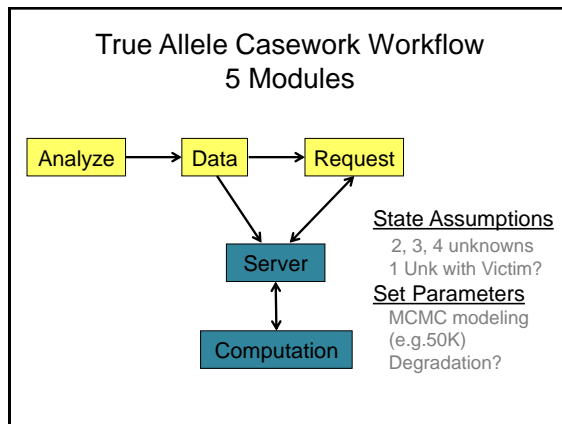
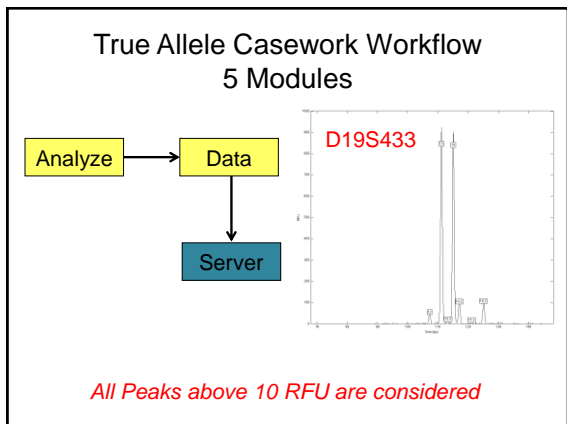
- Software runs on a Linux Server with a Mac interface.

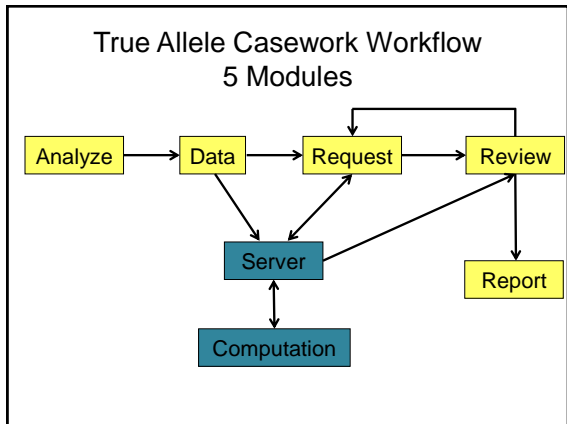


### True Allele Casework Workflow 5 Modules

Analyze

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





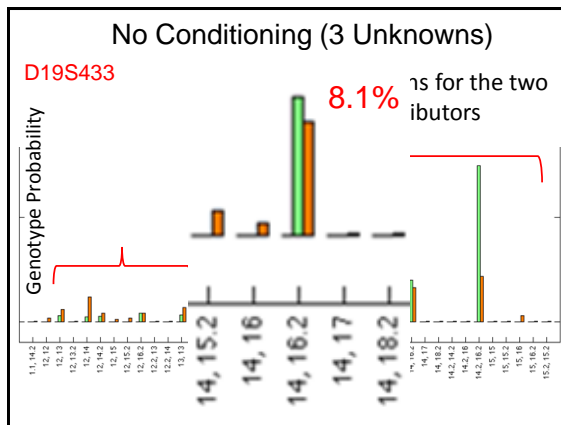
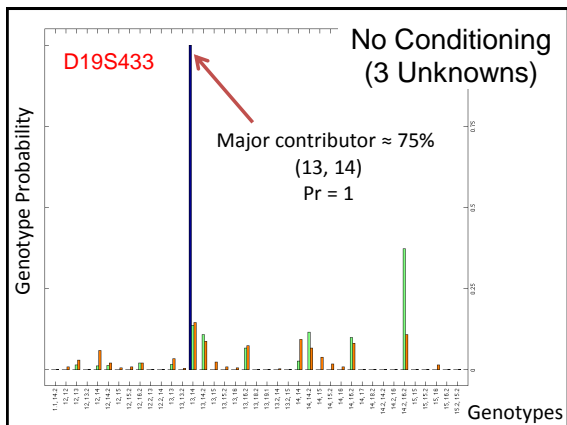
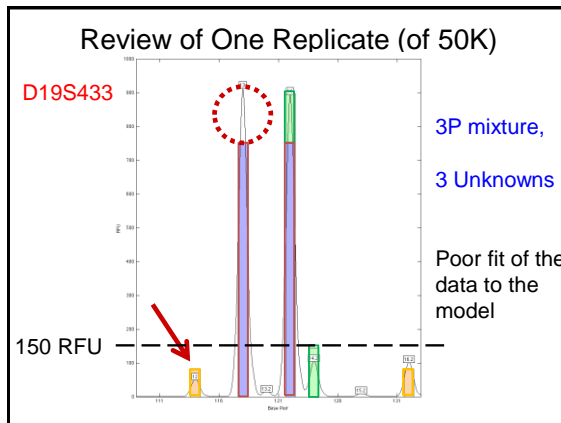
### Combined LR = 5.6 Quintillion

locus	allele pair x	Likelihood l(x)	Genotype Probability Distribution		Reference r(x)	Suspect s(x)	Weighted Likelihood		Likelihood Ratio LR	log(LR)
			Questioned q(x)	Reference			Numerator l(x)*s(x)	Denominator 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)  
 $\log(LR) = 6$

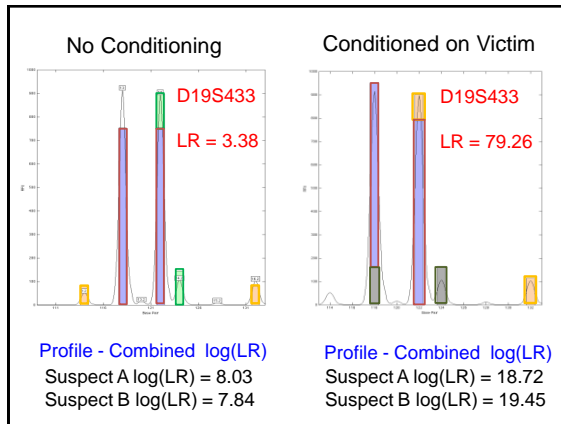


Source	allele pair	L	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	L <sub>1</sub> %	L <sub>2</sub> %	L <sub>3</sub>	log(LR)
D19S433	13 - 14	0.082	0.146	0.1882					0.00020	
	14,2 - 16,2	0.179	0.189	0.0004					0.00118	
	14 - 14	0.082	0.093	0.0498					0.00008	
	13 - 14,2	0.017	0.081	0.0302					0.00008	
	14 - 16,2	0.015	0.081	0.0239	1		0.01295		0.00016	
	13 - 16,2	0.018	0.074	0.0311					0.00013	
	14 - 14,2	0.089	0.087	0.0361					0.00011	
	12 - 14	0.082	0.059	0.0458					0.00012	
	14 - 15	0.081	0.038	0.0343					0.00002	
	13 - 13	0.081	0.034	0.0527					0.00007	
	12 - 13	0.082	0.029	0.0541					0.00019	
	13 - 15	0.081	0.024	0.0573					0.00002	
	12 - 16,2	0.017	0.021	0.0000					0.00019	
	12 - 14,2	0.015	0.020	0.0100					0.00013	
	14 - 15,2	0.081	0.018	0.0275					0.00003	
	15 - 16	0.082	0.015	0.0006					0.00000	
	13 - 15,2	0.081	0.009	0.0239					0.00001	
	12 - 15	0.083	0.009	0.0137					0.00001	
	14 - 16	0.080	0.009	0.0017					0.00000	
	12 - 12	0.084	0.009	0.0125					0.00004	
	12 - 13	0.081	0.006	0.0172					0.00001	
	13 - 16	0.080	0.006	0.0019					0.00000	
	13 - 13,2	0.081	0.004	0.0201					0.00003	
	13,2 - 14	0.081	0.003	0.0140					0.00002	
	13,2 - 15	0.081	0.002	0.0083					0.00001	
	14 - 18,2	0.082	0.002	0.0017					0.00000	
	13 - 13,1	0.019	0.002	0.0000					0.00000	
	12 - 13,2	0.082	0.002	0.0120					0.00003	
	14,2 - 16	0.081	0.002	0.0006					0.00000	
	12,2 - 13	0.081	0.002	0.0140					0.00002	
	13 - 13,1	0.082	0.001	0.0019					0.00000	
	12,2 - 14	0.081	0.001	0.0133					0.00001	
	14,2 - 14,2	0.084	0.001	0.0005					0.00001	
	15 - 15	0.080	0.001	0.0009					0.00000	
	15 - 15,2	0.080	0.001	0.0005					0.00000	
	14 - 17	0.081	0.001	0.0000					0.00000	
	13 - 16,2	0.080	0.001	0.0042					0.00000	
	15,2 - 15,2	0.081	0.001	0.0038					0.00000	
	1,1 - 14,2	0.072	0.001	0.0007					0.00000	
							0.01295	3.367	0.327	

Suspect "A" Genotype

39 probable genotypes

D19S433

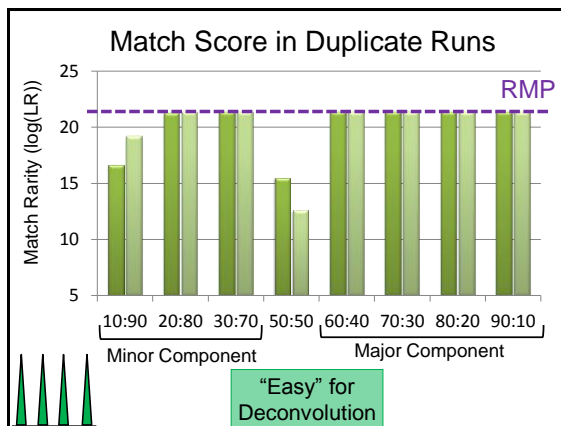
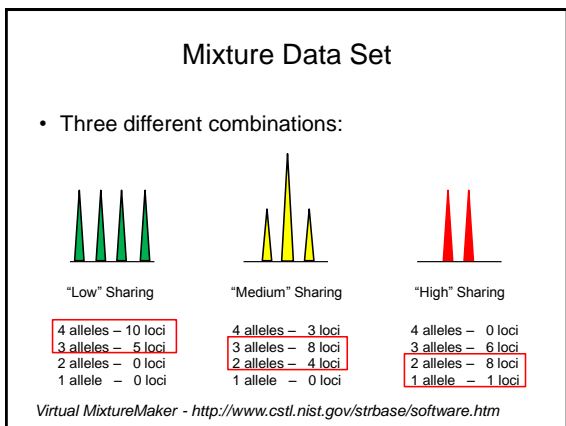


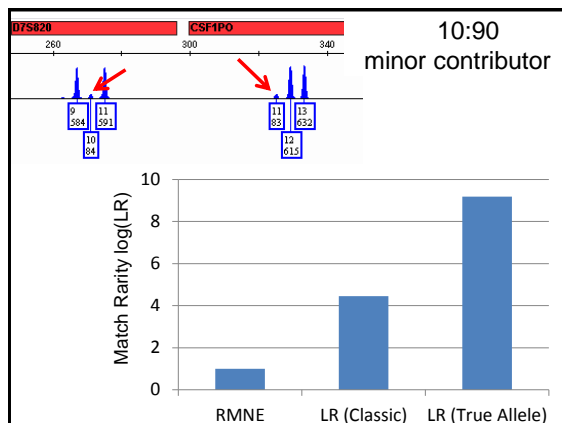
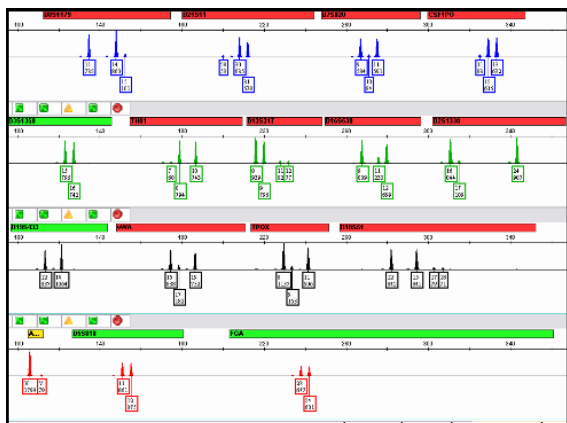
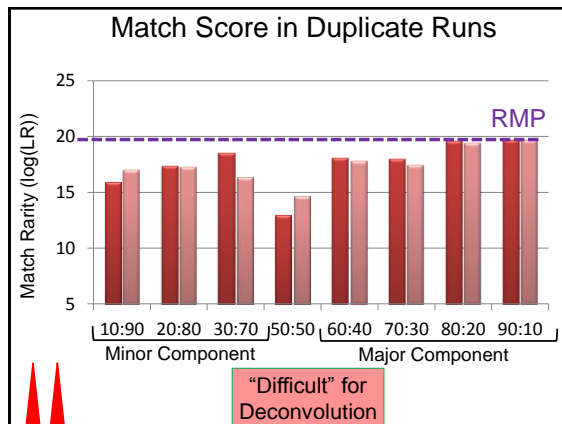
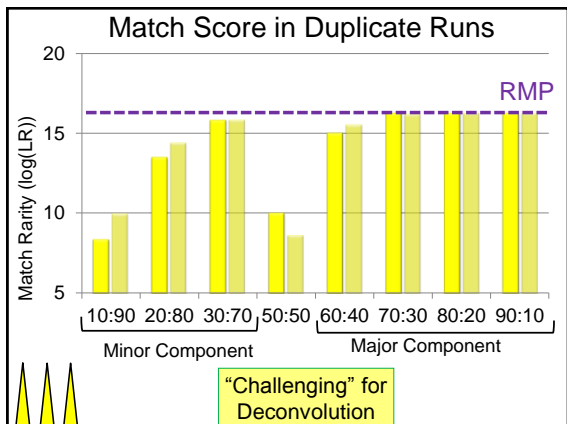
### Exploring the Capabilities

- Degree of Allele Sharing
- Mixture Ratios
- DNA Quantity

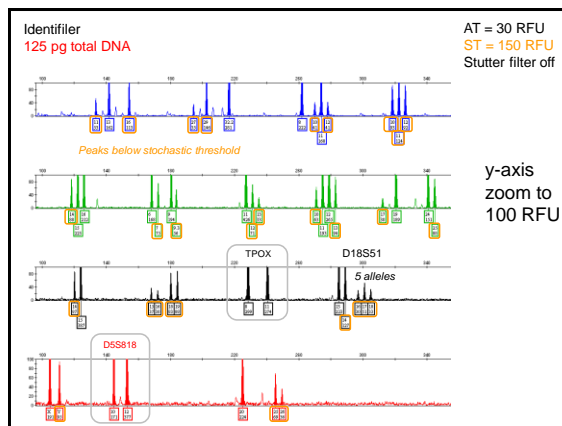
### Mixture Data Set

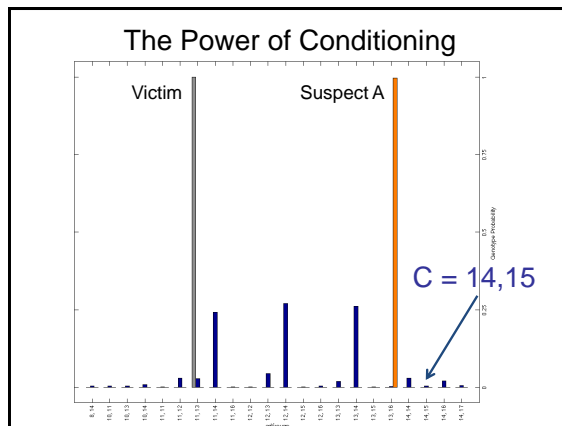
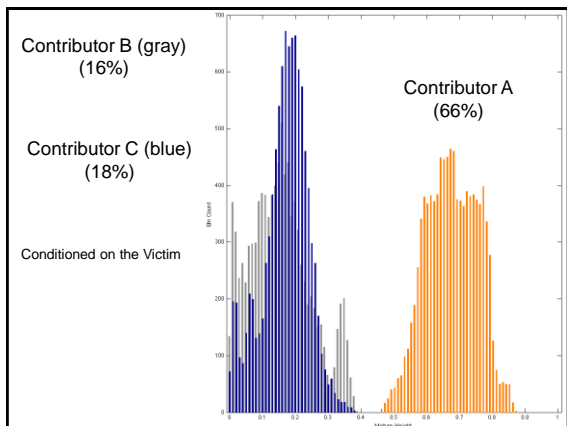
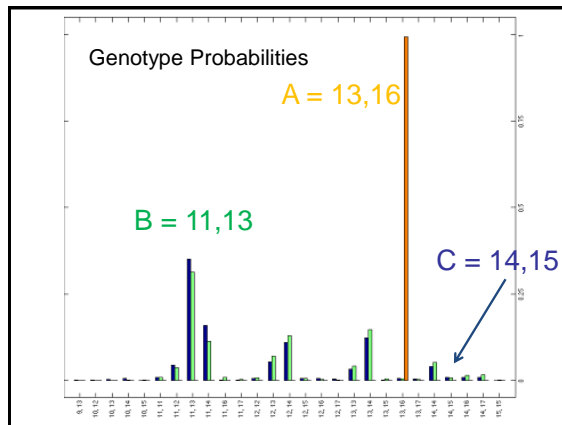
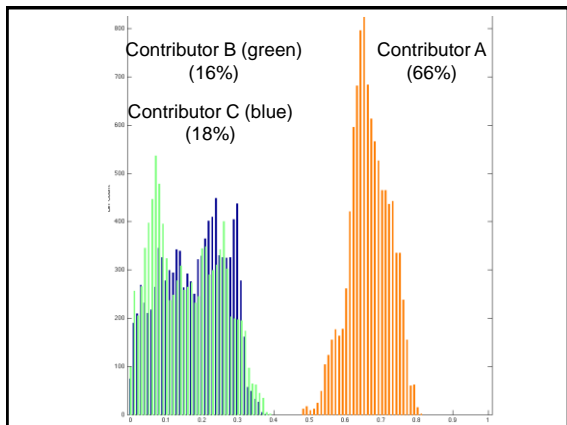
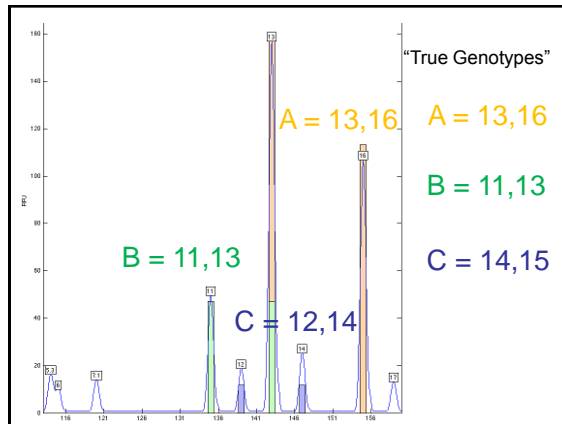
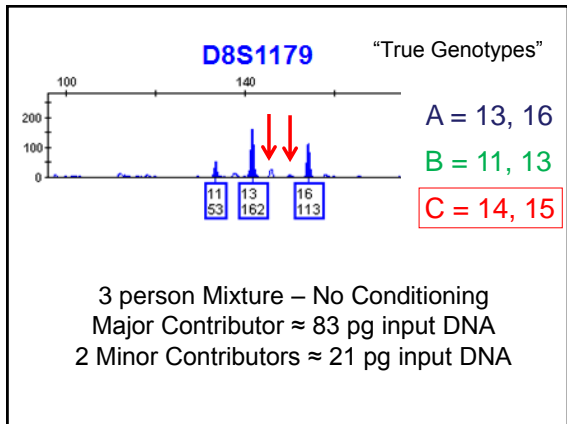
- Mixtures of pristine male and female DNA amplified at a total concentration of 1.0 ng/μL using Identifiler (standard conditions).
- Mixture ratios ranged from 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, and 10:90
- Each sample was amplified twice.





- ### Exploring the Capabilities
- Degree of Allele Sharing
  - Mixture Ratios
  - DNA Quantity







### 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).

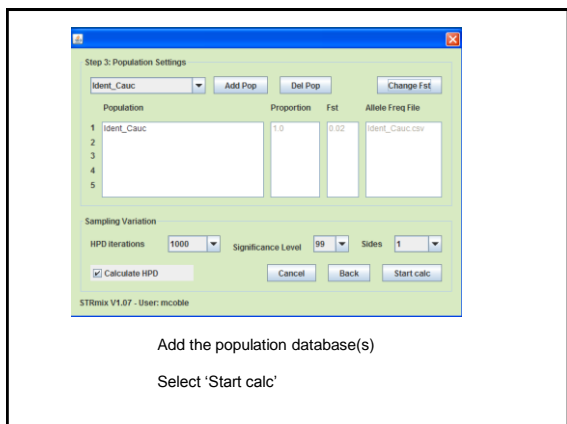
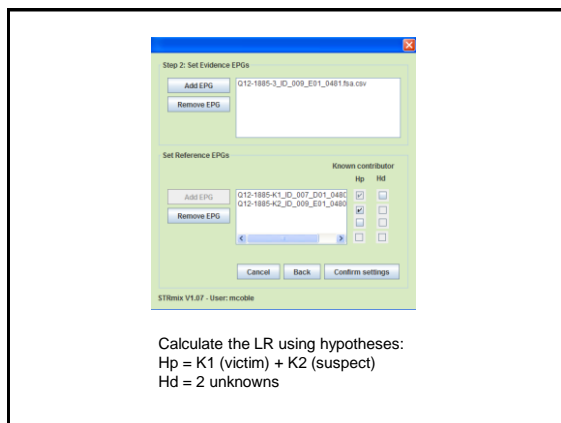
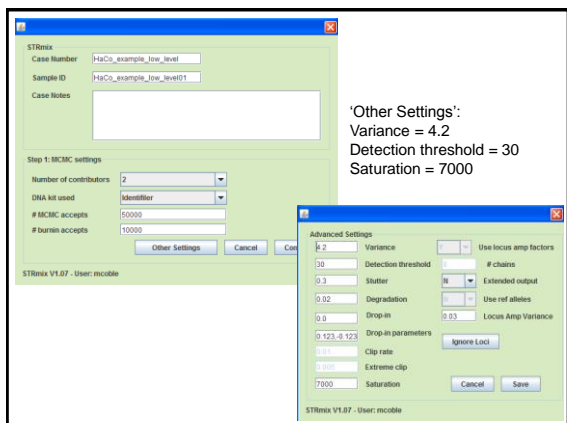
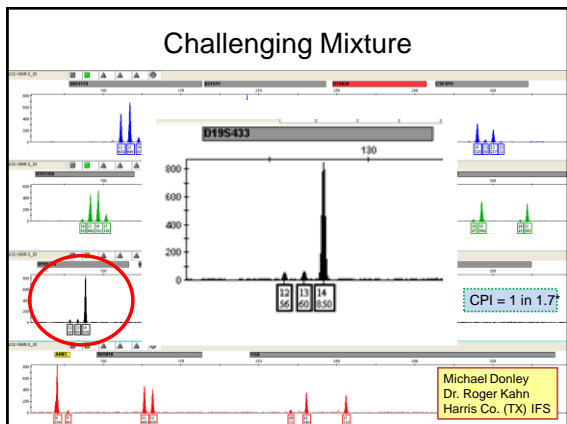
STRmix

<http://strmix.com/>



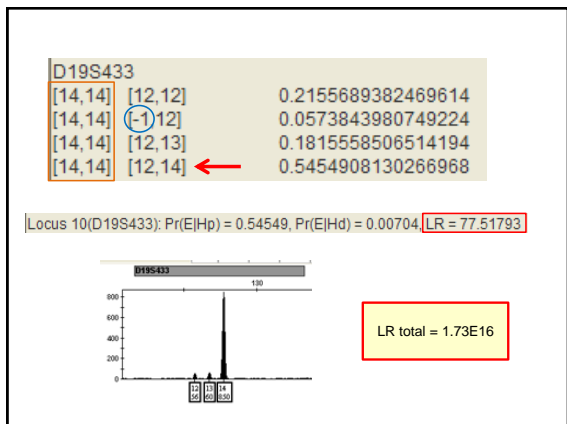
Government of South Australia  
Forensic Science SA



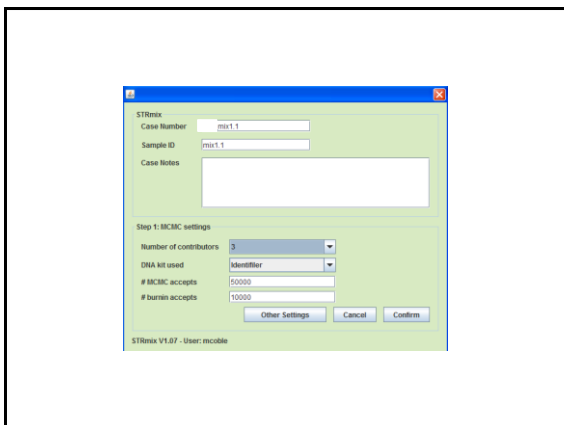
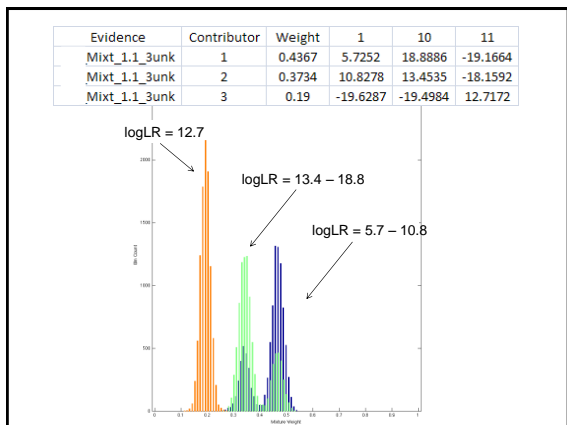
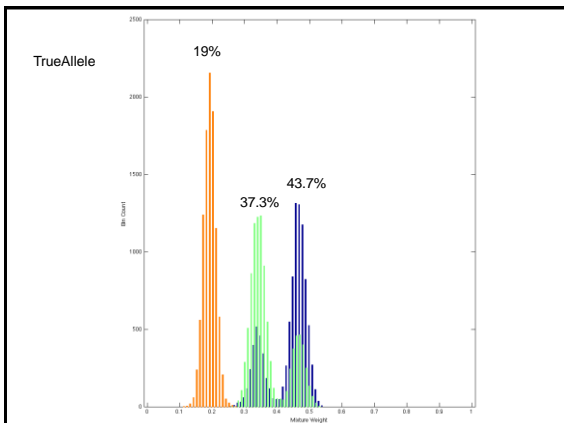
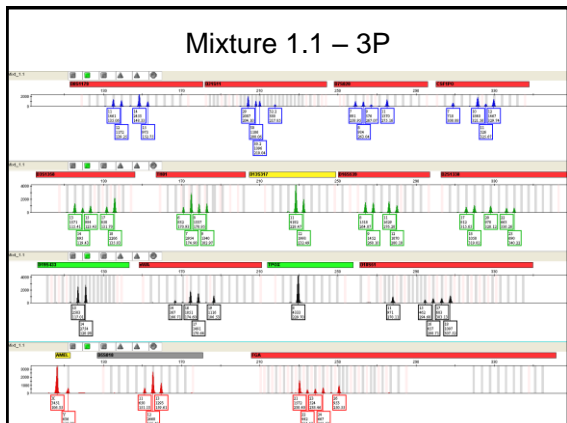


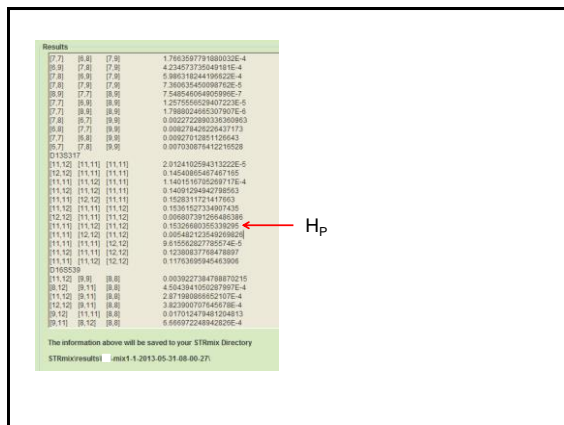
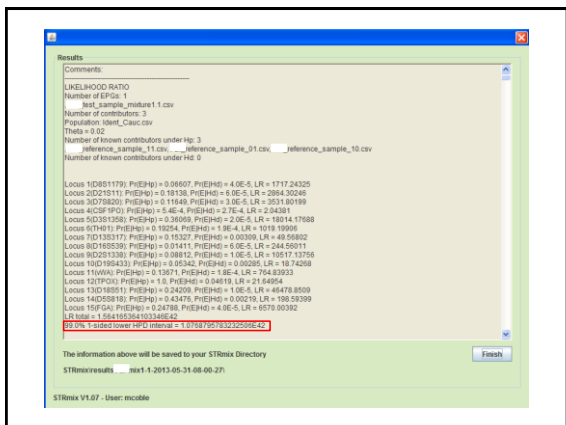
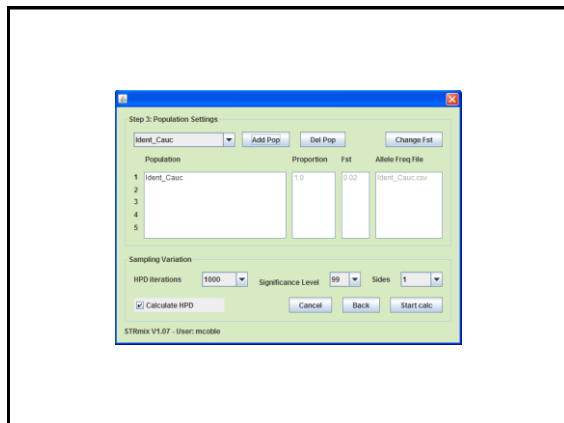
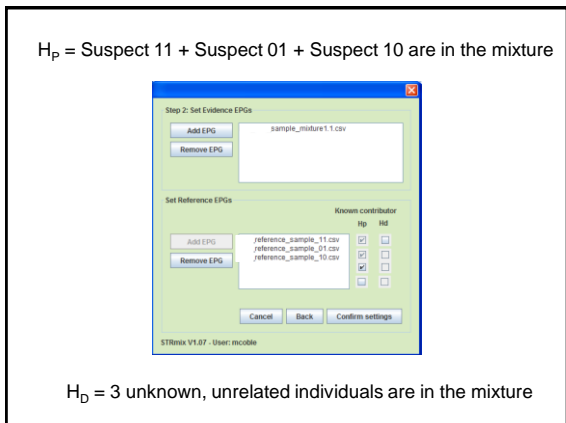
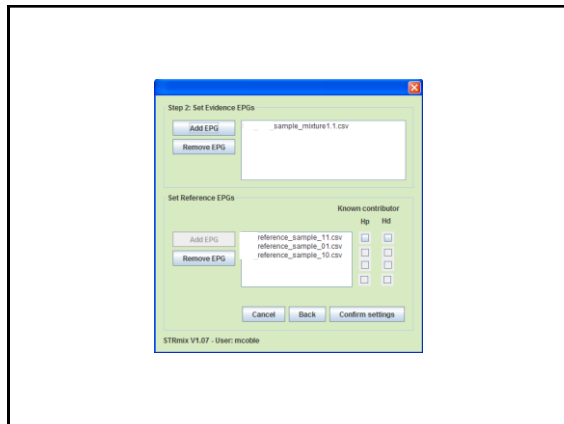
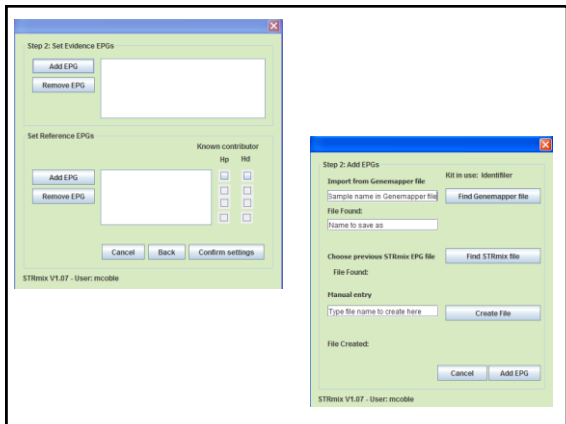
Add the population database(s)  
Select 'Start calc'

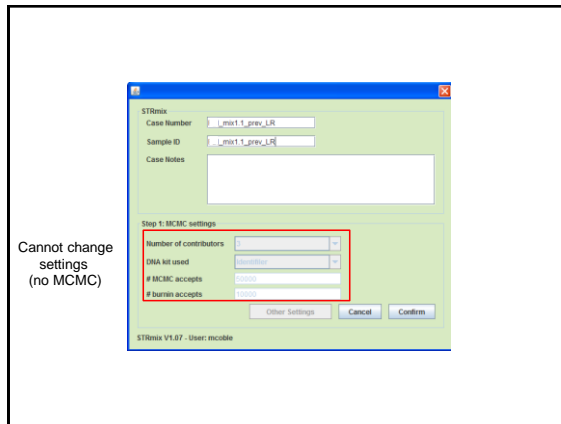
Mixture Proportions  
Contributor 1 - 87%  
Contributor 2 - 13%



- ### Fully continuous methods
- Use a Pr(DO) and LRs
  - Speed of analysis – can vary
  - Attempts to use all of the data







Cannot change settings (no MCMC)

