DNA Mixture Interpretation:
Where did we come from? What are we doing?
Where are we going?

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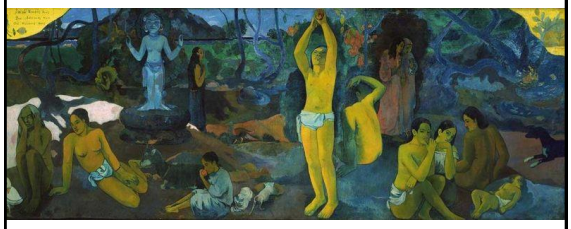
Where Do We Come From? What Are We? Where Are We Going?



Paul Gauguin, 1897

http://en.wikipedia.org/wiki/File:Woher_kommen_wir_Wer_sind_wir_Wohin_gehen_wir.jpg

Where are we going?
(2015 -)

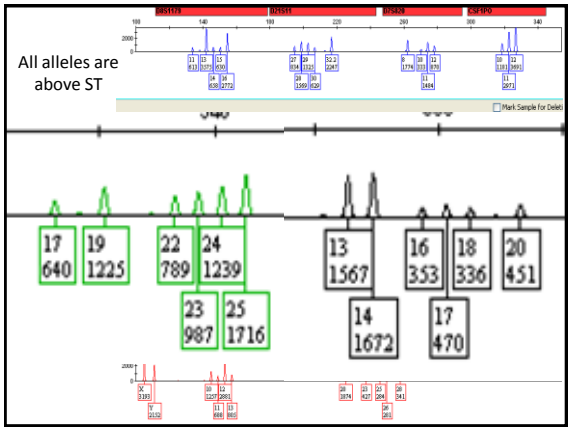
ASCLD Trivia Time!!!

Three Questions

- What were the last words of Julius Caesar before he died?
- Et tu, Brute? Then fall Caesar!
- What is the capital of Bangladesh?
- Dhaka

Three Questions

- How many people are in this mixture?



Do you have any uncertainty in your answer?

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

-Dennis Lindley

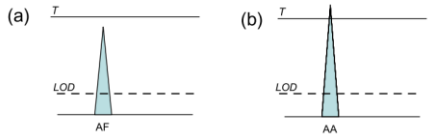
Handling Complex Mixtures

- Stochastic thresholds are necessary in combina… with CPI statistics
  - but a stochastic threshold may not hold much meaning for >2 person mixtures (due to potential allele sharing)
- Most labs are not adequately equipped to cope with complex mixtures
  - Extrapolating validation studies from simple mixtures will not be enough to create appropriate interpretation SOPs

David Balding (UK professor of statistical genetics): "LTDNA cases are coming to court **with limited abilities for sound interpretation.**" (Rome, April 2012 meeting)

"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 data below our Stochastic Threshold?

- Continue to use RMNE (CPI, CPE) (not optimal)
- Use the Binary LR with 2p (not optimal)
- Probabilistic Models

Probabilistic Approaches

- "Semi-Continuous" or "Fully Continuous"
- Semi-Continuous – information is determined from the alleles present – peak heights are not considered.
- Fully Continuous – incorporation of biological parameters (PHR [Hb], Mx ratio, Stutter percentage, etc…).

R. v Garside and Bates

- James Garside was accused of hiring Richard Bates to kill his estranged wife, Marilyn Garside.
- Marilyn was visiting her mother when someone knocked on the door. Marilyn answered and was stabbed to death.
- A profile from the crime scene stain gave a low-level DNA profile of the perpetrator.

Summary

Locus	Mrs Garside	Bates	CSP: minor component
D3	16,16	13,16	13
VWA	15,17	16,16	16
D16	11,12	11,12	–
D2	20,20	**19**,22	22
D8	12,13	8,13	8
D21	30,32.2	30,31.2	31.2
D18	14,14	**12,15**	–
D19	12,14	12,15	15
THO1	9.3,9.3	7,7	7
FGA	23,25	21,21	21

Three alleles from Bates were not present in the evidence

Court case

- The Crown expert dropped the D18 locus (gave a LR = 1) from the statistical results and used “2p” for D2 to give an overall odds for Bates of 1 in 610,000.
- David Balding argued for the defense that dropping loci is not conservative.

Balding and Buckleton (2009)

Forensic Science International: Genetics 4 (2009) 1–10

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Interpreting low template DNA profiles

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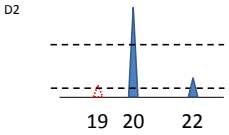
Present the "Drop model" for interpreting LT-DNA profiles

Drop Model



V = 20, 20
S = 19, 22

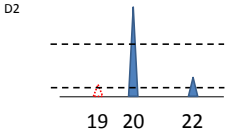
Pr(Drop-out) = 0.05
Pr(Drop-in) = 0.01

$P(E|H_1)$ = Pr(no Drop-out at 22) Pr(Drop-out at 19) Pr(No Drop-in)

= 0.95 0.05 0.99

= 0.047

Drop Model



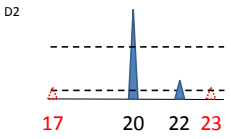
V = 20, 20
S = 19, 22

Pr(Drop-out) = 0.05
Pr(Drop-in) = 0.01

$$\frac{P(E|H_1)}{P(E|H_2)} = \frac{0.047}{}$$

The defense can now argue that someone else in the population unrelated to Bates was the true perpetrator!

Drop Model



V = 20, 20
UC = 17, 23

Pr(Drop-out) = 0.05
Pr(Drop-in) = 0.01

$P(E|H_2)$

Pr(Drop-out at 17)	Pr(Drop-out at 23)	Pr(Drop-in at 22)
0.05	0.05	0.01

= **0.000025** x **$2pq_{17,23}$** **(0.027)** = **0.000000675**

Summary

- Using "2p" for D2 gave a LR = 11. This is non-conservative compared to the probabilistic approach where a Pr(D) was incorporated into the calculation, the LR = 2.8
- The use of a probabilistic approach uses all of the information in the profile.
- The final LR in favor of the Hp was ≈ 400,000.

Some Semi-Continuous 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)
- Armed Expert (NicheVision)
- Puch-Solis *et al.* (LiRa and LiRaHT)
- GenoProof Mixture (Qualitype)

Semi-continuous methods

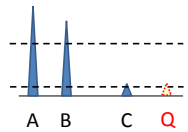
- Use a Pr(DO) and LRs
- Speed of analysis – "relatively fast" depending on the mixture.
- The methods do not make full use of data - only the alleles present.

What should we do with data below our Stochastic Threshold?

- 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)
- DNA-View Mixture Solution (Charles Brenner)
- DNAmixtures (Graversen 2013a,b) – open source, but requires HUGIN.

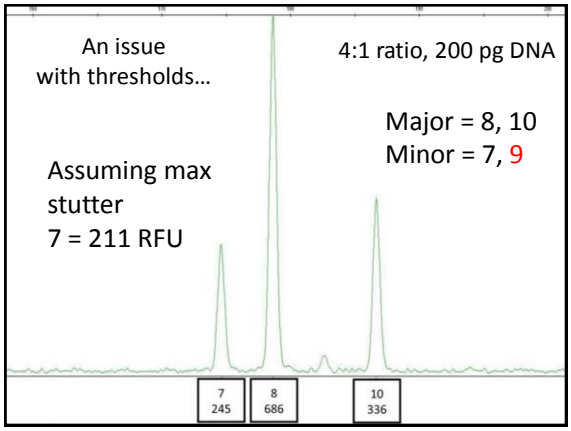
Weights may be determined by performing simulations of the data (Markov Chain Monte Carlo - MCMC).

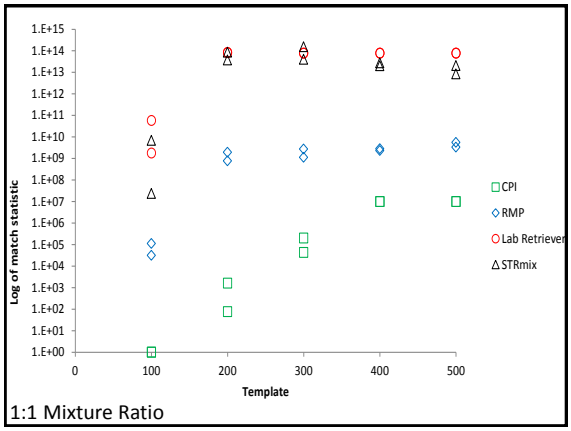
Fully continuous methods

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

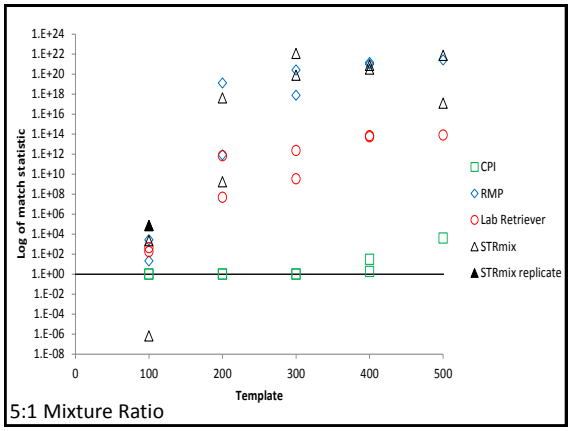
Advantages of Probabilistic Models

- Bille et al. *Electrophoresis*
- Used two samples with low allele sharing (10 markers – 4 alleles, 5 markers – 3 alleles). 2 PCR amplifications.
- 1:1, 2:1, 3:1, 4:1, and 5:1
- 500, 400, 300, 200, 100 pg input DNA
- CPI, RMP (2p), Lab Retriever, STRmix



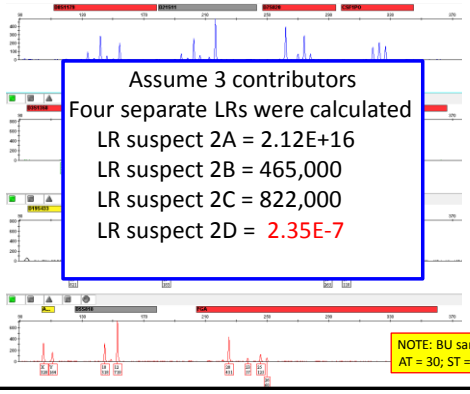


1:1 Mixture Ratio



5:1 Mixture Ratio

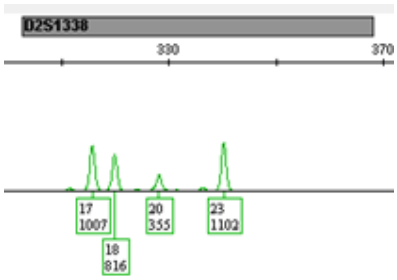
Case 02 – IDFiler



Case 05

"Couldn't help but note the need for mix deconvolution software tools for case 05"

(a) Deconvolution as 3p mixture



GENOTYPE PROBABILITY DISTRIBUTION
D2S1338

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[18,23]	[20,23]	[17,18]	1.1378843915710276E-5
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[17,20]	[17,18]	[18,23]	5.501858596607167E-6
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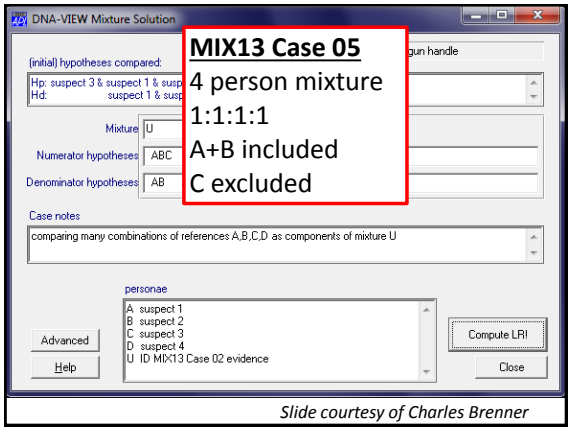
[17,17]	[18,20]	[23,23]	2.1663568224140716E-5

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



Slide courtesy of Charles Brenner

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 to interpret 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 only logical approach to evaluate complex, low-level mixture evidence. Probabilistic genotyping software can overcome ***many (noticed I didn't say "ALL")*** of the limitations we are facing today.
- Software solutions will be helpful – but it's also important that we understand the limitations of these programs.

Concluding Thoughts

- Despite the improvements in protocols and interpretation guidelines since 2010, mixture interpretation tends to be all over the place.
- Some of this is a consequence of using a statistical approach that is inappropriate for complex mixture interpretation – CPI is often being used as a substitute for interpretation.
- Software solutions can greatly assist in the statistical evaluation and removal of bias.

Acknowledgments

National Institute of Justice and NIST Law Enforcement Standards Office

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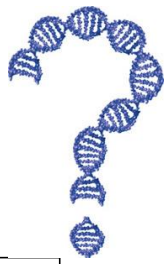
Dr. Robin Cotton
Dr. John Butler

Dan Katz



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