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ISFG DNA commission recommendations

(how software can be used to address the implications)

Peter Gill

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

P. Gill [a,*], C.H. Brenner [b], J.S. Buckleton [c], A. Carracedo [d], M. Krawczak [e], W.R. Mayr [f], N. Morling [g], M. Prinz [h], P.M. Schneider [i], B.S. Weir [j]

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- *Recommendation 1: The likelihood ratio is the preferred approach to mixture interpretation. The RMNE approach is restricted to DNA profiles where the profiles are unambiguous. If the DNA crime stain profile is low level and some minor alleles are the same size as stutters of major alleles, and/or if drop-out is possible, then the RMNE method may not be conservative.*

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- *Recommendation 2: Even if the legal system does not implicitly appear to support the use of the likelihood ratio, it is recommended that the scientist is trained in the methodology and routinely uses it in case notes, advising the court in the preferred method before reporting the evidence in line with the court requirements. The scientific community has a responsibility to support improvement of standards of scientific reasoning in the court-room.*

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- *Recommendation 3: The methods to calculate likelihood ratios of mixtures (not considering peak area) described by Evett et al [13] and Weir et al [14] are recommended.*

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- *Recommendation 4: If peak height or area information is used to eliminate various genotypes from the unrestricted combinatorial method, this can be carried out by following a sequence of guidelines based on Clayton et al* [17].
- *Recommendation 5: The probability of the evidence under Hp is the province of the prosecution and the probability of the evidence under Hd is the province of the defence. The prosecution and defence both seek to maximise their respective probabilities of the evidence profile. To do this both Hp and Hd require propositions. There is no reason why multiple pairs of propositions may not be evaluated* (Appendix 3).

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- *Recommendation 6: If the crime-profile is a major/ minor mixture, where minor alleles are the same size (height or area) as stutters of major alleles, then stutters and minor alleles are indistinguishable. Under these circumstances alleles in stutter positions that do <u>not</u> support Hp should be included in the assessment.*

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- *Recommendation 9: When a DNA profile is at a level that is dominated by background noise, then a biostatistical interpretation should not be attempted.*

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- *Recommendation 11: In relation to low copy number, stochastic effects limit the usefulness of heterozygous balance and mixture proportion estimates. In addition, allelic drop-out and allelic drop-in (contamination) should be taken into consideration of any assessment.*

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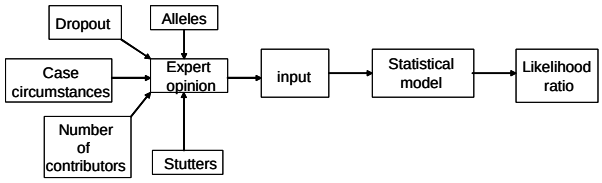
Interpretation process is an interaction of the expert with a statistical model



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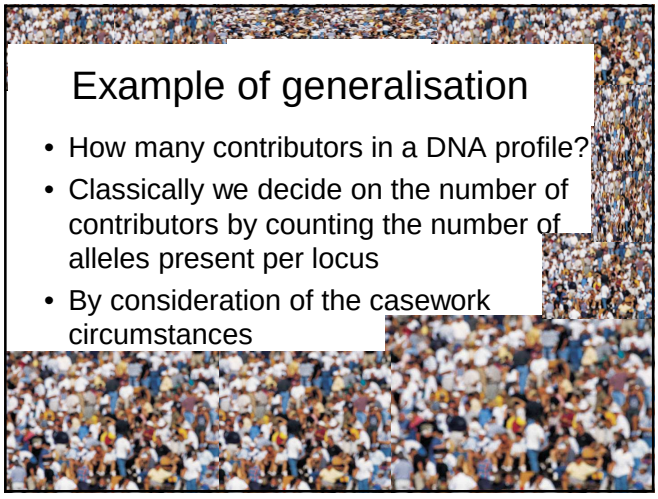

Example of generalisation

- How many contributors in a DNA profile?
- Classically we decide on the number of contributors by counting the number of alleles present per locus
- By consideration of the casework circumstances

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ISFG DNA commission recommendation 5 (anchoring the hypothesis)

- The probability of the evidence under the prosecution hypothesis is the province of the prosecution
- The probability of the evidence under the defence hypothesis is the province of the defence
- *There is no reason why multiple pairs of propositions may not be evaluated*
- BUT how can we apply this in practice?

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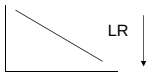
Anchoring the prosecution hypothesis

$$LR = \frac{\Pr E \mid Hp}{\Pr E \mid Hd}$$

Not anchored – the number of propositions is the same in numerator and denominator:

$$\frac{S+U_1+U_2+U_3}{U_0+U_1+U_2+U_3}$$



Anchored - the number of propositions is different in numerator and denominator:

$$\frac{S+U_1}{U_0+U_1+U_2+U_3}$$

Contributors under Hd

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How does this help?

- Usually the scientist decides the number of contributors on behalf of both prosecution and defence
- Minimising the number of contributors usually maximises the Probability on behalf of the defence
- The foregoing is a *generalisation* which may not always be true (Buckleton et al 2007).
- Is the generalisation true in this case?
- **check the *trend:***

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Establishing the trend when increased numbers of contributors are considered

No. Contributors under *Hd*	*Pr|Hd*	LR<
1	.02	50
2	$.2^4$	625
3	$.2^6$	15625
4	$.2^8$	390625
5	$.2^{10}$	9765625

Conditioned with 1 contributor under Hp
(we vary number of contributors under Hd)

The LR minimises when the number of contributors under Hd=1
We can easily demonstrate this. This is also the fairest calculation for the defence proposition. The probability PrHd is maximised when the number of contributors is minimised.

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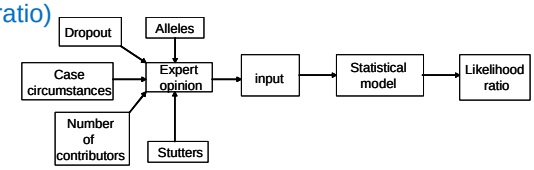
Establishing the 'robustness' of a reported likelihood ratio

- Our idea is to introduce software that allows *exploratory data analysis* to enable an interaction between expert and the software system (we can use *'what-if'* analysis to determine the scenarios that can be accommodated by a given likelihood ratio)



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A useful generalisation

- It is necessary to carry out at least 2 calculations in order to establish the general trend of the LR relative to the alternative sets of propositions. This way, we can establish the minimum likelihood of multiple sets of propositions.

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Towards understanding the effect of uncertainty in the number of contributors to DNA stains

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Numbers of contributors

- There is no need to anchor the number of contributors to be the same under Hp and Hd – they will often be different
- There will be differences between prosecution and defence hypotheses that courts will wish to explore. Software will facilitate the exploration

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Dropout

- Recommendation 7: If drop-out of an allele is required to explain the evidence under *Hp*: (*S = ab; E = a),* then the allele should be small enough (height/area) to justify this *(i.e. the allele should be below a predetermined threshold).*
- Basically, this means that if an allele found in the reference sample is missing in the crime stain then it is not necessarily neutral evidence.
- Reworking the sample is always important to see if we can recover the missing alleles.
- But we now have a method to evaluate the effect of PrD on the likelihood ratio

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More generalisations

- Don't ignore inconvenient (to the prosecution) events.
- Use statistical tools to explore the data so we can understand what is going on
- The statistical analysis may suggest that samples need to be reworked as a preferable option

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New ISFG DNA commission

- New commission recently reported and recommends the incorporation of dropin and drop-out into probabilistic calculations

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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

P. Gill [a,b,*], L. Gusmão [c], H. Haned [d], W.R. Mayr [e], N. Morling [f], W. Parson [g], L. Prieto [h], M. Prinz [i], H. Schneider [j], P.M. Schneider [k], B.S. Weir [l]

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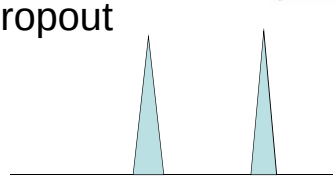
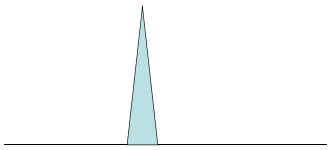
Dropout

- Suspect



- Crime stain



Match??

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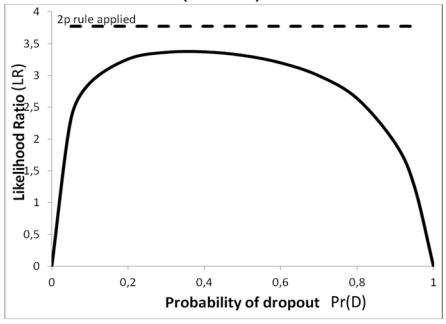
Fig. Locus D18S51 frequencies are used as an example, where allele a corresponds to D18S51 allele 13 (frequency: 0.135). Using the 2p rule: LR=1/3.Effect of Pr(D) on LR. S is ab, E is a. The likelihood ratio LR=Pr(E|S)/Pr(E|U) is plotted as a function of Pr(D) ∈ [0,1]. (2pa) = 1/(2x0.135) = 3.8 (dashed line).



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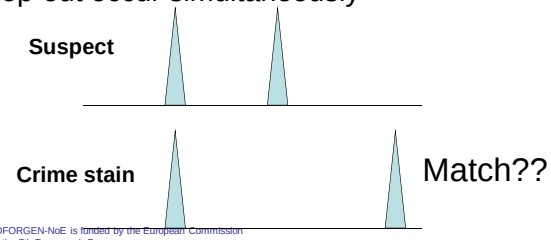
Drop-in

- An additional band(s) is present in the profile that are not in the suspect
- It gets complicated if both drop-in and drop-out occur simultaneously



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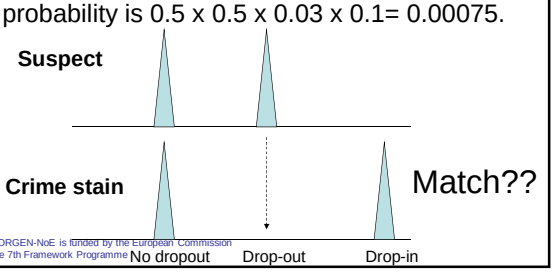
How can this be a match?

If we have a reasonable estimate of the chance of drop-out (*PrD*) and the chance of drop-in (*PrC*) then we can assess the chance of the event below:

If Pr(*D*)=0.5 and Pr(*C*)=0.03, *f*=0.1 then the combined (Hp) probability is 0.5 x 0.5 x 0.03 x 0.1= 0.00075.



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How can this be a match?

- The numerator =0.00075 (instead of 1)
- The denominator =.02
- The LR=.00075/.02 = 0.0375 (strongly favours defence)
- But the important point is that: it is not an exclusion.
- We can provide a LR to any DNA profile – they don't need to be scored as 'inconclusive'
- An answer is always possible even for the most complex of cases.
- If we want to use words like *exclusion* etc we can at least use a parallel numeric scale which makes these terms much more meaningful

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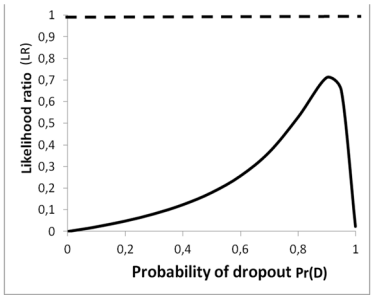
Fig. 5. Effect of Pr(D) on LR. Suspect is ab and crime stain evidence is ac. Locus D18S51allele 13 frequency was used to calculate the LR example (pa=0.135). Since LR<1, then this favours Hd. The dashed line indicates LR = 1.



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Putting theory into practice: Analysis of a complex mixture using *LRmix*

- New tool that can be used for low copy number and for conventional DNA profiles
- A method that can take account of drop-out and drop-in.
- An exploratory tool to evaluate evidence in relation to multiple case-work 'what-if' scenarios
- *We show how the expert can be an expert.*

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Summary of New ISFG DNA commission recommendations

- Probabilistic methods following the *'basic model'* described here can be used to evaluate the evidential weight of DNA results considering drop-out and/or drop-in.
- Estimates of drop-out and drop-in probabilities should be based on validation studies that are representative of the method used.
- The weight of the evidence should be expressed following likelihood ratio principles.
- The use of appropriate software is highly recommended to avoid hand-calculation errors.

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What ~~is~~ *was* low-copy-number

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Low-level DNA profiling

- This talk will examine the reasons for the apparent 'contentious' nature of LCN DNA (now termed LT-DNA)
- This 'debate' has been bought to a conclusion and we show a constructive way forward to interpret partial DNA profiles

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What is Low Copy Number?

- Let's make a list of what LCN is <u>not</u>
  - Its not related to an overall quantity of DNA (such as 200pg)
  - Its not restricted to 'touch DNA'
  - Its not related to any particular technique
- What is it then?
- Before we can answer this question lets examine effects we expect with LCN

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What about the effects?

- Low levels of DNA are typically associated with phenomena of 'drop-out' and 'drop-in'
- BUT these effects are universally observed across all DNA profiling strategies – i.e. not restricted to Low-levels of DNA

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Drop-out with progressive dilution (from Gill et al 2000) FSI, 112,17-40

	Am	THO	D21	D18	D8	VWA	FGA	D19	D16	D2	D3
1ng	X Y	6 7	61 68	12 13	11 12	16 17	23 25	14 15	11 13	17 22	15 17
100pg	X Y	6 7	61 68	12 13	11 12	16 17	23 25	14 15	11 13	17 22	15 17
50pg	X Y	6 7	61 68	12 13	11 12	16 17	23 25	14 15	11 13	17 22	15 17
25pg	X Y	6 7	61 68	12 13	11 12	16 17	23 25	14 15	11 13	17 22	15 17
12pg	X Y	6 7	- 68	12 13	11 -	16 17	- 25	14 15	11 13	17 -	- 17
6.4pg	X Y	6 7	61 68	12 -	11 12	- 17	23 25	14 15	- -	- -	15 17
3.2pg	X Y	- -	- -	- -	11 -	- -	23 -	14 -	- 13	- 22	15 -
1.6pg	- Y	- -	- -	12 -	- -	16 -	23 25	- -	- -	17 -	- -
0.8pg	- -	- -	- -	- -	- -	16 -	23 -	- -	- -	- -	- -
0.4pg	X -	- -	- 68	- -	- -	- -	- -	14 -	- -	- -	- 17
0.2pg	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
0.1pg	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
PCR -ve	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -

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Negative controls - showing drop-in

	Amelo	D19	D3	D8	THO	VWA	D21	FGA	D16	D18	D2
1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
2	- -	- -	15	- -	- -	- -	- -	- -	- -	- -	- -
3	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
4	- -	- -	17	- -	- -	- -	- -	- -	- -	- -	- -
5	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
6	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
7	- -	14	- -	- -	- -	- -	- -	- -	- -	- -	- -
8	X	- -	- -	13	- -	- -	- -	- -	- -	- -	- -
9	- -	- -	14	- -	- -	- -	- -	- -	- -	- -	- -
10	X	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
11	X	- -	- -	- -	- -	16	- -	- -	- -	- -	- -
12	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
13	- -	13	- -	- -	- -	- -	- -	- -	- -	- -	- -
14	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
15	- -	- -	16	- -	- -	- -	- -	- -	- -	- -	- -
16	- -	- -	- -	15	- -	- -	- -	- -	- -	- -	- -
17	X	15	- -	- -	- -	- -	- -	- -	- -	- -	- -
18	X	14	- -	14	- -	- -	- -	- -	- -	- -	- -
19	- -	- -	- -	- -	- -	- -	28	- -	- -	- -	- -
20	- -	- -	- -	- -	- -	- -	- -	- -	- -	13	- -
21	- -	- -	- -	- -	- -	- -	33.2	- -	- -	- -	- -
22	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
23	- -	- -	- -	10	- -	- -	25 27	- -	- -	- -	- -
24	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
25	- -	- -	15	- -	- -	- -	- -	- -	- -	- -	- -
26	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
27	X	- -	- -	10	- -	- -	- -	- -	- -	- -	- -
28	- -	15	- -	- -	- -	- -	- -	- -	- -	- -	- -
29	- -	- -	15	- -	- -	16	- -	- -	- -	- -	- -
30	- -	15	- -	- -	- -	- -	- -	- -	- -	- -	- -
+ ve	X Y	14 15	15 17	11 12	6 7	16 17	28 31.2	23 25	11 13	12 13	17 22
- ve	- -	- -	- -	- -	9.3	- -	- -	- -	- -	- -	- -

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What is drop-in

- Independent allelic events – no more than one or two per profile.
- Important not to confuse this with gross contamination i.e. a profile from a single individual (dependent events).

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Interpretation

- Full statistical method
- Biological consensus method
- Validation was provided by comparison of the two methods.
- No need to decide on optimum number of replicates (as claimed by some) since the statistical model can be used to 'validate' any number of replciates.

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Interpretation

- There has been much confusion which we can trace to the constraints of the RMNE method being wrongly assumed for the LR framework.
- Typically interpretation of evidence follows two different methods
  - RMNE or LR
- What are the main differences between the two methods?

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RMNE

- Two consecutive step process
  - (1) Is the suspect included or excluded?
  - (2) What is the strength of evidence **IF** the suspect is included?
  - Note that there is often a third category
  - Is the profile 'inconclusive'?
- Note that the RMNE statistic exists independently of a 'match' with the suspect – no conditioning needed

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LR

- LR is a one step-process
- Philosophically quite different from the LR method
- Suspect anchored – this means that the strength of the evidence is always tested against two alternative pairs hypotheses
- Consequently there is no need to decide if there is an inclusion or an exclusion.
- We simultaneously test the strength of the evidence that favours the prosecution hypothesis and the defence hypothesis.

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LR

- Instead of saying the suspect is included or excluded or inconclusive – we would say:
  - "the evidence is x times more likely if the prosecution hypothesis is true than if the defence hypothesis is true"
- Ie the strength of evidence is on a sliding scale
- The LR only exists as a result of conditioning
- This is the fundamental difference between RMNE and the LR

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Thresholds

- To decide whether something is a match or isn't – or to decide if something is LCN or not LCN requires decisions based on thresholds.
- Typical thresholds include:
  - LOD (limit of detection) at 50rfu
  - The stochastic threshold at 150rfu (Why?)
- Thresholds are important because important decisions are dependent upon them
- But are thresholds used logically?

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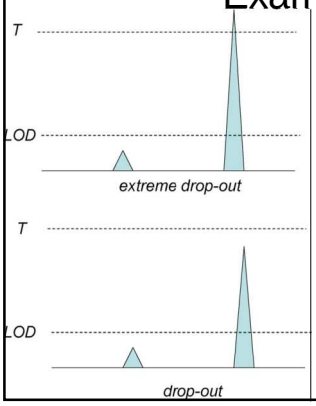
Example



- T is the stochastic threshold used to signify PrD≈0
- It is designed to capture the event S=ab C=aa.
- If allele<T then it is given the F designation
- If allele>T it is designated as a homozygote
- The threshold wont capture all events (unless set to infinity)
- If it's too high then too many samples are rejected to make it feasible
- So all thresholds will be subject to some error
- How much error can be tolerated
- Who decides this?
- Scientist as gatekeeper?

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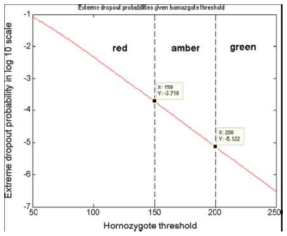
There is risk associated with any threshold

- Who decides how much risk is acceptable?
- See: Gill, Puch-Solis, Curran The *low-template-DNA* (stochastic) threshold—Its determination relative to risk analysis for national DNA databases (FSI Genetics, 3,104-111, 2009)



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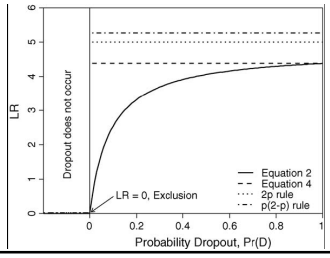
The 2p rule

- Suppose S=ab and C=aa and a>T
- This cannot be viewed as neutral evidence (Buckleton) – can be very anticonservative



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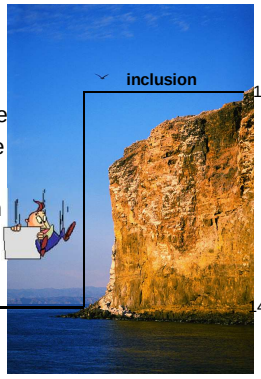
Thresholds

- Falling off the cliff
- E.g. if we have a

Rule that states:
150rfu – no dropout is possible
V. 149rfu – dropout is possible

- There is nothing in between



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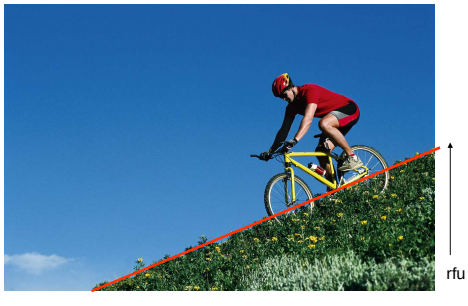
In reality it's a gentle ride downhill



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On the threshold of a dilemma

- A recent commentator provides the following definition of an 'exclusion':
- "An exclusion is declared when the reference sample has alleles that are not observed in the evidence and these unobserved alleles cannot be due to degradation within the evidence sample"
- OK so what's an 'inclusion'??
- "An inclusion is declared when the genetic results obtained from a mixture is such that the reference sample(s) can not be excluded as a part contributor of the mixed profile"
- Hang on!!! – so an inclusion is something that cannot be excluded!!!??
- **BUT there's more:**

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"An inconclusive call can be divided into two categories: (i) those profiles that are unsuitable for comparison (other than for exculpatory purposes); and (ii) an interpretation where the profile or portion of a profile is not used for statistical purposes such as for any locus of an indistinguishable mixture when any potentially attributable allele to a single contributor(s) is below the empirically established MIT."

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What does this mean?

- It is very difficult to define the meaning of the following words:
- match, inclusion, exclusion, inconclusive
- This is because the context of the words carries a meaning that is definitive
- We always encounter the 'threshold dilemma'

included	inconclusive	exclusion
match	Cannot be excluded	Non-match

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The underlying model is continuous

- Thresholds are difficult to apply and cannot be used in a definitive way unless associated with an estimate of (acceptable) risk.
- It is tempting to use the 'inconclusive' category and to use statements like ' the suspect cannot be excluded'.
- But this kind of statement may be prosecution biased – especially if a proper analysis favours the defence hypothesis.
- Therefore it is not possible to demonstrate that such guidelines are always more conservative, simply by increasing the number of inconclusive calls.

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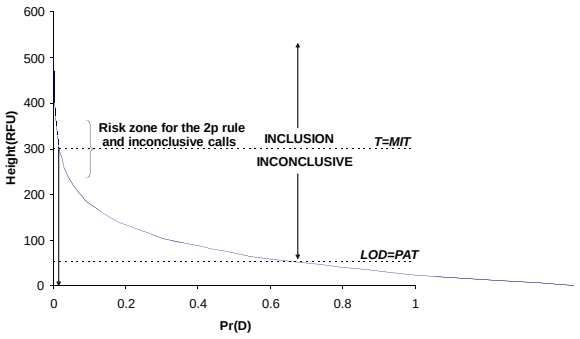
On the threshold of a dilemma



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Consensus profiling

Sample ID	Am		D19		D3		D8		VW		TH		D21		FG		D16		D18		D2	
Amp 1	X	Y	12	F	15	F	11	15	16	F	7	9.3	31	F	23	24	9	F	15	F	16	F
Amp 2	X	F	12	14	17	F	14	15	16	20	7	9.3			24	F	11	F	18	F	16	23
Amp 3	X	Y	12	F	15	17	11	15	20	F	9.3	F	30	F	24	F			15	20	16	23
CONSENSUS	**X**	**Y**	**12**	F	**15**	**17**	**11**	**15**	**16**	**20**	**7**	**9.3**			**24**	F			**15**	F	**16**	**23**

- An allele can only be scored if it is present in TWO separate amplifications
- Note there is some variation on this method (Benschop et al 2011, FSI Genetics,5,316-328)
- An 'F' designation is used with loci displaying only one allele *(in all profiles including consensus)*
  - Indicates that there may be allele drop-out
    - Disregards 150 rfu peak height rule used in standard STR profiling

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Consensus profiling (example)

Sample ID	Am		D19		D3		D8		VW		TH		D21		FG		D16		D18		D2	
Amp 1	X	Y	14	15	17	18	13	F	14	F	6	F	30	F	20	21	13	F	14	F		
Amp 2	X	F	14	F	17	18	13	F	15	18	9	F	30	31	21	24	13	14	13	14	24	F
Amp 3	X	Y			18	F	13	14	14	18	9	F	31	F	21	F	13	14	14	F		
CONSENSUS																						

X, Y | 14, F | 17, 18 | 13, F | 14, 18 | 9, F | 30, 31 | 21, F | 13, 14 | 14, F | ?

- 'F' designations means the locus is treated as 'could be a homozygote or could be a heterozygous' in match probability calculations
  - i.e. p^2 AND 2pq (p^2 + 2pq)

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The consensus method

- There are limitations to the consensus method.
  - It is ad-hoc (not a proper statistical method)
  - It is difficult to analyse mixtures
  - It wastes information
  - The theory to provide a statistical model has been around for more than ten years
  - We have never stated that the consensus model is preferable to the full statistical model
  - The 2p (F designation) method can be anti-conservative
  - Time to move forward to the next generation software

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A different calculation is needed

- If the profile is unambiguous (ie matches suspect then the numerator =1
- If the profile is ambiguous (ie does not match suspect completely) then the numerator is less than one
- i.e. we are used to calculating

$$\frac{1}{2ab}$$

The bottom line:
If this is less than one then the strength of evidence decreases

AND

If there is any uncertainty about
The prosecution hypothesis then
This must be less than one (not neutral)

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Probability of dropout/dropin can be built into the LR model without any problem

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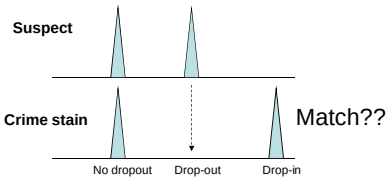
No need to decide if a profile is an exclusion/inconclusive/included



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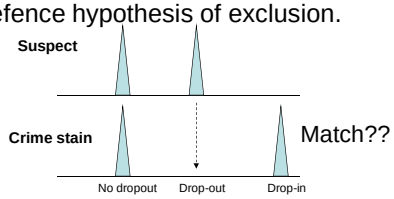
This is not an exclusion!

Its not neutral! But the evidence strongly supports the defence hypothesis of exclusion.



Assume D=0.5, Cp=0.03 p(a,b,c)=0.1

Possible random men	*Pr(genotype)*	*Pr(E=ac|genotype)*	multiply columns	*Denominator*	*Numerator*
ab	$2p_a p_b$	$\frac{DDCp_c}{D^2C}$	$2p_a p_b \frac{DDCp_c}{D^2C}$	0.000015	0.00075
ac	$2p_a p_c$		$2p_a p_c \frac{D^2C}{D^2C}$	0.005	
			sum	***0.005***	
			LR=	0.15	

This is our (incomplete) conditioning list. It can be expanded to include all possible genotypes. There is no bias in the method. This format can be easily expanded to interpret mixtures and can include stutters. **THIS LOOKS COMPLEX, BUT IT IS EASY TO FOLLOW**

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A list of advantages of the LR framework

- No need for definitive thresholds
- The framework can easily accommodate any set of probabilities – eg. Pr*D*, stutter, drop-in.
- Method advocated by ISFG DNA mixtures commission
- The framework can be expanded to include replicates (used in the biological model)
- The LR method was used to validate the biological model
- Discussions on optimum number of replicates are redundant.
- The correct question is “how does the biological model perform when compared to the statistical model”?

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Some of our recent publications

[1] P. Gill, L. Gusmao, H. Haned, W.R. Mayr, N. Morling, W. Parson, L. Prieto, M. Prinz, H. Schneider, P.M. Schneider, B.S. Weir, 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, Forensic Sci Int Genet 6 (2012) 679-688.

[2] H. Haned, K. Slooten, P. Gill, Exploratory data analysis for the interpretation of low template DNA mixtures, Forensic Science International: Genetics 6 (2012) 762-774.

[3] A. Kirkham, J. Haley, Y. Haile, A. Grout, C. Kimpton, A. Al-Marzouqi, P. Gill, High-throughput analysis using AmpFlSTR® Identifiler® with the Applied Biosystems 3500 xl Genetic Analyser, Forensic Science International: Genetics 7 (2012) 92-97.

[4] P. Gill, H. Haned, A new methodological framework to interpret complex DNA profiles using likelihood ratios, Forensic Science International: Genetics 7 (2013) 251-263.

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Evaluation of the evidence in the murder of Meredith Kercher

(implications and recommendations for forensic laboratories)

Peter Gill (NIPH) and Hinda Haned (NFI)

(with special thanks to Carla Vecchiotti for discussions. This paper is an appraisal of the evidence from the Conti-Vecchiotti report)
http://knoxdnareport.wordpress.com/

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Background of the case

- Brutal murder by stabbing of Meredith Kercher in Perugia in November 2007
- Key evidence in the case:
  - Item 36 (a knife found at Sollecito's flat in kitchen drawer)
  - Item 165 (bra-clasps forcibly removed at the crime-scene)
- Led to the conviction of *Amanda Knox* and *Raffaele Sollecito*
- Conviction was quashed in 2011 after successful appeal (Evidence of Carla Vecchiotti and Stefano Conti was crucial to the appeal)

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There were two important aspects to the challenge

- The interpretation of the DNA profiling evidence
- The meaning or the relevance of the DNA evidence
- What lessons can be learned from this experience?
- How does it impact on current casework?
- What are the emerging issues in forensic biology
- Does practice need to change (if so how?)

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Exhibit 36 –knife retrieved from drawer in Sollecitos flat

- Allegedly had traces of DNA from Amanda Knox on the handle and of Meredith Kercher on the blade.
- The DNA alleged to have come from Knox was not disputed, but the profile alleged to have come from Kercher was very low level
- Furthermore, there was no evidence that the DNA was from blood

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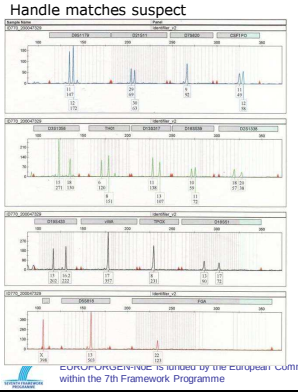
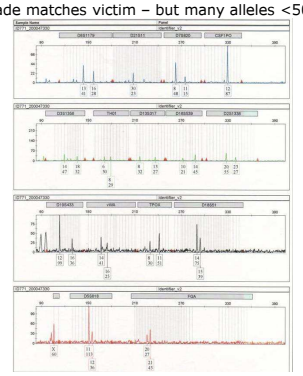
Item 36 (knife, epg)

Handle matches suspect



Blade matches victim – but many alleles <50



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Item 165 (bra-clasp)

- This item displayed clear major/minor(s)
- The major profile came from the victim (undisputed)
- A minor profile was alleged to have come from Sollecito
- Y chromosome analysis indicated presence of at least 3 males

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Table of alleles

Scientific police Appeal court experts

DNA	RTIGF	Interpretazione elettroferogramma (ISFG: Raee. 6)	Interpretazione elettroferogramma (Raee. 6/ solo picchi altezza superiore 50 RFU)
D8S1179	13 15 16	11-12-13-14-15-16	12-13-14-15-16
D21S11	30 32.2 33.2	29-30-32.2-33.2	29-30-32.2-33.2
D7S820	8 11	8-10-11	8-10-11
CSF1PO	10 12	10-11-12	10-11-12
D3S1358	14 16 17 18	14-15-16-17-18	14-16-17-18
TH01	6 8 9 9.3	6-8-9-9.3	6-8-9-9.3
D13S317	8 12 13	8-12-13	8-12-13
D16S539	10 11 14	10-11-13-14	10-11-13-14
D2S1338	16 20 23 24	16-18-19-20-22-23-24	16-19-20-22-23-24
D19S433	12 13 15.2 16	11-12-13-14-15-15.2-16	12-13-14-15-15.2-16
VWA	12 14 15 16	12-14-15-16	12-14-15-16
TPOX	8 9 11	8-9-11	8-9-11
D18S51	14 15 16 17	13-14-15-16-17	13-14-15-16-17
D5S818	11 12	11-12-13	11-12-13
FGA	20 21	19-20-21-22	20 21

➔Original allele scores (scientific police) were 'filtered' to remove stutter etc

➔The profiles were re-analysed by appeal court experts and the profiles show at least three contributors

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The profiles are complex and the propositions are also uncertain, so how should analysis proceed?

- The process is 'exploratory'
- Suitable software is needed that can accommodate:
  - Complex mixtures
  - Drop-out (alleles that are missing)
  - Drop-in (additional alleles)

It is strongly suggested that there should be agreement between defence and prosecution on propositions *before analysis proceeds* ➔ the statistical model should be able to evaluate the differing positions

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The profiles are complex and the propositions are also uncertain, so how should analysis proceed?

It is strongly suggested that there should be agreement between defence and prosecution on propositions *before analysis proceeds* ➔ the statistical model should be able to evaluate the differing positions

- Then it is for the court to decide
- The scientist is a facilitator of the discussion – there are strict boundaries to observe
- The scientist's purpose is to clearly define and separate the issues of relevance – to prevent confusion between the fact of the DNA profile and the circumstances whereby it came to be deposited at the crime scene

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Interpretation of DNA evidence

- Concepts such as '*exclusion*' or '*inclusion*' or '*inconclusive*' are vague and difficult to define as they cannot be enumerated
- Conversely, the *likelihood ratio* allows evidence to be evaluated on a sliding scale where a number less than one favours the *defence* hypothesis of exclusion
- And a number greater than one favours the *prosecution* hypothesis of inclusion
- The main problem with conventional statistical methods is that they cannot calculate strength of evidence when LR<1 (i.e. when the strength of evidence favours the defence hypothesis)

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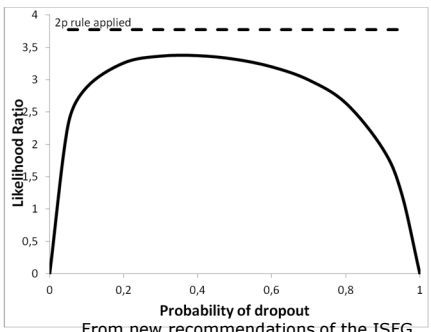
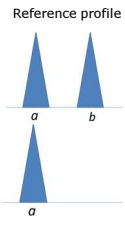
Dropout

(the 2p rule is always anti-conservative when this scenario is considered)





From new recommendations of the ISFG

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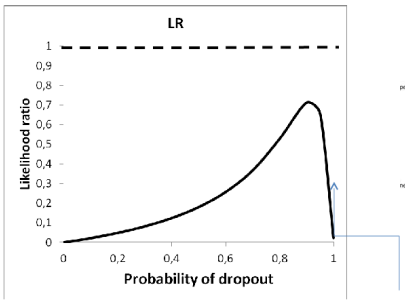
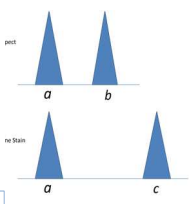
Drop-in





From new recommendations of the ISFG

This always favours the defence Hypothesis of exclusion – LR<1

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Ignoring alleles is anti-conservative as the evidence cannot be neutral (i.e. LR is not one)

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Method

Open-source software project FORENSIM

- http://forensim.r-forge.r-project.org/
- Supported by Euroforgen-NoE open-source software initiative.
- LRmixTK() module based on:

Curran, J. M.; Gill, P. & Bill, M. R. Interpretation of repeat measurement DNA evidence allowing for multiple contributors and population substructure Forensic Science International, 2005, 148, 47-53

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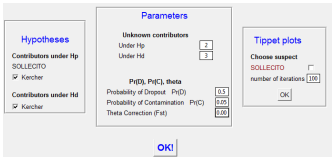
Analysis of item 165 (bra-clasp) using the exploratory approach

Step 1: Putting all issues of relevance to one side, discuss with the defence and prosecution the number of contributors in the profile.



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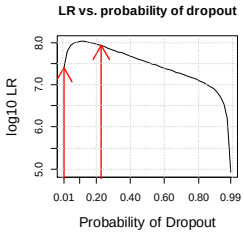
Step 2: How sensitive is the LR to the dropout probability and what is a reasonable range to consider?



"--------- drop-out ranges: under Hp------"
"5% percentile 0.03"
"95% percentile 0.23"
"--------- drop-out ranges: under Hd------"
"5% percentile 0.01"
"95% percentile 0.23"

```
PrD" "log10LR"
0.01 7.401
0.03 7.792
0.05 7.929
0.07 7.993
0.09 8.023
0.11 8.032
0.13 8.029
0.15 8.017
0.17 8
0.19 7.979
0.21 7.954
0.23 7.928
0.25 7.9
```

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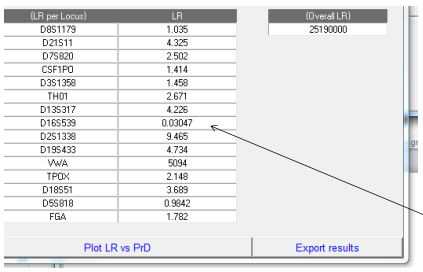
Step 3: Review LR relative to PrD=0.01



LR<1

Refer back to the epg – is further work indicated??
The process is exploratory – can further work be carried out? – ensure that the results are properly evaluated – we are not blindly generating numbers!

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Step 4: How robust is the answer?

- So far we have generated a number but we must be aware that different models will produced different answers
- Therefore we must make sure that the 'number' is meaningful
- How do we do this?
  - What happens with a different number of contributors?
  - What happens if we evaluate random individual(s)?
  - Is the LR always less than one if Hd is true?

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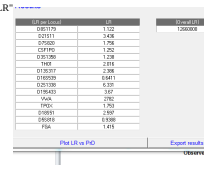
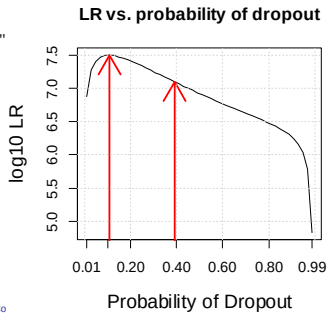
5 contributors – LR is same order of magnitude (10e8) (ie is relatively insensitive to no. of contributors)

"--------- drop-out ranges: under Hp------"
"5% percentile 0.13"
"95% percentile 0.37"
"--------- drop-out ranges: under Hd------"
"5% percentile 0.11"
"95% percentile 0.39"

"PrD" "log10LR"
0.01 6.884
0.03 7.272
0.05 7.407
0.07 7.468
0.09 7.494
0.11 7.5
0.13 7.494
0.15 7.479
0.17 7.458
0.19 7.432
0.21 7.404
0.23 7.374
0.25 7.342
0.27 7.308
0.29 7.275
0.31 7.24
0.33 7.206
0.35 7.171
0.37 7.137
0.39 7.102





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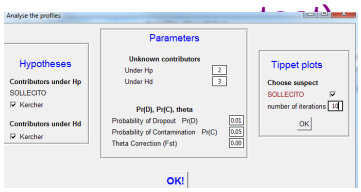
Now try new 'random suspects' (non- contributor robustness



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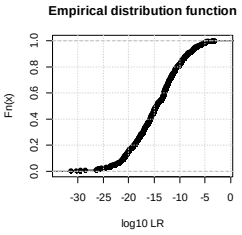
Simulation of 500 random individuals



Recall actual LR given by the model

(Overall LR)
25190000

"quantile" "value"
"min" "-31.3232"
"0.01" "-26.4818"
"0.05" "-22.7483"
"0.5" "-14.5926"
"0.95" "-6.8534"
"0.99" "-4.7681"
"max" "-3.1271"

Very remote chance of random man giving a LR=10e8

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Models to analyse complex STRs must be considered 'exploratory'

- There is not a single answer!
- And there isn't a single model!!!
- A likelihood ratio approach is used
- What are the (basic) model requirements:
  - Must be able to analyse multiple contributors
  - Must be able to incorporate *drop-out* and *drop-in*
- Robustness measurements are important
  - Replacing the defendant with a random man should give a markedly lower LR and we can plot the cdf.

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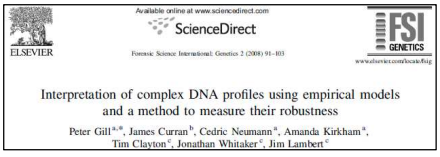
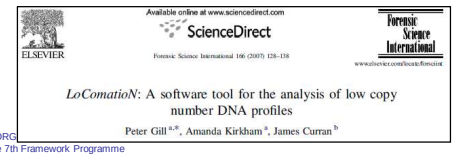
Description of the theory of the exploratory approach


Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
FSI GENETICS
Forensic Science International: Genetics 2 (2008) 91–103
www.elsevier.com/locate/fsig

Interpretation of complex DNA profiles using empirical models and a method to measure their robustness

Peter Gill [a,*], James Curran [b], Cedric Neumann [a], Amanda Kirkham [a], Tim Clayton [c], Jonathan Whitaker [c], Jim Lambert [c]


Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Forensic Science International
Forensic Science International 166 (2007) 128–138
www.elsevier.com/locate/forsciint

LoComatioN: A software tool for the analysis of low copy number DNA profiles

Peter Gill [a,*], Amanda Kirkham [a], James Curran [b]

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Relevance of evidence

- The analysis of the profile is valid but the issue of relevance is a separate question that is often confused.
- This is known as the 'CSI effect'.
- Many uncertainties remain about the relevance of the evidence
- Advice for scientists reporting DNA profiles (not just Low-level DNA)
  - The court first needs to be aware of the possible methods of transfer

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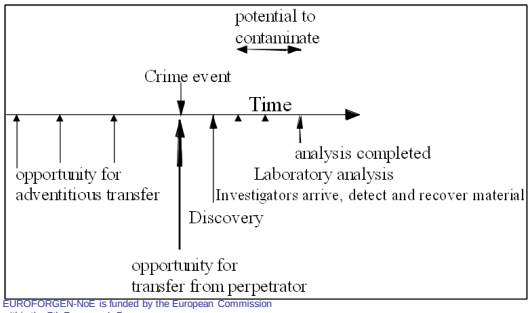
Generalised Timeline (this applies to all casework)



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Cross transfer issues

- Suspects and victim knew each-other and had access to each others premises on a regular basis
- The knife
  - Found in a kitchen cutlery drawer
  - No evidence of blood *(described as:"extremely clean")*
  - Not obvious why the knife was believed to be evidential
  - Questions raised about handling and packaging

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The bra clasp (found on the floor of the apartment)

- "The item was recovered 46 days after the crime, in a context highly suggestive of environmental contamination."
- **".....the documentation regarding possible contamination of the item, both before and after recovery, is inadequate**. The mere fact that the amplification control — which was not provided — was negative is not enough to rule out environmental contamination of the item previous to the extraction and amplification of the DNA. **It would have been necessary to obtain the allele profiles present in the surrounding environment**."
- **"extremely strict control protocols including the analysis of extracts from sterile cotton swabs soaked with sterile buffer that have passed on ambient surfaces to take dust samples (*Toothman MH et al., 2008*)."**

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Conclusions

- There is a general lack of understanding on the process of contamination

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Controls

- All scientific experiments rely on the use of adequate controls in order to demonstrate a meaningful result
- Forensic science is no different
- This case shows that it is necessary to screen for the prevalence of background DNA in order to provide meaningful results on the question of relevance
- However, much more research is needed to define the parameters, and procedures that should be followed
- This research needs to simulate casework environments (and the entire process of investigation) as closely as possible

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Final conclusion

- Transfer of DNA is much 'easier' than previously believed
- Introduction of AB 3500 and new multiplexes has greatly increased the opportunities to detect low level DNA using conventional methods (no definition of LtDNA is possible)
- Often these will be full profiles
- Therefore, much more caution is needed in reporting
- E.g. the association of an *activity* such as *stabbing*, with a DNA profile can never be definitively inferred simply by the presence of a DNA profile on a knife handle
- The relevance of the evidence and the probative value of the DNA profile are *two separate issues* to be dealt with.
- Unexpected ease of spread of DNA profiles means that scientists should be very cautious in reporting – it is suggested that evidence should not be inadvertently weighted to suggest that an *activity* is associated with a profile in the absence of other corroborating evidence
- Collection of background controls makes a lot of sense (but currently it is unlikely that this procedure is ever followed in practice).

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