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ISFG DNA commission recommendations

Peter Gill

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Forensic Science International 160 (2006) 90–101

Forensic Science International

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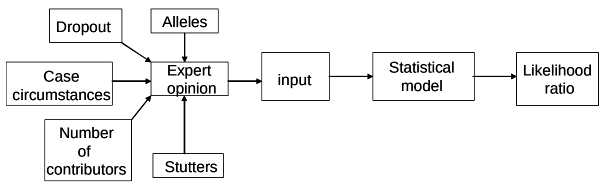
Interpretation process is an interaction of the expert with a statistical model



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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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Is it possible for a non-mixture to be confused with a mixture?

- A mixture may be identified by presence at 3 or 4 bands at each locus
- Masking will occur - this happens when two individuals share alleles
- Therefore it is possible for a mixture to have just one or two alleles at a locus
- is it possible for only 1 or 2 alleles to be seen at every locus in the multiplex?

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This experiment was carried out on the old SGM system

- Only 4 samples out of 200,000 showed 1 or 2 alleles per locus

Locu	D18S51	D18S51	D21S11	D21S11	HUMTH01	HUMTH01
Allele	1	2	1	2	1	2
Allele designations (1)	14	14	61	63	8	9.3
Allele designations (2)	14	17	63	63	8	9.3

- Note imbalance. If mixture is 1:1 then peaks for 2 loci will show 3:1 peak area imbalance. Only THO is balanced

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Forensic Bioinformatics Article

http://www.bioforensics.com/articles/empirical_mixtures.pdf

J Forensic Sci, Nov. 2005, Vol. 50, No. 6
Paper ID JFS2004475
Available online at: www.astm.org

David R. Paoletti,[1] M.S.; Travis E. Doom,[1,2] Ph.D.; Carissa M. Krane,[3] Ph.D.; Michael L. Raymer,[1,2] Ph.D.; and Dan E. Krane,[4] Ph.D.

Empirical Analysis of the STR Profiles Resulting from Conceptual Mixtures

TABLE 2—*Count and percent of three-person mixtures in which a particular number of unique alleles was the maximum observed across all loci, both for the original and randomized individuals*[+].

Unique Alleles	Count	Percent (%)
2	0	0.00%
3	78	0.00%
4	4,967,034	3.39%
5	93,037,010	63.49%
6	48,532,037	33.12%

Using 959 complete 13-locus STR profiles from FBI dataset

146,536,159 possible combinations **with 3-person mixtures**

3.39 % (4,967,034 combinations) would only show a maximum of four alleles (i.e., appear based on maximum allele count alone to be a 2-person mixture)

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Article by Buckleton et al.

Available online at www.sciencedirect.com

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Forensic Science International: Genetics 1 (2007) 20–28

FSI GENETICS

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

John S. Buckleton[a], James M. Curran[b,*], Peter Gill[c]

[a] *The Institute of Environmental Science and Research Ltd., Private Bag 92021, Auckland, New Zealand*
[b] *Department of Statistics, University of Auckland, Private Bag 92019, Auckland, New Zealand*
[c] *The Forensic Science Service, Trident Court, Solihull Parkway, Birmingham Business Park, Solihull B37 7YN, UK*

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Abstract

DNA evidence recovered from a scene or collected in relation to a case is generally declared as a mixture when more than two alleles are observed at several loci. However, in principle, all DNA profiles may be considered to be potentially mixtures, even those that show not more than two alleles at any locus. When using a likelihood ratio approach to the interpretation of mixed DNA profiles it is necessary to postulate the number of potential contributors. However, this number is never known with certainty. The possibility of a, say three-person mixture, presenting four or fewer peaks at each locus of the CODIS set was explored by Paoletti et al. [D.R. Paoletti, T.E. Doom, C.M. Krane, M.L. Raymer, D.E. Krane, Empirical analysis of the STR profiles resulting from conceptual mixtures, J. Forensic Sci. 50 (2005) 1361–1366]. In this work we extend this analysis to consider the profiler plus and SGM plus multiplices. We begin the assessment of the risk associated with current practice in the calculation of LR's. We open the discussion of possible ways to surmount this ambiguity.

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Two-Person Mixtures for Simulated Profiles:

Probability by Locus of A Particular Number of Alleles Being Observed

Table 1
The probability of observing a given number of alleles in a two-person mixtures for simulated profiles at the SGM^{+TM} loci

Loci	No. of alleles			
	1	2	3	4
D3	0.011	0.240	0.559	0.190
vWA	0.008	0.194	0.548	0.250
D16	0.016	0.287	0.533	0.164
D2	0.003	0.094	0.462	0.441
D8	0.011	0.194	0.521	0.274
D21	0.007	0.147	0.505	0.341
D18	0.003	0.095	0.472	0.430
D19	0.020	0.261	0.516	0.203
THO	0.016	0.271	0.547	0.166
FGA	0.003	0.116	0.500	0.381

Buckleton et al. (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *FSI Genetics* 1:20-28

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Three-Person Mixtures for Simulated Profiles: Probability by Locus of A Particular Number of Alleles Being Observed

Table 2
The probability of observing a given number of alleles in a three-person mixtures for simulated profiles at the SGM^{+TM} loci

Loci	No. of alleles showing					
	1	2	3	4	5	6
D3	0.000	0.053	0.366	0.463	0.115	0.002
vWA	0.000	0.037	0.285	0.468	0.194	0.016
D16	0.001	0.086	0.397	0.411	0.100	0.005
D2	0.000	0.008	0.104	0.385	0.393	0.110
D8	0.001	0.041	0.258	0.436	0.236	0.029
D21	0.000	0.023	0.192	0.428	0.302	0.055
D18	0.000	0.007	0.109	0.392	0.396	0.096
D19	0.003	0.078	0.352	0.401	0.152	0.014
THO	0.001	0.074	0.395	0.439	0.088	0.002
FGA	0.000	0.012	0.144	0.424	0.346	0.074

Buckleton et al. (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *FSI Genetics* 1:20-28

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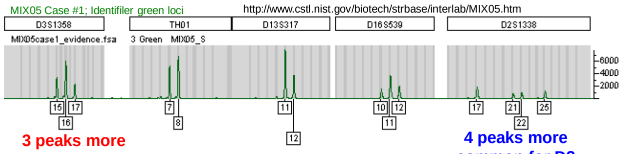
Levels of Locus Heterozygosity Impact Number of Alleles Observed in Mixtures (slide from J Butler)

Loci	No. of alleles 1	2	3	4
D3	0.011	0.240	0.559	0.190
vWA	0.008	0.194	0.548	0.250
D16	0.016	0.287	0.533	0.164
D2	0.003	0.094	0.462	0.441



3 peaks more common for D3

4 peaks more common for D2

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Number of Alleles Observed with Simulated Four-Person Mixtures

- The simulation of four person mixtures suggests that 0.014% of four person mixtures would show four or fewer alleles and that 66% would show six or fewer alleles for the SGM Plus loci.
- The results for the Profiler Plus loci were 0.6% and 75%.
- The equivalent values for the CODIS set from Paoletti et al. were 0.02% showing four or fewer and 76.35% showing six or fewer.

Buckleton et al. (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *FSI Genetics* 1:20-28

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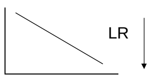
Anchoring the prosecution hypothesis

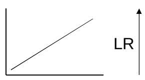
$$LR = \frac{\Pr E \mid Hp}{\Pr E \mid Hd}$$

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:



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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

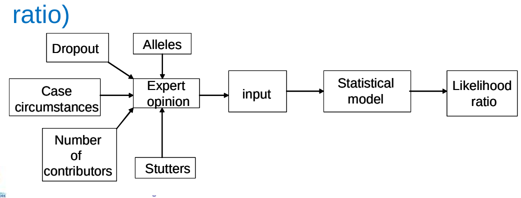
No. Contributors under *Hd*	*Pr|Hd*	LR<
1	.02	50
2	$.2^4$	625
3	$.2^6$	15625
4	$.2^8$	390625
5	$.2^{10}$	9765625

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.

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)



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

John S. Buckleton [a], James M. Curran [b,*], Peter Gill [c]
[a] The Institute of Environmental Science and Research Ltd., Private Bag 92021, Auckland, New Zealand
[b] Department of Statistics, University of Auckland, Private Bag 92019, Auckland, New Zealand
[c] The Forensic Science Service, Trident Court, Solihull Parkway, Birmingham Business Park, Solihull B37 7YN, UK

Received 31 May 2006; received in revised form 12 September 2006; accepted 13 September 2006

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

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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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LT-DNA

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

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]

[a] Norwegian Institute of Public Health, Oslo, Norway
[b] University of Oslo, Oslo, Norway

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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
- NY court found it to be a simple extension of an existing technique
- R. v. Reed accepted that the 34 cycle definition was not relevant to any definition of LT-DNA
- Why can't a definition be adduced?

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Stochastic variation

- In a heterozygous sample, one allele is amplified more than the other
- Leads to heterozygous imbalance or allele drop-out
  - Good quality DNA will always give heterozygous balance >60%
  - i.e. both target alleles are amplified with similar efficiency
- Much more pronounced with low level DNA as there is less template DNA
- If one target gets amplified more in the first rounds of PCR then it becomes preferentially amplified in later rounds

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Heterozygous imbalance OR allele drop-out (from J Butler, NIST)

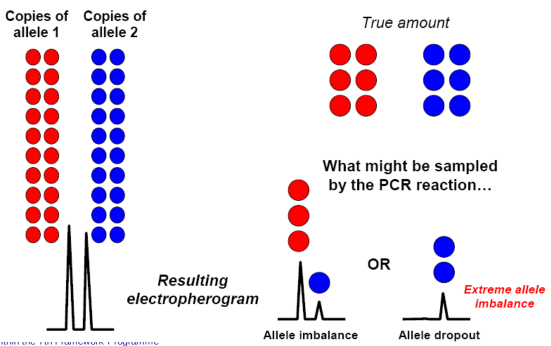

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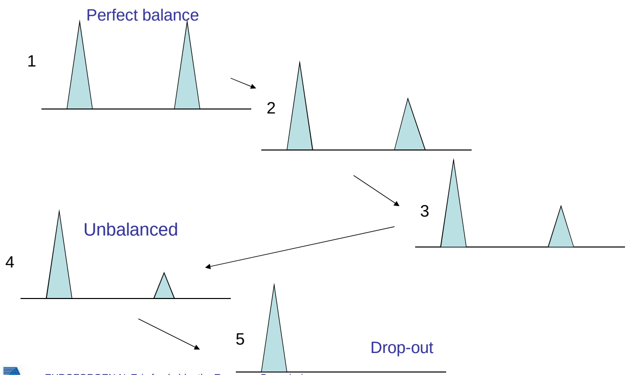
Illustration of heterozygous balance



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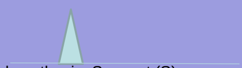
Explanation of drop-out

The DNA profile from the crime stain looks like this:

a b

And the DNA profile from the reference sample (Suspect) looks like this:



Prosecution hypothesis: Suspect (S)

Defence hypothesis: Unknown(U)

So IF Hp is true then dropout of allele b must have occurred

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An example

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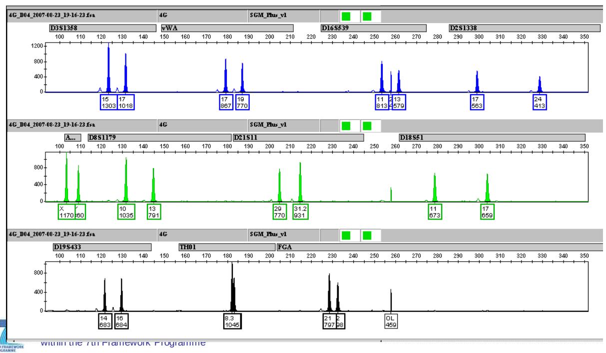
Good quality DNA (2)



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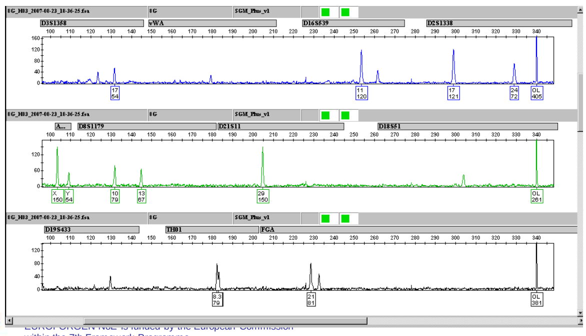
Low level DNA



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Illogical use of thresholds

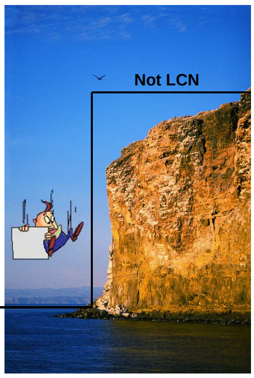
- Falling off the cliff
- E.g. if we have a

Rule that states:

150rfu – This is conventional

V. 149rfu – This is LCN

- There is nothing in between



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In reality it’s a gentle ride downhill

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Continuum of change

- The peak height rules break down when the quantity of DNA becomes very low – in particular the Hb guideline will no longer hold true
- Allele drop-out can lead to a heterozygous locus being genotyped as a homozygous locus
  - In standard DNA profiling, a homozygous peak height of 150 rfu is often used (stochastic threshold)
    - i.e. single peaks <150 rfu are labelled 'F' indicating allele drop-out may have occurred

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This is why we prefer a universal method

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A universal strategy to interpret DNA profiles that does not require a definition of *low-copy-number*

Peter Gill[a,b,*], John Buckleton[c]

[a] University of Strathclyde, Glasgow, UK
[b] Institute of Legal Medicine, University of Oslo, Oslo, Norway
[c] ESR, Auckland, New Zealand

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Papers outlining heterozygous balance

- Holt CL, Buoncristiani M, Wallin JM, Nguyen T, Lazaruk KD, Walsh PS. (2002) TWGDAM validation of AmpFlSTR PCR amplification kits for forensic DNA casework. J. Forensic Sci. 47(1): 66-96.
- Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. (2004) Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR amplification kit. J. Forensic Sci. 49(6): 1265-1277.
- L.A. Dixon, C.M. Murray, E.J. Archer, A.E. Dobbins, P. Koumi & P. Gill (2005) Validation of a 21-locus autosomal SNP multiplex for forensic identification purposes. For. Sci. Int. 154 (1): 62-77
- Gill, P., Sparkes, R. and Kimpton, C. (1997). "Development of guidelines to designate alleles using an STR multiplex system." *Forensic Sci Int* **89**(3): 185-197

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Degradation

- Occurs with fragmented / degraded DNA as there are more of the small target molecules available for amplification
- Leads to a distinctive slope in peak heights across the profile

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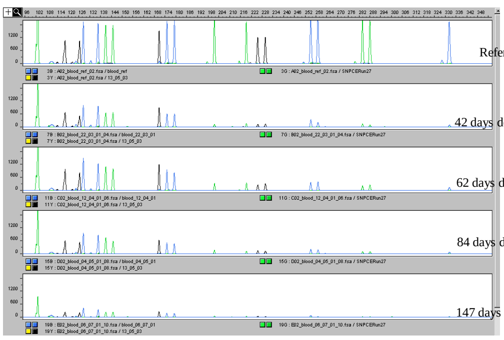
Effect of degradation



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Allele drop-in

- A contamination event resulting in only one or two foreign alleles
- Independent from gross contamination in that it comes from different sources

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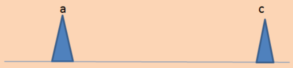
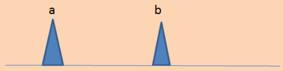
Drop-in

The DNA profile from the crime stain looks like this:



And the DNA profile from the reference sample (Suspect) looks like this:



Prosecution hypothesis: Suspect (S)
Defence hypothesis: Unknown(U)
So IF Hp is true then dropout of allele b must have occurred and drop-in of allele c must have occurred (of course this is unlikely and the LR should reflect this

It is not known if there is dropout in the crime-stain -alelels a and c are visible but other alleles may have dropped out and we consider this using the Q-allele where any allele (other than those visualised int he crime stain are considered possible)

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Contamination

- Gross contamination is identified as being from a single contributing source
- Dependent on transfer event as to when contamination occurred
- Could be pre-incident or post-incident

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LCN DNA profiling (34 cycles)

- Extracted and amplified in duplicate or triplicate using 50 uL reaction volume (20 uL DNA extract)
  - Most standard tests are now run at 25 uL

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LCN DNA consensus profiling

- Due to stochastic variation, some alleles may be amplified in one or two reactions but not another
- Consensus profiling allows a profile to be produced from different amplifications of the *same* DNA extract
- This is usually carried out with low level DNA profiling, regardless of the technique

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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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Is the 2p rule always conservative?

John Buckleton[a,*], Christopher Triggs[b]
[a] ESR, Mount Albert Science Center, Private Bag 92021, Auckland, New Zealand
[b] Department of Statistics, University of Auckland, Private Bag 92019, Auckland, New Zealand

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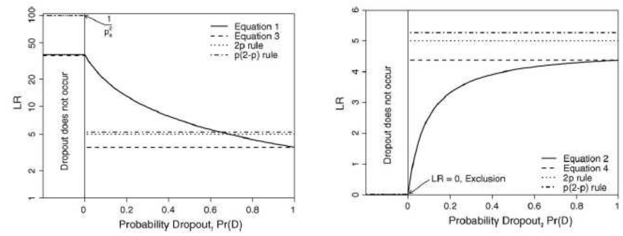

Fig. 1. Values of the LR for a homozygous profile derived using the 2p rule and Eq. (1) as the probability of dropout, Pr(D) increases from 0 to 1.

Fig. 2. Values of the LR for a heterozygous profile derived using the 2p rule and Eq. (2) as the probability of dropout, Pr(D) increases from 0 to 1.

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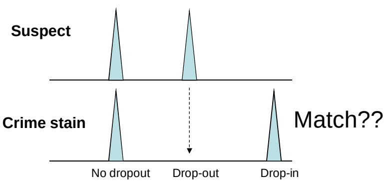
No need to decide if a profile is an exclusion/inconclusive/included



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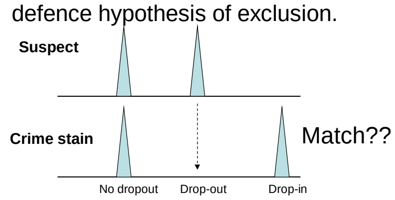
This is not an exclusion!
Its not neutral! But the evidence strongly supports the defence hypothesis of exclusion.



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	$2p_ap_b$	$\bar{D}DCp_c$	$2p_ap_b\bar{D}DCp_c$	0.000015	0.00075
ac	$2p_ap_c$	$\bar{D}^2\bar{C}$	$2p_ap_c\bar{D}^2\bar{C}$	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 interpre mixtures and can include stutters. **THIS LOOKS COMPLEX, BUT IT IS EASY TO FOLLOW**

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New methods

- Incorporate probability of dropout and dropin
- Uses statistical theory that is well established
- The theory can be used to evaluate complex mixtures
- No limitation on number of contributors
- No limitation on number of replicates that can be combined to form a single LR

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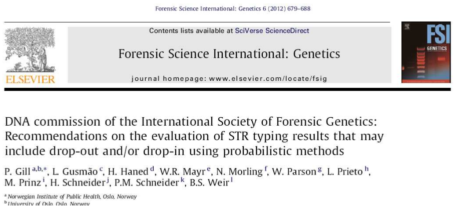
New ISFG DNA commission

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


Forensic Science International: Genetics 6 (2012) 679–688

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

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]

[a] Norwegian Institute of Public Health, Oslo, Norway
[b] University of Oslo, Oslo, Norway

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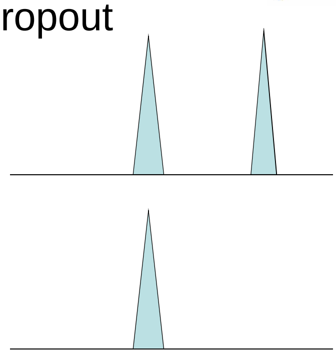
Dropout

- Suspect

- Crime stain



Match??

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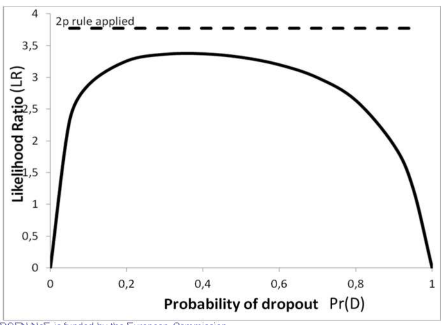
Fig. Locus D18S51 frequencies are used as an example, where allele a corresponds to D18S51 allele 13 (frequency: 0.135). Using the 2p rule: LR=1/3.Effect of Pr(D) on LR. S is ab, E is a. The likelihood ratio LR=Pr(E|S)/Pr(E|U) is plotted as a function of Pr(D) ∊ [0,1]. (2pa) = 1/(2x0.135) = 3.8 (dashed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



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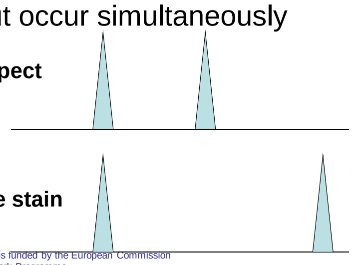
Drop-in

- 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

Suspect

Crime stain



Match??

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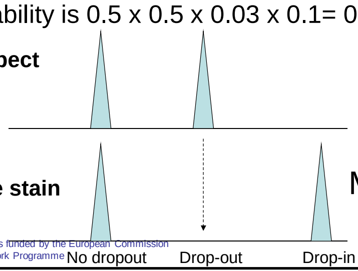
How can this be a match?

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.

Suspect

Crime stain



Match??

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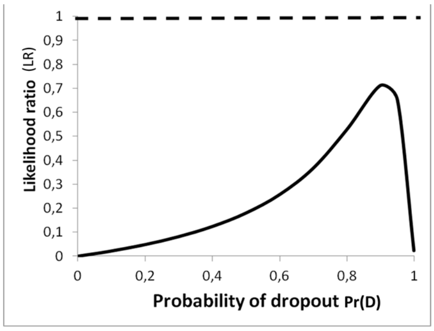
Fig. 5. Effect of Pr(D) on LR. Suspect is ab and crime stain evidence is ac. Locus D18S51allele 13 frequency was used to calculate the LR example (pa=0.135). Since LR<1, then this favours Hd. The dashed line indicates LR = 1.



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Putting theory into practice: Analysis of a complex mixture using *new genetic models*

- New tools that can be used for low copy number and for conventional DNA profiles
- Methods 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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