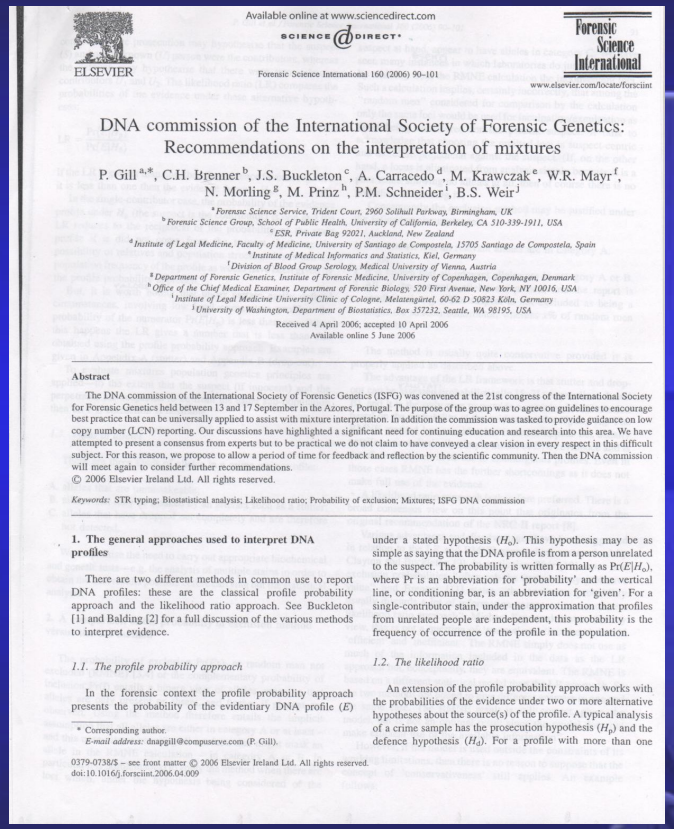
Le raccomandazioni dell'ISFG: la definizione di *stutter bands*



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Forensic Science International 160 (2006) 90–101
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DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

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Received 4 April 2006; accepted 10 April 2006
Available online 5 June 2006

Abstract

The DNA commission of the International Society of Forensic Genetics (ISFG) was convened at the 21st congress of the International Society for Forensic Genetics held between 13 and 17 September in the Azores, Portugal. The purpose of the group was to agree on guidelines to encourage best practice that can be universally applied to assist with mixture interpretation. In addition the commission was tasked to provide guidance on low copy number (LCN) reporting. Our discussions have highlighted a significant need for continuing education and research into this area. We have attempted to present a consensus from experts but to be practical we do not claim to have conveyed a clear vision in every respect in this difficult subject. For this reason, we propose to allow a period of time for feedback and reflection by the scientific community. Then the DNA commission will meet again to consider further recommendations.

Keywords: STR typing; Biostatistical analysis; Likelihood ratio; Probability of exclusion; Mixtures; ISFG DNA commission

1. The general approaches used to interpret DNA profiles

There are two different methods in common use to report DNA profiles: these are the classical profile probability approach and the likelihood ratio approach. See Buckleton [1] and Balding [2] for a full discussion of the various methods to interpret evidence.

1.1. The profile probability approach

In the forensic context the profile probability approach presents the probability of the evidentiary DNA profile (E) under a stated hypothesis (H_a). This hypothesis may be as simple as saying that the DNA profile is from a person unrelated to the suspect. The probability is written formally as $\Pr(E|H_a)$, where Pr is an abbreviation for 'probability' and the vertical line, or conditioning bar, is an abbreviation for 'given'. For a single-contributor stain, under the approximation that profiles from unrelated people are independent, this probability is the frequency of occurrence of the profile in the population.

1.2. The likelihood ratio

An extension of the profile probability approach works with the probabilities of the evidence under two or more alternative hypotheses about the source(s) of the profile. A typical analysis of a crime sample has the prosecution hypothesis (H_p) and the defence hypothesis (H_d). For a profile with more than one

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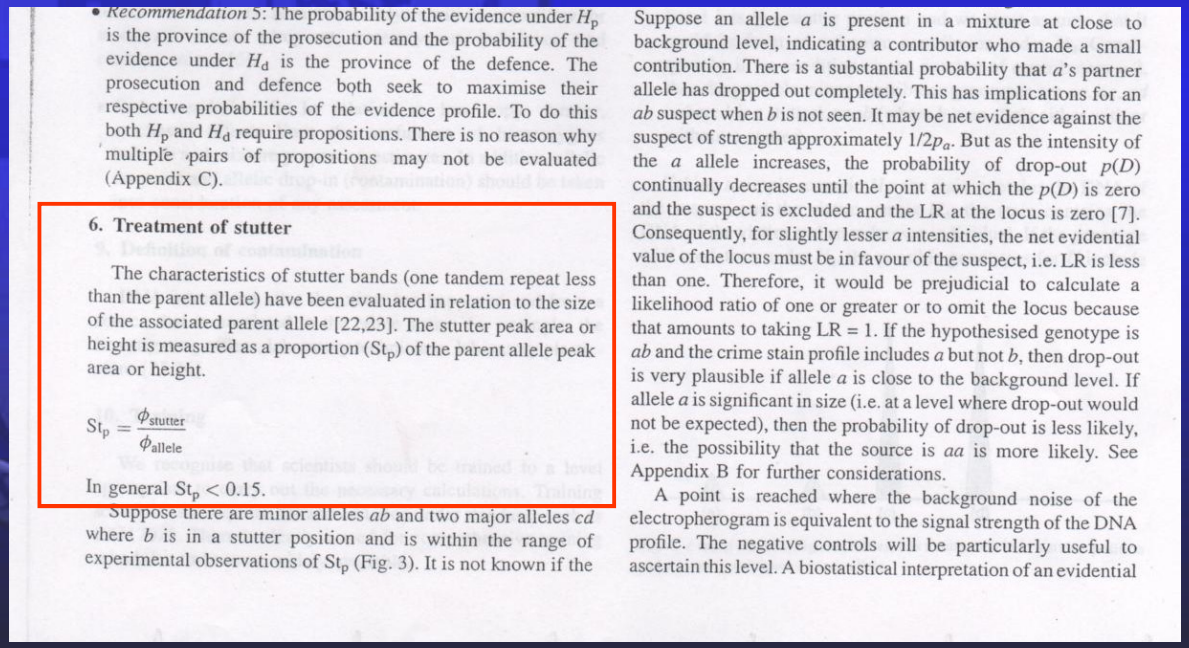
doi:10.1016/j.forsciint.2006.04.009



• *Recommendation* 5: The probability of the evidence under H_p is the province of the prosecution and the probability of the evidence under H_d 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 H_p and H_d require propositions. There is no reason why multiple pairs of propositions may not be evaluated (Appendix C).

6. Treatment of stutter

The characteristics of stutter bands (one tandem repeat less than the parent allele) have been evaluated in relation to the size of the associated parent allele [22,23]. The stutter peak area or height is measured as a proportion (St_p) of the parent allele peak area or height.

$$St_p = \frac{\phi_{stutter}}{\phi_{allele}}$$

In general $St_p < 0.15$.

Suppose there are minor alleles *ab* and two major alleles *cd* where *b* is in a stutter position and is within the range of experimental observations of St_p (Fig. 3). It is not known if the

Suppose an allele *a* is present in a mixture at close to background level, indicating a contributor who made a small contribution. There is a substantial probability that *a*'s partner allele has dropped out completely. This has implications for an *ab* suspect when *b* is not seen. It may be net evidence against the suspect of strength approximately $1/2p_a$. But as the intensity of the *a* allele increases, the probability of drop-out $p(D)$ continually decreases until the point at which the $p(D)$ is zero and the suspect is excluded and the LR at the locus is zero [7]. Consequently, for slightly lesser *a* intensities, the net evidential value of the locus must be in favour of the suspect, i.e. LR is less than one. Therefore, it would be prejudicial to calculate a likelihood ratio of one or greater or to omit the locus because that amounts to taking LR = 1. If the hypothesised genotype is *ab* and the crime stain profile includes *a* but not *b*, then drop-out is very plausible if allele *a* is close to the background level. If allele *a* is significant in size (i.e. at a level where drop-out would not be expected), then the probability of drop-out is less likely, i.e. the possibility that the source is *aa* is more likely. See Appendix B for further considerations.

A point is reached where the background noise of the electropherogram is equivalent to the signal strength of the DNA profile. The negative controls will be particularly useful to ascertain this level. A biostatistical interpretation of an evidential

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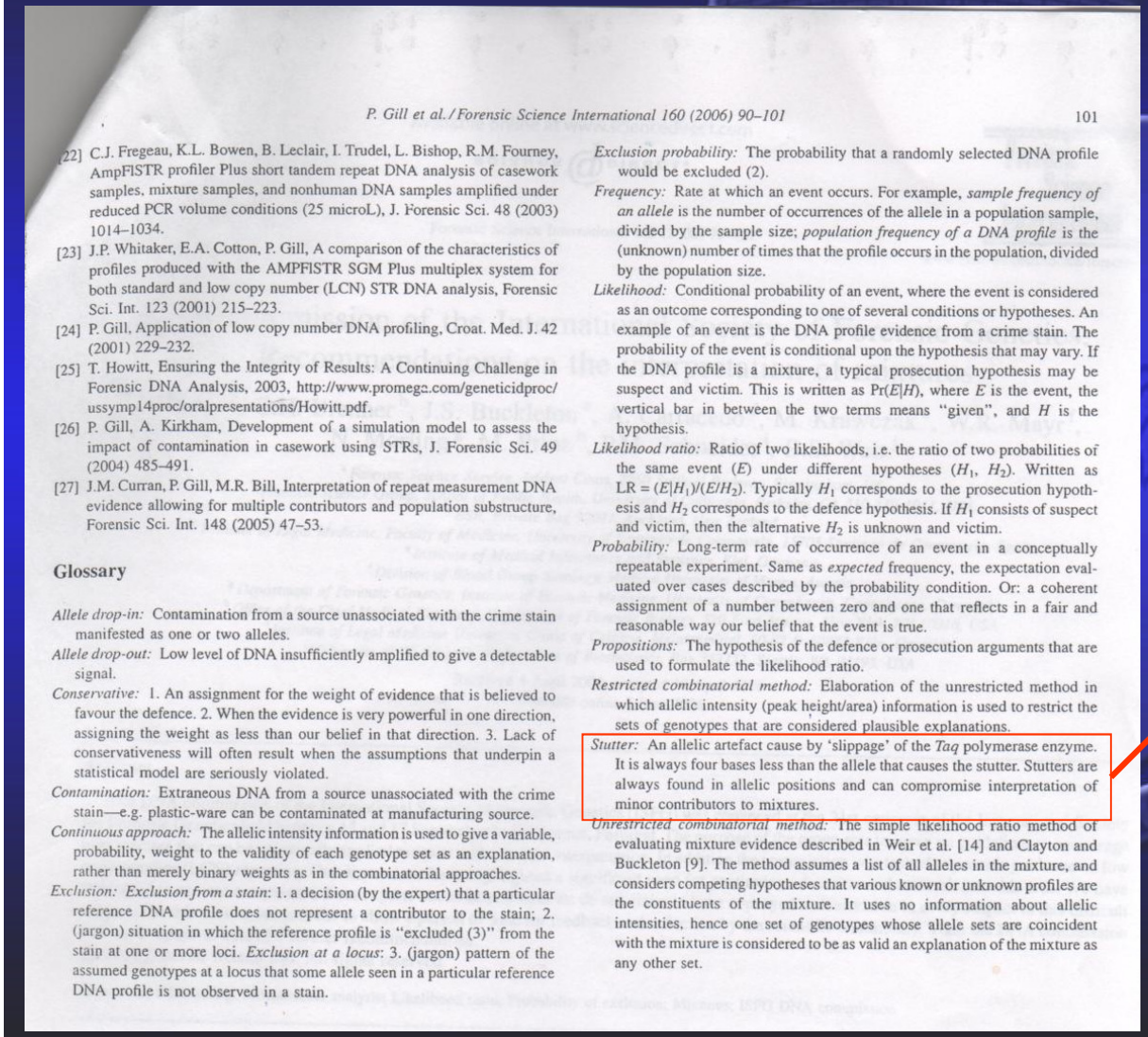

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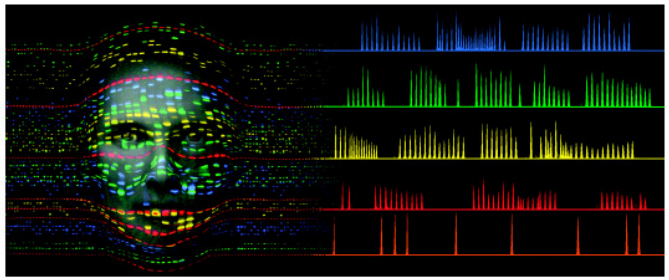
Glossary

Allele drop-in: Contamination from a source unassociated with the crime stain manifested as one or two alleles.
Allele drop-out: Low level of DNA insufficiently amplified to give a detectable signal.
Conservative: 1. An assignment for the weight of evidence that is believed to favour the defence. 2. When the evidence is very powerful in one direction, assigning the weight as less than our belief in that direction. 3. Lack of conservativeness will often result when the assumptions that underpin a statistical model are seriously violated.
Contamination: Extraneous DNA from a source unassociated with the crime stain—e.g. plastic-ware can be contaminated at manufacturing source.
Continuous approach: The allelic intensity information is used to give a variable, probability, weight to the validity of each genotype set as an explanation, rather than merely binary weights as in the combinatorial approaches.
Exclusion: Exclusion from a stain: 1. a decision (by the expert) that a particular reference DNA profile does not represent a contributor to the stain; 2. (jargon) situation in which the reference profile is "excluded (3)" from the stain at one or more loci. *Exclusion at a locus*: 3. (jargon) pattern of the assumed genotypes at a locus that some allele seen in a particular reference DNA profile is not observed in a stain.
Exclusion probability: The probability that a randomly selected DNA profile would be excluded (2).
Frequency: Rate at which an event occurs. For example, *sample frequency of an allele* is the number of occurrences of the allele in a population sample, divided by the sample size; *population frequency of a DNA profile* is the (unknown) number of times that the profile occurs in the population, divided by the population size.
Likelihood: Conditional probability of an event, where the event is considered as an outcome corresponding to one of several conditions or hypotheses. An example of an event is the DNA profile evidence from a crime stain. The probability of the event is conditional upon the hypothesis that may vary. If the DNA profile is a mixture, a typical prosecution hypothesis may be suspect and victim. This is written as $Pr(E|H)$, where E is the event, the vertical bar in between the two terms means "given", and H is the hypothesis.
Likelihood ratio: Ratio of two likelihoods, i.e. the ratio of two probabilities of the same event (E) under different hypotheses (H_1, H_2). Written as $LR = (E|H_1)/(E/H_2)$. Typically H_1 corresponds to the prosecution hypothesis and H_2 corresponds to the defence hypothesis. If H_1 consists of suspect and victim, then the alternative H_2 is unknown and victim.
Probability: Long-term rate of occurrence of an event in a conceptually repeatable experiment. Same as *expected* frequency, the expectation evaluated over cases described by the probability condition. Or: a coherent assignment of a number between zero and one that reflects in a fair and reasonable way our belief that the event is true.
Propositions: The hypothesis of the defence or prosecution arguments that are used to formulate the likelihood ratio.
Restricted combinatorial method: Elaboration of the unrestricted method in which allelic intensity (peak height/area) information is used to restrict the sets of genotypes that are considered plausible explanations.
Stutter: An allelic artefact cause by 'slippage' of the *Taq* polymerase enzyme. It is always four bases less than the allele that causes the stutter. Stutters are always found in allelic positions and can compromise interpretation of minor contributors to mixtures.
Unrestricted combinatorial method: The simple likelihood ratio method of evaluating mixture evidence described in Weir et al. [14] and Clayton and Buckleton [9]. The method assumes a list of all alleles in the mixture, and considers competing hypotheses that various known or unknown profiles are the constituents of the mixture. It uses no information about allelic intensities, hence one set of genotypes whose allele sets are coincident with the mixture is considered to be as valid an explanation of the mixture as any other set.

Un artefatto allelico causato dallo "scivolamento" dell'enzima Taq polimerasi. Esso si presenta sempre 4 basi più piccolo dell'allele che causa la "stutter".Le bande "stutter" si ritrovano sempre in una posizione allelica e ciò può compromettere l'interpretazione dei contributi minori nelle misture

Le specifiche tecniche del kit di amplificazione



AmpFℓSTR® Identifiler®

PCR Amplification Kit

User's Manual

Applied Biosystems

Extra Peaks in the Electropherogram

Causes of Extra Peaks

To further demonstrate reproducibility, 1187 population database DNA samples have been typed using the AmpFℓSTR Identifiler PCR Amplification Kit. These samples have been previously genotyped with concordant results of the same loci using other AmpFℓSTR kits.

Peaks other than the target alleles may be detected on the electropherogram displays. Several causes for the appearance of extra peaks, including the stutter product (found at the n–4 position), incomplete 3´ A nucleotide addition (found at the n–1 position), artifacts and mixed DNA samples (see 8.1.2.2).

Stutter Products

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter (n–4) than the corresponding main allele peak. This is referred to as the stutter peak or product. Sequence analysis of stutter products at tetranucleotide STR loci has revealed that the stutter product is missing a single tetranucleotide core repeat unit relative to the main allele (Walsh *et al.*,1996).

The proportion of the stutter product relative to the main allele (percent stutter) is measured by dividing the height of the stutter peak by the height of the main allele peak. Such measurements have been made for amplified samples at the loci used in the AmpFℓSTR Identifiler kit. All data were generated on the ABI PRISM 310 Genetic Analyzer.

Some of the general conclusions from these measurements and observations are as follows:

- For each AmpFℓSTR Identifiler kit locus, the percent stutter generally increases with allele length, as shown in Figures 4-4, 4-5, 4-7 and 4-8. Smaller alleles display a lower level of stutter relative to the longer alleles within each locus. This is reflected in Figures 4-4 through 4-7, where minimal data points are plotted for some smaller alleles, as stutter could not be detected for many of these samples.
- For the alleles within a particular locus, the percent stutter is generally greater for the longer allele in a heterozygous sample (this is related to the first point above).
- Each allele within a locus displays percent stutter that is reproducible.
- The highest percent stutter observed for each allele is as follows: CSF1PO, 9.2%; D2S1338, 11.1%; D3S1358, 10.7%; D5S818, 6.8%; D7S820, 8.2%; D8S1179, 8.2%; D13S317, 8.0%; D16S539,