CHAPTER

12

DNA Mixture Statistics

"There is a considerable aura to DNA evidence. Because of this aura it is vital that weak evidence is correctly represented as weak or not presented at all."

Buckleton & Curran (2008)

INTRODUCTION

The points that have been addressed in the previous chapter involve obtaining a "clean" full DNA profile from a single-source. However, crime scene evidence can produce mixed DNA profiles from more than one individual (see Chapters 6 and 7). These mixtures can be challenging to interpret and may never be unambiguously separated depending on the short tandem repeat (STR) alleles present in the profile of the individual components. An indistinguishable mixture is a DNA profile where major and minor(s) components cannot be ascertained with confidence. In addition, partial profiles where entire loci have dropped out sometimes occur due to the presence of degraded DNA or polymerase chain reaction (PCR) inhibitors.

Many forensic cases involve multiple pieces of DNA evidence, and not all of these will be mixtures. Thus, if additional samples can be tested that are easier to interpret, they should be sought after versus complicated mixtures. Reference samples from individuals (e.g. victims or suspects) may unexpectedly exhibit a mixed STR profile. Natural DNA mixtures can exist in some people from birth or temporarily following a bone marrow transplant (D.N.A. Box 12.1).

At the turn of the 21st century, mixtures did not represent a majority of cases in forensic DNA laboratories, especially if a good differential extraction was performed in a sexual assault case where the sperm fraction could be fully separated from the victim's DNA (see Butler 2012). As an example, over a four-year period from 1997 to 2000, one forensic laboratory in Spain worked 1,547 criminal cases that involved a total of 2,424 samples, yet only 163 showed a mixed profile or 6.7% (Torres et al. 2003). In more recent years, however, crimes in which touch DNA evidence is present, such as burglaries, are investigated, and therefore an increasing number of mixtures are being observed (Roman et al. 2008).

The statistical methods described in this chapter will focus on two-person mixtures that contain a relatively high amount of DNA such that allele drop-out is not expected. Sexual assault evidence with a mixture of DNA from a victim and a perpetrator is an example of a sample in this category (Figure 12.1). Chapter 13 will cover situations where allele drop-out is a possibility, such as exists in complex mixtures with three or more contributors or compromised DNA samples.

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D.N.A. BOX 12.1

NATURAL MIXTURES AND CHIMERIC INDIVIDUALS

In May 2002, the *New England Journal of Medicine* published a report of the genetic analysis of a phenotypically normal chimeric individual who was unexpectedly identified because histocompatibility testing of family members suggested that she was not the biological mother of two of her three children (Yu et al. 2002). The doctors examining this chimeric individual proposed that her condition had arisen because two fertilized eggs, destined originally to be fraternal twins, had fused to form a zygote that possessed DNA of two different types. Thus, from a genetic perspective she was both her children's mother and their aunt.

Among the various genetic tests performed on this chimeric individual was analysis of 22 STR loci. All of the 13 U.S. core loci except CSF1PO were examined in this study. This unusual patient possessed some differences in her STR profiles among various tissues tested. While the buccal and blood samples that were tested matched exactly, a mixture containing another type was present as the minor component in her thyroid, hair, and skin cells.

While chimeric individuals such as the one described above are most likely extremely rare in the general population, it is possible in theory for DNA testing from different tissues of a chimeric individual to not match one another and thus lead to a false exclusion. This situation may increase in frequency with the rise of in vitro fertilization since multiple eggs are sometimes fertilized in order to increase the success rate of the procedure.

STR profiles from chimeric individuals have been seen in forensic cases and observed in single-source reference samples. An individual may exhibit chimeric characteristics, where some body tissues have a natural mixture of DNA from the donor and the recipient, following a bone marrow transplant. A study of DNA from buccal swabs of 77 recipients of allogeneic hematopoietic cell transplantation found relative donor chimerism levels between 0% and 100%, although blood always exhibited the donor's STR genotypes and hair samples always provided the recipient's genotypes (Berger et al. 2013). This study points out that if blood stem cell transplantation has occurred between a donor and a recipient then plucked hair samples (with roots) should be collected in addition to standard buccal swabs to obtain the recipient's true DNA profile.

Sources: *Yu* et al. *(2002)* New England Journal of Medicine, 346, *1545–1552; David Baron, "DNA tests shed light on hybrid human," National Public Radio-Morning Edition, August 11, 2003 (http://www.npr.org); Berger, B., et al. (2013). Chimerism in DNA of buccal swabs from recipients after allogeneic hematopoietic stem cell transplantations: implications for forensic DNA testing.* International Journal of Legal Medicine, 127, *49–54.*

Genotypes – Not Alleles – Matter in Profiles!

Since humans are diploid and typically possess two alleles at each locus, it is important to keep in mind that it is the genotype, or specific combination of alleles (the allele pair) found at each locus in an individual, that matters in both interpretation and statistical analysis. Simply describing and determining the statistical rarity of alleles present at a locus and in the entire DNA profile does not reflect the true nature of the sample contributor(s). Thus, a fundamental tenet of mixture interpretation involves effort to decipher possible *genotype* combinations of contributors, not simply the evaluation of whether or not *alleles* are present.

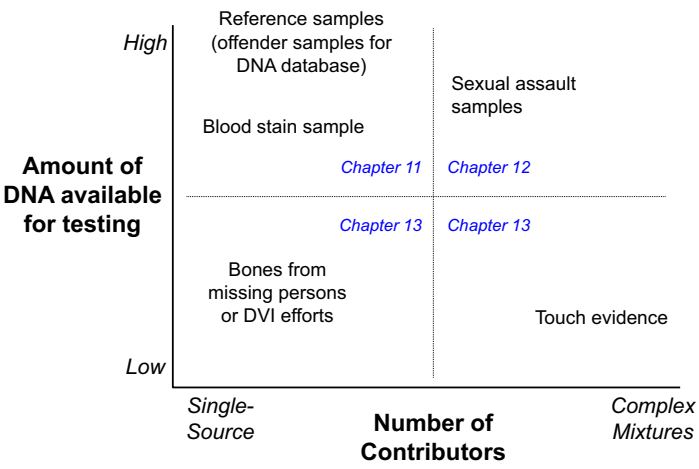


FIGURE 12.1 Comparison of sample types and challenges in terms of the amount of DNA available for testing and the potential number of contributors. Sexual assault samples typically involve DNA mixtures from people who are not related, whereas touch evidence (e.g. an item from a home) may very well have DNA from related people in a complex mixture. Statistical interpretation information in Chapter 11 focused on single-source samples with high amounts of DNA, and information in Chapter 12 is directed primarily to two-person mixtures with high amounts of DNA. Principles explained in Chapter 13 deal with low amounts of DNA that may be seen in complex mixtures or compromised samples from disaster victim identification (DVI) efforts.

D.N.A. Box 12.2 provides a simple test to see whether a person is thinking in terms of genotypes or alleles when it comes to mixture interpretration and evaluation. Specific assumptions as to the number of potential contributors in a mixture informs the interpretation process. These decisions are made in the context of examining a DNA profile as a whole and not focusing on a single locus.

An essential part of any DNA interpretation effort involves assuming the number of contributors. Making a specific assumption is sometimes referred to as *conditioning*. Quantitative information in the form of peak heights can aid efforts to reduce the number of genotype combinations that are reasonable in a mixture. While *unrestricted* approaches permit all possible combinations of genotypes with the alleles observed at a locus, *restricted* approaches limit the number of genotypes under consideration. As discussed in Chapter 6, use of peak height ratios (PHRs) and examination of mixture ratios across the profile are important elements in forming the assumptions regarding what can be restricted (Clayton et al. 1998). Appropriate statistical methods should be applied that correspond to the interpretation an analyst has made of the evidence.

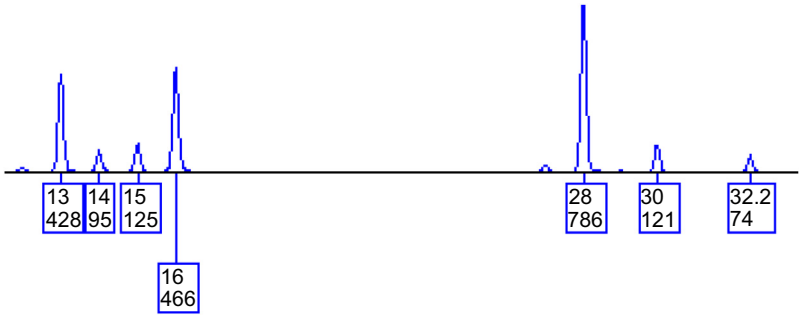
Examples help improve understanding of concepts. A few simple illustrations are provided in this chapter to introduce concepts, and a more detailed worked example is contained in Appendix 4 at the back of the book.

Figure 12.2 illustrates some potential mixture data from a single STR locus. Since alleles P and Q are much taller than alleles R and S, they may be grouped into a major genotype of PQ and a minor genotype of RS. With this example, the major component is a 14,16 genotype and the minor component is an 18,19 genotype. This mixture deconvolution used to create these inferred allele pairs is conditioned on an assumption of two contributors.

If more than two contributors are assumed, then the other peaks labeled "stutter?" could be from additional contributors to the original biological sample. The decision as to the number of potential contributors comes from examining the STR profile as a whole, not just this single locus (see Chapter 5). The process of considering restricting potential genotype combinations by using relative peak height information and mixture proportions is reviewed in the example found in Appendix 4.

D.N.A. BOX 12.2

ARE YOU THINKING IN TERMS OF ALLELES RATHER THAN GENOTYPES?



This profile can be used as a simple test to see if an analyst is thinking in terms of alleles or genotypes. **Would you include or exclude a reference sample that is 13,14 and 28,30 at these two loci?** The alleles 13 and 14 and 28 and 30 are present, but, are allele pairs (genotypes) of 13,14 and 28,30 reasonable for contributors to this mixture result? Of course the answer depends on information from additional loci and careful consideration of locus peak height ratios (PHRs) and mixture proportions (M_x).

If (because of information from the entire profile) it can be assumed that there are only two contributors, then the reasonable major contributor for the locus on the left would be 13,16 (PHR = 0.92) and the reasonable minor contributor would be 14,15 (PHR = 0.76), although this value may be slightly skewed due to some stutter contribution in the allele 15 position from allele 16. Thus, the genotype 13,14, which has an unreasonable PHR of 0.22, would be excluded from this two-person mixture. Likewise, for the locus on the right, a 28,30 genotype possesses an unreasonable PHR of 0.15. It would be more reasonable to assume that the major contributor is a 28,28 genotype and the minor contributor is a 30,32.2 genotype. Assessment of the number of contributors and likely genotypes present in this mixture should be completed *prior to comparison to a reference sample*. The important point to note here is that under a specific assumption of two contributors the genotypes of 13,14 and 28,30 are not reasonable, and therefore these genotypes should be excluded as possibilities even though the alleles are present!

If, instead, an analyst is thinking only in terms of alleles without conditioning on the number of contributors, which is often done when approaching a mixture interpretation scenario with a combined probability of inclusion (CPI) viewpoint, then the presence of alleles 13 and 14 for the locus on the left and alleles 28 and 30 for the locus on the right can lead to inclusion of unreasonable genotypes.

Genotypes of possible contributors matter — not simply the presence of corresponding alleles between reference samples and the evidentiary mixture profile. Likewise, assumptions as to the number of contributors are crucial to effective mixture interpretation. If more than two contributors can be reasonably assumed based on review of the profile as a whole, then some low-level allele information may be missing in this situation. For

example, a possible pairing allele may not be amplified and detected to complete the true genotype with the 95 RFU allele 14 in the left locus or with the 74 RFU allele 32.2 in the right locus. If all alleles and genotypes are not represented at a locus, then an inconclusive result may need to be reported at least for the minor component(s) due to insufficient data being available to render a reliable conclusion on the evidentiary result.

Source: *Charlotte Word presentation at the 2012 International Symposium on Human Identification mixture workshop; see http://www.cstl.nist.gov/strbase/training/ISHI2012-MixtureWorkshop-DifferentAssumptions.pdf (slide 16).*

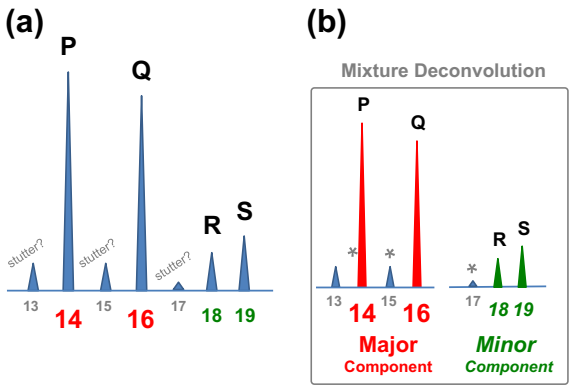


FIGURE 12.2 Hypothetical example of STR mixture data at the D18S51 locus. (a) The crime scene evidence data possesses alleles 14, 16, 18, and 19. Additional peaks at allele positions 13, 15, and 17 could be stutter products of the observed alleles or potentially alleles from other contributor(s), depending on the number of contributors present in the mixture. The suspect is 14,16 and the victim is 18,19. (b) Assuming two contributors, the mixture profile from (a) can be divided (deconvoluted) into major and minor components using relative peak height ratios to determine genotype combinations. Assuming this result is from a two-person mixture, the best explanation for peaks labeled with asterisks (*) is that they are stutter products.

Importance of Quantitative Assessments for Mixture Results

In the past, laboratory reports have provided qualitative assessment of mixture results with statements such as "the profile of the suspect cannot be excluded as being a possible contributor to the crime scene DNA mixture." Qualitative statements, such as "cannot be excluded" without a numerical qualifier, can be dangerous because an investigator or the court may assume that this situation with "failure to exclude" is equivalent to a DNA "match" with astronomical numbers like those described in Chapter 11 for single-source evidence and reference sample associations.

Presenting a simple qualitative statement of inclusion or exclusion rather than performing any calculations is often not satisfactory in a court of law where a judge typically requires some kind of numerical estimate to give statistical weight to the evidence. As pointed out in *The Evaluation of Forensic DNA Evidence*, "to make appropriate use of DNA technology in the courtroom, the trier of fact must give the DNA evidence appropriate weight" (NRC 1996, p. 203).

As John Buckleton and James Curran have observed: "There is a considerable aura to DNA evidence. Because of this aura it is vital that weak evidence is correctly represented as weak or not presented at all" (Buckleton & Curran 2008). With some mixtures where "failure to exclude" a particular suspect is the conclusion reached, a very large percentage of the population could also be part of the mixture evidence. In such a situation, the inclusionary statement may be weak, such as 1 in 10 or 1 in 100 randomly selected, unrelated individuals could also be part of the mixture result.

For this reason, the Scientific Working Group on DNA Analysis Methods (SWGDAM) in their 2010 interpretation guidelines clearly state in section 4.1: "The laboratory must perform statistical analysis in support of any inclusion that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis" (SWGDAM 2010). Furthermore, the SWGDAM 2010 guidelines state that "the genetic loci and assumptions used for statistical calculations must be documented, at a minimum, in the case notes." Approaches to providing a statistic to the DNA mixture are provided below.

DIFFERENT STATISTICAL APPROACHES TO MIXTURE INTERPRETATION

As stated in earlier chapters, there is no one universal formula that fits all situations when interpreting DNA evidence. This reality certainly applies for mixture interpretation and reporting. Various laboratories have adopted different approaches to these challenging situations. There are three primary approaches to interpretating two-person DNA mixtures (Ladd et al. 2001).

First, if major and minor components can be separated into individual profiles due to sufficient differences in peak heights (see Chapter 6), then it may be possible to compute the random match probability (RMP) for the individual profiles as if each component was from a single source. However, since RMP technically refers to single-source samples, use of this statistic for deduced profiles is typically referred to as a modified RMP (mRMP) (see SWGDAM 2010). Genotypes are restricted to specific combinations using allele peak heights and mixture proportions, as described in Chapter 6 (see D.N.A. Box 6.4).

With the mRMP approach, it is sometimes only possible to interpret the major profile confidently as ambiguity may prevent complete deciphering of the minor component genotype(s) due to potential allele masking by the major alleles or potential allele drop-out with low-level minor components. Example calculations for two-person and three-person mixtures with mRMP have been published (Bille et al. 2013). This approach requires specific assumptions as to the number of contributors in the mixture.

Second, for indistinguishable mixtures, where allele peak heights and mixture proportions are similar, exclusion (or inclusion) probabilities may be calculated from the evidence profile. The random man not excluded (RMNE) approach utilizes combined probability of inclusion (CPI) statistics where all possible genotype combinations are given equal weight. A probability of inclusion (PI) calculation involves summing all of the observed alleles at a locus and then squaring this value to obtain the combination of all possible genotypes.

Individual locus PI values are multiplied to obtain an overall profile CPI. A CPI calculation is performed on the evidence and is therefore independent of any suspect's STR profile genotypes. A CPI statistic does not need any assumptions as to the number of contributors because it is calculating all possible combinations of genotypes based on the evidence profile results. A combined probability of exclusion (CPE) can also be determined, which is 1 − CPI (SWGDAM 2010).

Because each possible genotype is given an equal weight, this approach wastes information and is not very efficient when genotypes are in fact distinguishable.

Third, likelihood ratios (LRs) can be calculated where the weight of evidence is ascertained by examining the probability of observing the mixture data under one hypothesis compared to the probability of observing the mixture data under a second, mutually exclusive hypothesis. LRs can be unrestricted (if based on allele calls only) or restricted (if peak height information is used to limit the number of possible genotype combinations). When applied to two-person mixtures, a restricted LR is equivalent to the inverse of the mRMP except when there is no known contributor (Bille et al. 2013). LR calculations require an assumption as to the number of contributors in the mixture.

Table 12.1 compares these three methods in terms of the questions addressed, how a statistical answer is estimated, and specific requirements. More detail will be provided on these approaches later in the chapter.

TABLE 12.1 Comparison of Methods Used to Statistically Evaluate DNA Mixtures

Method	What Question Is Asked?	How Is the Answer Estimated?	Specific Requirements
Modified random match probability (mRMP)	Having inferred a specific major component (and possibly a minor component), what is the probability of the inferred profile occurring in a specific population based on population genetic models and STR allele frequencies? *Note*: This approach is dependent on the ability to reliably infer specific genotypes based on peak height information and will generally not work well with low-level data where stochastic effects occur.	Following mixture deconvolution where major and minor components are deduced using peak height information, the random match probability is calculated for deduced genotypes as if they originated from a single-source sample	Typically an assumption of two contributors; usually a 4:1 or greater major-to-minor component ratio to provide confidence that a clean separation can be obtained between the major and minor components so that specific genotypes can be inferred
Random man not excluded (RMNE)	What fraction of the population would not be excluded (i.e. would be included) as a potential contributor to the crime scene evidence mixture? *Note*: This statistic, which is calculated from the evidence result (Q profile), does not directly answer whether or not a specific suspect (known K profile) can be included in the mixture result. The Q-to-K profile comparison can be somewhat subjective with more complex mixtures.	Combined probability of inclusion (CPI) which involves giving equal weight to all potential genotypes based on alleles observed; is easily calculated by squaring the sum of frequencies of observed alleles	Alleles are all present (i.e. no allele drop-out), contributors are not related
Likelihood ratio (LR)	What is the weight of evidence for or against a specific suspect being in the crime scene evidence mixture? *Note*: LRs can be challenging to formulate with mixtures containing more than two contributors	Formulating a ratio of probabilities for the evidence under two different hypotheses with specific genotype combinations	An assumption as to the number of contributors and specification of a hypothesis for the prosecution (H_p) and a hypothesis for the defense (H_d)

See also Buckleton & Curran (2008), Bille et al. (2013).

NRC I and NRC II on DNA Mixtures

As described in Appendix 2, there have been two National Research Council (NRC) reports written on forensic DNA analysis: (NRC I) "DNA Technology in Forensic Science" published in 1992 and (NRC II) "The Evaluation of Forensic DNA Evidence" released in 1996. An examination of their discussions on DNA mixture interpretation is instructive. Some of these topics are still not fully appreciated by forensic DNA scientists almost two decades later.

On page 59 of NRC I (NRC 1992): "If the samples are mixtures from more than one person, one should see additional bands for all or most polymorphic probes, but not for a single-copy monomorphic probe. Mixed samples can be very difficult to interpret because the components can be present in different quantities and states of degradation. It is important to examine the results of multiple RFLPs, as a consistency check. Typically, it will be impossible to distinguish the individual genotypes of each contributor. If a suspect's pattern is found within the mixed pattern, the appropriate frequency to assign such a 'match' is *the sum of the frequencies of all genotypes that are contained within (i.e. that are a subset of) the mixed pattern*." Emphasis has been added here to show what is a description of the combined probability of inclusion (CPI) statistic.

In NRC II (NRC 1996) the discussion of mixed samples is found on pages 129 and 130: "Mixed samples are sometimes found in crime situations — for instance, blood from two or more persons at the scene of a crime, victim and assailant samples on a vaginal swab, and material from multiple sexual assailants. … when the contributors to a mixture are not known or cannot otherwise be distinguished, *a likelihood ratio approach offers a clear advantage and is particularly suitable*" (emphasis added). The NRC II text continues by quoting the 1992 report page 59 statement (shown above) and an example CPI calculation is illustrated.

The top of page 130 begins (NRC 1996): "That [CPI] calculation is hard to justify, because it does not make use of some of the information available, namely, the genotype of the suspect. The correct procedure [i.e. the likelihood ratio approach], we believe, was described by Evett et al. (1991)." A simple example is then illustrated for the likelihood ratio approach. The text continues: "This LR [likelihood ratio], compared with that derived from the recommendation of the 1992 NRC report [i.e. CPI], is larger when the suspect bands are relatively rare and smaller when the suspect bands are relatively common. The reason is that *we have taken account of the information in the genotype of the suspect rather than averaging over the set of possible genotypes* consistent with the four-band evidence-sample profile" (emphasis added).

The text then notes (NRC 1996): "We have considered only simple cases. With VNTRs [variable number of tandem repeats], it is possible, though very unlikely, that the four bands were contributed by more than two persons, who were either homozygous or shared rare alleles. With multiple loci, it will usually be evident if the sample was contributed by more than two persons. Calculations taking those possibilities into account could be made if there were reason to believe that more than two persons contributed to the sample. …The problem is complex, and some forensic experts follow the practice of making several reasonable assumptions and then using the calculation that is most conservative. For a fuller treatment of mixed samples, see Weir et al. (1997)."

The final section of NRC II that deals with mixtures is found on pages 162 and 163 (NRC 1996): "Mixed stains introduce a number of complexities. We limit our consideration to cases in which the stain comes from two persons, but only one suspect is identified." …[In the case of potential allele drop-out], "the 2p rule may be needed." A table (NRC II, Table 5.1) provides some likelihood ratio formulas for each potential combination of crime scene and suspect genotypes in which there is either

TABLE 12.2 Likelihood Ratios for Mixed Stains Using p^2 Rule for True Homozygotes Assuming No Allele Drop-Out or $2p$ Rule for a False Homozygote Where Allele Drop-Out Has Occurred

Crime Scene (question genotype)	Suspect (known genotype)	Likelihood Ratio (assuming no drop-out, p^2)	Likelihood Ratio (assuming drop-out, $2p$)
PQRS	PQ	$\frac{1}{12pq}$	$\frac{1}{12pq}$
PQR	QR	$\frac{p+2q+2r}{12qr(p+q+r)}$	$\frac{1+q+r}{12qr(p+q+r)}$
PQR	P	$\frac{1}{6p(p+q+r)}$	$\frac{1}{4p(3+p+q+r)}$
PQ	PQ	$\frac{(p+q)^2}{2pq(3pq+2p^2+2q^2)}$	$\frac{p+q+pq}{2pq(2+2p+2q+pq)}$
PQ	P	$\frac{2p+q}{2p(3pq+2p^2+2q^2)}$	$\frac{1+p}{2p(2+2p+2q+pq)}$

Allele frequencies (p, q, r, s) for observed alleles (*P, Q, R, S*) can be obtained from population databases, such as Appendix 1. Adapted from (NRC 1996, p. 163).

a false homozygote (2p) or a true homozygote (p^2). The information in this table has been reorganized and included in Table 12.2.

DNA Advisory Board 2000 Statement on Mixtures

The DNA Advisory Board (DAB) was a 13-member, congressionally mandated entity created and funded by the United States Congress DNA Identification Act of 1994. During its 1995–2000 tenure, the DAB discussed challenges facing forensic DNA and issued guidance to the community. Bruce Budowle (FBI Laboratory), Fred Bieber (Harvard Medical School, Boston), Ranajit Chakraborty (University of Texas Health Science Center, Houston), and George Carmody (Carleton University, Ottawa) formed the statistical subcommittee of the DAB and thus were involved in writing the February 2000 statistical issues statement that was published later in *Forensic Science Communications* (see Appendix 3).

A few excerpts from the DAB statistical issues document regarding mixtures are included below:

> *"…When intensity differences are sufficient to identify the major contributor in the mixed profile, it can be treated statistically as a single-source sample. At times, when alleles are not masked, a minor contributor to the mixed profile may be elucidated. Almost always in a mixture interpretation, certain possible genotypes can be excluded.* ***It may be difficult to be confident regarding the number of contributors in some complex mixtures of more than two individuals****; however, the number of contributors often can be inferred by reviewing the data at all loci in a profile.*

> "…*When the contributors of a DNA mixture profile cannot be distinguished, two calculations convey the probative value of the evidence… The PE [probability of exclusion] provides an estimate of the portion of the population that has a genotype composed of at least one allele not observed in the mixed profile. Knowledge of the accused and/or victim profiles is not used (or needed) in the calculation. The calculation is particularly useful in analyses involving complex mixtures because it requires no assumptions about the identity or number of contributors to a mixture. The probabilities derived are valid and for all practical purposes are conservative. However*, the PE does not make use of all of the available genetic data.

> "*Calculation of an LR considers the identity and actual number of contributors to the observed DNA mixture.* Certainly, LR makes better use of the available genetic data than does the PE. … The DAB finds either one or both PE or LR calculations acceptable *and strongly recommends that one or both calculations be carried out whenever feasible and a mixture is indicated*" (DAB 2000, see Appendix 3, emphasis added).

When this statement was made in 2000 by the DAB, the types of mixtures being encountered in laboratories were mostly simple two-person mixtures primarily from sexual assault evidence (Clayton et al. 1998, Torres et al. 2003). Complex mixtures with three or more contributors were not routinely encountered by forensic DNA laboratories at that time. Therefore trying to extrapolate the DAB statement to the challenges faced with touch evidence today, in which allele drop-out is common, (see Chapter 13) is probably not appropriate.

ISFG 2006 Recommendations and SWGDAM 2010 Interpretation Guidelines

Chapter 6 introduces the July 2006 International Society for Forensic Genetics (ISFG) nine recommendations covering mixture interpretation principles and the SWGDAM 2010 interpretation guidelines (see Table 6.4).

The ISFG DNA Commission that issued the July 2006 mixture recommendations was chaired by Peter Gill (UK Forensic Science Service) and had as co-authors mathematicians and statisticians Charles Brenner (DNA View, Berkeley, California), John Buckleton (ESR, New Zealand), Michael Krawczak (Institute of Medical Informatics and Statistics, Kiel, Germany), and Bruce Weir (University of Washington, Seattle) in addition to the ISFG Board members Angel Carracedo (Spain), Wolfgang Mayr (Austria), Niels Morling (Denmark), Mecki Prinz (USA), and Peter Schneider (Germany).

A primary emphasis of the ISFG recommendations was the value of using the likelihood ratio as a preferred approach to mixture interpretation (Gill et al. 2006). Recommendation 1 notes that RMNE may not be conservative with low-level profiles (see Buckleton & Triggs 2006). Recommendation 2 encourages scientists to be trained in the likelihood ratio methodology and reminds the scientific community that it "has a responsibility to support improvement of standards of scientific reasoning in the court-room" (Gill et al. 2006).

As noted in Chapter 6, published support for the principles underlying the ISFG recommendations has come from the European DNA Profiling Group (EDNAP) and the European Network of Forensic Science Institutes (ENFSI) DNA working group (Morling et al. 2007), the UK DNA working group (Gill et al. 2008), an FBI-led mixture committee (Budowle et al. 2009), Australian and New Zealand forensic leadership (Stringer et al. 2009), and the German Stain Commission (Schneider et al. 2009).

Further guidance on the use of probabilistic genotyping for statistical analysis of DNA mixtures having the potential of allele drop-out and drop-in was published by the ISFG DNA Commission in December 2012 (Gill et al. 2012). This material will be discussed in Chapter 13.

SWGDAM approved interpretation guidelines in January 2010 that address mixture interpretation and statistical analysis. As reviewed earlier in this chapter, these SWGDAM guidelines require statistical analysis in support of any inclusion and documentation for assumptions and data used in generating numerical support for a conclusion. The SWGDAM guidelines review statistical principles and formulas for mRMP, CPI, and LR (SWGDAM 2010).

The "frequently asked questions" section of the SWGDAM website (SWGDAM FAQs 2014) states that the 2010 guidelines were "written with single-source samples and two-person mixtures in mind," and that while "the *basic concepts* outlined in the 2010 SWGDAM Mixture Interpretation Guidelines hold true as they relate to DNA mixtures of three or more contributors, low-level DNA samples, and mixtures containing biologically related individuals…there are nuances and limitations to the interpretation of these more complex mixtures, which are not fully explored in the 2010 guidelines" (emphasis in the original). This statement by SWGDAM also encourages laboratories to "perform additional validation studies of complex mixtures to further their understanding of the issues related to these challenging samples."

Allele Masking and Handling Alleles in the Stutter Position

Two primary challenges with interpreting DNA mixtures involve allele masking and handling potential minor contributor alleles in the stutter position of major contributor alleles. Allele masking results when two STR alleles from different contributors are indistinguishable in terms of size. Since these same-size alleles comigrate through the capillary electrophoresis separation process, their individual signals stack on top of each other. This is known as *allele sharing* or *allele stacking*. The outcome for a minor allele is *allele masking* by a major allele. In other words, the smaller height minor allele is hidden within the taller peak height of the major allele.

Sometimes multiple minor alleles can add up to produce the appearance of a false apparent major allele (see Figure 6.6). Allele masking and stacking present a problem for mixture interpretation because it is impossible to know exactly how much of the peak-height signal arises from one mixture component versus another. Assumptions can be made to partition part of a potential shared allele peak height to different potential contributor genotypes through assuming a certain peak height ratio with a potential sister allele peak (see example in Appendix 4 and Figure A4.4).

In the Figure 12.2 example, the "stutter?" labeled peaks can be categorized as *indistinguishable from stutter* (Heidebrecht 2013). When minor alleles have peak heights that are similar in amount to stutters of major alleles, then these stutter peaks and minor alleles are indistinguishable and may need to be accounted for in the interpretation of the profile (Gill et al. 2006, SWGDAM 2010). Depending on the assumed number of contributors in the STR profile, those indistinguishable from stutter peaks could be part of a minor component.

In the case of Figure 12.2, if we assume two contributors, then the PQ (major) and RS (minor) account for all of the possible genotypes and the peaks at allele positions 13, 15, and 17 can be assumed to be only stutter peaks of alleles 14, 16, and 18. However, if the number of contributors is not assumed (as with a CPI calculation) or if the number of potential contributors can reasonably be assumed to be greater than two, then these peaks have to be considered as alleles that could: (1) potentially pair with alleles masked by the major allele peaks (P and Q) or (2) potentially form genotypes with the minor allele peaks (R and S) or alleles masked by these minor peaks. Ambiguity increases as the potential number of contributors increases.

REPORTING MATCH PROBABILITIES ON DEDUCED COMPONENTS

In some cases it may be possible to confidently pull apart the alleles from individual contributors to a mixture. In cases of sexual assault, the victim's DNA profile is typically compared to the mixed profile and this comparison may be helpful in identifying the STR alleles present in the perpetrator's DNA profile.

The DNA Advisory Board recommendations on statistics issued in February 2000 state that "when intensity differences are sufficient to identify the major contributor in the mixed profile, it can be treated statistically as a single source sample" (DAB 2000; see Appendix 3). In such a situation, after deciphering the individual components for the major and minor contributors, the statistical treatment of their profiles could be conducted as described in Chapter 11 for single-source samples.

Interpretation of genotypes present in a mixture is much more complicated when the contributions of the donors are approximately equal and thus a major contributor cannot be definitively determined, or when true alleles for a contributor are masked by stutter products (see Chapter 6) or other alleles in the mixture. It is not always possible to unambiguously determine all of the alleles present in a mixture, especially with a partial profile from a degraded DNA sample. Likewise, it is not always possible to infer the complete genotypes of all contributors with a high degree of confidence because the mixture combination may be too complex to easily decipher.

Computer programs using probabilistic genotyping approaches (see Chapter 13) have been developed to help interpret DNA mixtures that are too complex to easily solve by manually considering all potential combinations (Perlin et al. 2013, Balding 2013, Taylor et al. 2013).

RANDOM MAN NOT EXCLUDED (RMNE)

A statistical significance can be placed on a DNA mixture following the interpretation process using the combined probability of inclusion (CPI) method (DAB 2000, Ladd et al. 2001). The random man not excluded (RMNE) concept, which utilizes the CPI calculation, was originally developed for paternity testing (Devlin 1993). This probability-of-inclusion statistic provides an estimate of the portion of the population that has a genotype composed of at least one allele observed in the mixed profile. Alternatively, results can be reported from the other perspective with the combined probability of exclusion (CPE). CPE and CPI add up to 1, so as mentioned earlier, $CPE = 1 - CPI$.

In the example data illustrated in Figure 12.2, determination of CPI would involve calculation of the frequency of genotypes that possess the alleles 14, 16, 18, and 19 present in the evidentiary mixed profile. Depending on the peak heights of the "stutter?" peaks in Figure 12.2, they may need to also be considered in the CPI calculation.

The CPI approach avoids the potential pitfalls associated with attempting to decipher specific genotype combinations of mixture contributors (e.g. overlooking a true allele because it is in a stutter position). No prior knowledge or assumptions regarding the number of possible contributors to the mixture are needed and results can still be reported without knowledge of a known profile, such as the victim's profile. Problems can arise when trying to associate the mixture profile to a specific suspect. Determining whether a suspect is included or excluded from the mixture is a separate step (Buckleton & Curran 2008) that can sometimes be subjective.

While calculating the CPI is not as powerful a technique as the likelihood ratio method that is discussed in the next section, a supposed advantage with the CPI approach is that the number of contributors to the crime scene DNA profile does not need to be taken into account. Simply all alleles

observed in the stain are considered in the CPI statistic. In principle, this approach is considered conservative because an individual can be excluded if he/she has any allele at any locus that is not detected in the stain. However, as will be seen in the next chapter, things are not always quite so simple when allele drop-out is a possibility, and attempts are being made to interpret low-level DNA results.

The inclusion probability reflects the combined frequency of all genotypes that can be included in a mixture, assuming Hardy–Weinberg equilibrium for the genotype frequencies (see Chapter 10). CPI is based on the evidence only. Selecting different loci for comparison purposes, something often referred to as "suspect-driven CPI" is inappropriate since decisions on which loci are suitable for comparison should be made prior to doing a comparison to reference sample(s).

What often makes CPI a subjective process with some DNA mixtures is that decisions about whether a reference sample is appropriately included in the mixture are made outside of the statistical framework. LR methods, on the other hand, actually address the question of the suspect's profile, locus-by-locus, genotype-by-genotype.

Example CPI Calculation

For the locus on the left in D.N.A. Box 12.2 with alleles 13, 14, 15, and 16, a CPI calculation accounts for genotypes 13,13 and 14,14 and 15,15 and 16,16 (four homozygotes) as well as 13,14 and 13,15 and 13,16 and 14,15 and 14,16 and 15,16 (six heterozygotes). Under a CPI approach, no consideration is given to the relative peak heights present in the four alleles. Alleles 13, 14, 15, and 16 for this locus are effectively being viewed as equal in intensity.

All possible genotypes are considered with equal probabilities in RMNE (Table 12.3). Thus, with a CPI calculation, a homozygote (such as r^2) is given the same weight as a heterozygote (e.g. $2pr$) that may fit the mixture pattern better. In the D.N.A. Box 12.2 (left locus) example, only two genotypes are reasonable due to relative peak height ratios when assuming two contributors: 13,16 and 14,15. The

TABLE 12.3 Possible Genotypes Considered with Equal Probabilities for Random Man Not Excluded (RMNE) Approach using Combined Probability of Inclusion (CPI) Calculations

Alleles Observed in Mixture	Possible Genotypes	Genotype Frequencies for RMNE = (sum of allele frequencies)^2
1 allele: P	PP	$p^2 = p^2$
2 alleles: P, Q	PP, QQ, PQ	$p^2 + q^2 + 2pq = (p + q)^2$
3 alleles: P, Q, R	PP, QQ, RR, PQ, PR, QR	$p^2 + q^2 + r^2 + 2pq + 2pr + 2qr = (p + q + r)^2$
4 alleles: P, Q, R, S	PP, QQ, RR, SS, PQ, PR, PS, QR, QS, RS	$p^2 + q^2 + r^2 + s^2 + 2pq + 2pr + 2ps + 2qr + 2qs + 2rs = (p + q + r + s)^2$
5 alleles: P, Q, R, S, T	PP, QQ, RR, SS, TT, PQ, PR, PS, PT, QR, QS, QT, RS, RT, ST	$p^2 + q^2 + r^2 + s^2 + t^2 + 2pq + 2pr + 2ps + 2pt + 2qr + 2qs + 2qt + 2rs + 2rt + 2st = (p + q + r + s + t)^2$
6 alleles: P, Q, R, S, T, U	PP, QQ, RR, SS, TT, UU, PQ, PR, PS, PT, PU, QR, QS, QT, QU, RS, RT, RU, ST, SU, TU	$p^2 + q^2 + r^2 + s^2 + t^2 + u^2 + 2pq + 2pr + 2ps + 2pt + 2pu + 2qr + 2qs + 2qt + 2qu + 2rs + 2rt + 2ru + 2st + 2su + 2tu = (p + q + r + s + t + u)^2$

use of additional genotypes in the statistical calculation effectively dilutes the overall statistic and robs it of its potential probative power. Note that the more observed alleles there are, the more unreasonable genotypes there are.

The CPE wastes a lot of information from the evidentiary sample because not all of these possibilities are equally probable. Likelihood ratios, on the other hand, are specific to the case scenario of interest. With LR calculations, data are evaluated to see how well they address a proposition that the mixture is composed of specific combinations of genotypes that happen to match the individuals of interest in the case.

Limitations of CPI/CPE

Since all possible genotype combinations need to be considered with CPI, all observed alleles need to be included in the calculation. SWGDAM 2010 Guideline 4.6.3 states: "When using CPE/CPI (with no assumptions of number of contributors) to calculate the probability that a randomly selected person would be excluded/included as a contributor to the mixture, loci with alleles below the stochastic threshold may not be used for statistical purposes to support an inclusion. In these instances, *the potential for allelic drop-out raises the possibility of contributors having genotypes not encompassed by the interpreted alleles*" (SWGDAM 2010, emphasis added).

CPI Is Not an Interpretation Tool!

CPI is a statistical calculation and should not be used as an interpretation tool. Analysts should interpret their evidentiary profiles by examining potential genotype combinations considering relative peak heights, potential minor alleles in stutter positions of major contributors, etc. Unfortunately, through simply looking at the presence of alleles and blindly applying a stochastic threshold (see Chapter 4) to decide when loci should be dropped from statistical calculations, some analysts have inappropriately used CPI as an *interpretation* tool.

One of the most significant deficiencies of the CPI calculation is that this approach does not take into account an alternative hypothesis. How can you make an informed opinion on a topic if you consider only a single point of view? This is why likelihood ratios are essential in mixture interpretation and why they have been strongly recommended by the ISFG DNA Commission (Gill et al. 2006) — because two different possibilities are compared in developing an opinion. In the words of Professor Max Baur of Bonn University in Germany: "RMNE is a deficient method and we should not use it!" (Author notes from ISFG 2009 conference).

LIKELIHOOD RATIOS

The likelihood ratio (LR) is the ratio of possibilities under alternative propositions and provides a reliable method that is able to make full use of available genetic data (Evett & Weir 1998, Buckleton & Curran 2008). Two competing hypotheses are set up: the hypothesis of the prosecution (H_p), which is that the defendant committed the crime, and the hypothesis of the defense (H_d), that some unknown individual committed the crime. Thus, the LR involves a ratio describing the probability of the evidence given the prosecution's hypothesis over the probability of the evidence given the defense's hypothesis:

$$LR = \frac{Pr(E|H_p)}{Pr(E|H_d)}$$

Unfortunately, determination of which hypotheses to consider is not necessarily straightforward. Interpretation of a mixture depends on the circumstances of the case and involves assumptions about the identity and number of contributors to the mixture in question. LR calculations are more widely used in Europe than in the United States for forensic applications. Paternity testing routinely uses LR calculations (see Chapter 14). The LR method makes better use of the available genetic data than does the CPI method discussed previously (Buckleton & Curran 2008).

If evidence contains four alleles at a locus (P, Q, R, and S) and the victim possesses R and S while the suspect exhibits P and Q, then the prosecution's hypothesis would be that the DNA evidence is from the victim and the suspect. On the other hand, the defense's hypothesis would be that the DNA evidence is from the victim and an unknown person. The probability of the prosecution's hypothesis is one because their position is that they are 100% confident (probability = 1) that the defendant committed the crime, which is why the trial is occurring in the first place.

The defense's hypothesis can vary depending on the circumstances of the case, such as the number of other possible contributors under consideration and the alleles present in the evidentiary DNA profile. The H_d considers that the suspect is truly innocent and the DNA profile came from an unknown, unrelated individual. The likelihood ratio describes the relative chance of observing a specific mixture and combination of STR alleles. Some LR examples for various scenarios are listed in Table 12.2. Hypothesis statements for three general categories of two-person mixtures are provided in Table 12.4.

TABLE 12.4 Three General Categories of Two-Person Mixture Likelihood Ratio (LR) Calculations

Category	Hypothesis Statements for Prosecution (H_p) and Defense (H_d)	Likelihood Ratio
1	**H_p**: mixture contains the DNA of the victim and the suspect **H_d**: mixture contains the DNA of the victim and an unknown, unrelated person	$LR = \frac{V+S}{V+U}$
	H_p: mixture contains the DNA of the suspect and the victim **H_d**: mixture contains the DNA of the suspect and an unknown, unrelated person	$LR = \frac{V+S}{S+U}$
2	**H_p**: mixture contains the DNA of suspect 1 and suspect 2 **H_d**: mixture contains the DNA of two unknown, unrelated people	$LR = \frac{S1+S2}{U1+U2}$
	H_p: mixture contains the DNA of victim 1 and victim 2 **H_d**: mixture contains the DNA of two unknown, unrelated people	$LR = \frac{V1+V2}{U1+U2}$
3	**H_p**: mixture contains the DNA of the suspect and an unknown, unrelated person **H_d**: mixture contains the DNA of two unknown, unrelated people	$LR = \frac{S+U}{U1+U2}$
	H_p: mixture contains the DNA of the victim and an unknown, unrelated person **H_d**: mixture contains the DNA of two unknown, unrelated people	$LR = \frac{V+U}{U1+U2}$

LRs are formulated from possible genotype combinations of match probabilities for victim (V), suspect (S), and unknown, unrelated person (U). Adapted from Buckleton et al. (2005), p. 226.

TABLE 12.5 Various Likelihood Ratio (LR) Formats

LR Formats	Verbal Description of Equation Involved
Hypothesis form	$\text{information gain in hypothesis} = \frac{\text{Odds}(hypothesis \mid data)}{\text{Odds}(hypothesis)}$
Likelihood form	$\text{information gain in likelihood} = \frac{\Pr(data \mid identification\ hypothesis)}{\Pr(data \mid alternative\ hypothesis)}$
Genotype form	$\text{information gain in genotype} = \frac{\Pr(evidence\ genotype)}{\Pr(coincidental\ genotype)}$
Match form	$\text{information gain in match} = \frac{\Pr(evidence\ match)}{\Pr(coincidental\ match)}$

The likelihood form is what is commonly used in forensic DNA literature. TrueAllele software uses the match form. Adapted from Perlin (2010).

As introduced previously in Chapter 9 and Chapter 11, likelihood ratios evaluate "what if" scenarios by comparing two opinions or hypotheses against one another. These two hypotheses (H_p and H_d) must be mutually exclusive, which means that their proposed ideas do not overlap. All LR calculations make assumptions about the number of contributors. These calculations are all "conditional" — that is, they make specific assumptions. If peak height information is utilized to confidently pair alleles into genotypes, then "restricted" sets of genotypes are deduced. If the mixture does not contain a discernible major and minor contributor, then an "unrestricted" approach is typically used where all possible genotype combinations are permitted for the detected alleles (see D.N.A. Box 6.4).

There are multiple formats whereby likelihood ratios can be reported (Table 12.5). The most commonly used form in forensic DNA publications is the likelihood form. The TrueAllele software developed by Cybergenetics (Pittsburgh, PA) uses the match form in which the LR is calculated by evaluating the probability of the evidence match (i.e. between the mixture and the suspect) against the probability of a coincidental match (i.e. between the mixture and an unknown, unrelated individual) (Perlin et al. 2009, Perlin 2010).

Example LR Calculation

Table 12.6 contains a simple worked example using the illustrated data from Figure 12.2. Readers are referred to a more detailed example that is provided in Appendix 4.

Rather than reporting very large LR values, many computer programs, such as TrueAllele (Perlin et al. 2013) or likeLTD (Balding 2013) prefer to report their weight of evidence values on a logarithmic

TABLE 12.6 Example of mRMP, CPI, and LR Calculations Using Data Illustrated in Figure 12.2

Method	Peak Height Ratios Used	Genotype Combinations Considered	Calculations Suspect = 14,16 Victim = 18,19
mRMP	Yes	Major: PQ ($2pq$) = 14,16 Minor: RS ($2rs$) = 18,19	Inferred genotype 14,16 matches suspect genotype 14,16 $2pq = 2(0.134)(0.147)$ $= 0.0394$ **1 in 25.4**
RMNE (CPI)	No	Depending on peak heights: P, Q, R, S plus peaks in stutter positions (13, 15, 17) allele frequencies to be used in CPI calculations = 13, 14, 15, 16, 17, 18, 19	***Inconclusive*** *if any peaks in stutter position are below stochastic threshold (and assuming potential contributor alleles are a portion of these peak heights)* If CPI calculation is performed: $(0.123 + 0.134 + 0.170 + 0.147 + 0.139 + 0.0776 + 0.0402)^2 = (0.831)^2 = 0.691$ = 69.1% of population could be included **1 in 1.45**
LR (restricted)	Yes	PQ with RS	$\mathrm{LR} = \frac{\cancel{V} + S}{\cancel{V} + U} = \frac{1}{2pq} = \frac{1}{2(0.134)(0.147)}$ LR = **25.4**
LR (unrestricted)	No	When conditioning on the victim's genotype, in this case the unrestricted LR is the same as the restricted LR because victim and suspect are fully represented without any ambiguity in possible genotype combinations	$\mathrm{LR} = \frac{\cancel{V} + S}{\cancel{V} + U} = \frac{1}{2pq} = \frac{1}{2(0.134)(0.147)}$ LR = **25.4**

D18S51 allele frequencies were from U.S. Caucasians in Appendix 1.

scale to compress the data and make it a little easier to compare among sample results. The log(LR) is sometimes referred to as a "ban" (D.N.A. Box 12.3).

ADDITIONAL TOPICS

Interpretation of mixtures can be quite complicated, such as in the case of *People of the State of California versus Orenthal James Simpson* (Weir & Buckleton 1996). However, interpretations in that particular case would probably have been simplified if STR testing had been available at the time; STRs have more possible alleles than the dot blot methods used to recover DNA evidence in the O.J. Simpson trial.

Evett and Weir (1998) note that the essence of mixture interpretation is to first identify the alleles in the crime scene evidence sample and alleles carried by the known contributor(s) to the sample, such as the victim. Then any alleles present in the evidence sample that are not provided by the known

D.N.A. BOX 12.3

THE "BAN" AS A WAY TO REPRESENT LARGE NUMBERS

In 1940, while working to crack the communication code used by the German navy with their Enigma cipher machine, British mathematician Alan Turing and his colleague Irving John (I.J.) "Jack" Good proposed a unit of measure to simplify weight-of-evidence (WoE) descriptions. Their method involved condensing numerical information reflecting the WoE towards a particular hypothesis (i.e. a likelihood ratio) through applying base 10 logarithms, where $\log_{10}$(number) = x ban. (Computers store information in "bits" (binary digits) based on powers of 2 and base 2 logarithms). Thus, instead of having to record the number one million (1,000,000) with seven digits, it can be reduced to 10^6 or simply 6 ban. Because the long sheets of paper used to work on code breaking came from Banbury, England, the cryptanalysis process was referred to as Banburismus. Thus, Turing's logarithmic unit of measurement for the WoE became the "**ban**." This unit of measurement has also been referred to as a **hartley** (named for Ralph Hartley who proposed it in 1928) or a **dit** (decimal digit).

A few examples are worked below to show the relationship between a number and its ban value.

Number	Number in powers of 10	Ban
10	10^1	1
100	10^2	2
1,000,000	10^6	6
1,547,623	1.547623×10^6	6.189665

TrueAllele software (Cybergenetics, Pittsburgh, PA) utilizes the ban in its mixture match statistics (Perlin 2010), as does David Balding with his likeLTD program (Balding 2013). Thus, a likelihood ratio (LR) of 10^{12} is reported by its $\log_{10}$(LR) value, which is 12.

Sources: *Good, I.J. (1985). Weight-of-evidence: a brief survey.* Bayesian Statistics, 2, *249–270; http://en.wikipedia.org/wiki/Ban_(information); http://en.wikipedia.org/wiki/Banburismus; Perlin, M.W. (2010). Explaining the likelihood ratio in DNA mixture interpretation.* Proceedings of the 21st International Symposium on Human Identification *(Promega Corporation). Available at http://www.cybgen.com/information/publication/page.shtml.; Balding, D.J. (2013). Evaluation of mixed-source, low-template DNA profiles in forensic science.* Proceedings of the National Academy of Sciences of the United States of America, 110(30), *12241–12246.*

contributor(s) must be carried by one or more unknown contributors, which may or may not include the suspect.

The DNA Advisory Board recommends that either or both CPE and LR calculations be performed whenever feasible when a mixture exists (DAB 2000). However, there will be mixture results for which no interpretation of the profile can be made due to low-copy number stochastic limits, DNA template degradation or PCR inhibition. In the end, the interpretation of results in forensic casework, whether arising from single-source samples or mixtures, is a matter of professional judgment and expertise.

Mixtures will be complicated by the fact that some loci will possess intensity differences that permit contributors to be deciphered, while other loci may not be fully interpretable due to overlapping allele combinations. With STRs and peak intensity differences, some loci may be interpretable so that contributors can be statistically treated as single sources, while other loci may be too

complex to confidently attribute alleles to their sources. Thus, when performing mixture interpretation, analysts should do everything possible to first eliminate artifacts such as stutter products from consideration and then interpret remaining alleles to determine how many contributors are present.

Partial DNA Profiles

Interpretation of a DNA profile can only be performed on loci for which there are results. Unfortunately, with degraded DNA specimens or low-copy number samples (see Chapter 7) the PCR amplification may fail to generate signals above the detection threshold of the instrument, and individual alleles and entire loci may be lost from the final DNA profile. Foreman and Evett (2001) note that partial profiles occur in approximately 20% of cases seen by the Forensic Science Service. Given that it is often not possible to know what alleles would have been present had the sample not been degraded, the standard practice is to interpret only the detected alleles.

Obtaining matching alleles between a full-profile suspect and a partial-profile evidentiary sample is not as powerful as a full-profile to full-profile match. However, any data is better than none. Even if results are obtained from only a few STR loci, this information may provide ample assistance to either include or exclude the suspect and therefore aid in resolving the case.

Occasionally results from additional loci may be recovered from degraded DNA samples through the use of miniSTR primer sets or other genetic systems such as single nucleotide polymorphisms that amplify smaller regions of the DNA template (see Butler 2012). Finally, in most cases, the forensic sample has been divided into two or more parts so that unused portions are retained to permit additional tests as desired by the court according to NRC II recommendation 3.3 (see Appendix 2). These retained samples can be tested as occasion warrants in order to verify previous test results.

Software

GeneMapper*ID-X* has a mixture interpretation function that can be used for calculating two-person LRs and CPI with more than two-person mixtures (Hansson & Gill 2011). However, laboratories often create their own spreadsheet programs to perform LR or CPI calculations. Validation can be tedious as manual calculations are often performed to evaluate formulas used and mathematical operations performed by the software.

A number of software programs have been developed recently to perform probabilistic genotyping and to cope with potential allele drop-out (see Table 13.1). ISFG supports an open-source software resource page on forensic statistics packages (ISFG 2014). A list of available programs is available on the NIST STRBase website mixture section (NIST 2014).

Historical Perspectives on Approaches Used for Mixture Statistics

Historical perspectives are often valuable to provide an understanding of where we as a forensic DNA community have come over the past several decades. Hopefully we can learn from the past as we try to move forward into the future in a productive manner. Table 12.7 reviews the use of LR and CPI approaches with some key events in each area. Publications cited in the table can be found in the reference list at the back of the chapter.

TABLE 12.7 Brief Historical Timeline Comparison of Likelihood Ratio (LR) and Combined Probability of Inclusion (CPI)/Random Man Not Excluded (RMNE) Approaches to DNA Mixture Interpretation

LR	
1977	Dennis Lindley describes value of LRs in forensic science interpretation
1991	Ian Evett et al. publish first work with LRs for DNA mixtures
1995	Bruce Weir uses LRs in OJ Simpson case
1996	NRC II (p. 130) endorses Evett et al. 1991 LR approach
1997	Bruce Weir et al. describe LRs for mixtures
1998	Ian Evett & Bruce Weir publish their book *Interpreting DNA Evidence*
2005	John Buckleton et al. publish book *Forensic DNA Evidence Interpretation*
2005	David Balding publishes book *Weight-of-evidence for Forensic DNA Profiles*
2006	ISFG DNA Commission recommends LR over CPI
2009	Mark Perlin describes his match likelihood ratio approach used in TrueAllele
2009	ISFG session in Buenos Aires debates LR and CPI
2010	SWGDAM guidelines provide RMP, CPI, and LR as possibilities
2012	ISFG DNA Commission discusses LR with drop-out
2013	Articles are published describing probabilistic genotyping software approaches (TrueAllele and STRmix)
RMNE (CPI)	
1982	RMNE (CPI) used for paternity testing
1992	NRC I (p. 59) mentions CPI calculation for mixtures
1993	Bernie Devlin article discusses CPI for paternity testing
2000	DAB Stats document (see Appendix 3) states that either CPI or LR can be used with DNA mixtures and uses Devlin 1993 article as support for CPI; simple two-person mixtures are implied as this was what all laboratories were doing at the time
2001	Carl Ladd et al. publish review article that promotes use of CPI
2008	John Buckleton & James Curran discuss CPI and LR pros and cons
2009	Bruce Budowle et al. defend CPI in *Journal of Forensic Sciences* article on mixtures
2009	Max Baur mentions deficiencies of CPI at ISFG session
2010	Buckleton & Curran publish article on possibility of false inclusion with CPI
2010	SWGDAM guidelines provide RMP, CPI, and LR as possibilities
2011	Charles Brenner attacks CPI deficiencies at AAFS meeting
2013	Problems with CPI are reviewed at DNA Technical Leader Summit

As DNA testing has shown its value to the criminal justice community, more samples and more mixtures have been added to the docket of DNA analysts. As has been stated multiple times in this book, increased detection sensitivity means more challenges for interpretation. Complex mixtures containing more than two contributors add to the challenge of determining if a suspect should be included or excluded, especially if allele drop-out is possible. This is the subject of the next chapter.

Reading List and Internet Resources

General Information

Balding, D. J. (2005). *Weight-of-evidence for Forensic DNA Profiles*. John Wiley & Sons. see mixture section on pp. 101–110.

Butler, J. M. (2012). *DNA extraction methods. Chapter 2 in* Advanced Topics in Forensic DNA Typing: Methodology. San Diego: Elsevier Academic Press. pp. 29–47.

Clayton, T. M., et al. (1998). Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Science International, 91*, 55–70.

Clayton, T., & Buckleton, J. (2005). *Mixtures. Chapter 7 in* Forensic DNA Evidence Interpretation, J. Buckleton, C. M. Triggs, & S. J. Walsh (Eds.) (pp. 217–274). Boca Raton, Florida: CRC Press.

Crespillo, M., et al. (2014). GHEP-ISFG collaborative exercise on mixture profiles of autosomal STRs (GHEP-MIX01, GHEP-MIX02 and GHEP-MIX03): results and evaluation. *Forensic Science International: Genetics, 10*, 64–72.

DNA Advisory Board. (2000). Statistical and population genetics issues affecting the evaluation of the frequency of occurrence of DNA profiles calculated from pertinent population database(s). *Forensic Science Communications*, 2(3). Available at http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2000/dnastat.htm/. Accessed April 2, 2014.

Gill, P., et al. (2006). DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Science International, 160*, 90–101.

Gill, P., et al. (2008). National recommendations of the technical UK DNA working group on mixture interpretation for the NDNAD and for court going purposes. *Forensic Science International: Genetics, 2*, 76–82.

Gill, P., et al. (2012). 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 Science International: Genetics, 6*(6), 679–688.

Hansson, O., & Gill, P. (2011). Evaluation of GeneMapperID-X mixture analysis tool. *Forensic Science International: Genetics Supplement Series, 3*, e11–e12.

Heidebrecht, B. J. (2013). *Mixture interpretation (interpretation of mixed DNA profiles with STRs only). Encyclopedia of Forensic Sciences, Second Edition*. San Diego: Elsevier Academic Press. pp. 243–251.

International Society for Forensic Genetics (ISFG). (2014). *Forensic Software Resources*. http://www.isfg.org/software. Accessed April 2, 2014.

Ladd, C., et al. (2001). Interpretation of complex forensic DNA mixtures. *Croatian Medical Journal, 42*, 244–246.

National Research Council. (1992). *DNA Technology in Forensic Science*. Washington, DC: National Academy Press.

National Research Council (NRCII) Committee on DNA Forensic Science. (1996). *The Evaluation of Forensic DNA Evidence*. Washington, DC: National Academy Press.

NIST. (2014). DNA Mixture Interpretation. http://www.cstl.nist.gov/strbase/mixture.htm. Accessed April 2, 2014.

Puch-Solis, R., et al. (2012). *Assessing the probative value of DNA evidence: guidance for judges, lawyers, forensic scientists and expert witnesses. Practitioner Guide No. 2. Prepared under the auspices of the Royal Statistical Society's Working Group on Statistics and the Law (Chairman: Colin Aitken)*. Available at http://www.rss.org.uk/uploadedfiles/userfiles/files/Practitioner-Guide-2-WEB.pdf. Accessed April 2, 2014.

Robertson, B., & Vignaux, G. A. (1995). *DNA evidence: wrong answers or wrong questions?* Human Identification: The Use of DNA Markers. In B. S. Weir (Ed.) (pp. 145–152). The Netherlands: Kluwer Academic Publishers.

Roman, J. K., et al. (2008). *The DNA field experiment: cost-effectiveness analysis of the use of DNA in the investigation of high-volume crimes*. Washington, DC: Urban Institute Justice Policy Center. Available at https://www.ncjrs.gov/pdffiles1/nij/grants/222318.pdf. Accessed April 2, 2014.

Schneider, P. M., et al. (2009). The German Stain Commission: recommendations for the interpretation of mixed stains. *International Journal of Legal Medicine, 123*, 1–5 (originally published in German in 2006 – Rechtsmedizin 16:401–404).

Stringer, P., et al. (2009). Interpretation of DNA mixtures – Australian and New Zealand consensus on principles. *Forensic Science International: Genetics, 3*, 144–145.
SWGDAM. (2014). *Frequently Asked Questions (FAQs)*. http://swgdam.org/faq.html. Accessed April 2, 2014.
SWGDAM. (2010). *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories*. Available at http://swgdam.org/Interpretation_Guidelines_January_2010.pdf. Accessed April 2, 2014.
Torres, Y., et al. (2003). DNA mixtures in forensic casework: a 4-year retrospective study. *Forensic Science International, 134*, 180–186.
Yu, N., et al. (2002). Disputed maternity leading to identification of tetragametic chimerism. *New England Journal of Medicine, 346*, 1545–1552.

Random Match Probability

Bille, T., et al. (2013). Application of random match probability calculations to mixed STR profiles. *Journal of Forensic Sciences, 58*(2), 474–485.
Buckleton, J., & Triggs, C. (2006). Is the 2p rule always conservative? *Forensic Science International, 159*, 206–209.

Random Man Not Excluded

Buckleton, J. S., & Curran, J. M. (2008). A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Science International: Genetics, 2*, 343–348.
Buckleton, J., Triggs, C. M., & Walsh, S. J. (2005). *Forensic DNA Evidence Interpretation*. Boca Raton, Florida: CRC Press.
Budowle, B., et al. (2009). Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. *Journal of Forensic Sciences, 54*, 810–821.
Curran, J. M., & Buckleton, J. (2010). Inclusion probabilities and dropout. *Journal of Forensic Science, 55*, 1171–1173.
Devlin, B. (1993). Forensic inference from genetic markers. *Statistical Methods in Medical Research, 2*, 241–262.
Morling, N., et al. (2007). Interpretation of DNA mixtures – European consensus on principles. *Forensic Science International: Genetics, 1*, 291–292.
Van Nieuwerburgh, F., et al. (2009a). Impact of allelic dropout on evidential value of forensic DNA profiles using RMNE. *Bioinformatics, 25*, 225–229.
Van Nieuwerburgh, F., et al. (2009b). RMNE probability of forensic DNA profiles with allelic drop-out. *Forensic Science International: Genetics Supplement Series, 2*, 462–463.

Likelihood Ratios

Brenner, C. H. (1997). Proof of a mixed stain formula of Weir. *Journal of Forensic Sciences, 42*, 221–222.
Curran, J. M., et al. (1999). Interpreting DNA mixtures in structured populations. *Journal of Forensic Sciences, 44*, 987–995.
Curran, J. M., et al. (2005). Interpretation of repeat measurement DNA evidence allowing for multiple contributors and population substructure. *Forensic Science International, 148*, 47–53.
Cybergenetics publications: http://www.cybgen.com/information/publication/page.shtml. Accessed April 2, 2014.
Dørum, G., et al. (2014). Exact computation of the distribution of likelihood ratios with forensic applications. *Forensic Science International: Genetics, 9*, 93–101.
Evett, I. W., et al. (1991). A guide to interpreting single locus profiles of DNA mixtures in forensic cases. *Journal of Forensic Science Society, 31*(1), 41–47.
Evett, I. W., & Weir, B. S. (1998). *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists*. Sunderland, MA: Sinauer Associates.
Fung, W. K., & Hu, Y.-Q. (2008). *Statistical DNA Forensics: Theory, Methods and Computation*. Hoboken, NJ: Wiley.
Fung, W. K., & Hu, Y.-Q. (2001). The evaluation of mixed stains from different ethnic origins: general result and common cases. *International Journal of Legal Medicine, 115*, 48–53.
Fung, W. K., & Hu, Y.-Q. (2002). Evaluating mixed stains with contributors of different ethnic groups under the NRC-II Recommendation 4.1. *Statistics in Medicine, 21*, 3583–3593.
Fung, W. K., & Hu, Y.-Q. (2002). The statistical evaluation of DNA mixtures with contributors from different ethnic groups. *International Journal of Legal Medicine, 116*, 79–86.
Fung, W. K., et al. (2006). On statistical analysis of forensic DNA: theory, methods and computer programs. *Forensic Science International, 162*, 17–23.

Hu, Y.-Q., & Fung, W. K. (2003). Evaluating forensic DNA mixtures with contributors of different structured ethnic origins: a computer software. *International Journal of Legal Medicine, 117*, 248–249.
Lindley, D. V. (1977). A problem in forensic science. *Biometrika, 64*, 207–213.
Pascali, V. L., & Merigioli, S. (2012). Joint Bayesian analysis of forensic mixtures. *Forensic Science International: Genetics, 6*, 735–748.
Perlin, M. W. (2006). Scientific validation of mixture interpretation methods. *Proceedings of Promega's Seventeenth International Symposium on Human Identification*. Available at http://www.promega.com/products/pm/genetic-identity/ishi-conference-proceedings/17th-ishi-oral-presentations/. Accessed April 2, 2014.
Perlin, M. W., et al. (2009). Match likelihood ratio for uncertain genotypes. *Law, Probability and Risk, 8*, 289–302.
Perlin, M. W., & Sinelnikov, A. (2009). An information gap in DNA evidence interpretation. *PLoS ONE, 4*(12), e8327.
Perlin, M. W. (2010). *Explaining the likelihood ratio in DNA mixture interpretation*. Proceedings of the 21st International Symposium on Human Identification (Promega Corporation). Available at http://www.cybgen.com/information/publication/page.shtml. Accessed April 2, 2014.
Perlin, M. W., et al. (2011). Validating TrueAllele® DNA mixture interpretation. *Journal of Forensic Sciences, 56*, 1430–1447.
Perlin, M. W., et al. (2013). New York State TrueAllele® Casework validation study. *Journal of Forensic Sciences, 58*, 1458–1466.
Perlin, M. W., et al. (2014). TrueAllele Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLoS ONE, 9*(3), e92837.
Weir, B. S., et al. (1997). Interpreting DNA mixtures. *Journal of Forensic Sciences, 42*, 213–222.

Additional Topics

Foreman, L. A., & Evett, I. W. (2001). Statistical analyses to support forensic interpretation for a new ten-locus STR profiling system. *International Journal of Legal Medicine, 114*, 147–155.
Kelly, H., et al. (2014). A comparison of statistical models for the analysis of complex forensic DNA profiles. *Science & Justice, 54*, 66–70.
Weir, B. S., & Buckleton, J. S. (1996). *Statistical issues in DNA profiling*. In A. Carracedo, B. Brinkmann, & W. Bar (Eds.), *Advances in Forensic Haemogenetics, 6*; (pp. 457–464). New York: Springer-Verlag.

Estimating the Number of Contributors

Biedermann, A., et al. (2012). Inference about the number of contributors to a DNA mixture: comparative analyses of a Bayesian network approach and the maximum allele count method. *Forensic Science International: Genetics, 6*, 689–696.
Brenner, C. H., et al. (1996). Likelihood ratios for mixed stains when the number of donors cannot be agreed. *International Journal of Legal Medicine, 109*, 218–219.
Buckleton, J. S., et al. (1998). Setting bounds for the likelihood ratio when multiple hypotheses are postulated. *Science & Justice, 38*, 23–26.
Buckleton, J. S., et al. (2007). Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *Forensic Science International: Genetics, 1*, 20–28.
Egeland, T., et al. (2003). Estimating the number of contributors to a DNA profile. *International Journal of Legal Medicine, 117*, 271–275.
Haned, H., et al. (2011). The predictive value of the maximum likelihood estimator of the number of contributors to a DNA mixture. *Forensic Science International: Genetics, 5*, 281–284.
Haned, H., et al. (2011b). Estimating the number of contributors to forensic DNA mixtures: does maximum likelihood perform better than maximum allele count? *Journal of Forensic Sciences, 56*, 23–28.
Lauritzen, S. L., & Mortera, J. (2002). Bounding the number of contributors to mixed DNA stains. *Forensic Science International, 130*, 125–126.
Manabe, S., et al. (2013). Simulated approach to estimate the number and combination of known/unknown contributors in mixed DNA samples using 15 short tandem repeat loci. *Forensic Science International: Genetics Supplement Series, 4*, e154–e155.
Paoletti, D. R., et al. (2005). Empirical analysis of the STR profiles resulting from conceptual mixtures. *Journal of Forensic Sciences, 50*, 1361–1366.
Paoletti, D. R., et al. (2012). Inferring the number of contributors to mixed DNA profiles. *IEEE/ACM Transactions on Computational Biology and Bioinformatics, 9*, 113–122.

Perez, J., et al. (2011). Estimating the number of contributors to two-, three-, and four-person mixtures containing DNA in high template and low template amounts. *Croatian Medical Journal, 52*(3), 314–326.
Presciuttini, S., et al. (2003). Allele sharing in first-degree and unrelated pairs of individuals in the Ge.F.I. AmpFlSTR Profiler Plus database. *Forensic Science International, 131*, 85–89.

Dealing with Potential Relatives

Egeland, T., et al. (2014). Mixtures with relatives: a pedigree perspective. *Forensic Science International: Genetics, 10*, 49–54.
Hu, Y.-Q., & Fung, W. K. (2003). Interpreting DNA mixtures with the presence of relatives. *International Journal of Legal Medicine, 117*, 39–45.
Hu, Y.-Q., & Fung, W. K. (2005). Evaluation of DNA mixtures involving two pairs of relatives. *International Journal of Legal Medicine, 119*, 251–259.
Puch-Solis, R., et al. (2010). Calculating likelihood ratios for a mixed DNA profile when a contribution from a genetic relative of a suspect is proposed. *Science & Justice, 50*, 205–209.

Dealing with Low-Level DNA Mixtures

Balding, D. J. (2013). Evaluation of mixed-source, low-template DNA profiles in forensic science. *Proceedings of the National Academy of Sciences of the United States of America, 110*(30), 12241–12246.
Taylor, D., et al. (2013). The interpretation of single source and mixed DNA profiles. *Forensic Science International: Genetics, 7*(5), 516–528.