CHAPTER

6

DNA Mixtures

"Don't do mixture interpretation unless you have to."

Peter Gill (Mixture Workshop Conducted in Annapolis, Maryland in July 1998)

GENERAL INFORMATION

DNA mixtures arise when two or more individuals contribute to the sample being tested. Mixtures can be challenging to detect and interpret without extensive experience and careful training. DNA detection technologies have become more sensitive through polymerase chain reaction (PCR) amplification coupled with fluorescent measurements using capillary electrophoresis (CE). This detection sensitivity has improved the ability to see minor components in the DNA profile of mixed samples over that which was available with early DNA methods.

Likewise, the theoretical framework for statistical calculations involved in mixture interpretation has expanded in recent years (e.g. Clayton & Buckleton 2005, Gill et al. 2012). This chapter will focus on mixture detection and evaluation while Chapter 12 will cover statistical approaches to interpreting mixture comparisons to reference profiles. Chapter 13 discusses efforts to cope with allele drop-out that occurs in low-level and complex DNA mixtures.

Determining the Presence of a Mixture

Figure 6.1 provides an example of a DNA mixture from two contributors. This profile is part of the training data set available from (Boston University 2014). It represents the PCR amplification of 1 ng DNA from a two-person mixture (with a 4:1 mixture ratio) using the Identifiler STR kit. In Appendix 4, Dr. Michael Coble, from the National Institute of Standards and Technology (NIST) Applied Genetics Group, explores aspects of this mixture example and approaches for statistical analysis.

Several characteristics of this profile suggest that a DNA mixture is present. First, there are more than two alleles present at multiple loci (e.g. D8S1179 and D21S11). In fact, D2S1338 has four detected alleles. Second, several loci with only two detected alleles (e.g. D5S818 and D7S820) exhibit fairly extreme peak imbalance (peak height ratios of 13% and 17%, respectively), which exceeds typical peak height ratios expected for single-source samples. Third, the amelogenin X and Y alleles are imbalanced, which suggests a male-female mixture with the male as the minor contributor.

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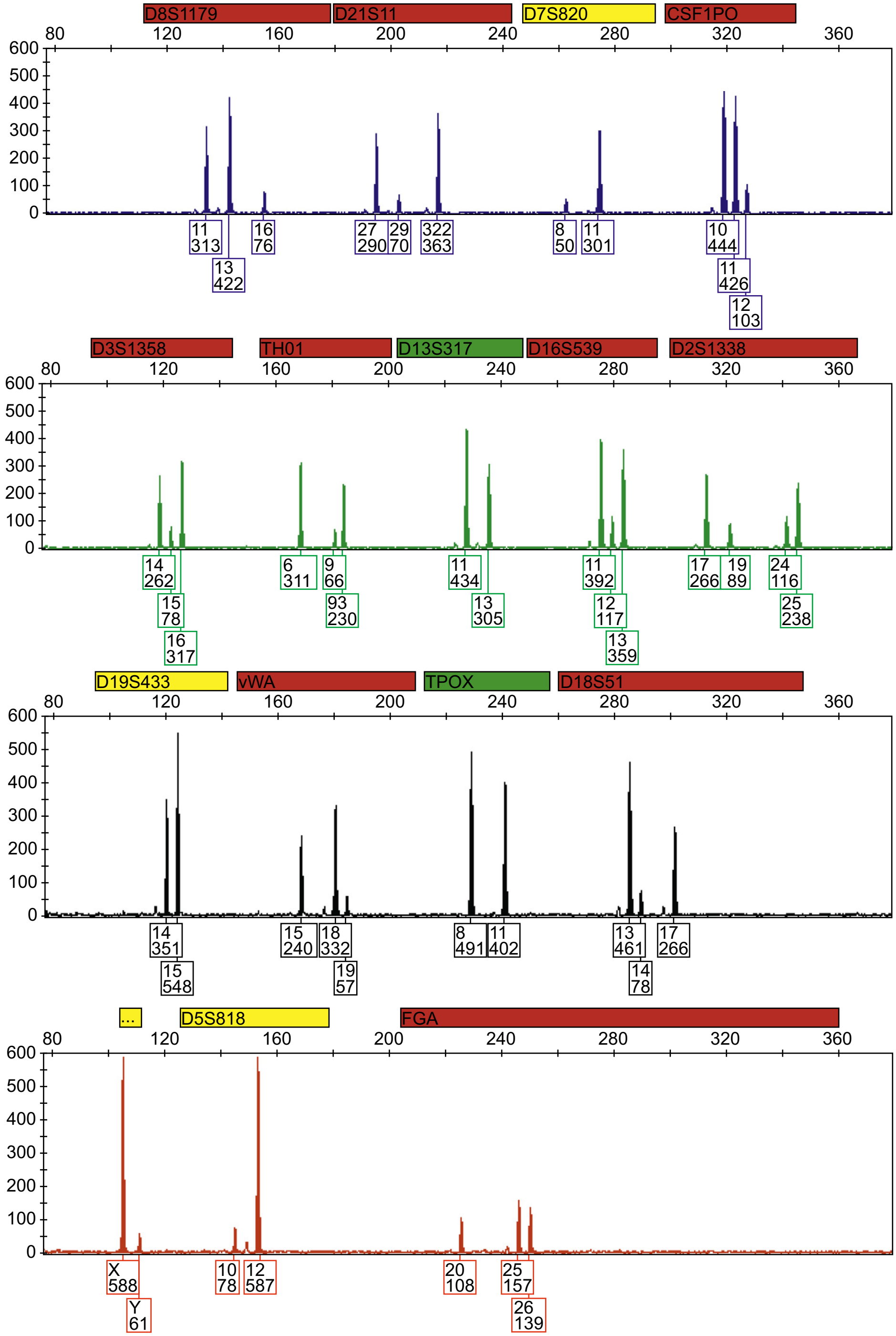


FIGURE 6.1 Example two-person DNA mixture with Identifiler. Output from GeneMapperID-X color codes STR loci names with green (appearance of single-source), yellow (imbalance with two or less peaks), and red (mixture evident). This example is worked in greater detail in Appendix 4. Data courtesy of Robin Cotton and Catherine Grgicak, Boston University.

Evaluation of the entire profile, rather than trying to focus on a single locus in isolation, is important for determining the presence of a mixture. In Figure 6.1 three loci have only two alleles, which are fairly well-balanced and if considered in isolation these loci could appropriately fit expected heterozygote peak height ratios (PHRs) for single-source samples. TPOX has a PHR of 81.9%, D13S317 has a PHR of 70.3%, and D19S433 has a PHR of 64.1%. However, this STR profile is clearly a mixture due to the aforementioned imbalances in other two-allele loci and the fact that nine loci exhibit three alleles and D2S1338 has four alleles.

The probability that a mixture will be detected improves with the use of more STR loci and genetic markers that have a high incidence of heterozygotes. Using highly polymorphic STR markers with more possible alleles translates to a greater chance of seeing differences between the two components of a mixture. For example, D18S51 has 73 possible alleles while TPOX only has 19 known alleles (Butler 2012), making D18S51 a more useful marker for detecting mixtures. D2S1338 is one of the most polymorphic loci in the Identifiler STR kit, and thus observing four alleles at this locus in the Figure 6.1 example is not unexpected. However, because D18S51 and D2S1338 are some of the larger-size loci, they may experience allele drop-out on a low-quality or low-quantity evidence sample.

The detectability of multiple DNA sources in a sample relates to the ratio of DNA present from each source, the specific combinations of genotypes, and the total amount of DNA amplified. In other words, some mixtures will not be as easily detectable as other mixtures.

Chromosomal abnormalities do exist and can give rise to extra allele peaks at a particular STR locus as discussed in Chapter 5 with tri-allelic patterns. Chromosomal translocations, somatic mutations, and trisomies may occur in the cells of the donor of a forensic stain. However, the STR profile from the individual with the chromosomal abnormality would most likely show only a single extra peak and the same pattern would be present in both the forensic stain and the reference sample from the matching suspect (Clayton et al. 1998). The rare cases where a chromosomal abnormality is observed can even help strengthen the final conclusions.

An excellent example of a chromosomal abnormality is found in the standard cell line K562. Three peaks are obtained at the D21S11 locus and at least five other STR loci have heterozygous peak patterns that are not balanced. At first glance, this sample might be suspected to have arisen from more than one source rather than a sample with an abnormal number of chromosomes. More than 240 different tri-allelic patterns have been reported spanning the 13 core STR loci used in the United States (NIST STRBase Tri-Allelic Patterns 2014).

Potential Sources of Mixtures

Several potential scenarios can result in a mixture DNA profile where cells from more than one individual are sampled. A vaginal swab following a sexual assault can contain cells from the victim as well as the perpetrator. There may also be residual cells from a prior consensual sexual partner as sperm can survive several days in the vaginal cavity. Likewise, blood or saliva from a perpetrator collected from the surface of a victim's skin is likely to contain cellular material from both the perpetrator and the victim. As will be discussed further, the collection of victim and consensual sexual partner reference samples can help decipher the expected components of a mixture result. With this additional information, it may thus be easier to derive the perpetrator portion of the mixture that can be evidence that a crime was committed.

It is also possible for a single-source sample to be contaminated at the crime scene by police or evidence collection staff or in the laboratory by staff handling the sample. For this reason, reference samples are commonly taken of crime scene investigators, DNA examiners, and people with access to the laboratory. DNA profiles developed from these reference samples then become part of an "elimination database" that can be examined following the observation of a mixture in order to eliminate contamination as a possible reason for the mixture result. Ideally, anyone with legitimate potential exposure to the biological evidence should have a reference sample on file for elimination purposes.

Finally, it has been observed that PCR reagents, tubes, pipet tips, or other plastic disposables used in the DNA testing process may contain contaminating DNA, usually at low levels (Gill & Kirkham 2004, Gefrides et al. 2010). Mixtures may then result when a single-source DNA sample is added to a tube containing a few contaminating cells from the manufacturer. For this reason, best practices encourage manufacturers of disposable plasticware to provide reference samples of employees to compare to any profiles found in negative controls, reagent blanks, or potential contamination mixtures (Gill et al. 2010). When evidentiary material is extremely limited, then a mixture from a few contaminating manufacturer cells becomes more significant.

Types of Mixtures Encountered Most Frequently

As an example of the number of mixture samples encountered in typical casework, a forensic laboratory in Spain reported on casework encountered over the four-year time period of 1997 to 2000 (Torres et al. 2003). Of 1,547 criminal cases involving a total of 2,424 samples, only 163 (6.7% of total samples) showed a mixed profile during this time period. Almost all of the observed mixtures (95%) were two-person mixtures. Only 8 of 163 samples (0.3% of the total 2,424 examined) had DNA profiles that suggested more than two individuals contributed to the biological sample (Torres et al. 2003).

A collection of casework sample data in 2007 and 2008 from 14 laboratories in North America found the majority of mixtures at that time were two-person mixtures (D.N.A. Box 6.1). From a total of 4,541 samples, about 55% were single-source (N = 2,489), 34% were two-person mixtures (N = 1,526), and just over 11% were DNA mixtures with more than two contributors (N = 526).

The types of samples being collected can make a difference in the types of DNA mixtures being observed in the laboratory. An examination of 8,470 casework profiles (either body fluids or trace evidence) from Lyon, France found that 71% were comprised of one contributor, 6% were two-person stains, and 23% were classified as unresolvable mixtures presumably containing more than two contributors (Haned et al. 2011a,b). The trace samples (N = 3,310) had 51% of DNA profiles with more than two contributors compared to the body fluids (N = 5,169) that only had 6% in this category (Haned et al. 2011a,b).

The number of complex mixtures containing more than two contributors has increased in recent years, probably in large measure due to the success of touch DNA evidence in aiding work with burglary cases (Roman et al. 2008). Higher volumes of sample submissions involving poor-quality and low-quantity DNA samples can create backlogs and lead to lower success rates (see Chapter 7 for further discussion).

Alternative Solutions to Avoid Mixture Results

Mixed sample stains are present in many forensic investigations and STR typing procedures have been demonstrated to be an effective means of differentiating components of a mixed sample. However, a case may contain multiple stains and not all of these will be mixtures. In fact, the proportions of a mixture can vary across the forensic stain itself. Thus, if additional samples can be tested that are

D.N.A. BOX 6.1

DATA COLLECTION ON MIXTURE TYPES IN 2007 AND 2008

In order to understand the types of samples and complexity of DNA mixtures that forensic DNA laboratories were processing when the Scientific Working Group on DNA Analysis Methods (SWGDAM) began work on revising the autosomal STR interpretation guidelines, case summary data were collected. From July 2007 to February 2008, Ann Marie Gross of the Minnesota Bureau of Criminal Apprehension, on behalf of the SWGDAM Mixture Interpretation Committee, coordinated the collection of case summary data. Participating forensic DNA laboratories were requested to fill out a spreadsheet with type of sample, type of substrate, quantity of DNA amplified, a minimum number of contributors (1, 2, 3, 4, or >4), if a predominant type or major profile was determined, if statistics were reported, and any comments as needed.

A total of 14 laboratories submitted information on 4780 samples: Centre for Forensic Sciences-Toronto (N = 276), Connecticut State Police (N = 610), Washington State Police (N = 419), Illinois State Police (N = 76), Montana State Crime Laboratory (N = 408), New Jersey State Police (N = 101), Georgia Bureau of Investigation (N = 19), Royal Canadian Mounted Police (N = 1555), U.S. Army Criminal Investigation Laboratory (N = 119), Michigan State Police (N = 225), California Department of Justice (N = 285), Kern County California Crime Lab (N = 31), Anne Arundel County Maryland Police Crime Laboratory (N = 322), and the Minnesota Bureau of Criminal Apprehension (N = 334). Some of the provided sample information was not complete which is why the total number of samples in the table (N = 4551) differs from the 4780 sample information provided by the participating laboratories. The information in the table is divided by crime classifications: sexual assault, major crime (homicide), and high volume (burglary). Over half of the samples examined were single-source and ≈75% of all reported mixtures involved two contributors. The results of this case sample survey are why the SWGDAM 2010 interpretation guidelines were developed to focus on single-source samples and two-person mixtures.

	Minimum # of contributors					
Crime Class	1	2	3	4	>4	N
Sexual Assault	884	787	145	11	0	1,827
Major Crime	1,261	519	182	32	0	1,994
High Volume	344	220	140	11	5	720
Total	2,489	1,526	467	54	5	4,541
	54.8%	33.6%	10.3%	1.2%	0.1%	

Source: *Ann Marie Gross presentation at AAFS Feb 2008 mixture workshop available at http://www.cstl.nist.gov/strbase/training/AAFS2008_1_CaseworkSurvey.pdf; NIST 2008 poster available at http://www.cstl.nist.gov/strbase/pub_pres/Promega2008poster.pdf; Torres, Y., et al. (2003). DNA mixtures in forensic casework: a 4-year retrospective study.* Forensic Science International, 134, *180–186; Clayton, T.M., et al. (1998). Analysis and interpretation of mixed forensic stains using DNA STR profiling.* Forensic Science International, 91, *55–70.*

easier to interpret, they should be sought after versus complicated mixtures (Gill et al. 1998b). Dr. Peter Gill, who at the time was head of research at the UK Forensic Science Service, provided some of the best advice on DNA mixtures in a workshop he gave in July 1998: "Don't do mixture interpretation unless you have to!"

Differential extraction can help in sexual assault cases to separate perpetrator sperm cells from victim vaginal epithelial cells (see Chapter 2 in Butler 2012). Laser microdissection combined with fluorescent *in-situ* hybridization (see Chapter 1 in Butler 2012) can also aid separation of male and female cells in some instances. Likewise, PCR amplification of Y-chromosome STRs (see Chapter 13 in Butler 2012) can enable differential amplification of male DNA components over female DNA, which may be in excess in biological samples recovered in sexual assault cases.

However, touch DNA samples do not have the same advantages of a physical or biological property that can be used to separate individual components prior to or during PCR amplification.

DNA Advisory Board Comments on DNA Mixtures

The DNA Advisory Board (DAB) was established by the United States Congress to provide guidance to the FBI Laboratory. The DAB issued the initial Quality Assurance Standards for U.S. forensic DNA laboratories. At the end of its five-year term in February 2000, the DAB provided recommendations on statistical and population genetic issues (DAB, 2000). Exerpts from these recommendations related to DNA mixtures are included here. Appendix 3 contains the entire text of the February 2000 document.

> *Mixtures, which for our purposes are DNA samples derived from two or more contributors, are sometimes encountered in forensic biological evidence. The presence of a mixture is evidenced typically by the presence of three or more peaks, bands, dots, and/or notable differences in intensities of the alleles for at least one locus in the profile. In some situations, elucidation of a contributor profile is straightforward. An example would be the analysis of DNA from an intimate swab revealing a mixture consistent with the composition of the perpetrator and the victim. 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.*
>
> *Interpretation of genotypes is complicated when the contributions of the donors are approximately equal (i.e. when a major contributor cannot be determined unequivocally) or when alleles overlap. Also, stochastic fluctuation during polymerase chain reaction (PCR) arising from a low quantity of DNA template can make typing of a minor contributor complicated. When the contributors of a DNA mixture profile cannot be distinguished, two calculations [probability of exclusion or likelihood ratio] convey the probative value of the evidence (DAB 2000).*

It is useful to keep in mind that at the turn of the century when the DAB issued its recommendations simple two-person mixtures, mostly from sexual assault evidence, were the norm (e.g. Torres et al. 2003). In fact, all of the early theory and processes for DNA mixture interpretation were developed for two-person mixtures rather than more complex ones such as discussed in the next chapter. Therefore, this chapter will focus on principles involved with two-person mixture analysis.

TOOLS FOR MIXTURE INTERPRETATION

STR data obtained in CE electropherograms are a reflection of both the true sample components (i.e. what exists in the original biological sample) and the sample processing involved (Figure 6.2). PCR enables copying the original DNA sample material to a detectable level. However, the PCR amplification process can sometimes distort the allelic components. With low levels of DNA, alleles may fail to be copied due to stochastic variation in primer binding during the early PCR cycles. A primary purpose of validation studies where heterozygote balance (Chapter 4) and stutter ratios (Chapter 3) are characterized is to help establish the limits in accurately conveying true sample component *genotype information* through the sample processing steps for single-source samples. From the available CE electropherogram data, which depicts *decoupled allele information*, the goal of interpretation is to infer possible genotypes and determine the sample components. This task becomes more challenging as the number of contributors to a sample profile increases.

Potential allele overlap can lead to stacking of signal from multiple components. While there are many possible alleles with each STR locus, some alleles are more common than others — and thus more likely to be shared. In Figure 6.2 the true sample component genotypes at D18S51 are 13,17 and 13,14. Thus, the allele 13 is shared between two contributors. The electropherogram data exhibits allele 13 with a signal of 461 relative fluorescence units (RFU). The mixture ratio also impacts the relative allele signal that is produced from the combination of genotypes present in the original sample. Finally, because the total amount of DNA amplified is typically fixed at something like 1 ng (since an analyst will not know *a priori* how many

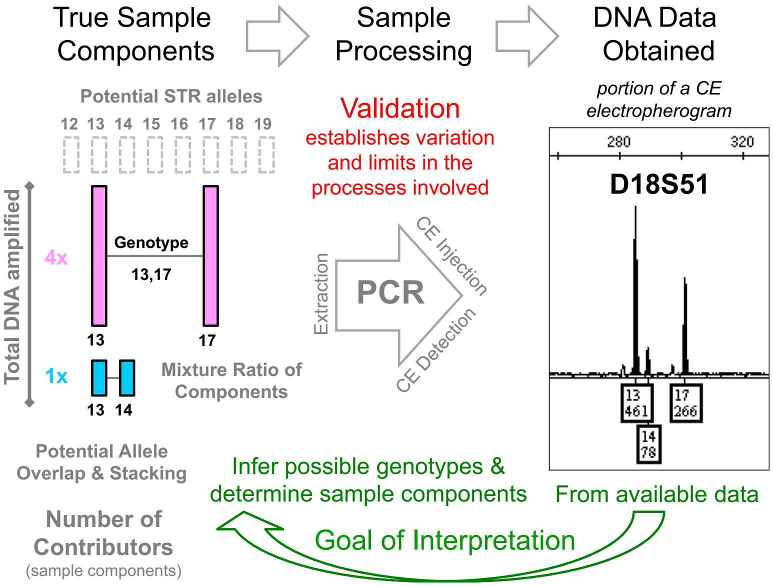


FIGURE 6.2 DNA data obtained in the form of STR peak heights in electropherograms are a reflection of the true sample components and the sample processing involved. Validation studies help establish the expected variation and limits in the processes. These processes include DNA extraction (that impacts the amount of DNA available for testing), PCR amplification (with potential stochastic effects that can unevenly sample elements of the true components) and CE injection and detection (that impact the amount of signal obtained). Aspects of the true sample components that influence the available biological material to be evaluated include the number of contributors, mixture ratio of the components, potential allele overlap and stacking, and the total amount of DNA template amplified.

contributors are present in a sample), the number of contributors (i.e. individual sample components) impact the DNA amount of each contributor.

Determining If a DNA Profile Contains a Mixture

Several clues exist to help determine that a mixture is present. Answers to the following questions can help ascertain the genotypes that make up the composite DNA profile of the mixture:

- Do any of the loci show more than two peaks in the expected allele size range?
- Is there a severe peak height imbalance between heterozygous alleles at a locus?
- Does the stutter product appear abnormally high (e.g. <15% to 20%)?

If the answer to any one of these three questions is yes, then the DNA profile may very well have resulted from a mixed sample. Mixture interpretation has been examined extensively by the Forensic Science Service (Clayton et al. 1998, Gill et al. 1998a, 1998b) and many of their strategies have been incorporated into this chapter's material.

Quantitative Capabilities with Peak Heights

The ability to obtain quantitative information from peaks in an electropherogram using an ABI Genetic Analyzer (see Chapter 2) permits relative peak heights or areas of STR alleles to be measured. This peak information can then be used to decipher the possible genotypes of the contributors to the mixed sample. Due to peak shape variation with slab gel analysis, use of peak areas was originally advocated as being superior to use of peak heights when comparing allele peak information (Gill et al. 1998a). However, STR allele peaks are much more symmetrical on capillary electrophoresis systems and thus peak heights are now more routinely used in most laboratories for mixture interpretation.

Figure 6.3 illustrates how typical single-source samples differ from mixed samples in their STR profiles. STR allele peak patterns for heterozygous samples will generally have stutter products that are less than 15% of the associated allele peak height. In addition, the peak height ratio, as measured by dividing the height of the lower quantity peak in relative fluorescence units by the height of the higher quantity allele peak, should be greater than approximately 60% in a single-source sample (Gill et al. 1997, Gill et al. 2006). Thus, if peaks fall in the region between 15% and 60% of the highest peak at a particular STR locus, a mixed sample that has resulted from two or more contributors is probable. The observation of three or more alleles at multiple loci is also a strong indicator of the presence of a mixture.

Mixture Ratios (Proportion)

A mixture ratio is the relative ratio of the DNA contributions of multiple individuals to a mixed DNA typing result, as determined by the use of quantitative peak height information (SWGDAM 2010). It may also be expressed as a percentage of one contributor in the overall sample mixture.

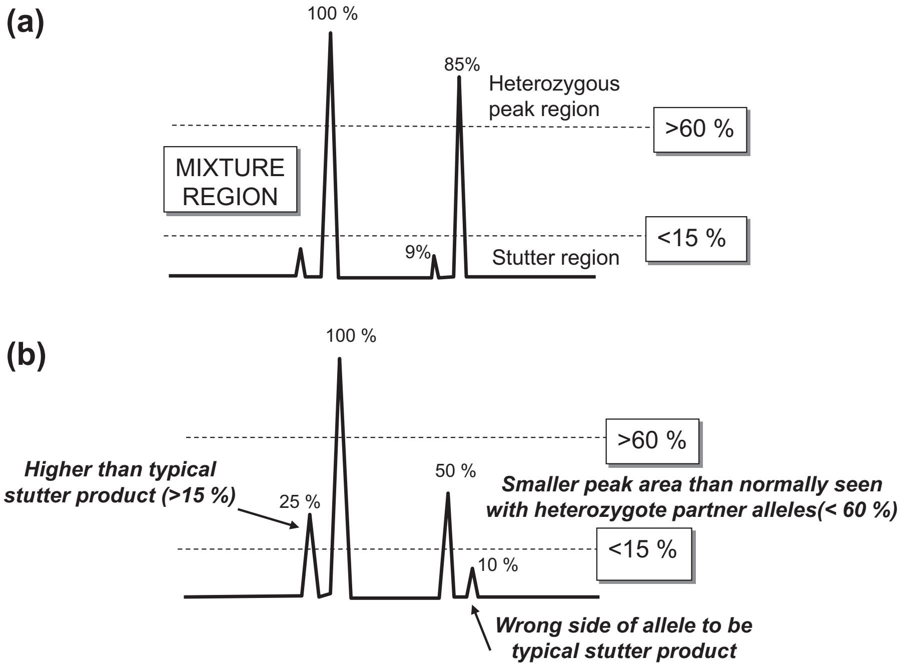


FIGURE 6.3 Illustration of typical single-source (a) versus mixed sample (b) heterozygote peak patterns. The relative peak heights due to the measured fluorescent signal are useful indicators to decipher the presence of a sample mixture. If the highest peak at a locus is set at 100%, then heterozygous alleles in single-source samples should have peak heights that are greater than about 60% of the highest alleles. Stutter products are typically less than 15% of their corresponding allele peak and shorter by four base pairs for tetranucleotide repeats.

As illustrated in Figure 6.2, there is an actual mixture ratio that exists in the true sample components which can only be estimated from relative peak height information in the observed electropherogram data. The deduced mixture proportion(s) can be expressed as a mixture ratio of the sum of peak heights for major alleles over minor alleles or mixture proportion (M_X), which has been referred to as the mass ratio (Wang et al. 2006) (D.N.A. Box 6.2).

Major and Minor Components

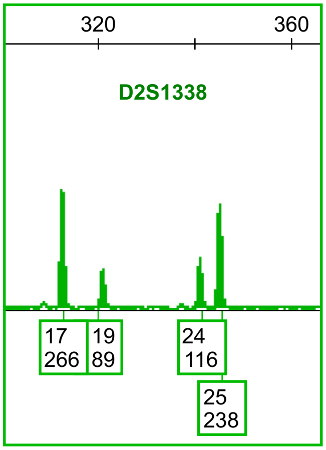
The quantity of each component in a mixture makes a difference in the ability to detect all contributors to the mixed sample. For example, if the two DNA sources are in similar quantities they will be much easier to detect than if one is present at only a fraction of the other.

Figure 6.2 illustrates the D18S51 genotype components of the mixture example in Figure 6.1. The 13,17 genotype is approximately four times the amount of the 13,14 genotype. The genotype in higher quantity is commonly called the “major” component and the genotype in the small quantity is called the “minor” component. The minor component of a mixture is usually not detectable for mixture ratios below the 5% level or 1:20. When the minor component is at a low level it is subject to stochastic effects that will be discussed in greater detail in the next chapter.

D.N.A. BOX 6.2

MIXTURE PROPORTION AND MIXTURE RATIO

With two-person mixtures, it is easiest to calculate a mixture proportion (M_x) or mixture ratio (M_R) by examining peak heights in STR loci with four alleles. The only locus in Figure 6.1 that has four alleles is D2S1338 (see figure insert). It may also be possible to calculate the M_x by using a three-allele locus where a high homozygote is present with two smaller alleles that can be assumed to be a heterozygous minor contributor genotype.



$$M_R = \frac{\varphi_{17} + \varphi_{25}}{\varphi_{19} + \varphi_{24}} = \frac{266 + 238}{89 + 116} = 2.46$$

$$M_x = \frac{\varphi_{17} + \varphi_{25}}{\varphi_{17} + \varphi_{25} + \varphi_{19} + \varphi_{24}}$$

$$= \frac{266 + 238}{266 + 238 + 89 + 116} = 0.711 = 71\ \%$$

In the equations, lower-case Greek letter phi (φ) represents peak height. The peak heights for alleles in the major component were put in the numerator. The calculation can also be done for the minor contributor, but it is typically easier to think in terms of the amount of the major contributor. This example is also discussed in Appendix 4 (see Table A4.1).

Impact of Allele Sharing

Usually a mixture is first identified by the presence of three or more prominent peaks at one or more loci. At a single locus, a sample containing DNA from two sources can exhibit one, two, three, or four peaks due to the possible genotype combinations listed below and illustrated in Figure 6.4. Table 6.1 displays the various genotype combinations using P, Q, R, and S as possible alleles.

When two contributors to a mixed stain share one or more alleles, the alleles are “masked” (i.e. covered up) and the contributing genotypes may not be easily decipherable. For example, if two

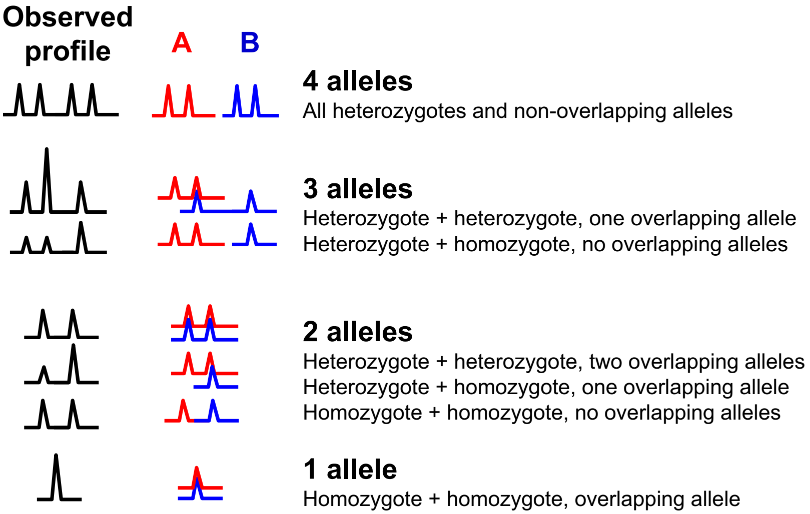


FIGURE 6.4 With mixtures containing DNA from two contributors, each individual STR locus can exhibit 1, 2, 3, or 4 different alleles. There are a total of 14 different combinations of homozgyotes and heterozygotes not considering the reciprocal possibilities (see Table 6.1). Hypothetical results from individual A are in red with individual B results in blue. In this example, the observed profile reflects relative peak height changes that would occur with allele stacking due to shared alleles and a 1:1 mixture ratio.

TABLE 6.1 Possible Genotype Combinations with Two-Person Mixtures Organized by the Number of Observed Alleles at a Locus

2 — 1 allele (P)

2 hom, 1 shared

1	PP	PP

8 — 2 alleles (P, Q)

hom + hom, 0 shared

1	PP	QQ

hom + het, 1 shared

2	PP	PQ
	QQ	PQ

2 het, 2 shared

1	PQ	PQ

12 — 3 alleles (P, Q, R)

hom + het, 1 shared

3	PP	QR
	QQ	PR
	RR	PQ

het + het, 1 shared

3	PQ	PR
	PR	QR
	QR	PQ

6 — 4 alleles (P, Q, R, S)

het + het, 0 shared

3	PQ	RS
	PR	QS
	QR	PS

Reciprocal genotype combinations

2 hom, 1 shared

1	PP	PP

hom + hom, 0 shared

1	QQ	PP

hom + het, 1 shared

2	PQ	PP
	PQ	QQ

2 het, 2 shared

1	PQ	PQ

hom + het, 1 shared

3	QR	PP
	PR	QQ
	PQ	RR

het + het, 1 shared

3	PR	PQ
	QR	PR
	PQ	QR

het + het, 0 shared

3	RS	PQ
	QS	PR
	PS	QR

As illustrated in Figure 6.4, there are a total of 14 different genotype pairs of combinations of homozgyotes (hom) and heterozygotes (het). Below the line are the reciprocal genotype combinations when contributor order is important. Adapted from Overson (2009).

individuals at the FGA locus have genotypes 23,24 and 24,24, then a mixture ratio of 1:1 will produce a ratio of 1:3 for the 23:24 peak areas. In this particular case, the mixture could be interpreted as a homozygous allele with a large stutter product without further information. However, by examining the STR profiles at other loci that have unshared alleles, i.e. three or four peaks per locus, an analyst may be able to dissect or "deconvolute" this sample properly into its components.

In an effort to see whether it was possible for masking to occur at every locus in a multiplex, the Forensic Science Service conducted a simulated-mixture study with 120,000 individual STR profiles in their Caucasian database (Gill et al. 1997). This study found that the vast majority of these artificial mixtures showed 15 to 22 peaks across a 6-plex STR assay. The maximum number in a mixture of two heterozygous individuals with no overlapping alleles at six STRs would be 24 peaks. Thus, in this example with unrelated individuals, simple mixtures can be identified by the presence of three or more alleles at several loci. Out of more than 212,000 pairwise comparisons, there were only four examples where one or two alleles were observed at each locus in the 6-plex, and these could be designated mixtures because of peak imbalances (Gill et al. 1997).

The ability to confidently estimate the number of contributors goes down as the mixture becomes more complex (see Chapter 7).

German Classification Scheme for Mixtures

The German Stain Commission, which is composed of leaders from Institutes of Legal Medicine in Germany, proposed use of a simple classification system for mixture types (Schneider et al. 2009). This system also discusses how to handle the various types of mixtures from a statistical analysis point-of-view. This German classification scheme focuses on two-person mixtures and is based on earlier work by Tim Clayton and John Buckleton (Clayton & Buckleton 2005).

The three German mixture categories are Type A (where there is no obvious major contributor and no evidence of stochastic effects), Type B (where there is a clearly distinguishable major and minor and no stochastic effects), and Type C (where mixtures have no major contributor(s) and there is evidence of stochastic effects) (D.N.A. Box 6.3). The SWGDAM 2010 guidelines calls Type A mixtures "indistinguishable," Type B mixtures "distinguishable," and Type C mixtures "uninterpretable."

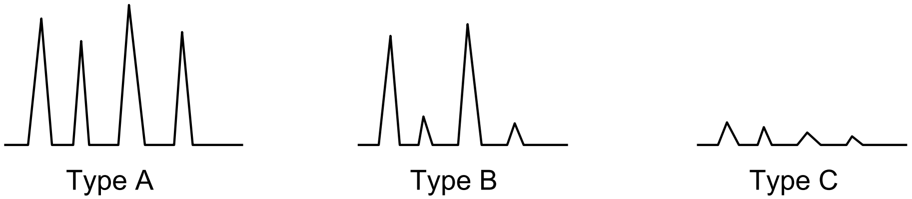
ELEMENTS OF MIXTURE INTERPRETATION

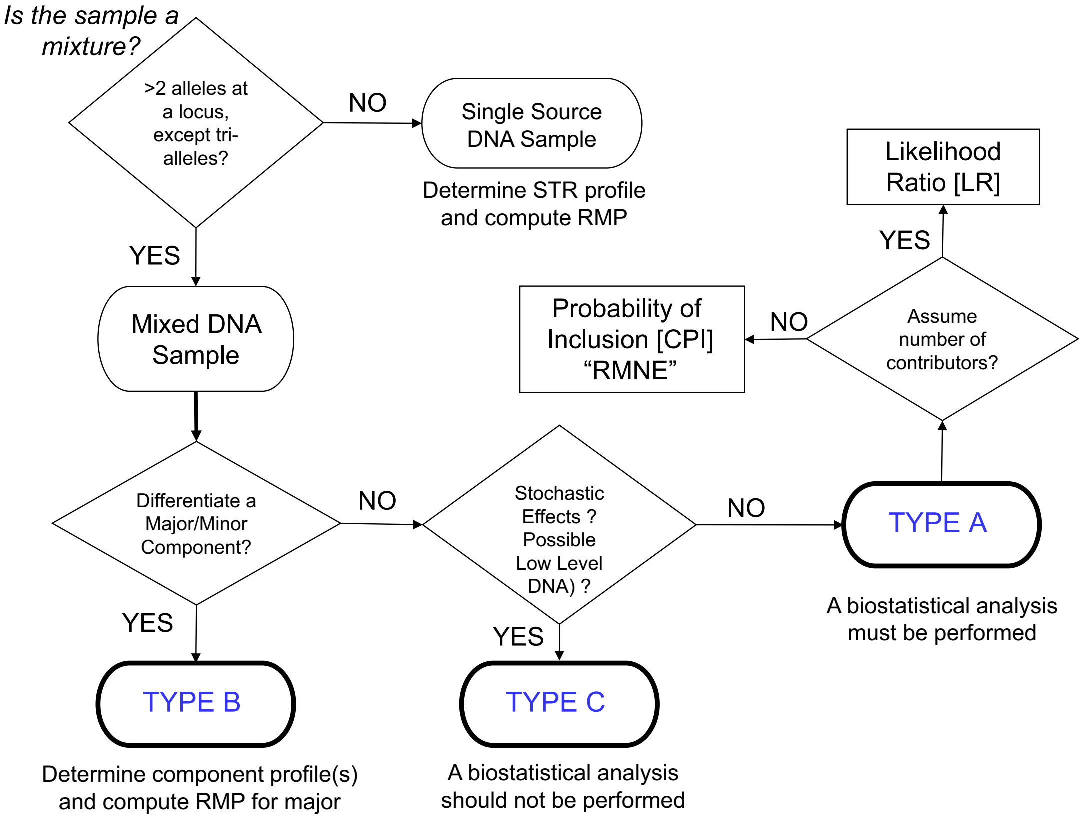
Mixture interpretation involves *interpretation of alleles and possible genotypes* from contributors followed by an *assessment of the statistical weight of evidence* if an association between the evidence DNA profile and the suspect's DNA profile can be made. With some two-person mixtures involving a major and a minor profile, it may be possible to reliably decipher the genotypes of the individual contributors at each tested genetic marker through a mixture deconvolution process. Good practice is driven by well-written protocols and a correct understanding of principles involved.

D.N.A. BOX 6.3

MIXTURE CLASSIFICATION SCHEME FROM THE GERMAN STAIN COMMISSION

Several years ago the German Stain Commission, a group of scientists from Germany's Institutes of Legal Medicine, developed a three-part classification scheme for DNA mixtures. Simple examples of what a Type A (no major contributor), Type B (major and minor contributors distinguishable), or Type C (low level DNA with stochastic effects) mixture might look like at a single STR locus are shown below:





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D.N.A. BOX 6.3—(*cont'd*)

Type A mixtures require a biostatistical analysis that can be performed with a likelihood ratio (LR) or combined probabiilty of inclusion (CPI), which is also known as random man not excluded (RMNE). Type B mixtures can be deconvoluted into the major and minor components, usually if they are present with consistent peak height ratios of approximately 4:1. The major component following deconvolution can be treated as a single-source profile with a random match probability (RMP) being calculated. For Type C mixtures, where all alleles may not be seen due to allele drop-out, a biostatistical interpretation is not appropriate, and a clear decision about whether to include or exclude a suspect may be difficult to reach.

Depending on laboratory interpretation thresholds, the DNA mixture in Figure 6.1 would probably be classified as a Type B or a Type C mixture. A mixture classification flowchart is shown on the previous page based on the three types of mixtures.

Source: *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* Rechtsmedizin *(2006)* 16:401–404*].*

Mixture Solving Strategies

This next section will review the principles described by Gill et al. (1998b) and Clayton et al. (1998) for interpreting mixed forensic stains using STR typing results. Seven primary steps for interpreting mixtures are outlined in Figure 6.5. The interpretation steps are discussed in the context of Figure 6.1, which is covered in more detail in the worked example shown in Appendix 4.

An understanding of how non-mixtures behave is essential to being able to proceed with mixture interpretation. Mixed DNA profiles need to be interpreted against a background of biological and technological artifacts. Chapter 3 and Chapter 4 discussed some of the prominent biological artifacts that exist for STR markers. These include stutter products and null alleles. In addition, chromosomal abnormalities, such as tri-allelic (three-banded) patterns resulting from trisomy (the presence of three chromosomes instead of the normal two) or duplication of specific chromosomal regions can occur. In addition, non-specific amplification products can occasionally occur and must be considered prior to making an attempt to decipher a mixed profile.

Stutter products represent the greatest challenge in confidently interpreting a mixture and designating the appropriate alleles. It is not always possible to exclude stutters since they are allelic products and differ from their associated allele by a single repeat unit. The general guideline for stutter identification of one repeat unit less than the corresponding allele and less than 15% of that allele's peak area is typically a useful one and can be used to mark suspected stutter products. The introduction of pentanucleotide repeat markers with stutter products of less than 1% or 2% has greatly simplified mixture interpretation for these loci (Bacher et al. 1999; Krenke et al. 2002).

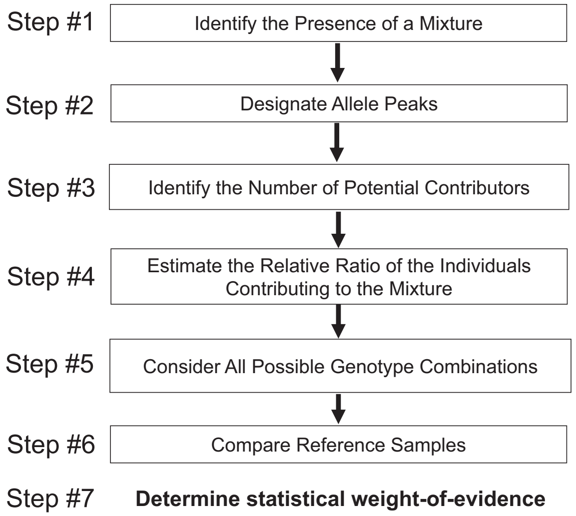

FIGURE 6.5 Steps in mixture interpretation. Adapted from Clayton et al. (1998).

After a mixture has been identified as such and all of the alleles have been called, the next step (Figure 6.5, step #3) is to identify the number of potential contributors. For a two-person mixture, the maximum number of alleles at any given locus is four if both individuals are heterozygous and there is no allele overlap. Thus, if more than four alleles are observed at a locus then a complex mixture consisting of more than two individuals is possible. In the past, the overwhelming majority of mixtures encountered in forensic casework involved two-person mixtures (Clayton et al. 1998).

Mixtures can range from equal proportions of each component to one component being greatly in excess. The varying proportions of a mixture are usually referred to in a ratio format (e.g. 1:1 or 1:5). Mixtures of known quantities of DNA templates have shown that the mixture ratio is approximately preserved during PCR amplification (Gill et al. 1998a, Perlin & Szabady 2001). Thus, the peak areas and heights observed in an electropherogram can in most cases be related back to the amount of DNA template components included in the mixed sample.

An approximate mixture ratio can be best determined by considering the profile as a whole and looking at all of the information from each locus. The ratio of mixture components is most easily determined when there are no shared alleles at a locus. Thus, it is best to first examine loci with four alleles as a starting point for estimating the relative ratio of the two individuals contributing to the mixture. Determining the ratio when there are shared alleles is more complex because there may be more than one possible combination of alleles that could explain the observed peak patterns (Clayton et al. 1998).

The possible combinations of alleles for two-, three-, and four-allele peak patterns are illustrated in Figure 6.4. For four alleles at a locus, there are three possible pairwise comparisons that exist, if one does not worry about the reciprocal cases, i.e. which allele combinations belong to the minor

TABLE 6.2 Possible Amelogenin X and Y Allele Peak Height Ratios with Varying Quantities of DNA

Mixture ratio		Allele combination		Ratio of X:Y peak heights
Female (X,X)	**Male (X,Y)**	**X**	**Y**	**X:Y**
20	1	41	1	41:1
10	1	21	1	21:1
5	1	11	1	11:1
4	1	9	1	9:1
3	1	7	1	7:1
2	1	5	1	5:1
1	1	3	1	3:1
1	2	4	2	2:1
1	3	5	3	1.7:1
1	4	6	4	1.5:1
1	5	7	5	1.4:1
1	10	12	10	1.2:1
1	20	22	20	1.1:1

In Figure 6.1, the amelogenin X:Y peak height ratio (588 RFU: 61 RFU) is 9.6, which suggests that the relative ratio of female (major) to male (minor) is approximately 4:1.

contributor and which belong to the major contributor. For three alleles at a locus, there are six possible pairwise combinations, and for two alleles at a locus there are four possible pairwise combinations (Table 6.1).

Amelogenin, the sex-typing marker, is an effective marker for deciphering the contributions of genetically normal male and female individuals. The predicted X and Y allele peak ratios for a number of possible male and female mixture ratios are listed in Table 6.2. The amelogenin X and Y peak areas are especially useful in determining whether the major contributor to the mixture is male or female.

The next step in examining a mixture is to consider all possible genotype combinations at each locus (Figure 6.5, step #5). Peaks representing the allele calls at each locus are labeled with the designations P, Q, R, and so forth. The possible pairwise combinations from Table 6.1 are considered using the peak heights for each called allele. Each particular combination of alleles at the different loci is considered in light of the information determined previously regarding the mixture ratio for the sample under investigation (step #4). By stepping through each STR locus in this manner, the genotypes of the major and minor contributors to the mixture can be deciphered. In the example shown in Appendix 4 some of these calculations are demonstrated. Table 6.3 illustrates the possible major and minor genotypes for the D18S51 locus in Figure 6.1.

Many times specific combinations of genotypes can be eliminated or restricted due to the possible allele pair not meeting the expected levels of heterozygote balance or the needed mixture ratio for an

TABLE 6.3 Possible Genotype Combinations with D18S51 Data from Figure 6.1 Assuming Two Contributors

If the major genotype is	then the minor genotype is…
13,13	14,17
13,14	17,17 *or* 13,17 *or* 14,17
13,17	14,14 *or* **13,14** *or* 14,17
14,14	13,17
14,17	13,13 *or* 13,14 *or* 13,17
17,17	13,14

There are 12 possible genotype combinations with three alleles (see Table 6.1). Use of peak height information can help eliminate many of these possibilities. Making assumptions regarding mixture ratios can eliminate some other possibilities. In this situation with a 4:1 mixture, the correct genotypes are 13,17 for the major contributor and 13,14 for the minor contributor (shown in bold font).

overall STR profile. A restricted approach utilizes peak height information to help deconvolve a mixture (D.N.A. Box 6.4).

The sixth step in the interpretation of a mixture is to compare the resultant genotype profiles for the possible components of the mixture with the genotypes of reference samples (Figure 6.5, step #6). This is also known as the question-to-known (Q-to-K) profile comparison. In a sexual assault case, this reference sample could be the suspect and/or the victim. If the DNA profile from the suspect's reference sample matches the major or minor component of the mixture, then that person cannot be eliminated as a possible contributor to the mixed stain (Clayton et al. 1998).

Finally, with Q-to-K comparisons where the known profile cannot be excluded, a statistical weight-of-evidence is generated to provide context to the association being made. Chapter 12 will discuss several statistical approaches that can be taken.

RESOURCES AND GUIDANCE ON MIXTURE INTERPRETATION

ISFG 2006 and SWGDAM 2010 Recommendations

In July 2006, the DNA Commission of the International Society of Forensic Genetics (ISFG) published nine recommendations covering mixture interpretation principles (Gill et al. 2006). The DNA Commission endorsed the mixture interpretation steps covered in this chapter that were published in 1998 by the Forensic Science Service (Clayton et al. 1998). Since a number of prominent

D.N.A. BOX 6.4

UNRESTRICTED VERSUS RESTRICTED GENOTYPE COMBINATIONS

An *unrestricted* approach considers all combinations of genotypes possible because relative peak height differences are not utilized. It is as though all of the allele peaks are the same height in the electropherogram. With data near the limit of detection or where stochastic effects may cause allele drop-out, the ability to confidently eliminate some genotype possibilities may be reduced.

Quantitative data from allele peak heights can be used in some cases to eliminate possible genotype combinations (see figure). A *restricted* approach eliminates some of the possible genotype combinations based on relative peak heights. Alleles are paired into potential genotypes only where specific combinations are deemed possible using relative peak height information and validated heterozygote balance and stutter ratio ranges. The ability to restrict genotype combinations of possible contributors is based on assumptions made regarding the number of contributors, consideration of quantitative peak height information, and inference of contributor mixture ratios (SWGDAM 2010).

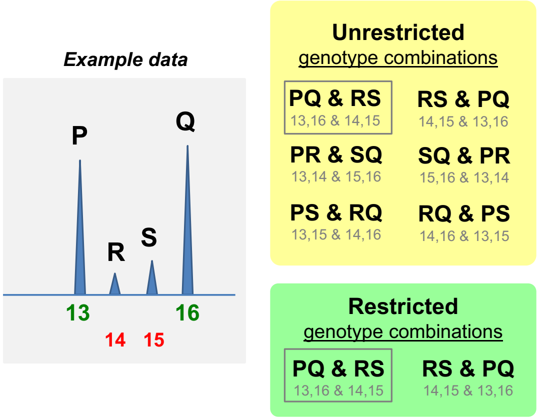

Figure caption: Example data exhibits what appears to be a PQ (13,16) major and a RS (14,15) minor. An unrestricted approach generates six possible genotype combinations, many of which are unreasonable in terms of heterozygote balance (e.g. PR and SQ). The restricted approach eliminates combinations that do have have reasonable heterozygote balance. Mixture ratio information, based on data from multiple loci, could also be used to help eliminate and restrict possible genotype combinations.

statisticians were part of this paper, these recommendations favor use of likelihood ratios (Evett et al. 1991, Weir et al. 1997).

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.

In January 2010 SWGDAM approved interpretation guidelines covering autosomal STR markers with guidance on mixture interpretation (SWGDAM 2010). Among other topics, these guidelines emphasized the importance of using a stochastic threshold in conjunction with a combined probability of inclusion statistical approach. Table 6.4 compares the ISFG 2006 recommendations for mixtures with sections of the SWGDAM 2010 guidelines that cover the same areas.

Software for Deciphering Mixture Components

Computer programs can be used to aid the process of deciphering mixture components and determining mixture ratios. Many laboratories have created their own spreadsheet programs to perform calculations needed in mixture interpretation.

A linear mixture analysis approach can derive estimated mixture ratios from quantitative STR peak information that are similar to known input mixture proportions (Perlin & Szabady 2001). This approach combined with probabilistic genotyping is the basis of the TrueAllele program from Cybergenetics (Perlin et al. 2011). Researchers at the University of Tennessee developed a least-squares deconvolution approach to decipher mixture components in an automated fashion (Wang et al. 2006). The UK Forensic Science Service developed a program named PENDULUM that evaluated potential genotype combinations with mixture ratios and heterozygote peak height balance to restrict possible genotypes (Bill et al. 2005). GeneMapper*ID-X* has a mixture analysis tool (Oldroyd & Shade 2008) that has been evaluated by researchers in Norway (Hansson & Gill 2011). A researcher at the U.S. Army Criminal Investigation Laboratory (USACIL) created a spreadsheet program called DNA_DataAnalysis (Overson 2009) that has been commercialized by NicheVision Forensics into Armed Xpert™ (2014). Other software tools for mixture deconvolution and statistical analysis are also listed on the STRBase website (NIST STRBase Mixture Information 2014).

Interlaboratory Studies

Five exploratory interlaboratory studies assessing DNA mixtures have been conducted by NIST over the time period of 1997 to 2013 (D.N.A. Box 6.5). Interlaboratory studies provide an interesting window into the practices and protocols under use at the time of a study. In addition, results from these studies expand the view of the community beyond each laboratory's focus on their own protocol and analyst performance.

STRBase Website and Training Materials

Since the NIST MIX05 interlaboratory study identified a large variability in DNA mixture interpretation results, a great deal of effort has gone into training and informing the community (NIST MIX05 2005). Over 50 workshops and presentations have been given by the author and NIST colleagues (Table 6.5). Our understanding of mixture interpretation has grown over the years and evolved with experiences in teaching the principles involved.

A few years ago a mixture section was established as part of the NIST STRBase website (NIST STRBase Mixture Information 2014). This website contains workshop materials and presentations given to help with mixture training, a listing of available software programs, and literature references.

TABLE 6.4 The DNA Commission of the International Society for Forensic Genetics (ISFG) 2006 Recommendations on DNA Mixture Interpretation (Gill et al. 2006) Are Compared with Corresponding Portions of the SWGDAM 2010 Guidelines (SWGDAM 2010)

ISFG (2006) recommendations	SWGDAM (2010) guidelines
Recommendation 1: The likelihood ratio is the preferred approach to mixture interpretation. The RMNE (probability of exclusion) 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.	**4. Statistical Analysis of DNA Typing Results** In forensic DNA testing, calculations are performed on evidentiary DNA profiles that are established as relevant in the context of the case to aid in the assessment of the significance of an inclusion. These calculations are based on the random match probability (RMP), the likelihood ratio (LR), or the combined probability of exclusion/inclusion (CPE/CPI). **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.
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 courtroom.	No equivalent guideline.
Recommendation 3: The methods to calculate likelihood ratios of mixtures (not considering peak area) described by Evett *et al.* (*J. Forensic Sci. Soc.* 1991;31:41—47) and Weir *et al.* (*J. Forensic Sci.* 1997;42:213—222) are recommended.	The Evett et al. (1991) and Weir et al. (1997) references are included in section 7 (Additional Suggested Readings). **5.4.2.3** Additional formulae for restricted and unrestricted LRs can be found in Fung and Hu (2008).
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.* (*Forensic Sci. Int.* 1998; 91:55-70).	The Clayton et al. 1998 reference is included in section 7 (Additional Suggested Readings).

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 defense. The prosecution and defense both seek to maximize 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.	**5.4.2.** The calculation of the LR in a mixture is dependent upon the evidence profile, the comparison reference profile(s), and the individual hypotheses.
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 not support H_p should be included in the assessment.	**3.5.8.3.** If a peak is at or below [a laboratory's established stutter percentage expectation], it is generally designated as a stutter peak. However, it should also be considered as a possible allelic peak, particularly if the peak height of the potential stutter peak(s) is consistent with (or greater than) the heights observed for any allelic peaks that are conclusively attributed (i.e. peaks in non-stutter positions) to the minor contributor(s).
Recommendation 7: If drop-out of an allele is required to explain the evidence under H_p: ($S = ab$; $E = a$), then the allele should be small enough (height/area) to justify this. Conversely, if a full crime stain profile is obtained where alleles are well above the background level, and the probability of drop-out approaches $\Pr(D) \approx 0$, then H_p is not supported.	No equivalent guideline.
Recommendation 8: If the alleles of certain loci in the DNA profile are at a level that is dominated by background noise, then a biostatistical interpretation for these alleles should not be attempted.	No equivalent guideline.
Recommendation 9: 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.	[from Preamble] …this document is not intended to address the interpretation of analytical results from enhanced low template DNA techniques.

D.N.A. BOX 6.5

NIST INTERLABORATORY STUDIES INVOLVING DNA MIXTURES

Over the past two decades, the National Institute of Standards and Technology (NIST) has conducted 13 exploratory interlaboratory studies addressing DNA typing issues. Five of these studies (see table below) have dealt with DNA mixtures. Interlaboratory studies involve multiple labs examining the same samples or data to look for consistencies or differences in results, trends in laboratory practice, and potential opportunities for further training and reference material development. Mixed stain study 1 (MSS1) was conducted from April 1997 through November 1997 and focused on assessing how well participating laboratories could determine donor types given a complete set of reference sources. Data from MSS2 was collected from January 1999 to May 1999 with a focus on evaluating donor types given an incomplete set of references. The impact of DNA quantitation was also explored. MSS3, which ran from December 2000 to October 2001, examined the effect of DNA quantitation on STR typing performance.

These interlaboratory studies provide a brief snapshot in laboratory technology, methods, and practices used at the time of the study. For example, while only 20% of MSS1 participants used the ABI 310 single-capillary instrument, this number jumped to 47% of MSS2 and 72% of MSS3 participants as more laboratories moved to STR typing with capillary electrophoresis. Since there are inherent laboratory differences in DNA quantitation, PCR amplification, and instrument detection sensitivity, the MIX05 and MIX13 studies have only involved data interpretation based on supplied .fsa files from DNA mixtures created at NIST with commonly used STR kits. Data files were then posted on the STRBase website for laboratories to download and test. This data-only approach significantly reduces the costs and logistics of preparing, packaging, and mailing DNA samples to participating laboratories. DNA samples were prepared with Profiler Plus, COfiler, SGM Plus, Identifiler, and PowerPlex 16 data for MIX05 and only Identifiler and PowerPlex 16 data for MIX13. These data sets reflect the STR kits in general use at the time the studies were conducted. A primary goal of MIX05 was to evaluate the "lay of the land" in order to determine future needs for training and tools for better mixture interpretation. Due to the wide range of variation seen within and among MIX05 participating laboratories, extensive mixture training has been conducted since 2005 (see Table 6.5). An important purpose of the MIX13 results was to examine whether consistency in mixture interpretation has improved across North America since the publication of the 2010 SWGDAM guidelines on autosomal STR interpretation. The MIX13 results were presented at the Technical Leader Summit held in Norman, Oklahoma in November 2013. A publication describing these results is planned.

Year (study)	# Labs	What was supplied
1997 (MSS1)	22	Mixed buffy coat cells on paper; six single-source, four two-person, one three-person (stains on paper).

Year (study)	# Labs	What was supplied
1999 (MSS2)	45	Bloodstains & semen on cotton cloth; Part A: four single-source, one two-person, one three-person (stains on paper); Part B: one two-person mixture at five different concentrations.
2001 (MSS3)	74	Extracted DNA samples supplied; one single-source, five two-person, one three-person (all extracts).
2005 (MIX05)	69	Electronic data supplied (covering five STR kits) mimicking sexual assault evidence; four two-person mixtures (all unrelated, male/female, various major/minor ratios).
2013 (MIX13)	108	Electronic data (covering two STR kits) and case scenarios supplied mimicking sexual assault and touch evidence; five cases (two-, three-, >three-person; relatives, low-template, inclusion/exclusion).

Sources: *http://www.cstl.nist.gov/strbase/interlab.htm; Duewer, D.L., et al. (2001). NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples.* Journal of Forensic Sciences, *46, 1199–1210; Kline, M.C., et al. (2003). NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity.* Analytical Chemistry, *75, 2463–2469; Duewer, D.L., et al. (2004). NIST Mixed Stain Study #3: signal intensity balance in commercial short tandem repeat multiplexes,* Analytical Chemistry, *76, 6928–6934; http://www.cstl.nist.gov/strbase/interlab/MIX05.htm; http://www.cstl.nist.gov/strbase/interlab/MIX13.htm.*

TABLE 6.5 Summary of 51 DNA Mixture Workshops and Presentations Given by the Author or NIST Colleagues to More Than 7,000 Attendees Since 2005

Date	Meeting	Location	#Attendees (approximate)
27–28 September 2005	ISHI 2005 poster presentation	Grapevine, TX	poster
30 September 2005	ISHI 2005 HITA workshop	Grapevine, TX	70
20 February 2006	AAFS 2006	Seattle, WA	200
6 June 2006	NYC OCME training	New York City, NY	120
7 August 2006	MN BCA training	St. Paul, Mn	35
23 October 2006	CODIS Conference talk	Arlington, VA	400
5–6 December 2006	NJSP training	Hamilton, NJ	100
3–4 April 2007	Houston PD training	Houston, TX	50
11 September 2007	SAFS workshop	Atlanta, GA	50

(Continued)

TABLE 6.5 Summary of 51 DNA Mixture Workshops and Presentations Given by the Author or NIST Colleagues to More Than 7,000 Attendees Since 2005 (*cont'd*)

Date	Meeting	Location	#Attendees (approximate)
2–3 November 2007	NEAFS workshop	Bolton Landing, NY	40
19 February 2008	AAFS 2008	Washington, DC	200
10 April 2008	CE Users' Group	Ammendale, MD	40
25 April 2008	Virginia criminal defense seminar	Richmond, VA	650
12–13 May 2008	Florida statewide training	Indian Rocks Beach, FL	80
11 November 2008	CODIS Conference workshop	Crystal City, VA	350
23 January 2009	AFDIL training	Rockville, MD	20
27–28 January 2009	Harris County Texas training	Houston, TX	80
25 March 2009	NYC OCME training	New York City, NY	150
7 April 2009	Towson University class	Towson, MD	25
12 May 2009	Wisconsin DNA training	Milwaukee, WI	75
13–14 May 2009	Utah DNA training	Salt Lake City, UT	40
20 May 2009	NDAA lawyer training	Columbia, SC	50
20–24 July 2009	FIU DNA workshop	Miami, FL	15
22 September 2009	NWAFS workshop	Ft. Collins, CO	30
15 October 2009	ISHI TL session	Las Vegas, NV	50
29 October 2009	George Washington University	Washington, DC	40
11 October 2010	ISHI 2010 Mixture workshop	San Antonio, TX	200
22 February 2011	AAFS 2011 Mixture workshop	Chicago, IL	220
15–17 March 2011	NFSTC mixture workshop	Largo, FL	120
28 March 2011	Indiana State Police training	Indianapolis, IN	65
12 April 2011	Maryland DNA training	Pikesville, MD	60
26–27 April 2011	Florida DNA training	Palm Beach, FL	50
9–10 May 2011	Texas DNA training	Houston, TX	50
16–17 May 2011	Michigan DNA training	Lansing, MI	50
6–7 June 2011	Arizona DNA training	Mesa, AZ	50
1–3 August 2011	Hawaii DNA training	Honolulu, HI	10
3 October 2011	ISHI 2011 mixture workshop	Washington, DC	160
25 October 2011	CAC fall meeting	Sacramento, CA	100
18 April 2012	New York/New Jersey	New York City, NY	150
8 May 2012	Canada/RCMP training	Vancouver, BC	40

TABLE 6.5 Summary of 51 DNA Mixture Workshops and Presentations Given by the Author or NIST Colleagues to More Than 7,000 Attendees Since 2005 (*cont'd*)

Date	Meeting	Location	#Attendees (approximate)
6–7 June 2012	Taiwan DNA training	Taipei, Taiwan	60
20 June 2012	NIJ Conference mini-workshop	Crystal City, VA	150
13 September 2012	Washington State Patrol training	Seattle, WA	50
25 September 2012	MAFS workshop	Milwaukee, WI	50
15 October 2012	ISHI 2012 mixture workshop	Nashville, TN	120
12 April 2013	**NIST webcast**	Gaithersburg, MD	1100
18 April 2013	NACDL post-Conviction conference	Charlotte, NC	120
7 June 2013	ABA Fordham Law School talk	New York City, NY	150
2–3 Sept 2013	ISFG 2013 workshops	Melbourne, Australia	100
20–21 Nov 2013	**Technical Leaders' Summit**	Norman, OK	550
21 February 2014	AAFS 2014 talk	Seattle, WA	400

Feedback was collected from some meeting participants through audience response systems or written surveys. Slides and handouts are available on the NIST STRBase website (see http://www.cstl.nist.gov/strbase/NISTpub.htm or http://www.cstl.nist.gov/strbase/mixture.htm).

A number of the workshops were conducted in collaboration with Dr. Michael Coble from the NIST Applied Genetics Group, Boston University researchers Dr. Robin Cotton and Dr. Catherine Grgicak, and independent consultant Dr. Charlotte Word. Boston University received a training grant from the National Institute of Justice that funded the workshops performed in 2010, 2011, and 2012. In addition, Boston University (BU) has created a training website (Boston University DNA Mixture Training 2014) with about a dozen lessons that take a student through the various steps of mixture interpretation. The BU website also contains more than 2,700 .fsa files with single-source, two-person, three-person, and four-person mixtures at different mixture ratios and DNA amounts that can be downloaded and used in training programs.

COMPLICATING FACTORS IN MIXTURE DECONVOLUTION

Two of the primary complicating factors in deciphering mixture components are the potential for allele sharing that results in peak signal stacking (Figure 6.6) and handling potential alleles in the stutter position (Figure 6.7). The potential of N+4 forward stutter (see Chapter 3) can also complicate mixture interpretation.

These complicating factors increase interpretation uncertainty in some STR profiles. Some forensic DNA laboratories may decide not to go through the trouble of fully deciphering the genotype possibilities and assigning them to the major and minor contributors even for simple two-person

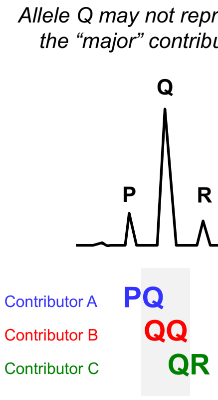

FIGURE 6.6 Illustration of the impact of allele sharing. This type of result where allele Q appears to be from a "major" contributor is possible with a three-person mixture containing fairly equal amounts of each genotype (PQ + QQ + QR).

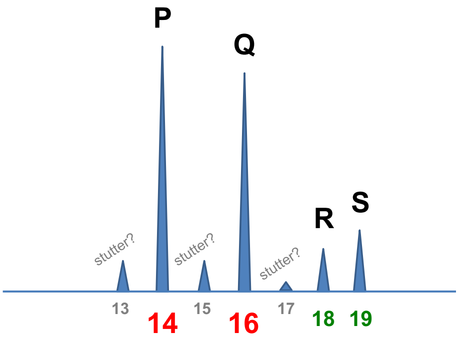

FIGURE 6.7 Hypothetical example of STR mixture data at the D18S51 locus illustrating that when minor components are similar in peak height to stutter products of the major alleles these stutter products need to be considered as possible alleles from another minor contributor.

mixtures. An easier approach is to simply include or exclude a suspect's DNA profile from the crime scene mixture profile. If all of the alleles from a suspect's DNA profile are represented in the crime scene mixture, then the suspect cannot be excluded as contributing to the crime scene stain. Likewise, the alleles in a victim's DNA profile could be subtracted from the mixture profile to simplify the alleles that need to be present in the perpetrator's DNA profile. Approaches to attaching a statistical value to mixture results are presented in Chapter 12.

Reading List and Internet Resources

General Information

Bacher, J. W., et al. (1999). Pentanucleotide repeats: highly polymorphic genetic markers displaying minimal stutter artifact. *Proceedings of the 9th International Symposium on Human Identification*, 24–37.

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Gonzalez-Andrade, F., et al. (2006). DNA mixtures in forensic casework resolved with autosomic STRs. *Progress in Forensic Genetics 11, ICS, 1288*, 580–582.

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