ELSEVIER

Forensic Science International
91 (1998) 55–70

Forensic Science International

Analysis and interpretation of mixed forensic stains using DNA STR profiling

T.M. Clayton[a,*], J.P. Whitaker[a], R. Sparkes[b], P. Gill[b]

[a]*Forensic Science Service, Wetherby Laboratory, Sandbeck Way, Audby Lane, Wetherby, West Yorkshire LS22 4DN, UK*
[b]*Forensic Science Service, Priory House, Gooch Street North, Birmingham B56QQ, UK*

Received 13 May 1997; received in revised form 9 October 1997; accepted 27 October 1997

Abstract

The use of multiplex PCR and fluorescent dye technology in the automated detection and analysis of short tandem repeat loci provides not only qualitative information about the profile—i.e. which alleles are present—but can also provide quantitative information on the relative intensities of the bands, and is therefore a measure of the amount of amplified DNA. The availability of this quantitative information allows for the interpretation of mixtures in a detailed way which has not been previously possible with many other human identification systems. In this paper we present a simple approach to the resolution and analysis of mixed STR profiles resulting from the testing of mixed biological stains in forensic casework and highlight factors which can affect it. This approach requires a detailed knowledge—gained through a mixture of experiments and validation studies—of the behaviour of each locus within the multiplex systems described. We summarise the available data from previously published experimental work and validation studies to examine the general principles underlying this approach. © 1998 Elsevier Science Ireland Ltd

Keywords: PCR; Fluorescent dyes; STR profiling; Mixtures; Forensic casework; Interpretation

1. Introduction

When DNA is extracted from a biological stain which contains body fluids or tissue

*Corresponding author

PII S0379-0738(97)00175-8

from more than one individual, the result is often a mixed STR profile. This consists essentially of one person's STR profile superimposed on that of another. During routine casework, mixed STR profiles can be anticipated in a number of situations depending on the extraction protocol employed and/or the type of body fluids under test. Post-transfusion blood is an obvious example but, more commonly, a mixed profile stems from testing body fluids present on intimate samples, such as fingernail clippings or swabs taken from the skin and body orifices. This is because, in such instances, it is not usually possible to separate the various cell types, e.g. blood mixed with saliva. One exception to this is spermatozoa, which can be preferentially extracted from other cell types. However, on some occasions the preferential extraction procedure is not totally effective in removing all traces of non-seminal DNA, in which event the DNA derived from the seminal fraction will still contain a proportion of non-seminal DNA. Furthermore, as PCR-based tests can be sufficiently sensitive to detect background cellular material deposited on the fabric of clothes by the wearer, a single blood spot on a garment, for instance, could also yield a mixed STR profile when tested.

For use in forensic casework, the Forensic Science Service (FSS) has developed two multiplexed STR systems, both of which utilise fluorescently labeled primers in conjunction with a Perkin-Elmer/Applied Biosystems 373/377 Genesequencer, for automated detection and sizing of the amplified DNA [1]. The 373/377 Genesequencer software can display the detected bands in a graphical format as peaks by plotting band size (on the *x*-axis) against the degree of fluorescence. This is known as an electropherogram.

As well as sizing the detected bands for allelic designation, the software also displays their peak heights and automatically calculates the area under each peak and displays it in tabulated form. Peak area is a more accurate indicator of the relative amount of DNA present in a fragment than peak height, since the former takes account of the morphology of the band. Thus, the peak area values accompanying the band sizes provide the scientist with valuable quantitative information about the profile. As fluorescent signals are known to be linear over a much greater range of intensities than film-based detection methods, fluorescence detection is a more accurate measure of the amount of amplified DNA present [3].

The first system developed by the FSS was a quadruplex test [2,4] based on the tetrameric STR loci HUMVWFA [5], HUMTHO1 [6], HUMF13A1 [7] and HUMFES/FPS [8]. This has been in use in the Criminal Justice system of England and Wales since mid-1994. Recently, following a change in legislation to allow the formation of a National Criminal Intelligence DNA Database, a second generation system has been developed using a heptaplex test [9]. The heptaplex system comprises a gender test—utilising the X–Y homologous gene amelogenin [10,11]—in conjunction with six tetrameric STR loci. Two of the loci are in common with its quadruplex predecessor (HUMVWFA and HUMTHO1). The remaining four loci are HUMFIBRA(FGA) [12], D8S1179 [9], D21S11 [13] and D18S51 [14].

We advocate that the analysis and interpretation of a mixed STR profile follows a series of logical steps.

2. Step 1: identify the presence of a mixture

2.1. By the presence of extra bands

As previously stated, the presence of a mixed STR profile can often be anticipated from the type of sample under test. However, interpretation must be carried out in isolation of any prior information about the case. This is usually straightforward as a mixed STR profile is indicated by the presence of three (or more) bands at any locus (see Appendix B and Appendix C). Simulation experiments using the heptaplex system described above have shown that, for full STR profiles, the number of occasions in which a two-person mixture would **not** be detected on account of the lack of extra bands in the whole profile, is remote [15]. The propensity for this situation to occur will be increased when partial STR profiles are encountered and/or persons who are related to each other are involved. However, the presence of additional bands at any particular locus is not necessarily diagnostic of a mixture because other circumstances can lead to extra bands, giving the (wrong) impression of a mixed STR profile. A short consideration whereby this can occur is given in this paper: comprehensive details are given by Gill et al. [15] and Sparkes et al. [16].

2.1.1. Stutter bands

The first and most common cause of extra bands are usually termed 'stutters' and are caused by slippage of the Taq polymerase enzyme during copying of the STR allele. In simple, tetramerically repeating STR loci the position of a stutter will correspond to one full repeat unit shorter than the main band (Fig. 1). Stutter bands occur frequently when tetrameric STR loci are co-amplified in a multiplexed system and are a normal consequence of amplification reactions which are not optimal for all of the constituent loci. The frequency of stuttering varies not only between loci—depending on the multiplex system in use—but may also vary between the alleles within a locus, depending upon sequence variation in the repeats. Stutter bands have smaller peak area in relation to the main band; usually of the order of 15% or less of the peak area of the

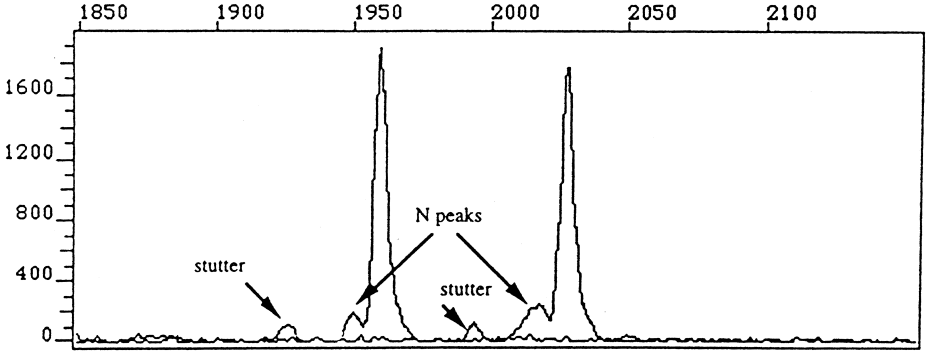

Fig. 1. Stuttering and *N* banding at a heterozygous tetrameric STR locus.

main band [15]. This may complicate the interpretation of mixtures where one component is at low level and for this reason are noteworthy.

2.1.2. Non-specific artefacts

Non-specific artefacts are usually the result of non-specific priming in a multiplex system [15]. In general, the more loci that are co-amplified, the greater will be the propensity for non-specific priming to occur because there will more primer pairs in the reaction mixture. However, non-specific artefacts are also seen when relatively few loci are co-amplified especially when the DNA in the sample is degraded [18]. This has been attributed to the exposure of short sequences of complementarity in the degraded template. Almost all of the artefacts encountered to date have low peak areas, many have an aberrant peak morphology and, moreover, most do not fall within the allelic range of the locus or loci with the appropriate coloured fluorescent dye. Furthermore, the position of artefact bands are rarely consistent with band shift expectations, calculated as described by Gill et al. [19]; for these reasons they are easily recognised [15].

2.1.3. Chromosomal abnormalities

Extra DNA bands can arise in rare cases due to chromosomal abnormalities, such as chromosomal translocations, somatic mutations and trisomies, in the cells of the donor of the forensic stain. However, the resultant STR profile would most probably only show a single extra band. Furthermore, the same pattern of DNA bands should be present in both the forensic stain and the reference sample from the matching suspect. For these reasons, the rare cases in which chromosomal abnormalities are encountered should not be problematic and, paradoxically, may serve to strengthen the final conclusions.

2.1.4. 'N' bands

Taq polymerase has an additional activity that causes the addition of a single nucleotide to the terminus of a newly synthesised DNA molecule. Following amplification, this can result in the formation of two species of DNA molecule generated from the same target sequence, and which differ in size by one base (see Fig. 1). This is because not all copies ('N' fragments) have undergone base addition to form '$N+1$' fragments. Incomplete conversion of N into $N+1$ bands may be prevalent at some loci but not others [16]. When designating alleles in a profile, the presence of a prominent N band in conjunction with an $N+1$ band must be distinguished from the presence of two alleles differing in size by a single base pair. Some tetrameric STR loci have alleles with fractions of a repeat unit. For example, at the HUMTHO1 locus, two commonly occurring alleles (9.3 and 10) are separated by a single base pair [17].

2.1.5. Software issues

The dyes used to label amplified DNA fragments fluoresce at different wavelengths. However, there is some overlap in the emission spectra of dyes and, therefore, a blue-labeled DNA fragment, for example, will also emit a small proportion of green fluorescence. A software tool called a 'matrix file' mathematically compensates for the spectral overlap between fluorescent dyes by subtracting minor colours from the main colour. If an overamplified sample is analysed this can saturate the matrix and can result

in a 'pull-up peak' [16] of another colour under the main peak. Since that other colour may also be used to label another locus within the same multiplex, these pull-up peaks need to be differentiated from true alleles. Position and peak morphology can resolve this ambiguity or, alternatively, a further analysis, such as singleplexing the locus of interest, may be performed.

In summary, additional DNA bands at several loci in the multiplex are generally a good indication that a mixture of body fluids is present. Other possible explanations for additional bands can be resolved by a process of repeat analysis and/or further testing. By considering all of the loci in the STR profile, and by drawing on information gained from validation studies, it is possible to separate artefactual bands from those which are true alleles and the result of co-amplification of DNA from more than one source. The factors considered above are, in fact, an integral part of the process of designating alleles in any profile. Accordingly, interpretation of all bands in an STR profile is a fundamental part of the analysis, irrespective of whether the sample is a mixture.

2.2. By the presence of allele peak area asymmetry

In some circumstances, particularly in those cases where the individuals involved are closely related to one another, or where a partial profile is obtained, a mixed stain may exhibit no additional bands at all (see Appendix A). Although no additional bands may be present at a locus, the presence of a mixed STR profile can sometimes be inferred if the allele peak areas are asymmetric at an apparently heterozygous locus. A significant perturbation in the peak balance could arise due to a number of factors:

2.2.1. Differential amplification of the alleles

Any variation could be the result of slight differences in the efficiency of amplification between two alleles. For example, very low levels of template DNA might cause stochastic variation in early cycles of PCR, leading to preferential amplification of one allele. Starting a PCR with multiple copies of the target sequence can counteract this effect. Alternatively, preferential amplification of one of a pair of alleles could also occur when two alleles have markedly different molecular weights. In general, within any given STR locus, a low molecular weight allele will amplify with more efficiency than a high molecular weight allele. However, the STR loci described above have only limited size ranges (100–350 bp); during the PCR process, alleles at a heterozygous locus have been found to amplify to approximately equal proportions, hence peak areas are approximately the same [2,15] (normally the lower peak area is greater than 60% the higher peak area).

2.2.2. Effect of primer-binding site mutations

The primer-binding site of an allele may contain a mutation which renders the annealing phase of its amplification less efficient. If the mutation is at the 5′ end of the primer site template, extension could be blocked completely and the allele would become 'null' and a pseudohomozygote would be observed. If the mutation occurs elsewhere in the primer binding site, then the annealing temperature of the primer would be affected, resulting in reduced signal. Examples of both have been observed in our

hands (unpublished data). Because of the rarity of the occurrence of primer site mutations (approximately estimated between 0.01 and 0.001 per locus), it is unlikely that a single individual will show an effect at two different loci. Furthermore, unless the mutation at the primer site is somatic, different body fluids and tissues should give consistent results. Currently, we have no data to suggest somatic mutations at primer binding sites.

In forensic casework, the most common reason for a large perturbation in the peak area balance is due to the presence of shared alleles in a mixed stain rather than as a result of preferential amplification of alleles. It should be noted that the observed symmetry between peak areas is not exact, and that after amplification of reference DNA there is a degree of natural variation between the peak areas at a heterozygous locus. The degree of natural variation between heterozygote peaks can be assessed by experimental observation. To measure the variation of peak areas within individual heterozygotes, at each locus for each sample, Gill et al. [15] calculated the proportion of: the allele with the smallest peak area/the allele with the largest peak area—a value termed (ϕ). In the systems described here, differences of up to 40% have occasionally been observed but, more usually, differences are less than 20% [15].

3. Step 2: identify the number of contributors to a mixture

Once the scientist has assessed the possibilities outlined in step 1, if a mixed STR profile is the likeliest alternative then the possible number of contributors must be assessed. The number of extra alleles at each locus, and their relative proportions are useful indicators. The maximum number of alleles that would be detected at a locus for a mixture of two heterozygotes is four. Five or six alleles at a locus are indicative of three or more contributors. The factual circumstances surrounding the case will be an important factor in any assessment and the subsequent interpretation will, in turn, be conditioned on the working assumptions. It is important for the court to either agree, or to put forward alternative working assumptions so that the evidence can be reassessed if necessary.

In our experience two-person mixtures account for the overwhelming majority of mixtures encountered during casework, but occasionally three-person mixtures are seen. For this reason, the remaining text deals with the interpretation of two-person mixtures and the issue of higher-order mixtures will be discussed in a later section.

4. Step 3: determine the approximate ‘ratio’ of the components in the mixture

A mixture can range from the two components being present in equal proportions to one component being greatly in excess. Hereafter, the varying proportions of a mixture will be referred to as the ratio.

Experiments conducted using known STR profiles, in which the DNA is admixed in a variety of ratios, have shown that the admixture ratio at each locus is approximately preserved after co-amplification. Thus, if two DNA templates are mixed 2:1, then this

approximate ratio will be maintained when the peak areas of the different component alleles within a locus are compared [2,20]. Note that this is only true when comparing peak areas within a locus (intralocus ratio) and does not hold when comparing peak areas between different loci (interlocus ratios) which can vary markedly between consecutive amplifications.

When there are no shared alleles at one or more of the loci (because both of the genotypes are heterozygous for different alleles), determining the approximate ratio of the mixture is a relatively straightforward task. This is because the alleles can be segregated into two distinct component pairs on the basis of peak area. If this is not possible and the peak areas of all four alleles are similar, then it must be because the mixture components are approaching a ratio of 1:1 (see Table 1).

Determining the ratio when there are shared alleles is more complex because there may be more than one combination of alleles which could explain the observation. However, it is still possible to calculate a mixture ratio because the allele peak areas are additive for any shared alleles (see Tables 1 and 2). Hence, at any locus where there is a single shared allele, if the two DNA templates were admixed in a 1:1 ratio, then the peak area of the shared allele should be about twice those of the unshared alleles [2].

Table 1 exemplifies this situation using a series of hypothetical genotypes to illustrate the effect of none, one, two, three and four shared alleles at an imaginary locus. From this it may be noted that certain genotype combinations can yield more than one possible alternative for the ratio of the mixture at that locus, whereas others provide no information at all about the ratio of the mixture at the locus.

Table 2 illustrates the effect on the ratio of the X and Y homologues after mixing DNA in differing proportions from a genetically normal male and female. Thus, at the amelogenin locus described in Section 1 (or indeed any other locus where there are three

Table 1
Hypothetical example to show the effect of none, one, two, three and four shared alleles on the peak area ratios at an imaginary locus

Example genotype	No. of shared alleles	Hypothetical peak area for allele		Inferred ratio (drawn from peak areas)
		Allele	Peak area	
6,7 and 8,9	0	6	1000	1:1
		7	1000	
		8	1000	
		9	1000	
6,7 and 7,8	1	6	1000	1:1
		7	2000	
		8	1000	
6,7 and 6,7	2	6,7	2000	None
			2000	
6,7 and 7,7	3	6	1000	1:1[a]
		7	3000	
6,6 and 6,6	4	6	4000	None

[a]Key: this deduction relies on an advance knowledge of the genotypes, otherwise two possibilities exist either: (1) 6,7+7,7 in a 1:1 ratio or (2) 6,6+7,7 in a 1:3 ratio.

Table 2
The effect of varying quantities of DNA from a genetically normal male and female on the peak area ratios

Mixture ratio		Dosage of alleles observed		Ratio of peak areas X:Y
Male (XY)	Female (XX)	X	Y	X:Y
10	1	12	10	1.2:1
5	1	7	5	1.4:1
4	1	6	4	1.5:1
3	1	5	3	1.6:1
2	1	4	2	2:1
1	1	3	1	3:1
1	2	5	1	5:1
1	3	7	1	7:1
1	4	9	1	9:1
1	5	11	1	11:1
1	10	21	1	21:1

shared alleles) the peak area ratios can change rapidly when the mixture ratio switches from being predominantly male (or heterozygous) to female (or homozygous).

Considering the profile as a whole (i.e. all the information from each locus) commonly allows the scientist to determine an approximate mixture ratio.

As the ratio of a mixture increases, the additive contribution of the peak area of a shared allele from the minor component, may become indistinct. Consider, for example, a locus where there is a single shared allele and the two DNA templates are admixed in a 10:1 ratio. For the major component, the peak area of the shared allele should then be increased by about a tenth relative to the peak area of the unshared allele. As already pointed out, this degree of peak imbalance in a heterozygote is not uncommon, and thus the additive contribution will be no longer discernible. This effect—which we refer to as ‘masking’—frequently arises when attempting to interpret the minor component in mixture ratios of about 3:1 or greater.

When the minor component of a mixture falls to about 5:1 or less, there are additional complications in interpretation due to problems in discerning true alleles—present as minor bands—from other artefacts of the system. Conversely, as the ratio of a mixture increases interpreting the major component becomes less complicated.

5. Step 4: determine the possible pairwise combinations for the components of the mixture

At this point, given prior knowledge of the results from the reference samples, it would be a simple task to determine whether or not the observed mixed profile was consistent with a mixture of body fluids from the persons concerned. We do not endorse this approach and recommend that interpretation of the mixed profile is conducted independently of knowledge of the results of reference samples. Indeed, in those cases where there are no reference samples from victim or suspects (or both), this is the only possible approach.

In addition, for PCR-based tests, it is good scientific practice and makes economical sense to generate a result from the forensic sample before any reference samples are analysed.

The advantages of this approach are obvious: (a) the interpretation cannot be influenced by the reference sample results, and is therefore demonstrably objective; (b) since the scientist is unhindered by prior knowledge of the results from the reference samples, the various alternative interpretations can be more easily considered.

We support a method described by Evett et al. [21] for use in single locus profiling, whereby all pairwise combinations for allelic pairs at each locus are listed. This is done qualitatively and, initially, without regard to the quantitative aspects of the profile. Thus, with four alleles at a locus, and allowing for reciprocal combinations, three pairwise combinations of alleles exist. Similarly, with three alleles at a locus, there are six pairwise combinations and with two alleles, given that a full profile has been obtained for **both** components of the mixture, there are four possible combinations. With one allele, the only possibility is that both individuals are homozygous for the same allele. These combinations are listed in Table 3.

Using the quantitative information drawn from the peak areas in the profile and the approximate ratio of the mixture, some of the pairwise possibilities can then be discounted. This is straightforward where there are high ratio mixtures and the major component is the profile of interest as it invariably results in one combination per locus. This is because the peak areas will be such that there will be an easily distinguishable allele pair for the main component and therefore no ambiguity in the resultant profile (see Appendix B). When it is the minor component which is of interest, the reverse is usually true and the resultant profile is seldom unambiguous. This is a direct result of minor bands being masked by major bands and difficulty in deciding whether or not a small peak is a true allele or has some other explanation (see above).

For mixture ratios approaching parity, some of the possible combinations can be eliminated by taking into account the additive effects of peak areas when shared alleles

Table 3
Pairwise combinations of two, three and four alleles

Four alleles (a,b,c,d)		Three alleles (a,b,c)		Two alleles (a,b)	
a,b	c,d	a,a	b,c	a,a	a,b
a,c	b,d	b,b	a,c	a,b	a,b
a,d	b,c	c,c	a,b	a,a	b,b
c,d	**a,b**	a,b	a,c	a,b	b,b
b,d	**a,c**	b,c	a,c	**a,b**	**a,a**
b,c	**a,d**	a,b	b,c	**b,b**	**a,a**
		b,c	**a,a**	**b,b**	**a,b**
		a,c	**b,b**		
		a,b	**c,c**		
		a,c	**a,b**		
		a,c	**b,c**		
		b,c	**a,b**		

Key: bold entries represent reciprocal combinations.

are present. Since the mixture ratio is low, these effects should be substantial in comparison to the natural peak variation between heterozygote alleles (see Appendix C).

With experience, the elimination of certain combinations becomes automatic and the procedures outlined in step 4 can be performed simultaneously.

6. Step 5: compare the resultant profiles for the possible components of the mixture with those from the reference samples

If the profiles from the suspect's reference sample matches one or other of the alternatives, then that person cannot be eliminated as a possible contributor of one component of the mixed stain. If the factual circumstances of a case are such that the profile from the donor of the sample might also be anticipated, then one might expect this individual's profile to complete the match and account for all of the remaining alleles.

For instance, in Appendix B, conditioning on the presence of the female victim within the mixture leaves only one possible genotype for her assailant at the THO1 locus: 7,7. A matching suspect should therefore be 7,7 at THO1 and not 5,5.

It follows that the more ambiguity in the predicted profile for the component of interest, the lower will be the evidential significance of any subsequent match. Consequently, when the minor component of a high-ratio mixture is under consideration, the evidential significance is often lower as a result. The mathematics supporting a statistical evaluation of the evidential significance for a mixture can be complex and are beyond the scope of this article [22].

7. Higher-order mixtures

Thus far we have dealt with a suggested manual approach to two-person mixtures as they are, by far, the most commonly encountered during forensic casework. However, the application of this manual approach to three-person mixtures is complicated and unwieldy. Unless there is a distinct major component for one of the constituent profiles, or the third profile is so weak that it does not complicate the interpretation of the other two, then the number of possible combinations for the constituent profiles causes computational difficulties. In such instances, the only recourse may be to interpret the mixture by using computer software to assist the analyst. It may be appropriate in some circumstances to condition on the assumption that the victim's DNA or DNA from another specified individual (e.g. a spouse or boyfriend) is present and therefore subtract that profile from the mixture.

8. Factors which can affect mixture interpretation

The presence of degraded DNA can affect mixture interpretation. Previous work has indicated that degradation is processive with the DNA molecule being broken into

successively smaller pieces [18]. The net result of this is that higher molecular weight loci are the first affected, and so the electropherogram exhibits a gradual loss of signal (see the STR profile in Appendix A, which shows this effect). Depending on the extent of the degradation, the result can be a full profile with reduced signal at the higher molecular weight end or a partial profile at one or more loci or, in the extreme, no profile at all.

With a two-component mixture it is possible that degradation of the DNA, could affect the components unequally. However, in many of the mixtures where DNA showing signs of degradation has been tested, both components appear to have been equally affected. In Appendix A, for example, the approximate 1:1 ratio of the two components was preserved at the HUMFES locus, even though the signal intensity at this locus had been drastically reduced.

Acknowledgements

The authors would like to thank the Forensic Science Service Research and Development Division Birmingham for access to prepublication manuscripts and unpublished data. We are grateful to colleagues at the Forensic Science Service Wetherby Laboratory for helpful discussions and comments on the manuscript.

Appendix A

Case history (Fig. 2)

Fingernail debris (recovered as scrapings) was taken from a man accused of sexually assaulting his biological daughter by digitally penetrating her vagina.

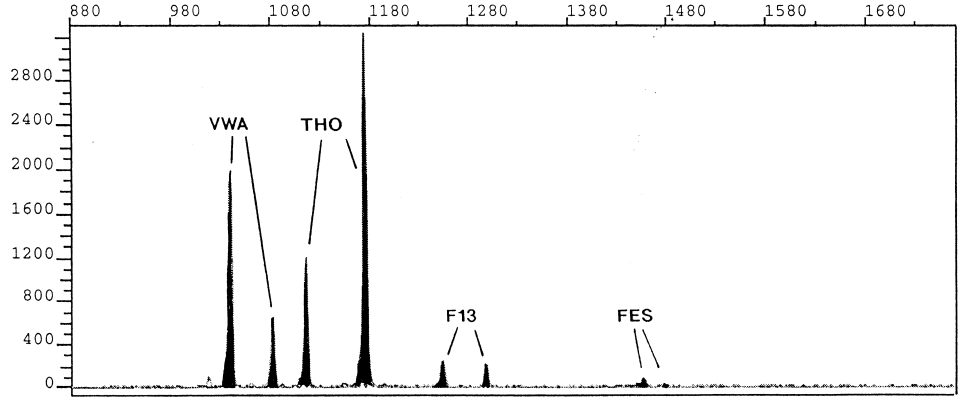

Fig. 2. Partial STR profile obtained from cellular debris under fingernails.

The allelic designations for the partial STR profile obtained from the forensic sample and the results from corresponding loci for the accused and his daughter are displayed beneath the electropherogram. Note the high degree of band sharing encountered as a result of the biological relationship between accused and victim. In the sample profile no more than two bands are identifiable at each locus and all are represented in the profile from the donor's reference sample. However, two loci, VWA and THO1 show marked peak imbalance. The signal from locus FES was considered too weak to report, although on higher magnification a FES:11 allele was present as was a FES:12 allele at a third of the intensity of the FES:11 allele. The ratio of the peak areas at VWA and THO1 was about 3:1 (data not shown). This was consistent with an even proportion mixture of DNA from **both** donor **and** victim.

Fingernail scrapings	VWA	THO1	F13
	16,18	7,9.3	5,7
	VWA	THO1	F13
Suspect	16,18	7,9.3	5,7
Daughter	16,16	9.3,9.3	5,7

Appendix B

Case history (Fig. 3)

A woman was raped, her assailant using a condom. A condom was recovered from the scene. It became important to show not only that the cellular material on the condom could have come from the victim but also that the semen on the condom could have come from the suspect.

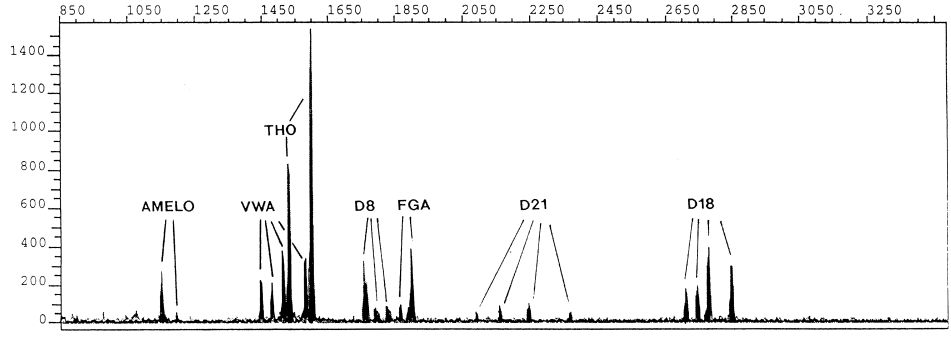

Fig. 3. Worked example 1.

LOCUS	ALLELE	PEAK AREA	Possible component profiles giving rise to observed mixed profile		INTERPRETATION AND COMMENT
			A (major)	B (minor)	
Amelo	X Y	1277 262	XX XY	XY XY	Female to male ratio 2:1 - acceptable Excessive peak asymmetry - reject (NB gene product ratio 5:1 X:Y See table 2)
VWA	15 16 17 19	1247 1193 2279 2000	17,19 15,17 15,19	15,16 16,19 16,17	Ratio - 2:1 acceptable Excessive peak asymmetry - reject Excessive peak asymmetry - reject
THO	5 7	5735 10769	7,7 5,7 5,7	5,5 7,7 5,7	Ratio 2:1 - acceptable Ratio 2:1 - acceptable Heterozygote peak imbalance - reject
D8	13 14 15	3234 752 894	13,13 14,14 15,15 13,14 13,14 13,15	14,15 13,15 13,15 14,15 13,15 14,15	Ratio 2:1 - acceptable Excessive peak asymmetry - reject Excessive peak asymmetry - reject Excessive peak asymmetry - reject Excessive peak asymmetry - reject Excessive peak asymmetry - reject
FGA	22 23	534 2792	23,23 23,23 22,23	22,23 22,22 22,23	Ratio 2:1 - acceptable Inconsistent ratio 5:1 - reject Excessive peak asymmetry - reject
D21	61 65 70 77	373 590 615 356	65,70 61,70 61,65	61,77 65,77 70,77	Ratio 2:1 - acceptable Excessive peak asymmetry - reject Excessive peak asymmetry - reject
D18	14 15 16 18	1339 1465 2895 2288	16,18 14,16 14,18	14,15 15,18 15,16	Ratio 2:1 - acceptable Excessive peak asymmetry - reject Excessive peak asymmetry - reject

Analysis procedure

(1) Profile shows multiple bands at D21, D18 and VWA. Significant peak asymmetry at amelogenin, THO, D8 and FGA.

(2) Assume that the profile mixture is from two individuals. This is supported by maximum number of bands (4) exhibited at D21, D18, VWA and the case history.

(3) Approximate ratio of the mixture can easily be determined from peak areas as

~2:1. Additionally, amelogenin indicates a female (XX) to male (XY) mixture of about 2:1.

(4) Consider all possible combinations of genotypes which could give rise to the observed alleles.

(5) Certain combinations can now be rejected based on heterozygote peak imbalance and an inconsistent ratio.

(6) Examine reference samples.

(7) Further discrimination of possibilities at THO can be made once reference samples are known, if it is assumed that the female victim is one part of the profile. This leaves only one possible genotype for the assailant at this locus as 7,7.

Reference profiles

	Amelo	VWA	THO	D8	FGA	D21	D18
Female victim	XX	17,19	5,7	13,13	23,23	65,70	16,18
Male suspect	XY	15,16	7,7	14,15	22,23	61,77	14,15

Appendix C

Case history (Fig. 4)

A parcel of heroin was discovered in an inmate's prison cell. The heroin had been packaged into clingfilm and may have been concealed within body cavities of both the inmate and the visitor who had allegedly smuggled it into the prison.

Analysis procedure

(1) Profile shows multiple bands at VWA and THO1.

(2) Assume that the mixture profile is from two individuals. This is supported by maximum number of bands (4) exhibited at THO1 and the case history.

(3) Approximate ratio of the mixture can easily be determined from peak areas at THO1 as ~1.5:1

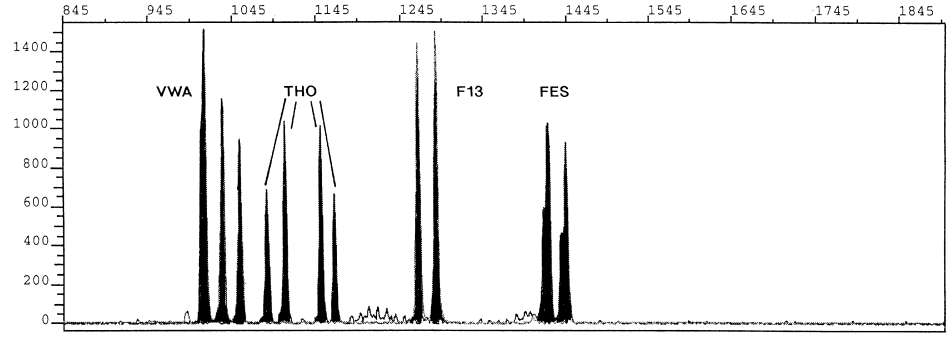

Fig. 4. Worked example 2.

LOCUS	ALLELE	PEAK AREA	Possible component profiles giving rise to observed mixed profile		INTERPRETATION AND COMMENT
			A (major)	B (minor)	
VWA	15	7312	16,17	15,15	Ratio acceptable
	16	5831	15,16	17,17	Ratio higher. Possible but unlikely
	17	4633	15,17	16,16	Ratio higher. Possible but unlikely
THO	6	3098	7,9	6,9.3	
	7	4571			
	9	4440	7,9.3	6,9	Excessive peak imbalance - reject
	9.3	2862	9,9.3	6,7	Excessive peak imbalance - reject
F13	6*	7357	7,7	6,6	Ratio slightly low but acceptable
	7*	7790	6,7	6,7	Any ratio possible - acceptable
FES	10*	8584	10,10	11,11	Ratio slightly low but acceptable
	11*	7154	10,11	10,11	Any ratio possible - acceptable

*** NB An added consideration here must be as to whether the second component is a full profile. For instance, one component could be degraded thus causing loss of signal at the higher molecular weight loci (F13 and FES). See text for further discussion.**

(4) Consider all possible combinations of genotypes which could give rise to the observed alleles.

(5) Certain combinations can now be rejected based on heterozygote peak imbalance and an inconsistent ratio.

(6) Examine reference samples.

Reference profiles

	VWA	THO1	F13	FES
Suspect 1	16,17	7,9	6,7	10,11
Suspect 2	15,15	6,9.3	6,7	10,11

References

[1] R.R.E. Frazier, E.S. Millican, S.K. Watson, N.J. Oldroyd, R.L. Sparkes, K.M. Taylor, S. Panchal, L. Bark, C.P. Kimpton, P.D. Gill, Validation of the Applied Biosystems Prism 377 Automated Sequencer for forensic short tandem repeat analysis, Electrophoresis 17 (1996) 1550–1552.

[2] J.E. Lygo, P.E. Johnson, D.J. Holdaway, S. Woodroffe, J.P. Whitaker, T.M. Clayton, C.P. Kimpton, P. Gill, The validation of short tandem repeat (STR) loci for use in forensic casework, Int. J. Leg. Med. 107 (1994) 77–89.
[3] J.S. Ziegle, Y. Su, K.P. Corcoran, L. Nie, P.E. Mayrand, L.B. Hoff, L.J. McBride, M.N. Kronick, S.R. Diehl, Application of automated DNA sizing technology for genotyping microsatellite loci, Genomics 14 (1992) 1026–1031.
[4] C.P. Kimpton, D. Fisher, S.K. Watson, M. Adams, A. Urquhart, J.E. Lygo, P.D. Gill, Evaluation of an automated DNA profiling employing multiplex amplification of four tetrameric STR loci, Int. J. Leg. Med. 106 (1994) 302–311.
[5] C.P. Kimpton, A. Walton, P.D. Gill, A further tetranucleotide repeat polymorphism in the vWF gene, Hum. Mol. Genet. 1 (1992) 287.
[6] M.H. Polymeropoulos, H. Xiao, D.S. Rath, C.R. Merril, Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (TH), Nucleic Acids Res. 19 (1991) 3753.
[7] M.H. Polymeropoulos, H. Xiao, D.S. Rath, C.R. Merril, Tetranucleotide repeat polymorphism at the human coagulation factor XIII gene, Nucleic Acids Res. 19 (1991) 4036.
[8] M.H. Polymeropoulos, H. Xiao, D.S. Rath, C.R. Merril, Tetranucleotide repeat polymorphism at the c-fes/fps proto-oncogene (FES), Nucleic Acids Res. 19 (1991) 4018.
[9] C.P. Kimpton, N.J. Oldroyd, S.K. Watson, R.R.E. Frazier, P.E. Johnson, E.S. Millican, A. Urquhart, R.L. Sparkes, P.D. Gill, Validation of highly discriminating short tandem repeat amplification systems for individual identification, Electrophoresis 17 (1996) 1283–1293.
[10] A. Mannucci, K.M. Sullivan, P.L. Ivanov, P. Gill, Forensic application of a rapid and quantitative DNA sex test by amplification of the X–Y homologous gene amelogenin, Int. J. Leg. Med. 106 (1994) 1990–1993.
[11] K.M. Sullivan, A. Mannucci, C.P. Kimpton, P. Gill, A rapid and quantitative DNA sex test: Fluorescence-based PCR analysis of X–Y homologous gene amelogenin, BioTechniques 15 (1993) 636–641.
[12] K.A. Mills, D. Even, J.C. Murray, Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA), Hum. Mol. Genet. 1 (1992) 779.
[13] V. Sharma, M. Litt, Tetranucleotide repeat polymorphism at the D21S11 locus (FGA), Hum. Mol. Genet. 1 (1992) 67.
[14] R.E. Straub, M.C. Speer, Y. Luo, K. Rojas, J. Overhauser, J. Ott, T.C. Gilliam, A microsatellite genetic linkage map of human chromosome 18, Genomics 15 (1993) 48–56.
[15] P.D. Gill, R. Sparkes, C.P. Kimpton, Development of guidelines to designate alleles using an STR multiplex system, Forensic Sci. Int. 89 (1997) 185–197.
[16] R. Sparkes, C.P. Kimpton, S. Gilbard, P. Carne, J. Anderson, N. Oldroyd, D. Thomas, A. Urquhart, P.D. Gill, The validation of a 7-locus multiplex STR test for use in forensic casework. (II) Artefacts, casework studies and success rates, Int. J. Leg. Med. 109 (1996) 195–204.
[17] A. Urquhart, C.P. Kimpton, T.J. Downes, P. Gill, Variation in short tandem repeat sequences: A suvey of twelve microsatellite loci for use as forensic identification markers, Int. J. Leg. Med. 107 (1994) 13–20.
[18] J.P. Whitaker, T.M. Clayton, A.J. Urquhart, E.S. Millican, T.J. Downes, P.D. Gill, C.P. Kimpton, STR typing of bodies from the scene of a mass disaster: High success rate and characteristic amplification patterns in highly degraded samples, BioTechniques 18(4) (1995) 670–677.
[19] P. Gill, A. Urquhart, E. Millican, N. Oldroyd, S. Watson, R. Sparkes, C.P. Kimpton, A new method of STR interpretation using inferential logic—development of a criminal intelligence database, Int. J. Leg. Med. 109 (1996) 14–22.
[20] R. Sparkes, C.P. Kimpton, S. Gilbard, P. Carne, J. Anderson, N. Oldroyd, D. Thomas, A. Urquhart, P.D. Gill, The validation of a 7-locus multiplex STR test for use in forensic casework. (I) Mixtures, degradation and species studies, Int. J. Leg. Med. 109 (1996) 186–194.
[21] I.W. Evett, C. Buffery, G. Willott, D. Stoney, A guide to interpreting single locus profiles of DNA mixtures in forensic cases, J. Forensic Sci. 31(1) (1990) 41–47.
[22] I.W. Evett, P.D. Gill, J.A. Lambert, J.S. Buckleton, C.S. Triggs, Taking account of peak areas when interpreting mixed DNA profiles, Sci. Justice (1997) submitted.