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A new method of STR interpretation using inferential logic – development of a criminal intelligence database

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Abstract A short tandem repeat (STR) system consisting of seven multiplexed loci has recently been introduced in the UK to support a National strategy to create large DNA databases for criminal intelligence purposes. The process uses automated sequencers, employing dye-labelled primers. Identification of tetrameric loci such as HUMTH01 are straightforward. Sizing windows are estimated by running a series of control allelic ladders on several gels and 'unknown' samples are designated if they fall within a defined window. However, utilisation of complex STRs (eg. D21S11) characteristically have common variants which differ by just 2 bp. In addition, rare alleles are encountered which may differ by just 1 bp from a common variant. To assist with the identification of alleles, we have introduced a series of allelic ladders, so that direct comparisons with 'unknown' samples can be made on the same gel. To designate an allele, it should be within 0.5 bp of an allelic ladder marker. Not all alleles (in particular rare alleles) can be included within an allelic ladder, however their expected positions can be easily calculated by reference to existing alleles in the ladder. Measurement of band shift is also a useful diagnostic tool. A series of guidelines are described to enable reliable allelic identification. These guidelines can be converted into computer programmes, which form the basis of an expert system.

Key words Short tandem repeats (STRs) · Allelic ladders · Multiplex · DNA databases · Automation · Expert systems

Introduction

DNA profiling in forensic science in the UK is focussed on the analysis of short tandem repeat (STR) loci using PCR. It is the technique of choice for the National strategy to create criminal intelligence databases. Apart from the increased sensitivity inherent with any PCR technique, with STRs there is also the advantage of definitive allelic identification. This is a consequence of lower measurement errors associated with the use of polyacrylamide gel electrophoresis to detect DNA fragments ranging between 200–400 bp in size (Ziegle et al. 1992). Because of their small sizes STRs are more likely to be successful on old or badly degraded material (Hagelberg et al. 1991; Jeffreys et al. 1992; Wiegand et al. 1993, Gill et al. 1994), an important aspect of forensic casework.

The National DNA database unit

Recently, a change in the UK legislation allowed the formation of a national DNA database. The purpose is to store DNA profiles derived from individuals either suspected or convicted of crimes. DNA is sampled from either buccal scrapes or from hair roots. The aim is to store 135,000 DNA profiles per year and it is envisaged that the database may eventually contain 5 million profiles. This is a significant proportion of the UK population of 60 million people. As the custodian of the DNA database, the Forensic Science Service (FSS) has constructed a unit at the FSS headquarters, Birmingham, UK, consisting of ca. 80 scientists, four 373A ABD automated sequencers and twelve 377 ABD automated sequencers.

Method

To decide the method of choice, the following requirements must be fulfilled:

- It must be reliable (the quality of results must be high)
- Throughput must be high
- The process must be cost-effective

Automation is the key to achieving all three requirements

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Choice of loci

Several factors are considered when choosing candidate loci:

- Discriminating power (DP: Jones 1972) of > 0.9 (Observed heterozygosity > 70%)
- The predicted length of alleles must be approximately between 90–500 bp (the higher the molecular weight the lower the precision of measurement). Also the lower the size of the STR locus, the less chance of locus or allelic drop-out because of degradation of the sample
- Chromosomal location (to ensure that closely linked loci are not chosen)
- Robustness and reproducibility of results, low stuttering characteristics

The loci used in the National DNA database include the sex-test Amelogenin and the simple STRs HUMVWFA31/A, HUMTH01 and D8S1179. In addition, there are three highly discriminating complex STRs-D18S51 (15 common alleles), D21S11 (21 common alleles), HUMFIBRA(FGA) (21 common alleles). The latter three STRs have alleles which differ in size by 2 bp, and consequently have DPs greater than simple STRs (Urquhart et al. 1994).

Multiplex conditions are described by Oldroyd et al. (1995). The main purpose of using multiplexes, is to speed the process of analysis. Inevitably, there may be some loss of efficiency of amplification since the conditions used are a compromise. This has no significant implications for the database, since the operator has large quantities of undegraded DNA available for analysis. Furthermore, the DNA is never a mixture. In casework, where the sample is less predictable, singleplexes may sometimes be used to identify difficult (e.g. degraded) samples (i.e. singleplexing and multiplexing are not mutually exclusive techniques).

Nomenclature

Nomenclature follows the recommendations of the DNA Commission of the ISFH (1994) where the number of complete tandem repeats observed are designated by digit(s). If a partial repeat is present then it is designated by the number of bases prefixed by a decimal point, thus HUMTH01 9.3 consists of 9 complete repeat units and an incomplete repeat of 3 bases. The exception is D21S11; nomenclature is based on the number of dimeric repeats (instead of tetrameric repeats) and is described by Urquhart et al. (1994). In addition, the following terms are used in the text to describe STR alleles and their associated artefacts:

'n' band: is the actual size of the allele

'n + 1' band: is the actual size of the allele + 1 bp; it is characteristic of the Taq enzyme to add an additional base during the amplification process.

stutter band: is an artefact which is 1 repeat unit (or 4 bp) less than the allele.

Determining the size of STR alleles

To determine the sizes of DNA fragments, standard marker ladders consisting of all the common alleles of a given locus can be used for comparison on an electrophoretic gel (Puers et al. 1993, 1994). They are made by mixing together DNA from different individuals displaying the entire range of alleles for comparison and carrying out PCR on the mixture. Ladder markers are widely utilised and available throughout the forensic community.

Allelic ladders in use for multiplexed systems

Three sets of allelic ladder cocktails have been prepared (Figs. 1–3). In principle, several loci can be labelled with a single dye provided that there is no overlap in size of common alleles. Occasionally, there may be overlap of rare alleles. If a possible ambiguity arises, the simplest solution is to analyse the relevant loci using two separate singleplex reactions.

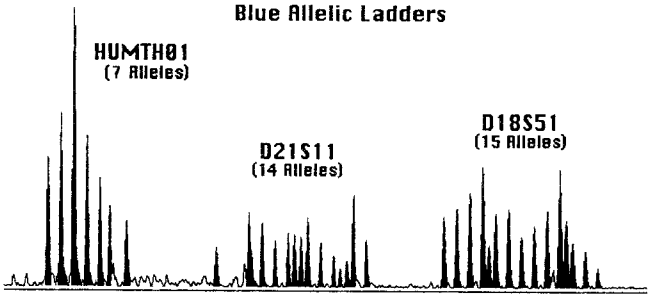

Fig. 1 Blue allelic ladders consisting of HUMTH01, D21S11 and D18S51 alleles

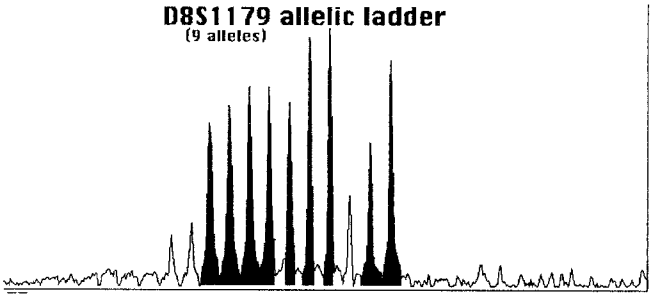

Fig. 2 (The peaks not highlighted in the ladder e.g. allele 15 are artefactual and are probably stutters)

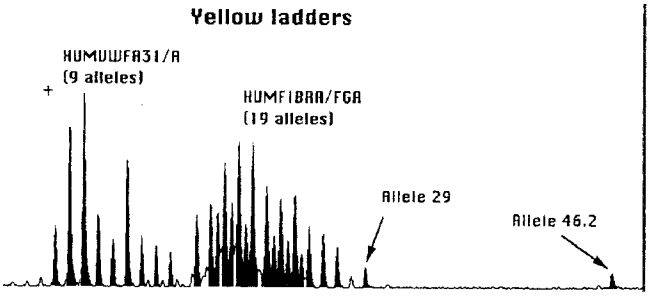

Fig. 3 HUMVWFA31, HUMFIBRA(FGA) ladders

Locus HUMTH01

Dye = 6-FAM

A simple 4 bp repeating sequence; repeat motif is (TCAT)n; range 150–180 bp.

The following alleles are present in the ladder (7 in total): 5, 6, 7, 8, 9, 9.3, 11

Allele 10 is 1 bp larger than 9.3.

Locus D21S11

Dye = 6-FAM

This is a complex tetrameric repeat with alleles which may be 2 bp apart (range 205–255 bp).

The following alleles are present in the ladder: (14 in total): 54, 59, 61, 63, 65, 66, 67, 68, 70, 72, 73, 74, 75, 77.

The sizes (bp) of the following alleles which have been identified, not included in the ladder marker are calculated as follows:

Allele 56: Allele 54 + 4 bp
Allele 57: ((Allele 54 + 6 bp) + (Allele 59 – 4 bp))/2
Allele 62: (allele 61 + allele 63)/2
Allele 64: (allele 63 + allele 65)/2
Allele 69: (allele 68 + allele 70)/2
Allele 71: (allele 70 + allele 72)/2
Allele 76: (allele 75 + allele 77)/2
Allele 79: (allele 77 + 4 bp)

Locus D18S51

Dye = 6 FAM

This is a compound repeat with some alleles which differ by 2 bp (range 270–332 bp).

The following alleles are present in the ladder (15 in total): 10, 11, 12, 13, 13.2, 14, 15, 16, 17, 18, 19, 19.2, 20, 21, 22

Sizes (bp) of the following alleles, not included in the ladder marker are calculated as follows:

allele 9: subtract 4 bp from allele 10
allele 17.2: [allele 17 (bp) + allele 18 (bp)]/2
allele 23: allele 22 (bp) + 4 bp
allele 24: allele 22 (bp) + 8 bp

Locus D8S1179

Dye = TET

This is a simple 4 bp repeat; repeat motif is (TCTA)n; range 160–210 bp.

In extracted samples, the locus is prone to producing 'n + 1' and 'n' peaks such that the 'n + 1' component may be low level or absent. However, the allelic ladder should always be designated using the 'n + 1' peaks. A window is then constructed ranging from + 0.5 bp greater than the appropriate ladder marker to – 1.5 bp less than the ladder marker. This incorporates the range of both 'n' and 'n + 1' peaks. Within each gel, however, samples from a given batch should consist of either 'n' or 'n + 1' peaks. Once identified, an appropriate ± 0.5 bp window can be used.

The following alleles are present in the ladder: (9 alleles in total): 8, 9, 10, 11, 12, 13, 14, 16, 17

Size (bp) of the following allele, not included in the ladder marker is calculated as follows:

Allele 15: (Allele 14 + allele 16)/2
Allele 18: (Allele 17 + 4 bp)

HUMFIBRA(FGA)

Dye = HEX

This is a complex tetranucleotide with alleles which are 2 bp apart; range 168–294 bp. The region between alleles 29 and 46.2 must be examined with care for new alleles.

The following alleles are present in the ladder (19 alleles): 17, 18, 18.2, 19, 19.2, 20, 20.2, 21, 22, 22.2, 23, 23.2, 24, 24.2, 25, 26, 27, 29, 46.2

Sizes (bp) of the following alleles, not included in the ladder marker are calculated as follows:

Allele 15†: (allele 17 – 8 bp)
Allele 16 : (allele 17 – 4 bp)
Allele 21.2: (allele 21 + allele 22)/2
Allele 25.2: (allele 25 + allele 26)/2
Allele 26.2: (allele 26 + allele 27)/2
Allele 27.2: (allele 27 + 2 bp)
Allele 28 : (allele 27 + allele 29)/2
Allele 28.2: allele 29 – 2 bp
Allele 30.2: allele 29 + 6 bp

HUMVWFA31/A

Dye = HEX

This is a 4 bp repeat TCTA(TCTG)n(TCTA)m; size range 120–170 bp.

The following alleles are present in the ladder (9 alleles): 13, 14, 15, 16, 17, 18, 19, 20, 21†

Sizes (bp) of the following alleles, not included in the ladder marker are calculated as follows:

Allele 11: allele 13 – 8 bp
Allele 12: allele 13 – 4 bp
Allele 15.2: (allele 15 + allele 16)/2
Allele 22: allele 21 + 4 bp

† Allele 21 (VWA) coincides with a rare allele 15 (FGA). If there is any ambiguity, then the sample must be reanalysed using singleplexes (or the quadruplex system described by Kimpton et al. 1994) to resolve the allelic designation of the sample.

Allele 22 (VWA) is very rare (< 1 in 4000) but coincides with allele 16 (FGA) which is also very rare (it is very unlikely that either will exist as a homozygote). If an allele appears in this position where both FGA and VWA are heterozygous then three bands will appear in the FGA zone (VWA will appear homozygous). Identification can be confirmed by singleplex reaction.

Suppose that there was a homozygote FGA and an extremely rare (VWA 22,22 genotype present in the FGA read region, then the profile would appear to be a heterozygous FGA, but atypically the VWA read region would be empty, and therefore further investigation would be needed (regardless of initial designations, the crime sample and control from the suspect would still appear the same).

Amelogenenin

Dye = 6-FAM
The X-specific product is 106 bp.
The Y-specific product is 112 bp.

Internal size markers

The major advantage of fluorescence is that internal size markers can be incorporated and this improves sizing accuracy both within and between gels (Mayrand et al. 1991; Ziegle et al. 1992; Kimpton et al. 1993). Whereas the use of allelic ladders is indispensable with non-automated systems, fluorescently tagging STR loci enables inclusion of size standards as an internal size marker within each lane. This reduces measurement errors and allows automatic sizing of STR-PCR products with GENESCAN™ 672 analysis software. For the majority of loci, reliable sizing can be achieved by direct comparison with lambda Pst I restriction digests (ABD GS2500 or GS350). For calibration purposes allelic ladders are used in our laboratory (if new software is installed, for example).

The consistency of automatic size calling against the GS2500 ladder marker was evaluated for each locus studied by examination of the distribution of computer-generated band sizes of allelic ladders for a large number of samples (72 allelic ladders run across 6 different gels). These experiments are used to set windows based on the range of observed sizes of any given allele. Accuracy has been demonstrated to be within a range of approximately 1.2 bp (see Table 2 and Fig. 2 of Kimpton et al. 1993). Ranges based on the observations are programmed into the ABD GENOTYPER™ software, enabling automatic allele designations to be made. This is called the 'absolute window method'.

A new method of guideline-based interpretation using allelic ladder controls (the floating window method)

HUMFIBRA/FGA (Mills et al. 1992) is a complex repeat locus with 21 common alleles differing by increments of just 2 bp. If complex loci were perfect 2 bp repeats, then the window established to encompass measurement error for any given allele would be just ± 1 bp of its mean. However, intermediate alleles differing by 1 bp may also occur. For example, we have recently discovered a 22.3 allele in HUMFIBRA(FGA). This means that to identify these very rare ($P < 0.001$) alleles, windows must be no greater than ± 0.5 bp. The difficulty is that measurement errors may exceed this value, hence a different approach is needed to use complex STRs as discrete systems. An alternative approach is to calculate the relative difference in size between a questioned sample and an allelic ladder marker control on the same gel. All sizes, in base pairs, are calculated by reference to the internal GS350 standard. If the distance is within a predetermined range, then the allele can be designated. This is a fundamentally different approach to that previously described. Whereas the former method calculates 'absolute' windows based upon repeated running of allelic standard markers, the new method calculated windows relative to ladder markers on the same gel – windows are therefore set for each individual gel.

Demonstration of robustness of an electrophoretic system by measuring errors relative to the allelic ladder

There are 2 types of measurement error which contribute to an estimate of the size of an allele (Fig. 4):

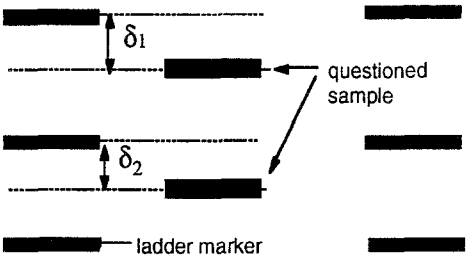

Fig. 4 Exaggerated diagrammatic representation of band shift correlation. The questioned sample is compared to ladder markers on the gel

- Deviation of an allele relative to its respective ladder marker (δ_1 and δ_2)
- The relative band shift ($c = \delta_1 - \delta_2$) which is a measure of the correlation of 2 fragments in a heterozygote.

The relative band shift (c) is measured as an absolute value (the sign is ignored). The proper statistical notation is given as $c = |\delta_1 - \delta_2|$. This notation is used throughout this paper whenever absolute values are indicated.

It is important to estimate the measurement errors inherent within the system of choice. One way to demonstrate robustness and reproducibility of a protocol is to analyse replicate samples of allelic ladders within and between a number of different gels (Fig. 5). Analysis proceeds by pairwise comparisons of individual alleles – this is a convenient way to generate a large amount of data which takes account of the entire length of the gel.

In general, the greater the molecular weight of PCR fragments, the greater the measurement error. D18S51 has the greatest molecular weight (270–332 bp), and is the locus most likely to fall outside measurement guidelines.

Estimation of δ

The size of each allele in the allelic ladder (Fig. 5) was measured and compared in turn to the size of all identical alleles in the gel. The differences in measurements were compiled. From Fig. 5, allele A in lane 1 (A_1) is compared to A_2 and the absolute difference in size ($\delta = |A_1 - A_2|$) is recorded. Then A_1 is compared to A_3, $A_4 \ldots A_n$. Then A_2

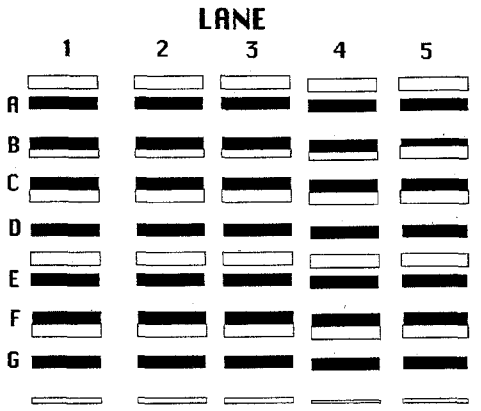

Fig. 5 A representation of allelic ladders run across a gel in order to carry out pairwise comparisons of measurement errors (The light bands represent the internal ROX-labelled GS350 marker; the dark bands represent the allelic ladder marker)

is compared to A_3, $A_4 \ldots A_n$, and so on. This gives a*[(n*n–1)/2)] pairwise comparisons where a is the number of alleles in the allelic ladder and n = number of ladders.

Estimation of band shift (c)

The purpose of this experiment was to measure the correlation of band shift in heterozygotes. Heterozygotes were simulated as follows:

Again, referring to Fig. 5, lane 1 was designated the reference lane. In lane 2, the sizes of bands A and B were compared to the reference sample A, B and the differences in size $c = |\delta_{A1} - \delta_{A2}|$ were calculated. Then lane 1 was compared to A and B in lanes 3, 4, 5, 6...n. Next, lane 2 was designated the reference lane and bands A, B were compared to A, B in lanes 3, 4, 5, 6...n. The process continued until all lanes in turn had been designated the reference (except for lane n).

The entire procedure was repeated, choosing a different pair of alleles A, C, then A, D and so on. This gave a large number of pairwise comparisons [(a*a–1)/2]* [(n*n–1)/2] where a = number of alleles in the allelic ladder and n = number of ladders. Typically there may be 15 alleles and 35 ladders which gives 62475 possible heterozygote comparisons in just one gel.

Analysis of three gels consisting of allelic ladders

Three gels consisting of allelic ladders were analysed by carrying out pairwise comparisons. Figure 6 illustrates the proportion of alleles where |δ| > the present window size (thus for gel no. 1, approximately 16% of alleles fall outside a window |δ| > 0.3 bp). All alleles known to be identical in gels 2 and 3 are within a range of 0.5 bp, whereas in gel 1, a total of 3.9 % of comparisons of alleles known to be identical failed the 0.5 bp test.

Figure 7 illustrates the proportion of heterozygotes where the band-shift |c| is greater than that given on the

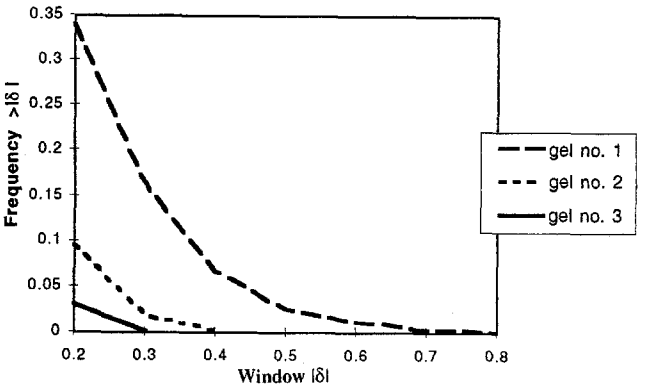

Fig. 6 Pairwise comparisons of alleles in D18S51 ladder demonstrating the proportion of alleles which do not match within the window |δ| stated

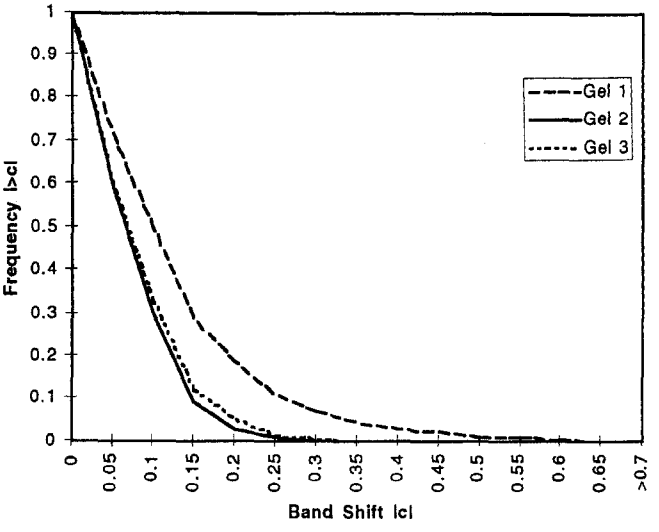

Fig. 7 Pairwise comparisons of heterozygotes simulated from D18S51 allelic ladders, illustrating the proportion where the band shift |c| is greater than that indicated

x-axis. For example, in gel 1 approximately 50% have a band shift |c| > 0.1 bp. There is a strong correlation. In gels 2 and 3 there were no examples of band shift |c| > 0.35 bp, whereas in gel 1 only 1.1 % of comparisons of identical homozygotes were inconclusive when compared to the 0.5 bp guideline.

Pairwise comparisons using the guidelines that both bands must be within 0.5 bp of their respective allelic ladder marker, *and* the shift |c| must be = < 0.5 bp resulted in a total of 5.1% of comparisons of identical heterozygotes failing the test in gel 1 (there were no failures in gels 2 or 3).

Gel 1 is a poor quality gel. normally such a gel would be repeated as a matter of course, the purpose of including it in this experiment was to examine the properties of band deviation |δ| and band shift |c| under extreme conditions. It is important to note that the correlation of band shift |c| is very tight even in poor quality gels (< 1% of comparisons fail the 0.5 bp guideline) and in practice, the number of samples which must be repeated will be rare. The system appears to be robust. Furthermore, the proce-

dure described is useful to test a protocol or to test the effect of changes to a protocol.

Knowledge of the population frequency distribution of a locus affects interpretation of alleles

Examination of the population distribution of HUMFIBRA/FGA (Fig. 8) shows that the most common alleles such as 19, 20, 21 are complete tetramers, whereas intermediates (19.2, 20.2, 21.2) are much rarer ($P < 0.02$). If α.0 represents a complete-number of repeats, we can generalise that α.0 repeats are common, α.1 and α.3 alleles are always very rare ($P < 0.001$) and α.2 repeats are intermediate. In some systems we have examined e.g. D21S11, some α.2 variants are relatively common, yet the extreme rarity of α.1 and α.3 alleles holds true for complex loci utilised in the multiplex.

For HUMFIBRA(FGA), the following conditional logic applies:

- *If* an α.1 or α.3 allele is observed then this is an extremely rare occurrence
- Given that the size distribution of individual STR alleles may be in the region of ± 0.6 bp, an explanation may be that an α.0 or α.2 variant is in a tail of its measurement error distribution such that it now resides in an adjacent window normally occupied by an α.1 or α.3 variant
- To test this possibility, re-run the sample to determine if the result is reproducible
- *If* the allele is a true α.1 or α.3 variant then the locus would normally be heterozygous (unless, the population from which the sample is derived is atypical, or inbred). It follows that the partner allele must also normally be a common α.0 or α.2 variant
- *If* a heterozygous sample is observed where both alleles are apparently α.1 or α.3 variants then that result suggests the strong possibility that two common variants are shifted into the tails of their error distributions. In fact band shifts are strongly correlated (Fig. 7).

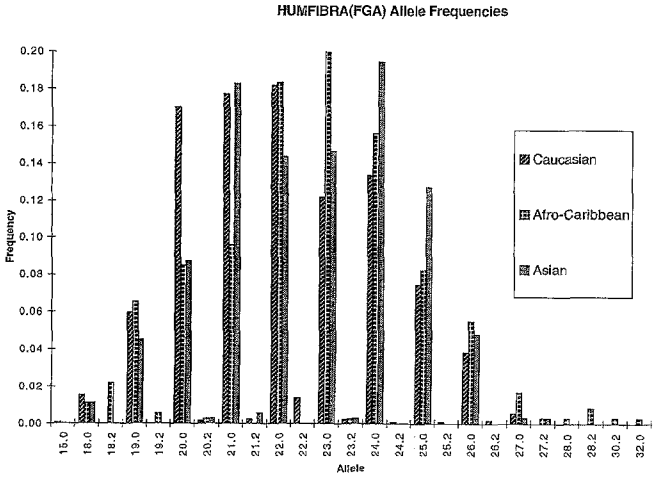

Fig. 8 HUMFIBRA(FGA) population survey of three different ethnic groups

- *If* the locus is homozygous, then band shift (c) cannot be determined as previously described. However, an approximation of (c) can still be obtained by reference to the positive control (where δ_1 is determined from the homozygote band and δ_2 is estimated from the average δs in the positive control)

Correlation of band shift (c) is a useful diagnostic tool:

- Determine *if* both alleles are < 0.5 bp from the closest α.0 or α.2 ladder marker then continue to the next test
- Measure the correlation of band shift ($c = |\delta_1 - \delta_2|$). *If* $|c| < 0.5$ then the alleles may be designated
- Extremely rare α.1 or α.3 variants can only be designated *if* the sample has been separately analysed and the same results obtained

The utility of this procedure is best illustrated by reference to an actual example (Table 1; Fig. 9):

Table 1 compilation of allele sizes illustrated in Fig. 9

Conditional allele designation	Size in questioned sample (bp)	Size in allelic ladder (bp)	Difference (δ)
22.3	197.42	197.48[t]	− 0.06 (δ_1)
22.2	197.42	196.48	+ 0.98*
23	197.42	198.48	− 0.98*
24	202.38	202.50	− 0.12 (δ_2)

* *If* the questioned allele (designated 22.3) is truly a common allele it must be either 22.2 or 23. The test fails this condition since $|\delta| > 0.5$

[t] The 22.3 allele is not in the allelic ladder, but its size can be estimated as the mean of alleles 22.2 and 23. The difference passes the condition $|\delta| < 0.5$, *if* the allele is 22.3

Identification of a rare HUMFIBRA(FGA) 22.3 allele

Band shift (c) measurement

- *If* the questioned allele is 22.3 then the observed correlation of band shift with allele 24 ($c = |\delta_1 - \delta_2|$) is 0.06 bp, passing the condition since $|c| < 0.5$
- *If* the questioned allele is 22, then the observed correlation of band shift with allele 24 is 1.1 bp, failing the condition since $|c| > 0.5$
- *If* the questioned allele is 22.2, then the observed correlation of band shift with allele 24 is –0.86 bp, failing the condition since $|c| > 0.5$
- Because the putative allele is an α.3 variant, the sample was separately reanalysed, confirming the inference that the allele was a rare 22.3 variant

Identification of a rare HUMTH01 8.3 allele where the measurement error exceeded ± 0.5 bp

In the following example (Fig. 10), all HUMTH01 alleles in the gel were shifted > 0.5 bp relative to the allelic ladder. On closer examination, the shift was observed to be caused by a problematical ladder marker which appeared

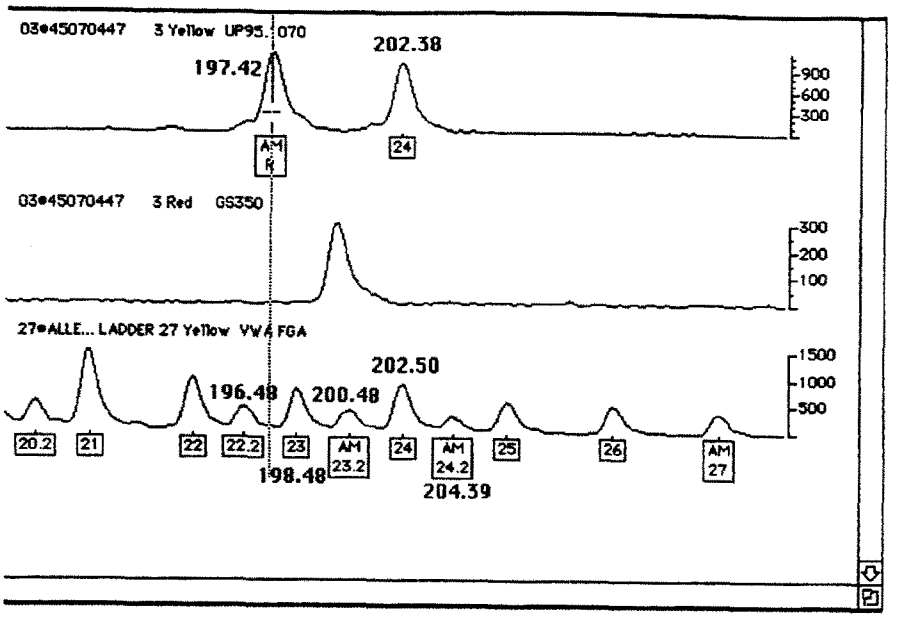

Fig. 9 Interpretation of an extremely rare variant allele in HUMFIBRA/FGA-showing designations made by GENOTYPER. The top lane is the questioned sample; the bottom lane is the allelic ladder

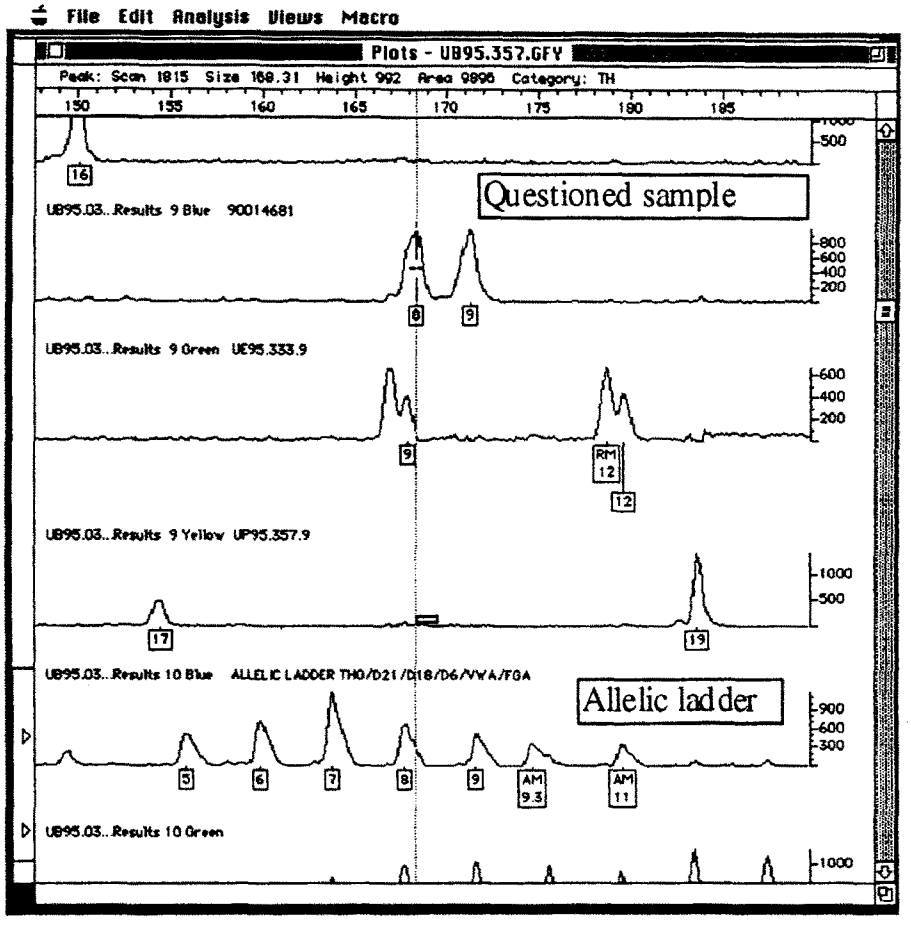

Fig. 10 GENOTYPER analysis of 8, 8.3 HUMTH01 genotype

to be saw-toothed and therefore displaced towards the low molecular weight. This effect was confined to the HUMTH01 locus only (i.e. the correlation of band shift may not apply between different loci). In the example given, a rare 8.3 variant was discovered.

Interpretation proceeds by conditional or inferential logic:

- If the locus is 8,9 then the 8 allele fails $|\delta| < 0.5$ bp condition (Table 2), the 9 allele passes, but the shift lcl is almost 1 bp (i.e. the two alleles are separated by 3 bp)
- If the locus is 8,8.3 then the 8 allele fails the 0.5 bp condition, the position of the 8.3 allele in the ladder marker

Table 2 Analysis of recorded sizes of 8, 8.3 HUMTH01 genotype

Inference – If the locus is 8,9				
Allele	Ladder	Sample	Difference (δ)	Result |δ| or |c| < 0.5
8	167.72	168.31	– 0.59	fail
9	171.61	171.24	0.37	pass
Correlation (c)			– 0.96	fail
Inference – If the locus is 8, 8.3				
Allele	Ladder	Sample	Difference (δ)	Result |δ| or |c| < 0.5
8	167.72	168.31	– 0.59	fail
8.3	170.51	171.24	– 0.73	fail
Correlation (c)			0.14	pass

Table 3 (HUMTH01 6,8 Positive control)

The locus designation is 6.8

Allele	Ladder	Sample	Difference (δ)	Result |δ| or |c| < 0.5
6	159.89	160.53	– 0.64	fail
8	167.72	168.41	– 0.69	fail
Correlation |c|			0.05	pass

is estimated as a + [(a–b)*0.75] where a = size of 8 allele (bp) and b = size of the 9 allele in the ladder marker; the band-shift |c| = 0.14 (passes test).

In addition, the positive control demonstrates a shift to the right of the allelic ladder markers (Table 3). Although the shift is excessive, it is in the same direction and magnitude if the genotype of the HUMTH01 locus is 8,8.3.

Results of reanalysis

When the sample was reanalysed on a different gel, it was confirmed that the questioned sample was indeed in 8,8.3 genotype (Fig. 11). Both alleles were within range, where |δ| < 0.5 bp (if the high molecular weight allele was type 9 then it failed the 0.5 bp test). In addition, the observed 8.3 allele was 0.92 bp smaller than the 9 allele. If the genotype is 8,8.3 then the band shift correlation is well within range (|c| = 0.13), confirming this inference.

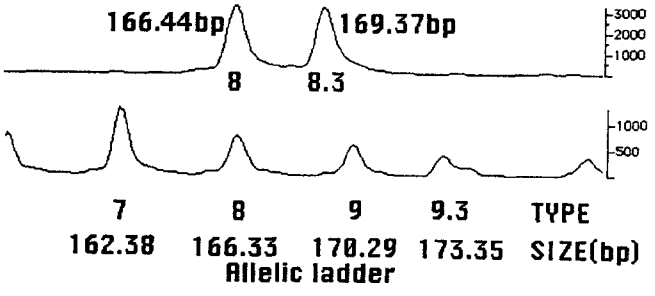

Fig. 11 Reanalysis of rare 8, 8.3 HUMTH01 genotype

Modification of the above procedure may be needed for different loci but the same principles can be applied, given knowledge of rare and common variants; for example in HUMTH01, both 9.3 and 10 alleles are common, whereas 9.2 and 10.1 alleles are not observed (or extremely rare). Otherwise all remaining common alleles are α.0 variants.

Development of an expert system

A computer program has been written which interacts directly with the ABD GENOTYPER programme, automatically designating alleles based upon the δ and c guidelines already outlined. A total of 257 database STR profiles were examined to compare the efficacy of the absolute window method with the floating window method. Using the absolute window method, there was one example where a rare allele was initially designated as the adjacent common allele (differing in size by 1 bp). Using the floating window method, no mis-designations were recorded.

Universal application of the floating window method

These principles, incorporating use of the 0.5 bp guide, can be universally applied to interpretation of complex STRs where common alleles differ by 2 bp, regardless of the platform or method used, provided that allele sizes are always cross-referenced to allelic ladders or to known control standards. The number of inconclusive results (apparent extremely rare alleles observed) is dependent upon the resolving power of the system used, but it is not a prerequisite that the true measurement error is < 0.5 bp. The main advantage of the floating window method is that it will easily recognise the presence of rare alleles which may differ by just 1 bp from a common allele.

Interpretation when allelic ladders are not in use

The method described has a particular application for use in criminal intelligence databases where it is normal practice that only control samples from suspects are analysed. This is different to use in normal casework where both control and crime stains are available. In the system described by Kimpton et al. (1994) and Lygo et al. (1994) allelic ladders are not used, only the internal GS2500 standard is utilised. However, in principle the same interpretation methods can be used. If the control sample is used as the reference (analogous to the allelic ladders described previously) then the crime stain can be compared directly to the control and estimates of deviation (δ and c) can be reliably made. Useful information can also be obtained by reference to the positive control.

In our hands the 'absolute' method of window estimation suffers from the drawback that windows must be reset when new acrylamide batches are prepared – other factors may necessitate the use of different windows in different laboratories. On the other hand, the floating window approach has a universal applicability because the windows are constant for each locus and independent of the method used. The principle of guideline-based interpretation packages built upon experimental observations can be extended to enable construction of algorithms or computer-based expert systems. This will automate the interpretation procedure, guiding the scientist to interpret results in the presence of artefacts, such as stutters, and to distinguish the components of mixtures.

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