APPLICATION OF GenePrint® PowerPlex™ 16 SYSTEM IN ANALYSING OF FORENSIC MIX STAINS

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ABSTRACT: The use of STR Multiplexes has become a routine procedure in profiling forensic stains. The GenePrint® PowerPlex™ 16 System allows the simultaneous coamplification and three-color detection of sixteen loci. It contains two new low-stutter, highly polymorphic pentanucleotide repeat loci, Penta E and Penta D, what makes it ideal for evaluation of DNA mixtures often encountered in forensic casework. The statistical treatment of mixed stains has been formulated in general mathematical approach by Weir et [7]. The strength of such evidence is represented by the Likelihood Ratio. The analysis requires the assignment of probabilities of all of the combinations of genotypes. Taking into consideration the area of peaks enhances the interpretation of DNA mix stains but involves the need to consider possible artefacts such as stutters [6].

DNA analyses were performed in the range of alleles included in PowerPlex™ 16 System. In the case when major/minor components cannot be distinguished, we used “dnamix” program of Weir. When the major/minor components can be established, the mixture proportions were estimated for all loci. The inference about suspect/victim match to the profile was described with Likelihood Ratio.

We describe here three forensic cases where the above-mentioned analyses were successfully performed. At the first case the suspect set of alleles was find in the mix stain DNA profile taken from rubbery mask and the probability of this hypothesis was estimated. At the second case, the victims set of alleles was found in the mixed DNA profile from the suspect’s night suit. It was estimated that the explanation that it was the victim’s material in the mixed profile was 50 000 more probable than under the hypothesis that it was of random origin. In the third case each of three assailants cell material were revealed in the mixed DNA profiles taken from three different fragments of stockings found at the crime scene.

The presented examples of cases from our routine work examinations indicate usefulness of likelihood ratio approach, as theoretically justified by Evett and Gill [4], to estimation the value of DNA evidence, in the case of mixed stain.

KEY WORDS: Forensic mix stain; Multiplex; Interpretation.

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INTRODUCTION

During routine casework, there are a number of situations where it is impossible to separate the various cell types present on evidential material preserved on the crime scene, e.g. when blood is mixed with saliva or male and female material found on swabs taken from body orifices. Furthermore, PCR based analysis is sufficiently sensitive to detect background cellular material deposited on the fabric of clothes by the wearer, minute amounts of victim's blood from suspect's fingernail scrapes or dandruff deposited on rubbery mask. However, the presence of additional bands at particular locus is not necessarily diagnostic of a mixture because of other reasons such as the presence of stutter bands, "N" bands, "pull-up" peaks [6] or even chromosomal abnormalities (Figure 1).

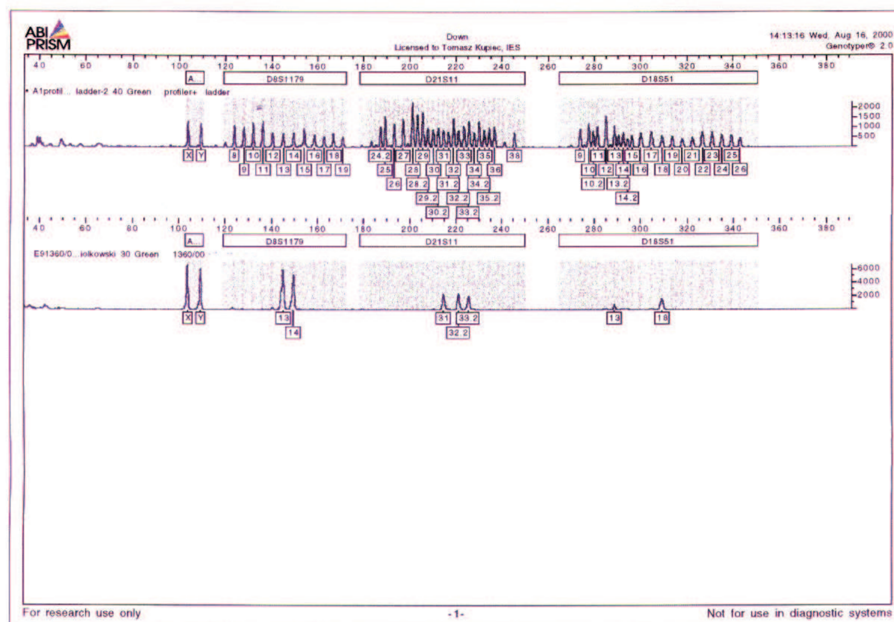

Fig. 1. Example of chromosomal abnormality – three peaks from chromosome 21 of the victim with Down syndrome.

In forensic casework we use Profiler Plus kit which contain fluorescently labelled primers in conjunction with a Perkin-Elmer Applied Biosystems ABI 310 Sequencer, for automated detection and sizing the amplified DNA. The software also calculates the peak areas of detected alleles, which are accurate quantitative indicator of the relative amount of amplified DNA.

Taking into consideration the area of peaks enhances the interpretation of DNA mix stains but involves the need to consider possible artefacts [3].

A comparison of a stain profile with the single persons's DNA profiles is performed with the aim to identify the person who had a contact with the evidence. However, interpretation should be carried out in isolation of any prior information about the case. So one has to consider more than one mutually excluding hypotheses concerning the circumstances of the crime. At a trial there will be alternative propositions about who contributed to this evidence: the prosecution will have proposition that the assailant left his own material at the stain, and defence alternative is that the random person contributed to the stain. The strength of such evidence is represented by the Likelihood Ratio. The analysis requires the assignment of probabilities of all of the combinations of genotype [1, 2, 5].

The presented work is a part of validation study of the GenePrint® Power Plex™ 16 System performed in our Haemogenetic Section laboratory.

The Gene Print Power Plex 16 System allows the coamplification and three-colour detection of sixteen loci: fifteen STR loci and gender marker Amelogenin. All sixteen loci are amplified simultaneously in a single tube and analysed in a single injection or gel line. The GenePrint® PowerPlex™ 16 System is designed specifically for use with the ABI PRISM 310 Genetic Analyser.

The loci included in the Power Plex 16 System satisfy the needs of several major standandardization bodies throughout the word. The European Police network – INTERPOL, has established a set of four STR loci: FGA, D21S11, TH01, VWA, as a pan-European standard.

The European Network of Forensic Science Institutes (ENFSI) has recommended seven STR loci: FGA, D21S11, TH01, VWA, D8S1179, D18S51, and D3S317.

GITAD has recommended six loci (CSF1PO, TH01, TPOX, D16S539, D7S820 and D13S317).

The FBI has selected 13 STR core loci to be typed prior to searching or submitting samples in the CODIS (Combined DNA Index System), the US national database of convicted offender profiles. The loci amplified in the GenePrint Power Plex System include all of these standard loci. The Power Plex 16 system also contains two new low-stutter highly polymorphic pentanucleotide repeat loci, Penta E and Penta D. These additional loci were added significantly to the discrimination of the system, in addition the extremely low stutter seen with Penta E and Penta D makes them ideal loci for evaluation of DNA mixtures often encountered in forensic casework.

MATERIAL AND METHODS

DNA from trace material and reference blood samples was extracted using standard phenol-chloroform method. The amounts of template DNA were measured fluorimetrically with Pico Green using Fluoroscan Ascent Fl.

Experimental mixtures were prepared from known amounts of standard DNA templates: 9947A (PPLX positive control) and SGM positive Control DNA supplied by the manufacturers. Amplification of template DNA was performed at 25 μl volume according to manual provided by the supplier on PE GeneAmp 9700 Thermal Cycler. The electrophoretic separation of the PCR products was performed in a Perkin Elmer ABI 310 Genetic Analyser. A threshold value for peak detection was 150 RFU.

RESULTS AND DISCUSSION

To analyse the natural variation between the peak areas at a heterozygous loci we performed some amplifications of known control samples mixed with proportion: 0.5:0.5, 1:1, 1.5:0.5 and 1.8:0.2 ng per reaction, respectively. The results are shown at Table I for three completely heterozygous loci D7, D16 and vWA.

TABLE I. THE RESULTS OF ANALYSIS A CONTROLLED MIXED SAMPLES OF DNA. AMPLIFICATION OF TEMPLATE DNA WAS PERFORMED WITH POWER PLEX SYSTEM FOLLOWED BY ABI 310 CAPILLARY ELECTROPHORESIS OF AMPLIFIED ALLELES

Locus	Alleles of A	Alleles of B	A / A + B ratio			
			0.5**	0.5	0.75	0.9
D7		7	0.46	0.49	0.57	0.8
	10					
	11					
		12				
D16		9	0.37	0.41	0.39	0.79
		10				
	11					
	12					
vWA		14	0.41	0.36	0.6	0.8
		16				
	17					
	18					
Amelo*	X	X	1.9	2.0	4.8	10.2
		Y				

*Ratio of peak areas X/Y; the expected peak area ratio in bold letters.
** DNA template are mixed at 0.5:0.5 ng, 1:1 ng, 1.5:0.5 and 1.8:0.2 ng, respectively.

One can see that the observed symmetry between peak areas is not exact. It may be a result of preferential amplification of shorter alleles and superimposing a stutter peaks with alleles peaks. It is interested that even in the range of 3:1 ratio of template amount the A / A + B ratio of peak area approaches the 1:1 ratio. Thus the admixture ratio is approximately preserved after co-amplification in mixture ratios of about 5:1 or greater (data not shown).

In forensic casework the most common reason for a variation in peak area balance is due to the presence of shared alleles in mixed stain. Hence, determining the ratio when there are shared alleles is very complex because there may be more than one combination of alleles, which could explain the observation. However, when the DNA template ratio approaches 1:1 and there is a single shared allele it is possible to assess the mixture ratio. Examining the X:Y ratio may be helpful in determination an approximate mixture ratio. As the ratio of mixture increases, the additive contribution of minor component can be indistinct from, for example, stutter contribution.

In every cases it is essential to consider the contribution in a mix stain the known reference set of alleles. First of all, it is necessary to evaluate the nominator of the likelihood ratio. And it is the only possible approach, because it is impossible to assess the likelihood ratio independently from the knowledge of the results of reference samples. In any case, the various alternatives are considered in assessing a denominator of likelihood ratio.

Using the approximate quantitative information drawn from the peak areas in the examined profile some of the alternative allele combinations can be discounted.

In our treatment of forensic DNA mix profile we have to consider just two hypotheses to explain the evidence: the prosecution and defence alternatives. This means that the odds form of Bayes' theorem can be used and the DNA evidence, summarised by the likelihood ratio, can be maintained distinct from the non-DNA evidence expressed by the prior odds.

The interpretation of a mixture depends very much on the circumstances surrounding the crime. For our first example we present a case where there is very good reason for the victim's DNA to be present in the mixed profile.

Case 1. A man was murdered in his bad and a trace amount of his blood was found on his wife's dirty night-shirt.

At the evidence mixed profile, one can see complete set of the victim's alleles together with those matched to his wife's.

The prosecution proposition is:

H_1 – the crime sample contains DNA both from the victim and the suspect.

On the other hand, the defence proposition may of the kind:

H_2 – the crime sample contains DNA from the suspect (her cell material on the night-shirt) and an unknown person.

The numerator is one, because the crime sample profile is exactly as expected if H_1 is true.

The denominator is the probability that the unknown person and the suspect contributed in a mixed DNA profile. In our expertise (Table II), we included a following sentence:

"The evidence is about 1 300 000 000 times more probable if the first of these hypotheses is true than if the second is true."

The judgement of whether or not a wife killed her husband depends not only on the DNA evidence but also on other circumstances that the court will take into account.

TABLE II. COMPARISON OF DNA PROFILES OBTAINED FROM THE VICTIM, SUSPECT AND EVIDENCE MATERIAL. LIKELIHOOD RATIO CALCULATED WITH DNAMIX

Locus	Suspect	Victim	Evidence sample	LR
D3S1358	18	14/15	14/15/18	3.77
TH01	9.3/10	6/9.3	6/9.3/10	51.02
D21S11	29/30.2	30/32.2	29/30/30.2/32.2	37.6
D18S51	15/16	15/16	15/16	1
Penta E	12/17	9/15	9/15	*
D5S818	11/13	10/12	10/11/12/13	11.12
D13S317	11	12	11/12	3.26
D7S820	8/10	11/12	8/10/11/12	7.06
D16S539	12	11/12	11/12	1
CSF1PO	10/12	10	10/12	3.87
Penta D	9/12	9/11	9/11	*
Amelogenina	X	X/Y	X/Y	
VWA	17	14/17	14/17	1
D8S1179	11/15	13/15	11/13/15	3.52
TPOX	8	8/11	8/11	1
FGA	19/20	23/27	19/20/23/27	51.28
Combined likelihood ratio (about)				1 300 000 000

*Could not be calculated because of the lack of population data (September 2000).

Case 2. Suspect and the unknown person. Some crime samples contain DNA from more than one person, but only one known person is suspected of being a contributor.

Four pieces of stockings were found around the place of robbery. The police raised an objection against four persons. One of them matched to the DNA profile obtained from one of the stockings. Three other analysed evidence samples revealed mixed DNA profiles. Here is an example of one of them.

It was impossible to exclude the contribution of suspect Nb 3 at the allele set, so at the first stage the likelihood ratio on the probability of hypothesis,

that the suspect left his cell material on the stocking was calculated with DNAMIX.

TABLE III. AN ILLUSTRATION OF CASE 2 ANALYSIS

Locus	Suspect 1	Suspect 2	Suspect 3	Suspect 4	Evidence sample (suspect 3?)	LR
D3S1358	15/16	15/16	15/17	12/16	15/16/17	2.64
TH01	6/9.3	6/9.3	6/9	9.3	6/9/9.3	3.6
D21S11	30/32.2	29/30	29/31.2	30/31.2	28/29/30/31.2	5.7
D18S51	13/14	13/14	14/17	15/16	13/14/17/18	3.64
Penta E	11/18	14/18	10/12	11/16	10/12	*
D5S818	11/12	11	10/11	11	10/11/12	4.73
D13S317	12/14	12/14	8/11	11	8/11/12/14	1.73
D7S820	9/12	10	9/11	9/12	9/10/11/12	3.47
D16S539	11/12	11/13	12/14	11	11/12/14	14.24
CSF1PO	10/11	10/11	9/11	12	9/10/11	9.67
Penta D	9/17	12/14	12	8/14	12	*
Amelo	X/Y	X/Y	X/Y	X/Y	X/Y	
VWA	15/17	15/16	18/20	16/18	15/17/18/20	36.23
D8S1179	13/14	12/13	13/14	13/14	13/14/15	2.0
TPOX	8/11	9/11	8/11	8	8/11	3.5
FGA	20/21	21/23.2	21	20/25	21	30.5
Combined likelihood ratio (about)						2 965 400 000

As can be seen from the electropherogram at each locus there is obvious excess of one component. After examination of the ratio of major component, peak area to the minor one, it was found a match between the Suspect 3 and the major component from evidence sample profile.

Case 3. A man was found dead in his house. There were lot cigarettes ends on the floor near the victim. DNA profiles obtained from some of them matched DNA profile of the victim, but on two of them there were trace amounts of blood. These cigarettes ends revealed mixed DNA profiles. One could not exclude that one of the contributor of the stain was a victim, and another an unknown, still not found suspect.

Once more, we face the problem that the likelihood ratio formulation can be used only if we restrict attention to two proposition at a time (Table IV).

Proposition of prosecution:

H_1: The crime sample contains DNA from victim and suspect.

H_2: The crime sample contains DNA from two unknown people.

TABLE IV. COMPARISON OF DNA PROFILES TAKEN FROM THE VICTIM AND EVIDENCE SAMPLE

Locus	Victim	Evidence sample	Mixture ratio	LR
D3S1358	16, 18	14, 16, 18		4.29
TH01	7,9	7, 9, 9.3		4.0
D21S11	32.2, 33.2	28, 30, 32.2, 33.2	0.83	20.8
D18S51	14, 15	14, 15, 16, 17	0.74	3.8
Penta E	7,11	7, 9, 11		*
D5S818	12	11, 12		1.6
D13S317	11, 14	8, 11, 14		8.21
D7S820	9,12	8, 9, 11, 12	0.79	3.09
D16S539	12	11, 12, 13		0.69
CSF1PO	10, 15	10, 12		42.13
Penta D	12, 13	9, 12, 13		*
Amelogenine	X,Y	X/Y	1.05	
VWA	17, 18	14, 17, 18		2.46
D8S1179	12, 15	12, 14, 15		8.61
TPOX	8	8, 11		1.01
FGA	20, 24	20, 22, 24		7.5
Combined Likelihood Ratio			256 771 000	

Because alleles 11 and 13 at D16S539 locus are common in the population, the LR is less than one, so the evidence favours the defence proposition. Analysis of combined loci allowed a statement: "It is about 257 million times more probable that the victim's material is present at the mixed stain, than the mixture origins from two random men."

It is also an example, that it is important to use the principles of evidence interpretation instead of simplistic rules of the "random man not excluded"

CONCLUSIONS

1. The interpretation of mixed stains is possible only in the context of likelihood ratios. Unlike single contributor stains, the sample profile may not be certain under either of two alternative propositions, so the likelihood ratio is the ratio of two probabilities that are less than one.
2. The advantage of using PowerPlex™ 16 relies on robust and simultaneous typing a large set of loci.
3. Taking account of peak areas can increase discrimination, but:
   - There are too many factors influencing exact mixture interpretation (stutter peaks, superimposing of peaks, pull-up, N-bands, chromosomal abnormalities, sequence changes etc.).

– Examination of electropherogram's peak areas can increase expert's conviction about the contributors of the mixed DNA profile, but it is rather a matter of subjectivity resembling fingerprinting.

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