ANALYSIS AND CONCLUSION OF THE CASE MIXED DNA SAMPLES IN FORENSIC EXAMINATION BY RELATIVE FLUORESCENSE UNIT METHOD

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ABSTRACT

Purpose: *We aimed to extract mixed DNA samples collected from the crime scene into DNA profiles and provided an accurate assessment of which contributor DNA belongs to the culprit.*

Methods: *We analyze the nuclear DNA (nDNA) from DNA mixtures and amplify genes with the GlobalFiler 24 Locus Gene (Thermo Fisher, USA) kit, followed by capillary electrophoresis using the Genetic Analyzer 3500 System. Data analysis was performed using Genemapper ID_X software and interpreting the results by calculating the Relative Fluorescence Unit (RFU).*

Conclusion: *The method of analyzing the results using RFU for short repeat sequences (STRs) of the nuclear genome is highly reliable and current, applied in most of the advanced countries in the world. Therefore, the International Society of Forensic Genetics has encouraged and prioritized this method.*

Keywords: DNA mixture, Relative Fluorescence Unit, DNA profile, Interpretation.

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1. INTRODUCTIONS

DNA identification is constantly advancing and can be extracted from low-DNA template samples (e.g., sweat stains, body fluids left in clothes, nails, or crime items). In a forensic examination, mixed DNA samples collected from the crime scene were separated into DNA profiles and provided an accurate assessment of which contributor DNA belongs to the culprit [2] [5].

In DNA assessment, and individual identification at the Military Forensic Institute, forensic samples with mixed DNA from many people are biological traces left over from crime scenes. Analyzing samples with mixed DNA from many people is one of the more complicated experiments, always asking two main questions: “How many contributors are in the biomarker sample ?” and “What is the genotype of each contributor?” [1] [3] [4]. When analyzing this type of sample, the examiner encounters many difficulties due to the complex genetic analysis results, which need to be scientifically interpreted.

2. SUBJECTS AND METHODS

2.1. Subjects

A common case in DNA identification is mixed samples from multiple contributors. In this study, we selected a mixed sample of two contributors from the nail biomarker sample, we denote: HT569.4

2.2. Equipment and chemicals used

The DNA was extracted using a QIAamp Investigator DNA kit (Qiagen - Germany) and quantified by Quantifiler® Trio DNA Quantification Kit on a 7500 PCR Realtime System. DNA amplification was performed using a GlobalFiler 24 gene loci (Thermo Fisher, USA), followed by capillary electrophoresis using ABI 3500 Genetic Analyzer and interpreting results using a Genemapper ID_X software.

2.3. Methods of analyzing DNA data and interpreting individual identification results

The steps for analyzing and interpreting the results of individual identification in the case of a mixed-DNA sample were first proposed by the British forensic geneticist T.M. Clayton et al. (1998)

and was standardized by the International Society for Forensic Genetics ISFG as a step-by-step process in 2006 [1] [6], interpreting the results by calculating the Relative Fluorescence Unit.

Fluorescence unit (RFU): Value for measuring the luminescence intensity of alleles (when fluorescently stained alleles are electrophoresed through a capillary electrophoresis system).

- Step 1: Identify alleles of a mixed DNA profile:

+ Through DNA analysis records, determine the number of allele peaks at each gene locus.

+ Exclude the case of Tri-Allen due to mutation.

+ Exclude secondary peaks and stutters, RFU height to primary peak ratio < 15%.

+ Eliminate the extra peak pulled up by spectral saturation.

+ Criteria for determining true alleles in DNA profiles is based on the relative fluorescence unit elevation ratio (RFU).

* Rate of RFU elevation in heterozygous allele pairs is not balanced, with a value < 60%.

* Rate of RFU elevation in suspected stutter locations > 15%.

- Step 2: Identify the number of contributors to a mixture:

+ Based on investigation documents to hypothesize the number of individuals who can contribute DNA in the test sample.

+ Based on the number of alleles appearing in a locus to determine the number of contributors in a mixture sample.

* If 2, 3, or 4 alleles per locus then 2 contributors.

* If 5 or 6 alleles per locus then 3 contributors.

* If > 6 alleles per locus, then > 4 contributors.

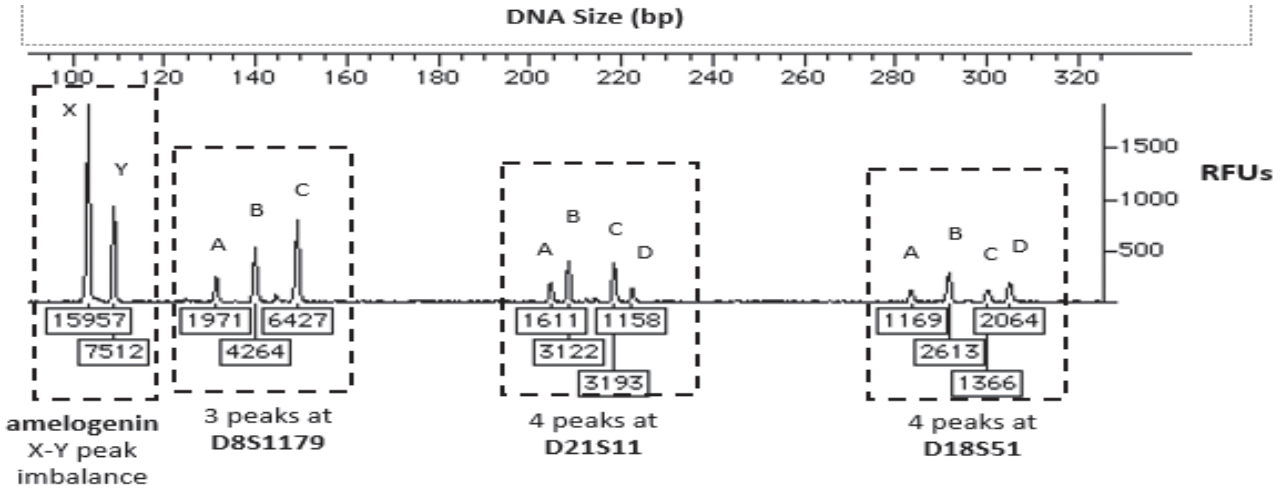

Figure 1. Mixed DNA from 2 contributors

- Step 3: Estimate the relative ratio of the individuals contributing to the mixed sample:

Locus D13S317 there are four alleles: 8, 9, 11, 13.

There are RFU fluorescence unit values of 4 alleles: 6333, 6006, 2753, 2307.

The total RFU fluorescence unit value of allele pairs 11 and 13 (sub-contribution) is: 2753 + 2307 = 5060.

The total RFU fluorescence unit value of the 8, 9 allele pair (main contributor) is: 6333 + 6006 = 12339.

The ratio of the RFU elevation between the central contributing allele pair and the minor contributing allele pair is: 12339: 5060 = 2.4.

Comment: The genotype composition of the main contributor is about 2.4 times higher than that of the minor contributor.

- Step 4: Consider all possible genotype combinations:

Locus D13S317 (Figure 2) has a genotype of 4 alleles: 8,9,11,13 with RFU fluorescence unit values: 6333, 6006, 2753, and 2307.

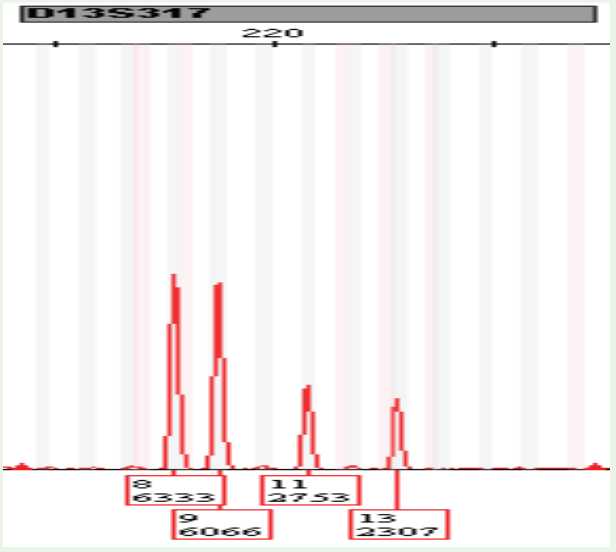

Figure 2. Possible genotype combination (Locus D13S317)

There is a ratio of RFU elevation between heterozygous allele pairs: 8.9 = 96%; 8.11 = 43%; 8.13 = 36%; 9.11 = 45%; 9.13 = 38%; 11.13 = 84%.

Considering the relative elevation ratios of RFUs of possible gene combinations:

8, 9 + 11.13 have 8, 9 = 96% and 11.13 = 84%.

8.11 + 9.13 has 8.11 = 43% and 9.13 = 38%.

8.13 + 9.11 has 8.13 = 36% and 9.11 = 45%.

The allele pair 8, 9 + 11.13 is the most feasible combination because the allele pairs have the most balanced fluorescence unit ratio in the considered cases.

- Step 5: Compare with reference samples.

Each DNA profile contributor is analyzed from a mixed DNA profile, which can be compared with a reference sample's DNA profile or archived DNA profile for comparison with a DNA databank.

3. RESULTS

3.1. Determination of alleles and fluorescence unit heights of alleles in the mixed DNA

Table 1. Alleles and fluorescence unit heights of alleles in sample HT569.4

Locus	D3S1358	vWA	D16S539	CSF1PO	TPOX	IN-DEL
Allele	15 (18385) 16 (4001)	14 (4592) 16 (10278) 18 (6061)	9 (5517) 11 (2209) 13 (6347)	11 (4690) 12 (2496) 13 (1222)	8 (720) 9 (930) 11 (2737)	1 (16853)
Locus	**SEX**	**D8S1179**	**D21S11**	**D18S51**	**DYS391**	**D2S441**
Allele	X (26784) Y (5601)	11 (13192) 14 (17818) 15 (6308)	28 (5365) 30.3 (2585) 32.2 (6849)	15 (2106) 16 (4752) 20 (2445)	11 (1518)	11 (10755) 11.3 (13116)
Locus	**D19S433**	**TH01**	**FGA**	**D22S1045**	**D5S818**	**D13S317**
Allele	13 (6222) 14 (8009) 18.2 (1750)	7 (3572) 9 (10077) 9.3 (6407)	19 (2696) 21 (2992) 22 (9483)	15 (5093) 16 (5543) 17 (13825)	9 (3835) 11 (11125) 12 (5967)	8 (6333) 9 (6066) 11 (2753) 13 (2307)
Locus	**D7S820**	**SE33**	**D10S1248**	**D1S1656**	**D12S391**	**D2S1338**
Allele	8 (3851) 11 (3950) 12 (3298)	18 (1980) 19 (2998) 23.2 (2634) 25.2 (1203)	13 (7540) 14 (4308) 15 (6715) 16 (3352)	11 (9900) 13 (4980) 14 (4682) 15 (7476)	20 (5019) 25 (7531)	19 (6370) 23 (1469) 24 (1140)

Table 1 shows the identified alleles and the relative height of the fluorescence unit corresponding to that allele; for example, Locus D13S317 has 02 alleles, 15 and 16, with the relative height of the fluorescence unit times. 18385 and 4001.

3.2. Results of the number of contributors with genotype and the proportion of genotypes in the mixed DNA

In Table 1, results of the number of people with genotype in the mixture DNA: There are 02 individuals contributing genotype in the mixture DNA because the results show on gene locus with 2, 3, or 4 alleles.

The resulting genotype ratio in the DNA: the genotype component of the main contributor is approximately 2.4 times higher than the genotype of the minor contributor.

3.3. Results of identifying possible allele combinations

Table 2. Determination of possible allele combinations

Locus	D3S1358	vWA	D16S539	CSF1PO	TPOX	IN-DEL
Possible Allele Combination	15,15 + 15,16 15,15 + 16,16 16,16 + 15,16 15,16 + 15,16	14,14 + 16,18 16,16 + 14,18 18,18 + 14,16 14,16 + 14,18 14,16 + 16,18 14,18 + 16,18	9,9 + 11,13 11,11 + 9,13 13,13 + 9,11 9,11 + 9,13 9,11 + 11,13 9,13 + 11, 13	11,11 + 12,13 12,12 + 11,13 13,13 + 11,12 11,12 + 11,13 11,12 + 12,13 11,13 + 12,13	8,8 + 9,11 9,9 + 8,11 11,11 + 8,9 8,9 + 8,11 8,9 + 9,11 8,11 + 9,11	1
Locus	**SEX**	**D8S1179**	**D21S11**	**D18S51**	**DYS391**	**D2S441**
Possible Allele Combination	X X + X Y	11,11 + 14,15 14,14 + 11,15 15,15 + 11,14 11,14 + 11,15 11,14 + 14,15 11,15 + 14,15	28,28 +30.3,32.2 30.3, 30.3 + 28, 32.2 32.2, 32.2 + 28, 30.3 28, 30.3 + 28, 32.2 28, 30.3 + 30.3, 32.2 28, 32.2 + 30.3, 32.2	15,15 + 16,20 16,16 + 15,20 20,20 + 15,16 15,16 + 15,20 15,16 + 16,20 15,20 + 16,20	11	11,11 +11.3,11.3 11,11 + 11,11.3 11,11.3+11.3,11.3 11,11.3 + 11, 11.3
Locus	**D19S433**	**TH01**	**FGA**	**D22S1045**	**D5S818**	**D13S317**
Possible Allele Combination	13,13 + 14,18.2 14,14 + 13,18.2 18.2,18.2+13,14 13,14 + 13,18.2 13,14 + 14,18.2 13,18.2+14,18.2	7,7 + 9,9.3 9,9 + 7,9.3 9.3,9.3 + 7,9 7,9 + 7,9.3 7,9 + 9,9.3 7,9.3 + 9,9.3	19,19 + 21,22 21,21 + 19,22 22,22 + 19,21 19,21 + 19,22 19,21 + 21,22 19,22 + 21,22	15,15 + 16,17 16,16 + 15,17 17,17 + 15,16 15,16 + 15,17 15,16 + 16,17 15,17 + 16,17	9,9 + 11,12 11,11+ 9,12 12,12 +9, 11 9,11 + 9,12 9,11+11,12 9,12 +11,12	8, 9 + 11, 13 8, 11 + 9, 13 8, 13 + 9, 11
Locus	**D7S820**	**SE33**	**D10S1248**	**D1S1656**	**D12S391**	**D2S1338**
Possible Allele Combination	8,8 + 11,12 11,11 + 8,12 12,12 + 8,11 8,11 + 8,12 8,11 + 11,12 8,12 + 11,12	18,19+23.2,25.2 18,23.2+19,25.2 18,25.2+19,23.2	13,14 + 15,16 13, 15 + 14,16 13, 16 + 14, 15	11,13 + 14,15 11,14 + 13,15 11,15 + 13, 14	20,20+20,25 20,20+25,25 20,25+25,25 20,25+20,25	19, 19 + 23, 24 23, 23 + 19, 24 24, 24 + 19, 23 19, 23 + 19, 24 19, 24 + 23, 24 19,23 + 23,24

The possible allele combinations (in bold) in table 2 are the selected allele combinations, having the most balanced relative fluorescence unit elevation ratio and correct range of relative elevation ratio between the main contributing allele pair versus the minor contributing allele pairs is approximately 2.4 times.

Table 3. Relative fluorescence unit ratios of the possible allele combinations corresponding to table 2

Locus	D3S1358	vWA	D16S539	CSF1PO	TPOX	IN-DEL
Ratio	100% + 100% 100% + 100% 100% + 22% 22% + 22%	100% + 59% 100% + 76% 100% + 45% 45% + 1% 100% + 94% 100% + 14%	100% + 35% 100% + 87% 100% + 40% 100% + 52% 100% + 1% 75% + 99%	100% + 49% 100% + 26% 100% + 53% 100% + 100% 100% + 1% 100% + 1%	100% + 34% 100% + 26% 100% + 77% 100% + 1% 100% + 5% 100% + 46%	1
Locus	**SEX**	**D8S1179**	**D21S11**	**D18S51**	**DYS391**	**D2S441**
Ratio	X, Y = 21%	100% + 35% 100% + 48% 100% + 74% 100% + 1% 100% + 73% 100% + 1%	100% + 38% 100% + 78% 100% + 48% 100% + 41% 47% + 1% 100% + 57%	100% + 51% 100% + 87% 100% + 44% 100% + 41% 47% + 1% 100% + 57%	11	100% + 100% 100% + 28% 100% + 100% 82% + 82%
Locus	**D19S433**	**TH01**	**FGA**	**D22S1045**	**D5S818**	**D13S317**
Ratio	100% + 22% 100% + 28% 100% + 78% 77% + 1% 98% + 100% 27% + 1%	100% + 35% 100% + 58% 100% + 35% 34% + 1% 100% + 98% 100% + 28%	100% + 32% 100% + 28% 100% + 90% 34% + 1% 99% + 1% 100% + 44%	100% + 40% 100% + 37% 100% + 92% 99% + 1% 99% + 1% 100% + 63%	100% + 53% 100% + 64% 100% + 34% 33% + 1% 100% + 82% 100% + 19%	96% + 84% 43% + 38% 36% + 45%
Locus	**D7S820**	**SE33**	**D10S1248**	**D1S1656**	**D12S391**	**D2S1338**
Ratio	100% + 84% 100% + 86% 100% + 98% 99% + 1% 99% + 11% 99% + 1%	66% + 46% 75% + 40% 61% + 89%	57% + 50% 89% + 78% 44% + 64%	50% + 63% 49% + 67% 76% + 94%	99% + 1% 100% + 100% 100% + 100% 71% + 71%	100% + 78% 100% + 18% 100% + 23% 100% + 23% 17% + 1% 22% + 1%

The relative fluorescence unit ratios (in bold) in table 3 are the most balanced values selected for the selected allele combinations. Allele pairs are selected when the relative fluorescence unit elevation ratio is the most balanced and the relative elevation ratio between the main contributing allele pair and the minor contributing allele pair is approximately 2.4 times.

At locus D16S539, there are two allele combinations with a balanced RFU ratio: the allele combination 11, 11 + 9, 13 has a relative height ratio between the main contributing allele pair compared to the minor contributing allele pair about 5.4 times and allele combination 9, 13 + 11, 13 has a relative height ratio between the main contributing allele pair compared to the secondary contributing allele pair about 2.2 times. Therefore, a combination of alleles 9 and 13, 11 and 13 are selected, in which allele pairs 9 and 13 are the main contributor, and allele pairs 11 and 13 are minor contributor in the mixed DNA. Analysis of 24 locus resulted in complete genotyping of contributor 1 and contributor 2 of the mixed DNA profile.

The analyzed HT569.4 sample by RFU method, which is a mixed DNA profile with the highest probability of 02 contributors, identifying the DNA profile contributor 1 and DNA profile contributor 2.

3.4. Results of comparing reference samples

Table 4. Comparison results: DNA profile of contributor 1, 2, and reference samples

Locus	D3S1358	vWA	D16S539	CSF1PO	TPOX	IN-DEL	SEX	D8S1179
Contributor 1, Reference 1	15 15	16 18	9 13	11 12	11 11	-	X X	11 14
Contributor 2, Reference 2	15 16	14 16	11 13	11 13	8 9	1	X Y	14 15
Locus	**D21S11**	**D18S51**	**DYS391**	**D2S441**	**D19S433**	**TH01**	**FGA**	**D22S1045**
Contributor 1, Reference 1	28 32.2	16 20	-	11 11.3	13 14	9 9.3	22 22	17 17
Contributor 2, Reference 2	30.3 30.3	15 16	11	11 11.3	14 18.2	7 9	19 21	15 16
Locus	**D5S818**	**D13S317**	**D7S820**	**SE33**	**D10S1248**	**D1S1656**	**D12S391**	**D2S1338**
Contributor 1, Reference 1	11 12	8 9	8 12	19 23.2	13 15	11 15	25 25	23 24

Comparing 24 locus in Table 4: with DNA profiles of contributor 1, contributor 2 with DNA profiles of 2 reference individuals (reference 1, reference 2) are completely matched.

4. DISCUSSIONS

There are many methods to analyze and interpret mixed DNA samples, but the method of analyzing the results using RFU for short repeat sequence of the nuclear genome is highly reliable and current, applied in most of the advanced countries in the world. Therefore, the International Society of Forensic Genetics has encouraged and prioritized this method.

Our study analyzed a mixture sample from 2 contributors, which is a common pattern case in forensic examination, our interpretation of the mixed DNA profile is guided by data processing methods. the mixed DNA profile based on relative fluorescence unit rates developed by British forensic geneticist T.M. Clayton et al. 1998; was re-standardized by the International Society of Forensic Genetics - ISFG in 2006; as well as the guiding procedure of scientists in the United States and Canada in the field of forensic DNA analysis SWGDAM - The Scientific Working Group on DNA Analysis Methods was released in 2010 and updated in 2017.

We have applied the method of calculating the RFU ratio when analyzing and interpreting the mixed DNA profiles in the examination cases at the Military Insitute of Forensic Medicine's Molecular Biology Laboratory, which is a solution to the problem of complex DNA tests, involving multiple sample contributors. This method is feasible and highly effective when it has been applied to analyze mixed samples in complex cases: such as gang rape, murder cases that need to find the perpetrator whose traces are left behind mixed DNA profiles.

5. CONCLUSIONS

We have analyzed, interpreted, and fully determined the number of gene loci of the test sample HT569.4. By the method of calculating the relative fluorescence unit ratio, we have determined that the identification sample HT569.4 is the sample with mixed DNA with the highest probability of 2 contributors, get the DNA profile of contributor 1, contributor 2 in the mixed DNA profile of sample HT569.4.

When comparing the DNA profiles of contributor 1, and contributor 2 with the DNA profiles of 2 reference individuals, they match perfectly.

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