Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

New Off-Ladder Alleles Detected by Using the Power Plex® Fusion System during Establishment of Genetic Fingerprint Database for the Iraqi Security Forces

Esam Ghazi Mohammed Salih[1], Mohammed Ibrahim Nader[2] , Majeed Arsheed Sabbah[3] , Mohammed Mahdi Al-Zubaidi[3] , Hanan Khaleel Mahmood[4] , Khalifah Mahdi Salih[4] , Dhurgham Hameed Hasan[4] .

[1]Ministry of Defense, Directorate of Military Medical Affairs.
[2]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.
[3]Forensic DNA center Research and Training Al-Nahrain University, Baghdad, Iraq.
[4]Ministry of Health/Medical-legal Directorate, Paternity and Kinship Section.

Abstract

In present study, we detected thirty two off-ladder alleles during establishment of genetic fingerprint database for 354 individual from Iraqi security forces volunteers. These individuals had been typed by using the PowerPlex® Fusion System for the following 22 autosomal STRs: D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA and D22S1045. Eight of them are located at the D12S391 locus, seven are located in Penta E, three are located in D1S1656, D2S441, D7S820 and FGA respectively, two are located in D18S51, one occurred at the D16S539 locus, Penta D locus and D8S1179 locus respectively. The results showed that 10 of 22 STR loci have non-ladder alleles, and the number of observed OL alleles was 32, with a percentage of 4.5%. Totally, we detected 16 different OL allele types from the 32 alleles, 2 located at outside the range of commercial allelic ladder, and 14 at inside this range. The OL allele data can help to increase the power of cases identification when samples are involved with those variant loci.

Keywords: Off-ladder allele; rare allele; DNA typing; Autosomal; Short tandem repeat; PowerPlex® Fusion System; Iraqi security forces; Iraq.

Introduction

Rare alleles are encountered in the human population that may differ from common allele variants at tested DNA markers by one or more base pairs. Sequence variation between STR alleles can take the form of insertions, deletions, or nucleotide changes. Alleles containing some form of sequence variation compared to more commonly observed alleles are often referred to as variant alleles or microvariants because they are only slightly different from full repeat alleles. Because microvariant alleles often do not size the same as the commonly observed consensus alleles present in the reference allelic ladder, they can be referred to as "off-ladder" alleles (Butler, 2015).

STR typing is typically performed using size comparisons to standardized allelic ladders that possess the most common alleles, which have been sequenced to reveal the true number of repeats.

Different STR kit manufacturers may supply allelic ladders with slightly different allele

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

ranges. As more samples are run with STR loci, new alleles are constantly being discovered that do not size exactly with the ladder alleles (Brinkmann *et al.,* 1998).

These "off-ladder" alleles can be variants with more or less of the core repeat unit than present in the common alleles found in the commercially available allelic ladder. Alternatively, these variant alleles may contain partial repeats or insertions/deletions in the nearby flanking region to the repeat. Insertion/deletion event that creates off-ladder alleles is found in new alleles can be discovered that occur outside the range defined by the commercially available allelic ladder. In many instances, these alleles are simply classified as greater than the largest allele (or smaller than the smallest allele) in the ladder rather than attempting to extrapolate to a predicted number of repeats.

One example of a common microvariant is allele 9.3 at the STR locus TH01. The repeat region of TH01 allele 9.3 contains nine full repeats (AATG) and a partial repeat of three bases (ATG). The 9.3 allele differs from the 10 allele by a single base deletion of adenine in the seventh repeat (Puers, *et al*. 1993). Microvariants exist for most STR loci and are being identified in greater numbers as more samples are being examined around the world. For example, in one study, 42 apparent microvariants were seen in over 10,000 samples examined at the CSF1PO, TPOX, and TH01 loci (Crouse, *et al*. 1999).

Microvariants are most commonly found in more polymorphic STR loci, such as FGA, D21S11, and D18S51 that possess the largest and most complex repeat structures compared to simple repeat loci, such as TPOX and CSF1PO.

Table (1) contains a list of variant or "off-ladder" alleles that have been reported to the NIST STRBase website as of April 2005.

Off-ladder alleles are rare alleles that are not represented in the locus-specific allelic ladders. These off-ladder alleles do not fit within the 0.5 bp range of corresponding alleles in the allelic ladder. Since such alleles cannot be sized by direct comparisons to the reference alleles in the allelic ladders, genotyping software will often designate them as "off-ladder alleles". An off-ladder allele may occur between two alleles in the allelic ladder or it may be smaller (or larger) than the smallest (or largest) allele in the allelic ladder (Butler, 2005).To identify an off-ladder allele, the size (in base pairs) of the off-ladder allele is compared to the sizes of the two closest alleles in the allelic ladder.

Material and methods

Iraqi Security Forces (ISF) samples (354) were collected. Whole blood samples, buccal swabs and hair follicles were the sample types of choice. Blood is one of the richest sources of DNA. All samples were collected from donors volunteering in the AL-Muthana Military hospital by dedicated medical personnel. Three milliliters of blood were collected through venipuncture into 3 ml sterile EDTA blood collection tubes which were labeled with the subject's name, rank, age, sex and the date. Afterwards, the blood stains were made from the collected blood on a FTA™ Classic Card (Whatman™).

FTA cards were packed into individual clean paper envelopes for easy transfer to their final destination in the forensic genetic laboratories in DNA Typing Department of Medico-legal Directorate / Ministry of Health / Baghdad / Iraq. All samples were made anonymous, given serial numbers after collection and analyzed according to standard operating procedures. All possible measures were taken to prevent contamination.

DNA profiles consisting of the following 22 autosomal STRs were determined:, D3S1358,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA and D22S1045, using the PowerPlex® Fusion System (Applied Biosystems).

PCR products were separated by capillary electrophoresis on the ABI 3130xl Genetic Analyzer (Applied Biosystems) and alleles were identified using ABI's Genemapper software (Promega Corporation, 2017).

Results and Discussion

A total of thirty two off-ladder alleles were detected in present study Table (1). Eight of them are located at the D12S391 locus Figure (1 – A, B, C, D, E, F, G and H), seven are located in Penta E Figure (2 – A, B, C, D, E, F and G), three are located in D1S1656 Figure (3 – A, B and C), three are located in D2S441 Figure (4 – A, B and C), three are located in D7S820 Figure (5 – A, B and C), three are located in FGA Figure (6 – A, B and C), two are located in D18S51 Figure (7 – A and B), one occurred at the D16S539 locus Figure (8), one occurred at the Penta D locus Figure (9) and one occurred at the D8S1179 locus Figure (10). All of the samples containing off-ladder alleles were analyzed twice on the Genetic analyzer 3130xl to confirm the data. Alleles' sizes in base pairs are all generated by the GeneScan® software.

No two humans are genetically identical. Even monozygotic twins, who develop from one zygote, have infrequent genetic differences due to mutations occurring during development and gene copy number variation (Bruder, 2008).

Differences between individuals, even closely related individuals, are the key to techniques such as genetic fingerprinting. Alleles occur at different frequencies in different human populations, with populations that are more geographically and ancestrally remote tending to differ more. Causes of differences between individuals include the exchange of genes during meiosis and various mutational events. There are at least two reasons why genetic variation exists between populations. Natural selection may confer an adaptive advantage to individuals in a specific environment if an allele provides a competitive advantage. Alleles under selection are likely to occur only in those geographic regions where they confer an advantage. The second main cause of genetic variation is due to the high degree of neutrality of most mutations. Most mutations do not appear to have any selective effect one way or the other on the organism. The main cause is genetic drift; this is the effect of random changes in the gene pool.

Table 1: New variant or "off-ladder" discovered in present study and comparison with alleles reported in STRBase**.**

STRBase			**In present study**	
STR Locus	**Number Reported**	**Variant alleles reported as of Apr 2005**	**STR Locus**	**Variant alleles**
D1S1656	0	None reported yet in STRBase	**D1S1656**	**8(3)**
D2S441	0	None reported yet in STRBase	**D2S441**	**10.1, 12.3, 13.3**
Penta E	13	9.4, 11.4, 12.1, 12.2, 13.2, 14.4, 15.2, 15.4, 16.4, 17.4, 18.4, 19.4, 23.4	**Penta E**	**16.4(5), 17.4(2)**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

D16S539	10	6, 7, 9.3, 11.3, 12.1, 12.2, 13.1, 13.3, 14.3, 16	**D16S539**	**8.3**
D18S51	30	7, 8, 9, 11.2, 12.2, 12.3, 13.1, 13.3, 14.2, 15.1, 15.2, 16.1, 16.2, 16.3, 17.2,17.3, 18.1, 18.2, 19.2, 20.1, 20.2, 21.2, 22.1, 22.2, 23.2, 24.2, 27, 28.1, 28.3, 40	**D18S51**	**12.2, 16.2**
Penta D	14	6, 6.4, 7.1, 7.4, 9.4, 10.3, 11.1, 11.2, 12.2, 12.4, 13.2, 13.4, 14.1, 14.4	**Penta D**	**9.4**
D7S820	22	5, 5.2, 6.3, 7.1, 7.3, 8.1, 8.2, 8.3, 9.1, 9.2, 9.3, 10.1, 10.3, 11.1, 11.3, 12.1, 12.2, 12.3, 13.1, 14.1, 15, 16	**D7S820**	**9.1(2), 9.3**
D8S1179	4	7, 15.3, 18, 20	**D8S1179**	**20**
D12S391	0	None reported yet in STRBase	**D12S391**	**19.2(3), 19.3(5)**
FGA	69	12.2, 13.2, 14, 14.3, 15, 15.3, 16,16.1, 16.2, "<17", 17, 17.2, 18.2,19.1, 19.2, 19.3, 20.1, 20.2, 20.3, 21.1, 21.2, 21.3, 22.1, 22.2, 22.3, 23.1, 23.2, 23.3,24.1, 24.2, 24.3, 25.1, 25.2, 25.3, 26.1,26.2, 26.3, 27.3, 29.2, 30.2, 31, 31.2, 32.1, 32.2, 33.1, 34.1, 34.2, 35.2, 41.1, 41.2, 42.1, 42.2, 43.1, 43.2, 44, 44.1, 44.2, 44.3, 45.1, 45.2, 46.1, 46.2,47.2, 48.2, 49, 49.1, 49.2, 50.2, 50.3	**FGA**	**16.1(3)**

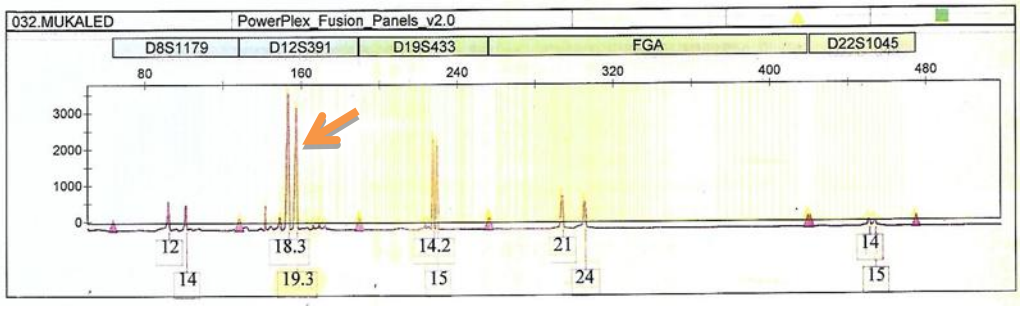

Figure (1 – A): Off-ladder allele (19.3) located at the D12S391 locus from sample number 32.

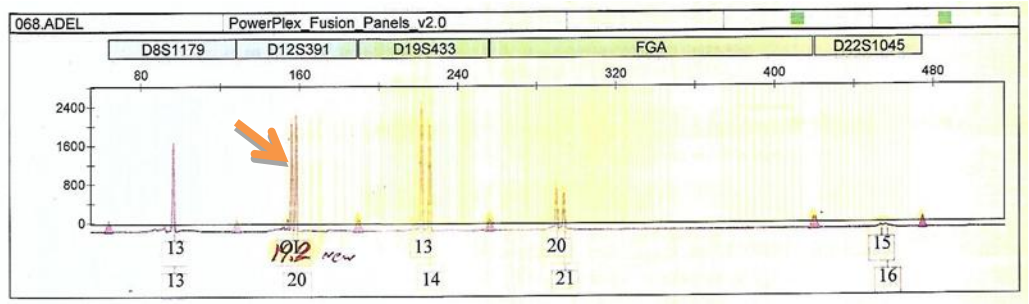

Figure (1 – B): Off-ladder allele (19.2) located at the D12S391 locus from sample number 68.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

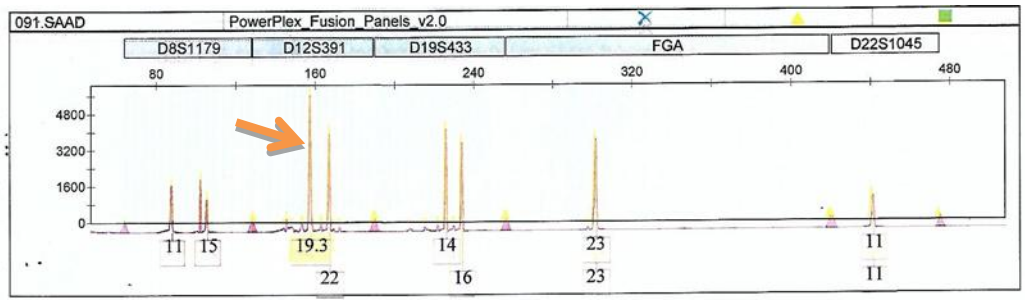

Figure (1 – C): Off-ladder allele (19.3) located at the D12S391 locus from sample number 91.

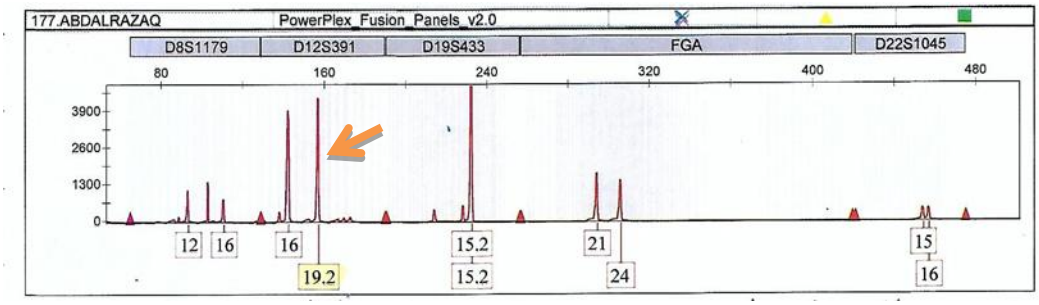

Figure (1 – D): Off-ladder allele (19.2) located at the D12S391 locus from sample number 177.

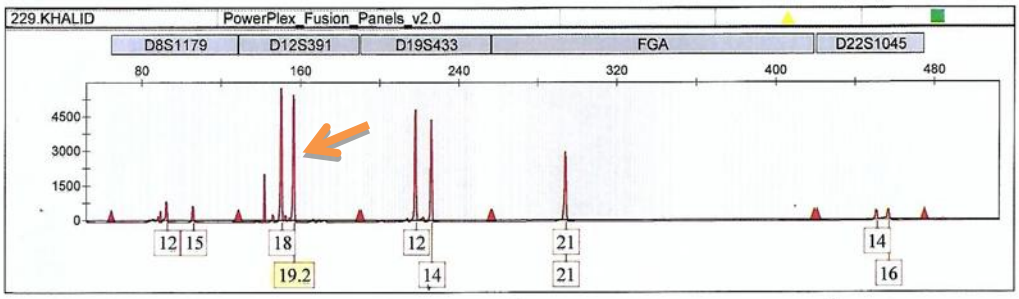

Figure (1 – E): Off-ladder allele (19.2) located at the D12S391 locus from sample number 229.

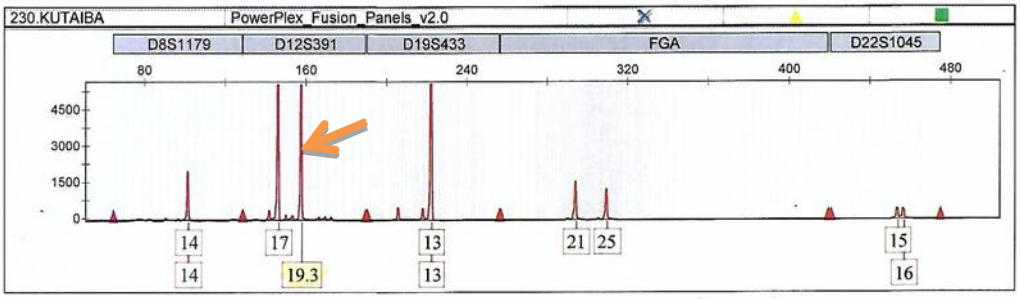

Figure (1 – F): Off-ladder allele (19.3) located at the D12S391 locus from sample number 230.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

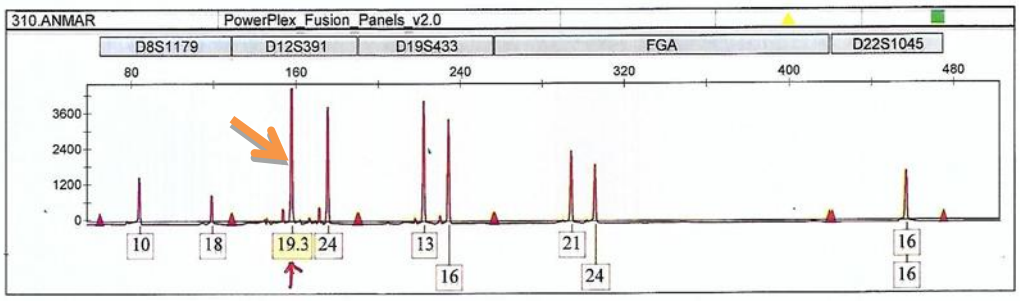

Figure (1 – G): Off-ladder allele (19.3) located at the D12S391 locus from sample number 310.

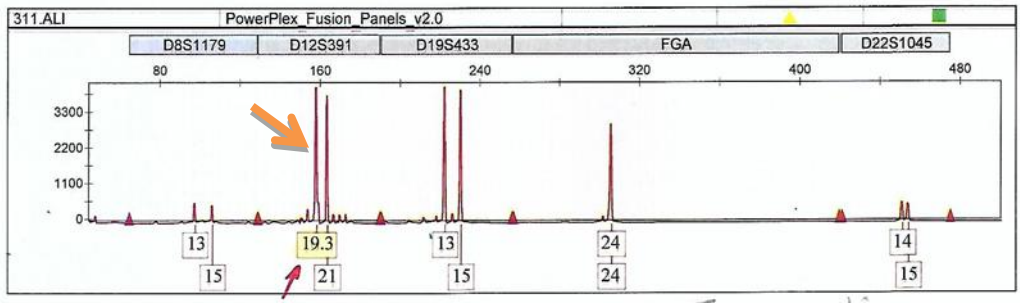

Figure (1 – H): Off-ladder allele (19.3) located at the D12S391 locus from sample number 311.

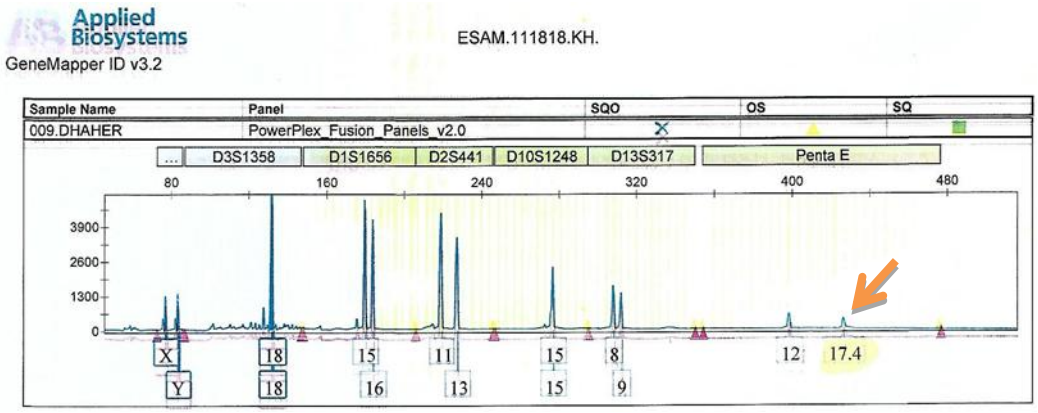

Figure (2 – A): Off-ladder allele (17.4) located at the Penta E locus from sample number 9.

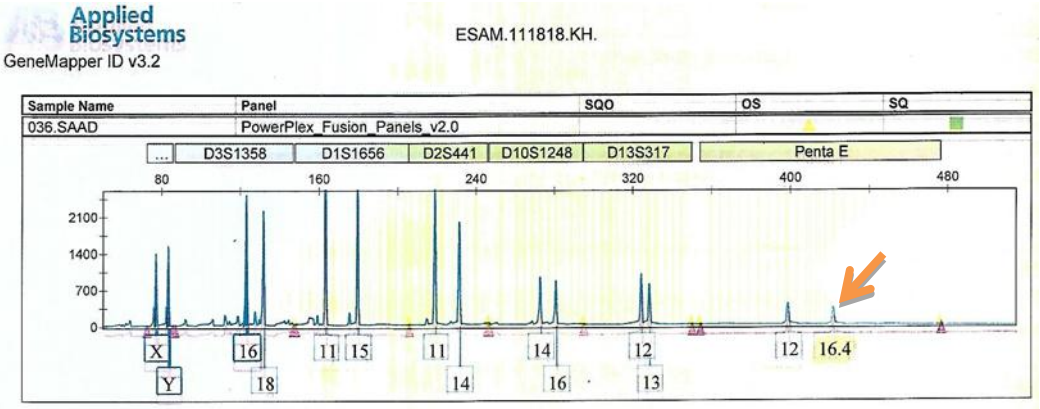

Figure (2 – B): Off-ladder allele (16.4) located at the Penta E locus from sample number 36.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

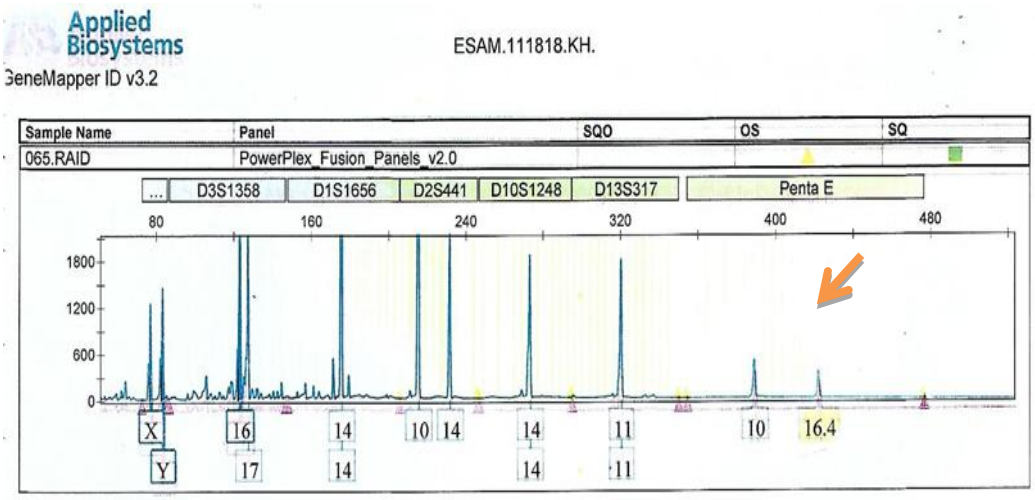

Figure (2 – C): Off-ladder allele (16.4) located at the Penta E locus from sample number 65.

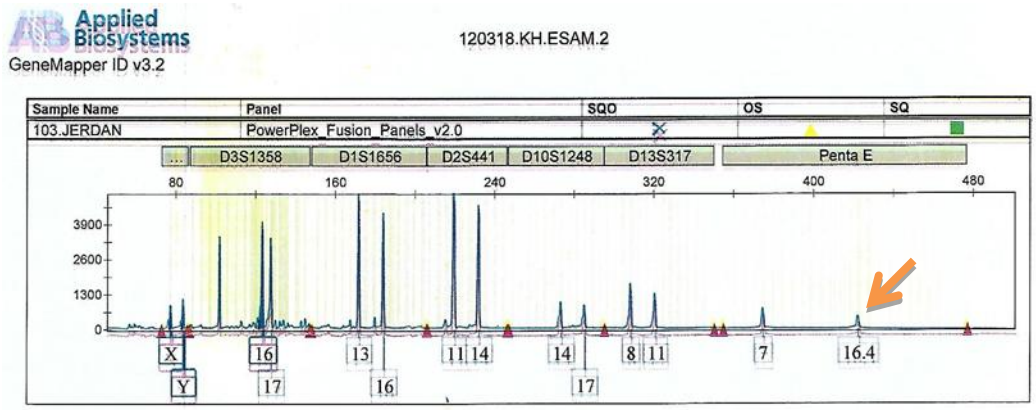

Figure (2 – D): Off-ladder allele (16.4) located at the Penta E locus from sample number 103.

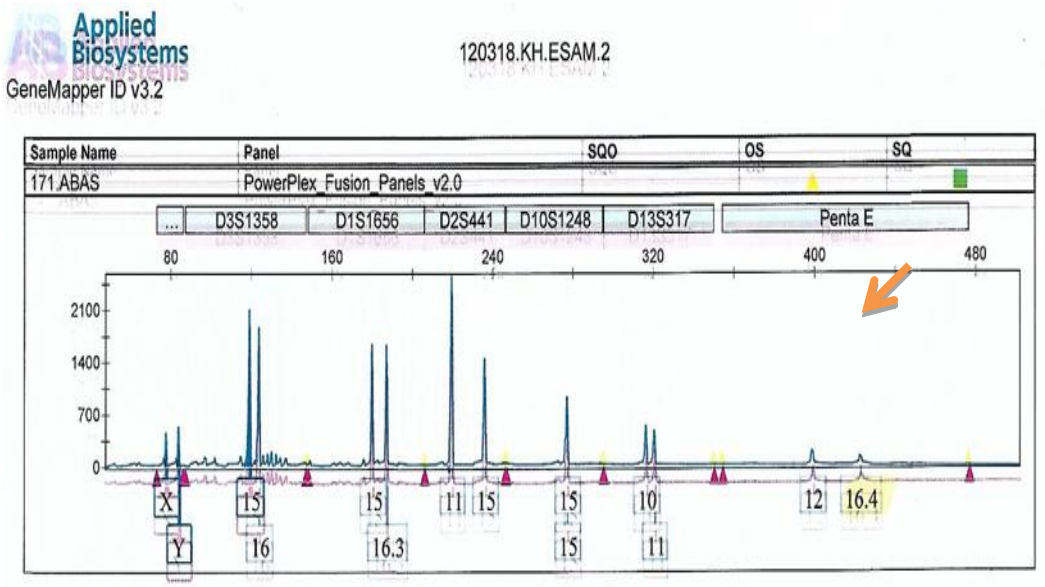

Figure (2 – E): Off-ladder allele (16.4) located at the Penta E locus from sample number 171.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

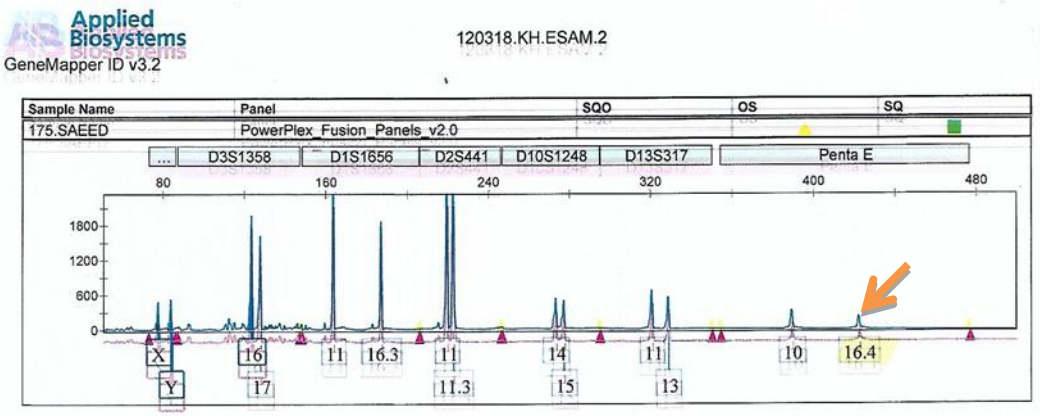

Figure (2 – F): Off-ladder allele (16.4) located at the Penta E locus from sample number 175.

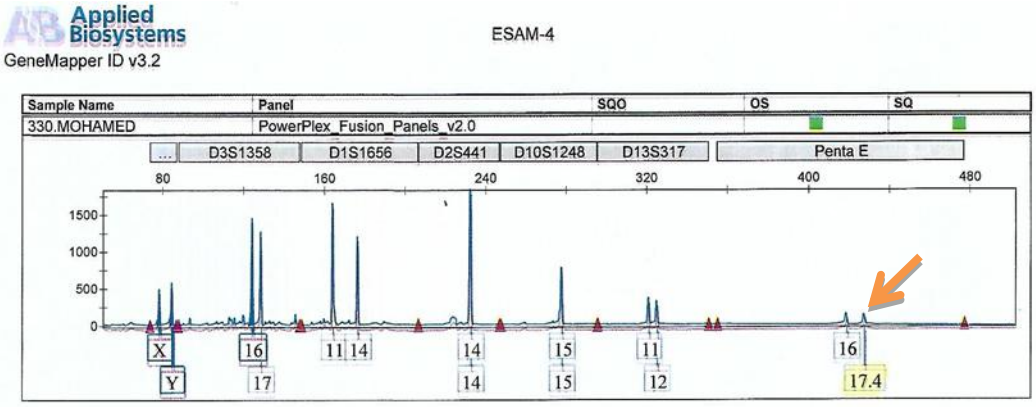

Figure (2 – G): Off-ladder allele (17.4) located at the Penta E locus from sample number 330.

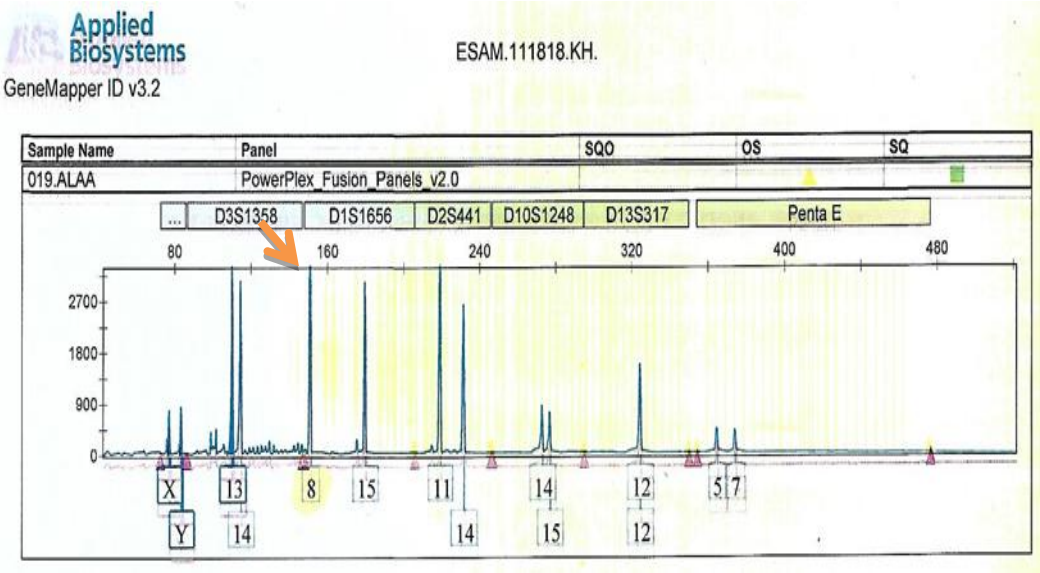

Figure (3 – A): Off-ladder allele (8) located at the D1S1656 locus from sample number 19.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

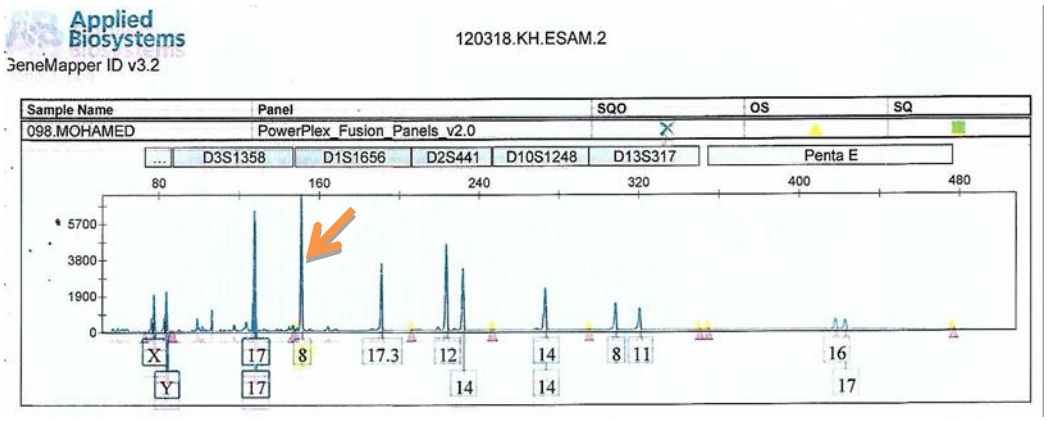

Figure (3 – B): Off-ladder allele (8) located at the D1S1656 locus from sample number 98.

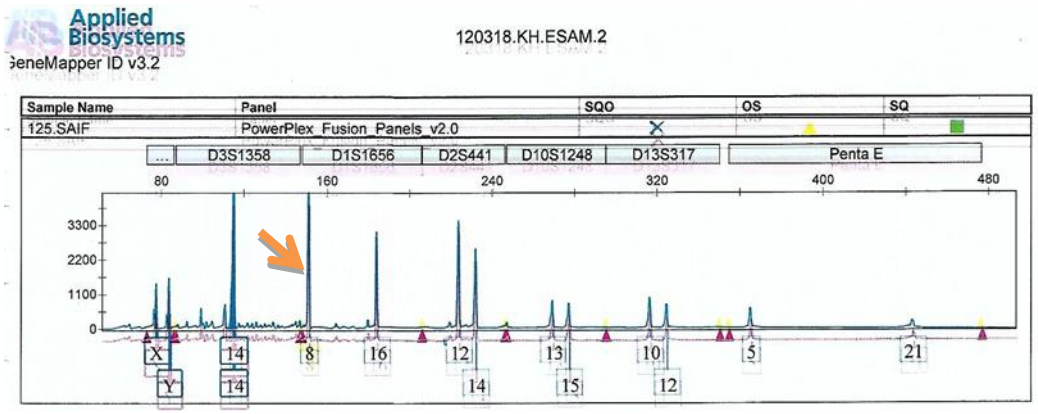

Figure (3 – C): Off-ladder allele (8) located at the D1S1656 locus from sample number 125.

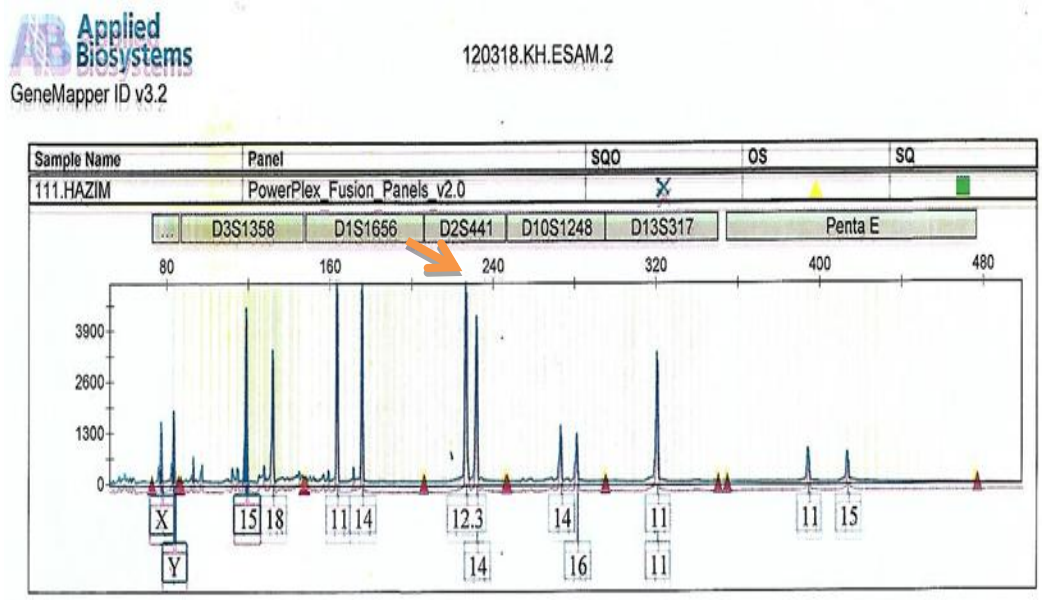

Figure (4 – A): Off-ladder allele (12.3) located at the D2S441 locus from sample number 111.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

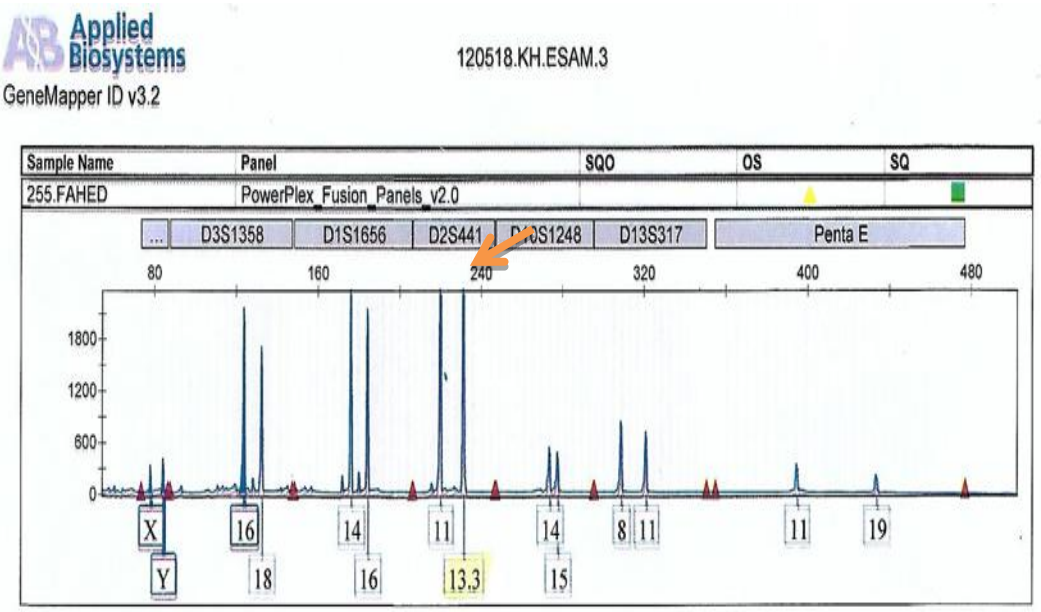

Figure (4 – B): Off-ladder allele (13.3) located at the D2S441 locus from sample number 255.

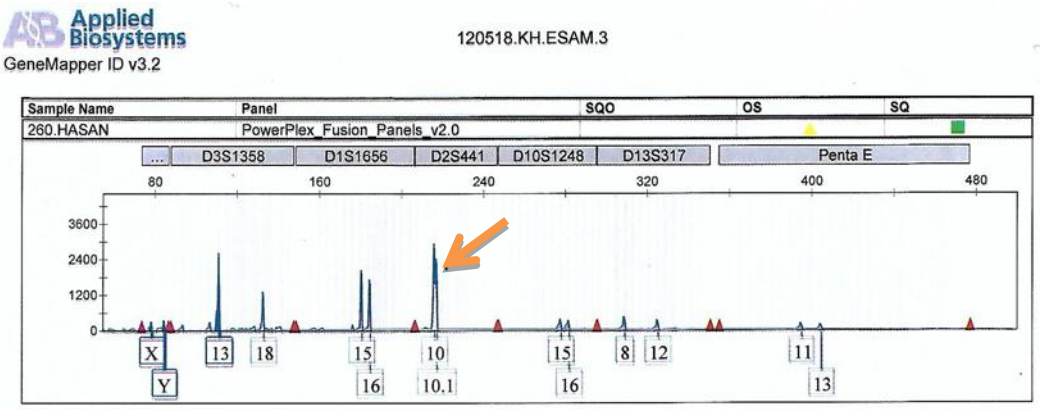

Figure (4 – C): Off-ladder allele (10.1) located at the D2S441 locus from sample number 260.

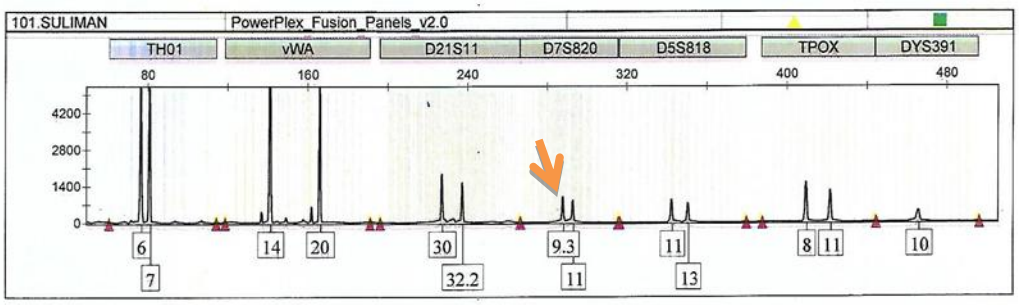

Figure (5 – A): Off-ladder allele (9.3) located at the D7S820 locus from sample number 101.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

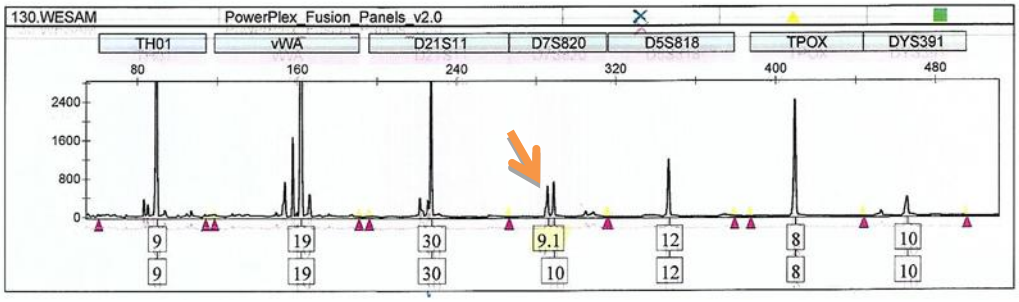

Figure (5 – B): Off-ladder allele (9.1) located at the D7S820 locus from sample number 130.

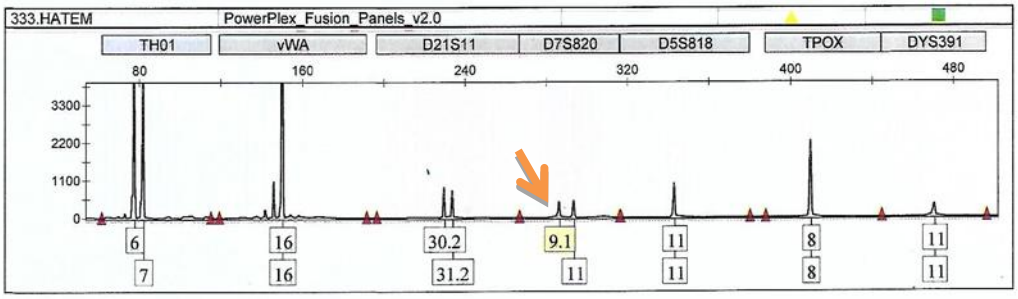

Figure (5 – C): Off-ladder allele (9.1) located at the D7S820 locus from sample number 333.

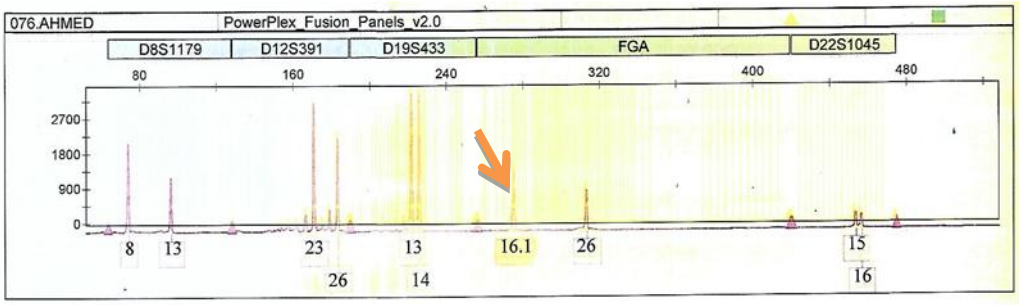

Figure (6 – A): Off-ladder allele (16.1) located at the FGA locus from sample number 76.

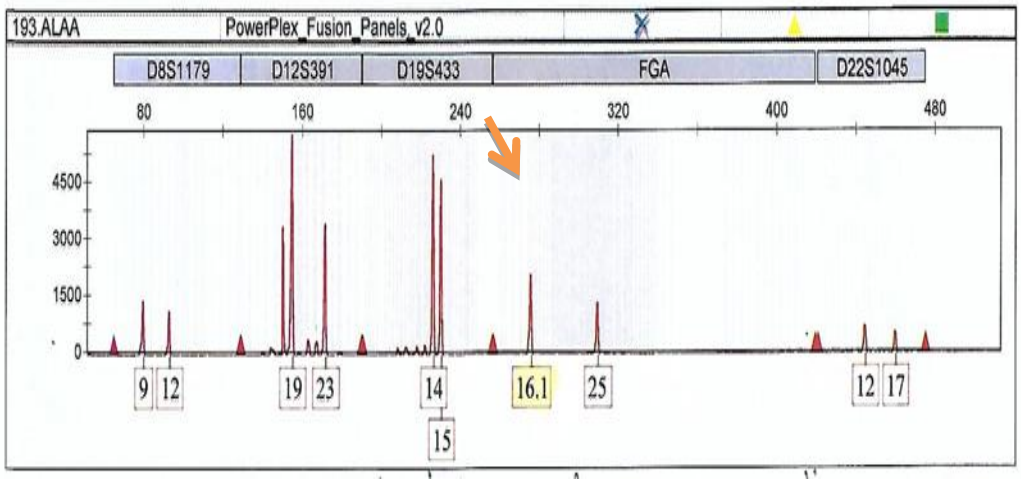

Figure (6 – B): Off-ladder allele (16.1) located at the FGA locus from sample number 193.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

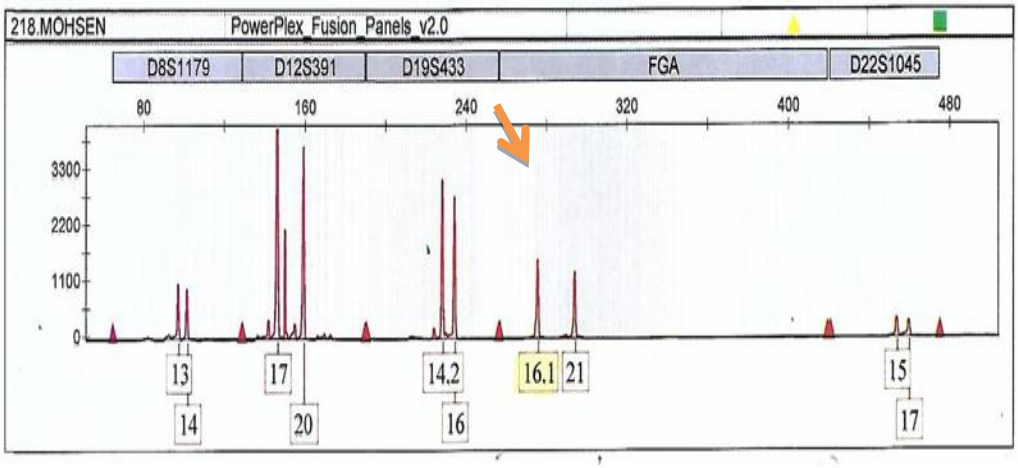

Figure (6 – C): Off-ladder allele (16.1) located at the FGA locus from sample number 218.

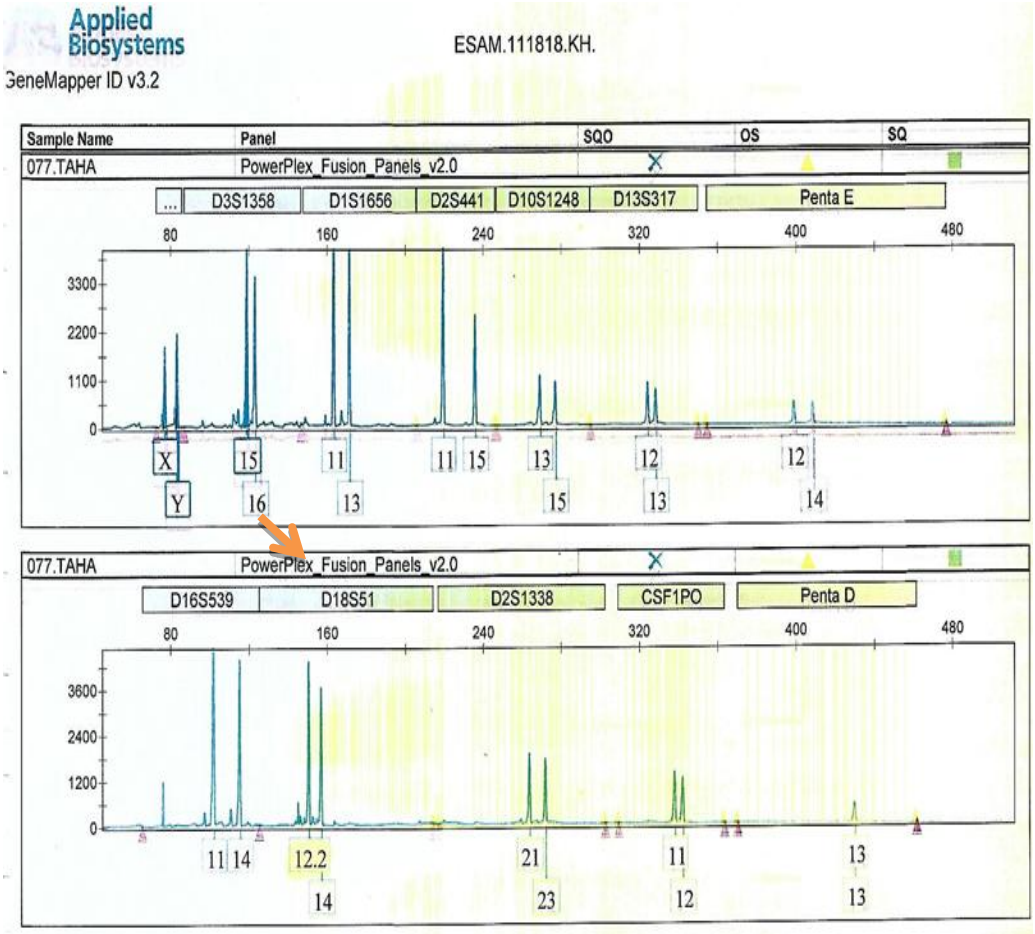

Figure (7 – A): Off-ladder allele (12.2) located at the D18S51 locus from sample number 77.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

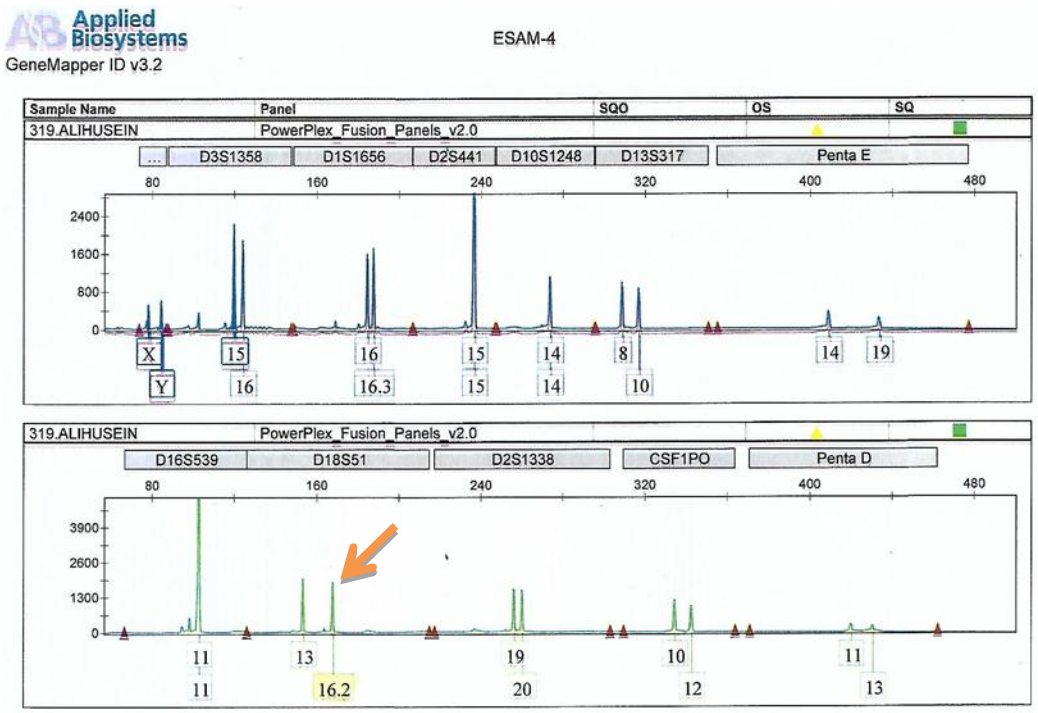

Figure (7 – B): Off-ladder allele (16.2) located at the D18S51 locus from sample number 319.

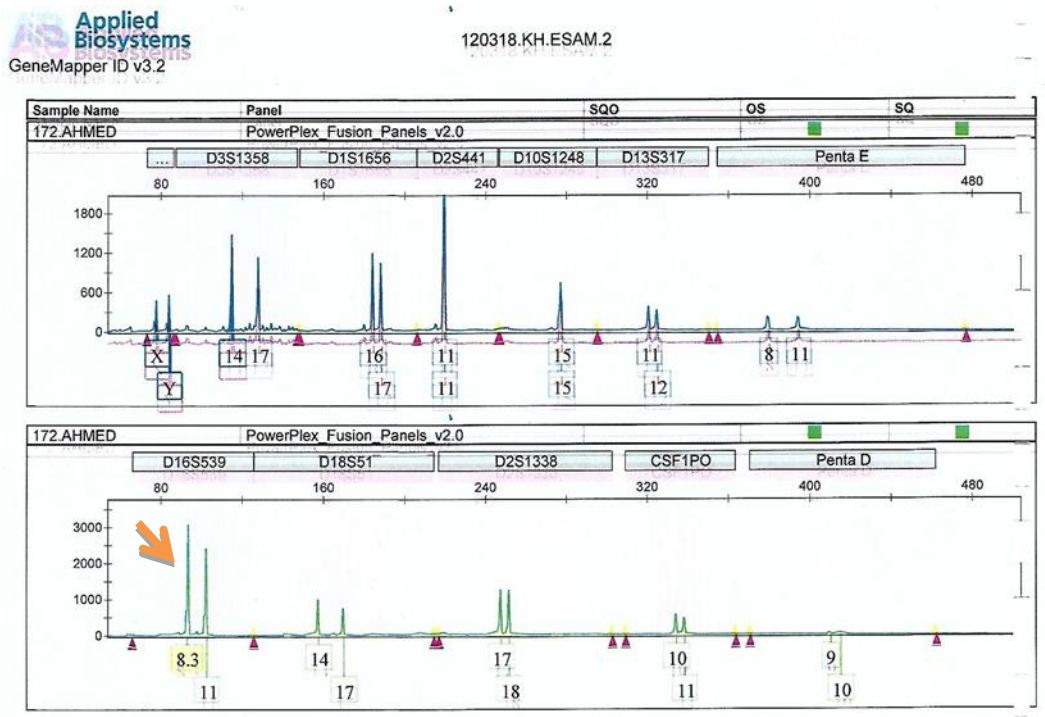

Figure 8: Off-ladder allele (8.3) located at the D16S539 locus from sample number 172.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

Figure 9: Off-ladder allele (9.4) located at the Penta D locus from sample number 65.

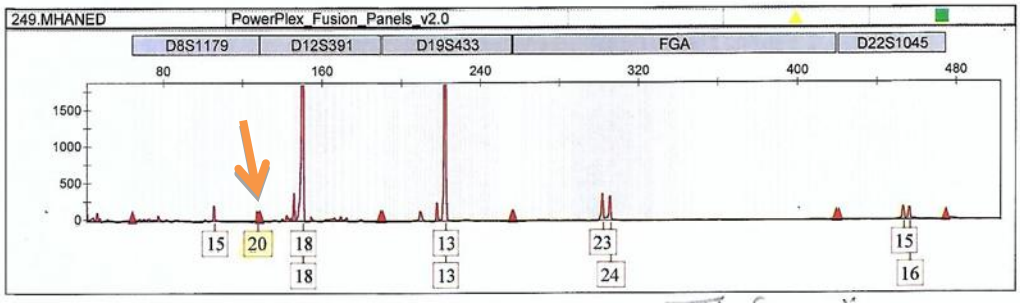

Figure 10: Off-ladder allele (20) located at the D8S1179 locus from sample number 249.

References

1. Brinkmann, B.; Klintschar; M.; Neuhuber, F.; Huhne, J. and Rolf, B. (1998). Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat, Am. J. Hum. Genet. 62(6): 1408 – 1415.

2. Bruder, C. E. (2008). "Phenotypically Concordant and Discordant Monozygotic Twins Display Different DNA Copy-Number- Variation Profiles". The American Journal of Human Genetics. 82(3): 763.

3. Butler, J. M. (2005). Forensic DNA Typing. Biology, Technology, and Genetics of STR Markers (2nd ed.), Elsevier Academic Press, New York.

4. Butler, J. M. (2015). Advance topics in forensic DNA typing: interpretation. Elsevier Academic Press. USA.

5. Crouse, C. A.; Rogers, S.; Amiott, E.; Gibson, S. and Masibay, A. (1999). Analysis and interpretation of short tandem repeat microvariants and three banded patterns

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1055 - 1069
Received 05 May 2021; Accepted 01 June 2021.

using multiple allele detection systems. Journal Forensic Sciences, 44(1): 87 – 94.

6. Promega Corporation (2017). PowerPlex® Fusion System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual. Instructions for Use of Product DC2402. 2800 Woods Hollow Road. Madison, WI 53711-5399 USA.

7. Puers, C.; Hammond, H. A.; Jin, L.; Caskey, C. T. and Schumm, J. W. (1993). Identification of repeat sequence heterogeneity at the polymorphic short tandem repeat locus HUMTH01 [AATG]n and reassignment of alleles in population analysis by using a locus-specific allelic ladder. American Journal Human Genetics, 53(4): 953 – 958.