Forensic Science International: Genetics 7 (2013) 494–498

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Case Report

Can brothers share the same STR profile?

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ARTICLE INFO

Article history:
Received 9 February 2013
Received in revised form 18 April 2013
Accepted 24 April 2013

Keywords:
Forensic science
Siblings
Allele sharing
Likelihood ratio
Short tandem repeats

ABSTRACT

This report demonstrates the limits of DNA identification when siblings are involved.

The Israeli DNA database routinely amplifies suspects samples using the PowerPlex® ESI16 system (Promega). While uploading a series of suspects into the database software, we found an unusual high number of shared alleles between two suspects 31 out of 32 alleles. Verification of their demographic data identified them as brothers. After confirmation of their paternity affiliation using the AmpFlSTR®YFiler™ (Applied Biosystems), we used two other multiplexes kits to improve the differentiation rate. The PowerPlex® ESX17 System (Promega) added one locus, SE33, who exhibits four different alleles. The second kit, the AmpFlSTR®MiniFiler™ (Applied Biosystems) added three more loci. Only one allele difference was found.

In order to increase the discrimination power between related and unrelated individuals, we recommend that the DNA laboratories consider using a larger multiplex typing kit in cases like the one informed here.

1. Introduction

The Israel Police DNA Database Laboratory has been founded in 2007, and by October 2011 analyzed and uploaded about 145,000 DNA profiles using a rapid, semi-automated analysis system for the AmpFLSTR SGM Plus™ kit (Applied Biosystems) [1]. This genotyping system contains 10 STRs (Short Tandem Repeats) loci and the sex-determining marker Amelogenin.

Following a partial match in which a pair of siblings differing in only one allele was found using this process, among other reasons, it was realized that 11 loci were not enough to provide the required differentiation ability. Therefore it was decided that the Israeli DNA database and casework laboratories should be upgraded to a kit that would provide better discrimination power for items of evidence and reference samples.

As a consequence of the formation of The European Union, the growth of national databases and data sharing between countries, the forensic community recommended generating kits that will both improve performance and reduce incidence of adventitious matches [2,3]. The forensic commercial manufacturers responded with new and improved kits. These systems, "The new generation kits", include five new loci raising the total number of analyzed loci to 16.

After testing four kits manufactured by three different companies which duplicate the same 16 loci, the PowerPlex® ESI16 system (Promega) won a tender and it was validated according to the work flow in the lab [4–6].

Since the transition, about 80,000 profiles were analyzed with the new kit, and updated statistical data was established for the Israeli population. As expected, the amplification of 16 loci increased the differentiation ability between individuals not only in the entire population, but also within families (Fig. 1).

This article describes a case where the advanced 16 loci kit was used and a surprising partial match was found between two individuals who shared 31 out of 32 alleles. Background check confirms that these two individuals were siblings. The details of this case are presented as well as non-conventional methods in our lab, taken in order to increase the differentiation between the brothers. The possible consequences of this case in the context of evidence in criminal trials are discussed here.

2. Materials and methods

2.1. DNA preparation, amplification and analysis

The collection of buccal cells samples is part of the routine procedure at local police stations. The samples are collected on FTA

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http://dx.doi.org/10.1016/j.fsigen.2013.04.011

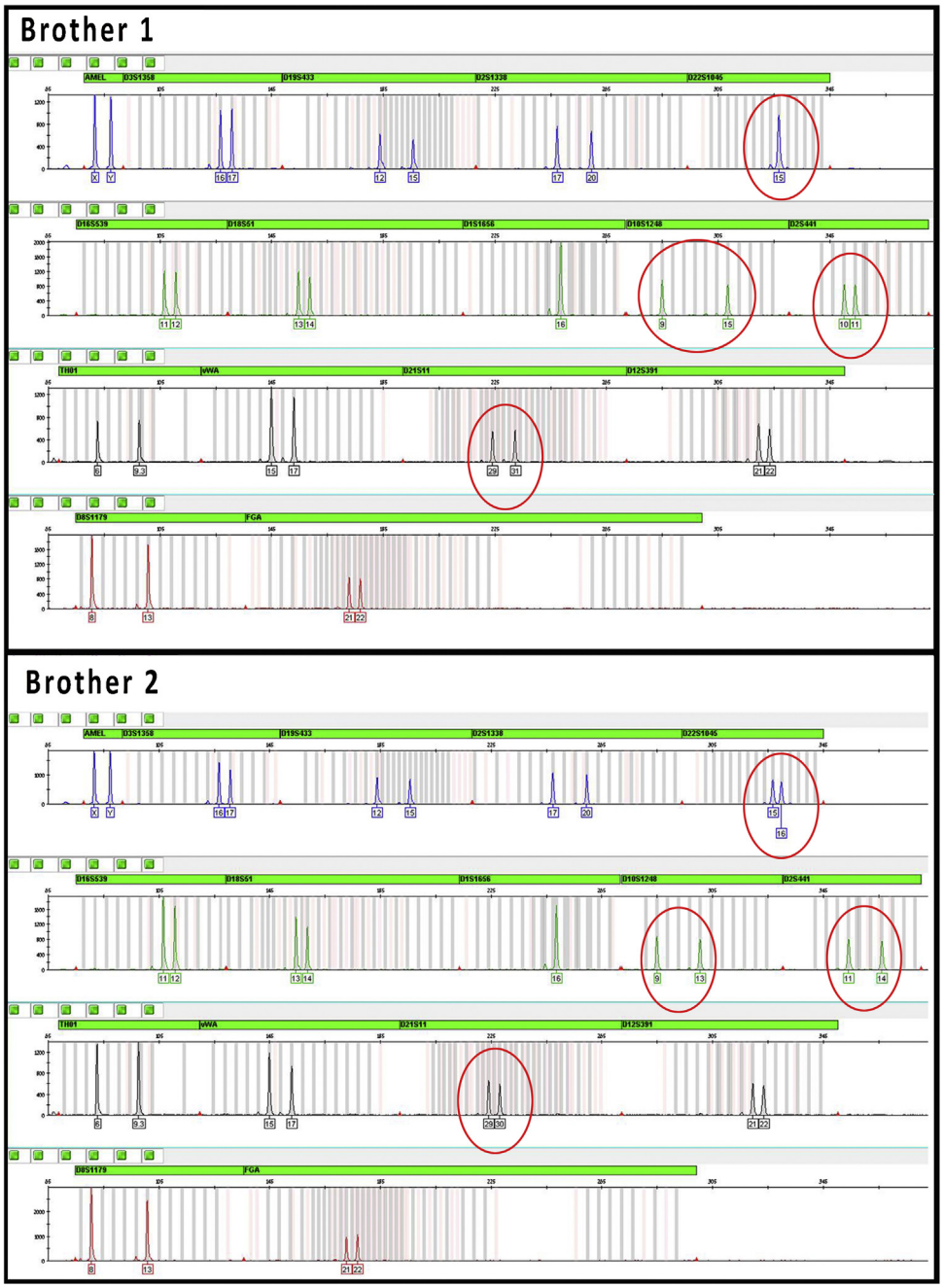

Fig. 1. PowerPlex® ESI 16 System (Promega) genetic profiles of two brothers who share 10 out of 11 loci using the AmpFLSTR SGM Plus™ kit (Applied Biosystems). The differences are shown in red circles.

cards and are processed, profiled and stored in Israel Police DNA Database Laboratory.

Four different kits were used to amplify these two samples. Pre-PCR preparations for three kits were carried out on a single 1.2 mm punch per kit (BSD600 Duet semi-automated puncher by BSD Robotics). An in-house developed method employing 7 min water HPLC rinses, with no necessity for quantification steps prior to PCR was used. Thermal cycling was performed in a GeneAmp PCR System 9700 (Applied Biosystems). The PCR processes were according to kits manufacturer's recommendations with minimal variations regarding the number of cycles and final volumes: AmpFlSTR®YFiler™ (28 cycles, final volume 10 μl) kit by Applied Biosystems, Powerplex® ESI and Powerplex® ESX17 systems (24 cycles, final volume 10 μl) kits by Promega [7].

The Pre-PCR preparation for the fourth kit, AmpFlSTR®MiniFiler™ by Applied Biosystems, carried out on two disks of 1.2 mm manually punched from the FTA cards with UNI-CORE™ (Harris) and CHELEX™ extracted. Quantitation process using Quantifiler®Duo was done in 7500 Real Time PCR System (Applied Biosystems). Amplification parameters were 30 cycles, final volume 25 μl.

The amplified products were separated and detected by capillary electrophoresis on the ABI Prism 3130xl and 3500xl Genetic Analyzer. Each kit products were run with the kit's respective internal size standard and allelic ladders according to manufacturer's recommendation. Fragment analysis was performed using GeneMapperID-X software (Applied Biosystems).

2.2. Fingerprints comparison on the FTA cards (Whatman)

Suspect's name, ID and two index fingerprints are taken on the FTA card during sampling procedure. The brothers' fingerprints on their FTA cards were compared with the fingerprints present in the AFIS database (Automated Fingerprints Identification System) using standard procedures.

2.3. Demographic data comparison

In order to dismiss the possibility of twin brothers with a mutation occurrence, demographic data was used. The Israeli demographic database was checked for date of birth, family relationship and ethnic information.

3. Results

More than 4000 profiles are analyzed every month using the PowerPlex® ESI16 System (Promega) in the Israel Police DNA Database Laboratory. During a routine procedure, two almost identical profiles were found (Fig. 2). The two profiles agreed on 31 out of 32 alleles, where the only difference was found at D18S51 locus. In order to confirm the finding, the two samples were re-amplified, and verified.

Given the similarity between the two profiles, a statistical test was performed to check the hypothesis that the samples belong to two different siblings. The Likelihood Ratio – the ratio between the probability of obtaining this match under the hypothesis that these samples belong to two siblings and the probability of obtaining this

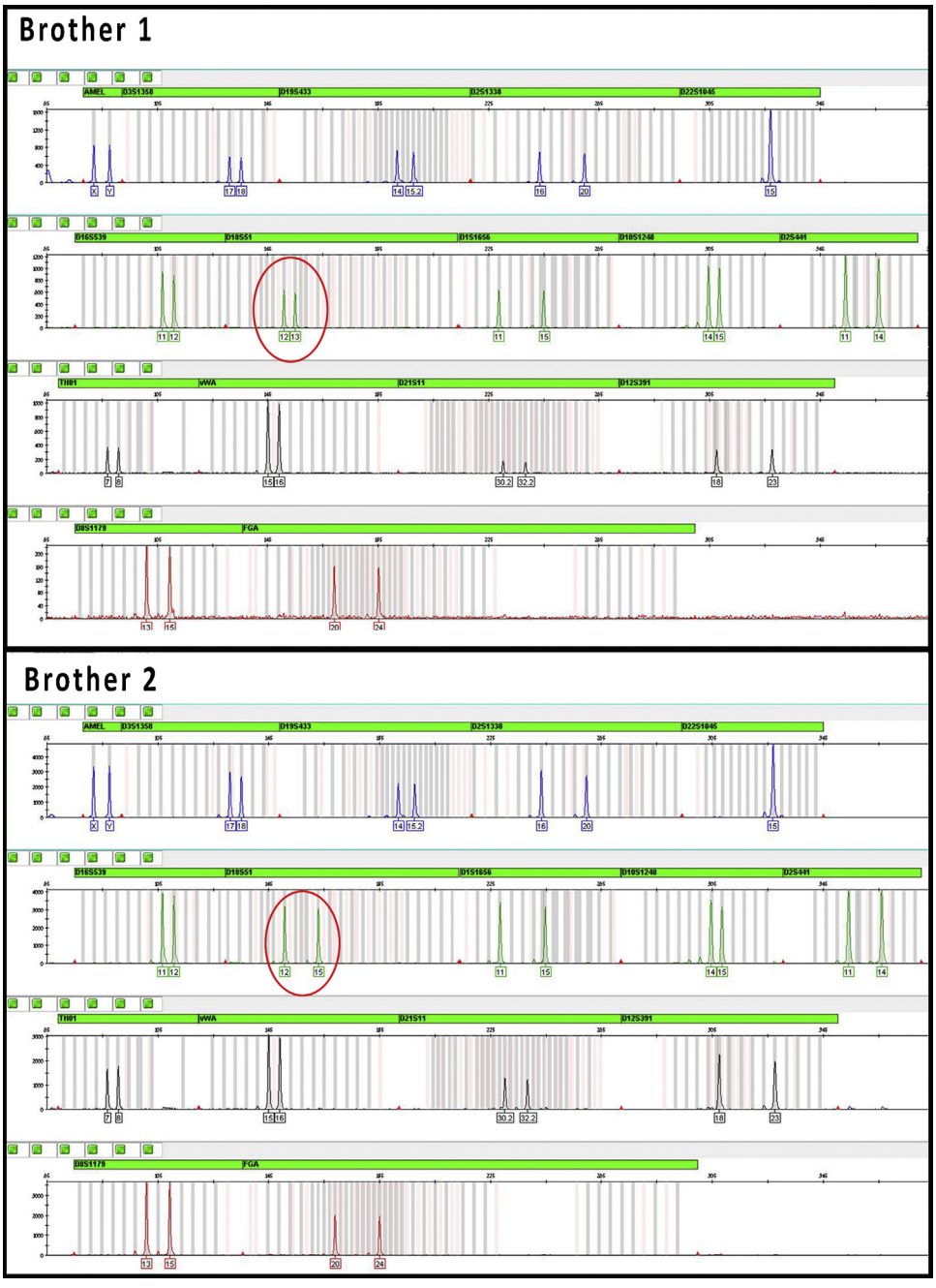

Fig. 2. Two profiles obtained using PowerPlex® ESI 16 System (Promega) showing 31 identical loci. The difference in the D18S51 locus is shown in a red circle.

Table 1
Chromosome Y profiles of the brothers characterized usingAmpFLSTR®Yfiler™ kit (Applied Biosystems).

	B_DYS 456	B_DYS 389I	B_DYS 390	B_DYS 389II	G_DYS 458	G_DYS 19	G_DYS 385	Y_DYS 393	Y_DYS 391	Y_DYS 439	Y_DYS 635	Y_DYS 392	R_Y_GATA	R_DYS 437	R_DYS 438	R_DYS 448
Brother 1	15	14	23	31	17	14	15,16	13	10	11	21	13	11	14	9	19
Brother 2	15	14	23	31	17	14	15,16	13	10	11	21	13	11	14	9	19

match under the hypothesis that these samples belong to two unrelated individuals – was calculated and determined to be 1.4×10^{11}.

During the sampling process, fingerprints of the two index fingers were imprinted on the sampling FTA cards and were positively identified by the AFIS experts in the fingerprints comparison lab. The fingerprints of the suspects were different and each suspect matched his previous taken fingerprints.

Demographic data verified that the samples belong to brothers, one of them was born in January 1973 and his brother in November 1974, therefore the possibility of identical twins with a mutation in one of them was rejected. The demographic data also indicated that the brothers belong to a small ethno-religious community, numbering 130,000 persons in Israel.

Further investigation was done by amplifying 16 loci located on the Y chromosome of the brothers in order to check their paternity affiliation. AmpFLSTR®Yfiler™ kit (Applied Biosystems) was used, and the profiles obtained (Table 1) demonstrated that the two suspects indeed shared the same paternity affiliation.

The surprising and unexpected similarity between the two profiles had led us to check whether it is possible to increase the discriminability between the two samples. The samples were amplified using other kits that are not routinely used in the DNA database lab. AmpFLSTR®Minifiler™ kit (Applied Biosystems) added three more loci to the 16 loci of PowerPlex® ESI16 System (Promega) D13S317, D7S820 and CSF1PO. One of those loci, D13S317 added one more distinguishing allele (Table 2).

The SE33 locus was analyzed using PowerPlex® ESX17 Systems (Promega). One of the alleles was termed OL (Off Ladder) by the GeneMapper ID-X software and manually designated as allele 33 (Table 2). The SE33 locus profiles showed four different alleles in the DNA profile of these two brothers.

Table 2
Alleles of the different loci and kits used. The differential alleles are in bold.

Kit	Site	Brother 1	Brother 2
PowerPlex® ESI System Promega	AMEL	X,Y	X,Y
	D3S1358	17,18	17,18
	D19S433	14,15.2	14,15.2
	D2S1338	16,20	16,20
	D22S1045	15,15	15,15
	D16S539	11,12	11,12
	D18S51	12,**13**	12,**15**
	D1S1656	11,15	11,15
	D10S1248	14,15	14,15
	D2S441	11,14	11,14
	THO1	7,8	7,8
	vWA	15,16	15,16
	D21S11	30.2,32.2	30.2,32.2
	D12S391	18,23	18,23
	D8S1179	13,15	13,15
	FGA	20,24	20,24
AmpFLSTR®Minifiler™ kit Applied Biosystems	D13S317	**8**,11	11,**12**
	D7S820	8,8	8,8
	CSF1PO	10,11	10,11
PowerPlex® ESX17 System Applied Biosystems	SE33	**16**,26.**2**	**21**,**33**

4. Discussion

The discriminating power between random individuals in the population has significantly increased after employing the PowerPlex® ESI16 Systems (Promega). Using this new generation system was supposed to provide a unique identification of an individual in different scenarios, including those where family members are involved. Consequently, finding two brothers sharing 31 of 32 alleles' profile, demands further consideration.

These brothers belong to a small ethno-religious community, genetically isolated, where customs strongly favor marriage within the same village, geographical area or family. Furthermore, this religion is strictly closed to new adherents, thus precluding admixture with other populations [8–10]. The genetic differentiation in such populations decreases thus increasing the probability of genetic resemblance. Despite the likelihood that these population's characteristics may play a role in this case, the lack of genealogy data and DNA samples from the parents makes it impossible to determine that indeed these are the reasons for the brother's allele sharing.

In the reported case, both profiles were complete, and only one allele indicated them as two different samples. The clear discrimination of true versus false relationship is important for complex kinship cases, such as familial searches, paternity testing, missing persons work, and immigration testing [11]. The use of other DNA amplification kits was crucial in order to increase the differentiation rate of the brothers.

The SE33 locus (PowerPlex® ESX17 System Promega), was the only locus that indicated four different alleles – maximum variation possible among full siblings. It is known as a highly polymorphic locus (58 observed alleles), high heterozygosity index (93.7%) and high mutation rate in the population (0.64%). SE33 characteristics provide advantage for analyzing mixture profiles (many alleles seen).[12–14].

Differential allele was also found at the D13S317 locus in the AmpFLSTR®Minifiler™ (Applied Biosystems) and thus increased differentiation between the two brothers.

Forensic laboratories analyze loci to identify a perpetrator of a crime by directly matching an evidence profile and a suspect profile, or by searching an evidence profile against an offender database [11]. The brothers' profiles were uploaded and searched against the database and no match was found. In the future, if an item of evidence match occurs with one of these brothers, assuming the evidence's profile will be analyzed with PowerPlex® ESI16 system (Promega) routinely used, it is recommended that the evidence's profile would be first amplified with PowerPlex® ESX17 System (Promega) so SE33 locus can be designated, and then with AmpFLSTR®Minifiler™ (Applied Biosystems) as well. However, additional analysis may not be possible with crime samples since the entire sample might be consumed during analysis.

Casework samples might contain degraded DNA or PCR inhibitors. In those samples, the larger STR loci in a multiplex reaction will be the first to fail [15]. D18S51, the only differential locus between the brothers, is one of the smaller loci in the PowerPlex® ESI16 Systems (Promega), therefore we expect to obtain designation even in a partial STR profile. Still, given the possibility that due to technical reasons some loci will not provide data, the evidence might match the two brothers. Search results

will greatly diminish the importance of these matches and they could only be used as an investigation support like in cases of identical twin brothers.

Mixture interpretation where relatives share similar alleles can make individual identification more difficult [16]. For example, the DNA mixture of both brothers in the reported case can mistakenly be interpreted as a single source profile with triallelic pattern at D18S51. Also, mixtures involving one of the brothers with another random individual might match the other brother as well.

Considering the PowerPlex® ESI16 system (Promega), and applying the Israeli allele frequencies database, one can calculate the expected probability of a complete match and of partial matches between the profiles of two full siblings. It turns out that a complete match (i.e., identity in all 15 STR genotypes) between two full siblings of the same sex is expected to occur in one out of 5.82 million pairs (the maximal probability is 1 in 835 thousand pairs). A partial match, in 14 out of 15 STR genotypes, is expected to occur in one out of 137 thousand same-sex pairs (the maximum is 1 in 22 thousand pairs). A partial match of 29 out of 30 alleles at the 15 STR loci (as the one observed in our particular case) is expected to occur in one out of 283 thousand pairs of full, same-sex siblings (the maximum is 1 in 48 thousand pairs). All these are calculated under the assumption of a randomly mated population. The markedly uncommon occurrence of the match described here, can probably be attributed to the genetic isolation and the strong endogamy of this particular community.

Increasing the number of STR loci resulted in increased discrimination of true and unrelated pairs [11]. Consequently, we recommend, in cases like the one described here, consider using larger multiplex kits, with preference to kits containing the highly polymorphic locus SE33.

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