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ARTICLE

# Paternity testing and forensic DNA typing by multiplex STR analysis using ABI PRISM 310 Genetic Analyzer

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**Abstract** Short tandem repeats (STRs) are widespread throughout the human genome and are a rich source of highly polymorphic markers which can be detected by PCR. To gain a better appreciation for how the polymorphism at a particular locus impacts the individual identity, the present study was undertaken to explore the use of 15 STR loci in forensic investigation and paternity testing. Multiplex STR typing was used to study the 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) in addition to a gender identification marker, amelogenin, by capillary electrophoresis on 310 Genetic Analyzer. Samples from 85 trio and duo cases of disputed paternity were investigated. The data were analyzed to give information on paternity index, probability of paternity, frequency of number of exclusions and rate of mismatch at each STR locus. The method was also successfully applied to forensic personal identification in theft and murder cases. The results demonstrated that the STR typing is a reliable and robust tool for analyzing the forensic practice as well as for paternity testing. The advantages of using multiplex STR analysis over other conventional methods are discussed.



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## 1. Introduction

Modern DNA typing technology has opened new possibilities to perform human identity testing. Individual identification is imperative in a number of situations including the determination of perpetrators of violent crimes such as murder and sexual assault, and identification of remains of missing persons or victims of mass disasters [21,12,25]. Forensic DNA typing has an important impact on the society by providing reliable evidence both for convicting the offenders and for exonerating the innocent suspects. Another field of application of DNA

typing is paternity testing and testing for relatedness on the maternal or the paternal lineage using autosomal, mitochondrial or Y-chromosomal DNA markers [35,36,28]. In many ways, DNA typing methods have increased the ability to solve problems of family relationships and forensic cases that might have otherwise been left unsolved.

DNA fingerprinting or DNA typing (profiling) was first described by Jeffreys et al. [23]. They found that certain regions of DNA contained repeated DNA sequences. These repeated DNA sequences come in all types of sizes and collectively referred to as variable number of tandem repeats (VNTRs). They are typically designated by the length of the core repeat unit and the number of contiguous repeat units [18].

DNA regions with repeat units that are 2–6 bp in length are called microsatellites or short tandem repeats (STRs). The number of repeats in STR markers is highly variable among individuals, making these markers effective for human identification purposes. They are scattered throughout the genome [17,13]. The small size of STR alleles compared to minisatellites used by Jeffreys et al. [23] makes STR markers better candidates for use in forensic applications [9].

Polymorphic STR loci can be copied simultaneously by multiplex PCR, simply by adding more than one primer set to the reaction mixture [16]. One of the first STR multiplexes developed was a quadruplex created by the Forensic Science Service (FSS) that comprised four STR loci [24]. The FSS followed with a second-generation multiplex (SGM) made up of six polymorphic STRs and a gender identification marker [33]. As the technology has evolved quickly, the number of STRs that can be simultaneously amplified has increased from three or four with silver stained systems to over 15 STRs using multiplex-color fluorescent tags. The present study was conducted in order to assess the utility and feasibility of 15 STR markers for identification of individual identity in disputed paternity and forensic cases by way of capillary electrophoresis on 310 Genetic Analyzer. The advantages of using multiplex STR analysis over other conventional methods are discussed.

## 2. Materials and methods

### 2.1. Materials

Peripheral blood samples (drawn in EDTA) were taken from 85 paternity cases, 48 trios and 37 duos. Each trio consisted of mother, one child and one alleged father, whereas each duo consisted of one child and one alleged father (motherless case). Two criminal cases were also investigated. In one case, theft case, a blood stain was taken from a crime scene in a big company and was matched with two peripheral blood samples obtained from two suspects who were thought to be the thieves. In the other case, murder case, a piece of femur bone was taken from skeletonized remains of a missing man and a peripheral blood sample was taken from a man who was thought to be the son of the victim. Spots of blood stains found on a mattress were lifted from the crime scene, and a peripheral blood sample of a man suspected to be the perpetrator of murder was also used for forensic identity test. Samples for construction of population frequency database were obtained from 170 unrelated Egyptian individuals submitted to the forensic science laboratory.

### 2.2. DNA extraction

The peripheral blood samples and blood stains were treated with SDS and proteinase K. The hard bone tissue was fragmented and pulverized in liquid nitrogen using tissue lizer, then treated with SDS, proteinase K and DTT (Dithiothreitol) for 2 days. DNA from lysate was extracted by automated purification procedure using BioRobot® EZ1 and EZ1 DNA investigator kit (QIAGEN, California, USA) according to the instructions provided by the manufacturer [1]. This silica-based DNA purification method was used to obtain high quality DNA devoid of PCR inhibitors. This purification method depends on binding of DNA to silica-coated magnetic beads in the presence of chaotropic salts such as guanidine hydrochloride, sodium iodide and sodium perchlorate. The beads were separated from the lysate using a magnet. The DNA was then washed and eluted in TE buffer (10 mM Tris–HCl, 1 mM EDTA pH 8). DNA quantity and quality were assessed by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, Delaware, USA), and stored at −20 °C until analysis.

### 2.3. PCR amplification

Multiplexed PCR amplification of 15 polymorphic STR loci was performed using the AmpFlSTR® identifiler™ amplification kit (Applied Biosystems, Foster City, CA, USA). The amelogenin marker was also included in this kit to enable gender identification. It amplifies specific products at the X and Y chromosomes which are 107 bp and 113 bp long, respectively. Primer sequences are commercial information and are not available for publication. The characteristics of the 15 STR loci are shown in Fig. 1 and Table 1.

For each locus, one fluorescently labeled primer pair was used [either 6-FAM (blue), VIC (green), NED (yellow) or PET (red)]. PCR was carried out in a 25 μL volume using 1–5 ng template DNA, 2.5 μL of 10× reaction buffer, 5.5 μL of primer set and 4 units of AmpliTaq Gold DNA polymerase following the procedure recommended by the manufacturer [6]. A negative control with no DNA template was used. PCR was performed using GeneAmp-9700 thermal cycler (Applied Biosystems) with the following amplification conditions: an initial denaturation at 95 °C for 11 min; 28 cycles of 94 °C for 1 min, 59 °C for 1 min and 72 °C for 1 min, and a final extension of 60 °C for 60 min to enable full A-addition to the PCR products.

### 2.4. Electrophoresis and analysis

The amplified fragments were analyzed with the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). One microliter of each PCR reaction product was added to 24 μL of deionized formamide (Sigma, St Louis, MO, USA) and 0.5 μL of GeneScan 500 LIZ size standard (Applied Biosystems). PCR products were denatured at 95 °C for 3 min and chilled for 3 min. Electrophoresis was performed using the Performance Optimized Polymer 4 (Applied Biosystems) with a 47 cm/50 μm capillary at 15 kV for 30 min at 60 °C.

Data were analyzed using GeneScan and GenoTyper Analysis software (Applied Biosystems) to assess the quality of PCR amplification and to assign the specific alleles to each fragment analyzed.

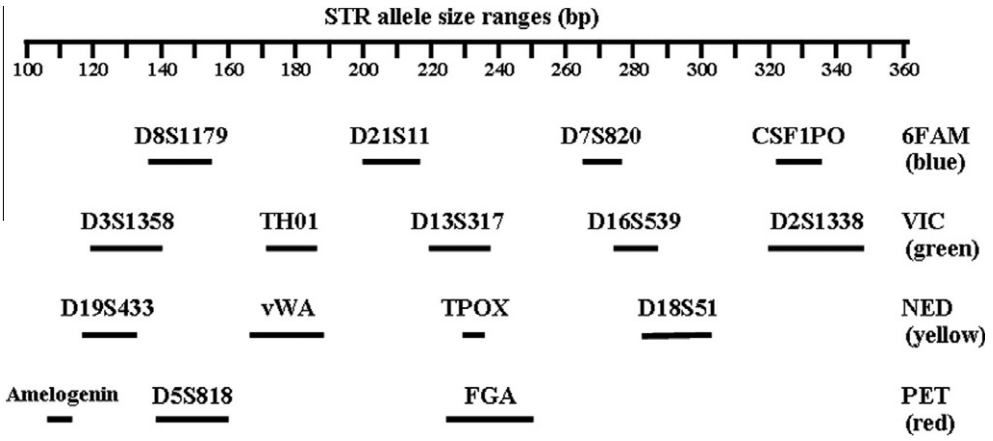


**Figure 1** A schematic drawing of the allele size ranges of 15 STR loci and the gender identification marker, amelogenin. For each locus, one fluorescently labeled primer pair is used [either 6-FAM (blue), VIC (green), NED (yellow) or PET (red)]. Each STR allele is defined and distinguished from the other in the amplification reaction based on its size and color.

### *2.5. Statistical analysis*

Population allele frequency for the STR loci was calculated according to Botstein et al. [7]. Paternity index which measures the weight of the scientific evidence obtained from the paternity test was calculated using the method described by Brenner and Morris [8]. Paternity index (PI) was calculated for each STR locus, then the combined paternity index (CPI) was estimated by multiplying the individual paternity index with the others. For simply, the paternity index was used to give the meaning of combined paternity index. Probability of paternity (POP), a conditional probability of whether an alleged father is the biological father of a child, was calculated using the following equation: $CPI \times 100/CPI + (1 - 0.5)$, where CPI is the combined paternity index and 0.5 is the prior probability [8]. In addition, the frequency of paternity of indices, rate of mismatch (exclusion) at each STR locus and the frequency of number of exclusions were determined according to Thomson et al. [35].

**Table 1** The characteristics of the 15 STR loci examined in this study.

| Locus | Chromosome location | Repeat motif | Primer label |
|---|---|---|---|
| D8S1179 | 8q | TCTA | 6-FAM |
| D21S11 | 21q11-21 | TCTA | 6-FAM |
| D7S820 | 7q11.21-22 | GATA | 6-FAM |
| CSF1PO | 5q33.3-34 | AGAT | 6-FAM |
| D3S1358 | 3p | TCTA | VIC |
| TH01 | 11p15.5 | AATG | VIC |
| D13S317 | 13q22-31 | TATC | VIC |
| D16S539 | 16q24-qter | GATA | VIC |
| D2S1338 | 2q35-37.1 | TGCC | VIC |
| D19S433 | 19q12-13.1 | AAGG | NED |
| vWA | 12p12-pter | TCTA | NED |
| TPOX | 2p23-pter | AATG | NED |
| D18S51 | 18q21.3 | AGAA | NED |
| D5S818 | 5q23.3-32 | AGAT | PET |
| FGA | 4q28 | TTTC | PET |

## 3. Results

### *3.1. Population database*

Database of allele frequencies for the 15 STR loci was constructed from 170 unrelated Egyptian individuals. Figs. 2 and 3 showed the estimated allele frequencies at the 15 STR loci. The loci D21S11, D2S1338 and FGA were highly polymorphic with their alleles 29, 17 and 24 most frequent (at 0.282, 0.247 and 0.200, respectively). The allele 8 of TPOX locus was the most frequent (at 0.530) among all alleles of the 15 STR loci in the examined population. The D3S1358 locus had the lowest number of alleles (5) with the allele 15 most frequent (at 0.306).

### *3.2. Paternity cases*

The paternity testing is based on matches of the alleles at the 15 STR loci between the child and the mother and the alleged father (trio cases). For motherless cases or duo cases, matches of the alleles of the STR loci were performed between the child and the alleged father. If the DNA profiles of the child and the alleged father were matched, the test was considered a non-excluded paternity case. But, if the DNA profiles of the child and the alleged father were not matched, the result was considered an excluded paternity case. The present study was applied to 85 paternity cases (48 trios and 37 duos). Of the 85 cases analyzed, 65 alleged fathers were not excluded from paternity. The remaining 20 alleged fathers (13 from trios and 7 from duos) were excluded from paternity.

Figs. 4–6 showed a non-excluded paternity case in which a mother required paternity testing. In this case, no genetic discrepancy for the alleged father–child relationship was observed in any of the 15 STR loci. All alleles of the child at all STR loci monitored were detected in both the mother and the alleged father. The paternity index and the probability of paternity were calculated to be 184,184,249 and 0.999999997, respectively. The results are summarized in Table 2.

The paternity index (PI) values for the 35 non-excluded trio cases ranged from 150,413 to 3,772,229,625, whereas those of the 30 non-excluded duo cases ranged from 1594 to 2,651,740

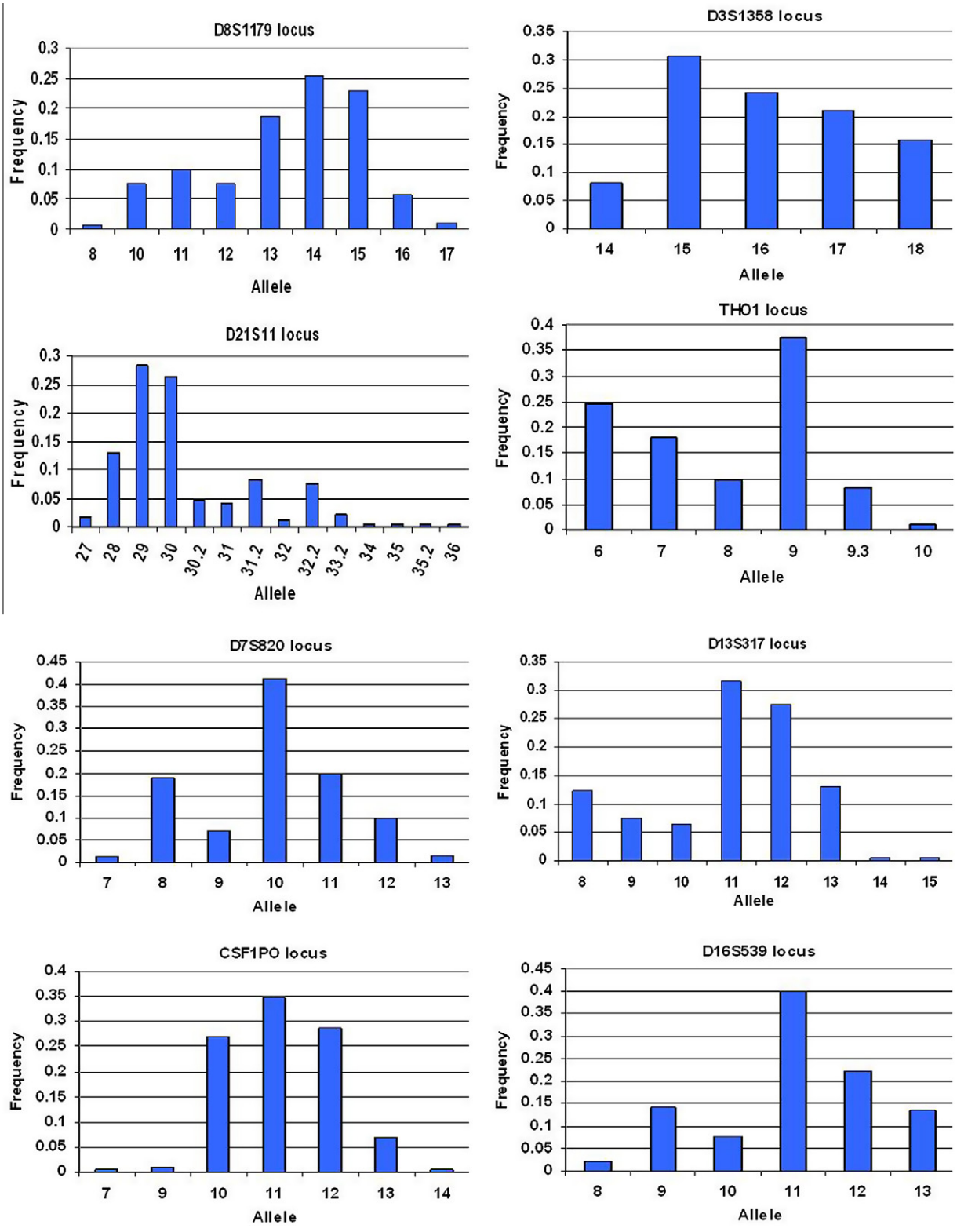


**Figure 2** Allele frequencies at the loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317 and D16S539. About 170 Egyptian individuals were examined by the present method.

(Table 3). About 83% of the trio cases showed paternity index values more than 1 million, 46% of the trio cases showed very high paternity index, PI > 10 million (Fig. 7), 0.06% of the duo cases showed paternity index values more than 1 million, and 73% of the duo cases showed paternity index values less than 100,000 (Fig. 7).

In the 20 excluded cases, paternity was excluded in 5–13 of the present 15 STR loci. Seventy five percent of the alleged

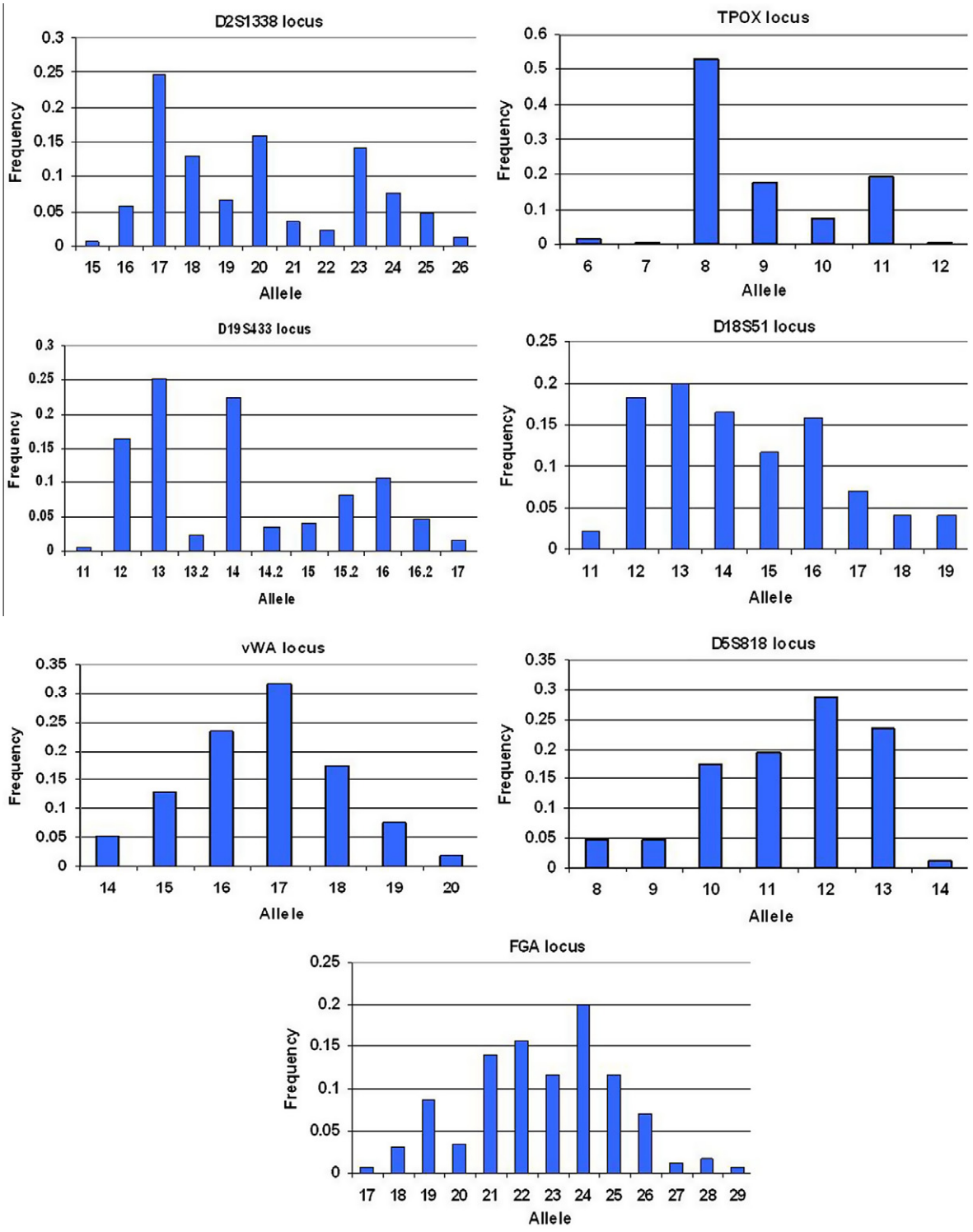


**Figure 3** Allele frequencies at the loci D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA estimated from 170 Egyptian individuals.

fathers were excluded at 8–10 loci, whereas 10% of the alleged fathers were excluded at 11–13 loci (Fig. 8). STR results were also examined to determine the rate of mismatch (exclusion) at each locus against each non-father (Fig. 9). The D2S1338 locus showed the highest rate of mismatch (0.80), whereas the CSF1PO locus showed the lowest rate of mismatch (0.35).

### *3.3. Criminal cases*

#### *3.3.1. Theft case*

In the criminal cases, to determine the perpetrators of the crimes, there should be complete matches for each STR marker. Figs. 10–12 showed a forensic theft case. No genetic

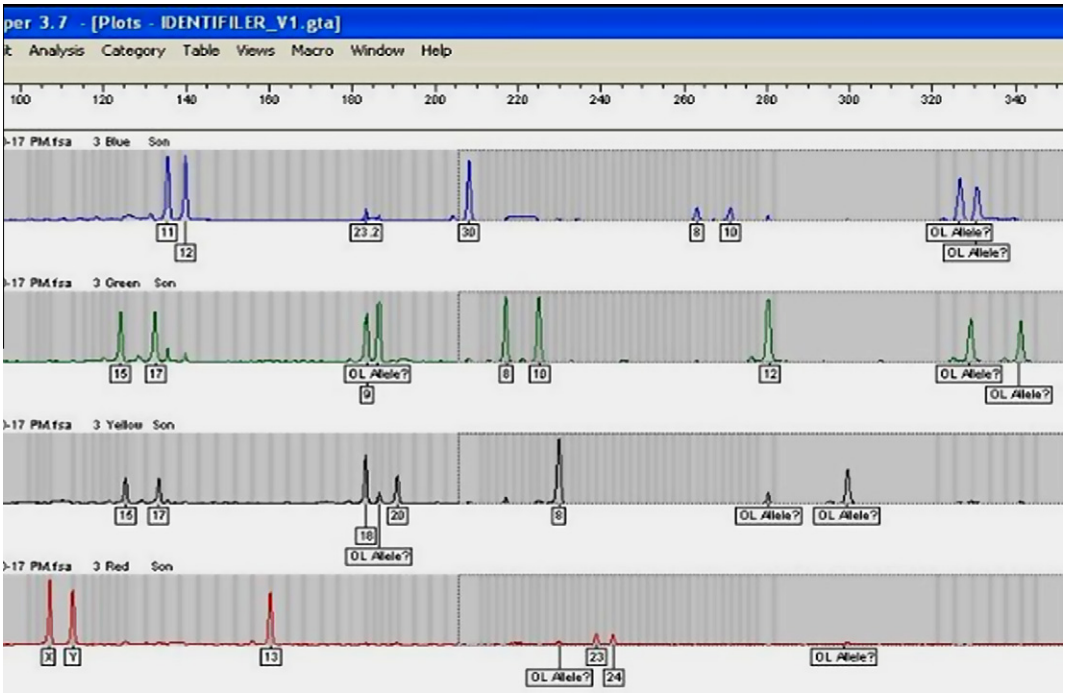


**Figure 4** The electrophoretic analysis of 15 STR loci and the amelogenin gene of the child.

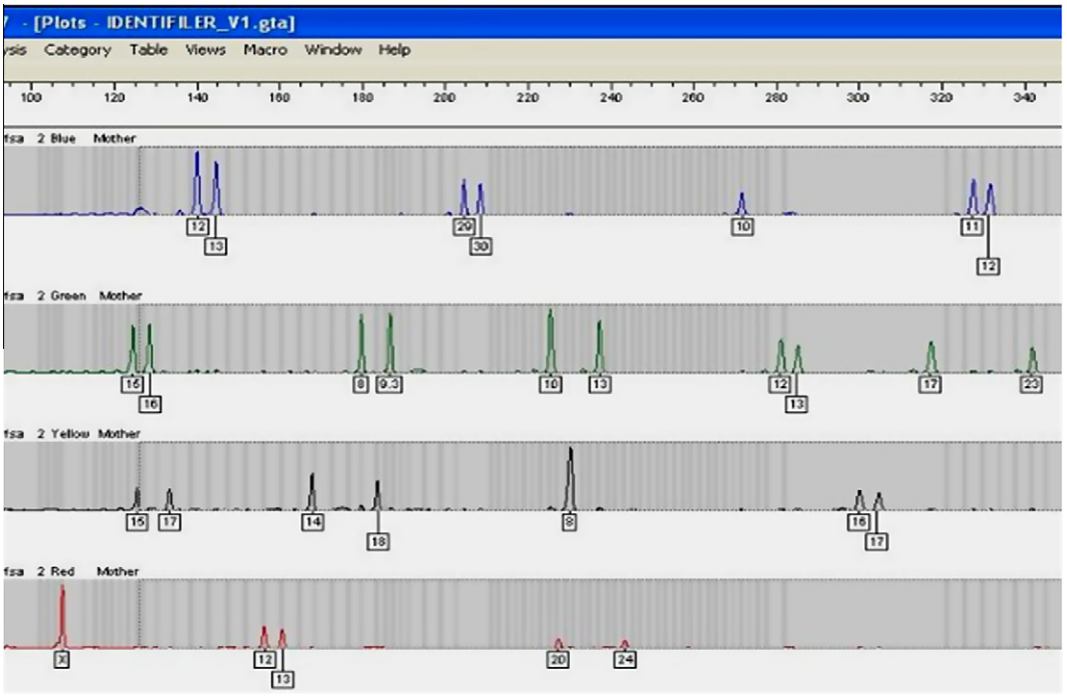


**Figure 5** STR typing of the mother. No genetic discrepancy for the mother–child relationship was observed in any of the 15 STR loci.

discrepancy between the crime scene sample and the suspect 2 sample was observed in any of the 15 STR loci. Identical patterns in the two DNA samples due to similar allele sizes at the 15 STR markers were obtained. On the contrary, genetic variation was detected between the DNA sample of the crime scene and that of the suspect 1 at all STR loci examined except for the locus TPOX. The latter marker produced one peak corresponded to the allele 8 in the three samples. The results are summarized in Table 4.

### *3.3.2. Long unsolved murder case*

Human skeletal remains found buried in courtyard house for 1 year in a suspected murder case were discovered and identified using DNA from a relative. The skeletal remains were

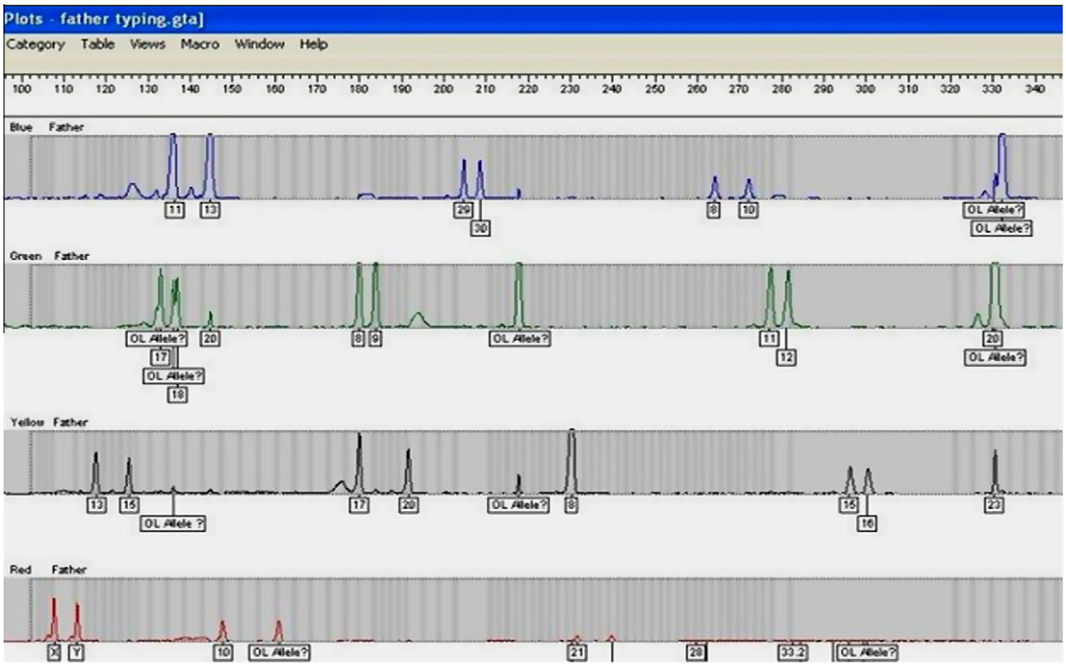


**Figure 6** STR typing of the alleged father. In this non-excluding paternity case, no genetic discrepancy for the alleged father–child relationship was detected in any of the 15 STR loci.

**Table 2** The alleles scored at 15 STR loci in the child and the mother and the alleged father. At each locus, the child received one STR allele from his mother and the other from his father (alleged father).

| Locus | Allele designations | | |
|---|---|---|---|
| | Child | Mother | Alleged father |
| D8S1179 | 11,12 | 12,13 | 11,13 |
| D21S11 | 30,30 | 29,30 | 29,30 |
| D7S820 | 8,10 | 10,10 | 8,10 |
| CSF1PO | 11,12 | 11,12 | 12,12 |
| D3S1358 | 15,17 | 15,16 | 17,18 |
| TH01 | 9,9.3 | 8,9.3 | 8,9 |
| D13S317 | 8,10 | 10,13 | 8,8 |
| D16S539 | 12,12 | 12,13 | 11,12 |
| D2S1338 | 20,23 | 17,23 | 20,20 |
| D19S433 | 15,17 | 15,17 | 13,15 |
| vWA | 18,20 | 14,18 | 17,20 |
| TPOX | 8,8 | 8,8 | 8,8 |
| D18S51 | 16,16 | 16,17 | 15,16 |
| D5S818 | 13,13 | 12,13 | 10,13 |
| FGA | 23,24 | 20,24 | 21,23 |
| Amelogenin | X,Y | X,X | X,Y |

almost completely devoid of remaining flesh. A review of police investigations pointed to the case of a 52-year-old man who had been missing for 1 year. DNA analysis was carried out from a piece of the femur bone and the blood of a man who was thought to be the son of the missing person. The STR typing strongly suggested that the skeletal remains were those of the missing man. The paternity index and probability of paternity calculated between the skeletonized man and the son were 4264 and 0.9998827, respectively. STR profiling tests were carried out for many tiny blood stains found on a mattress lifted from the crime scene inside the house and compared with the suspect's blood. Some blood stains were found to match the suspect's blood, whereas the others were found to match the skeletonized remains of the victim, the missing man. The results are summarized in Table 4.

## 4. Discussion

Technologies used for performing forensic DNA analysis differ in their ability to differentiate two individuals and in the sample processing speed. Forensic DNA analysis has used a variety of techniques including single-locus and multi-locus probe RFLP methods [23,5] and more recently PCR-based assays [21,10]. Multi-locus RFLP probes are highly variable between individuals but require large amounts of sample, a great deal of labor, time and expertise to produce a DNA profile. Moreover, analysis of RFLP cannot be easily automated. A rapid analysis has been achieved with short tandem repeat (STR) DNA markers. Most STR loci have been detected inadvertently in flanking regions or non-coding introns of expressed genes sequenced in the context of human genome projects [34]. Short tandem repeat markers are polymorphic as the number of copies of the repeat sequence present at a given STR locus varies between individuals. Characteristics of polymorphism and abundance have earned these repeated units widespread usage as genetic markers in population studies [3,2,4,32]. Short tandem repeat analysis can handle forensic samples that are of low quantity and of poor quality. In addition, Short tandem repeats can be multiplexed [16] and their detection can be easily automated [26,22].

Among the various types of STR systems, tetranucleotide repeats have become more popular than di-, tri-, penta- and hexanucleotide repeats. Tetranucleotide repeats reduce formation of

**Table 3** The paternity index (PI) and the probability of paternity (POP) in some non-excluded paternity cases, standard trios (mother, alleged father and child) and duos (motherless cases).

| Case no | Case type | PI | POP | Case no | Case type | PI | POP |
|---|---|---|---|---|---|---|---|
| 1 | Trio | 1,971,800,547 | 0.9999999995 | 11 | Trio | 1,972,818 | 0.999999746 |
| 2 | Trio | 194,665 | 0.999997431 | 12 | Duo | 16,114 | 0.999968972 |
| 3 | Trio | 150,413 | 0.999996675 | 13 | Duo | 7665 | 0.999934772 |
| 4 | Trio | 2,650,753 | 0.999999811 | 14 | Duo | 2,651,740 | 0.999999811 |
| 5 | Trio | 9,218,742 | 0.999999945 | 15 | Duo | 53,218 | 0.999990604 |
| 6 | Trio | 1,352,374,672 | 0.9999999993 | 16 | Duo | 1594 | 0.999686422 |
| 7 | Trio | 3,772,229,625 | 0.9999999997 | 17 | Duo | 106,320 | 0.999995297 |
| 8 | Trio | 184,184,249 | 0.999999997 | 18 | Duo | 4324 | 0.999884379 |
| 9 | Trio | 5,647,718 | 0.999999911 | 19 | Duo | 89,299 | 0.9999944 |
| 10 | Trio | 46,803,912 | 0.999999989 | 20 | Duo | 275,773 | 0.999998186 |

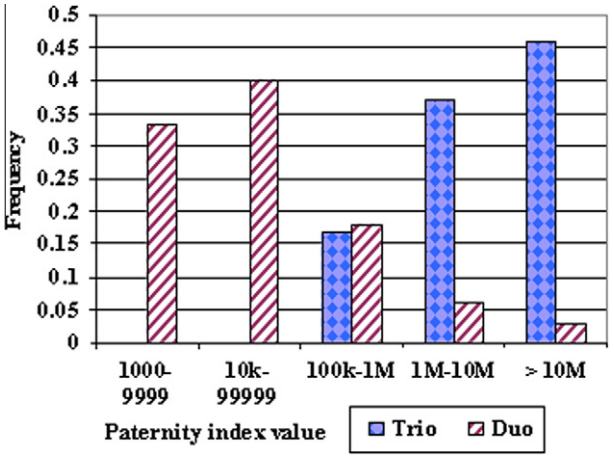


**Figure 7** The frequency of paternity index values deduced from 35 trio cases and 30 duo cases.

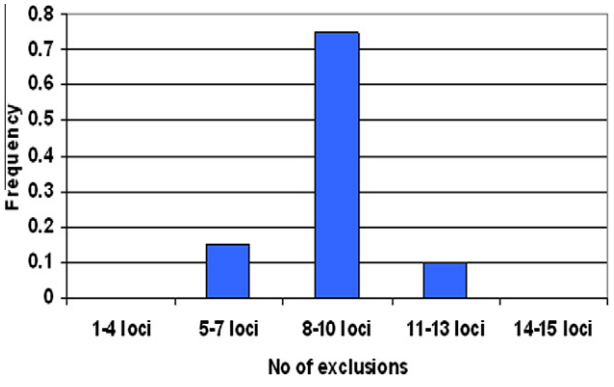


**Figure 8** The frequency of number of exclusions deduced from 20 excluded paternity cases.

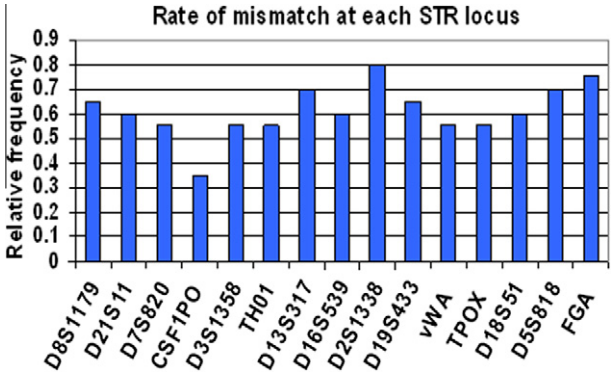


**Figure 9** The rate of mismatch for all STR loci deduced from 20 excluded paternity cases.

stutter products, amplicons that are typically one or more repeat units less in size than the true allele, which arise during PCR amplification because of strand slippage (slipped strand mispairing) [37,14,19]. In the present study, 15 tetranucleotide markers or STR loci in addition to a gender identification marker were examined for human identification purposes in the paternity testing and in the crime case investigation. The more DNA markers examined and compared, the greater the chance that two unrelated individuals will have different genotypes. Alternatively, each piece of matching information adds to the confidence in connecting two matching DNA profiles from the same individual.

DNA variation is exhibited in the form of different alleles or various possibilities at a particular locus. Two forms of variations are possible at the DNA level: sequence polymorphisms and length polymorphisms [30]. Each STR allele is defined and distinguished from the other in the amplification reaction based on its length and color. The color results from the fluorescent dyes that are attached during the amplification reaction. A sample containing two alleles (two peaks in the electropherogram), one with 13 and the other with 15 repeat units would be said to have a genotype of 13,15 at a particular locus. The gender identification marker resulted in two peaks for a male sample (XY) and a single peak for a female sample (XX).

DNA profiling in cases of disputed parentage has come behind its use in forensic casework. In contrast to forensic practice, in which a match at both alleles is required, only one allele at each locus is informative in cases of disputed parentage. Individuals have thousands of genes, half from each parent. The randomness of this combination makes each person's genetic make up unique, except for monozygotic twins. The continuing development and validation of STR systems have now resulted in twenty or more suitable STR systems being available for use in paternity testing. In the present study, 15 STR loci were multiplexed and used for their great


**Figure 10** STR typing of the crime scene DNA sample (theft case).

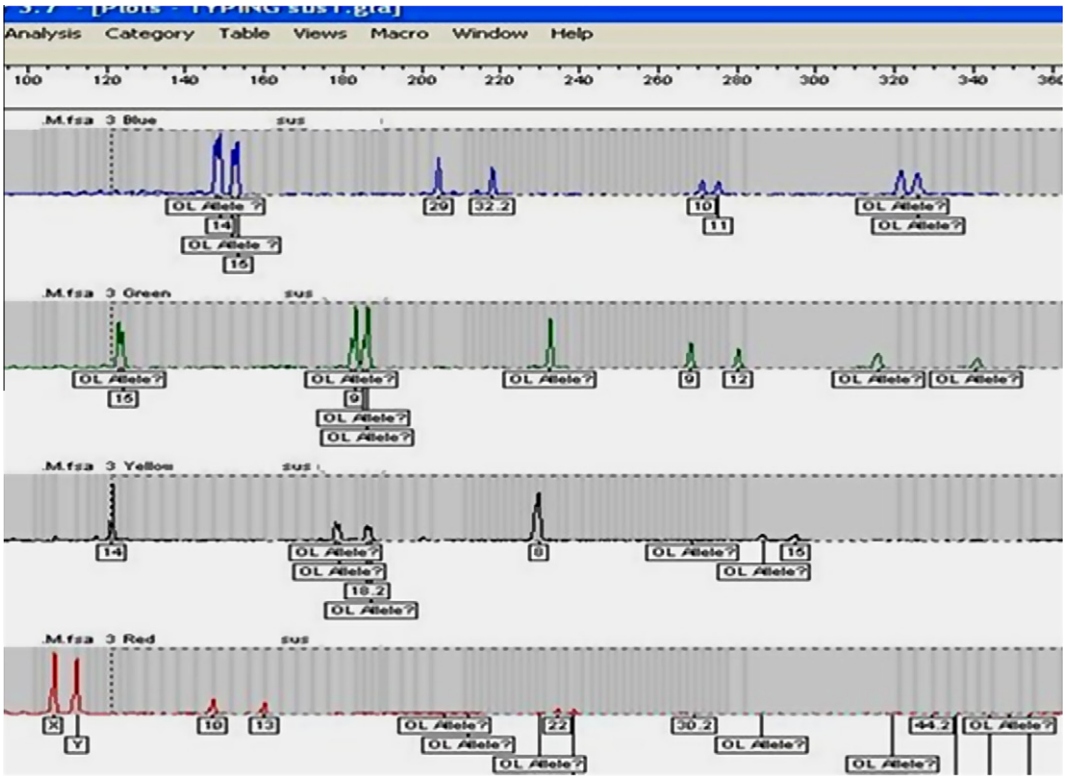


**Figure 11** STR typing of the suspect 1. Genetic variation was detected between the DNA sample of the suspect 1 and the crime scene one at all STR loci examined except for the allele 8 at the locus TPOX.

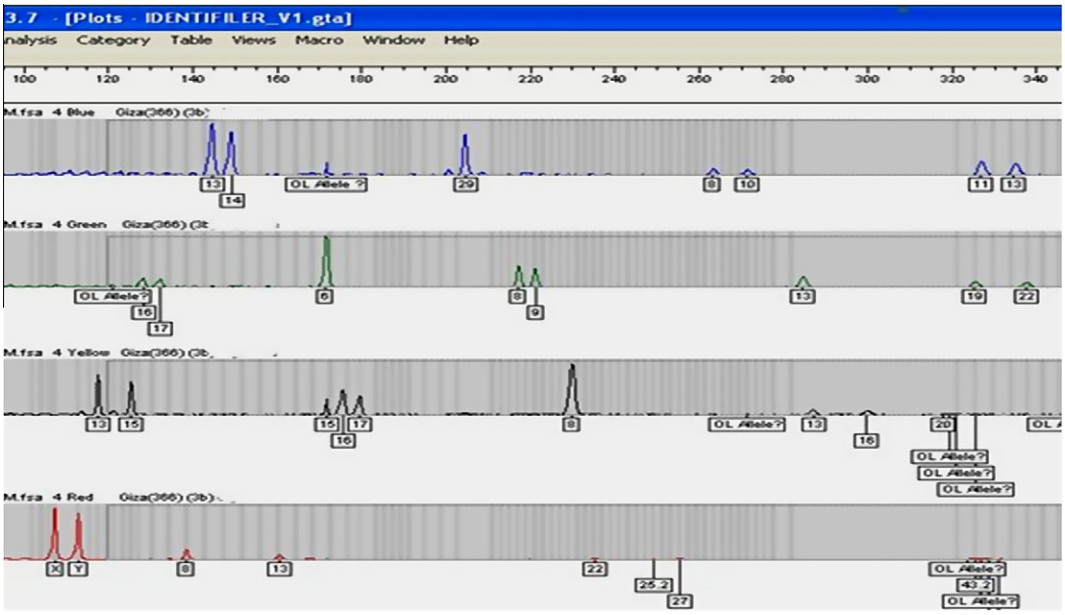


**Figure 12** STR typing of the suspect 2. This suspect showed an identical pattern to the crime scene sample at the 15 STR markers.

**Table 4** The alleles investigated at 15 STR loci from the crime scene sample and the two suspects (theft case), and from the missing man, son of the missing man, crime scene samples and the suspect (murder case).

| Locus | Allele designations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Theft case | | | Murder case | | | | |
| | Crime scene sample | Suspect 1 | Suspect 2 | Killed missing man | Son of the missing man | Crime scene sample 1 | Crime scene sample 2 | Suspect |
| D8S1179 | 13,14 | 14,15 | 13,14 | 13,15 | 13,14 | 13,15 | 10,14 | 10,14 |
| D21S11 | 29,29 | 29,32.2 | 29,29 | 28,31 | 28,31.2 | 28,31 | 29,30 | 29,30 |
| D7S820 | 8,10 | 10,11 | 8,10 | 8,11 | 11,12 | 8,11 | 8,10 | 8,10 |
| CSF1PO | 11,13 | 10,11 | 11,13 | 10,11 | 10,11 | 10,11 | 10,10 | 10,10 |
| D3S1358 | 16,17 | 15,15 | 16,17 | 14,16 | 16,17 | 14,16 | 17,18 | 17,18 |
| TH01 | 6,6 | 9,9.3 | 6,6 | 7,7 | 7,9 | 7,7 | 7,9 | 7,9 |
| D13S317 | 8,9 | 12,12 | 8,9 | 11,12 | 11,11 | 11,12 | 11,14 | 11,14 |
| D16S539 | 13,13 | 9,12 | 13,13 | 11,11 | 11,11 | 11,11 | 9,11 | 9,11 |
| D2S1338 | 19,22 | 17,23 | 19,22 | 24,25 | 24,25 | 24,25 | 20,23 | 20,23 |
| D19S433 | 13,15 | 14,14 | 13,15 | 13,14 | 14,15 | 13,14 | 14,16 | 14,16 |
| vWA | 16,17 | 17,19 | 16,17 | 14,16 | 16,16 | 14,16 | 16,17 | 16,17 |
| TPOX | 8,8 | 8,8 | 8,8 | 8,11 | 8,10 | 8,11 | 10,11 | 10,11 |
| D18S51 | 13,16 | 13,15 | 13,16 | 13,16 | 16,18 | 13,16 | 17,18 | 17,18 |
| D5S818 | 8,13 | 10,13 | 8,13 | 11,12 | 11,11 | 11,12 | 12,13 | 12,13 |
| FGA | 22,27 | 22,23 | 22,27 | 21,22 | 21,23 | 21,22 | 24,26 | 24,26 |
| Amelogenin | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y |

utility in paternity testing. Multiplex STR analysis conferred a greater sensitivity on the test. An ABO blood group typing, which was the first genetic tool used for distinguishing between individuals, can be performed in a few minutes, but it is not very informative [9]. In ABO blood group system, three alleles are possible and thus, six possible genotypes are present in the human population. In contrast, multiplex STR markers produce a greater number of possible genotypes as large numbers of alleles are present for each STR locus. Thus, while the ABO blood group typing is useful for excluding an individual from the paternity test or being the source of a crime scene evidence, the test is not useful when an inclusion has been made.

It has been suggested that STR loci do not offer sufficient levels of discrimination to replace the commonly used single locus probe (SLP) analysis in paternity testing [27]. The present study has shown that a battery of 15 polymorphic STR loci offers a discriminating power sufficient to exclude or include an alleged father in disputed paternity cases. This is consistent with many reports which described the usefulness and discrimination power of STR markers [35,20,15,11]. In paternity and identity testing, the allele frequency database obtained from the population to which the person in question belongs should be used in the calculations for forensic evaluations. The calculated paternity index values and the probability of paternity,

which are of importance in applying the DNA typing method to paternity testing, were high with the present STR typing. The paternity index measures the strength of a genetic match between an alleged father and a particular child. If the alleged father and the child share an allele with low frequency, the match is considered strong and will give a high paternity index. Okamoto et al. [25] have estimated the extremely high paternity index values and the probability of paternity obtained from STR typing compared with those obtained from conventional blood groups. The present results showed that 83% of trio cases gave paternity index (PI) values > 1 million, while 100% gave PI values > 100,000. Thomson et al. [35] have reported that 90% of cases gave PI values > 10,000, while 100% gave PI values > 1000. In paternity testing, the results of probability of parentage would be either 100% to exclude someone as a biological parent of a child or 99% or more to confirm someone as a biological parent of a child. Legally, 99% or greater probability of a biological relationship is considered proof of paternity.

Empirical and theoretical studies investigating the number of excluding loci against non-fathers show that when 12–15 STR loci are analyzed, less than 1 in 2000 of non-fathers would be excluded at only a single locus and all others (>99.96%) would be excluded at two or more loci [35,38]. The present study that investigated the number of excluding loci in the excluded paternity cases showed that 75% of the alleged fathers were excluded at 8–10 loci. Alleged fathers excluded only at a single locus were not observed in the present study. Therefore, when the biological father of a child is in doubt, a PCR-based STR assay is the most accurate choice available for paternity testing.

Concerning the crime cases in the present study, the STR analysis was also very useful for personal identification. For theft case, all markers examined in the DNA profiles were found to match between the crime scene evidence and one of the two suspects working in the company indicating that the two DNA types are from the same individual. The present study also applied STR typing method to forensic personal identification from skeletal remains. Although the soft tissue was almost completely decayed, STR loci could be detected from DNA extracted from a piece of the femur bone. This may have been due to the DNA contained in the femur bone was protected from degradation by the hard bone tissue consistent with the findings of Okamoto et al. [25]. Moreover, the STR loci detected by the present study are relatively small in size (117–345 bp). This is an advantage when analyzing forensic specimens, which are often contain severely damaged and minute DNA [33,30,25]. In the United States, a core set of 13 STR markers are being used to generate a nationwide DNA database called the FBI Combined DNA Index System (CODIS). The CODIS database and similar DNA databases around the world have been successful at linking DNA profiles from convicted offenders and crime scene evidence [29,31].

On conclusion, STR typing is a reliable and robust genetic tool which has an important central role in the society to solve problems of family relationships and forensic caseworks.

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