

Evaluation of the Full-sibling Kinship Regarding Attendance of Multiple Full-siblings

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ABSTRACT The Identity By Descent (IBD) method and Identity By Sharing (IBS) scoring methods implement individual-to-individual comparison based on statistical data. Here, the researchers used the integrative information of known-siblings to evaluate the identity of a suspect-the Multi-participants retro-genetic (MPR) method. MPR uses the genetic laws to deduce the possible alleles of offspring from the same ancestor. The full-sibling relationship was ruled out if 3 or more STRs disobey the genetic laws. To compare the MPR and IBS method, the calculation of 100 unrelated individuals and the pairs of 2 or 3-known full-siblings were performed using 15 or 19 STR. The exclusion rate of MPR method was much higher than the IBS method. Those STRs offering the exclusion allele patterns were termed efficient STRs, and the number of efficient STR is crucial in determining the exclusion power of the system. The MPR method may serve as a valuable adjuvant tool for handling ambiguous results of the IBS method.

INTRODUCTION

In forensic practice, kinship inference is requested in many situations: identification of the victims of disasters, familial searching for suspects in a forensic DNA databank, and daily case-work of matching missing relatives (Maguire et al. 2014; Kaur et al. 2016; Dorum et al. 2017). The polymorphisms of Y-chromosome Short Tandem Repeats (STRs), and the mitochondrial DNA analysis from the other kinship are used in this area, albeit, they only offer explicit results for certain pedigrees (Gjertson et al. 2007; Rohlf et al. 2013). To infer a kinship, such as full-sibling and half-sibling relationship, many scientists have developed suitable formulas based on the Identity By Descent (IBD, also named exact method) or Identity By Sharing (IBS) scoring (Wenk et al. 1996; Brenner 1997; Bieber et al. 2006; Lu et al. 2009). Both of them are based on statistics-

by calculating the chances of sharing allele from the same ancestry. This tactic results in some degree of false positive or false negative rate due to their statistical nature. The threshold, such as likelihood Ratio (LR) or IBS scoring, could be set higher to bring about a decline in the misjudgment rate (Pu et al. 2008; Lu et al. 2009; Musanovic et al. 2012; Tamura et al. 2013; Inoue et al. 2016).

However, when more than two known kinships are involved, the identity of the suspect can be determined theoretically by the integrated information of known kinships. Thus, the application of the whole information of reference sibs would improve sensitivity and down-regulate false judgement (Lee et al. 2012). A paper recently tried to identify some offspring's genotypes by the reconstruction of pedigree (Huang et al. 2017). The principle of this method could be described as follows. Based on the known siblings' genetic information, it is possible to infer according to genetic laws their ancestor's alleles and the offspring's alleles of the same ancestor(s). If the observed allele(s) of the suspect disobeys the genetic laws, he would be ruled out of one kind of kinship of the known siblings. Since this method requires going back

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and forth to find the possibility of some kinds of alleles, it was termed the multi-participants' retro-genetic (MPR) method. The principle of this method is totally different from the individual-to-individual comparison methods. It uses the integrative information of known siblings and the genetic laws other than the statistical data. The simplest kinship involves full-siblings who share the same father and mother. To establish this method and evaluate the examinational power, 2-known full-siblings and 3-known full-siblings were used for comparison with unrelated individuals using sets of STRs. Recently, China released a standard for full-sibling identification based on the IBS method, which suggested that 19, 29 or 39 STRs should be used (Li et al. 2014). Till date, no literature comparing MPR method with IBS and IBD methods has been published.

Objective

The aim of this work is to assess the possibility of using genetic information from several known full siblings to determine the suspect's identity. Moreover, the strategy was compared with IBS method to evaluate its putative application.

MATERIAL AND METHODS

Population and Materials

The blood or swabs of Chinese Han ethnic population were collected from the daily casework of Forensic Judicial Identification Center of Shanxi Medical University, Forensic Judicial Identification Center of Southwest University of Politics and Law. All the research procedures were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). DNA was extracted using the Chelex-100 method. PCR amplification was conducted on GeneAmp PCR System 9700 (Applied Biosystems, USA) using Goldeneye™20A kit (Peoplespot Inc. China) or PowerPlex® 16 System (Promega Corporation, USA). All the procedures were followed according to the manufacturer's instructions. PowerPlex® 16 System comprises 15 autosome STR loci: Penta D, Penta E and 13 CODIS STRs. Goldeneye™20A kit comprises 19 autosome STRs: D2S1338, D19S433, D6S1043, D12S391 and 15 STRs from PowerPlex® 16 System. The PCR products were separated

and analyzed by ABI 310 DNA analyzer and GeneMapper ID software v3.2 (Applied Biosystems, USA). The relationship of full-siblings was confirmed by analyzing their parents' DNA. To assess the exclusion rate, the identified 2- or 3-full-siblings were compared with 100 unrelated individuals whose STR typing had been obtained from the researchers' daily cases. The IBS scores were calculated by directly numbering the identical gene alleles between the known full siblings and unrelated individuals.

Exclusion Patterns of MPR Method

Since the full siblings share the same parents, the possible allele number of autosomal STR in their parents should not be more than 4. Despite the number of alleles of one STR from the 2 or more full-siblings, the different alleles should not exceed 4. The possible pattern of the known-full siblings and the deduced parents' allele of one STR could be A, A/B, A/B/C or A/B/C/D. According to Mendel's law of genetics, the exclusion patterns for an unrelated individual allele types could be deduced (Table 1). Those STRs offering the exclusion patterns were termed efficient STRs (Table 1). For MPR method, only efficient STR can provide STR genotypes regardless of genetic laws.

If one STR of a tested individual was against Mendel's hereditary law, it should be noted that he/she might not be a full-sibling of the known full-siblings. Considering the occurrence of mutation, the kinship was excluded only if more than 3 STRs (including 3) meet the exclusion pattern listed in Table 1. To evaluate the exclusion rate of MPR method, 38 groups of 2-known and 7 groups of 3-known full-siblings were compared with 100 unrelated individuals' DNA.

Statistics

The number of STR indicating exclusion pattern compared with unrelated individuals were presented with the Mean±SD formula. IBM SPSS Statistics 23.0 software was used to explore the distribution of exclusion rate and the Kruskal-Wallis non-parametric test was applied to examine the discrepancy among groups. To describe the relationship between the number of efficient STRs and exclusion rate, a box plot was presented using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego California USA).

Table 1: The patterns of exclusion from full-sibling relationship when 2 or more known full-siblings' STR typing were identified

<i>Detected alleles from known siblings</i>	<i>Possible STR typing combinations by 2 known sibling</i>	<i>The possible STR typing of the parents of the 2-knowns full siblings</i>	<i>The impossible STR types appeared in the full sibling(s) of known-full-siblings</i>	<i>Efficient or not</i>
A	A/A + A/A	A/X+A/X	None	Not efficient
A,B	A/A+ A/B or A/B+ A/B or A/A +B/B	A/B+A/X A/X+B/X A/B+A/B	M/N, M/M, N/N None M/M, M/N, N/N, A/M, B/M, A/N,B/N	Not efficient Not efficient
A,B,C	A/A+ B/C or A/B+A/C	A/B+A/C	M/N, A/M, B/M, C/M, B/B, C/C,M/M,N/N,A/N,B/N,C/N	Efficient
A,B,C,D	A/B+A/C A/B+C/D	A/X+B/C or A/C+A/B A/C+B/D or A/D+B/C	M/N, M/M, A/M,A/N,N/N M/N, M/M, A/A, B/B, C/C, D/D,A/M,B/M,C/M,D/M,N/N, A/N,B/N, C/N,D/N	Efficient Efficient

Note: A, B, C, D, M, N, X presents any allele. +, indicating the “company with”.

RESULTS

The Exclusion Rate of MPR Method

The STR profiles of 38 pairs of 2-known full-siblings was compared separately with 100 unrelated individuals (Table 2). Each comparison would produce series of STRs meeting or not meeting the exclusion pattern(s) listed in Table 1. Among the 3,800 comparisons, Goldeneye™ 20A kit containing 19 STRs was used for matching each time. There were 3,665 individuals who had 3 or more STRs meeting the exclusion patterns, with a total exclusion rate of 96.447%. Twenty-eight unrelated individuals (0.737%) showed 1 STR and 105 individuals (2.763%) with 2 STRs that met the exclusion pattern, which meant their identities could not be determined according to the researchers' standard (Table 2). Two unrelated individuals could not be detected with any STR meeting the exclusion pattern, indicating the limit of exclusion power of this method. The information obtained from each pair comparison is shown in Table S1.

IBS scores were obtained by accounting for the number of shared alleles. The exclusive and inclusive standards were set according to the biological full sibling identification code for practice in China (Li et al. 2014). The score of $IBS \geq 22$ was classified as “intend to be full siblings”, $IBS \leq 13$ as “intend to be unrelated individuals” and IBS between 13 and 22 as “not determined”. Twenty individuals of the known-full sibling pairs were compared with 100 unrelated individuals using Goldeneye™ 20A kit. The exclusion rate was 71.75 percent (Table 3, Table S2 gives the detailed information of each comparison).

If the number of tested STRs is reduced to 15 (PowerPlex® 16 System), the exclusion rate was reduced to 86.395 percent (MPR method, Table 2). (Table S3 gives the information in details). The researchers did not list the IBS scoring data of 15 STRs due to the unavailable corresponding standard in China. A comparable result reported $LR > 9$ in 95 percent and $LR < 3$ in 4 percent of sibling pairs, using Powerplex® 16 STRs (von Wurmb-Schwark et al. 2015). In another study, the log LR range varied from -2.24

Table 2: The unrelated individuals meeting the exclusion pattern with varied number of STRs. The result below shows the comparison between 38 pairs of 2-known full-siblings and 100 unrelated individuals (e”3 STRs was taken as the exclusion criteria)

<i>Individuals meeting the exclusion pattern</i>	<i>0 STR number (%)</i>	<i>1 STR number (%)</i>	<i>2 STRs number (%)</i>	<i>≥ 3 STRs number (%)</i>	<i>Total comparisons</i>
Goldeneye™20A kit	2(0.053%)	28(0.737%)	105(2.763%)	3665(96.447%)	3800(100%)
PowerPlex®16 System	14(0.368%)	118(3.105%)	385(10.132%)	3283(86.395%)	3800(100%)
Results	Not determined	Not determined	Not determined	Exclusion	

Table 3: The IBS scoring of 20 known full-siblings compared with 100 unrelated individuals within 19 STR typing (IBSd²13 was taken as the exclusion criteria)

<i>IBS score</i>	≤ 13 (%)	$13 > \text{IBS} < 22$ (%)	> 22 (%)	<i>Total comparisons</i>
Goldeneye™ 20A kit Results	1435(71.75%) Intend to be unrelated individuals	561 (28.05%) Not determined	4(0.20%) Intend to be full-siblings	2000(100%)

to 15.96 for the combination of simulated information of 2 reference sibs using 15 STRs, which is a much higher value than the individual-to-individual comparison (Lee et al. 2012).

In addition to the examined STR numbers, another determinant for the exclusion rate using the MPR method was the number of known full-siblings that participated. The researchers assessed the exclusion capability using 3-known full-siblings. Seven groups made up of 3-known full-siblings were tested with 19 or 15 STRs. The exclusion rate was 100 percent for 19 STRs (Goldeneye™20A kit) and 99.429 percent for 15 STRs (See Table S4, Table S5 and Table 4 for detailed information).

Number of Efficient STRs and Exclusion Rate

Unlike IBS scoring and IBD method, the number of efficient STRs was vital to the exclusion capability using the MPR method, because those inefficient STRs offered no valuable genetic information for exclusion of an unrelated person. In determining the relationship between the efficient STR number and exclusion ability, 2-known full-siblings whose DNA were typed with 15 or 19 STRs were analyzed. The exclusion rates increased when the number of efficient STR number enlarged. The Kruskal-Wallis test showed the value of p among groups to be $p < 0.0001$. Since some groups have fewer numbers, the group to group discrepancies are not reported.

While the efficient STR number was above 10, the exclusion rate varied from 77 to 100 per-

cent. Furthermore, the exclusion rate improved to about 95-100 percent when the efficient number was above 12. The exclusion rate varies vastly (65-96%) when the efficient STR number was 7-9. When the efficient number was below 7, this method was not recommended for use in drawing conclusions because of its poor exclusion power (Fig.1).

Application of MPR in Forensic Case

A pair of known full-siblings asked for the identification of the person who contacted them from the internet. They reported that their eldest sister was given up for adoption about 50 years ago because of the family's poor financial condition. The full sibling relationship was referred using MPR method. The STR typing was obtained using Goldeneye™20A kit. The IBS scoring method, LR calculation and MPR method were applied to evaluate the full-sibling relationship in this case. This person was identified as being unrelated with all this 3 methods (Table S6).

DISCUSSION

The huge number of polymorphic STRs and automatic detecting method make it possible and easier to obtain as much as STR typing. Since the non-classical paternity tests are often required in forensic practice, it is necessary to establish the standard(s) for all kinds of kinship relations. The calculation of paternity index and

Table 4: Seven groups of 3-known-full-siblings with varied exclusion STR numbers compared with unrelated individuals

<i>Individuals meeting the exclusion pattern</i>	<i>0 STR (%)</i>	<i>1 STR (%)</i>	<i>2 STR_s (%)</i>	<i>≥3 STR_s (%)</i>	<i>Total comparisons</i>
Goldeneye™20A kit	0	0	0	100	700
PowerPlex® 16 System	0	0	4(0.571%)	596 (99.429%)	700
Conclusion	Not determined	Not determined	Not determined	Exclusion	

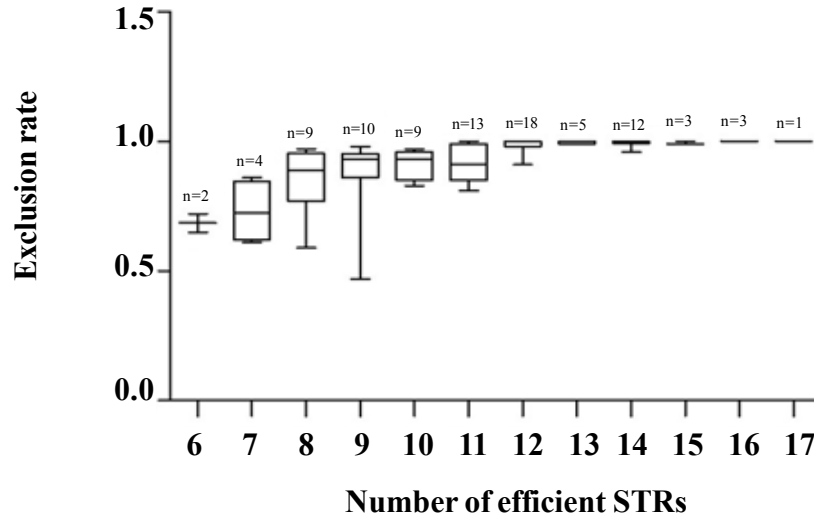


Fig. 1. The relation of the exclusion rate and the number of efficient STRs

LR was established based on the infinite polymorphism of STRs. IBS calculation is another method of evaluating the kinship relations. Both of them are based on the statics, leading to a certain degree of false positive and false negative results. In order to fulfill the requirement of precision for forensic identification, the LR threshold has to be set higher to avoid the false exclusive, however, this policy will increase the possibility of false inclusion results. So the balance of false positive and false negative is a paradox for IBD and IBS methods (Rohlf et al. 2013; Tamura et al. 2013; Inoue et al. 2016). Four false positive results were observed in this study using IBS scoring method and 19 STRs. Based on the genetic laws, the more information obtained from as many kinship as possible, the more clues it would provide. So the MPR was designed based on genetic laws. Although, only full-sibling relationship was assessed here, it appeared to be more powerful than the IBS scoring method.

Principle of MPR Method and Exclusion Pattern

The principle of this method differs from that of LR calculation or IBS scoring method. The effectiveness of LR calculation depends on the

allele frequencies. The performance of IBS is determined by the locus heterozygosity (Wen et al. 1996; Presciutti et al. 2002). However, the effectiveness of the researchers' MPR is based on the number of efficient STRs.

The formation of exclusion pattern depends on the STR allele pattern and allele composition of known full-siblings. Since the STR biomarkers are co-dominantly inherited, when only one allele is examined in the known full-siblings, this STR cannot be used for exclusion because their parents should have two free positions at each chromosome and it could be any allele. So their genetic offspring may present any allele combination. When two alleles are found in the known full-siblings, the allele pattern determines the exclusion pattern. If the alleles of the known full-siblings are A/B and A/B, their parents should have 2 free positions on the chromosome and their other offspring could have any allele combination. If the known full-siblings alleles are A/A and A/B, their parents should have only 1 free position for other alleles, so the typing of M/N, M/M, N/N should be excluded (Table 1). When 3 or 4 alleles are found in known full-siblings, their possible full-siblings allele should follow the same pattern. The STR having an allele pattern that could be used to exclude the full-siblings relationship was termed an efficient STR

(Table1). As the full-siblings share the same parents, theoretically, one STR locus does not have more than 4 alleles among all the possible full-siblings. The possible compositions of alleles of the known full-siblings are listed in Table 1. For example, if 2 alleles were detected from all the known full-siblings: A and B, and one shows the allele A/A and the other A/B, as they are full-siblings, such an allele pattern is efficient because it may be used to exclude a possible claim of full-sibling, that is, the M/N, M/M, N/N allele. On the other hand, if the full-siblings show A/B and A/B on one STR locus, such a composition of alleles is not efficient because it contributes nothing for exclusion regarding any unrelated individual. When the number of detected alleles are 3, two patterns are helpful; the A/B-A/C grouping or A/A-B/C grouping. Each showed different exclusion patterns as listed in Table1. When 4 alleles have been detected, which is usually A/B-C/D, the suspects presenting any allele not equal to A, B, C and D would violate the Mendel's law and should be excluded for a full-sibling relationship to the known full-siblings.

It should be noted that the detected alleles from the known full-siblings are stochastic. Thus, increasing the number of examined STRs would promote the chances of more efficient STRs, so as to enhance the examination power of this method. This is totally different from the IBD and IBS methods in principle, although, a rise in the number of tested STRs would increase the examined power on all these 3 methods. Using the latter two methods, the efficient STRs are meaningless because they did not use any allele information to back-step the parent allele.

The Exclusion Pattern and Exclusion Criteria

The exclusion criteria were set where more than 3 STRs provide the exclusion information. The false exclusion rate should be noted when using MPR method-the chance of exponential of 3 of the STR mutation rate. The average STR mutation rate varies from 5×10^{-5} to 1×10^{-2} (Gjertson et al. 2007). The false exclusion rate of this method should vary from 1.25×10^{-13} to 1×10^{-6} .

The IBS scoring method with 19 STRs have an average exclusion rate of 75 percent for unrelated individuals, which is comparable to the IBS scoring data in this study (71.75% in Table 3). The MPR method worked much more efficiently (exclusion rate of 96.447%) than the IBS scoring

method. Additionally, the IBS scoring method has a higher frequency of obtaining false positive results (false inclusive). The researchers found 4 unrelated individuals (0.20%) who were taken as full-siblings ($IBS \geq 22$) among the 2000 comparisons conducted in this study. Their IBS scores were 22, 23, 23, and 24, respectively (data not shown). Since vast data in many studies were obtained by computer-simulated program other than real STR typing, the false positive results might be inappropriately estimated. At least in this study, the false positive result is high for the IBS method. This might be caused by the statistics-based nature of this method. IBS method has a false positive rate of 0.1 percent, according to the Chinese standard (Li et al. 2014). It reminds us to be cautious in making a decision of having or not having a full sibling relationship using the IBS method. The false inclusion of full-sibling relationship would impair the precision level of appraisal in forensic practice. For comparison of exact method, researchers (Rohlf et al. 2013) described the false positive rates of parent-offspring and sibling identification on the order of 1×10^{-5} to 1×10^{-9} using autosome STRs combined with Y-STRs, varying among populations.

In this study, 2 unrelated individuals showed 0 STR (0.053%) meeting the exclusion pattern in 19 STRs. This reminds the researchers that 19 STRs of 2-known full-sibling participant is not enough for the exclusion of all unrelated individuals. This might be solved by the following ways: (1) By increasing the number of tested STRs, which would in turn increase the number of efficient STRs; (2) By increasing the number of participants of known full-siblings, which would also increase the number of efficient STRs.

The exclusion rate increased to 100 percent, while the full-siblings participants were 3 when tested by 19 STRs. While the number of tested STRs is less than 19, the exclusion rate for unrelated individuals is reduced rapidly for 2- or 3-known full-siblings (Tables 2 and 4). It is highly recommended that the number of tested STRs should be more than 19 for 2-known full-siblings and not less than 19 for 3-known full-siblings participants when using the MPR method.

The Efficient STRs and Exclusion Rate

The efficient STRs' number is crucial in determining the exclusion rate of MPR method. The

exclusion rate varied vastly when the efficient STR number was 10-12. This may be caused by the allele pattern and allele frequencies distribution. Chinese population has a relatively poor allele distribution and discrimination power for many STRs, such as TH01 and TPOX (Huang et al. 2013; Zhang et al. 2013). This method is highly powerful when the efficient STR number was above 12.

Compared with IBD or IBS scoring method, the MPR method is concise and shows clarity. But they need to be evaluated with more population data and more STRs, such as 29 STRs or 39 STRs, of which the IBS scoring method have been used for further evaluation. Because this method is based on genetic laws, the more STRs tested, the more efficient STRs should be accumulated. In this paper, those individuals cannot be excluded with this method using 19 STRs are recommended to be tested for further examinations with more STRs and/or known full-siblings.

CONCLUSION

In this study, the MPR method was assessed for full-sibling identification: the exclusion patterns, exclusion standard, and exclusion rate. Unlike the IBD and IBS scoring methods, MPR uses at least 2-known full-siblings to assess the possibility of full-sibling relationship of an individual. This method is based on the genetic information and has explicit results. The exclusion power of this method is higher than the IBS scoring method. Those cases which cannot get a conclusion using the IBS method may have a clear conclusion result using the MPR method.

RECOMMENDATIONS

The STRs discussed in this paper are co-dominant autosomal STRs. The STRs at X- or Y-chromosome were not discussed, which should be another issue of concern. Another limitation of this study was the inadequacy of data. The authors addressed the establishment of methods and principles in this paper. For a large population, more groups of full-sibling pairs and more STRs should be studied for more accurate results. In the future, the automatic or computer-aided programs should be developed for calculation and comparison with unrelated individ-

uals. The IBD, IBS and MPR methods are complementary in determining the kinships. The genetic order and possible alleles for other relationships under the same ancestor should be developed using the underlying principles of the MPR method.

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SUPPORTING MATERIALS

Supplemental data: Tables S1–6

Table S1: The number of STRs that met the exclusion pattern for 38 groups of 2-known full-siblings after comparing with 100 unrelated individuals using 19 STRs consisted of Goldeneye™ 20A kit

STR number	0	1	2	≥3
1	0	0	0	100
2	0	0	0	100
3	0	0	0	100
4	0	0	1	99
5	0	2	11	87
6	0	0	4	96
7	0	0	0	100
8	0	0	0	100
9	1	0	2	97
10	0	0	2	98
11	0	1	0	99
12	0	2	7	91
13	0	1	3	96
14	0	1	5	94
15	0	1	14	85
16	0	0	0	100
17	0	0	6	94
18	0	0	0	100
19	0	2	3	95
20	0	0	1	99
21	0	0	1	99
22	0	8	15	77
23	0	0	0	100
24	0	0	0	100
25	1	5	13	81
26	0	0	1	99
27	0	1	8	91
28	0	0	0	100
29	0	0	0	100
30	0	0	0	100
31	0	1	0	99
32	0	0	4	96
33	0	0	0	100
34	0	0	1	99
35	0	1	0	99
36	0	1	1	98
37	0	0	0	100
38	0	1	2	97
Sum	2	28	105	3665
Percent (%)	0.053	0.737	2.763	96.447
Mean	0.053	0.737	2.763	96.447
SD	0.226	1.554	4.233	5.564

Table S2: IBS scoring of 20 individuals from known full-siblings after comparing with 100 unrelated individuals using 19 STRs

IBS scoring	IBS≤13	13<IBS<22	≥22
1	70	30	0
2	96	4	0
3	87	13	0
4	66	34	0

Table S2: Contd...

IBS scoring	1	BS≤13	13<IBS<22	≥22
5		69	30	1
6		36	64	0
7		59	41	0
8		84	16	0
9		62	38	0
10		98	2	0
11		75	25	0
12		79	19	2
13		71	29	0
14		71	29	0
15		54	46	0
16		72	28	0
17		68	31	1
18		84	16	0
19		75	25	0
20		59	41	0
Sum		1435	561	4
Percent (%)		71.75	28.05	0.20
mean		71.75	28.05	0.20
SD		14.429	14.468	0.523

Table S3: The number of STRs that met the exclusion pattern for 38 groups of 2-known full-siblings after comparing with 100 unrelated individuals using PowerPlex® 16 System containing 15 STRs

STR number	0	1	2	≥3
1	0	4	15	81
2	0	1	3	96
3	0	0	0	100
4	0	3	14	83
5	1	12	22	65
6	0	3	20	77
7	0	0	2	98
8	0	1	4	95
9	2	5	9	84
10	0	0	14	86
11	1	6	13	80
12	2	4	9	85
13	0	3	4	93
14	0	4	12	84
15	0	5	34	61
16	1	0	2	97
17	0	3	14	83
18	0	1	7	92
19	0	2	3	95
20	0	0	1	99
21	0	2	4	94
22	3	7	25	65
23	0	0	0	100
24	0	0	5	95
25	3	21	29	47
26	0	3	8	89
27	0	12	29	59
28	0	0	0	100
29	0	0	10	90
30	0	0	7	93
31	1	0	8	91
32	0	2	13	85
33	0	2	7	91

Table S3: Contd...

<i>STR number</i>	0	1	2	≥ 3
34	0	1	5	94
35	0	2	0	98
36	0	3	11	86
37	0	0	0	100
38	0	6	22	72
Sum	14	118	385	3283
%	0.368	3.105	10.132	86.395
Mean	0.368	3.105	10.132	86.395
SD	0.819	4.222	9.017	19.902

Table S4: The number of STRs that met the exclusion pattern for 7 groups of 3-known full-siblings after comparing with 100 unrelated individuals using 19 STRs consisted of Goldeneye™ 20A kit

<i>Group STRs</i>	0	1	2	≥ 3
1	0	0	0	100
2	0	0	0	100
3	0	0	0	100
4	0	0	0	100
5	0	0	0	100
6	0	0	0	100
7	0	0	0	100
Percent (%)	0	0	0	100
mean	0	0	0	100
sd	0	0	0	0

Table S5: The number of STRs that met the exclusion pattern for 7 groups of 3-known full-siblings after comparing with 100 unrelated individuals using 15 STRs which consisted of PowerPlex® 16 System

<i>Group STRs</i>	0	1	2	≥ 3
1	0	0	1	99
2	0	0	0	100
3	0	0	0	100
4	0	0	3	97
5	0	0	0	100
6	0	0	0	100
7	0	0	0	100
Sum	0	0	4	696
Percent (%)	0	0	0.571	99.429
Mean	0	0	0.571	99.429
SD	0	0	1.134	1.134

Table S6: One case that excluded an unrelated person with 2-known-siblings. A and B were known full-siblings, L was the suspect who contacted them from the internet. L was identified as an unrelated individual of A and B. Those italicized and bold fonts of STRs were the efficient STRs for this case

<i>STR loci</i>	<i>A</i>	<i>B</i>	<i>L</i>	<i>L vs.BIBS</i> <i>scoring</i> <i>FSI=LR</i>	<i>L vs.AIBS</i> <i>scoring</i> <i>FSI=LR</i>	<i>A vs.BIBS</i> <i>scoring</i> <i>FSI=LR</i>	<i>A/B</i> <i>vs.LSTR</i> <i>meeting</i> <i>Exclusive</i> <i>pattern</i>
<i>D19S433</i>	13	13/17	14/15	0 (0.25)	0(0.5)	1(1.10)	YES
<i>D5S818</i>	11/12	11	9/12	0 (0.25)	0(0.76)	1(1.06)	NOT
<i>D21S11</i>	29/30	29/30	29/31	1(0.70)	1(0.70)	2(2.71)	NOT
<i>D18S51</i>	12/13	13	13/15	1(1.31)	1(0.77)	1(1.31)	NOT
<i>D6S1043</i>	12/18	11/20	11/14	1(1.71)	0(0.25)	0(0.25)	YES
<i>D3S1358</i>	15	15/16	15	1(0.94)	2(3.55)	1(0.94)	NOT
<i>D13S317</i>	10/12	8	8	2(4.69)	0(0.25)	0(0.25)	NOT
<i>D7S820</i>	10/12	10/11	10/12	1(1.09)	2(5.01)	1(1.09)	NOT
<i>D16S539</i>	9/13	9/13	9/12	1(0.67)	1(0.67)	2(4.94)	NOT
<i>CSF1PO</i>	11/12	11/12	10	0(0.25)	0(0.25)	2(2.37)	NOT
<i>PENTA D</i>	10/13	10/13	12	0(0.25)	0(0.25)	2(9.68)	NOT
<i>VWA</i>	14	14	16/19	0(0.25)	0(0.25)	2(6.50)	NOT
<i>D8S1179</i>	11/14	11/12	13/15	0(0.25)	0(0.25)	1(1.29)	YES
<i>TPOX</i>	8/9	8/11	8	1(0.73)	1(0.73)	1(0.50)	NOT
<i>PENTA E</i>	18/23	11/18	10/11	1(1.24)	0(0.25)	1(1.80)	NOT
<i>TH01</i>	7/8	6/7	7/9	1(0.70)	1(0.70)	1(0.70)	YES
<i>D12S391</i>	19/23	17	18/22	0(0.25)	0(0.25)	0(0.25)	YES
<i>D2S1338</i>	17/23	18/24	23/26	0(0.25)	1(0.80)	0(0.25)	YES
<i>FGA</i>	23/24	23	23/27	1(1.08)	1(0.67)	1(1.08)	NOT
<i>AMG</i>	X/Y	X	X				
Total				IBS=12 FSI=8.4 $\times 10^{-4}$	IBS=12 FSI=5.1 $\times 10^{-6}$	IBS=20 FSI=10.48	6 STRs meeting exclusive pattern