COMPARISON OF TWO DIFFERENT MULTIPLEX SYSTEMS IN CALCULATING KINSHIP AMONG CLOSE RELATIVES

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Abstract

This study compares the results obtained using two multiplex systems, PowerPlex® 16 System and PowerPlex® Fusion System, to evaluate the probability of a specific kinship relationship between the offspring of three pairs of identical twins, such as full kinship (siblings), first-degree relatives (first cousins) and half-siblings. Genomic DNA was isolated and amplified from buccal swab and selected short tandem repeat (STR) markers were detected. Electropherograms were generated and analyzed for all persons, using two multiplex systems. Paternity testing for every nine offspring of six examined couples was performed and in all cases the probability that the alleged father is the true father, was over 99.9999%. Kinship analyses were performed setting up two different hypotheses and calculating the likelihood ratio (LR) and kinship probability. Determining the degree of kinship between persons who were full siblings, likelihood ratio showed the highest values contrary to other two types of kinship. Kinship analyses between first cousins showed a higher probability that the examined persons are half-siblings, rather than they are first cousins. In most cases, the introduction of additional seven loci included in PowerPlex® Fusion System increased the values of average likelihood ratios. It is recommendable to use over 20 STR loci in complex kinship analyses.

Key words: *kinship, identical twins, PowerPlex® 16 System, PowerPlex® Fusion System, likelihood ratio*

Introduction

Personal identification or complex kinship analyses represents the most challenging tasks for forensic investigators. Determining sibship or half sibship among close relatives is a frequent issue in kinship cases. These analyses can be carried out by testing a set of STR (Short Tandem Repeat) loci. STR allelic variants vary from person to person, so it is not unusual that two persons share the same alleles at STR loci, or even to match at two or three STR loci. However, the minimum theoretical probability

that two individuals share identical alleles at all 15 STR loci included in the commercial multiplex STR system PowerPlex® 16 for the Caucasian population is 1/1.83 x 10^{17} (Marjanovic and Primorac, 2013).

STR markers became very popular in forensic practice because they are based on PCR technology, and they can be used when it comes to a small amount, or degraded DNA. STR markers are suitable for multiplex amplification and include sensitive fluorescent detection, which allows researchers to quickly collect the data based on the markers. STR markers have a great power of discrimination between persons who are not related, but also between closely related individuals (Butler, 2015).

A large number of multiplex STR systems have been developed and they are used in forensic practice for many purposes, like confirming or excluding paternity, but also, for other types of relationships between individuals. For example, Thomson et al. (2001) analyzed the kinship relations using multiplex STR loci and Gaytmenn et al. (2002) studied the sensitivity and specificity of kinship analysis. Reid et al. (2004) compared the probability of sibship for pairs of full siblings versus unrelated individuals, using Identifiler multiplex STR kit (15 STR loci plus amelogenin). In this study, LR values for known full siblings ranged from 4.6 to over 1 billion and for unrelated individuals from 0.000000045 to 0.12. All full siblings had an LR > 1 and all nonsiblings had an LR < 1.

PowerPlex® 16 System allows co-amplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin) including Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818 (Promega Corporation, 2013). The PowerPlex® Fusion System is a 24-locus multiplex for human identification applications and allows co-amplification and fluorescent detection of the 13 core CODIS loci (CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11), the 12 core European Standard Set loci (TH01, vWA, FGA, D21S11, D3S1358, D8S1179, D18S51, D10S1248, D22S1045, D2S441, D1S1656 and D12S391) and Amelogenin for gender determination. This system also includes DYS391, as a male specific locus. Penta D and Penta E loci are included in order to increase the discriminatory power and enable searching the databases that contain profiles with these loci. Finally, D2S1338 and D19S433 loci, which are contained within large number of databases, are incorporated to further increase the power of discrimination (Promega Corporation, 2014).

Appropriate application of statistical model plays an important role in the determination of kinship between individuals. To calculate the kinship between two individuals, it is necessary to define the relatedness coefficients for each type of relations. The relatedness coefficients are commonly referred as k_0, $2k_1$, k_2 (for two observed persons, X and Y): k_0 = P (none of allele X is identical by descent to Y alleles); $2k_1$ = P (one allele X is identical by descent to one of the Y allele, but the second one is not); k_2 = P (both X alleles are identical by descent to Y alleles), where P is the probability for each individually analyzed locus (Fung and Hu, 2008). The probability of kinship is calculated based on these coefficients.

The aim of this study was to compare the performance of two multiplex systems, PowerPlex® 16 and PowerPlex® Fusion, in the process of statistical determination of kinship between the descendants of three pairs of identical twins. The specificity of this study is that the descendants of identical twins are first cousins, but regarding their DNA profiles, they are half siblings. The main objective was to

investigate whether larger number of loci contained within PowerPlex® Fusion System, increases the possibility of discrimination between three types of kinship: full sibship, first cousins and half sibship.

Materials and methods

Samples were collected from 21 individuals, of which three pairs of identical twins with their spouses and with a total of nine offspring (Fig. 1). The first twin pair is labeled as D and N, their spouses are A and T, and their children are DA1, DA2, NT1 and NT2. The second twin pair is labeled as M and B, their spouses as V and T1, and children as MV, BT1 and BT2. The third twin pair is labeled as AD and AD1, their spouses as SA and ME and their children as ASA and AMS. All persons investigated gave informed consent.

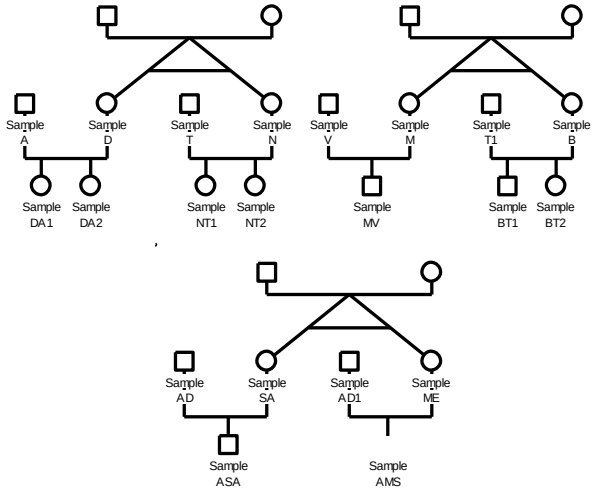

Figure 1. Pedigrees for three pairs of identical twins

DNA was isolated and amplified from buccal swab of all persons, according to the modified Miller's protocol (Miller, Dykes and Polesky, 1987) and selected STR markers were detected using ABI PRISM 310 Genetic Analyzer and two multiplex systems.

Data analysis was performed using GeneMapper® ID software, ver. 3.2. Paternity testing for all the descendants of six examined couples was performed. Kinship analyses were performed by setting up two different hypotheses and calculating the LR. Calculations were performed using EasyDNA_2Persons software, suggested by Fung and Hu (2008). Before starting the software analysis, it was necessary to make one input file, which contains a list of all 15 loci, allelic variants and their frequencies for PowerPlex® 16 System, or 22 for PowerPlex® Fusion System. This software uses relatedness coefficients to describe the type of relationship between two persons under the hypothesis (Tab. 1).

Table 1. Relatedness coefficients (k_0, $2k_1$, k_2) for some common relationships between two persons (Fung and Hu, 2008)

Relationship	k_0	$2k_1$	k_2
Parent-child	0	1	0
Full siblings	1/4	1/2	1/4
Half siblings	1/2	1/2	0
Grandparent-grandchild	1/2	1/2	0
Uncle-nephew	1/2	1/2	0
First cousins	3/4	1/4	0
Second cousins	15/16	1/16	0

Hypothesis testing and calculation of LR was performed for each locus individually, using the formulas for the calculation of probability, as shown in Table 2.

Table 2. Likelihood ratio from with two opposing hypotheses Hp: (Y, Z) ~ (x0, 2x1, k2) versus Hd: (Y, Z) ~ (1, 0, 0) (Fung & Hu, 2008)

Y	Z	Likelihood ratio
A_iA_i	A_iA_i	$k_0 + 2k_1/p_i + k_2/p_i^2$
A_iA_i	A_iA_j	$k_0 + k_1/p_i$
A_iA_i	A_jA_j	k_0
A_iA_i	A_jA_k	k_0
A_iA_j	A_iA_j	$k_0 + k_1(p_i+p_j)/(2p_ip_j) + k_2/(2p_ip_j)$
A_iA_j	A_iA_k	$k_0 + k_1/(2p_i)$
A_iA_j	A_kA_l	k_0

As an input file for EasyDNA_2Persons software, we used the list of loci and allelic variants for the Bosnian-Herzegovinian population (Marjanovic et al., 2006). Profiles, obtained using PowerPlex® Fusion System, include seven additional loci, whose allelic frequencies were taken from the data for Croatian population (Curic, Gasic, Pluzaric and Smiljcic, 2012).

Results and discussion

Electropherograms were generated and analyzed using two multiplex systems, where both multiplex systems gave satisfactory results for all individuals. DNA samples were profiled using PowerPlex® 16 System and PowerPlex® Fusion System. Samples were analyzed using the ABI PRISM 310 Genetic Analyzer and the profiles were obtained using GeneMapper® ID software, ver. 3.2.

In all the cases of paternity testing using the PowerPlex® 16 System, probability that the alleged father is the true father, was higher than 99.9999%. There were some variations in the values of CPI in some samples, which reflected on the probability of paternity. The chart shows that the lowest probability of paternity had parental couple labeled as M and V, for the child labeled as sample MV (99.99998%) and a parental couple labeled as B and T1, for the child labeled as sample BT1 (99.999975%) (Fig. 2).

Other samples had similar values, except for the pair of samples AD and AD1, where in both cases of paternity testing were obtained much higher probabilities, compared to the other tested samples. High CPI values resulted from rare allelic variants present at several loci. In this case, Penta E locus was especially interesting, since twin pairs with the highest probability of paternity, had allelic variant 20. According to Marjanovic et al. (2006), this allelic variant was not observed in a sample of 100 individuals in the Bosnian-Herzegovinian population, so allelic frequency of variant 20 is considered as 0.005. Another allelic variant that was not observed by Marjanovic et al. (2006) is 14.3 at the D1S1656 locus, which was found in the sample T.

Having confirmed the paternity for every descendant of all twin pairs, calculation of the degree of kinship could be performed. The examined types of kinship were: full sibship, first-degree relatives and half sibship. It

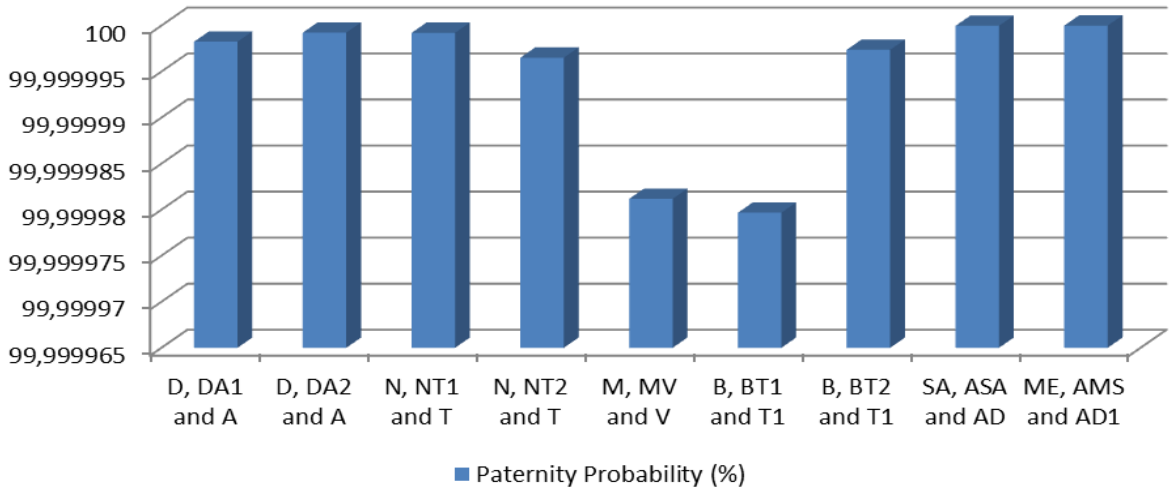

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Figure 2. Paternity testing for all samples using the PowerPlex® 16 System

Table 3. Analysis of the relationships of full siblings versus first cousins, for all descendants

	PowerPlex® 16 System		**PowerPlex® Fusion System**	
	Average LR	Kinship Probability (%)	Average LR	Kinship Probability (%)
Samples DA1 and DA2	2.9458	74.66%	10.1532	91.03%
Samples DA1 and NT1	0.5106	33.80%	0.9545	48.84%
Samples DA1 and NT2	1.234	55.24%	0.2656	20.99%
Samples DA2 and NT1	0.1114	10.02%	0.0021	0.2%
Samples DA2 and NT2	0.0789	7.31%	0.0058	0.58%
Samples NT1 and NT2	921.7813	99.89%	34707.44	99.99%
Samples BT1 and BT2	2399.483	99.96%	499822.1	99.99%
Samples MV and BT1	0.748	43.95%	0.1962	16.4%
Samples MV and BT2	0.0377	3.36%	0.0333	3.22%
Samples ASA and AMS	0.0337	3.26%	0.0054	0.54%

necessary to set up two hypotheses (Hp and Hd) and to calculate LR or probability factor. If the value of LR is greater than 1, it supports the probability of Hp hypothesis, and if the value is less than 1, it supports probability of Hd hypothesis.

Firstly, we tested the following two hypotheses: Hp - The relationship between the examined persons is full kinship (brothers and sisters) and Hd - The relationship between the examined persons is first-degree relatives (first cousins). The obtained average LRs for both multiplex systems are presented in Table 3.

Average LRs for fully related samples using PowerPlex® 16 were higher than 1, but varied in their values. LR for DA1 and DA2 samples was 2.9458, for NT1 and NT2 samples was 921.7813 and for BT1 and BT2 samples was 2399.483. These differences between LRs resulted from the fact that some samples have a high number of loci where they share the same alleles, whether they are homozygote or heterozygote, or they share some rare alleles with low allelic frequencies. For the same reason, samples DA1 and NT2 have an average LR over 1 (1.234). This result could indicate that there is a probability that samples DA1 and NT2 are full siblings, even though they are first cousins. The use of 22 loci in PowerPlex® Fusion System gave better confirmation of supposed relations in 9 out of 10 sample pairs. Additional loci solved the dilemma about the samples DA1 and NT2, where the average LR decreased under 1 (0.2656), so hypothesis Hp could be rejected and the samples DA1 and NT2 could be considered as not full siblings. Distinguishing between full siblings and first degree relatives requires larger number of STR loci than 15. The PowerPlex® Fusion profiles also confirmed the full kinship relations between samples DA1 and DA2, NT1 and NT2 and BT1 and BT2, but with much greater reliability (10.1532, 34707.44 and 499822.1, respectively). PowerPlex® Fusion System contains two loci (D2S1338 and D12S391) that are recommended by Yuan et al. (2017) as loci with higher discrimination power. STRs with higher discrimination power values should be included in analyses when additional autosomal markers are required for full sibship identification. Next, we tested the following two hypotheses: Hp - The relationship between the examined persons is full kinship (brothers and sisters) and Hd - The relationship between the examined samples is half siblings (half-brothers and half-sisters). Results are presented in Table 4. Regarding the probabilities of full sibship

Table 4. Analysis of the relationships of full siblings versus half siblings, for all descendants

	PowerPlex® 16 System		**PowerPlex® Fusion System**	
	Average LR	Kinship Probability (%)	Average LR	Kinship Probability (%)
Samples DA1 and DA2	1.0358	50.88%	1.9523	66.13%
Samples DA1 and NT1	0.1258	11.17%	0.0647	6.08%
Samples DA1 and NT2	0.4324	30.19%	0.1055	9.54%
Samples DA2 and NT1	0.0579	5.47%	0.0027	0.27%
Samples DA2 and NT2	0.0487	4.64%	0.004	0.4%
Samples NT1 and NT2	43.376	97.75%	307.191	99.67%
Samples BT1 and BT2	188.626	99.47%	4963.224	99.98%
Samples MV and BT1	0.3554	26.22%	0.0534	5.07%
Samples MV and BT2	0.0130	1.28%	0.0033	0.33%
Samples ASA and AMS	0.039	3.75%	0.0043	0.43%

versus half sibship, average LRs in all samples decreased (Tab. 4), comparing to analysis of full sibship versus full cousins (Tab 3). This is the result of relatedness coefficients being lower for first degree relatives, than for half sibling. It was also demonstrated in the study of Von Wumb-Schwark et al. (2015) that LR decreased in full related individuals after analyzing full sibship versus half sibship, comparing to the full sibship versus unrelated persons.

All cases of full sibship were again confirmed, in both multiplex systems. It is also noticable that in all analyses, additional loci in the PowerPlex® Fusion System gave higher support to supposed relations, ie. higher values for full sibship in fully related individuals and lower values of full sibship in half related individuals.

Studies suggest that genotyping more than 20 autosomal STR loci improve forensic personal identification, especially in the sibship analyses (Allen et al., 2007; Carboni et al., 2014; Von Wumb-Schwark et al., 2015; Tamura et al., 2015; Turrina et al., 2016), which corresponds to our findings.

Finally, two hypotheses were tested: Hp - The relationship between the examined samples is half siblings (half-brothers and half-sisters) and Hd - The relationship between the examined persons is first-degree relatives (first cousins). The fully related individuals were excluded from this analysis (Tab. 5). All results obtained with the PowerPlex® 16 System gave average LR values

Table 5. Analysis of the relationships of half siblings versus first cousins, for all descendants

	PowerPlex® 16 System		**PowerPlex® Fusion System**	
	Average LR	Kinship Probability (%)	Average LR	Kinship Probability (%)
Samples DA1 and NT1	4.0609	80.24%	10.2145	91.08%
Samples DA1 and NT2	2.8555	74.06%	2.5191	71.58%
Samples DA2 and NT1	1.9246	65.81%	0.7757	43.68%
Samples DA2 and NT2	1.6202	61.83%	1.4643	59.42%
Samples MV and BT1	2.1098	67.84%	3.6839	78.65%
Samples MV and BT2	2.8924	74.31%	10.0634	90.96%
Samples ASA and AMS	1.3949	58.24%	2.0016	66.68%

higher than 1, indicating the confirmation of half sibship. Considering the fact that identical twins share the same DNA profile, their descendants can be perceived as half siblings. The introduction of additional seven loci used in the PowerPlex® Fusion System increased the values of LR in 5 out of 7 analyzed pairs. Carboni et al. (2014) reported a case study of two women who were cousins and they suspected to be daughters of the same father. Their mothers were sisters and were not available for kinship testing. The analysis with a commercial kit showed a probability value of about 15, to support the hypothesis that they were half-sisters. Including the additional 26Plex markers, the total of 41 loci, probability value increased to 435, supporting the hypothesis that the subjects were half-sisters. Our results also suggest the use of larger number of loci when distinguishing between full and half sibship.

Conclusions

In this study, we compared two multiplex systems, PowerPlex® 16 System and PowerPlex® Fusion System, regarding the determination of kinship between the descendants of identical twins. Likelihood ratios obtained by calculating the degree of kinship between first cousins provided greater support to the hypothesis that they are half siblings. In all cases of full siblings, likelihood ratio the most strongly supported the hypothesis of full sibship.

Additional seven loci in PowerPlex® Fusion System gave more accurate results, so it is recommendable to use 20 or more STR loci in analysis of different types of kinships to avoid ambiguous results and to facilitate interpretation of obtained results.

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