J Forensic Sci, Nov. 2004, Vol. 49, No. 6
Paper ID JFS2004041
Available online at: www.astm.org

TECHNICAL NOTE

Thomas M. Reid,[1] Ph.D.; Caitlin A. Wolf,[1] B.S.; Christopher M. Kraemer,[1] B.S.; Susannie C. Lee,[1] Ph.D.; Michael L. Baird,[1] Ph.D.; and Richard F. Lee,[1] Ph.D.

Specificity of Sibship Determination Using the ABI Identifiler Multiplex System

ABSTRACT: Fifty known siblings and fifty unrelated pairs were genotyped using the ABI Identifiler STR system and sibship indices computed for each pair. Combined sibship indices (CSIs) for the known siblings ranged from less than 10 to greater than 1 billion. CSIs for the unrelated pairs ranged from 4.5×10^{-8} to 0.12. In the known sibling group the percentage of loci where both alleles matched was approximately 40%, while the percentage of loci where neither matched was approximately 10%. In the non-sibling group, the percentage of loci where both alleles matched was approximately 6%, while the percentage of loci where neither matched was approximately 45%. Interestingly, the percentage of loci where a single allele matched was the same in both the known siblings and unrelated pairs, approximately 50%.

KEYWORDS: forensic science, human identification, short tandem repeats, sibship

Sibship analyses can be more problematic than parentage testing in that there are no obligatory alleles between siblings that make it possible to absolutely exclude a biological relationship. In addition, full siblings are as likely to possess two alleles identical by descent from common ancestors at a given locus as they are to possess zero alleles. Thus, a lack of shared alleles at any particular locus does not exclude two individuals from being related (1).

The combination of PCR and short tandem repeats (STRs) is by far the most common technology used to determine biological relationships. More than 90% of parentage tests performed in the U.S. utilize this technology (2), and its use in the forensic field is well documented (3). Gaytmenn et al. reported on the analysis of 19 sibling pairs using the ABI Profiler Plus STR system that utilizes nine independent STR loci (4). We extend that study using 50 full and 50 non-sibling pairs analyzed with the ABI Identifiler STR system that utilizes 15 independent STR loci.

Methods

Samples from previously analyzed parentage tests were used as a source of full siblings and non-siblings. For full siblings, results from fifty cases that included the mother, two children (non-identical twins), and an alleged father were used. In each of these cases neither the mother nor alleged father was excluded as biological parents of either child, and no mutational events were detected. One hundred unrelated individuals were selected randomly from independent parentage cases and matched by race into fifty pairs as non-sibling controls.

DNA samples were isolated from whole blood or buccal swabs using a modified alkaline lysis method (5). DNA samples were amplified using the ABI AmpFLSTR Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations. This kit consists of the 13 CODIS loci plus D2S1338, D19S433, and the amelogenin locus. Amplified DNA was analyzed with an ABI Prism 3100 Genetic Analyzer. Full STR profiles were generated for all 15 loci for each full and non-sibling pair analyzed.

Likelihood ratios, in the form of combined sibship indices (1), were calculated for each pair using DNA-View software v25.05 (6). An in-house population database was used to compute allele frequencies. Locus heterozygosity was calculated with DNA-View.

Results and Discussion

As part of the validation of new STR systems being utilized in our laboratory, we undertook studies to determine the sensitivity and specificity of the ABI Identifiler PCR system to identify full siblings and to discriminate between full and non-siblings. Fifty full siblings previously tested in parentage cases that included two children and both putative parents and fifty non-sibling pairs selected at random were used in this study. As part of this analysis, likelihood ratios, in the form of combined sibship indices (CSIs), were calculated for each pair along with the extent of allele sharing among full siblings and non-siblings at each STR locus.

CSIs ranged from 4.6 to greater than 1 billion in the full sib pairs and from 4.5×10^{-8} to 0.12 in the non-sib controls (Fig. 1). None of the non-sibling pairs had CSIs high enough to be classified as siblings (i.e., CSIs greater than 1); all but two of the known sibling pairs had CSIs greater than 10, with the majority (80%) having CSIs greater than 1000.

The extent of shared alleles at each locus is shown in Figure 2. Full siblings are expected to share zero or two alleles identical

[1] DNA Diagnostics Center, Fairfield, OH.

Received 8 Feb. 2004; and in revised form 18 May 2004; accepted 5 June 2004; published 5 Oct. 2004.

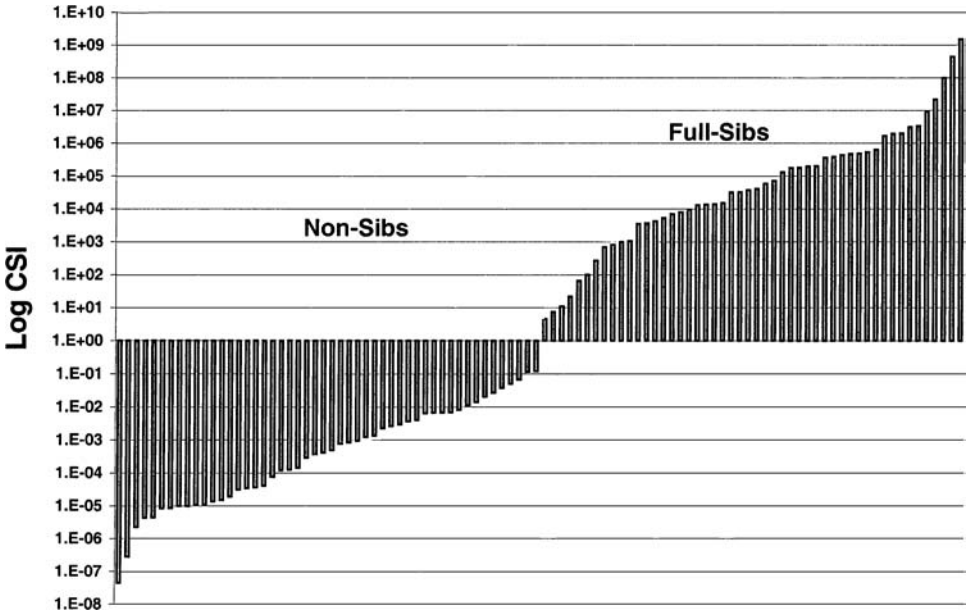

FIG. 1—*Combined sibship indices of known full sibling and non-sibling pairs. Y-axis: log scale.*

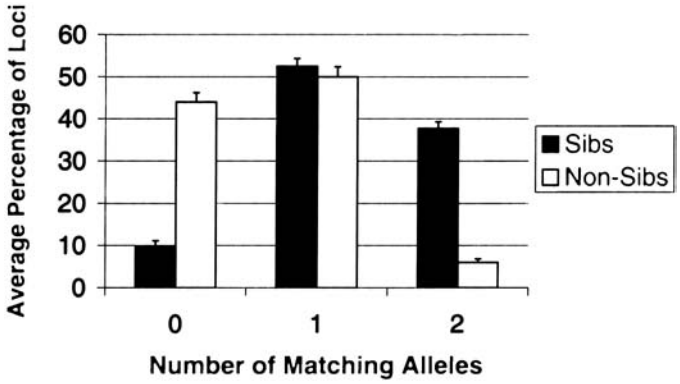

FIG. 2—*Average percentage of allele sharing in full sibling and non-sibling pairs. Error bars: standard deviation of the mean.*

TABLE 1—*Heterozygosities of Identifiler STR loci.*

Locus	Number of Individuals	% Heterozygosity
D8S1179	9601	0.812
D21S11	9736	0.839
D7S820	9694	0.793
CSF1PO	8515	0.740
D3S1358	8312	0.755
TH01	9515	0.763
D13S317	9648	0.784
D16S539	9633	0.793
D2S1338	9146	0.881
D19S433	6486	0.810
VWA	9184	0.803
TPOX	9692	0.683
D18S51	9247	0.876
D5S818	9697	0.738
FGA	9439	0.870
Average	9169	0.796

by descent at a given locus with an equal probability of 0.25 and share a single allele with a probability of 0.5 (1). However, in the present study full siblings shared 0, 1, and 2 alleles approximately

TABLE 2—*Probabilities of allele sharing in Sibling and non-sibling pairs.*

	No. Alleles	One Allele	Two Alleles
		Siblings	
Presciuttini, et al. (7)	0.095	0.523	0.376
Present Study	0.097	0.525	0.377
		Non-Siblings	
Presciuttini, et al. (7)	0.429	0.50	0.076
Present Study	0.440	0.50	0.060

10%, 50%, and 40% of the time, respectively. The extent of allele sharing may be due more to the heterozygosity of the STR systems being used than allele frequency distribution. Presciuttini et al. developed methods to predict the probability of allele sharing at a particular locus based solely on the locus heterozygosity (7). Using a calculated mean heterozygosity of the 15 STR systems in the Identifiler kit of 0.796 (Table 1), the degree of allele sharing in the present study compares quite well with that predicted by Presciuttini et al. (Table 2). In addition, the extent of allele sharing by non-siblings in this study is also quite similar to that predicted by Presciuttini (7). It is worth noting that in the present study using the Identifiler STR system both siblings and non-siblings shared single alleles approximately 50% of the time (Table 2). Tzeng et al. also reported this phenomenon with a similar 15 loci STR system (8).

In conclusion, the 15 loci STR system used in this study distinguished all 50 sibling pairs tested without difficulty; no false positives were identified among the non-sibling pairs. The fact that all but five of the sibling pairs had CSIs greater than 100 suggests that the use of this STR system will be very predictive for identifying siblings even without testing a commonn parent.

References

1. Wenk RE, Traver M, Chiafari FA. Determination of sibship in any two persons. Transfusion 1996;36:59–62.

2. Parentage testing unit. Annual report summary for testing in 2002. Bethesda: American Association of Blood Banks, 2003.
3. Butler JM. Forensic DNA typing. London: Academic Press, 2001.
4. Gaytmenn R, Hildebrand DP, Sweet D, Pretty IA. Determination of the sensitivity and specificity of sibship calculations using the AmpF/STR Profiler Plus. Int J Legal Med 2002;116:161–4. [PubMed]
5. Rudbeck L, Jorgen D. Rapid, simple alkaline extraction of human genomic DNA from whole blood, buccal epithelial cells, semen and forensic stains for PCR. Biotechniques 1998;25:588–92. [PubMed]
6. http://dna-view.com/
7. Presciuttini S, Toni C, Tempestini E, Verdiani S, Casarino L, Spinetti I, et al. Inferring relationships between pairs of individuals from locus heterozygosities. BMC Genetics 2002; http://www.biomedcentral.com/1471-2156/3/23. [PubMed]
8. Tzeng CH, Lyou JY, Chen YR, Hu HY, Lin JS, Wang SY, et al. Determination of sibship by PCR-amplified short tandem repeat analysis in Taiwan. Transfusion 2000;40:840–5. [PubMed]

Additional information and reprint requests:
Thomas Reid
DNA Diagnostics Center
205 Corporate Court
Fairfield, OH 45014
E-mail: tmr@dnacenter.com