Probability distribution of sibship determination with ABI Identifiler multiplex system using different software

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Abstract. Forensic laboratories may be asked to provide genetic evidence that two persons are related or not, when no other relatives are available for study. Sibship analysis of autosome polymorphisms are more complicated since there are no obligatory alleles between siblings that make it possible to exclude a biological relationship with absolute certainty.

In this preliminary study, seventy full-sib pairs were genotyped using the AmpFLSTR Identifiler PCR Amplification kit. All subjects belonged to families that included mother, two or three children, and an alleged father, in which neither the mother nor the alleged father were excluded as biological parents, and no mutational event was observed. In addition, the Y chromosome was investigated with AmpFLSTR Yfiler PCR Amplification kit to provide further support for the relationship. The probability that each pair was composed of full sibs rather than non-relatives was calculated by standard formulas, and was verified using different published software. The distribution of these probability values was used to ascertain the statistical power of the Identifiler kit to resolve sibship relationships to forensic purposes. © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

In forensic routine sibship analyses can be more problematic than parentage testing in that there are no obligatory alleles between siblings that make it possible to absolutely

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exclude a biological relationship. In addition, full siblings are as likely to possess two alleles identical by descent from common ancestors belonging to the same sub-population.

The combination of PCR and short tandem repeats (STRs) today is the most common technology used to investigate these relationships. Different authors report study on the statistical analysis of siblings pairs [1–3].

We made a preliminary study evaluating the usefulness of two software PATCAN [4] and the AlleleSharingSheet at http://statgen.dps.unipi.it/software.htm to evaluate the statistical information in siblings pairs.

In a second step we will evaluate the application of method for inferring relationships between pairs of individuals on a bigger number of cases.

2. Materials and methods

Samples from previously seventy analyzed parentage tests were used as a source of full siblings, all cases included: the mother, two or three male children (non-identical twins), and the alleged father. 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. DNA samples were isolated from whole blood or buccal swabs using the Chelex 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. Furthermore each sibling pairs were amplified with AmpFLSTR Yfiler PCR Amplification Kit (Applied Biosystems, Foster City, CA) or Powerplex Y system (Promega) to confirm the relationship between siblings. The amplified samples were analyzed analyzed with an ABI Prism 310 Genetic Analyzer. The Italian population database was used to compute allele frequencies [6–8]. The percentage of sibling relationship was calculated separately with two software: PATCAN [4] and the AlleleSharingSheet at http://statgen.dps.unipi.it/software.htm.

The second step will be the statistical evaluation on a larger sample of method for inferring relationships between pairs of individuals.

3. Results and discussion

In this study, which represents initial finding of a small sample size, we have evaluated the applicability of two software to determine full siblings. Seventy full siblings previously tested in parentage cases that included two children and both parents were used in this study. The percentage range obtained, based on the Italian frequencies, was the same for the two software, ranging from the minimum of 93.07% to 99.99999%, using all 15 STR loci. The number of loci is crucial to determining sibship relation to obtain a result with a high percentage of probability. In fact with one ore two systems less, the probability drastically falls down even to 10 points. In conclusion, the 15 loci STR systems used in this study distinguished all 70 sibling pairs tested with both software, without difficulty.

References

[1] S. Presciuttini, et al., Inferring relationships between pairs of individuals from locus heterozygosities, BMC Genet. 20 (2002) 23–34.
[2] S. Presciuttini, et al., Allele sharing in first-degree and unrelated pairs of individuals in the GeFI AmpFlSTR Profiler Plus database, Forensic Sci. Int. 131 (2003) 85–89.

[3] T.M. Reid, et al., Specificity of sibship determination using the ABI Identifiler multiplex system, J. Forensic Sci. 49 (2004) 1262–1264.

[4] J.A. Riancho, M.T. Zarrabeitia, A Windows-based software for common paternity and sibling analyses, Forensic Sci. Int. 135 (2003) 232–234.

[5] P.S. Walsh, D.A. Metzger, R. Higuchi, Chelex 100 as a medium for simple extraction of DNA, BioTechniques 10 (1991) 506–513.

[6] S. Presciuttini, et al., Validation of a large Italian Database of 15 STR loci, Forensic Sci. Int. (in press). Available online 22 April 2005.

[7] S. Presciuttini, et al., Y-chromosome haplotypes in Italy: the GEFI collaborative database, Forensic Sci. Int. 122 (2001) 184–188.

[8] N. Cerri, Population data for 12 Y-chromosome STRs in a sample from Brescia (northern Italy), Forensic Sci. Int. 15 (2005) 83–87.