Forensic Science International: Genetics 7 (2013) e21–e22

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Forensic Population Genetics – Letter to the Editor

Analysis of Investigator HDplex markers in Swedish and Somali populations

Dear Editor,

In this study we have performed population analyses of a Swedish and a Somali population for the twelve short tandem repeat (STR) markers included in the Investigator HDplex kit (Qiagen). The set of markers tested, was also evaluated for their efficiency in forensic casework.

A total of 217 unrelated Swedish individuals and 211 Somali male individuals were selected for the population analyses. The Swedes were selected on their Swedish surnames and the Somalis were collected from immigration casework performed at the Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden. The Swedish population samples consisted of blood samples extracted as earlier described [1]. The samples from the Somali individuals consisted of buccal cells on FTA-cards. For the blood samples, approximately 0.5 ng of purified DNA was used for the PCR amplification which was performed according to the manufacturer's protocol except for a reduced total reaction volume (total amount of 10 μl) and reduced number of PCR-cycles (from 30 to 27 cycles). The FTA samples were washed in water prior to the PCR. The PCR products were prepared for capillary electrophoresis by adding 1 μl PCR product to 8 μl of a HiDi Formamide/DNA size standard 550 BTO mix and separation and detection were performed on a ABI 3500XL genetic analyser with 50 cm capillary array and POP7 polymer (Applied Biosystems).

The Swedish population samples were also typed for the 15 loci included in the AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) and the 17 loci included in the PowerPlex® ESI 17 System kit (Promega) according the manufacturers' protocols, except for a reduced total PCR sample volume (10 μl).

The internal validation of the Investigator HDplex kit revealed one notable observation. The mean peak height balance for marker D21S2055 was estimated to 0.67 (±0.31), which was too low to be acceptable. Further analysis of this phenomenon showed that the peak height imbalance was restricted to heterozygous genotypes for which one allele was larger than allele 30.

Allele frequencies for the twelve STRs are shown in Supplementary Data 1 for the Swedish and the Somali populations. SE33 was the locus with the highest power of discrimination for both tested populations. For the Swedish population the power of exclusion ranged from 0.51 to 0.90 for the different markers, while the corresponding values varied between 0.48 and 0.82 for the Somali population. The overall match probability for the Swedish population was 1 in 7×10^{16}, and 1 in 2×10^{17} for the Somali population. These figures are in the same magnitude as for the Identifiler kit in both the Swedish and Somali populations [2,3]. No significant departure, tested by the exact test using genetic data analysis (GDA) [4], from Hardy Weinberg equilibrium (HWE) was detected for any of the markers (Supplementary Data 1).

The allele frequencies for the Swedish and Somali populations were compared with frequencies from a German population [5], a Polish population [6] and a Lithuanian population [7] using the exact test of population differentiation and were carried out using the Arlequin Software version 3.5 [8]. The test showed that the frequency distributions for the Somali population were significantly different from the Swedish population for all 12 STRs (Supplementary Data 2). No significant *P*-values were found (after correction for multiple testing [9,10]) between the Swedish and the German populations or between the Swedish and the Polish population. There were, however, differences between the Swedish and the Lithuanian population for three loci (D21S2055, D2S1360 and SE33).

To test the usefulness of the STR loci in paternity testing, 28 paternity trio cases were typed. 14 of these represented cases with a previous confirmed true father (paternity index (PI) > 100,000) and 14 trios represented cases where the alleged father had, by previous DNA-typing, been excluded as the true father of the child. The analysis of the paternity trios resulted in high PIs (1.9×10^5–5.0×10^{10}, median: 1.5×10^8) for the earlier confirmed true trios, and numerous genetic inconsistencies (6–12, median: 8) for the 14 non-paternity trios.

Investigator HDplex has been suggested to be suitable as a supplementary marker kit. Since many of the loci included in the kit are located on the same chromosomes as many of the core STRs, allelic association (or linkage disequilibrium, LD) and the effect of linkage need to be tested for. When it comes to linkage Phillips et al. [11] provided a map of the genetic distances between the commonly used STRs, including the markers in the Investigator HDplex kit. The effect of linkage is, apart from the genetic distance, also dependent on the pedigree and the homozygous/heterozygous status of the involved individuals, etc. [12,13].

No significant *P*-values were found when allelic association between alleles at the different loci in the Investigator HDplex kit was tested, using GDA [4], in the Swedish and in the Somali population (data not shown). No significant *P*-values were found when allelic association between pairs of loci were tested for all 30 STRs included in the Investigator HDplex kit, the Identifiler kit and the ESI 17 kit for the Swedish population (data not shown).

Simulations were performed to demonstrate the informative value and the effect of linkage for the combined use of the 30 STRs included in the Investigator HDplex kit, the Identifiler kit and the ESI 17 kit for a case scenario with a question of sibship. FamLink [12] was used for the simulation study and the hypotheses were H_1: individual A and individual B are full siblings and H_2: individual A and individual B are maternal half siblings. Data were available from individual A and B and their mother in common. 10,000 simulations were performed with Swedish allele frequencies and recombination rates from Phillips et al. [11]. The results from the simulations shown that the median likelihood ratio (LR) from such analysis can be expected around 10^5 and that the LR is, in general, only slightly overestimated when linkage is ignored

1872-4973/$ – see front matter
http://dx.doi.org/10.1016/j.fsigen.2012.08.006

(Supplementary Data 3). However, as shown in the box plot in Supplementary Data 3, the case specific effect of linkage can be considerable, resulting in up to over 100 times overestimation of the true LR when linkage is ignored.

In summary, the Investigator HDplex markers have shown to be highly informative in both the Swedish and the Somali populations and are well suitable for use as supplementary markers in relationship testing. Although no signs of allelic association, linkage should be accounted for when the Investigator HDplex markers are used in combination with closely located core STRs.

This paper follows the ISFG guidelines for publication of population data requested by the journal [14].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2012.08.006.

References

[1] B. Lindblom, G. Holmlund, Rapid DNA purification for restriction fragment length polymorphism analysis, Gene Anal. Tech. 5 (1988) 97–101.
[2] K. Montelius, A.O. Karlsson, G. Holmlund, STR data for the AmpFlSTR Identifiler loci from Swedish population in comparison to European, as well as with non-European population, Forensic Sci. Int. Genet. 2 (2008) e49–e52.
[3] A.O. Tillmar, G. Backstrom, K. Montelius, Genetic variation of 15 autosomal STR loci in a Somali population, Forensic Sci. Int. Genet. 4 (2009) e19–e20.
[4] P.O. Lewis, D. Zaykin, Genetic Data Analysis: Computer Program for the Analysis of Allelic Data, 2001.
[5] D. Schmid, K. Anslinger, B. Rolf, Allele frequencies of the ACTBP2 (=SE33), D18S51, D8S1132, D12S391, D2S1360, D3S1744, D5S2500, D7S1517, D10S2325 and D21S2055 loci in a German population sample, Forensic Sci. Int. 151 (2005) 303–305.
[6] P. Kuzniar, E. Jastrzebska, R. Ploski, Validation of nine non-CODIS STR loci for forensic use in a population from Central Poland, Forensic Sci. Int. 159 (2006) 258–260.
[7] W. Pepinski, A. Niemcunowicz-Janica, M. Skawronska, J. Janica, Polymorphism of 11 non-CODIS STRs in a population sample of Lithuanian minority residing in northeastern Poland, Forensic Sci. Int. Genet. 5 (2011) e37.
[8] L. Excoffier, G. Laval, S. Schneider, Arlequin (version 3.0): an integrated software package for population genetics data analysis, Evol. Bioinform. Online 1 (2005) 47–50.
[9] S. Holm, A simple sequentially rejective multiple test procedure, Scand. J. Stat. 6 (1979) 65–70.
[10] Z. Sidak, Rectangular confidence regions for the means of multivariate normal distributions, J. Am. Stat. Assoc. 62 (1967) 626–633.
[11] C. Phillips, D. Ballard, P. Gill, D.S. Court, A. Carracedo, M.V. Lareu, The recombination landscape around forensic STRs: accurate measurement of genetic distances between syntenic STR pairs using HapMap high density SNP data, Forensic Sci. Int. Genet. 6 (2012) 354–365.
[12] D. Kling, T. Egeland, A.O. Tillmar, FamLink – a user friendly software for linkage calculations in family genetics, Forensic Sci. Int. Genet. 6 (2012) 616–620.
[13] K.L. O'Connor, A.O. Tillmar, Effect of linkage between vWA and D12S391 in kinship analysis, Forensic Sci. Int. Genet. (2012), http://dx.doi.org/10.1016/j.fsigen.2012.03.008.
[14] A. Carracedo, J.M. Butler, L. Gusmao, W. Parson, L. Roewer, P.M. Schneider, Publication of population data for forensic purposes, Forensic Sci. Int. Genet. 4 (2010) 145–147.

Andreas O. Tillmar*
National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden

Helena Nilsson
National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden

Daniel Kling[a,b]
[a]*Norwegian Institute of Public Health, Department of Family Genetics, Oslo, Norway*
[b]*Norwegian University of Life Sciences, Department for Chemistry, Biotechnology and Food Science, Aas, Norway*

Kerstin Montelius
National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden

*Corresponding author. Tel.: +46 13 25 21 43; fax: +46 13 13 60 05
E-mail address: andreas.tillmar@rmv.se (A.O. Tillmar)

13 April 2012