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Correspondence

Population analysis and forensic evaluation of 21 autosomal loci included in GlobalFiler™ PCR Kit in Poland

Dear Editor,

The GlobalFiler™ is a recently developed 6-dye chemistry kit aimed at forensic DNA identification and has improved buffer systems, reduced inhibition effects, increased amplification efficiency and increased resistance to degradation. This commercially available megamultiplex kit incorporates a set of 24 loci, which have 21 autosomal STRs. In our population study, we used buccal samples collected from 600 unrelated adults of both genders from all over Poland who had well-documented Polish ancestry for at least two generations. The samples were catalogued in the Department of Forensic Genetics of the Pomeranian Medical University in Szczecin, Poland in PBGOT (www.pbgot.pl), which serves as a central repository for genetic information of the nearest living relatives and victims of totalitarianism, with the goal of positive identification of the greatest possible number of these types of individuals [1]. This laboratory works according to ISO/IEC 17025 and successfully participates in the national (PTMSIK) and international (GEDNAP) proficiency tests. Informed consent was obtained for the use of the samples. After anonymizing the samples, DNA was extracted with the PrepFiler™ BTA Forensic DNA Extraction Kit following the manufacturer's instructions (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Quantification was performed on a 7500 Real-Time PCR system using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). DNA samples were amplified on a 9700 thermal cycler with a 25-μl reaction volume using the GlobalFiler™ PCR Amplification Kit according to the protocol for assay preparation and using the cycling conditions recommended by the manufacturer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). DNA input was estimated to be 0.8 ng for each PCR reaction. Separation and detection of amplified products were performed using a 3500 Genetic Analyzer, with reference to the LIZ600 size standard v2 (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Alleles were identified, and DNA profiles were established according to the allelic ladder supplied by the manufacturer using GeneMapper ID-X software v.2.0 (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Statistical analyses were performed and evaluated the allele frequencies, agreement of the population with Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD) in the investigated pairs of loci, coancestry coefficient (φ) and the parameters that are forensically useful, i.e., the major allele frequency (MAF), observed heterozygosity (HET), gene diversity (GD), matching probability (MP), power of discrimination (PD), power of exclusion (PE), typical paternity index (TPI) and polymorphism information content (PIC). The statistical analyses carried out using the PowerStats v.1.2 spreadsheet [2], GDA software v.1.0 [3] and PowerMarker software v.3.25 [4]. The obtained data were tested for statistical significance with Fisher's exact test, with probability values (p-values) representing 0.05 as an acceptable significance level. Bonferroni's correction adjusted to multiple testing was applied [5].

The allele frequencies within the range of the overall 21 loci included in the GlobalFiler™ system evaluated in the Polish population are summarized in **SupplementaryTable 1**. No significant deviations of the HWE expectations were found (p-value >0.05), except for the loci D7S820 and TH01. However, after applying Bonferroni's correction for multiple tests, departures from the HWE expectations turned out to be statistically not significant. The forensic efficiency parameters found in the Polish population are included in **SupplementaryTable 2**. The observed heterozygosity (HET) values ranged from 0.617 to 0.962, power of discrimination (PD) value spanned from 0.785 to 0.994, and power of exclusion (PE) value stretched from 0.311 to 0.922, leading to accumulated values of PD = 0.99999999999999999999999998 and PE = 0.99999999899. The overall value of matching probability (MP) was be extremely low, 1.8×10^{-26}, i.e., specific multiallelic DNA profile appears in the population, on average, in 1 out of 5.7×10^{25} people. This value is much higher than European recommendations require, i.e., 1 in 1 billion is the minimum reported match probability in UK forensics practice [6].

Pairwise correlation analysis was carried out in syntenic pairs of the studied loci, D2S441, D2S1338, and TPOX (on chromosome 2); D5S818 and CSF1PO (on chromosome 5); and D12S391 and VWA (on chromosome 12), which showed no linkage disequilibrium (LD) (**SupplementaryTable 3**). This is particularly important in the case of syntenic loci that are close on the same chromosome's arm, i.e., CSF1PO/D5S818 with 28 cM (centimorgans) and D12S391/VWA with 12 cM recombination distances. Loci D2S1338/D2S441 and D2S1338/TPOX are located on different chromosome arms, and D2S441/TPOX, which are located on the same arm, are separated by a recombination distance of more than 50 cM, which is sufficient to assure independent assortment [7]. The fact that the association between all of the pairs of syntenic loci was not detectable at the population level, similar to previously reported data [8,9], allows the combined values of the forensic data to be determined by multiplying the values of the individual 21 markers included in the GlobalFiler™ kit. However, when relatives are involved, evaluation of the value of forensic evidence should take into account the close physical proximity between the loci VWA and D12S391, which are only 6.37 Mb apart [10].

The average value of φ for the studied population was established at a very low level, below 0.01 (**SupplementaryTable 2**). This value is recommended by the FBI as a conservative substructure correction factor for the majority of large populations

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in which there is the lowest likelihood of inbreeding [11]. According to the European recommendations (UK), this threshold should be even less restrictive, i.e., 0.03, which is sufficient to ensure that the results are conservative with any reference database [6].

The investigated set of 21 autosomal STR markers included in the 6-dye kit GlobalFiler Kit makes this kit an excellent tool for kinship analysis and provides a highly discriminating level of forensic genetic evaluation in Poland, which reduces the risk of adventitious matches of DNA profiles. This study followed the guidelines for population data requested by the journal [12,13] as well as the International Society for Forensic Genetics (ISFG) [14].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.fsigen.2017.05.003.

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