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Forensic and population genetic analyses of Danes, Greenlanders and Somalis typed with the Yfiler® Plus PCR amplification kit

Jill Katharina Olofsson *, Helle Smidt Mogensen, Anders Buchard, Claus Børsting, Niels Morling

Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

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ABSTRACT

Recently, the Yfiler® Plus PCR Amplification Kit (Yfiler® Plus, Thermo Fisher Scientific, Waltham, MA, USA) was introduced. Yfiler® Plus amplifies 27 Y-chromosomal short tandem repeat loci (Y-STRs) and adds ten new Y-STRs to those analysed with the commonly used AmpFlSTR® Yfiler® PCR Amplification Kit (Yfiler®, Thermo Fisher Scientific, Waltham, MA, USA). Seven of the new Y-STRs are rapidly mutating Y-STRs (RM Y-STRs). In this study, 551 male individuals from Denmark, Greenland and Somalia were typed with Yfiler® Plus. The results were compared to those obtained with Yfiler® in the same individuals. Forensic and population genetic parameters were estimated for Yfiler® Plus. Yfiler® Plus had a higher power of discrimination than Yfiler® in all three populations. Compared to Yfiler®, Yfiler® Plus offers increased power of discrimination, which is obviously an advantage in crime case investigations. However, the inclusion of seven RM Y-STRs in Yfiler® Plus makes it less attractive for relationship testing because of the relatively high combined mutation rate, approximately 15%.

1. Introduction

Y-chromosomal short tandem repeats (Y-STRs) are used in certain cases in forensic genetics. In relationship testing, the paternal inheritance of the Y chromosome is utilised to establish the paternal lineage [1,2]. In crime case work, Y-STRs are primarily used to investigate male-specific components of female–male DNA mixtures with small proportions of male DNA and large portions of female DNA [2]. Such mixtures are commonly encountered in sexual assault cases.

The minimal haplotype (MHT) with nine Y-STRs [3] and the haplotype of the Scientific Working Group for DNA Analysis Methods (SWGDAM) with 11 Y-STRs [4] were defined more than a decade ago. However, the SWGDAM haplotype is no longer supported. These loci are included in the commonly used Y-STR kits, AmpFlSTR® Yfiler® PCR Amplification Kit [5] (Yfiler®, Thermo Fisher Scientific, Waltham, MA, USA) and PowerPlex® Y System (PPY® 12, Promega, Madison, WI, USA) (Table S1). Yfiler® detects 17 Y-STRs [5] that are also included in the Yfiler® Plus PCR Amplification Kit (Yfiler® Plus, Thermo Fisher Scientific, Waltham, MA, USA), which has been supplemented with 10 Y-STRs in order to increase the power of discrimination among unrelated and related individuals.

The Y-STRs in the MHT and SWGDAM sets have mutation rates of the order of 10^{-3}. A set of Y-STR loci with mutation rates above 0.01 was recently described [6]. These rapidly mutating Y-STRs (RM Y-STRs) are very useful for the discrimination between closely related and unrelated males [6,7]. RM Y-STRs are included in the latest generation of commercial Y-STR multiplex kit. The 23 loci PowerPlex® Y23 System (PPY® 23, Promega Madison, WI, USA) includes two RM Y-STR loci [8,9] and the 27 loci Yfiler® Plus includes seven RM Y-STRs.

In this study, the results obtained with Yfiler® Plus and Yfiler® were compared. Population and forensic genetic parameters were estimated for Yfiler® Plus in Danish, Greenlandic and Somali males.

2. Materials and methods

2.1. Samples

A total of 551 male individuals, 185 from Denmark, 189 from Greenland and 177 from Somalia (Table S2) were investigated with Yfiler® Plus. The individuals from Greenland were born in Greenland and the individuals from Somalia were self-declared

* Corresponding author at: Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Frederik V's Vej 11, DK-2100 Copenhagen, Denmark.
Tel.: +45 35 32 73 01; fax: +45 35 32 62 70.
E-mail address: jill.olofsson@sund.ku.dk (J.K. Olofsson).

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Somalis. All the samples involved in the study were anonymised DNA extracts obtained from healthy unrelated individuals. The project was approved by the Danish ethical committee (KF-01-037/03, H-1-2011-081 and H3-2012-023) and complied with the ethical principles of the 2000 Helsinki Declaration of the 206 World Medical Association.

2.2. Y-STR analyses

All samples were investigated with Yfiler® Plus according to the manufacturer's protocol with modifications in the reaction volume (12.5 μl), the volume of input DNA (0.5 μl) and the number of PCR-cycles (25–29). Prior to electrophoresis, 1 μl of the amplified products and 0.5 μl of GeneScan™ 600 LIZ® Size Standard v. 2.0 were added to 9.5 μl of deionized Hi-Di™ formamide and denatured for 3 min at 95 °C. The samples were electrophoresed on an Applied Biosystems® 3500 × L Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) according to the recommendations of the manufacturer except for a slight modification of the injection time (12 s). The electropherograms were analysed using GeneMapper® IDX v. 1.4 (Thermo Fisher Scientific, Waltham, MA, USA) with the default settings except that the peak amplitude thresholds were set to 75 RFU for the blue and green, 125 RFU for the yellow, 175 RFU for the red and purple and 50 RFU for the orange colurs. All the results were compared with previously published data on the PPY® 12 [10–12], Yfiler® [13,14], RM-Y-STRs [13], and PPY® 23 [15] using an in-house written script in the statistical software program R v. 2.15.3 [16]. Additionally, 28 of the male Somalis were typed with Yfiler®. The results were reported to the Y-chromosomal haplotype reference database (YHRD) [17] in accordance with the updated guidelines of Forensic Science International Genetics under the accession numbers YA003283 (Danes) YA003999,YA004000, YA004001, YA004002 YA004003 (Greenlanders) and YA003284 (Somalis). The Yfiler® Plus haplotypes are presented in TableS7.

2.3. Statistical analyses

Forensic and population genetic parameters were estimated for the various sets of Y-STRs. The allele and haplotype frequencies were estimated by the counting method. The genetic diversities (GD) of the markers were calculated according to Nei [18]. The match probability (MP) was calculated as the sum of the squared haplotype frequencies, and the power of discrimination (PD) was calculated as the ratio between the number of different haplotypes and the total number of haplotypes. Haplotype diversities (HD) were calculated as one minus the MP times the number of haplotypes divided with the number of haplotypes minus one.

The genetic distance between populations was evaluated as R_{ST} in Arlequin v. 3.5.1.2 (10,000 permutations) [19] and visualised through multidimensional scaling (MDS) (cdmscale function) plots using the statistical software program R v. 2.15.3 [16]. The variances at the individual Y-STR loci were calculated as previously described [20]. The median joining networks of haplotypes (15 Yfiler® and 23 Yfiler® Plus Y-STR haplotypes) were constructed using the program Network v. 4.6.1.1 (http://www.fluxus-engineering.com) [21]. The weights (1–5) given to the loci were based on the inverse variance of the Y-STRs. The following weight groups were used; 1: inverse variance 0–0.5, 2: inverse variance 0.5–1, 3: inverse variance 1–1.5, 4: inverse variance 1.5–2, 5: inverse variance >2. The multi-copy loci DYS385 and DYF387S1 and the haplotypes with null or intermediate alleles and copy number variants were excluded from the R_{ST} and median joining network analyses.

For all statistical analyses, the alleles of the DYS389II locus were converted to the DYS389B nomenclature by subtracting the repeat number of the DYS389I locus from that of the DYS389II locus.

3. Results

3.1. Single-locus analyses

The Y-STR loci were divided into a group with one allele (single-allelic loci) and a group with two or more alleles (multi-copy loci). A total of 212 different alleles were detected among the 23 single-allelic loci typed with Yfiler® Plus. Of these, 35 (16%) were observed in only one population (Table S3). The median number of alleles per locus was 7.5 (range: three for DYS437 to 15 for DYS458). Seven different microvariants were found in four loci. Two null alleles, one in DYS448 and one in DYS570, were found among the male Danes. A duplication event in YGATA H4 was noted in a male Greenlander (Table S3).

The number of allelic combinations for the two multi-copy loci DYS385 and DYF387S1 are presented in Table S3. Six microvariants, all population specific, were found in DYF387S1. The ratio between the peak heights was calculated. Combinations of two unbalanced alleles with height ratio >1.9:1 were considered to be three alleles. Nine Danes and Greenlanders had two unbalanced alleles in DYF387S1 that were interpreted as three alleles (Table S4). One Dane and two Greenlanders had three alleles in DYF387S1 (Table S4).

The genetic diversity (GD) was below 0.50 at two loci (8%) in Danes, one locus (4%) in Greenlanders and 11 loci (44%) in Somalis (Fig. 1 and Table S3). The GD was above 0.70 at 11 loci (44%) in Danes, 10 loci (40%) in Greenlanders and seven loci (28%) in Somalis (Fig. 1 and Table S3). As expected, the RM Y-STR loci showed high GD values (>0.70) in all three populations (Fig. 1 and Table S3).

3.2. Haplotype analyses

Typing with Yfiler® Plus resulted in 185, 149 and 167 different haplotypes in the male Danish, Greenlandic and Somali populations, respectively (Table 1). This was three, 42 and 59 haplotypes, respectively, more than the number of haplotypes only considering the 17 loci in Yfiler® (Table 1). The frequency of unique haplotypes (singletons) was increased when the 10 additional Y-STRs in the Yfiler® Plus kit were typed (Danes: 97% to 100%, Greenlanders: 44% to 67% and Somalis: 51% to 91%) (Table 1).

The Yfiler® Plus results from this study were combined with previously published Y-STR data for Danes, Greenlanders and

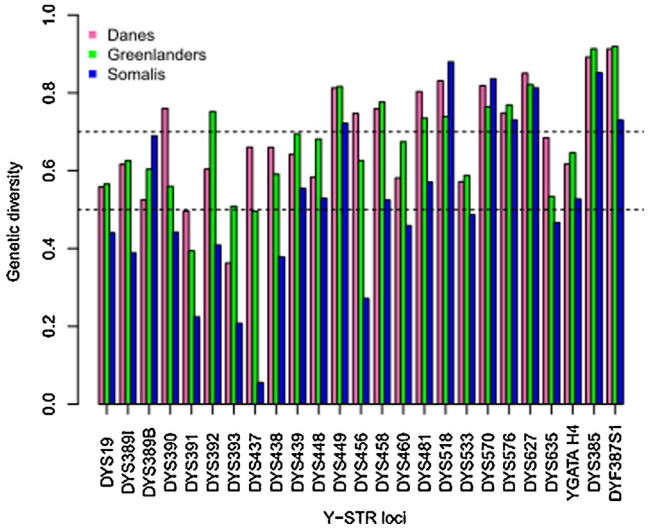

Fig. 1. The genetic diversities of the 27 loci typed with Yfiler® Plus. Broken lines represent genetic diversity of 0.50 and 0.70.