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Short communication

Forensic genetic value of a 27 Y-STR loci multiplex (Yfiler® Plus kit) in an Italian population sample

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ABSTRACT

The analysis of Y chromosome short tandem repeat (Y-STR) haplotypes provides important information that can be used for investigative purposes and in population studies. The Yfiler® Plus PCR Amplification kit (Yfiler® Plus, Thermo Fisher Scientific, Waltham, MA, USA) allows the multiplex amplification of 27 Y-STRs, including 7 rapidly mutating markers (RM Y-STRs). In this study, 203 unrelated males from Italy, which were subdivided into 4 different geographical groups (North, Center, South and Sardinia) were analyzed. Several intra-population diversity indexes were computed and compared to those obtained using only loci either from the minimal haplotype or the 17-plex (Yfiler®, Thermo Fisher Scientific, Waltham, MA, USA). In addition, inter-population diversity analysis (R_{ST}) among the four Italian samples was performed. The same analysis was also used to compare the Italian sub-sets to other European populations where the Yfiler® Plus haplotype frequency data were available.

The Sardinians were significantly differentiated from the other three Italian groups, thus requiring a specific sub-national Y-STR haplotype database. The Yfiler® Plus kit showed a high power of discrimination which is useful for criminal investigations, principally due to the inclusion of RM Y-STRs.

1. Introduction

Analysis of Y-chromosomal short tandem repeat (Y-STR) markers has been widely accepted as a valuable method to be used in the identification of individuals in specific forensic cases such as sexual assaults where a very low amount of male DNA is mixed with a high amount of female DNA [1]. Y-STRs have also been used in a variety of kinship testing [2,3] and to infer ancient human migration trajectories and timing [4,5].

The Yfiler® Plus kit, recently released by Thermo Fisher, is a 6-dye multiplex PCR kit that simultaneously amplify 27 Y-STR targets and includes 7 rapidly mutating loci (RM Y-STRs). In addition, Yfiler® Plus kit includes 3 additional Y-STRs (DYS460, DYS481 and DYS533) that were added to the original set of markers of the Yfiler® kit [6] (ThermoFisher Scientific, Waltham, MA, USA). The Y-STR markers that are included in both kits have the same amplification primers in order to be consistent in the allele frequency. RM Y-STRs increase the discrimination power not only between unrelated individuals but also between males of the same patrilineage [7,8] making it possible, in some cases, to discriminate between first degree relatives [8,9].

In the present study, we used the Yfiler® Plus kit to type a cohort of 203 unrelated individuals from 4 geographically different Italian areas (northern Italy, central Italy, southern Italy and Sardinia). Genetic diversity indexes and haplotype frequencies were evaluated and the data were used to compare the Italian populations with other European populations that had been previously typed with the same multiplex.

2. Materials and methods

2.1. Samples

A total of 203 male individuals here analyzed for the first time (52 from northern Italy, 50 from the central area, 50 from southern Italy and 51 from Sardinia. Supplementary Fig. 1) were investigated

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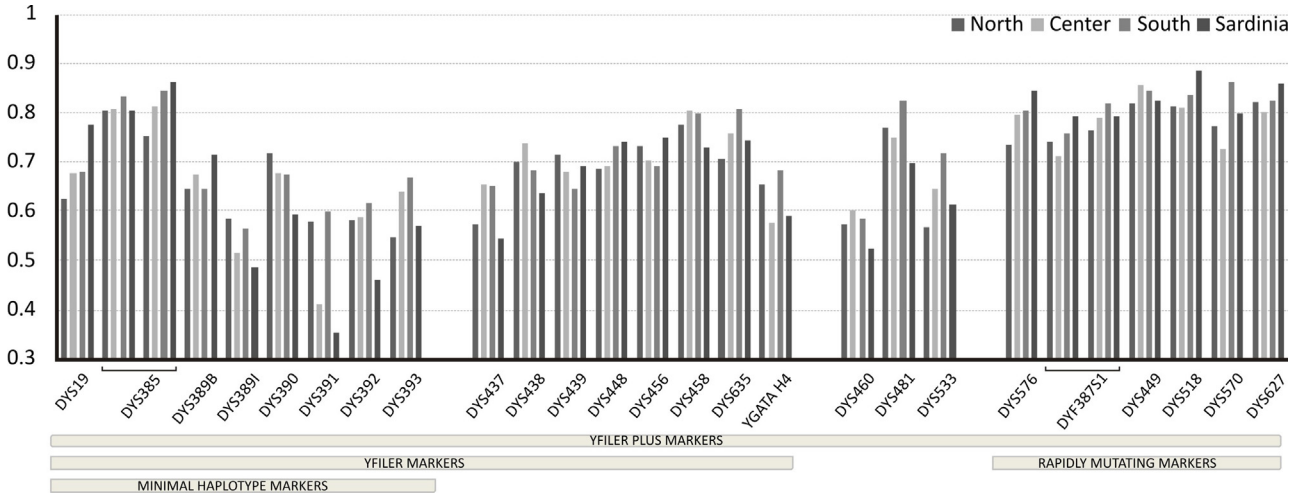

Fig. 1. Diagram showing the genetic diversity (top panel) and the total number of alleles (bottom panel) for each marker in the four geographic areas analyzed. Both DYS385 and DYF387S1 multilocus markers were treated as two separate loci (see text for details).

with Yfiler® Plus. All DNA samples were obtained from healthy unrelated individuals, born and resident in the selected areas for at least three generations and all voluntary participants gave an informed consent. The research project has been approved by the General Command of the Arma dei Carabinieri under the Ministry of Defense.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2015.11.006.

2.2. DNA extraction and Y-STRs analysis

Genomic DNA was extracted from blood or buccal swabs on an EZ1 Advanced XL instrument with a Qiagen's EZ1 DNA Investigator kit (Qiagen Inc., Valencia, CA) and quantified with a Quantifiler® Trio Quantification Kit (ThermoFisher Scientific, Waltham, MA, USA).

Table 1
Diversity indexes for the global sample from Italy.

	Italy (203)		
Observed haplotype	MH	Yfiler® kit	Yfiler® plus kit
Once	152	191	197
Twice	18	6	3
3 times	3	–	–
6 times	1	–	–
HD	0.9980	0.9997	0.9999
HMP	0.0070	0.0052	0.0051
DC	0.8571	0.9704	0.9852

HD = Haplotype diversity; HMP = Haplotype match probability; DC = Discrimination capacity; MH = Minimal haplotype.

Y-STR amplification was performed on an Applied Biosystems® GeneAmp® PCR System 9700 Silver Block Thermal Cycler (ThermoFisher Scientific, Waltham, MA, USA) using the Yfiler® Plus PCR Amplification Kit (Yfiler® Plus, ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol from 1 ng of total DNA. Amplified DNAs were electrophoresed on a 3500 XL Genetic Analyzer (ThermoFisher Scientific, Waltham, MA, USA) and the fragment analysis was performed with a GeneMapper® IDX v.1.4 (Thermo Fisher Scientific, Waltham, MA, USA). The Authors followed ISFG recommendations for the analysis of the polymorphisms. Haplotype data were submitted to the Y-chromosomal haplotype reference database (www.yhrd.org) [10] (YHRD accession number: YA004045 for Sardinia and YA003983 for northern, central and southern Italy). The contributor successfully passed the quality control test.

2.3. Statistical analysis

The genetic diversity (GD) for each locus was calculated according to Nei and Tajima [11]. Haplotype frequencies were determined using the counting method. The haplotype diversity (HD) was calculated using the formula $HD = n(1 - \Sigma p_i^2)/(n - 1)$, where n is the sample size and p_i the frequency of the ith haplotype. The haplotype match probability (HMP) was estimated using the formula $HMP = \Sigma p_i^2$, whereas the discrimination capacity (DC) was obtained dividing the total number of observed haplotypes by the total number of individuals in the dataset. All the above diversity indexes were computed using the Arlequin software ver. 3.5.1.3 [12]. Between-population genetic distances (R_{ST}) and multidimensional scaling (MDS) analyses were obtained

Table 2
Diversity indexes for the four Italian sub-groups.

	Italy North (52)			Italy Center (50)			Italy South (50)			Italy Sardinia (51)		
Observed haplotype	MH	Yfiler® kit	Yfiler® Plus kit	MH	Yfiler® kit	Yfiler® Plus kit	MH	Yfiler® kit	Yfiler® Plus kit	MH	Yfiler® kit	Yfiler® Plus kit
Once	39	46	48	48	48	48	44	50	50	41	49	51
Twice	5	3	2	1	1	1	3	–	–	5	1	–
3 times	1	–	–	–	–	–	–	–	–	–	–	–
HD	0.9940	0.9977	0.9985	0.9992	0.9992	0.9992	0.9976	1	1	0.9961	0.9992	1
HMP	0.0251	0.0214	0.0207	0.0208	0.0208	0.0208	0.0224	0.0200	0.0200	0.0235	0.0204	0.0196
DC	0.8654	0.9423	0.9615	0.9800	0.9800	0.9800	0.9400	1	1	0.9020	0.9804	1

HD = Haplotype diversity; HMP = Haplotype match probability; DC = Discrimination capacity; MH = Minimal haplotype.