Int J Legal Med (2006) 120: 56–59
DOI 10.1007/s00414-005-0054-x

SHORT COMMUNICATION

Stefania Turrina · Renzo Atzei · Domenico De Leo

Y-chromosomal STR haplotypes in a Northeast Italian population sample using 17plex loci PCR assay

Received: 11 March 2005 / Accepted: 28 September 2005 / Published online: 19 November 2005

Abstract One hundred fifty-five unrelated, autochthonous healthy males from Northeast Italy were typed for the 17 Y-chromosome short tandem repeat (STR) (Y-STR) loci DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438, DYS448 using the AmpFLSTR Yfiler polymerase chain reaction amplification kit. A total of 153 different haplotypes were observed, and among these, 151 were unique, while 2 were found two times. The overall haplotype diversity was 0.9997. Furthermore, 50 father–son pairs, previously confirmed by autosomal STR analysis, were typed using the same set of 17 Y-STR loci, and, among 850 allele transfers, three mutation events were identified, giving an average mutation rate of 3.53×10^{-3} per locus per generation (95% confidence interval 0.73–1.03).

Keywords Y chromosome · Haplotype diversity · Population genetics · North Italy

Introduction

Polymorphic Y-chromosome-specific short tandem repeats (STRs) (Y-STRs) have become a very useful tool both in evolutionary studies and in forensic casework. In particular, Y-STRs can be very useful in paternity testing involving male children to exclude or establish paternity or paternal lineage [6, 10]. Furthermore, the use of Y-STR in sexual assault cases containing a mixture of male and female DNA can improve the chances of detecting low levels of male DNA in a high background of female DNA [5, 9, 12]. Nevertheless, because of the lack of Y-chromosome recombination, Y-STR haplotype diversity (HD) is lower than that of similar autosomal STR panels. Thus, to achieve a power of discrimination (PD) similar to that for autosomal STR sets, a large number of Y-chromosome markers are needed.

A practical approach to improve the potential DC of Y-STR haplotype is to combine the most polymorphic markers into a Y-STR multiplex.

In this study, we report allele frequencies and basic forensic parameters for a new 17 Y-STR multiplex set commercially available as the AmpFLSTR Yfiler polymerase chain reaction (PCR) amplification kit (Applied Biosystems, Forest City, CA, USA) and includes all nine loci of the European minimal haplotype (DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393), two loci recommended by SWGDAM (DYS438, DYS439), and six other markers: DYS456, DYS458, DYS635 (Y GATA C4), Y GATA H4, DYS437, DYS448.

Electronic supplementary material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00414-005-0054-x

S. Turrina · R. Atzei · D. De Leo (✉)
Department of Medicine and Public Health,
Institute of Legal Medicine, Forensic Genetic Laboratory,
University of Verona, Policlinico G.B. Rossi,
Piazzale L.A. Scuro,
37134 Verona, Italy
e-mail: deleodom@tin.it
Fax: +39-045-505259

Materials and methods

DNA samples

A total of 155 unrelated, autochthonous healthy males, living in Northeast Italy and the male children of 50 among these, confirmed by autosomal STR analysis using AmpFLSTR Identifiler PCR amplification kit (Applied Biosystems), with paternity probability >99.9%, were studied. Genomic DNA was extracted from peripheral blood by GenomicPrep Blood DNA Isolation Kit (Amersham Biosciences, Piscataway, NJ, USA). The amount of human DNA was determined by spectrophotometry.

Y-STR typing

Aliquots of 10–20 ng DNA were amplified in a total reaction volume of 25 μl consisting of 9.2 μl AmpFLSTR

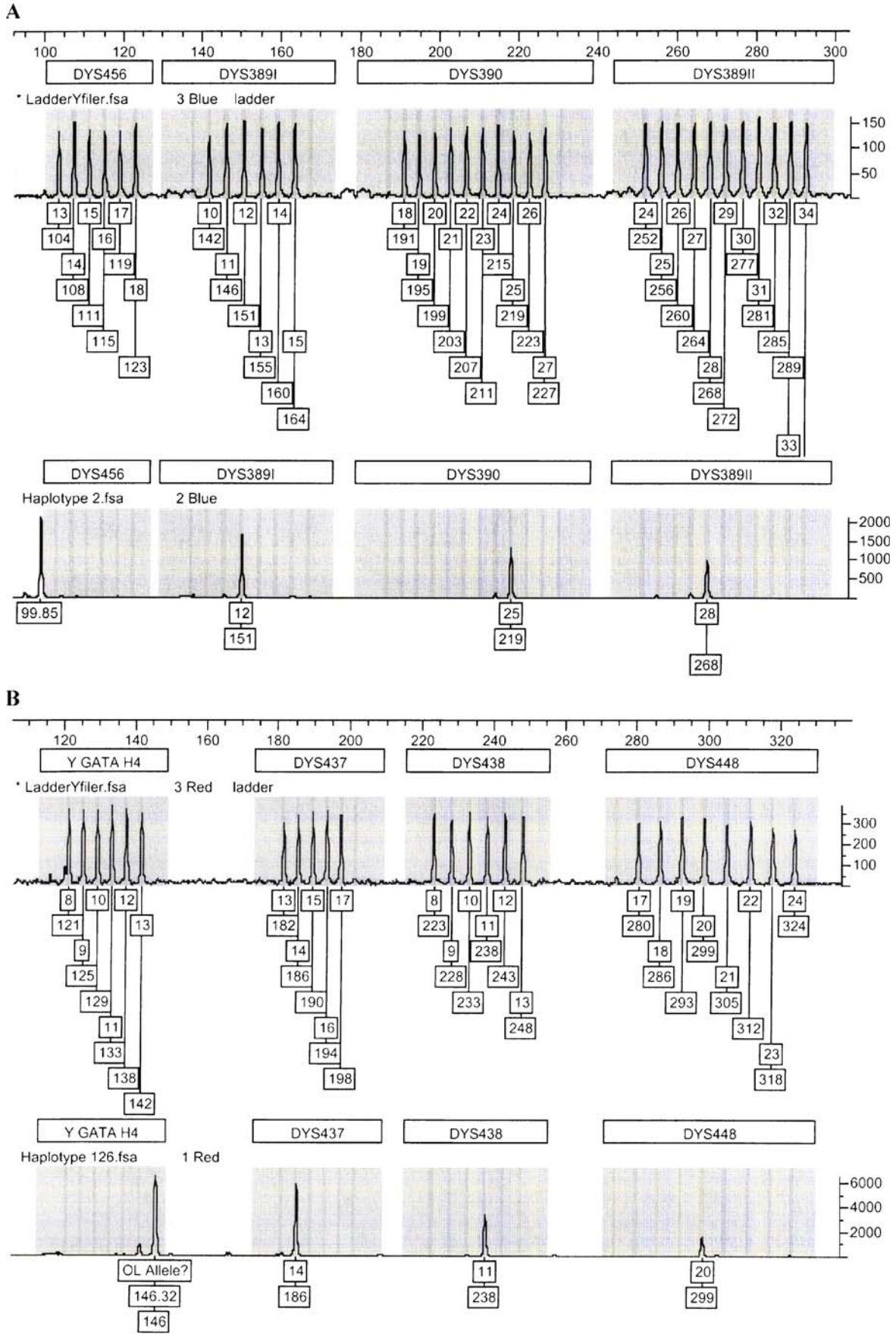

Fig. 1 An example of two off-ladder alleles: allele 12 with 99.85 bp at DYS456 locus (**a**) and allele 14 with 146 bp at Y GATA H4 locus (**b**). These alleles are not included in the specific allelic ladders that are shown in the upper part of the picture. The estimated size in base pair of the amplification products is reported below the allele designation

Table 1 Y-STR mutations observed in three father–son pairs

Father–son pairs	Locus allele	Repetitive sequence structure	Fathers' age
Father	DYS398I 12	(TCTG)3(TCTA)9	39
	DYS389II 30	(TCTG)4(TCTA)14… ..(TCTG)3(TCTA)9	
Son	DYS398I 11	(TCTG)3(TCTA)8	
	DYS389II 29	(TCTG)4(TCTA)14… ..(TCTG)3(TCTA)8	
Father	DYS456 16	(AGAT)16	28
Son	DYS456 15	(AGAT)15	
Father	DYS458 17	(GAAA)17	23
Son	DYS458 18	(GAAA)18	

Table 3 Number of different haplotypes, unique haplotypes, and HD values found for three combinations of Y-STR loci in 155 unrelated individuals

	European minimal haplotype nine Y-STR loci	PowerPlex Y System[a] 12 Y-STR loci	AmpFLSTR Yfiler[b] 17 Y-STR loci
No. of different haplotypes	130	143	153
No. of unique haplotypes	115	134	151
HD	0.9967	0.9986	0.9998

[a]Promega
[b]Applied Biosystems

Yfiler PCR reaction mix, 5.0 µl of AmpFLSTR Yfiler kit primer set, and 0.8 µl of AmpliTaq Gold DNA polymerase. Amplification was carried out in a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems) under the conditions reported in the user's manual of the AmpFLSTR Yfiler PCR amplification kit.

Samples were prepared by mixing 8.7 µl of Hi-Di formamide with 0.3 µl of GeneScan-500 LIZ size standard (Applied Biosystems) and 1 µl of PCR product. Separation and detection of Y-STR 17plex PCR products were accomplished with the ABI Prism 3100 Avant Genetic Analyzer four-capillary array system (Applied Biosystems) following the manufacturer's protocols using POP4 polymer, 36 cm×50 µm capillary array, and the G5 matrix filter set to detect the five dyes 6FAM, VIC, NED, PET, and LIZ. Prior to sample analysis, a spectral matrix was established using matrix standard set DS-33 (Applied Biosystems). Following data collection, genotyping of each samples was carried out automatically using the allelic ladders provided with the Y-17plex amplification kit and AmpFLSTR Yfiler kit template 9 macro (Applied Biosystems).

Statistical analysis

Allele and haplotype frequencies were estimated by gene/haplotype counting. DYS385 shows variation at two loci simultaneously which cannot be differentiated, and it is analyzed as a "genotype," as recommended by DNA Commission of International Society of Forensic Genetics [4]. Gene diversity (GD) was calculated for each locus using the equation $GD = (1 - \sum q_i^2)(n/n-1)$, where n is the sample size and q_I is the allelic frequency. The HD for all 17 Y-STR loci, corresponding to the PD or chance of exclusion for unrelated males, was computed with the same formula using haplotype frequencies instead of allele frequencies. The haplotype match probability (HMP), i.e., the probability of finding an identical haplotype in a pair of randomly unrelated males, was calculated as HMP=1−HD [2]. The discriminatory capacity (DC) was determined by dividing the number of different haplotypes by the number of samples in a given population [6]. Mutation rate was estimated as the number of mutations, divided by the number of meiosis analyzed, and statistical calculations were carried out using StatCalc 1.1 (http://www.ucs.louisiana.edu/kxk4695).

Table 2 Age distribution of 47 fathers without mutation events

Fathers' age groups	Number of fathers
20–25	10
26–30	8
31–35	12
36–40	7
41–45	5
46–50	3
51–55	2

Results and discussion

In our sample of 155 unrelated, autochthonous healthy males living in Northeast Italy, we observed a total of 153 distinct haplotypes, among which, 151 were unique (97.40%) and 2 occurred two times (Table S1). Allele frequencies and GD for each Y-STR loci analyzed in this population are shown in Table S2. The number of alleles found for each locus ranged from 3 to 7 with the exception of DYS385 loci, which presented 33 genotypes (Table S3). Almost all Y-STR systems reached values for GD higher than 0.5, except DYS393 (GD=0.492). The present haplotype data were compared with those reported by [1] for Austrian population, the only Caucasian data available for the same set of the 17 Y-STR loci, and no significant differences were observed.

Furthermore, in our population study, alleles 12 of 99.85 bp at DYS456 locus and 14 of 146 bp at Y GATA H4 locus were observed two times (haplotypes n.1, n.2 and n.126, n.128 in Table S1, respectively, as shown in Fig. 1). These alleles are not included in the allelic ladders provided with the AmpFLSTR Yfiler PCR amplification kit (Applied Biosystems) but have been described in other population studies [11, 13, 14].

In the 50 father–son pairs analyzed, three mutation events were identified at the DYS389I, DYS456, and DY

S458 loci, and, in two events, a reduction of allele size by a single unit repeat was observed (Table 1). In one father–son pair, two mutation events at the DYS389I (allele 12 to allele 11) and DYS389II (allele 30 to allele 29) loci were observed. In this case, both DYS389I and DYS389II differed by a single-unit repeat change in the same direction, and, because the primers designed for these two Y-STR loci encompass the PCR product of DYS389I within that of DYS389II, mutations in DYS389I would automatically lead to lengthening of the whole locus. In other words, this apparent double mutation is a single original event and should be counted as a single mutation. For the three fathers with a mutation, their age at the child's birth was 23–39 years [average age 30±8.19 (DS) years], while for the 47 fathers without mutations (Table 2), their age at the child's birth was 20–54 years [average age 33.30±10.14 (DS) years]. A nonparametric test (Mann–Whitney *U* test) showed that the difference in age between the two groups is not significant (p=0.182). The average mutation rate was estimated at 3.52×10^{-3} per locus per generation (3/850), with a 95% confidence limit of 0.73×10^{-3} to 1.03×10^{-2}. This rate is not significantly different (p>0.05) from that reported in other Y-STR studies [3, 7, 8].

With regard to the correlation between mutation rate and Y-STR's structure, our observations fitted in with those of [7]: the mutation always occurred either in the most frequent alleles (as for locus DYS458 when a single repeat is gained) or in alleles longer than the most frequent ones (as for DYS456 and DYS89II when a single repeat is lost). Furthermore, it was noted that mutations occurred mostly in the Y-STR loci with less than 170 bp: DYS456, DYS458, and DYS389I.

The most common way to measure the ability of a Y-STR assay to resolve two unrelated male samples is determined by the HD value and corresponding random HMP. For this 17 Y-plex, HD was 0.9998, HMP was 0.0002, while the overall DC was 98.70%.

Comparing the number of haplotypes produced and the respective HD between the nine Y loci of the European minimal haplotype, 12 Y-plex (PowerPlex Y System, Promega), and the new 17 Y-STR set (Table 3), we can see that the 17 Y-plex has a higher PD and is therefore more informative. The possibility of erroneous sample identification with few Y-STR markers is high if compared with a panel of 17 Y-STRs. This means that it is very important to increase the number of Y-microsatellites for individual identification and paternity testing in forensic genetics to improve the discrimination power.

Acknowledgements This work was supported by a grant from M.I.U.R. and "Donazione Miria Cherubini e Amleto Loro."

References

1. Berger B, Lindinger A, Niederstätter H, Grubwieser P, Parson W (2005) Y-STR typing of an Austrian population sample using a 17-loci multiplex PCR assay. Int J Legal Med. DOI 10.1007/s0041400505468
2. Bosch E, Lee AC, Calafell F, Arroyo E, Henneman P, De Knijff P, Jobling MA (2002) High resolution Y chromosome typing: 19 STRs amplified in three multiplex reactions. Forensic Sci Int 125:42–51
3. Dupuy BM, Andreassen R, Flones AG et al (2001) Y chromosome variation in a Norwegian population sample. Forensic Sci Int 117:163–173
4. Gusmão L, Butler ML, Carracedo A et al (2005) DNA Commission of the International Society of Forensic Genetics (ISFG): an update of the recommendations on the use of Y-STRs in forensic analysis. Int J Legal Med. DOI 10.1007/s0041400500261
5. Hall A, Ballantyne J (2003) Novel Y-STR typing strategies reveal the genetic profile of the semen donor in extended interval post-coital cervicovaginal samples. Forensic Sci Int 136:58–72
6. Kayser M, Caglià A, Corache D et al (1997) Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med 110:125–133
7. Kayser M, Roewer L, Hedman M et al (2000) Characteristics and frequency of germline mutations at microsatellite loci from human Y chromosome, as revealed by direct observation in father/son pairs. Am J Hum Genet 66:1580–1588
8. Kurihara R, Yamamoto T, Uchihi R et al (2004) Mutations in 14 Y-STR loci among Japanese father–son haplotypes. Int J Legal Med 118:125–131
9. Parson W, Niederstätter H, Kochl S, Steinlechner M, Berger B (2001) When autosomal tandem repeats fail: optimized primer and reaction design for Y-chromosome short tandem repeat analysis in forensic casework. Croat Med J 42:285–287
10. Redd AJ, Agellon AB, Kearney VA, Contreras VA et al (2002) Forensic value of 14 novel STRs on the human Y chromosome. Forensic Sci Int 130:97–111
11. Schoske R, Vallone PM, Kline MC, Redman JW, Butler JM (2004) High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays. Forensic Sci Int 139:107–121
12. Sibille I, Duverneuil C, De La Grandmaison GL, Guerrouache K, Teissière F, Durigon M, De Mazancourt P (2002) Y-STR DNA amplification as biological evidence in sexually assaulted female victims with no cytological detection of spermatozoa. Forensic Sci Int 125:212–216
13. Tang JP, Hou YP, Li YB, Wu J, Zhang J, Zhang HJ (2003) Characterization of eight Y-STR loci and haplotypes in a Chinese Han population. Int J Legal Med 117:263–270
14. Uchihi R, Yamamoto T, Usuda K et al (2003) Haplotype analysis with 14 Y-STR loci using 2 multiplex amplification and typing systems in 2 regional populations in Japan. Int J Legal Med 117:34-38