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Short Communication

Population and mutation analysis of Y-STR loci in a sample from the city of São Paulo (Brazil)

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Abstract

The haplotypes of seven Y-chromosome STR loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393) were determined in a sample of 634 healthy Brazilian males (190 adult individuals and 222 father-son pairs). The 412 adults were unrelated, and the 222 father-son pairs had their biological relationship confirmed using autosomal STRs (LR > 10,000). Among the 412 adults, a total of 264 different 7-loci haplotypes were identified, 210 of which were unique. The most frequent haplotype was detected in 31 instances, occurring with a frequency of 7.52%. The haplotype diversity index was calculated as 98.83%. Upon transmission of the 1,554 alleles, in 222 father-son pairs, six mutations were observed, with an average overall rate of 3.86×10^{-3} per locus. A haplotype with a duplicated DYS389I locus, and another with duplicated DYS389I, DYS389II, and DYS439 loci were detected in both fathers and their respective sons.

Key words: Y-STR population data, São Paulo (Brazil), mutation rates, duplications.

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Y-chromosome STR typing has become an important tool in forensic analysis (Betz *et al.*, 2001; Sibille *et al.*, 2002; Cerri *et al.*, 2003; Shewale *et al.*, 2003; Shewale *et al.*, 2004; Delfin *et al.*, 2005; Johnson *et al.*, 2005). Recently, the DNA Commission of the International Society of Forensic Genetics (ISFG) has published guidelines and recomendations concerning the use of Y-STRs polymorphisms in human identification and kinship analysis (Gusmão *et al.*, 2005). According to a recent Brazilian government census (IBGE), 54% of Brazilians were self-declared as white, 38% as mixed (mulatto), and 6% as black; 2% were classified in other categories that include Orientals and Amerindians, with striking regional differences. For instance, mixed tri-hybrid types are overwhelmingly predominant (almost 100%) in some parts of the northeastern region, whereas whites vastly predominate in the southern states (almost 100% in some inner regions of the states of Santa Catarina and Rio Grande do Sul). Several studies performed in different population samples from Brazil (Costa *et al.*, 2002; Grattapaglia *et al.*, 2005; Cainé *et al.*, 2005;; Carvalho-Silva *et al.*, 2006; Silva *et al.*, 2006; Domingues *et al.*, 2007; Palha *et al.*, 2007) have shown, however, that in spite of this racial melting pot, genes carried on the Y chromosome are almost exclusively of European origin (Iberian, Mediterranean, and Central-European), while analyses of mtDNA variability in Brazilian samples revealed that about 60% of the maternal lineages are Amerindian and African (Carvalho-Silva *et al.*, 2006). Therefore, regardless of this intense gene flow and high degree of genetic heterogeneity, Y chromosome polymorphisms in Brazilian males have a distribution typical of a mixed European population. This paper presents data on 7 Y-STR loci DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393 in a Brazilian mixed population sample from the city of São Paulo.

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Whole blood samples were collected from 634 healthy Brazilian males (190 adult individuals and 222 pairs of fathers and respective sons), under written informed consent. The 412 adults were unrelated and the 222 father-son pairs had their biological relationship confirmed by paternity index values larger than 10,000 obtained by means of autosomal STRs typing. DNA was extracted from 5 mL of peripheral blood by a salting-out procedure (Miller *et al.*, 1988), and quantified by spectrometry (Ultrospec III, Pharmacia, Piscataway, NJ, USA). The amplification of DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393 loci was performed according to Kayser *et al.* (1997), in two multiplex reactions, one triplex (DYS391,DYS392, DYS393), and one tetraplex (DYS19, DYS389I, DYS389II and DYS390). One primer of each pair was labeled with a fluorescent dye. In a final volume of 25 μL, 50 ng of genomic DNA was mixed with 200 μM of dNTP, 2.0 mM $MgCl_2$, 2.5 U of Taq polymerase (Amersham Biosciences, Piscataway, NJ, USA), 2.5 μL of the10X reaction buffer provided by the manufacturer, and with the forward and reverse primers. In the triplex reaction, the concentrations of primers were 7.0 pmol for DYS391, 8.5 pmol for DYS392 and 3.0 pmol for DYS393; in the tetraplex reaction, 7.0 pmol for DYS19, 6.0 pmol for DYS389I/II and 4.0 pmol for DYS390. The samples were subjected to 30 cycles of amplification in a 9700 thermal cycler (Applied Biosystems, Foster City, CA). The amplification conditions were 94 °C, 5 min; 35 cycles of 94 °C 1 min, 55 °C 1 min, and 72 °C 1 min; followed by 72 °C 30 min, and 12 °C until the samples were removed from the thermal cycler. Fragment size analysis was performed using the GeneScan 2.1 software. Two microliters of the amplification products were mixed with 24 μL of Hi-Di Formamide (Applied Biosystems), 1 μL of the size standard TAMRA-350 (triplex reaction) or TAMRA-500 (tetraplex reaction) and subjected to capillary electrophoresis on the ABI 310 Genetic Analyzer (Applied Biosystems) using POP-4 (performance optimized polymer), filter set C and an injection time of 5 s. The electrophoresis time was 24 min for the triplex reaction and 28 min for the tetraplex reaction. Four samples were reanalyzed using the AmpFlSTR YFiler kit (Applied Biosystems) as recommended by the manufacturer. Since Y-STRs are haploid, allele and haplotype frequencies, as well as linkage disequilibrium values between pairs of genes at different loci and mutation rates per locus, were estimated by direct counting methods, using computer programs prepared by us. The significance of association measurements [linkage disequilibrium values (ldv) estimated between all possible pairs of alleles belonging to two out of the seven loci here studied] was verified through Fisher exact tests performed on 2x2 contingency tables. All the methods here mentioned are detailed in standard text-books on statistical and population genetic methodology (Zar, 1999; Weir, 2001; Sham, 2002).

Table 1 lists the observed relative frequencies of different Y-STR alleles segregating in each of the seven loci. Table S1 (Supplementary Material) lists the observed absolute and relative frequencies of the 7-loci haplotypes among the 412 unrelated adult subjects.

Table 1 - Observed relative frequencies of Y-STR alleles segregating at each of the seven loci here reported.

Allele	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393
8						0.0024	
9					0.0558		
10		0.0024			0.5073	0.0097	
11					0.4053	0.3835	0.0073
12	0.0121	0.1772			0.0316	0.0437	0.1893
13	0.1117	0.6432				0.5073	0.6626
13/14		0.0024					
14	0.5655	0.1699				0.0461	0.1165
15	0.2354	0.0049				0.0024	0.0243
16	0.0607					0.0049	
17	0.0146						
18							
19							
20							
21				0.0704			
22				0.0777			
23				0.2524			
24				0.4733			

Table 1 (cont.)

Allele	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393
25				0.1189			
26			0.0049	0.0049			
27			0.0121	0.0024			
28			0.1408				
29			0.4223				
29/30			0.0049				
30			0.3010				
31			0.0947				
32			0.0170				
33			0.0024				

A total of 264 different haplotypes were identified, 210 of which were unique. The most frequent haplotype (DYS19 14/DYS389I 13/DYS389II 29/DYS390 24/DYS391 11/DYS392 13/DYS393 13) was found in 31 instances (7.52%). The second most frequent haplotype (14/13/29/24/10/13/13), which differed from the previous haplotype by only a single DYS391 repeat, was shared by 14 individuals (3.39%).

Table 2 lists the estimates of the diversity index obtained for each of the 7 Y-STR loci, the average figure calculated for these 7 loci and for the set of 7-loci haplotypes, together with their corresponding standard errors and approximate 95% confidence intervals. While diversity indices for isolated loci ranged from 0.51 to 0.70 with an overall average value of 0.60, the 7-loci haplotype diversity index was of the order of 0.99, as expected, since 210/264 (79.5%) of all haplotypes were unique, each occurring with a frequency of about 0.002.

Table 3 lists the results of association tests performed between the genes of possible pairs of Y-STR loci. Since the number of different statistical tests performed was 859, the critical alpha rejection level (for testing the null hypothesis of ldv = 0) was adjusted following Bonferroni's method, giving a corrected alpha critical value of 0.00006. Therefore, Table 3 lists only the pairs of linked Y-STR loci (out of the 859 tested for linkage disequilibrium) with linkage disequilibrium values [D(i,j)] significantly different from zero at the level of 6 x 10^{-5} or less. As expected, many (if not most) of these very significant values occurred preferentially between pairs of contiguous loci.

Table 2 - Estimates of Nei's diversity index (di), with corresponding standard errors [se(di)] and approximate 95% confidence intervals for each individual locus, for their average value, and for the complete 7-loci haplotypes.

	di	se(di)	95% c.i.(di)
locus 393	0.5109	0.1550	0.2071-0.8148
locus 19	0.6083	0.1184	0.3762-0.8404
locus 391	0.5743	0.0897	0.3985-0.7501
locus 389I	0.5260	0.1315	0.2682-0.7837
locus 389II	0.7018	0.0620	0.5803-0.8233
locus 390	0.6871	0.0874	0.5159-0.8583
locus 392	0.5914	0.0711	0.4521-0.7307
average	0.6000	0.1068	0.3905-0.8094
all 7 loci	0.9883	0.0017	0.9850-0.9916

In forensic cases, in which multiple male aggressors are involved, the autosomal STR profiles often provide inconclusive results. Y-STR markers are being increasingly used as potential tools for distinguishing low levels of male DNA in the presence of excess female DNA, which occurs in many sexual assault samples. Due to the haploid nature of Y-STRs, in cases where multiple males are contributors, the number of donors can be estimated by the presence of additional alleles in a Y chromosome profile, usually being interpreted as an admixture of more than one contributor. The most commonly used Y-STR markers are single-copy loci, with the exception of the DYS385 locus. However, many regions of the Y-chromosome are duplicated or even triplicated in some individuals and this fact can thus complicate potential mixture interpretation (Kayser *et al.*, 2000; Bosch and Jobling, 2003; Çakir *et al.*, 2004; Kurihara *et al.*, 2004; Butler *et al.*, 2005; Diederiche *et al.*, 2005;). The precise estimation of the frequency of duplicated mutated Y-STR alleles is thus very important in forensic genetic analyses, because the presence of multiple peaks can be misinterpreted as mixed profiles (Diederiche *et al.*, 2005). In the present study, one sample had a 7 Y-STR haplotype with double peaks at the DYS389I locus, and another presented a 7 Y-STR haplotype with double peaks at DYS389I and DYS389II. The analysis of these two samples was increased to 16 markers, using the AmpF/STR Yfiler Kit, and a double peak was also found in the locus DYS439 of the second sample. Each one of the two pairs of father and son had the same haplotype (Table S2).

The set of 7 Y-STR loci was typed in 222 father-son pairs. Upon 1,554 allele transmissions, six *de novo* muta-

Table 3 - Results of association tests and estimates of linkage disequilibrium values between possible pairs of Y-STR loci. Only pairs of loci for which the hypothesis of no association was discarded with a probability value less than 0.00006 are shown (see text).

loci	hap		observ. freq.	expect. freq.	D(i, j)	p (Fisher)
	i	j				
393(i) *vs.* 391(j)	13	11	0.3228	0.2686	0.0542	<0.000005
393(i) *vs.* 392(j)	12	11	0.1189	0.0726	0.0463	<0.000005
	12	13	0.0534	0.0960	-0.0426	0.00001
	13	11	0.1820	0.2541	-0.0721	<0.000005
	13	13	0.4199	0.3361	0.0838	<0.000005
19(i) *vs.* 391(j)	14	11	0.2791	0.2292	0.0499	0.00003
19(i) *vs.* 390(j)	14	21	0.0049	0.0398	-0.0350	<0.000005
	15	21	0.0437	0.0166	0.0271	<0.000005
19(i) *vs.* 392(j)	14	11	0.1214	0.2169	-0.0955	<0.000005
	14	13	0.3908	0.2869	0.1039	<0.000005
	16	13	0.0024	0.0308	-0.0284	<0.000005
391(i) *vs.* 389I(j)	10	12	0.1383	0.0899	0.0485	<0.000005
	11	12	0.0316	0.0718	-0.0403	0.00001
	10	12	0.0704	0.0394	0.0310	<0.000005
	10	1314	0.1699	0.2401	-0.0702	<0.000005
	11	12	0.0049	0.0315	-0.0266	0.00002
	11	1314	0.2597	0.1918	0.0679	<0.000005
391(i) *vs.* 392(j)	9	11	0.0510	0.0214	0.0296	<0.000005
	9	13	0.0049	0.0283	-0.0235	0.00002
	10	11	0.2767	0.1945	0.0822	<0.000005
	10	13	0.1723	0.2573	-0.0850	<0.000005
	11	11	0.0534	0.1554	-0.1020	<0.000005
	11	13	0.3010	0.2056	0.0953	<0.000005
389I(i) *vs.* 389II(j)	12	28	0.1044	0.0249	0.0794	<0.000005
	12	30	0.0097	0.0533	-0.0436	<0.000005
	13	28	0.0364	0.0905	-0.0541	<0.000005
	13	29	0.3544	0.2716	0.0827	<0.000005
	14	28	0.0000	0.0239	-0.0239	0.00001
	14	29	0.0170	0.0718	-0.0548	<0.000005
	15	30	0.1044	0.0511	0.0532	<0.000005
389I(i) *vs.* 390(j)	12	22	0.0413	0.0138	0.0275	<0.000005
	12	24	0.0364	0.0839	-0.0475	<0.000005
	13	24	0.3544	0.3044	0.0499	0.00002
389I(i) *vs.* 392(j)	12	11	0.1141	0.0679	0.0461	<0.000005
	12	13	0.0413	0.0899	-0.0486	<0.000005
389II(i) *vs.* 392(j)	29	11	0.1019	0.1620	-0.0600	<0.000005
	29	13	0.2718	0.2142	0.0576	<0.000005
390(i) *vs.* 392(j)	21	11	0.0631	0.0270	0.0361	<0.000005
	21	13	0.0024	0.0357	-0.0333	<0.000005
	22	11	0.0680	0.0298	0.0382	<0.000005
	22	13	0.0073	0.0394	-0.0321	<0.000005
	24	11	0.0947	0.1815	-0.0868	<0.000005
	24	13	0.3228	0.2401	0.0827	<0.000005
	24	14	0.0437	0.0218	0.0219	0.00001

Table 4 - Estimated mutation rates for Y-STR individual loci, together with an overall estimate (average 7-loci figure) and the corresponding 95% binomial exact confidence intervals for each estimate.

System	Allelic transfers	Mutations	Mutation rate ($x10^{-3}$)	95% C.I. ($x10^{-3}$)
DYS19	222	0	0	
DYS389 I	222	0	0	
DYS389 II	222	0	0	
DYS390	222	2	9.01	0.00-32.15
DYS391	222	3	13.51	2.83-38.97
DYS392	222	1	4.50	0.04-24.82
DYS393	222	0	0	
Total	1554	6	3.86	1.49-8.34

tions were observed, one mutation had occurred at the DYS392 locus (14 to 13), two mutations at the DYS390 locus (24 to 23; 22 to 24), and three mutations took place at the DYS391 locus (12 to 10; 12 to 11; 11 to 10) (Table 4). Except for two cases (DYS390 and DYS391), all were single-step mutations, and only a single mutation occurred during each father-son transmission.

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Internet Resources

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Supplementary Material

The following online material is available for this article:

- Table S1. Observed absolute and relative frequencies of STR-Y 7-loci haplotypes.

- Table S2. Y-STR haplotype profiles showing the presence of additional alleles.

This material is available as part of the online article from http://www.scielo.br.gmb.

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TABLE S1 – Observed absolute and relative frequencies of STR-Y 7-loci haplotypes.

H	**19**	**389I**	**389II**	**390**	**391**	**392**	**393**	**obs.no.**	**Freq.**
1	12	12	28	22	10	11	13	1	0.00243
2	12	12	28	23	11	16	11	1	0.00243
3	12	13	29/30	24	11	13	13	1	0.00243
4	12	13	31	24	10	11	13	1	0.00243
5	12	14	30	25	11	13	13	1	0.00243
6	13	12	28	23	10	11	13	1	0.00243
7	13	12	28	24	10	14	12	1	0.00243
8	13	12	29	24	10	11	13	1	0.00243
9	13	12	30	24	10	14	13	1	0.00243
10	13	13	28	23	11	13	13	1	0.00243
11	13	13	28	24	11	13	13	1	0.00243
12	13	13	29	23	11	13	13	1	0.00243
13	13	13	29	24	9	11	13	2	0.00485
14	13	13	29	24	10	11	12	1	0.00243
15	13	13	29	24	10	13	13	2	0.00485
16	13	13	29	24	11	13	12	1	0.00243
17	13	13	29	24	11	13	13	2	0.00485
18	13	13	29	24	12	13	12	1	0.00243
19	13	13	29	25	10	13	13	1	0.00243
20	13	13	29	25	11	13	13	1	0.00243
21	13	13	30	23	10	11	13	3	0.00728
22	13	13	30	23	10	11	14	1	0.00243
23	13	13	30	23	10	11	12	1	0.00243
24	13	13	30	23	10	11	13	5	0.01214
25	13	13	30	23	10	11	14	1	0.00243
26	13	13	30	25	9	11	14	1	0.00243
27	13	13	30	25	10	11	13	1	0.00243
28	13	13	31	23	10	11	13	1	0.00243
29	13	13	31	23	10	15	13	1	0.00243
30	13	13	31	24	10	11	12	1	0.00243
31	13	13	31	24	10	11	13	1	0.00243
32	13	13	31	24	10	14	13	1	0.00243
33	13	14	30	23	10	13	13	1	0.00243
34	13	14	30	24	9	11	13	5	0.01214
35	13	14	30	24	9	11	14	1	0.00243
36	13	14	30	24	10	14	13	1	0.00243
37	13	14	30	25	10	11	13	1	0.00243
38	13	14	33	23	10	11	12	1	0.00243
39	14	12	27	23	10	13	13	1	0.00243
40	14	12	27	24	10	14	12	1	0.00243
41	14	12	27	24	11	13	13	1	0.00243
42	14	12	27	25	10	13	14	1	0.00243
43	14	12	28	22	10	11	13	1	0.00243
44	14	12	28	22	10	11	15	1	0.00243
45	14	12	28	23	9	13	13	1	0.00243
46	14	12	28	23	10	11	12	2	0.00485
47	14	12	28	23	10	11	13	5	0.01214
48	14	12	28	23	10	13	13	1	0.00243
49	14	12	28	23	11	11	13	1	0.00243
50	14	12	28	23	11	12	13	1	0.00243
51	14	12	28	23	12	13	13	1	0.00243
52	14	12	28	24	10	13	13	2	0.00485

TABLE S1 (Continued)

H	19	389I	389II	390	391	392	393	obs.no.	Freq.
53	14	12	28	24	11	13	13	2	0.00485
54	14	12	28	24	11	14	13	1	0.00243
55	14	12	28	24	12	13	13	1	0.00243
56	14	12	28	27	10	13	13	1	0.00243
57	14	12	29	22	10	11	12	1	0.00243
58	14	12	29	22	10	11	14	1	0.00243
59	14	12	29	23	10	11	12	1	0.00243
60	14	12	29	25	10	13	13	1	0.00243
61	14	12	29	25	11	12	13	1	0.00243
62	14	12	30	22	10	11	13	1	0.00243
63	14	12	30	23	10	12	14	1	0.00243
64	14	13	26	23	10	13	13	1	0.00243
65	14	13	28	23	11	13	13	1	0.00243
66	14	13	28	24	10	13	13	1	0.00243
67	14	13	28	24	11	13	13	4	0.00971
68	14	13	28	24	11	13	14	1	0.00243
69	14	13	28	24	11	14	12	1	0.00243
70	14	13	29	21	11	13	14	1	0.00243
71	14	13	29	22	9	11	12	1	0.00243
72	14	13	29	22	10	11	12	1	0.00243
73	14	13	29	22	10	11	13	1	0.00243
74	14	13	29	23	10	11	12	3	0.00728
75	14	13	29	23	10	13	13	2	0.00485
76	14	13	29	23	11	11	12	3	0.00728
77	14	13	29	23	11	11	13	1	0.00243
78	14	13	29	23	11	12	13	1	0.00243
79	14	13	29	23	11	13	13	4	0.00971
80	14	13	29	24	9	13	13	1	0.00243
81	14	13	29	24	10	11	12	1	0.00243
82	14	13	29	24	10	13	12	7	0.01699
83	14	13	29	24	10	13	13	14	0.03398
84	14	13	29	24	10	14	13	2	0.00485
85	14	13	29	24	11	12	13	1	0.00243
86	14	13	29	24	11	13	12	3	0.00728
87	14	13	29	24	11	13	13	31	0.07524
88	14	13	29	24	11	13	14	2	0.00485
89	14	13	29	24	11	14	12	1	0.00243
90	14	13	29	24	11	14	13	1	0.00243
91	14	13	29	24	12	13	13	1	0.00243
92	14	13	29	25	10	13	13	3	0.00728
93	14	13	29	25	11	11	12	1	0.00243
94	14	13	29	25	11	13	11	1	0.00243
95	14	13	29	25	11	13	13	6	0.01456
96	14	13	29	25	12	13	13	1	0.00243
97	14	13	29	26	10	11	12	1	0.00243
98	14	13	29	26	11	13	12	1	0.00243
99	14	13	30	21	10	11	13	1	0.00243
100	14	13	30	22	10	11	13	1	0.00243
101	14	13	30	22	10	11	14	1	0.00243
102	14	13	30	22	10	13	13	1	0.00243
103	14	13	30	23	10	11	11	1	0.00243
104	14	13	30	23	10	11	12	5	0.01214
105	14	13	30	23	10	13	13	3	0.00728
106	14	13	30	23	11	11	12	1	0.00243

TABLE S1 (Continued)

H	19	389I	389II	390	391	392	393	obs.no.	Freq.
107	14	13	30	23	11	13	13	1	0.00243
108	14	13	30	24	10	10	13	1	0.00243
109	14	13	30	24	10	11	12	2	0.00485
110	14	13	30	24	10	13	12	1	0.00243
111	14	13	30	24	10	13	13	2	0.00485
112	14	13	30	24	10	13	14	1	0.00243
113	14	13	30	24	11	12	13	1	0.00243
114	14	13	30	24	11	13	12	1	0.00243
115	14	13	30	24	11	13	13	7	0.01699
116	14	13	30	24	11	13	14	1	0.00243
117	14	13	30	24	12	13	13	2	0.00485
118	14	13	30	25	10	13	13	1	0.00243
119	14	13	30	25	10	16	13	1	0.00243
120	14	13	30	25	11	13	13	3	0.00728
121	14	13	31	23	10	11	12	1	0.00243
122	14	13	31	23	10	13	13	2	0.00485
123	14	13	31	24	9	11	13	1	0.00243
124	14	13	31	24	11	13	13	1	0.00243
125	14	14	29	23	10	10	14	1	0.00243
126	14	14	29	23	10	13	12	1	0.00243
127	14	14	29	23	10	13	13	1	0.00243
128	14	14	29	23	11	13	13	1	0.00243
129	14	14	29	24	11	14	12	2	0.00485
130	14	14	30	22	10	13	13	1	0.00243
131	14	14	30	23	9	11	13	1	0.00243
132	14	14	30	23	10	11	12	1	0.00243
133	14	14	30	23	10	13	13	1	0.00243
134	14	14	30	23	11	13	12	1	0.00243
135	14	14	30	23	11	13	13	2	0.00485
136	14	14	30	23	12	13	13	1	0.00243
137	14	14	30	24	10	13	12	1	0.00243
138	14	14	30	24	10	13	13	2	0.00485
139	14	14	30	24	11	13	12	1	0.00243
140	14	14	30	24	11	13	13	7	0.01699
141	14	14	30	24	11	13	14	2	0.00485
142	14	14	30	24	11	14	13	2	0.00485
143	14	14	30	24	12	13	13	1	0.00243
144	14	14	30	24	12	13	14	1	0.00243
145	14	14	30	25	10	11	12	1	0.00243
146	14	14	30	25	10	13	12	1	0.00243
147	14	14	30	25	10	13	13	2	0.00485
148	14	14	30	25	11	12	13	1	0.00243
149	14	14	30	25	11	13	13	1	0.00243
150	14	14	31	23	9	11	12	1	0.00243
151	14	14	31	23	9	11	14	1	0.00243
152	14	14	31	23	10	11	13	1	0.00243
153	14	14	31	23	10	13	13	1	0.00243
154	14	14	31	24	11	11	13	1	0.00243
155	14	14	31	24	11	13	13	2	0.00485
156	14	14	31	25	11	11	13	1	0.00243
157	14	14	31	25	11	13	13	1	0.00243
158	14	14	32	23	10	11	13	1	0.00243
159	14	14	32	24	11	13	13	1	0.00243
160	14	14	32	25	10	12	14	1	0.00243

TABLE S1 (Continued)

H	19	389I	389II	390	391	392	393	obs.no.	Freq.
161	14	15	31	25	11	13	13	1	0.00243
162	14	15	32	23	10	13	13	1	0.00243
163	15	10	29	21	10	10	13	1	0.00243
164	15	12	26	21	10	11	13	1	0.00243
165	15	12	27	23	10	13	13	1	0.00243
166	15	12	28	21	10	11	14	1	0.00243
167	15	12	28	22	10	11	13	1	0.00243
168	15	12	28	22	10	11	14	2	0.00485
169	15	12	28	23	10	11	12	1	0.00243
170	15	12	28	23	10	11	14	1	0.00243
171	15	12	28	24	10	11	12	1	0.00243
172	15	12	28	24	10	11	13	2	0.00485
173	15	12	28	24	11	11	12	1	0.00243
174	15	12	28	25	10	11	12	1	0.00243
175	15	12	28	25	11	13	12	1	0.00243
176	15	12	28	25	11	13	13	1	0.00243
177	15	12	29	21	10	11	14	1	0.00243
178	15	12	29	22	10	11	12	1	0.00243
179	15	12	29	22	10	11	14	2	0.00485
180	15	12	29	23	10	11	12	2	0.00485
181	15	12	29	23	10	11	13	1	0.00243
182	15	12	29	23	10	11	15	1	0.00243
183	15	12	29	23	10	12	14	1	0.00243
184	15	12	29	23	10	13	13	1	0.00243
185	15	12	30	22	10	11	14	1	0.00243
186	15	13	28	22	11	11	13	1	0.00243
187	15	13	28	23	10	11	13	1	0.00243
188	15	13	28	25	10	13	13	1	0.00243
189	15	13	28	25	11	13	13	1	0.00243
190	15	13	29	21	10	11	14	1	0.00243
191	15	13	29	22	10	11	13	1	0.00243
192	15	13	29	22	11	13	12	1	0.00243
193	15	13	29	23	9	11	12	3	0.00728
194	15	13	29	23	10	11	12	1	0.00243
195	15	13	29	23	10	11	13	1	0.00243
196	15	13	29	23	10	11	14	1	0.00243
197	15	13	29	23	10	12	14	1	0.00243
198	15	13	29	23	10	13	13	2	0.00485
199	15	13	29	23	10	12	12	1	0.00243
200	15	13	29	23	10	13	13	3	0.00728
201	15	13	29	23	11	13	13	9	0.02184
202	15	13	29	23	11	14	13	2	0.00485
203	15	13	29	23	12	13	14	1	0.00243
204	15	13	29	25	10	13	13	1	0.00243
205	15	13	29	25	11	13	13	1	0.00243
206	15	13	29	25	12	13	13	1	0.00243
207	15	13	30	21	9	11	14	1	0.00243
208	15	13	30	21	10	11	13	1	0.00243
209	15	13	30	21	10	11	14	1	0.00243
210	15	13	30	21	10	11	15	1	0.00243
211	15	13	30	21	11	11	13	1	0.00243
212	15	13	30	21	11	11	14	1	0.00243
213	15	13	30	21	12	11	13	1	0.00243
214	15	13	30	22	10	11	12	1	0.00243

TABLE S1 (Continued)

H	19	389I	389II	390	391	392	393	obs.no.	Freq.
215	15	13	30	23	9	11	12	2	0.00485
216	15	13	30	23	11	13	13	1	0.00243
217	15	13	30	24	10	11	13	1	0.00243
218	15	13	30	24	10	12	15	1	0.00243
219	15	13	30	24	11	13	13	1	0.00243
220	15	13	30	24	11	13	14	2	0.00485
221	15	13	30	24	11	14	13	1	0.00243
222	15	13	31	21	10	10	13	1	0.00243
223	15	13	31	21	10	11	13	3	0.00728
224	15	13	31	21	10	11	15	1	0.00243
225	15	13	31	21	11	11	13	1	0.00243
226	15	13	31	24	10	11	12	1	0.00243
227	15	13	31	24	10	11	13	1	0.00243
228	15	13	31	24	11	13	13	1	0.00243
229	15	13	31	25	10	11	13	1	0.00243
230	15	13	32	22	10	11	14	1	0.00243
231	15	14	29	24	11	13	13	1	0.00243
232	15	14	30	24	11	13	13	1	0.00243
233	15	14	31	22	10	11	14	1	0.00243
234	15	14	31	23	10	12	14	1	0.00243
235	15	14	31	23	10	12	15	1	0.00243
236	15	14	32	24	10	11	12	1	0.00243
237	15	14	32	24	11	13	13	1	0.00243
238	16	12	28	21	10	11	13	1	0.00243
239	16	12	28	22	10	11	13	3	0.00728
240	16	12	28	22	10	11	14	1	0.00243
241	16	12	29	21	10	11	15	1	0.00243
242	16	12	29	21	11	11	13	2	0.00485
243	16	12	29	25	10	11	13	1	0.00243
244	16	13	29	22	10	8	15	1	0.00243
245	16	13	29	23	9	11	12	1	0.00243
246	16	13	29	23	10	12	14	1	0.00243
247	16	13	29	23	11	11	12	1	0.00243
248	16	13	29	23	11	12	14	1	0.00243
249	16	13	29	25	11	12	13	1	0.00243
250	16	13	30	21	10	11	15	1	0.00243
251	16	13	30	23	11	12	13	1	0.00243
252	16	13	30	24	10	11	13	1	0.00243
253	16	13	30	24	10	11	14	1	0.00243
254	16	13	30	24	11	11	13	1	0.00243
255	16	13	30	25	11	11	13	1	0.00243
256	16	13	31	23	10	14	14	1	0.00243
257	16	13	31	24	11	11	13	2	0.00485
258	16	13/14	29/30	25	11	13	13	1	0.00243
259	17	13	28	22	10	11	13	1	0.00243
260	17	13	30	21	10	11	14	1	0.00243
261	17	13	30	24	11	11	13	1	0.00243
262	17	14	30	21	10	11	15	1	0.00243
263	17	14	31	21	10	11	13	1	0.00243
264	17	14	31	21	10	11	14	1	0.00243

TABLE S2 – Y-STR haplotype profiles showing the presence of additional alleles at DYS389II (case 1) and DYS389I, DYS389II and DYS439 (case 2), after amplification with the AmpF/STR Yfiler kit.

Samples	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385	DYS393	DYS391	DYS439	DYS635	DYS392	GATA H4	DYS437	DYS438	DYS448
Case 1 father	15	13	24	29-30	16	12	12-14	13	11	11	23	13	21	15	12	20
Case 1 son	15	13	24	29-30	16	12	12-14	13	11	11	23	13	21	15	12	20
Case 2 father	15	13-14	25	29-30	17	16	13-14	13	11	10-13	23	13	21	15	12	19
Case 2 son	15	13-14	25	29-30	17	16	13-14	13	11	10-13	23	13	21	15	12	19