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SHORT COMMUNICATION

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Haplotype frequencies of eight Y-chromosome STR loci in Barcelona (North-East Spain)

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Abstract Haplotype frequencies for eight Y-chromosomal short tandem repeat (STR) loci were determined in a population sample from Barcelona (NE Spain). After PCR amplification and denaturing PAGE electrophoresis, DYS19, DYS388, DYS389 I/II, DYS390, DYS391, DYS392 and DYS393 loci were typed. Complete eight Y-chromosomal STRs haplotypes could be formed for 223 subjects, among which 137 different haplotypes were observed. The most common haplotype was shared by 13% of the sample, while 108 haplotypes were unique. The discrimination capacity was 61.5% and the gene diversity was 0.978. From the forensic point of view the combined polymorphisms provide a high diagnostic efficiency.

Key words STR polymorphisms · Y-chromosome · Y-haplotype · Forensic genetics · Population genetics

Introduction

The aim of this study is to report a haplotype frequency database for DYS19, DYS388, DYS389 (I and II), DYS390, DYS391, DYS392 and DYS393 loci, useful for forensic genetic diagnosis in Barcelona. The establishment of well defined local databases is an essential for using Y-chromosomal short tandem repeats (STR) in routine practice [1].

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Material and methods

Population samples

DNA was extracted from blood samples (n = 239) from healthy unrelated individuals (males) living in the metropolitan area of Barcelona using the phenol-chloroform-isoamyl alcohol method [2].

Amplification conditions

Two triplex PCR amplifications were performed. Triplex I: DYS19 and DYS389 I/II; Triplex II: DYS390, DYS391 and DYS393.

Hot start PCR reactions were carried out in a 20 µl volume containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.4 mM $MgCl_2$, 200 µM each dNTP, 10–20 pmol each primer, 0.9 units of AmpliTaq Gold polymerase and 1–5 ng template DNA.

Singleplex amplifications of DYS388 and DYS392 were carried out in a 20 µl volume containing 50 mM KCl, 10 mM Tris (pH 9), 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 µM each dNTP, 4–8 pmols of each primer, 0.6 units of Taq DNA polymerase and 20 ng template DNA.

All primers were those described by Kayser et al. [1] and de Knijff et al. [3] and one of each pair was fluorescent labelled.

Cycling conditions (PTC-200, MJ Research, Inc.)

Triplex I: 95 °C – 11 min soak, 94 °C – 50 s, 58 °C – 50 s, 72 °C – 1 min, 5 cycles; 94 °C – 50 s, 54 °C – 50 s, 72 °C – 1 min, 33 cycles; 72 °C – 5 min soak

Triplex II: 95 °C – 10 min soak, 94 °C – 50 s, 57 °C – 50 s, 72 °C – 1 min 30 s, 5 cycles; 94 °C – 50 s, 55 °C – 1 min, 72 °C – 2 min, 33 cycles; 72 °C – 5 min soak

DYS388: 94 °C – 3 min soak, 95 °C – 50 s, 63 °C – 50 s decreasing 1 °C each cycle, 72 °C – 50 s, 6 cycles; 95 °C – 1 min, 57 °C – 50 s, 72 °C – 1 min 20 s, 29 cycles; 72 °C – 5 min soak

DYS392: 94 °C – 1 min soak, 94 °C – 50 s, 60 °C – 50 s, 72 °C – 1 min 30 s, 5 cycles; 94 °C –50 s, 57 °C – 1 min, 72 °C – 1 min 30 s, 33 cycles; 72 °C – 5 min soak

Electrophoresis conditions

Detection of the alleles from PCR-amplified products was carried out on 6% (w/v acrylamide/bisacrylamide) polyacrylamide denaturing high-performance DNA sequencing gels (Ready Mix Gel ALF grade, Pharmacia). The electrophoresis was carried out on the Automated Laser Fluorescent (ALF) DNA sequencer (Pharmacia)

Table 1 A list of the 137 eight Y-chromosomal STRs haplotypes made from 223 subjects from Barcelona. N: number of subjects

DYS19	DYS388	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	N
13	1	10	29	24	10	11	13	1
13	2	9	27	24	10	14	13	1
13	2	9	28	25	10	11	13	1
13	2	10	27	24	9	11	13	1
13	2	10	27	24	11	13	13	4
13	2	10	27	25	9	14	13	1
13	2	10	28	22	10	13	13	1
13	2	10	28	23	10	11	13	1
13	2	10	28	24	11	11	13	1
13	2	10	29	24	10	11	13	3
13	2	10	30	23	10	11	13	1
13	2	10	31	26	10	11	13	1
13	2	11	28	23	9	12	13	1
13	2	11	28	24	9	11	13	3
13	2	11	28	25	9	11	13	1
13	2	11	28	25	10	13	13	1
13	2	11	28	25	11	13	13	1
13	2	11	29	25	10	11	13	1
13	2	12	30	25	9	11	13	1
13	3	10	27	24	10	13	13	1
14	2	9	26	23	11	14	13	1
14	2	9	26	24	10	13	13	1
14	2	9	26	24	10	13	13	1
14	2	9	26	24	11	13	13	2
14	2	9	26	25	11	13	13	1
14	2	9	27	24	10	13	13	1
14	2	9	27	24	11	13	13	1
14	2	9	28	22	10	14	14	1
14	2	10	25	23	11	13	13	1
14	2	10	25	24	11	13	13	1
14	2	10	26	24	11	13	13	3
14	2	10	26	25	11	13	13	1
14	2	10	27	21	11	13	13	1
14	2	10	27	23	10	13	13	2
14	2	10	27	23	11	13	13	3
14	2	10	27	23	11	14	13	1
14	2	10	27	24	10	13	13	11
14	2	10	27	24	10	13	14	1
14	2	10	27	24	10	14	13	1
14	2	10	27	24	11	12	13	1
14	2	10	27	24	11	13	10	1
14	2	10	27	24	11	13	12	1
14	2	10	27	24	11	13	13	28
14	2	10	27	24	11	13	14	1
14	2	10	27	24	11	14	13	2
14	2	10	27	24	12	13	13	2
14	2	10	27	25	11	13	13	1
14	2	10	27	26	11	13	13	1
14	2	10	28	23	11	13	13	3
14	2	10	28	24	10	11	13	2
14	2	10	28	24	11	13	13	4
14	2	10	28	24	11	14	13	1
14	2	10	29	24	10	12	14	1

Table 1 (continued)

DYS19	DYS388	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	N
14	2	11	27	23	10	13	13	2
14	2	11	27	24	11	13	13	2
14	2	11	28	23	10	13	13	3
14	2	11	28	23	10	14	13	1
14	2	11	28	23	11	13	13	3
14	2	11	28	24	10	13	13	3
14	2	11	28	24	11	13	13	11
14	2	11	28	24	11	14	13	1
14	2	11	28	25	11	13	13	1
14	2	11	29	23	10	13	13	2
14	2	11	29	23	10	13	14	1
14	2	11	29	24	10	13	13	1
14	2	11	29	24	10	13	14	1
14	2	11	29	24	11	11	13	1
14	2	11	29	24	11	13	13	2
14	2	11	30	24	10	13	13	1
14	2	11	30	24	10	13	14	1
14	2	11	30	24	11	13	13	1
14	2	12	29	24	10	13	13	1
14	3	10	27	23	11	13	13	1
14	3	10	28	24	10	11	12	1
14	4	9	26	22	10	11	13	2
14	4	9	26	23	10	11	13	1
14	4	9	26	24	10	11	13	1
14	4	10	27	23	10	11	12	1
14	5	9	26	23	10	11	12	1
14	5	9	27	24	10	11	13	1
14	5	10	27	23	10	11	12	3
14	5	10	28	24	10	11	12	1
14	6	10	29	24	10	11	12	1
14	6	10	30	23	10	13	12	1
14	6	11	29	23	11	11	12	1
14	7	10	29	23	10	11	12	1
15	2	9	27	23	10	14	13	1
15	2	9	28	22	9	12	13	1
15	2	9	28	23	10	11	14	1
15	2	10	26	24	11	13	13	1
15	2	10	26	25	11	13	13	1
15	2	10	27	22	10	14	12	1
15	2	10	27	23	10	13	13	1
15	2	10	27	23	11	13	13	2
15	2	10	27	24	11	13	12	1
15	2	10	27	24	11	13	13	2
15	2	10	27	25	11	13	13	1
15	2	10	28	23	10	13	13	1
15	2	10	28	23	10	15	13	1
15	2	10	28	23	11	13	13	1
15	2	10	28	24	11	13	13	2
15	2	10	29	25	11	11	13	1
15	2	11	28	22	10	12	12	1
15	2	11	28	23	10	11	12	1
15	2	11	28	24	10	13	13	1
15	2	11	28	24	11	13	13	1
15	2	11	28	25	11	13	13	1

Table 1 (continued)

DYS19	DYS388	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	N
15	2	11	29	24	11	13	13	1
15	3	9	26	22	10	11	13	1
15	3	9	27	22	10	11	12	1
15	3	9	27	22	10	11	14	1
15	3	10	26	23	10	11	13	1
15	3	10	27	22	10	11	12	1
15	3	10	27	22	10	11	15	1
15	3	10	27	22	10	12	14	1
15	3	10	27	22	11	11	13	1
15	3	10	28	22	10	12	14	1
15	3	10	30	23	10	12	14	1
15	5	9	26	22	10	11	13	1
15	5	10	27	23	10	11	12	1
15	6	11	28	23	9	11	12	1
16	2	10	27	24	10	13	13	1
16	2	11	29	23	10	13	13	1
16	2	11	29	25	10	11	13	1
16	3	9	26	26	10	11	13	1
16	3	9	27	22	10	11	14	2
16	3	10	26	23	10	11	13	1
16	3	10	27	24	11	11	13	1
16	3	11	27	22	11	11	13	1
16	3	12	28	23	9	11	13	1
17	2	11	27	24	9	11	13	1
17	3	10	26	23	10	11	13	2
17	3	10	26	23	11	11	13	1
17	3	10	27	23	10	13	14	1
17	3	11	27	24	9	11	13	1
17	3	11	27	25	9	11	12	1
17	3	11	28	23	10	11	13	1
17	5	11	29	25	10	12	14	1

under standard conditions. The Triplex I PCR product was loaded with the DYS388 product in a single electrophoretic lane using a monochrome sequencer due to the absence of overlapping size ranges. The Triplex II products were run along with DYS392 product for the same reason. Allelic ladders were used for each system as recommended by the DNA Commission of the International Society for Forensic Haemogenetics [4, 5]. Alleles were scored following the nomenclature described by Kayser et al. [1] based on the number of repeat units, according to the recommendations of the International Society for Forensic Haemogenetics [5], except for DYS388 locus since the alleles are not yet completely sequenced [1, 3].

Statistical methods

The gene diversity (D) was calculated as $D = 1 - \Sigma q_i^2$, where q_i is the frequency of the i^{th} haplotype [6, 7]. The power of exclusion for Y-linked systems is identical with the gene diversity. The discrimination capacity was calculated as the percentage proportion of different haplotypes. The program ARLEQUIN 1.1 was used for all these calculations [8].

Results and discussion

Allele distributions and gene diversities for each locus (data not shown) were similar to those previously reported for European and Catalan data [1, 9]. As shown in Table 1, complete eight Y-chromosomal STRs haplotypes could be obtained for 223 individuals, among which 137 different haplotypes were observed, 108 of them being unique (discrimination capacity: 61.5%). The gene diversity was 0.978 (standard error: 0.005).

From a forensic point of view, the theoretical values obtained revealed that the eight combined systems have a high diagnostic efficiency. The database reported here improves previously published papers [1, 3] by including complete haplotype information, as was recently done by Caglià et al. [10].

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