

Y-chromosomal Short Tandem Repeat Haplotypes in Southern Croatian Male Population Defined by 17 Loci

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Aim To define the Y-chromosome genetic structure in a sample of men from southern Croatia.

Methods Blood samples were collected from 166 unrelated healthy men from southern Croatia at the Department of Forensic Medicine and Biochemical Laboratory of University Hospital Split between 2004 and 2007. Genomic DNA was extracted using the standard procedures. Seventeen Y-chromosome short tandem repeat (Y-STR) polymorphic loci (DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385, DYS393, DYS391, DYS439, DYS635, DYS392, GATAH4, DYS437, DYS438, and DYS448) were analyzed using AmpFlSTR Yfiler Polymerase Chain Reaction Amplification Kit.

Results We observed 152 different haplotypes. Total haplotype diversity was 0.997289 and 141 haplotypes (84.49%) were unique. The most common haplotype was shared by only 4 men in the study sample. The locus diversity ranged between 0.21292 for DYS392 and 0.75546 for DYS439 locus.

Conclusion The Y-chromosome structure in men from southern Croatia is very diverse. Combination of Y chromosome 17 STR loci may be used as a powerful tool for individual identification and parentage analysis in the southern Croatian male population.

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Except for occasional mutations, the major part of the male-specific Y chromosome is inherited from father to son unchanged, as a haplotype of physically linked markers, because it is not involved in recombination with X chromosome. Genotyping of this Y-specific non-recombining region (NRY) can be used to track paternal lineage, since all patrilineal relatives share the same NRY haplotype. Thus, NRY multiple marker genotyping is used to infer paternity when the putative father is not available for DNA analysis or to validate biological relationships when one of the relatives is unable to take part in the DNA typing test. NRY multiple marker genotyping is also used in forensic casework to identify a male perpetrator's DNA in mixed samples with an excess of female victim's DNA. In such cases, it is a better option than polymerase chain reaction (PCR)-based amplification of female autosomal DNA markers or potential overlap of autosomal DNA marker profiles (1).

Genotyping small tandem repeats (STR) or microsatellite repeats or simple sequence repeats represents one of the most reliable and reproducible DNA profiling methods in forensic investigation. Genotyping multiple Y-chromosomal STR (Y-STR) markers is widely used in forensic and population studies in spite of its lower discrimination potential in comparison with autosomal STR genotyping. The constantly growing number of Y-STR markers available for forensic, evolutionary, and population studies increases Y-haplotype differentiation potential in distinct male populations (2).

The aim of our study was to use 17 Y-STRs to define the genetic structure of Y chromosome in a sample of men from southern Croatia. Our results may contribute to the development of a population database that may be used to estimate the haplotype frequency, ie, the probability that two or more unrelated men share the same Y-STR haplotype. Fur-

thermore, a Y-STR haplotype database for Croatian population may serve for assigning geographic origin to male individuals, since Y chromosomal markers are more prone to genetic drift than autosomal markers due to smaller effective population size, which enhances geographically-based differentiation of Y lineages.

Materials and methods

Population

The blood samples were collected between 2004 and 2007 at the Department of Forensic Medicine, Split University Hospital, from unrelated healthy male volunteers ($n = 66$) and in Biochemical Laboratory of the Split University Hospital from unrelated male individuals ($n = 100$) who came for regular medical examinations. All known ancestors of participants were of southern Croatian origin (Figure 1). All individuals gave their informed consent and the conduct of the study was approved by the institution's Ethics Committee.

DNA analysis

DNA was extracted from peripheral blood by the Chelex method (3). From 0.5 to 1 ng



Figure 1. The map of the Republic of Croatia. The circle indicates the region of southern Croatia.

target DNA was amplified using AmpFISTR Yfiler PCR Amplification Kit in accordance with the AmpFISTR Yfiler PCR Amplification Kit User Manual in a GeneAmp PCR System (Applied Biosystems, Foster City, CA, USA). The Kit includes a 9-marker European minimal haplotype (minHt), an 11-marker SWGDAM core set (minHt plus DYS438 and DYS439), and markers DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4), and Y GATA H4, allowing single-tube PCR co-amplification of multiple STR loci and four-color detection of the resulting length-variant STR alleles. PCR reactions were carried out in the final volume of 12.5 µL on a GeneAmp PCR System 9700 (Applied Biosystems). Separation of STR alleles, together with GeneScan-500 Internal Lane Size Standard (LIZ-500), was performed on the ABI PRISM 310 sequencer (Applied Biosystems) using GeneScan, version 3.7, software (Applied Biosystems) in order to determine their size by applying Yfiler

Allelic Ladder. Alleles were named as suggested by the DNA Commission (ISFG) (4).

Data analysis

Allelic frequencies were estimated by direct counting. Haplotype and locus diversity within the population was calculated using the Arlequin 2000 package (5), according to the formula:

$$H = \frac{n}{n-1} \left(1 - \sum_{i=1}^k p_i^2 \right)$$

where *n* is the number of samples, *k* the number of haplotypes, and *p_i* is the frequency of the *i*-th haplotype. Locus diversity was estimated according to the same equation as haplotype diversity (the number of alleles was used instead of the number of haplotypes and allele frequencies were used instead of haplotype frequencies).

Presentation of data follows the guidelines for publication of population data as required

Table 1. Allele frequencies and locus diversity at seventeen Y chromosome short tandem repeats in southern Croatian population

Allele	Loci																
	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS393	DYS391	DYS439	DYS635	DYS392	GATA H4	DYS437	DYS438	DYS448	Genotype	DYS385
8																10-14	0.012
9							0.006	0.036							0.060	11-13	0.036
10							0.006	0.337	0.139			0.048	0.012		0.681	11-14	0.090
11							0.054	0.596	0.187			0.886	0.645		0.229	11-15	0.090
12		0.078					0.090	0.030	0.319			0.030	0.289		0.024	12-15	0.018
13	0.006	0.740			0.006	0.048	0.831	0.301	0.301			0.030	0.048		0.006	13-14	0.036
14	0.060	0.169			0.036	0.175	0.012		0.048				0.006	0.337		13-15	0.024
15	0.633	0.012			0.150	0.235			0.006			0.006		0.608		13-16	0.018
16	0.240				0.187	0.463							0.054			13-17	0.018
17	0.042				0.440	0.072										13-19	0.006
18	0.012				0.157	0.006									0.024	14-14	0.024
19	0.006				0.018										0.452	14-15	0.446
20					0.006					0.024					0.452	14-16	0.030
21										0.120					0.060	14-17	0.018
22			0.078							0.205					0.012	15-15	0.048
23			0.084							0.536						15-18	0.006
24			0.627							0.115						15-19	0.012
25			0.199													16-18	0.024
26			0.012													17-18	0.018
27																11-16	0.006
28				0.030												12-14	0.006
29				0.108												15-17	0.006
30				0.313												17-17	0.006
31				0.446													
32				0.090													
33				0.012													
LDV*	0.53956	0.41877	0.55794	0.68631	0.72721	0.69573	0.29941	0.53151	0.75546	0.64629	0.21292	0.50142	0.51617	0.48287	0.59094		0.74134

*LDV – locus diversity value.

Table 2. Y short tandem repeat haplotypes in southern Croatian population*

H	n	f	Loci															
			DYS 456	DYS 389I	DYS 390	DYS 389II	DYS 458	DYS 19	DYS 385	DYS 393	DYS 391	DYS 439	DYS 635	DYS 392	DYS H4	DYS 437	DYS 438	DYS 448
1	1	0.006	15	13	24	30	18	17	14-15	13	11	13	24	11	11	15	10	19
2	1	0.006	15	13	24	31	18	16	14-16	13	11	14	22	11	12	15	10	19
3	1	0.006	14	13	24	31	17	16	14-15	13	11	13	22	11	11	15	10	19
4	2	0.012	16	14	25	31	17	16	11-15	13	11	10	23	11	12	14	11	20
5	1	0.006	15	13	25	31	17	14	14-15	13	11	13	23	11	11	15	10	19
6	1	0.006	15	13	24	30	18	16	14-15	13	12	12	24	11	11	15	10	19
7	2	0.012	16	14	25	31	16	16	11-15	12	11	10	23	11	11	14	11	20
8	1	0.006	15	14	24	32	17	16	14-16	13	11	13	23	11	11	15	10	19
9	1	0.006	16	13	23	30	17	13	15-18	13	10	12	23	11	12	14	10	20
10	1	0.006	15	13	24	31	17	14	14-15	13	11	13	23	11	11	15	10	19
11	1	0.006	15	14	24	32	17	14	14-15	13	11	13	21	11	12	15	10	19
12	1	0.006	14	12	23	28	15	14	13-14	13	10	11	23	11	11	16	10	19
13	1	0.006	17	13	24	30	16	13	16-18	13	10	12	21	11	12	14	10	20
14	1	0.006	18	14	26	31	15	16	11-13	14	10	10	23	11	12	14	11	20
15	1	0.006	15	13	24	30	15	13	17-18	13	11	11	23	11	12	14	10	20
16	3	0.018	16	14	25	31	15	16	11-15	13	11	10	23	11	12	14	11	20
17	1	0.006	15	13	24	29	16	14	13-16	12	10	10	21	11	12	14	9	20
18	1	0.006	16	13	25	30	16	17	10-14	13	10	11	23	11	12	14	11	20
19	1	0.006	15	13	24	29	17	15	11-14	13	11	12	23	13	13	15	12	20
20	1	0.006	15	13	24	30	18	16	15-15	13	10	13	22	11	11	15	10	19
21	1	0.006	15	13	24	31	17	16	14-15	13	11	12	24	11	11	15	10	20
22	2	0.012	15	13	24	31	18	16	14-15	13	11	12	23	11	11	15	10	19
23	1	0.006	16	14	24	31	15	16	11-15	13	11	11	23	11	13	14	11	20
24	1	0.006	15	13	24	31	16	14	14-15	13	11	13	22	11	11	15	10	19
25	1	0.006	14	12	23	28	16	15	16-18	14	12	12	22	12	11	14	10	20
26	2	0.012	15	13	24	31	17	16	14-15	13	11	12	22	11	11	15	10	19
27	4	0.024	15	13	24	31	17	15	14-15	13	11	12	23	11	11	15	10	19
28	1	0.006	17	15	25	32	15	15	11-15	13	11	10	23	11	12	14	11	21
29	1	0.006	15	13	24	31	18	14	14-15	13	11	15	24	11	11	15	10	19
30	1	0.006	15	15	24	30	17	16	14-16	13	10	12	22	11	11	15	10	18
31	1	0.006	15	13	22	29	18	13	14-17	13	10	12	22	15	10	15	11	19
32	1	0.006	15	13	24	31	16	16	14-15	13	10	12	23	11	11	15	10	19
33	1	0.006	16	13	25	30	15	16	11-14	13	10	10	23	11	12	14	11	20
34	1	0.006	16	14	25	31	15	16	11-15	13	11	10	23	11	12	14	11	21
35	1	0.006	15	13	24	31	18	17	14-15	13	11	12	23	11	11	15	10	19
36	1	0.006	15	13	24	29	16	14	14-15	13	11	13	23	11	11	15	10	19
37	1	0.006	16	13	25	31	17	16	14-15	13	10	14	22	11	11	15	10	19
38	1	0.006	16	13	24	30	15	16	11-13	13	10	11	23	11	12	14	11	21
39	1	0.006	15	13	24	31	17	14	14-15	12	11	13	22	11	11	15	10	18
40	1	0.006	15	13	24	30	18	16	14-15	13	11	11	22	11	11	15	10	19
41	1	0.006	14	12	22	28	14	15	13-14	13	10	11	20	11	11	16	10	20
42	1	0.006	15	14	24	32	17	16	14-15	13	10	13	22	11	11	14	10	19
43	1	0.006	15	13	24	31	17	15	14-15	13	11	13	23	11	11	15	10	20
44	1	0.006	16	13	24	30	14	15	13-17	12	9	12	21	11	12	14	9	21
45	1	0.006	15	14	24	32	17	14	14-15	13	11	13	23	11	12	15	10	19
46	1	0.006	15	13	24	31	18	16	14-14	13	11	13	23	11	11	15	10	19
47	1	0.006	15	13	24	31	17	14	14-15	13	11	14	21	11	11	15	10	19
48	1	0.006	16	14	25	30	15	16	11-15	13	10	10	23	11	12	14	11	20
49	1	0.006	14	12	24	28	16	15	15-19	12	10	12	21	12	12	14	9	19
50	1	0.006	15	14	24	32	17	16	14-15	13	10	13	24	11	11	15	10	20
51	1	0.006	16	14	25	30	15	16	11-15	13	11	10	23	11	12	14	11	20
52	1	0.006	15	13	22	30	17	16	14-15	13	11	13	23	11	11	15	10	19
53	1	0.006	16	14	24	31	15	17	11-13	13	11	11	23	11	14	14	11	20
54	1	0.006	15	12	24	30	17	16	14-15	13	11	12	23	11	11	15	10	19
55	1	0.006	15	13	24	31	18	16	14-15	13	11	13	23	11	11	15	10	19
56	1	0.006	15	13	24	30	18	16	14-15	13	11	13	23	11	11	15	10	19
57	1	0.006	15	14	24	32	17	14	14-15	13	11	13	22	11	11	15	11	19
58	2	0.012	15	13	24	30	17	16	14-15	13	10	13	23	11	11	15	10	19
59	1	0.006	15	13	24	32	18	15	14-15	13	12	12	23	11	11	15	10	20
60	2	0.012	16	14	25	31	16	16	11-15	13	11	10	23	11	12	14	11	20
61	1	0.006	15	13	24	31	16	16	14-16	13	11	12	22	11	11	15	10	19
62	1	0.006	15	13	24	30	18	15	14-15	13	11	12	24	11	11	15	10	19
63	1	0.006	14	12	23	30	17	15	13-14	13	10	11	21	11	11	16	10	20
64	1	0.006	15	13	24	31	17	16	14-15	13	11	12	24	12	11	15	10	19
65	1	0.006	15	13	24	31	17	15	14-15	13	11	13	23	12	11	15	10	20
66	1	0.006	15	14	24	32	17	14	14-15	13	11	12	22	11	11	15	10	19
67	1	0.006	15	13	22	30	17	16	13-15	13	11	12	23	11	11	15	10	19
68	1	0.006	15	13	24	31	16	16	14-15	13	11	13	23	11	11	15	10	20
69	1	0.006	15	13	24	31	15	15	14-15	13	11	13	23	11	11	15	10	19
70	1	0.006	15	13	24	32	17	16	14-15	13	11	12	24	11	11	15	10	20
71	1	0.006	16	13	25	30	15	17	11-13	13	12	11	23	11	13	14	11	20
72	1	0.006	15	13	24	31	17	16	14-15	13	11	14	21	11	12	15	10	20
73	1	0.006	16	13	25	30	15	17	11-13	13	11	11	23	11	13	14	11	20
74	1	0.006	15	13	24	30	17	15	14-15	13	11	13	23	11	11	15	10	20
75	1	0.006	15	13	23	30	19	14	13-17	12	10	12	20	11	11	14	10	20
76	1	0.006	16	13	24	31	17	15	14-15	13	11	13	22	11	11	15	10	20

Table 2. Y short tandem repeat haplotypes in southern Croatian population* - continued

H	n	f	Loci															
			DYS 456	DYS 389I	DYS 390	DYS 389II	DYS 458	DYS 19	DYS 385	DYS 393	DYS 391	DYS 439	DYS 635	DYS 392	DYS H4	DYS 437	DYS 438	DYS 448
77	1	0.006	15	13	24	30	17	15	14-15	13	11	12	23	11	11	15	10	19
78	1	0.006	15	13	24	30	17	17	14-15	13	10	12	24	11	11	14	10	20
78	1	0.006	14	12	23	29	16	15	13-14	13	10	11	21	11	11	16	10	20
80	1	0.006	15	12	24	31	17	16	14-14	13	10	13	23	11	12	15	10	19
81	1	0.006	16	13	23	29	14	15	13-16	12	9	12	22	11	12	14	9	22
82	1	0.006	15	13	24	30	17	15	14-15	13	11	13	22	11	11	15	10	20
83	1	0.006	15	13	23	31	17	16	14-15	13	10	13	23	11	11	15	10	19
84	1	0.006	17	13	25	30	16	16	11-14	13	11	10	23	11	12	14	11	20
85	1	0.006	15	12	22	29	17	15	13-14	13	10	12	20	11	11	16	10	20
86	1	0.006	15	14	23	30	17	14	13-19	12	10	13	22	11	12	15	9	21
87	1	0.006	15	13	22	30	17	16	14-15	13	11	12	23	11	11	15	10	19
88	1	0.006	16	13	24	30	17	14	11-14	13	11	12	23	13	12	15	12	19
89	1	0.006	15	13	24	30	16	13	17-18	13	11	11	23	11	12	14	10	20
90	1	0.006	15	13	24	31	16	16	14-15	13	10	13	22	11	11	15	10	19
91	1	0.006	15	12	23	30	16	14	13-14	13	10	11	21	11	11	16	10	20
92	1	0.006	15	13	24	30	17	14	15-15	13	11	13	22	11	11	15	10	19
93	1	0.006	16	13	25	29	15	16	11-15	13	10	10	23	11	12	14	11	20
94	1	0.006	15	13	22	29	14	14	14-14	13	10	11	23	10	10	16	10	20
95	1	0.006	16	13	24	29	18	14	11-14	13	12	12	23	12	11	15	11	19
96	1	0.006	15	13	24	31	17	15	14-15	13	11	13	22	11	11	15	10	20
97	1	0.006	16	13	25	30	17	16	11-14	13	10	10	23	11	13	14	11	20
98	1	0.006	15	13	24	31	18	16	14-15	13	11	13	24	11	11	15	10	19
99	1	0.006	16	13	24	31	18	14	11-14	13	11	12	23	13	12	15	12	19
100	1	0.006	16	13	25	29	13	17	11-14	13	11	10	23	11	13	14	11	20
101	1	0.006	15	13	24	32	17	16	14-15	13	11	13	23	11	11	15	10	20
102	1	0.006	14	14	23	30	17	15	12-15	13	10	11	21	11	11	14	10	*
103	1	0.006	16	13	24	30	17	14	11-14	13	10	12	23	13	12	15	12	19
104	1	0.006	15	14	24	32	17	15	14-15	13	11	11	22	11	11	15	10	19
105	2	0.012	15	13	24	31	18	16	14-15	13	11	14	22	11	11	15	10	19
106	2	0.012	15	13	24	33	17	16	15-15	13	10	12	21	11	11	15	10	20
107	1	0.006	16	13	25	30	15	16	11-13	13	10	11	23	11	12	14	11	21
108	1	0.006	16	13	24	30	16	14	17-17	13	10	12	21	11	12	14	10	20
109	1	0.006	15	13	24	31	18	15	14-16	13	11	13	24	11	11	15	10	18
110	1	0.006	15	13	24	31	17	14	14-15	13	11	13	22	11	11	15	10	19
111	1	0.006	16	13	25	30	16	16	10-14	13	10	11	23	11	12	14	11	20
112	1	0.006	16	13	24	30	18	16	14-15	13	11	14	23	11	11	15	10	19
113	1	0.006	15	13	24	31	17	16	14-15	13	11	13	24	11	11	15	10	19
114	1	0.006	15	13	24	31	19	15	14-15	13	11	13	23	11	11	15	10	19
115	2	0.012	17	13	25	29	16	16	11-14	13	10	11	23	11	12	14	11	20
116	1	0.006	19	13	24	30	15	13	16-18	13	10	12	21	11	12	14	10	20
117	1	0.006	15	13	24	30	20	13	17-18	13	11	11	23	11	12	14	10	20
118	1	0.006	15	13	24	31	17	17	14-15	13	11	13	23	11	11	15	10	19
119	1	0.006	15	13	24	31	16	15	15-15	13	10	13	24	11	11	15	10	20
120	1	0.006	15	13	24	31	16	16	15-15	13	10	13	24	11	11	15	10	20
121	1	0.006	15	13	24	30	18	16	15-15	13	11	12	22	11	11	15	10	20
122	1	0.006	15	13	24	32	17	16	15-15	11	10	12	21	11	11	15	10	19
123	1	0.006	17	13	25	29	17	15	11-14	13	10	11	23	11	12	14	11	20
124	1	0.006	16	13	25	30	16	15	11-14	13	10	11	23	11	13	14	11	20
125	1	0.006	15	13	24	32	18	16	14-15	13	11	13	22	10	11	15	10	20
126	1	0.006	15	13	23	31	17	15	14-15	13	11	13	22	11	11	15	10	20
127	1	0.006	15	13	24	32	17	16	13-15	13	11	13	23	11	11	15	10	20
128	1	0.006	15	13	22	30	15	15	12-15	12	10	11	21	11	11	14	9	21
129	1	0.006	15	13	24	31	17	18	13-15	13	11	12	22	11	11	15	10	20
130	1	0.006	15	13	24	31	18	16	14-15	13	10	12	23	11	11	15	10	19
131	1	0.006	15	13	24	31	17	17	14-15	13	11	13	23	11	11	15	10	20
132	1	0.006	16	13	24	30	14	15	13-17	11	9	12	21	11	12	14	9	21
133	1	0.006	15	13	24	31	17	14	14-15	12	11	14	23	11	11	15	10	19
134	1	0.006	15	13	24	31	17	15	14-15	12	11	12	23	11	11	15	10	19
135	1	0.006	15	13	24	31	17	17	14-15	12	11	12	23	11	11	15	10	19
136	1	0.006	14	13	25	31	19	16	14-15	13	10	11	23	11	11	15	10	19
137	1	0.006	15	14	26	31	15	15	11-16	13	9	10	23	11	12	14	11	20
138	1	0.006	13	12	24	29	16	15	13-15	13	9	11	21	11	11	16	10	20
139	1	0.006	16	14	25	31	17	16	12-15	11	11	10	24	10	12	14	11	20
140	1	0.006	15	13	25	31	17	17	14-15	13	11	13	23	11	11	15	10	20
141	1	0.006	15	13	23	31	17	16	14-15	11	11	12	21	10	11	15	10	19
142	1	0.006	17	14	25	31	15	16	12-14	11	10	10	24	10	12	14	11	20
143	1	0.006	16	13	22	29	15	15	13-16	9	9	12	23	10	12	14	9	22
144	1	0.006	15	12	22	29	16	15	14-14	13	10	11	20	11	11	16	10	21
145	1	0.006	15	13	23	31	17	14	14-15	11	11	13	22	10	12	15	10	19
146	1	0.006	14	12	22	28	16	16	15-19	10	10	12	22	11	12	14	9	19
147	1	0.006	15	13	22	29	17	14	11-14	11	11	11	24	13	11	15	13	19
148	1	0.006	15	13	24	31	16	14	14-15	13	11	13	23	11	11	15	10	19
149	1	0.006	14	13	22	30	14	14	14-17	12	10	11	24	11	11	15	9	21
150	1	0.006	15	13	24	31	18	16	15-17	11	11	13	24	10	11	15	10	19
151	1	0.006	18	13	24	30	15	13	16-18	11	10	12	22	11	11	14	10	20
152	1	0.006	16	13	24	30	16	16	11-14	12	11	11	23	11	13	14	11	20

*Abbreviations: n – number of individuals; f – haplotype frequency.

by the journal (6) and the DNA Commission of the International Society of Forensic Genetics (6,7).

Results and discussion

STR locus diversity for 17 Y-specific markers was analyzed in 166 unrelated men from southern Croatia. Allele frequencies and locus diversity across 17 Y-STR loci are shown in Table 1, while 17 Y-STR marker haplotypes are shown in Table 2. We observed 152 different haplotypes. Total haplotype diversity was 0.997289 and 141 haplotypes (84.49%) were unique. Haplotype No. 27 was the most common haplotype, shared by 4 men in our study sample (Table 2). The locus diversity ranged from 0.21292 for DYS392 to 0.75546 for DYS439 locus.

Our results are in accordance with the results reported in the previous study of 457 unrelated Croatian men, of whom 274 belonged to southern Croatian population (8). In that study, Y-STR polymorphisms across 8 loci were analyzed, 7 of which were identical to the loci analyzed in our study. However, we used additional 10 Y-STR markers, thus providing further population data for southern Croatian men.

Our results differ significantly from the results reported for western Croatian population (9). The differences were found across all loci, both in allele frequency and in locus diversity values. Furthermore, not a single haplotype that appeared more than once in western Croatian population (17 out of 101) matched the haplotypes that appeared more than once in southern Croatian population (25 out of

166). The combination of Y-chromosome 17 STR loci may be used as a powerful tool for individual identification and parentage analysis in the southern Croatian male population.

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