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# Haplotype data for 23 Y-chromosome markers in a reference sample from Bosnia and Herzegovina

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**Aim** To detect polymorphisms of 23 Y-chromosomal short tandem repeat (STR) loci, including 6 new loci, in a reference database of male population of Bosnia and Herzegovina, as well as to assess the importance of increasing the number of Y-STR loci utilized in forensic DNA analysis.

**Methods** The reference sample consisted of 100 healthy, unrelated men originating from Bosnia and Herzegovina. Sample collection using buccal swabs was performed in all geographical regions of Bosnia and Herzegovina in the period from 2010 to 2011. DNA samples were typed for 23 Y STR loci, including 6 new loci: DYS576, DYS481, DYS549, DYS533, DYS570, and DYS643, which are included in the new PowerPlex® Y 23 amplification kit.

**Results** The absolute frequency of generated haplotypes was calculated and results showed that 98 samples had unique Y 23 haplotypes, and that only two samples shared the same haplotype. The most polymorphic locus was DYS418, with 14 detected alleles and the least polymorphic loci were DYS389I, DYS391, DYS437, and DYS393.

**Conclusion** This study showed that by increasing the number of highly polymorphic Y STR markers, to include those tested in our analysis, leads to a reduction of repeating haplotypes, which is very important in the application of forensic DNA analysis.

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The highly polymorphic short tandem repeat (STR) loci located on the Y chromosome in the male human genome are widely used for forensic and paternity testing and population genetic studies (1-3). Currently, in response to the requirement for increasing the number of Y-STR markers included in some Y-STR multiplex kits, Promega developed the PowerPlex® Y 23 amplification kit (Promega Corporation, Madison, WI, USA), which we used in this study.

Previously, population studies of the male reference sample of Bosnia and Herzegovina were performed by analyzing 12 Y-chromosomal STR loci incorporated in the PowerPlex® Y 12 amplification kit (Promega Corporation) (4), various numbers of Y-SNP markers (5), as well as autosomal (6,7), and X-STR markers (8). All obtained results were included in the reference database of Bosnia and Herzegovina. However, these studies used different referent samples. In order to contribute to the development of this database we decided to analyze 23 Y-STR loci, which included 11 additional loci compared to the previous number of Y-STRs, among which there were 6 new loci incorporated for the first time in the Y-STR multiplex kit (DYS576, DYS481, DYS549, DYS533, DYS570, DYS643).

## MATERIALS AND METHODS

### Sampling and extraction

This study was conducted on a population sample of 100 unrelated men from Bosnia and Herzegovina during 2012. The reference sample approximately proportionaly included the three main ethnic groups in Bosnia and Herzegovina: Bosnian Muslims (45%), Bosnian Serbs (34%), and Bosnian Croats (21%), with the M/F ratio of 0.97. The tested individuals were voluntary participants and gave the informed consent. Sample collection using buccal swabs was done in all geographical regions in Bosnia and Herzegovina. Genomic DNA was extracted from the buccal swabs using the salting out method (9), as well as Qiagen DNeasyTM Tissue Kit (10) (Qiagen, GmbH, Hilden, Germany).

### Genotyping

Polymerase chain reaction (PCR) was performed using the PowerPlex® Y23 System (Promega Corporation) according to the manufacturer's recommendations (11), which includes the loci DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS481,DYS533,DYS549, DYS570, DYS576, DYS635, DYS643, and Y-GATA-H4. The PCR amplifications were carried out in PE GeneAmp PCR System Thermal Cycler (ABI, Foster City, CA, USA) according to the manufacturer's recommendations. The 23 Y-chromosomal STR markers were typed using the ABI 310 Genetic Analyser (ABI, Foster City, CA, USA).

### Statistical analysis

Haplotype and allele frequencies were estimated by gene counting. Gene and haplotype diversities were calculated according to Nei using the Arlequin software, V. 3.5 (12,13).

## RESULTS

A total of 98 unique haplotypes were detected, and 1 (ID1) appeared two times (Supplementary Table). The most polymorphic locus was DYS418, with 14 detected alleles (Table 1). This locus is one of 6 new loci added to the PowerPlex® Y23 kit, which confirms the importance of increasing the number of Y STR loci included in forensic analysis. Furthermore, at the locus DYS418, we detected allele 33, which was not incorporated in the allelic ladder provided by the PowerPlex® Y23 kit. The least polymorphic loci in our study were DYS389I, DYS391, DYS437, and DYS393.

## DISCUSSION

A previous Y-STR study (4) was conducted on a different reference sample comprising 100 men from Bosnia and Herzegovina and including 12 loci. This study showed 69 unique haplotypes, 7 appeared twice, 4 appeared three times, and 1 appeared five times. Our study was done on the same number of reference samples from Bosnia and Herzegovina, but included 11 additional loci. The results showed 98 unique haplotypes and only one repetition. This indicates that by increasing the number of STR loci, the number of unique haplotypes increases and the number of repetitions decreases.

Furthermore, in the study of Y-STR diversity in Sarajevo region (14), which analyzed 12 loci using the PowerPlex® Y kit, the most polymorphic loci were DYS385a and DYS385b. The least polymorphic loci were DYS 391, DYS389I, and DYS437. In our study, the least polymorphic loci were DYS 391, DYS389I, and DYS437, which confirms the previous results (4,14), but includes the DYS393 locus in the group of the least polymorphic loci. A quality control check was performed using the proficiency testing of the Y-STR Haplotyping Quality Assurance Exercise 2012 (15).

TABLE 1. Allele frequency distribution and average gene diversity for the PowerPlex® Y23 System in a population sample from Bosnia and Herzegovina

| Locus | Allele | Frequency | Locus | Allele | Frequency | Locus | Allele | Frequency | Locus | Allele | Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DYS576 | 11 | | DYS389 I | 9 | | DYS448 | 14 | | DYS389 II | 24 | |
| | 12 | | | 10 | | | 15 | | | 25 | |
| | 13 | | | 11 | | | 16 | | | 26 | |
| | 14 | | | 12 | 0.110 | | 17 | | | 27 | |
| | 15 | | | **13** | **0.700** | | 18 | 0.040 | | 28 | 0.050 |
| | 16 | 0.060 | | 14 | 0.190 | | 19 | 0.440 | | 29 | 0.190 |
| | 17 | 0.280 | | 15 | | | **20** | **0.460** | | 30 | 0.330 |
| | **18** | **0.390** | | 16 | | | 21 | 0.060 | | **31** | **0.340** |
| | 19 | 0.170 | | 17 | | | 22 | | | 32 | 0.080 |
| | 20 | 0.070 | | | | | 23 | | | 33 | 0.010 |
| | 21 | 0.030 | | | | | 24 | | | 34 | |
| | 22 | | | | | | | | | 35 | |
| | 23 | | | | | | | | | | |
| DYS19 | 9 | | DYS391 | 5 | | DYS481 | 17 | | DYS549 | 7 | |
| | 10 | | | 6 | | | 18 | | | 8 | |
| | 11 | | | 7 | | | 19 | | | 9 | |
| | 12 | 0.020 | | 8 | | | 20 | 0.010 | | 10 | |
| | 13 | 0.160 | | 9 | 0.040 | | 21 | 0.030 | | **11** | **0.460** |
| | 14 | 0.150 | | 10 | 0.440 | | 22 | 0.160 | | 12 | 0.420 |
| | 15 | 0.240 | | **11** | **0.520** | | 23 | 0.180 | | 13 | 0.070 |
| | **16** | **0.370** | | 12 | | | 24 | 0.070 | | 14 | 0.050 |
| | 17 | 0.060 | | 13 | | | 25 | 0.050 | | 15 | |
| | 18 | | | 14 | | | 26 | 0.020 | | 16 | |
| | 19 | | | 15 | | | 27 | 0.040 | | 17 | |
| | | | | 16 | | | 28 | 0.020 | | | |
| | | | | | | | 29 | 0.040 | | | |
| | | | | | | | 30 | 0.160 | | | |
| | | | | | | | **31** | **0.200** | | | |
| | | | | | | | 32 | 0.010 | | | |
| | | | | | | | 33 | 0.010 | | | |
| DYS533 | 7 | | DYS438 | 6 | | DYS437 | 11 | | DYS570 | 10 | |
| | 8 | | | 7 | | | 12 | | | 11 | |
| | 9 | 0.010 | | 8 | | | 13 | | | 12 | |
| | 10 | 0.010 | | 9 | 0.070 | | 14 | 0.440 | | 13 | |
| | 11 | 0.160 | | **10** | **0.710** | | **15** | **0.510** | | 14 | |
| | **12** | **0.610** | | 11 | 0.180 | | 16 | 0.050 | | 15 | |
| | 13 | 0.210 | | 12 | 0.040 | | 17 | | | 16 | |
| | 14 | | | 13 | | | 18 | | | 17 | 0.110 |
| | 15 | | | 14 | | | | | | **18** | **0.430** |
| | 16 | | | 15 | | | | | | 19 | 0.310 |
| | 17 | | | 16 | | | | | | 20 | 0.090 |
| | | | | | | | | | | 21 | 0.050 |
| | | | | | | | | | | 22 | 0.010 |
| | | | | | | | | | | 23 | |
| | | | | | | | | | | 24 | |
| | | | | | | | | | | 25 | |
| DYS635 | 15 | | DYS390 | 17 | | DYS439 | 6 | | DYS392 | 4 | |
| | 16 | | | 18 | | | 7 | | | 5 | |
| | 17 | | | 19 | | | 8 | | | 6 | |
| | 18 | | | 20 | | | 9 | 0.030 | | 7 | |

TABLE 1. Continued. Allele frequency distribution and average gene diversity for the PowerPlex® Y23 System in a population sample from Bosnia and Herzegovina

| Locus | Allele | Frequency | Locus | Allele | Frequency | Locus | Allele | Frequency | Locus | Allele | Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 19 | | | 21 | | | 10 | 0.100 | | 8 | |
| | 20 | 0.060 | | 22 | 0.050 | | 11 | 0.180 | | 9 | |
| | 21 | 0.170 | | 23 | 0.070 | | **12** | **0.430** | | 10 | |
| | 22 | 0.300 | | **24** | **0.610** | | 13 | 0.220 | | **11** | **0.880** |
| | **23** | **0.410** | | 24.3 | 0.050 | | 14 | 0.040 | | 12 | 0.020 |
| | 24 | 0.060 | | 25 | 0.220 | | 15 | | | 13 | 0.040 |
| | 25 | | | 26 | | | 16 | | | 14 | 0.050 |
| | 26 | | | 27 | | | 17 | | | 15 | |
| | 27 | | | 28 | | | | | | 16 | 0.010 |
| | 28 | | | 29 | | | | | | 17 | |
| | | | | | | | | | | 18 | |
| | | | | | | | | | | 19 | |
| | | | | | | | | | | 20 | |
| DYS643 | 6 | | DYS393 | 7 | | DYS458 | 10 | | DYS385a/b | 7 | |
| | 7 | | | 8 | | | 11 | | | 8 | |
| | 8 | 0.010 | | 9 | | | 12 | | | 9 | |
| | 9 | 0.080 | | 10 | | | 13 | 0.010 | | 10 | |
| | **10** | **0.620** | | 11 | | | 14 | 0.030 | | 11 | 0.130 |
| | 11 | 0.090 | | 12 | 0.080 | | 15 | 0.230 | | 12 | 0.020 |
| | 12 | 0.180 | | **13** | **0.840** | | 16 | 0.160 | | 13 | 0.070 |
| | 13 | 0.020 | | 14 | 0.080 | | **17** | **0.300** | | **14** | **0.310** |
| | 14 | | | 15 | | | 17.2 | 0.010 | | 15 | 0.220 |
| | 15 | | | 16 | | | 18 | 0.220 | | 16 | 0.140 |
| | 16 | | | 17 | | | 19 | 0.030 | | 17 | 0.035 |
| | 17 | | | 18 | | | 20 | | | 18 | 0.045 |
| | | | | | | | 21 | 0.010 | | 19 | 0.030 |
| | | | | | | | 22 | | | 20 | |
| | | | | | | | 23 | | | 21 | |
| | | | | | | | 24 | | | 22 | |
| | | | | | | | | | | 23 | |
| | | | | | | | | | | 24 | |
| | | | | | | | | | | 25 | |
| | | | | | | | | | | 26 | |
| | | | | | | | | | | 27 | |
| | | | | | | | | | | 28 | |
| DYS456 | 11 | | YGATAH4 | 8 | | | | | | | |
| | 12 | | | 9 | | | | | | | |
| | 13 | 0.010 | | 10 | 0.010 | | | | | | |
| | 14 | 0.120 | | **11** | **0.540** | | | | | | |
| | **15** | **0.500** | | 12 | 0.370 | | | | | | |
| | 16 | 0.230 | | 13 | 0.050 | | | | | | |
| | 17 | 0.110 | | 14 | | | | | | | |
| | 18 | 0.020 | | 15 | 0.030 | | | | | | |
| | 19 | 0.010 | | 16 | | | | | | | |
| | 20 | | | 17 | | | | | | | |
| | 21 | | | 18 | | | | | | | |
| | 22 | | | | | | | | | | |
| | 23 | | | | | | | | | | |

*Average gene diversity per locus: 0.619166 ± 0.309990. Major allele frequencies per locus are in bold.

This study showed that increasing the number of highly polymorphic Y-STR markers, to include those tested in our analysis, leads to a reduction of repeating haplotypes, which is very important in the application of forensic DNA analysis.

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**Competing interests** All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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