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# Analysis of 12 X-chromosomal markers in the population of central Croatia

Josip Crnjac [a], Petar Ozretić [b], Siniša Merkaš [c,d], Martina Ratko [c], Mateja Lozančić [c], Sara Rožić [c,d], Daniel Špoljarić [e], Marina Korolija [c,d], Maja Popović [e], Gordan Mršić [c,d,*]

[a] University Department for Forensic Sciences, University of Split, Split, Croatia
[b] Ruđer Bošković Institute, Zagreb, Croatia
[c] Forensic Science Centre "Ivan Vučetić", Zagreb, Croatia
[d] Forensic Science Office, University of Zagreb, Zagreb, Croatia
[e] Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

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ABSTRACT

Investigator® Argus X-12 Kit is a commercially available set that allows simultaneous PCR amplification of 12 X-STR markers belonging to four linkage groups (LG). To assess the forensic efficiency of these markers for the population of central Croatia and consequent applicability in routine forensic casework, DNA from 200 blood samples of unrelated donors (100 female and 100 male) was amplified by Investigator® Argus X-12 Kit and analyzed by capillary electrophoresis. Statistical computations based on allele and haplotype frequencies for LG1 – LG4 were performed using Arlequin 3.5 software and on-line tool available at ChrX-STR.org. In female samples, all X-STR markers were in Hardy-Weinberg equilibrium (HWE). The most informative marker for central Croatia population was DXS10135 with polymorphism information content (PIC) 0.9296. The least polymorphic locus was DXS8378 (PIC = 0.6363). Power of discrimination (PD) varied from 0.6968 to 0.9336 in male and from 0.8476 to 0.9916 in female samples. Combined PD exceeded 0.999999999 in both men and women. In male samples, linkage disequilibrium (LD) test revealed significant association (P = 0.0000) of one marker pair in LG4 and two marker pairs in LG3. Portion of observed haplotypes in the number of possible haplotypes varied from 2.86% to 7.47% across all LGs. LG1 was the most informative with haplotype diversity (H) 0.9972. High PD of all analyzed markers exhibited for central Croatia population confirms suitability of Investigator® Argus X-12 for forensic pertinence. Moreover, results of this study will be included in establishing a national reference X-STR database based on 12 X-STR loci, which is necessary for the correct interpretation of the forensic casework results.

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## 1. Population

Total of 200 samples (100 males and 100 females) from central Croatia region (Fig. 1) were analyzed. Sampling was performed in an attempt to account for all subpopulation variations by choosing unrelated participants from the entire region. All samples were collected during routine forensic work by the Forensic Science Centre "Ivan Vučetić" and their use in the study is approved by the Ethics Committee of the Institute for Medical Research and Occupational Health, Zagreb, Croatia.

## 2. Extraction

Blood samples were collected by finger puncture and applied on Flinder's Technology Associates (FTA) cards (Whatman, Maidstone, Kent, UK). Chelex 100 was used to extract DNA from FTA cards [1].

## 3. DNA quantification

For quantification, Investigator Quantiplex Kit (Qiagen GmbH, Hilden, Germany) was used and normalization of samples to approximately 1 ng/μL was carried out subsequently.

## 4. Amplification

Investigator® Argus X-12 Kit (Qiagen GmbH, Hilden, Germany) was used for amplification of amelogenin for sex determination

* Corresponding author at: Forensic Science Centre "Ivan Vučetić", Zagreb, Croatia.
*E-mail address:* gmrsic@mup.hr (G. Mršić).

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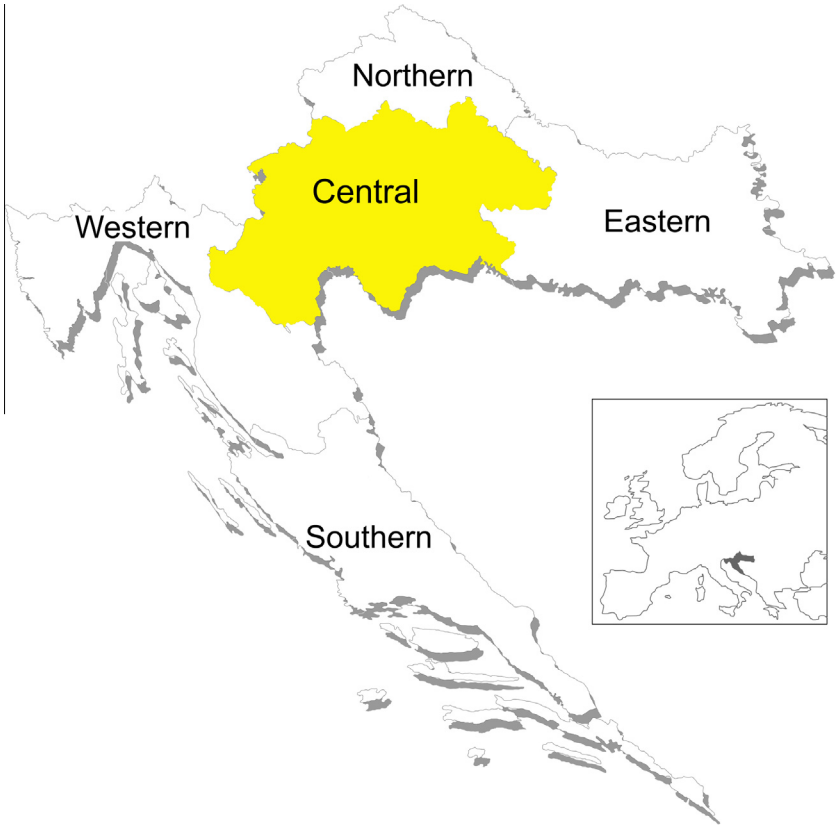


**Fig. 1.** Regions of Croatia. In this study samples from the population of central Croatia were analyzed. Central Croatia region (yellow) comprises five counties (Zagreb, Sisak-Moslavina, Karlovac, Bjelovar-Bilogora and City of Zagreb County) with the total of 1.5 million inhabitants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and 12 X-STR markers belonging to four different LGs: LG1 (DXS10148, DXS10135, DXS8378), LG2 (DXS7132, DXS10079, DXS10074), LG3 (DXS10103, HPRTB, DXS10101), and LG4 (DXS10146, DXS10134, DXS7423).

## 5. Typing

Amplification products were analyzed on 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Data obtained from capillary electrophoresis were analyzed using Genemapper ID-X software (version 1.4, Applied Biosystems). All procedures and protocols were carried out following manufacturer's instructions.

## 6. Analysis of data

The allele frequencies for all samples and haplotype frequencies for male samples were determined by counting. For biallelic male and triallelic female samples, allele(s) with highest frequencies were selected for further calculations [2]. Testing for a departure from Hardy-Weinberg Equilibrium (HWE), including observed heterozygosity (Ho) and expected heterozygosity (He), was performed only for female samples. Linkage disequilibrium (LD) test for pair-wise loci was performed for female and male samples separately. For DXS10134-DXS10079 pair of loci $r^2$ correlation coefficients between all allele combinations were also computed. Pair-wise genetic distances ($F_{ST}$) were calculated for inter-population comparison of haplotype frequencies between central Croatian population and Czech [3], German [4], Hungarian [5], Swedish [6], Danish, Somalian, Greenlandic [7], Chinese and Japanese [8] populations. Genetic heterogeneity within population was estimated as gene i.e. haplotype diversity (H) for male haplotype data. All aforementioned computations were performed using Arlequin software v3.5.2.1 [9] and significance level for all statistical tests was set to 0.05, corrected for multiple comparisons using Bonferroni adjustment.

Forensic parameters encompassing polymorphism information content (PIC), power of exclusion (PE), power of discrimination (PD) for males and for females, mean exclusion chance (MEC) for deficiency cases (Krüger's formula), MEC for normal trios consisting of a mother, a daughter and a putative father (Kishida's formula), and MEC for duos consisting of a daughter and a putative father (Desmarais' formula), were computed based on allele frequencies data using on-line tool available at ChrX-STR.org web page [10].

## 7. Results and discussion

We determined allele frequencies, observed heterozygosity, expected heterozygosity and P values for the HWE of 12 X-STR in the population of central Croatia (Table 1). We found no statistically significant deviations from HWE at any locus after Bonferroni correction, which makes our data set suitable for match probability calculations in forensic work. Variant alleles were observed at the DXS10103 (14), DXS10134 (35.2, 37.1), DXS10135 (15, 26.2, 47.2), DXS10146 (37.2, 47.2, 48.2,) and DXS10148 (17.1, 31.1, 32.1) loci. Two triallelic patterns were observed on DXS10079 locus (14, 20, 21 and 18, 20, 23). One biallelic pattern was found in male sample on DXS10079 locus (21, 22).

Based on allele frequencies, we further determined forensic parameters (Table 2) and compared results with another population study of 8 X-chromosomal markers for population of central

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