

# Forensic Efficiency Parameters for the 15 STR Loci in the Population of the Island of Cres (Croatia)

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## ABSTRACT

Forensic parameters based on 15 AmpFlSTR Identifiler short tandem repeat (STR) loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818 and FGA) were evaluated in the sample of 122 unrelated, autochthonous, adult individuals from the Island of Cres (Croatia). PCR amplification was performed with the AmpFlSTR Identifiler PCR Amplification Kit and the amplified products were separated and detected using the ABI 3130 DNA genetic analyzer. The agreement with Hardy Weinberg Equilibrium (HWE) was confirmed for all loci ( $p > 0.05$ ). The combined power of discrimination (PD) and the combined power of exclusion (PE) for the 15 tested STR loci were 0.99999999999999997988728679 and 0.999997397, respectively. According to the presented data, D18S51 proved to be the most informative marker followed by markers D2S1338 and D21S11. Inter-population comparisons in allele frequencies with other East Adriatic Islands revealed significant differences for all analyzed population pairs ranging from 4 loci (Cres vs. Hvar) to 1 locus (Cres vs. Krk). Furthermore, allele frequencies comparisons of Cres and Croatian mainland revealed the lack of statistically significant differences at all studied loci. The results of the current study indicate that the examined fifteen STR loci are useful genetic markers for individual identification and paternity testing in Croatian population from the Island of Cres.

**Key word:** STRs, AmpFlSTR identifiler, forensic parameters, the Island of Cres, Croatia

## Introduction

Short tandem repeats (STRs) are loci with alleles composed of tandemly repeated short DNA sequences of 2–7 base pair in length<sup>1</sup>. There are hundreds of these STR regions in the human genome, but 10 to 15 regions are sufficient to give high levels of discrimination between individuals<sup>2</sup>. Numerous previous studies have demonstrated that STRs have become the choice of loci for determination of biological and parentage relationship of individuals as well as for forensic analysis<sup>2–10</sup>.

The island of Cres is the second largest island in the Croatian section of the Adriatic sea (Figure 1). The last

official population census (2001) lists a total population of 2946 inhabitants living in 26 settlements. Cres is a hilly island, 66 km long and ranging in width from 2 to 12 km.

The current population structure of Cres was formed through several immigratory episodes of genetically distant populations. The earliest available archeological data show that this area was continuously inhabited since the middle Paleolithic. The oldest western Balkan population of a clearly Indo European-speaking Illyrians remained the basic population after which other popula-



**TABLE 1**  
FORENSIC STATISTICAL PARAMETERS AT THE 15 STR LOCI IN THE POPULATION FROM THE ISLAND OF CRES (CROATIA)

| Allele                        | D8S1179 | D21S11 | D7S820 | CSF1PO | D3S1358 | TH01  | D13S317 | D16S539 | D2S1338 | D19S433 | VWA   | TPOX  | D18S51 | D5S818 | FGA   |
|-------------------------------|---------|--------|--------|--------|---------|-------|---------|---------|---------|---------|-------|-------|--------|--------|-------|
| H <sub>obs</sub> <sup>a</sup> | 0.795   | 0.844  | 0.786  | 0.721  | 0.819   | 0.762 | 0.795   | 0.672   | 0.868   | 0.745   | 0.819 | 0.59  | 0.877  | 0.713  | 0.795 |
| H <sub>exp</sub> <sup>b</sup> | 0.808   | 0.841  | 0.817  | 0.7    | 0.779   | 0.758 | 0.796   | 0.744   | 0.863   | 0.78    | 0.81  | 0.626 | 0.871  | 0.737  | 0.836 |
| $\chi^2$ -test                | 0.064   | 0.001  | 0.565  | 0.17   | 0.917   | 0     | 0.005   | 2.985   | 0.002   | 0.67    | 0.02  | 0.556 | 0.003  | 0.267  | 1.218 |
| Exact test (p)                | 0.524   | 0.592  | 0.107  | 0.274  | 0.45    | 0.647 | 0.099   | 0.06    | 0.352   | 0.29    | 0.579 | 0.348 | 0.662  | 0.073  | 0.723 |
| PM <sup>c</sup>               | 0.071   | 0.048  | 0.068  | 0.145  | 0.089   | 0.098 | 0.079   | 0.108   | 0.040   | 0.076   | 0.071 | 0.195 | 0.035  | 0.105  | 0.049 |
| PD <sup>d</sup>               | 0.929   | 0.952  | 0.932  | 0.855  | 0.911   | 0.902 | 0.921   | 0.892   | 0.960   | 0.924   | 0.929 | 0.805 | 0.965  | 0.895  | 0.951 |
| PE <sup>e</sup>               | 0.590   | 0.684  | 0.575  | 0.462  | 0.636   | 0.531 | 0.590   | 0.386   | 0.732   | 0.503   | 0.636 | 0.279 | 0.749  | 0.449  | 0.590 |
| PIC <sup>f</sup>              | 0.78    | 0.82   | 0.79   | 0.65   | 0.75    | 0.72  | 0.77    | 0.70    | 0.85    | 0.75    | 0.78  | 0.57  | 0.86   | 0.70   | 0.82  |

<sup>a</sup>Hobs – observed heterozygosity

<sup>b</sup>H<sub>exp</sub> – expected heterozygosity

<sup>c</sup>PM – probability of match

<sup>d</sup>PD – power of discrimination

<sup>e</sup>PE – power of exclusion

<sup>f</sup>PIC – polymorphism information content

share the same DNA profile presented in a paternity case<sup>16</sup>. Single locus PE values ranged from 0.279 (TPOX) to 0.749 (D18S51), whereas combined value using all fifteen loci has increased the forensic utility to 0.999997397 (99.99%).

The agreement with Hardy-Weinberg equilibrium (Table 1), tested by the  $\chi^2$ -test based on the number of observed and expected heterozygotes and the exact test based on the number of observed and expected genotypes, is confirmed for all tested loci ( $p > 0.05$ ).

In this study we also compared allele frequencies of fifteen loci of the Cres population with the same number of loci obtained from Croatian mainland<sup>17</sup>, whereas nine available loci were compared with East Adriatic Islands<sup>18–22</sup>. These exact tests of population differentiation<sup>23</sup> show statistically significant differences (significance level was 0.05) in allele frequencies between Cres and Hvar at 4 loci (D7S820, CSF, TPOX and D5S818), followed by Cres and Korčula at 3 loci (D7S820, CSF and D3S1358), Cres and Vis at 3 loci (D3S1358, D13S317 and FGA), Cres and Brač at 2 loci (TH01 and FGA). There are significant differences at 1 locus (D7S820) only between two

geographically closest islands Cres and Krk. Significant differences in allele frequencies between the Island of Cres and Croatian mainland were not observed at any of the studied loci.

In conclusion, the analysis of fifteen AmpFlSTR Identifier short tandem repeat (STR) loci yielded reliable forensic parameters and was highly differentiating in studied isolated rural populations from Eastern Adriatic islands, thus proving the effectiveness of multiple STR locus profiles even in small rural populations.

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## REFERENCES

- WEBER JL, MAY PE, Am J Hum Genet, 44 (1989) 388. — 2. HAMMOND HA, JIN L, ZHONG Y, CASKEY CT, CHAKRABORTY S, Am J Hum Genet, 55 (1994) 175. — 3. SÃO-BENTO M, CARVALHO M, ANDRADE L, LOPES V, SERRA A, GAMERO JJ, CORTE-REAL F, VIDE MC, ANJOS MJ, Forensic Sci Int: Genetics Supplement Series, 1 (2008) 367. — 4. BUTLER JM, J Forensic Sci, 51 (2006) 253. — 5. SHIMADA I, BRINKMANN B, TUYEN NQ, HOHOFF C, Int J Legal Med, 116 (2002) 246. — 6. BUDOWLE B, CHAKRABORTY R, Legal Medicine, 3 (2001) 29. — 7. SPARKES R, KIMPTON C, GILBARD S, CARNE P, ANDERSON J, OLDROYD N, THOMAS D, URQUHART A, GILL P, Int J Legal Med, 109 (1996) 195. — 8. HAGELBERG E, GRAY IC, JEFFREYS AD, Nature, 352 (1991) 427. — 9. JEFFREYS AJ, ALLEN MJ, HAGELBERG E, SONNBERG A, Forensic Sci Int, 56 (1992) 65. — 10. MARJANOVIĆ D, KAPUR L, DROBNIĆ K, BUDOWLE B, POJSKIĆ, HADŽISELIMOVIC R, Human Biology, 76 (2004) 15. — 11. FORENBAHER S, Coll Antropol, 23 (1999) 521. — 12. RUDAN P, FINKA B, JANIČIJEVIĆ B, JOVANOVIĆ V, KUŠEC V, MILIČIĆ J, MIŠIČIĆ-DURAKOVIĆ M, ROBERTS DF, SCHMUTZER LJ, SMOLEJ NARANČIĆ N, SUJOLDŽIĆ A, SZIROVICZA L, ŠIMIĆ D, ŠIMUNOVIĆ P, ŠPOLJAR-VRŽINA SM, Anthropological investigations of Eastern Adriatic. Biological and cultural microdifferentiation of the rural populations on the island of Hvar (in Croatian) 1990, Zagreb, HAD. — 13. GUO SW AND THOMPSON EA, Biometrics, 48 (1992) 361. — 14. TEREBA A, Tools for analysis of population statistics, Profiles in DNA 3, Promega Corporation, 1999, Available from: URL: <http://promega.com/geneticidtools/powerstats/>. — 15. SCHNEIDER S, ROESSLI SD, AND EXCOFFIER L, A software for population ge-

netics data analysis, Arlequin version 2.0, Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland, 2000. — 16. BRENNER C, MORCIS JW, Paternity index calculations in single locus hypervariable DNA probes: validation and other studies. The International Symposium on Human Identification, (Promega Corporation, Madison, WI, 1990). — 17. PROJIĆ P, ŠKARO V, ŠAMLIJA I, POJSKIĆ N, DURMIĆ-PAŠIĆ A, KOVAČEVIĆ L, BAKAL N, PRIMORAC D, MARIJANOVIĆ D, Croat Med J, 48 (2007) 473. — 18. MARTINOVIĆ I, BARAĆ L, FURAĆ I, JANIĆIJEVIĆ B, KUBAT M, PERIČIĆ M, VIDOVIĆ B, RUDAN P, Human Biology, 71 (1999) 341. — 19. MARTINOVIĆ KLARIĆ I, BARAĆ L, BUKOVIĆ D,

FURAĆ I, GEBER G, JANIĆIJEVIĆ B, KUBAT M, PERIČIĆ M, VIDOVIĆ PUPIĆ B, RUDAN P, Annals of Human Biology, 28 (2001) 281. — 20. MARTINOVIĆ KLARIĆ I, BARAĆ L, BUKOVIĆ D, FURAĆ I, GEBER G, HOMO – Journal of Comparative Human Biology, 51 (2000) 141. — 21. MARTINOVIĆ KLARIĆ I, PERIČIĆ M, BARAĆ LAUC L, JANIĆIJEVIĆ B, KUBAT M, PAVIČIĆ D, RUDAN I, WANG N, JIN L, CHAKRABORTY R, DEKA R, RUDAN P, Human Biology, 77 (2000) 471. — 22. MARTINOVIĆ KLARIĆ I, American Journal of Human Biology, 12 (2000) 509. — 23. RAYMOND M, ROUSSET F, Evolution, 49 (1995) 1280.

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## UČINKOVITOST FORENZIČKIH PARAMETARA 15 STR LOKUSA U POPULACIJI OTOKA CRESA (HRVATSKA)

### SAŽETAK

Forenzički parametri određeni su na uzorku od 122 nesrodne, odrasle osobe podrijetlom sa otoka Cresa, na temelju 15 AmpF/STR Identifiler (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818 and FGA) kratkih uzastopno ponavljajućih sljedova DNK (STR). Lančana reakcija polimerazom provedena je upotrebom AmpF/STR Identifiler PCR Amplification Kit sustava. Umnoženi produkti razdvojeni su i određeni pomoću instrumenta ABI 3130 DNK *Genetic Analyzer*. Slaganje s Hardy-Weinbergovom ravnotežom (HWE) potvrđeno je za sve analizirane lokuse ( $p > 0,05$ ). Zajednička snaga diskriminacije (PD) i zajednička snaga isključivanja (PE) za 15 analiziranih lokusa iznosila je 0,99999999999999997988728679 odnosno 0,999997397. Prema dobitim rezultatima, lokus D18S51 je najinformativniji, a zatim slijede lokusi D2S1338 i D21S11. Na temelju usporedbe učestalosti alela između populacije otoka Cresa i ostalih istraživanih otoka istočnog Jadrana, utvrđene su značajne razlike između svih analiziranih otočnih parova. Najveća razlika utvrđena je između otoka Cresa i Hvara (na 4 lokusa), dok je najmanja razlika utvrđena između otoka Cresa i Krka (na 1 lokusu). Nadalje, usporedba otoka Cresa i kontinentalne Hrvatske nije pokazala statistički značajnu razliku niti na jednom od analiziranih lokusa. Rezultati provedenog istraživanja ukazali su na značajnu učinkovitost 15 analiziranih STR lokusa kao važnih genetičkih biljega za utvrđivanje identiteta osoba i dokazivanje oćinstva u populaciji otoka Cresa.