

Prof.dr.sc Dragan Primorac

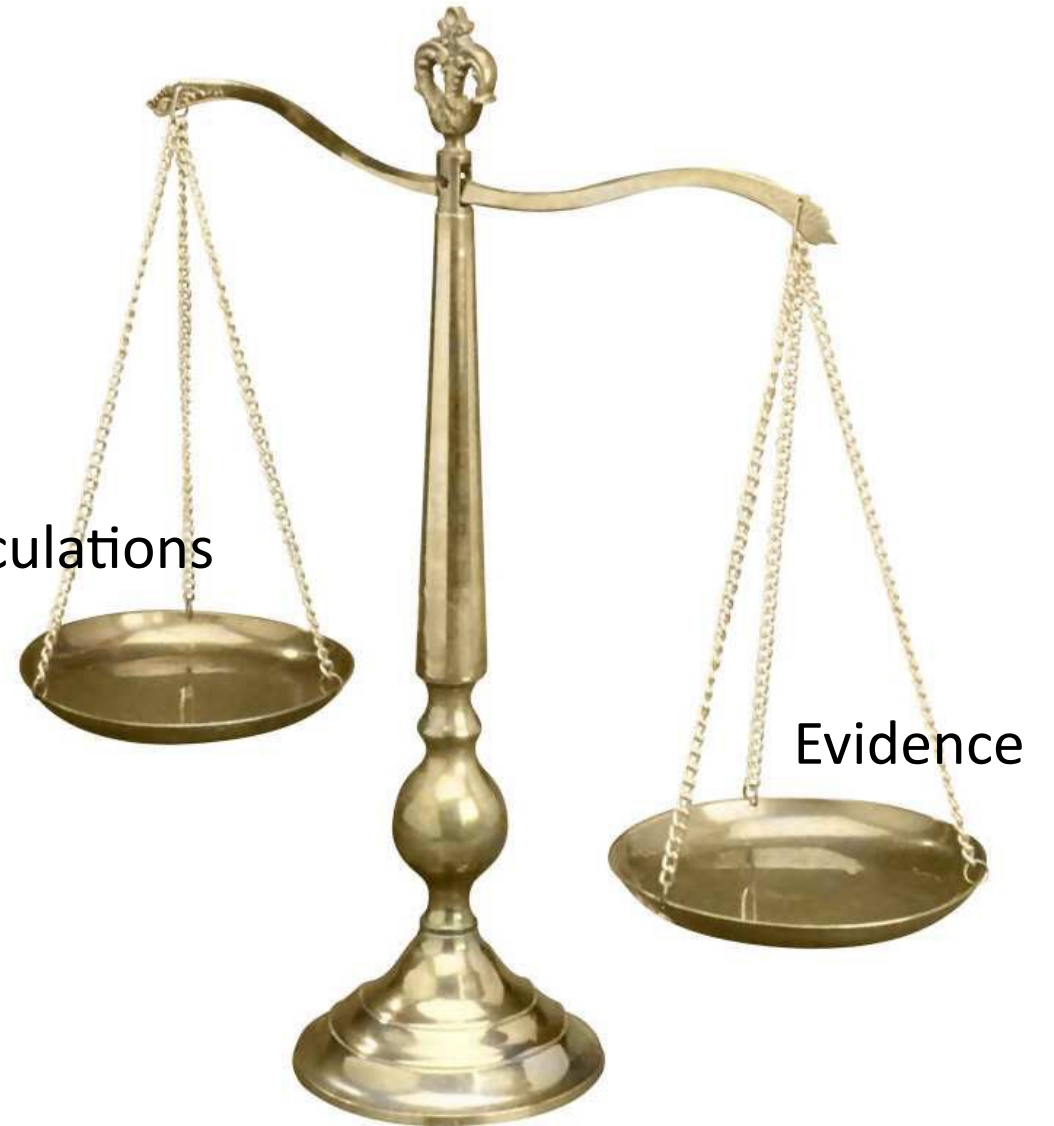
**STRATEGIJA RAZVOJA PRIMJENJENE GENOMIKE U
HRVATSKOJ**

FORENZIČKA GENOMIKA



“EVIDENCE-BASED SCIENCE (FORENSICS)”

Speculations

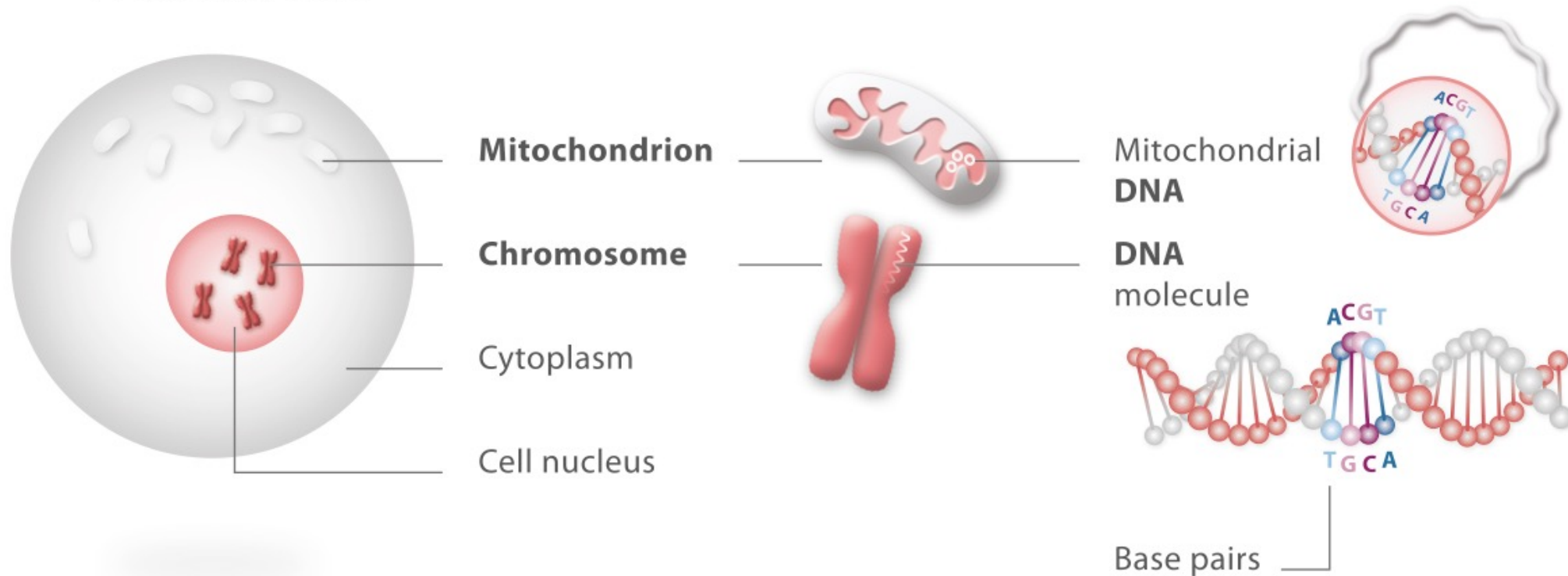


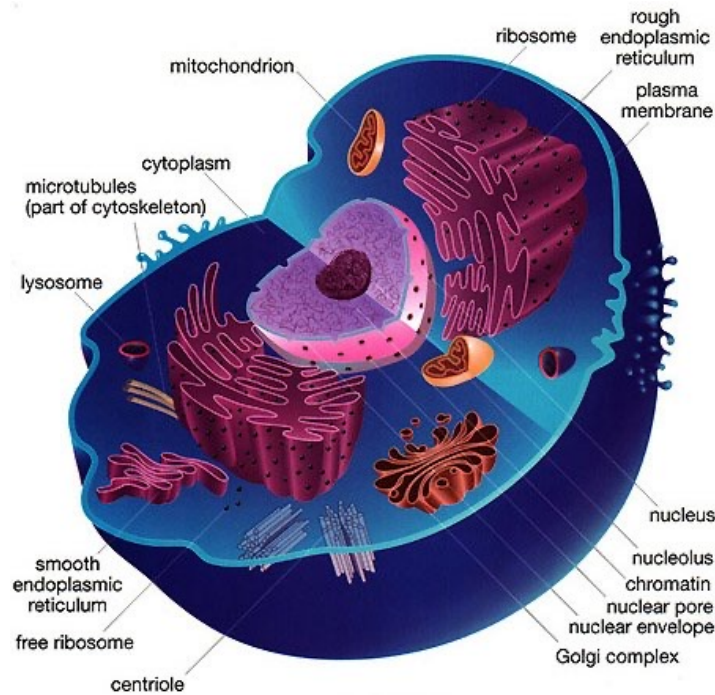
**ABSENCE OF EVIDENCE IS NOT
EVIDENCE OF ABSENCE**



ANALIZA HUMANE DNA

Human cell



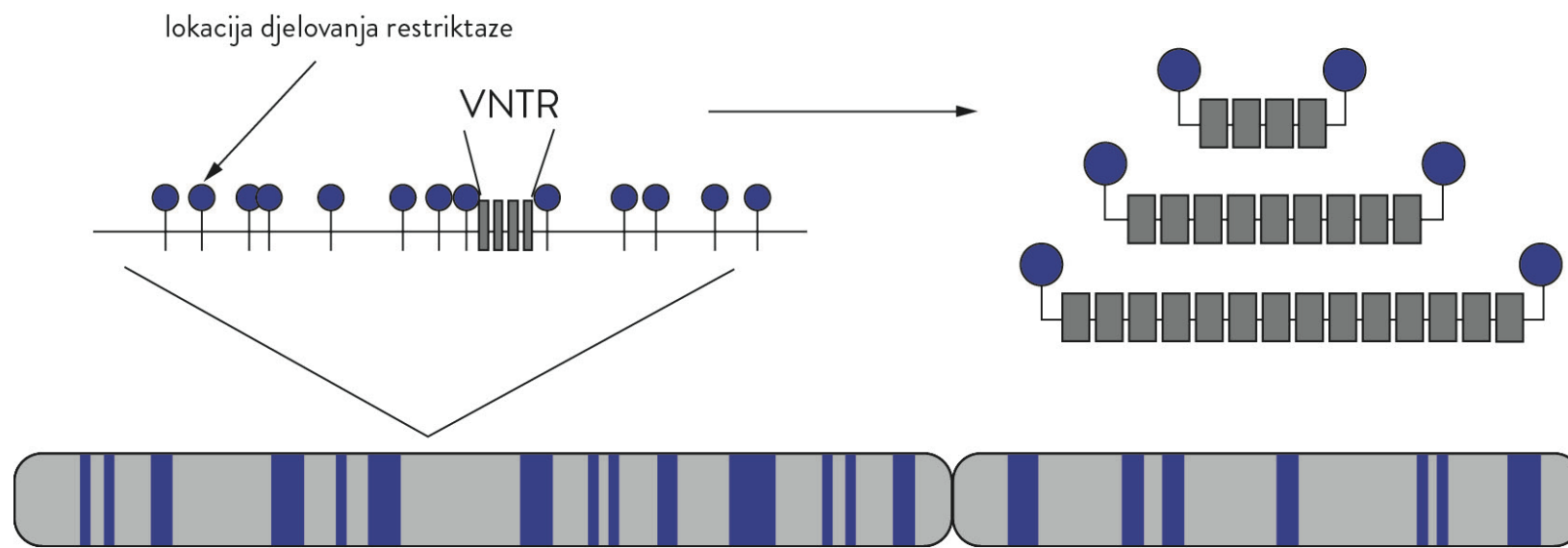


For a human diploid cell, the amount of DNA per cell is 6 pg

1 pg = 978 Mb*

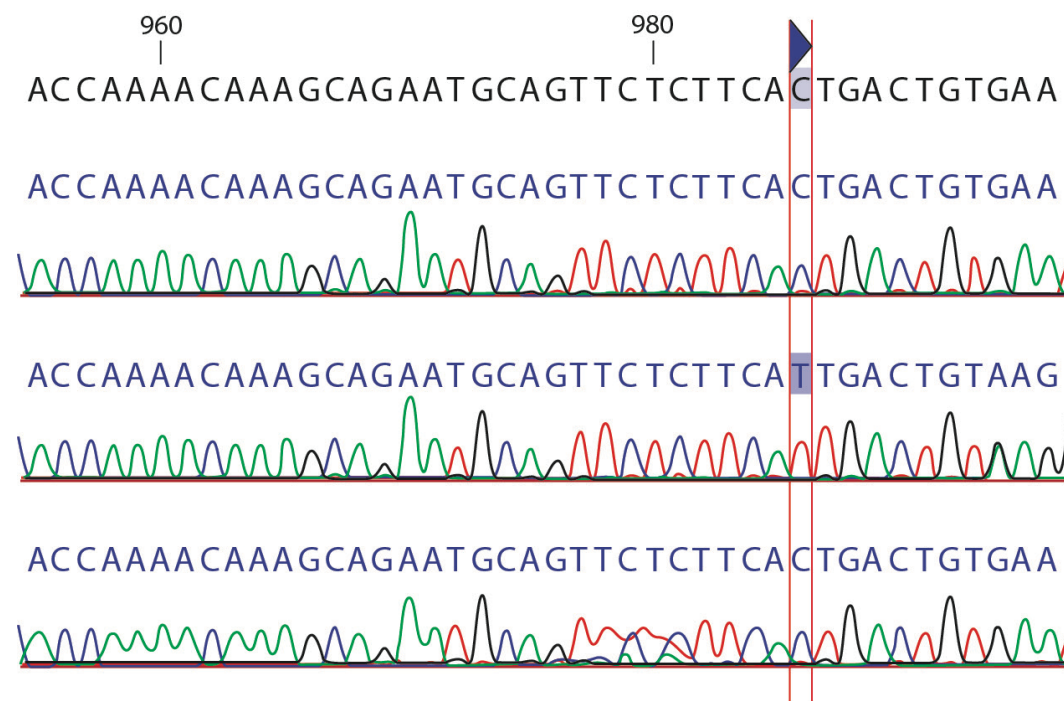
Since the entire human genome (haploid) is 3 000 Mb, all together in one human diploide cell there is 3 pg of DNA per haploid genome or 6 pg per the entire genom (diploid).

*Dolezel J, Bartoš J, Voglmayr H, Greilhuber J (2003). "Nuclear DNA content and genome size of trout and human". *Cytometry A* **51** (2): 127–128.



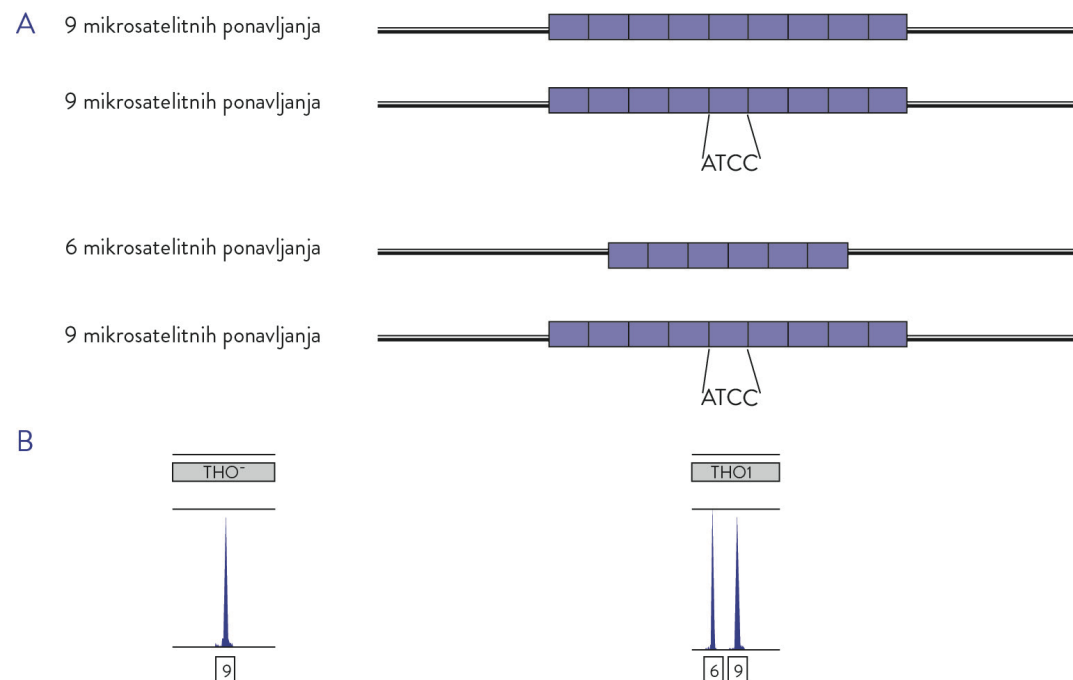
Shematski prikaz VNTR biljega



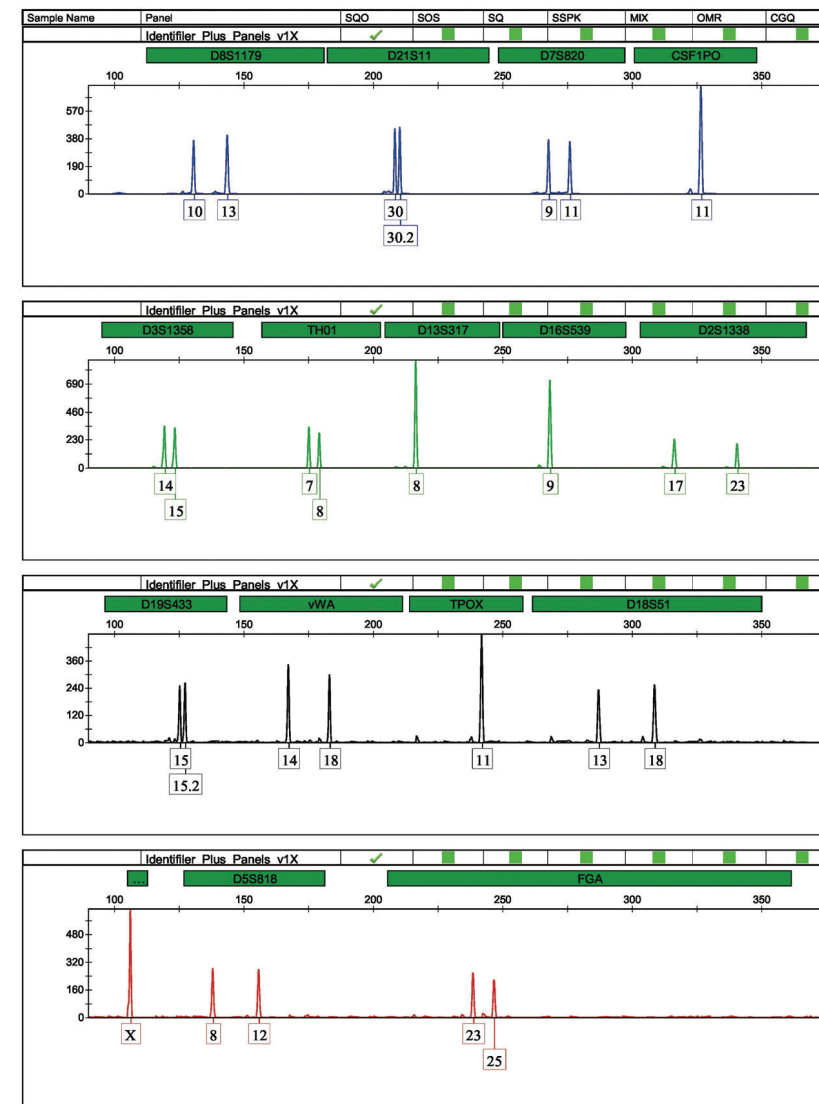


Shematski prikaz SNP biljega





Shematski prikaz STR biljega



Prikaz rezultata AmpFLSTR® Identifier® Plus PCR Amplification sustava.

Primorac D, Marjanović D, Škaro V, Projić P, Džijan S, Topić E, Primorac D, Janković S, Štefanović M, Forenzička analiza DNA.

(Ur: Topić E, Primorac D, Janković S, Štefanović M. 2., dopunjeno izdanje. Zagreb: Medicinska naklada; 2018.

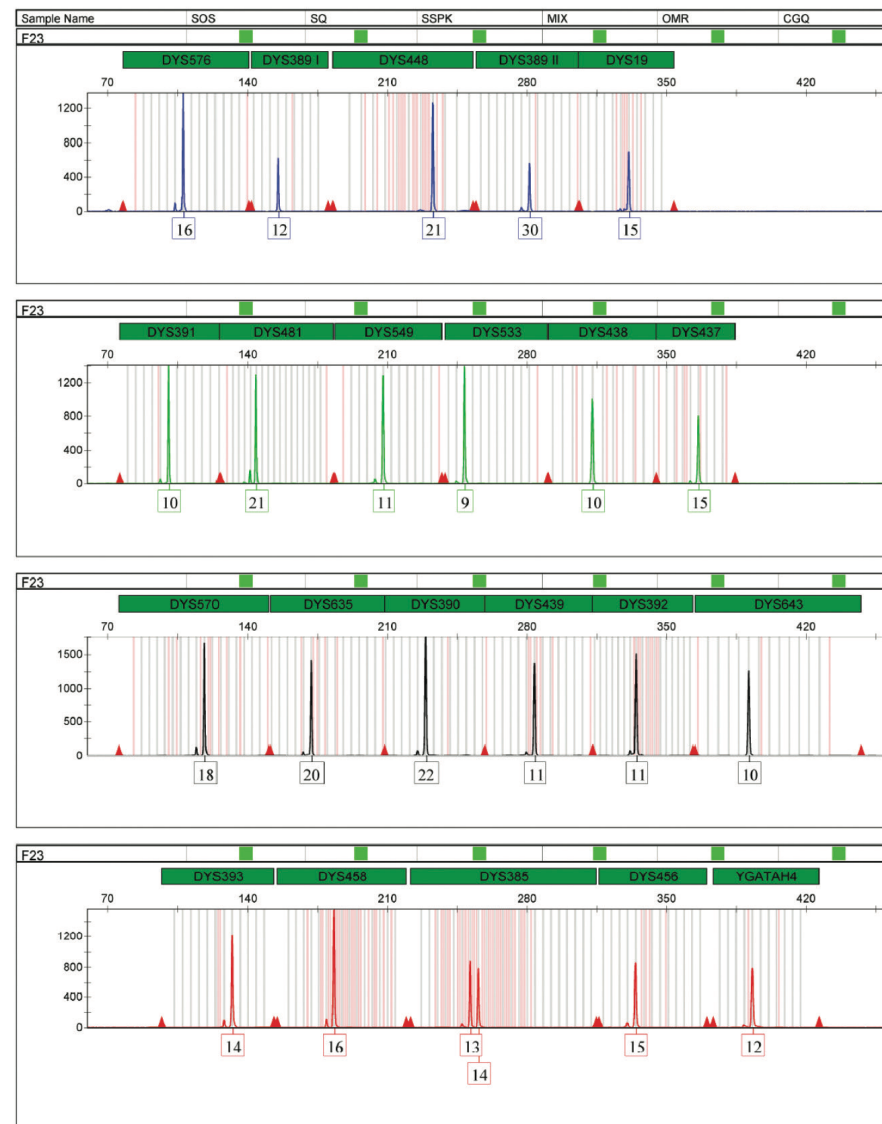


Analiza genskih biljega na Y kromosomu

Njegov veći dio (oko 95 %) ne podliježe homolognoj rekombinaciji tijekom mejoze - nerekombinirajući dio Y-kromosoma (engl. *non-recombining region of the Y chromosome*, NRY).

Samo se 5 % Y-kromosoma potencijalno može rekombinirati s X-kromosomom i ta regija se naziva pseudoautosomnom regijom X i Y-kromosoma.

Primjena Y-kromosoma u forenzičkoj znanosti može značajno pomoći u rješavanju slučajeva kao što su prenatalno testiranja očinstva (ukoliko potencijalni očevi nisu u srodstvu putem muške linije), postupak identifikacije i testiranje srodstva po muškoj liniji u slučajevima kad drugačiji tip analize nije moguć, silovanje (posebice kada je uključeno više muškaraca), studije migracija stanovništva i evolucijske studije.



Y-STR profil generiran primjenom seta reagencija PowerPlex Y23 (Promega).

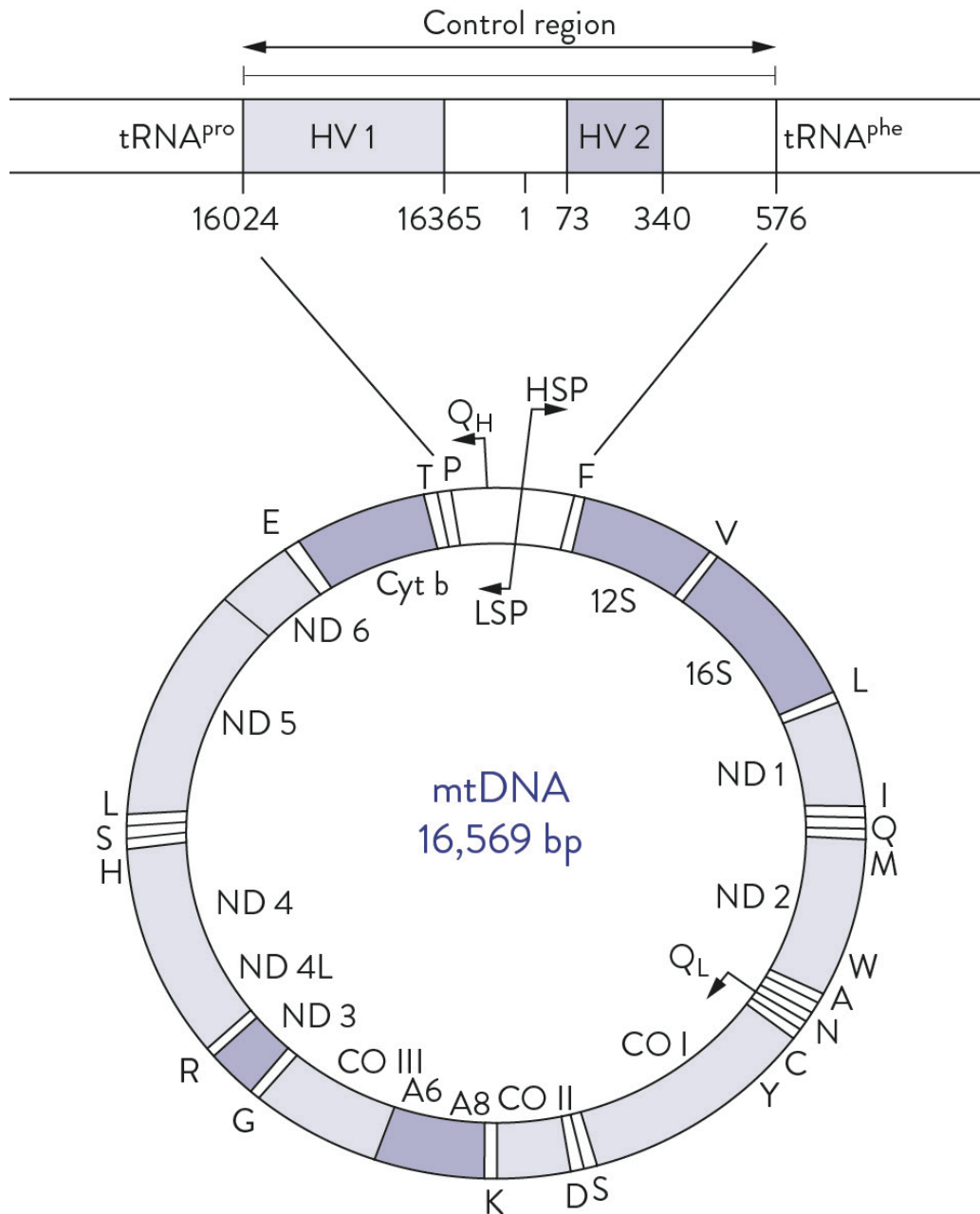


Analiza genskih biljega na X kromosomu

Za X-STR biljege najznačajniji multipleksni sustav koji se primjenjuje je Investigator® Argus X-12 (Qiagen), koji uključuje 12 X-STR biljega (DXS8378, DS10135, DXS10148, DXS7132, DXS10074, DXS10079, HPRTB, DXS10101, DXS10103, DXS7423, DXS10134, DXS10146).



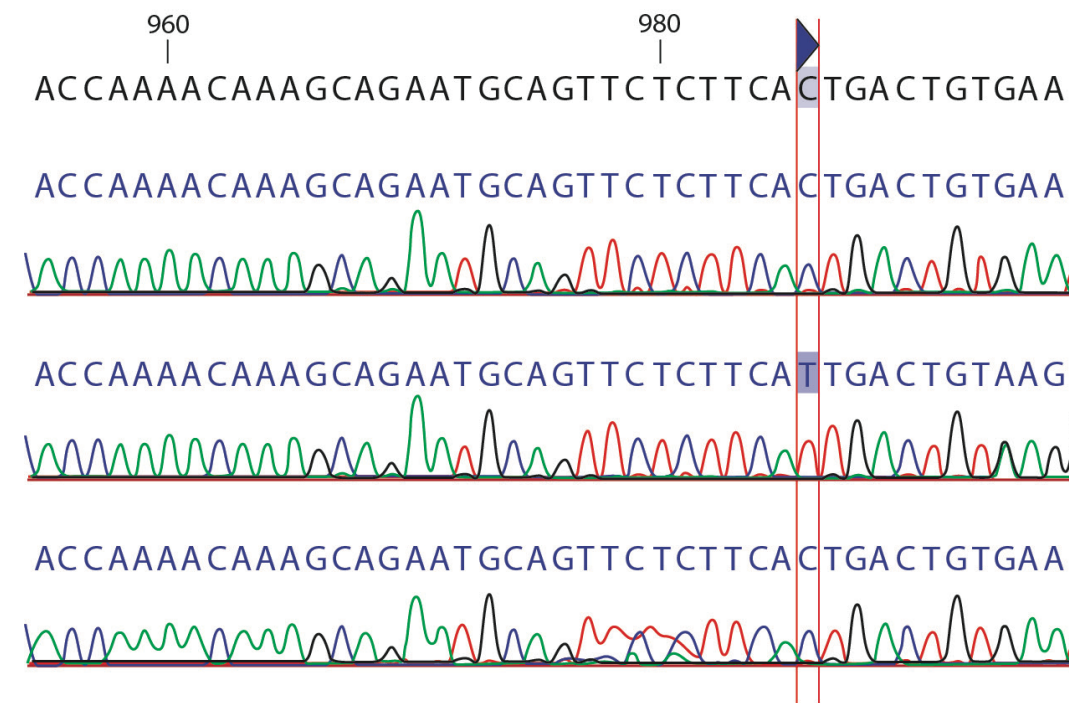
ANALIZA mt DNA



Mt DNA

- Nasljeđivanje putem majčine linije
- Slabija snaga diskriminacije
- Heteroplazmija

Primarno područje mtDNA, od interesa za forenzičku analizu, jest kontrolna regija koja nema kodirajuću funkciju. Unutar te regije su hipervarijabilna regija I (HVI) i hipervarijabilna regija II (HVII) koje su bogate polimorfizmima



Primorac D, Marjanović D, Škaro V, Projić P, Džijan S, Topić E, Primorac D, Janković S, Štefanović M, Forenzička analiza DNA.

(Ur: Topić E, Primorac D, Janković S, Štefanović M. 3., dopunjeno izdanje. Zagreb: Medicinska naklada; 2024.



METODOLOGIJE KOJE TREBA SUSTAVNO INTEGRIRATI U FORENZIČKOJ GENETICI

- Sekvenciranja nove generacije (engl. Next-generation sequencing) s naglaskom na cijelogenomsku analizu,
- Brze (rapidne) DNA tehnologije,
- mtDNA tehnika linearnih tračica sa svrhom brze analize informativnih polimorfizama u cjelokupnom mitohondrijskom genomu u svrhu forenzičkih vještačenja.



POSTUPCI KOJE TREBA SUSTAVNO INTEGRIRATI U FORENZIČKU GENETIKU

- 1.Forenzička identifikacija tkiva analizom nukleinskih kiselina,
- 2.DNA fenotipizacija,
- 3.Molekularna obdukcija,
- 4.Utvrđivanje starosti biološkog traga,
- 5.Forenzičke analiza RNA,
- 6.Forenzičke analiza biljne DNA,
- 7.Forenzičke analiza životinjske DNA,
- 8.Forenzička analiza DNA kukaca,
- 9.Farmakogenomika,
- 10.Brze rapidne detekcije mikroorganizama

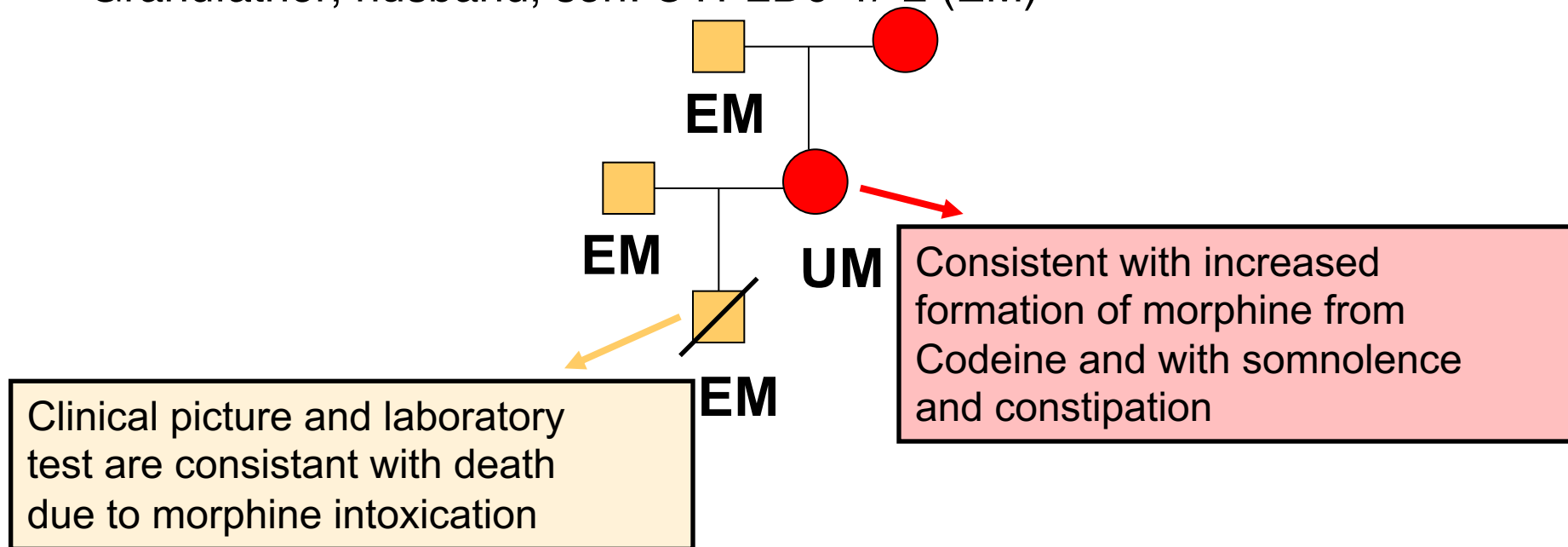


NEKOLIKO PRIMJERA

FARMAKOGENOMSKA ANALIZA U FORENZICI

MORPHINE POISONING IN A BREASFD NEONATE

- Genetics for cytochrome P450 2D6 (CYP2D6), the enzyme catalysing the O-demethylation of codeine to morphine.
- Mother: was heterozygous for a CYP2D6*2A allele with CP2D6*2x2 gene duplication, classified as an **ultra-rapid metabolizer (UM)**
- Grandfather, husband, son: CYP2D6*1/*2 (EM)





Personalized medicine is an important topic in today's health care. Adjusting the dose of frequently prescribed medicines to the genetic profile of patients is a tool which is nowadays available: pharmacogenetics. Yet, it is not always clear how this genetic information can be used in health care settings. Or for which drugs this information can be helpful. The current book "Pharmacogenetics in Clinical Practice" by Dragan Primorac and Wolfgang Höppner excellently provides this information in a clear and accessible manner. In 210 pages, an overview of 55 drugs, their important gene-drug interactions are presented, including background, genes involved, test descriptions, indications for using pharmacogenetic testing, and the consequences of test results. It is especially the included tables with specific dose recommendations based on genotype that make this book an essential tool for every health care practitioner who want to achieve optimal and safe drug treatment of their patients. Highly recommended.

Professor Ron van Schaik, Ph.D.

Erasmus University Medical Center, The Netherlands

Modern medicine is unthinkable without modern drugs. Pharmacogenetics is lifting their clinical use to new levels of efficacy and safety. This book delivers the basis of personalized medicine for them – it has been of great significance for me personally. Clinical medicine of the future will strongly depend on knowledgeable physicians capable of applying pharmacogenetics to the benefit of their patients. This book serves as an outstanding tool to, provide the essentials of pharmacogenomics for all clinicians.

Prof. Johannes Brachmann, M.D., Ph.D.,

Medical School REGIOMED, Coburg, Germany

For those keen on bringing pharmacogenomics into their daily clinical practice, this book is sure to serve as a reliable and informative resource. I highly recommend this publication to physicians, pharmacists, nurses, and other healthcare providers who are committed to bringing personalized medicine to their patients' bedside. It quickly became one of the most frequent resources to consult in my pharmacogenomics practice because it is easy to use, quick to reference, and relevant to practice.

Dr. Adrijana Kekić, Pharm. D.,

Mayo Clinic, Phoenix, Arizona, United States

This compendium on "Pharmacogenetics in clinical practice" by Primorac and Höppner has compiled clinically highly relevant contemporary information on the pharmacogenetics of 55 often prescribed drugs for physicians and other health care professionals. The great value of this work lies in the efficient communication of the essential information on how the specific genetic makeup determines drug actions and adverse reactions. Physicians and health care professionals can immediately come up with sound decisions on the use of these drugs. Primorac and Höppner give the state of the art knowledge and advice that enables personalized medicine to harvest the latest progress in the fields of molecular biology and genetics for the practice of drug treatment.

Prof. Burkhard Poeggeler, Ph.D.,

Georg-August-University of Göttingen, Göttingen, Germany

The book "Pharmacogenetics in Clinical Practice" by Dragan Primorac and Wolfgang Höppner, which is available in 3 languages, deals with the pharmacokinetics of 55 frequently used drugs against the background of pharmacogenetics. It takes up in a clear form the difficulties that the practicing physician experiences in his everyday life when he repeatedly finds that the same drug in apparently identical patients (age, size, gender) has a completely different effect and thus potentially one has an adverse effect. The work shows that the way to personalized medicine, as we would like it to be in the future, is through understanding pharmacogenetics.

Dr. Jan Oliver Schönfeldt, M.D.,

Institut für Kinderneurologie, Hamburg, Germany

The second edition of this book, written in English, German and Croatian, presents a systematic approach to patient pharmacogenetics. The recommendations are based on the CPIC ones and have the highest clinical level of evidence. This book certainly represents a contribution to safer treatment with minimal or no side effects, along with its role in the current and future efforts to develop fully personalized medicine.

Prof. Elizabeta Topić, Ph.D.,

Croatian Society of Medical Biochemists, Zagreb, Croatia

This text is excerpted from original summary statements by reviewers.



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Pharmacogenetics in clinical practice / Farmakogenetika u kliničkoj praksi

Pharmacogenetics in clinical practice

Experience with 55 commonly used drugs

Pharmakogenetik in der klinischen Praxis

Die Erfahrungen mit 55 häufig verwendeten Medikamenten

Farmakogenetika u kliničkoj praksi

Iskustvo s 55 lijekova korištenih u kliničkoj praksi

Dragan Primorac and Wolfgang Höppner

Pharmacogenomics in Clinical Practice

Dragan Primorac
Wolfgang Höppner
Lidija Bach-Rojecky
Editors

 Springer



MOLEKULARNA OBDUKCIJA

FORENSIC DNA APPLICATIONS

An Interdisciplinary Perspective

Forensic DNA Applications: An Interdisciplinary Perspective, Second Edition is fully updated to outline the latest advances in forensic DNA testing techniques and applications. It continues to fill the need for a reference book for people working in the field of forensic molecular biology testing and research as well as individuals investigating and adjudicating cases involving DNA evidence, whether they be civil or criminal cases.

DNA techniques have greatly impacted obvious traditional forensic areas, but such advances have also positively affected myriad new areas of research and inquiry. It is possible today to think about solving forensic problems that were simply unheard of even a few years ago. As such, the book pulls all relevant research and applied science together into a detailed and comprehensive collection.

Part I begins with the history and development of DNA typing and profiling for criminal and civil purposes. It discusses the statistical interpretation of results with case examples, mitochondrial DNA testing, Y single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs), and X-SNP and STR testing. It also explores low copy number DNA typing, mixtures, and quality assurance and control. Part II moves on to cover the various uses and applications of analyzing collected physical evidence, victim identification in mass disasters, analyzing animal DNA, forensic botany, and other unique applications. Part III is dedicated to the latest advances and developments in human molecular biology and Part IV looks at policies and laws and ethics governing DNA evidence, and its utilization in various cases and the courts.

Forensic DNA Applications, Second Edition covers cutting-edge research and advancements in the field and is the most up-to-date reference available. Edited and contributed to by the world's foremost leaders in the field, it is a must-have reference for established professionals, and an essential resource to legal professionals—lawyers and judges dealing with civil and criminal cases involving DNA technology—as well as students entering the fields of genetics and forensic DNA analysis.

FORENSIC SCIENCE/GENETICS



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FORENSIC DNA APPLICATIONS
SECOND EDITION

Edited by Dragan Primorac
and Moses S. Schanfield



FORENSIC DNA APPLICATIONS

An Interdisciplinary Perspective

SECOND EDITION

Edited by
Dragan Primorac
Moses S. Schanfield



IZNENADNA SRČANA SMRT

These athletes, in alphabetical order, experienced sudden cardiac death by age 40

Mohamed Abdelwahab, 22 (2006), soccer	Lyle Downs, 24 (1921), Austral. football	Cătălin Hîldan, 24 (2000), soccer	Scott Mason, 28 (2005), cricket	Bernardo Ribeiro, 26 (2016), soccer
Gaines Adams, 26 (2010), Amer. football	Patrick Ekeng, 26 (2016), soccer	Dixie Howell, 40 (1960), baseball	Stan Mauldin, 27 (1948), Amer. football	Darcy Robinson, 26 (2007), ice hockey
Jaouad Akaddar, 28 (2012), soccer	Bobsam Elejiko, 30 (2011), soccer	Chuck Hughes, 28 (1971), Amer. football	Cormac McAnallen, 24 (2004), Gaelic football	Brad Rone, 34 (2003), boxing
Davide Astori, 31 (2018), soccer	Derrick Faison, 36 (2004), Amer. football	Flo Hyman, 31 (1986), volleyball	Conrad McRae, 29 (2000), basketball	Omar Sahnoun, 24 (1980), soccer
Víctor Hugo Ávalos, 37 (2009), soccer	Sebastian Faisst, 20 (2009), handball	Endurance Idahor, 25 (2010), soccer	Fab Melo, 26 (2017), basketball	Serginho, 30 (2004), soccer
Heath Benedict, 24 (2008), Amer. football	Miklós Fehér, 24 (2004), soccer	Robbie James, 40 (1998), soccer	Nilton Pereira Mendes, 30 (2006), soccer	Ryan Shay, 28 (2007), marathon
Hédi Berkhis, 24 (1997), soccer	Neil Fingleton, 36 (2017), basketball	Daniel Jarque, 26 (2009), soccer	Igor Misko, 23 (2010), ice hockey	Dave Sparks, 26 (1954), Amer. football
Viktor Blinov, 22 (1968), ice hockey	Marc-Vivien Foé, 28 (2003), soccer	Cristiano Júnior, 25 (2004), soccer	Stéphane Morin, 29 (1998), ice hockey	Cheick Tioté, 30 (2017), soccer
Gilbert Bulawan, 29 (2016), basketball	Matt Gadsby, 27 (2006), soccer	Joe Kennedy, 28 (2007), baseball	Piermario Morosini, 25 (2012), soccer	Robert Traylor, 34 (2011), basketball
J. V. Cain, 28 (1979), Amer. football	Hank Gathers, 23 (1990), basketball	Darryl Kile, 33 (2002), baseball	Carl Morton, 39 (1983), baseball	Zeke Upshaw, 26 (2018), basketball
Sékou Camara, 27 (2013), soccer	Cristian Gómez, 27 (2015), soccer	John Kirkby, 23 (1953), soccer	Damien Nash, 24 (2007), Amer. football	Luciano Vendemini, 24 (1977), basketball
Alexei Cherepanov, 19 (2008), ice hockey	Michael Goolaerts, 23 (2018), cycling	Michael Klein, 33 (1993), soccer	Frederiek Nolf, 21 (2009), cycling	Ginty Vrede, 22 (2008), kickboxing
Mitchell Cole, 27 (2012), soccer	Larry Gordon, 28 (1983), Amer. football	György Kolonics, 36 (2008), canoeing	Chaswe Nsofwa, 28 (2007), soccer	Frank Warfield, 35 (1932), baseball
Jason Collier, 28 (2005), basketball	Herb Gorman, 28 (1953), baseball	Wayne Larkin, 29 (1968), ice hockey	Gábor Ocskay, 33 (2009), ice hockey	Chandler Williams, 27 (2013), Amer. football
Hugo Cunha, 28 (2005), soccer	Rasmus Green, 26 (2006), soccer	Rauli Levonen, 28 (1981), ice hockey	Phil O'Donnell, 35 (2007), soccer	David "Soldier" Wilson, 23 (1906), soccer
Renato Curi, 24 (1977), soccer	Sergei Grinkov, 28 (1995), figure skating	Reggie Lewis, 27 (1993), basketball	Samuel Okwaraji, 25 (1989), soccer	Sergejs Žoltoks, 31 (2004), ice hockey
Alexander Dale Oen, 26 (2012), swimming	Eddie Guerrero, 38 (2005), wrestling	José Lima, 37 (2010), baseball	David Oniya, 30 (2015), soccer	
Shane del Rosario, 30 (2013), MMA	Frank Hayes, 35 (1923), horse racing	David Longhurst, 25 (1990), soccer	Alen Pamić, 23 (2013), soccer	
Ben Idrissa Dermé, 34 (2016), soccer	Thomas Herrion, 23 (2005), Amer. football	Nikola Mantov, 23 (1973), soccer	Pavão, 26 (1973), soccer	
		Pete Maravich, 40 (1988), basketball	Bruno Pezzey, 39 (1994), soccer	
		Alex Marques, 20 (2013), soccer	Pheidippides, c. 40 (490 BC), marathon	
		Jesse Marunde, 27 (2007), weightlifting	Petar Radaković, 29 (1966), soccer	
			Mickey Renaud, 19 (2008), ice hockey	



HYPOTHESIS AND THEORY

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Sudden Cardiac Death—A New Insight Into Potentially Fatal Genetic Markers

Dragan Primorac^{1,2,3,4,5,6,7,8,9*}, Ljubica Odak^{1,10}, Vitorio Perić¹, Jasmina Ćatić^{1,11}, Jozica Šikić¹², Vjekoslav Radeljić¹³, Šime Manola¹³, Robert Nussbaum¹⁴, Matteo Vatta¹⁴, Swaroop Aradhya¹⁴, Tanja Sofrenović¹⁴, Vid Matišić¹, Vilim Molnar¹, Andrea Skelin¹, Jure Mirat⁶ and Johannes Brachmann^{4,8}

¹ St. Catherine Specialty Hospital, Zagreb, Croatia, ² Eberly College of Science, The Pennsylvania State University, University Park, State College, Philadelphia, PA, United States, ³ The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, United States, ⁴ Medical School, University of Split, Split, Croatia, ⁵ Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, ⁶ Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, ⁷ Medical School, University of Rijeka, Rijeka, Croatia, ⁸ Medical School REGIOMED, Coburg, Germany, ⁹ Medical School, University of Mostar, Mostar, Bosnia and Herzegovina, ¹⁰ Children's Hospital Zagreb, Zagreb, Croatia, ¹¹ Department of Cardiology, Clinical Hospital Dubrava, Zagreb, Croatia, ¹² Department of Cardiology, Clinical Hospital Sveti Duh, Zagreb, Croatia, ¹³ Department of Cardiology, Clinical Hospital Center Sestre Milosrdnice, Zagreb, Croatia, ¹⁴ Invitae, San Francisco, CA, United States

IDENTIFIKACIJE ŽRTAVA DOMOVINSKOG RATA

Prema stanju evidencija na dan 28. siječnja 2024. godine (podatci dostupni putem <https://branitelji.gov.hr/o-ministarstvu/djelokrug/mjere/nestale-osobe/nestale-osobe-u-domovinskom-ratu-834/834>) još je uvijek nepoznata sudbina **1.409 osoba** te mjesto ukopa posmrtnih ostataka **394 smrtno stradale osobe**, što **ukupno čini 1.803 neriješenih slučajeva iz Domovinskog rata**.

Uprava za zatočene i nestale pri Ministarstvu hrvatskih branitelja organizira, koordinira i i sudjeluje u terenskim izvidima, istraživanjima i ekshumacijama posmrtnih ostataka iz pojedinačnih, masovnih i asanacijskih grobnica osoba nestalih u Domovinskom ratu i posmrtnih ostataka smrtno stradalih osoba u Domovinskom ratu za koje nije poznato mjesto ukopa; organizira i koordinira obradu posmrtnih ostataka osoba nestalih u Domovinskom ratu i posmrtnih ostataka smrtno stradalih osoba u Domovinskom ratu za koje nije poznato mjesto ukopa ekshumiranih na području Republike Hrvatske te posmrtnih ostataka preuzetih s područja drugih država i njihovu identifikaciju.

Republika Hrvatska mora iskazati bezuvjetnu predanost i posvećenost rješavanju pitanja nestalih i smrtno stradalih osoba u Domovinskom ratu za koje nije poznato mjesto ukopa.







Sample Selection Preferences

Teeth



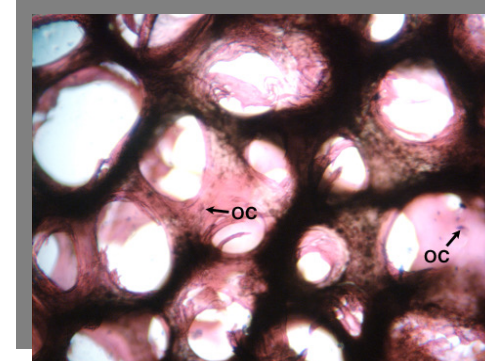
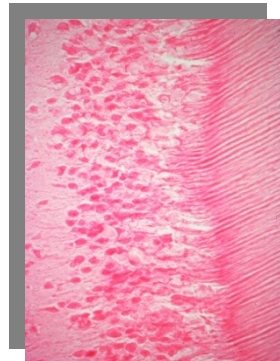
Long Bones



Flat Bones



**DNA Is Better Preserved In A High Density Matrix
Like Dentine & Compact Bone than in Spongy Bone**







Primorac D., Andelinovic S., Definis-Gojanovic M., Drmic I., Rezic B., Baden MM., Kennedy MA., Schanfield MS., Skakel SB., Lee CH. Identification of war victims from mass graves in Croatia, Bosnia and Herzegovina by use of standard forensic methods and DNA typing. *J Forensic Sci* 1996;41:891-894.

ORGANIZACIJA FORENZIČKE GENETIKE U REPUBLICI HRVATSKOJ

Uspješna primjena forenzičke genomike zahtjeva interakciju između znanstveno-nastavnih institucija (Sveučilišni Odjel za forenzične znanosti, Sveučilišta u Splitu, Stručni studij – Kriminalistika pri Policijskoj akademiji, pravni fakulteti hrvatskih sveučilišta, Zavod za sudsku medicinu i kriminalistiku Medicinskog fakulteta Sveučilišta u Zagrebu, Klinički zavod za patologiju, sudsku medicinu i citologiju KBC Split, Zavod za sudsku medicinu i kriminalistiku Medicinskog fakulteta Sveučilišta u Rijeci, Klinički zavod za patološku anatomiju i sudsku medicinu KBC Osijek, i druge srodne institucije, Centra za forenzička vještačenja i ispitivanja “Ivan Vučetić” pri MUP-u, MORH-a, sigurnosno-obavještajnih agencija (SOA, VSOA), Ureda Vijeća za nacionalnu sigurnost, Institut za antropologiju, Genos d.o.o. itd.

Suradnja s znanstvenim organizacijama poput Hrvatskog društva za humanu genetiku te institucijama iz inozemstva (Američka Akademija za forenzičke znanosti (AAFS), Međunarodno društvo primijenjenih bioloških znanosti (ISABS)), Međunarodno društvo za forenzičku genetiku (ISFG), itd., nužna je kako bi se hrvatski forenzičari upoznali s najnovijim dostignućima iz područja forenzičke genetike, ali prezentirali i rezultate vlastitog rada i istraživanja.



NEKA OD ETIČKIH PITANJA U FORENZIČKOJ GENETICI

- Koja kaznena djela trebaju aktivirati pretraživanje DNA baza podataka?
- U kojem trenutku bi se uzorci trebali prikupljati (nakon podizanju optužnice ili nakon pritvora ili pak po rođenju za osnivanje široke (preventivne) populacijske baze podataka)?
- Koje lokuse u genomu treba analizirati da bi se generirali genetski profili nužni za forenzičku obradu?
- Tko bi trebao imati pristup uzorcima i DNA profilima i u koje svrhe?
- Koliko dugo DNA profile i uzorke treba zadržati u DNA bazi podataka



DNA BAZE PODATAKA U RH

- Evidencija DNA osumnjičenih i osuđenih osoba, Centar za forenzična ispitivanja, istraživanja i vještačenja „Ivan Vučetić"
- DNA baza podataka pri Zavodu za sudsku medicinu i kriminalistiku Medicinskog fakulteta Sveučilišta u Zagrebu koja sadrži DNA profile srodnika nestalih osoba i DNA profile ekshumiranih posmrtnih ostataka



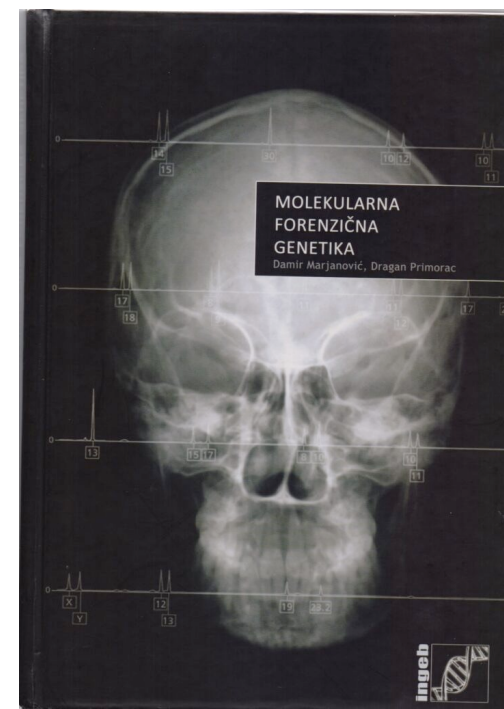
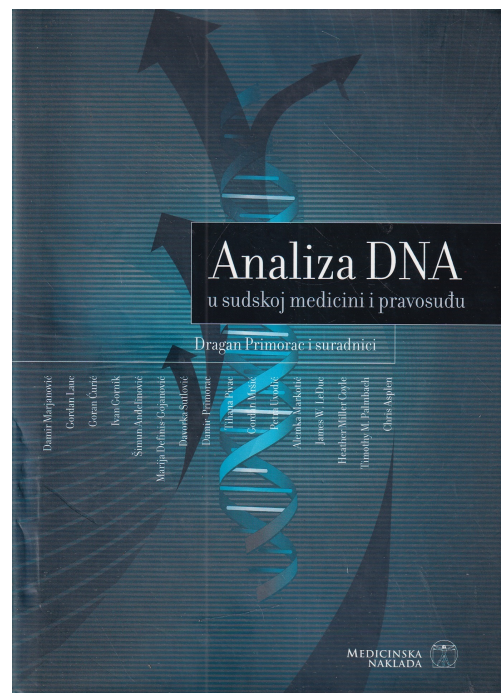
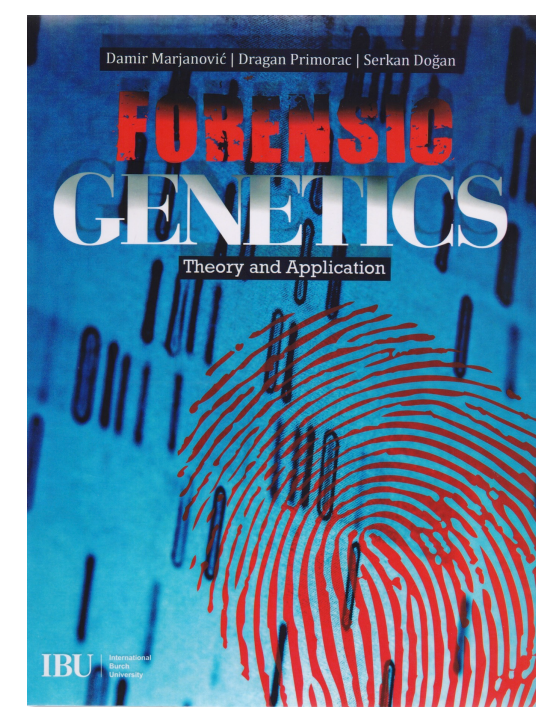
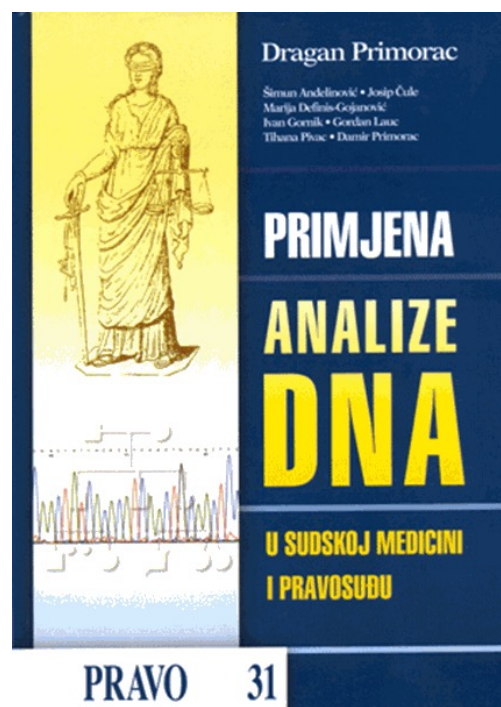
Molekularno-genetska analiza u kaznenom postupku Republike Hrvatske uređena je:

- Zakonom o kaznenom postupku,
- Pravilnikom o načinu uzimanja uzoraka biološkog materijala i provođenja molekularno-genetske analize,
- Pravilnikom o ustrojstvu i načinu vođenja zbirke s automatskom obradom podataka o utvrđivanju istovjetnosti osumnjičenika,
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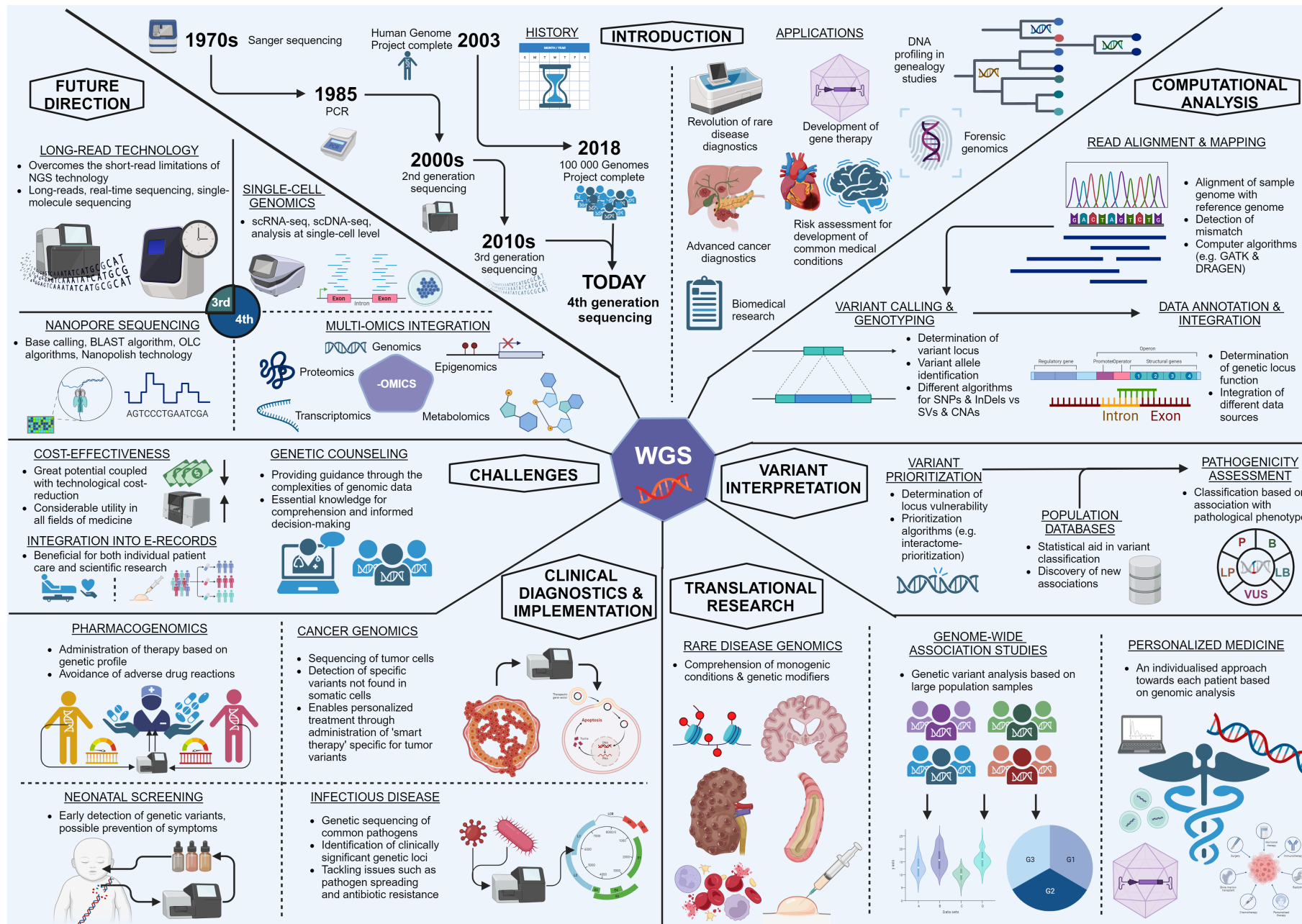


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Petar Brlek, Luka Bulić, Matea Bračić, Petar Projić, Vedrana Škaro, Dragan Primorac. Implementing Whole Genome Sequencing (WGS) in Clinical Practice: Challenges and Future Perspectives (in press)