

Damir Marjanović | Dragan Primorac | Serkan Doğan

FORENSIC GENETICS

Theory and Application



FORENSIC **GENETICS**

Theory and Application

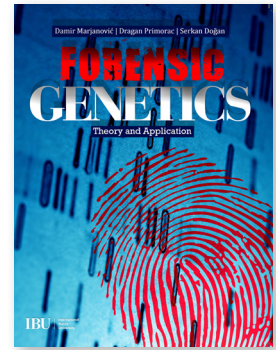
IBU

International
Burch
University

FORENSIC GENETICS

THEORY AND APPLICATION

Damir Marjanović, Ph.D.
Dragan Primorac, M. D. , Ph.D.
Serkan Doğan, Ph.D.



Publisher:

International Burch University, Sarajevo, Bosnia and Herzegovina

Printed by:

Sabah Print d.o.o.

DTP-Design:

Veysel Cebe

Circulation:

500 copies

Place of publication:

Sarajevo, Bosnia and Herzegovina

Copyright:

International Burch University, 2018

CIP - Katalogizacija u publikaciji

Nacionalna i univerzitetska biblioteka

Bosne i Hercegovine, Sarajevo

577.21:340.64

MARJANOVIĆ, Damir

Forensic genetics : theory and application / Damir Marjanović, Dragan Primorac, Serkan Doğan. - Sarajevo : International Burch University, 2018. - 289 str. : ilustr. ; 28 cm

Bibliografija uz tekst. - Registar.

ISBN 978-9958-834-63-9

1. Primorac, Dragan 2. Doğan, Serkan

COBISS.BH-ID 26376198

FORENSIC GENETICS

Theory and Application

Damir Marjanović, Ph.D.

Professor of Forensic Genetics and Molecular Anthropology, Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina

Scientific Associate, Institute for Anthropological Research, University of Zagreb, Zagreb, Croatia

Dragan Primorac, M.D., Ph.D.

President of the Board of Trustees, St. Catherine Specialty Hospital, Zagreb and Zabok, Croatia

Adjunct Professor of Forensic Science, Eberly College of Science, The Pennsylvania State University, University Park, PA, USA

Adjunct Professor of Forensic Science, The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA

Professor of Pediatric Medicine, Medical School, University of Split, Split, Croatia

Professor of Pediatric Medicine, Medical School, University of Osijek, Osijek, Croatia

Professor, Medical School, University of Rijeka, Rijeka, Croatia

Serkan Doğan, Ph.D.

Assistant Professor of Population Genetics and Forensic Genetics, Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina

Contributors

Adna Ašić

Department of Genetics and Bioengineering
Faculty of Engineering and Natural Sciences
International Burch University
Sarajevo, Bosnia and Herzegovina

Serkan Doğan

Department of Genetics and Bioengineering
Faculty of Engineering and Natural Sciences
International Burch University
Sarajevo, Bosnia and Herzegovina

Monia Avdić

Department of Genetics and Bioengineering
Faculty of Engineering and Natural Sciences
International Burch University
Sarajevo, Bosnia and Herzegovina

Mirela Džehverović

Institute for Genetic Engineering and
Biotechnology
University of Sarajevo

Larisa Bešić

Department of Genetics and Bioengineering
Faculty of Engineering and Natural Sciences
International Burch University
Sarajevo, Bosnia and Herzegovina

Mirsada Hukić

Institute for Biomedical Diagnostics and
Research NALAZ
Sarajevo, Bosnia and Herzegovina
Academy of Sciences and Arts of Bosnia and
Herzegovina
Sarajevo, Bosnia and Herzegovina

Lada Lukić Bilela

Department of Biology
Faculty of Natural Sciences and Mathematics
University of Sarajevo
Sarajevo, Bosnia and Herzegovina
BIOSPEL – Biospeleological Society of Bosnia
and Herzegovina
Sarajevo, Bosnia and Herzegovina
ADIPA – Society for Research and Conservation
of Croatian Natural Diversity
Zagreb, Croatia

Damir Marjanović

Department of Genetics and Bioengineering
Faculty of Engineering and Natural Sciences
International Burch University
Sarajevo, Bosnia and Herzegovina
Institute for Anthropological Research
University of Zagreb
Zagreb, Croatia

Elma Ferić Bojić

Department of Genetics and Bioengineering
Faculty of Engineering and Natural Sciences
International Burch University
Sarajevo, Bosnia and Herzegovina

Enisa Omanović Mikličanin

Faculty of Agriculture and Food Science
University of Sarajevo
Sarajevo, Bosnia and Herzegovina

Jasmina Čakar

Institute for Genetic Engineering and
Biotechnology
University of Sarajevo
Sarajevo, Bosnia and Herzegovina

Imer Muhović

Ascidea Genomics & Bioinformatics CRO
Barcelona, Spain

Lejla Kovačević Mulahasanović

Center for Genomics and Transcriptomics,
CeGaT, GmbH
Tübingen, Germany

Dragan Primorac

St. Catherine Specialty Hospital
Zagreb and Zabok, Croatia
Eberly College of Science
The Pennsylvania State University
University Park, PA, USA
The Henry C. Lee College of Criminal Justice
and Forensic Sciences
University of New Haven
West Haven, CT, USA
Medical School
University of Split
Split, Croatia
Medical School
University of Osijek
Osijek, Croatia
Medical School
University of Rijeka
Rijeka, Croatia

Lejla Smajlović Skenderagić

Department of Genetics and Bioengineering
Faculty of Engineering and Natural Sciences
International Burch University
Sarajevo, Bosnia and Herzegovina

Table of Contents and Authors

Edited by:

Damir Marjanović, Ph.D., Dragan Primorac, M. D.,
Ph.D., Serkan Doğan, Ph.D.

1. Introductory concepts and facts
Damir Marjanović, Dragan Primorac, Serkan Doğan
2. Historical Development of Forensic Genetics
Damir Marjanović, Dragan Primorac, Serkan Doğan
3. The variability of DNA and molecular markers in forensic genetics
Serkan Doğan, Adna Ašić, Dragan Primorac, Damir Marjanović
4. Basic models and phases of the process of DNA analysis
Damir Marjanović, Jasmina Čakar, Lejla Smajlović Skenderagić, Larisa Bešić, Serkan Doğan, Dragan Primorac
5. Application of lineage markers and the X chromosome analyses in forensic genetics
Adna Ašić, Larisa Bešić, Lejla Kovačević Mulahasanović, Elma Ferić Bojić, Serkan Doğan, Dragan Primorac
6. Technological development trends in forensic genetics
Lejla Smajlović Skenderagić, Damir Marjanović, Serkan Doğan, Imer Muhović, Larisa Bešić, Adna Ašić
7. Basic biostatistical rules in forensic genetics
Serkan Doğan, Imer Muhović, Adna Ašić, Larisa Bešić, Dragan Primorac, Damir Marjanović
8. DNA database and missing persons identification
Damir Marjanović, Imer Muhović, Monia Avdić, Serkan Doğan, Lejla Smajlović Skenderagić
9. Forensic DNA analysis of plant and animal biological traces
Larisa Bešić, Imer Muhović, Adna Ašić, Jasmina Čakar, Lada Lukić Bilela, Mirela Džehverović, Monia Avdić
10. Food Forensics
Enisa Omanović Mikličanin
11. Microbiomes as tools in human identification
Monia Avdić, Lejla Smajlović Skenderagić, Mirsada Hukić
12. Supplement: Procedures for the collection and labeling of biological traces meant for DNA analysis
Imer Muhović, Adna Ašić, Larisa Bešić, Mirela Džehverović, Damir Marjanović

Contents

Chapter 1

INTRODUCTORY CONCEPTS AND FACTS	15
1. Fundamentals of forensic science	19
1.1. What is forensic science?	19
1.2. Fields of forensic science	19
1.3. Classical criminalistics methods for the identification of human individuals and the individualization of human traces	25
1.3.1. Identification and individualization via phenotypic marker analysis	27
1.3.2. Identification and individualization using fingerprints	29
1.3.3. Identification using dental records	30
1.3.4. Identification and individualization using skeletal remains	34
2. Basic models of molecular genetics	38
2.1. The cell	38
2.2. Chromosomes	39
2.3. Deoxyribonucleic acid (DNA)	42

Chapter 2

HISTORICAL DEVELOPMENT OF FORENSIC GENETICS	47
--	----

Chapter 3

VARIABILITY OF DNA AND MOLECULAR MARKERS IN FORENSIC GENETICS	57
3.1. Variable Number Tandem Repeats – VNTR molecular markers	60
3.1.1. RFLP analysis of minisatellite markers	61
3.2. STR – Short Tandem Repeat molecular markers	62
3.2.1. The structure and nomenclature of STR markers	63
3.2.2. Standard systems of STR loci	64
3.2.2.1. CODIS (COMbined DNA Indexing System) loci	64
3.2.2.2. INTERPOL Standard Set of Loci (ISSOL)	65
3.2.2.3. European Standard Set of Loci (ESS)	65
3.3. Alu repeats	66
3.4. SNP – Single Nucleotide Polymorphism	66
3.5. mtDNA hypervariable regions	70

Chapter 4

BASIC MODELS AND PHASES OF THE PROCESS OF DNA ANALYSIS	75
4.1. Biological traces	78

4.2. Collection and storage of samples	79
4.3. Identification of type of biological trace	81
4.3.1. Testing and identification of bodily fluids	82
4.3.1.1. Testing and identification of blood traces	82
4.3.1.2. Testing and identification of sperm traces	85
4.3.1.3. Testing and identification of saliva traces	87
4.3.1.4. Testing and identification of urine traces	88
4.3.2. Testing and identification of hair traces	88
4.4. DNA isolation methods	91
4.4.1. DNA isolation using organic solvents	92
4.4.2. DNA isolation using “Chelex® 100” method	93
4.4.3. DNA isolation using Qiagen method	94
4.4.4. Promega DNA IQ™ System	95
4.4.5. Prepfiler™ Forensic DNA Extraction Kit	96
4.4.6. Invitrogen ChargeSwitch® System	96
4.4.7. Other DNA isolation methods	97
4.5. DNA quantification methods	98
4.5.1. Determination of DNA quantity in a sample using spectrophotometry	98
4.5.2. Determination of concentration, yield and purity of DNA sample using the agarose gel electrophoresis method	99
4.5.3. Hybridization (slot blot) method	100
4.5.3.1. QuantiBlot® Human DNA Quantitation Kit	100
4.5.3.2. AluQuant™ Human DNA Quantitation System	101
4.5.4. qRT-PCR quantification	102
4.5.4.1. Quantifiler® Human DNA Quantification Kit	102
4.5.4.2. Quantifiler® Duo DNA Quantification Kit	103
4.5.4.2. Quantifiler™ HP DNA Quantification Kit	104
4.5.4.3. Quantifiler® Trio DNA Quantification Kit	104
4.5.4.4. Plexor® HY System	105
4.5.4.5. Investigator® Quantiplex Kit	106
4.6. Polymerase Chain Reaction	107
4.6.1. Basic premises of the PCR and its biochemistry	107
4.6.2. Optimization of basic parameters in application of PCR in forensic genetics	109
4.7. Detection of PCR results	110
4.7.1. Detection of allelic variants on STR loci	110
4.7.1.1. DNA profile	112
4.7.2. DNA sequencing	115
4.8. Application of autosomal multiplex PCR-based systems in forensic genetics	116
4.8.1. Early PCR-based systems	117
4.8.1.1. AmpliFLP® D1S80 PCR Amplification Kit	117
4.8.1.2. AmpliType® PM+DQA1 PCR Amplification and Typing Kit	117

Contents

4.8.2. Commercial autosomal multiplex PCR-based STR systems	118
4.8.2.1. PowerPlex® 16 System	120
4.8.2.2. PowerPlex® 21 System	121
4.8.2.3. AmpFISTR® Identifiler® PCR Amplification Kit	122
4.8.2.4. Investigator IDplex Kit	123
4.8.2.5 PowerPlex® ESX and ESI System	124
4.8.2.6 AmpFISTR® NGM and NGM Select PCR Amplification kits	126
4.8.2.7. GlobalFiler® PCR Amplification Kit	126
4.8.2.8. Investigator ESSplex Kit	128
4.8.3. MiniSTR Systems	128
4.8.4. Direct Amplification STR Systems	131
4.9. Most frequent challenges in forensic DNA analysis	134
4.9.1. Low copy number DNA – LCN DNA analysis	134
4.9.2 Mixed sample analysis	137
4.9.3 Degraded DNA	138
4.9.4. Microvariants	138
4.9.5. Mutations	139
4.9.7. Challenges in the interpretation of results in court	141

Chapter 5

APPLICATION OF LINEAGE MARKERS AND X CHROMOSOME ANALYSES IN FORENSIC GENETICS	147
5.1. Y chromosome analysis in forensic genetics	150
5.1.1. Cytogenetic structure of the Y chromosome and its genes	151
5.1.2. Application of the Y chromosome in forensic and population genetics	152
5.1.2.1. Minimal haplotype	154
5.1.2.2. Commercial Y-STR multiplex kits	155
5.2. Mitochondrial DNA analysis	155
5.2.1. Inheritance of mitochondrial DNA	157
5.2.2. The importance of mtDNA analysis in court medicine	158
5.2.3. Heteroplasmy	159
5.2.4 Mitochondrial DNA Haplogroups	160
5.2.5 Mitochondrial SNPs	160
5.3. Characteristics of the X-chromosome and its application in forensic genetics	161
5.3.1. Main characteristics of the X chromosome	161
5.3.2. Cytogenetic comparison of human X and Y gonosomes	161
5.3.3. Genes on the X chromosome and the molecular and genetic determination of sexes	163
5.3.4. X-linked STR (Short Tandem Repeat) Markers	165
5.3.5. Applications of X-STR Markers in Forensic DNA Analysis	166
5.3.6. Possibilities of the application of X-STR markers in cases of kinship testing	167
5.3.6.1. Paternity testing on human skeletal remains and their postmortem identification	167
5.3.6.2. Paternity testing in cases where potential fathers are closely related	167

5.3.6.3. Paternity testing in cases where the potential father is not available	167
5.3.6.4. Paternity testing in cases of rape and incest	168
5.3.6.5. Maternity testing	168

Chapter 6

DEVELOPMENT TRENDS IN FORENSIC GENETICS

TECHNOLOGY	171
6.1. Automation of DNA extraction	174
6.1.1. Promega Maxwell®16 System	174
6.1.2. Maxwell® Forensic Sample Concentrator (FSC) Instrument.....	175
6.1.3. Qiagen EZ1 Advanced Instrument	175
6.1.4. Qiagen QIA Symphony® SPInstrument	175
6.1.5 Qiagen STAR Q SP Instrument	176
6.1.6. AutoMateExpress™Forensic DNA Extraction System	176
6.1.7. Freedom EVO®	176
6.1.8. Biomek® 3000 Laboratory Automation Workstation	177
6.1.9. iPrep™ Purification Instrument	177
6.2. Development and trends in PCR technology	178
6.2.1. Applied Biosystems PCR machines	179
6.2.2. Eppendorf PCR machines	180
6.2.3. Rotor-Gene Q	180
6.2.4. Other PCR machines	181
6.3. Method and technology development in DNA marker analysis in forensic genetics	181
6.3.1. Fluorescent labeling and detection	181
6.3.2. Development of DNA sequencers and genetic analyzers	183
6.3.2.1. Automatic genetic gel analyzers	183
6.3.2.1.1. ABI PRISM 373 DNA SEQUENCER	183
6.3.2.1.2. ABI PRISM 377 DNA SEQUENCER	184
6.3.2.2. Automated capillary genetic analyzers	184
6.3.2.2.1. ABI PRISM 310 GENETIC ANALYZER	184
6.3.2.2.2. Other systems for molecular marker detection	185
6.3.2.3. Recent developments in automatic capillary genetic analyzers	185
6.3.2.3.1. Spectrum CE System	185
6.3.2.3.2. Spectrum Compact CE System	185
6.4. New technological fields in forensic genetics	186
6.4.1. DNA phenotyping	186
6.4.2. Genetic methods for determining age and type of biological traces	189
6.4.3. Application of INDELS in forensic genetics	190
6.4.4. Next generation sequencing	191
6.4.4.1. Pyrosequencing	196
6.4.5. Third generation sequencing	197

Contents

Chapter 7

BASIC BIOSTATISTICS RULES IN FORENSIC GENETICS	201
7.1. Mendelian inheritance	204
7.2. Rules in parentage testing	204
7.3. Hardy-Weinberg equilibrium	204
7.4. Linkage disequilibrium	205
7.5. Creating a population database	205
7.6. Paternity testing	207
7.6.1. Statistical procedures and paternity testing - Paternity Index or Combined Paternity Index	207
7.6.2. Probability of Paternity (W)	209
7.6.3. Random Man Not Excluded (RMNE)	210
7.6.4. Motherless paternity testing	211
7.6.5. Maternity testing	212
7.6.6. Parentage testing as opposed to forensic DNA analysis	214
7.7. Forensic individualization	214
7.8. Statistical analysis of mixed and low copy number traces	216
7.8.1. Statistical analysis of mixed traces	216
7.8.2. Statistical analysis of low copy number traces	216
7.9. Statistical testing of biological kinship	217
7.10. Identification of mass disaster victims	217
7.11. Statistical rules in the analysis of sex-linked markers	218
7.11.1. Presenting results obtained by the usage of Y-STR systems	218
7.11.2. Presenting results obtained by the usage of X-STR systems	218

Chapter 8

DNA DATABASES	221
8.1. Criteria for Creating Legislation Regarding DNA Databases	224
8.1.1. Criteria for Profile Archiving	224
8.1.1.1. Profiles of Convicted Felons	224
8.1.1.2. Profiles of Suspects	224
8.1.1.3. Profiles Obtained from Traces from the Crime Scene	224
8.1.2. Criteria for Removal of Profiles from the Database	225
8.1.2.1. Profiles of Convicted Felons	225
8.1.2.2. Suspects/Arrested Persons	225
8.1.3. Sample Storage	225
8.2. Forensic DNA Databases in the World	225
8.2.1. CODIS Database – United States of America	225
8.2.2. NDNAD Database – England	226
8.2.3. Application of DNA Analysis in Germany	227
8.2.4. European Exchange of DNA Data	228
8.2.5. INTERPOL's Global DNA Gateway	228
8.2.6. Y Chromosome Databases	229
8.3. Current Situation in the Region	229

Chapter 9

FORENSIC DNA ANALYSIS OF PLANT AND ANIMAL BIOLOGICAL TRACES

	231
9.1. Forensic botany	234
9.1.1. Fundamentals of the molecular and genetic techniques in plant material analysis	234
9.1.1.1. Analysis of STR molecular markers on plant traces	234
9.1.2. Analysis of random/unknown markers	235
9.1.2.1. Analysis of randomly amplified polymorphic DNA	235
9.1.2.2. Amplified fragment length polymorphisms	236
9.1.2.3. Species identification	237
9.1.3. Palynology and Mycology	238
9.2. Fundamentals of animal forensic DNA analysis	238
9.2.1. Forensic entomology	238
9.2.1.1. Analysis of human DNA isolated from insects	240
9.2.1.2. Ribonucleic acid analysis (RNA analysis)	240
9.2.2. Forensic DNA analysis of vertebrates	240
9.2.2.1. Analysis of animal nuclear DNA	240
9.2.2.2. Analysis of animal mitochondrial DNA	242
9.2.2. Forensic DNA analysis in the control of food products	242

Chapter 10

FOOD FORENSICS

	245
10.1. Food Fraud	248
10.2. Definitions	249
10.3. Methods used in food forensics	249
10.3.1. DNA analysis in food forensics	250
10.3.2. Applications of DNA analysis in food forensics	250
10.3.2.1. Identification of meat and fish origin in food products	250
10.3.2.2. Tracing of botanical origin and adulteration identification in basmati rice	253
10.3.2.3. The provenance of olive oil	253
10.3.2.4. Durum wheat pasta adulteration	253
10.3.3. Forensic toxicology	254
10.3.4. Genetically modified organisms (GMO)	254
10.3.5. Trace elements	255
10.4. Conclusions	255

Chapter 11

MICROBIOMES AS TOOLS IN HUMAN IDENTIFICATION

	259
11.1. Microbial forensics – an introduction	262
11.2. Human microbial „ID Cards“	263
11.3. Abundance of microbial species can be individualized	264

Contents

11.4. 16S rDNA as microbial signature	266
11.5. Metagenomics reveals complex microbial information	267
 Chapter 12	
SUPPLEMENT: SUGGESTED PROCEDURES FOR COLLECTION AND LABELING OF BIOLOGICAL TRACES FOR DNA ANALYSIS	271
12.1. Collection of blood samples	274
12.1.1. Collection of wet blood samples from objects that cannot be delivered for analysis	274
12.1.2. Collection of wet blood samples from objects that can be delivered for analysis	274
12.1.3. Collection of dry blood samples from objects that cannot be delivered for analysis	275
12.1.4. Collection of dry blood samples from objects that can be delivered for analysis	276
12.1.5. Blood samples found on wet or moist clothes and shoes	276
12.2. Collection of sperm samples	276
12.2.1. Time period in which it is necessary to collect a sperm sample	276
12.2.2. Collection of wet (fresh) sperm samples from objects that cannot be delivered for analysis	277
12.2.3. Collection of wet (fresh) sperm traces from objects which can be delivered for analysis	277
12.2.4. Collection of dry sperm samples from objects that cannot be delivered for analysis	278
12.2.5. Collection of dry sperm samples from objects that can be delivered for analysis	278
12.2.6. Collection of sperm samples found inside the victim's body (vaginal and anal smear)	278
12.2.7. Collection of sperm samples found on the victim's body	279
12.2.8. Collection of Sperm Samples from the Mouth (Buccal Swab)	279
12.2.9. Early Paternity Dispute Testing	279
12.3. Collection of Saliva Samples	279
12.3.1. Collection of wet (fresh) saliva traces from objects that cannot be delivered for analysis	279
12.3.2. Collection of wet (fresh) saliva traces from objects that can be delivered for analysis	280
12.3.3. Collection of dry saliva traces from objects that cannot be delivered for analysis	280
12.3.4. Collection of dry saliva traces from objects that can be delivered for analysis	280
12.3.5. Collection of Saliva Samples from the Body	281
12.4. Collection of Hair Samples	281
12.4.1. Collection of Hair Samples Found at the Crime Scene	281
12.4.2. Collection of Hair Samples from the Victim's Body	281
12.4.3. Collection of Hair Samples from the Body of a Victim Potentially Containing Sperm or Blood Traces	282
12.5. Collection of Biological Samples under the Nails and from Feces	282
12.6. Collection of Reference Biological Samples	282
12.6.1. Collection of Reference Buccal Mucous Membrane Samples	283
12.6.2. Collection of Reference Blood Samples	283
12.6.3. Collection of Skeletal Remains	283
12.6.4. Collection of Personal Objects for Use as Reference Samples	284
Index	285

FOREWORD

The science of the 21st century has not given up on its rapid development. The pace of evident progress in certain scientific disciplines, especially those relying on applied genetics, does not allow for a breakthrough in the collection, sorting and presentation of the latest achievements made in hundreds of laboratories around the world. Continuous education of scientists, professors, experts, and users of scientific achievements has never been this prominent and observable.

After a brief analysis of the development of forensic genetics in the past five years, we have decided that it would be wise to approach the complementation of existing material available with, as we then thought, “some new information”. But when we included everything we wanted to add onto the previous edition, we found that the new facts, hypotheses and models have been generated, as well as a promising direction for potential development established. Soon upon this realization, we had nothing left to do but to, significantly influenced by young and enthusiastic associates, “roll up our sleeves” and prepare a new textbook. As a result, this book was created, which at the moment of its creation is probably the only existing edition that includes the most up-to-date information, especially related to the new multiplex STR systems, next-generation sequencing platforms and lineage markers, as well as new approaches in forensic DNA analysis in general. Two completely new chapters have been prepared, including the topics of food forensics and microbiology in forensic investigations. We are especially proud of the last chapter of this book that gives brief, understandable and highly applicable guidelines for proper sample handling, collection and storage, and overall model of behavior at the crime scene.

As in the previous editions of this material, we tried to present the basic molecular biological, biochemical, statistical and technological knowledge, and other principles that must be known in order to comprehend the application of fundamental scientific knowledge in forensic genetics. Also, we aimed at adding everything that is important into this book, and also what is written within the best books of the world, and everything that we have learnt from our practical work in the past decade. By preparing this edition in English language, we have thought of potential international readers of our book and tried our best to make this text as accessible worldwide as possible.

Damir Marjanović

Dragan Primorac

Serkan Doğan

Sarajevo/Zagreb, Summer of 2018