ADVANCED TOPICS IN FORENSIC DNA TYPING

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ADVANCED TOPICS IN FORENSIC DNA TYPING: METHODOLOGY

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Dedication

To the hardworking professionals
throughout the forensic DNA community
and the individuals and families
impacted by your service
– your work makes a difference!

Contents

Foreword

Once again, John Butler has provided the forensic community with a much needed definitive text. Several editions of the original book have appeared since 2000. It has now evolved into a project, as the original will no longer fit easily into a single volume. This is now the second book in the series—*Advanced Topics in Forensic DNA Typing: Methodology*.

The information provided is easily amenable to a wide audience, from scientists and lawyers to the interested public. The comprehensive referencing makes it a handy document to refer to when "giving evidence" in court, as a definitive authority on the "state of the science."

The new volume is organized in the same order as the work flow, beginning with "sample collection and storage." The second and subsequent chapters provide comprehensive reviews of extraction methods, quantitation, amplification, and separation. One important topic considered for the first time is the importance of manufacturing controls to prevent potential contamination of plasticware and other reagents, a problem first highlighted by the "German phantom." The STR marker section is completely up to date, describing the European Standard Set (ESS) of markers and referring to a discussion on the proposed expansion of US core loci. Each locus is described in turn, with details of their molecular structures and listing of aberrant alleles such as a point mutation in D16S539. There is a very useful comparison of all STR typing kits along with their respective dye colors and sizes mapped for individual loci.

There are comprehensive reviews of non-autosomal DNA: mitochondrial DNA, Y-chromosomal DNA, and a brand new chapter on X-chromosomal DNA.

The chapter on degraded DNA leads into a discussion on the development of mini-STRs and their incorporation into the new multiplexes. John provides a timeline that stretches back 17 years to 1994, where the first STR multiplex was used in the Waco disaster. The subsequent chapter is a discussion on low-copy-number (or low template) DNA analysis. A balanced review is provided of this sometimes contentious area.

The appendices are particularly interesting. They provide an updated compilation of all the rare, and common, alleles currently observed and sequenced in the systems. The final appendix contains interviews with highly experienced expert witnesses and attorneys, providing valuable perspectives on how to be a good witness. John's books have become an essential adjunct to this objective.

I look forward to the third volume in the series, and marvel that John can physically find the time to do so much good work. I wonder what his secret is?

Peter Gill, Ph.D.
University of Oslo, Norway

Introduction

Since the second edition of *Forensic DNA Typing* was written in 2004, a great deal has happened in the field of forensic DNA analysis. Hence, the need to update the information contained in the book in as comprehensive a manner as possible. In forensic science review articles published in 2005, 2007, and 2009 in the journal *Analytical Chemistry*, I briefly described topics from hundreds of articles published during the time frame of 2003–2008. In my own laboratory at the National Institute of Standards and Technology (NIST), we have published over 75 articles since 2004 on a variety of subjects including miniSTRs, Y-STRs, mtDNA, SNPs, validation, and DNA quantitation (see http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm).

Since 2004, I have also had the privilege of teaching more than three dozen workshops (see http://www.cstl.nist.gov/biotech/strbase/training.htm) to thousands of scientists and lawyers either at conferences or individual laboratories. In addition, I have responded to hundreds of email requests for more information on various topics. These interactions with forensic scientists, lawyers, and the general public have provided me with a valuable perspective on topics that need further clarification and questions that have not been answered with the information in the first or second edition of *Forensic DNA Typing*.

I have divided what is essentially the third edition of *Forensic DNA Typing* up into three volumes: a basic volume for students and beginners in the field and two advanced volumes for professionals/practitioners who may be interested in more detail. The basic volume was released in September 2009 (with a publication date of 2010) and is entitled *Fundamentals of Forensic DNA Typing*. The present book, *Advanced Topics in Forensic DNA Typing: Methodology*, is volume 2. A forthcoming book, to be titled *Advanced Topics in Forensic DNA Typing: Interpretation*, will be volume 3.

Several reasons exist for dividing the material. First and foremost, people use books more frequently if they are less bulky. I have heard from more than one colleague at conferences that they prefer to carry the smaller first edition with them to court or other teaching situations. Second, by having multiple books, each volume can be focused on its intended audience rather than trying to be all things to all readers. Third, the books will enable both undergraduate and graduate studies with each building upon the previous volumes.

With a vast majority of the topics, there is only minor overlap in subject matter between the various volumes. The basic *Fundamentals* volume contains the simpler "starter" information while most of the "updates" to the field are found in the *Advanced Topics* volumes. It is my intention that the three volumes together provide a comprehensive view of the current state of forensic DNA analysis.

NEW MATERIAL IN THIS VOLUME

In many ways, this is a completely new book. Those familiar with the previous editions of my book will find that *Advanced Topics in Forensic DNA Typing: Methodology* is substantially enhanced with additional information. Since the first edition was written in the winter months of 2000, the published literature on STR typing and its use in forensic DNA testing has grown dramatically. With more than 3,500 papers now available describing STR markers, technology for typing these STRs, and allele frequencies in various populations around the world, the scientific basis for forensic DNA typing is sound. The foundational material in the previous editions is still relevant and thus has remained essentially unchanged. However, as with every scientific field, advances are being made and thus new information needs to be shared to bring the book up-to-date.

In addition to updating information on essentially every topic in the second edition of *Forensic DNA Typing*, I have included new chapters on X-chromosome markers (Chapter 15) and legal aspects of serving as an expert witness in a U.S. court of law (Chapter 18). The chapter on DNA databases (Chapter 8) is significantly expanded and new information on familial DNA searches is included (Appendix 2).

At the end of each chapter throughout the book, I have included a fairly comprehensive list of references that serve as a foundation for citations found throughout the chapter as well as a launching point where interested readers can go for additional information. More than 2800 references are provided enabling readers to expand their study beyond the information contained between the covers of this book. References to journal articles include titles to enhance value.

In this edition, I again utilize **D**ata, **N**otes, and **A**pplications (D.N.A.) Boxes to cover specific topics of general interest, to review example calculations, or to cover a topic that serves to highlight information needed by a DNA analyst.

OVERVIEW OF BOOK CHAPTERS

Many times information within chapters and even the order of the chapters themselves have been changed from the second edition. These structural changes reflect changes in my way of thinking about how to present the information to the intended audience. Note that new topics are being added and old ones phased out. A brief "cross-walk" of major topics covered across the various editions of *Forensic DNA Typing* is shown below with chapters (Ch.) and appendices (App.) indicated. Note that although topics are defined for the forthcoming *Interpretation* volume, final chapter numbers are still to be determined (TBD).

Topic	1st Edition (2001)	2nd Edition (2005)	Fundamentals (2009)	Advanced Topics: Methodology (2011)	Advanced Topics: Interpretation (forthcoming)
History of DNA	Ch. 1	Ch. 1	Ch. 1 & 3	–	–
DNA basics	Ch. 2	Ch. 2	Ch. 2	–	–
Sample collection	Ch. 3	Ch. 3	Ch. 4	Ch. 1	–

(*Continued*)

(*Cont'd*)

Topic	1st Edition (2001)	2nd Edition (2005)	Fundamentals (2009)	Advanced Topics: Methodology (2011)	Advanced Topics: Interpretation (forthcoming)
DNA extraction	Ch. 3	Ch. 3	Ch. 5	Ch. 2	–
DNA quantitation	Ch. 3	Ch. 3	Ch. 6	Ch. 3	–
PCR	Ch. 4	Ch. 4	Ch. 7	Ch. 4	–
STR markers	Ch. 5	Ch. 5	Ch. 8	Ch. 5	–
Data interpretation	Ch. 6 & 13	Ch. 6 & 15	Ch. 10	–	TBD
DNA databases	Ch. 16	Ch. 18	Ch. 12	Ch. 8	–
Capillary electrophoresis	Ch. 9 & 11	Ch. 12 & 14	Ch. 9	Ch. 6	TBD
FMBIO gel imaging system	Ch. 12	Ch. 14	DNA Box 9.2	–	–
Random match probability calculations	–	Ch. 21	Ch. 11	–	TBD
Statistics & probability basics	–	Ch. 19	App. 3	–	TBD
Familial searching	–	–	Ch. 12 (p. 282)	App. 2	–
DNA mixtures	Ch. 7	Ch. 7	Ch. 14	–	TBD
Low copy number DNA testing	–	Ch. 7	Ch. 14	Ch. 11	TBD
Validation	Ch. 14	Ch. 16	Ch. 13	Ch. 7	–
SNPs	Ch. 8	Ch. 8	Ch. 15	Ch. 12	–
Y-STRs	Ch. 8	Ch. 9	Ch. 16	Ch. 13	–
mtDNA	Ch. 8	Ch. 10	Ch. 16	Ch. 14	–
X-STRs	–	–	–	Ch. 15	–
Non-human DNA	Ch. 8	Ch. 11	Ch. 15	Ch. 16	–
New technologies	Ch. 15	Ch. 17	Ch. 18	Ch. 17	–
Disaster victim identification	Ch. 17	Ch. 24	Ch. 17	Ch. 9	–
Expert witness testimony	–	–	–	Ch. 18, App. 4	–
Reported STR alleles	App. 1	App. 1	–	App. 1	–
FBI QAS	App. 3 (1998/99)	App. 4 (1998/99)	–	–	TBD
Glossary	–	–	App. 1	–	–

Appendices

There are four appendices at the back of the book that provide supplemental material.

- Appendix 1 describes all reported alleles for the 13 CODIS and other commonly used STR loci as of December 2010. Sequence information, where available, has been included along with the reference that first described the noted allele. As most laboratories now use either a Promega or an Applied Biosystems STR typing kit for PCR amplification, we have listed the expected size for each allele based on the sequence information.
- Appendix 2 discusses familial DNA searching and the potential, pitfalls, and privacy concerns surrounding this controversial technique.
- Appendix 3 is a compilation of companies and organizations that are suppliers of DNA analysis equipment, products, and services. Over 80 companies are listed along with their addresses, phone numbers, Internet web pages, and a brief description of their products and/or services.
- Appendix 4 is a compilation of responses to interview questions asked of several scientists and lawyers relating to issues faced when serving as an expert witness.

Acknowledgments

I express a special thanks to colleagues and fellow researchers who kindly provided important information and supplied some of the figures for this book or previous editions of *Forensic DNA Typing*. These individuals include Michael Baird, Susan Ballou, Brad Bannon, Martin Bill, Theresa Caragine, George Carmody, Mike Coble, Robin Cotton, David Duewer, Dan Ehrlich, Nicky Fildes, Lisa Forman, Ron Fourney, Lee Fraser, Richard Guerrieri, Chip Harding, Doug Hares, Bruce Heidebrecht, Debbie Hobson, Bill Hudlow, Ted Hunt, Dennis Kilcoyne, Margaret Kline, Ken Konzak, Carll Ladd, Steve Lee, Dina Mattes, Bruce McCord, Ruth Montgomery, Steven Myers, Steve Niezgoda, Thomas Schnibbe, Richard Schoske, Jim Schumm, Scott Scoville (and the Orange County DA's DNA Unit), Bob Shaler, Michelle Shepherd, Gary Sims, Melissa Smrz, Amanda Sozer, Jill Spriggs, Mark Stolorow, Kevin Sullivan, Lois Tully, and Charlotte Word.

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Several other people deserve specific recognition for their support of this endeavor. The information reported in this book was in large measure made possible by a comprehensive collection of references on the STR markers used in forensic DNA typing. For this collection now numbering more than 3000 references, I am indebted to the initial work of Christian Ruitberg for tirelessly collecting and cataloging these papers and the steady efforts of Jan Redman to monthly update this STR reference database. A complete listing of these references may be found at http://www.cstl.nist.gov/biotech/strbase.

My wife Terilynne, who carefully reviewed the manuscript and made helpful suggestions, was always a constant support in the many hours that this project took away from our family. As the initial editor of all my written materials, Terilynne helped make the book more coherent and readable. In addition, David Duewer and Katherine Sharpless provided a fine technical review of the *Fundamentals* book as well as this one. Review of materials and input from Mary Satterfield and several members of my research group was also very helpful. The support of NIST management especially Laurie Locascio and Willie May made completion of this book possible.

I was first exposed to forensic DNA typing in 1990 when a friend gave me a copy of Joseph Wambaugh's *The Blooding* to read, and since then I have watched with wonder as the forensic DNA community has rapidly evolved. DNA testing that once took weeks can now be performed in a matter of hours. I enjoy being a part of the developments in this field and hope that this book will help many others come to better understand the fundamental principles behind the biology, technology, and genetics of STR markers.

About the Author

John Marshall Butler grew up in the U.S. midwest and, enjoying science and law, decided to pursue a career in forensic science at an early age. After completing an undergraduate education at Brigham Young University in chemistry, he moved east to pursue graduate studies at the University of Virginia. While a graduate student, he enjoyed the unique opportunity of serving as an FBI Honors Intern and guest researcher for more than two years in the FBI Laboratory's Forensic Science Research Unit. His Ph.D. dissertation research, which was conducted at the FBI Academy in Quantico, Virginia, involved pioneering work in applying capillary electrophoresis to STR typing. After completing his Ph.D. in 1995, Dr. Butler obtained a prestigious National Research Council postdoctoral fellowship to the National Institute of Standards and Technology (NIST). While a postdoc at NIST, he designed and built STRBase, the widely used Short Tandem Repeat Internet Database (http://www.cstl.nist.gov/biotech/strbase) that contains a wealth of standardized information on STRs used in human identity applications. He worked for several years as a staff scientist and project leader at a California startup company named GeneTrace System developing rapid DNA analysis technologies involving time-of-flight mass spectrometry. In the fall of 1999, he returned to NIST to lead their efforts in human identity testing with funding from the National Institute of Justice.

Dr. Butler is currently a NIST Fellow and Group Leader of Applied Genetics in the Biochemical Science Division at the National Institute of Standards and Technology. He is a regular invited guest of the FBI's Scientific Working Group on DNA Analysis Methods (SWGDAM) and a member of the Department of Defense Quality Assurance Oversight Committee for DNA Analysis. Following the terrorist attacks of September 11, 2001, he aided the DNA identification efforts and served as part of the distinguished World Trade Center Kinship and Data Analysis Panel (WTC KADAP). He is a member of the International Society of Forensic Genetics and serves as an Associate Editor for *Forensic Science International: Genetics*.

Dr. Butler has received numerous awards including the Presidential Early Career Award for Scientists and Engineers (2002), the Department of Commerce Silver Medal (2002) and Gold Medal (2008), the Arthur S. Flemming Award (2007), the Edward Uhler Condon Award (2010), Brigham Young University's College of Physical and Mathematical Sciences Honored Alumnus (2005), and the Scientific Prize of the International Society of Forensic Genetics (2003).

He has more than 100 publications describing aspects of forensic DNA testing and is one of the most prolific active authors in the field with articles appearing regularly in every major forensic science journal. Dr. Butler has been an invited speaker to numerous national and international forensic DNA meetings and in the past few years has spoken in Germany, France, England, Canada, Mexico, Denmark, Belgium, Poland, Portugal, Cyprus, The Netherlands, Argentina, Japan, and Australia. Much of the content in this book has come from his Group's research efforts over the past two decades. In addition to his busy scientific career, he and his wife serve in their community and church and are the proud parents of six children, all of whom have been proven to be theirs through the power of DNA typing.