

# Fundamentals of Forensic DNA Typing

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# Fundamentals of Forensic DNA Typing

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and to my wife, Terilynne, and six children  
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*References are provided at the end of each chapter by subtopic (but without direct citation within the text).*

*High-profile cases and other interesting information are included as D.N.A. (Data, Notes, and Applications) boxes scattered throughout the book in the chapter pertaining to a particular subject.*



# Foreword

In the fast-moving field of forensic DNA, there is always the danger that a textbook will become quickly out of date. It is a tribute to John's remarkable tenacity and attention to detail that his work has never been endangered in this way.

In his latest book, John explains how and why the organization of his texts has evolved into this latest iteration. Not wishing to write an encyclopedia, he explains that his solution has necessitated splitting the original format of *Forensic DNA Typing* into two volumes. The first volume is entitled *Fundamentals of Forensic DNA Typing*. There is a forthcoming companion volume entitled *Advanced Topics in Forensic DNA Typing*.

Forensic science attracts a very broad audience from a wide diversity of backgrounds. Devising a framework that is able to appeal to such an audience is perhaps the biggest challenge. The scientist, the lawyer, the law enforcement officer, and the student will all expect a different emphasis from a book that deals with all aspects of DNA profiling.

Dividing *Forensic DNA Typing* into two volumes is a masterful stroke. The first volume, *Fundamentals of Forensic DNA Typing*, is written from a more general perspective. It is subdivided into 18 chapters ordered into a logical sequence. Thus we begin with an extensive overview and history in Chapter 1 and end with Chapter 18 on future trends. In between, chapters progress through sample collection, DNA extraction, PCR, and interpretation. There is a brand new section on DNA databases that describes the growth of this 'industry' since the first U.K. use in 1995. Ethical concerns are discussed in relation to the retention of DNA profiles, and the proposed expansion of databases to allow for close relatives to be searched for (so-called familial searching). Chapter 13 provides an expanded section on quality assurance. What is meant by validation? What procedures are in place that maintain public confidence? How can we learn from mistakes that have been made in the past?

Although slightly shorter than *Forensic DNA Typing*, skillful arrangement of chapters and extensive use of cross-referencing enhance the reading

experience. It can easily be read from cover to cover. The specialist reader requiring more information is directed to the companion volume, *Advanced Topics in Forensic DNA Typing*. In addition, there are extensive references to material that is freely available on the Internet (the NIST Web site is itself a tremendous resource). *Fundamentals of Forensic DNA Typing* admirably succeeds in its goal of reaching a diverse audience. It does this by acting as a portal—if a specific piece of information isn't explicitly written down, it will tell you where to find it. Consequently, it fulfills the same function as an encyclopedia but in a very user-friendly way.

**Peter Gill, PhD FIBiol**

**Strathclyde, UK**

# Introduction

An expert is one who knows more and more about less and less until they know absolutely everything about nothing...

—Nicholas Butler, *Bartlett's*, 585:10

Being directly involved in the forensic DNA typing community over the past 15 years has been rewarding yet challenging as the field continues to grow rapidly. The popularity of television shows like *CSI: Crime Scene Investigation* and *Law & Order* have sparked interest by the general public. Since the first edition of *Forensic DNA Typing* was published in January 2001 and expanded on in the second edition in February 2005, I have had the opportunity to directly teach thousands of scientists, students, and lawyers regarding the fundamentals of forensic DNA analysis. Questions raised during my lectures and discussions held as part of training workshops conducted have aided in refinement of the information provided herein.

A number of forensic science programs have arisen on college campuses around the world to meet the needs and interests of students. The purpose of this book is to aid students, beginning scientists, and members of the legal community in gaining an introductory understanding of and a fundamental foundation to forensic DNA testing. A companion volume for practitioners (*Advanced Topics in Forensic DNA Typing, 3rd Edition*) touches on more advanced topics and provides further details to the basic information in this volume. Rather than creating an encyclopedic single text to be all things to all people, we have decided with the third edition of *Forensic DNA Typing* to divide the material into fundamental information and advanced topics.

Several significant things have happened since the first edition of *Forensic DNA Typing* was published in January 2001. The Human Genome Project published a draft sequence of the human genome in February 2001 and completed the reference sequence in April 2003. In addition, human mitochondrial DNA population genomics is under way and thousands of full mitochondrial genomes have been published. Soon hundreds if not thousands of full

human genomes will be available due to next-generation sequencing capabilities. Technology for DNA sequencing and typing continues to advance as does our understanding of genetic variation in various population groups around the world. These milestones are a tribute to the progress of science and will benefit the field of forensic DNA typing.

The literature on the short tandem repeat (STR) markers used in forensic DNA testing has more than tripled in the 8 years since the first edition of this book became available. More than 3500 publications now detail the technology and report the allele frequencies for forensically informative STR loci. Hundreds of different population groups have been studied. New technologies for rapidly typing DNA samples have been developed. Standard protocols have been validated in laboratories worldwide. Yet DNA results are still sometimes challenged in court—not usually because of the technology, which is sound—but rather to question the ability of practitioners to perform the tests carefully and correctly. A major purpose of this book is to help in the training of professionals in the field of forensic DNA testing. The knowledge of forensic scientists, lawyers, and students coming into the field will be enhanced by careful review of the materials found herein.

In the past few years, the general public has become more familiar with the power of DNA typing as the media has covered efforts to identify remains from victims of the World Trade Center Twin Towers collapse following the terrorist attacks of 11 September 2001, the O.J. Simpson murder trial in 1994 and 1995, the parentage testing of Anna-Nicole Smith's daughter in 2007, and the ongoing Innocence Project that has led to the exoneration of over 200 wrongfully convicted individuals. News stories featuring the value of forensic DNA analysis in solving crime seem commonplace today. In another popular application, DNA testing with Y-chromosome markers is now used routinely to aid genealogical investigations. In addition, the medical community is poised to benefit from the tremendous amount of genomic DNA sequence information being generated. DNA testing has an important role in our society that is likely to grow in significance and scope in the future.

Though high-profile cases have certainly attracted widespread media attention in recent years, they are only a small fraction of the thousands of forensic DNA and paternity cases that are conducted each year by public and private laboratories around the world. The technology for performing these tests has evolved rapidly over the past two decades to the point where it is now possible to obtain results in a few hours on samples with only the smallest amount of biological material.

This book will examine the science of current forensic DNA typing methods by focusing on the biology, technology, and genetic interpretation of short

tandem repeat (STR) markers, which encompass the most common forensic DNA analysis methods used today. The materials in this book are intended primarily for two audiences: (1) students learning about forensic DNA analysis in an academic environment and (2) forensic science professionals and members of the law enforcement and legal communities who want to gain a better understanding of the fundamentals behind STR typing. Further information on each of the subjects presented here is available in the second volume, *Advanced Topics in Forensic DNA Typing, 3rd Edition*.

## NEW MATERIAL IN THIS EDITION

In many ways, this is a completely new book. Those familiar with the previous two editions of my book will come to find that *Fundamentals of Forensic DNA Typing* is substantially enhanced with additional information. A chapter has been added reviewing historical methods used in the first two decades of forensic DNA testing (Chapter 3). Topics have been reordered to reflect the DNA testing process, which progresses from sample collection (Chapter 4) to statistical interpretation of a DNA match (Chapter 11). A number of new figures have been added, including Figure 1.3, which provides an overview of the entire DNA testing process. A chapter discussing my perspectives on future trends in the field has also been added (Chapter 18). A number of additional Data, Notes, and Applications (D.N.A.) boxes have been added as well as an extensive glossary to aid newcomers to the field (Appendix 1). Major updates have been made to all of the content, bringing the information contained herein current as of the time of publication.

At the end of each chapter are points for discussion to foster thought on the topics covered. To avoid interrupting the flow of ideas, the references and Web sites listed at the end of each chapter are not cited within the text but are provided to indicate sources of material as well as to enable readers to be aware of additional resources on each topic. These reference lists are subdivided by topic to enable easy access to further information desired on a specific subject. The citation format has also changed. Author lists have been shortened to only the first author, and titles for each article have been added to make it more meaningful. Links are provided to Web sites including the online training available from <http://www.dna.gov> (Appendix 2).

In this edition, we again utilize D.N.A. boxes to cover specific topics of general interest. These D.N.A. boxes include high-profile cases—such as the O.J. Simpson trial (D.N.A. Box 4.2)—along with other topics of interest relevant to a specific section of the book. These are scattered throughout the book near the sections dealing with the science or issues behind these cases. It is hoped that these D.N.A. boxes will help readers see the practical value of forensic DNA typing.



# 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, Martin Bill, George Carmody, Mike Coble, David Duewer, Dan Ehrlich, Nicky Fildes, Lisa Forman, Ron Fourny, Lee Fraser, Chip Harding, Doug Hares, Debbie Hobson, Bill Hudlow, Margaret Kline, Carl Ladd, Steve Lee, Bruce McCord, Ruth Montgomery, Steve Niezgoda, Richard Schoske, Jim Schumm, Bob Shaler, Melissa Smrz, Amanda Sozer, Mark Stolorow, Kevin Sullivan, and Lois Tully. I am indebted to the dedicated project team members, past and present, who work with me at the U.S. National Institute of Standards and Technology: Pete Vallone, Mike Coble, Margaret Kline, Jan Redman, David Duewer, Jill Appleby, Amy Decker, Becky Hill, Dennis Reeder, Christian Ruitberg, and Richard Schoske. It is a pleasure to work with such supportive and hard-working scientists.

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 update this STR reference database monthly. 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. 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 in chemistry at Brigham Young University, he moved east to pursue his 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 2 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 receiving 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. Dr. Butler then went to California for several years to work as a staff scientist and project leader at a startup company named GeneTrace System to develop 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 NIST. He is a regular invited guest of the FBI's Scientific Working Group on DNA Analysis Methods (SWGDM) and a member of the Department of Defense Quality Assurance Oversight Committee for DNA Analysis. Following the terrorist attacks of 11 September 2001, Dr. Butler's expertise was sought to aid the DNA identification efforts, and he 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*.

He has received numerous awards during his career for his work in advancing forensic DNA typing, 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), 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).

Dr. Butler has more than 100 publications describing aspects of forensic DNA testing, making him one of the most prolific active authors in the field with articles appearing regularly in every major forensic science journal. He 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, and Australia. He is well qualified to present the information found in this book, much of which has come from his own research efforts over the past 15 years. In addition to his busy scientific career, Dr. Butler and his wife serve in their community and church and are the parents of six children, all of whom have been proven to be theirs through the power of DNA typing.