

# METHODS IN MOLECULAR BIOLOGY

*Series Editor*  
**John M. Walker**  
**School of Life and Medical Sciences**  
**University of Hertfordshire**  
**Hatfield, Hertfordshire, AL10 9AB, UK**

For further volumes:  
<http://www.springer.com/series/7651>



# **Mitochondrial DNA**

**Methods and Protocols**

**Third Edition**

Edited by

**Matthew McKenzie**

*Hudson Institute of Medical Research, Clayton, VIC, Melbourne, Australia*

 **Humana Press**

*Editor*

Matthew McKenzie  
Hudson Institute of Medical Research  
Clayton, VIC, Melbourne, Australia

ISSN 1064-3745                      ISSN 1940-6029 (electronic)  
Methods in Molecular Biology  
ISBN 978-1-4939-3039-5              ISBN 978-1-4939-3040-1 (eBook)  
DOI 10.1007/978-1-4939-3040-1

Library of Congress Control Number: 2015947277

Springer New York Heidelberg Dordrecht London  
© Springer Science+Business Media New York 2016

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Humana Press is a brand of Springer  
Springer Science+Business Media LLC New York is part of Springer Science+Business Media ([www.springer.com](http://www.springer.com))

---

## Preface

Since the publication of the second edition of *Mitochondrial DNA: Methods and Protocols*, major technological advances have been made in high-throughput DNA sequencing, high-resolution cellular imaging, and the use of targeted nucleases for genetic manipulation. These new scientific tools have revolutionized the study of human genetics and biology and apply equally to the investigation of the mitochondrial genome. This has allowed researchers to gain new insights into how mitochondrial DNA modulates mitochondrial metabolism and also the association between mitochondrial DNA variation and human disease.

This third edition of *Mitochondrial DNA: Methods and Protocols* describes these new methodologies for mitochondrial DNA analysis and, combined with well-established protocols that are considered the gold standard in the field, comprises a compendium of detailed methods for the study of mitochondrial DNA biology. The first part describes protocols for detecting mutations in mitochondrial DNA by next-generation massive parallel sequencing as well as techniques to assess mitochondrial DNA damage. The second part describes the visualization of mitochondrial DNA in situ and the detection of mitochondrial DNA nucleoids within the mitochondria. The third part describes methods for analyzing mitochondrial DNA replication, mitochondrial DNA-encoded protein translation, and mitochondrial DNA copy number. The fourth part describes the latest technology for modifying the mitochondrial genome, while the fifth part describes how to purify proteins involved in the replication and transcription of mitochondrial DNA.

These protocols will prove highly useful, not only for mitochondrial researchers but also for scientists studying human diseases where mitochondrial DNA variation has been recognized as an important pathogenic factor, including cancer and neurodegeneration. As such, this book will be a valuable addition to the *Mitochondrial DNA: Methods and Protocols* series title, and I would like to thank all of the authors for their excellent contributions to this publication.

*Clayton, VIC, Melbourne, Australia*

*Matthew McKenzie*



---

# Contents

<i>Preface</i> . . . . .	<i>v</i>
<i>Contributors</i> . . . . .	<i>ix</i>
PART I DETECTION OF MITOCHONDRIAL DNA MUTATIONS AND MITOCHONDRIAL DNA DAMAGE	
1 Comprehensive Mitochondrial Genome Analysis by Massively Parallel Sequencing . . . . . <i>Meagan E. Palculict, Victor Wei Zhang, Lee-Jun Wong, and Jing Wang</i>	3
2 Analysis of Translesion DNA Synthesis by the Mitochondrial DNA Polymerase $\gamma$ . . . . . <i>William C. Copeland, Rajesh Kasiviswanathan, and Matthew J. Longley</i>	19
3 Quantification of DNA Damage by Real-Time qPCR . . . . . <i>Wei Wang, Katja Scheffler, Ying Esbensen, and Lars Eide</i>	27
4 Quantitation of Mitochondrial DNA Deletions Via Restriction Digestion/Long-Range Single-Molecule PCR . . . . . <i>Yevgenya Kravtsov, Xinhong Guo, Saisai Tao, Alexandra Kuznetsov, Catherine MacLean, Daniel Ehrlich, Evan Feldman, Igor Dombrovsky, Deye Yang, Gregory J. Cloutier, Carmen Castaneda-Sceppa, and Konstantin Khrapko</i>	33
PART II VISUALIZATION OF MITOCHONDRIAL DNA AND MITOCHONDRIAL DNA NUCLEOIDS	
5 A Single-Cell Resolution Imaging Protocol of Mitochondrial DNA Dynamics in Physiopathology, mTRIP, Which Also Evaluates Sublethal Cytotoxicity . . . . . <i>Laurent Chatre, Benjamin Montagne, and Miria Ricchetti</i>	49
6 Scalable Isolation of Mammalian Mitochondria for Nucleic Acid and Nucleoid Analysis . . . . . <i>Ken-Wing Lee and Daniel F. Bogenhagen</i>	67
7 Tracking Mitochondrial DNA <i>In Situ</i> . . . . . <i>Anna Ligasová and Karel Koberna</i>	81
PART III ANALYSIS OF MITOCHONDRIAL DNA REPLICATION, MITOCHONDRIAL DNA-ENCODED PROTEIN TRANSLATION AND MITOCHONDRIAL DNA COPY NUMBER	
8 Analysis of Replicating Mitochondrial DNA by <i>In Organello</i> Labeling and Two-Dimensional Agarose Gel Electrophoresis . . . . . <i>Ian J. Holt, Lawrence Kazak, Aurelio Reyes, and Stuart R. Wood</i>	95

9	Translation and Assembly of Radiolabeled Mitochondrial DNA-Encoded Protein Subunits from Cultured Cells and Isolated Mitochondria . . . . .	115
	<i>Luke E. Formosa, Annette Hofer, Christin Tischner, Tina Wenz, and Michael T. Ryan</i>	
10	Analysis of Mitochondrial DNA Copy Number and Its Regulation Through DNA Methylation of <i>POLGA</i> . . . . .	131
	<i>Xin Sun, William Lee, Vijesh Vaghjiani, and Justin C. St. John</i>	
PART IV MODIFICATION OF MITOCHONDRIAL DNA		
11	Engineered mtZFNs for Manipulation of Human Mitochondrial DNA Heteroplasmy . . . . .	145
	<i>Payam A. Gammage, Lindsey Van Haute, and Michal Minczuk</i>	
12	Generation of Xenomitochondrial Embryonic Stem Cells for the Production of Live Xenomitochondrial Mice . . . . .	163
	<i>Ian A. Trounce, Jessica Ackerley, and Matthew McKenzie</i>	
13	Import of Fluorescent RNA into Mitochondria of Living Cells . . . . .	175
	<i>Jaroslav Zelenka and Petr Ježek</i>	
PART V PURIFICATION OF PROTEINS INVOLVED IN MITOCHONDRIAL DNA REPLICATION AND TRANSCRIPTION		
14	Purification and Comparative Assay of the Human Mitochondrial Replicative DNA Helicase . . . . .	185
	<i>Fernando A. Rosado-Ruiz, Minyoung So, and Laurie S. Kaguni</i>	
15	Expression and Purification of Mitochondrial RNA Polymerase and Transcription Factor A from <i>Drosophila melanogaster</i> . . . . .	199
	<i>John P. Gajewski, Jamie J. Arnold, Tiina S. Salminen, Laurie S. Kaguni, and Craig E. Cameron</i>	
16	Purification and Comparative Assay of Human Mitochondrial Single-Stranded DNA-Binding Protein . . . . .	211
	<i>Grzegorz L. Ciesielski, Fernando A. Rosado-Ruiz, and Laurie S. Kaguni</i>	
17	Biolayer Interferometry: A Novel Method to Elucidate Protein–Protein and Protein–DNA Interactions in the Mitochondrial DNA Replisome. . . . .	223
	<i>Grzegorz L. Ciesielski, Vesa P. Hytönen, and Laurie S. Kaguni</i>	
	Erratum to . . . . .	<i>E1</i>
	<i>Index</i> . . . . .	233



---

## Contributors

JESSICA ACKERLEY • *Centre for Genetic Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia*

JAMIE J. ARNOLD • *Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA*

DANIEL F. BOGENHAGEN • *Department of Pharmacological Sciences, Stony Brook University, Stony Brook, NY, USA*

CRAIG E. CAMERON • *Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA*

CARMEN CASTANEDA-SCEPPA • *Bouve College of Health Sciences, Northeastern University, Boston, MA, USA*

LAURENT CHATRE • *Team “Stability of Nuclear and Mitochondrial DNA”, Paris, France; Stem Cells and Development, Department of Developmental and Stem Cell Biology, Institut Pasteur, Paris, France*

GRZEGORZ L. CIESIELSKI • *Institute of Biosciences and Medical Technology, University of Tampere, Tampere, Finland*

GREGORY J. CLOUTIER • *Bouve College of Health Sciences, Northeastern University, Boston, MA, USA*

WILLIAM C. COPELAND • *Mitochondrial DNA Replication Group, Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA*

IGOR DOMBROVSKY • *Beth Israel Deaconess Medical Center, Boston, MA, USA*

DANIEL EHRlich • *Beth Israel Deaconess Medical Center, Boston, MA, USA*

LARS EIDE • *Department of Medical Biochemistry, Institute of Clinical Medicine, Oslo University Hospital and University of Oslo, Oslo, Norway*

YING ESBENSEN • *Institute of Clinical Epidemiology and Molecular Biology, Akershus University Hospital and University of Oslo, Lørenskog, Norway*

EVAN FELDMAN • *Beth Israel Deaconess Medical Center, Boston, MA, USA*

LUKE E. FORMOSA • *Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC, Australia; Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia*

JOHN P. GAJEWSKI • *Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA*

PAYAM A. GAMMAGE • *MRC Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Cambridge, UK*

XINHONG GUO • *College of Biology, Hunan University, Changsha, People’s Republic of China*

LINDSEY VAN HAUTE • *MRC Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Cambridge, UK*

ANNETTE HOFER • *Institute for Genetics and Cluster of Excellence: Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany*

IAN J. HOLT • *MRC-National Institute for Medical Research, London, UK*

VESA P. HYTÖNEN • *Institute of Biosciences and Medical Technology, University of Tampere, Tampere, Finland; Fimlab Laboratories, Tampere, Finland*

- PETR JEŽEK • *Department No.75, Membrane Transport Biophysics, Academy of Sciences of the Czech Republic, Institute of Physiology, Prague, Czech Republic*
- LAURIE S. KAGUNI • *Institute of Biosciences and Medical Technology, University of Tampere, Tampere, Finland; Department of Biochemistry and Molecular Biology, and Center for Mitochondrial Science and Medicine, Michigan State University, East Lansing, MI, USA*
- RAJESH KASIVISWANATHAN • *Mitochondrial DNA Replication Group, Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA*
- LAWRENCE KAZAK • *MRC-Mitochondrial Biology Unit, Cambridge, UK*
- KONSTANTIN KHRAPKO • *Department of Biology, Northeastern University, Boston, MA, USA*
- KAREL KOBERNA • *Faculty of Medicine, Institute of Molecular and Translational Medicine, Palacký University, Olomouc, Czech Republic*
- YEVGENYA KRAYTSBERG • *Beth Israel Deaconess Medical Center, Boston, MA, USA*
- ALEXANDRA KUZNETSOV • *Beth Israel Deaconess Medical Center, Boston, MA, USA*
- KEN-WING LEE • *Department of Pharmacological Sciences, Stony Brook University, Stony Brook, NY, USA; Memorial Sloan Kettering Cancer Institute, New York, NY, USA*
- WILLIAM LEE • *Mitochondrial Genetics Group, Centre for Genetic Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia; The Department of Molecular and Translational Science, Monash University, Clayton, VIC, Australia*
- ANNA LIGASOVÁ • *Faculty of Medicine, Institute of Molecular and Translational Medicine, Palacký University, Olomouc, Czech Republic*
- MATTHEW J. LONGLEY • *Mitochondrial DNA Replication Group, Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA*
- MATTHEW MCKENZIE • *Hudson Institute of Medical Research, Clayton, VIC, Melbourne, Australia*
- CATHERINE MACLEAN • *Beth Israel Deaconess Medical Center, Boston, MA, USA*
- MICHAL MINCZUK • *MRC Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Cambridge, UK*
- BENJAMIN MONTAGNE • *Team “Stability of Nuclear and Mitochondrial DNA”, Paris, France; Stem Cells and Development, Department of Developmental & Stem Cell Biology, Institut Pasteur, Paris, France*
- MEAGAN E. PALCULICT • *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA*
- AURELIO REYES • *MRC-Mitochondrial Biology Unit, Cambridge, UK*
- MIRIA RICCHETTI • *Team “Stability of Nuclear and Mitochondrial DNA”, Paris, France; Stem Cells and Development, Department of Developmental & Stem Cell Biology, Institut Pasteur, Paris, France*
- FERNANDO A. ROSADO-RUIZ • *Department of Biochemistry and Molecular Biology, Center for Mitochondrial Science and Medicine, Michigan State University, East Lansing, MI, USA*
- MICHAEL T. RYAN • *Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia*
- TIINA S. SALMINEN • *Institute of Biosciences and Medical Technology, University of Tampere, Tampere, Finland*

- KATJA SCHEFFLER • *Department of Medical Biochemistry, Oslo University Hospital and University of Oslo, Oslo, Norway; Department of Microbiology, Oslo University Hospital and University of Oslo, Oslo, Norway*
- MINYOUNG SO • *Department of Biochemistry and Molecular Biology, Center for Mitochondrial Science and Medicine, Michigan State University, East Lansing, MI, USA*
- JUSTIN C. ST. JOHN • *Mitochondrial Genetics Group, Centre for Genetic Diseases, Hudson Institute of Medical Research and The Department of Molecular and Translational Science, Monash University, Clayton, VIC, Australia*
- XIN SUN • *Mitochondrial Genetics Group, Centre for Genetic Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia; The Department of Molecular and Translational Science, Monash University, Clayton, VIC, Australia*
- SAISAI TAO • *Beth Israel Deaconess Medical Center, Boston, MA, USA*
- CHRISTIN TISCHNER • *Institute for Genetics and Cluster of Excellence: Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany*
- IAN A. TROUNCE • *Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, University of Melbourne, East Melbourne, VIC, Australia*
- VIJESH VAGHJANI • *Mitochondrial Genetics Group, Centre for Genetic Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia; The Department of Molecular and Translational Science, Monash University, Clayton, VIC, Australia*
- JING WANG • *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA*
- WEI WANG • *Department of Medical Biochemistry, Oslo University Hospital and University of Oslo, Oslo, Norway; Department of Microbiology, Oslo University Hospital and University of Oslo, Oslo, Norway*
- TINA WENZ • *Institute for Genetics and Cluster of Excellence: Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany*
- LEE-JUN WONG • *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA*
- STUART R. WOOD • *MRC-Mitochondrial Biology Unit, Cambridge, UK*
- DEYE YANG • *Heart Centre, The Affiliated Hospital, Hangzhou Normal University, Hangzhou, People's Republic of China*
- JAROSLAV ZELENKA • *Department No.75, Membrane Transport Biophysics, Academy of Sciences of the Czech Republic, Institute of Physiology, Prague, Czech Republic*
- VICTOR WEI ZHANG • *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA*