METHODS IN MOLECULAR BIOLOGY

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

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Chromatin Protocols

Third Edition

Edited by

Srikumar P. Chellappan

H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, USA

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Editor
Srikumar P. Chellappan
H. Lee Moffitt Cancer Center & Research Institute
Tampa, FL, USA

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Cover illustration: Primary rat cortical neurons undergoing apoptosis stained with P-Ser10 Histone H3 and MAP2 antibodies; nuclei were visualized using Hoechst staining. *Protocol described in Chapter 13.*

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Preface

Spectacular advancements have been made in our knowledge of chromatin structure and function in recent years. The recent development of several novel technologies to interrogate various biological processes has impacted the field of chromatin biology in a remarkable fashion. Thus, the ability to sequence large stretches of DNA, the enhanced capabilities to study protein-DNA interactions at very high resolutions, novel techniques to study histone modifications and other epigenetic changes have all shed new light on our understanding of chromatin structure as well as processes like transcriptional regulation, DNA replication and repair. In addition, the ability to edit genes using CRISPR/CAS technology has opened up new avenues and methodologies to study chromatin biology. It has also become increasingly clear that noncoding RNA molecules might play a vital role in the regulation of chromatin organization and function. The third edition of *Chromatin Protocols* compiles many of these techniques.

The first edition of *Chromatin Protocols* that was published in 1999 became the staple of laboratories studying chromatin structure and function. Significant advances in the field as well as development of novel techniques led to the publication of the second edition in 2009, which was received very well by the scientific community. The field has moved at a remarkable pace in the 5 years since the publication of the second edition, calling for a third edition. This edition carries over certain chapters and protocols from the previous edition; these have been updated. Many additional protocols that have been recently developed have been included in the volume. Thus, we have now included protocols for chromatin imaging at a very high resolution; determination of DNA methylation using Illumina BeadChips; identification and characterization of nonhistone chromatin proteins; fluorescent in situ hybridization on comets; an enChIP protocol using CRISPR; analysis of transcription using spFRET, to mention a few. Detailed protocols for these novel techniques, along with the protocols for established and time-tested methods for isolation of nucleosomes, analysis of histone modifications and chromatin function using ChIP assays etc., make this volume a handy source for information needed to study chromatin biology.

This volume is organized such that the initial part of this volume describes techniques related to the study of chromatin structure. Protocols for reconstitution of chromatin on solid supports for analysis, preparation of positioned mononucleosomes, techniques to study premature chromatin condensation, and the use of comparative genomic hybridization to assess genomic aberration are included here. Novel techniques for imaging chromatin using atomic force microscopy and the isolation of specific genomic regions using engineered DNA-binding molecules generated by CRISPR are included here. This section is followed by protocols to analyze DNA and histone modifications; eight different protocols are presented here. The third section includes methods to study DNA replication and repair, in the context of chromatin. Last but not the least, protocols for studying chromatin and its relation with transcriptional regulation are presented in a fourth section.

We believe that this updated edition of *Chromatin Protocols* will be as useful as the first two editions and will facilitate in-depth molecular analysis of various aspects of chromatin structure and function. This volume would not have been possible without the valuable

contributions from a truly international panel of highly talented and accomplished scientists. My sincere thanks to them for taking the time and effort to pen down the intricate details of their favorite techniques and for generously sharing them with the scientific community. I would also like to express my thanks to Dr. John Walker, the Series Editor, without whose valuable input and suggestions this volume would not have taken shape.

Tampa, FL, USA *Srikumar P. Chellappan*

Contents

Contributors

Salomé Adam • *Epigenetics and Cell Fate Centre, Paris Diderot University, Paris, France; Chromatin Dynamics Laboratory, Institut Curie Research Centre, Paris, France*

Andrés Aguilera • *CABIMER, Universidad de Sevilla-CSIC, Sevilla, Spain*

Mark G. Alexandrow • *Department of Molecular Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*

Mahesh Bachu • *HIV-AIDS Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India*

Siau-Kun Bai • *Epigenetics and Cell Fate Centre, Paris Diderot University, Paris, France*

Partha P. Banerjee • *Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, Washington, DC, USA*

Peter B. Becker • *Molékularbiologie, Adolf-Butenandt-Institut Ludwig-Maximilians-Universität München (LMU), Munich, Germany*

Victoria L. Bryant • *Department of Molecular Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*

Sergiy Borysov • *Department of Molecular Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*

Melanie A. Carless • *Texas Biomedical Research Institute, San Antonio, TX, USA*

Srikumar P. Chellappan, • *Department of Tumor Biology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*

Oleg V. Chertkov • *Biology Faculty, Lomonosov Moscow State University, Moscow, Russia*

Andrew R. Collins • *Department of Nutrition, Faculty of Medicine, University of Oslo, Oslo, Norway*

Juliette Dabin • *Epigenetics and Cell Fate Centre, Paris Diderot University, Paris, France*

Qian Dai • *Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA*

Russell P. Darst • *Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, FL, USA; UF Health Cancer Center Program in Cancer Genetics, Epigenetics, and Tumor Virology, University of Florida College of Medicine, Gainesville, FL, USA*

Chandrima Das • *Biophysics & Structural Genomics Division, Saha Institute of Nuclear Physics, Kolkata, India*

Piyali Dasgupta • *Department of Pharmacology, Physiology, and Toxicology, Marshall University, Huntington, WV, USA*

Anastasija V. Efremenko • *Biology Faculty, Lomonosov Moscow State University, Moscow, Russia*

Jia Fang • *Department of Tumor Biology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*

Alexey V. Feofanov • *Biology Faculty, Lomonosov Moscow State University, Moscow, Russia; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*

Hodaka Fujii • *Chromatin and Biochemistry Research Group, Combined Program on Microbiology and Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan*
Toshitsugu Fujita • *Chromatin and Biochemistry Research Group, Combined Program on Microbiology and Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan*
Shrikanth S. Gadad • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, Karnataka, India*
Hélène Gaillard • *CABIMER, Universidad de Sevilla-CSIC, Sevilla, Spain*
Edgar González-Buendía • *Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad de México, DF, Mexico*
Eisuke Gotoh, • *Department of Radiology, Jikei University School of Medicine, Tokyo, Japan*
Nobuo Horikoshi • *Department of Radiation Oncology, Houston Methodist Research Institute, University of Texas, Houston, TX, USA*
Clayton Hunt • *Department of Radiation Oncology, Houston Methodist Research Institute, University of Texas, Houston, TX, USA*
Shankar Jagadeesh • *Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, Washington, DC, USA*
Amanda E. Jones • *Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA*
Heui-Yun Joo • *Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA*
Mikhail P. Kirpichnikov • *Biology Faculty, Lomonosov Moscow State University, Moscow, Russia; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*
Michael P. Kladde • *Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, FL, USA; UF Health Cancer Center Program in Cancer Genetics, Epigenetics, and Tumor Virology, University of Florida College of Medicine, Gainesville, FL, USA*
Kenji Kokura • *Tumor Biology Department, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Kseniya S. Kudryashova • *Biology Faculty, Lomonosov Moscow State University, Moscow, Russia; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*
Olga I. Kulaeva • *Cancer Epigenetics Program, Fox Chase Cancer Center, Philadelphia, PA, USA*
Manoj Kumar • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India*
Sujata Kumari • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India*
Tapas K. Kundu • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India*
Yuri L. Lyubchenko • *Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE, USA*
Karin Meier • *Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad de México, DF, Mexico*

Lopa Mishra • *Department of Gastroenterology, University of Texas MD Anderson Cancer Center, Houston, TX, USA*
Nancy H. Nabilsi • *Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, FL, USA; UF Health Cancer Center Program in Cancer Genetics, Epigenetics, and Tumor Virology, University of Florida College of Medicine, Gainesville, FL, USA*
Dmitry V. Nikitin • *Biology Faculty, Lomonosov Moscow State University, Moscow, Russia; G.K. Skryabin's Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia*
Jaya Padmanabhan • *Johnnie B. Byrd Sr. Alzheimer's Center and Research Institute, Tampa, FL, USA*
Tej K. Pandita, • *Department of Radiation Oncology, Houston Methodist Research Institute, University of Texas, Houston, TX, USA*
Carolina E. Pardo • *Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, FL, USA; UF Health Cancer Center Program in Cancer Genetics, Epigenetics, and Tumor Virology, University of Florida College of Medicine, Gainesville, FL, USA*
Lirong Peng • *Department of Molecular Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Nikolai A. Pestov • *Biology Faculty, Lomonosov Moscow State University, Moscow, Russia*
Smitha Pillai • *Department of Tumor Biology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Sophie E. Polo • *Epigenetics and Cell Fate Centre, Paris Diderot University, Paris, France*
Udaykumar Ranga • *HIV-AIDS Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India*
Félix Recillas-Targa • *Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad de México, DF, Mexico*
Wasia Rizwani • *Department of Biochemistry, Osmania University, Hyderabad, Telangana, India*
Ricardo Saldaña-Meyer • *Department of Biochemistry and Molecular Pharmacology, Howard Hughes Medical Institute, New York University School of Medicine, New York, NY, USA*
Raphael Sandaltzopoulos • *Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece*
Parijat Senapati • *Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India*
Edward Seto • *Department of Molecular Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Jayasha Shandilya • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, Karnataka, India*
Sergey Shaposhnikov • *Department of Nutrition, Faculty of Medicine, University of Oslo, Oslo, Norway; NorGenoTech AS, Skreia, Norway*
Luda S. Shlyakhtenko • *Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE, USA*
Sweta Sikder • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India*

Alexander S. Solonin • *G.K. Skryabin's Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia*
Vasily M. Studitsky • *Biology Faculty, Lomonosov Moscow State University, Moscow, Russia; Cancer Epigenetics Program, Fox Chase Cancer Center, Philadelphia, PA, USA*
Deepthi Sudarshan • *Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India*
Lidong Sun • *Department of Tumor Biology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Venkatesh Swaminathan • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, Karnataka, India*
Durga Udayakumar • *Department of Radiation Oncology, Houston Methodist Research Institute, University of Texas, Houston, TX, USA*
Hengbin Wang • *Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA*
Ralf Erik Wellinger • *CABIMER, Universidad de Sevilla-CSIC, Sevilla, Spain*
Naouale El Yamani • *Department of Nutrition, Faculty of Medicine, University of Oslo, Oslo, Norway*
Sean J. Yoder • *Molecular Genomics Core Facility, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Zhigang Yuan • *H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Ling Zhai • *Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA*