

METHODS IN MOLECULAR BIOLOGY

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Chromatin Protocols

Third Edition

Edited by

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 **Humana Press**

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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

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