
Preface

Significant advancements have been made in the study of chromatin structure and function over the past 50 years, but few as spectacular as those made in the last decade. Development of novel techniques and the ability to sequence large stretches of DNA has facilitated in-depth analysis of DNA structure and modifications as well as proteins associated with it. It has been well established that histones and non-histone proteins associated with DNA in chromatin modulate many vital cellular functions, including transcription, replication, repair and gene silencing. Moreover, the modifications of the proteins associated with chromatin have been correlated with many of these functions. Development of novel molecular techniques in the past decade has provided immense momentum to detailed analysis of chromatin, which can now be carried out with remarkable precision. The second edition of *Chromatin Protocols* compiles many of these techniques that facilitated the explosion of information on chromatin structure and function.

The first edition of *Chromatin Protocols* was published in 1999 and had become the staple of laboratories studying chromatin structure and function. Since its publication, the landscape of chromatin biology has changed immensely. The concept of 'histone code' was proposed during the earlier part of this decade and has found universal acceptance. Now it is possible not only to analyze specific histone modifications on each specific nucleosome but such analysis can also be carried out on a genome-wide scale. To a great extent, these advances in the analysis of histone modifications in relation to transcriptional regulation and gene expression were facilitated by the development of chromatin immunoprecipitation (ChIP) assays. Development and refinement of the ChIP assay have led to an understanding of molecular changes associated with histone modifications, transcription factor binding and gene expression in the cell at a level that could only be imagined a decade ago; the progress this technique and its variants have brought to the field of chromatin biology is similar to the progress electrophoretic mobility shift assays brought to transcription factor biology in the early 1980s. These are examples of techniques developed and fine-tuned in a handful of labs changing the landscape of an entire area of biology.

The second edition of *Chromatin Protocols* encompasses a wide array of techniques spanning from isolation of nucleosomes, assembly of nucleosomes and study of the basic chromatin structure to detailed analysis of histone modifications and chromatin function. Techniques to prepare chromatin assembly extracts, analyze chromatin decondensation and drug-induced premature chromatin condensation, study of reconstituted chromatin, assess DNA topology in chromatin, analyze unique mononucleosomes and a variety of other protocols to study chromatin structure form the initial part of this volume.

This section is followed by techniques to study DNA damage and repair in the context of chromatin; techniques for monitoring DNA breaks, detailed methods to study transcription-coupled repair in chromatin, techniques for analysis of H2AX phosphorylation to assess DNA damage as well as monitoring DNA breaks in chromatin are included here. A technique to study DNA damage induced by various drugs as well as radiation is included, in addition to a detailed protocol to study genomic aberrations by comparative genomic hybridization. These techniques to study DNA damage in chromatin are followed by an assay to study DNA replication *in vitro* using mammalian cell extracts. This technique and its variations allow the analysis of the role of different histone and non-histone proteins involved in the replication of DNA.

The above sections are followed by detailed protocols to study DNA methylation as well as histone modifications including methylation, acetylation, phosphorylation and ubiquitination. In addition to the analysis of histone modifications, protocols are also provided to study the activities of many of the enzymes involved, including histone demethylases and deacetylases. An elegant technique to study histone phosphorylation by immunostaining is provided, which can be modified to study other histone modifications and chromatin-associated proteins as well. Further, detailed methods to successfully carry out chromatin immunoprecipitation assays are presented. A protocol is presented which describes how genome-wide ChIP analysis can be conducted, using a ChIP-on-chip technique; success of such studies depends to a great extent on the analysis of the data generated and methods to analyze ChIP-on-chip tiling arrays are presented. Imaginative utilization of these ChIP based techniques has immense potential to generate information on various aspects of chromatin biology.

The role of telomeres in maintaining chromatin integrity and how alterations in telomerase activities lead to oncogenesis as well as other proliferative disorders have gained substantial interest in recent times. Two protocols are given in this broad context, one describing techniques to study telomerase activity and telomere length and one to study ATM-dependent chromatin modifications. These techniques, we believe, would be very useful for those working in these areas in the context of normal biology of chromatin as well as proliferative disorders associated with telomere function.

We believe that these protocols, as well as their creative modifications, will facilitate in-depth molecular analysis of various aspects of chromatin structure and function including transcriptional regulation, DNA repair and replication. As can be imagined, this volume would not have been made possible without valuable contributions from a truly international panel of authors, who are all experts in their fields. My sincere thanks to them for taking the time and effort to pen down the intricate details of their favorite techniques and for their willingness to share them with the readers. In addition, my thanks to Dr. John Walker, the series editor, without whose helpful suggestions and persistence this volume would not have been completed in its present form; it was a real pleasure working with John. It is our genuine belief that the second edition of *Chromatin Protocols* will be a valuable tool for studying various aspects of chromatin function and would contribute significantly to the development of new techniques as well as ideas in the field of chromatin biology.