

CONTENTS

Contributors

xi

Preface

xv

Part I

Epigenetic Technologies

1. Identification and Quantification of Histone PTMs Using High-Resolution Mass Spectrometry	3
K.R. Karch, S. Sidoli, and B.A. Garcia	
1. Introduction	4
2. Histone Extraction from Cells	6
3. Bottom-Up Mass Spectrometry	9
4. Offline Fractionation of Histone Species	18
5. Middle-Down Mass Spectrometry	19
6. Top-Down Mass Spectrometry	24
References	28
2. Substrate Specificity Profiling of Histone-Modifying Enzymes by Peptide Microarray	31
E.M. Cornett, B.M. Dickson, R.M. Vaughan, S. Krishnan, R.C. Trievel, B.D. Strahl, and S.B. Rothbart	
1. Introduction	32
2. Assay Optimization	34
3. Assay Methodology	37
4. Enzyme Specificity Profiling by Microarray	42
5. Summary and Perspectives	49
Acknowledgments	50
References	50
3. ArrayNinja: An Open Source Platform for Unified Planning and Analysis of Microarray Experiments	53
B.M. Dickson, E.M. Cornett, Z. Ramjan, and S.B. Rothbart	
1. Introduction	54
2. ArrayNinja	55
3. Planning Custom Microarrays with ArrayNinja	61

4. Data Analysis Features of ArrayNinja	64
5. Benchmarking ArrayNinja Against ImageQuant TL	69
6. Methodological Details	72
7. Limitations, Assumptions, Other Features, and Future Development	74
8. Summary	76
Acknowledgments	76
References	76
4. Chemical Biology Approaches for Characterization of Epigenetic Regulators	79
D. Barsyte-Lovejoy, M.M. Szewczyk, P. Prinos, E. Lima-Fernandes, S. Ackloo, and C.H. Arrowsmith	
1. Introduction	80
2. Validation of Chemical Probes for Use in Cell-Based Experiments	81
3. Inhibitor Enabled Discovery	92
4. Conclusions	99
Acknowledgments	100
References	100
5. Mapping Lysine Acetyltransferase-Ligand Interactions by Activity-Based Capture	105
D.C. Montgomery and J.L. Meier	
1. Introduction	106
2. Technical Aspects	110
3. Discussion	118
References	121
6. Investigating Histone Acetylation Stoichiometry and Turnover Rate	125
J. Fan, J. Baeza, and J.M. Denu	
1. Introduction	126
2. Labeling and Methods for Sample Preparation	128
3. Sample Analysis	132
4. Data Analysis and Kinetic Modeling	140
5. Discussion and Perspective	145
Acknowledgments	146
References	146

7. Rapid Semisynthesis of Acetylated and Sumoylated Histone Analogs 149

A. Dhall, C.E. Weller, and C. Chatterjee

1. Introduction 150
2. Materials and Methods 152
3. Semisynthesis of Sumoylated Histone H4 153
4. Preparation of Acetylated Histone H3 Analogs 156
5. Generation of Designer MNs 159
6. Summary and Conclusions 163
- Acknowledgments 164
- References 164

8. An IF-FISH Approach for Covisualization of Gene Loci and Nuclear Architecture in Fission Yeast 167

K.-D. Kim, O. Iwasaki, and K. Noma

1. Introduction 168
2. Case Studies in the Application of the IF-FISH Approach 168
3. Supplies 171
4. Protocol 174
5. Notes 178
- Acknowledgments 179
- References 179

Part II**Small Molecule Epigenetic Regulators****9. Biology, Chemistry, and Pharmacology of Sirtuins** 183

A. Bedalov, S. Chowdhury, and J.A. Simon

1. Introduction 184
2. Sirtuins and Metabolism 187
3. Sirtuins and Regulation of Cellular NAD⁺ Levels 189
4. Sirtuin Functions 189
5. Sirtuins and Metabolic Disorders 190
6. Sirtuins and Cancer 191
7. Sirtuin Activity Assays 194
8. Identification of First-Generation Sirtuin Inhibitors 197
9. Second-Generation Splitomicin Inhibitors 200
10. Other Sirtuin Inhibitors 201
11. Concluding Remarks 205
- References 205

10. Synthesis and Assay of SIRT1-Activating Compounds	213
H. Dai, J.L. Ellis, D.A. Sinclair, and B.P. Hubbard	
1. Introduction	214
2. Materials	215
3. Methods	217
4. Notes	240
Acknowledgments	242
References	242
11. Synthesis and Assays of Inhibitors of Methyltransferases	245
X.-C. Cai, K. Kapilashrami, and M. Luo	
1. Introduction to Methyltransferases	246
2. Designing and Synthesizing Inhibitors of Methyltransferases	249
3. Evaluating Methyltransferase Inhibitors	283
4. Conclusion	296
References	298
 Part III	
Epigenetics and Biological Connections	
12. Exploring the Dynamic Relationship Between Cellular Metabolism and Chromatin Structure Using SILAC-Mass Spec and ChIP-Sequencing	311
P. Mews and S.L. Berger	
1. Introduction	312
2. Analyzing the Turnover Dynamics of Histone Modifications	314
3. Genome-Wide Mapping of Histone Modifications	320
4. Summary	327
References	327
13. Current Proteomic Methods to Investigate the Dynamics of Histone Turnover in the Central Nervous System	331
L.A. Farrelly, B.D. Dill, H. Molina, M.R. Birtwistle, and I. Maze	
1. Introduction	332
2. Early Methods to Study Histone Turnover in Brain	334
3. Current Proteomic Methods to Study Histone Turnover in Brain	337
4. Retrospective Birth Dating of Histones in Human Postmortem Brain	342
5. Conclusion	347

6. Methodology: Preparing Chromatin from Neurons for Mass Spectrometry Analysis of Histone Variants and Turnover	348
Acknowledgments	352
References	352
14. ChIP-Sequencing to Map the Epigenome of Senescent Cells Using Benzonase Endonuclease	355
T.S. Rai and P.D. Adams	
1. Introduction	356
2. Buffer Compositions	358
3. Protocol	359
4. Conclusion	362
References	363
15. Exploiting Chromatin Biology to Understand Immunology	365
J.L. Johnson and G. Vahedi	
1. Introduction: Design Principle of Immune Responses	366
2. Epigenome: Our Software	366
3. Mapping DNA Methylation	367
4. Mapping Histone Modifications by Chromatin Immunoprecipitation and Sequencing	368
5. Limitations of ChIP-seq	369
6. MNase-seq for Nucleosome Positioning	370
7. DNase-seq and ATAC-seq for DNA Accessibility	371
8. Analysis of NGS Data	372
9. Painting an Enhancer Landscape	376
10. Chromatin Biology to Understand Gene Regulation in Immune Cells	377
11. Role of Intrinsic and Extrinsic Signals on Enhancer Formation	378
12. Conclusions	379
Acknowledgments	380
References	380
Author Index	385
Subject Index	413