
Contents

| | |
|--|-----------|
| <i>Preface</i> | <i>v</i> |
| <i>Contributors</i> | <i>xi</i> |
| PART I NUCLEAR ENVELOPE ISOLATION | |
| 1 Isolation, Proteomic Analysis, and Microscopy Confirmation of the Liver Nuclear Envelope Proteome | 3 |
| <i>Nadia Korfali, Laurence Florens, and Eric C. Schirmer</i> | |
| 2 Exploring the Protein Composition of the Plant Nuclear Envelope | 45 |
| <i>Xiao Zhou, Kentaro Tamura, Katja Graumann, and Iris Meier</i> | |
| 3 High-Efficiency Isolation of Nuclear Envelope Protein Complexes from Trypanosomes | 67 |
| <i>Samson O. Obado, Mark C. Field, Brian T. Chait, and Michael P. Rout</i> | |
| PART II NUCLEAR ENVELOPE PROTEIN INTERACTIONS, LOCALIZATION, AND DYNAMICS | |
| 4 Superresolution Microscopy of the Nuclear Envelope and Associated Proteins. | 83 |
| <i>Wei Xie, Henning F. Horn, and Graham D. Wright</i> | |
| 5 Analyses of the Dynamic Properties of Nuclear Lamins by Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Correlation Spectroscopy (FCS). | 99 |
| <i>Shimi Takeshi, Chan-Gi Pack, and Robert D. Goldman</i> | |
| 6 Probing Protein Distribution Along the Nuclear Envelope In Vivo by Using Single-Point FRAP | 113 |
| <i>Krishna C. Mudumbi and Weidong Yang</i> | |
| 7 The Use of Two-Photon FRET–FLIM to Study Protein Interactions During Nuclear Envelope Fusion In Vivo and In Vitro | 123 |
| <i>Richard D. Byrne, Banafshé Larijani, and Dominic L. Poccia</i> | |
| 8 Identifying Protein-Protein Associations at the Nuclear Envelope with BioID | 133 |
| <i>Dae In Kim, Samuel C. Jensen, and Kyle J. Roux</i> | |
| 9 In Situ Detection of Interactions Between Nuclear Envelope Proteins and Partners | 147 |
| <i>Alice Barateau and Brigitte Buendia</i> | |
| 10 Methods for Single-Cell Pulse-Chase Analysis of Nuclear Components | 159 |
| <i>Marek Drozd and David J. Vaux</i> | |

- 11 Analysis of Nuclear Lamina Proteins in Myoblast Differentiation
by Functional Complementation 177
Olga Tapia and Larry Gerace
- 12 Analysis of Meiotic Telomere Behavior in the Mouse 195
Jana Link, Ricardo Benavente, and Manfred Alsheimer

PART III NUCLEAR ENVELOPE INTERACTIONS WITH THE CYTOSKELETON

- 13 Identification and Validation of Putative Nesprin Variants 211
Flavia Autore, Catherine M. Shanahan, and Qiuping Zhang
- 14 Detection of Diverse and High Molecular Weight Nesprin-1
and Nesprin-2 Isoforms Using Western Blotting 221
James Carthew and Iakowos Karakesisoglou
- 15 The Use of Polyacrylamide Hydrogels to Study the Effects
of Matrix Stiffness on Nuclear Envelope Properties 233
Rose-Marie Minaisah, Susan Cox, and Derek T. Warren
- 16 Cell Microharpooning to Study Nucleo-Cytoskeletal Coupling 241
Gregory Fedorchak and Jan Lammerding
- 17 Wound-Healing Assays to Study Mechanisms of Nuclear
Movement in Fibroblasts and Myoblasts 255
Wakam Chang, Susumu Antoku, and Gregg G. Gundersen
- 18 Methods for Assessing Nuclear Rotation and Nuclear Positioning
in Developing Skeletal Muscle Cells 269
Meredith H. Wilson, Matthew G. Bray, and Erika L.F. Holzbaur
- 19 Imaging Approaches to Investigate Myonuclear
Positioning in *Drosophila*. 291
*Mafalda Azevedo, Victoria K. Schulman, Eric Folker,
Mridula Balakrishnan, and Mary Baylies*

PART IV NUCLEAR ENVELOPE-CHROMATIN INTERACTIONS

- 20 Mapping Nuclear Lamin-Genome Interactions by Chromatin
Immunoprecipitation of Nuclear Lamins 315
Anja R. Oldenburg and Philippe Collas
- 21 Lamin ChIP from Chromatin Prepared by Micrococcal
Nuclease Digestion 325
Isabelle Duband-Goulet
- 22 DamID Analysis of Nuclear Organization in *Caenorhabditis elegans* 341
Georgina Gómez-Saldivar, Peter Meister, and Peter Askjaer
- 23 The Application of DamID to Identify Peripheral Gene Sequences in
Differentiated and Primary Cells 359
Michael I. Robson and Eric C. Schirmer
- 24 Visualizing the Spatial Relationship of the Genome
with the Nuclear Envelope Using Fluorescence In Situ Hybridization 387
*Craig S. Clements, Ural Bikkul, Mai Hassan Ahmed,
Helen A. Foster, Lauren S. Godwin, and Joanna M. Bridger*

| | | |
|-------------------------------------|--|-----|
| 25 | Visualization of Genomic Loci in Living Cells with a Fluorescent CRISPR/Cas9 System | 407 |
| | <i>Tobias Anton, Heinrich Leonhardt, and Yolanda Markaki</i> | |
| 26 | Methods to Monitor DNA Repair Defects and Genomic Instability in the Context of a Disrupted Nuclear Lamina | 419 |
| | <i>Susana Gonzalo and Ray Kreienkamp</i> | |
| PART V NUCLEO-CYTOPLASMIC TRANSPORT | | |
| 27 | High-Resolution Scanning Electron Microscopy and Immuno-Gold Labeling of the Nuclear Lamina and Nuclear Pore Complex | 441 |
| | <i>Martin W. Goldberg</i> | |
| 28 | An In Vitro Assay to Study Targeting of Membrane Proteins to the Inner Nuclear Membrane | 461 |
| | <i>Rosemarie Ungricht, Sumit Pawar, and Ulrike Kutay</i> | |
| 29 | Nuclear Protein Transport in Digitonin Permeabilized Cells | 479 |
| | <i>Stephen A. Adam</i> | |
| 30 | Analysis of CRM1-Dependent Nuclear Export in Permeabilized Cells | 489 |
| | <i>Ralph H. Kehlenbach and Sarah A. Port</i> | |
| 31 | SPEED Microscopy and Its Application in Nucleocytoplasmic Transport. | 503 |
| | <i>Jiong Ma, Joseph M. Kelich, and Weidong Yang</i> | |
| | <i>Index</i> | 519 |

The Nuclear Envelope

Methods and Protocols

Shackleton, S.; Collas, P.; Schirmer, E.C. (Eds.)

2016, XIV, 523 p. 103 illus., 77 illus. in color., Hardcover

ISBN: 978-1-4939-3528-4

A product of Humana Press