

CONTENTS

<i>Contributors</i>	xv
<i>Preface</i>	xxiii
<i>Volumes in Series</i>	xxv

Section I. Functional Genomics 1

1. Analysis of Gene Function Using DNA Microarrays 3

Andrew P. Capaldi

1. Introduction and Experimental Design	4
2. Methods	8
References	16

2. An Introduction to Microarray Data Analysis and Visualization 19

Gregg B. Whitworth

1. Introduction	20
2. Experimental Design: Single-Sample Versus Competitive Hybridization	23
3. Image Analysis	26
4. Preprocessing	29
5. Visualizing Data Using Cluster Analysis	38
6. Assessing the Statistical Evidence for Differential Expression	41
7. Exploring Gene Sets	42
8. Managing Data	44
Acknowledgments	49
References	49

3. Genome-Wide Approaches to Monitor Pre-mRNA Splicing 51

Maki Inada and Jeffrey A. Pleiss

1. Introduction	52
2. Microarray Design	53
3. Sample Preparation	56
4. Microarray Data Collection	68
5. Microarray Data Analysis	69
6. Future Methodologies	72
Acknowledgments	73
References	74

4. ChIP-Seq: Using High-Throughput DNA Sequencing for Genome-Wide Identification of Transcription Factor Binding Sites	77
Philippe Lefrançois, Wei Zheng, and Michael Snyder	
1. Introduction	78
2. Protocols	81
3. Sequencing Data Management	93
4. Genome Analysis Pipeline	94
5. Examining Data Quality and Parsing Barcoded Data	95
6. Visualization in Genome Browser	95
7. Conclusion and Future Directions	102
Acknowledgments	102
References	102
5. Genome-Wide Mapping of Nucleosomes in Yeast	105
Oliver J. Rando	
1. Introduction	105
2. Isolation of Mononucleosomal DNA	107
3. Variation in Titration Level Used for Nucleosome Purification	112
4. Labeling of Mononucleosomal DNA for Tiling Microarray Analysis	113
5. Generation of Nucleosomal DNA Libraries for Deep Sequencing	115
References	117
6. Genome-Wide Translational Profiling by Ribosome Footprinting	119
Nicholas T. Ingolia	
1. Introduction	120
2. Ribosome Footprint Generation and Purification	121
3. Sequencing Library Preparation	128
4. Data Analysis	133
5. Solutions and Common Procedures	138
Acknowledgments	140
References	140
Section II. Systematic Genetic Analysis	143
7. Synthetic Genetic Array (SGA) Analysis in <i>Saccharomyces cerevisiae</i> and <i>Schizosaccharomyces pombe</i>	145
Anastasia Baryshnikova, Michael Costanzo, Scott Dixon, Franco J. Vizeacoumar, Chad L. Myers, Brenda Andrews, and Charles Boone	
1. Introduction	146
2. Methodology	148
3. Media and Stock Solutions	168
4. Applications of SGA Methodology	171
References	176

8. Making Temperature-Sensitive Mutants	181
Shay Ben-Aroya, Xuewen Pan, Jef D. Boeke, and Philip Hieter	
1. Introduction	182
2. Diploid Shuffle—Plasmid Method	183
3. Diploid Shuffle—Chromosome Method	194
4. Perspectives	202
References	203
9. Quantitative Genetic Interaction Mapping Using the E-MAP Approach	205
Sean R. Collins, Assen Roguev, and Nevan J. Krogan	
1. Introduction	206
2. Selection of Mutations for Genetic Analysis	209
3. Generation and Measurement of Double Mutant Strains	211
4. Data Processing and Computation of Scores	221
5. Extraction of Biological Hypotheses	224
6. Perspective	229
References	229
10. Exploring Gene Function and Drug Action Using Chemogenomic Dosage Assays	233
Elke Ericson, Shawn Hoon, Robert P. St. Onge, Guri Giaever, and Corey Nislow	
1. Introduction	234
2. Methodology	237
3. Experimental Considerations	252
4. Perspectives	253
Acknowledgments	254
References	254
Section III. Proteomics	257
11. Yeast Expression Proteomics by High-Resolution Mass Spectrometry	259
Tobias C. Walther, Jesper V. Olsen, and Matthias Mann	
1. Introduction	260
2. Background, Methods, and Applications	261
3. Protocols	272
Acknowledgments	278
References	278

12. High-Quality Binary Interactome Mapping	281
Matija Dreze, Dario Monachello, Claire Lurin, Michael E. Cusick, David E. Hill, Marc Vidal, and Pascal Braun	
1. Introduction	282
2. High-Quality Binary Interactome Mapping	283
3. High-Throughput Y2H Pipeline	292
4. Validation Using Orthogonal Binary Interaction Assays	309
5. Conclusion	312
Acknowledgments	312
References	313
13. Quantitative Analysis of Protein Phosphorylation on a System-Wide Scale by Mass Spectrometry-Based Proteomics	317
Bernd Bodenmiller and Ruedi Aebersold	
1. Introduction	318
2. Protocols	319
Acknowledgments	331
References	332
14. A Toolkit of Protein-Fragment Complementation Assays for Studying and Dissecting Large-Scale and Dynamic Protein-Protein Interactions in Living Cells	335
Stephen W. Michnick, Po Hien Ear, Christian Landry, Mohan K. Malleshaiah, and Vincent Messier	
1. Introduction	336
2. General Considerations in Using PCA	337
3. DHFR PCA Survival-Selection for Large-Scale Analysis of PPIs	339
4. A Life and Death Selection PCA Based on the Prodrug-Converting Cytosine Deaminase for Dissection of PPIs	348
5. Visualizing the Localization of PPIs with GFP Family Fluorescent Protein PCAs	356
6. Studying Dynamics of PPIs with Luciferase Reporter PCAs	361
References	366
15. Yeast Lipid Analysis and Quantification by Mass Spectrometry	369
Xue Li Guan, Isabelle Riezman, Markus R. Wenk, and Howard Riezman	
1. Introduction	370
2. Methods	373
Acknowledgments	389
References	389

16. Mass Spectrometry-Based Metabolomics of Yeast	393
Christopher A. Crutchfield, Wenyun Lu, Eugene Melamud, and Joshua D. Rabinowitz	
1. Introduction	394
2. LC-MS Basics	395
3. Experimental Design	395
4. Strains	398
5. Culture Conditions	398
6. Metabolite Extraction	402
7. Chemical Derivatization of Metabolites	405
8. LC-MS for Mixture Analysis	406
9. Liquid Chromatography	407
10. Electrospray Ionization	410
11. Mass Spectrometry	411
12. Targeted Data Analysis	415
13. Untargeted Data Analysis	418
14. Future Outlook	421
References	422
Section IV. Systems Analysis	427
17. Imaging Single mRNA Molecules in Yeast	429
Hyun Youk, Arjun Raj, and Alexander van Oudenaarden	
1. Introduction	429
2. RNA FISH Protocol	432
3. Example: <i>STL1</i> mRNA Detection in Response to NaCl Shock	444
4. Conclusions	445
Acknowledgments	445
References	445
18. Reconstructing Gene Histories in <i>Ascomycota</i> Fungi	447
Ilan Wapinski and Aviv Regev	
1. Introduction	448
2. Synergy	451
3. Evaluating Orthogroup Quality	459
4. Biological Analysis of Gene Histories	466
5. Analysis of Paralogous Genes	478
6. Discussion and General Applicability	480
References	482

19. Experimental Evolution in Yeast: A Practical Guide	487
Maitreja J. Dunham	
1. Introduction	488
2. Experiment Rationale	488
3. Experimental Evolution Approaches	490
4. Experimental Design	493
5. Practical Considerations	496
6. Analysis Techniques	500
7. Example Protocol	502
8. Conclusions	504
Acknowledgments	505
References	505
20. Enhancing Stress Resistance and Production Phenotypes Through Transcriptome Engineering	509
Felix H. Lam, Franz S. Hartner, Gerald R. Fink, and Gregory Stephanopoulos	
1. Introduction	510
2. Transcription Factor Selection	511
3. Plasmid Library Construction	512
4. Assessment of Phenotypic Diversity	519
5. Selecting for Phenotypes of Interest	524
6. Validation	528
7. Concluding Remarks	529
References	530
Section V. Advances in Cytology/Biochemistry	533
21. Visualizing Yeast Chromosomes and Nuclear Architecture	535
Peter Meister, Lutz R. Gehlen, Elisa Varela, Véronique Kalck, and Susan M. Gasser	
1. Introduction	536
2. Strain Constructions and Image Acquisition for Nuclear Architecture Analysis in Living Cells	537
3. Data Analysis and Quantitative Measurements	547
4. IF and FISH on Fixed Samples	557
References	566
22. Quantitative Localization of Chromosomal Loci by Immunofluorescence	569
Donna Garvey Brickner, William Light, and Jason H. Brickner	
1. Yeast Strain Construction	570
2. Immunofluorescence	572

3. Fixing Cells	573
4. Spheroplasting	574
5. Preparing Slides	574
6. Antibody Incubations	575
7. Mounting and Storage of Slides	575
8. Microscopy and Analysis	576
Acknowledgments	578
References	579
23. Spinning-Disk Confocal Microscopy of Yeast	581
Kurt Thorn	
1. Introduction	582
2. Building a Spinning-Disk Confocal Microscope	583
3. Sample Preparation	594
Acknowledgments	600
References	600
24. Correlative GFP-Immunoelectron Microscopy in Yeast	603
Christopher Buser and Kent McDonald	
1. Introduction	604
2. Recent Advances in High-Pressure Freezing and Freeze-Substitution	605
3. How to Prepare Yeast by HPF/FS	606
4. Immunolabeling	612
5. Conclusions	613
6. Protocols	614
Acknowledgments	617
References	617
25. Analyzing P-Bodies and Stress Granules in <i>Saccharomyces cerevisiae</i>	619
J. Ross Buchan, Tracy Nissan, and Roy Parker	
1. Introduction	620
2. Determining If a Specific Protein can Accumulate in P-Bodies or Stress Granules	622
3. Monitoring Messenger RNA in P-Bodies	630
4. Determining If a Mutation/Perturbation Affects P-Body or Stress Granule Size and Number	631
5. Quantification of P-Body Size and Number	635
Acknowledgments	637
References	637

26. Analyzing mRNA Expression Using Single mRNA Resolution Fluorescent <i>In Situ</i> Hybridization	641
Daniel Zenklusen and Robert H. Singer	
1. Introduction	642
2. Probe Design	643
3. Probe Labeling	645
4. Cell Fixation, Preparation, and Storage	647
5. Hybridization	650
6. Image Acquisition	653
7. Image Analysis	654
8. Summary and Perspectives	657
Acknowledgments	658
References	658
27. The Use of <i>In Vitro</i> Assays to Measure Endoplasmic Reticulum-Associated Degradation	661
Jeffrey L. Brodsky	
1. Introduction	662
2. <i>In Vitro</i> ERAD Assays Using a Soluble Substrate, p α F	665
3. <i>In Vitro</i> Assays for Integral Membrane Proteins that are ERAD Substrates	673
References	677
28. A Protein Transformation Protocol for Introducing Yeast Prion Particles into Yeast	681
Motomasa Tanaka	
1. Introduction	682
2. Purification of Bacterially Expressed Sup-NM	683
3. Preparation of Different Conformations of <i>In Vitro</i> Sup-NM Amyloid	684
4. Preparation of <i>In Vivo</i> Prions from Yeast	685
5. Preparation of Lyticase	686
6. Protein Transformation	686
7. Determination of Prion Conversion Efficiency and Prion Strain Phenotypes	688
Acknowledgments	692
References	692
29. Overexpression and Purification of Integral Membrane Proteins in Yeast	695
Franklin A. Hays, Zygy Roe-Zurz, and Robert M. Stroud	
1. Introduction	696
2. General Considerations	696

3. Protocol—Molecular Biology	697
4. Protocol—Cell Growth	699
5. Protocol—Membrane Preparation and Solubilization	699
6. Protocol—Protein Purification	701
7. Protocol—Protein Characterization	705
8. Conclusion	706
Acknowledgments	706
References	706
30. Biochemical, Cell Biological, and Genetic Assays to Analyze Amyloid and Prion Aggregation in Yeast	709
Simon Alberti, Randal Halfmann, and Susan Lindquist	
1. Introduction	710
2. Methods	712
3. Concluding Remarks	731
References	731
Section VI. Other Fungi	735
31. Genetics and Molecular Biology in <i>Candida albicans</i>	737
Aaron. D. Hernday, Suzanne. M. Noble, Quinn. M. Mitrovich, and Alexander. D. Johnson	
1. Homozygous Gene Disruption in <i>C. albicans</i>	738
2. <i>C. albicans</i> DNA Transformation	743
3. <i>C. albicans</i> Total RNA Purification	745
4. C-Terminal Epitope Tagging in <i>C. albicans</i>	746
5. <i>C. albicans</i> Chromatin Immunoprecipitation	750
References	758
32. Molecular Genetics of <i>Schizosaccharomyces pombe</i>	759
Sarah A. Sabatinos and Susan L. Forsburg	
1. Introduction	760
2. Biology, Growth, and Maintenance of Fission Yeast	761
3. Genetics and Physiology	767
4. Molecular Analysis	776
5. Cell Biology	785
6. Conclusion	791
References	793

33. Applying Genetics and Molecular Biology to the Study of the Human Pathogen <i>Cryptococcus neoformans</i>	797
Cheryl D. Chun and Hiten D. Madhani	
1. Introduction	798
2. Serotypes, Strains, and Sequences	799
3. Life Cycle	800
4. Techniques for Basic Culture	802
5. Basic Molecular Biology Techniques	804
6. Methods for Assaying Pathogenesis	817
7. Concluding Remarks	828
Acknowledgments	829
References	829
34. The Fungal Genome Initiative and Lessons Learned from Genome Sequencing	833
Christina A. Cuomo and Bruce W. Birren	
1. Introduction: Yeast Genomes and Beyond	834
2. Computational Prediction of Genes and Noncoding Elements	841
3. Mechanisms of Genome Evolution	843
4. Genomic Potential for Sex	847
5. Gene Family Conservation and Evolution	848
6. Impact of Next-Generation Sequencing	849
7. Future Directions	851
Acknowledgments	852
References	853
35. Ultradian Metabolic Cycles in Yeast	857
Benjamin P. Tu	
1. Introduction	857
2. Induction of Ultradian Cycles of Oxygen Consumption Using a Chemostat	858
3. Long-Period Cycles	861
4. Short-Period Cycles	862
5. Significance of Ultradian Cycles	863
Acknowledgments	865
References	865
<i>Author Index</i>	<i>867</i>
<i>Subject Index</i>	<i>879</i>