

CONTENTS

<i>Contributors</i>	xiii
<i>Preface</i>	xxi
<i>Volumes in Series</i>	xxiii

Section I. Measuring and Engineering Central Dogma Processes **1**

1. Sequence-Specificity and Energy Landscapes of DNA-Binding Molecules **3**

Joshua R. Tietjen, Leslie J. Donato, Devesh Bhimisaria,
and Aseem Z. Ansari

1. Introduction	4
2. Array-Based Cognate Sequence Identification	6
3. Solution-Based Cognate Sequence Identification	13
4. Data Analysis and Visualization of Specificity, Binding Energy, and Genomic-Association Landscapes	18
References	27

2. Promoter Reliability in Modular Transcriptional Networks **31**

Rajat Anand, Navneet Rai, and Mukund Thattai

1. Results	33
2. Conclusion	44
3. Methods	45
Acknowledgments	47
References	47

3. The Analysis of ChIP-Seq Data **51**

Wenxiu Ma and Wing Hung Wong

1. Introduction	52
2. Planning of ChIP-Seq Experiments	53
3. Processing and Analyzing ChIP-Seq Datasets	58

4. Discussion	68
Acknowledgment	70
References	71
4. Using DNA Microarrays to Assay Part Function	75
Virgil A. Rhodius and Carol A. Gross	
1. Introduction	76
2. Different Microarray Platforms	77
3. Experimental Design	81
4. Experimental Variation	82
5. Sample Preparation	85
6. Microarray Preprocessing	93
7. Clustering	99
8. Differential Expression Analysis	104
9. Data Analysis: Understanding the Perturbation	106
10. Closing Remarks	108
References	109
5. Orthogonal Gene Expression in <i>Escherichia coli</i>	115
Wenlin An and Jason W. Chin	
1. Introduction	116
2. High-Throughput Screening for Orthogonal T7 Promoter O-rbs System	119
3. Integration of Orthogonal Pairs to Synthesize Transcription-Translation FFL	122
4. Engineering the FFL Delay via the Discovery of a Minimal O-rRNA	123
5. Discussion	126
6. Material and Methods	129
Acknowledgments	132
References	132
6. Directed Evolution of Promoters and Tandem Gene Arrays for Customizing RNA Synthesis Rates and Regulation	135
Keith E. J. Tyo, Elke Nevoigt, and Gregory Stephanopoulos	
1. Introduction	136
2. Promoter Modification by Error-Prone PCR	138
3. Generating Stable Tandem Gene Arrays for Controlling RNA Synthesis Rate	150
4. Concluding Remarks	153
References	154

Section II. Device and System Design, Optimization, and Debugging	157
7. Design and Connection of Robust Genetic Circuits	159
Adrian Randall, Patrick Guye, Saurabh Gupta, Xavier Duportet, and Ron Weiss	
1. Introduction	160
2. Sources of Failure	161
3. Robustness Principles and Examples in Natural Systems	163
4. Methods for Obtaining Robust Synthetic Circuits	165
5. Robustness Trade-Offs	181
6. Conclusion	182
References	182
8. Engineering RNAi Circuits	187
Yaakov Benenson	
1. Introduction	188
2. Constructing a Computational Logic Core for the RNAi-Based DNF Circuit	189
3. Constructing a Computational Logic Core for the RNAi-Based CNF Circuit	200
4. Transition from siRNA to miRNA	202
Acknowledgments	204
References	204
9. From SELEX to Cell: Dual Selections for Synthetic Riboswitches	207
Joy Sinha, Shana Topp, and Justin P. Gallivan	
1. Introduction	207
2. General Precautions	208
3. <i>In Vitro</i> Selection	208
4. <i>In Vivo</i> Selection	212
References	219
10. Using Noisy Gene Expression Mediated by Engineered Adenovirus to Probe Signaling Dynamics in Mammalian Cells	221
Jeffrey V. Wong, Guang Yao, Joseph R. Nevins, and Lingchong You	
1. Introduction	222
2. Design and Construction	224
3. Measurement	230
4. Broader Applications	234
References	234

11. <i>De novo</i> Design and Construction of an Inducible Gene Expression System in Mammalian Cells	239
Maria Karlsson, Wilfried Weber, and Martin Fussenegger	
1. Introduction	240
2. Selection of a Conditional DNA-Binding Protein	243
3. Establishment of the Inducible Expression System	244
4. Optimization of the Expression System	248
5. Summary	250
Acknowledgments	251
References	251
12. BioBuilding: Using Banana-Scented Bacteria to Teach Synthetic Biology	255
James Dixon and Natalie Kuldell	
1. Introduction	256
2. Eau d'coli	257
3. "Eau That Smell" Teaching Lab Using the MIT iGEM Team's Eau d'coli Cells	259
4. Teaching Labs Modified for Resource-Stretched Settings	267
5. Summary	269
Acknowledgments	270
References	270
Section III. Device Measurement, Optimization, and Debugging	273
13. Use of Fluorescence Microscopy to Analyze Genetic Circuit Dynamics	275
Gürol Süel	
1. Fluorescent Reporters	276
2. Constructing and Using Genetic Fluorescent Reporters	277
3. Fluorescent Time-Lapse Microscopy	281
4. Measuring and Interpreting Dynamics	284
5. Applications for Measurement of Circuit Dynamics	286
References	292
14. Microfluidics for Synthetic Biology: From Design to Execution	295
M. S. Ferry, I. A. Razinkov, and J. Hasty	
1. Part I: Introduction	296
2. Part II: Fabrication	338

3. Part III: Experiments	358
Acknowledgments	371
References	371
15. Plate-Based Assays for Light-Regulated Gene Expression Systems	373
Jeffrey J. Tabor	
1. Bacterial Photography Protocol	374
2. Bacterial Edge Detection Protocol	379
3. Setting up a Projector-Incubator	381
4. The β -Galactosidase/S-Gal Reporter System	384
5. Quantifying Signal Intensity on the Plates	385
6. Microscopic Imaging of Agarose Slabs	385
7. Properties of Relevant Strains	386
8. Properties of Relevant Plasmids	387
References	390
16. Spatiotemporal Control of Small GTPases with Light Using the LOV Domain	393
Yi I. Wu, Xiaobo Wang, Li He, Denise Montell, and Klaus M. Hahn	
1. Introduction	394
2. The LOV Domain as a Tool for Protein Caging	395
3. Design and Structure Optimization of PA-Rac	395
4. Activation of PA-Rac in Living Cells	397
5. Application of PA-Rac in <i>Drosophila</i> Ovarian Border Cell Migration	400
References	407
17. Light Control of Plasma Membrane Recruitment Using the Phy-PIF System	409
Jared E. Toettcher, Delquin Gong, Wendell A. Lim, and Orion D. Weiner	
1. Introduction	410
2. Light-Controlled Phy-PIF Interaction	411
3. Genetic Constructs Encoding Phy and PIF Components	412
4. Purification of PCB from <i>Spirulina</i>	415
5. Cell Culture Preparation for Phy-PIF Translocation	418
6. Imaging PIF Translocation Using Spinning Disk Confocal Microscopy	419
Acknowledgments	421
References	421

18. Synthetic Physiology: Strategies for Adapting Tools from Nature for Genetically Targeted Control of Fast Biological Processes	425
Brian Y. Chow, Amy S. Chuong, Nathan C. Klapoetke, and Edward S. Boyden	
1. Introduction	426
2. Molecular Design and Construction	429
3. Transduction of Microbial Opsins into Cells for Heterologous Expression	432
4. Physiological Assays	435
5. Conclusion	438
Acknowledgments	439
References	439
 Section IV. Devices for Metabolic Engineering	 445
19. Metabolic Pathway Flux Enhancement by Synthetic Protein Scaffolding	447
Weston R. Whitaker and John E. Dueber	
1. Introduction	448
2. Method—How to Build Modular Protein Scaffolds Systems for Metabolic Engineering Applications	454
3. Systems that May Benefit from Scaffolding	465
4. Concluding Remarks	465
Acknowledgments	466
References	466
 20. A Synthetic Iterative Pathway for Ketoacid Elongation	 469
C. R. Shen and J. C. Liao	
1. Introduction	470
2. Natural Pathways Involving Ketoacid Chain Elongations Catalyzed by the LeuABCD-Dependent Mechanisms	471
3. IPMS and Similar Enzymes	473
4. Expansion to Nonnatural Pathways	475
5. Transfer of Citramalate Pathway to <i>E. coli</i> for Ketoacid Chain Elongation	478
6. Conclusion Remarks	480
References	480

Section V. Expanding Chassis	483
21. Synthetic Biology in <i>Streptomyces</i> Bacteria	485
Marnix H. Medema, Rainer Breitling, and Eriko Takano	
1. Synthetic Biology for Novel Compound Discovery in <i>Streptomyces</i>	486
2. Practical Considerations for Synthetic Biology in <i>Streptomyces</i>	488
3. Iterative Reengineering of Secondary Metabolite Gene Clusters	489
4. The Molecular Toolbox for <i>Streptomyces</i> Synthetic Biology	491
5. Transcriptional Control	492
6. Translational Control	494
7. Vectors	494
Acknowledgments	497
References	497
22. Methods for Engineering Sulfate Reducing Bacteria of the Genus <i>Desulfovibrio</i>	503
Kimberly L. Keller, Judy D. Wall, and Swapnil Chhabra	
1. Introduction	504
2. Chromosomal Modifications Through Homologous Recombination	505
3. Culturing Conditions and Antibiotic Selection	507
4. DNA Transformation	510
5. Screening Colonies for Proper Integration	513
6. Complementing Gene Deletions	514
7. Concluding Remarks	515
Acknowledgments	516
References	516
23. Modification of the Genome of <i>Rhodobacter sphaeroides</i> and Construction of Synthetic Operons	519
Paul R. Jaschke, Rafael G. Saer, Stephan Noll, and J. Thomas Beatty	
1. Introduction	520
2. Gene Disruption and Deletion	522
3. Construction of Synthetic Operons	527
4. Future Directions	532
References	533

24. Synthetic Biology in Cyanobacteria: Engineering and Analyzing Novel Functions	539
Thorsten Heidorn, Daniel Camsund, Hsin-Ho Huang, Pia Lindberg, Paulo Oliveira, Karin Stensjö, and Peter Lindblad	
1. Introduction	540
2. Cyanobacterial Chassis	542
3. Biological Parts in Cyanobacteria	544
4. Genetic Engineering of Cyanobacteria	550
5. Molecular Analysis of Cyanobacteria	562
6. Conclusion and Outlook	571
Acknowledgments	572
References	572
25. Developing a Synthetic Signal Transduction System in Plants	581
Kevin J. Morey, Mauricio S. Antunes, Kirk D. Albrecht, Tessa A. Bowen, Jared F. Troupe, Keira L. Havens, and June I. Medford	
1. Introduction	582
2. Foundation for Developing a Molecular Testing Platform for HK Systems	586
3. Technical Considerations in Developing a Eukaryotic Synthetic Signal Transduction System Based on Bacterial TCS Components	589
4. A Partial Synthetic Signal Transduction System Using Cytokinin Input	592
5. A Eukaryotic Synthetic Signal Transduction Pathway	593
6. Conclusions	595
7. Protocols	597
Acknowledgments	599
References	599
26. Lentiviral Vectors to Study Stochastic Noise in Gene Expression	603
Kate Franz, Abhyudai Singh, and Leor S. Weinberger	
1. Introduction	604
2. The Lentiviral-Vector Approach	605
3. Production of Lentiviral Vectors and Transduced Cell Lines	609
4. Procedure for Constructing a CV ² Versus Mean Plot	616
5. Inferring Promoter Regulatory Architecture from CV ² Versus Mean Analysis	616
6. Conclusion	620
Acknowledgments	620
References	620
<i>Author Index</i>	623
<i>Subject Index</i>	651