Contents

Preface ..... *v*
Contributors ..... *ix*

1 Pathway and Gene Discovery from Natural Hosts and Organisms 1
Jing Ke and Yasuo Yoshikuni

2 Metabolic In Silico Network Expansions to Predict and Exploit Enzyme Promiscuity 11
James Jeffryes, Jonathan Strutz, Chris Henry, and Keith E. J. Tyo

3 Antisense RNA Elements for Downregulating Expression 23
Yaping Yang, Jian Wang, Ruihua Zhang, and Yajun Yan

4 Chemically Inducible Chromosomal Evolution (CIChE) for Multicopy Metabolic Pathway Engineering 37
Aaron M. Love, Bradley W. Biggs, Keith E. J. Tyo, and Parayil Kumaran Ajikumar

5 Bacterial Expression of Membrane-Associated Cytochrome P450s and Their Activity Assay in Nanodiscs 47
Demetrios Maroutsos, Hannah Huff, and Aditi Das

6 Delta Integration CRISPR-Cas (Di-CRISPR) in *Saccharomyces cerevisiae* 73
Shuobo Shi, Youyun Liang, Ee Lui Ang, and Huimin Zhao

7 Assembly of Complex Pathways Using Type IIs Restriction Enzymes 93
Sylvestre Marillonnet and Stefan Werner

8 Combinatorial Assembly of Multigene Pathways by Combining Single-Strand Assembly with Golden Gate Assembly 111
D. Bauwens, P. Coussement, J. Maertens, and Marjan De Mey

9 Checks and Balances with Use of the Keio Collection for Phenotype Testing 125
Sandra J. Aedo, Helena R. Ma, and Mark P. Brynildsen

10 Genetic Engineering of Cyanobacteria: Design, Implementation, and Characterization of Recombinant *Synechocystis* sp. PCC 6803 139
Jacob Sebesta, Allison Werner, and Christie Ann Marie Peebles

11 Genetic Tools for Streamlined and Accelerated Pathway Engineering in *Yarrowia lipolytica* 155
Lynn Wong, Benjamin Holdridge, Jake Engel, and Peng Xu

12 Gas Chromatography–Mass Spectrometry Microbial Metabolomics for Applications in Strain Optimization 179
McKenzie L. Smith, April M. Miguez, and Mark P. Styczynski

13 Targeted Mass Spectrometry-Based Proteomics Tools for Strain Optimization 191
Hsien-Chung Tseng and Christine Nicole S. Santos

14 A Protocol for the Construction and Curation of Genome-Scale Integrated Metabolic and Regulatory Network Models 203
Sriram Chandrasekaran

15 ^{13}C-Fingerprinting and Metabolic Flux Analysis of Bacterial Metabolisms 215
Whitney Hollinshead, Lian He, and Yinjie J. Tang

16 A Genetic Screen for the Isolation of Mutants with Increased Flux in the Isoprenoid Pathway of Yeast 231
Manisha Wadhwa and Anand K. Bachhawat

Index *247*