Contents

About the Series Editors  xv

Part I  DNA Synthesis and Genome Engineering  1

1  Competition and the Future of Reading and Writing DNA  3
   Robert Carlson
   1.1  Productivity Improvements in Biological Technologies  3
   1.2  The Origin of Moore’s Law and Its Implications for Biological Technologies  5
   1.3  Lessons from Other Technologies  6
   1.4  Pricing Improvements in Biological Technologies  7
   1.5  Prospects for New Assembly Technologies  8
   1.6  Beyond Programming Genetic Instruction Sets  10
   1.7  Future Prospects  10
   References  11

2  Trackable Multiplex Recombineering (TRMR) and Next-Generation Genome Design Technologies: Modifying Gene Expression in E. coli by Inserting Synthetic DNA Cassettes and Molecular Barcodes  15
   Emily F. Freed, Gur Pines, Carrie A. Eckert, and Ryan T. Gill
   2.1  Introduction  15
   2.2  Current Recombineering Techniques  16
   2.2.1  Recombineering Systems  17
   2.2.2  Current Model of Recombination  17
   2.3  Trackable Multiplex Recombineering  19
   2.3.1  TRMR and T²RMR Library Design and Construction  19
   2.3.2  Experimental Procedure  23
   2.3.3  Analysis of Results  24
   2.4  Current Challenges  25
   2.4.1  TRMR and T²RMR are Currently Not Recursive  26
   2.4.2  Need for More Predictable Models  26
   2.5  Complementing Technologies  27
   2.5.1  MAGE  27
   2.5.2  CREATE  27
2.6 Conclusions 28
Definitions 28
References 29

3 Site-Directed Genome Modification with Engineered Zinc Finger Proteins 33
Lauren E. Woodard, Daniel L. Galvan, and Matthew H. Wilson
3.1 Introduction to Zinc Finger DNA-Binding Domains and Cellular Repair Mechanisms 33
3.1.1 Zinc Finger Proteins 33
3.1.2 Homologous Recombination 34
3.1.3 Non-homologous End Joining 35
3.2 Approaches for Engineering or Acquiring Zinc Finger Proteins 36
3.2.1 Modular Assembly 37
3.2.2 OPEN and CoDA Selection Systems 37
3.2.3 Purchase via Commercial Avenues 38
3.3 Genome Modification with Zinc Finger Nucleases 38
3.4 Validating Zinc Finger Nuclease-Induced Genome Alteration and Specificity 40
3.5 Methods for Delivering Engineered Zinc Finger Nucleases into Cells 41
3.6 Zinc Finger Fusions to Transposases and Recombinases 41
3.7 Conclusions 42
References 43

4 Rational Efforts to Streamline the Escherichia coli Genome 49
Gabriella Balikó, Viktor Vernyik, Ildikó Karcagi, Zsuzsanna Györfy, Gábor Draskovits, Tamás Fehér, and György Pósfai
4.1 Introduction 49
4.2 The Concept of a Streamlined Chassis 50
4.3 The E. coli Genome 51
4.4 Random versus Targeted Streamlining 54
4.5 Selecting Deletion Targets 55
4.5.1 General Considerations 55
4.5.1.1 Naturally Evolved Minimal Genomes 55
4.5.1.2 Gene Essentiality Studies 55
4.5.1.3 Comparative Genomics 56
4.5.1.4 In silico Models 56
4.5.1.5 Architectural Studies 56
4.5.2 Primary Deletion Targets 57
4.5.2.1 Prophages 57
4.5.2.2 Insertion Sequences (ISs) 57
4.5.2.3 Defense Systems 57
Contents

4.5.2.4 Genes of Unknown and Exotic Functions 58
4.5.2.5 Repeat Sequences 58
4.5.2.6 Virulence Factors and Surface Structures 58
4.5.2.7 Genetic Diversity-Generating Factors 59
4.5.2.8 Redundant and Overlapping Functions 59
4.6 Targeted Deletion Techniques 59
4.6.1 General Considerations 59
4.6.2 Basic Methods and Strategies 60
4.6.2.1 Circular DNA-Based Method 60
4.6.2.2 Linear DNA-Based Method 62
4.6.2.3 Strategy for Piling Deletions 62
4.6.2.4 New Variations on Deletion Construction 63
4.7 Genome-Reducing Efforts and the Impact of Streamlining 64
4.7.1 Comparative Genomics-Based Genome Stabilization and Improvement 64
4.7.2 Genome Reduction Based on Gene Essentiality 66
4.7.3 Complex Streamlining Efforts Based on Growth Properties 67
4.7.4 Additional Genome Reduction Studies 68
4.8 Selected Research Applications of Streamlined-Genome E. coli 68
4.8.1 Testing Genome Streamlining Hypotheses 68
4.8.2 Mobile Genetic Elements, Mutations, and Evolution 69
4.8.3 Gene Function and Network Regulation 69
4.8.4 Codon Reassignment 70
4.8.5 Genome Architecture 70
4.9 Concluding Remarks, Challenges, and Future Directions 71
References 73

5 Functional Requirements in the Program and the Cell Chassis for Next-Generation Synthetic Biology 81
Antoine Danchin, Agnieszka Sekowska, and Stanislas Noria
5.1 A Prerequisite to Synthetic Biology: An Engineering Definition of What Life Is 81
5.2 Functional Analysis: Master Function and Helper Functions 83
5.3 A Life-Specific Master Function: Building Up a Progeny 85
5.4 Helper Functions 86
5.4.1 Matter: Building Blocks and Structures (with Emphasis on DNA) 87
5.4.2 Energy 91
5.4.3 Managing Space 92
5.4.4 Time 95
5.4.5 Information 96
5.5 Conclusion 97
Acknowledgments 98
References 98
Part II  Parts and Devices Supporting Control of Protein Expression and Activity  107

6  Constitutive and Regulated Promoters in Yeast: How to Design and Make Use of Promoters in *S. cerevisiae*  109
*Diana S. M. Ottoz and Fabian Rudolf*

6.1 Introduction  109
6.2 Yeast Promoters  110
6.3 Natural Yeast Promoters  113
6.3.1 Regulated Promoters  113
6.3.2 Constitutive Promoters  115
6.4 Synthetic Yeast Promoters  116
6.4.1 Modified Natural Promoters  116
6.4.2 Synthetic Hybrid Promoters  117
6.5 Conclusions  121
Definitions  122
References  122

7  Splicing and Alternative Splicing Impact on Gene Design  131
*Beatrix Suess, Katrin Kemmerer, and Julia E. Weigand*

7.1 The Discovery of “Split Genes”  131
7.2 Nuclear Pre-mRNA Splicing in Mammals  132
7.2.1 Introns and Exons: A Definition  132
7.2.2 The Catalytic Mechanism of Splicing  132
7.2.3 A Complex Machinery to Remove Nuclear Introns: The Spliceosome  132
7.2.4 Exon Definition  134
7.3 Splicing in Yeast  135
7.3.1 Organization and Distribution of Yeast Introns  135
7.4 Splicing without the Spliceosome  136
7.4.1 Group I and Group II Self-Splicing Introns  136
7.4.2 tRNA Splicing  137
7.5 Alternative Splicing in Mammals  137
7.5.1 Different Mechanisms of Alternative Splicing  137
7.5.2 Auxiliary Regulatory Elements  139
7.5.3 Mechanisms of Splicing Regulation  140
7.5.4 Transcription-Coupled Alternative Splicing  142
7.5.5 Alternative Splicing and Nonsense-Mediated Decay  143
7.5.6 Alternative Splicing and Disease  144
7.6 Controlled Splicing in *S. cerevisiae*  145
7.6.1 Alternative Splicing  145
7.6.2 Regulated Splicing  146
7.6.3 Function of Splicing in *S. cerevisiae*  147
7.7 Splicing Regulation by Riboswitches  147
7.7.1 Regulation of Group I Intron Splicing in Bacteria  148
7.7.2 Regulation of Alternative Splicing by Riboswitches in Eukaryotes  148
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.8</td>
<td>Splicing and Synthetic Biology</td>
<td>150</td>
</tr>
<tr>
<td>7.8.1</td>
<td>Impact of Introns on Gene Expression</td>
<td>150</td>
</tr>
<tr>
<td>7.8.2</td>
<td>Control of Splicing by Engineered RNA-Based Devices</td>
<td>151</td>
</tr>
<tr>
<td>7.9</td>
<td>Conclusion</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Acknowledgments</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Definitions</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>153</td>
</tr>
<tr>
<td>8</td>
<td>Design of Ligand-Controlled Genetic Switches Based on RNA Interference</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td><em>Shunichi Kashida and Hirohide Saito</em></td>
<td></td>
</tr>
<tr>
<td>8.1</td>
<td>Utility of the RNAi Pathway for Application in Mammalian Cells</td>
<td>169</td>
</tr>
<tr>
<td>8.2</td>
<td>Development of RNAi Switches that Respond to Trigger Molecules</td>
<td>170</td>
</tr>
<tr>
<td>8.2.1</td>
<td>Small Molecule-Triggered RNAi Switches</td>
<td>171</td>
</tr>
<tr>
<td>8.2.2</td>
<td>Oligonucleotide-Triggered RNAi Switches</td>
<td>173</td>
</tr>
<tr>
<td>8.2.3</td>
<td>Protein-Triggered RNAi Switches</td>
<td>174</td>
</tr>
<tr>
<td>8.3</td>
<td>Rational Design of Functional RNAi Switches</td>
<td>174</td>
</tr>
<tr>
<td>8.4</td>
<td>Application of the RNAi Switches</td>
<td>175</td>
</tr>
<tr>
<td>8.5</td>
<td>Future Perspectives</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>Definitions</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>178</td>
</tr>
<tr>
<td>9</td>
<td>Small Molecule-Responsive RNA Switches (Bacteria): Important Element of Programming Gene Expression in Response to Environmental Signals in Bacteria</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td><em>Yohei Yokobayashi</em></td>
<td></td>
</tr>
<tr>
<td>9.1</td>
<td>Introduction</td>
<td>181</td>
</tr>
<tr>
<td>9.2</td>
<td>Design Strategies</td>
<td>181</td>
</tr>
<tr>
<td>9.2.1</td>
<td>Aptamers</td>
<td>181</td>
</tr>
<tr>
<td>9.2.2</td>
<td>Screening and Genetic Selection</td>
<td>182</td>
</tr>
<tr>
<td>9.2.3</td>
<td>Rational Design</td>
<td>183</td>
</tr>
<tr>
<td>9.3</td>
<td>Mechanisms</td>
<td>183</td>
</tr>
<tr>
<td>9.3.1</td>
<td>Translational Regulation</td>
<td>183</td>
</tr>
<tr>
<td>9.3.2</td>
<td>Transcriptional Regulation</td>
<td>184</td>
</tr>
<tr>
<td>9.4</td>
<td>Complex Riboswitches</td>
<td>185</td>
</tr>
<tr>
<td>9.5</td>
<td>Conclusions</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>Keywords with Definitions</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>186</td>
</tr>
<tr>
<td>10</td>
<td>Programming Gene Expression by Engineering Transcript Stability</td>
<td>189</td>
</tr>
<tr>
<td></td>
<td><em>Jason T. Stevens and James M. Carothers</em></td>
<td></td>
</tr>
<tr>
<td>10.1</td>
<td>An Introduction to Transcript Control</td>
<td>189</td>
</tr>
<tr>
<td>10.1.1</td>
<td>Why Consider Transcript Control?</td>
<td>189</td>
</tr>
<tr>
<td>10.1.2</td>
<td>The RNA Degradation Process in <em>E. coli</em></td>
<td>190</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>10.1.3</td>
<td>The Effects of Translation on Transcript Stability</td>
<td>192</td>
</tr>
<tr>
<td>10.1.4</td>
<td>Structural and Noncoding RNA-Mediated Transcript Control</td>
<td>193</td>
</tr>
<tr>
<td>10.1.5</td>
<td>Polyadenylation and Transcript Stability</td>
<td>195</td>
</tr>
<tr>
<td>10.2</td>
<td>Synthetic Control of Transcript Stability</td>
<td>195</td>
</tr>
<tr>
<td>10.2.1</td>
<td>Transcript Stability Control as a “Tuning Knob”</td>
<td>195</td>
</tr>
<tr>
<td>10.2.2</td>
<td>Secondary Structure at the 5’ and 3’ Ends</td>
<td>196</td>
</tr>
<tr>
<td>10.2.3</td>
<td>Noncoding RNA-Mediated</td>
<td>197</td>
</tr>
<tr>
<td>10.2.4</td>
<td>Model-Driven Transcript Stability Control for Metabolic Pathway Engineering</td>
<td>198</td>
</tr>
<tr>
<td>10.3</td>
<td>Managing Transcript Stability</td>
<td>201</td>
</tr>
<tr>
<td>10.3.1</td>
<td>Transcript Stability as a Confounding Factor</td>
<td>201</td>
</tr>
<tr>
<td>10.3.2</td>
<td>Anticipating Transcript Stability Issues</td>
<td>201</td>
</tr>
<tr>
<td>10.3.3</td>
<td>Uniformity of 5’ and 3’ Ends</td>
<td>202</td>
</tr>
<tr>
<td>10.3.4</td>
<td>RBS Sequestration by Riboregulators and Riboswitches</td>
<td>203</td>
</tr>
<tr>
<td>10.3.5</td>
<td>Experimentally Probing Transcript Stability</td>
<td>204</td>
</tr>
<tr>
<td>10.4</td>
<td>Potential Mechanisms for Transcript Control</td>
<td>205</td>
</tr>
<tr>
<td>10.4.1</td>
<td>Leveraging New Tools</td>
<td>205</td>
</tr>
<tr>
<td>10.4.2</td>
<td>Unused Mechanisms Found in Nature</td>
<td>206</td>
</tr>
<tr>
<td>10.5</td>
<td>Conclusions and Discussion</td>
<td>207</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td></td>
<td>208</td>
</tr>
<tr>
<td>Definitions</td>
<td></td>
<td>208</td>
</tr>
<tr>
<td>References</td>
<td></td>
<td>209</td>
</tr>
</tbody>
</table>

11 Small Functional Peptides and Their Application in Superfunctionalizing Proteins 217

Sonja Billerbeck

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.1</td>
<td>Introduction</td>
<td>217</td>
</tr>
<tr>
<td>11.2</td>
<td>Permissive Sites and Their Identification in a Protein</td>
<td>218</td>
</tr>
<tr>
<td>11.3</td>
<td>Functional Peptides</td>
<td>220</td>
</tr>
<tr>
<td>11.3.1</td>
<td>Functional Peptides that Act as Binders</td>
<td>220</td>
</tr>
<tr>
<td>11.3.2</td>
<td>Peptide Motifs that are Recognized by Labeling Enzymes</td>
<td>221</td>
</tr>
<tr>
<td>11.3.3</td>
<td>Peptides as Protease Cleavage Sites</td>
<td>222</td>
</tr>
<tr>
<td>11.3.4</td>
<td>Reactive Peptides</td>
<td>223</td>
</tr>
<tr>
<td>11.3.5</td>
<td>Pharmaceutically Relevant Peptides: Peptide Epitopes, Sugar Epitope Mimics, and Antimicrobial Peptides</td>
<td>223</td>
</tr>
<tr>
<td>11.3.5.1</td>
<td>Peptide Epitopes</td>
<td>224</td>
</tr>
<tr>
<td>11.3.5.2</td>
<td>Peptide Mimotopes</td>
<td>224</td>
</tr>
<tr>
<td>11.3.5.3</td>
<td>Antimicrobial Peptides</td>
<td>225</td>
</tr>
<tr>
<td>11.4</td>
<td>Conclusions</td>
<td>227</td>
</tr>
<tr>
<td>Definitions</td>
<td></td>
<td>228</td>
</tr>
<tr>
<td>Abbreviations</td>
<td></td>
<td>229</td>
</tr>
<tr>
<td>Acknowledgment</td>
<td></td>
<td>229</td>
</tr>
<tr>
<td>References</td>
<td></td>
<td>229</td>
</tr>
</tbody>
</table>
Part III  Parts and Devices Supporting Spatial Engineering  237

12  Metabolic Channeling Using DNA as a Scaffold  239
    Mojca Benčina, Jerneja Mori, Rok Gaber, and Roman Jerala
12.1  Introduction  239
12.2  Biosynthetic Applications of DNA Scaffold  242
12.2.1  l-Threonine  242
12.2.2  trans-Resveratrol  245
12.2.3  1,2-Propanediol  246
12.2.4  Mevalonate  246
12.3  Design of DNA-Binding Proteins and Target Sites  247
12.3.1  Zinc Finger Domains  248
12.3.2  TAL-DNA Binding Domains  249
12.3.3  Other DNA-Binding Proteins  250
12.4  DNA Program  250
12.4.1  Spacers between DNA-Target Sites  250
12.4.2  Number of DNA Scaffold Repeats  252
12.4.3  DNA-Target Site Arrangement  253
12.5  Applications of DNA-Guided Programming  254
    Definitions  255
    References  256

13  Synthetic RNA Scaffolds for Spatial Engineering in Cells  261
    Gairik Sachdeva, Cameron Myhrvold, Peng Yin, and Pamela A. Silver
13.1  Introduction  261
13.2  Structural Roles of Natural RNA  261
13.2.1  RNA as a Natural Catalyst  262
13.2.2  RNA Scaffolds in Nature  263
13.3  Design Principles for RNA Are Well Understood  263
13.3.1  RNA Secondary Structure is Predictable  264
13.3.2  RNA can Self-Assemble into Structures  265
13.3.3  Dynamic RNAs can be Rationally Designed  265
13.3.4  RNA can be Selected in vitro to Enhance Its Function  266
13.4  Applications of Designed RNA Scaffolds  266
13.4.1  Tools for RNA Research  266
13.4.2  Localizing Metabolic Enzymes on RNA  267
13.4.3  Packaging Therapeutics on RNA Scaffolds  269
13.4.4  Recombinant RNA Technology  269
13.5  Conclusion  270
13.5.1  New Applications  270
13.5.2  Technological Advances  270
    Definitions  271
    References  271
14 Sequestered: Design and Construction of Synthetic Organelles
Thawatchai Chaijarasphong and David F. Savage

14.1 Introduction
14.2 On Organelles
14.3 Protein-Based Organelles
14.3.1 Bacterial Microcompartments
14.3.1.1 Targeting
14.3.1.2 Permeability
14.3.1.3 Chemical Environment
14.3.1.4 Biogenesis
14.3.2 Alternative Protein Organelles: A Minimal System
14.4 Lipid-Based Organelles
14.4.1 Repurposing Existing Organelles
14.4.1.1 The Mitochondrion
14.4.1.2 The Vacuole
14.5 De novo Organelle Construction and Future Directions

Acknowledgments
References

Part IV Early Applications of Synthetic Biology: Pathways, Therapies, and Cell-Free Synthesis

15 Cell-Free Protein Synthesis: An Emerging Technology for Understanding, Harnessing, and Expanding the Capabilities of Biological Systems
Jennifer A. Schoborg and Michael C. Jewett

15.1 Introduction
15.2 Background/Current Status
15.2.1 Platforms
15.2.1.1 Prokaryotic Platforms
15.2.1.2 Eukaryotic Platforms
15.2.2 Trends
15.3 Products
15.3.1 Noncanonical Amino Acids
15.3.2 Glycosylation
15.3.3 Antibodies
15.3.4 Membrane Proteins
15.4 High-Throughput Applications
15.4.1 Protein Production and Screening
15.4.2 Genetic Circuit Optimization
15.5 Future of the Field
Definitions
Acknowledgments
References
### 16 Applying Advanced DNA Assembly Methods to Generate Pathway Libraries

*Dawn T. Eriksen, Ran Chao, and Huimin Zhao*

16.1 Introduction 331
16.2 Advanced DNA Assembly Methods 333
16.3 Generation of Pathway Libraries 334
16.3.1 *In vitro* Assembly Methods 335
16.3.2 *In vivo* Assembly Methods 339
16.3.2.1 *In vivo* Chromosomal Integration 339
16.3.2.2 *In vivo* Plasmid Assembly and One-Step Optimization Libraries 340
16.3.2.3 *In vivo* Plasmid Assembly and Iterative Multi-step Optimization Libraries 341
16.4 Conclusions and Prospects 343
Definitions 343
References 344

### 17 Synthetic Biology in Immunotherapy

*Patrick Ho and Yvonne Y. Chen*

17.1 The Need for a New Therapeutic Paradigm 349
17.2 Rationale for Cellular Therapies 350
17.3 Synthetic Biology Approaches to Cellular Immunotherapy Engineering 351
17.3.1 CAR Engineering for Adoptive T-Cell Therapy 352
17.3.2 Genetic Engineering to Enhance T-Cell Therapeutic Function 357
17.3.3 Generating Safer T-Cell Therapeutics with Synthetic Biology 359
17.4 Challenges and Future Outlook 362
Acknowledgment 364
Definitions 364
References 365

### Part V Societal Ramifications of Synthetic Biology

### 18 Synthetic Biology: From Genetic Engineering 2.0 to Responsible Research and Innovation

*Lei Pei and Markus Schmidt*

18.1 Introduction 375
18.2 Public Perception of the Nascent Field of Synthetic Biology 376
18.2.1 Perception of Synthetic Biology in the United States 377
18.2.2 Perception of Synthetic Biology in Europe 379
18.2.2.1 European Union 379
18.2.2.2 Austria 379
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.2.2.3 Germany</td>
<td>381</td>
</tr>
<tr>
<td>18.2.2.4 Netherlands</td>
<td>382</td>
</tr>
<tr>
<td>18.2.2.5 United Kingdom</td>
<td>383</td>
</tr>
<tr>
<td>18.2.3 Opinions from Concerned Civil Society Groups</td>
<td>384</td>
</tr>
<tr>
<td>18.3 Frames and Comparators</td>
<td>384</td>
</tr>
<tr>
<td>18.3.1 Genetic Engineering: Technology as Conflict</td>
<td>386</td>
</tr>
<tr>
<td>18.3.2 Nanotechnology: Technology as Progress</td>
<td>387</td>
</tr>
<tr>
<td>18.3.3 Information Technology: Technology as Gadget</td>
<td>387</td>
</tr>
<tr>
<td>18.3.4 SB: Which Debate to Come?</td>
<td>388</td>
</tr>
<tr>
<td>18.4 Toward Responsible Research and Innovation (RRI) in Synthetic Biology</td>
<td>389</td>
</tr>
<tr>
<td>18.4.1 Engagement of All Societal Actors – Researchers, Industry, Policy Makers, and Civil Society – and Their Joint Participation in the Research and Innovation</td>
<td>390</td>
</tr>
<tr>
<td>18.4.2 Gender Equality</td>
<td>391</td>
</tr>
<tr>
<td>18.4.3 Science Education</td>
<td>392</td>
</tr>
<tr>
<td>18.4.4 Open Access</td>
<td>392</td>
</tr>
<tr>
<td>18.4.5 Ethics</td>
<td>394</td>
</tr>
<tr>
<td>18.4.6 Governance</td>
<td>395</td>
</tr>
<tr>
<td>18.5 Conclusion</td>
<td>396</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>397</td>
</tr>
<tr>
<td>References</td>
<td>397</td>
</tr>
<tr>
<td>Index</td>
<td>403</td>
</tr>
</tbody>
</table>