

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 T²RMRLibrary 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 T²RMRL 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
	<i>Lauren E. Woodard, Daniel L. Galvan, and Matthew H. Wilson</i>	
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 <i>Escherichia coli</i> Genome	49
	<i>Gabriella Balikó, Viktor Vernyik, Ildikó Karcagi, Zsuzsanna Györfy, Gábor Draskovits, Tamás Fehér, and György Pósfai</i>	
4.1	Introduction	49
4.2	The Concept of a Streamlined Chassis	50
4.3	The <i>E. coli</i> 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	<i>In silico</i> 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

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 <i>E. coli</i>	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 <i>S. cerevisiae</i>	109
	<i>Diana S. M. Ottoz and Fabian Rudolf</i>	
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
	<i>Beatrix Suess, Katrin Kemmerer, and Julia E. Weigand</i>	
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 <i>S. cerevisiae</i>	145
7.6.1	Alternative Splicing	145
7.6.2	Regulated Splicing	146
7.6.3	Function of Splicing in <i>S. cerevisiae</i>	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

7.8	Splicing and Synthetic Biology	150
7.8.1	Impact of Introns on Gene Expression	150
7.8.2	Control of Splicing by Engineered RNA-Based Devices	151
7.9	Conclusion	153
	Acknowledgments	153
	Definitions	153
	References	153
8	Design of Ligand-Controlled Genetic Switches Based on RNA Interference	169
	<i>Shunnichi Kashida and Hirohide Saito</i>	
8.1	Utility of the RNAi Pathway for Application in Mammalian Cells	169
8.2	Development of RNAi Switches that Respond to Trigger Molecules	170
8.2.1	Small Molecule-Triggered RNAi Switches	171
8.2.2	Oligonucleotide-Triggered RNAi Switches	173
8.2.3	Protein-Triggered RNAi Switches	174
8.3	Rational Design of Functional RNAi Switches	174
8.4	Application of the RNAi Switches	175
8.5	Future Perspectives	177
	Definitions	178
	References	178
9	Small Molecule-Responsive RNA Switches (Bacteria): Important Element of Programming Gene Expression in Response to Environmental Signals in Bacteria	181
	<i>Yohei Yokobayashi</i>	
9.1	Introduction	181
9.2	Design Strategies	181
9.2.1	Aptamers	181
9.2.2	Screening and Genetic Selection	182
9.2.3	Rational Design	183
9.3	Mechanisms	183
9.3.1	Translational Regulation	183
9.3.2	Transcriptional Regulation	184
9.4	Complex Riboswitches	185
9.5	Conclusions	185
	Keywords with Definitions	185
	References	186
10	Programming Gene Expression by Engineering Transcript Stability Control and Processing in Bacteria	189
	<i>Jason T. Stevens and James M. Carothers</i>	
10.1	An Introduction to Transcript Control	189
10.1.1	Why Consider Transcript Control?	189
10.1.2	The RNA Degradation Process in <i>E. coli</i>	190

10.1.3	The Effects of Translation on Transcript Stability	192
10.1.4	Structural and Noncoding RNA-Mediated Transcript Control	193
10.1.5	Polyadenylation and Transcript Stability	195
10.2	Synthetic Control of Transcript Stability	195
10.2.1	Transcript Stability Control as a “Tuning Knob”	195
10.2.2	Secondary Structure at the 5′ and 3′ Ends	196
10.2.3	Noncoding RNA-Mediated	197
10.2.4	Model-Driven Transcript Stability Control for Metabolic Pathway Engineering	198
10.3	Managing Transcript Stability	201
10.3.1	Transcript Stability as a Confounding Factor	201
10.3.2	Anticipating Transcript Stability Issues	201
10.3.3	Uniformity of 5′ and 3′ Ends	202
10.3.4	RBS Sequestration by Riboregulators and Riboswitches	203
10.3.5	Experimentally Probing Transcript Stability	204
10.4	Potential Mechanisms for Transcript Control	205
10.4.1	Leveraging New Tools	205
10.4.2	Unused Mechanisms Found in Nature	206
10.5	Conclusions and Discussion	207
	Acknowledgments	208
	Definitions	208
	References	209

11 **Small Functional Peptides and Their Application in Superfunctionalizing Proteins** 217

Sonja Billerbeck

11.1	Introduction	217
11.2	Permissive Sites and Their Identification in a Protein	218
11.3	Functional Peptides	220
11.3.1	Functional Peptides that Act as Binders	220
11.3.2	Peptide Motifs that are Recognized by Labeling Enzymes	221
11.3.3	Peptides as Protease Cleavage Sites	222
11.3.4	Reactive Peptides	223
11.3.5	Pharmaceutically Relevant Peptides: Peptide Epitopes, Sugar Epitope Mimics, and Antimicrobial Peptides	223
11.3.5.1	Peptide Epitopes	224
11.3.5.2	Peptide Mimotopes	224
11.3.5.3	Antimicrobial Peptides	225
11.4	Conclusions	227
	Definitions	228
	Abbreviations	228
	Acknowledgment	229
	References	229

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	279
	<i>Thawatchai Chaijarasphong and David F. Savage</i>	
14.1	Introduction	279
14.2	On Organelles	281
14.3	Protein-Based Organelles	283
14.3.1	Bacterial Microcompartments	283
14.3.1.1	Targeting	285
14.3.1.2	Permeability	287
14.3.1.3	Chemical Environment	288
14.3.1.4	Biogenesis	289
14.3.2	Alternative Protein Organelles: A Minimal System	290
14.4	Lipid-Based Organelles	292
14.4.1	Repurposing Existing Organelles	293
14.4.1.1	The Mitochondrion	293
14.4.1.2	The Vacuole	294
14.5	<i>De novo</i> Organelle Construction and Future Directions	295
	Acknowledgments	297
	References	297

Part IV Early Applications of Synthetic Biology: Pathways, Therapies, and Cell-Free Synthesis 307

15	Cell-Free Protein Synthesis: An Emerging Technology for Understanding, Harnessing, and Expanding the Capabilities of Biological Systems	309
	<i>Jennifer A. Schoborg and Michael C. Jewett</i>	
15.1	Introduction	309
15.2	Background/Current Status	311
15.2.1	Platforms	311
15.2.1.1	Prokaryotic Platforms	311
15.2.1.2	Eukaryotic Platforms	312
15.2.2	Trends	314
15.3	Products	316
15.3.1	Noncanonical Amino Acids	316
15.3.2	Glycosylation	316
15.3.3	Antibodies	318
15.3.4	Membrane Proteins	318
15.4	High-Throughput Applications	320
15.4.1	Protein Production and Screening	320
15.4.2	Genetic Circuit Optimization	321
15.5	Future of the Field	321
	Definitions	322
	Acknowledgments	322
	References	323

16	Applying Advanced DNA Assembly Methods to Generate Pathway Libraries	331
	<i>Dawn T. Eriksen, Ran Chao, and Huimin Zhao</i>	
16.1	Introduction	331
16.2	Advanced DNA Assembly Methods	333
16.3	Generation of Pathway Libraries	334
16.3.1	<i>In vitro</i> Assembly Methods	335
16.3.2	<i>In vivo</i> Assembly Methods	339
16.3.2.1	<i>In vivo</i> Chromosomal Integration	339
16.3.2.2	<i>In vivo</i> Plasmid Assembly and One-Step Optimization Libraries	340
16.3.2.3	<i>In vivo</i> Plasmid Assembly and Iterative Multi-step Optimization Libraries	341
16.4	Conclusions and Prospects	343
	Definitions	343
	References	344
17	Synthetic Biology in Immunotherapy and Stem Cell Therapy Engineering	349
	<i>Patrick Ho and Yvonne Y. Chen</i>	
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 373

18	Synthetic Biology: From Genetic Engineering 2.0 to Responsible Research and Innovation	375
	<i>Lei Pei and Markus Schmidt</i>	
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
18.2.2.3	Germany	381
18.2.2.4	Netherlands	382
18.2.2.5	United Kingdom	383
18.2.3	Opinions from Concerned Civil Society Groups	384
18.3	Frames and Comparators	384
18.3.1	Genetic Engineering: Technology as Conflict	386
18.3.2	Nanotechnology: Technology as Progress	387
18.3.3	Information Technology: Technology as Gadget	387
18.3.4	SB: Which Debate to Come?	388
18.4	Toward Responsible Research and Innovation (RRI) in Synthetic Biology	389
18.4.1	Engagement of All Societal Actors – Researchers, Industry, Policy Makers, and Civil Society – and Their Joint Participation in the Research and Innovation	390
18.4.2	Gender Equality	391
18.4.3	Science Education	392
18.4.4	Open Access	392
18.4.5	Ethics	394
18.4.6	Governance	395
18.5	Conclusion	396
	Acknowledgments	397
	References	397
	Index	403