



VOLUME SIX HUNDRED AND TWENTY ONE

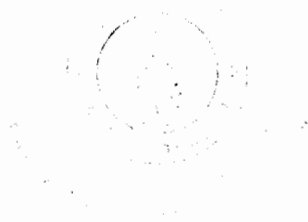
METHODS IN ENZYMOLGY

Chemical and Synthetic Biology
Approaches To Understand Cellular
Functions - Part A

Edited by

ARUN K. SHUKLA

*Associate Professor, Joy Gill Chair Professor
and EMBO Young Investigator,
Intermediate Fellow, Wellcome Trust DBT India Alliance,
Department of Biological Sciences and Bioengineering,
Indian Institute of Technology, Kanpur 208016, India*



ACADEMIC PRESS

An imprint of Elsevier

Contents

<i>Contributors</i>	<i>xi</i>
<i>Preface</i>	<i>xvii</i>
1. Targeted m⁶A reader proteins to study the epitranscriptome	1
Simone Rauch and Bryan C. Dickinson	
1. Introduction	2
2. Programmable RNA reader proteins to study the epitranscriptome	3
3. Design and applications of targeted RNA reader proteins	7
4. Summary and conclusions	13
Acknowledgments	14
References	14
2. Synthetic gene expression circuits regulating sexual reproduction	17
Nobuo Fukuda and Shinya Honda	
1. Introduction	18
2. Control of sexual reproduction	18
3. Experimental design and protocol	25
4. Conclusions	29
Acknowledgments	29
References	29
3. Nucleotide resolution sequencing of N4-acetylcytidine in RNA	31
Justin M. Thomas, Keri M. Bryson, and Jordan L. Meier	
1. Introduction	32
2. Technical aspects	37
3. Discussion	46
Acknowledgments	48
References	48
4. A guide for drug inducible genome editing with HIT systems	53
Chen Zhao, Shixian Wei, and Yu Wang	
1. Introduction	53
2. Drug inducible genome editing by transient transfection	57

3. Drug inducible genome editing by lentiviral transduction	62
4. Summary and conclusions	66
Acknowledgments	66
References	66
Further reading	67
5. A guide for drug inducible transcriptional activation with HIT systems	69
Chen Zhao, Shixian Wei, and Yu Wang	
1. Introduction	70
2. Drug inducible transcriptional activation	74
3. Simultaneous transcriptional activation and genome editing in a drug inducible manner	80
4. Summary and conclusions	83
Acknowledgments	84
References	84
6. Direct cloning and heterologous expression of natural product biosynthetic gene clusters by transformation-associated recombination	87
Jia Jia Zhang, Kazuya Yamanaka, Xiaoyu Tang, and Bradley S. Moore	
1. Introduction	88
2. Vector design	91
3. TAR cloning	93
4. Heterologous hosts	101
5. Conclusions	105
Acknowledgments	108
References	108
7. Salt-sensitive intein for large-scale polypeptide production	111
Yi-Zong Lee and Shih-Che Sue	
1. Introduction	112
2. Intein	113
3. Polypeptide preparation using salt-sensitive intein	120
4. Protocol	122
5. Extension of application	124
6. Discussion	127
7. Summary	128
Acknowledgments	129
References	129

8. Methods for the recombinant expression of active tyrosine kinase domains: Guidelines and pitfalls	131
M. Escarlet Díaz Galicia, Abdullah Aldehaiman, SeungBeom Hong, Stefan T. Arold, and Raik Grünberg	
1. Introduction	132
2. Expression of PTKs in eukaryotic systems	134
3. PTK expression from <i>E. coli</i>	135
4. <i>In-vitro</i> kinase activity assays	138
5. Practical guidelines and hidden pitfalls	141
6. Conclusion	146
Acknowledgments	147
References	147
9. Design, cloning and characterization of transcription factor-based inducible gene expression systems	153
Erik K.R. Hanko, Nigel P. Minton, and Naglis Malys	
1. Introduction	154
2. Generation of reporter constructs	156
3. Fluorescence reporter assays	161
4. Data analysis	163
5. Concluding remarks	167
Acknowledgments	168
References	168
10. Design, construction, and validation of optogenetic proteins	171
Colin P. O'Banion, Anwasha Goswami, and David S. Lawrence	
1. Introduction	172
2. Preparing low level, constitutively active POIs	174
3. Fusion of the POI to Cry2	176
4. Characterization and validation of light mediated translocation	179
5. Characterization and validation of light-mediated biological activity of optogenetic constructs	183
6. Summary	188
Acknowledgments	189
References	189
11. Overcoming component limitations in synthetic biology through transposon-mediated protein engineering	191
Joshua T. Atkinson, Bingyan Wu, Laura Segatori, and Jonathan J. Silberg	
1. Component limitations in synthetic biology	192

2. Overcoming component limitations with transposon mutagenesis	193
3. Overview of the library construction workflow	195
4. Choosing an artificial transposon	196
5. Library construction	200
6. Sampling considerations when screening versus selecting	205
7. Profiling protein tolerance to topological changes	206
8. Conclusions	209
Acknowledgments	209
References	209
12. Chemical biology of glycoproteins: From chemical synthesis to biological impact	213
Yaohao Li, Amy H. Tran, Samuel J. Danishefsky, and Zhongping Tan	
1. Introduction	214
2. Chemical synthesis of homogeneous glycoforms	218
3. Biological impact of protein glycosylation	224
4. Summary	226
Acknowledgments	227
References	227
13. <i>In bulla</i> functional channel expression systems that mimic bacterial synthetic membranes	231
Masayuki Iwamoto and Shigetoshi Oiki	
1. Introduction	232
2. Experimental tips for <i>in bulla</i> functional channel expression	236
3. Materials	237
4. Protocol	238
5. Nascent channel activity	239
6. Summary of additional experimental results	240
7. Summary	241
Acknowledgment	242
References	243
14. A mass spectrometry-based isotope-coded mass tag method to map thiol accessibility in biological systems	245
John E. Gadbery and Nicole S. Sampson	
1. Introduction	246
2. ICMT probe synthesis	252
3. ICMT labeling applications	252

4. Acquisition and analysis of MALDI-TOF mass spectra	257
5. Conclusions and future perspectives	259
References	259
15. Quick-soaking of crystals reveals unprecedented insights into the catalytic mechanism of glycosyltransferases	261
David Albesa-Jové, Javier O. Cifuentes, Beatriz Trastoy, and Marcelo E. Guerin	
1. Introduction	262
2. Reaction mechanisms of inverting and retaining glycosyltransferases	262
3. GpgS, a retaining glycosyltransferase lacking a putative catalytic nucleophile	265
4. α 3GalT, a retaining glycosyltransferase containing a putative catalytic nucleophile	266
5. Quick-soaking protocol	267
6. A model for the reaction mechanism of retaining glycosyltransferases	271
7. Summary	275
Acknowledgments	275
Competing financial interest	276
References	276
16. Cysteine-ethylation of tissue-extracted membrane proteins as a tool to detect conformational states by solid-state NMR spectroscopy	281
Daniel K. Weber, Taysir Bader, Erik K. Larsen, Songlin Wang, Tata Gopinath, Mark Distefano, and Gianluigi Veglia	
1. Introduction	282
2. Protocols for using ^{13}C -EMTS	283
3. Solid-state NMR spectroscopy	291
4. Conclusions and perspectives	297
Acknowledgment	299
References	299
17. Improved sensitivity and resolution of in-cell NMR spectra	305
David S. Burz, Leonard Breindel, and Alexander Shekhtman	
1. Introduction	306
2. Materials	311
3. Methods	312

4. Results	322
5. Summary	324
Acknowledgment	325
References	325
18. High-throughput methods in aptamer discovery and analysis	329
Kyle H. Cole and Andrej Lupták	
1. Introduction	330
2. Post-selection sequence analysis	331
3. High-throughput characterization methods	338
4. Conclusions	342
References	342
19. Drop-in-well chamber for droplet interface bilayer with built-in electrodes	347
Kazuhiro Urakubo, Masayuki Iwamoto, and Shigetoshi Oiki	
1. Introduction	348
2. DIB formation in the drop-in-wells	349
3. Electrical measurements	352
4. Thin-walled sheet for recordings with low background noise	354
5. Materials	355
6. Protocol	357
7. Single-channel current recordings	360
8. Summary	360
Acknowledgment	361
References	361