Contents

Preface .......................................................... v
Contributors ....................................................... xi

SECTION I  GENE AND GENOME-EDITING METHODS PART I

1 Design and Validation of CRISPR/Cas9 Systems for Targeted Gene Modification in Induced Pluripotent Stem Cells .................... 3
   Ciaran M. Lee, Haibao Zhu, Timothy H. Davis, Harshahardhan Deshmukh, and Gang Bao

2 Mutagenesis and Genome Engineering of Epstein–Barr Virus in Cultured Human Cells by CRISPR/Cas9 .......................... 23
   Kit-San Yuen, Chi-Ping Chan, Kin-Hang Kok, and Dong-Yan Jin

3 Use of CRISPR/Cas Genome Editing Technology for Targeted Mutagenesis in Rice .................................. 33
   Rongfang Xu, Pengcheng Wei, and Jianbo Tang

4 All-in-One CRISPR-Cas9/FokI-dCas9 Vector-Mediated Multiplex Genome Engineering in Cultured Cells ...................... 41
   Tetsushi Sakuma, Takuya Sakamoto, and Takashi Yamamoto

5 CRISPR/Cas9-Mediated Mutagenesis of Human Pluripotent Stem Cells in Defined Xeno-Free E8 Medium ......................... 57
   Chew-Li Soh and Danwei Huangfu

6 Development of CRISPR/Cas9 for Efficient Genome Editing in Toxoplasma gondii ............................................ 79
   Bang Shen, Kevin Brown, Shaojun Long, and L. David Sibley

SECTION II  GENE AND GENOME-EDITING METHODS PART II

7 Generation of Stable Knockout Mammalian Cells by TALEN-Mediated Locus-Specific Gene Editing ............................... 107
   Barun Mahata and Kaushik Biswas

8 Efficient Generation of Gene-Modified Mice by Haploid Embryonic Stem Cell-Mediated Semi-cloned Technology ................ 121
   Cuiqing Zhong and Jinsong Li

9 Insertion of Group II Intron-Based Ribozyme Switches into Homing Endonuclease Genes ........................................... 135
   Tuhin Kumar Guha and Georg Hausner

10 Generating a Genome Editing Nuclease for Targeted Mutagenesis in Human Cells ............................................. 153
    Zhenyu He and Kehkooi Kee
11 Use of Group II Intron Technology for Targeted Mutagenesis
in Chlamydia trachomatis ........................................  163
Charlotte E. Key and Derek J. Fisher

SECTION III  BIOINFORMATICS APPROACHES FOR IDENTIFYING
AND ANALYZING MUTAGENESIS TARGETS

12 In Silico Approaches to Identify Mutagenesis Targets to Probe
and Alter Protein–Cofactor and Protein–Protein Functional Relationships . . .  181
Brian A. Dow, Esha Sehanobish, and Victor L. Davidson

13 In Silico Prediction of Deleteriousness for Nonsynonymous
and Splice-Altering Single Nucleotide Variants in the Human Genome ......  191
Xueqiu Jian and Xiaoming Liu

14 In Silico Methods for Analyzing Mutagenesis Targets .................  199
Troy C. Messina

15 Methods for Detecting Critical Residues in Proteins ...............  227
Nurit Haspel and Filip Jagodzinski

16 A Method for Bioinformatic Analysis of Transposon Insertion
Sequencing (INSeq) Results for Identification of Microbial
Fitness Determinants. .....................................................  243
Nengding Wang and Egon A. Ozer

SECTION IV  IN VITRO TRANSPORTON MUTAGENESIS METHODS
IN DIVERSE PROKARYOTIC SYSTEMS

17 Application of In Vitro Transposon Mutagenesis to Erythromycin
Strain Improvement in Saccharopolyspora erythraea .................  257
J. Mark Weber, Andrew Reeves, William H. Cernota, and Roy K. Wesley

18 Engineering Gram-Negative Microbial Cell Factories
Using Transposon Vectors ...........................................  273
Esteban Martínez-García, Tomás Aparicio, Victor de Lorenzo,
and Pablo I. Nikel

19 PERMutation Using Transposase Engineering (PERMUTE):
A Simple Approach for Constructing Circularly Permuted Protein Libraries . . .  295
Alicia M. Jones, Joshua T. Atkinson, and Jonathan J. Silberg

20 Transposon Insertion Mutagenesis for Archaeal Gene Discovery ...........  309
Saija Kiljunen, Maria I. Pajunen, and Harri Savilahti

21 Genome-Wide Transposon Mutagenesis in Mycobacterium tuberculosis
and Mycobacterium smegmatis ........................................  321
Gaurav Majumdar, Rendani Mbau, Vinayak Singh, Digby F. Warner,
Marte Singsås Dragset, and Raju Mukherjee

SECTION V  SITE-DIRECTED MUTAGENESIS: PCR
AND DNA POLYMERASE-BASED METHODS

22 Multiple Site-Directed and Saturation Mutagenesis
by the Patch Cloning Method ........................................  339
Naohiro Taniguchi and Hiroshi Murakami
<table>
<thead>
<tr>
<th>Section VI</th>
<th>In Vitro Mutagenesis for Studies of Protein Structure and Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>Step-By-Step In Vitro Mutagenesis: Lessons From Fucose-Binding Lectin PA-IIL</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Jana Mrázková, Lenka Malinovská, and Michaela Wimmerová**

| 29         | Analytical Methods for Assessing the Effects of Site-Directed Mutagenesis on Protein–Cofactor and Protein–Protein Functional Relationships | 421 |
|            | 

**Esha Sehanobish, Brian A. Dow, and Victor L. Davidson**

| 30         | Biochemical and Biophysical Methods to Examine the Effects of Site-Directed Mutagenesis on Enzymatic Activities and Interprotein Interactions | 439 |
|            | 

**Misaki Kinoshita, Ju Yae Kim, Yuxi Lin, Natalia Markova, Toshiharu Hase, and Young-Ho Lee**

| 31         | Use of Random and Site-Directed Mutagenesis to Probe Protein Structure–Function Relationships: Applied Techniques in the Study of Helicobacter pylori | 461 |
|            | 

**Jeannette M. Whitmire and D. Scott Merrell**

<table>
<thead>
<tr>
<th>Section VII</th>
<th>Random Mutagenesis: Novel PCR-Based Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>Novel Random Mutagenesis Method for Directed Evolution</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Hong Feng, Hai-Tan Wang, and Hong-Yan Zhao**

| 33          | Random Mutagenesis by Error-Prone Polymerase Chain Reaction Using a Heavy Water Solvent | 491 |
|             | 

**Toshifumi Minamoto**
In Vitro Mutagenesis
Methods and Protocols
Reeves, A. (Ed.)
2017, XV, 511 p. 96 illus., 52 illus. in color., Hardcover
ISBN: 978-1-4939-6470-3
A product of Humana Press