## Contents

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

### Part I Mutagenesis in Various Microbial Backgrounds

1. Mutagenesis Protocols in *Saccharomyces cerevisiae* by In Vivo Overlap Extension .......................... 3
   - Miguel Alcalde

2. In Vitro Mutagenesis of *Brucella* Species ................................................. 15
   - Thomas A. Ficht, Jianwu Pei, and Melissa Kahl-McDonagh

3. Random Mutagenesis Strategies for *Campylobacter* and *Helicobacter* Species .......................... 37
   - Duncan J.H. Gaskin and Arnoud H.M. van Vliet

4. Mutagenesis of the Repeat Regions of Herpesviruses Cloned as Bacterial Artificial Chromosomes .......... 53
   - Yuguang Zhao and Venugopal Nair

5. An Efficient Protocol for VZV BAC-Based Mutagenesis ......................................................... 75
   - Zhen Zhang, Ying Huang, and Hua Zhu

6. A Method for Rapid Genetic Integration into *Plasmodium falciparum* Utilizing Mycobacteriophage Bxb1 Integrase .............................................. 87
   - Sophie H. Ad jalley, Marcus C.S. Lee, and David A. Fidock

### Part II PCR Mutagenesis

7. Random Mutagenesis by Error-Prone PCR .................................................................................. 103
   - Elizabeth O. McCullum, Berea A.R. Williams, Jinglei Zhang, and John C. Chaput

8. A Rapid and Versatile PCR-Based Site-Directed Mutagenesis Protocol for Generation of Mutations Along the Entire Length of a Cloned cDNA .......................... 111
   - Vincent Dammai

9. Rapid Sequence Scanning Mutagenesis Using *In Silico* Oligo Design and the Megaprimer PCR of Whole Plasmid Method (MegaWHOP) ............................................. 127
   - Ulrich Krauss, Karl-Erich Jaeger, and Thorsten Eggert

10. Insertion and Deletion Mutagenesis by Overlap Extension PCR .................................................. 137
    - Jehan Lee, Myeong-Kyun Shin, Dong-Kyun Ryu, Seahk Kim, and Wang-Shick Ryu
Contents

11 Targeted Amplification of Mutant Strands for Efficient Site-Directed Mutagenesis and Mutant Screening ........................................ 147
   Lei Young and Qihan Dong

12 A Modified Inverse PCR Procedure for Insertion, Deletion, or Replacement of a DNA Fragment in a Target Sequence and Its Application in the Ligand Interaction Scan Method for Generation of Ligand-Regulated Proteins ........................................ 157
   Oran Erster and Moti Liscovitch

13 Amplification of Orthologous Genes Using Degenerate Primers ........................................ 175
   Samya Chakravorty and Jim O. Vigoreaux

Part III Reviews

14 Computational Evaluation of Protein Stability Change upon Mutations ........................................ 189
   Shuangye Yin, Feng Ding, and Nikolay V. Dokholyan

15 Approaches for Using Animal Models to Identify Loci That Genetically Interact with Human Disease-Causing Point Mutations ........................................ 203
   Josef D. Franke

Part IV Protein Evolution Mutagenesis

16 Using Peptide Loop Insertion Mutagenesis for the Evolution of Proteins ........................................ 217
   Christian Heinis and Kai Johnsson

17 Massive Mutagenesis®: High-Throughput Combinatorial Site-Directed Mutagenesis ........................................ 233
   Julien Sylvestre

18 Directed In Vitro Evolution of Reporter Genes Based on Semi-Rational Design and High-Throughput Screening ........................................ 239
   Ai-Sheng Xiong, Quan-Hong Yao, Ri-He Peng, and Zong-Ming Cheng

19 Ribosome Display for Rapid Protein Evolution by Consecutive Rounds of Mutation and Selection ........................................ 257
   Hayato Yanagida, Tomoaki Matsuura, and Tetsuya Yomo

Part V Protein Structure and Function Mutagenesis

20 Fine-Tuning Enzyme Activity Through Saturation Mutagenesis ........................................ 271
   Holly H. Hogrefe

21 Characterization of Structural Determinants of Type 1 Corticotropin Releasing Hormone (CRH) Receptor Signalling Properties ........................................ 285
   Danijela Markovic and Dimitris K. Grammatopoulos

22 Site-Directed Mutagenesis for Improving Biophysical Properties of V<sub>H</sub> Domains ........................................ 309
   Mehdi Arbabi-Ghabroud, Roger MacKenzie, and Jamshid Tanha

23 Phenotype Based Functional Gene Screening Using Retrovirus-Mediated Gene Trapping in Quasi-Haploid RAW 264.7 Cells ........................................ 331
   Sung Ouk Kim and Soon-Duck Ha
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Site-Directed Disulfide Cross-Linking to Probe Conformational Changes of a Transporter During Its Functional Cycle: <em>Escherichia coli</em> AcrB Multidrug Exporter as an Example</td>
<td>Yumiko Takatsuka and Hiroshi Nikaido</td>
<td>Part VI Random Mutagenesis</td>
<td>343</td>
</tr>
<tr>
<td>25</td>
<td>Site-Specific Incorporation of Extra Components into RNA by Transcription Using Unnatural Base Pair Systems</td>
<td>Michiko Kimoto and Ichiro Hirao</td>
<td></td>
<td>355</td>
</tr>
<tr>
<td></td>
<td><strong>PART VI</strong> <strong>RANDOM MUTAGENESIS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Mutagen™: A Random Mutagenesis Method Providing a Complementary Diversity Generated by Human Error-Prone DNA Polymerases</td>
<td>Philippe Mondon, David Grand, Nathalie Souyris, Stéphane Emond, Khalil Bouayadi, and Hakim Kharrat</td>
<td></td>
<td>373</td>
</tr>
<tr>
<td>27</td>
<td>Random-Scanning Mutagenesis</td>
<td>Robert A. Smith</td>
<td></td>
<td>387</td>
</tr>
<tr>
<td>28</td>
<td>Easy Two-Step Method for Randomizing and Cloning Gene Fragments</td>
<td>Vivian Q. Zhang and Holly H. Hogrefe</td>
<td></td>
<td>399</td>
</tr>
<tr>
<td></td>
<td><strong>PART VII</strong> <strong>MUTATOR BACTERIAL STRAIN MUTAGENESIS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Random Mutagenesis Using a Mutator Strain</td>
<td>Ghazala Muteeb and Ranjan Sen</td>
<td></td>
<td>411</td>
</tr>
<tr>
<td>30</td>
<td><em>En Passant</em> Mutagenesis: A Two Step Markerless Red Recombination System</td>
<td>B. Karsten Tischer, Gregory A. Smith, and Nikolaus Osterrieder</td>
<td></td>
<td>421</td>
</tr>
<tr>
<td></td>
<td><strong>Index</strong></td>
<td></td>
<td></td>
<td>431</td>
</tr>
</tbody>
</table>