CONTENTS

VOLUME III

Methods in Gene Engineering **1**
Chanan Angsuthanasombat, *Mahidol University, Thailand*

1. Introduction
2. Gene Engineering with Polymerase Chain Reaction
   2.1 Principles and Limitations of PCR
   2.2 Applications of PCR
      2.2.1 Gene Fusion via Splicing by Overlap Extension
      2.2.2 *In Vitro* Mutagenesis via Amplification of Entire Plasmid
      2.2.3 Dideoxy-Based Directed DNA Sequencing
3. Gene Expression Strategies
   3.1 High-Level of Protein Expression in *Escherichia coli*
   3.2 Detection of Expressed Gene Products
   3.3 Expression of Cloned Genes as Fusion Proteins

Genetic Manipulation of Bacteria **16**
Christopher Morton Thomas, *University of Birmingham, UK*

1. Introduction
2. Genetic manipulation in bacteria
   2.1 Vectors
      2.1.1 Plasmid vectors
      2.1.2 Phage vectors
   2.2 *In vitro* recombination
3. Introduction of DNA into bacteria
4. Library screening
5. Polymerase chain reaction (PCR)
6. Expression and protein purification
7. Site directed mutagenesis
8. Genetic manipulation of bacteria
   8.1 Cloning specific genes
   8.2 DNA shuffling
9. Examples of genetic manipulation
   9.1 Producing antibodies in bacteria
   9.2 Manipulation of bacterial metabolism
   9.3 Manipulation of biodegradative capacity
   9.4 Bacteria as biosensors
   9.5 Manipulation of antibiotic biosynthesis
10. Biosafety

Genetic Engineering of Fungal Cells **36**
Margo M. Moore,*Department of Biological Sciences, Simon Fraser University, Burnaby, Canada*

1. Introduction
   1.1. Industrial importance of fungi
   1.2. Purpose and range of topics covered
2. Generation of transforming constructs
   2.1. Autonomously-replicating plasmids
   2.2. Promoters
      2.2.1. Constitutive promoters

2.2.2. Inducible promoters
2.3. Selectable markers
2.3.1. Dominant selectable markers
2.3.2. Auxotrophic markers
2.4. Fusion PCR and Ligation PCR
2.5. Gateway technology
3. Transformation methods
3.1. Natural competence and protoplast formation
3.2. $CaCl_2$ and PEG
3.3. Electroporation
3.4. *Agrobacterium*-mediated Ti plasmid
3.5. Biolistics
3.6. Homo- versus heterokaryotic selection
4. Gene disruption and gene replacement
4.1. Targeted gene disruption
4.1.1. Ectopic versus homologous recombination
4.1.2. NHEJ-deficient strains
4.1.3. AMT and homologous recombination
4.1.4. RNA interference
4.2. Random gene disruption
4.2.1. Restriction enzyme-mediated integration (REMI)
4.2.1.1.1. 4.2.2. T-DNA tagging using AMT
4.2.2. Transposon mutagenesis & TAGKO
5. Concluding statement

Genetic Engineering of Algal Species **67**
Anna Godhe, *University of Göteborg, Sweden*
Ann-Sofi Rehnstam-Holm, *G'teborg University, Sweden*

1. Introduction
1.1 What are Algae?
1.2 What is Genetic Engineering?
1.3 The Importance of Algae
2. Classification of Algae
3. Principles of Microalga Culture
4. Gene Technology
4.1 Polymerase Chain Reaction
4.2 Cloning
4.3 Hybridization
5. Genetical Identification and Phylogeny
5.1 Origin of Chloroplasts
5.2 Use of Conserved Genes
5.3 Molecular Identification of Algae
5.4 Molecular Identification of Algal Populations
6. Genetic Engineering as a Tool to understand the Physiology, Biochemistry and Molecular Biology of Algae
6.1 Model Organisms
6.2 Genetic Studies of Photosynthesis
6.3 Genetic Studies of Photoprotection
6.4 Genetic Studies on the Function of Flagellae
6.5 Genetic Studies on Transport of Proteins into Plastids
6.6 Markers used for Growth Studies
6.7 Processes Regulated by the Circadian Clock
7. Genetic Engineering of Algae: Examples of Environmental and Industrial Applications
7.1 Cyanophyceae as N-fertilizers and Bioremediators
7.2 Commercially Attractive Compounds from Algae
7.3 Cultivation of Marine Macro Algae

Genetic Engineering of Plants **94**
Jennifer Ann Thomson, *University of Cape Town, South Africa*

1. Introduction
2. Transformation of dicotyledonous plants
   2.1 Transformation using *Agrobacterium tumefaciens*
   2.2 Other transformation methods
3. Transformation of monocotyledonous plants
   3.1 Biolistic transformation
   3.2 *Agrobacterium* transformation
4. Transformation of algae
5. Promoter efficiency and tissue specificity
6. Targeting genes to organelles
7. Integration and stability of transgenes

Genetic Engineering of Mammalian Cells **110**
Hugo H. Montaldo, *Universidad Nacional Autonoma de Mexico (UNAM), México*

1. Introduction
2. Expression and Regulation of Eukaryotic Genes
3. Recombinant DNA Technology
   3.1 Restriction Enzymes
   3.2 DNA Vectors
   3.3 DNA Libraries
4. Genetic Maps\
   4.1 Genetic Linkage Maps
   4.2 Physical Maps
      4.2.1 Chromosomal Map
      4.2.2 cDNA Map
   4.3 Sequencing Technologies and the Human Genome Project
   4.4 Gene Function and Expression in Mammals
5. Use of Genomic Information in Animal Improvement
6. Cloning Adult Mammals
   6.1 Cloning Methods
   6.2 Problems
   6.3 Use of Cloning in Animal Breeding
7. Transgenic Animals
   7.1 Transgenic Methods
   7.2 Transgenesis in the Improvement of Production Traits
      7.2.1 Growth and meat traits
      7.2.2 Milk Composition
   7.3 Animal Models for Human Diseases
   7.4 Synthesis of Biomedical Products (Bioreactors)
   7.5 Commercial Transgenic Products
   7.6 Organs for transplant
   7.7 Future Perspectives of transgenesis
8. Gene Therapy
9. Ethical and Social Issues
10. Conclusions

Protein Engineering **140**
Robert A. Grayling, *The Procter & Gamble Company, USA*
Donn N. Rubingh, *The Procter & Gamble Company, USA*

1. Introduction
2. Strategies for Protein Engineering

2.1 Rational Methods
2.1.1 Rational Design
2.1.2 De Novo Protein Design
2.2 Directed Evolution
2.2.1 Introduction and Background
2.2.2 DNA Shuffling
2.2.3 Screening and Selection
3. Commercial Applications of Protein Engineering
3.1 The Cleaning Products Industry
3.2 The Starch Processing Industry
3.3 Other Enzymes
3.4 Other Proteins
4. Future Possibilities
4.1 Economics and Production
4.2 Future Directions
4.2.1 Bioremediation
4.2.2 Chemical Processing
4.2.3 Pharmaceuticals
4.3 Emerging Technologies
4.4 Perspectives on Directed Evolution and Biodiversity
5. Conclusions

Health and Gene Sciences **166**
Yongyuth Yuthavong, *National Science & Technology Development Agency, Thailand*

1. Introduction
2. Relevance of genomes to human health
3. Diagnosis
4. Drugs and Vaccines: pharmacogenomics
5. Gene Therapy
6. Conclusion

GMO-Technology and Malnutrition **176**
Ingo Potrykus, *Swiss Federal Institute of Technology(ETH), Switzerland*

1. Micronutrient malnutrition
2. Cost-effective and sustained production of nutritious food
3. Why do we have GMO regulations?
4. Traditional breeding
5. The paradox of GMO regulation
5.1 What is the ‘status quo’ of GMO regulation?
5.2 What are the consequences of the ‘status quo’?
5.3 What does the author consider ‘rational regulation?
6. Is GMO over-regulation costing lives?

New opportunities revealed by biotechnological Exploration of Extremophiles **187**
Anna-Louise Reysenbach, *Portland State University, USA*
Mircea Podar, ,

1. Introduction
2. Extremophiles and biomolecules
3. Extremophile genomics exposing the biotechnological potential
4. Tapping into the hidden biotechnological potential through metagenomics
5. Unexplored frontiers and future prospects

The Challenges of Genetic Information **198**
Lisa Austin, *University of Toronto, Canada*
Trudo Lemmens, *University of Toronto, Canada*

1. Introduction
2. What is Genetic Information?
   2.1 DNA Testing
   2.2 Indirect Genetic Testing
   2.3 Family History
   2.4 Differentiation of Genetic Testing According to its Health Care Purpose and its Timing
   2.5 Identification Purposes: Forensic DNA and Military DNA banks
   2.6 Genetic Information Distinguished According to the Way it is Stored
   2.7 Which Definition?
3. Characteristics of Genetic Information: The Claim of Genetic Exceptionalism
   3.1 Genetic Prophecy
   3.2 Lack of Control Over One's Genome
   3.3 Family and Ethnic Community
4. How Genetics Highlights Existing Problems
   4.1 Family and Disclosure of Risk Information
   4.2 Information, Longevity and Identification
   4.3 Uses of Genetic Information
5. Conclusion

Index **227**

About EOLSS **233**