

Contents

<i>Message</i>	vi
<i>Foreword</i>	vii
<i>Preface</i>	ix
<i>Acknowledgements</i>	xi
1. Introduction to Genome Editing	1.1
Advent of genetic engineering	1.1
Genome engineering	1.3
Genome-editing tools	1.8
Genome-editing versus transgenic technologies	1.9
References	1.10
Problems	1.13
2. DNA Repair Mechanisms in Genome Editing	2.1
Cell's response to DNA double-stranded breaks	2.1
Homology-dependent repair	2.3
Nonhomologous end-joining	2.9
Interaction between HDR and NHEJ DNA repair pathways	2.18
Mismatch repair	2.20
Base excision repair	2.25
DNA repair in cell organelles	2.26
Homology-dependent repair in genome editing	2.27
Nonhomologous end-joining in genome editing	2.29
References	2.31
Problems	2.34
3. Gene Knockout, Gene Knockin and Gene Knockdown	3.1
Gene knockout	3.1
Gene knockin	3.10
Gene knockout and gene knockin in plants	3.15
Gene knockdown	3.18
Recent gene-targeting approaches	3.19
References	3.20
Problems	3.21

4. Meganucleases	4.1
Introns	4.1
Nomenclature of homing meganucleases	4.2
Homing	4.2
Structure-function insights into homing endonucleases	4.4
Homing cycle	4.9
Genome editing using meganucleases	4.10
Synthesis of artificial meganucleases	4.13
Screening assays for meganuclease activity	4.20
ARCUS technology	4.21
Applications of homing endonucleases	4.21
References	4.28
Problems	4.30
 5. Zinc-Finger Nucleases	 5.1
Composition of zinc-finger nucleases	5.1
Designer zinc-finger proteins	5.4
Engineering zinc-finger nucleases with extended specificity	5.11
Gene exchange using zinc-finger nucleases	5.13
Zinc-finger nuclease-mediated genome editing	5.14
Limitations of zinc-finger nuclease technology	5.18
References	5.19
Problems	5.22
 6. Transcription Activator-Like Effector Nucleases	 6.1
Transcription activator-like effector gene	6.1
Transcription activator-like effectors	6.1
Transcription activator-like effector nucleases	6.3
TALE protein secretion signals	6.4
TALEN scaffold	6.5
Engineered TALENs	6.6
Design and assembly of TALE arrays	6.9
Considerations for TALEN design	6.11
Functional testing of assembled designer TALEs	6.12
Large-scale TALEN construction and testing	6.14
Variant TALENs	6.17
Applications of TALENs in genome editing	6.20
Limitations of TALENs	6.27
References	6.29
Problems	6.32

7. RNA-Guided Nucleases – The CRISPRs	7.1
CRISPR-Cas as an adaptive immune system	7.4
Components of CRISPR-Cas systems	7.6
Classification of CRISPR-Cas systems	7.7
Working of CRISPR-Cas systems	7.9
Development of CRISPR-Cas technology	7.11
Real-space and real-time dynamics of CRISPR-Cas9	7.12
DNA recognition and cleavage with CRISPR-Cas9	7.12
Orthogonal Cas9 proteins for genome editing	7.14
Harnessing HDR for precise genome editing with CRISPR-Cas	7.20
Expanding target range of CRISPR-Cas systems	7.21
RNA targeting and cleavage	7.22
Bioinformatics tools for CRISPR-Cas9 applications	7.23
How to Plan your CRISPR experiment?	7.24
Applications of CRISPR systems	7.27
Limitations of CRISPR systems	7.52
Technical pitfalls in using the CRISPR-Cas9 system	7.54
Advantages of CRISPR systems	7.55
Disadvantages of CRISPR systems	7.55
Unanswered questions about CRISPR technology	7.55
Future prospects of CRISPR technology	7.58
Genome editing B.C. and A.C.	7.59
Late but welcome recognition	7.59
References	7.67
Problems	
8. DNA-Guided Nucleases – Argonautes	8.1
Eukaryotic Argonautes	8.1
Bacterial Argonautes	8.6
Archaeal Argonautes	8.6
Salient features of NgAgo system	8.9
Advantages of NgAgo system for genome editing	8.11
Limitations of NgAgo system	8.12
Future of NgAgo as genome-editing tool	8.12
References	8.14
Problems	8.17

9. Site-Specific Recombinases	9.1
Site-specific recombination	9.1
Site-specific recombination systems	9.2
Utility of site-specific recombinases in genome editing	9.20
Hybrid site-specific recombinases	9.27
Intrachromosomal homologous recombination	9.32
Recombinase-mediated site-specific gene integration	9.33
Advantages of site-specific recombinases in genome editing	9.36
Problems of site-specific recombinases in genome editing	9.37
References	9.37
Problems	
10. Transposons	10.1
DNA transposons	10.1
Group II retrotransposons	10.8
Trans-splicing group II introns	10.25
References	10.26
Problems	10.27
11. Recombineering	11.1
Substrates for recombineering	11.1
Recombineering using double-stranded DNA	11.2
Mosberg's single-stranded DNA intermediate model	11.4
Recombineering using single-strand oligodeoxynucleotides	11.4
Strategies for DNA engineering in <i>E. coli</i>	11.5
Genome editing via recombineering	11.11
"Hit and fix" method of generating point mutations	11.13
Applications of recombineering	11.14
Advantages of recombineering over genetic engineering	11.16
References	11.18
Problems	11.20
12. Oligonucleotides as Delivery Vehicles	12.1
Oligonucleotide-directed mutagenesis	12.2
Strategies of oligonucleotide-directed mutagenesis	12.6
Synthesis of oligonucleotides	12.6
Types of oligonucleotides	12.6
Applications of oligonucleotides in genome editing	12.35
References	12.37
Problems	12.41

13. Engineered Viruses as Delivery Vehicles	13.1
Lentiviruses	13.2
Adeno-associated viruses	13.8
Recombinant adeno-associated viruses	13.13
Adenoviruses	13.27
Comparison of different animal viral vector systems	13.35
Tobacco rattle virus	13.39
Geminiviruses	13.40
Usefulness of engineered viruses in genome editing	13.44
Is there an ideal virus for genome editing?	13.46
References	13.46
Problems	13.49
14. Comparing Genome-Editing Tools	14.1
Adoption of site-specific nucleases	14.1
Efficiencies of site-specific nucleases	14.5
Will NgAgo-gDNA replace CRISPR-Cas9 for genome editing?	14.8
Advantages of different site-specific nucleases	14.8
Which genome-editing tool is the best?	14.10
References	14.13
Problems	14.14
15. Gene-Editing Approaches in Bacteria and Yeasts	15.1
Site-specific recombination systems	15.1
The "pop-in/pop-out" method	15.3
SceI-assisted method	15.5
Custom endonucleases-assisted genome-editing method	15.5
Tandem repeat-assisted genome-editing method	15.6
Genome Editing with CRISPR-Cas9 without PAM Sequences	15.11
Genome-scale engineering	15.11
Mutagenic inverted repeat-assisted genome engineering	15.12
Seamless genome editing in yeast	15.15
Synthetic RNA polymerase III promoters plus tRNA	15.17
Haploid engineering and replacement protocol	15.20
Delitto Perfetto	15.23
Inducing templated insertions and strand-specific insertions/deletions in yeast	15.24
Multiplexed accurate genome editing with short, trackable, integrated cellular barcodes (MAGESTIC)	15.24
References	15.25
Problems	15.26

16. Multiplex Genome Editing	16.1
Forms of multiplex genome editing	16.1
Multiplex genome editing by natural transformation	16.3
CRISPR-Cas9-mediated multiplex genome editing	16.5
Boosting CRISPR-Cas9 multiplex editing capability	16.8
RNA nuclease-based multiplex systems in mammals	16.9
Multiplex genome editing in microbes	16.10
Multiplex genome editing in animals	16.18
Multiplex genome editing in human cells	16.21
Multiplex genome editing in plants	16.21
Future prospects of multiplex genome editing	16.28
References	16.28
Problems	16.30
 17. Gene Drive Technology	 17.1
Characteristics of gene drive	17.1
Molecular mechanism of gene drive	17.2
Natural gene drive	17.3
Engineered gene drive	17.6
New models of CRISPR gene drives	17.11
Applications of gene drive	17.14
Resistance allele formation and gene drive efficiency	17.17
Integrated risk management	17.20
Limitations of gene drive technology	17.20
Gene drive technology – Is it safe?	17.21
Risk assessment and management of gene drive technology	17.21
Regulatory aspect of gene drives	17.22
Key questions in gene drives	17.23
Future prospects of gene drive	17.25
References	17.26
Problems	17.28
 18. Genome Editing in Mitochondria and Plastids	 18.1
Mitochondrial genome editing	18.1
Applications of mitochondrial genome editing	18.10
mtDNA editing vis-à-vis mitochondrial replacement therapy	18.10
Plastid genome editing	18.11
Seamless editing of the chloroplast genome	18.13
Applicability of the chloroplast genome-editing	18.15
References	18.15
Problems	18.16

19. Repurposing Genome-Editing Tools for Gene Expression Control	19.1
Zinc-finger-based designer proteins	19.2
Transcription activator-like effector-based designer proteins	19.2
Modified versions of CRISPR-Cas9 Protein	19.9
Genomewide screens	19.19
Epigenome editing	19.20
Post-transcriptional gene expression control	19.27
Future applications of gene expression control	19.30
References	19.31
Problems	19.33
20. Safety Issues of Genome-Editing Technology	20.1
Genotoxicity	20.1
Cytotoxicity	20.19
Testing the designer nucleases before use	20.34
Regulatory switches to reduce off-target activities	20.35
Tools for regulating working of Cas9	20.39
References	20.43
Problems	20.48
21. Regulatory Issues and Future of Genome-Editing Technology	21.1
Are the genome edited organisms GMOs?	21.1
Opinions on the use of site-specific nucleases	21.1
Proposed regulatory framework for genome edited crops	21.2
Genome editing in gene therapy	21.5
Legal interpretation of genome-editing techniques	21.6
Genome edited organisms sidestep USDA's regulatory system	21.7
Genome editing intellectual property	21.8
Consumer acceptance of genome edited crops	21.13
Possible regulations on DNA-free genome edited crops	21.14
Genome editing technology – future prospects	21.15
References	21.16
Problems	21.18
Subject Index	S.1