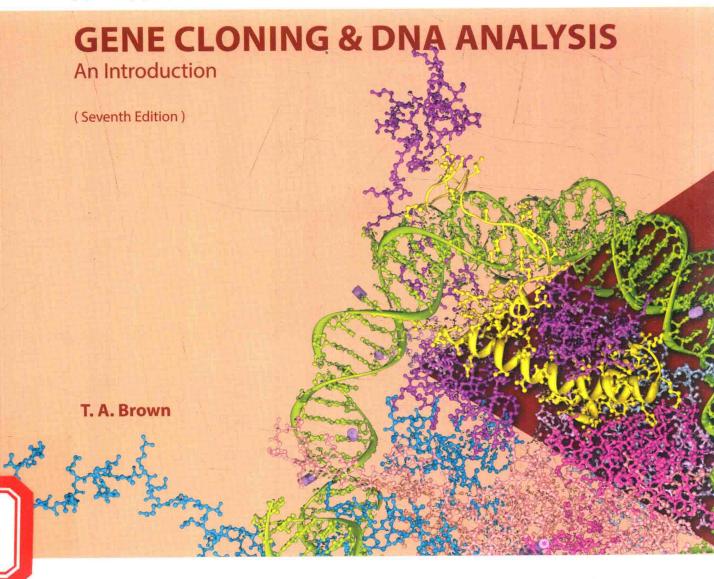
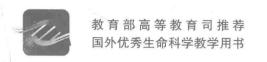


基因克隆和 DNA 分析

(第7版)(影印版)





基因克隆和 DNA 分析

(第7版)(影印版)

GENE CLONING & DNA ANALYSIS

An Introduction

(Seventh Edition)

T. A. Brown

高等教育出版社·北京

图字:01-2017-6697号

Gene Cloning and DNA Analysis: An Introduction, Seventh Edition/by T. A. Brown

Copyright © 2016 by John Wiley & Sons, Ltd1

All Rights Reserved. Authorized reprint of the edition published by John Wiley & Sons Ltd. Responsibility for the accuracy of the reprint rests solely with the Publisher and is not the responsibility of John Wiley & Sons Ltd. No part of this book may be reproduced in any form without the written permission of the original copyright holder, John Wiley & Sons Ltd.

图书在版编目(CIP)数据

基因克隆和 DNA 分析:第7版=Gene Cloning and DNA Analysis: An Introduction,7th edition:英文/(英)布朗(T.A.Brown)著. -- 影印本. -- 北京 : 高等教育出版社,2018.1

ISBN 978-7-04-048913-2

I. ①基··· Ⅱ. ①布··· Ⅲ. ①基因克隆-英文②脱氧核糖核酸-英文 Ⅳ. ①Q785②Q523

中国版本图书馆 CIP 数据核字(2018)第 000991 号

策划编辑 高新景 责任编辑 高新景 封面设计 张 楠 责任印制 赵义民

400-810-0598 出版发行 高等教育出版社 咨询电话 http://www.hep.edu.cn 社 址 北京市西城区德外大街 4号 http://www.hep.com.cn 邮政编码 100120 大厂益利印刷有限公司 网上订购 http://www.hepmall.com.cn 印 http://www.hepmall.com http://www.hepmall.cn 850mm×1168mm 1/16 开 本 23.25 版 2018年1月第1版 张 2018年1月第1次印刷 字 印 次 数 600 干字 010-58581118

本书如有缺页、倒页、脱页等质量问题,请到所购图书销售部门联系调换

版权所有 侵权必究 物料号 48913-00

GENE CLONING& DNA ANALYSIS

An Introduction

Seventh Edition

Terry Brown

Manchester Institute of Biotechnology, University of Manchester, UK

Known worldwide as the standard introductory text to this important and exciting area, the Seventh Edition of *Gene Cloning and DNA Analysis* addresses new and growing areas of research whilst retaining the philosophy of the previous editions. Assuming that the reader has little prior knowledge of the subject, its importance, the principles of the techniques used and their applications are all carefully laid out, with over 250 clearly presented four-colour illustrations.

In addition to a number of informative changes in the text throughout the book, the chapters on DNA sequencing and genome studies have been rewritten to reflect the continuing rapid developments in this area of DNA analysis:

- · In-depth descriptions of next-generation sequencing methods and their applications in studying genomes and transcriptomes.
- · New material on the use of ChiP-seq to locate protein-binding sites.
- · Extended coverage of the strategies used to assemble genome sequences.
- An account of sequencing the Neanderthal genome, and what it reveals about interbreeding with Homo sapiens.

Gene Cloning and DNA Analysis remains an essential introductory text to a wide range of biological sciences students; including genetics and genomics, molecular biology, biochemistry, immunology, and applied biology. It is also a perfect introductory text for any professional needing to learn the basics of the subject. All libraries in universities where medical, life and biological sciences are studied and taught should have copies available on their shelves.

数字课程(基础版)

基因克隆和 DNA分析

(影印版)

T. A. Brown

登录方法:

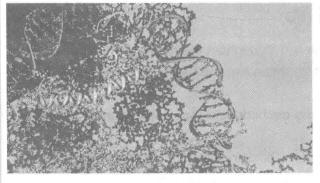
- 1. 电脑访问 http://abook.hep.com.cn/48913, 或手机扫描下方二维码、下载并安装 Abook 应用。
- 2. 注册并登录, 进入"我的课程"。
- 3. 输入封底数字课程账号(20 位密码, 刮开涂层可见), 或通过 Abook 应用扫描封底数字课程账号二维码,完成课程绑定。
- 4. 点击"进入学习", 开始本数字课程的学习。

课程绑定后一年为数字课程使用有效期。如有使用问题,请发邮件至:

lifescience@pub.hep.cn







基因克隆和 DNA 分析 (影印版)

"基因克隆和DNA分析"数字课程与纸质教材紧密配合。数字课程包括彩图、表格等内容,充分运用多种形式的媒体资源,为师生提供教学参考。

用户名:

密码

验证码:

0.922

忠记密码?

登录

22-00

http://abook.hep.com.cn/48913



扫描二维码,下载Abook应用

Preface to the Seventh Edition

nyone who works with DNA is well aware of the dramatic changes that have taken place during the past few years in DNA sequencing methodology. To reflect these advances, in this new edition of Gene Cloning and DNA Analysis: An Introduction I have completely remodelled the chapter on DNA sequencing to give the new 'next-generation' methods equal prominence alongside the traditional approaches to DNA sequencing, and also to modernize the description of the ways in which genome sequences are generated. Elsewhere, I have stressed the importance of RNA-seq as a means of studying transcriptomes, and ChIP-seq for locating protein-binding sites. These changes correct the major weakness of the Sixth Edition, which was written just before these methods came into mainstream use.

Elsewhere, I have made the usual updates, especially in Part III where I have tried to keep pace with the increasingly rapid developments in the applications of gene cloning and DNA analysis in industry, medicine and agriculture. I have also rewritten the last part of the final chapter, on archaeogenetics, in order to present some of the new information on the human past that has been revealed by the Neanderthal and Denisovan genome sequences. As always, my primary aim is to ensure that *Gene Cloning* remains an introductory text that begins at the beginning and does not assume that the reader has any prior knowledge of the techniques used to study genes and genomes.

For the n-th time I must thank my wife Keri for the unending support that she has given to me in my decision to use up evenings and weekends writing this and other books.

T.A. Brown University of Manchester

Contents in Brief CONTENTS IN BRIEF

Part I The Basic Principles of Gene Cloning and DNA Analysis 1

- 1 Why Gene Cloning and DNA Analysis are Important 3
- 2 Vectors for Gene Cloning: Plasmids and Bacteriophages 13
- 3 Purification of DNA from Living Cells 25
- 4 Manipulation of Purified DNA 47
- 5 Introduction of DNA into Living Cells 75
- 6 Cloning Vectors for Escherichia coli 93
- 7 Cloning Vectors for Eukaryotes 111
- 8 How to Obtain a Clone of a Specific Gene 135
- 9 The Polymerase Chain Reaction 157

Part II The Applications of Gene Cloning and DNA Analysis in Research 173

- 10 Sequencing Genes and Genomes 175
- 11 Studying Gene Expression and Function 201
- 12 Studying Genomes 225

Part III The Applications of Gene Cloning and DNA Analysis in Biotechnology 245

- 13 Production of Protein from Cloned Genes 247
- 14 Gene Cloning and DNA Analysis in Medicine 269
- 15 Gene Cloning and DNA Analysis in Agriculture 291
- 16 Gene Cloning and DNA Analysis in Forensic Science and Archaeology 311

Glossary 329 Index 345

Contents CONTENTS

Preface to the Seventh Edition xvii About the Companion Website xix

Part I The Basic Principles of Gene Cloning and DNA Analysis 1

1 Why Gene Cloning and DNA Analysis are Important 3

- 1.1 The early development of genetics 3
- 1.2 The advent of gene cloning and the polymerase chain reaction 4
- 1.3 What is gene cloning? 5
- 1.4 What is PCR? 6
- 1.5 Why gene cloning and PCR are so important 71.5.1 Obtaining a pure sample of a gene by cloning 71.5.2 PCR can also be used to purify a gene 8
- 1.6 How to find your way through this book 11 Further reading 12

2 Vectors for Gene Cloning: Plasmids and Bacteriophages 13

- 2.1 Plasmids 13
 - 2.1.1 Size and copy number 14
 - 2.1.2 Conjugation and compatibility 16
 - 2.1.3 Plasmid classification 16
 - 2.1.4 Plasmids in organisms other than bacteria 17
- 2.2 Bacteriophages 17
 - 2.2.1 The phage infection cycle 18
 - 2.2.2 Lysogenic phages 19
 Gene organization in the λ DNA molecule 19
 The linear and circular forms of λ DNA 19
 M13 a filamentous phage 22
 - 2.2.3 Viruses as cloning vectors for other organisms 24 Further reading 24

3 Purification of DNA from Living Cells 25

- 3.1 Preparation of total cell DNA 25
 - 3.1.1 Growing and harvesting a bacterial culture 26
 - 3.1.2 Preparation of a cell extract 28
 - 3.1.3 Purification of DNA from a cell extract 29
 Removing contaminants by organic extraction and enzyme digestion 29
 Using ion-exchange chromatography to purify DNA from a cell extract 30
 Using silica to purify DNA from a cell extract 30
 - 3.1.4 Concentration of DNA samples 32
 - 3.1.5 Measurement of DNA concentration 33
 - 3.1.6 Other methods for the preparation of total cell DNA 34
- 3.2 Preparation of plasmid DNA 35
 - 3.2.1 Separation on the basis of size 35
 - 3.2.2 Separation on the basis of conformation 37
 Alkaline denaturation 37
 Ethidium bromide–caesium chloride density gradient centrifugation 38
 - 3.2.3 Plasmid amplification 39
- 3.3 Preparation of bacteriophage DNA 40
 - 3.3.1 Growth of cultures to obtain a high λ titre 41
 - 3.3.2 Preparation of non-lysogenic λ phages 41
 - 3.3.3 Collection of phages from an infected culture 43
 - 3.3.4 Purification of DNA from λ phage particles 43
 - **3.3.5** Purification of M13 DNA causes few problems 43 Further reading 45

3

- 4 Manipulation of Purified DNA 47
 - 4.1 The range of DNA manipulative enzymes 48
 4.1.1 Nucleases 48
 - 4.1.2 Ligases 50
 - 4.1.3 Polymerases 51
 - 4.1.4 DNA-modifying enzymes 52
 - 4.2 Enzymes for cutting DNA: Restriction endonucleases 53
 - 4.2.1 The discovery and function of restriction endonucleases 54
 - 4.2.2 Type II restriction endonucleases cut DNA at specific nucleotide sequences 55
 - 4.2.3 Blunt ends and sticky ends 55
 - 4.2.4 The frequency of recognition sequences in a DNA molecule 57
 - 4.2.5 Performing a restriction digest in the laboratory 58
 - 4.2.6 Analysing the result of restriction endonuclease cleavage 59 Separation of molecules by gel electrophoresis 59 Visualizing DNA molecules in an agarose gel 60
 - 4.2.7 Estimation of the sizes of DNA molecules 61
 - 4.2.8 Mapping the positions of different restriction sites in a DNA molecule 62

4.2.9	Special ge	l electrophoresis	methods	for	separating	larger
	molecules	63				

4.3 Ligation: Joining DNA molecules together 66

- 4.3.1 The mode of action of DNA ligase 66
- 4.3.2 Sticky ends increase the efficiency of ligation 67
- 4.3.3 Putting sticky ends on to a blunt-ended molecule 67 Linkers 68 Adaptors 68 Homopolymer tailing 70

4.3.4 Blunt end ligation with a DNA topoisomerase 71 Further reading 74

5 Introduction of DNA into Living Cells 75

- 5.1 Transformation: The uptake of DNA by bacterial cells 76
 - 5.1.1 Not all species of bacteria are equally efficient at DNA uptake 77
 - 5.1.2 Preparation of competent E. coli cells 78
 - 5.1.3 Selection for transformed cells 78
- 5.2 Identification of recombinants 79
 - 5.2.1 Recombinant selection with pBR322: Insertional inactivation of an antibiotic resistance gene 80
 - 5.2.2 Insertional inactivation does not always involve antibiotic resistance 81
- 5.3 Introduction of phage DNA into bacterial cells 83
 - 5.3.1 Transfection 83
 - 5.3.2 In vitro packaging of λ cloning vectors 83
 - 5.3.3 Phage infection is visualized as plaques on an agar medium 86
- 5.4 Identification of recombinant phages 86
 - 5.4.1 Insertional inactivation of a *lacZ'* gene carried by the phage vector 87
 - 5.4.2 Insertional inactivation of the λ cl gene 87
 - 5.4.3 Selection using the Spi phenotype 88
 - 5.4.4 Selection on the basis of λ genome size 88
- 5.5 Introduction of DNA into non-bacterial cells 88
 - 5.5.1 Transformation of individual cells 88
 - 5.5.2 Transformation of whole organisms 90

Further reading 90

6 Cloning Vectors for Escherichia coli 93

- 6.1 Cloning vectors based on E. coli plasmids 94
 - 6.1.1 The nomenclature of plasmid cloning vectors 94
 - 6.1.2 The useful properties of pBR322 94
 - 6.1.3 The pedigree of pBR322 95
 - 6.1.4 More sophisticated E. coli plasmid cloning vectors 95 pUC8: A Lac selection plasmid 97 pGEM3Z: In vitro transcription of cloned DNA 98

- 6.2 Cloning vectors based on λ bacteriophage 99
 - 6.2.1 Segments of the λ genome can be deleted without impairing viability 99
 - 6.2.2 Natural selection was used to isolate modified λ that lack certain restriction sites 100
 - 6.2.3 Insertion and replacement vectors 102
 Insertion vectors 102
 Replacement vectors 102
 - 6.2.4 Cloning experiments with λ insertion or replacement vectors 103
 - 6.2.5 Long DNA fragments can be cloned using a cosmid 103
 - 6.2.6 λ and other high-capacity vectors enable genomic libraries to be constructed 104
- 6.3 Cloning vectors for the synthesis of single-stranded DNA 106
 - 6.3.1 Vectors based on M13 bacteriophage 107
 - 6.3.2 Hybrid plasmid-M13 vectors 108
- 6.4 Vectors for other bacteria 109
 Further reading 110

7 Cloning Vectors for Eukaryotes 111

- 7.1 Vectors for yeast and other fungi 111
 - 7.1.1 Selectable markers for the 2 µm plasmid 112
 - 7.1.2 Vectors based on the 2 µm plasmid: Yeast episomal plasmids
 - 7.1.3 A YEp may insert into yeast chromosomal DNA 113
 - 7.1.4 Other types of yeast cloning vector 115
 - 7.1.5 Artificial chromosomes can be used to clone long pieces of DNA in yeast 116
 The structure and use of a YAC vector 116
 Applications for YAC vectors 118
 - 7.1.6 Vectors for other yeasts and fungi 118
- 7.2 Cloning vectors for higher plants 119
 - 7.2.1 Agrobacterium tumefaciens: nature's smallest genetic engineer 119
 Using the Ti plasmid to introduce new genes into a plant cell 120
 Production of transformed plants with the Ti plasmid, 122

Production of transformed plants with the Ti plasmid 122 The Ri plasmid 123

- Limitations of cloning with Agrobacterium plasmids 123
- 7.2.2 Cloning genes in plants by direct gene transfer 124
 Direct gene transfer into the nucleus 125
 Transfer of genes into the chloroplast genome 125
- 7.2.3 Attempts to use plant viruses as cloning vectors 126
 Caulimovirus vectors 127
 Geminivirus vectors 127
- 7.3 Cloning vectors for animals 127
 - 7.3.1 Cloning vectors for insects 128
 P elements as cloning vectors for *Drosophila* 128
 Cloning vectors based on insect viruses 129

7.3.2 Cloning in mammals 130
Viruses as cloning vectors for mammals 130
Gene cloning without a vector 131
Further reading 132

8 How to Obtain a Clone of a Specific Gene 135

- 8.1 The problem of selection 135
 - 8.1.1 There are two basic strategies for obtaining the clone you want 136
- 8.2 Direct selection 137
 - 8.2.1 Marker rescue extends the scope of direct selection 138
 - 8.2.2 The scope and limitations of marker rescue 139
- 8.3 Identification of a clone from a gene library 140
 - 8.3.1 Gene libraries 140

Not all genes are expressed at the same time 140 mRNA can be cloned as complementary DNA 142

- 8.4 Methods for clone identification 143
 - 8.4.1 Complementary nucleic acid strands hybridize to each other 143
 - 8.4.2 Colony and plaque hybridization probing 144
 Labelling with a radioactive marker 145
 Non-radioactive labelling 146
 - 8.4.3 Examples of the practical use of hybridization probing 146
 Abundancy probing to analyse a cDNA library 147
 Oligonucleotide probes for genes whose translation products have been characterized 148
 Heterologous probing allows related genes to be identified 150
 Southern hybridization enables a specific restriction fragment containing a gene to be identified 151
 - 8.4.4 Identification methods based on detection of the translation product of the cloned gene 153

 Antibodies are required for immunological detection methods 153

 Using a purified antibody to detect protein in recombinant colonies 154

The problem of gene expression 155

Further reading 155

9 The Polymerase Chain Reaction 157

- 9.1 PCR in outline 157
- 9.2 PCR in more detail 159
 - 9.2.1 Designing the oligonucleotide primers for a PCR 159
 - 9.2.2 Working out the correct temperatures to use 162
- 9.3 After the PCR: Studying PCR products 164
 - 9.3.1 Gel electrophoresis of PCR products 164
 - 9.3.2 Cloning PCR products 166
 - 9.3.3 Problems with the error rate of Tag polymerase 167

- 9.4 Real-time PCR enables the amount of starting material to be quantified 169
 - 9.4.1 Carrying out a quantitative PCR experiment 169
 - 9.4.2 Real-time PCR can also quantify RNA 171

Further reading 171

Part II The Applications of Gene Cloning and DNA Analysis in Research 173

10 Sequencing Genes and Genomes 175

- 10.1 Chain-termination DNA sequencing 176
 - 10.1.1 Chain-termination sequencing in outline 176
 - 10.1.2 Not all DNA polymerases can be used for sequencing 178
 - 10.1.3 Chain-termination sequencing with Tag polymerase 179
 - 10.1.4 Limitations of chain-termination sequencing 180
- 10.2 Next-generation sequencing 182
 - 10.2.1 Preparation of a next-generation sequencing library 182
 DNA fragmentation 183
 Immobilization of the library 184
 Amplification of the library 184
 - 10.2.2 Next-generation sequencing methods 185
 Reversible terminator sequencing 186
 Pyrosequencing 187
 - 10.2.3 Third-generation sequencing 188
 - 10.2.4 Directing next-generation sequencing at specific sets of genes 188
- 10.3 How to sequence a genome 189
 - Shotgun sequencing of prokaryotic genomes 190
 Shotgun sequencing of the Haemophilus influenzae genome 190
 Shotgun sequencing of other prokaryotic genomes 193
 - 10.3.2 Sequencing of eukaryotic genomes 194
 The hierarchical shotgun approach 194
 Shotgun sequencing of eukaryotic genomes 196
 What do we mean by 'genome sequence'? 198

Further reading 198

11 Studying Gene Expression and Function 201

- 11.1 Studying the RNA transcript of a gene 202
 - 11.1.1 Detecting the presence of a transcript and determining its nucleotide sequence 203
 - 11.1.2 Transcript mapping by hybridization between gene and RNA 204
 - 11.1.3 Transcript analysis by primer extension 205
 - 11.1.4 Transcript analysis by PCR 206

11.2 Studying the regulation of gene expression 207

- 11.2.1 Identifying protein binding sites on a DNA molecule 209
 Gel retardation of DNA-protein complexes 209
 Footprinting with DNase I 210
 Modification interference assays 212
- 11.2.2 Identifying control sequences by deletion analysis 212
 Reporter genes 213
 Carrying out a deletion analysis 215
- 11.3 Identifying and studying the translation product of a cloned gene
 - 11.3.1 HRT and HART can identify the translation product of a cloned gene 216
 - 11.3.2 Analysis of proteins by in vitro mutagenesis 216
 Different types of in vitro mutagenesis techniques 218
 Using an oligonucleotide to create a point mutation in a cloned gene 220
 Other methods of creating a point mutation in a cloned gene 220
 The potential of in vitro mutagenesis 223

Further reading 223

12 Studying Genomes 225

- 12.1 Genome annotation 225
 - 12.1.1 Identifying the genes in a genome sequence 226
 Searching for open reading frames 226
 Simple ORF scans are less effective at locating genes in eukaryotic genomes 227
 Gene location is aided by homology searching 228
 Comparing the sequences of related genomes 229
 Identifying the binding sites for regulatory proteins in a genome sequence 230
 - 12.1.2 Determining the function of an unknown gene 231
 Assigning gene function by experimental analysis requires a reverse approach to genetics 231
 Specific genes can be inactivated by homologous recombination 232
- 12.2 Studies of the transcriptome and proteome 233
 - 12.2.1 Studying the transcriptome 234
 Studying transcriptomes by microarray or chip analysis 234
 Studying a transcriptome by SAGE 235
 Sequencing a transcriptome by RNA-seq 236
 Advantages of the different methods for transcriptome analysis 237
 - 12.2.2 Studying the proteome 237
 Separating the proteins in a proteome 238
 Identifying the individual proteins after separation 239
 - 12.2.3 Studying protein-protein interactions 240 Phage display 241 The yeast two-hybrid system 242

Further reading 243

Part III The Applications of Gene Cloning and DNA Analysis in Biotechnology 245

13	Production	of	Protein	from	Cloned	Ganas	247

- 13.1 Special vectors for the expression of foreign genes in E. coli 249
 - 13.1.1 The promoter is the critical component of an expression vector 251

The promoter must be chosen with care 251
Examples of promoters used in expression vectors 253

13.1.2 Cassettes and gene fusions 254

- 13.2 General problems with the production of recombinant protein in E. coli 257
 - 13.2.1 Problems resulting from the sequence of the foreign gene 257

13.2.2 Problems caused by E. coli 258

13.3 Production of recombinant protein by eukaryotic cells 259

- 13.3.1 Recombinant protein from yeasts and filamentous fungi 260
 Saccharomyces cerevisiae as the host for recombinant protein synthesis 260
 Other yeasts and fungi 261
- 13.3.2 Using animal cells for recombinant protein production 262
 Protein production in mammalian cells 262
 Protein production in insect cells 262
- 13.3.3 Pharming: Recombinant protein from live animals and plants
 263
 Pharming in animals 263

Recombinant proteins from plants 265 Ethical concerns raised by pharming 265

Further reading 266

14 Gene Cloning and DNA Analysis in Medicine 269

- 14.1 Production of recombinant pharmaceuticals 269
 - 14.1.1 Recombinant insulin 270

Synthesis and expression of artificial insulin genes 270

- 14.1.2 Synthesis of human growth hormones in E. coli 271
- 14.1.3 Recombinant factor VIII 274
- 14.1.4 Synthesis of other recombinant human proteins 275
- 14.1.5 Recombinant vaccines 275
 Producing vaccines as recombinant proteins 276
 Recombinant vaccines in transgenic plants 277
 Live recombinant virus vaccines 279
- 14.2 Identification of genes responsible for human diseases 280
 - 14.2.1 How to identify a gene for a genetic disease 282
 Locating the approximate position of the gene in the human genome 282
 Linkage analysis of the human *BRCA1* gene 283
 Identification of candidates for the disease gene 284

14.3	Gene	therapy	286
------	------	---------	-----

- 14.3.1 Gene therapy for inherited diseases 286
- 14.3.2 Gene therapy and cancer 288
- 14.3.3 The ethical issues raised by gene therapy 288 Further reading 290

15 Gene Cloning and DNA Analysis in Agriculture 291

- 15,1 The gene addition approach to plant genetic engineering 292
 - 15.1.1 Plants that make their own insecticides 292
 The δ-endotoxins of Bacillus thuringiensis 292
 Cloning a δ-endotoxin gene in maize 293
 Cloning δ-endotoxin genes in chloroplasts 295
 Countering insect resistance to δ-endotoxin crops 296
 - 15.1.2 Herbicide-resistant crops 298
 'Roundup Ready' crops 298
 A new generation of glyphosate-resistant crops 299
 - 15.1.3 Other gene addition projects 300
- 15.2 Gene subtraction 302
 - 15.2.1 Antisense RNA and the engineering of fruit ripening in tomato 302
 Using antisense RNA to inactivate the polygalacturonase gene 302
 Using antisense RNA to inactivate ethylene synthesis 304
 - 15.2.2 Other examples of the use of antisense RNA in plant genetic engineering 304
- 15.3 Problems with genetically modified plants 305
 - 15.3.1 Safety concerns with selectable markers 305
 - 15.3.2 The terminator technology 306
 - 15.3.3 The possibility of harmful effects on the environment 307 Further reading 308

16 Gene Cloning and DNA Analysis in Forensic Science and Archaeology 311

- 16.1 DNA analysis in the identification of crime suspects 312
 - 16.1.1 Genetic fingerprinting by hybridization probing 312
 - 16.1.2 DNA profiling by PCR of short tandem repeats 312
- 16.2 Studying kinship by DNA profiling 315
 - 16.2.1 Related individuals have similar DNA profiles 315
 - 16.2.2 DNA profiling and the remains of the Romanovs 315
 STR analysis of the Romanov bones 315
 Mitochondrial DNA was used to link the Romanov skeletons with living relatives 317
 The missing children 318
- 16.3 Sex identification by DNA analysis 318
 - 16.3.1 PCRs directed at Y chromosome-specific sequences 318
 - 16.3.2 PCR of the amelogenin gene 319

16.4 Archaeogenetics: Using DNA to study human prehistory 320

16.4.1 The origins of modern humans 320
DNA analysis has challenged the multiregional hypothesis 321
DNA analysis shows that Neanderthals are not the direct ancestors of modern Europeans 322
The Neanderthal genome sequence suggests there was interbreeding with *H. sapiens* 323

16.4.2 DNA can also be used to study prehistoric human migrations 324

Modern humans may have migrated from Ethiopia to Arabia 324

Colonization of the New World 325

Further reading 328

Glossary 329 Index 345