

An Introduction to Genetic Engineering Fourth Edition

The fourth edition of this popular textbook retains its focus on the fundamental principles of gene manipulation, providing an accessible and broad-based introduction to the subject for beginning undergraduate students. It has been brought thoroughly up to date with new chapters on the story of DNA and genome editing, and new sections on bioethics, significant developments in sequencing technology and structural, functional and comparative genomics and proteomics, and the impact of transgenic plants. In addition to chapter summaries, learning objectives, concept maps, glossary and key word lists, the book now also features new concluding sections, further reading lists and websearch activities for each chapter to provide a comprehensive suite of learning resources to help students develop a flexible and critical approach to the study of genetic engineering.

Desmond S. T. Nicholl was Senior Lecturer in Biological Sciences, Head of Bioscience, Head of Quality Enhancement and Assistant Dean for Education at the University of the West of Scotland. As well as three previous editions of *An Introduction to Genetic Engineering*, he also authored *Cell and Molecular Biology* (Learning & Teaching Scotland, 2000).



'Genetic engineering represents a toolbox that all students within the basic and applied biology fields must get acquainted with. The fourth edition of *An Introduction to Genetic Engineering* is an excellent up-to-date version of a classic textbook. This ambitious book excellently balances the molecular biology knowledge required to grasp the comprehensive gene technology toolbox with a discussion of its impact on society.'

Per Amstrup Pedersen, University of Copenhagen

'As a biomedical engineering professor teaching an undergraduate Genetic Engineering course for close to 10 years, I use Dr Nicholl's *An Introduction to Genetic Engineering* as my go-to textbook. It is not one of those overly thick textbooks that overwhelm students. Its comprehensiveness captures readers' attention with succinct fundamental concepts that truly promote one's interest in exploring the wonder of many genetic engineering techniques and applications. To facilitate that further, the material provided at the end of each chapter encourages readers to expand their learning with relevant resources ... Many of my students become so interested that they pursue graduate degrees and have a career in this field. Dr Nicholl's textbook has a long-term influence on its readers.'

M. Ete Chan, State University of New York at Stony Brook

'Dr Nicholl's book covers all the basic material that one would expect from its title, but what particularly impressed me was how it isn't afraid to move into political and socio-economic arenas. In Chapter 16, for example, balanced arguments are presented for and against the development of transgenic organisms, and these don't always come out in favour of the science.'

Neil Crickmore, University of Sussex



An Introduction to Genetic EngineeringFourth Edition

Desmond S. T. Nicholl







Shaftesbury Road, Cambridge CB2 8EA, United Kingdom

One Liberty Plaza, 20th Floor, New York, NY 10006, USA

477 Williamstown Road, Port Melbourne, VIC 3207, Australia

314–321, 3rd Floor, Plot 3, Splendor Forum, Jasola District Centre, New Delhi-110025, India

103 Penang Road, #05-06/07, Visioncrest Commercial, Singapore 238467

Cambridge University Press is part of Cambridge University Press & Assessment, a department of the University of Cambridge.

We share the University's mission to contribute to society through the pursuit of education, learning and research at the highest international levels of excellence.

www.cambridge.org

Information on this title: www.cambridge.org/highereducation/isbn/9781009180597

DOI: 10.1017/9781009180610

First and second editions © Cambridge University Press 1994, 2002

Third and fourth editions © Desmond S. T. Nicholl 2008, 2023

This publication is in copyright. Subject to statutory exception and to the provisions of relevant collective licensing agreements, no reproduction of any part may take place without the written permission of Cambridge University Press & Assessment.

First published 1994 Second edition 2002 Third edition 2008 Fourth edition 2023

Printed in the United Kingdom by TJ Books Limited, Padstow, Cornwall, 2023

A catalogue record for this publication is available from the British Library.

A Cataloging-in-Publication data record for this book is available from the Library of Congress

ISBN 978-1-009-18059-7 Hardback ISBN 978-1-009-18060-3 Paperback

Additional resources for this publication at www.cambridge.org/nicholl4

Cambridge University Press & Assessment has no responsibility for the persistence or accuracy of URLs for external or third-party internet websites referred to in this publication and does not guarantee that any content on such websites is, or will remain, accurate or appropriate.



Contents

Preface		page xv
Part I Ge	netic Engineering in Context	
Chapter I	Introduction	2
Chapter 2	The Story of DNA	16
Chapter 3	Brave New World or Genetic Nightmare?	36
Part 2 The	e Basis of Genetic Engineering	
Chapter 4	Introducing Molecular Biology	52
Chapter 5	The Tools of the Trade	78
Chapter 6	Working with Nucleic Acids	94
Part 3 The	e Methodology of Gene Manipulation	
Chapter 7	Host Cells and Vectors	134
Chapter 8	Cloning Strategies	160
Chapter 9	The Polymerase Chain Reaction	188
Chapter 10	Selection, Screening and Analysis of Recombinants	210
Chapter II	Bioinformatics	230
Chapter 12	Genome Editing	248
Part 4 Ge	netic Engineering in Action	
Chapter 13	Investigating Genes, Genomes and 'Otheromes'	264
Chapter 14	Genetic Engineering and Biotechnology	296
Chapter 15	Medical and Forensic Applications of Gene Manipulation	326



vi

CONTENTS

Chapter 16	Transgenic Plants and Animals	362
Chapter 17	The Other Sort of Cloning	390
Glossary Index		405 439



Detailed Contents

Prej	face	page xv
Par	Ct I Genetic Engineering in Context	
Ch	apter I Introduction	2
	Chapter Summary	2
1.1	What Is Genetic Engineering?	3
1.2	Laying the Foundations	5
1.3	First Steps in DNA Cloning	6
	Using the Web to Support Your Studies	8
1.5	Conclusion: The Breadth and Scope of Genetic Engineering	12
	Further Reading	13
	Websearch	14
	Concept Map	15
Ch	apter 2 The Story of DNA	16
	Chapter Summary	16
2.1	How Science Works	17
	2.1.1 A Simple Model for the Scientific Method	23
	2.1.2 A More Realistic Model for How Science Works	24
2.2	DNA: A Biographical Timeline	25
2.3	People, Places and Progress: Paradigm Shifts or Step-	
	Changes?	28
2.4	Conclusion: The Scientific Landscape	32
	Further Reading	33
	Websearch	34
	Concept Map	35
Ch	apter 3 Brave New World or Genetic Nightmare?	36
	Chapter Summary	36
3.1	What Is Ethics?	37
	3.1.1 The Ethical Framework	38
	3.1.2 Is Science Ethically and Morally Neutral?	39
	3.1.3 The Scope of Bioethics	40
3.2	Elements of the Ethics Debate	42
	3.2.1 The Role of the Scientist	42
	3.2.2 The Role of Society	43
	3.2.3 Current Issues in Bioethics	43
3.3	Conclusion: Has Frankenstein's Monster Escaped from	
	Pandora's Box?	46
	Further Reading	47
	Websearch	47
	Concept Map	49



viii

Pai	rt 2 The Basis of Genetic Engi	neering	
Ch	napter 4 Introducing Molecular Bio	ology	52
	Chapter Summary		52
4.1	How Living Systems Are Organised		53
4.2	The Flow of Genetic Information		55
4.3	The Structure of DNA and RNA		57
4.4	Gene Organisation		60
	4.4.1 The Anatomy of a Gene		61
	4.4.2 Gene Structure in Prokaryotes		62
	4.4.3 Gene Structure in Eukaryotes		63
4.5	Gene Expression		64
	4.5.1 From Genes to Proteins		65
	4.5.2 Transcription and Translation		66
	4.5.3 Regulation of Gene Expression		67
4.6	Genes and Genomes		69
	4.6.1 Genome Size and Complexity		70
	4.6.2 Genome Organisation		71
	4.6.3 The Transcriptome and Proteome		72
4.7	Conclusion: Structure and Function		73
	Further Reading		74
	Websearch		75
	Concept Map		76
Ch	The Tools of the Trade		78
	Chapter Summary		78
5.1	Restriction Enzymes – Cutting DNA		79
	5.1.1 Type II Restriction Endonucleases		80
	5.1.2 Use of Restriction Endonucleases		81
	5.1.3 Restriction Mapping		84
5.2	DNA Modifying Enzymes		84
	5.2.1 Nucleases		85
	5.2.2 Polymerases		86
	5.2.3 Enzymes That Modify the Ends of DNA	A Molecules	87
	DNA Ligase – Joining DNA Molecules	44.4	88
5.4	Conclusion: The Genetic Engineer's T	oolkit	88
	Further Reading		90
	Websearch		91
	Concept Map		92
Ch	napter 6 Working with Nucleic A	cids	94
	Chapter Summary		94
	Evolution of the Laboratory		95
	Isolation of DNA and RNA		99
6.3	Handling and Quantification of Nucle	eic Acids	100



6.6 Gel Electrophoresis 6.7 DNA Sequencing: The First Generation 6.7.1 Principles of First-Generation DNA Sequencing 6.7.2 Sanger (Dideoxy or Enzymatic) Sequencing 6.7.3 Electrophoresis and Reading of Sequences 6.7.4 Automation and Scale-Up of DNA Sequencing 6.8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors
6.4.2 End Labelling 6.4.3 Nick Translation 6.4.4 Labelling by Primer Extension 6.4.4 Labelling by Primer Extension 6.5 Nucleic Acid Hybridisation 6.6 Gel Electrophoresis 7 DNA Sequencing: The First Generation 6.7.1 Principles of First-Generation DNA Sequencing 6.7.2 Sanger (Dideoxy or Enzymatic) Sequencing 6.7.3 Electrophoresis and Reading of Sequences 6.7.4 Automation and Scale-Up of DNA Sequencing 8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors 13 14 15 17 17 17 17 17 17 17 17 17 17 17 17 17
6.4.3 Nick Translation 6.4.4 Labelling by Primer Extension 10 6.4.4 Labelling by Primer Extension 11 12 15 Nucleic Acid Hybridisation 16 16 Gel Electrophoresis 17 17 18 19 19 10 11 11 11 11 11 11 11 11 11 11 11 11
6.4.4 Labelling by Primer Extension 6.5 Nucleic Acid Hybridisation 6.6 Gel Electrophoresis 6.7 DNA Sequencing: The First Generation 6.7.1 Principles of First-Generation DNA Sequencing 6.7.2 Sanger (Dideoxy or Enzymatic) Sequencing 6.7.3 Electrophoresis and Reading of Sequences 6.7.4 Automation and Scale-Up of DNA Sequencing 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bascteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.5 Nucleic Acid Hybridisation 6.6 Gel Electrophoresis 6.7 DNA Sequencing: The First Generation 6.7.1 Principles of First-Generation DNA Sequencing 6.7.2 Sanger (Dideoxy or Enzymatic) Sequencing 6.7.3 Electrophoresis and Reading of Sequences 6.7.4 Automation and Scale-Up of DNA Sequencing 6.8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.6 Gel Electrophoresis 6.7 DNA Sequencing: The First Generation 6.7.1 Principles of First-Generation DNA Sequencing 6.7.2 Sanger (Dideoxy or Enzymatic) Sequencing 6.7.3 Electrophoresis and Reading of Sequences 6.7.4 Automation and Scale-Up of DNA Sequencing 6.8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2.2 Plasmid Vectors for Use in E. coli 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
5.7 DNA Sequencing: The First Generation 6.7.1 Principles of First-Generation DNA Sequencing 6.7.2 Sanger (Dideoxy or Enzymatic) Sequencing 6.7.3 Electrophoresis and Reading of Sequences 6.7.4 Automation and Scale-Up of DNA Sequencing 6.8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.7.1 Principles of First-Generation DNA Sequencing 6.7.2 Sanger (Dideoxy or Enzymatic) Sequencing 6.7.3 Electrophoresis and Reading of Sequences 6.7.4 Automation and Scale-Up of DNA Sequencing 6.8.8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.7.2 Sanger (Dideoxy or Enzymatic) Sequencing 6.7.3 Electrophoresis and Reading of Sequences 6.7.4 Automation and Scale-Up of DNA Sequencing 5.8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 5.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.3.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.7.3 Electrophoresis and Reading of Sequences 6.7.4 Automation and Scale-Up of DNA Sequencing 5.8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 5.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 13 15 16 17 17 18 18 19 19 10 10 11 11 12 13 14 15 15 16 17 17 17 17 18 18 18 18 18 18 18 18 18 18 18 18 18
6.7.4 Automation and Scale-Up of DNA Sequencing 6.8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.8 Next-Generation Sequencing Technologies 6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.8.1 NGS – A Step-Change in DNA Sequencing 6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.8.2 Principles of NGS 6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.8.3 NGS Methodologies 6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
6.9 Conclusion: Essential Techniques and Methods Further Reading Websearch Concept Map The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
Further Reading Websearch Concept Map The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
Websearch Concept Map The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
Concept Map The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
 Part 3 The Methodology of Gene Manipulation Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
Chapter 7 Host Cells and Vectors Chapter Summary 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 13 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
 7.1 Types of Host Cell 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in <i>E. coli</i> 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in <i>E. coli</i> 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
 7.1.1 Prokaryotic Hosts 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
 7.1.2 Eukaryotic Hosts 7.2 Plasmid Vectors for Use in E. coli 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in E. coli 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
 7.2 Plasmid Vectors for Use in <i>E. coli</i> 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in <i>E. coli</i> 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ
 7.2.1 What Are Plasmids? 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in <i>E. coli</i> 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ 14 15 16 17 18 19 19 19 19 19 10 10 10 11 12 12 13 14 15 16 17 18 19 19 19 19 10 10<!--</td-->
 7.2.2 Basic Cloning Plasmids 7.2.3 Slightly More Exotic Plasmid Vectors 7.3 Bacteriophage Vectors for Use in <i>E. coli</i> 7.3.1 What Are Bacteriophages? 7.3.2 Vectors Based on Bacteriophage λ 14 15 16 17 18 19 19
7.2.3 Slightly More Exotic Plasmid Vectors 13 7.3 Bacteriophage Vectors for Use in <i>E. coli</i> 14 7.3.1 What Are Bacteriophages? 14 7.3.2 Vectors Based on Bacteriophage λ 14
7.3 Bacteriophage Vectors for Use in <i>E. coli</i> 14 7.3.1 What Are Bacteriophages? 14 7.3.2 Vectors Based on Bacteriophage λ 14
7.3.1 What Are Bacteriophages? 14 7.3.2 Vectors Based on Bacteriophage λ 14
7.3.2 Vectors Based on Bacteriophage λ
7.4 Other Vectors
7.4.1 Hybrid Plasmid/Phage Vectors 14
7.4.2 Vectors for Use in Eukaryotic Cells
7.4.3 Artificial Chromosomes
7.5 Getting DNA into Cells
7.5.1 Transformation and Transfection
7.5.2 Packaging Phage DNA In Vitro
7.5.3 Alternative DNA Delivery Methods
7.5.3 Alternative DNA Delivery Methods 15 7.6 Conclusion: From <i>In Vitro</i> to <i>In Vivo</i> 15
· · · · · · · · · · · · · · · · · · ·
7.6 Conclusion: From In Vitro to In Vivo



Ch	apter 8	Cloning Strategies	160
	Chapter S	ummary	160
8.1	Which Ap	pproach Is Best?	161
	8.1.1 Cloni	ng in the Pre-genomic Era	162
	8.1.2 Cloni	ng (or Not) in the Genomic and Post-genomic Eras	162
8.2	Generatin	g DNA Fragments for Cloning	165
	8.2.1 Geno	mic DNA	165
	8.2.2 Synth	nesis of cDNA	165
	8.2.3 PCR I	Fragments	168
	8.2.4 Synth	etic Biology: Making Genes from Scratch	168
8.3	Inserting	DNA fragments into Vectors	169
	8.3.1 Ligati	on of Blunt/Cohesive-Ended Fragments	169
	8.3.2 Homo	ppolymer Tailing	170
	8.3.3 Linke	rs and Adapters	170
	8.3.4 Other	Methods for Joining DNA Fragments and Vectors	173
8.4	Putting It	All Together	175
	8.4.1 Cloni	ng in a λ Replacement Vector	176
	8.4.2 Expre	ession of Cloned cDNA Molecules	177
	8.4.3 Cloni	ng Large DNA Fragments in BAC and YAC Vectors	178
	8.4.4 Gatev	vay Cloning Technology	180
	8.4.5 Golde	n Gate Cloning and Assembly	180
	8.4.6 The C	Gibson Assembly Method	183
8.5	Conclusio	n: Designing a Cloning Strategy	184
	Further R	eading	184
	Websearc	·h	185
	Concept N	Мар	186
Ch	apter 9	The Polymerase Chain Reaction	188
	Chapter S	ummary	188
9.1	History of	f the PCR	189
9.2	The Meth	odology of the PCR	190
	9.2.1 Essen	tial Features of the PCR	190
	9.2.2 Desig	ning Primers for the PCR	192
	9.2.3 DNA	Polymerases for the PCR	194
9.3	More Exo	tic PCR Techniques	195
	9.3.1 PCR U	Jsing mRNA Templates	195
	9.3.2 Neste	d PCR	198
	9.3.3 Inver	se PCR	199
	9.3.4 Quan	titative and Digital PCR	199
	9.3.5 RAPD	and Several Other Acronyms	202
9.4	Processing	g and Analysing PCR Products	205
9.5	Conclusio	n: The Game-Changing Impact of the PCR	205
	Further R	eading	206
	Websearc	h	207
	Concept N	Man	208



DETAILED CONTENTS

Χİ

Cha	pter 10	Selection, Screening and Analysis	
		of Recombinants	210
	Chapter S	ummary	210
10.1	_	election and Screening Methods	212
		of Chromogenic Substrates	212
		rtional Inactivation	213
	10.1.3 Com	plementation of Defined Mutations	214
	10.1.4 Othe	er Genetic Selection Methods	215
10.2	Screening	Using Nucleic Acid Hybridisation	216
	10.2.1 Nucl	eic Acid Probes	216
	10.2.2 Scree	ening Clone Banks	218
10.3	Use of the	PCR in Screening Protocols	220
10.4	Immunolo	ogical Screening for Expressed Genes	221
10.5	Analysis o	of Cloned Genes	222
	10.5.1 Resta	riction Mapping	222
	10.5.2 Blott	ring Techniques	223
	10.5.3 Sub-	cloning	225
	10.5.4 DNA	Sequencing	225
10.6	Conclusio	n: Needles in Haystacks	226
	Further R	eading	227
	Websearc	h	227
	Concept M	Лар	229
Cha	pter II	Bioinformatics	230
	Chapter S	ummary	230
11.1	What Is B	ioinformatics?	231
	11.1.1 Com	puting Technology	232
	11.1.2 The	Impact of the Internet and World Wide Web	234
11.2	Biological	Data Sets	234
	11.2.1 Gene	eration and Organisation of Information	234
	11.2.2 Prim	ary and Secondary Databases	235
	11.2.3 Nucl	eic Acid Databases	236
	11.2.4 Prote	ein Databases	237
	11.2.5 Othe	er Bioinformatics Resources	239
11.3	Using Bioi	informatics as a Tool	241
	11.3.1 Avoi	ding the 'GIGO' Effect – Real Experiments	241
	11.3.2 Avoi	ding the Test Tube – Computational Experimentation	242
	11.3.3 Prese	entation of Database Information	243
11.4	Conclusio	n: Bioscience and 'Big Data'	244
	Further R	eading	245
	Websearc	h	246
	Concept M	Мар	247
Cha	pter I2	Genome Editing	248
	Chapter S	ummary	248
12.1	Gene Targ	b and a second s	250
		Editing Using Engineered Nucleases	251
		Finger Nucleases	251



xii

	12.2.2 TALENS	253
	12.2.3 The CRISPR-Cas9 System	253
	12.2.4 Prime Editing	256
12.3	Editing RNA as an Option	258
12.4	Where Can Genome Editing Take Us?	258
12.5	Conclusion: From Genome Read to Genome Write	259
	Further Reading	260
	Websearch	260
	Concept Map	261
Part	Genetic Engineering in Action	
Cha	pter 13 Investigating Genes, Genomes	
	and 'Otheromes'	264
	Chapter Summary	264
13.1	Analysis of Gene Structure and Function	265
	13.1.1 A Closer Look at Sequences	265
	13.1.2 Finding Important Regions of Genes	266
10.0	13.1.3 Investigating Gene Expression	270
13.2	Understanding Genomes	272
	13.2.1 Analysing and Mapping Genomes	273
	13.2.2 An Audacious Idea	276
	13.2.3 The Human Genome Project	277
199	13.2.4 Other Genome Projects 'Otheromes'	281 282
13.3		
	13.3.1 The Transcriptome 13.3.2 The Proteome	282 285
	13.3.3 Metabolomes, Interactomes and More	285 286
19.4		
13.4	Life in the Post-genomic Era 13.4.1 Structural Genomics and Proteomics	288
	13.4.1 Structural Genomics and Proteomics	289
	13.4.3 Comparative Genomics	289 289
12.5	Conclusion: The Central Role of the Genome	289 291
13.3	Further Reading	291
	Websearch	292
	Concept Map	294
Cha	pter 14 Genetic Engineering and Biotechnology	296
	Chapter Summary	296
14.1	Making Proteins	297
1 1.1	14.1.1 Native and Fusion Proteins	299
	14.1.2 Yeast Expression Systems	300
	14.1.3 The Baculovirus Expression System	301
	14.1.4 Mammalian Cell Lines	302
14.2	Protein Engineering	303
- 1.4	14.2.1 Rational Design	303
	14.2.2 Directed Evolution	305



DETAILED CONTENTS

xiii

14.3	From Laboratory to Production Plant	308
	14.3.1 Thinking Big – The Biotechnology Industry	308
	14.3.2 Production Systems	310
	14.3.3 Scale-Up Considerations	310
	14.3.4 Downstream Processing	312
14.4	Examples of Biotechnological Applications of	
	rDNA Technology	312
	14.4.1 Production of Enzymes	313
	14.4.2 The BST Story	314
	14.4.3 Therapeutic Products for Use in Human Healthcare	316
	14.4.4 Meeting the COVID-19 Challenge	320
14.5	Conclusion: Industrial-Scale Biology	322
	Further Reading	323
	Websearch	324
	Concept Map	325
Cha	pter 15 Medical and Forensic Applications	
	of Gene Manipulation	326
	Chapter Summary	326
15.1	Diagnosis and Treatment of Medical Conditions	327
	15.1.1 Diagnosis of Infection	327
	15.1.2 Patterns of Inheritance	328
	15.1.3 Genetically Based Disease Conditions	330
	15.1.4 Investigating Disease Alleles Using Comparative Genomics	337
	15.1.5 Vaccine Development Using rDNA	338
	15.1.6 Therapeutic Antibodies	339
	15.1.7 Xenotransplantation	341
15.2	Treatment Using rDNA Technology – Gene Therapy	342
	15.2.1 Getting Transgenes into Patients	343
	15.2.2 Gene Therapy for Adenosine Deaminase Deficiency	344
	15.2.3 Gene Therapy for Cystic Fibrosis	346
	15.2.4 What Does the Future Hold for Gene Therapy?	346
15.3	RNA Interference	347
	15.3.1 What Is RNAi?	347
	15.3.2 Using RNAi as a Tool for Studying Gene Expression	348
	15.3.3 RNAi as a Potential Therapy	348
	15.3.4 Antisense Oligonucleotides	350
15.4	Medical Applications of Genome Editing	350
	15.4.1 Disease Targets for Genome Editing	350
	15.4.2 Sickle-Cell Success	351
	15.4.3 CRISPR-Cas9 – CAR T-Cell Therapies in Cancer Treatment	352
	15.4.4 The CCR5 Controversy	353
15.5	DNA Profiling	354
	15.5.1 The History of 'Genetic Fingerprinting'	354
	15.5.2 DNA Profiling and the Law	356
	15.5.3 Mysteries of the Past Revealed by Genetic Detectives	356
15.6	Conclusion: rDNA in Diagnosis, Analysis and Treatment	358
	Further Reading	359
	Websearch	360
	Concept Map	361



xiv

Chapter 16 Tran	nsgenic Plants and Animals	362
Chapter Summa		362
16.1 A Complex Land	•	363
16.2 Transgenic Plan	-	365
16.2.1 Why Transg		365
16.2.2 Making Trai		365
=	Technology to Work	369
_	genic Plants Delivered or Disappointed?	377
16.3 Transgenic Anin	= =	378
16.3.1 Why Transg		378
16.3.2 Producing T		379
_	s of Transgenic Animal Technology	380
16.4 Future Trends	or transgeme rammar recamology	383
	s or Genome Editing?	384
16.4.2 Gene Drives	_	384
	nging Genomes and Attitudes	385
Further Reading		386
Websearch	•	387
Concept Map		389
Chapter 17 The	Other Sort of Cloning	390
Chapter Summa	_	390
17.1 Early Thoughts	-	391
17.1.1 First Steps t	-	393
17.1.2 Nuclear Tot	C	393
17.2 Frogs and Toads		394
-	– The Breakthrough Achieved	396
17.4 Beyond Dolly	The Breaking againement	398
17.4.1 Potential Ur	nfulfilled?	399
	of Organismal Cloning	400
	m Genome to Organism	401
Further Reading	=	402
Websearch	•	403
Concept Map		404
Glossary		405
Index		439
IIIUCA		439



Preface

Advances in genetics continue to be made at an ever increasing rate, which presents something of a dilemma when writing an introductory text on the subject. In the years since the third edition was published, many new applications of gene manipulation technology have been developed; genome sequencing has become available at bench-top scale and cost, and gene editing can be achieved using very modest laboratory infrastructure. Personal genome profiling is available from a range of companies, and genetic technology has played a major role in managing many aspects of the COVID-19 pandemic, from diagnostic testing to rapid development of safe and effective vaccines.

Information technology resources, coupled with the internet and World Wide Web, have been critical parts of all these developments, providing tools for the analysis of DNA sequences and instant sharing of data across the globe. At the same time, a level of mistrust has developed among some sections of society, largely driven by misinformation on social media channels, which has illustrated the power of the internet in a less positive way. It is against this background that some themes began to emerge for the fourth edition, reflecting the aim of encouraging students to use the excellent resources on the web, whilst retaining a level of critical assessment of the information. Aspects around the ethics of using genetic technology are perhaps now even more important than before, so these are discussed early in the text to enable the applications to be placed within an appreciation of the ethical framework.

Whilst aiming for a slight broadening in scope, I remain convinced that a basic technical introduction to the subject should be the major focus of the text. Thus, some of the original methods used in gene manipulation have been kept as examples of how the technology developed, even though some of these have become little used or even obsolete. From the educational point of view, this should help the reader cope with more advanced information about the subject, as a sound grasp of the basic principles is an important part of any introduction to genetic engineering. I have been gratified by the many positive comments about the third edition of the text, and I hope that this new edition continues to serve a useful purpose as part of the introductory literature on this fascinating subject.

This book is organised as four parts. Part 1 (Genetic Engineering in Context; Chapters 1–3) sets the scene and brings the discussion of the ethical issues around DNA technology to the start of the book. Part 2 (The Basis of Genetic Engineering; Chapters 4–6) provides an introduction to molecular biology and outlines the tools available to the genetic engineer, and Part 3 (The Methodology of Gene Manipulation; Chapters 7–12) extends this theme further by examining how these tools enable



xvi

PREFACE

sophisticated experiments and procedures to be carried out. Finally, in *Part 4 (Genetic Engineering in Action*; Chapters 13–17), we look at the impact of DNA technology across a range of key areas.

In the fourth edition, I have expanded the range of features that should be useful as study aids where the text is used to support a particular academic course. In the book, there are text boxes sprinkled throughout the chapters. These highlight key points on the way through the text, and can be used as a means of summarising the content. At the start of each chapter, the aims of the chapter are presented, along with a chapter summary in the form of learning objectives. These have been written quite generally, so that an instructor can modify them to suit the level of detail required. A list of the key words in each chapter is also provided for reference. These are shown as bold in the text; terms in blue can also be found in the Glossary. A new addition to the end of each chapter is a websearch page that provides some structured web-based search exercises that help to set the chapter in context and act as a start point for further study using the resources available online. As in previous editions, a concept map has been generated for each chapter, showing how the main topics are linked. The concept maps provided here are essentially summaries of the chapters, and may be examined either before or after reading the chapter.

As this remains an introductory text, no in-text reference has been made to the primary (research) literature, but some suggestions for *further reading* are given at the end of each chapter. Most of these are available in open-access format or may be available through an institution's library subscription service. A *glossary* of terms used has also been provided.

A new development for the fourth edition is a set of *online resources* at www.cambridge.org/nicholl4. This provides access to a range of materials from the book (and additional information) that I hope will be useful in building a learning system to suit your preferred learning style. The resources have been provided in electronic format as a *study guide* to enable collation into a set of student-generated notes.

My thanks go to the anonymous (but appreciated) reviewers of the proposal and the early versions of the manuscript. Their comments and suggestions have made the book better; any errors of fact or interpretation of course remain my own responsibility. Special thanks to Megan Keirnan, Susan Francis, Helen Shannon and Rachel Norridge at Cambridge University Press, and to Joyce Cheung, for their cheerful advice, support, encouragement and patience, which helped bring the project to its conclusion.

My final and biggest thank you goes as ever to my wife Linda and to Charlotte, Thomas and Anna, who have grown up along with the various editions of 'IGE'. I dedicate this new edition to them.