Molecular Biology and Biotechnology

Edited by John M Walker and Ralph Rapley



RSCPublishing

Molecular Biology and Biotechnology
5th Edition

Edited by

John M Walker

School of Life Sciences, University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK

Ralph Raply

School of Life Sciences, University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK





RSCPublishing

ISBN: 978-0-85404-125-1

A catalogue record for this book is available from the British Library

© Royal Society of Chemistry, 2009

All rights reserved

Apart from fair dealing for the purposes of research for non-commercial purposes or for private study, criticism or review, as permitted under the Copyright, Designs and Patents Act 1988 and the Copyright and Related Rights Regulations 2003, this publication may not be reproduced, stored or transmitted, in any form or by any means, without the prior permission in writing of The Royal Society of Chemistry or the copyright owner, or in the case of reproduction in accordance with the terms of licences issued by the Copyright Licensing Agency in the UK, or in accordance with the terms of the licences issued by the appropriate Reproduction Rights Organization outside the UK. Enquiries concerning reproduction outside the terms stated here should be sent to The Royal Society of Chemistry at the address printed on this page.

Published by The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our website at www.rsc.org

Molecular Biology and Biotechnology 5th Edition

Preface

One of the exciting aspects of being involved in the field of molecular biology is the ever-accelerating rate of progress, both in the development of new methodologies and in the practical applications of these methodologies. Indeed, such developments led to the idea of the first edition of *Molecular Biology and Biotechnology* and subsequent editions have reflected the fast-moving nature of the area, not least this latest edition, which continues to reflect recent developments, with new chapters in developing areas such as genome technology, nanobiotechnology, regenerative medicine and biofuels.

The first six chapters deal with the technology used in current molecular biology and biotechnology. These deal primarily with core nucleic acid techniques and protein expression through microbial and genetic detection methods. Further chapters address the huge advances made in gene and genome analysis and update the rapid advances into yeast analysis, which is providing very exciting insights into molecular pathways. Molecular biology also continues to affect profoundly progress in biotechnology in areas such as vaccine development, use and application of monoclonal antibodies, clinical treatment and diagnosis, the production of transgenic animals, and many other areas of research relevant to the pharmaceutical industry. Chapters on all these areas have been retained and fully updated in this new edition and new chapters introduced on the applications of molecular biology in the areas of drug design and diseases, and regenerative medicine. In addition, we continue to ensure that biotechnology is not just considered at the gene level and full consideration continues to be given to applications

Molecular Biology and Biotechnology, 5th Edition Edited by John M Walker and Ralph Rapley © Royal Society of Chemistry 2009 Published by the Royal Society of Chemistry, www.rsc.org manufacturing, with chapters on downstream processing, biosensors, the applications of immobilised biocatalysts, and a new chapter on the developing area of biofuels.

Our continued intention is that this book should primarily have a teaching function. As such, this book should prove of interest both to undergraduates studying for biological or chemical qualifications and to postgraduate and other scientific workers who need a sound introduction to this ever rapidly advancing and expanding area.

John M. Walker Ralph Rapley

Chapter 1	Basic Molecular Biology Techniques <i>Ralph Rapley</i>				
	 1.1 Enzymes Used in Molecular Biology 1.2 Isolation and Separation of Nucleic Acids 1.2.1 Isolation of DNA 1.2.2 Isolation of RNA 1.3 Electrophoresis of Nucleic Acids 1.4 Restriction Mapping of DNA Fragments 1.5 Nucleic Acid Analysis Methods 1.5.1 DNA Blotting 	1 3 3 5 6 8 8			
Chapter 2	1.5.2 RNA Blotting 1.6 Gene Probe Derivation 1.7 Labelling DNA Gene Probe Molecules 1.7.1 End Labelling of DNA Molecules 1.7.2 Random Primer Labelling 1.7.3 Nick Translation 1.8 The Polymerase Chain Reaction References Molecular Cloning and Protein Expression	10 11 12 13 14 15 15			
•	Stuart Harbron 2.1 Introduction 2.2 Host-related Issues 2.3 Vectors 2.4 Expression Systems 2.4.1 The pET Expression System 2.4.2 The pBAD Expression System	20 21 24 30 30 33			

Molecular Biology and Biotechnology, 5th Edition Edited by John M Walker and Ralph Rapley © Royal Society of Chemistry 2009 Published by the Royal Society of Chemistry, www.rsc.org viii Contents

	2.5 Problems	34
	2.6 Fusion Proteins	37
	2.6.1 Solubility-enhancing Tags	37
	2.6.2 Purification-facilitating Tags	40
	2.6.3 HT Approaches	43
	2.7 Other Hosts	44
	2.8 Cell-free Systems	44
	2.9 Conclusion	45
	References	45
Chapter 3	Molecular Diagnostics	
	Laura J. Tafe, Claudine L. Bartels, Joel A. Lefferts and	
	Gregory J. Tsongalis	
	3.1 Introduction	51
	3.2 Technologies	52
	3.3 The Infectious Disease Paradigm	53
	3.4 Genetics	55
	3.5 Hematology	59
	3.6 Oncology	62
	3.7 Pharmacogenomics	65 71
	3.8 Conclusion References	71
	References	/1
Chapter 4	Molecular Microbial Diagnostics	
	Karl-Henning Kalland	
	4.1 Introduction	76
	4.2 Classical Microbiological Diagnosis	78
	4.3 Sample Collection and Nucleic Acid Purification	79
	4.3.1 Sample Collection and Transport	79
	4.3.2 Extraction of Nucleic Acids	80
	4.3.3 Manual Extraction of Nucleic Acids	81
	4.3.4 Automated Extraction of Nucleic Acids	81
	4.4 Nucleic Acid Amplification Techniques	81
	4.4.1 Polymerase Chain Reaction (PCR)	81
	4.4.2 The Contamination Problem	82
	4.4.3 Reverse PCR – cDNA Synthesis	82
	4.4.4 Nested PCR	83
	4.4.5 Real-time PCR	83
	4.4.6 Visualisation of Real-time PCR Amplification	84
	4.4.7 Real-time PCR Equipment4.4.8 Real-time Quantitative PCR	86 86
	4.4.8 Real-time Quantitative PCR 4.4.9 Determination of 'Viral Load' in Clinical	80
	Microbiology	87
	Microbiology	0/

		4.4.10 Internal Controls in Microbiological	
		Real-time qPCR	8′
		4.4.11 Multiplex Real-time PCR	88
		4.4.12 Melting Curve Analysis	88
		4.4.13 Genotyping	89
	4.5	Other Techniques Used in Clinical Microbiology	90
		4.5.1 Hybridisation Techniques	90
		4.5.2 Nucleic Acid-based Typing of Bacteria	93
		4.5.3 Pyrosequencing	97
		4.5.4 TaqMan Low-density Arrays (TLDAs)	98
	4.6	Selected Examples of Clinical Nucleic	
		Acid-based Diagnosis	99
		4.6.1 Central Nervous System (CNS) Disease	99
		4.6.2 Respiratory Infections	100
		4.6.3 Hepatitis	100
		4.6.4 Gastroenteritis	101
		4.6.5 Sexually Transmitted Diseases	102
		4.6.6 HIV Infection and AIDS	103
		4.6.7 Bacterial Antibiotic Resistance and Virulence	ce
		Factor Genes	104
	4.7	Conclusion and Future Challenges	106
	Ref	erences	107
Chapter 5	Gen	nes and Genomes	
-		vid B. Whitehouse	
	5.1	Introduction	112
		5.1.1 Background	112
	5.2	Key DNA Technologies	115
		5.2.1 Molecular Cloning Outline	115
		5.2.2 Cloning Vectors	115
		5.2.3 The Cloning Process	118
		5.2.4 DNA Libraries	120
	5.3	The Polymerase Chain Reaction (PCR)	122
		5.3.1 Steps in the PCR	123
		5.3.2 PCR Primer Design and Bioinformatics	125
		5.3.3 Reverse Transcriptase PCR (RT-PCR)	126
		5.3.4 Quantitative or Real-time PCR	126
	5.4	DNA Sequencing	128
		5.4.1 Dideoxynucleotide Chain	
		Terminators	129
		5.4.2 Sequencing Double-stranded DNA	129
		5.4.3 PCR Cycle Sequencing	130
		, , , , , , , , , , , , , , , , , , , ,	150
		5.4.4 Automated DNA Sequencing 5.4.5 Pyrosequencing	130

X Contents

	5.5	Genome Analysis	131
		5.5.1 Mapping and Identifying Genes	131
		5.5.2 Tools for Genetic Mapping	132
		5.5.3 Mutation Detection	139
	5.6	Genome Projects Background	144
		5.6.1 Mapping and Sequencing Strategies	144
	5.7	Gene Discovery and Localisation	149
		5.7.1 Laboratory Approaches	149
		5.7.2 Bioinformatics Approaches	150
	5.8	Future Directions	152
	Refe	erences	153
Chapter 6	The	Biotechnology and Molecular Biology of Yeast	
Chapter 0		ndan P. G. Curran and Virginia C. Bugeja	
	6.1	Introduction	159
	6.2	The Production of Heterologous Proteins by Yeast	161
		6.2.1 The Yeast Hosts	161
		6.2.2 Assembling and Transforming Appropriate	
		DNA Constructs into the Hosts	162
		6.2.3 Ensuring Optimal Expression of the Desired	
		Protein	165
	6.3	From Re-engineering Genomes to Constructing Novel	
		Signal and Biochemical Pathways	170
		6.3.1 Large-scale Manipulation of Mammalian and	
		Bacterial DNA	170
		6.3.2 Novel Biological Reporter Systems	176
		6.3.3 Novel Biochemical Products Include	
		Humanised EPO	179
	6.4	Yeast as a Paradigm of Eukaryotic Cellular Biology	187
		6.4.1 Genomic Insights	187
		6.4.2 Transcriptomes, Proteomes and Metabolomes	
		and Drug Development	188
		6.4.3 Systems Biology	190
	6.5	Future Prospects	191
		erences	191
Chapter 7	Met	abolic Engineering	
P /		fan Kempa, Dirk Walther, Oliver Ebenhoeh and Wolfram	
		ckwerth	
	7.1	Introduction	196
	7.2	Theoretical Approaches for Metabolic Networks	197
		7.2.1 Kinetic Modelling	198

		7.2.2 Metabolic Control Analysis (MCA),	
		Elementary Flux Modes (EFM) and	
		Flux-balance Analysis (FBA)	202
	7.3	1	g 208
		7.3.1 Tools for Metabolic Engineering	208
		7.3.2 Metabolomics	209
		7.3.3 Metabolomics in the Context of Metaboli	c
		Engineering	210
	7.4	I amount Engineering	211
		7.4.1 Metabolic Engineering of Plants	211
		7.4.2 Acetate Metabolism and Recombinant Protei	
		Synthesis in E. coli – a Test Case for Metaboli	c
		Engineering	213
		7.4.3 Metabolic Flux Analysis and a Bioartificial Live	er 213
	7.5	Bree open frem felispeetives for	
		Metabolic Engineering	214
	7.6		215
	Ref	ferences	215
~! ·			
Chapter 8		nanotechnology	
	Da	vid W. Wright	
	8.1	Introduction	220
	8.2	Semiconductor Quantum Dots	222
		8.2.1 Quantum Confinement Effects	222
		8.2.2 Biotechnological Applications of Fluorescen	
		Semiconductor Quantum Dots	224
	8.3	Magnetic Nanoparticles	226
		8.3.1 Nanoscaling Laws and Magnetism	226
		8.3.2 Biotechnological Applications of Magnetic	
		Nanoparticles	228
	8.4		232
		8.4.1 Nanoscale Properties of Zerovalent Nobel	232
		Metal Nanoparticles	232
		8.4.2 Bionanotechnology Application of Zerovalen	
		Noble Metal Nanoparticles	234
	8.5	Making Nanoscale Structures Using Biotechnology	236
	8.6	Conclusions	241
	Refe	erences	241
Chapter 9	Mol	ecular Engineering of Antibodies	
pres y		nes D. Marks	
	9.1	Introduction	245
	9.2	Antibodies as Therapeutics	246

xii Contents

	9.3	Antibody	Structure and Function	250
	9.4		Antibodies	251
	9.5		/ Humanization	255
	9.6		es from Diversity Libraries and Display	
		Technolo		256
			ntibody Phage Display	257
			lternative Display Technologies	262
	9.7		ing Antibody Affinity	263
	9.8		ng Antibody Potency	264
	9.9	Conclusi		265
		rences		265
Chapter 10				
	Mici	hael G. K.	Jones	
	10.1	Introdu	ction	272
	10.2		tions of Molecular Biology to Speed Up the	
			es of Crop Improvement	273
		10.2.1	Molecular Maps of Crop Plants	273
		10.2.2	Molecular Markers	274
		10.2.3	Types of Molecular Markers	274
		10.2.4	Marker-assisted Selection	275
		10.2.5	Examples of Marker-assisted Selection	27ϵ
		10.2.6	Molecular Diagnostics	277
		10.2.7	DNA Fingerprinting, Variety Identification	278
		10.2.8	DNA Microarrays	279
		10.2.9	Bioinformatics	279
	10.3	Transge	enic Technologies	279
		10.3.1	Agrobacterium-mediated Transformation	280
		10.3.2	Selectable Marker and Reporter Genes	280
		10.3.3	Particle Bombardment	281
	10.4	Applica	tions of Transgenic Technologies	281
	10.5	Enginee	ering Crop Resistance to Herbicides	283
	10.6	Enginee	ering Resistance to Pests And Diseases	284
		10.6.1	Insect Resistance	284
		10.6.2	Engineered Resistance to Plant Viruses	285
		10.6.3	Resistance to Fungal Pathogens	287
		10.6.4	Natural Resistance Genes	288
		10.6.5	Engineering Resistance to Fungal Pathogens	290
		10.6.6	Resistance to Bacterial Pathogens	291
		10.6.7	Resistance to Nematode Pathogens	292
	10.7	-	lating Male Sterility	292
	10.8		ice to Abiotic Stresses	293
	10.9		lating Quality	294
		10.9.1	Prolonging Shelf Life	294

10.9.2	Nutritional and Technological Properties	294
10.9.3	Manipulation of Metabolic Partitioning	297
10.10 Produ	ction of Plant Polymers and Biodegradable	
Plastic		298
10.11 Plants	as Bioreactors: Biopharming and	270
	aceuticals	298
	l Edible Vaccines	
	2 Production of Antibodies in Plants	298
	3 Plant Neutraceuticals	299
	Biotechnology in Forestry	299
	ctual Property	300
10.14 Public	Acceptance	300
10.14 Fublic	Progrante	301
References	Frospects	302
References		303
Chapter 11 Biotechnology	hasad Drug Disasyary	
K. K. Jain	based Ding Discovery	
11.1 Introdu	ction to Drug Discovery	307
11.1.1	Basics of Drug Discovery in the Biopharma-	507
	ceutical Industry	307
11.1.2	Historical Landmarks in Drug Discovery and	307
	Development Development	308
11.1.3	Current Status of Drug Discovery	309
11.2 New Bio	otechnologies for Drug Discovery	310
	c Technologies for Drug Discovery	310
11.3.1	SNPs in Drug Discovery	311
11.3.2	Gene Expression Profiling	312
	Limitations of Genomics for Drug Discovery	312
	and Need for Other Omics	312
11.4 Role of	Proteomics in Drug Discovery	
11.4.1	Proteins as Drug Targets	313
11.4.2	Protein Expression Mapping by 2D Gel	313
	Electrophoresis	214
	Liquid Chromatography-based Drug	314
	Discovery	214
		314
	Matrix-assisted Laser Desorption/Ionisation	
	Mass Spectrometry	314
	Protein—Protein Interactions	315
11.4.0	Use of Proteomic Technologies for	
11.5 Metabol	Important Drug Targets	316
	omic and Metabonomic Technologies for	
Drug Di		317
	Nanobiotechnology	
in Drug	Discovery	318

xiv Contents

	11.6.1	Nanobiotechnology for Target Validation	318
	11.6.2	Nanotechnology-based Drug Design at	
		Cell Level	318
	11.6.3	Nanomaterials as Drug Candidates	319
11.7	Role of E	Biomarkers in Drug Discovery	320
11.8		g in Drug Discovery	320
		Cell-based Screening System	321
	11.8.2	Receptor Targets: Human versus Animal	
		Tissues	321
	11.8.3		322
11.9		alidation Technologies	322
	11.9.1	Animal Models for Genomics-based	
		Target Validation Methods	322
	11.9.2	Role of Knockout Mice in Drug Discovery	323
11.10		se for Drug Discovery	323
	11.10.1	Antisense Oligonucleotides for Drug	
		Target Validation	324
	11.10.2		324
		RNA as a Drug Target	325
	11.10.4	Ribozymes	325
11.11		or Drug Discovery	326
	11.11.1	Use of siRNA Libraries to Identify Genes	
		as Therapeutic Targets	327
	11.11.2	RNAi as a Tool for Assay Development	328
	11.11.3	Challenges of Drug Discovery with	
		RNAi	328
	11.11.4	Role of MicroRNA in Drug Discovery	329
11.12		s and Microarrays	
	_	g Discovery	329
	11.12.1	Finding Lead Compounds	330
	11.12.2	High-throughput cDNA Microarrays	330
	11.12.3	Use of Gene Expression Data to Find New	220
	11.12.5	Drug Targets	330
	11.12.4	Investigation of the Mechanism of Drug	
	11.12.1	Action	331
11.13	Applica	tions of Bioinformatics	551
11.13		g Discovery	331
	_	Combination of <i>In Silico</i> and <i>In vitro</i> Studies	332
11.14		Model Organisms in Drug Discovery	333
11.15		genomic Approach to Drug Discovery	334
11.16		Drug Development	334
11.17		f Biotechnology in Lead Generation and	557
11.1/	Validati		335
11.18	Conclus		335
Refer		51011	336
INVIV	CITCO		220

Chapter	12	Vaccines

Chapter 13

Niall McMullan

12.1	An Ov	erview of Vaccines and	
	Vaccin	ation	337
12.2	Types	of Vaccines in Current Use	338
		Live, Attenuated Vaccines	338
		Inactivated Vaccines	339
	12.2.3	Subunit Vaccines	340
12.3	The N	eed for New Vaccines	342
12.4	New A	approaches to Vaccine Development	343
	12.4.1	Recombinant Live Vectors	343
	12.4.2	Recombinant BCG Vectors	343
	12.4.3		344
	12.4.4	Recombinant Adenovirus Vectors	345
	12.4.5		346
	12.4.6	DNA Vaccines	346
12.5	Adjuva	ants	347
	12.5.1	Immune-stimulating Complexes (ISCOMs)	
		and Liposomes	347
	12.5.2	Freund-type Adjuvants	347
	12.5.3	CpG Oligonucleotides (CpG ODNs)	348
Refe	rences		349
	e Engine Link and	e ering I Martin Fussenegger	
13.1	Introdu	action	351
1011		Economic Impact of Healthcare	351
	13.1.2		352
	13.1.3	Treating Disease Through Tissue	332
	10.110	Engineering	353
13.2	Cell Ty		356
		Embryonic Stem Cells	356
	13.2.2	Adult Stem Cells	360
		Mature Cells	361
13.3		ellular Matrix	362
	13.3.1	Biological Extracellular Matrices	362
	13.3.2	Artificial Extracellular Matrices	364
13.4	Tissue	Engineering Concepts	369
		Cultivation of Artificial Tissues	369
	13.4.2		372
13.5	Conclu		373
Refer	ences		373

Chapter	14	Transgene	sis
---------	----	-----------	-----

Elizabeth J.	Cartwright	and Xin	Wano
Duzuotin o.	Curtivitgini	unu Am	" ung

	14.1	Introduction		390
		14.1.1	From Gene to Function	390
	14.2		enesis by DNA Pronuclear Injection	391
		14.2.1	Generation of a Transgenic Mouse	391
		14.2.2	Summary of Advantages and Disadvantages	
			of Generating Transgenic Mice by	
			Pronuclear Injection of DNA	397
	14.3	8 8 9		
		in Emb	oryonic Stem Cells	397
		14.3.1	Basic Principles	398
		14.3.2		400
		14.3.3	Summary of Advantages and Disadvantages	
			of Generating Gene Knockout Mice	404
	14.4	Condit	ional Gene Targeting	404
		14.4.1	Generation of a Conditional Knockout	
			Mouse Using the Cre-loxP System	406
		14.4.2	Chromosomal Engineering Using the	
			Cre-loxP System	410
		14.4.3	,	
			Disadvantages of Conditional Gene	
			Targeting	410
	14.5		ypic Analysis of Genetically	
			ed Mice	411
	14.6	Ethical	and Animal Welfare Considerations	412
		Conclu		414
	14.8		vledgements	414
	Refer	ences		415
Chapter 15	Prote	in Engin	eering	
-			nd Duncan McGregor	
	15.1	Introdu		418
		15.1.1	Protein Structures	419
	15.2	Tools o	f the Trade	420
		15.2.1	Sequence Identification	420
		15.2.2		420
		15.2.3	Sequence Modification	421
		15.2.4	Production	432
		15.2.5	Analysis	433
	15.3	Applications		434
		15.3.1	Point Mutations	434
		15.3.2	Domain Shuffling (Linking, Swapping	
			and Deleting)	435

Contents			xvii		
		15.3.3 Whole Protein Shuffling	441		
		15.3.4 Protein-Ligand Interactions	441		
		15.3.5 Towards De Novo Design	442		
	15.4	_	443		
	References				
Chapter 10		obilisation of Enzymes and Cells don F. Bickerstaff			
	Gordon 1. Bickerstay				
	16.1	Introduction	454		
	16.2	J	455		
		16.2.1 Enzymes	455		
		16.2.2 Ribozymes, Deoxyribozymes and Ribosomes	459		
		16.2.3 Splicesomes	460		
		16.2.4 Abzymes	461		
		16.2.5 Multienzyme Complexes	462		
		16.2.6 Cells	466		
	160	16.2.7 Biocatalyst Selection	468		
	16.3		469		
		16.3.1 Choice of Support Material	470		
	16.4	16.3.2 Choice of Immobilisation Procedures	474		
	16.4	F or million brocatary sts	483		
		16.4.1 Stability	483		
		16.4.2 Catalytic Activity	484		
	16.5	16.4.3 Coenzyme Regeneration	485		
	16.5	F F	487		
	Kefe	rences	489		
Chapter 17	Dow	nstream Processing			
	Daniel G. Bracewell, Mohammad Ali S. Mumtaz and				
	C. M.	Tark Smales			
	17.1	Introduction	492		
	17.2	Initial Considerations and Primary Recovery	493		
		17.2.1 Centrifugation and Filtration	494		
		17.2.2 Cell Lysis	494		
		17.2.3 Recovery of Material from Inclusion Bodies	495		
	17.3	Protein Precipitation	496		
	17.4	Chromatography	497		
		17.4.1 Ion-exchange Chromatography (IEX)	499		
		17.4.2 Affinity Chromatography	500		
		17.4.3 Hydrophobic Interaction Chromatography			
		(HIC)	501		
		17.4.4 Gel Filtration Chromatography	501		
	17.5	Alternatives to Packed Bed Chromatography	502		