

5th Edition

# Molecular Biology and Biotechnology

Edited by John M Walker and Ralph Rapley



RSC Publishing

# Contents

## Chapter 1 Basic Molecular Biology Techniques

*Ralph Rapley*

1.1	Enzymes Used in Molecular Biology	1
1.2	Isolation and Separation of Nucleic Acids	3
1.2.1	Isolation of DNA	3
1.2.2	Isolation of RNA	5
1.3	Electrophoresis of Nucleic Acids	6
1.4	Restriction Mapping of DNA Fragments	8
1.5	Nucleic Acid Analysis Methods	8
1.5.1	DNA Blotting	9
1.5.2	RNA Blotting	10
1.6	Gene Probe Derivation	11
1.7	Labelling DNA Gene Probe Molecules	12
1.7.1	End Labelling of DNA Molecules	13
1.7.2	Random Primer Labelling	14
1.7.3	Nick Translation	15
1.8	The Polymerase Chain Reaction	15
	References	18

## Chapter 2 Molecular Cloning and Protein Expression

*Stuart Harbron*

2.1	Introduction	20
2.2	Host-related Issues	21
2.3	Vectors	24
2.4	Expression Systems	30
2.4.1	The pET Expression System	30
2.4.2	The pBAD Expression System	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](http://www.rsc.org)

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
<b>Chapter 3</b>	<b>Molecular Diagnostics</b>	
	<i>Laura J. Tafe, Claudine L. Bartels, Joel A. Lefferts and Gregory J. Tsongalis</i>	
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
3.8	Conclusion	71
	References	71
<b>Chapter 4</b>	<b>Molecular Microbial Diagnostics</b>	
	<i>Karl-Henning Kalland</i>	
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 Equipment	86
4.4.8	Real-time Quantitative PCR	86
4.4.9	Determination of ‘Viral Load’ in Clinical Microbiology	87

4.4.10	Internal Controls in Microbiological Real-time qPCR	87
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 (TLDA)s	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 Factor Genes	104
4.7	Conclusion and Future Challenges	106
	References	107

## Chapter 5 Genes and Genomes

*David 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
5.4.4	Automated DNA Sequencing	130
5.4.5	Pyrosequencing	131



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
	References	153

## **Chapter 6 The Biotechnology and Molecular Biology of Yeast**

*Brendan 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
	References	191

## **Chapter 7 Metabolic Engineering**

*Stefan Kempa, Dirk Walther, Oliver Ebenhoeh and Wolfram Weckwerth*

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	Experimental Approaches for Metabolic Engineering	208
7.3.1	Tools for Metabolic Engineering	208
7.3.2	Metabolomics	209
7.3.3	Metabolomics in the Context of Metabolic Engineering	210
7.4	Examples in Metabolic Engineering	211
7.4.1	Metabolic Engineering of Plants	211
7.4.2	Acetate Metabolism and Recombinant Protein Synthesis in <i>E. coli</i> – a Test Case for Metabolic Engineering	213
7.4.3	Metabolic Flux Analysis and a Bioartificial Liver	213
7.5	Omics Technologies Open New Perspectives for Metabolic Engineering	214
7.6	Acknowledgement	215
	References	215
<b>Chapter 8</b>	<b>Bionanotechnology</b>	
	<i>David W. Wright</i>	
8.1	Introduction	220
8.2	Semiconductor Quantum Dots	222
8.2.1	Quantum Confinement Effects	222
8.2.2	Biotechnological Applications of Fluorescent 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	Zerovalent Noble Metal Nanoparticles	232
8.4.1	Nanoscale Properties of Zerovalent Nobel Metal Nanoparticles	232
8.4.2	Bionanotechnology Application of Zerovalent Noble Metal Nanoparticles	234
8.5	Making Nanoscale Structures Using Biotechnology	236
8.6	Conclusions	241
	References	241
<b>Chapter 9</b>	<b>Molecular Engineering of Antibodies</b>	
	<i>James D. Marks</i>	
9.1	Introduction	245
9.2	Antibodies as Therapeutics	246

9.3	Antibody Structure and Function	250
9.4	Chimeric Antibodies	251
9.5	Antibody Humanization	255
9.6	Antibodies from Diversity Libraries and Display Technologies	256
	9.6.1 Antibody Phage Display	257
	9.6.2 Alternative Display Technologies	262
9.7	Engineering Antibody Affinity	263
9.8	Enhancing Antibody Potency	264
9.9	Conclusion	265
	References	265

## Chapter 10 Plant Biotechnology

*Michael G. K. Jones*

10.1	Introduction	272
10.2	Applications of Molecular Biology to Speed Up the Processes 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	276
	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	Transgenic Technologies	279
	10.3.1 <i>Agrobacterium</i> -mediated Transformation	280
	10.3.2 Selectable Marker and Reporter Genes	280
	10.3.3 Particle Bombardment	281
10.4	Applications of Transgenic Technologies	281
10.5	Engineering Crop Resistance to Herbicides	283
10.6	Engineering 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	Manipulating Male Sterility	292
10.8	Tolerance to Abiotic Stresses	293
10.9	Manipulating 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	Production of Plant Polymers and Biodegradable Plastics	298
10.11	Plants as Bioreactors: Biopharming and Neutraceuticals	298
10.11.1	Edible Vaccines	298
10.11.2	Production of Antibodies in Plants	299
10.11.3	Plant Neutraceuticals	299
10.12	Plant Biotechnology in Forestry	300
10.13	Intellectual Property	300
10.14	Public Acceptance	301
10.15	Future Prospects	302
	References	303

## Chapter 11 Biotechnology-based Drug Discovery

*K. K. Jain*

11.1	Introduction to Drug Discovery	307
11.1.1	Basics of Drug Discovery in the Biopharmaceutical Industry	307
11.1.2	Historical Landmarks in Drug Discovery and Development	308
11.1.3	Current Status of Drug Discovery	309
11.2	New Biotechnologies for Drug Discovery	310
11.3	Genomic Technologies for Drug Discovery	310
11.3.1	SNPs in Drug Discovery	311
11.3.2	Gene Expression Profiling	312
11.3.3	Limitations of Genomics for Drug Discovery and Need for Other Omics	312
11.4	Role of Proteomics in Drug Discovery	313
11.4.1	Proteins as Drug Targets	313
11.4.2	Protein Expression Mapping by 2D Gel Electrophoresis	314
11.4.3	Liquid Chromatography-based Drug Discovery	314
11.4.4	Matrix-assisted Laser Desorption/Ionisation Mass Spectrometry	314
11.4.5	Protein-Protein Interactions	315
11.4.6	Use of Proteomic Technologies for Important Drug Targets	316
11.5	Metabolomic and Metabonomic Technologies for Drug Discovery	317
11.6	Role of Nanobiotechnology in Drug Discovery	318



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 Biomarkers in Drug Discovery	320
11.8	Screening in Drug Discovery	320
11.8.1	Cell-based Screening System	321
11.8.2	Receptor Targets: Human <i>versus</i> Animal Tissues	321
11.8.3	Tissue Screening	322
11.9	Target Validation 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	Antisense for Drug Discovery	323
11.10.1	Antisense Oligonucleotides for Drug Target Validation	324
11.10.2	Aptamers	324
11.10.3	RNA as a Drug Target	325
11.10.4	Ribozymes	325
11.11	RNAi for 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	Biochips and Microarrays for Drug 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 Drug Targets	330
11.12.4	Investigation of the Mechanism of Drug Action	331
11.13	Applications of Bioinformatics in Drug Discovery	331
11.13.1	Combination of <i>In Silico</i> and <i>In vitro</i> Studies	332
11.14	Role of Model Organisms in Drug Discovery	333
11.15	Chemogenomic Approach to Drug Discovery	334
11.16	Virtual Drug Development	334
11.17	Role of Biotechnology in Lead Generation and Validation	335
11.18	Conclusion	335
	References	336

**Chapter 12 Vaccines***Niall McMullan*

12.1	An Overview of Vaccines and Vaccination	337
12.2	Types of Vaccines in Current Use	338
12.2.1	Live, Attenuated Vaccines	338
12.2.2	Inactivated Vaccines	339
12.2.3	Subunit Vaccines	340
12.3	The Need for New Vaccines	342
12.4	New Approaches to Vaccine Development	343
12.4.1	Recombinant Live Vectors	343
12.4.2	Recombinant BCG Vectors	343
12.4.3	Recombinant <i>Salmonella</i> Vectors	344
12.4.4	Recombinant Adenovirus Vectors	345
12.4.5	Recombinant Vaccinia Vectors	346
12.4.6	DNA Vaccines	346
12.5	Adjuvants	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
	References	349

**Chapter 13 Tissue Engineering***Nils Link and Martin Fussenegger*

13.1	Introduction	351
13.1.1	Economic Impact of Healthcare	351
13.1.2	Tissue Engineering	352
13.1.3	Treating Disease Through Tissue Engineering	353
13.2	Cell Types	356
13.2.1	Embryonic Stem Cells	356
13.2.2	Adult Stem Cells	360
13.2.3	Mature Cells	361
13.3	Extracellular Matrix	362
13.3.1	Biological Extracellular Matrices	362
13.3.2	Artificial Extracellular Matrices	364
13.4	Tissue Engineering Concepts	369
13.4.1	Cultivation of Artificial Tissues	369
13.4.2	Design of Scaffold-free Tissues	372
13.5	Conclusions	373
	References	373

**Chapter 14 Transgenesis***Elizabeth J. Cartwright and Xin Wang*

14.1	Introduction	390
14.1.1	From Gene to Function	390
14.2	Transgenesis 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	Gene Targeting by Homologous Recombination in Embryonic Stem Cells	397
14.3.1	Basic Principles	398
14.3.2	Generation of a Knockout Mouse	400
14.3.3	Summary of Advantages and Disadvantages of Generating Gene Knockout Mice	404
14.4	Conditional 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	Summary of Advantages and Disadvantages of Conditional Gene Targeting	410
14.5	Phenotypic Analysis of Genetically Modified Mice	411
14.6	Ethical and Animal Welfare Considerations	412
14.7	Conclusions	414
14.8	Acknowledgements	414
	References	415

**Chapter 15 Protein Engineering***John Adair and Duncan McGregor*

15.1	Introduction	418
15.1.1	Protein Structures	419
15.2	Tools of the Trade	420
15.2.1	Sequence Identification	420
15.2.2	Structure Determination and Modelling	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

15.3.3	Whole Protein Shuffling	441
15.3.4	Protein–Ligand Interactions	441
15.3.5	Towards <i>De Novo</i> Design	442
15.4	Conclusions and Future Directions	443
	References	447

## Chapter 16 Immobilisation of Enzymes and Cells

*Gordon F. Bickerstaff*

16.1	Introduction	454
16.2	Biocatalysts	455
16.2.1	Enzymes	455
16.2.2	Ribozymes, Deoxyribozymes and Ribosomes	459
16.2.3	Splicosomes	460
16.2.4	Abzymes	461
16.2.5	Multienzyme Complexes	462
16.2.6	Cells	466
16.2.7	Biocatalyst Selection	468
16.3	Immobilisation	469
16.3.1	Choice of Support Material	470
16.3.2	Choice of Immobilisation Procedures	474
16.4	Properties of Immobilised Biocatalysts	483
16.4.1	Stability	483
16.4.2	Catalytic Activity	484
16.4.3	Coenzyme Regeneration	485
16.5	Applications	487
	References	489

## Chapter 17 Downstream Processing

*Daniel G. Bracewell, Mohammad Ali S. Mumtaz and  
C. Mark 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

17.5.1	Expanded Bed Adsorption	502
17.5.2	Aqueous Two-phase Extraction	503
17.5.3	Membrane Chromatography and Filtration	503
17.5.4	Crystallisation	504
17.5.5	Monolith Columns	505
17.6	Design of Biomolecules for Downstream Processing	505
17.7	Scaledown Methods	506
17.8	Validation and Robustness	506
17.9	Formulation and Antiviral Treatments	507
17.9.1	Formulation	507
17.9.2	Antiviral Treatments	508
17.10	Current Developments and Future Directions	509
	References	510

## Chapter 18 Biosensors

*Martin F. Chaplin*

18.1	Introduction	513
18.2	The Biological Reaction	518
18.3	Theory	519
18.4	Electrochemical Methods	522
18.4.1	Amperometric Biosensors	522
18.4.2	Potentiometric Biosensors	531
18.4.3	Conductimetric Biosensors	533
18.5	Piezoelectric Biosensors	534
18.6	Optical Biosensors	536
18.6.1	Evanescence Wave Biosensors	538
18.6.2	Surface Plasmon Resonance	540
18.7	Whole Cell Biosensors	542
18.8	Receptor-based Sensors	543
18.9	Conclusion	545
	References	546

## Chapter 19 Biofuels and Biotechnology

*Jonathan R. Mielenz*

19.1	Introduction	548
19.2	Production of the Major Biofuels	549
19.2.1	Corn Processing and Ethanol	550
19.2.2	Biomass Conversion for Ethanol	552
19.2.3	Biodiesel	556
19.3	Application of Biotechnology Tools to Biofuels Processes	557
19.3.1	Improved Production of Corn Ethanol	559
19.3.2	Ethanol Production from Biomass	561

*Contents*

xix

19.3.3	Biobutanol	572
19.3.4	Biodiesel	573
19.3.5	New Concepts	573
19.4	Future Perspectives	574
	References	576

**Subject Index**

585