

Molecular Analysis and Genome Discovery

Edited by

Ralph Rapley

University of Hertfordshire, UK

and

Stuart Harbron

The Enzyme Technology Consultancy, UK



JOHN WILEY & SONS, LTD

Contents

Preface	xi
List of contributors	xiii
1 Pharmacogenetics and Pharmacogenomics: An Overview	1
<i>W. Kalow</i>	
The origins of pharmacogenetics	1
The initial progress of pharmacogenetics	2
Molecular genetic methods enlarged pharmacogenetics	3
Multifactorial variation	7
New problems and opportunities	9
Summary and conclusions	12
References	13
2 Quantitative TaqMan Real-time PCR: Diagnostic and Scientific Applications	17
<i>Jörg Dötsch, Ellen Schoof and Wolfgang Rascher</i>	
Summary	17
Introduction	18
Principles of TaqMan real-time PCR	18
Reliability and validation of TaqMan real-time PCR	19
Applications for TaqMan real-time PCR	20
Limitations and pitfalls in the use of TaqMan real-time PCR	23
Alternative real-time PCR methods	24
Future developments	25
Conclusions	25
References	26
3 Hybridization Probes for Real-time PCR	29
<i>Elaine Lyon</i>	
Introduction	29
Probe chemistry	29
Fluorescence resonance energy transfer	30
Hybridization probe design	31
FRET applications	33

Summary	39
References	39
4 An Overview of Genotyping and Single Nucleotide Polymorphisms (SNP)	43
<i>Ivo Glynne Gut</i>	
Introduction	43
Genotyping of single nucleotide polymorphisms	44
Methods for interrogating SNPs	47
Analysis formats	53
How can SNP genotyping be made more economic?	
Miniaturization – Multiplexing	59
Haplotyping	59
DNA methylation analysis – analysis of methylation variable positions (MVPs)	60
SNP genotyping and quantitation	60
Emerging methods	61
High-throughput SNP genotyping methods	62
The next generation	62
Conclusions	63
Acknowledgements	63
References	64
5 High-throughput Mutation Screening	71
<i>Paal Skytt Andersen and Lars Allan Larsen</i>	
Introduction	71
Automated DNA sequencing for mutation analysis	72
Methods for detection of specific mutations	73
Methods for mutation scanning	87
References	98
6 Determination of Nucleic Acid Sequences by Pyrosequencing	101
<i>Elahe Elahi and Mostafa Ronaghi</i>	
Introduction	101
General principles	102
Methodology	103
Applications	104
Future perspectives	107
References	109
7 An Introduction to DNA Chips	113
<i>Magdalena Gabig-Ciminska and Andrzej Ciminski</i>	
Introduction	113
DNA chip structure and operating principles	115
Achievements and future research directions	122

Application and potential use of DNA chips	124
Concluding remarks	125
References	126
8 Overview of Microarrays in Genomic Analysis	127
<i>Janette K. Burgess</i>	
Introduction	128
What is a microarray?	129
History	129
Production of a cDNA microarray	131
Sources of cDNA	134
Production of an oligonucleotide microarray	135
Array substrates	138
Commercial sources of arrays	138
Isolation of RNA	139
Amplification	140
Target labelling	140
Hybridization	142
Image acquisition	143
Image analysis	146
Data storage	147
Reporting standards	149
Applications to genomic analysis	150
The future?	158
References	158
9 Overview of Differential Gene Expression by High-throughput Analysis	167
<i>Kin- Ying To</i>	
Introduction	167
Differential display	168
cDNA-amplified fragment length polymorphism	168
Representational difference analysis	172
Subtractive hybridization	172
Expressed sequence tags	173
Serial analysis of gene expression	175
DNA microarrays and plant functional genomics	177
Discussion	187
References	188
10 Aptamers: Powerful Molecular Tools for Therapeutics and Diagnostics	191
<i>Eva Baldrich Rubio, Mònica Campàs i Homs and Ciara K. O'Sullivan</i>	
Introduction	191
Aptamer selection	192

Aptamers for therapeutics	195
Aptamers in analysis	199
Aptamers for proteomic applications	206
Perspectives and future outlook	208
Acknowledgements	209
Abbreviations	209
References	210
11 Chip-based Proteomics Technology	217
<i>Mikhail Soloviev, Richard Barry and Jon Terrett</i>	
Introduction	217
Protein separation and purification approaches	219
Chip-based proteomics	220
Protein arrays	225
Content	228
Microfluidics	243
Other chip-based technologies in proteomics	244
Conclusion	246
References	247
12 Infectomics Overview: Holistic and Integrative Studies of Infectious Diseases	251
<i>Sheng-He Huang and Ambrose Jong</i>	
Introduction	251
Investigating microbial infection with 'omic' approaches	252
Dissecting the microbiome: ecological solutions to infectious diseases	262
Animal models and gene knockin/knockout technologies	263
Computational and mathematic tools for infectomics	264
Prospects of infectomics	265
References	267
13 The Drug Discovery Process	271
<i>Roberto Solari</i>	
Introduction	271
The process	272
Target discovery	274
Target validation	276
Discovery of chemical leads	277
Hits to leads	282
Lead molecular optimization	282
Lead to development candidate	284
Biological candidates	284
Pre-clinical development	285
Development issues for biological drugs	289
Clinical trials	290
Intellectual property	292

Summary	293
References	293
Appendix	294
14 Structure-based Drug Discovery	295
<i>Chen-Chen Kan, Kevin Hambly and Derek A. Debe</i>	
Overview of drug discovery and development	295
Principles of structure-based drug discovery	297
Case studies of structure-based drug discovery	301
Structure-based drug discovery in the post-genomic era	313
Acknowledgements	318
References	318
15 Protein Interaction-targeted Drug Discovery	323
<i>Gary Hudes, Sanjay Menon and Erica A. Golemis</i>	
Introduction	323
PPIs: classes, functional relationships and regulation	324
Protein interactions in disease-related signalling: categories of disruption	327
Selecting targets and screens	330
Progress in cancer treatment	334
Summary	341
References	341
16 Overview of Quantitative Structure–Activity Relationships (QSAR)	347
<i>David A. Winkler</i>	
Modelling structure–activity relationships	347
The QSAR method	349
Generation of descriptors	352
Descriptor selection	356
Structure–activity mapping	357
Validation and testing	361
Applications and conclusions	362
References	362
Index	369