

Contents

Preface	xiii
Preface to <i>Molecular Biology in Histopathology</i>	xv
List of Contributors	xvii
1 Blotting Techniques: Methodology and Applications	1
<i>Fiona Watson and C. Simon Herrington</i>	
1.1 Introduction	1
1.2 Blotting techniques	1
1.3 References	15
2 <i>In-situ</i> Hybridisation in Histopathology	19
<i>Gerald Niedobitek and Hermann Herbst</i>	
2.1 Introduction	19
2.2 Experimental conditions	20
2.3 Probes and labels	23
2.4 Controls and pitfalls	27
2.5 Double-labelling	29
2.6 Increasing the sensitivity of ISH	31
2.7 What we do in our laboratories	33
2.8 Applications of ISH: examples	35
2.9 Perspective	39
2.10 References	40
3 DNA Flow Cytometry	49
<i>M.G. Ormerod</i>	
3.1 Introduction	49
3.2 Definitions and terms	49
3.3 Dye used for DNA analysis	50
3.4 Sample preparation for DNA analysis	52

3.5 Analysis of the DNA histogram	53
3.6 Quality control	53
3.7 Computer analysis of the DNA histogram	55
3.8 Multiparametric measurement	57
3.9 Acknowledgements	59
3.10 References	59
4 Interphase Cytogenetics	61
<i>Sara A. Dyer and Jonathan J. Waters</i>	
4.1 Introduction	61
4.2 Interphase cytogenetics	62
4.3 Applications	67
4.4 Conclusion	76
4.5 References	77
5 Oncogenes	79
<i>Fiona Macdonald</i>	
5.1 Introduction	79
5.2 Identification of the oncogenes	79
5.3 Functions of the proto-oncogenes	80
5.4 Mechanism of oncogene activation	89
5.5 Oncogenes in colorectal cancer	91
5.6 Oncogenes in breast cancer	94
5.7 Oncogenes in lung cancer	95
5.8 Oncogenes in haematological malignancies	96
5.9 Other cancers	99
5.10 Conclusion	100
5.11 References	100
6 Molecular and Immunological Aspects of Cell Proliferation	105
<i>Karl Baumforth and John Crocker</i>	
6.1 The cell cycle and its importance in clinical pathology	105
6.2 Molecular control of the cell cycle	108
6.3 Cell cycle control	111
6.4 The cell cycle and cancer	112
6.5 Immunocytochemical markers of proliferating cells	115
6.6 References	133
6.7 Further Reading	135
7 Interphase Nucleolar Organiser Regions in Tumour Pathology	137
<i>Massimo Derenzini, Davide Treré, Marie-Françoise O'Donohue and Dominique Ploton</i>	

7.1 Introduction	137
7.2 The AgNORs	138
7.3 NOR silver-staining	142
7.4 Quantitative AgNOR analysis	145
7.5 AgNORs as a parameter of the level of cell proliferation	146
7.6 Application of the AgNOR technique to tumour pathology	147
7.7 What future for AgNORs in tumour pathology?	151
7.8 References	152
8 Apoptosis and Cell Senescence	153
<i>Lee B. Jordan and David J. Harrison</i>	
8.1 Introduction	153
8.2 Apoptosis	153
8.3 Cell senescence	174
8.4 Summary	178
8.5 References	179
9 The Polymerase Chain Reaction	193
<i>Timothy Diss</i>	
9.1 Introduction	193
9.2 Principles	194
9.3 Analysis of products	197
9.4 RT-PCR	199
9.5 Quantitative PCR	200
9.6 DNA and RNA extraction	200
9.7 Correlation of the PCR with morphology	201
9.8 Problems	202
9.9 Applications	202
9.10 Diagnostic applications	203
9.11 Infectious diseases	209
9.12 Identity	209
9.13 The future	210
9.14 References	210
9.15 Online information	212
10 Laser Capture Microdissection: Techniques and Applications in the Molecular Analysis of the Cancer Cell	213
<i>Amanda Dutton, Victor Lopes and Paul G. Murray</i>	
10.1 Introduction	213
10.2 The principle of LCM	214
10.3 Technical considerations	216
10.4 Advantages and disadvantages of LCM	217

10.5 Applications of LCM	222
10.6 Future perspectives	229
10.7 Acknowledgements	229
10.8 References	229
11 The <i>In-situ</i> Polymerase Chain Reaction	233
<i>John J. O’Leary, Cara Martin and Orla Sheils</i>	
11.1 Introduction	233
11.2 Overview of the methodology	234
11.3 In-cell PCR technologies	235
11.4 In-cell amplification of DNA	238
11.5 Detection of amplicons	242
11.6 Reaction, tissue and detection controls for use with in-cell DNA PCR assays	243
11.7 In-cell RNA amplification	244
11.8 Problems encountered with in-cell PCR amplification	246
11.9 Amplicon diffusion and back diffusion	247
11.10 Future work with in-cell PCR-based assays	247
11.11 References	249
12 TaqMan® Technology and Real-Time Polymerase Chain Reaction	251
<i>John J. O’Leary, Orla Sheils, Cara Martin, and Aoife Crowley</i>	
12.1 Introduction	251
12.2 Probe technologies	252
12.3 TaqMan® probe and chemistry (first generation)	254
12.4 Second generation TaqMan® probes	256
12.5 Hybridisation	258
12.6 TaqMan® PCR conditions	259
12.7 Standards for quantitative PCR	260
12.8 Interpretation of results	261
12.9 End-point detection	262
12.10 Real-time detection	263
12.11 Relative quantitation	263
12.12 Reference genes	264
12.13 Specific TaqMan® PCR applications	265
12.14 References	268
13 Gene Expression Analysis Using Microarrays	269
<i>Sophie E. Wildsmith and Fiona J. Spence</i>	
13.1 Introduction	269
13.2 Microarray experiments	269

13.3 Data analysis	273
13.4 Recent examples of microarray applications	284
13.5 Conclusions	284
13.6 Acknowledgements	284
13.7 References	284
13.8 Further Reading	286
13.9 Useful websites	286
14 Comparative Genomic Hybridisation in Pathology	287
<i>Marjan M. Weiss, Mario A.J.A. Hermsen, Antoine Snijders, Horst Buerger, Werner Boecker, Ernst J. Kuipers, Paul J. van Diest and Gerrit A. Meijer</i>	
14.1 Introduction	287
14.2 Technique	289
14.3 Data analysis	292
14.4 Applications	293
14.5 Clinical applications	299
14.6 Screening for chromosomal abnormalities in fetal and neonatal genomes	299
14.7 Future perspectives	300
14.8 Acknowledgements	301
14.9 References	301
15 DNA Sequencing and the Human Genome Project	307
<i>Philip Bennett</i>	
15.1 Introduction	307
15.2 DNA sequencing: the basics	308
15.3 Applications of DNA sequencing	318
15.4 The Human Genome Project	320
15.5 References	327
15.6 Further Reading	327
15.7 Useful websites	328
16 Monoclonal Antibodies: The Generation and Application of 'Tools of the Trade' Within Biomedical Science	329
<i>Paul N. Nelson, S. Jane Astley and Philip Warren</i>	
16.1 Introduction	329
16.2 Antibodies and antigens	331
16.3 Polyclonal antibodies	332
16.4 Monoclonal antibody development	333
16.5 Monoclonal antibody variants	338
16.6 Monoclonal antibody applications	341

16.7 Therapy	345
16.8 Specific applications	346
16.9 Conclusions	347
16.10 Acknowledgements	347
16.11 References	347
17 Proteomics	351
<i>Kathryn Lilley, Azam Razzaq and Michael J. Deery</i>	
17.1 Introduction	351
17.2 Definitions and applications	352
17.3 Stages in proteome analysis	352
17.4 Future directions	368
17.5 References	368
Index	371