



FISH

Edited by
Barbara Beatty
Sabine Mai
Jeremy Squire

**PRACTICAL
APPROACH**

Contents

List of protocols page *xiii*

Abbreviations *xvii*

1 Introduction 1

Sabine Mai, Barbara G. Beatty, and Jeremy A. Squire

References 4

2 FISH probes and labelling techniques 5

Patricia Bray-Ward

1 Introduction 5

2 Fluorescence principles 5

Fluors and haptens 6

Commonly used fluors 7

Choice of filter sets 7

3 Nucleic acid probes 8

Types of probes 8

Preparation of cloned probes 9

Enzymatically amplified probes 15

Synthetic oligonucleotide probes 16

4 Coupling of fluors/haptens to nucleotides 17

5 Labelling of probes 19

Nick translation 20

Random primer labelling 21

RNA transcription labelling 22

PCR labelling 24

6 Post-labelling DNA processing and purification 25

DNase treatment (for FISH and other hybridization protocols) 25

Removal of unincorporated nucleotides and BSA from the reaction mix prior to probe precipitation 25

7 Other labelling systems 26

Coupling of fluors or haptens to amine-modified nucleic acids 26

Other chemical coupling systems 26

Direct chemical coupling of fluors or haptens to proteins 26

References 27

3 Human chromosome mapping of single copy genes 29

Barbara G. Beatty and Stephen W. Scherer

- 1 Introduction 29
- 2 DNA probes for FISH mapping 30
 - Identification of FISH probes from WWW sites 30
 - Preparation of probes for FISH mapping 32
- 3 Target DNA preparation 34
 - Metaphase chromosomes 34
 - Mapping with interphase nuclei 36
 - Hypotonic treatment and fixation 38
- 4 Slide preparation 39
 - Target slide pre-treatment 41
- 5 Denaturation and hybridization of probe and target DNA 42
- 6 Post-hybridization washes 44
- 7 Immunodetection 45
- 8 Chromosome counterstaining and banding 47
- 9 Microscopy and image analysis 48
- 10 FISH mapping points to consider 49
 - FISH mapping of single probes to metaphase chromosomes 49
 - Relational mapping with multiple probes 51
- References 53

4 Murine chromosome preparation 55

Sabine Mai and Francis Wiener

- 1 Murine chromosome preparation for banding and *in situ* hybridization procedures 55
 - Introduction 55
- 2 Giemsa-trypsin banding of mouse chromosomes 63
- 3 Molecular cytogenetic approaches for murine chromosomes 66
 - References 76

5 High resolution FISH mapping using chromatin and DNA fibre 77

Henry H. Q. Heng

- 1 Introduction 77
- 2 Practical considerations for fibre preparation 78
- 3 General equipment required for fibre FISH 78
- 4 Chromatin fibre preparation 79
- 5 DNA fibre preparation 83
- 6 FISH 85
 - DNA probe labelling 85
 - Hybridization 86
 - Wash 88
 - Detection and amplification 89
 - Counterstaining and antifade 90
- 7 Photography 91
 - Acknowledgements 91
 - References 91

6 Applications of RNA FISH for visualizing gene expression and nuclear architecture 93

Rose Tam, Lindsay S. Shopland, Carol V. Johnson, John A. McNeil, and Jeanne B. Lawrence

- 1 Introduction 93
- 2 Cell preparation 96
 - Detergent-extracted cell preparation 96
 - Cytogenetic preparations 97
- 3 Probe preparation 99
- 4 Hybridization to RNA 100
 - Basic RNA hybridization procedure 101
 - Oligonucleotide hybridization 102
- 5 Hybridization to DNA 103
 - Detecting heat denatured cellular DNA 104
 - DNA FISH using NaOH denaturation and RNA hydrolysis 105
- 6 Multiple label techniques and applications 105
 - Coupling the detection of RNA with DNA 105
 - Coupling protein detection with FISH 107
 - Chromosome paints and RNA FISH 108
 - Differentiating transcripts with intron and cDNA probes 109
 - Exon suppression hybridization: an example of the use of specific competition 110
- 7 Visualizing and analysing results 112
 - Microscopy 113
 - Digital imaging 114
- 8 Concluding remarks 116
- Acknowledgements 117
- References 117

7 FISH on three-dimensionally preserved nuclei 119

I. Solovei, J. Walter, M. Cremer, F. Habermann, L. Schermelleh, and T. Cremer

- 1 Introduction 119
- 2 Preparation and fixation of cells 123
 - Preparation of slides 123
 - Cultivation and fixation of adherent cells 125
 - Preparation, attachment, and fixation of cells growing in suspension 126
- 3 Preparation of cells directly isolated from peripheral blood 127
- 4 Pre-treatments needed for hybridization 128
- 5 Hybridization set-up 131
 - Probe labelling 131
 - Probe preparation 132
 - DNA denaturation and hybridization 132
- 6 Post-hybridization washes, detection, nuclei counterstaining, and slide mounting 134
 - Post-hybridization washes 134
 - Detection of hybridized probes 135
 - Counterstaining of nuclei and mounting cells in antifade medium 137
- 7 Combined 3D FISH and replication labelling 139
 - Replication labelling 139
 - Detection of incorporated halogenated deoxyuridines after FISH 140

8	Combined protein immunodetection and 3D FISH	142
9	Preservation of the chromatin structure during 3D FISH	144
10	Confocal microscopy	144
	Selection of the filter configuration	147
	Conditions of image acquisition	149
	Calibration of the instrument	150
	Visualization	150
	Quantitative measurements and deconvolution	152
	References	154
8	Comparative genomic hybridization on metaphase chromosomes and DNA chips	159
	<i>Stefan Joos, Carsten Schwänen, and Peter Lichter</i>	
1	Introduction	159
2	Preparation of metaphase chromosomes	161
3	Isolation of genomic DNA	161
4	Isolation of single cells by micromanipulation	167
5	Amplification of genomic DNA from small cell populations by universal polymerase chain reaction (PCR)	168
6	Probe labelling	172
7	Comparative genomic hybridization	174
	Denaturation of metaphase chromosomes	174
	Probe mixture	175
	In situ hybridization and signal detection	176
8	Image acquisition and evaluation	177
9	Troubleshooting of CGH experiments	178
10	Troubleshooting of CGH experiments in combination with universal PCR	179
11	New developments: matrix-CGH	180
	References	181
9	FISH in clinical cytogenetics	183
	<i>Jeremy A. Squire, P. Marrano, and E. Kolomietz</i>	
1	Introduction	183
2	Probes commonly used for FISH in the clinical laboratory	184
3	Preparation of clinical samples for FISH analysis	185
	Preparation of metaphase chromosomes for FISH	185
	Preparation of interphase nuclei derived from clinical specimens for FISH	187
4	Criteria for assessing and reporting FISH results	194
	General considerations when selecting cells for FISH microscopy	195
	Scoring criteria for interphase FISH signal evaluation and enumeration	196
	Special considerations concerning interphase FISH interpretation	197
5	Some of the commonly used FISH probes in clinical cytogenetics	198
	FISH analysis of microdeletion syndromes	198
	Use of the three-colour fusion (translocation/inversion) probes	199
	Use of FISH probes in assessing gene amplification	201
6	Appendix (useful web sites for molecular cytogenetics clinical sources)	202
	References	202

10 Multicolour FISH and spectral karyotyping 205*Jane Bayani and Jeremy A. Squire*

- 1 Introduction 205
- 2 Spectral karyotyping (SKY) 206
- 3 M-FISH 208
- 4 M-FISH and SKY protocols 208
- 5 General considerations for image acquisition and analysis 214
 - Image analysis using SKY 215
 - Image analysis using M-FISH 216
- 6 Troubleshooting 216
- References 219

11 cDNA microarrays for fluorescent hybridization analysis of gene expression 221*Javed Khan, Lao H. Saal, Michael L. Bittner, Yuan Jiang, Gerald C. Gooden, Arthur A. Glatfelter, and Paul S. Meltzer*

- 1 Introduction 221
 - Serial analysis of gene expression 221
 - Oligonucleotide arrays 222
 - cDNA microarrays 222
- 2 Fabrication of cDNA microarrays 223
 - Culturing cDNA bacterial clones 223
 - Microarray slide printing 230
- 3 Target production 232
- 4 Hybridization 235
- 5 Image acquisition 237
- 6 Image analysis and normalization 237
- 7 Sensitivity and specificity 238
- 8 Data mining and statistical analysis 238
- 9 Summary 239
- References 239

List of suppliers 241**Index 251**