

CONTENTS

PREFACE	xv
INTRODUCTION	xvii
CHAPTER 1	
PRE-TREATMENT, HYPOTONIC TREATMENT, FIXATION AND STAINING	1
PRE-TREATMENT AND HYPOTONIC TREATMENT	1
Pre-Treatment for Clearing the Cytoplasm and Softening the Tissue	1
Separation of Chromosome and Clarification of Constrictions	2
Colchicine	2
Chloral Hydrate	3
Gammexane	4
Coumarin and its Derivatives	4
Oxyquinoline (OQ)	5
<i>p</i> -Dichlorobenzene (C ₆ H ₄ Cl ₂)	5
Monobromonaphthalene	5
Veratrine	8
FIXATION	8
Non-Metallic Fixatives	10
Metallic Fixatives	12
Fixing Mixtures	18
STAINING	18
Fuchsin	20
Carmine	22
Orcein	24
Lacmoid	25
Chlorazol Black	25
Crystal Violet	26
Haematoxylin	28
Toluidine Blue (Michrome No. 641)	28
Giemsa (Michrome No. 144)	28
Fluorochromes	29
DNA-Binding Nucleoside Specific Antibiotics	31
Some Miscellaneous Double Stains	31

MOUNTING MEDIA	31
Temporary and Permanent Mounts	31
CHAPTER 2	
MICROSCOPY, MICROSPECTROPHOTOMETRY, FLOW CYTOMETRY, IMAGE ANALYSIS, CONFOCAL MICROSCOPY	33
A. ORDINARY LIGHT MICROSCOPY	33
B. PHASE AND INTERFERENCE MICROSCOPY	35
C. FLUORESCENCE MICROSCOPY	37
Schedule for the Study of Chromosome Fluorescence Following Treatment with Anti-DNA Serum	39
D. MICROSPECTROPHOTOMETRY	40
Under Ultraviolet Light	40
Under Visible Light	43
E. FLOW CYTOMETRY	46
F. CHROMOSOME IMAGE ANALYSIS	47
G. CONFOCAL MICROSCOPY	48
CHAPTER 3	
STUDY OF BANDING PATTERNS OF CHROMOSOMES	49
FLUORESCENT BANDING OR Q-BANDING	50
With Quinacrine Mustard (QM) or Quinacrine Dihydrochloride (Q)	50
With Hoechst 33258	51
Reverse Fluorescent Banding with Chromomycin and DAPI	52
D-Bands with Antibiotics	54
Ethidium Bromide as Counterstain for Quinacrine	54
Banding with Acridine Orange (AO)	54
GIEMSA OR G-BANDING	55
G-Banding in Plants	55
Giemsa G-Banding in Animals	56
Feulgen-Bands	59

C-BANDING	60
Giemsa C-Banding in Animals	60
Giemsa C-Banding in Plants	61
CT-BANDING	63
Cd-BANDING	63
O-BANDING OR ORCEIN BANDING	64
HKG BANDING TECHNIQUE FOR PLANTS	64
REVERSE BANDING OR R-BANDING	65
R-Banding in Animals	65
NOR-BANDING	66
A. Giemsa Staining for Human Chromosomes	66
B. Giemsa Staining for Animal and Plant Chromosomes	66
C. Silver Staining Methods	66
HY-BANDING	67
RESTRICTION ENDONUCLEASE BANDING	67
RE Banding in Human Chromosomes	68
RE Banding for <i>Drosophila</i>	69
RE Banding in Mammalian Chromosomes <i>in Vitro</i>	69
RE Bands in Plants (<i>Allium cepa</i> , <i>Hordeum vulgare</i> , <i>Vicia faba</i>)	70
RE Bands in Plants (Barley)	70
RESTRICTION ENDONUCLEASE/NICK TRANSLATION	71
In Fixed Mouse Chromosomes	71
<i>In Situ</i> Nick Translation of Human Chromosomes Directed by Restriction Endonucleases	72
RE-Banding and Electron Microscopy of Fixed Mouse Metaphase Chromosomes	72
Antibody Binding Methods for Human Chromosomes	73
SISTER CHROMATID EXCHANGE	74
Fluorescence Plus Giemsa (FPG) Technique	75
Acridine Orange Staining Method	76
4'-6-Diamidino-2 Phenylindole Staining	76
Giemsa Staining	77
Combined SCD Staining and G-Banding	77
'Reverse' Staining Method	78
BrdU-33258 H Analysis of DNA Replication	79
Dot Formation on Chromosomes	79

CHAPTER 4**AUTORADIOGRAPHY—LIGHT, FIBRE AND
HIGH RESOLUTION**

A. LIGHT MICROSCOPE AUTORADIOGRAPHY	82
Technical Steps in the Preparation of Autoradiographs	84
Representative Schedules for Light Microscopy Autoradiography	85
B. FIBER AUTORADIOGRAPHY	88
Protocol	88
C. HIGH RESOLUTION AUTORADIOGRAPHY	88
Fixation and Embedding	89
Section Cutting	89
Coating of Ultra Thin Sections	90
Thickness of Emulsion Layer	91
Developing	91
Staining	92
Protocol for Spreading and Autoradiography	93

CHAPTER 5**ELECTRON MICROSCOPY**

FIXATION	94
DEHYDRATION	96
EMBEDDING	96
Schedule for Embedding with Epoxy Resin Media	97
SECTIONING	98
MOUNTING	100
STAINING	100
<i>IN SITU</i> FIXATION AND EMBEDDING	101
REPRESENTATIVE SCHEDULES	101
EM Chromosome Analysis of Slime Mold	101
EM Chromosome Analysis of Nematode	102
EM Chromosome Analysis of Algae	102
EM Analysis of Dinoflagellate Chromosomes	102

EM Analysis from Whole Mount Preparation of Protozoa	102
EM Analysis of Whole Mount Alkali Urea Treated Polytene Chromosomes	103
Study of Synaptonemal Complex	104
EM Study of Nucleosome	105
Study of Chromatin in Animals ("Miller" Spread)	106
EM Study of Spheroid Chromatin Units (v Bodies)	108
EM Study of Scaffold	108
Study of Chromosome Core of Trilophidia	108
Scanning Electron Microscopy	109
EM Study Following <i>in Situ</i> Hybridization to Metaphase Chromosomes in Animals	110
Reagents for EM	113

CHAPTER 6

STUDY OF CHROMOSOMES FROM TISSUE AND PROTOPLAST CULTURE, CELL FUSION AND GENE TRANSFER IN PLANTS

A. TISSUE CULTURE	115
Principal Steps Involved in Tissue Culture	115
Representative Schedules for Plant Tissue Culture	119
B. ISOLATION AND CULTURE OF PROTOPLASTS	120
Techniques for Protoplast Isolation	121
C. ANTHOR AND POLLEN CULTURE	123
D. FUSION BETWEEN PLANT CELLS	123
Techniques for Cell Fusion in Plants	124
E. SOMATIC CELL FUSION AND GENE TRANSFER	125
Methods for Gene Transfer	125

CHAPTER 7

CHROMOSOME ANALYSIS FOLLOWING SHORT- AND LONG-TERM CULTURES IN MAMMALIAN AND HUMAN SYSTEMS, FROM NORMAL AND MALIGNANT TISSUES

A. CHROMOSOME STUDY FROM NORMAL TISSUES	130
Study of Chromosomes from Male Germ Cells	131
Study of Chromosomes from Female Germ Cells	136
Study of Chromosomes from Somatic Cells	137
Sex Chromatin Studies	147

B. CHROMOSOME STUDY FROM MALIGNANT TISSUES	149
Study of Cancer Tissue in Culture	151
Schedules for Preparation of Tissue	152
Schedules	153
Conclusions	155
 CHAPTER 8	
SOMATIC CELL FUSION IN ANIMALS	161
SCHEDULE FOR SOMATIC FUSION BETWEEN CELL LINES DERIVED FROM DIFFERENT MAMMALIAN SPECIES	161
A. Use of Sendai Virus	161
B. Virus Induced Fusion Mediated by Lectin	162
C. Use of Lipid Vesicles in Cell Fusion	163
D. Use of Lysolecithin in Cell Fusion	164
E. Use of Polyethylene Glycol in Cell Fusion	164
F. Use of Microcells for Cell Fusion	165
FUSION BETWEEN PLANT AND ANIMAL CELLS	166
 CHAPTER 9	
EFFECTS OF EXTERNAL AGENTS AND MONITORING FOR ENVIRONMENTAL TOXICANTS	168
SECTION A: EFFECTS OF PHYSICAL AND CHEMICAL AGENTS ON CHROMOSOMES	168
Chemical Agents	169
Schedules for Inducing Chromosome Breaks in Plants	171
Physical Agents	174
Treatment of Tissue with Both Physical and Chemical Agents	179
SECTION B: METHODS FOR MONITORING CYTOGENETIC EFFECTS OF ENVIRONMENTAL TOXICANTS	182
Some Common Test Systems Used	184
 CHAPTER 10	
ISOLATION AND EXTRACTION OF NUCLEI, CHROMOSOMES AND COMPONENTS	197
A. ISOLATION OF NUCLEI	197
A) For Animal Cells	197
B) For Plant Tissues	200

B. ISOLATION OF CHROMOSOMES	201
Separation of DNA and Protein after Chromosome Isolation	201
Isolation and Separation of Heterochromatin and Euchromatin of Chromosomes	203
C. REPRESENTATIVE SCHEDULES FOR ISOLATION OF CHROMOSOMES AND DNA	205
A) Isolation of Chromosomes in Animals	205
B) Isolation of Chromosomes in Plants	208
Mass Isolation of Chromosomes from Plant Protoplasts	210
C) Isolation of DNA from Animals	211
D) Isolation of DNA from Plants: Minipreparation	217
 CHAPTER 11	
MICRURGY	221
MICRURGICAL METHOD OF ISOLATION OF NUCLEI, CHROMOSOMES AND MICRODISSECTION	221
ISOLATION AND CULTURE OF NUCLEI IN MEDIUM UNDER OIL	222
Isolation of Chromosomes	223
MICRODISSECTION OF CHROMOSOMES	223
Protocol for Microdissection	224
Microdissection of Animal Chromosomes	225
Microdissection of Plant Chromosomes	225
 CHAPTER 12	
IDENTIFICATION OF CHROMOSOME SEGMENTS AND DNA SEQUENCES BY <i>IN SITU</i> MOLECULAR HYBRIDIZATION	227
REPRESENTATIVE SCHEDULES	235
<i>In Situ</i> Hybridization in Mammals Using Autoradiography	235
DNA <i>In Situ</i> Hybridization (Human)	242
<i>In Situ</i> Hybridization and SCE Combined Method in Human Leucocyte	243
<i>In Situ</i> Hybridization in Insects	244
<i>In Situ</i> Hybridization for Insects	245
Two-Colour Hybridization in Human Leucocyte Culture	245
Multiple Banding and <i>In Situ</i> Hybridization in Mammal	248
MICRO-FISH—Rapid Regeneration of Probes by Chromosome Microdissection	249
<i>In Situ</i> Hybridization Using Radioactive Probe in Plant Pachytene	251
Genomic <i>In Situ</i> Hybridization with Non-Radioactive Probe (GISH) for Plants	252

<i>In Situ</i> Hybridization with Automated Chromosome Denaturation in Plants	256
Mapping of Low Copy DNA Sequence in Plants	258
<i>In Situ</i> Hybridization (Plants) with Biotin-Labelled Probe	259
Two Colour Mapping of Plant DNA Sequences	260
Genome <i>In Situ</i> Hybridization in Sectioned Plant Nuclei	261
<i>In Situ</i> Hybridization with Immunofluorescent Antibodies	261
Immunofluorescent Staining of Kinetochores in Human Micronuclei	262
Monoclonal Antibody Against Kinetochores Component — Schedule for Preparation in CHO Cells	262
Indirect Immunofluorescence in Mammalian Sperm	264
Modified Banding of Human Chromosomes for Electron Microscopy	265
Antibody Labelling and Flow Cytometry in CHO Cell Line	266
Antigen Reaction with Human Autoantibody in Diptera	267
Immunofluorescence in Nematodes	268
Anti-Topoisomerase II in Recognising Meiotic Chromosome Core in Bird	268
BrdU Antibody Technique (BAT)	269
Products Available for <i>In Situ</i> Hybridization	270
Kinetochores Visualization with Crest Serum in Plants	271
Antibody Method for Replicating Bands in Plants	272
Methylation of DNA	272

CHAPTER 13

SPECIAL MOLECULAR TECHNIQUES NECESSARY FOR CHROMOSOME ANALYSIS

SECTION A: RESTRICTION FRAGMENT LENGTH POLYMORPHISM	274
Preparation and Examination of Agarose Gels	278
Separation of Restriction Fragments of Mammalian Genomic DNA by Agarose Gel Electrophoresis	280
Hybridization of Radiolabeled Probes to Nucleic Acids Immobilized on Nitrocellulose Filters or Nylon Membranes	283
SECTION B: THE DNA FINGERPRINT	285
SECTION C: POLYMERASE CHAIN REACTION	288
Chromosome Crawling	291
SECTION D: CHROMOSOME WALKING	291
SECTION E: FOOT PRINT	296

CHAPTER 14

REPRESENTATIVE SCHEDULES FOR DIRECT OBSERVATION OF CHROMOSOMES FROM PLANTS AND ANIMALS <i>IN VIVO</i> AND FROM SPECIAL MATERIALS	297
A. STUDY FROM NORMAL TISSUES	297
Schedules for the Study of Mitotic Chromosomes	297
Schedules for the Study of Meiotic Chromosomes	307
B. STUDY FROM SPECIAL MATERIAL	311
1. Chromosome Segments	311
2. Special Chromosomes	313
3. Special Groups	319
4. Components-Nucleic Acids	325
REFERENCES	333
INDEX	365