Contents

Contributing authors xxvii
Preface xxix
Acknowledgments xxxi

1 The cell and cell division 1
Margaret J. Barch and Helen J. Lawce

1.1 The cell 1
1.1.1 Cell membrane
1.1.2 Cytoplasm
1.1.3 Nucleus
1.1.4 Chromosomes and their proteins
1.1.5 X inactivation
1.1.6 Satellite DNA

1.2 The cell cycle 14
1.2.1 Interphase
1.2.2 Cell division
1.2.3 Mitosis
1.2.4 Meiosis

1.3 Recombinant DNA techniques 19
1.3.1 Bacterial-plasmid cloning
1.3.2 Electrophoresis
1.3.3 Southern blotting
1.3.4 Synthetic oligonucleotides
1.3.5 Polymerase chain reaction

1.4 The human genome 21
1.4.1 Genomic DNA variations

References 22

2 Cytogenetics: an overview 25
Helen J. Lawce and Michael G. Brown

2.1 Introduction 25
2.2 History of human cytogenetics 25
2.3 Cytogenetics methods 29
2.3.1 Work flow 29
2.3.2 Culture methods 33
2.3.3 Harvesting 35
2.3.4 Removal of attached cells and centrifugation steps 35
2.3.5 Mitotic arrest: Colcemid® 37
2.3.6 Hypotonic treatment 37
2.3.7 Fixation 40
2.3.8 In situ harvesting 41
2.3.9 Chromosome anticontraction methods 43
2.3.10 Mechanism of action of synchrony chemicals 46
2.3.11 Additives to prevent chromosome contraction 48
2.3.12 Combination of synchrony and additives for longer chromosomes 49
2.3.13 Automatic harvesting devices and slide-making chambers/drying chambers 49
### 2.4 Slide-making

2.4.1 History of slide-making
2.4.2 Theory of slide-making
2.4.3 Slide-making variables
2.4.4 Wet versus dry slides
2.4.5 Angle of the slide
2.4.6 Ambient humidity and temperature
2.4.7 Fixative ratio
2.4.8 Quality and freshness of fixative
2.4.9 Height from which cells are dropped
2.4.10 Wicking effects
2.4.11 Air flow
2.4.12 Dilution factor
2.4.13 Slide cleaning and labeling
2.4.14 Slide type
2.4.15 Cell and culture type
2.4.16 Culturing and harvesting techniques
2.4.17 In situ cell drying
2.4.18 Slide-making for FISH studies
2.4.19 Slide aging

### 2.5 Chromosome staining

### 2.6 Chromosome microscopy/analysis

2.6.1 Chromosome abnormalities
2.6.2 Mosaicism
2.6.3 Chromosome breakage
2.6.4 Karyotyping a cell
2.6.5 Banded karyograms
2.6.6 Haploid band number and band levels
2.6.7 The complete cytogenetic study
2.6.8 Karyograms, karyotypes, and the final report
2.6.9 Sources of error in analysis and reporting

### 2.7 Laboratory procedure manual

References

Contributed protocols

Protocol 2.1 Slide-making
Protocol 2.2 Slide-making
Protocol 2.3 Making wet slides for chromosome analysis
Protocol 2.4 Slide-making
Protocol 2.5 Slide preparation
Protocol 2.6 Slide preparation procedure

---

### 3 Peripheral blood cytogenetic methods

*Helen J. Lawce and Michael G. Brown*

3.1 Using peripheral blood for cytogenetic analysis
3.2 Special uses of peripheral blood cultures
   3.2.1 Chromosome instability syndromes
   3.2.2 Fragile sites
3.3 Peripheral blood constituents
3.4 Specimen handling
   3.4.1 Stimulants
   3.4.2 Anticoagulants
   3.4.3 Culturing
3.5 Cell culture equipment and supplies
   3.5.1 Materials for cell culture
   3.5.2 Solutions for tissue culture
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6 Harvesting peripheral blood cultures</td>
<td>95</td>
</tr>
<tr>
<td>3.7 Chromosome analysis of peripheral blood</td>
<td>95</td>
</tr>
<tr>
<td>3.8 Storage of fixed specimens</td>
<td>95</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>95</td>
</tr>
<tr>
<td>References</td>
<td>95</td>
</tr>
<tr>
<td>Contributed protocols</td>
<td>98</td>
</tr>
<tr>
<td>Protocol 3.1 Blood culture and harvest procedure</td>
<td>98</td>
</tr>
<tr>
<td>Protocol 3.2 High-resolution peripheral blood method</td>
<td>100</td>
</tr>
<tr>
<td>Protocol 3.3 Constitutional cytogenetic studies on peripheral blood</td>
<td>108</td>
</tr>
<tr>
<td>Protocol 3.4 Blood culture and harvest procedure for microarray confirmation studies</td>
<td>115</td>
</tr>
<tr>
<td>4 General cell culture principles and fibroblast culture</td>
<td>119</td>
</tr>
<tr>
<td>Debra F. Saxe, Kristin M. May and Jean H. Priest</td>
<td></td>
</tr>
<tr>
<td>4.1 Definitions of a culture</td>
<td>119</td>
</tr>
<tr>
<td>4.1.1 Time in culture</td>
<td>119</td>
</tr>
<tr>
<td>4.1.2 Growth characteristics</td>
<td>120</td>
</tr>
<tr>
<td>4.1.3 Morphology</td>
<td>120</td>
</tr>
<tr>
<td>4.1.4 Tissue source</td>
<td>121</td>
</tr>
<tr>
<td>4.2 Basic considerations in cell culture</td>
<td>121</td>
</tr>
<tr>
<td>4.2.1 Culture containers</td>
<td>122</td>
</tr>
<tr>
<td>4.2.2 Sterilization and washing principles of cell culture</td>
<td>123</td>
</tr>
<tr>
<td>4.2.3 Water for cell culture</td>
<td>124</td>
</tr>
<tr>
<td>4.2.4 Temperature, pH, CO₂, and humidity control</td>
<td>124</td>
</tr>
<tr>
<td>4.2.5 Media</td>
<td>125</td>
</tr>
<tr>
<td>4.2.6 Balanced salt solution</td>
<td>126</td>
</tr>
<tr>
<td>4.2.7 Dispersion of monolayer cells for subculture</td>
<td>127</td>
</tr>
<tr>
<td>4.2.8 Antimicrobial agents and monitoring for contamination</td>
<td>127</td>
</tr>
<tr>
<td>4.3 Fibroblast culture</td>
<td>128</td>
</tr>
<tr>
<td>4.3.1 Tissue sampling and transport</td>
<td>128</td>
</tr>
<tr>
<td>4.3.2 Tissue sampling to exclude mosaicism</td>
<td>128</td>
</tr>
<tr>
<td>4.3.3 Specimen setup</td>
<td>128</td>
</tr>
<tr>
<td>4.3.4 Routine handling and maintenance of monolayer cultures</td>
<td>129</td>
</tr>
<tr>
<td>4.3.5 Preparation of cultured cells for analysis</td>
<td>130</td>
</tr>
<tr>
<td>4.3.6 Tracking culture age</td>
<td>131</td>
</tr>
<tr>
<td>4.3.7 Freezing and storage of cell lines</td>
<td>131</td>
</tr>
<tr>
<td>4.3.8 Transport and shipping cultured cells</td>
<td>132</td>
</tr>
<tr>
<td>4.4 Lymphoblastoid cell lines</td>
<td>132</td>
</tr>
<tr>
<td>Glossary</td>
<td>132</td>
</tr>
<tr>
<td>Reference</td>
<td>133</td>
</tr>
<tr>
<td>Additional readings</td>
<td>133</td>
</tr>
<tr>
<td>Contributed protocols section</td>
<td>134</td>
</tr>
<tr>
<td>Protocol 4.1 Solid tissue collection for establishing cultures</td>
<td>134</td>
</tr>
<tr>
<td>Protocol 4.2 Solid tissue transport and sendout media</td>
<td>135</td>
</tr>
<tr>
<td>Protocol 4.3 Tissue culture reagents</td>
<td>138</td>
</tr>
<tr>
<td>Protocol 4.4 Phosphate buffer solution deficient in Ca²⁺ and Mg²⁺</td>
<td>141</td>
</tr>
<tr>
<td>Protocol 4.5 Solid tissue and fibroblast culture setup</td>
<td>141</td>
</tr>
<tr>
<td>Protocol 4.6 Solid tissue setup and processing</td>
<td>142</td>
</tr>
<tr>
<td>Protocol 4.7 Flask and coverslip setup for POC/fibroblast cultures</td>
<td>145</td>
</tr>
<tr>
<td>Protocol 4.8 Coverslip setup for solid tissue biopsy specimens</td>
<td>147</td>
</tr>
<tr>
<td>Protocol 4.9 Solid tissue (fibroblast) culturing and harvesting</td>
<td>150</td>
</tr>
<tr>
<td>Protocol 4.10 Fibroblast culture maintenance: media feeding and changing</td>
<td>154</td>
</tr>
<tr>
<td>Protocol 4.11 Routine subculture of fibroblast cultures</td>
<td>155</td>
</tr>
<tr>
<td>Protocol 4.12 Manual harvest for flasks</td>
<td>157</td>
</tr>
<tr>
<td>Protocol 4.13 Treated media for contamination</td>
<td>158</td>
</tr>
<tr>
<td>Protocol 4.14 Fungizone–mycostatin solution for treatment of fungus/yeast contaminated cultures</td>
<td>158</td>
</tr>
</tbody>
</table>
Protocol 4.15 Mycoplasma testing 159
Protocol 4.16 Plating efficiency of serum 160
Protocol 4.17 Routine replication plating for human diploid cells 160
Protocol 4.18 Cell counting chamber method 161
Protocol 4.19 Cell viability by dye exclusion 161
Protocol 4.20 Mitotic index 161
Protocol 4.21 Growth rate—estimation of mean population doubling time during logarithmic growth 162
Protocol 4.22 Maintenance of fibroblast cultures as non-mitotic population 163
Protocol 4.23 Synchronization at S-phase with BrdU 163
Protocol 4.24 Making direct FISH preparations from abortus tissue 164
Protocol 4.25 Cryopreservation 165
Protocol 4.26 Cryopreservation with Nalgene cryogenic container 166
Protocol 4.27 Lymphoblastoid lines 167
Protocol 4.28 Freezing tissue cultures (cryopreservation) 171

5 Prenatal chromosome diagnosis 173

Kristin M. May, Debra F. Saxe and Jean H. Priest

5.1 Introduction 173
5.2 Amniotic fluid 173
5.2.1 Amniocentesis 173
5.2.2 Amniotic fluid characteristics 173
5.2.3 Cells in amniotic fluid 174
5.2.4 Specimen size 175
5.2.5 Collection container 175
5.2.6 Specimen transport 175
5.3 Culture of amniotic fluid 175
5.3.1 Open versus closed system for culture 175
5.3.2 In situ versus flask growth methods 176
5.3.3 Media and additives 176
5.3.4 Number of cultures per specimen 176
5.3.5 Number of days to first culture check 177
5.3.6 Medium addition or changes 177
5.3.7 Treatment of precipitation in cultures 177
5.3.8 Treatment of microbial contamination 177
5.3.9 Treatment of bloody specimens 177
5.3.10 Treatment of poor growth 178
5.3.11 Notifying the physician about poor growth or no growth 178
5.4 Analysis of amniotic fluid 178
5.4.1 Determining harvest times 178
5.4.2 Steps involved in harvest and slide-making 178
5.4.3 Number of slides per patient 179
5.4.4 Stains performed 179
5.4.5 Analysis of amniotic fluid 179
5.4.6 Average time to report cases 179
5.4.7 Maternal cell contamination 180
5.4.8 Mosaicism 180
5.5 Chorionic villus sampling 180
5.5.1 Amniocentesis versus chorionic villus sampling 180
5.5.2 Chorionic villus sampling procedures 181
5.5.3 Structure and cell types 181
5.5.4 Specimen transport 182
5.5.5 Specimen cleaning 182
5.5.6 Direct harvest 184
5.5.7 Villus culture 184
5.6 Analysis of chorionic villi
  5.6.1 Routine chromosome analysis 184
  5.6.2 Confined placental mosaicism and uniparental disomy 185
  5.6.3 Maternal cell contamination 186
  5.6.4 Fetal blood sampling 186
References 186
Contributed protocols section 188
Protocol 5.1 Amniotic fluid culture setup and routine maintenance 188
Protocol 5.2 Coverslip (in situ) harvest procedure for chromosome preparations from amniotic fluid, CVS, or tissues (manual method) 191
Protocol 5.3 Harvest of flask amniocyte cultures 193
Protocol 5.4 Amniotic fluid culturing, subculturing, and harvesting (flask method) 195
Protocol 5.5 Criteria for interpreting mosaic amniotic fluid cultures 198
Protocol 5.6 Chorionic villi sampling – setup, direct harvest, and culture 199
Protocol 5.7 Chorionic villus sampling 204
Protocol 5.8 G-Banding with Leishman's stain (GTL) 208
Protocol 5.9 Cystic hygroma fluid protocol 209

6 Chromosome stains 213
  Helen J. Lawce

6.1 Introduction 213
  6.1.1 Definitions of banding patterns and reference tables 215
  6.1.2 Slide mounting 215
  6.1.3 Mounting media 216
  6.1.4 Slide aging for chromosome staining methods 218
  6.1.5 Conventional (solid) staining methods 219
6.2 Chromosome banding methods 220
  6.2.1 Quinacrine banding (QFQ) 220
  6.2.2 Giemsa banding (GTG, GTW, GAG, GTL) 222
  6.2.3 C-banding (CBG) 230
  6.2.4 G-11 staining 233
  6.2.5 Centromere/kinetochore staining 235
  6.2.6 Reverse banding (R-bands) 238
  6.2.7 DAPI/distamycin A staining (DA-DAPI) 241
  6.2.8 Silver staining (AgNOR) for nucleolus organizing regions 243
6.3 5-bromo-2′-deoxyuridine methodologies 246
  6.3.1 Historical and theoretical perspectives 249
  6.3.2 Replication banding 250
  6.3.3 Sister chromatid exchanges 251
  6.3.4 Technical considerations for replication banding and SCEs 251
6.4 T-banding/CT-banding 252
  6.4.1 Historical and theoretical perspectives 252
  6.4.2 Technical considerations 252
6.5 Antibody banding and restriction endonuclease banding 252
6.6 Destaining slides 252
6.7 FISH DAPI bands 252
6.8 Sequential staining 253
Acknowledgments 253
References 253
Contributed protocols section 266
Protocol 6.1 Conventional Giemsa staining (unbanded) 266
Protocol 6.2 Leishman's stain 266
Protocol 6.3 Quinacrine mustard chromosome staining (Q-bands) 266
Protocol 6.4 C-banding 268
8.3 Karyotype
  8.3.1 Chromosome count 364
  8.3.2 Event symbols 365
  8.3.3 Structural breakpoint 367
  8.3.4 Spaces in a karyotype 372
  8.3.5 Short form karyotype 372
  8.3.6 Detailed karyotype 374
  8.3.7 Karyotype order priority 374
  8.3.8 Repeated description 376
  8.3.9 Amending a cytogenetic karyotype 377

8.4 Numerical events 378
  8.4.1 Polyploidy and endoreduplication 378
  8.4.2 Near ploidy 379
  8.4.3 Autosome aneuploidy 379
  8.4.4 Sex chromosome aneuploidy 379
  8.4.5 Pseudodiploid 380

8.5 Structural events 380
  8.5.1 Deletion (del) 380
  8.5.2 Dicentric (dic) 381
  8.5.3 Duplication (dup) 383
  8.5.4 Insertion (ins) 385
  8.5.5 Inversion (inv) 385
  8.5.6 Isochromosome (i) 386
  8.5.7 Isodicentric (idic) 387
  8.5.8 Constitutional origin (mat, pat, dn, inh, and c) 388
  8.5.9 Recombinant (rec) 389
  8.5.10 Ring of known centric origin (r) 390
  8.5.11 Telomeric association (tas) 391
  8.5.12 Translocation (t) 391
  8.5.13 Uniparental disomy (upd) and loss of heterozygosity (LOH) 394

8.6 Derivative chromosomes (der) 394
  8.6.1 One centric derivative 394
  8.6.2 Homologue derivative 394
  8.6.3 Isoderivative 395
  8.6.4 Multiple events derivative 395
  8.6.5 Neocentromere derivative 396
  8.6.6 Ring derivative involving more than one chromosome 396
  8.6.7 Whole arm derivative 396

8.7 Symbols of uncertainty 397
  8.7.1 Uncertainty (?) 397
  8.7.2 Additional material (add) 399
  8.7.3 Homogeneously staining region (hsr) 399
  8.7.4 Or 399
  8.7.5 Approximation (-) range 399
  8.7.6 Rings of unknown origin (r) 400
  8.7.7 Marker (mar) 401
  8.7.8 Double minute (dmin) 402
  8.7.9 Incomplete (inc) 402
  8.7.10 Composite karyotype [cp20] 402

8.8 Random versus reportable 403
  8.8.1 Guidelines for reporting an abnormality 403
  8.8.2 Heteromorphic variations 403
  8.8.3 Common fragile sites 404
  8.8.4 Rare fragile sites 404
8.9 Multiple cell lines and clones
  8.9.1 Constitutional mosaicism
  8.9.2 Neoplastic clonal evolvement
  8.9.3 Mainline
  8.9.4 Stemline (sl), sideline (sdl) and idem
  8.9.5 Neoplastic polyploidy
  8.9.6 Multiple stemlines
  8.9.7 Jumping translocations
8.10 Fluorescence in situ hybridization
  8.10.1 Probe validation and normal cut-off values
  8.10.2 Signal patterns
  8.10.3 Probe name
  8.10.4 Metaphase FISH karyotype
  8.10.5 Metaphase fusion FISH strategy
  8.10.6 Chromosome paints
  8.10.7 Interphase FISH karyotype
  8.10.8 Interphase fusion karyotype
  8.10.9 Break-apart probe strategy
  8.10.10 Building a string of interphase FISH results
  8.10.11 Paraffin-embedded malignant tissue
  8.10.12 Bone marrow transplant chimerism
8.11 Microarray (arr) and region-specific assay (rsa)
8.12 Conclusion
Acknowledgments
Addendum for ISCN 2016 updates
References

9 Constitutional chromosome abnormalities
  Kathleen Kaiser-Rogers

9.1 Numerical abnormalities
  9.1.1 Clinical consequences of numerical aneuploidy
  9.1.2 Mechanisms of aneuploidy
  9.1.3 Mosaicism
  9.1.4 Trisomy
  9.1.5 Monosomy
  9.1.6 Euploidy
  9.1.7 Triploidy
  9.1.8 Mosaic triploidy
  9.1.9 Tetraploidy
9.2 Structural rearrangements
  9.2.1 Mechanism for structural rearrangements
  9.2.2 Deletions
  9.2.3 Duplications
  9.2.4 Inversions
  9.2.5 Neocentromeric or analphoid chromosomes
  9.2.6 Dicentric chromosomes
  9.2.7 Isochromosomes
  9.2.8 Rings
  9.2.9 Marker chromosomes
  9.2.10 Reciprocal translocations
  9.2.11 Robertsonian translocations
  9.2.12 Insertions
  9.2.13 Complex chromosome rearrangements
References
10 Genomic imprinting

R. Ellen Magenis

10.1 Introduction 481
10.2 Human genomic disease and imprinting
   10.2.1 Chromosomal syndromes 488
   10.2.2 Whole chromosome uniparental disomies 490
   10.2.3 Partial (segmental) uniparental disomies 492
10.3 Germ cell tumors – UPD and imprinting
   10.3.1 Common chromosome abnormalities 493
   10.3.2 Imprinting status 493
Glossary 494
References 496

11 Cytogenetic analysis of hematologic malignant diseases

Nyla A. Heerema

11.1 Introduction 499
11.2 Myeloid leukemias
   11.2.1 Acute myeloid leukemia (AML) 508
   11.2.2 Common recurring cytogenetic abnormalities in AML 510
   11.2.3 Other abnormalities recognized by the WHO 514
   11.2.4 Therapy-related MDS and AML (t-MDS and t-AML) 514
11.3 Myelodysplastic syndromes 514
11.4 Myeloproliferative neoplasms
   11.4.1 Chronic myelogenous leukemia 515
   11.4.2 Chronic myelomonocytic leukemia 515
   11.4.3 Polycythemia vera 516
   11.4.4 Essential thrombocytemia 516
   11.4.5 Primary myelofibrosis 516
   11.4.6 Chronic neutrophilic leukemia 516
   11.4.7 Chronic eosinophilic leukemia 516
   11.4.8 Other myeloid and lymphoid disorders 516
11.5 B- and T-cell lymphoid neoplasms
   11.5.1 Acute lymphoblastic leukemia 517
   11.5.2 Chronic lymphocytic neoplasias 521
11.6 Lymphomas
   11.6.1 B-cell lymphomas 522
   11.6.2 T-cell lymphomas 525
11.7 Laboratory practices
   11.7.1 Common cytogenetic aberrations 525
   11.7.2 Cytogenetic methodology 525
Acknowledgments 533
Glossary of hematopoietic malignancies 533
References 535
Contributed protocols section
Protocol 11.1 Cancer cytogenetics procedure 553
Protocol 11.2 Bone marrow/leukemic peripheral blood setup
   and harvest procedure 553
Protocol 11.3 Bone marrow and leukemic blood culture and harvest procedure using DSP30 CPG
   oligonucleotide/interleukin-2 for B-cell mitogenic stimulation 558
Protocol 11.4 Culture of CpG-stimulated peripheral blood and bone marrow in chronic
   lymphocytic leukemia 560
Protocol 11.5 Plasma cell separation and harvest procedure for FISH analysis 567
Protocol 11.6 Plasma cell separation and harvest procedure for FISH 569
12 Cytogenetic methods and findings in human solid tumors

Marilu Nelson

12.1 Introduction
  12.1.1 Historical review
  12.1.2 Tumor classification

12.2 Processing tumor specimens
  12.2.1 Culture of tumor cells
  12.2.2 Specimen requirements
  12.2.3 Specimen transport
  12.2.4 Specimen receipt
  12.2.5 Culture media and supplementation
  12.2.6 Culture vessels
  12.2.7 Tissue dissociation
  12.2.8 Cell dilution and culture initiation
  12.2.9 Culture maintenance and duration
  12.2.10 Mitotic arrest
  12.2.11 Cell synchrony
  12.2.12 Determination of harvest time
  12.2.13 Hypotonic treatment
  12.2.14 Fixation
  12.2.15 Slide preparation and staining
  12.2.16 Chromosome analysis

12.3 Recurrent cytogenetic abnormalities
  12.3.1 Benign adipose tumors
  12.3.2 Malignant adipocytic tumors
  12.3.3 Skeletal muscle tumors
  12.3.4 Tumors of uncertain differentiation
  12.3.5 Bone tumors
  12.3.6 Tumors of the nervous system
  12.3.7 Tumors of the lung
  12.3.8 Tumors of the liver
  12.3.9 Tumors of the thymus
  12.3.10 Tumors of the salivary glands
  12.3.11 Tumors of the prostate
  12.3.12 Tumors of the kidney
  12.3.13 Tumors involving germ cells of testicular or ovarian origin
  12.3.14 Tumors of the pleura

12.4 Molecular genetic and cytogenetic techniques
  12.4.1 FISH applications
  12.4.2 RT–PCR
  12.4.3 Chromosome microarray analysis and multicolor karyotyping

12.5 Conclusion

Glossary

References

Contributed protocol section

Protocol 12.1 Solid tumor cell culture and harvest
Protocol 12.2 Solid tumor cell culture and harvest
Protocol 12.3 Solid tumor culture
Protocol 12.4 Solid tumor harvest: monolayer and flask methods
Protocol 12.5 Solid tumor culturing and harvesting
13 Chromosome instability syndromes 653
Yassmine Akkari

13.1 Introduction 653
  13.1.1 Cytogenetics versus molecular diagnosis 654
13.2 Fanconi anemia 656
  13.2.1 Cytogenetic diagnosis of Fanconi anemia 656
  13.2.2 Somatic mosaicism in Fanconi anemia 658
13.3 Bloom syndrome 658
13.4 Ataxia–telangiectasia 658
13.5 Nijmegen breakage syndrome 659
13.6 Immunodeficiency, centromeric instability, and facial anomalies syndrome 660
13.7 Roberts syndrome 661
13.8 Werner syndrome 661
13.9 Rothmund–Thomson syndrome 662
13.10 Proficiency testing 662
Glossary 662
References 667
Contributed protocol section 671
Protocol 13.1 Fanconi anemia chromosome breakage procedure for whole blood 671
Protocol 13.2 Supplemental procedure; Ficoll separation of whole blood 675
Protocol 13.3 Fanconi anemia fibroblast set up, culture, subculture, and harvest procedure 676
Protocol 13.4 Fanconi anemia chromosome breakage analysis policy 681
Protocol 13.5 Table for breakage studies result interpretation 682
Protocol 13.6 Fanconi anemia 684

14 Microscopy and imaging 687
Margaret J. Barch and Helen J. Lawce

14.1 The standard microscope 687
  14.1.1 The light path 688
  14.1.2 Magnification, numerical aperture, and resolution 689
  14.1.3 Lenses 690
  14.1.4 Condensers 692
  14.1.5 Eyepieces (or oculars) 693
  14.1.6 Homogenous system 694
  14.1.7 Mechanical stages 694
  14.1.8 Practical microscopy 694
  14.1.9 Cleaning the microscope 694
14.2 Brightfield microscopy 695
  14.2.1 Köhler illumination 695
  14.2.2 Filters 695
  14.2.3 Immersion oil 696
  14.2.4 Coverglass 696
  14.2.5 Slides 696
  14.2.6 Eyepiece adjustment 697
14.3 Fluorescence microscopy 697
  14.3.1 Light sources for fluorescence 697
  14.3.2 Filters for fluorescence 699
  14.3.3 Practical advice for fluorescence 699
14.4 Specialized microscopy 699
  14.4.1 Phase contrast microscopy 699
  14.4.2 Inverted microscope 701
14.5 Capturing the microscopic image 701
  14.5.1 Brightfield photography 701
  14.5.2 Digital imaging 702
15 Computer imaging

Christine E. Haessig

15.1 Introduction 705
15.2 Techniques to improve karyogram image quality 705
15.3 Metaphase preparation 706
15.3.1 Use of phase contrast 706
15.3.2 Chromosome length and staining 706
15.3.3 Band resolution 706
15.4 Microscopy 706
15.4.1 Köhler illumination 706
15.4.2 Magnification 707
15.4.3 Focus 707
15.5 Image capture 707
15.5.1 Computer gray levels 707
15.5.2 Gray scale 708
15.5.3 Indicator chromosomes 710
15.6 Enhancement 710
15.6.1 Sharpening 710
15.6.2 Contrast 710
15.7 Advanced contrast 710
15.7.1 Pink/blue sliders 711
15.7.2 Brighten/darken contrast slider 711
15.7.3 Cutoff % sliders 711
15.8 Macro programming 712
15.9 FISH imaging 713
15.9.1 Microscope setup 713
15.9.2 Thresholding 714
15.9.3 Probe enhancement tips 714
15.10 Printing 715
15.11 Quality control 715
15.12 Archiving 715
Acknowledgments 715
References 715

16 Fluorescence in situ hybridization (FISH)

Helen J. Lawce and Jeffrey S. Sanford

16.1 Introduction 717
16.1.1 Molecular mechanisms of FISH 717
16.1.2 Historical development of FISH 717
16.1.3 FISH of today 719
16.2 Clinical applications of FISH probes 722
16.2.1 In vitro diagnostics versus analyte-specific reagents 722
16.2.2 Probe designs 722
16.3 Deletion/duplication probes for constitutional abnormalities 730
16.3.1 Subtelomeric-specific probes 732
16.3.2 All-human telomere probes 733
16.4 Hematology/oncology and solid tumor probes 734
16.4.1 Cancer-related deletion probes 734
16.4.2 Cancer-related enumeration probes 735
16.14 Other applications

16.14.1 Array CGH confirmation FISH
16.14.2 FISH for bladder cancer

16.15 Variants in FISH signal patterns

16.15.1 Alpha satellite DNA probes
16.15.2 Subtelomere-specific probes
16.15.3 Locus-specific probes

16.16 Conclusion

Acknowledgments

Glossary

References

Contributed protocols

Protocol 16.1 FISH (fluorescence in situ hybridization) methods
Protocol 16.2 LSI, CEP, and paint probe protocol
Protocol 16.3 FISH protocol for multiprobe® FISH panels
Protocol 16.4 Slide pretreatment with pepsin for FISH
Protocol 16.5 Interphase FISH for amniotic fluid specimen aneuploidy
Protocol 16.6 FISH on direct preparations from abortus tissue
Protocol 16.7 FISH on cultured non-mitotic abortus tissue
Protocol 16.8 FISH on smears
Protocol 16.9 FISH on very small samples
Protocol 16.10 Paraffin-embedded tissue FISH method
Protocol 16.11 VP2000 automated slide processor method for FFPE FISH
Protocol 16.12 Plasma cell targeted FISH
Protocol 16.13 Plasma cell separation for interphase FISH using easy SEP magnet method
Protocol 16.14 Preimplantation genetic testing (PGD) for aneuploidy
Protocol 16.15 Preimplantation genetic testing (PGD) FISH for translocations
Protocol 16.16 Post-FISH BrdU antibody detection
Protocol 16.17 Same-day HER2 IQ-FISH pharmDx™ for breast tissue

17 Multicolor FISH (SKY and M-FISH) and CGH

Turid Knutsen

17.1 Introduction

17.1.1 Online databases

17.2 Multicolor FISH (SKY/M-FISH)

17.2.1 Introduction
17.2.2 Other multicolor FISH techniques
17.2.3 Theory
17.2.4 Applications of multicolor FISH
17.2.5 Methodology

17.3 Comparative genomic hybridization

17.3.1 Introduction
17.3.2 Theory
17.3.3 Advantages and limitations
17.3.4 Applications of CGH
17.3.5 Methodology

17.4 Conclusion

Acknowledgments

References

Contributed protocols section

Protocol 17.1 Spectral karyotyping (SKY)
Protocol 17.2 Spectral karyotyping (SKY)
Protocol 17.3 DNA spectral karyotyping
### Protocol 17.4 Multicolor-FISH method (M-FISH) I

- 881

### Protocol 17.5 Multicolor FISH (M-FISH) or 24-color FISH II

- 884

### Protocol 17.6 Multicolor FISH (M-FISH) III

- 888

### Protocol 17.7 Comparative genomic hybridization I

- 891

### Protocol 17.8 Comparative genomic hybridization II

- 898

## 18 Genomic microarray technologies for the cytogenetics laboratory

_Bhavna J. Davé and Warren G. Sanger_

### 18.1 Introduction

- 903
  18.1.1 Principle and advances
  - 903
  18.1.2 Advantages
  - 905
  18.1.3 Methods
  - 906

### 18.2 Applications

- 907
  18.2.1 CMA for identification of congenital genetic defects (constitutional abnormalities)
  - 907
  18.2.2 CMA for characterization of acquired genetic changes
  - 909
  18.2.3 Use of CMA in prenatal diagnostics
  - 911
  18.2.4 CMA in determination of genomic variations and polymorphisms
  - 911
  18.2.5 Evolutionary characterization with CMA
  - 912
  18.2.6 Limitations
  - 912

### 18.3 Genomic microarray in a cytogenetics laboratory

- 913
  18.3.1 General considerations
  - 913
  18.3.2 Specimens
  - 913
  18.3.3 Types of microarray used in the clinical laboratory
  - 914
  18.3.4 Microarray data analysis
  - 914
  18.3.5 Aspects specific to the validation of home-brew microarrays
  - 914
  18.3.6 Validation of FDA-approved commercial, IVD commercial, or home-brew microarrays
  - 914
  18.3.7 Confirmation of abnormal CMA results
  - 915
  18.3.8 Genomic polymorphisms and variations
  - 915
  18.3.9 Reporting CMA results
  - 915
  18.3.10 Examples
  - 915

### 18.4 Conclusion

- 922

Acknowledgment

- 922

Authors’ note

- 923

References

- 923

## 19 Mathematics for the cytogenetic technologist

_Patricia K. Dowling_

### 19.1 General concepts

- 937
  19.1.1 Scientific notation
  - 937
  19.1.2 Manipulating numbers written in scientific notation
  - 939
  19.1.3 Unit conversion
  - 940

### 19.2 Solutions

- 942
  19.2.1 Definitions
  - 942
  19.2.2 Moles and molarity
  - 943
  19.2.3 Preparing a molar solution
  - 943
  19.2.4 Making molar solutions using hydrated compounds
  - 945
  19.2.5 Making percent solutions
  - 946
  19.2.6 Stock solutions
  - 947
  19.2.7 Diluting molar solutions
  - 947
  19.2.8 Diluting a stock molar solution to a specific concentration
  - 947
  19.2.9 Using dilution ratios
  - 949
  19.2.10 Converting molarity to percent
  - 951
  19.2.11 Converting percent to molarity
  - 951
  19.2.12 Serial dilutions (application for dose–response experiments)
  - 951
19.3 Statistical tools
  19.3.1 Determining the normal cut-off for FISH validation 956
  19.3.2 Other statistics of use in the cytogenetics laboratory 961
  19.3.3 Choosing the right method to fit the situation 965
19.4 Using a hemacytometer
  19.4.1 Determining cell count by using a hemacytometer 968
  19.4.2 Determining cell viability using a hemacytometer 972
19.5 Quantification and purity determination of DNA using spectroscopy 973

Reference 974
Additional readings 974

20 Selected topics on safety, equipment maintenance, and compliance for the cytogenetics laboratory 975

20.1 Introduction 975
20.2 Biological hazard safety
  20.2.1 Safety program 975
  20.2.2 Work practice controls 977
  20.2.3 Personal protective equipment 977
  20.2.4 Universal (or standard) precautions 977
  20.2.5 Engineering controls 979
  20.2.6 Housekeeping procedures 979
  20.2.7 Biological hazard spills 979
20.3 Chemical safety
  20.3.1 Know your chemicals 980
  20.3.2 Proper chemical storage 983
  20.3.3 Fume hoods 984
  20.3.4 Working with chemicals 985
  20.3.5 Chemical emergencies 985
20.4 Fire safety
  20.4.1 Fire prevention unit 986
  20.4.2 Fire drills 986
  20.4.3 Fire extinguishers 986
20.5 Electrical safety 987
20.6 Disaster plan 988
20.7 Equipment operation, maintenance, and safety
  20.7.1 Autoclaves 990
  20.7.2 Compressed gas cylinders 990
  20.7.3 Centrifuges 991
  20.7.4 Thermometers 991
  20.7.5 Refrigerators/freezers 991
  20.7.6 Ovens 992
  20.7.7 Water baths 992
  20.7.8 Microscopes 992
  20.7.9 Environmental control chamber 992
  20.7.10 Biological safety cabinets 993
  20.7.11 Pipettes 993
  20.7.12 pH meter 994
  20.7.13 Scales 994
  20.7.14 Timers 994
  20.7.15 Incubators 994
  20.7.16 Fume hoods 995
  20.7.17 Automated hybridization system 995
  20.7.18 Robotic harvester 995
20.8 Ergonomics 996
21 A system approach to quality  

*Peggy J. Stupca and Sheryl A. Tran*

21.1 Quality system  
21.1.1 What is the quality system?  
21.1.2 Organization  
21.1.3 Facilities and safety  
21.1.4 Personnel  
21.1.5 Purchasing and inventory  
21.1.6 Equipment  
21.1.7 Process management  
21.1.8 Documents and records  
21.1.9 Nonconforming event management  
21.1.10 Assessments  
21.1.11 Customer focus  
21.1.12 Information management  
21.1.13 Continual improvement  

21.2 Process management  
21.2.1 Validating new tests, changed tests, and new or moved equipment  
21.2.2 Validation plan  
21.2.3 Specimen number for validation  
21.2.4 Data analytical evaluation  
21.2.5 Reference range maintenance  

21.3 Documents and records  
21.3.1 Document creation and control  
21.3.2 Records  

21.4 Assessments  
21.4.1 Internal assessments  
21.4.2 External assessments  

21.5 Continual improvement  
21.6 Summary  

References  

Contributed protocols section  
Protocol 21.1 Quality control overview document  
Protocol 21.2 Monitoring specimen quality from off-hill sites  

22 Laboratory management  

*Mervat S. Ayad and Adam Sheiti*  

22.1 Introduction  

22.2 Management concepts and functions  
22.2.1 Planning (goal setting)  
22.2.2 Organizing/staffing needs
## Contents

### 22.2.3 Directing (leading) 1032
### 22.2.4 Monitoring (controlling) 1032

### 22.3 Personnel management 1033
  - 22.3.1 Staff level assessment 1033
  - 22.3.2 Job descriptions 1033
  - 22.3.3 Selecting your team 1033
  - 22.3.4 Training 1034
  - 22.3.5 Competency 1034
  - 22.3.6 Performance evaluation and appraisals 1035
  - 22.3.7 Staff motivation and retention 1035
  - 22.3.8 Policy manual 1036

### 22.4 Quality management and control 1036
  - 22.4.1 Technology assessment and implementation 1036
  - 22.4.2 Financial management 1037
  - 22.4.3 Cost per test 1038
  - 22.4.4 Productivity and workload 1038

### 22.5 Budget development and monitoring 1039
  - 22.5.1 Forecast 1039
  - 22.5.2 Budgeting 1040
  - 22.5.3 Capital expenditure 1041
  - 22.5.4 Trends and measures 1042

### 22.6 Conclusion 1043

References 1043
Suggested reading 1043

### 23 Laboratory information system 1045

**Peining Li and Richard Van Rheeden**

- 23.1 Historical perspective 1045
- 23.2 General description of LIS 1045
  - 23.2.1 LIS concept 1045
  - 23.2.2 Software architecture and hardware considerations 1046
  - 23.2.3 Validation and implementation 1047
  - 23.2.4 Compliance and security 1048
- 23.3 LIS in cytogenetics laboratories 1048
  - 23.3.1 The CytoGen system from Washington University in St. Louis 1048
  - 23.3.2 Examples of other cytogenetic LIS systems 1051
- 23.4 Trends for the future LIS 1051

References 1052

### 24 Animal cytogenetics 1055

**Marlys L. Houck, Teri L. Lear and Suellen J. Charter**

- 24.1 Introduction 1055
- 24.2 Domestic animal fertility 1056
  - 24.2.1 Cattle 1056
  - 24.2.2 Horses 1056
- 24.3 Captive management 1057
  - 24.3.1 Species integrity 1057
  - 24.3.2 Chromosome abnormalities 1057
  - 24.3.3 Studbooks 1058
- 24.4 Wildlife conservation 1059
- 24.5 General sample collection considerations 1060
  - 24.5.1 Regulations 1060
  - 24.5.2 Record keeping 1062
24.6 Fibroblast cell culture
   24.6.1 Bioresource banking
   24.6.2 Fibroblast sample sources
   24.6.3 Fibroblast culture conditions
24.7 Peripheral blood culture
24.8 Chromosome analysis
   24.8.1 Conventional staining
   24.8.2 Banding
   24.8.3 Digital imaging
   24.8.4 Karyotyping
   24.8.5 Karyotype standards and precedents
24.9 Molecular and comparative cytogenetics
   24.9.1 Zoo-FISH
   24.9.2 Reciprocal chromosome painting
   24.9.3 BAC maps
   24.9.4 Future directions
Acknowledgments
Glossary
References
Contributed protocol section
Protocol 24.1 Blood feather collection
Protocol 24.2 Avian lymphocyte culture (for large birds)
Protocol 24.3 Lymphocyte culture using whole blood
Protocol 24.4 Lymphocyte culture using autologous plasma/buffy coat (AP/BC)
Protocol 24.5 Horse lymphocyte culture method
Protocol 24.6 Rhino blood culture
Protocol 24.7 Organ tissue collection protocol from carcass
Protocol 24.8 Skin biopsy procedure
Protocol 24.9 Placenta biopsy procedure
Protocol 24.10 Freezing of fibroblast cell cultures
Protocol 24.11 Freezing tissue biopsy samples for later initiation of cell culture (tissue piecing)
Protocol 24.12 Preparation of primary cultures from feather pulp
Protocol 24.13 Preparation of primary cultures from solid tissue (explants)
Protocol 24.14 Preparation of primary cultures using enzyme digestion
Protocol 24.15 Harvesting of fibroblast cell cultures
Protocol 24.16 Preparation of competitor DNA for FISH hybridization
Protocol 24.17 In situ hybridization of BAC clones labeled with spectrum fluorochromes: probe and slide preparation
Protocol 24.18 Labeling DNA with spectrum fluorochromes

25 Online genetic resources and references
   Wahab A. Khan

   25.1 Introduction
   25.2 Resource information
      25.2.1 Databases, laboratory tools and educational tutorials
      25.2.2 Bioinformatic resources
      25.2.3 Links to cytogenetics and genomics support groups
      25.2.4 Prominent peer-reviewed journals pertaining to genetics
      25.2.5 Cytogenetics and medical genetics textbooks
      25.2.6 Vendor products/equipment and lab support
      25.2.7 Credentialing and guidelines
      25.2.8 Genetics training programs and courses
      25.2.9 Professional organizations
      25.2.10 Job search

Index