

J. Mitsuhashi

Invertebrate Tissue Culture Methods



Springer
Lab Manual

Contents

Part 1 General Methods

Chapter 1 Facilities and Equipment	3
1 Facilities	3
2 Equipment	3
3 Tools	10
4 Culture System	13
Chapter 2 Basic Information and Overview	15
1 Routine Precautions for Sterile Work	15
2 Culture Media	15
3 Primary Culture	22
Chapter 3 Preparation of Media	25
1 Direct Method of Preparing Media Consisting of a Few Chemicals	25
2 Indirect Method of Preparing Media Consisting of a Few Chemicals .	26
3 Preparation of Complex Media	27
4 Preparation of Media Containing Water-Insoluble Substances	29
5 Sterilization of Culture Media	30
Chapter 4 General Cell Culture Methods	33
1 Trypsin Treatment for Dissociation of Cell Masses	33
2 Pancreatin Treatment for Dissociation of Cell Masses	34
3 Dispase Treatment for Dissociation of Cell Masses	35
4 Methods for Subculturing	36
5 Hybrid-Cell Technique for Obtaining Growing Cells	37
6 Introduction of Oncogenes to Primary Cultured Cells to Obtain Growing Cells	39
7 Cryopreservation of Cells	41
8 How to Get Cell Lines and How to Ship Cell Lines	43

Part 2 Methods for Setting Up Primary Cultures Specific to Animal Groups

Chapter 5 Insecta (Lepidoptera)	47
1 Ovaries	47
2 Testes	50
3 The Gut	51
4 Stem Cells from the Midgut	52
5 Fat Bodies	54
6 Wing Discs	56

7	Circulatory Organs	57
8	Hemocytes	59
9	Hemocytopoietic Organs	61
10	Malpighian Tubes	63
11	Nervous Tissues	64
12	Embryos	66
13	Neonate Larvae	69
Chapter 6 Insecta (Diptera)		73
1	Embryos	73
2	Neonate Larvae	75
3	Ovaries	77
4	Imaginal Discs	78
5	Nervous Tissues	81
6	Hemocytes	84
Chapter 7 Insecta (Coleoptera)		87
1	Embryos	87
2	Hemocytes	88
Chapter 8 Insecta (Hemiptera)		91
1	Embryos in Oviposited Eggs	91
2	Parthenogenetic Embryos in Ovaries	94
3	Larval Tissues	95
4	Larval and Imaginal Organs	97
Chapter 9 Insecta (Hymenoptera)		101
1	Ovaries	101
2	Embryos	102
3	Parasitized Embryos (Endoparasitoid Wasp)	104
Chapter 10 Insecta (Blattaria)		107
1	Embryos	107
2	Hemocytes	108
3	Nervous Tissues	109
4	Neurosecretory Cells	111
5	Corpora Cardiac and Corpora Allata	112
Chapter 11 Insecta (Orthoptera)		117
1	Dorsal Vessels	117
2	Embryos	118
Chapter 12 Arthropods Other Than Insects		121
1	Tick (Arachnida) Embryos	121
2	Crayfish (Crustacea) Embryos	123
3	Developing Adult Tissues from Ticks (Arachnida)	124
4	Tick (Arachnida) Hemocytes	126
5	Tick (Arachnida) Preimaginal Bodies	128
6	Shrimp (Crustacea) Hepatopancreas	130
7	Shrimp (Crustacea) Lymphoid Organs (Oka Organs) and Ovaries	132
8	Crustacean (Crustacea) Testicular Cells	135

9	Prawn (Crustacea) Gonad Cells	137
10	Cirriped (Crustacea) Ovaries	138
Chapter 13 Prochordata		143
1	Pharyngeal Tissues of the Solitary Protochordates (Tunicata)	143
2	Circulatory Hemocytes of Colonial Protochordates (Tunicata)	145
3	Embryos of the Colonial Protochordates (Tunicata)	148
4	Palleal Buds of Colonial Protochordates (Tunicata)	149
Chapter 14 Echinodermata		153
1	Starfish (Asteroidea) Embryos	153
2	Sea Urchin (Echinoidea) Embryos	156
3	Coelomocytes from Starfish (Asteroidea)	157
4	Coelomocytes from Sea Urchins (Echinoidea)	158
Chapter 15 Mollusca		161
1	Blastomeres of <i>Ilyanassa obsoleta</i> (Gastropoda)	161
2	Snail (Pulmonata) Embryos	162
3	Mantle Tissues of Bivalves (Bivalvia)	165
4	Foot and Mantle Tissues of Land Slugs (Pulmonata)	167
5	Outer Epithelial Cells of Mantle Tissues of Pearl Oysters (Bivalvia) ...	169
6	Cardiac Tissues of Clams (Bivalvia)	171
7	Snail (Pulmonata) Hearts	173
8	Heart Ventricles of Bivalves (Bivalvia)	175
9	Abalone (Gastropoda) Hemocytes	179
10	Amoebocytes from Oysters (Bivalvia)	180
11	Cells from Larval Tissues of Bivalves (Bivalvia)	181
12	Cells from Larval Tissues of Abalones (Gastropoda)	182
13	Gills of the Hard Clams (Bivalvia)	184
14	Snail (Pulmonata) Neurons	186
15	Neurons of the Nudibranches (Opisthobranchia)	188
16	Midgut Glands of <i>Oncomelania</i> (Gastropoda)	189
17	Snail (Pulmonata) Ootestis	190
18	Octopus (Cephalopoda) White Bodies	193
Chapter 16 Annelida		197
1	Oocytes of <i>Nereis virens</i> (Polychaeta)	197
2	Neurons of Leeches (Hirudinea)	198
3	Cell Culture of Earthworms (Oligochaeta)	199
Chapter 17 Nematoda		203
1	Whole Bodies of Plant Parasitic Nematodes (Tylenchida)	203
2	Ovaries of Rat Parasitic Nematodes (Rhabditida)	205
Chapter 18 Platyhelminthes		209
1	Cyst Cells from <i>Echinococcus</i> (Cestoda)	209
2	Hybridoma Technique for Obtaining Growing Metacystode Germinal Cells (Cestoda)	211
3	Cells from <i>Schistosoma mansoni</i> (Trematoda)	213
4	Dissociated Cells from <i>Planaria</i> (Turbellaria)	214

5 Hanging Drop Culture of Explants from <i>Planaria</i> (Turbellaria)	216
6 Neoblasts from <i>Planaria</i> (Turbellaria)	217
Chapter 19 Coelenterata	221
1 Interstitial Cells from <i>Hydra</i> (Hydroidea)	221
2 Coenosarc of <i>Tubularia</i> (Hydrozoa)	224
3 Septa of Sea Anemones (Anthozoa)	226
4 Cells from Colonial Cnidarians (Anthozoa and Hydrozoa)	227
Chapter 20 Porifera	231
1 Cells from Gemmules of Fresh Water Sponges (Demospongia)	231
2 Cell Culture from Seawater Sponges (Calcarea and Demospongia)	232
3 Embryos of Hermaphroditic Sponges (Demospongia)	235

Part 3 Organ Culture

Chapter 21 Insecta	239
1 Imaginal Discs of Caterpillars (Lepidoptera)	239
2 Imaginal Discs of Flies (Diptera)	240
3 Integuments of Lepidopteran Insects (Lepidoptera)	242
4 Nervous System of Cockroach Embryos (Blattaria)	243
5 Nervous System of Adult Cockroaches (Blattaria)	245
6 Stomatogastric Ganglia of Nymphal Cockroaches (Blattaria)	246
7 Brains of Lepidopteran Insects (Lepidoptera)	248
8 Corpora Cardiaca-Corpora Allata Complex of Nymphal Cockroaches (Blattaria)	249
9 Cephalic Complex of Flies (Diptera)	251
10 Prothoracic Glands of Lepidopteran Insects (Lepidoptera)	252
11 Salivary Glands of Dipteran Insects (Diptera)	253
12 Silk Glands of Lepidopteran Insects (Lepidoptera)	254
13 Pheromone Glands of the Indian Meal Moth (Lepidoptera)	255
14 Proventriculus of the Blowfly (Diptera)	256
15 Testes of Various Insects	257
16 Ovaries of Various Insects	259
17 Ovaries and Fat Bodies of the Honeybee (Hymenoptera)	260
18 Regenerating Tissues from Cockroach Legs (Blattaria)	261
19 Tumor Tissues of <i>Drosophila</i> (Diptera)	262
20 Dechorionated Egg Culture and Further Development up to Imaginal Stage in the Silkworm (Lepidoptera)	263
21 In Vivo Culture of <i>Drosophila</i> Imaginal Discs (Diptera)	265
Chapter 22 Arthropoda Other Than Insecta	269
1 Integument of Crayfish (Crustacea)	269
2 Pre-Exuvial Integument of Crabs (Crustacea)	270
3 Mantle Tissue of Cirripeds (Crustacea)	271
4 X- and Y-Organs of Crustaceans (Crustacea)	272
5 Ventral Eye of <i>Limulus</i> (Merostomacea)	274
6 Ovaries of Cirripeds (Crustacea)	275

7 Ovaries of Crabs (Crustacea)	276
8 Dorsal Vessels, Nervous Systems, Pancreases, Gonads, and Intestines of <i>Limulus</i> (Merostomacea)	277
Chapter 23 Prochordata	279
1 Gonads of Protochordates (Tunicata)	279
Chapter 24 Echinodermata	281
1 Gonads of Starfish (Asteroidea)	281
Chapter 25 Mollusca	283
1 Gonads of Slugs (Pulmonata)	283
2 Ovaries of Cuttlefish (Cephalopoda)	284
Chapter 26 Annelida	287
1 Cephalic and Esophageal Regions of Earthworms (Oligochaeta)	287
2 Integuments of Earthworms (Oligochaeta)	288
3 Various Organs of Leeches (Hirudinea)	288
4 Parapodia of Nereids (Polychaeta)	289
5 Enigmatic Vesicles and Urns of <i>Sipunculus</i> (Sipunculoidea)	290
Chapter 27 Nematoda	293
1 Female Reproductive Organs of Root Knot Nematodes (Tylenchida)	293
Chapter 28 Platyhelminthes	295
1 Regeneration Blastema of Fresh-Water Planaria (Turbellaria)	295
2 Scolex of Cyclophyllidean Cestodes (Cestoda)	296
Chapter 29 Coelenterata	299
1 Ectodermal Fragments of Hydra (Hydrozoa)	299
2 Stalks of Hydra (Hydrozoa)	300
3 Gorgonian Tissues of Hydra (Hydrozoa)	301
Chapter 30 Porifera	303
1 Ciliated Layer of Sponge Larva (Demospongia)	303

Part 4 Related Techniques

Chapter 31 Cell Cloning	307
1 Single Cell Cloning	307
2 Capillary Method	309
3 Oil Overlay Method	310
4 Fibrin Clot Method	311
5 Colony Formation for Substrate-Dependent Cells	312
6 Colony Formation in Soft Agar	314
7 Dilution Limit Method	315
8 Feeder Layer Culture	316
9 Nurse Culture	318

Chapter 32 Karyotype Analysis	321
Chapter 33 Identification of Cell Lines	325
1 Isozyme Analysis by Agar Gel Electrophoresis	325
2 Phosphoglucosomerase Isozyme Pattern on Isoelectric Focusing	328
3 DNA Amplification Fingerprinting (DAF)	329
4 RAPD-PCR	331
Chapter 34 Viability of Cells	337
1 Vital Staining Method	337
2 Dye-Exclusion Method	337
Chapter 35 Viable Cell Enumeration	341
1 Hemocytometer Count	341
2 Counting Cells Using a Grid Projected onto a Microscope Field	342
3 MTT Method	343
4 Protein Measurement	344
Chapter 36 Growth Rate	347
1 Growth Curves	347
2 Population Doubling Time	350
Chapter 37 Microscope Photography	353
Chapter 38 Virus Inoculation-Plaque Assay	357
1 Preparation of Inoculum from Hemolymph	357
2 Preparation of Inoculum from Whole Bodies or Tissues	358
3 Preparation of Inoculum from Isolated Occlusion Bodies	359
4 Preparation of Inoculum from an Infected Cell Culture	360
5 Qualitative Inoculation	360
6 Quantitative Inoculation (Infectivity and Multiplicity of Infection) ...	361
7 Plaque Assay Method	363
Chapter 39 Examination of Drug Effects	367
Chapter 40 Cell Fusion	369
1 Cell Fusion by Viruses	369
2 Cell Fusion with Polyethyleneglycol	370
3 Electrofusioin of Cells	371
4 Method for the Selection of Hybrid Cells: Use of Auxotrophic Cells	374
5 Method for the Selection of Hybrid Cells: Use of ts Mutants	375
Chapter 41 Gene Technology	379
1 DNA-Calcium Phosphate Coprecipitation Method	379
2 Polybrene-DMSO Method	380
3 Lipofection Method	381
4 Electroporation Method	382
5 Selection of Transfected Cells with G418	383

6	Selection of Transfected Cells with Puromycin	384
7	Selection of Transfected Cells with Hygromycin B	384
8	Selection of Transfected Cells with α -Amanitin	385
 Chapter 42 Large-Scale Cell Culture		387
1	Anchorage-Dependent Cell Culture	388
2	Shaking Flask Culture	390
3	Spinner Culture	390
4	Stirred Tank Bioreactor Culture	391
5	Internal Membrane Perfusion Culture	393
6	External Membrane Perfusion Culture	394
7	Air-Sparged Bioreactor Culture (Air-Lift Bioreactor Culture)	395
8	Immobilization of Cells by the Microcapsule Method	396

Appendix

1	Composition of Salt Solutions and Culture Media	401
2	List of Reported Continuous Invertebrate Cell Lines	421
3	Commercially Available Culture Medium Products and List of Suppliers	435
 Subject Index		439