Index

Page references followed by f denote figures; those followed by t denote tables.

A

Acids
  disposal of, 197
  safe handling of, 196, 199
Amacrine cells, 17
American Type Culture Collection (ATCC), 199
Angiogenesis, 112
Animals, humane treatment of, 197

B

Bacteria
  biological safety procedures, 198–199
  shipping requirements, 199
Bandeira simplifolia lectin I (BSL-1). See \textit{Bandeira simplifolia} lectin I

Bases
  \( g/\, mL \) (recipe), 123
  \( g/m\, mL \) (recipe), 18, 53, 142, 156, 173

Binding
  of antibody markers, history of development of, 197
  selection for immunopanning, 192, 193t

Antibody
  immunolabeling for identifying cell types, 192t
  monoclonal antibody production, culturing hybridoma cell lines for (protocol), 21–23
  primary for immunopanning purification of neural cells, 192t
  secondary, coating plates with, 13, 49–50, 76–77, 102, 116, 143, 148, 169, 182, 192
  for astrocytes, 71–95
  addition to myelinating cocultures of oligodendrocyte lineage cells and retinal ganglion cells, 163, 164
  coculture with endothelial cells, 112
dissociation of cells, 79–80
flow diagram for, 183
  for astrocytes, 71–95
  for purification of astrocytes from transgenic rodents by fluorescence-activated cell sorting (protocol), 90
  for purification of rat and mouse astrocytes by immunopanning (protocol), 74–85
  flow diagram for, 77f
  materials, 74–76
  method, 76–83
  brain dissection, 78–79
  dissociation of cells, 79–80
  modifications for mouse immunopanning plates, 82–83
  panning, 81–82
  preparation of panning dishes, 76–77
  preparation of solutions and panning dishes, 77–78
  recipes, 83–85

ATCC (American Type Culture Collection), 199
Bases
  \( g/m\, mL \) (recipe), 18, 53, 142, 156, 173

CD31 antigen, 120, 122
Collagenase
  collagenase \( \text{C1} \) (recipe), 18, 40, 65
  collagenase \( \text{C2} \) (recipe), 18, 40, 65
  collagenase \( \text{D (collagenase 4)} \) enzyme cocktail, for Schwann cell isolation, 183
  dispase (C\text{1}–2\text{D}) enzyme cocktail, for Schwann cell isolation, 183

Chemicals, properties of common hazardous, 199–200
Cholera toxin B subunit (CTB), 26–27, 28–29
Chun, Linda, 1, 189
Ciliary neurotrophic factor (10 \( \mu \text{g/mL} \) (recipe), 18, 53, 142, 156, 173
Ciliary neurotrophic factor (CNTF), as growth factor for retinal ganglion cells, 9
Claudin 5, as endothelial cell marker, 122, 122f
Cnp1 gene, 26
CNP, 155f
CNTF (ciliary neurotrophic factor), as growth factor for retinal ganglion cells, 9
CNTF stock (10 \( \mu \text{g/mL} \) (recipe), 18, 53, 142, 156, 173
CO\textsubscript{2} incubators, problems with, 191
Collagenase/ dispase cocktail (recipe), 185
Collagenase/ dispase (C/D) enzyme cocktail, for Schwann cell isolation, 183
Collagen IV, 118, 123
Corticospinal motor neurons (CSMNs), 25–44
  anatomical and morphological characterization, 25
  function and survival in model systems, 26–27
  generation and development, 25–26
  immunopanning of retrograde-labeled corticospinal motor neurons from early postnatal rodents (protocol), 31–44
  discussion, 39
  flow diagram for, 34f
  materials, 31–32
  method, 33–39, 39f
  dissection, 35
  dissociation, 35–36

©2014 by Cold Spring Harbor Laboratory Press
Corticospinal motor neurons (CSMNs), (Continued)

epitope recovery and buffer
transition, 36–37
panning, 37–38
preparation, 33–35
trypsinization and plating, 38–39
recipes, 39–43
laminar identity, 26
retrograde labeling from early postnatal
rodents (protocol), 28–30
materials, 28–29
method, 29, 29f
Cory, David, 1, 189

Cytosine arabinoside (AraC), 52, 177

DMEM (Dulbecco’s modified Eagle’s medium), 47–50

Density gradient centrifugation, in spinal motor
neurons, 55–69

Dorsal root ganglion neurons (DRGs), 55–69

DNase I (recipe), 157

DMEM-SATO base growth medium (with NB)

See specific protocols

Dissection.

Diap3 gene, 26

Cysteine.

Culturing hybridoma cell lines for monoclonal
antibody production (protocol), 21–23

materials, 21–22

method, 22

recipes, 23

Culturing Nerve Cells (Banker and Goslin), 2

Cutting devices, safe handling of, 197

Cox2 gene, 26

Cyclo AMP (cAMP), retinal ganglion cell
survival and, 9

Cysteine. See L-cysteine, for papain activation

Cytosine arabinoside (araC), 52, 177

D

Density gradient centrifugation, in spinal motor
neuron purification, 50

Department of Health, Education, and Welfare
(HEW), U.S., 199

Diap3 gene, 26

Disposal

general cautions, 195–197

of laboratory waste, 197

Dissection. See specific protocols

Dissociation of cells. See specific protocols

DMEM (Dulbecco’s modified Eagle’s medium), 191

DMEM-SATO base growth medium (recipe), 142–143, 156–157

DMEM-SATO base growth medium (with NB)

(recipe), 18

DNase, problems with, 190

DNase I (recipe), 157

Dorsal root ganglion neurons (DRGs), 55–69

nonprospective purification strategies, 55

prospective isolation, 55–56

purification from rat by immunopanning

(Protocol), 37–69, 60f

flow diagram for, 59f

materials, 57–59

method
dissection, 61–62
dissociation, 62–63
feeding and culturing, 65

panning, 63–64

plating, 64

preparation of coverslips, solutions, and

panning dishes, 59–61

recipes, 65–69

DRG base medium (recipe), 65

DRGs. See Dorsal root ganglion neurons

(CSMNs), 26

Dukes, hazards of, 199

E

EBSS stock (10 x) (recipe), 123, 143, 157

Efflux transporters, 112

Endocytotes, immunolabeling antibodies for
identification of, 192t

Endothelial cell growth medium (recipe), 124

Endothelial cells, CNS, 111–126

coculture with astrocytes, 112

properties and functions of, 111–112

purification from rodent brain by

immunopanning (protocol), 114–126

flow diagram for, 117f

materials, 114–116

method, 116–122

dissociation, 119

preparation of panning dishes, coverslips, and solutions, 116–119

trypsinization, 121–122

troubleshooting, 123

purification strategies, 112

Endothelial cells, CNS, 111–126

coculture with astrocytes, 112

properties and functions of, 111–112

purification from rodent brain by

immunopanning (protocol), 114–126

flow diagram for, 117f

materials, 114–116

method, 116–122

dissociation, 119

preparation of panning dishes, coverslips, and solutions, 116–119

trypsinization, 121–122

troubleshooting, 123

purification strategies, 112

Environmental Protection Agency (EPA), U.S., 197

Enzyme stock solution (recipe), 83, 94

Ethanol-washed glass coverslips (recipe), 19, 41, 66, 108, 143, 157, 185

F

FACS (fluorescence-activated cell sorting)

GFP (green fluorescent protein) and, 86

FACS compared, 3–4, 26, 129

FACS (fluorescence-activated cell sorting)

GFP (green fluorescent protein) and, 86

FACS compared, 3–4, 26, 129

FACS (fluorescence-activated cell sorting)

GFP (green fluorescent protein) and, 86

FACS compared, 3–4, 26, 129

FACS (fluorescence-activated cell sorting)

GFP (green fluorescent protein) and, 86

FACS compared, 3–4, 26, 129

FACS (fluorescence-activated cell sorting)

GFP (green fluorescent protein) and, 86

FACS compared, 3–4, 26, 129

FACS (fluorescence-activated cell sorting)

GFP (green fluorescent protein) and, 86

FACS compared, 3–4, 26, 129

FACS (fluorescence-activated cell sorting)

GFP (green fluorescent protein) and, 86

FACS compared, 3–4, 26, 129

FACS (fluorescence-activated cell sorting)

GFP (green fluorescent protein) and, 86

FACS compared, 3–4, 26, 129
SATO supplement, NB-based (100×), 43, 68, 84–85, 125, 186–187
Schwann cell growth medium, 186
thyroxine (T3) stock (4 μg/mL), 20, 54, 69, 76–77, 126, 159, 176, 187
Retinal ganglion cells (RGCs), 7–23
advantages of RGCs as a model system, 7–8
anatomy, function, and development of RGCs, 7
mRNA profiling of RGCs, 8
mRNA expression of RGCs, 8
immunopanning of RGCs, 8
retina formation of RGCs, 8
migration of RGCs, 8
purification of RGCs, 8
growth factors for purified RGCs, 8
in vivo and in vitro assays for RGCs, 8
myelinating cocultures of RGCs, 8
in vitro expansion and culture for RGCs, 8
immunopanning of purified RGCs, 8
retrograde labeling of corticospinal motor neurons from early postnatal rodents, 8–20
immunopanning, 51
plating and culturing, 52, 52f
preparation of cell culture growth substrate and immunopanning dish, 49–50
recipes, 53–54
Svet1 gene, 26

T
T3. See Tri-iodothyronine
T3 (4 μg/mL stock) (recipe), 20, 54, 69, 126, 159, 176, 187
Temperature, for immunopanning, 192
Tyr-1, on retinal ganglion cell surface, 8
Thyroxine, in Bottenstein–Sato serum-free additive, 191
Tie2GFP transgenic mouse line, 112
Tight junctions, 111
Titration, 193
Toxic compounds, 200
Transcytotic vesicles, 111
Trypsin. See also Trypsinization
cell release by, 193
preparation and storage of, 190
Trypsinization in astrocyte purification, 81
in CNS endothelial cell purification, 121–122
in corticospinal motor neuron purification, 38–39
in oligodendrocyte precursor cell purification, 139–140, 172
in oligodendrocyte purification, 153–154
in pericytes purification from rodent optic nerve, 106
in retinal ganglion cell isolation, 17
in Schwann cell isolation and purification protocol, 184
in spinal motor neuron purification, 50

U
Ultrasonicators, 196–197

V
Vascular endothelial growth factor (VEGF), for corticospinal motor neuron growth, 26
Vascular smooth muscle cells, 97
Vasculogenesis, 112
VCL.1 (amacrine surface antigen), 17
VE-Cadherin, as endothelial cell marker, 122
VWF, as endothelial cell marker, 122

W
Waste, disposal of, 197, 198