

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
- 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, 134, 148, 169, 182, 192
 - selection for immunopanning, 192, 193t
- Antibody markers, history of development of, 1–2
- Anti-CD31 antibodies, 112, 114
- Anti-macrophage antisera, 8
- Anti-oxidants (AO) (1000×) (recipe), 39–40
- APV (DL-2-amino-5-phosphonopentanoic acid) stock (25 mM) (recipe), 39–40
- AraC (cytosine arabinoside), 52, 177
- Astrocytes, 71–95
 - addition to myelinating cocultures of oligodendrocyte lineage cells and retinal ganglion cells, 163, 164
 - coculture with endothelial cells, 112
 - heterogeneity of, 2
 - immunolabeling antibodies for identification of, 192t
 - limitations of standard astrocyte preparations, 71–72
 - prospective isolation, 72
 - purification of astrocytes from transgenic rodents by fluorescence-activated cell sorting (protocol), 86–95
 - flow diagram for, 89f
 - materials, 86–88
 - method, 88–93
 - additional depletion of oligodendrocytes and myelin for animals older than P8, 93
 - brain dissection, 90
 - dissociation of cells, 90–92
 - FACS, 93
 - panning, 92–93
 - plating cells, 93
 - preparation of panning dishes, 88
 - preparation of solutions and panning dishes, 88–90
 - recipes, 94–95

- 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
- Autoclave safety, 196
- Axonal–glial interactions, 161

B

- Bacteria
 - biological safety procedures, 198–199
 - shipping requirements, 199
- Bandeira simplicifolia* lectin I (BSL-1). *See* BSL-1
- Banker, Gary, 2
- Barres, Ben, 46, 52
- Bases
 - disposal of, 197
 - safe handling of, 196, 199
- Basic fibroblast growth factor (bFGF),
 - for CNS endothelial cells, 122
 - for retinal ganglion cells, 9
- BDNF stock (10 µg/mL) (recipe), 53
- BDNF stock (50 µg/mL) (recipe), 18, 40, 65
- bFGF (50 µg/mL) (recipe), 123
- Biological safety procedures, 198–199
- Biotin stock (500×) (recipe), 173
- Blood–brain barrier (BBB)
 - CNS endothelial cells and, 111
 - pericytes and, 98
- BNDF. *See* Brain-derived neurotrophic factor
- Bottenstein–Sato serum-free additive, 9, 191
- Bovine serum albumin (BSA)
 - for blocking nonspecific binding on panning dishes, 183
 - in Bottenstein–Sato serum-free additive, 191
- Brain-derived neurotrophic factor (BDNF)
 - for corticospinal motor neurons, 26
 - for dorsal root ganglion neurons, 64
 - for retinal ganglion cells, 9
- Brain dissection
 - for endothelial cell purification (protocol), 119
 - for oligodendrocyte precursor cells (protocol), 137, 170
 - for oligodendrocytes (protocol), 151
 - for pericyte purification from rodent optic nerve (protocol), 104

- for purification of astrocytes from transgenic rodents by fluorescence-activated cell sorting (protocol), 90
- for purification of rat and mouse astrocytes by immunopanning (protocol), 78–79
- BSA. *See* Bovine serum albumin
- BSA stock (4%) (recipe), 40, 173, 185
- BSL-1, 8, 15, 56, 60, 150, 154–155, 192
- B-27 supplement, 9, 191

C

- cAMP (cyclic AMP), retinal ganglion cell survival and, 9
- CD31 antigen, 120, 122
- C/D (collagenase/dispase) enzyme cocktail, for Schwann cell isolation, 183
- CD45 monoclonal antibody, 192
- CD9 protein, 56, 60
- Cell types, immunolabeling antibodies for identifying, 192t
- Chemicals, properties of common hazardous, 199–200
- Cholera toxin B subunit (CTB), 26–27, 28–29
- Chun, Linda, 1, 189
- Ciliary neurotrophic factor (10 µg/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
- Clim1* gene, 26
- CNP, 155f
- CNTF (ciliary neurotrophic factor), as growth factor for retinal ganglion cells, 9
- CNTF stock (10 µg/mL) (recipe), 18, 53, 142, 156, 173
- CO₂ 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

- 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
- Coverslips
 coating with collagen IV, 118
 coating with poly-D-lysine, 33, 49, 59–60, 102, 118, 136, 149, 167, 182
 ethanol-washed glass, 19, 41, 108, 143, 157, 185
- C5 panning for pericyte purification, 106
- Crim1* gene, 26
- CSMN growth medium (recipe), 40
- CSMNs. *See* Corticospinal motor neurons
- CSMN survival medium (recipe), 41
- CTB (cholera toxin B subunit), 26–27, 28–29
- Ctip2* gene, 26
- Culture medium
 limitations of, 3
 osmolarity of, 191
 selection of, 191
- 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
- Cux2* gene, 26
- Cyclic 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), 57–69, 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
- Dulbecco's modified Eagle's medium (DMEM), 191
- Dyes, hazards of, 199
- ## E
- EBSS stock (10×) (recipe), 123, 143, 157
- Efflux transporters, 112
- Endocytes, 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
 dissection, 119
 dissociation, 119–120
 panning, 120–121
 plating, 122, 122f
 preparation of panning dishes, coverslips, and solutions, 116–119
 trypsinization, 121–122
 troubleshooting, 123
 purification strategies, 112
- Environmental Protection Agency (EPA), U.S., 197
- Enzymes. *See specific enzyme names; specific protocols*
- Enzyme stock solution (recipe), 83, 94
- Ethanol-washed glass coverslips (recipe), 19, 41, 108, 124, 143, 157, 185
- Euthanasia. *See specific protocols*
- ## F
- FACS (fluorescence-activated cell sorting)
 GFP (green fluorescent protein) and, 86
 immunopanning compared, 3–4, 26, 129
 for pericyte isolation, 98
 purification of astrocytes from transgenic rodents by fluorescence-activated cell sorting (protocol), 86–95
- Fetal calf serum, 3
- Fezl* gene, 26
- Fibroblasts
 complement-mediated lysis of, 177
 growth limitation with cytosine arabinoside (AraC), 177
- Fluorescence-activated cell sorting. *See* FACS
- Forskolin
 for dorsal root ganglion neurons, 64
 for retinal ganglion cells growth, 9
- Forskolin stock (4.2 mg/mL) (recipe), 19, 53, 66, 124, 143, 157, 173, 186
- Freezing hybridoma cells, 22
- FUDR (5-fluoro-2'-deoxyuridine), 65
- ## G
- γ-secretase inhibitor addition to coculture of oligodendrocyte lineage cells and retinal ganglion cells, 163, 163f, 172
- Gas containers, safe handling of, 196
- GDNF stock (10 μg/mL) (recipe), 53
- GFP (green fluorescent protein), FACS and, 86
- Glial cell line-derived growth factor (GDNF), for corticospinal motor neurons (CSMNs), 26
- Goslin, Kimberly, 2
- Griffonia simplicifolia* lectin. *See* BSL-1
- ## H
- Hazardous chemicals, properties of common, 199–200
- High-ovomucoid stock solution (6×) (recipe), 19, 41, 66, 108, 124–125, 143, 157, 174
- High-ovomucoid stock solution (10×) (recipe), 83, 94
- Hormone mix (200×) (recipe), 174
- Hybridoma, culturing cell lines for monoclonal antibody production (protocol), 21–23
 materials, 21–22
 method, 22
 recipes, 23
- Hybridoma cell line medium (recipe), 23
- ## I
- IGF-1. *See* Insulin-like growth factor 1
- Igfbp4* gene, 26
- Immunolabeling antibodies for identifying cell types, 192t
- Immunopanning, 1–2, 5, 189. *See also specific cell types*
 for astrocytes, 72, 72f, 74–95
 for CNS endothelial cells, 112–126
 for corticospinal motor neurons, 26, 31–44
 designing and troubleshooting protocols, 189–194
 antibody selection, 192, 193t
 common errors, 190
 low yield problems, 190–191
 removing cells from final dish, 193
 temperature, 192
 for dorsal root ganglion neurons, 57–69
 FACS compared, 3–4, 26, 129
 for oligodendrocytes, 129, 132–159, 169–172
 for pericytes, 98–109
 for retinal ganglion cells, 8, 11–20
 for Schwann cells, 177–178, 180–187
 for spinal motor neurons, 47–54
- Incubators, problems with, 191
- Inhibitor stock solution (recipe), 84, 94, 125
- Institutional safety office, 195
- Insulin, as growth factor for retinal ganglion cells, 9
- Insulin-like growth factor 1 (IGF-1)
 for corticospinal motor neurons, 26
 for retinal ganglion cells, 9
- Insulin stock (0.5 mg/mL) (recipe), 19, 23, 41, 53, 66, 108, 125, 143, 158, 174, 186
- IP-astrocyte base medium (recipe), 84, 94
- Isopropanol, for freezing hybridoma cells, 22

- K**
Ky stock (0.8 M) (recipe), 41
- L**
Laminin
 in corticospinal motor neuron protocol, 33
 in dorsal root ganglion neuron protocol, 59–60
 human placental, 50
 in retinal ganglion cell protocol, 13
 in Schwann cell protocol, 182
 in spinal motor neuron, 50
Laser safety, 196
L-cysteine, for papain activation, 15, 35, 78, 105
Leukemia inhibitory factor (LIF)
 for corticospinal motor neurons, 26
 for retinal ganglion cells, 9
Lower motor neurons, 45–46. *See also* Spinal motor neurons
Low-ovomucoid stock solution (10×) (recipe), 19, 41, 66, 84, 95, 108, 125, 144, 158, 174, 186
Lumafuor Retrobeads IX, 28–29
Lysine. *See* Poly-D-lysine, coating coverslips with
- M**
Material safety data sheets (MSDSs), 195
MBP (myelin basic protein), 141f
Medical Pathological Waste (MPW), 197
Metabolomics, 4–5
Microglia, depletion by BSL-1, 154–155
Microwave safety, 196
Mitchison, Avrion, 1
Mitogens, oligodendrocyte precursor cells (OPCs) proliferation and, 128
Molecular transporters of CNS endothelial cells, 111
Monoclonal antibody production, culturing hybridoma cell lines for (protocol), 21–23
 materials, 21–22
 method, 22
 recipes, 23
Motor neuron growth medium (recipe), 53–54
Motor neurons, 45–54. *See also* Corticospinal motor neurons; Spinal motor neurons
 characteristics of, 45
 in culture, 46
 disease and trauma of lower motor neurons, 45–46
Mouse. *See also* Rodents
 production and culture of retinal ganglion cells from rodents (protocol), 11–20
 purification of astrocytes from transgenic rodents by fluorescence-activated cell sorting (protocol), 86–95
 purification of endothelial cells from rodent brain by immunopanning (protocol), 114–126
 purification of oligodendrocyte lineage cells from mouse cortices by immunopanning (protocol), 146–160
 purification of rat and mouse astrocytes by immunopanning (protocol), 74–85
 Schwann cell purification from neonatal and injured adult peripheral nerve (protocol), 180–187
 MPW (Medical Pathological Waste), 197
 MSDSs (material safety data sheets), 195
 Myelinating cocultures of purified oligodendrocyte lineage cells and retinal ganglion cells, 161–176
 establishing coculture, 161–163, 162f, 163f
 γ-secretase inhibitor addition to coculture, 163, 163f, 172
 maturation of coculture, 163–164, 164f
 optic nerve astrocyte addition to coculture, 163, 164
 protocol, 165–176
 flow diagram of, 169f
 materials, 165–167
 method, 167–173, 167f, 169f
 dissection, 170
 dissociation, 170–171
 nucleofection of optic nerve cells, 172–173
 optic nerve OPC isolation by immunopanning, 169–172
 trypsinization, 172
 recipes, 173–176
 Myelination, regulation of, 127–128
 Myelination medium, 162, 163
 Myelination medium (recipe), 174
 Myelin basic protein (MBP), 141f
 Myelin debris, in Schwann cell isolation and purification protocol, 185
 MyM base medium (recipe), 175
- N**
NAC stock (5 mg/mL) (recipe), 19, 43, 54, 66, 84, 125, 175, 186
National Institute of Environmental Health and Human Services, 199
NBS buffer (recipe), 42–43
NB-sucrose buffer (recipe), 42
ND-growth medium (recipe), 175
ND-SATO base medium (recipe), 175
Nerve growth factor (NGF)
 for corticospinal motor neurons, 26
 for dorsal root ganglion neurons, 64
 p75 receptor, 47
Nestin, 107f
Neurobasal, 18, 43, 68, 85, 126, 186–187, 191
Neuronal-glia interactions, 4, 161
Neurotrophin-3 (NT3)
 for corticospinal motor neurons, 26
 for dorsal root ganglion neurons, 64
 for oligodendrocyte lineage cells, 141, 154
NG2, as pericyte marker, 107
NGF. *See* Nerve growth factor
Nkx2.2 gene, 128
Notch, oligodendrocyte differentiation and, 128
NS21 (50×) (recipe), 67–68
NS21 additive, 191
NT-3. *See* Neurotrophin-3
NT-3 (1 μg/mL stock) (recipe), 68, 144, 158
NT-4/5 (neurotrophin-4/5), as growth factor for retinal ganglion cells, 9
Nucleofection of optic nerve cells, 172–173
- O**
Occludin, as endothelial cell marker, 122, 122f
Occupational Safety and Health Administration (OSHA), 195
Olig1 gene, 128
Olig2 gene, 128
Oligodendrocyte precursor cells (OPCs)
 heterogeneity in, 129
 immunolabeling antibodies for identification of, 192t
 immunopanning for, 132–145, 169–172
 origin of, 127
 proliferation, mitogens and, 128
 purification of oligodendrocyte precursor cells from rat cortices by immunopanning (protocol), 132–145
 flow diagram for, 135f
 materials, 132–134
 method, 134–141
 dissection, 137
 panning, 139
 plating, 140–141, 141f
 preparation for cell purification, 136–137
 preparation of plates and reagents, 134–136
 tissue dissociation, 137–139
 trypsinization, 139–140
 recipes, 142–145
 troubleshooting, 141–142
 from rats and mice, 130
 T3 promotion of clock mediated differentiation, 128–130
Oligodendrocytes, 127–160
 development of, 127–129
 mitogens and, 128
 regulation of differentiation and myelination, 127–128
 T3 promotion of clock mediated differentiation, 128–129
 immunolabeling antibodies for identification of, 192t
 methods for purification and culture of, 129–130
 myelinating cocultures of purified oligodendrocyte lineage cells and retinal ganglion cells, 161–176
 establishing coculture, 161–163, 162f, 163f
 γ-secretase inhibitor addition to coculture, 163, 163f, 172
 maturation of coculture, 163–164, 164f
 optic nerve astrocyte addition to coculture, 163, 164
 protocol, 165–176
 dissection, 170
 dissociation, 170–171
 flow diagram of, 169f
 materials, 165–167
 method, 167–173, 167f, 169f
 nucleofection of optic nerve cells, 172–173
 optic nerve OPC isolation by immunopanning, 169–172
 recipes, 173–176
 trypsinization, 172
 purification of oligodendrocyte lineage cells from mouse cortices by immunopanning (protocol), 146–160
 flow diagram for, 149f
 materials, 146–148
 method, 148–154
 dissection, 151
 panning, 152–153

- Oligodendrocytes, (*Continued*)
 plating, 154, 155f
 preparation of cell purification, 150–151
 preparation of plates and reagents, 148–150
 tissue dissociation, 151–152
 trypsinization, 153–154
 recipes, 156–159
 troubleshooting, 154–156
 purification of oligodendrocyte precursor cells from rat cortices by immunopanning (protocol), 132–145
 flow diagram for, 135f
 materials, 132–134
 method, 134–141
 recipes, 142–145
 troubleshooting, 141–142
 OPC culture medium (recipe), 144, 158
 OPCs. *See* Oligodendrocyte precursor cells
 Optic nerve
 myelinating cocultures of rat retinal ganglion cell reagggregates and optic nerve oligodendrocyte precursor cells (protocol), 165–176
 nucleofection of optic nerve cells, 172–173
 purification of oligodendrocyte precursor cells from rat by immunopanning (protocol), 132–145
 purification of pericytes from rodent by immunopanning (protocol), 100–109
 OSHA (Occupational Safety and Health Administration), 195
 Osmolarity, of culture medium, 191
Otx1 gene, 26
- P**
 p75 (neurotrophin receptor)
 Schwann cell, 177
 spinal motor neuron, 47
 Papain, 8
 in CNS endothelial cell protocol, 119–120
 in corticospinal motor neuron protocol, 35–36
 in dorsal root ganglion neuron protocol, 62
 in oligodendrocyte protocols, 137–138, 151, 170–171
 in pericyte protocol, 105
 in retinal ganglion cell protocol, 15
 source of, 190
 Papain buffer (recipe), 125, 144, 158
Pcp4 gene, 26
 PDGF, for oligodendrocyte lineage cells, 141, 154, 155f
 PDGF-AA (platelet-derived growth factor-AA), 128
 PDGF-BB (platelet-derived growth factor-BB), 97–98
 PDGFR α (platelet-derived growth factor receptor alpha), 155, 155f
 PDGFR β (platelet-derived growth factor receptor beta), 97–98, 100, 105–106, 122, 123
 PDGF stock (10 μ g/mL) (recipe), 144–145, 159
 PDL. *See* Poly-D-lysine, coating coverslips with
 Pericytes, 97–109
 function, 97–98
 heterogeneity of, 98
 immunolabeling antibodies for identification of, 192t
 morphology, 97
 purification from rodent optic nerve by immunopanning (protocol), 100–109
 flow diagram for, 103f
 materials, 100–102
 method, 102–107
 dissection, 104
 dissociation, 105–106
 panning, 106
 plating, 107, 107f
 preparation of panning dishes, culture dishes, coverslips, and solutions, 102–104
 trypsinization, 106
 recipes, 108–109
 purification strategies, 98–99
 Platelet-derived growth factor-AA (PDGF-AA), 128
 Platelet-derived growth factor-BB (PDGF-BB), 97–98
 Platelet-derived growth factor receptor alpha (PDGFR α), 155, 155f
 Platelet-derived growth factor receptor beta (PDGFR β), 97–98, 100, 105–106, 122, 123
 Poly-D-lysine, coating coverslips with, 33, 49, 59–60, 102, 118, 136, 149, 167, 182
 Poly-D-lysine (PDL) stock (1 mg/mL) (recipe), 68, 109, 126, 145, 159
 Progesterone, 191
 Protein A, 193
 Protein G, 193
 Puromycin, 112, 122, 123
 Putrescine, 191
- R**
 Radiation
 safety procedures, 198
 waste disposal, 197, 198
 Raff, Martin, 1, 2
 Rat. *See also* Rodents
 immunopanning of retrograde-labeled corticospinal motor neurons from early postnatal rodents (protocol), 31–44
 myelinating cocultures of retinal ganglion cell reagggregates and optic nerve oligodendrocyte precursor cells (protocol), 165–176
 production and culture of retinal ganglion cells from rodents (protocol), 11–20
 purification and culture of spinal motor neurons from rat embryos (protocol), 47–54
 purification of dorsal root ganglion neurons from rat by immunopanning (protocol), 57–69
 purification of endothelial cells from rodent brain by immunopanning (protocol), 114–126
 purification of oligodendrocyte precursor cells from rat cortices by immunopanning (protocol), 132–145
 purification of pericytes from rodent optic nerve by immunopanning (protocol), 100–109
 purification of rat and mouse astrocytes by immunopanning (protocol), 74–85
 retrograde labeling of corticospinal motor neurons from early postnatal rodents (protocol), 28–30, 29f
- Recipes
 anti-oxidants (AO) (1000 \times), 39–40
 APV (DL-2-amino-5-phosphonopentanoic acid) stock (25 mM), 39–40
 BDNF stock (10 μ g/mL), 53
 BDNF stock (50 μ g/mL), 18, 40, 65
 bFGF (50 μ g/mL), 123
 biotin stock (500 \times), 173
 BSA stock (4%), 40, 173, 185
 ciliary neurotrophic factor (10 μ g/mL), 18, 53, 142, 156, 173
 CNTF stock (10 μ g/mL), 18, 53, 142, 156, 173
 collagenase/dispase cocktail, 185
 CSMN growth medium, 40
 CSMN survival medium, 41
 DMEM-SATO base growth medium, 142–143, 156–157
 DMEM-SATO base growth medium (with NB), 18
 DNase I, 157
 DRG base medium, 65
 EBSS stock (10 \times), 123, 143, 157
 endothelial cell growth medium, 124
 enzyme stock solution, 83, 94
 ethanol-washed glass coverslips, 19, 41, 108, 124, 157, 185
 forskolin stock (4.2 mg/mL), 19, 53, 66, 124, 143, 157, 173, 186
 GDNF stock (10 μ g/mL), 53
 high-ovomuroid stock solution (6 \times), 19, 41, 66, 108, 124–125, 143, 157, 174
 high-ovomuroid stock solution (10 \times), 83, 94
 hormone mix (200 \times), 174
 hybridoma cell line medium, 23
 inhibitor stock solution, 84, 94, 125
 insulin stock (0.5 mg/mL), 19, 23, 41, 53, 66, 108, 125, 143, 158, 174, 186
 IP-astrocyte base medium, 84, 94
 Ky stock (0.8 M), 41
 low-ovomuroid stock solution (10 \times), 19, 41, 66, 84, 95, 109, 125, 144, 158, 174, 186
 motor neuron growth medium, 53–54
 myelination medium, 174
 MyM base medium, 175
 NAC stock (5 mg/mL), 19, 43, 54, 66, 84, 125, 175, 186
 NBS buffer, 42–43
 NB-sucrose buffer, 42
 ND-growth medium, 175
 ND-SATO base medium, 175
 NS21 (50 \times), 67–68
 NT-3 stock (1 μ g/mL), 68, 144, 158
 OPC culture medium, 144, 158
 papain buffer, 125, 144, 158
 PDGF stock (10 μ g/mL), 144–145, 159
 poly-D-lysine (PDL) stock (1 mg/mL), 68, 109, 126, 145, 159
 RGC growth medium, 19
 SATO supplement (100 \times), 20, 54, 145, 159, 176

- SATO supplement, NB-based (100×), 43, 68, 84–85, 126, 186–187
- Schwann cell growth medium, 186
- thyroxine (T3) stock (4 µg/mL), 20, 54, 69, 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
- culturing hybridoma cell lines for monoclonal antibody production (protocol), 21–23
- materials, 21–22
- method, 22
- recipes, 23
- in defined, serum-free growth medium, 8, 8f
- growth factors for, 8–9
- myelinating cocultures of purified oligodendrocyte lineage cells and retinal ganglion cells, 161–176
- establishing coculture, 161–163, 162f, 163f
- γ-secretase inhibitor addition to coculture, 163, 163f, 172
- maturation of coculture, 163–164, 164f
- optic nerve astrocyte addition to coculture, 163, 164
- protocol, 165–176
- dissection, 170
- dissociation, 170–171
- flow diagram of, 169f
- materials, 165–167
- method, 167–173, 167f, 169f
- nucleofection of optic nerve cells, 172–173
- optic nerve OPC isolation by immunopanning, 169–172
- recipes, 173–176
- trypsinization, 172
- principles of isolation and culture, 8–9
- production and culture from rodents (protocol), 11–20
- flow diagram for, 14f
- materials, 11–13
- method, 13–18
- dissection, 15–16
- panning, 16–17
- plating, 17–18
- preparation, 13–15
- trituration, 16
- trypsinization, 17
- recipes, 18–20
- Retinoic acid, 128
- Retrobeads, 28–29
- Retrograde labeling of corticospinal motor neurons from early postnatal rodents, 28–30, 29f
- RGC growth medium (recipe), 19
- RGCs. *See* Retinal ganglion cells
- RGS5, pericyte marker, 107
- Rodents. *See also* Mouse; Rat
- immunopanning of retrograde-labeled corticospinal motor neurons from early postnatal rodents (protocol), 31–44
- production and culture of retinal ganglion cells from rodents (protocol), 11–20
- purification of astrocytes from transgenic rodents by fluorescence-activated cell sorting (protocol), 86–95
- purification of endothelial cells from rodent brain by immunopanning (protocol), 114–126
- purification of pericytes from rodent optic nerve by immunopanning (protocol), 100–109
- retrograde labeling of corticospinal motor neurons from early postnatal rodents, 28–30, 29f
- S**
- Safety, 195–200
- biological safety procedures, 198–199
- general cautions, 195–197
- institutional safety office, 195
- material safety data sheets (MSDSs), 195
- properties of common hazardous chemicals, 199–200
- radioactive safety procedures, 198
- waste disposal, 197
- S100a10* gene, 26
- Saltatory action potential propagation, 127
- SATO, as serum-free supplement for retinal ganglion cells, 9
- SATO supplement (100×) (recipe), 20, 54, 145, 159, 176
- SATO supplement, NB-based (100×) (recipe), 43, 68, 84–85, 126, 186–187
- Schwann cell growth medium (recipe), 186
- Schwann cells, purification of, 177–187
- culturing cells, 178
- isolating cells, 177–178, 178f
- protocol, 180–187
- flow diagram of, 182f
- materials, 180–181
- method
- dissection and dissociation, 183
- immunopanning, 184
- preparation, 182–183
- trypsinization and plating, 184
- recipes, 185–187
- troubleshooting, 185
- Sciatic nerve, Schwann cell isolation from, 183
- Secondary antibody, coating plates with, 13, 49–50, 76–77, 102, 116, 134, 148, 169, 182, 192
- Selenite, 191
- Serum, fetal calf, 3
- Serum-free medium, defined, 3
- for oligodendrocytes, 129
- for retinal ganglion cells, 8, 8f
- Smooth muscle actin, pericyte marker, 107, 107f, 122
- Sodium selenite, 191
- Solvents, safe handling of, 196, 199
- Sox10* gene, 128
- Spinal motor neurons, 45–54
- characteristics of, 45
- in culture, 46
- disease and trauma of lower motor neurons, 45–46
- purification and culture from rat embryos (protocol), 47–54
- flow diagram for, 49f
- materials, 47–48
- method, 49–52
- cell separation by density gradient centrifugation, 51
- dissection, 50
- dissociation of spinal cord tissue, 50–51
- 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
- Thy-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
- Transferrin, 191
- Transporters of CNS endothelial cells, 111, 112
- Tri-iodothyronine (T3)
- in Bottenstein–Sato serum-free additive, 191
- for promotion of clock mediated oligodendrocyte differentiation, 128–130, 141, 154, 155f
- as serum-free supplement for retinal ganglion cells, 9
- T3 stock (4 µg/mL) (recipe), 20, 54, 69, 126, 159, 176, 187
- Trituration. *See specific protocols*
- Trypan blue, 63, 120
- 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 preparation, 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 neurons, 26
- Vascular smooth muscle cells, 97
- Vasculogenesis, 112
- VC1.1 (amacrine surface antigen), 17
- VE-Cadherin, as endothelial cell marker, 122
- VWF, as endothelial cell marker, 122
- W**
- Waste, disposal of, 197, 198