

Index

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

A

- Acousto-optical tunable filter (AOTF), 477, 481
aCSF for hippocampus (recipe), 506
ACSF for two-photon imaging (recipe), 592
Action potentials, calcium dynamics and, 508, 510, 513–515
Adeno-associated virus (AAV)
 AAV-G-CaMP13, 513
 construction and packaging of herpes simplex virus/adeno-associated virus (HSV/AAV) hybrid amplicon vectors (protocol), 441–445, 442t, 443f
 discussion, 444–445
 materials, 441–442
 method, 442–444, 442t, 443f
 cotransfection of packaging-defective HSV-1 helper DNA and vector DNA, 442–443, 442t, 443f
 harvesting packaged vectors, 443–444
 titration of amplicon stocks, 444
Cre-dependent reporter, 406
integration of genome into human chromosome 19, 441
optogenetics and, 530–531
stable producer cell lines for adeno-associated virus (AAV) assembly (protocol), 427–431, 429f–430f
 materials, 427–428
 method, 428–430, 429f–430f
 generation of stable packaging lines, 428–429
 generation of stable producer clones, 430
 screening clones for *rep* and/or *cap* DNA, 429–430, 430f
 selection procedure, 429f
 recipes, 431
 troubleshooting, 430
Aerosols, avoiding generation of, 62
Affymetrix microarrays
 processing data, 292–293
 tips on hybridizing, washing, and scanning, 285–290
 materials, 285–286
 method, 286–290
Agar or agarose containing media (recipes), 87, 102
Agarose gel electrophoresis
 denaturation and electrophoresis of RNA with formaldehyde (protocol), 212–215
 gel purification of amplified cDNA, 307–308, 308f
 in generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct protocol, 386–387, 387f, 390–391
 northern blots: capillary transfer of RNA from agarose gels and filter hybridization using standard stringency conditions (protocol), 216–223
 protocol, 124–127
 materials, 124–125, 125t
 method, 125–126
 recipes, 126–127
Airy disk, 464, 465
Albumin-agarose (recipe), 573–574
Alexa Fluor 488 dextran 3000, 544
Alkaline lysis
 isolation of BAC DNA from small-scale cultures (protocol), 103–105
 materials, 103–104
 method, 104
 recipes, 104–105
 preparation of plasmid DNA by (protocol), 81–84
 materials, 81
 method, 82
 recipes, 83–84
Alkaline lysis solution I (recipe), 83, 104
Alkaline lysis solution II (recipe), 83, 105
Alkaline lysis solution III (recipe), 83, 105
Alkaline transfer buffer (recipe), 132
Allen Institute for Brain Science, 121
Alternative splicing events, detection of, 311
Amacrine cells, 31
Ambion MEGAScript T7 In Vitro Transcription (IVT) Kit, aRNA synthesis using, 204–205
Ambion Megclear Kit, clean up of aRNA with, 205–206
 binding RNA to column, 205
 concentration of RNA by ethanol precipitation, 205–206
 elute RNA from column, 205
 wash RNA bound to column, 205
American Type Culture Collection (ATCC), 64
Amidine, 356t
Amino acids
 in cell culture media, 19
 essential, 19
Amphotropic viruses, 338
Ampicillin (100 mg/mL stock solution) recipe, 68t
Amplicon vectors, construction and packaging of herpes simplex virus/adeno-associated virus (HSV/AAV) hybrid, 441–445, 442t, 443f
Amplification buffer (10 \times) (recipe), 92–93
Amplification buffer for HCR (recipe), 232
AMPure XP beads, 316–318
Anesthesia
 of rats and mice, 370, 535, 594, 597, 604
 recovering the animal, 601
 of tadpoles, 548, 549
Annealing buffer (5 \times) (recipe), 336
Antarctic phosphatase, dephosphorylation with, 391
Antibiotics
 in cell culture media, 20
 for selection of plasmid-carrying strains, 68t, 69
Antibody. *See also* Immunopanning
 coupling antibody to Dynabeads, 154
 for fusion protein localization analysis, 361
 oligonucleotide conjugation to, 248
 restriction enzyme digestion of cDNA from, 251
Antibody-positioned RNA amplification (APRA), identification of RNA cargoes by (protocol), 246–253
 discussion, 252
 materials, 246–248
 methods, 248–251
 APRA reaction, 249–250, 249f
 conjugation of oligonucleotide to antibody, 248
 preparation of cells for APRA, 248
 removing antibody-conjugated cDNA from tissue, 250
 restriction enzyme digestion of cDNA from antibody, 251
 second-strand cDNA synthesis, 250–251
 recipes, 252–253
 troubleshooting, 251–252
Antisense RNA amplification for target assessment of total mRNA from a single cell (protocol), 200–211
 discussion, 210–211
 materials, 200–201
 method, 201–208, 202f, 207f, 209f
 clean up aRNA with Ambion Megclear Kit, 205–206
 binding RNA to column, 205
 concentration of RNA by ethanol precipitation, 205–206
 elute RNA from column, 205
 wash RNA bound to column, 205
 clean up with MinElute Kit, 203–204
 DNA binding to column, 203–204
 elute DNA bound to column, 204
 wash DNA bound to column, 204
 PCR analysis of cDNA, 208–209
 round 1, 201–206, 202f
 aRNA synthesis using Ambion MEGAScript T7 In Vitro Transcription (IVT) Kit, 204–205
 clean up aRNA with Ambion Megclear Kit, 205–206
 clean up dsDNA with MinElute Kit, 203–204
 first-strand cDNA synthesis, 201–203
 second-strand cDNA synthesis, 203
 round 2, 202f, 206–207, 207f
 first-strand cDNA synthesis, 206
 second-strand cDNA synthesis, 206–207
 round 3, 207–208
 first-strand cDNA synthesis, 207–208
 second-strand cDNA synthesis, 208
 recipe, 211
 troubleshooting, 209–210
Arabinose-induced excision of selectable marker, 114
Aseptic technique. *See* Sterile technique
Aspirating fluids with sterile technique (protocol), 11–12
 materials, 11
 method, 11–12
Astrocytes
 piggyBac transposon-mediated cellular transgenesis by in utero electroporation, 374f, 375
 purification and culture of, 35–49
 prospective isolation of astrocytes, 36, 36f
 purification of rat and mouse astrocytes by immunopanning (protocol), 38–49, 41f
 brain dissection, 42–43
 dissociation of cells, 43–44
 materials, 38–40
 method, 40–47, 41f
 modifications for mouse immunopanning, 46–47
 panning, 45–46
 preparation of panning dishes, 40–41

Index

- Astrocytes (*Continued*)
preparation of solutions and panning dishes, 41–42
recipes, 47–49
standard preparations and their limitations, 35–36
- Auromine, 460
- Autoclaving, 17, 63, 67
- Autoradiography, 219, 219f, 263, 263f, 301, 302f, 303, 304f, 305, 306f
- ## B
- Bacteria
classification, 61
culture media, 64
disposal of infectious waste, 63
growth and maintenance, 64
obtaining, 63–64
phenotypic drift, 64
protocols
freezing bacteria for long-term storage, 78–80
discussion, 79–80
materials, 78–79
methods, 79
reviving a frozen culture, 79
making media for bacterial culture, 66–69
antibiotics, 68t, 69
liquid medium, 67
materials, 66–67
method, 67–69
plates of solid medium (pour plates), 67–69
tubes of solid medium (slants), 69
measurement of bacterial growth by spectrophotometry, 75–77, 76f
calculation of generation time, 77
discussion, 76–77
materials, 75
method, 75–76
normal growth in liquid culture, 76f
obtaining isolated colonies of bacteria, 70–72, 71f
discussion, 72
materials, 70
methods, 71–72, 71f
streaking agar plates, 71–72, 71f
streaking and stabbing slants using isolated colonies, 72
transferring isolated colonies to plates, 72
Petroff-Hausser counting chamber use, 73–74, 74f
example calculation, 74
materials, 73
method, 73–74, 74f
preparation and transformation of competent *E. coli* using calcium chloride, 85–88
materials, 85
method, 86
recipes, 87–88
preparation of plasmid DNA by alkaline lysis with sodium dodecyl sulfate: minipreparation, 81–84
materials, 81
method, 82
recipes, 83–84
setting up for working with, 62
storage, 65, 78–80
working rules, 62–63
- Bacterial artificial chromosomes (BACs)
GENSAT database of engineered mouse strains, 117–122
advantages of BACs to label genetically defined cell types, 119–120, 120f
application of reporter mice to neuroimaging studies, 120–122, 121f
considerations for choosing a mouse line, 121–122
generation of BAC mice, 118–119, 119f
GENSAT website, 122
ordering mouse lines, 122
homologous recombination using BACs (protocol), 106–116, 109f–110f
materials, 106–108
method, 108–114, 109f–110f
analysis of putative recombinant BACs, 113–114
arabinose-induced excision of the selectable marker, 114
assembly and preparation of the ECFP-Kan cassette, 108–112, 109f–110f
electroporation of the ECFP-Kan insert into electrocompetent cells, 113
preparation of electrocompetent cells, 112–113
recipes, 115–116
troubleshooting, 114–115
isolation of BAC DNA from small-scale cultures, 103–105
materials, 103–104
method, 104
recipes, 104–105
working with BACs (protocol), 100–102
materials, 100
method, 101
recipes, 101–102
- Bandeira simplicifolia* lectin I (BSL-I), 22, 29, 45
- Barcode, 313, 316–319, 321, 324, 326–327
- Barres, Ben, 2
- BDNF (brain-derived neurotrophic factor), 23
- BDNF stock (50 µg/mL) (recipe), 32
- bFGF (basic fibroblast growth factor), 23
- Bimolecular fluorescence complementation (BiFC) assay, 489
- Binding buffer (2×) (recipe), 322
- Binding buffer for mobility shift assays (recipe), 238
- Bioconductor, 324, 328, 329, 330
- Biosafety cabinet
classes of, 13–15, 15t
working sterilely in (protocol), 13–15, 15t
discussion, 14–15
materials, 13
method, 13–14
- Biosafety hood. *See* Biosafety cabinet
- Biosafety Level 2 (BL2), 14
- Biosafety Level 3 (BL3), 61
- Biosafety Level 4 (BL4), 61
- Bleaching, 461. *See also* Photobleaching
- Blocking buffer for microarrays (recipe), 279
- Blunting of DNA ends, 162
- Bottenstein-Sato supplement (SATO), 23
- Bowtie, 329
- Brain. *See also* Neurons
piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by *in utero* electroporation, 374f, 375
two-photon imaging of microglia in mouse cortex *in vivo*, 583–593
- Brainbow* mice, generating and imaging multicolor, 575–582
- Brainbow* strategies, 577–578, 577t
color-assisted circuit tracing, 578
colors, 578
generation of *Brainbow* transgenes, 578–579
elements for optimized expression, 579
FRT site, 579
lox sites, 579
promoter, 578
subcellular localization signals, 579
XFPs, 579
overview of approach, 575–577, 576f–577f
pros and cons of *Brainbow* imaging, 580–581
transgenic mice, 579–580
- Bright-field microscopy, 450
- B-27 supplement, 23
- Buffered HBSS for APRA (recipe), 252
- Buffer L1 (recipe), 152
- Buffer L2 (recipe), 152
- Buffer L3 (recipe), 152
- Bulk electroporation of retinal ganglion cells in live *Xenopus* tadpoles (protocol), 558–562
discussion, 560–561, 561f
materials, 558–559
method, 559–560, 560f
recipe, 562
- Bump-hole calmodulin/M13 pairs, 513
- Buprenorphine, 537, 594, 597
- ## C
- Ca²⁺, chelation of, 176
- CAG promoter, in lentiviral vectors, 404–408
- Calcium channels, 510
- Calcium chloride, transformation of competent *E. coli* using (protocol), 85–88
- Calcium indicators. *See* Genetically encoded calcium indicators (GECIs)
- Calcium phosphate transfection, 407, 428
- Calf intestinal alkaline phosphatase (CIP), 261
- Calibrated slides, 459
- Camera
digital scanned laser light sheet fluorescence microscopy (DSLM), 479, 485–486
dynamic range, 486
- cAMP (cyclic AMP), 23
- Cannulation, 534–539, 537f, 598f, 599, 601, 604
- cap* gene, AAV, 427–430, 444
- Capillary transfer of RNA from agarose gels and filter hybridization using standard stringency conditions (protocol), 216–223
- Carbocyanine dyes, 553
- Carprofen, 594, 597
- Carriers, for RNA precipitation, 180, 181f
- Cation-conducting channelrhodopsins (ChRs), 527t, 529–531, 594
- CCD. *See* Charge-coupled device
- cDNA. *See also* cDNA library
in antibody-positioned RNA amplification (APRA) protocol, 250–252
gel purification of amplified cDNA, 307–308, 308f
microarray slide hybridization using fluorescently labeled cDNA (protocol), 274–279
materials, 274–275
method, 275–279, 276f–278f
blocking slides, 275–276, 276f
hybridization of microarray slides, 276–279, 277f–278f
recipes, 279
- PCR amplification in preparation of small RNA libraries for high-throughput sequencing protocol, 307
- preparation of fluorescent-dye-labeled cDNA from RNA for microarray hybridization (protocol), 269–273
discussion, 272–273
materials, 269–270
method, 270–272
coupling reaction and purification, 271–272
purification of cDNA, 271
reverse transcription reaction, 270–271
troubleshooting, 272
- RACE (rapid amplification of cDNA ends), 336
- synthesis from RNA in aRNA amplification procedure, 200–209

- cdNA library
 - data processing for RNA-Seq, 323–331
 - classification of changes and associated biology, 330
 - experimental design, 324–328
 - library construction, 325–327
 - quantity of starting material, 324–325
 - library construction, 325–327
 - amplification, 325–326
 - bar coding, 326–327
 - directionality (strand selection), 326
 - normalization, 328
 - number of reads, 327
 - paired ends, 326
 - priming *versus* fragmentation, 325
 - read length, 326
 - replicates, 327–328
 - mapping, 329
 - measuring expression levels and changes in expression, 329
 - presentation of data, 330–331
 - raw reads, 328–329
 - systems for, 323
 - transcript (isoform) assembly, 329
 - validation, 329–330
- high-throughput Illumina strand-specific RNA sequencing library preparation (protocol), 313–322
 - discussion, 319
 - different multiplex sequencing strategies for GAI and HiSeq2000 instruments, 321
 - false antisense read derived from uracil-minus RNA, 320
 - purification and size selection on magnetic beads, 320–321
 - recipes, 322
 - strand-specific RNA-Seq vs. conventional RNA-Seq, 319–320, 320f
 - using phosphorothioate oligos to prevent PCR amplification of adapter dimers, 321
 - materials, 313–314
 - method, 314–319, 315f
 - dA-tailing, 316
 - end-repair, 316
 - first-strand cDNA synthesis, 315–316
 - general procedure for using AMPure beads, 318–319
 - mix barcoded libraries for multiplex sequencing, 318
 - PCR enrichment, 317–318, 318f
 - polyA RNA isolation and fragmentation, 314–315
 - second-strand synthesis with dUTP, 316
 - triple-SPRI purification and size selection, 317, 317f
 - Y-shape adapter ligation, 316–317
- mRNA-Seq libraries from poly(A)⁺ mRNA for Illumina transcriptome high-throughput sequencing, 310–312
 - notes and considerations before beginning, 311
 - overview, 310
 - tips and troubleshooting, 311–312
 - adapter ligation, 311
 - first gel purification, 311–312
 - optimization, 311
 - PCR enrichment, 312
 - second gel purification, 311–312
- Cell culture medium for virus production (recipe), 408, 419
- Cell cultures. *See also* Mammalian cell culture gene transfer protocols
 - DNA transfection by electroporation, 364–366
 - DNA transfection mediated by lipofection, 355–357, 356f
- single cell/cellular subregion-targeted phototransfection, 376–380, 378f–379f
- transfection of mammalian cells with fluorescent protein fusions, 358–363
- infection with short hairpin RNA (shRNA) retroviruses, 342–343
 - materials, 342
 - method, 342–343, 343t
- RNA extraction from tissues, 179
- Cell division, Hayflick limit and, 2
- Cell smear, making, 455
- Centers for Disease Control and Prevention (CDC) Select Agents Regulations, 63
- CF Achromat lens, 459
- CF Plan Achromat lens, 459
- CF Plan Apochromat lens, 459
- Channelrhodopsins (ChRs), 527t, 529–531, 594
- Charge-coupled device (CCD), 467
 - digital scanned laser light sheet fluorescence microscopy (DSLM) camera, 479, 485–486
 - dynamic range, 486
 - quantum efficiency of, 467
 - viewing stained RNA, 214
- Chelators, 176
- ChIP. *See* Chromatin immunoprecipitation
- ChIP-chip
 - overview, 143–144, 145t
 - protocol, 160–166
 - materials, 160–161
 - method, 161–165
 - amplification of ChIP DNA and input DNA, 162–163
 - hybridization, 164
 - hybridization preparation, 164
 - linker preparation, 162
 - microarray preparation, 164
 - preparation of fluorescently labeled DNA from LM-PCR products, 163
 - scanning, 165
 - washing, 164–165
 - recipes, 165–166
 - troubleshooting, 165
- ChIP-quantitative polymerase chain reaction (ChIP-qPCR)
 - overview, 144, 145t
 - protocol, 158–159
 - materials, 158
 - method, 158–159
- ChIP RIPA buffer (recipe), 156
- ChIP-seq
 - overview, 144, 145t
 - protocol, 167–171
 - materials, 167–168
 - method, 168–171
 - addition of A to 3' end of ChIP DNA, 169
 - end repair, 168
 - gel purification/size selection, 169–170
 - ligation, 169
 - recipes, 171
- Chlamydomonas reinhardtii*, opsins from, 527t, 529–531
- Chloramphenicol (20 mg/mL stock solution) recipe, 68t
- Chloroform-isoamyl alcohol, 184, 267
- Chloroquine, 339
- Chlorox, 63
- Choline acetyltransferase (ChAT), 106, 109f–110f
- Chromatic aberration, 473
- Chromatin immunoprecipitation (ChIP)
 - expected results and interpretation of ChIP-based data, 144–145
 - overview of methods, 143–144, 145t
 - antibody quality, 144
 - direct sequencing of ChIP DNA, 144
 - formaldehyde cross-linking, 143
- planning ChIP experiments, 144
- schematic outline, 144f
- protocols
 - basic ChIP, 153–157
 - ChIP DNA purification, 155–156
 - coupling antibody to Dynabeads, 154
 - immunoprecipitation, 155
 - materials, 153–154
 - method, 154–156
 - ChIP-chip, 160–166
 - materials, 160–161
 - method, 161–165
 - recipes, 165–166
 - troubleshooting, 165
 - ChIP-quantitative polymerase chain reaction (ChIP-qPCR), 158–159
 - materials, 158
 - method, 158–159
 - ChIP-seq, 167–171
 - materials, 167–168
 - method, 168–171
 - recipes, 171
 - formaldehyde cross-linking, 147–149
 - discussion, 148–149
 - materials, 147
 - method, 148
 - recipes, 149
 - preparation of cross-linked chromatin for ChIP, 150–152
 - materials, 150
 - method, 151
 - recipes, 152
 - sequential ChIP, 145
- ChRs (cation-conducting channelrhodopsins), 527t, 529–531
- Ciliary Neurotrophic factor (10 µg/mL) (recipe), 32
- Ciona intestinalis* voltage-sensing phosphatase (Ci-VSP), 518, 521–522
- Circuit tracing using *Brainbow* multicolor labeling, 577f, 578
- CLIP (cross-linking and immunoprecipitation) identification of RNAs bound by a specific protein, 254–268
 - discussion, 267
 - materials, 254–256
 - method, 256–267
 - addition of 454 capture linkers via second PCR step, 266
 - dynabead-antibody complexes, preparation of, 259
 - isolation of RNA from nitrocellulose, 264
 - ligation of RNA linkers to 5' ends of tags, 264
 - outline of protocol, 257f
 - phosphate removal from RNA ends, 261
 - phosphorylation of 5' ends of RNAs with PNK, 262
 - purification of PCR-amplified tag sequences, 266
 - recovering cross-linked RNA, 263–264
 - removal of DNA contaminants from RNA tags, 265
 - resolving of RNA-protein cross-linked complexes on gel, 262–263, 263f
 - RNA linker addition to 3' end of fragments with RNA ligase, 261–262
 - RNA tag trimming with micrococcal nuclease, 260–261
 - RNA tag trimming with RNase A and T1 mix, 259–260
 - RT-PCR amplification of linker-ligated tags, 265–266
 - UV cross-linking of cultured cells, 258–259
 - UV cross-linking of tissues, 257–258
 - recipes, 267–268
- CNTF (ciliary neurotrophic factor), 23
- CodeLink slides, 274–277
- CO₂ incubators, contamination of, 18
- Collection tube buffer (recipe), 198

Index

- Color space, 323, 328
Column chromatography, for RNA purification, 179
Combinatorial analysis of mRNA expression patterns in mouse embryos using hybridization chain reaction (protocol), 224–233
discussion, 230–232, 231f, 231t
materials, 224–225, 226f
method, 225–229, 227f
clearing and mounting embryos, 229
image acquisition, 229
in situ HCR, 225, 227f, 228–229
recipes, 232–233
troubleshooting, 229–230, 230f
Complete cell growth medium (recipe), 401
Condenser, microscope
aperture diaphragm, 457f
use of, 456
using immersion oil on lens, 457
Confocal microscopy, 463–470
advantage of, 465f
digital scanned laser light sheet fluorescence microscopy (DSLM) compared, 482–486
dynamic range, 486
flaws in, 471
illumination efficiency, 483
imaging speed, 485–486
implementation, 468–470, 468f
advances in, 470
laser-scanning design, 469
Nipkow disk design, 469
specimen-scanning design, 468
mRNA expression imaged by, 230f, 231f
multiphoton-excitation fluorescence microscopy compared, 471–473
detection, 472
laser pulse width, 473
localized excitation, 471–472
out-of-focus light rejection, 471
resolution, 473
scattered light, 472
photobleaching, 483–484
point-spread function (PSF), 464, 465, 484
resolution, factors influencing, 463–466
deconvolution, 465
depth of field, 465
object illumination and detector aperture, 464
point-spread function, 464
specimen thickness, 465
signal optimization, 466–467
detector quantum efficiency, 467
image digitization, 467
optical-transfer efficiency, 467
scanning mode, 467
signal intensity, 466
signal-to-noise ratio (SNR), 466
theory of confocal optics, 463–466
principle, 463, 464f
resolution, factors influencing, 463–466
Conformational actuator, in genetically encoded calcium indicators (GECIs), 509
Contamination, working without. *See* Sterile technique
Continuous cell lines, 2
Contrast, 449–450
Counting chamber use, 73–74, 74f
example calculation, 74
materials, 73
method, 73–74, 74f
Craniotomy, 599–600, 604
Cre
bulk electroporation of *Xenopus* retinal ganglion cells, 561, 561f
in multicolor *Brainbow* mice, 575, 576f, 577t, 578, 580
optogenetics and, 531
retrograde delivery of, 406–407
toxicity of overexpression, 407–408
Cross-linking, UV
of cultured cells, 258–259
DNA to membrane, 132
RNA to membrane, 218
of tissues, 257–258
Cross-linking buffer (recipe), 149
Cufflinks, 329
Culture medium (AAV) (recipe), 431
Cumate-inducible systems, 490
Cy3 dye, 272–273
Cy5 dye, 272–273
Cytomegalovirus (CMV) promoter, 336, 489, 555
in lentiviral vectors, 407–408
tet-CMV, 336, 350
- ## D
- DABCO (diaminobicyclo-octane), 461
DAB labeling, 497, 499
Dark-field microscopy, 450
Dark noise, 467
dA-tailing, 316
DC-cholesterol, 356t
D3cpVenus (D3cpV), 510, 512, 512t, 513
DDAB, 356t
Death phase, growth curve, 76, 76f
Deconvolution, 465
Defective interfering (DI) particles, 418–419
Denaturation and electrophoresis of RNA with formaldehyde (protocol), 212–215
discussion, 214
materials, 212–213
method, 213–214
electrophoresis and visualization of RNA, 214
preparation of formaldehyde-agarose gel, 213
preparation of RNA samples for electrophoresis, 213
recipes, 214–215
Denaturation of DNA, for Southern blotting, 130
Denaturation solution (recipe), 132
Dendrites, electron microscopy imaging of, 497–498, 499f
Denhardt's solution for northern blots (100×) (recipe), 221
DEPC-H₂O (recipe), 211, 380
Dephosphorylation
with Antarctic phosphatase, 391
with calf intestinal alkaline phosphatase (CIP), 261
Deproteinization, in RNA purification, 179
Depth of field, confocal microscopy and, 465
Depurination of DNA, 129–130
Descanning, 472
DiA, 553
Dialysis, extraction of RNA from gel slices by, 303
Diaphragm, microscope, 456, 456f–457f
DiD, 553
Diethyl pyrocarbonate (DEPC), 177, 182
Diethyl pyrocarbonate (DEPC)-treated H₂O (recipe), 189
Digital scanned laser light sheet fluorescence microscopy (DSLM), 476–488
advanced implementation, 488f
comparison to other microscopy methods, 482–486
cost efficiency, 486
dynamic range, 486
illumination efficiency, 482–483
illumination pattern quality and flexibility, 484–485
imaging speed, 485–486
performance in imaging large specimens, 486–487, 487t
photobleaching, 483–484
point-spread function (PSF), 484
components, 477–480, 479f
acousto-optical tunable filter (AOTF), 477, 481
computer, 480–481
control software, 481
detection system, 479, 481–482
illumination/excitation system, 477–478, 481
objective lens, 477–478
secondary observation system, 479–480
specimen-positioning system, 480
technical blueprint, 480f
temperature control system, 478
live imaging of embryonic development, 478f
overview, 476–477, 477f
DiI, 553
DiO, 553
DI (defective interfering) particles, 418–419
Direct immunofluorescence, 460
Disposal of infectious waste, 63
Dissection
in purification of rat and mouse astrocytes by immunopanning (protocol), 42–43
for retinal ganglion cell purification and culture from rodents, 29–30
Dithiothreitol (DTT), 177
Divalent metal ions, RNA degradation caused by, 175–176
DMEM/10% FBS for HSV (recipe), 439
DMEM-SATO Base Growth Medium with NB (recipe), 32
DMRIE, 356t
DNA. *See also* DNA isolation
blunting of DNA ends, 162
concentration measurement, 129
denaturation, 130
depurination of, 129–130
end repair, 168
ethanol precipitation of, 82, 129
gene transfer, nonviral methods of
DNA transfection by electroporation (protocol), 364–366
DNA transfection mediated by lipofection (protocol), 355–357, 356t
piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation (protocol), 367–375, 369f, 370t, 374f
transfection of mammalian cells with fluorescent protein fusions (protocol), 358–363
gene transfer payloads, 353
protocols
agarose gel electrophoresis, 124–127
basic polymerase chain reaction, 91–93
ChIP, 153–157
ChIP-chip, 160–166
ChIP-quantitative polymerase chain reaction (ChIP-qPCR), 158–159
ChIP-seq, 167–171
electrophoretic mobility-shift assays, 139–142
formaldehyde cross-linking, 147–149
freezing bacteria for long-term storage, 78–80
homologous recombination using bacterial artificial chromosomes, 106–116, 109f–110f
isolation of bacterial artificial chromosome DNA from small-scale cultures, 103–105
making media for bacterial culture, 66–69
measurement of bacterial growth by spectrophotometry, 75–77, 76f
obtaining isolated colonies of bacteria, 70–72, 71f
preparation and transformation of competent *E. coli* using calcium chloride, 85–88

- DNA (*Continued*)
preparation of cross-linked chromatin for ChIP, 150–152
preparation of plasmid DNA by alkaline lysis with sodium dodecyl sulfate: minipreparation, 81–84
Southern blotting: capillary transfer of DNA to membranes, 128–133
Southern hybridization of radiolabeled probes to nucleic acids immobilized on membranes, 134–138
using Petroff-Hausser counting chamber, 73–74, 74f
working with bacterial artificial chromosomes, 100–102
purification for ChIP, 155–156
quantitation of, 89–90
by ethidium bromide fluorescence emission, 90
with spectrophotometer, 89
DNA Clean & Concentrator-5 Kit, 271
DNA isolation
of BAC DNA from small-scale cultures (protocol), 103–105
materials, 103–104
method, 104
recipes, 104–105
of plasmid DNA by alkaline lysis (protocol), 81–84
materials, 81
method, 82
recipes, 83–84
DNA microarrays, 143. *See also* ChIP-chip scanning, 165
DNase
in CLIP (cross-linking and immunoprecipitation) protocol, 265
in purification and culture of retinal ganglion cells from rodents protocol, 25, 29–30
in purification of rat and mouse astrocytes by immunopanning protocol, 38, 43–44, 46–47
RQ1, 259, 265
dNTP solution (recipe), 93
DOGS (Transfectam), 355–356, 356t
DOSPA, 356t
DOSPER, 356t
DOTMA (Lipofectin), 355–357, 356t
Drying bacteria, 65
DSL. *See* Digital scanned laser light sheet fluorescence microscopy
Dual laser-scanning microscopy, for scanning microarray slides, 280–284
Durcupan, 495–496
dUTP, 315f, 316, 319–320
Dynabeads, 153–155, 259, 314–315
Dynamic range, 486, 511
- E**
E. coli
as facultative anaerobe, 64
phenotypic drift, 64
preparation and transformation of competent *E. coli* using calcium chloride (protocol), 85–88
materials, 85
method, 86
recipes, 887–88
Ecotropic viruses, 338
EDTA, for metal chelation, 176
EGTA, for metal chelation, 176
Electrocompetent cells, preparation of, 112–113
Electron microscopy
imaging green fluorescent protein-labeled neurons using light and electron microscopy (protocol), 492–500
discussion, 499
materials, 492–494
method, 494–498, 496f–499f
finding imaged neuron in sections, 496, 496f–497f
fixation and immunocytochemistry, 494
fixation of imaged slices, 494–495
imaging the dendrites in the EM, 497–498, 499f
resin embedding for EM, 495–496
serial sectioning and imaging in the EM, 496–497, 498f
recipes, 500
troubleshooting, 498–499
methods, 452
scanning electron microscopy (SEM), 452
transmission electron microscopy (TEM), 452
resin embedding for EM, 495–496
Electrophoresis. *See also* Agarose gel electrophoresis; Polyacrylamide gel electrophoresis
in CLIP (cross-linking and immunoprecipitation) protocol, 262–263, 263f, 266
denaturation and electrophoresis of RNA with formaldehyde (protocol), 212–215
electrophoretic mobility-shift assay for DNA-protein complexes (protocol), 139–142
electrophoretic mobility-shift assays for RNA-protein complexes (protocol), 234–239
in preparation of small RNA libraries for high-throughput sequencing (protocol), 299–309, 302f, 304f, 306f, 308f
prior to Southern blotting, 129
resolving of RNA-protein cross-linked complexes on gel, 262–263, 263f
RNA extraction from gel slices, 302–303
RNase footprinting to map sites of RNA-protein interactions (protocol), 240–244
Electrophoretic mobility-shift assay (EMSA) for DNA-protein complexes (protocol), 139–142
discussion, 141–142
materials, 139–140
method, 140–141
recipes, 142
troubleshooting, 141
for RNA-protein complexes (protocol), 234–239
discussion, 237–238
materials, 234–235
method, 235–237
large multicomponent RNA-protein complexes, 236–237
longer RNA forming small complex, 236
simple interactions, 235–236
recipes, 238–239
Electroporation, 101
bulk electroporation of retinal ganglion cells in live *Xenopus* tadpoles (protocol), 558–562
discussion, 560–561, 561f
materials, 558–559
method, 559–560, 560f
recipe, 562
DNA transfection by electroporation (protocol), 364–366
materials, 364
method, 364–365
recipes, 366
of ECFP-Kan insert into electrocompetent cells, 113
piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation (protocol), 367–375, 369f, 370t, 374f
discussion, 374–375
background, 374–375
expected results, 374f, 375
materials, 367–368
method, 368–372
animal preparation, 370–371
helper and donor plasmid combinations, 370, 370t
surgical station preparation, 368
suturing and animal recovery, 372
in utero electroporation, 371–372
in utero electroporation setup, 368–370, 369f
troubleshooting, 372–373
principles of, 540–541
single-cell electroporation of *Xenopus* tadpoles, 540–551
efficiency, factors influencing, 542–543
electrical stimulus parameters, 542
glass micropipettes, 542
microscopy, 542–543
implementation, 543–545, 544f
coelectroporation of fluorescent dye space fillers with morpholinos or peptides, 544f, 545
SCE of DNA for neuronal transfection, 543–544, 544f
SCE of fluorescent dyes, 544, 544f
SCE within *Xenopus* tadpole optic tectum, 543
microscopy of, 542–543
principles of, 540–541
protocol, 546–551
discussion, 550
expected results, 550
limitations and special considerations, 550
materials, 546–547, 547f
method, 548–549
setup, 547f, 548
troubleshooting, 549–550
setup, 541–542, 541f, 547f, 548
in transfection of mammalian cells with fluorescent protein fusions (protocol), 359
for transgene introduction into KH2 ES cells, 348–349
Elution buffer (recipe), 156
Embryonic quail development, 4D fluorescent imaging of, 563–574
overview, 563–564
image analysis, 564
microscopy equipment requirements, 564
in vitro culturing, 564
pre-imaging considerations, 565–566, 566t
data storage hardware, 565–566, 566t
environmental control, 565
fluorophore compatibility, 565
magnification, 565
protocol, 567–574
materials, 567–568
method, 568–572, 569f–572f
image analysis, 571–572
inverted imaging of in vitro embryos, 569
multitime macro imaging, 569–570
preparation of paper rings, 568
sample setup for in vitro imaging, 568, 569f
tiled z-stack time-lapse experiments, 570–571, 570f–572f
recipes, 573–574
troubleshooting, 572–573
Embryonic stem (ES) cells, 344–351
Embryos
4D fluorescent imaging of embryonic quail development, 563–574
isolating total RNA from mouse embryos or fetal tissues, 187–190
in utero electroporation, 371–373

Index

- Emission filter, 461, 473
EMSA. *See* Electrophoretic mobility-shift assay
End-It enzyme mix, 168
End repair, 168, 316
Enhanced green fluorescent protein (eGFP)
 bulk electroporation of *Xenopus* retinal ganglion cells, 560f, 561
 in GENSAT Project engineered mouse strains, 117–122
 in two-photon imaging of microglia in mouse cortex in vivo protocol, 586f, 588f
Enhanced yellow fluorescent protein (eYFP), in two-photon imaging of microglia in mouse cortex in vivo protocol, 586f, 588f
Enolase promoter, 555
EnvA, in rabies viral vectors, 416–417, 418
Enzyme stock solution (recipe), 47
Epifluorescence, 461, 467
Epigenetic modifications, 60
Ethanol
 fixation of frozen sections via dehydration in, 195
 precipitation
 of DNA, 82, 129
 of RNA, 180–181, 181f, 188, 205–206
Ethanol-washed glass coverslips (recipe), 33
Ethidium bromide
 quantitation of DNA and RNA by ethidium bromide fluorescence emission, 90
 RNA staining with, 214
Ethidium bromide (recipe), 126, 132
Eukaryotic initiation factor-1a (EIF-1a) promoter, 336
Euthanasia of rodents, 29
Excitation filter, 461
- F**
- Fast Green, 559, 560f
Feeder cells, preparation of, 347
Fetal bovine serum, in cell culture media, 19
Fiber-optic-based optical neural interface (ONI) (protocol), 534–539
 materials, 534–535
 method, 535–538, 537f
 infusion of the virus and surgery, 536–537
 optical stimulation, 537–538
 preparation for surgery, 535–536
 preparation of the bilateral ONI, 537f
 troubleshooting, 538–539
Filters for fluorescence microscopy, 461
Filter sterilization techniques (protocol)
 materials, 9
 method, 10
 filtration of large volume with a disposable cup filter, 10
 filtration of small volume with a syringe filter, 10
Fingerases, 178
Fixation, 455
 fixing DNA to membrane for hybridization, 131–132, 131t
 cross-link by UV irradiation, 132
 fix by baking in microwave oven, 131
 fix by baking in vacuum oven, 131
 of frozen sections via dehydration in ethanol, 195
 in imaging green fluorescent protein-labeled neurons using light and electron microscopy protocol, 494–495
FlaSh (fluorescent shaker), 519–520, 519t, 520f
FLaSH-EDT2, 362
Fluorescein, 461
Fluorescence microscopy, 460–461. *See also* Confocal microscopy
 artifacts, 461
 digital scanned laser light sheet fluorescence microscopy (DSLM), 476–488
 advanced implementation, 488f
 comparison to other microscopy methods, 482–486
 components, 477–480, 479f
 acousto-optical tunable filter (AOTF), 477, 481
 computer, 480–481
 control software, 481
 detection system, 479, 481–482
 illumination/excitation system, 477–478, 481
 objective lens, 477–478
 secondary observation system, 479–480
 specimen-positioning system, 480
 technical blueprint, 480f
 temperature control system, 478
 cost efficiency, 486
 dynamic range, 486
 illumination efficiency, 482–483
 illumination pattern quality and flexibility, 484–485
 imaging speed, 485–486
 live imaging of embryonic development, 478f
 overview, 476–477, 477f
 performance in imaging large specimens, 486–487, 487t
 photobleaching, 483–484
 point-spread function (PSF), 484
 features of, 461
 filters for, 461
4D fluorescent imaging of embryonic quail development, 563–574
 overview, 563–564
 pre-imaging considerations, 565–566, 566t
 protocol, 567–574
 materials, 567–568
 method, 568–572, 569f–572f
 recipes, 573–574
 troubleshooting, 572–573
multiphoton-excitation, 471–475
 confocal microscopy compared, 471–473
 detection, 472
 laser pulse width, 473
 localized excitation, 471–472
 out-of-focus light rejection, 471
 resolution, 473
 scattered light, 472
 instrumentation, 473–474
 excitation light source, 473
 wavelength selection, 473–474
 setting up the system, 474
 uses in neuroscience, 474
 overview of, 451
 photography, 461
 staining for, 461
 tips for, 461
 uses, 460–461
 virus titer determination using, 398–399
Fluorescence recovery after photobleaching (FRAP), 505–506
Fluorescence resonance energy transfer (FRET)
 genetically encoded calcium indicators (GECIs) and, 509–512
Fluorescent dyes
 dextrans for neuron labeling for in vivo imaging, 552–553
 microarray slide hybridization using fluorescently labeled cDNA (protocol), 274–279
 materials, 274–275
 method, 275–279, 276f–278f
 blocking slides, 275–276, 276f
 hybridization of microarray slides, 276–279, 277f–278f
 recipes, 279
 preparation of fluorescent-dye-labeled cDNA from RNA for microarray hybridization (protocol), 269–273
 discussion, 272–273
 materials, 269–270
 method, 270–272
 coupling reaction and purification, 271–272
 purification of cDNA, 271
 reverse transcription reaction, 270–271
 troubleshooting, 272
 for single-cell electroporation, 544–545
 in vivo time-lapse imaging of neuronal development in *Xenopus*, 552–555, 552–557, 554f
 fluorescent dextrans, 552–553
 genetic labeling by fluorescent protein expression, 553–555, 554f
 image analysis and morphometry, 555–557, 556f
 imaging labeled neurons, 555
 labeling neurons, 552–555
Fluorescent protein fusions (FPFs), 489–491
 constructing, 489–490
 development of stable mammalian cell lines expressing, 490–491
 antibiotic for selection of stable clones, 491
 cells for transfection, 491
 DNA for transfection, 491
 screening protocol, 491
 expressing, 490
 genetically encoded calcium indicators (GECIs), 508–517
 transfection of mammalian cells (protocol), 358–363
 discussion, 361–362
 functionality of fusion protein, 361–362
 location of fusion protein, 361
 materials, 358–359
 method, 359–361
 antibiotic selection (day 4), 360
 colony transfer and selection (days 14–21), 360
 DNA preparation and transfection (day 1), 359
 expanding stable clones, 361
 screening (days 21–28), 360
 trypsinization and splitting the cells (day 3), 360
 washing transfected cells (day 2), 359
 recipes, 363
Fluorescent proteins. *See also* Fluorescent protein fusions
 constructing, 489–490
 expressing, 490
 generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct, 381–402, 382f, 384f–385f, 387f, 394f, 396f–397f
 imaging neuronal activity with genetically encoded calcium indicators, 508–517
 measuring membrane voltage with, 518–525
 experimental setup and applications, 522–523, 523f
 fluorescent dyes as sensors, 518
 fluorescent shaker (FlaSh), 519–520, 519t, 520f
 improved voltage-sensing fluorescent proteins, 520–522, 521f
 mechanism of fluorescence change, 522
 protein-based sensors, 518–519
 in multicolor *Brainbow* mice, 575–582, 576f–577f
 origins of, 448
 voltage-sensing, 519t, 520–522, 520f
Fluorescent shaker (FlaSh), 519–520, 519t, 520f
Fluorochromes, 460–461
Formaldehyde
 cross-linking
 described, 143

- Formaldehyde (*Continued*)
 protocol, 147–149
 discussion, 148–149
 materials, 147
 method, 148
 recipes, 149
 denaturation and electrophoresis of RNA with formaldehyde (protocol), 212–215
- Formamide (recipe), 185
- Formamide gel-loading buffer (recipe), 244
- Forskolin stock (4.2 mg/mL) (recipe), 33
- Förster resonance energy transfer (FRET)
 membrane voltage measurement with fluorescent proteins, 518–525, 521f
- 4D fluorescent imaging of embryonic quail development, 563–574
- overview, 563–564
 image analysis, 564
 microscopy equipment requirements, 564
 in vitro culturing, 564
- pre-imaging considerations, 565–566, 566t
 data storage hardware, 565–566, 566t
 environmental control, 565
 fluorophore compatibility, 565
 magnification, 565
- protocol, 567–574
 materials, 567–568
 method, 568–572, 569f–572f
 image analysis, 571–572
 inverted imaging of in vitro embryos, 569
 multitime macro imaging, 569–570
 preparation of paper rings, 568
 sample setup for in vitro imaging, 568, 569f
 tiled z-stack time-lapse experiments, 570–571, 570f–572f
 recipes, 573–574
 troubleshooting, 572–573
- 454 sequencing, 323
- FPFs. *See* Fluorescent protein fusions
- FPKM (fragments per kilobase per million), 328
- Fragmentation, RNA, 314–315, 325
- Fragments per kilobase per million (FPKM), 328
- FRAP (fluorescence recovery after photobleaching), 505–506
- Freeze-drying bacteria, 65
- Freezing bacteria for storage, 65, 78–80
 discussion, 79–80
 materials, 78–79
 methods, 79
 reviving a frozen culture, 79
- FRET
 genetically encoded calcium indicators (GECIs) and, 509–512
 membrane voltage measurement with fluorescent proteins, 518–525, 521f
- FRT site, in *Brainbow* constructs, 579
- G**
- GAI1 instrument, different multiplex sequencing strategies for, 321
- Galaxy website, 331
- G-CaMP2, 511–513, 514f
- G-CaMP3, 511–513, 512t, 514f
- GECIs. *See* Genetically encoded calcium indicators
- Gel electrophoresis. *See* Electrophoresis
- Gel-loading buffer (6×) (recipe), 126
- Gel-loading buffer IV (6×) (recipe), 132
- Gel mobility shift assay. *See* Electrophoretic mobility-shift assay
- Gel purification, in library construction for mRNA-Seq, 311–312
- Gel shift. *See* Electrophoretic mobility-shift assay
- Gel slices, RNA extraction from, 302–303
- Gene expression and targeting systems, 530–531
- Gene Expression Nervous System Atlas (GENSAT) Project, 117–122
- Gene Expression Omnibus, 324
- Gene knockout, 60
- Gene Ontology (GO), 294, 330
- GenePix scanning software, 165, 280–284
- Generation time, calculation of, 77
- Genetically encoded calcium indicators (GECIs)
 classes, 509f
 fluorescence resonance energy transfer (FRET) and, 509–512
 future of design, 516
 imaging neuronal activity with, 508–517
 optimization, 512–514
 from G-CaMP2 to G-CaMP3, 513, 514f
 GECI expression, 513
 practical improvement, 512–513
 subcellular targeting, 513–514
 performance, properties influencing, 510–512
 calcium affinity, kinetics, and dynamic range, 511
 calcium dynamics, 510
 GECI expression level, 511–512
 GECI fluorescence properties, 512, 512t
 structure of, 508–509, 509f
 testing standardization, 515
 use to measure neural activity, 515
- Gene transfer, 353–445
 protocols, 355–445
 nonviral methods, 355–380
 DNA transfection by electroporation, 364–366
 DNA transfection mediated by lipofection, 355–357, 356t
 piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation, 367–375, 369f, 370t, 374f
 single cell/cellular subregion-targeted phototransfection, 376–380, 378f–379f
 transfection of mammalian cells with fluorescent protein fusions, 358–363
 viral methods, 381–445
 concentration and purification of rabies viral and lentiviral vectors, 421–426, 424f
 construction and packaging of herpes simplex virus/adeno-associated virus (HSV/AAV) hybrid amplicon vectors, 441–445, 442t, 443f
 generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct, 381–402, 382f, 384f–385f, 387f, 394f–397f
 generation of replication-competent and -defective HSV vectors, 432–440, 437f–438f
 lentiviral vectors for retrograde delivery of recombinases and transactivators, 403–409, 407f
 rabies viral vectors for monosynaptic tracing and targeted transgene expression in neurons, 410–420, 413f
 stable producer cell lines for adeno-associated virus (AAV) assembly, 427–431, 429f–430f
- Genomic Regions Enrichment of Annotations Tool (GREAT) program, 294, 330
- GENSAT (Gene Expression Nervous System Atlas) Project, 117–122
- Gentamicin, 20
- GFP. *See* Green fluorescent protein
- Giemsa stain, 460
- Glass micropipettes, for single-cell electroporation, 541–542, 541f, 547f, 548–549
- Glassware sterilization for mammalian cell culture, 17
- GLAST promoter, 375
- Gloves, disposable latex, 18
- Glutaraldehyde for fixation, 494, 498
- Glycerol, storing bacteria in, 78–80
- GlycoBlue, 180, 181f, 302, 304–305
- Gram stain, 460
- GREAT program, 294, 330
- Green fluorescent protein (GFP)
 bulk electroporation of *Xenopus* retinal ganglion cells, 560–561, 560f
 discovery of, 448
 imaging green fluorescent protein-labeled neurons using light and electron microscopy (protocol), 492–500
 discussion, 499
 materials, 492–494
 method, 494–498, 496f–499f
 finding imaged neuron in sections, 496, 496f–497f
 fixation and immunocytochemistry, 494
 fixation of imaged slices, 494–495
 imaging the dendrites in the EM, 497–498, 499f
 resin embedding for EM, 495–496
 serial sectioning and imaging in the EM, 496–497, 498f
 recipes, 500
 troubleshooting, 498–499
- imaging synaptic protein dynamics using photoactivatable green fluorescent protein (protocol), 501–507
 discussion, 505–506
 uses of method, 506
 in vitro and in vivo preparation, choosing, 505–506
- imaging setup, 502, 502f
 materials, 501–502
 method, 503–505
 analysis, 505
 choosing photoactivation and imaging wavelengths, 503
 neuronal transfection for in vitro and in vivo imaging preparations, 503
 photoactivation, 503–504, 504f
 preparation of PA-GFP-tagged synaptic proteins, 503
 time-lapse imaging of fluorescence decay, 504–505
 recipes, 506
- photoactivatable (PA-GFP), 501, 503–505, 504f, 561, 561f
- in vivo time-lapse imaging of neuronal development in *Xenopus*, 553–554, 554f
- Group velocity dispersion (GVD), 473
- Growth curve, 75–77, 76f
- Growth factors for retinal ganglion cells, 22–23
- Guanidine cesium chloride RNA purification method, 179
- Guanidine isothiocyanate, in RNA purification, 179
- H**
- Halogen light, 459
- Halorhodopsins (HRs), 528t, 529–530
- Hank's balanced salt solution (10×) (recipe), 252
- Harrison, Ross, 1
- Hayflick limit, 2
- HCl, for depurination of DNA, 129
- HeLa cells, 2
- Hemocytometer, 459
- HEPA (high-efficiency particle air) filter, 14, 16–17
- Heparin-binding EGF-like growth factor (HBEGF), 38, 45–46
- HEPES, in cell culture media, 19
- HEPES-buffered cell medium (recipe), 198
- HEPES-buffered saline (HEBS; 2×) (recipe), 340

Index

- Herpes simplex virus (HSV)
 construction and packaging of herpes simplex virus/adeno-associated virus (HSV/AAV) hybrid amplicon vectors (protocol), 441–445, 442t, 443f
 discussion, 444–445
 materials, 441–442
 method, 442–444, 442t, 443f
 cotransfection of packaging-defective HSV-1 helper DNA and vector DNA, 442–443, 442t, 443f
 harvesting packaged vectors, 443–444
 titration of amplicon stocks, 444
 generation of replication-competent and -defective HSV vectors (protocol), 432–440, 437f–438f
 discussion, 437–438
 materials, 432–433
 method, 434–437, 437f
 construction of recombination virus, 434–436
 isolation of viral DNA for transfection, 434
 viral stock preparation and purification, 436, 437f
 recipes, 439
 troubleshooting, 437
 life cycle, 437–438
 replication-defective HSV vectors, 438
Herring testis carrier DNA (recipe), 221
High-ovomucoid stock (6 \times) (recipe), 33
High-ovomucoid stock solution (10 \times) (recipe), 47
High-throughput sequencing (HTS)
 data processing, 323–331
 classification of changes and associated biology, 330
 experimental design, 324–328
 mapping, 329
 measuring expression levels and changes in expression, 329
 presentation of data, 330–331
 raw reads, 328–329
 transcript (isoform) assembly, 329
 validation, 329–330
 experimental design, 324–328
 library construction, 325–327
 quantity of starting material, 324–325
 fragmentation of whole-transcriptome RNA using *E. coli* RNase III for, 296–298
 library construction basics, 325–327
 amplification, 325–326
 bar coding, 326–327
 directionality (strand selection), 326
 normalization, 328
 number of reads, 327
 paired ends, 326
 priming *versus* fragmentation, 325
 read length, 326
 replicates, 327–328
 mRNA-Seq libraries from poly(A)⁺ mRNA for Illumina transcriptome high-throughput sequencing, 310–312
 notes and considerations before beginning, 311
 overview, 310
 tips and troubleshooting, 311–312
 adapter ligation, 311
 first gel purification, 311–312
 optimization, 311
 PCR enrichment, 312
 second gel purification, 311–312
 preparation of small RNA libraries (protocol), 299–309
 materials, 299–300
 method, 301–308
 5' linker ligation, 305
 gel purification of amplified cDNA, 307–308, 308f
 gel purification of 5'- and 3'-ligated RNA product, 305–306, 306f
 gel purification of 3'-ligated RNA product, 303–305, 304f
 PCR amplification of cDNA, 307
 PmeI digestion of radiolabeled oligonucleotides, 307
 reverse transcription, 306
 size selection and gel purification of RNA sample, 301–303, 302f
 3' linker ligation, 303
 recipes, 309
 strand-specific RNA sequencing library preparation (protocol), 313–322
 discussion, 319
 different multiplex sequencing strategies for GAI and HiSeq2000 instruments, 321
 false antisense read derived from uracil-minus RNA, 320
 purification and size selection on magnetic beads, 320–321
 recipes, 322
 strand-specific RNA-Seq vs. conventional RNA-Seq, 319–320, 320f
 using phosphorothioate oligos to prevent PCR amplification of adapter dimers, 321
 materials, 313–314
 method, 314–319, 315f
 dA-tailing, 316
 end-repair, 316
 first-strand cDNA synthesis, 315–316
 general procedure for using AMPure beads, 318–319
 mix barcoded libraries for multiplex sequencing, 318
 PCR enrichment, 317–318, 318f
 polyA RNA isolation and fragmentation, 314–315
 second-strand synthesis with dUTP, 316
 triple-SPRI purification and size selection, 317, 317f
 Y-shape adapter ligation, 316–317
 systems for, 323
HiSeq2000 instrument, different multiplex sequencing strategies for, 321
H2Ld allele, 489
Homologization of tissues, 184, 188
Homologous recombination using bacterial artificial chromosomes (protocol), 106–116, 109f–110f
 materials, 106–108
 method, 108–114, 109f–110f
 analysis of putative recombinant BACs, 113–114
 arabinose-induced excision of the selectable marker, 114
 assembly and preparation of the ECFP-Kan cassette, 108–112, 109f–110f
 electroporation of the ECFP-Kan insert into electrocompetent cells, 113
 preparation of electrocompetent cells, 112–113
 recipes, 115–116
 troubleshooting, 114–115
Hot-start PCR, 95–96
HSV. *See* Herpes simplex virus
HTS. *See* High-throughput sequencing
Humidified incubators, contamination of, 18
Hybrid amplicon vectors, construction and packaging of herpes simplex virus/adeno-associated virus (HSV/AAV), 441–445, 442t, 443f
Hybridization buffer for ChIP-chip (recipe), 165
Hybridization chain reaction
 combinatorial analysis of RNA expression patterns in mouse embryos using HCR (protocol), 224–233
 discussion, 230–232, 231f, 231t
 materials, 224–225, 226t
 method, 225–229, 227f
 clearing and mounting embryos, 229
 image acquisition, 229
 in situ HCR, 225, 227f, 228–229
 recipes, 232–233
 troubleshooting, 229–230, 230f
Hydrophobic interaction chromatography, in RNA purification, 179
Hygromycin, 349
- ## I
- IGF-1 (insulin-like growth factor 1), 23
Illumina Genome Analyzer, 171, 310
Illumina ssRNA-Seq kit, 319
Illumina TruSeq multiplex kit, 321
Image digitization, 467
Imaging
 knowing when specimen is unhealthy, 56
 maintaining live cells and tissue slices in imaging setup, 50–57, 52f–54f
 collecting images, 55–56
 medium considerations, 51
 mounting live specimens for microscopic observation, 51–54
 maintenance of gas and pH conditions after mounting, 53–54, 53f–54f
 technique for chamber construction and sample mounting, 51–53, 52f
 temperature considerations, 54–55
 overview of live imaging, 50–51
 photodamage, 55
 protocols
 light modulation of proteins
 establishing a fiber-optic-based optical neural interface, 534–539
 imaging green fluorescent protein-labeled neurons using light and electron microscopy, 492–500
 imaging synaptic protein dynamics using photoactivatable green fluorescent protein, 501–507
 microscopy basics
 using the light microscope, 453–462
 in vivo imaging
 bulk electroporation of retinal ganglion cells in live *Xenopus* tadpoles, 558–562
 mapping anatomy to behavior in Thy1:18 ChR2-YFP transgenic mice using optogenetics, 594–605
 preparation and 4D fluorescent imaging of quail embryos, 567–574
 single-cell electroporation of *Xenopus* tadpole tectal neurons, 546–551
 two-photon imaging of microglia in mouse cortex in vivo, 583–593
 topic introductions
 Brainbow mice, generating and imaging multicolor, 575–582
 confocal microscopy principles and practices, 463–470
 constructing and expressing fluorescent protein fusions, 489–491
 digital scanned laser light sheet fluorescence microscopy, 476–488
 4D fluorescent imaging of embryonic quail development, 563–566
 imaging neuronal activity with genetically encoded calcium indicators, 508–517
 measuring membrane voltage with fluorescent proteins, 518–525
 microscopy, 449–452
 multiphoton-excitation fluorescence microscopy, 471–475

- Imaging (*Continued*)
 optogenetics, 526–533
 single-cell electroporation in *Xenopus*, 540–545
 in vivo time-lapse imaging of neuronal development in *Xenopus*, 552–557
- Imaris software, 564
- Immersion oil, 449
 cleaning from lenses, 458
 Type A and Type B, 453
 using on the condenser lens, 457
 using on the objective lens, 457
- Immortal cell lines, 2
- Immunoblotting, for fusion protein analysis, 361–362
- Immunocytochemistry, in imaging green fluorescent protein-labeled neurons using light and electron microscopy protocol, 494
- Immunofluorescence, 460
- Immunopanning
 astrocytes, 38–49, 41f
 retinal ganglion cells, 22, 25–32, 28f
- Immunoprecipitation
 CLIP (cross-linking and immunoprecipitation) identification of RNAs bound by a specific protein (protocol), 254–268
 for fusion protein analysis, 361–362
- Incubators, contamination of, 18
- Indirect immunofluorescence, 460
- Inducible expression, 490
- Infectious waste, disposal of, 63
- Inhibitor stock solution (recipe), 48
- Insulin, 23
- Insulin stock (0.5 mg/mL) (recipe), 33
- Interference filters, 461
- Internal ribosomal entry site (IRES), 399
- in utero electroporation, *piggyBac* transposon-mediated cellular transgenesis in mammalian forebrain by, 367–375, 369f, 370t, 374f
 discussion, 374–375
 background, 374–375
 expected results, 374f, 375
 materials, 367–368
 method, 368–372
 animal preparation, 370–371
 helper and donor plasmid combinations, 370, 370t
 surgical station preparation, 368
 suturing and animal recovery, 372
 in utero electroporation, 371–372
 in utero electroporation setup, 368–370, 369f
 troubleshooting, 372–373
- Inverted microscopy, 451
- in vivo imaging protocols
 bulk electroporation of retinal ganglion cells in live *Xenopus* tadpoles, 558–562
 mapping anatomy to behavior in Thy1:18 Chr2-YFP transgenic mice using optogenetics, 594–605
 preparation and 4D fluorescent imaging of quail embryos, 567–574
 single-cell electroporation of *Xenopus* tadpole tectal neurons, 546–551
 two-photon imaging of microglia in mouse cortex in vivo, 583–593
- in vivo time-lapse imaging of neuronal development in *Xenopus*, 552–555, 552–557, 554f
 fluorescent dextran, 552–553
 genetic labeling by fluorescent protein expression, 553–555, 554f
 image analysis and morphometry, 555–557, 556f
 imaging labeled neurons, 555
 labeling neurons, 552–555
 lipophilic vital dyes, 553
- IP-astrocyte base medium (recipe), 48
- IRES (internal ribosomal entry site), 399
- IR irradiation, in single cell/cellular subregion-targeted transfection (protocol), 376–380
- Isoflurane, 370, 535, 594, 597, 604
- Isoform assembly, 329
- Isolated colonies of bacteria, obtaining, 70–72, 71f
- Isopropanol precipitation of RNA, 185
- ITGB5, 42, 45, 46–47
- J**
- Java, 564
- K**
- Kanamycin (30 mg/mL stock solution) recipe, 68t
- Kanamycin (50 mg/mL stock solution) recipe, 68t
- K⁺ channel, 518–519, 520t, 521
- Ketamine, 370, 535
- Klenow
 in antibody-positioned RNA amplification (APRA) protocol, 250
 in cDNA library preparation, 316
 in CHIP-seq protocol, 169
 in generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct protocol, 384f, 388–389
- Koehler illumination, 455–456, 456f–457f
- L**
- Lag phase, growth curve, 76, 76f
- Laminar flow hood, 16–18. *See also* Biosafety cabinet
- Laser
 mode-locked, 473
 in single cell/cellular subregion-targeted phototransfection (protocol), 376–380
- Laser capture microdissection (LCM), single-neuron isolation for RNA analysis using, 191, 195–196
- Laser pulse width, 473
- Laser-scanning confocal microscopy, 451. *See also* Confocal microscopy
 conversion to multiphoton-excitation fluorescence microscopy, 474
 laser-scanning design, 469
 optical-transfer efficiency, 467
- Lateral geniculate nucleus, 21
- LB freezing buffer (recipe), 101
- LB (Luria-Bertani) liquid medium (recipe), 83, 102, 105, 115, 401
- LB solid medium with 100 µg/mL ampicillin (recipe), 402
- Lead citrate for EM (recipe), 497, 500
- Lenses, 449. *See also* Objective lens
 numerical aperture (N.A.), 449, 459
 xylene for cleaning objective lens, 453, 458
- Lentivirus
 advantages as vehicle for gene delivery, 399
 concentration and purification of rabies viral and lentiviral vectors (protocol), 421–426, 424f
 discussion, 425
 materials, 421–422
 method, 422–425, 424f
 recipes, 425–426
 generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct (protocol), 381–402, 382f, 384f–385f, 387f, 394f, 396f–397f
 materials, 381–384, 382f
 method, 384–399, 384f–385f, 387f, 394f–397f
- bicistronic insert and lentiviral vector backbone preparation, 384–391, 384f–385f, 387f
 bicistronic lentiviral vector production, 391–394, 394f
 concentrating lentiviral supernatants, 398
 harvesting bicistronic viral supernatants and infecting cells, 396–398, 397f
 imaging infected 3T3 cells for bicistronic expression, 397–398, 397f
 plasmid DNA preparation, 393–394
 plasmid maps, 385f
 transfecting 293FT packaging cells for virus production, 394–396, 395f–396f
 transformation of *E. coli*, 391–393
 viral titer determination using fluorescent microscope, 398–399
- lentiviral vectors for retrograde delivery of recombinases and transactivators (protocol), 403–409, 407f
 discussion, 406–408, 407f
 materials, 403–404
 method, 405–406
 recipe, 408
- optogenetics and, 530
- Library
 high-throughput Illumina strand-specific RNA sequencing library preparation (protocol), 313–322
 discussion, 319
 different multiplex sequencing strategies for GAI and HiSeq2000 instruments, 321
 false antisense read derived from uracil-minus RNA, 320
 purification and size selection on magnetic beads, 320–321
 recipes, 322
 strand-specific RNA-Seq vs. conventional RNA-Seq, 319–320, 320f
 using phosphorothioate oligos to prevent PCR amplification of adapter dimers, 321
 materials, 313–314
 method, 314–319, 315f
 dA-tailing, 316
 end-repair, 316
 first-strand cDNA synthesis, 315–316
 general procedure for using AMPure beads, 318–319
 mix barcoded libraries for multiplex sequencing, 318
 PCR enrichment, 317–318, 318f
 polyA RNA isolation and fragmentation, 314–315
 second-strand synthesis with dUTP, 316
 triple-SPRI purification and size selection, 317, 317f
 Y-shape adapter ligation, 316–317
- mRNA-Seq libraries from poly(A)⁺ mRNA for Illumina transcriptome high-throughput sequencing, 310–312
 notes and considerations before beginning, 311
 overview, 310
 tips and troubleshooting, 311–312
 adapter ligation, 311
 first gel purification, 311–312
 optimization, 311
 PCR enrichment, 312
 second gel purification, 311–312
 preparing RNA for high-throughput sequencing, 299–309
 replicate, 327
- LiCl-urea solution for RNA isolation (recipe), 189
- LIF (leukemia inhibitory factor), 23

Index

- LifterSlip, 276–278
- Ligation
- in ChIP-seq protocol, 169
 - in generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct (protocol), 391
- Ligation-mediated polymerase chain reaction (LM-PCR), 162–163, 167
- Light
- halogen, 459
 - tungsten, 459
- Light microscopy. *See also* Fluorescence microscopy
- imaging green fluorescent protein-labeled neurons using light and electron microscopy (protocol), 492–500
 - discussion, 499
 - materials, 492–494
 - method, 494–498, 496f–499f
 - finding imaged neuron in sections, 496, 496f–497f
 - fixation and immunocytochemistry, 494
 - fixation of imaged slices, 494–495
 - imaging the dendrites in the EM, 497–498, 499f
 - resin embedding for EM, 495–496
 - serial sectioning and imaging in the EM, 496–497, 498f
 - recipes, 500
 - troubleshooting, 498–499
- methods, 450–451
- bright-field microscopy, 450
 - dark-field microscopy, 450
 - fluorescence microscopy, 451
 - inverted microscopy, 451
 - laser scanning confocal microscopy, 451
 - Nomarski imaging (differential interference contrast) microscopy, 451
 - phase-contrast microscopy, 450
 - using the light microscope (protocol), 453–462
 - components of a light microscope, 454f
 - considerations for light microscopy, 459–460
 - illumination type, 459
 - measurement, 459
 - objective lens type, 459
 - photography, 460
 - sizes of select biological samples, 460t
 - staining, 460
- discussion, 458–461
- fluorescence microscopy, 460–461
- materials, 453–454
- method
- cleaning the microscope, 455–457
 - fixing and staining specimen, 455
 - making a cell smear, 455
 - viewing a specimen, 455–457
- viewing a specimen, 455–457
- condenser aperture diaphragm, 457f
 - field diaphragm image, 456f
 - using aligned microscope to quickly examine samples, 456–457
 - using immersion oil on the condenser lens, 457
 - using immersion oil on the objective lens, 457
 - using Koehler illumination, 455–456, 456f–457f
 - working rules, 458–459
- Light modulation of proteins, protocols for
- establishing a fiber-optic-based optical neural interface, 534–539
- imaging green fluorescent protein-labeled neurons using light and electron microscopy, 492–500
- imaging synaptic protein dynamics using photoactivatable green fluorescent protein, 501–507
- Light-sheet fluorescence microscopy (LSFM). *See also* Digital scanned laser light sheet fluorescence microscopy (DSLM)
- optical sectioning, 476
 - point-spread function (PSF), 484
 - principles of, 476, 477
- Lipofectamine 2000, for transfection of 293FT packaging cells for virus production, 395, 395f
- Lipofectin (DOTMA), 355–357, 356t
- Lipofection
- DNA transfection mediated by lipofection (protocol), 355–357, 356t
 - materials, 355–356, 356t
 - method, 356–357
 - lipids used in, 356t
- Lipophilic vital dyes, 553
- Liposomes, 357
- LM-PCR mix (recipe), 166
- LNL-GFP, 561, 561f
- Loading buffer for mobility shift assays (recipe), 238
- Log phase, growth curve, 76, 76f
- Low-ovomuroid stock (10 \times) (recipe), 33
- Low-ovomuroid stock solution (10 \times) (recipe), 48
- lox sites, in *Brainbow* constructs, 579
- LSFM. *See* Light-sheet fluorescence microscopy
- ## M
- Magnetic concentrator, 155
- Magnification, 449
- Mammalian cell culture, 16–20
- gene transfer protocols
 - DNA transfection by electroporation, 364–366
 - DNA transfection mediated by lipofection, 355–357, 356t
 - single cell/cellular subregion-targeted phototransfection, 376–380, 378f–379f
 - transfection of mammalian cells with fluorescent protein fusions, 358–363
 - infection with short hairpin RNA (shRNA) retroviruses (protocol), 342–343
 - materials, 342
 - method, 342–343, 343t
 - medium formulation, 19–20
 - medium preparation, 20
 - purification and culture of retinal ganglion cells from rodents (protocol), 25–34, 28f
 - purification of rat and mouse astrocytes by immunopanning (protocol), 38–49, 41f
 - sterile technique, 16–18
- Mapping, 329
- MATLAB, 564
- mCherry, 561, 561f
- Medium formulation, for mammalian cell culture, 19–20
- Medium preparation, for mammalian cell culture, 20
- Membrane voltage, measuring with fluorescent proteins, 518–525
- experimental setup and applications, 522–523, 523f
 - fluorescent dyes as sensors, 518
 - fluorescent shaker (FlaSh), 519–520, 519t, 520f
 - improved voltage-sensing fluorescent proteins, 520–522, 521f
 - mechanism of fluorescence change, 522
 - protein-based sensors, 518–519
- Mermaid, 519t, 520, 522–523, 523f
- Metabond cement, 601, 604
- Metacam, 370
- Metal chelator, 176
- Metal ions, RNA degradation caused by, 175–176
- Methanol for fixation, 453, 455
- Methylcellulose overlay (recipe), 439
- Methylene blue stain, 460
- Mg²⁺, chelation of, 176
- MgCl₂-CaCl₂ solution (recipe), 87
- Microarray hybridization
- preparation of fluorescent-dye-labeled cDNA from RNA for microarray hybridization (protocol), 269–273
 - discussion, 272–273
 - materials, 269–270
 - method, 270–272
 - coupling reaction and purification, 271–272
 - purification of cDNA, 271
 - reverse transcription reaction, 270–271
 - troubleshooting, 272
 - slide hybridization using fluorescently labeled cDNA (protocol), 274–279
 - materials, 274–275
 - method, 275–279, 276f–278f
 - blocking slides, 275–276, 276f
 - hybridization of microarray slides, 276–279, 277f–278f
 - recipes, 279
- Microarrays. *See also* DNA microarrays; Microarray hybridization
- methods for processing microarray data, 291–295
 - analysis, 292–295
 - experimental design, 291–292
 - materials, 291
 - scanning microarray slides (protocol), 280–284, 283f
 - discussion, 283–284, 283f
 - materials, 280–281
 - method, 281–283
 - results file creation, 282–283
 - scanning slide, 281
 - spotfinding, 281–282
 - template for spotfinding, 281
 - tips on hybridizing, washing, and scanning Affymetrix microarrays (protocol), 285–290
 - materials, 285–286
 - method, 286–290
- Micrococcal nuclease, 260–261
- Micro-FastTrack mRNA isolation kit, 188
- Microglia, two-photon imaging in mouse cortex in vivo (protocol), 583–593
- Micropipettes, for single-cell electroporation, 541–542, 541f, 547f, 548–549
- Microscopy, 449–488
- contrast, 449–450
 - electron microscopy methods, 452
 - scanning electron microscopy (SEM), 452
 - transmission electron microscopy (TEM), 452
 - light microscopy methods, 450–451
 - bright-field microscopy, 450
 - dark-field microscopy, 450
 - fluorescence microscopy, 451
 - inverted microscopy, 451
 - laser scanning confocal microscopy, 451
 - Nomarski imaging (differential interference contrast) microscopy, 451
 - phase-contrast microscopy, 450
 - magnification, 449
 - numerical aperture (N.A.), 449
 - refractive index, 449
 - resolution, 449
 - for single-cell electroporation, 542–543, 549
 - using the light microscope (protocol), 453–462
 - components of a light microscope, 454f
 - considerations for light microscopy, 459–460
 - illumination type, 459
 - measurement, 459
 - objective lens type, 459
 - photography, 460
 - sizes of select biological samples, 460t
 - staining, 460
 - discussion, 458–461

- Microscopy (*Continued*)
fluorescence microscopy, 460–461
 features of, 461
 staining for, 461
 tips for, 461
 uses, 460–461
materials, 453–454
method
 cleaning the microscope, 455–457
 fixing and staining specimen, 455
 making a cell smear, 455
 viewing a specimen, 455–457
viewing a specimen, 455–457
 condenser aperture diaphragm, 457f
 field diaphragm image, 456f
 using aligned microscope to quickly examine samples, 456–457
 using immersion oil on the condenser lens, 457
 using immersion oil on the objective lens, 457
 using Koehler illumination, 455–456, 456f–457f
 working rules, 458–459
Microwave oven, fixing DNA to membrane, 131
MinElute Kit, clean up dsDNA with, 203–204
 DNA binding to column, 203–204
 elute DNA bound to column, 204
 wash DNA bound to column, 204
MiR30-based shRNA vector (protocol), 333–337
 discussion, 335–336
 examples of mammalian shRNA vectors, 334f
 materials, 333
 method, 334–335
 recipes, 336
MMRRC (Mutant Mouse Regional Resource Center), 122
Moloney murine leukemia virus (M-MuLV)
 promoter, 336
MOPS buffer (10×) (recipe), 214
Morpholinos, delivery by single-cell electroporation, 545
Motion artifacts, 588–589
Mounting live specimens for microscopic observation, 51–54
 maintenance of gas and pH conditions after mounting, 53–54, 53f–54f
 perfusion system, simple gravity-fed, 53, 53f
 simple integrated setup, 54f
 technique for chamber construction and sample mounting, 51–53, 52f
Mouse
 Brainbow mice, generating and imaging multicolor, 575–582
 GENSAT database of engineered mouse strains, 117–122
 isolating total RNA from mouse embryos or fetal tissues, 187–190
 purification and culture of retinal ganglion cells from, 25–34, 28f
 purification and culture of retinal ganglion cells from (protocol), 25–34, 28f
 purification of rat and mouse astrocytes by immunopanning, 38–49, 41f
 transgenic shRNA mouse creation by recombinase-mediated cassette exchange, 344–351
 two-photon imaging of microglia in mouse cortex in vivo, 583–593
Mouth pipetting, 18
mRNA
 antisense RNA amplification for target assessment of total mRNA from a single cell (protocol), 200–211
 combinatorial analysis of RNA expression patterns in mouse embryos using hybridization chain reaction (protocol), 224–233
 high-throughput Illumina strand-specific RNA sequencing library preparation (protocol), 313–322
 discussion, 319
 different multiplex sequencing strategies for GAI and HiSeq2000 instruments, 321
 false antisense read derived from uracil-minus RNA, 320
 purification and size selection on magnetic beads, 320–321
 recipes, 322
 strand-specific RNA-Seq vs. conventional RNA-Seq, 319–320, 320f
 using phosphorothioate oligos to prevent PCR amplification of adapter dimers, 321
materials, 313–314
method, 314–319, 315f
 dA-tailing, 316
 end-repair, 316
 first-strand cDNA synthesis, 315–316
 general procedure for using AMPure beads, 318–319
 mix barcoded libraries for multiplex sequencing, 318
 PCR enrichment, 317–318, 318f
 polyA RNA isolation and fragmentation, 314–315
 second-strand synthesis with dUTP, 316
 triple-SPRI purification and size selection, 317, 317f
 Y-shape adapter ligation, 316–317
mRNA-Seq libraries from poly(A)⁺ mRNA for Illumina transcriptome high-throughput sequencing, 310–312
 notes and considerations before beginning, 311
 overview, 310
 tips and troubleshooting, 311–312
 adapter ligation, 311
 first gel purification, 311–312
 optimization, 311
 PCR enrichment, 312
 second gel purification, 311–312
 number of distinct expressed, 174
 phototransfection of neurons with, 378–379, 379f
 single cell capture for mRNA reverse transcription, 194–195
MS-222, 548, 559
Multiphoton-excitation fluorescence microscopy, 471–475
 confocal microscopy compared, 471–473
 detection, 472
 laser pulse width, 473
 localized excitation, 471–472
 out-of-focus light rejection, 471
 resolution, 473
 scattered light, 472
 instrumentation, 473–474
 excitation light source, 473
 wavelength selection, 473–474
 setting up the system, 474
 uses in neuroscience, 474
Murine phosphoglycerate kinase promoter, 489
Murine RNA polymerase II promoter, 489
Murine stem cell virus (MSCV) long terminal repeat promoter, 336
Mutant Mouse Regional Resource Center (MMRRC), 122
Mycoplasma contamination
 from mouth pipetting, 18
 periodic analysis for, 20
N
NAC stock (5 mg/mL) (recipe), 33, 48
Nalidixic acid (100 mg/mL stock solution) recipe, 68t
NanoDrop, 271–272, 312, 388
Natronomonas pharaonis, opsins from, 528t, 529–530
Neomycin, 350
Nestin promoter, 375
Neurons
 gene transfer protocols
 lentiviral vectors for retrograde delivery of recombinases and transactivators (protocol), 403–409, 407f
 piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation (protocol), 367–375, 369f, 370t, 374f
 rabies viral vectors for monosynaptic tracing and targeted transgene expression in neurons (protocol), 410–420, 413f
 single cell/cellular subregion-targeted phototransfection (protocol), 376–380, 378f–379f
 imaging green fluorescent protein-labeled neurons using light and electron microscopy (protocol), 492–500
 imaging neuronal activity with genetically encoded calcium indicators, 508–517
 imaging synaptic protein dynamics using photoactivatable green fluorescent protein (protocol), 501–507
 labeling for in vivo imaging, 552–555, 554f
 fluorescent dextrans, 552–553
 genetic labeling by fluorescent protein expression, 553–555, 554f
 lipophilic vital dyes, 553
 number in adult human brain, 174
 single-cell electroporation of *Xenopus* tadpole tectal neurons (protocol), 546–551
 single-neuron isolation for RNA analysis using pipette capture and laser capture microdissection (protocol), 191–199
 synaptic connections, number of, 174
 in vivo time-lapse imaging of neuronal development in *Xenopus*, 552–557
Neutralization buffer I (recipe), 132
Neutralization buffer II (recipe), 132
NHS (N-hydroxysuccinimide) esters, 269, 271–273
Nitrocellulose, isolation of RNA from, 264
Nomarski imaging (differential interference contrast) microscopy, 451
Northern blots
 advantages, 221
 capillary transfer of RNA from agarose gels and filter hybridization using standard stringency conditions (protocol), 216–223
 discussion, 220–221
 materials, 216–217
 methods, 216–220
 autoradiography, 219, 219f
 capillary transfer, 217–218, 218f
 cross-linking and hybridization, 218–219
 stripping and reprobing, 220
 washing, 219
 recipes, 221–222
 troubleshooting, 220
 described, 220–221
 disadvantages, 221
NS21 supplement, 23
NT-4/5 (neurotrophin-4/5), 23
Numerical aperture (N.A.), 449, 459
 in digital scanned laser light sheet fluorescence microscopy (DSLM), 477
 in 4D fluorescent imaging of embryonic quail development, 563–566

Index

- Numerical aperture (N.A.) (*Continued*)
in light-sheet-based fluorescence microscopy (LSFM), 484
optical-transfer efficiency, 467
in point-spread function (PSF), 464
Nyquist criterion, 467
- O**
- Object-Image software, 556–557, 556f
Objective lens, 449
cleaning, 458
digital scanned laser light sheet fluorescence microscopy (DSLML), 477–478
numerical aperture (N.A.), 449, 459
phase-contrast, 459
using immersion oil on, 457
xylene for cleaning, 453, 458
- Ocular lens
cleaning, 458
micrometer, 459
- Oligodendrocytes, 36, 374f
Oligonucleotide, conjugation to antibody, 248
Opsins, 526–530, 527t–528t
Optical density (O.D.), 75–77, 89
Optical neural interface (ONI), 531
establishing a fiber-optic-based (protocol), 534–539
materials, 534–535
method, 535–538, 537f
infusion of the virus and surgery, 536–537
optical stimulation, 537–538
preparation for surgery, 535–536
preparation of the bilateral ONI, 537f
troubleshooting, 538–539
- Optical sectioning
laser light sheet fluorescence microscopy (LSFM), 476, 484
multiphoton-excitation fluorescence microscopy, 471
- Optical-transfer efficiency, 467
Opto- α 1AR/Opto- β 2AR, 528t
Optogenetics, 526–533
described, 448
establishing a fiber-optic-based optical neural interface (protocol), 534–539, 537f
gene expression and targeting systems, 530–531
mapping anatomy to behavior in Thy1:18 ChR2-YFP transgenic mice using optogenetics (protocol), 594–605
materials, 594–596
method, 596–603
analyzing the data, 603
closing the incision and recovering the animal, 601
identifying the target region, 596
performing craniotomy and introducing the fiber implant, 599–600
preparing for stimulation, 601–602, 602f
preparing surgical area, 596–597, 597f
preparing the mouse, 597–599, 598f
securing the implant, 600–601
stimulating the animal, 602–603, 603f
troubleshooting, 604
opsins, 526–530, 527t–528t
optical neural interface, 531, 534–539, 537f
outlook for, 532
tools, 527t–528t
- Oregon Green BAPTA, 552
Orthojet, 601
Osmolality, of cell culture media, 19
Out-of-focus light rejection, 471
Overview
digital scanned laser light sheet fluorescence microscopy (DSLML), 476–477, 477f
Oxygen radical scavengers, 461
- P**
- [32 P], in electrophoretic mobility-shift assays, 139–142
Paired-end reads, 311, 324, 326
Pancreatic RNase, 177
Papain
in purification and culture of retinal ganglion cells from rodents (protocol), 29–30
in purification of rat and mouse astrocytes by immunopanning (protocol), 42
Paraformaldehyde for fixation, 494
Paraformaldehyde (PFA) for HCR (4%) (recipe), 232
Pax2, 224, 226t
PBase, 374–375
PBS(P) (recipe), 500
PBS for CLIP (recipe), 267
PBS (pH 7.5) for HSV (recipe), 439
PBST for HCR (recipe), 232
PCR. *See* Polymerase chain reaction
Penicillin, 20
Pentobarbitone, 494
Peptides, delivery by single-cell electroporation, 545
Perfusion of rat tissue, 494
Petroff-Hausser counting chamber, 73–74, 74f, 459
pH, of cell culture media, 19
Phase-contrast microscopy, 450
Phase-contrast objectives, 459
Phenol (recipe), 185–186
Phenol:chloroform, 179, 183–185, 267
Phenol red dye, 19
Phenotypic drift in bacteria, 64
Phosphate-buffered saline (10 \times ; pH 7.35) (recipe), 253
Phosphate-buffered saline (PBS) (recipe), 149, 156, 363, 366
Phosphate-buffered saline (PBS) for HCR (10 \times , pH 7.4) (recipe), 232
Phosphate removal from RNA ends, 261
Phosphate-SDS washing solution 1 (recipe), 137
Phosphate-SDS washing solution 2 (recipe), 137
Phosphorothioate, 318, 318f, 321
Phosphorylation of 5' ends of RNAs with PNK, 262
Photoactivatable green fluorescent protein (PA-GFP), 501, 503–505, 504f, 561, 561f
- Photoactivation
imaging synaptic protein dynamics using photoactivatable green fluorescent protein (protocol), 501–507
discussion, 505–506
uses of method, 506
in vitro and in vivo preparation, choosing, 505–506
imaging setup, 502, 502f
materials, 501–502
method, 503–505
analysis, 505
choosing photoactivation and imaging wavelengths, 503
neuronal transfection for in vitro and in vivo imaging preparations, 503
photoactivation, 503–504, 504f
preparation of PA-GFP-tagged synaptic proteins, 503
time-lapse imaging of fluorescence decay, 504–505
recipes, 506
- Photobleaching
digital scanned laser light sheet fluorescence microscopy (DSLML), 483–484
fluorescence recovery after photobleaching (FRAP), 505–506
single plane illumination microscopy (SPIM), 483
Photocathode, confocal microscope, 467
Photodamage, 55
Photography
digital, 460
in fluorescence microscopy, 461
light microscopy and, 460
- Photomultiplier tubes, 467
Photoshop, 496
Phototoxicity, laser light sheet fluorescence microscopy and, 476
Phototransfection, single cell/cellular subregion-targeted (protocol), 376–380, 378f–379f
discussion, 379–380
materials, 376–377
method, 377–379, 378f
aligning multiphoton beam in the microscope, 377
configuring parameters for maximal cell viability, 378, 378f
phototransfection of neurons with mRNA, 378–379, 379f
recipes, 380
- PicoGreen dsDNA Assay Kit, 318
piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation (protocol), 367–375, 369f, 370t, 374f
discussion, 374–375
background, 374–375
expected results, 374f, 375
materials, 367–368
method, 368–372
animal preparation, 370–371
helper and donor plasmid combinations, 370, 370t
surgical station preparation, 368
suturing and animal recovery, 372
in utero electroporation, 371–372
in utero electroporation setup, 368–370, 369f
troubleshooting, 372–373
- Pipette capture, single-neuron isolation for RNA analysis using, 191–198, 193f–195f
Pipette solution (recipe), 198
Pipetting
mouth, 18
sterile technique (protocol), 6–8
discussion, 8
materials, 6
method, 6–8
opening and pipetting with individually wrapped disposable pipettes, 7–8
pipetting with packaged disposable pipettes, 7
pipetting with reusable glass pipettes, 6–7
- PK buffer (recipe), 267
Plasmids
AAV, 427
bulk electroporation of retinal ganglion cells in live *Xenopus* tadpoles (protocol), 558–562, 560f
DNA preparation (minipreps), 81–84, 393–394
in gene transfer protocols
DNA transfection by electroporation (protocol), 364–366
DNA transfection mediated by lipofection (protocol), 355–357, 356f
generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct (protocol), 384f–385f, 388–395, 394f
piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation (protocol), 367–375, 369f, 370t, 374f
preparation and transformation of competent *E. coli* using calcium chloride (protocol), 85–88
materials, 85

- Plasmids (*Continued*)
method, 86
recipes, 887–88
preparation of plasmid DNA by alkaline lysis with sodium dodecyl sulfate:
minipreparation (protocol), 81–84
materials, 81
method, 82
recipes, 83–84
transfection by single-cell electroporation of DNA for *Xenopus* neurons, 543–544, 548
- Plasticware sterilization for mammalian cell culture, 17
- PmeI digestion of radiolabeled oligonucleotides, 307
- PNK, 262, 301
- Point-spread function (PSF)
confocal microscopy, 464, 465, 484
digital scanned laser light sheet fluorescence microscopy (DSLIM), 484
lateral and axial extents of, 484
light-sheet-based fluorescence microscopy (LSFM), 484
two-photon fluorescence microscopy, 484
- Polyacrylamide gel electrophoresis
in ChIP-seq protocol, 169–170
in CLIP (cross-linking and immunoprecipitation) protocol, 262–263, 263f, 266
electrophoretic mobility-shift assay (EMSA), 139–142, 234–239
in preparation of small RNA libraries for high-throughput sequencing (protocol), 299–309, 302f, 304f, 306f, 308f
resolving of RNA-protein cross-linked complexes on gel, 262–263, 263f
RNA extraction from gel slices, 302–303
RNase footprinting to map sites of RNA-protein interactions (protocol), 240–244
- Polyacrylamide gel solution (8% in 1 × TBE) (recipe), 171
- PolyA RNA, isolation and fragmentation, 314–315
- Polybrene, 343
- Polymerase chain reaction (PCR), 91–99
antisense RNA amplification for target assessment of total mRNA from a single cell (protocol), 200–211
basic protocol, 91–93
materials, 91
method, 92
recipes, 92–93
in cDNA library preparation, 317–318, 325–326
for ChIP-chip, 162–163
ChIP-quantitative polymerase chain reaction (ChIP-qPCR)
overview, 144, 145t
protocol, 158–159
materials, 158
method, 158–159
ChIP-quantitative polymerase chain reaction (ChIP-qPCR) (protocol), 158–159
materials, 158
method, 158–159
in ChIP-seq protocol, 170–171
in generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct protocol, 384–390
in homologous recombination using bacterial artificial chromosomes protocol
analysis of putative recombinant BACs, 113–114
assembly and preparation of the ECFP-Kan cassette, 108–112
hot-start, 95–96
ligation-mediated (LM-PCR), 162–163, 167
optimization strategy, 96–97
in preparation of small RNA libraries for high-throughput sequencing protocol, 307
quantification of products by NanoDrop, 388
RT-PCR, 210, 256, 265–267, 330
stepdown, 95
sterile technique when setting up, 3
thermocycler programming, 95
touchdown PCR, 94–95
using phosphorothioate oligos to prevent PCR amplification of adapter dimers, 321
variables
conditions favoring enhanced specificity, 97t
cycle number, 98
enhancing agents, 97–98
inhibitors, 98
matrix analyses, 98
Mg²⁺ concentration, 98
product smearing, 98–99
reamplification, 99
Polymerase mix (recipe), 166
Polynucleotide kinase, 262, 301
Polynucleotide kinase (PNK) buffer (recipe), 268
Pouring, sterile technique for, 8
[³²P]phosphate, sterile technique when labeling cells with, 4
Prehybridization/hybridization solution for hybridization in aqueous buffer (recipe), 137
Prehybridization/hybridization solution for hybridization in formamide buffers (recipe), 138
Prehybridization/hybridization solution for hybridization in phosphate-SDS buffer (recipe), 138
Prehybridization/hybridization standard stringency mix (recipe), 221
Prehybridization solution (recipe), 166
Primary amines, to quench formaldehyde, 148
Primary cell culture, 1–2
Probe hybridization buffer for HCR (recipe), 232
Probe wash buffer for HCR (recipe), 233
Promoter. *See also specific promoters*
in *Brainbow* constructs, 578
neuron-specific, 555
for shRNA vectors, 336
Proteinase K, 98, 179
Protein-DNA interaction protocols
ChIP, 153–157
ChIP-chip, 160–166
ChIP-quantitative polymerase chain reaction (ChIP-qPCR), 158–159
ChIP-seq, 167–171
electrophoretic mobility-shift assays, 139–142
formaldehyde cross-linking, 147–149
preparation of cross-linked chromatin for ChIP, 150–152
Protein-RNA interactions
CLIP (cross-linking and immunoprecipitation)
identification of RNAs bound by a specific protein (protocol), 254–268
discussion, 267
materials, 254–256
method, 256–267
recipes, 267–268
electrophoretic mobility shift assays for
RNA-protein complexes (protocol), 234–239
discussion, 237–238
materials, 234–235
method, 235–237
large multicomponent RNA-protein complexes, 236–237
longer RNA forming small complex, 236
simple interactions, 235–236
recipes, 238–239
RNase footprinting to map sites of (protocol), 240–245
materials, 240–241
method, 241–243, 242f
establishing RNase digestion conditions, 241–242
footprinting with RNase A, RNase T1, or RNase VI, 243
footprinting with RNase I, 242–243
preparing markers, 241
typical experiment, 242f
recipes, 244
troubleshooting, 243
Proteins, light modulation of
establishing a fiber-optic-based optical neural interface, 534–539
imaging green fluorescent protein-labeled neurons using light and electron microscopy, 492–500
discussion, 499
materials, 492–494
method, 494–498, 496f–499f
recipes, 500
troubleshooting, 498–499
imaging synaptic protein dynamics using photoactivatable green fluorescent protein, 501–507
discussion, 505–506
imaging setup, 502, 502f
materials, 501–502
method, 503–505
recipes, 506
Protocols
gene transfer, 355–445
nonviral methods, 355–380
DNA transfection by electroporation, 364–366
DNA transfection mediated by lipofection, 355–357, 356t
piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation, 367–375, 369f, 370t, 374f
single cell/cellular subregion-targeted phototransfection, 376–380, 378f–379f
transfection of mammalian cells with fluorescent protein fusions, 358–363
viral methods, 381–445
concentration and purification of rabies viral and lentiviral vectors, 421–426, 424f
construction and packaging of herpes simplex virus/adeno-associated virus (HSV/AAV) hybrid amplicon vectors, 441–445, 442t, 443f
generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct, 381–402, 382f, 384f–385f, 387f, 394f–397f
generation of replication-competent and -defective HSV vectors, 432–440, 437f–438f
lentiviral vectors for retrograde delivery of recombinases and transactivators, 403–409, 407f
rabies viral vectors for monosynaptic tracing and targeted transgene expression in neurons, 410–420, 413f
stable producer cell lines for adeno-associated virus (AAV) assembly, 427–431, 429f–430f
imaging
light modulation of proteins
establishing a fiber-optic-based optical neural interface, 534–539
imaging green fluorescent protein-labeled neurons using light and electron microscopy, 492–500

Index

Protocols (Continued)

imaging synaptic protein dynamics
 using photoactivatable green fluorescent protein, 501–507

microscopy basics
 using the light microscope, 453–462

in vivo imaging
 bulk electroporation of retinal ganglion cells in live *Xenopus* tadpoles, 558–562

 4D fluorescent imaging of embryonic quail development, 563–566

 mapping anatomy to behavior in Thy1:18 Chr2-YFP transgenic mice using optogenetics, 594–605

 preparation and 4D fluorescent imaging of quail embryos, 567–574

 single-cell electroporation of *Xenopus* tadpole tectal neurons, 546–551

 two-photon imaging of microglia in mouse cortex in vivo, 583–593

working with cells
 aspirating fluids with sterile technique, 11–12

 filter sterilization techniques, 9–10

 purification and culture of retinal ganglion cells from rodents, 25–34, 28f

 purification of rat and mouse astrocytes by immunopanning, 38–49, 41f

 sterile pipetting and pouring techniques, 6–8

 working sterilely in a biosafety cabinet, 13–15, 15t

working with DNA
 agarose gel electrophoresis, 124–127

 basic polymerase chain reaction, 91–93

 ChIP, 153–157

 ChIP-chip, 160–166

 ChIP-quantitative polymerase chain reaction (ChIP-qPCR), 158–159

 ChIP-seq, 167–171

 electrophoretic mobility-shift assays, 139–142

 formaldehyde cross-linking, 147–149

 freezing bacteria for long-term storage, 78–80

 homologous recombination using bacterial artificial chromosomes, 106–116, 109f–110f

 isolation of bacterial artificial chromosome DNA from small-scale cultures, 103–105

 making media for bacterial culture, 66–69

 measurement of bacterial growth by spectrophotometry, 75–77, 76f

 obtaining isolated colonies of bacteria, 70–72, 71f

 preparation and transformation of competent *E. coli* using calcium chloride, 85–88

 preparation of cross-linked chromatin for ChIP, 150–152

 preparation of plasmid DNA by alkaline lysis with sodium dodecyl sulfate: minipreparation, 81–84

 Southern blotting: capillary transfer of DNA to membranes, 128–133

 Southern hybridization of radiolabeled probes to nucleic acids immobilized on membranes, 134–138

 using Petroff-Hausser counting chamber, 73–74, 74f

 working with bacterial artificial chromosomes, 100–102

working with RNA
 antisense RNA amplification for target assessment of total mRNA from a single cell, 200–211

 CLIP (cross-linking and immunoprecipitation) identification of RNAs bound by a specific protein, 254–268

combinatorial analysis of RNA expression patterns in mouse embryos using hybridization chain reaction, 224–233

creating an miR30-based shRNA vector, 333–337

creating transgenic shRNA mice by recombinase-mediated cassette exchange, 344–351

denaturation and electrophoresis of RNA with formaldehyde, 212–215

electrophoretic mobility shift assays for RNA-protein complexes, 234–239

fragmentation of whole-transcriptome RNA using *E. coli* RNase III, 296–298

high-throughput Illumina strand-specific RNA sequencing library preparation, 313–322

identification of RNA cargoes by antibody-positioned RNA amplification, 246–253

infection of mammalian cells with retroviral shRNAs, 342–343

isolating total RNA from mouse embryos or fetal tissues, 187–190

methods for processing microarray data, 291–295

microarray slide hybridization using fluorescently labeled cDNA, 274–279

northern blots: capillary transfer of RNA from agarose gels and filter hybridization using standard stringency conditions, 216–223

packaging shRNA retroviruses, 338–341

preparation of fluorescent-dye-labeled cDNA from RNA for microarray hybridization, 269–273

preparation of small RNA libraries for high-throughput sequencing, 299–309

purification of RNA from cells and tissues by acid phenol-guanidinium thiocyanate-chloroform extraction, 183–186

RNase footprinting to map sites of RNA-protein interactions, 240–245

scanning microarray slides, 280–284

single-neuron isolation for RNA analysis using pipette capture and laser capture microdissection, 191–199

tips on hybridizing, washing, and scanning Affymetrix microarrays, 285–290

PSF. *See* Point-spread function

PXL buffer (recipe), 268

Pyrosequencing, 323

Q

QIAGEN MinElute gel extraction kit, 312

qPCR. *See* ChIP-quantitative polymerase chain reaction

Quail embryos, preparation and 4D fluorescent imaging of, 567–574

Quantitation of DNA and RNA, 89–90
 by ethidium bromide fluorescence emission, 90
 with spectrophotometer, 89

Quantum efficiency, 467

Qubit dsDNA HS Assay Kit, 318

R

Rabies virus
 concentration and purification of rabies viral and lentiviral vectors (protocol), 421–426, 424f

 discussion, 425

 materials, 421–422

 method, 422–425, 424f

 recipes, 425–426

defective interfering (DI) particles, 418–419

rabies viral vectors for monosynaptic tracing and targeted transgene expression in neurons (protocol), 410–420, 413f

 discussion, 418–419

 materials, 410–412

 method, 412–418, 413f

 amplification from supernatants to titered stocks, 414–415

 amplification from titered stocks to more titered stocks, 415

 flowchart, 413f

 rescue from cDNA, 412–414

 stock production for concentration and use in vivo, 415–418

 recipe, 419

 stock production for concentration and use in vivo
 EnvA-enveloped version, 416–417

 RVG-enveloped version, 415–416

 VSVG-enveloped version, 417–418

 RV glycoprotein in lentiviral vectors, 404, 406–407

 RV glycoprotein in rabies viral vectors, 411, 415–416

Rapid amplification of cDNA ends (RACE), 336

Rats
 anesthesia for, 370

piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation (protocol), 367–375, 369f, 370t, 374f

 purification and culture of retinal ganglion cells from (protocol), 25–34, 28f

 purification of rat and mouse astrocytes by immunopanning (protocol), 38–49, 41f

Rayleigh criterion, 464

Read length, 311, 326

Reads, number of, 327

Reads per kilobase per million (RPKM), 328, 329

ReAsH-EDT2, 362

Receiver operator characteristics (ROC), 294

Recipes
 aCSF for hippocampus, 506

 ACSF for two-photon imaging, 592

 agar or agarose containing media, 87, 102

 albumin-agarose, 573–574

 alkaline lysis solution I, 83, 104

 alkaline lysis solution II, 83, 105

 alkaline lysis solution III, 83, 105

 alkaline transfer buffer, 132

 ampicillin (100 mg/mL stock solution), 68t

 amplification buffer (10 \times), 92–93

 amplification buffer for HCR, 232

 annealing buffer (5 \times), 336

 BDNF stock (50 μ g/mL), 32

 binding buffer (2 \times), 322

 binding buffer for mobility shift assays, 238

 blocking buffer for microarrays, 279

 buffered HBSS for APRA, 252

 buffer L1, 152

 buffer L2, 152

 buffer L3, 152

 cell culture medium for virus production, 408, 419

 ChIP RIPA buffer, 156

 chloramphenicol (20 mg/mL stock solution), 68t

 ciliary Neurotrophic factor (10 μ g/mL), 32

 collection tube buffer, 198

 complete cell growth medium, 401

 cross-linking buffer, 149

 culture medium (AAV), 431

 denaturation solution, 132

 Denhardt's solution for northern (100 \times), 221

 DEPC-H₂O, 211, 380

 diethyl pyrocarbonate (DEPC)-treated H₂O, 189

 DMEM/10% FBS for HSV, 439

- Recipes (*Continued*)
- DMEM-SATO Base Growth Medium with NB, 32
 - dNTP solution, 93
 - elution buffer, 156
 - enzyme stock solution, 47
 - ethanol-washed glass coverslips, 33
 - ethidium bromide, 126, 132
 - formamide, 185
 - formamide gel-loading buffer, 244
 - forskolin stock (4.2 mg/mL), 33
 - gel-loading buffer (6×), 126
 - gel-loading buffer IV (6×), 132
 - Hank's balanced salt solution (10×), 252
 - HEPES-buffered cell medium, 198
 - HEPES-buffered saline (HEBS; 2×), 340
 - herring testis carrier DNA, 221
 - high-ovomucoid stock (6×), 33
 - high-ovomucoid stock (10×), 47
 - hybridization buffer for ChIP-chip, 165
 - inhibitor stock solution, 48
 - insulin stock (0.5 mg/mL), 33
 - IP-astrocyte base medium, 48
 - kanamycin (30 mg/mL stock solution), 68t
 - kanamycin (50 mg/mL stock solution), 68t
 - LB freezing buffer, 101
 - LB (Luria-Bertani) liquid medium, 83, 102, 105, 115, 401
 - LB solid medium with 100 µg/mL ampicillin, 402
 - lead citrate for EM, 500
 - LiCl-urea solution for RNA isolation, 189
 - LM-PCR mix, 166
 - loading buffer for mobility shift assays, 238
 - low-ovomucoid stock (10×), 33, 48
 - methylcellulose overlay, 439
 - MgCl₂-CaCl₂ solution, 87
 - MOPS buffer (10×), 214
 - NAC stock (5 mg/mL), 33, 48
 - nalidixic acid (100 mg/mL stock solution), 68t
 - neutralization buffer I, 132
 - neutralization buffer II, 132
 - paraformaldehyde (PFA) for HCR (4%), 232
 - PBS(P), 500
 - PBS for CLIP, 267
 - PBS (pH 7.5) for HSV, 439
 - PBST for HCR, 232
 - phenol, 185–186
 - phosphate-buffered saline (10×; pH 7.35), 253
 - phosphate-buffered saline (PBS), 149, 156, 363, 366
 - phosphate-buffered saline (PBS) for HCR (10×, pH 7.4), 232
 - phosphate-SDS washing solution 1, 137
 - phosphate-SDS washing solution 2, 137
 - pipette solution, 198
 - PK buffer, 267
 - polyacrylamide gel solution (8% in 1× TBE), 171
 - polymerase mix, 166
 - polynucleotide kinase (PNK) buffer, 268
 - prehybridization/hybridization solution for hybridization in aqueous buffer, 137
 - prehybridization/hybridization solution for hybridization in formamide buffers, 138
 - prehybridization/hybridization solution for hybridization in phosphate-SDS buffer, 138
 - prehybridization/hybridization standard stringency mix, 221
 - prehybridization solution, 166
 - probe hybridization buffer for HCR, 232
 - probe wash buffer for HCR, 233
 - PXL buffer, 268
 - reverse transcriptase mix stock, 309
 - RGC growth medium, 33
 - rifampicin (50 mg/mL stock solution), 68t
 - RNase I dilution buffer, 244
 - RNase III buffer (10×), 298
 - RSB100 cell lysis buffer, 268
 - SATO supplement (100×), 34
 - SATO supplement, NB-based (100×), 48–49
 - scaleA2, 233
 - SDS, 138
 - SDS extraction buffer, 244
 - second-strand buffer (10×), 253
 - SET (20×), 222
 - SOB, 87
 - SOC, 88
 - sodium acetate, 189
 - solution D, 186
 - splicing mix for mobility shift assays (4×), 238
 - SSC, 132, 138, 279
 - SSC for northern (20×), 222
 - SSC (2.2×)/SDS (0.22%), 166
 - SSCT for HCR (5×), 233
 - SSPE, 133, 138
 - STE, 83, 105
 - Steinberg's rearing medium, 562
 - streptomycin (100 mg/mL stock solution), 68t
 - sucrose (20%), 425
 - sucrose (60%), 425
 - SYBR Gold, 126, 133
 - TAE, 116, 127, 171, 402
 - TBE buffer, 127, 171
 - TBE electrophoresis buffer (10×), 238
 - TBS (pH 7.5) for HSV, 439
 - T4 DNA ligase buffer (10×), 336
 - TE buffer (10×), 83, 105, 133, 156, 189
 - TE buffer for RNA isolation, 189
 - terrific broth, 84
 - tetracycline (15 mg/mL stock solution), 68t
 - 3T3 test cells for infection, 401
 - thyroxine (T3) stock (4 µg/mL), 34
 - TPE, 127
 - tracking dye, 215
 - trimethoprim (10 µg/mL stock solution), 68t
 - Tris-glycine buffer (10×), 239
 - T1 sequencing buffer, 244
 - 293FT cells for transfection, 400
 - urea loading dye, 244
 - wash buffer A, 222
 - wash buffer B, 222
 - wash buffer C, 222
 - wash buffer D, 222
 - washing buffer for ssRNA-Seq, 322
 - Xenopus* tadpole rearing solution, 550
 - x-gal staining solution for HSV, 439
 - YT, 84
 - YT medium (2×), 400
- Recombinase-mediated cassette exchange (RMCE), transgenic shRNA mouse creation by, 344–351
- materials, 344–346
 - method, 346–351, 346f
 - creation of transgenic mice, 351
 - culture of ES cells before electroporation, 347–348
 - electroporation of transgenes into KH2 ES cells, 348–349
 - feeder cell preparation, 347
 - plating irradiated feeder cells, 347
 - preparation of gelatin-coated ES culture plates, 346–347
 - selection of ES cell clones containing integrated shRNA cassettes, 349
 - Southern blotting for validation of clones, 350–351
 - testing for shRNA expression, 350
 - testing neomycin sensitivity, 349
- Recombinases, lentiviral vectors for retrograde delivery of (protocol), 403–409, 407f
- Refractive index, 449
- rep gene, AAV, 427–430, 430f, 441, 443f, 444–445
- Replication-defective HSV vectors, 438
- Reporter mouse strains, in GENSAT Project, 117–122
- Resin embedding for electron microscopy, 495–496
- Resolution, 449
- confocal microscopy, factors influencing, 463–466
 - digital scanned laser light sheet fluorescence microscopy (DSLM), 476
 - multiphoton-excitation fluorescence microscopy and confocal microscopy compared, 473
- Restriction digestion
- of cDNA from antibody, 251
 - sterile technique when setting up digests, 3
- Retinal ganglion cells (RGCs), 21–34
- advantages as a model system, 21–22, 22f
 - anatomy, function, and development, 21
 - bulk electroporation in live *Xenopus* tadpoles (protocol), 558–562
 - discussion, 560–561, 561f
 - materials, 558–559
 - method, 559–560, 560f
 - recipe, 562
 - principles of isolation and culture, 22–23
 - purification and culture from rodents (protocol), 25–34
 - materials, 25–27
 - methods, 27–32, 28f
 - dissection, 29–30
 - panning, 30–31
 - plating, 31–32
 - preparation, 27–29
 - trituration, 30
 - trypsinization, 31
 - recipes, 32–34
- Retrograde delivery of recombinases and transactivators, lentiviral vectors for, 403–409, 407f
- Retroviruses, short hairpin RNA (shRNA)
- infection of mammalian cells with (protocol), 342–343
 - materials, 342
 - method, 342–343, 343t
 - packaging (protocol), 338–341
 - materials, 338–339
 - method, 339–340
 - recipe, 340
- Reverse footprinting, 243
- Reverse transcriptase, lithium inhibition of, 315
- Reverse transcriptase mix stock (recipe), 309
- Reverse transcription
- cDNA preparation for microarray hybridization, 270–271
 - in preparation of small RNA libraries for high-throughput sequencing protocol, 306
 - single cell capture for mRNA, 194–195
- RGC growth medium (recipe), 33
- RGCs. *See* Retinal ganglion cells
- Rhodamine, 461
- Ribonucleases (RNases), 177–178. *See also specific enzymes*
- in CLIP protocol, 259–261
 - fragmentation of whole-transcriptome RNA using *E. coli* RNase III (protocol), 296–298
 - materials, 296–297
 - method, 297
 - recipe, 298
 - troubleshooting, 297
 - preventing damage from, 177–178, 182
- Ribonucleoprotein (RNP), 251, 259
- Rifampicin (50 mg/mL stock solution) recipe, 68t
- RNA, 173–351. *See also* mRNA
- chemical structure, 175, 176f
 - degradation causes
 - metal ions, 175–176
 - pH, 175
 - ribonucleases, 177–178, 182
 - extraction from gel slices, 302–303

Index

- RNA (*Continued*)
- fragmentation using *E. coli* RNase III, 296–298
 - gene transfer payloads, 353
 - library preparation, 299–309
 - precipitation
 - carriers, 180
 - ethanol, 180–181, 181f, 188, 205–206
 - guidelines for, 180–181, 181f
 - isopropanol, 185
 - protocols
 - antisense RNA amplification for target assessment of total mRNA from a single cell, 200–211
 - CLIP (cross-linking and immunoprecipitation) identification of RNAs bound by a specific protein, 254–268
 - combinatorial analysis of RNA expression patterns in mouse embryos using hybridization chain reaction, 224–233
 - creating an miR30-based shRNA vector, 333–337
 - creating transgenic shRNA mice by recombinase-mediated cassette exchange, 344–351
 - denaturation and electrophoresis of RNA with formaldehyde, 212–215
 - electrophoretic mobility shift assays for RNA-protein complexes, 234–239
 - fragmentation of whole-transcriptome RNA using *E. coli* RNase III, 296–298
 - high-throughput Illumina strand-specific RNA sequencing library preparation, 313–322
 - identification of RNA cargoes by antibody-positioned RNA amplification, 246–253
 - infection of mammalian cells with retroviral shRNAs, 342–343
 - isolating total RNA from mouse embryos or fetal tissues, 187–190
 - methods for processing microarray data, 291–295
 - microarray slide hybridization using fluorescently labeled cDNA, 274–279
 - northern blots: capillary transfer of RNA from agarose gels and filter hybridization using standard stringency conditions, 216–223
 - packaging shRNA retroviruses, 338–341
 - preparation of fluorescent-dye-labeled cDNA from RNA for microarray hybridization, 269–273
 - preparation of small RNA libraries for high-throughput sequencing, 299–309
 - purification of RNA from cells and tissues by acid phenol-guanidinium thiocyanate-chloroform extraction, 183–186
 - RNase footprinting to map sites of RNA-protein interactions, 240–245
 - scanning microarray slides, 280–284
 - single-neuron isolation for RNA analysis using pipette capture and laser capture microdissection, 191–199
 - tips on hybridizing, washing, and scanning Affymetrix microarrays, 285–290
 - purification
 - from cells and tissues by acid phenol-guanidinium thiocyanate-chloroform extraction (protocol), 183–186
 - for mammalian cells grown in monolayers, 184
 - for mammalian cells grown in suspension, 184
 - materials, 183–184
 - method, 184–185, 184t
 - recipes, 185–186
 - for tissues, 184
 - isolating total RNA from mouse embryos or fetal tissues (protocol), 187–190
 - materials, 187
 - method, 188
 - recipes, 189
 - troubleshooting, 188–189
 - methods, 178–179
 - column chromatography, 179
 - guanidine cesium chloride, 179
 - RNA extraction from tissues, 179
 - SDS/proteinase K/phenol:chloroform, 179
 - TRIzol reagent, 178
 - resuspending pelleted RNA, 181
 - quantitation of, 89–90
 - by ethidium bromide fluorescence emission, 90
 - with spectrophotometer, 89
 - single cell/cellular subregion-targeted phototransfection (protocol), 376–380, 378f–379f
 - spike-in, 311, 328
 - staining, 214
 - RNA-binding proteins (RBPs), 246, 249f, 252, 254.
See also RNA-protein interactions
 - RNA cargoes, identification by antibody-positioned RNA amplification, 246–253
 - RNAClean XP, 316
 - RNA-induced silencing complex (RISC), 353
 - RNA interference (RNAi), 335, 338, 344, 346f
 - RNA*later*, 179
 - RNA ligase, 261–262, 264, 303, 305
 - RNA-protein interactions
 - CLIP (cross-linking and immunoprecipitation) identification of RNAs bound by a specific protein (protocol), 254–268
 - discussion, 267
 - materials, 254–256
 - method, 256–267
 - recipes, 267–268
 - electrophoretic mobility shift assays for RNA-protein complexes (protocol), 234–239
 - discussion, 237–238
 - materials, 234–235
 - method, 235–237
 - large multicomponent RNA-protein complexes, 236–237
 - longer RNA forming small complex, 236
 - simple interactions, 235–236
 - recipes, 238–239
 - RNase footprinting to map sites of (protocol), 240–245
 - materials, 240–241
 - method, 241–243, 242f
 - establishing RNase digestion conditions, 241–242
 - footprinting with RNase A, RNaseT1, or RNase VI, 243
 - footprinting with RNase I, 242–243
 - preparing markers, 241
 - typical experiment, 242f
 - recipes, 244
 - troubleshooting, 243
 - RNase A, 177, 259–260
 - RNase assays, 178
 - RNase footprinting to map sites of RNA-protein interactions (protocol), 240–245
 - materials, 240–241
 - method, 241–243, 242f
 - establishing RNase digestion conditions, 241–242
 - footprinting with RNase A, RNaseT1, or RNase VI, 243
 - footprinting with RNase I, 242–243
 - preparing markers, 241
 - typical experiment, 242f
 - recipes, 244
 - troubleshooting, 243
 - RNase H, 203, 310, 316
 - RNase I, footprinting with, 242–243
 - RNase I dilution buffer (recipe), 244
 - RNase III, fragmentation of whole-transcriptome RNA using *E. coli* RNase III (protocol), 296–298
 - RNase III buffer (10 \times) (recipe), 298
 - RNase inhibitors, 177
 - RNaseOUT, 259, 270
 - RNA-Seq
 - data processing, 323–331
 - classification of changes and associated biology, 330
 - experimental design, 324–328
 - mapping, 329
 - measuring expression levels and changes in expression, 329
 - presentation of data, 330–331
 - raw reads, 328–329
 - transcript (isoform) assembly, 329
 - validation, 329–330
 - experimental design, 324–328
 - library construction, 325–327
 - quantity of starting material, 324–325
 - high-throughput Illumina strand-specific RNA sequencing library preparation (protocol), 313–322
 - discussion, 319
 - different multiplex sequencing strategies for GAI and HiSeq2000 instruments, 321
 - false antisense read derived from uracil-minus RNA, 320
 - purification and size selection on magnetic beads, 320–321
 - recipes, 322
 - strand-specific RNA-Seq vs. conventional RNA-Seq, 319–320, 320f
 - using phosphorothioate oligos to prevent PCR amplification of adapter dimers, 321
 - materials, 313–314
 - method, 314–319, 315f
 - dA-tailing, 316
 - end-repair, 316
 - first-strand cDNA synthesis, 315–316
 - general procedure for using AMPure beads, 318–319
 - mix barcoded libraries for multiplex sequencing, 318
 - PCR enrichment, 317–318, 318f
 - polyA RNA isolation and fragmentation, 314–315
 - second-strand synthesis with dUTP, 316
 - triple-SPRI purification and size selection, 317, 317f
 - Y-shape adapter ligation, 316–317
 - library construction basics, 325–327
 - amplification, 325–326
 - bar coding, 326–327
 - directionality (strand selection), 326
 - normalization, 328
 - number of reads, 327
 - paired ends, 326
 - priming *versus* fragmentation, 325
 - read length, 326
 - replicates, 327–328
 - mRNA-Seq libraries from poly(A)⁺ mRNA from Illumina transcriptome high-throughput sequencing, 310–312
 - notes and considerations before beginning, 311
 - overview, 310
 - tips and troubleshooting, 311–312
 - adapter ligation, 311
 - first gel purification, 311–312
 - optimization, 311
 - PCR enrichment, 312
 - second gel purification, 311–312

- RNA-Seq (*Continued*)
 strand-specific RNA-Seq (ssRNA-Seq) *versus*
 conventional RNA-Seq, 319–320,
 320f
 systems for, 323
RNase VI, 243
RNaseZap, 192, 225, 229
RNA shatter method, 310
RNasin, 177, 203, 206, 207, 249, 259, 265
RNasin Plus, 315
ROC (receiver operator characteristics), 294
Rodents. *See* Mouse; Rats
RPKM (reads per kilobase per million), 328, 329
RQ1 DNase, 259, 265
RSB100 cell lysis buffer (recipe), 268
RT-PCR, 210, 256, 265–267, 330
- S**
- SAM (significance analysis of microarrays), 294
SATO supplement (100×) (recipe), 34
SATO supplement, NB-based (100×) (recipe), 48–49
ScaleA2 (recipe), 233
Scanning electron microscopy (SEM), 452
Scanning microarrays, 165, 280–284
 discussion, 283–284, 283f
 materials, 280–281
 method, 281–283
 results file creation, 282–283
 scanning slide, 281
 spotfinding, 281–282
 template for spotfinding, 281
Schiff base, 148
SDS
 preparation of plasmid DNA by alkaline lysis
 with sodium dodecyl sulfate:
 minipreparation (protocol), 81–84
 in RNA purification, 179
SDS (recipe), 138
SDS extraction buffer (recipe), 244
Second-strand buffer (10×) (recipe), 253
Sequencing. *See* High-throughput sequencing (HTS)
Serum, in cell culture media, 19
SET (20×) (recipe), 222
SEVAG, in RNA purification, 179
SFOs (step-function opsins), 527t, 530
Short hairpin RNA (shRNA)
 creating miR30-based shRNA vector
 (protocol), 333–337
 discussion, 335–336
 examples of mammalian shRNA vectors, 334f
 materials, 333
 method, 334–335
 recipes, 336
 delivery by single-cell electroporation, 545
 retroviruses
 infection of mammalian cells with (proto-
 col), 342–343
 materials, 342
 method, 342–343, 343t
 packaging (protocol), 338–341
 materials, 338–339
 method, 339–340
 recipe, 340
 transgenic shRNA mouse creation by
 recombinase-mediated cassette
 exchange (protocol), 344–351
 materials, 344–346
 method, 346–351, 346f
 creation of transgenic mice, 351
 culture of ES cells before electropora-
 tion, 347–348
 electroporation of transgenes into KH2
 ES cells, 348–349
 feeder cell preparation, 347
 plating irradiated feeder cells, 347
 preparation of gelatin-coated ES culture
 plates, 346–347
 selection of ES cell clones containing
 integrated shRNA cassettes, 349
 Southern blotting for validation of
 clones, 350–351
 testing for shRNA expression, 350
 testing neomycin sensitivity, 349
 Short interfering RNAs (siRNAs), 145
 shRNA vectors
 miRNA-based design, 335–336
 promoters, 336
 Signal optimization, for confocal microscopy, 466–
 467
 Signal-to-noise ratio (SNR), 55
 confocal microscopy and, 465, 466
 genetically encoded calcium indicators (GECIs),
 510–513, 516
 Significance analysis of microarrays (SAM), 294
 Simian virus-40 (SV40) promoters, 489
 Single-cell electroporation of *Xenopus* tadpoles,
 540–551, 554f
 efficiency, factors influencing, 542–543
 electrical stimulus parameters, 542
 glass micropipettes, 542
 microscopy, 542–543
 implementation, 543–545, 544f
 coelectroporation of fluorescent dye space
 fillers with morpholinos or pep-
 tides, 544f, 545
 SCE of DNA for neuronal transfection,
 543–544, 544f
 SCE of fluorescent dyes, 544, 544f
 SCE within *Xenopus* tadpole optic tectum,
 543
 microscopy of, 542–543
 principles of, 540–541
 protocol, 546–551
 discussion, 550
 expected results, 550
 limitations and special considerations, 550
 materials, 546–547, 547f
 method, 548–549
 setup, 547f, 548
 troubleshooting, 549–550
 setup, 541–542, 541f, 547f, 548
 Single-neuron isolation for RNA analysis using
 pipette capture and laser capture
 microdissection (protocol), 191–
 199
 discussion, 197–198
 materials, 191–192
 method, 192–196
 laser capture microdissection, 195–196
 pipette capture, 192–195, 193f–195f
 recipes, 198
 troubleshooting, 196–197
 Single plane illumination microscopy (SPIM), 478f,
 481, 482–486
 dynamic range, 486
 illumination efficiency, 482–483
 illumination pattern quality and flexibility,
 484–485
 photobleaching, 483
 point-spread function (PSF), 484
 Slides, calibrated, 459
 Small RNA library, preparing for high-throughput
 sequencing, 299–309
 Smear, making cell, 455
 Snapback DNA, 251
 SNR. *See* Signal-to-noise ratio
 SOB (recipe), 87
 SOC (recipe), 88
 Society for In Vitro Biology, 18
 Sodium acetate (recipe), 189
 Sodium bicarbonate, in cell culture media, 19
 Sodium dodecyl sulfate. *See* SDS
 Sodium phosphate dibasic, 18
 Solid phase reversible immobilization (SPRI) beads,
 316–318, 321
 SOLiD sequencing, 323
 Solution D (recipe), 186
 Sonifier, 150–151
 Southern blotting, 350–351
 capillary transfer of DNA to membranes
 (protocol), 128–133
 materials, 128–129
 method, 129–132, 131t
 DNA denaturation, 130
 DNA digestion and fractionation by gel
 electrophoresis, 129
 DNA transfer, 130–131
 fixing DNA to membrane, 131–132, 131t
 recipes, 132–133
 hybridization of radiolabeled probes to nucleic
 acids immobilized on membranes
 (protocol), 134–138
 materials, 134–135
 method, 135–137
 hybridization, 136
 prehybridization, 135
 washing the membrane, 136–137
 recipes, 137–138
 SPARC, 519t, 521–522
 Specimen chamber, for mounting live specimens
 for microscopic observation, 51–54
 maintenance of gas and pH conditions after
 mounting, 53–54, 53f–54f
 perfusion system, simple gravity-fed, 53, 53f
 simple integrated setup, 54f
 technique for chamber construction and sample
 mounting, 51–53, 52f
 Spectrophotometry
 measurement of bacterial growth by (protocol),
 75–77, 76f
 calculation of generation time, 77
 discussion, 76–77
 materials, 75
 method, 75–76
 normal growth in liquid culture, 76f
 quantitation of DNA and RNA, 89
 SpeedVac, 180
 SPIM. *See* Single plane illumination microscopy
 Spinning-disk confocal fluorescence microscopy, 486
 Splicing mix for mobility shift assays (4×)
 (recipe), 238
 Spotfinding, 281–282
 SPRI (solid phase reversible immobilization) beads,
 316–318, 321
 SSC (recipe), 132, 138, 279
 SSC for northerns (20×) (recipe), 222
 SSC (2.2×)/SDS (0.22%) (recipe), 166
 SSCT for HCR (5×) (recipe), 233
 SSPE (recipe), 133, 138
 ssRNA-Seq. *See* Strand-specific RNA-Seq
 Staining, for light microscopy, 460
 Stationary phase, growth curve, 76, 76f
 STE (recipe), 83, 105
 Steinberg's rearing medium (recipe), 562
 Step-function opsins (SFOs), 527t, 530
 Sterile technique, 2, 3–15
 aspirating fluids with (protocol), 11–12
 materials, 11
 method, 11–12
 common mistakes that break sterility, 4–5
 filter sterilization techniques (protocol), 9–10
 materials, 9
 method, 10
 in mammalian cell culture, 16–18
 sterile pipetting and pouring techniques
 (protocol), 6–8
 discussion, 8
 materials, 6
 method, 6–8
 opening and pipetting with individually
 wrapped disposable pipettes, 7–8
 pipetting with packaged disposable
 pipettes, 7

Index

- Sterile technique (*Continued*)
 pipetting with reusable glass pipettes, 6–7
 pouring, 8
 technique tips, 4
 when to use, 3–4
 working sterily in a biosafety cabinet (protocol), 13–15, 15t
 discussion, 14–15
 materials, 13
 method, 13–14
- Stevens, Beth, 2
- Stir bars, 177
- Stoke's Law, 460
- Storage of bacteria, 65
 freezing bacteria for long-term storage (protocol), 78–80
 reviving a frozen culture, 79
- Strand-specific RNA-Seq (ssRNA-Seq)
 conventional RNA-Seq *versus*, 319–320, 320f
 library preparation (protocol), 313–322
 discussion, 319
 different multiplex sequencing strategies for GAI1 and HiSeq2000 instruments, 321
 false antisense read derived from uracil-minus RNA, 320
 purification and size selection on magnetic beads, 320–321
 recipes, 322
 strand-specific RNA-Seq *vs.* conventional RNA-Seq, 319–320, 320f
 using phosphorothioate oligos to prevent PCR amplification of adapter dimers, 321
 materials, 313–314
 method, 314–319, 315f
 dA-tailing, 316
 end-repair, 316
 first-strand cDNA synthesis, 315–316
 general procedure for using AMPure beads, 318–319
 mix barcoded libraries for multiplex sequencing, 318
 PCR enrichment, 317–318, 318f
 polyA RNA isolation and fragmentation, 314–315
 second-strand synthesis with dUTP, 316
 triple-SPRI purification and size selection, 317, 317f
 Y-shape adapter ligation, 316–317
- Streaking and stabbing slants using isolated colonies, 72
- Streaking bacteria over agar plates, 71–72, 71f
- Streptomycin, 20
- Streptomycin (100 mg/mL stock solution) recipe, 68t
- Stripping RNA from membrane, 220
- Subculturing bacteria, 65
- Sucrose (20%) (recipe), 425
- Sucrose (60%) (recipe), 425
- Sucrose gradients, for concentration and purification of rabies viral and lentiviral vectors (protocol), 421–426, 424f
- Supplements, in cell culture media, 20
- Surgery
 establishing fiber-optic-based optical neural interface (ONI) protocol, 534–539
 laser capture microdissection (LCM), 191, 195–196
- SV2 promoter, 489
- SYBR Gold (recipe), 126, 133
- Synaptic protein dynamics, imaging using photoactivatable green fluorescent protein (protocol), 501–507
 discussion, 505–506
 uses of method, 506
 in vitro and in vivo preparation, choosing, 505–506
 imaging setup, 502, 502f
 materials, 501–502
 method, 503–505
 analysis, 505
 choosing photoactivation and imaging wavelengths, 503
 neuronal transfection for in vitro and in vivo imaging preparations, 503
 photoactivation, 503–504, 504f
 preparation of PA-GFP-tagged synaptic proteins, 503
 time-lapse imaging of fluorescence decay, 504–505
 recipes, 506
- T**
- TAE (recipe), 116, 127, 171, 402
- Taguchi method, 98
- Taq polymerase
 antibody to, 96
 forms inactive at lower temperature, 96
 wax-encapsulated, 96
- T7 bacteriophage polymerase promoter, 246
- TBE buffer (recipe), 127, 171
- TBE electrophoresis buffer (10×) (recipe), 238
- TBS (pH 7.5) for HSV (recipe), 439
- T11D7 hybridoma line, 22
- T4 DNA ligase, 317, 335
- T4 DNA ligase buffer (10×) (recipe), 336
- T4 DNA polymerase, for blunting of DNA ends, 162
- TE buffer (10×) (recipe), 83, 105, 133, 156, 189
- TE buffer for RNA isolation (recipe), 189
- Telomerase, abnormal expression in HeLa cells, 2
- Telomeres, shortening of, 2
- Terrific broth (recipe), 84
- Tet-CMV promoter, 336, 350
- Tetracycline-inducible systems, 490
- Tetracycline (15 mg/mL stock solution) recipe, 68t
- Tetracysteine tag, 362
- Tg, 224, 226t
- Thermal cycler, programming, 95
- 3T3 test cells for infection (recipe), 401
- Thy-1, on retinal ganglion cells, 22, 28f
- Thyl1:18 Chr2-YFP transgenic mice, mapping anatomy to behavior in, 594–605
- Thyl1.2* promoter, in *Brainbow* constructs, 580
- Thyroxine (T3), 23
- Thyroxine (T3) stock (4 µg/mL) (recipe), 34
- Time-lapse imaging of fluorescence decay, 504–505
- Tissue culture, 1, 354
- TM-TPS, 356t
- TNA (trinitroacetate), 176
- TN-XXL, 510, 512–513, 512t
- Toluidine blue, RNA staining with, 214
- Tophat, 329
- Touchdown PCR, 94–95
- TOZ.1 vector, 432, 437f, 438
- TPE (recipe), 127
- T4 polynucleotide kinase, 262, 301
- Tracking dye (recipe), 215
- Transactivators, lentiviral vectors for retrograde delivery of (protocol), 403–409, 407f
- Transcript assembly, 329
- Transcriptome analysis protocols
 creating an miR30-based shRNA vector, 333–337
 creating transgenic shRNA mice by recombinase-mediated cassette exchange, 344–351
 fragmentation of whole-transcriptome RNA using *E. coli* RNase III, 296–298
 high-throughput Illumina strand-specific sequencing library preparation, 313–322
 infection of mammalian cells with retroviral shRNAs, 342–343
 methods for processing microarray data, 291–295
 microarray slide hybridization using fluorescently labeled cDNA, 274–279
 packaging shRNA retroviruses, 338–341
 preparation of fluorescent-dye-labeled cDNA from RNA for microarray hybridization, 269–273
 preparation of small RNA libraries for high-throughput sequencing, 299–309
 scanning microarray slides, 280–284
 tips on hybridizing, washing, and scanning Affymetrix microarrays, 285–290
- Transfectam (DOGS), 355–356, 356t
- Transfection
 calcium phosphate, 407, 428
 cotransfection of packaging-defective HSV-1 helper DNA and vector DNA, 442–443, 442t, 443f
 DNA transfection by electroporation (protocol), 364–366
 materials, 364
 method, 364–365
 recipes, 366
 DNA transfection mediated by lipofection (protocol), 355–357, 356t
 materials, 355–356, 356t
 method, 356–357
 in imaging synaptic protein dynamics using photoactivatable green fluorescent protein (protocol), 503
 lentiviral vectors for retrograde delivery of recombinases and transactivators (protocol), 403–409, 407f
 neuronal transfection for in vitro and in vivo imaging preparations using photoactivatable green fluorescent protein, 503
 overview, 353–354
 piggyBac transposon-mediated cellular transgenesis in mammalian forebrain by in utero electroporation (protocol), 367–375, 369f, 370t, 374f
 discussion, 374–375
 background, 374–375
 expected results, 374f, 375
 materials, 367–368
 method, 368–372
 animal preparation, 370–371
 helper and donor plasmid combinations, 370, 370t
 surgical station preparation, 368
 suturing and animal recovery, 372
 in utero electroporation, 371–372
 in utero electroporation setup, 368–370, 369f
 troubleshooting, 372–373
 single cell/cellular subregion-targeted phototransfection (protocol), 376–380, 378f–379f
 discussion, 379–380
 materials, 376–377
 method, 377–379, 378f
 aligning multiphoton beam in the microscope, 377
 configuring parameters for maximal cell viability, 378, 378f
 phototransfection of neurons with mRNA, 378–379, 379f
 recipes, 380
 single-cell electroporation of DNA for *Xenopus* neurons, 543–544
 transfection of mammalian cells with fluorescent protein fusions (protocol), 358–363
 discussion, 361–362
 functionality of fusion protein, 361–362
 location of fusion protein, 361
 materials, 358–359

- Transfection (*Continued*)
method, 359–361
antibiotic selection (day 4), 360
colony transfer and selection (days 14–21), 360
DNA preparation and transfection (day 1), 359
expanding stable clones, 361
screening (days 21–28), 360
trypsinization and splitting the cells (day 3), 360
washing transfected cells (day 2), 359
recipes, 363
of 293FT packaging cells for virus production, 394–396, 395f–396f
- Transformation
of competent *E. coli* using calcium chloride (protocol), 85–88
materials, 85
method, 86
recipes, 887–88
in generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct (protocol), 391–393
low efficiency of, 115
- Transgenic shRNA mouse creation by recombinase-mediated cassette exchange (protocol), 344–351
materials, 344–346
method, 346–351, 346f
creation of transgenic mice, 351
culture of ES cells before electroporation, 347–348
electroporation of transgenes into KH2 ES cells, 348–349
feeder cell preparation, 347
plating irradiated feeder cells, 347
preparation of gelatin-coated ES culture plates, 346–347
selection of ES cell clones containing integrated shRNA cassettes, 349
Southern blotting for validation of clones, 350–351
testing for shRNA expression, 350
testing neomycin sensitivity, 349
- Transmission electron microscopy (TEM), 452
- Transposon, *piggyBac*, 367–375, 369f, 370t, 374f
- T1 ribonuclease, 259–260
- Tricaine methanesulfonate, 548
- Trimethoprim (10 µg/mL stock solution) recipe, 68t
- Trinitroacetate (TNA), 176
- Tris-glycine buffer (10×) (recipe), 239
- TRIzol reagent, 178
- TrkB, 23
- T4 RNA ligase, 261–262, 264
- Trolox, 466
- Trypsinization
in purification and culture of retinal ganglion cells from rodents (protocol), 31
in purification of rat and mouse astrocytes by immunopanning (protocol), 45
in retinal ganglion cell purification and culture from rodents (protocol), 31
in transfection of mammalian cells with fluorescent protein fusions (protocol), 359, 360
- T1 sequencing buffer (recipe), 244
- Tungsten light, 459
- Twist1*, 224, 226t
- 2A peptide-linked sequences, generation and analysis of lentivirus expressing (protocol), 381–402, 382f, 384f–385f, 387f, 394f, 396f–397f
- 293FT cells for transfection (recipe), 400
- Two-photon fluorescence microscopy
digital scanned laser light sheet fluorescence microscopy (DSLM) compared, 482–486
dynamic range, 486
genetically encoded calcium indicators (GECIs) and, 515
illumination efficiency, 483
imaging speed, 485–486
imaging synaptic protein dynamics using photoactivatable green fluorescent protein (protocol), 501–507
photobleaching, 483–484
point-spread function (PSF), 484
single-cell electroporation of *Xenopus* tadpole neurons, 540, 547, 549
two-photon imaging of microglia in mouse cortex in vivo (protocol), 583–593
discussion, 590–591
applications, 591
choosing an optical window type, 590–591
data analysis, 591
potential effect of imaging, 591
materials, 583–585, 584f
method, 585–588, 586f, 588f
recipe, 592
setup, 584, 584f
troubleshooting, 588–589
- U**
- UCSC Genome Browser, 294, 330–331
- Uranyl acetate, 497
- Urea loading dye (recipe), 244
- U.S. Department of Health and Human Services hazard classification system, 61
- UV light
cross-linking DNA to membrane, 132
cross-linking in ChIP experiments, 143
cross-linking of cells, 258–259
cross-linking of tissues, 257–258
cross-linking RNA to membrane, 218
quantitation of DNA and RNA by ethidium fluorescence emission, 90
- V**
- Vacuum oven, fixing DNA to membrane, 131
- VChR1, 528t, 529
- Vesicular acetylcholine transporter (vAChT), 110f
- Vesicular stomatitis virus glycoprotein (VSV-G), 338–340, 342–343
in lentiviral vectors, 404, 406–407
in rabies viral vectors, 411, 417–418
- VGAT promoter, 555
- Viral gene transfer protocols, 381–445
concentration and purification of rabies viral and lentiviral vectors, 421–426, 424f
discussion, 425
materials, 421–422
method, 422–425, 424f
recipes, 425–426
construction and packaging of herpes simplex virus/adeno-associated virus (HSV/AAV) hybrid amplicon vectors, 441–445, 442t, 443f
discussion, 444–445
materials, 441–442
method, 442–444, 442t, 443f
cotransfection of packaging-defective HSV-1 helper DNA and vector DNA, 442–443, 442t, 443f
harvesting packaged vectors, 443–444
titration of amplicon stocks, 444
generation and analysis of lentivirus expressing a 2A peptide-linked bicistronic fluorescent construct, 381–402, 382f, 384f–385f, 387f, 394f–397f, 396f–397f
materials, 381–384, 382f
method, 384–399, 384f–385f, 387f, 394f–397f
bicistronic insert and lentiviral vector backbone preparation, 384–391, 384f–385f, 387f
bicistronic lentiviral vector production, 391–394, 394f
concentrating lentiviral supernatants, 398
harvesting bicistronic viral supernatants and infecting cells, 396–398, 397f
plasmid maps, 385f
transfecting 293FT packaging cells for virus production, 394–396, 395f–396f
viral titer determination using fluorescent microscope, 398–399
- generation of replication-competent and -defective HSV vectors, 432–440, 437f–438f
discussion, 437–438
materials, 432–433
method, 434–437, 437f
construction of recombination virus, 434–436
isolation of viral DNA for transfection, 434
viral stock preparation and purification, 436, 437f
recipes, 439
troubleshooting, 437
- lentiviral vectors for retrograde delivery of recombinases and transactivators, 403–409, 407f
discussion, 406–408, 407f
materials, 403–404
method, 405–406
recipe, 408
- rabies viral vectors for monosynaptic tracing and targeted transgene expression in neurons, 410–420, 413f
discussion, 418–419
materials, 410–412
method, 412–418, 413f
amplification from supernatants to titered stocks, 414–415
amplification from titered stocks to more titered stocks, 415
flowchart, 413f
rescue from cDNA, 412–414
stock production for concentration and use in vivo, 415–418
recipe, 419
stock production for concentration and use in vivo
EnvA-enveloped version, 416–417
RVG-enveloped version, 415–416
VSVG-enveloped version, 417–418
- stable producer cell lines for adeno-associated virus (AAV) assembly, 427–431, 429f–430f
materials, 427–428
method, 428–430, 429f–430f
generation of stable packaging lines, 428–429
generation of stable producer clones, 430
screening clones for *rep* and/or *cap* DNA, 429–430, 430f
selection procedure, 429f
recipes, 431
troubleshooting, 430
- Virus. *See also* Viral gene transfer protocols; *specific viruses*
infusion into a fiber-optic-based optical neural interface (protocol), 534–539, 537f
titer determination using fluorescent microscope, 398–399

Index

Voltage. *See* Membrane voltage
Voltage-sensing fluorescent proteins (VSFP), 519t, 520–522, 520f
Voltage-sensors, 518–525, 519t, 520f
Volvox carteri, opsins from, 528t, 529
VSFP1, 519t, 520–521
VSFP2, 519t, 522
VSFP3.1, 519t, 522
VSV-G. *See* Vesicular stomatitis virus glycoprotein

W

Wash buffer A (recipe), 222
Wash buffer B (recipe), 222
Wash buffer C (recipe), 222
Wash buffer D (recipe), 222
Washing buffer for ssRNA-Seq (recipe), 322
Water, RNase-free, 177
Wescodyne, 17
Wright's stain, 460

X

Xenopus
 bulk electroporation of retinal ganglion cells
 in live *Xenopus* tadpoles (protocol), 558–562
 discussion, 560–561, 561f

materials, 558–559
 method, 559–560, 560f
 recipe, 562
single-cell electroporation, 540–551, 554f
 efficiency, factors influencing, 542–543
 electrical stimulus parameters, 542
 glass micropipettes, 542
 microscopy, 542–543
implementation, 543–545, 544f
 coelectroporation of fluorescent dye
 space fillers with morpholinos or peptides, 544f, 545
 SCE of DNA for neuronal transfection, 543–544, 544f
 SCE of fluorescent dyes, 544, 544f
 SCE within *Xenopus* tadpole optic tectum, 543
microscopy of, 542–543
principles of, 540–541
protocol, 546–551
 discussion, 550
 expected results, 550
 limitations and special considerations, 550
 materials, 546–547, 547f
 method, 548–549
 setup, 547f, 548
 troubleshooting, 549–550
 setup, 541–542, 541f, 547f, 548

in vivo time-lapse imaging of neuronal development in *Xenopus*, 552–555, 552–557, 554f
 fluorescent dextrans, 552–553
 genetic labeling by fluorescent protein expression, 553–555, 554f
 image analysis and morphometry, 555–557, 556f
 imaging labeled neurons, 555
 labeling neurons, 552–555
 lipophilic vital dyes, 553
Xenopus tadpole rearing solution (recipe), 550
XFPs, in *Brainbow* mouse strategies, 575–580, 576f
X-gal staining solution for HSV (recipe), 439
Xylazine, 370, 535
Xylene, for cleaning objective lens, 453, 458

Y

YC3.60, 510, 512
Yeast, 63
YT (recipe), 84
YT medium (2×) (recipe), 400

Z

Zn²⁺, chelation of, 176