Index

Note: Page numbers followed by f, t, or b denote a figure, table, or box, respectively, on the corresponding page.

Α

AaAOP1, 645f Acoustic fitness, 459 Acoustic physiology, 459-479, 460f Acoustic-related mating behavior in tethered and free-flying mosquitoes, 259-272 overview, 259-263 recording and analysis (protocol), 265-272, 266f Acoustic traps and lures, 477 Acridine orange staining, 286, 294-295 Aedes courtship flight, 260 egg collection, 502-503, 502f harmonic convergence, 260 hatching embryos, 506 insemination in, 273-281, 279f mating, 259-260, 262 promoters, 578-579 puromycin resistance, 580 Aedes adult saline (recipe), 650 Aedes aegypti assessing blood and nectar meals, 117-128 attraction quantification, 205-209, 207f batch rearing (protocol), 8-17 blood feeding, 14 discussion, 15 egg conditioning and storage, 14-15 equipment, 9-10, 12f hatching eggs, 10 larvae, thinning and rearing, 11-12 materials, 8-9 method, 10-15, 11f-12f ovipositing materials, providing, 14 pupae collection, 12-13 rearing adults, 13-14 recipes, 16 troubleshooting, 15-16 blood feeding, 14, 21, 86, 102 evaluation of Aedes aegypti feeding status (protocol), 149–153, 150f evaluation of Aedes aegypti penetration, probing, and feeding times on mice (protocol), 154-157 calcium imaging, 449 chitinase-chymotrypsin digestion, 677

chromosome-level genome assemblies, 701 CRISPR-Cas9, 561-575 crosses (protocol), 18-25 blood feeding, 21 discussion, 23-24 egg conditioning, storage, and hatching, 22-23 establishing mating crosses, 20-21 materials, 18-19, 21f mating verification, 23 method, 19-23 oviposition, 21-22 pupal sexing, 19-20 recipes, 24-25 virginity maintenance, 20 cross-section immunohistochemistry (protocol), 636-642, 638f cytogenetics, 701 dextran amine-conjugated neural tracing (protocol), 614-617 egg collection, 502-503, 502f egg development and eggshell formation, 283-307, 286f embryo microinjection, 498 evaluation of Aedes aegypti feeding status (protocol), 149-153, 150f evolutionary history, biology, and ecology, 3 feeding time, 147 field-collected mosquitoes, establishing colonies from, 84-86 adults, 85 blood feeding, 86 eggs, 84-85, 95-98 hatching, 85 larvae, 85 overview, 84 oviposition, 86 genetics, 3 genome map, 700f, 701 host preference, 227-228 hybridization chain reaction RNA in situ hybridization, 667f insemination in, 273-281 knock-ins, success rate of, 526 laboratory care and maintenance, 1-25 laboratory strains, 4-5 landing assay, 355-356 life cycle, 1-3, 2f

membrane protein fractionation and analysis through western blot in Malpighian tubules (protocol), 643-652, 645f microinjection, 520t mitotic chromosomes, 707f, 711 NC82 for immunolabeling, 605 odor attraction, 355-357 olfaction, 197 as opportunistic (quick to bite) species, 353 opsins, 386, 388 oviposition preference, 309-328 probing time, 157 protease genes, 110 proteolytic enzymes, 109-110 PUb (polyubiquitin) promoter, 578, 580 quantifying host odor preference using two-point olfactometer, 211-225 RNAi, 545-547 salivary gland morphology, 654-655, 654f sensilla, 411 sex differences, 3-4 spectral sensitivity, 329, 387 sugar-baited delivery of siRNA for gene silencing (protocol), 539-544, 541t, 542f TALENs, 549 taste sensory responses, 411 transcript and protein detection, 619-652 transgenes, 5, 550-551 transposon-mediated transgenesis, 512, 519-523, 520t two-photon calcium imaging in brain of, 440-445, 442f validating single-guide RNA for gene editing, 528-533 vector competence, 4 visual responses, 330-338, 336f vitality, requirements for, 1-3 whole-mount immunofluorescent labeling of CNS (protocol), 607-613 whole-mount immunohistochemistry (protocol), 631-635, 632f wind direction, detection of, 350 Aedes aegypti aegypti, 84 Aedes aegypti formosus, 3, 5, 84, 444

Index

Aedes aegypti saline solution for imaging (recipe), 445 Aedes albopictus, 86 Aedes aegypti compared, 3 CRISPR-Cas9, 549 feeding time, 147 FISH-based cytogenetic mapping, 702 identifying transgenic larvae, 579f insemination in, 273-281 RNAi, 546, 548 taste sensory responses, 420 transgenes, 550 transposon-mediated transgenesis, 522 Wolbachia in, 4 Aedes baisasi, 174 Aedes bromeliae, 86 Aedes fluviatilis transgenes, 550 transposon-mediated transgenesis, 522 Aedes pertinax, 180f Aedes polynesiensis, 86 Aedes triseriatus, 522 Aedes vittatus, 86 AeSCP-2, 546 Affinity, antibody, 689 Air-driven assays, 349-357 Albumin-gelatin embedding medium (recipe), 467 Algae, use in delivery of RNAi, 548 Ammonia, 687-688 Amplification and identification of vertebrate host cytochrome c oxidase subunit I (COI) DNA barcoding templates from mosquito blood meals (protocol), 188-195 Anaconda virtual environment software, 322-323, 325 Anemometer, 214 Anesthetization Anopheles, 51 chemical, 51 CO₂, 51, 151, 345 cold, 51, 344 mouse, 151, 156-157 Annelid hosts, 174, 192-193 Anopheles, 89 autofluorescence, 585 calcium imaging of olfactory neurons, 449 chromatin architecture, 702 courtship flight, 260 cytogenetics, 701 egg collection, 502-503, 502f harmonic convergence, 260 hatching embryos, 506 larvae, 27 life cycle, 27-28 mating, 259-260, 262

oviposition preference, 309 promoters, 578-579 puromycin resistance, 580, 591-593, 592f species hybridization, 86-87 swarming, 259-260, 262 transposon-mediated transgenesis, 512 visualization of polytene chromatin using 3D fluorescence in situ hybridization (protocol), 713-719, 717f Anopheles, laboratory rearing of, 27-57 adult anesthesia, feeding, and sex separation (protocol), 48-53, 50f anesthesia, 51 blood feeding, 52 materials, 48-49 recipe, 53 sugar feeding, 49-50, 50f adults, 31-32 blood sources, 31-32 cage types and sizes, 31, 32f egg collection, disinfection, and hatching (protocol), 36-43 egg collection, 37-39, 38f egg counting, 41-42 egg disinfection, 39-40, 40f egg hatching, 40-41, 40f life-history traits, estimating, 42 materials, 36-37 eggs, 29 general environmental considerations, 28 larvae, 29-31 Anopheles coluzzii, 485 container size, 29 container type, 29 food amounts, 30-31 food types, 30 mounting, 486 larval rearing (protocol), 44-47, 46f pupa collection and sex identification (protocol), 54-57, 56f pupae, 31 water types used for, 28-29 Anopheles albimanus chromosome-level genome assemblies, 701 CRISPR-Cas9, 549 embryo microinjection, 498 polytene chromosomes, 700f, 701, 707f probing time, 157 transgenes, 550 Anopheles arabiensis, 86, 89 chromosome-level genome assemblies, 701 meiotic chromosome, 707f Anopheles atroparvus

polytene chromosomes, 701 visualization of polytene chromosomes using 3D-FISH, 717f Anopheles bwambae, 86 Anopheles coluzzii, 86, 89 calcium imaging of antennae expressing GCaMP6f (protocol), 451-457, 453f chromosome-level genome assemblies, 701 identifying transgenic larvae, 579f larval olfaction, 481-493 cup and pan behavioral assays for assessing Anopheles coluzzii larval volatile responses (protocol), 489-493 larval antennal sensory cone responses to volatile odorants, 484-488 puromycin selection, 591 wind-tunnel experiments, 352 Anopheles darlingi, 89 Anopheles fontenillei, 86 Anopheles funestus, 34 chromosome-level genome assemblies, 701 CRISPR-Cas9, 549 mitotic chromosomes, 711 polytene chromosomes, 701 Anopheles gambiae behavioral assay to quantify odorguided thermotaxis with Anopheles gambiae under semi-field conditions (protocol), 234-240, 235f chitinase-chymotrypsin digestion, 677 circadian entrainment, 475 egg hatching, 29 eggshell proteins, 285, 305 embryo microinjection, 498 field-collected mosquitoes, establishing colonies from, 86-89 adults, 88 blood feeding, 88 eggs, 87 hatching, 87 larvae/pupae, 87-88 mating, 88 overview, 86-87 oviposition, 89 gene drives, 551 host preference, 227-228 intravital video microscopy of feeding, 130 larvae and pupae transport from the field (protocol), 99-100 microinjection, 520t NC82 for immunolabeling, 605 olfactory cues, 350

opsins, 386-387 polytene chromosomes, 701, 710-711 pupae, 31, 33 puromycin selection, 591 quantification of Anopheles gambiae olfactory preferences under semi-field conditions (protocol), 241-249, 245f, 247f rearing, 27-57 RNAi, 546, 548 salivary gland morphology, 654-655 sensilla, 411, 448 spatial organization of chromosomes, 702 sugar-baited delivery of siRNA for gene silencing (protocol), 539-544, 541t TALENs, 549 transgenes, 550-551 transposon-mediated transgenesis, 519-523, 520t trypsin genes, 110 visualization of polytene chromosomes using 3D-FISH, 717f whole-mount immunofluorescent labeling of CNS (protocol), 607-613 X-chromosome physical genome map, 700f, 701 Anopheles melas, 86 Anopheles merus, 86, 89, 701 Anopheles minimus, 34 Anopheles moucheti, 89, 711 Anopheles nili, 89 Anopheles quadriannulatus, 86, 89, 411 Anopheles stephensi, 103 chromosome-level genome assemblies, 701 CRISPR-Cas9, 549 embryo microinjection, 498 microinjection, 520t Plasmodium in salivary glands, 655 polytene chromosomes, 700f, 701 RNAi, 546, 548 salivary gland morphology, 654-655 transgenes, 550-551 transposon-mediated transgenesis, 519-523, 520t UV sensitivity, 387 Antennae calcium imaging of Anopheles coluzzii antennae expressing GCaMP6f (protocol), 451-457, 453f combined gas chromatography and single-sensillum recording, 423, 427, 430-431, 431f, 433-434

electrophysiological measurements of compound action potential responses from antennal nerve in response to stimulation (protocol), 468-471, 471f heatmaps, 456 larval chemosensory system, 484-493, 486f visualizing gene expression in cryosectioning and immunohistochemistry (protocol), 671, 671f immunostaining whole-mount olfactory tissues (protocol), 674-679 RNA in situ hybridization of wholemount olfactory tissues (protocol), 680–686 Antennal lobe, 444 Antennal nerve, electrophysiological measurements of compound action potential responses in response to stimulation (protocol), 468-471, 471f Anthrone tests for sugar detection overview, 160 protocol, 163-167 cold anthrone test, 164-166 hot anthrone test, 165 Antibody buffer (recipe), 612 Antibody dilution buffer (ADB) for crosssection immunohistochemistry (recipe), 641 Antibody dilution buffer for whole-mount immunohistochemistry (recipe), 634 Antibody incubation buffer (recipe), 677 Antidiuretic hormone receptor transcript localization, 626f Anti-Plasmodium peptide, 554-555, 554f Apoptosis, follicle, 286, 289, 294-295, 296f Aquaporins, 688 Arbovirus transmission, 4 Artificial feeders, 101-108 Artificial meal (recipe), 144 Artificial meals, 131, 143 Aspirator, 417 ATP solution for mosquito feeding (recipe), 82 Attraction quantifying attraction behavior using olfactometry, 197-210 quantifying host odor preference using olfactometry, 211-225 Attractive targeted sugar baits (ATSBs), 536-537, 547 Audacity software, 262, 267, 270

Autofluorescence, 582, 584–585, 587, 589f Automated long-term monitoring of heat-seeking behavior (protocol), 254–258, 256f Avidin–biotin binding, 688

В

Bacteria, use in delivery of RNAi, 548 BApNA ($N\alpha$ -benzoyl-DL-arginine 4-nitroanilide hydrochloride) assay, 110, 112–116, 113f, 114t, 115f Barcode of Life Data System (BOLD), 175, 192-194 Batch rearing Aedes aegypti (protocol), 8-17, 11f-12f Beadle-Ephrussi Ringer solution (recipe), 42.0 Behavior biting behavior characterization (protocol), 134-144 chemosensory of Anopheles coluzzii larvae, 481-483 flight, analysis of, 339-347, 349-357 heat-seeking, 251-258, 256f host-seeking, 228-230, 249 introduction, 349-350 olfaction, role of, 397, 447 wind tunnels and airflow-driven assays, 349-357 mating, acoustic-related, 259-272 videography studies of, 361-384, 362f Behavioral assay to quantify odor-guided thermotaxis with Anopheles gambiae under semi-field conditions (protocol), 234-240, 235f β -tubulin, 547 Binary expression systems, 550-551 Bioactive volatile organic compound identification using combined gas chromatography and single-sensillum recording, 423-435 Bioassays. See Insecticide bioassays Biology, of Aedes aegypti, 3 biteOscope, 129-144, 148, 252 bite substrate and cage, 131 biting behavior characterization (protocol), 134-144 camera and lens choice, 131 illumination, 131 imaging considerations, 131 overview, 130-132 recording and detecting behavior, 131-132

Index

Biting behavior biteOscope, 129-144 characterization using biteOscope (protocol), 129-144 behavioral arena fabrication, 136-137 biteOscope construction, 136-138 bite substrate assembly and setup, 138-139 cage assembly, 137f data analysis, 140-143 data processing using conventional image analysis, 142-143 deep learning-based processing, 140 - 142discussion, 143 experimental procedure, 139-140 materials, 134-136 method, 136-143 recipes, 144 setup, 136f temperature controller, creating, 137-138, 138f overview, 129-130 Blocking buffer for immunostaining (recipe), 678 Blocking solution for immunostaining (recipe), 672 Blood as food source, 109 Blood digestion, 109–116 analysis (protocol), 112-116 peptidases, 109-110 Blood feeding Aedes aegypti artificial feeders, 102 evaluation of feeding status (protocol), 149-153, 150f evaluation of penetration, probing, and feeding times on mice (protocol), 154–157 field-collected colonies, 86 laboratory reared, 14, 21 Anopheles field-collected colonies, 88 glass bell blood-feeding system, 52 Hemotek blood-feeding system, 52 laboratory reared, 31-32, 52 live animal, 52 artificial feeders, 101-108, 102 building using a conical centrifuge tube, 102 elements of, 101-102 Glytube, 101-103, 105-108, 106f 3D printing technology, 103 Culex blood source, 63 field-collected colonies, 91-92 laboratory reared, 72, 72f, 79-81 multiple meals, 64

timing of blood meal, 63-64 disease transmission, 119 evaluation of Aedes aegypti feeding status (protocol), 149-153, 150f evaluation of Aedes aegypti penetration, probing, and feeding times on mice (protocol), 154-157 future steps in evaluation, 147-148 high-resolution characterization biting behavior, 129-144 osmoregulation, 688 overview, 118f, 119 phases feeding time, 147 penetration time, 146 probing time, 146-147 refeeding on multiple hosts, 119 Blood meal analysis, 173-195 introduction, 173-174 meal volume quantification, 119-127, 125f methods, 174-176 cytological, 174-175 DNA-based, 175-176 serological, 175 protocols, 178-195 amplification and identification of vertebrate host cvtochrome *c* oxidase subunit I (COI) DNA barcoding templates from mosquito blood meals, 188-195 extracting DNA from mosquito blood meals, 184-187 preservation of field-collected mosquito blood meals, 178-183, 180f-182f storage issues, 183 Blood meals assessing in Aedes aegypti, 117-128 overview, 117-118, 118f Blood meal size, 147 meal volume quantification, 119-127, 125f quantitative assessment, 151-152, 152f Body orientation of moving mosquitoes, 383 Body part tracking, 132, 140-142 BOLD (Barcode of Life Data System), 175, 192-194 Bouin's fixative for in situ hybridization (recipe), 641 Bradford assay for protein quantification, 647 Braid, 362, 364-365, 373-376 Brain anatomy, 604-605, 604f

dextran amine-conjugated neural tracing (protocol), 614–617 dissection, 609 whole-mount immunofluorescent labeling of CNS (protocol), 607–613 Bruchpilot (Brp) promoter, 449 Bruchpilot (Brp) protein, 605 Brugia malayi, 4 B-SOID, 143

С

Calcium imaging of Anopheles coluzzii antennae expressing GCaMP6f (protocol), 451-457, 453f antennal heatmaps, 456 intensity time traces, 457 materials, 451-452 method, 453-457, 453f chemical indicators, 448 genetically encoded calcium indicators (GECIs), 437-438, 447-457 Calcium-sensitive dyes, 437 Camera calibration, 365, 371, 375-376, 379-380 CAPA neuropeptide immunoreactivity, 620 Carbon dioxide (CO₂) anesthetization, 51 as attractant, 198, 206-209, 214, 253 CO2-activated heat-seeking behavior, 254-258, 256f components for delivery system in olfactometry, 223t contamination in wind tunnel assays, 352 detection (see Gustatory receptors (GRs) gating of attraction to dark colors, 385-386 plumes, 354 solenoid valve delivery system in olfactometry, 223 carboxypeptidase promoter, 550 Carnoy's solution (recipe), 711 Cas9 nuclease, 525-526, 549, 552-553, 561-563. See also CRISPR-Cas9 generating mutant strains with transgenic Cas9 (protocol), 568-575 Caspases, 286, 289, 291-292, 294-295, 296f Cell bodies, 604 Cell body rind, 604

Central nervous system overview, 604-605, 604f whole-mount immunofluorescent labeling (protocol), 607-613 Chemosensation, overview of, 423-424 Chemosensory genes, detection of, 666 Chemosensory methods of Anopheles coluzzii larvae, 481-493 Chemosensory organs, 205 Chemotactile cues, for oviposition site selection, 310 Chikungunya virus, Aedes aegypti transmission, 4 Chitinase-chymotrypsin digestion, 666, 675, 677, 681, 684 Chitinase-chymotrypsin dimethyl sulfoxide buffer (recipe), 678, 684 Chitin synthase-1 and -2, 547 Chitosan nanoparticle for RNAi delivery, 547-548 CHOPCHOP, 526, 529, 531, 564, 570 Chromatin, 3D organization of, 702 Chromation, in Golgi method, 596, 598-599 Chromosomal arms, 699, 700f Chromosomes linear organization, 699-702, 700f, 707f obtaining for cytogenetic analysis (protocol), 705-711, 707f, 710f discussion, 710-711, 710t materials, 705-706 method, 706-710, 707f recipes, 711 polytene, 699-702, 700f, 707f mitotic and meiotic compared, 710-711, 710t obtaining for cytogenetic analysis (protocol), 705-711 visualization of chromatin using 3D fluorescence in situ hybridization (protocol), 713-719, 717f sex-determining, 699 spatial organization, 702 visualization, 699-719 visualization of polytene chromatin using 3D fluorescence in situ hybridization (protocol), 713-719, 717f discussion, 716-717, 717f materials, 713, 715 method, 715-716 recipes, 718-719 troubleshooting, 716 Cibarium, 410, 410f Circadian entrainment, 475 Class II transposable elements, 511, 513f

Clustered regularly interspaced short palindromic repeats. See CRISPR-Cas9 CNN (convoluted neural network), 140 CO2. See Carbon dioxide Colors, attraction to dark, 385-386 Combined gas chromatography and singlesensillum recording (protocol), 427-435, 429f, 432f materials, 427-430, 429f method, 430-434 mounting mosquitos, 430-431, 431f odor stimulation, 434 recording, 433 sensillum functional type identification, 433-434 setup, 432f sharpening electrodes, 431 Complex Object Parametric Analyzer and Sorter (COPAS), 577-578, 587-590, 589f Compound eye, 386-387, 386f Contact chemoreception. See Taste system Convoluted neural network (CNN), 140 Coomassie blue/methanol solution (recipe), 650 Copulation, 260, 269-270 insemination, 273-281 mid-flight, 459, 460f multiple pairings, 274-275 process overview, 273-274 Courtship flight, 259-260 CRISPR-associated protein 9. See Cas9 CRISPR-Cas9 in Aedes aegypti, 561-575 basic principles, 561, 565f embryo microinjection, 496 gene drives, 551-554 controlling spread, 553-554 integral, 554-555, 554f overview, 551-552, 552f resistance to, 552-553 genome/gene editing, 438, 440, 549-550 guide RNA design and validation, 525-533 in nontraditional model organisms, 525-526 overview, 561 ReMOT control for component delivery, 526-527, 549-550, 562, 577 CRISPR-Cas9 in Aedes aegypti, 561-575 generating mutant strains with transgenic Cas9 (protocol), 568-575 discussion, 574 materials, 568-569

methods, 570-574, 572f recipe, 574 overview, 563 project planning decisions, 563-565 component forms, 563-564 donor DNA design, 564-565 repair mechanism, 563 sgRNA design, 564 target site selection, 564 CRISPR software, 564, 570 Crosses of Aedes aegypti (protocol), 18 - 25Cryoprotection solution (recipe), 673 Cryosectioning and immunohistochemistry of peripheral sensory tissue (protocol), 669-673, 671f discussion, 672 materials, 669-670 method, 670-672, 671f recipes, 672-673 troubleshooting, 672 Culex blood feeding, 72, 72f, 79-81 blood source, 63 multiple meals, 64 timing of blood meal, 63-64 challenges of working with, 59-60 collecting from the field, 60-61 considerations when establishing rearing protocols, 61-64 caring for adults, 62 larval growth and development, 61-62 pupal development, 62 diapause induction, 81 disease transmission, 59 establishing a colony from fieldcollected eggs (protocol), 67 - 74blood feeding, 72, 72f colony establishment, 72-73 discussion, 73 egg collecting, 68-69, 69f, 72 field collections, 68-69, 69f identifying larvae, 70-71 materials, 67-68 method, 68-73 rearing larvae to adulthood, 71 recipe, 73 harmonic convergence, 260 laboratory rearing, 59-82 blood feeding, 63-64 considerations when establishing protocols, 61-64 current protocols, 60 establishing a colony from fieldcollected eggs (protocol), 67-74

Culex (Continued) rearing and maintaining a colony (protocol), 75-82 larvae appearance, 70f collecting, 69 feeding, 61-62 growth and development, 61-62 identifying, 70-71 morphological identification, 70-71 PCR-based identification, 71 rearing to adulthood, 71, 77-79, 78t pupa appearance, 70f development, 62 rearing and maintaining a colony (protocol), 75-82 ATP solution for mosquito feeding (recipe), 82 blood feeding, 79-81 discussion, 81 feeding schedule, 78t materials, 75-76 methods, 77-81 rearing larvae to adulthood, 77-79, 78t sensilla, 411 species hybridization, 61, 64, 89 taste sensory responses, 411 Culex australicus, 89 Culex erraticus, 60-61 Culex globocoxitus, 89 Culex nigripalpus, 61 Culex pipiens collecting from the field, 60-61 CRISPR-Cas9, 549 dissection of mosquito midgut, 114f field-collected mosquitoes, establishing colonies from, 89-92 adults, 91 blood feeding, 91-92 eggs, 89-90 hatching, 90 overview, 89 oviposition, 92 rearing larvae and pupae, 90-91 life stages, 70f rearing and maintaining a colony (protocol), 75-82 RNAi, 546-547 temperature for rearing, 62 UV sensitivity, 387 Culex pipiens fatigans, 73 Culex pipiens molestus, 60, 64, 89, 91 Culex pipiens pallens, 89 Culex pipiens pipiens, 60, 64, 89 Culex quinquefasciatus, 60-61, 73, 89 blood substitute consumption, 63 CRISPR-Cas9, 549

embryo microinjection, 498 hybridization, 61, 64 microinjection, 520t mitotic chromosomes, 700f, 701 NC82 for immunolabeling, 605 odor attraction, 355 opsins, 386-387 probing and feeding times, 157 transposon-mediated transgenesis, 519-523, 520t UV sensitivity, 387 Culex restuans, 60-61, 73, 90 Culex salinarius, 60-61, 90, 92 Culex tarsalis, 60-61, 90, 92 Culex territans, 60-61 Culicine mosquitoes cytogenetic mapping, 701 mitotic chromosomes, 700f, 711 sex-determining chromosomes, 699 Cup and pan behavioral assays for assessing Anopheles coluzzii larval volatile responses (protocol), 489-493 cup assay, 491-492 discussion, 492-493 materials, 489-490 method, 490-492, 491f pan assay, 492-493 Cuticle, 665-666, 669, 672, 674 Cytochrome b (MT-CYB), 176, 194 Cytochrome c oxidase subunit I (COI), 176 amplification and identification of vertebrate host cytochrome *c* oxidase subunit I (COI) DNA barcoding templates from mosquito blood meals (protocol), 188-195 as DNA barcoding standard, 176 species determination, 183 Cytochrome P450s, 721, 724, 738, 744 Cytogenetics Aedes aegypti, 701 Anopheles, 701 Culicine mosquitoes, 701 visualization of chromosomes, 699-719 Cytological methods, of blood meal analysis, 174-175

D

Dark objects, attracting to, 385–386 Dechorionation of eggs, 521 Decoy traps, 229 DeepLabCut framework, 132, 140–142, 141f Deep learning analysis of biting behavior, 130, 132, 140–143 Deep learning-based processing, 140–142 Deep sequencing, 532 DEET, 199 DEF (S,S,S,-tributyl phosphorotrithioate), 721, 724, 738, 744 Degenerate oligonucleotide-primed PCR (DOP-PCR), 714b, 717f Dehydration ethanol, 693 methanol, 676, 682 DEM (diethyl maleate), 721, 724, 738, 744 Dengue virus, Aedes aegypti transmission, 4 Desistence time, 147 Desiccation, preventing embryo, 497 Detoxification, 721, 724, 738, 744 Development Studies Hybridoma Bank (DSHB), 605 Dextran amine-conjugated neural tracing (protocol), 614-617 materials, 614-615 methods, 615-616 recipe, 616 troubleshooting, 616 Diapause, in Culex, 81, 91 Diethyl maleate (DEM), 721, 724, 738, 744 Digestion of blood meals estimating extent of digestion, 180-181, 182f preservation of field-collected mosquito blood meals (protocol), 178-183, 181f Digital pulse generator, 371 Digoxigenin (DIG) RNA labeling, 626-627 *Dihydroxyphenylacetaldehyde (Dopal)* synthase, 547 Direct Linear Transformation data viewer (DLTdv), 362, 364-365, 377-380 Diuresis, 620 DLTdv, 362, 364-365, 377-380 DNA barcoding amplification and identification of vertebrate host cytochrome c oxidase subunit I (COI) DNA barcoding templates from mosquito blood meals (protocol), 188-195 cytochrome c oxidase subunit I (COI) as standard for, 176 DNA-based methods, of blood meal analysis, 175–176 DNA extraction HotSHOT (hot sodium hydroxide and Tris) method, 184-187 InstaGene, 187 from mosquito blood meals (protocol), 184-187 preservation of field-collected mosquito blood meals (protocol), 178-183, 181f

DNA preservation on FTA cards, 178-183, 181f Domestication, 83 Dopamine-1 receptor (dop1), 536-537, 547 DOP-PCR (degenerate oligonucleotideprimed PCR), 714b, 717f Dose-response relationship, 721-723, 722f, 732 doublesex, 3, 547-548, 553 Double-stranded RNAs. See dsRNAs Drabkin's reagent, Brij-supplemented (recipe), 153 Drosophila melanogaster, 283, 329, 331 Bruchpilot (Brp) protein, 605 CRISPR-Cas9 gene drives, 551-554 Development Studies Hybridoma Bank (DSHB), 605 embryo microinjection, 495-496 empty neuron system, 425, 482 GECI use in, 449 hsp70, 515 P element, 511 taste sensory responses, 409, 411-412 Wolbachia in, 4 Drosophila pseudoobscura hsp82, 515 DSHB (Development Studies Hybridoma Bank), 605 dsRNAs (double-stranded RNAs), 535-536, 544, 546 chitosan nanoparticle delivery, 547-548 feeding/soaking in solution of, 547 microbial delivery, 548 microiniection, 546 Durcupan ACM epoxy resin (recipe), 600 Dye-filling dextran-conjugated, 605-606 method, 605-606

Ε

EAG. See Electroantennography Ears flagellar, 459-461, 460f, 469-471, 471f immunohistochemical staining of ear (protocol), 463-467, 466f Ecology, of Aedes aegypti, 3 Egg brooding, 310 Egg collecting Aedes aegypti, 84-85, 95-98, 502-503, 502f Anopheles, 87, 502-503, 502f Culex, 68-69, 69f, 72, 89-90 Egg counting, 311, 318, 320, 322-323, 324f, 325–328 Anopheles, 41-42 automated counting, 42 manual counting, 41

automated approaches for Aedes aegypti oviposition experiments (protocol), 322-328, 324f Egg development, 283–298 Aedes aegypti, 1 dietary proteins, role of, 285-286, 285f, 294-295, 295f-296f images of eggs, 284f, 286f visualization of apoptotic ovarian follicles during Aedes aegypti mosquito egg maturation by fluorescent imaging studies (protocol), 289–298, 291f-293f, 295f-296f Egg-laying preference. See Oviposition preference Egg papers, 10, 12f, 14, 23, 39, 41, 317f, 318-320, 322-323, 324f, 325-328 Egg raft, Culex, 61-62, 64, 68-72, 69f-70f, 80-81, 89-90, 92 Eggs Aedes aegypti collecting, 84-85, 95-98 conditioning and storage, 14-15, 22 - 23hatching, 1, 10, 23 storage, 60 Anopheles, 29, 36-43 collecting, 87 egg collection, 29, 37-39, 38f egg counting, 41-42 egg disinfection, 29, 39-40, 40f egg hatching, 29, 40-41, 40f Culex, collecting, 68-69, 69f, 72, 89-90 dechorionation, 521 Eggshell formation, 299-307 hardening, 285, 287, 299 liquid chromatography with tandem mass spectrometry (LC-MS/ MS) analysis of eggshell proteins (protocol), 299-307 discussion, 304-305 protein extract preparation, 301-303 protein identification, 304 protein sample preparation for MS, 303-304 recipes, 305-307 ultrastructure characteristics, 286f Electroantennography (EAG), 397-408, 398f, 447-448 biological factors to consider feeding status, 399 physiological status, 399 time of day, 399 gas chromatography (GC), 402-407, 406f limitations to technique, 400

overview, 398 recordings (protocol), 402-407, 406f materials, 402-403 method, 404-407 mosquito saline (recipe), 407 technical details to maintain responsiveness, 399-400 gradual decrease in responsiveness, 399-400 noise-to-signal ratio, 400 whole-body mounting, 398 whole-head mounting, 399, 405-406, 406f Electrocution traps, 229 Electrode etching device, 428, 429f, 431 Electropenetrography, 148 Electrophysiological measurements of compound action potential responses from antennal nerve in response to stimulation (protocol), 468-471, 471f materials, 468-469 method, 469-470, 471f troubleshooting, 471 Electrophysiological responses of larval antennal sensory cone of Anopheles coluzzii to volatile odorants (protocol), 484-488 discussion, 487-488 materials, 484-485 method, 485-487, 486f Electroretinograms (ERGs) measuring spectral sensitivity (protocol), 390-395, 392f discussion, 394 materials, 390-391 method, 391-394, 392f Ringer's solution for mosquitoes (recipe), 395 overview, 387 ELISA (enzyme-linked immunosorbent assay), for blood meal analysis, 175 Embedding organs, 697 samples for cross-section immunohistochemistry, 639 whole mosquitoes, 694, 695f, 697, 697f Embryo desiccation, preventing, 497 Embryo microinjection, 495-509 alternatives to gene editing, 526-527 CRISPR, 525-527, 562 embryo desiccation, preventing, 497 generating mutant strains with transgenic Cas9 (protocol), 572f, 573

Embryo microinjection (Continued)

under Halocarbon oil or in aqueous solution (protocol), 500-509, 502f-504f discussion, 508 egg collection, 502-503, 502f hatching embryos, 506 injection process, 505-506 injection under aqueous solution, 504-505, 504f injection under oil, 503-504, 503f materials, 500-501 method, 501-507, 502f-504f rearing and mating transgenic mosquitoes, 506-507 troubleshooting, 507-508 injection process, 498, 498f injection timing and location, 495-496, 496f overview, 495 sharp needles, importance of, 496-497 strategies for alignment and injection, 498 for transposon-mediated transgenesis, 520-522, 520t Embryoplasm, 496-498 Empty neuron system, of Drosophila melanogaster, 425, 482 End-joining CRISPR-Cas9 in Aedes aegypti, 561-563, 565f, 568, 572f, 574 nonhomologous (NHEJ), 525-526, 533, 549, 552-553 Enhanced green fluorescent protein (eGFP), 449 Enhancer traps, 511-512, 518, 551 Environmental challenges, 687-688 Enzyme labels, 605 Epithelial cell membranes, localizing protein expression of transporters in (protocol), 691-698, 695f, 697f Epithelial transporters, 620 ERGs. See Electroretinograms Ethanol dehydration, 693 Evolutionary history, of Aedes aegypti, 3 External saline (recipe), 616 Extracting DNA from mosquito blood meals (protocol), 184-187 Eye, compound, 386-387, 386f

F

Faraday cage, 391–393, 392f, 400, 404, 406, 417 Fascicle, 146–147 Fecundity, estimating *Anopheles*, 42 Feeding status evaluation of *Aedes aegypti* (protocol), 149–153, 150f

Brij-supplemented Drabkin's reagent (recipe), 153 feeding success experiments, 150f, 151 materials, 149-150 method, 150-152 quantitative assessment of ingested blood, 151-152, 152f troubleshooting, 152-153 light microscopy of individual mosquitoes, 291f, 302f Feeding success experiments, 150f, 151 Feeding time, 147 evaluation of Aedes aegypti, 154-157 variability in, 157 Females Aedes aegypti appearance, 2f pupae, 2f, 3-4, 12, 19-20 Anopheles, 50f, 51 Culex pipiens, 70f ear anatomy, 471f flight tones, 461 olfaction, 198 Fertility rate, Anopheles, 42 FID (flame ionization detector), 424-425, 424f, 427, 434 Field-collected mosquitoes, establishing colonies from, 83-100 Aedes aegypti, 84–86 adults, 85 blood feeding, 86 eggs, 84-85 hatching, 85 larvae, 85 overview, 84 oviposition, 86 Aedes spp., 86 Anopheles gambiae complex, 86-89 adults, 88 blood feeding, 88 eggs, 87 hatching, 87 larvae/pupae, 87-88 mating, 88 overview, 86-87 oviposition, 89 Anopheles spp., 89 collection, storing, and hatching Aedes aegypti eggs (protocol), 95-98 Culex pipiens, 89-92 adults, 91 blood feeding, 91-92 eggs, 89-90 hatching, 90 overview, 89 oviposition, 92 rearing larvae and pupae, 90-91

field (protocol), 99-100 overview, 83-84 First fixative solution (recipe), 685 FISH. See Fluorescence in situ hybridization Fixative solution for immunostaining (recipe), 678 Fixative solution for mosquito tissues (recipe), 612 Flagellar ears, 459-461, 460f, 469-471, 471f Flagellar movement, 460, 469-471 Flame ionization detector (FID), 424-425, 424f, 427, 434 Flight audio analysis, 262, 270-271 recording, 262, 267-270 recording equipment, 262, 265-266, 266f Flight behavior analysis wind tunnels, 339-347 introduction, 339-341 protocol, 343-347, 345f-346f wind tunnels and airflow-driven assays, 349-357 Flight tones, 259-263, 265, 267-271, 459-462, 460f, 472-476, 479 phonotaxis assay (protocol), 477-479 recording and extraction of (protocol), 474-476, 475f sex differences, 461 Flinders Technology Associates (FTA) card preservation of field-collected mosquito blood meals, 178-183, 181f storing cards, 181-182 Flowers location by mosquitoes, 160 nectar feeding, 159-161, 163, 169-170 pollination, 160-161, 168-171, 170f as resources, 159 ultraviolet light cues to attraction, 386 Fluorescence binocular microscope, identifying and sorting transgenic larvae under (protocol), 582-585, 584f discussion, 585 materials, 582-583 methods, 583-584, 583f troubleshooting, 585 Fluorescence in situ hybridization (FISH), 620 cell-specific localization in Aedes aegypti tissues (protocol), 624-630, 627f materials, 624-625 methods, 626-629, 627f recipes, 629 immunohistochemistry combined, 621 probe preparation for 3D-FISH, 714b

larvae and pupae transport from the

Culex spp., 92

RNA probe template preparation, 626-627 visualization of polytene chromatin in cell nuclei (protocol), 713-719, 717f discussion, 716-717, 717f materials, 713, 715 method, 715-716 recipes, 718-719 troubleshooting, 716 Fluorescence lifetime imaging microscopy (FILM), 655 Fluorescence markers for identifying and sorting transgenic larvae, 578-580, 579f, 582-585, 588, 589f Fluorescent calcium chelators, 448 Fluorescent dye filling, coupled with immunofluorescent labeling, 605 Fluorescent-labeled inhibitor of caspases (FLICA), 286-287, 289-295, 296f Fluorescent probe preparation for 3D-FISH, 714b Fluorescent proteins in transposonmediated transgenesis, 515 Fluorophores dextran amine-conjugated neural tracing, 606, 614-617 for immunolabeling, 604-605 probe preparation for 3D-FISH, 714b Flydra, 364 FLYDRA software, 346 Flytrax, 334-335 Follicle resorption (oosorption), 283–286, 289, 294 fruitless gene, 3, 199 FTA card. See Flinders Technology Associates (FTA) card

G

GAL4/UAS, 550-551 Gas chromatography (GC) electroantennography (GC-EAG), 402-407, 406f, 423, 425 single-sensillum recording combined (GC-SSR), 423-435 factors affecting resolution, 424 protocol, 427-435, 429f, 432f using, 423-425, 424f GCaMP, components of, 448-449 GCaMP6, variants of, 449 GCaMP6f, 449, 451 GCaMP imaging of central nervous system, 437-445 key considerations, 438 overview, 437-439 strengths and weaknesses, 438

transgenic reagents available, 438 two-photon calcium imaging in brain of Aedes aegypti (protocol), 440-445, 442f Aedes aegypti saline solution for imaging (recipe), 445 discussion, 444 materials, 440-441 method, 441-443, 442f troubleshooting, 443-444 GECIs (genetically encoded calcium indicators), 437-438, 447-457 GenBank, 175 Gene drives, 551-554 controlling spread, 553-554 integral, 554-555, 554f overview, 551-552, 552f resistance to, 552-553 split drive system, 553 Gene editing, 549-550. See also CRISPR-Cas9 Gene expression visualizing in peripheral sensory tissue, 665-686 cryosectioning and immunohistochemistry (protocol), 669-673, 671f immunostaining whole-mount olfactory tissues (protocol), 674-679 improved methods to characterize, 666 RNA in situ hybridization of whole-mount olfactory tissues (protocol), 680-686 Gene gun bombardment, 577 Gene silencing by RNAi, 545-548 chitosan nanoparticle delivery, 547-548 feeding/soaking in ds/si RNA, 547 microbial delivery, 548 microinjection, 546 oral RNAi, 535-544 overview, 545-546 transgenic delivery, 548 Genetically encoded calcium indicators (GECIs), 437-438, 447-457 Genetically modified (GM) mosquitoes, 495-509 Genetics of Aedes aegypti, 3 Genetic toolbox, 545-555 binary expression systems, 550-551 future directions/outlook, 554 gene drives, 551-554 controlling spread, 553-554 integral, 554-555, 554f overview, 551-552, 552f resistance to, 552-553

gene editing, 549-550 gene silencing by RNAi, 545-548 chitosan nanoparticle delivery, 547-548 feeding/soaking in ds/si RNA, 547 microbial delivery, 548 microinjection, 546 overview, 545-546 transgenic delivery, 548 transgenes, 550 Genome editing. See CRISPR-Cas9 Germarium, 283, 294, 302, 305 GitHub, 140, 142-143, 316, 334 glmm TMB (R package), 215 Glutathione-S-transferases (GSTs), 721, 724, 738, 744 Glytube, 101-103, 105-108, 106f feeding element assembly, 106-107 heating element assembly, 107 illustration of, 106f protocol for use, 105-108 troubleshooting, 108 Golgi, Camillo, 585 Golgi method, 595-601 silver staining central neuropils (protocol), 597-601 materials, 597-598 method, 598-600 recipes, 600-601 stages, 595-596 chromation, 596, 598-599 metal impregnation, 596, 599 tissue fixation, 595-596, 599 uses in staining insects, 596 Gonotrophic cycle, 1, 283-285, 285f, 294-295, 295f Gouck-style two-point olfactometer, 221-222 G-protein-coupled receptors, 620-621 Gravid H₂O for mosquito larvae collection (recipe), 73 GSTs (glutathione-S-transferases), 721, 724, 738, 744 Gustatory receptors (GRs), 198, 397, 448

Н

Halocarbon oil embryo microinjection under, 498, 500–508, 503f recipe, 574 Harmonic convergence, 259–260, 262 Hatching eggs *Aedes aegypti*, 1, 10, 23, 85, 98 *Anopheles*, 29, 40–41, 40f, 87 *Culex*, 90 Hatching embryos *Aedes*, 506 *Anopheles*, 506 HCR (hybridization chain reaction), 680-686 HDR. See Homology-directed repair Health surveillance laboratory, 10 Hearing, 459-479, 460f audibility as complex, multivariate problem, 461 electrophysiological measurements of compound action potential responses from antennal nerve in response to stimulation (protocol), 468-471, 471f flagellar ears, 459-461, 460f, 469-471, 471f immunohistochemical staining of ear (protocol), 463-467, 466f overview, 459-461, 460f phonotaxis assay (protocol), 477-479 recording and extraction of flight tones (protocol), 474-476, 475f sensitivity, 460 vector control, 461-462, 477 Heat cues of host attraction, 349-350, 352 Heat detection. See Ionotropic receptors (IRs) Heat maps, 350, 456 Heat-seeking behavior automated long-term monitoring, 251-258, 256f carbon dioxide-activated, 254-258, 256f Heat shock protein genes, 515, 547 Heat source, as attractant, 253 HEGs (homing endonuclease genes), 551 Helper plasmids, for transposon-mediated transgenesis, 512, 513f, 515, 518, 520-522 Hemostasis, 145 Hemotek blood-feeding system, 52, 79-80, 91 HEPES larval buffer (recipe), 678, 685 Hermes, 512, 514t High-resolution characterization biting behavior, 129-144 HLCs (human landing catches), 229 Homing endonuclease genes (HEGs), 551 Homology-directed repair (HDR), 549, 551-553 CRISPR-Cas9 in Aedes aegypti, 561-565, 568, 570-574, 572f guide RNAs and, 525-527 Horseradish peroxidase, 605 Host associations blood meal analysis, 173-195 specialization, 174 Host odor as attractant, 198-199, 209, 228-229 lactic acid, 355

quantifying preference using two-point olfactometer, 211-225 wind tunnels and airflow-driven assays, 349-357 Host preference definitions, 227-228 interindividual differences in human attractiveness, 228, 231 plasticity in, 228 Host preference, quantifying, 227-250 Aedes aegypti host odor preference using two-point olfactometer, 211-225 behavioral assay to quantify odorguided thermotaxis with Anopheles gambiae under semi-field conditions (protocol), 234-240, 235f considerations for controlled assays, 229-231 air speed and plume dynamics, 230 assay configuration, 230 carrier air source, 230-231 environmental conditions, 229 equipment cleaning/decontamination, 231 interindividual variability in host attractiveness, 231 mosquito internal physiological state, 230 source of mosquitoes, 229 introduction, 227-231 methods, 228-229 quantification of Anopheles gambiae olfactory preferences under semi-field conditions (protocol), 241-249, 245f, 247f Host-seeking behavior, 228-230, 249 introduction, 349-350 olfaction, role of, 397, 447 wind tunnels and airflow-driven assays, 349-357 HotSHOT (hot sodium hydroxide and Tris) method, 184-187 House-entering behaviors, 356 hsp70, 515 hsp82, 515 hsp83, 547 hsp90, 547 Hull Reconstruction Motion Tracking (HRMT), 364 Human landing catches (HLCs), 229 Humidified airstream, 417-418 Humidity, in wind tunnel assays, 352 Hybridization Anopheles, 86-87 Culex, 61, 64, 89 FISH (see Fluorescence in situ hybridization)

RNA in situ hybridization of wholemount olfactory tissues (protocol), 680–686 Hybridization buffer for FISH (recipe), 718 Hybridization chain reaction (HCR), 680–686 Hydrolases, 721, 724, 738, 744

I

IFP2, 512 IHC. See Immunohistochemistry Image analysis, automated, 130, 132 ImageJ, 696 Imaginal discs, mitotic chromosomes from, 701, 705-709, 707f, 710t, 711 Immunofluorescent labeling fluorescent dye filling coupled with, 605 overview, 604-605 whole-mount labeling of CNS (protocol), 607-613 Immunohistochemical staining of ear (protocol), 463-467, 466f albumin-gelatin embedding medium (recipe), 467 materials, 463-464 method, 464-467, 466f Immunohistochemistry (IHC) cross-section in Aedes aegypti (protocol), 636-642, 638f materials, 636-638 method, 638-641, 638f recipes, 641-642 distribution of an antidiuretic neuropeptide, 633f fluorescent in situ hybridization (FISH) combined, 621 localizing protein expression of transporters in epithelial cell membranes (protocol), 691-698, 695f, 697f of salivary glands, 653-663 protocol, 657–663, 659f, 661f transporter identified by, 688-689 whole-mount and paraffin-embedded tissue sections, 620-621 whole-mount in Aedes aegypti (protocol), 631-635, 632f materials, 631-632 method, 632-634, 632f recipes, 634-635 Immunohistochemistry blocking buffer (recipe), 663 Immunohistochemistry washing buffer (recipe), 663 Immunostaining method, 605 Immunostaining whole-mount olfactory tissues (protocol), 674-679

discussion, 677 materials, 674-675 method, 675-677 recipes, 677-670 troubleshooting, 677 Infrared beam break method, 253-254 Injection buffer for mosquitoes (recipe), 523 Injection needles for embryo microinjection, 496-498 fabrication of, 497 Insecticide bioassays, 721-745, 723-724 adult bioassays (protocol), 732-737, 735f bottle bioassay, 735f, 736-737 materials, 732-733 method, 733-737, 735f topical application bioassay, 734, 735f, 736 dose-response relationship, 721-723, 722f, 732 larval bioassays (protocol), 726-731, 729f discussion, 730 materials, 726-727 method, 726-727, 727-729, 729f recipe, 730 schematic, 729f troubleshooting, 730 resistance surveillance, 723 schematic, 722f synergism study (protocol), 724, 738-745, 741f discussion, 744 materials, 738-739 method, 739-744, 741f recipe, 745 troubleshooting, 744 Insecticide resistance, 721-745 bioassays, 721-745 surveillance protocols, 723 CDC bottle bioassay, 723 WHO tube test, 723 Insecticides bioassays, 721-745 RNAi (RNA interference), 537 Insemination in Aedes, 273-281, 279f process overview, 273-274 resistance after initial, 273-274, 277 status of Aedes mosquitoes, determining (protocol), 277-281, 279f InstaGene, 187 Integral gene drives, 554-555, 554f Intensity assays, 723 Interprobing time, 147, 154 Introgressed genes, 194 Invertebrate hosts, 174, 192 Inverted terminal repeats (ITRs), 511-512, 515

Ionotropic receptors (IRs), 198, 397, 438, 448–449, 546 Isotonic buffer (recipe), 523

J

Johnston's organ, 260, 459–461, 470, 471f Juvenile hormone, 283, 285, 294

K

Killing mosquitoes by cold exposure, 179 by ethyl acetate exposure, 179 Kinefly, 334–335, 337 Kinematics quantifying and analyzing mosquito movement from video tracking results (protocol), 382-384, 383f tracking body, wing and leg of moving mosquitoes (protocol), 377-381, 378f discussion, 380 materials, 377-378 method, 378-380 workflow, 378f Klenow fragment of DNA polymerase I, 714b Klinotaxis, 356 Knock-ins, in Aedes aegypti, 526 kynurenine hydroxylase

L

Labellum, 146f, 409-411, 410f, 448 Labium, 146, 146f, 409, 410f Laboratory colonies Aedes aegypti, 1-25, 84-86 Anopheles, 27-57, 86-89 Culex, 59-82, 89-92 domestication, 83 field-collected mosquitoes, 83-100 Aedes aegypti, 84-86 Anopheles gambiae complex, 86-89 Culex pipiens, 89–92 Laboratory strains, of Aedes aegypti, 4-5 Lactic acid, as attractant, 355 Lamin, 702 Landing assays, 355-357 Landing platform, heated, 235f, 236-240, 246-247, 247f, 249 Larvae Aedes aegypti development, 2, 2f, 10 field-collected colonies, 85 thinning and rearing, 11-12 Anopheles chemosensory methods for, 481-493 field-collected colonies, 87-88

laboratory rearing, 29-31, 485, 490 mounting A coluzzii, 486 surface feeding, 482, 487-488 Anopheles, laboratory rearing, 29-31, 485 container size, 29 container type, 29 counting, 45 food amounts, 30-31 food types, 30 protocol, 44-47, 46f challenges to survival, 687-688 chemosensory methods for Anopheles, 481-493 Culex appearance, 70f collecting, 69 feeding, 61-62 field-collected colonies, 90-91 growth and development, 61-62 identifying, 70-71 morphological identification, 70-71 PCR-based identification, 71 rearing to adulthood, 71, 77-79, 78t identifying and sorting transgenic larvae, 577-592 with COPAS machine (protocol), 587-590, 589f under fluorescence binocular microscope (protocol), 582-585, 584f selecting with puromycin (protocol), 591-593, 592f olfaction, 481-493 cup and pan behavioral assays for assessing Anopheles coluzzii larval volatile responses (protocol), 489-493 overview, 481-483 peripheral electrophysiological responses of larval antennal sensory cone of Anopheles coluzzii to volatile odorants (protocol), 484-488 RNAi chitosan nanoparticle delivery, 547-548 feeding/soaking in ds/siRNA, 547 microbial delivery, 548 microinjection, 546 synergism study, 739-742, 741f transport from the field, 99-100 Larval development, 481-482 Larval insecticide bioassay (protocol), 726-731, 729f discussion, 730 materials, 726-727 method, 726-727, 727-729, 729f recipe, 730 schematic, 729f troubleshooting, 730

This is a free sample of content from Mosquitoes: A Laboratory Manual. Click here for more information on how to buy the book.

Larval mortality, estimating Anopheles, 42 Larvicides, RNAi (RNA interference) and, 537 Leg kinematics of moving mosquitoes, 384 Lethal concentration 50% (LC₅₀), 723, 726-727, 729-730, 736 Lethal concentration 90% (LC₉₀), 723, 726-727, 730, 732, 736 Lethal dose 50% (LD₅₀), 723, 732 Lethal dose 90% (LD₉₀), 723, 732 Life cycle Aedes aegypti, 1-3, 2f Anopheles, 27-28 Light, oviposition cue, 310 Liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis of eggshell proteins, 287, 299-207 Loading buffer $6 \times$ (recipe), 650

Μ

Machine learning biting behavior analysis, 130, 132, 134, 143 tracking behavior using, 252, 377 Machine vision, 131, 134, 377 Malaria, 27, 86, 227-228 Males Aedes aegypti appearance, 2f pupae, 2f, 3-4, 12, 19-20 Anopheles, 50f, 51 Culex pipiens, 70f ear anatomy, 471f flight tones, 461 olfaction, 199 rotation of genitalia, 1 swarming, 259-263, 267, 269, 271 Malpighian tubules aquaporins, 688 membrane protein fractionation and analysis through western blot in (protocol), 643-652, 645f Marker genes, for identifying and sorting transgenic larvae, 578-580, 579f Maternal deposition, 549 Mating, 1 field-collected Anopheles strains, 88 flight tones, 459-462, 460f, 472-476, 479 phonotactic chase, 459 transgenic mosquitoes, 506-507 Mating acoustics, 259-272 overview, 259-263 recording and analysis (protocol), 265-272, 266f Mating behavior acoustic-related, 259-272

overview, 259-263 recording and analysis (protocol), 265-272, 266f courtship flight, 259-260 swarming, 259-263, 267, 269, 271 Mating crosses of Aedes aegypti, 20-21, 21f Mating swarm, vector control and, 461 Mating verification, Aedes aegypti, 23 Maxillary palps. See Palps MCR (mutagenic chain reaction), 552, 552f Meal volume quantification, 119-127 applications, 120 method selection, 119-120, 120f protocol, 122-127, 125f discussion, 127 materials, 122-123 methods, 124-126, 125f troubleshooting, 127 Median lethal concentration (LC_{50}), 723, 726, 729–730, 736 Median lethal dose (LD₅₀), 723, 732 Meiotic chromosomes linear visualization, 700f, 707f obtaining for cytogenetic analysis (protocol), 705-711 polytene and mitotic compared, 710t from testes, 701, 705-709, 707f, 710t Membrane feeding devices, 101-108 Membrane protein fractionation and analysis through western blot in Aedes aegypti Malpighian tubules (protocol), 643-652, 645f materials, 643-645 method, 645-650, 645f recipes, 650-652 Membrane proteins, 621 Mesocosms, 198 Metabolic detoxification, 721, 724, 738, 744 Metal impregnation, in Golgi method, 596, 599 Methanol dehydration, 676, 682 Mice anesthetization, 151, 156-157 evaluation of Aedes aegypti feeding status (protocol), 149-153, 150f evaluation of Aedes aegypti penetration, probing, and feeding times on mice (protocol), 154-157 Microalgae, use in delivery of RNAi, 548 Microbial delivery of RNAi, 548 Microinjection embryo (see Embryo microinjection) larvae for RNAi delivery, 546 Micromanipulator, 424, 429f, 432f Microparticle image velocimetry, 147 Microscope

for GCaMP imaging experiments, 438 two-photon (2P), 438, 440-445 Midgut, dissection of mosquito, 114f Minos, 512, 514t Mitotic chromosomes from imaginal discs, 701, 705-709, 707f, 710t, 711 linear visualization, 700f, 707f obtaining for cytogenetic analysis (protocol), 705-711 polytene and meiotic compared, 710t Mobility assays, 511-512 Model-based 3D insect Tracker, 365 Mortality rate, corrected, 728, 736 Mos1, 512, 514t Mosquito larval food (recipe), 730, 745 Mosquito responses to odors during flight, 340-341 tools available for study, 341-342 in wind tunnel, 339-347 Mosquito saline (recipe), 407 Mosquito traps, visual cues used in, 385 Mounting mosquitoes, 430-431, 431f, 469-470, 473-474 Anopheles coluzzii larvae, 486 antennae for immunostaining, 676 for RNA in situ hybridization, 683 for immunofluorescent imaging of CNS, 610-611 for immunohistochemistry, 634, 659f, 660 palps for immunostaining, 676 for RNA in situ hybridization, 683 Mouthparts, 145-147, 146f Movement, videography of, 361-384 quantifying and analyzing mosquito movement from video tracking results (protocol), 382-384, 383f real-time tracking of multiple moving mosquitoes (protocol), 373-376, 374f tracking body, wing, and leg kinematics of moving mosquitoes (protocol), 377-381, 378f MultiCamSelfCal calibration routine, 375-376 Mutagenic chain reaction (MCR), 552, 552f MyTags oligonucleotide libraries, 714b

Ν

Na⁺/K⁺-ATPase, 638f, 689, 691 Navigation introduction, 339 wind tunnel experiments and analysis, 339–347

NC82, 605-606 NCBI-BLAST, 564 NEB Engen sgRNA synthesis kit, 529-530 Nectar feeding, 159-161, 163, 169-170 olfactory cues, 198, 209 overview, 118-119, 118f sweet taste receptors, 118, 120 Nectar meals assessing in Aedes aegypti, 117-128 overview, 117-118, 118f Needle pullers, 497 Neural circuitry dextran amine-conjugated neural tracing (protocol), 614-617 techniques used to study, 603-617 Neural tracing dextran amine-conjugated (protocol), 614-617 overview, 605, 606 Neuroanatomy techniques used to study, 603-617 dye-filling method, 605-606 immunostaining method, 605 overview, 604-605 whole-mount immunofluorescent labeling of CNS (protocol), 607-613 Neurocircuitry, techniques used to study dye-filling method, 605-606 immunostaining method, 605 overview, 604-605 Neuromeres, 604 Neurons dye-filling, 605-606 olfactory sensory neurons (OSNs), 423-425, 427, 433-434, 674-679 sensory cone neurons, larval antennal, 484-488, 486f silver staining by Golgi method, 595-601 Neuropeptides, 620 Neuropil, 595-601 NGG sequence, 564 NHEJ (nonhomologous end joining), 525-526, 533, 549, 552-553 Nick translation, 714b Nix, 3 No-choice assay, 355 Nonhomologous end joining (NHEJ), 525-526, 533, 549, 552-553 Nonlinear distortion, 459, 461 Nonvertebrate hosts, 174, 192

0

Odorant receptor co-receptor (Orco), 425, 448–449, 551 Odorant receptor neurons (ORBs), in Drosophila melanogaster, 482

Odorant receptors (ORs), 397, 425, 438, 448-449, 546 Odor cues, in wind tunnels and airflowdriven assays, 349-357 Odor-guided thermotaxis (OGTA) behavioral assay to quantify with Anopheles gambiae under semi-field conditions (protocol), 234-240, 235f quantification of Anopheles gambiae olfactory preferences under semi-field conditions (protocol), 241-249, 245f, 247f Odor plume creating defined in still air, 356 influence on flight, 353-354 structure in wind tunnel assays, 353-354 visualization, 354 OEH1 (ovarian ecdysteroidogenic hormone 1), 294 Olfaction, 197-210 anatomy of system, 448 Anopheles larval, 481-493 overview, 481-483 peripheral electrophysiological responses of larval antennal sensory cone of Anopheles coluzzii to volatile odorants (protocol), 484-488 electroantennography (EAG) studies, 397-408, 398f, 406f female, 198 functional imaging of olfactory neurons, 447-457 genetically encoded calcium indicators (GECIs) for, 437-438, 447-457 host odor preference, quantifying, 211-225 host preference, quantifying, 227-250 immunostaining whole-mount olfactory tissues (protocol), 674-679 introduction, 197-198 male, 198-199 mosquito responses during flight, 340-341 odor-guided thermotaxis (OGTA) behavioral assay to quantify with Anopheles gambiae under semi-field conditions (protocol), 234-240, 235f quantification of Anopheles gambiae olfactory preferences under semi-field conditions (protocol), 241-249, 245f, 247f overview, 447-448

oviposition site selection, 309-310 repellants, 199 vision tied to, 331 wind tunnels and airflow-driven assays, 349-357 Olfactometers building uniport (protocol), 201-204, 203f-204f introduction, 197-198 multiport, 341 overview, 252 quantifying attraction using uniport (protocol), 205-210, 207f quantifying host odor preference using two-point olfactometer, 211-225 Y-tube, 341 Olfactometry, 197-210 Olfactory glomeruli, 604 Olfactory neurons axonal projections of, 604 calcium imaging of Anopheles coluzzii antennae expressing GCaMP6f (protocol), 451-457, 453f GECIs for functional imaging of olfactory neurons, 447-457 immunostaining whole-mount olfactory tissues (protocol), 674-679 Olfactory plumes, in wind tunnels, 339-340 Olfactory receptors, 397. See also Odorant receptors (ORs) Olfactory sensory neurons (OSNs), 423-425, 427, 433-434, 674-679 OligoArray 2.0, 714b Ommatidia, 386, 386f Oogenesis, 283-284, 294, 299 Oosorption (follicle resorption), 283-286, 289, 294 OpenCV software, 322-323, 325-328 OpIE2 promoter, 580, 585 Opsin-deficient mutants, 388 Opsins, 330, 386-388 Optomotor responses, 329-338 analysis of, 330-331 tethered preparation for analysis of visual-motor responses using modular visual displays (protocol), 333-338, 336f tools for study, 331-332 LED-based systems, 331-332 projector-based systems, 331 wind tunnel assays, 349-350, 355

Oral RNAi, 535-544 for functional characterization of mosquito genes, 536-537 sugar-baited delivery of siRNA for gene silencing (protocol), 539-544, 541t, 542f materials, 539-540 method, 540-543, 541t, 542f troubleshooting, 543-544 Orco (odorant receptor co-receptor), 425, 448-449, 551 Osmoregulatory challenges and adaptations, 687-698 OSNs (olfactory sensory neurons), 423-425, 427, 433-434, 674-679 Ovarian ecdysteroidogenic hormone 1 (OEH1), 294 Ovaries dissection of, 292, 292f, 301-302, 302f images of dissected, 284f, 302f preparation for microscopy, 292-294, 293f Ovicups, 84-85, 96-97, 96f Ovipositing materials, providing, 14 Oviposition Aedes aegypti field-collected colonies, 86 laboratory crosses, 21-22 Anopheles, 29 en masse oviposition, 29, 38-39 field-collected colonies, 89 single-mosquito oviposition, 37-38 Culex field-collected colonies, 92 overview, 309-310 preference for dark sites, 386 skip, 309 Oviposition papers, 11, 12f, 14, 22-24. See also Egg papers Oviposition preference, 309-328 Anopheles, 309 automated egg-counting approaches for Aedes aegypti oviposition experiments (protocol), 322-328, 324f diversity in site selection, 309 evaluating egg-laying preference of Aedes aegypti mosquitoes (protocol), 314–321, 317f long-range oviposition site selection behavior, 310 measurement techniques, 311-312, 312t long-range site selection, 311 short-range site selection, 311 single animal versus groups, experiments with, 311-312, 312t overview, 309-310 short-range oviposition site selection behavior, 310

Р

Palps mounting, 676, 683 visualizing gene expression in immunostaining whole-mount olfactory tissues (protocol), 674-679 RNA in situ hybridization of wholemount olfactory tissues (protocol), 680-686 PAM (protospacer-associated motif), 526, 552, 564, 570 Paraformaldehyde 4% (recipe), 673 Pathogen transmission, probing and, 147 pattB PBO (piperonyl butoxide), 721, 724, 738, 744 PBT buffer for FISH (recipe), 629 PBT buffer with block (recipe), 629 PBT for FISH (recipe), 718 PBTr (recipe), 719 PBT with RNase A (recipe), 719 PBTx for immunostaining (recipe), 679 PCR. See Polymerase chain reaction P element, 511 Peltier element, 138-139, 138f, 235f, 236-237, 246, 248-249, 253, 256-258, 256f Penetration time, 146, 154-157 Peptidases, 109-110 Peripheral electrophysiological responses of larval antennal sensory cone of Anopheles coluzzii to volatile odorants (protocol), 484-488 Peripheral sensory tissue background, 665-666 visualizing gene expression in, 665-686 cryosectioning and immunohistochemistry (protocol), 669-673, 671f immunostaining whole-mount olfactory tissues (protocol), 674-679 improved methods to characterize, 666 RNA in situ hybridization of wholemount olfactory tissues (protocol), 680-686 Permeabilization solution for immunohistochemistry (recipe), 635 Permeabilization solution for mosquito tissues (recipe), 612 Pesticides, RNAi (RNA interference) and, 537 Phonotactic chase, 459 Phonotaxis assay (protocol), 477-479 materials, 477-478

method, 478-479 troubleshooting, 479 Phosphate-buffered saline with Triton X-100 (recipe), 673 Phosphate-buffered saline with Tween 20 for mosquito RNA-FISH (recipe), 685 Photo-ionization detector (PID), for visualizing odor plume, 354 Photoreceptors, 386-387, 386f Phytochemical lures, 160 piggyBac, 511-512, 514t, 551, 578 Piperonyl butoxide (PBO), 721, 724, 738, 744 Plants, mosquito interactions with, 159-171 floral location, 160 flowers as resources, 159 olfactory cues to attraction, 198 pollination, 160-161, 168-171, 170f sugar detection in mosquitoes via anthrone tests, 160, 163-167 Plasmid mobility assays, 511 Plasmodium P. falciparum, 228, 552 in salivary glands, 655 Plasticine, 398 Pollination assessment of mosquito pollination (protocol), 168–171, 170f mosquito contribution to, 160-161 Polyandry, 1 Polymerase chain reaction (PCR) amplification and identification of vertebrate host cytochrome c oxidase subunit I (COI) DNA barcoding templates from mosquito blood meals (protocol), 188–195 in blood meal analysis, 175-176 fluorescent probe preparation for 3D-FISH, 714b inverse (iPCR), 523 transgenesis confirmation, methods for, 522 validation of sgRNA activity, 531-532 Polytene chromosomes, 699-702 linear visualization, 700f, 707f mitotic and meiotic compared, 710-711, 710t obtaining for cytogenetic analysis (protocol), 705-711 visualization of chromatin using 3D fluorescence in situ hybridization (protocol), 713-719, 717f Polyubiquitin (PUb) promoter, 449 Population replacement gene drives, 552-554

Population suppression gene drives, 552-554 Potassium dichromate with glutaraldehyde and sucrose (recipe), 600 Potassium dichromate with osmium tetroxide (recipe), 601 Potassium dichromate with sucrose (recipe), 601 ppk301, 551 Precipitin test, 175 Preservation of field-collected mosquito blood meals (protocol), 178-183, 180f-182f Primary follicles, 283-284, 284f, 286-287, 294-295, 302f, 305 Probing time, 146-147 evaluation of Aedes aegypti, 154-157, 155f experiments, 155f, 156-157 pathogen transmission, 147 variability in, 157 Probit (probability unit) analysis, 722f, 723, 729, 736 Promoters functional promoter analysis studies, 512 heat shock protein gene, 515 for identifying and sorting transgenic larvae, 578-580, 579f for transgenesis, 514t Protein diet, artificial (recipe), 296 Protein localization cross-section immunohistochemistry (protocol), 636-642, 638f materials, 636-638 method, 638-641, 638f recipes, 641-642 localizing expression of transporters in epithelial cell membranes (protocol), 691-698, 695f, 697f discussion, 697, 697f materials, 691-693 methods, 693-696, 695f recipes, 698 overview, 619-622 whole-mount immunohistochemistry (protocol), 631-635, 632f materials, 631-632 method, 632-634, 632f recipes, 634-635 Protein quantification Bradford assay for, 647 total protein, 650 Proteolytic enzymes, 109-116 Protospacer-associated motif (PAM), 526, 552, 564, 570

PUb (*polyubiquitin*) promoter, 578, 580 Pupae

Aedes aegypti, 2, 12 appearance, 2, 2f collection, 12-13 sex differences, 2f, 3-4, 12, 19-20 Anopheles, 31, 54-57, 56f collection, 55-56, 56f field-collected colonies, 87-88 sex identification and sorting, 57 Culex appearance, 70f development, 62 field-collected colonies, 90-91 transport from the field, 99-100 Puromycin, selecting transgenic larvae with (protocol), 591-593, 592f materials, 591 methods, 592, 592f troubleshooting, 593 Puromycin acetyltransferase, 580 Pyrethroid resistance, 744

Q

R

Raspberry Pi, 135, 138-139, 234, 238-240, 246, 248-249, 317f, 318-319 Raven software, 262, 267, 270 Rearing mosquitoes Aedes aegypti batch rearing (protocol), 8-17, 11f-12f laboratory care and maintenance, 1 - 25Anopheles, 27-57 Anopheles coluzzii larvae, 485, 490 for behavior, 212-213 Culex, 59-82 field-collected mosquitoes, 83-100 transgenic, 506-507 Recording and analysis of mosquito acoustic-related mating behavior (protocol), 265-272, 266f Recording and extraction of flight tones (protocol), 474-476, 475f materials, 472-473 method, 473-475, 475f troubleshooting, 475 ReMOT control (receptor-mediated ovary transduction of cargo), 526-527, 549-550, 562, 577

Renal excretory processes, 620 Repellants, 199 Repetitive DNA, 701, 714b, 717f Reporter gene, 550 Resistance ratio, 723-724, 727, 729, 733 calculating, 729 data analysis of, 736-737 synergism (SRR), 738-739, 744 Rhabdomere, 386, 386f Rhodopsin, 329-330 Ribonuclease III, 546 Ringer's solution for mosquitoes (recipe), 395 RISC (RNA-induced silencing complex), 546 RNA hybridization solution (recipe), 629 RNAi (RNA interference), 545-548 chitosan nanoparticle delivery, 547-548 duration of effects, 546 feeding/soaking in ds/si RNA, 547 microbial delivery, 548 microinjection, 546 oral, 535–544 overview, 545-546 overview of use in mosquitoes, 535-536 pesticides, 537 stages of process, 546 transgenic delivery, 548 RNA-induced silencing complex (RISC), 546 RNA in situ hybridization of whole-mount olfactory tissues (protocol), 680-686 materials, 680-681 method, 681-684 recipes, 684-685 troubleshooting, 684 RNA probe template preparation for FISH, 626-627 RNAscope, 620

S

Saliva, 145-147, 154 Salivary glands compartmentalization of salivary proteins, 653-654 dissection, 658, 659f immunohistochemistry (IHC), 653-663 immunohistochemistry and imaging (protocol), 657-663, 659f, 661f materials, 657-658 method, 658-662, 659f, 661f recipes, 663 troubleshooting, 662 immunohistochemistry technique, 655-656 immunostaining, 658-660

Salivary glands (Continued) morphology and cellular organization, 653, 654-655 mounting, 659f, 660 pathogens in, 655 polytene chromosomes from, 699, 705–709, 707f, 710t Salt balance, regulation of, 687-688, 691 SciTrackS, 252 Scorpine (Sco), 554-555, 554f Secondary follicles, 283, 294, 302, 305 Second fixative solution (recipe), 685 Sectioning samples, 640 Seed set, testing, 169-170, 170f Self-sustained oscillation, 459, 461 Semaphorin-1a, 547 Semi-field cage, 243-246, 244f, 249-250 Semifield environment, oviposition site selection, 311 Semifield system (SFS), 252 Semiochemicals, 160, 310, 481 Semitethering, 261 Sensilla, 409-413, 410f, 415, 419-420, 448, 604,674 bioactive volatile organic compound identification using combined gas chromatography and single-sensillum recording, 423-435 functional type identification, 433-434 single-sensillum electrophysiology, 447-448 Sensory cone neurons, larval antennal, 484-488, 486f Sensory receptors. See Gustatory receptors (GRs); Ionotropic receptors (IRs); Odorant receptors (ORs); Olfactory receptors Sensory tissues and organs, of mosquito head, 398f Separation/resolving gel mixture 12% (recipe), 651 Serine proteases, 109-110 Serological methods, of blood meal analysis, 175 Sewage water, larvae in, 687-688 Sex differences Aedes aegypti, 3-4 ears, 471f flight tones, 461 Sex separation, in Anopheles, 50f, 51 SFS (semifield system), 252 sgRNA. See Single-guide RNA (sgRNA) Shaker (Sh), 536-537, 547 Sharpening electrodes, 431 shRNAs (short hairpin RNAs), 535-536, 546 Silver staining by Golgi method, 595-601 Single-guide RNA (sgRNA), 525, 549

CRISPR-Cas9 in Aedes aegypti, 561-564, 570-571, 572f design and selection, 526, 529, 564 molecular validation of activity, 531-532 synthesis and cleanup, 529-530 testing, 526 validating for Aedes aegypti gene editing (protocol), 528-533 discussion, 532-533 materials, 528-529 method, 529-531 troubleshooting, 532 Single-pair and small-group crosses of Aedes aegypti (protocol), 18 - 25Single-stranded oligo donors, 564 siRNA (small interfering RNA), 535-537, 539-544, 546 sugar-baited delivery of siRNA for gene silencing (protocol), 539–544, 541t, 542f SIT (sterile insect technique), 577-578 Site-specific recombination (SSR), 515 Sodium chloride-sodium citrate with Tween 20 (SSCT) (recipe), 685 Sodium citrate buffer (recipe), 642 Sorting adult Anopheles mosquitoes, 50f Sound perception, 260 Spectral sensitivity measuring using electroretinograms (protocol), 390-395 overview, 387 Spermathecae, 1 Anopheles, 28 dissection, 277-281, 279f filling after insemination, 274-275 mating verification, 23 Sperm motility, 274 Sperm numbers, estimating, 274 Sperm viability, 274 SR (synergism ratio), 738, 743-744 SRR (synergism resistance ratio), 738-739, 744 SSCT $2 \times$ (recipe), 718 SSCT $2 \times +50\%$ formamide (recipe), 718 ssDNA, 562, 564 S,S,S,-tributyl phosphorotrithioate (DEF), 721, 724, 738, 744 Stacking gel mixture 4% (recipe), 651 Stereomicroscope for sorting mosquitoes, 179 Sterile insect technique (SIT), 577-578 Still-air flight chamber, 355 Streptococcus pyogenes Cas9, 526 Stylets, 145-147, 154, 409, 410f, 449 Sucrose solution (10%) (recipe), 53, 144

Sugar detection in mosquitoes via anthrone tests (protocol), 163-167 cold anthrone test, 164-166 hot anthrone test, 165 Sugar feeding Anopheles, 49-50, 50f assessing nectar meals in Aedes aegypti, 117-128 Culex, 62, 91 meal volume quantification, 119-127, 125f sugar solution (10%) (recipe), 53, 144 Sugar solution (10%) (recipe), 53, 144 Survival systems, 688 Swarming, 259-263, 267, 269, 271 Synaptic neuropil, labeling, 605-606 Synchrotron X-ray technology, 147 Synergism ratio (SR), 738, 743-744 Synergism resistance ratio (SRR), 738-739, 744 Synergism study adult mosquito-bottle bioassay, 743 adult mosquito-topical application, 741f, 742-743 larval assay, 739-742, 741f overview, 724 protocol, 738-745, 741f data analysis, 743-744 discussion, 744 materials, 738-739 method, 739-744, 741f recipe, 745 troubleshooting, 744

Т

TADs (topologically associated domains), 702 TALENs (transcription activator-like effector nucleases), 549 Taste receptors, 118, 120 Taste sensory responses, 409-421 analysis, 411 single-sensillum taste recordings (protocol), 413-421 Beadle-Ephrussi Ringer solution (recipe), 420 data analysis, 420 discussion, 420 materials, 413-415 method, 415-419, 416f Taste system, 409-411, 410f TBS-T (recipe), 651 10× running buffer (recipe), 650 Terminal inverted repeats (TIRs), 511 Testes, meiotic chromosomes from, 701, 705-709, 707f, 710t Tethered assays, 355

acoustic-related mating behavior in tethered and free-flying mosquitoes, 259–272 visual-motor responses, 329-338, 336f Tethering mosquitoes, 261, 266f, 268-269, 336, 336f TetOn-Off, 550 Thermotaxis. See Odor-guided thermotaxis (OGTA) 3D FISH. See Fluorescence in situ hybridization 3D printing technology and artificial feeders, 103 3xP3 promoter, 578-580, 579f, 584 TIRs (terminal inverted repeats), 511 Tissue fixation, in Golgi method, 595-596, 599 Tissue fixation solution (recipe), 719 Titanium tetrachloride, for visualizing odor plume, 354 Topologically associated domains (TADs), 702 Toxorhynchites, 85, 260 Tracer gas, for visualizing odor plume, 354 Track3D, 364 Tracker Video Analysis and Modeling Tool, 364 Tracking analysis software packages, 364-365 Braid, 362, 364-365, 373-376 Deep Lab Cut (DLC), 365 Direct Linear Transformation data viewer (DLTdv), 362, 364-365, 377-380 Flydra, 364 Hull Reconstruction Motion Tracking (HRMT), 364 Model-based 3D insect Tracker, 365 Track3D, 364 Tracker Video Analysis and Modeling Tool, 364 automated, 129-130 of body, wing, and leg kinematics of moving mosquitoes (protocol), 377-381, 378f flight behavior in wind tunnel, 340-341, 343-346, 345f-346f videography, 361-384 real-time tracking of multiple moving mosquitoes (protocol), 373-376, 374f Transcript localization cell-specific in Aedes aegypti tissues with FISH (protocol), 624-630, 627f materials, 624-625 methods, 626-629, 627f recipes, 629

overview, 619-622 Transfer buffer 1x (recipe), 651 Transformation efficiency, in transposon-mediated transgenesis, 522-523 Transgenes binary expression systems, 550-551 insertion, 550 Transgenesis identifying and sorting transgenic larvae, 577-592 with COPAS machine (protocol), 587-590, 589f under fluorescence binocular microscope (protocol), 582-585, 584f marker genes, 578-580, 579f promoters, 578-580, 579f selecting with puromycin (protocol), 591–593, 592f introduction to transgenic production techniques, 577-578 transposon-mediated, 511-524 Transient receptor potential channel A1 (TRPA1), 198 Transporters, 687-689 ammonia, 688 identified by immunohistochemistry (IHC), 688-689 localizing expression in epithelial cell membranes (protocol), 691-698, 695f, 697f discussion, 697, 697f materials, 691-693 methods, 693-696, 695f recipes, 698 Transposable elements Class II, 511, 513f P element, 511 used in transgenesis, 514t Transposase, 512, 513f, 515 Transposon-mediated transgenesis, 511-524 background, 511-512 embryo microinjection, 496 experimental design considerations, 512-515 generating and validating transgenic mosquitoes (protocol), 518-523 discussion, 522-523 materials, 518-519 method, 519-522 recipes, 523 troubleshooting, 522 Transposon tagging, 518 Tris-HCl 1.0M, pH 6.8 (recipe), 651 Tris-HCl 1.5M, pH 8.8 (recipe), 652

TRPA1 (transient receptor potential channel A1), 198 Trypsin, 109-116 assay, 110, 112-116 estimation of active levels in mosquito midgut (protocol), 112-116 discussion, 116 materials, 112-113 method, 113-115, 113f-115f, 114t recipes, 116 troubleshooting, 115-116 genes, 110 stock (recipe), 116 T-tube for splitting air supply, 224 TUNEL assay, 286, 294-295 Two-photon calcium imaging in brain of Aedes aegypti (protocol), 440-445, 442f Aedes aegypti saline solution for imaging (recipe), 445 discussion, 444 materials, 440-441 method, 441-443, 442f troubleshooting, 443-444 Two-point olfactometer components for building, 222t components for carbon dioxide delivery system, 223t Gouck-style, 221-222 quantifying host odor preference using, 211-225 animal rearing, 212-213 assay conditions, 213-214 assay variations, 214-215 data analysis, 215-216 experimental design, 212 open versus closed air system, 215 performing behavioral assays, 224-225 protocol, 218-225, 219f troubleshooting, 216-217 solenoid valve carbon dioxide delivery system with controller, 223 T-tube for splitting air supply, 224 washing, 220 Tyramide signal amplification, 629

U

Uniport olfactometer building to assess responses to odors (protocol), 201–204, 203f–204f assembly, 202, 204f fabrication, 202, 203f cleaning, 208 overview, 197–198 quantifying attraction using (protocol), 205–210, 207f Uranotaenia lateralis, 174 Uranotaenia lowii, 174 Uranotaenia sapphirina, 174, 192

V

VAME, 143 vasa, 552, 554 Vector competence, of Aedes aegypti, 4 Vector control hearing and, 461-462, 477 insecticide resistance, 721-745 mating swarm, 461 Ventral nerve cord (VNC) anatomy, 604-605, 604f dextran amine-conjugated neural tracing (protocol), 614-617 dissection, 609 whole-mount immunofluorescent labeling of CNS (protocol), 607-613 Vestigial gene, 547 Vg (vitellogenin), 550 Vibrometer, 468-470 Videography, 361-384 analysis software packages, 364-365 Braid, 362, 364-365, 373-376 Deep Lab Cut (DLC), 365 Direct Linear Transformation data viewer (DLTdv), 362, 364-365, 377-380 Flydra, 364 Hull Reconstruction Motion Tracking (HRMT), 364 Model-based 3D insect Tracker, 365 Track3D, 364 Tracker Video Analysis and Modeling Tool, 364 camera calibration, 365, 371, 375-376, 379-380 designing generic experiment for studying mosquito behavior (protocol), 367-372, 369f high-contrast lightening, 371 image brightness and depth of field, 370 image brightness and motion blur, 370 materials, 367-368 method, 368-372 monochrome versus color cameras, 370 nocturnal mosquito species, 370-371 number of cameras, 368-370 setups, 369f spatial resolution and temporal resolution, 370

spatial resolution and volume of interest, 370 experimental design, 363-365 analysis software requirements, 364-365 hardware requirements, 363-364 overview, 361-362 quantifying and analyzing mosquito movement from video tracking results (protocol), 382-384, 383f body orientation of moving mosquito, 383 leg kinematics, 384 position of moving mosquito, 383 wingbeat kinematics, 384 real-time tracking of multiple moving mosquitoes (protocol), 373-376, 374f materials, 373-374 method, 374-376, 374f workflow, 374f tracking behavior using, 252 tracking body, wing, and leg kinematics of moving mosquitoes (protocol), 377-381, 378f discussion, 380 materials, 377-380 method, 378-380 workflow, 378f workflow for designing, 362f Viral RNA preservation on FTA cards, 182 Virginity, maintaining, 20 Virus, use in delivery of RNAi, 548 Vision analysis of visual responses, 330-331 history of study, 329-330 measuring spectral sensitivity using electroretinograms (protocol), 390-395, 392f olfaction tied to, 331 review of, 385-387 behavior, 385-386 photoreceptors, 386-387, 386f spectral sensitivity, 387 techniques to analyze, 387-388 electroretinograms (ERGs), 387, 390-395 intracellular recordings, 387-388 opsin-deficient mutants, 388 Visual cues for flower location, 160 to gauge direction of airflow, 349 oviposition site selection, 309-310 ultraviolet light dark floral cues, 386 use in mosquito traps, 385 in wind tunnel assays, 354 Visual-motor responses, 329-338

analysis of, 330-331 tethered preparation for analysis using modular visual displays (protocol), 333-338, 336f tools for study, 331-332 Vitellogenesis, 1, 284f, 294 Vitellogenin yolk, 283, 285, 294, 302, 305 VNC. See Ventral nerve cord Volatile odorants cup and pan behavioral assays for assessing Anopheles coluzzii larval volatile responses (protocol), 489-493 identification of VOCs using combined gas chromatography and single-sensillum recording, 423-435 peripheral electrophysiological responses of larval antennal sensory cone of Anopheles coluzzii to volatile odorants (protocol), 484-488 V-type H⁺-ATPase, 689, 691

W

Wash solution for mosquito tissues (recipe), 612 Water balance, regulation of, 687-688, 691 West Nile Virus, 59 Whole-body mounting, 398 Whole-head mounting, 399, 405-406, 406f Whole-mount immunofluorescent labeling of CNS (protocol), 607-613 materials, 607-608 method, 608-611 recipes, 612 Whole-mount immunohistochemistry (protocol), 631-635, 632f materials, 631-632 method, 632-634, 632f recipes, 634-635 Wild mosquito strains, establishing colonies from field-collected, 83-100 Wind tunnel assays, 349-357, 351f alternative assays, 355 assay caveats, 354 assay duration, 353 available protocols, 352 contamination, 352 general design considerations and standardization, 351-352 initiation of flight, 353 plume structure, 353-354 visual cues, 354 wind-tunnel size, 352-353

Wind tunnels, 211, 228, 230–231, 252 classic systems, 341–342 conducting an analysis of mosquito flight behaviors in (protocol), 343–347, 345f–346f discussion, 346–347 materials, 343–344 method, 344–346 schematic of low-speed, 345f design features, 340, 344 free flight assays, 330 mosquito responses in, 339–347 strengths and applications, 350–351 Y-tube and multiport olfactometers compared, 341 WinEDR, 334, 337 Wingbeat frequency, 361–362, 461, 472–475, 474f, 477–479 Wingbeat kinematics of moving mosquitoes, 384 Wingbeat tones. See Flight tones Wing-tracking system, 335, 337 Wolbachia pipientis, 4 Wuchereria bancrofti, 4 Y

Yeast, use in delivery of RNAi, 548 Yellow fever virus, *Aedes aegypti* transmission, 4 Y-mazes, 341 Y-tube choice chamber, 355

Ζ

zero population growth (zpg) promoter, 552 Zika virus, Aedes aegypti transmission, 4 zinc carboxypeptidase A1, 555 Zinc-finger nucleases (ZFNs), 525, 549