What follows are week-by-week lists that describe the materials required, what an instructor must prepare ahead, and student activities for each lab. Week 7 (Spring Break) is omitted.

Week 1: Introduction to Working with *C. elegans*

Materials Used in Lab

- Worm picks (Tritech Research or made using platinum wire fused to glass Pasteur pipette)
- Ethanol burners with 95% ethanol (to sterilize picks)
- Halocarbon oil (for transferring worms without food onto RNAi knockdown plates)
- Aluminum foil (for covering growth plates containing all-trans retinal or IPTG
- Dissecting microscopes (ideally including one with a camera/display capabilities to demonstrate techniques and point out worm stages)
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli*
- 60 mm standard NGM plates seeded with OP50 containing 500 μM all-trans retinal (ATR)
- 60 mm RNAi NGM knockdown plates containing IPTG and ampicillin (see Supplemental RNAi Protocol) seeded with HT115 (DE3) *E. coli* containing RNAi targeting *dpy-11*
- 60 mm unseeded RNAi NGM plates containing IPTG and ampicillin (see Supplemental RNAi Protocol)
- Liquid cultures of HT115 (DE3) bacteria containing RNAi targeting genes of interest (for seeding on RNAi NGM plates (see Supplemental RNAi Protocol))
- P1000 pipettes and tips
- N2 *C. elegans*
- AQ2235 *C. elegans*
- FJ1282 *C. elegans*
- 15˚C incubator (to store plates students pick to)
- Rubber bands (for wrapping stacks of worm plates)
- Markers (for labeling worm plates)

Instructor Preparation

- Introductory lecture on the goals and approach of this CURE
- Materials, plates and bacterial cultures listed above
- Well-fed plates of *C. elegans* strains with mixed stages
- Picking demonstration

Student Activities

- Learn the nomenclature used to describe *C. elegans* genes, transgenes, alleles, and strains
- Identify and distinguish between developmental stages of *C. elegans* worms
- Use a wire pick to transfer individual L4 stage worms from one plate to another without damaging the worms or the plates
    - Pick to maintain worm strains to be used in behavioral assays next week AQ2235 worms to standard NGM plates with OP50 but no ATR
    - AQ2235 worms to standard NGM plates with OP50 and ATR
    - FJ1282 worms to standard NGM plates with OP50 and ATR
- Add 450 μl RNAi-expressing bacteria to 60 mm knockdown plates to set up RNAi screen

Week 2: RNAi Screen Set-up and optoASH Assay

Materials Used in Lab

- Worm picks and ethanol burners with 95% ethanol (to sterilize picks)
- Halocarbon oil (for transferring worms without food onto RNAi knockdown plates)
- LED rig supplies and ring stand and clamps for mounting LED rigs (see main text)
- ThorLabs power meter (see main text)
- Aluminum foil (for covering growth plates containing all-trans retinal or IPTG
- Dissecting microscopes
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli* (for routine maintenance)
- 60 mm RNAi NGM knockdown plates containing IPTG and ampicillin (see Supplemental RNAi Protocol) seeded with HT115 (DE3) *E. coli* containing RNAi targeting genes of interest (prepared by students the previous week)
- 60 mm unseeded RNAi knockdown plates containing IPTG and ampicillin (see Supplemental RNAi Protocol)
- Shared RNAi colony growth plate (for students to use when inoculating liquid growth cultures with their RNAi clones of interest (see Supplemental RNAi Protocol))
- Liquid culture medium containing 100 μg/ml ampicillin for HT115 (DE3) bacteria containing RNAi targeting genes of interest (for students to inoculate using colonies from shared RNAi growth plate (see Supplemental RNAi Protocol))
- P1000 pipettes and tips
- N2 *C. elegans*
- AQ2235 *C. elegans* (maintained on NGM plates with all-trans retinal (ATR) by students)
- AQ2235 *C. elegans* (maintained on NGM plates without ATR by students)
- FJ1282 *C. elegans* (maintained on NGM plates with ATR by students)
- FJ1300 *C. elegans*
- 15˚C incubator (to store plates students pick to)
- Rubber bands (for wrapping stacks of worm plates)
- Markers (for labeling worm plates)

Instructor Preparation

- Materials, plates and bacterial cultures listed above
- Shared RNAi colony growth plate (for students to use when inoculating liquid growth cultures with their RNAi clones of interest (see Supplemental RNAi Protocol))
- Ensure RNAi NGM plates seeded last week by students are dried and ready for worm transfer
- Well-fed plates of N2 and FJ1300 *C. elegans* strains with mixed stages
- Continue maintaining AQ2235 and FJ1282 strains for next week

Student Activities

- Recap previous week & review the nomenclature used in this project
- Begin RNAi screen by picking 3x L4 FJ1300 worms to RNAi NGM knockdown plates seeded with RNAi bacterial clones
- Maintain the FJ1300 strain for future experiments by picking 3 x L4 to standard NGM plates
- Build LED illuminator and test light-activated behavior of “wildtype” (AQ2235) and *eat-4* mutant (FJ1282) worms
- Inoculate liquid media containing 100 μg/ml ampicillin with RNAi bacteria corresponding to set of genes to be tested. The following day, after cultures have grown, add RNAi-expressing bacteria to 60 mm worm knockdown plates to set up RNAi screen (see Supplemental RNAi protocol for additional details)
- Update and share lab notebook

Week 3: optoASH RNAi Screening and Thrashing Behavior

Materials Used in Lab

- Worm picks and ethanol burners with 95% ethanol (to sterilize picks)
- Halocarbon oil (for transferring worms without food onto RNAi knockdown plates)
- LED rigs, stands, clamps, and power meter
- Aluminum foil (for covering growth plates containing all-trans retinal or IPTG
- Dissecting microscopes
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli* (for routine maintenance)
- 60 mm RNAi knockdown plates containing IPTG and ampicillin (see Supplemental RNAi Protocol) seeded with HT115 (DE3) *E. coli* containing RNAi targeting genes of interest (prepared by students the previous week)
- 60 mm unseeded RNAi knockdown plates containing IPTG and ampicillin (see Supplemental RNAi Protocol)
- Shared RNAi colony growth plate (for students to use when inoculating liquid growth cultures with their RNAi clones of interest (see Supplemental RNAi Protocol))
- Liquid culture medium containing 100 μg/ml ampicillin for HT115 (DE3) bacteria containing RNAi targeting genes of interest (for students to inoculate using colonies from shared RNAi growth plate (see Supplemental RNAi Protocol))
- P1000 and P20 pipettes and tips
- Glass coverslips (22 mm x 22 mm or similar)
- M9 buffer (5 μl drops used on coverslips for neuromuscular thrashing assay)
- Timers
- N2, CB1264, AQ2235, and FJ1282 *C. elegans* (for thrashing assay)
- Student-maintained worm growth plates (including FJ1300 on standard NGM and FJ1300 on RNAi knockdown screening plates)
- 15˚C incubator (to store plates students pick to)
- Rubber bands (for wrapping stacks of worm plates)
- Markers (for labeling worm plates)

Instructor Preparation

- Materials, plates and bacterial cultures listed above
- Ensure RNAi knockdown plates seeded last week by students are dried and ready for worm transfer
- Well-fed plates of N2, AQ2235, FJ1282, and CB1263 *C. elegans* (maintained on standard NGM plates without ATR)
- Thrashing assay demonstration

Student Activities

- Repeat RNAi screen set-up by picking 3x L4 FJ1300 worms to RNAi NGM knockdown plates seeded with RNAi bacterial clones
- Maintain the FJ1300 strain for future experiments by picking 3 x L4 to standard NGM plates
- Use the optoASH assay and scoring rubric (see main text) to assess worm locomotion and lght response after RNAi knockdown
- Practice assessing neuromuscular junction function using the thrashing assay with N2 wildtype and *unc-104*/kinesin mutants (count thrashes per 30 seconds in droplet of M9 buffer)
- Develop plans to either repeat population-based optoASH screening or proceed to more quantitative single-worm optoASH assay with a subset of RNAi clones. Prepare cultures and plates as needed (see previous week activity)
- Update lab notebook

Week 4: Quantitative optoASH & Thrashing and Slide Preparation

Materials Used in Lab

- Worm picks and ethanol burners with 95% ethanol (to sterilize picks)
- Halocarbon oil (for transferring worms without food onto RNAi knockdown plates)
- LED rigs, stands, clamps, and power meter
- Aluminum foil (for covering growth plates containing all-trans retinal or IPTG
- Dissecting microscopes
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli* (for routine maintenance)
- 60 mm RNAi knockdown plates containing IPTG and ampicillin (see Supplemental RNAi Protocol) seeded with HT115 (DE3) *E. coli* containing RNAi targeting genes of interest (prepared by students the previous week)
- 60 mm unseeded RNAi NGM knockdown plates containing IPTG and ampicillin (see Supplemental RNAi Protocol)
- Shared RNAi colony growth plate (for students to use when inoculating liquid growth cultures with their RNAi clones of interest (see Supplemental RNAi Protocol))
- Liquid culture medium containing 100 μg/ml ampicillin for HT115 (DE3) bacteria containing RNAi targeting genes of interest (for students to inoculate using colonies from shared RNAi growth plate (see Supplemental RNAi Protocol))
- P1000 and P20 pipettes and tips
- Glass coverslips
- Glass slides for fluorescence microscopy
- Glass coverslips for fluorescence microscopy (no. 1.5 thickness, depending on microscope)
- 30 mg/ml 2,3-butanedione monoxime and heated 2% agarose (see Instructor Preparation below)
- M9 buffer (5 μl drops used on coverslips for neuromuscular thrashing assay)
- Timers
- N2 and CB1264 *C. elegans* (for thrashing assay)
- Student-maintained worm growth plates (including FJ1300 on standard NGM and FJ1300 on RNAi knockdown screening plates)
- 15˚C incubator (to store plates students pick to)
- Rubber bands (for wrapping stacks of worm plates)
- Markers (for labeling worm plates)
- Kimwipes

Instructor Preparation

- Materials, plates and bacterial cultures listed above
- Ensure RNAi NGM plates seeded last week by students are dried and ready for worm transfer
- Well-fed plates of N2 and CB1263 *C. elegans*
- Worm slide preparation demonstration
- Prepare 2% agarose in water, kept in glass tubes at 65˚C in a heat block (for creating pads on which to image worms)
- Prepare 30 mg/ml 2,3-butanedione monoxime (BDM) in M9 buffer (to paralyze worms for imaging)

Student Activities

- Maintain the FJ1300 strain and other needed strains for future experiments by picking 3 x L4 to standard NGM plates
- Use the single-worm optoASH and thrashing assays to quantify glutamatergic behavior and neuromuscular junction activity, respectively for RNAi knockdown and control worms
- Learn to prepare slides of immobilized worms for fluorescence microscopy
- Prepare cultures of RNAi bacteria as needed
- Update lab notebook

Week 5: Continued RNAi Follow-up and Slide Preparation

Materials Used in Lab

- Worm picks and ethanol burners with 95% ethanol (to sterilize picks)
- Halocarbon oil (for transferring worms without food onto RNAi knockdown plates)
- LED rigs, stands, clamps, and power meter
- Aluminum foil (for covering growth plates containing all-trans retinal or IPTG
- Dissecting microscopes
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli* (for routine maintenance)
- 60 mm RNAi knockdown plates containing IPTG and ampicillin (see Supplemental RNAi Protocol) seeded with HT115 (DE3) *E. coli* containing RNAi targeting genes of interest (prepared by students the previous week)
- P1000 and P20 pipettes and tips
- Glass coverslips (22 mm x 22 mm or similar)
- Glass slides for fluorescence microscopy
- Glass coverslips for fluorescence microscopy (no. 1.5 thickness, depending on microscope)
- 30 mg/ml 2,3-butanedione monoxime and heated 2% agarose (see Instructor Preparation below)
- M9 buffer (5 µl drops used on coverslips for neuromuscular thrashing assay)
- Timers
- N2 and CB1264 *C. elegans* (for thrashing assay)
- Student-maintained worm growth plates (including FJ1300 on standard NGM and FJ1300 on RNAi knockdown screening plates)
- 15˚C incubator (to store plates students pick to)
- Rubber bands and markers (for wrapping and labeling plates)
- Kimwipes

Instructor Preparation

- Materials, plates and bacterial cultures listed above
- Ensure RNAi NGM knockdown plates seeded last week by students are dried and ready for worm transfer
- Well-fed plates of N2 and CB1263 *C. elegans*
- Worm slide preparation demonstration
- Prepare 2% agarose in water, kept in glass tubes at 65˚C in a heat block (for creating pads on which to image worms)
- Prepare 30 mg/ml 2,3-butanedione monoxime (BDM) in M9 buffer (to paralyze worms for imaging)
- Order mutant *C. elegans* strains from the Caenorhabditis Genetics Center and necessary genotyping PCR primers following discussions with students during the lab session this week

Student Activities

- Maintain needed strains as done in previous weeks
- Complete RNAi experiments by collecting more optoASH and thrashing data
- Learn about genes, strains, and alleles of interest through Wormbase.org and the Caenorhabditis Genetics Center
- Practice designing primers and annotating DNA sequence files using SnapGene Viewer
- Plan follow-up experiments (including necessary transgenic strains) and discuss with instructor
- Practice preparing slides of immobilized worms for fluorescence microscopy (microscopy on new slides will be performed the following week)
- Prepare cultures of RNAi bacteria as needed
- Update lab notebook

Week 6: Fluorescence Microscopy

Materials Used in Lab

- Worm picks and ethanol burners with 95% ethanol (to sterilize picks)
- Halocarbon oil (for transferring worms without food onto RNAi knockdown plates)
- LED rigs, stands, clamps, and power meter
- Aluminum foil (for covering growth plates containing all-trans retinal or IPTG
- Dissecting microscopes
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli* (for routine maintenance)
- 60 mm RNAi NGM knockdown plates containing IPTG and ampicillin (see Supplemental RNAi Protocol) seeded with HT115 (DE3) *E. coli* containing RNAi targeting genes of interest (prepared by students the previous week)
- P1000 and P20 pipettes and tips
- Glass coverslips (22 mm x 22 mm or similar)
- Glass slides for fluorescence microscopy
- Glass coverslips for fluorescence microscopy (no. 1.5 thickness, depending on microscope)
- 30 mg/ml 2,3-butanedione monoxime and heated 2% agarose (see Instructor Preparation below)
- M9 buffer (5 µl drops used on coverslips for thrashing assay)
- Timers
- N2 and CB1264 *C. elegans* (for thrashing assay) and AQ2235 *C. elegans* for microscopy
- Compound fluorescence microscope with at least 40x magnification objective and z-stack capability (instrument requirements will vary based on instructor goals)
- Student-maintained worm growth plates (including FJ1300 on standard NGM and FJ1300 on RNAi knockdown screening plates)
- 15˚C incubator (to store plates students pick to)
- Rubber bands and markers (for wrapping and labeling plates)
- Kimwipes

Instructor Preparation

- Materials, plates and bacterial cultures listed above
- Well-fed plates of N2 and CB1263 *C. elegans*
- Ensure access to and familiarity with compound fluorescence microscope with at least 40x magnification objective and z-stack capability (instrument requirements will vary based on instructor goals)
- Create a schedule for staggered small group microscope training during the lab period. All groups not actively being trained can work on their proposal assignments or complete any remaining RNAi experiments
- Prepare 2% agarose in water, kept in glass tubes at 65˚C in a heat block (for creating pads on which to image worms)
- Prepare 30 mg/ml 2,3-butanedione monoxime (BDM) in M9 (to paralyze worms for imaging)

Student Activities

- Maintain needed strains as done in previous weeks
- Complete needed RNAi experiments (if necessary)
- Begin working on proposal assignment with group
- Prepare slides of immobilized AQ2235 worms for fluorescence microscopy of GFP-tagged glutamate receptors in the ventral nerve cord (other strains can also be used depending on instructor goals)
- Take detailed notes during microscopy training
- Update lab notebook

Week 8: Review and Intro to Genetic Crosses

Materials Used in Lab

- Picking materials and microscopes
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli* (for routine maintenance)
- 15˚C incubator (to store plates students pick to)
- Rubber bands and markers (for wrapping and labeling plates)
- P1000, P200, P20, and P2 pipettes and tips
- N2 *C. elegans* (for generating worm lysate)
- Worm lysis buffer containing proteinase K (see below)
- PCR tubes (for lysis: 20 worms in 20 μl lysis buffer)
- Buckets of ice
- -80˚C freezer (to freeze worms for 15 minutes before heating in a thermal cycler)
- Thermal cycler (to incubate worms at 65˚C for 60 minutes and then 95˚C for 15 minutes for lysis)
- Tubes of lyophilized primers for genotyping alleles of interest
- Ultrapure distilled water (for reconstituting primers to 100 μM and further diluting them to 10 μM)
- Microcentrifuge tubes for preparing working solutions of 10 μM primers

Instructor Preparation

- Materials and plates listed above
- Begin preparing necessary transgenic strains for follow-up experiments (not needed this week)
- Well-fed plates of N2 and mutant *C. elegans* ordered from the Caenorhabditis Genetics Center (if available)
- Worm lysis buffer: (50 mM KCl, 10 mM Tris pH 8.3, 2.5 mM $MgCl_2$, 0.45% Tween20, 0.45% NP40, before use add proteinase K to 200 μg/ml)
- Prepared lecture/discussion of the following:
    - Objectives and approach for the overall lab and individual experiments
    - Differences between using RNAi and working with mutant strains
    - Rationale and strategy for performing a genetic cross
- Following lab session, store lysates at -20˚C

Student Activities

- Obtain and maintain your mutant strain of interest (if available)
- Maintain needed strains as done in previous weeks
- Review objectives and approach for the overall lab and individual experiments
- Discuss the theory behind performing a genetic cross
- Prepare worm lysate and working concentrations of PCR primers
- Revise your mid-semester lab proposal
- Update lab notebook

Week 9: PCR Practice and Working with Transgenic Strains

Materials Used in Lab

- Picking materials and microscopes
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli* (for routine maintenance)
- 20˚C incubator (to store plates students pick to – maintenance should now be twice per week)
- Rubber bands and markers (for wrapping and labeling plates)
- P1000, P200, P20, and P2 pipettes and tips
- PCR tubes
- Buckets of ice
- Thermal cycler capable of generating temperature gradients
- Lysates (prepared by students the previous week)
- PCR reagents including dNTPs, Taq polymerase, Taq buffer, ultrapure water, $MgCl_2$ (if necessary) and 10 μM primers prepared the previous week
- Microcentrifuge tubes for preparing PCR master mix

Instructor Preparation

- Materials and plates listed above
- Well-fed plates of N2 and mutant *C. elegans* ordered from the Caenorhabditis Genetics Center (if available)
- Small aliquots of necessary PCR reagents
- Following lab session, store PCR reactions at -20˚C

Student Activities

- Obtain and maintain your mutant strain of interest (if available)
- Maintain needed strains – now to be done twice per week
- Determine expected PCR amplicon sizes using SnapGene software
- Perform a gradient PCR to test primer sets
- Plan out and schedule specific experiments using transgenic worms of interest
- Update lab notebook

Week 10: Gel Electrophoresis and Follow-up Experiments

Materials Used in Lab

- Picking materials and microscopes
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli* (for routine maintenance)
- 20˚C incubator (to store plates students pick to – maintenance should now be twice per week)
- Rubber bands and markers (for wrapping and labeling plates)
- P1000, P200, P20, and P2 pipettes and tips
- PCR tubes
- Buckets of ice
- DNA loading dye
- PCR reactions (prepared by students the previous week)
- 1% agarose gels and gel running apparatus
- 1 kb DNA ladder (or similar)
- UV transilluminator or other method of visualizing gels following electrophoresis
- Other materials needed depending on experiments planned by each group

Instructor Preparation

- Materials and plates listed above
- Well-fed plates of N2, transgenic, and mutant *C. elegans*
- Well-fed plate containing *C. elegans* males for use in genetic cross
- 1% agarose gel(s) in TAE containing ethidium bromide or alternative DNA binding dye

Student Activities

- Maintain needed strains (done twice per week)
- Analyze your PCR reactions using gel electrophoresis and discuss troubleshooting if necessary
- Begin your genetic cross
- Begin your planned experiments with transgenic and/or mutant worms
- Update lab notebook

Week 11-13: Continued Experiments

Materials Used in Lab

- Picking materials and microscopes, including one with fluorescence capabilities to aid in genotyping transgene-containing worms during the genetic cross
- 60 mm standard nematode growth medium (NGM) plates seeded with OP50 *E. coli* (for routine maintenance)
- 20˚C incubator (to store plates students pick to – maintenance should now be twice per week)
- Rubber bands and markers (for wrapping and labeling plates)
- Materials for worm lysis, PCR, and gel electrophoresis
- Other materials needed depending on experiments planned by each group

Instructor Preparation

- Materials and plates listed above
- Worms from student genetic crosses in progress

Student Activities

- Maintain needed strains (done twice per week)
- Freeze mutant strains and any crossed strains for long-term storage at -80˚C or in liquid nitrogen
- Continue your planned experiments with transgenic and/or mutant worms
- Update lab notebook, making sure to collect all necessary images and data for the final lab write-up

Week 14: Wrap-up and Presentations

Materials Used in Lab

- Materials needed depending on remaining experiments planned by each group

Instructor Preparation

- Materials depending on group needs

Student Activities

- Jigsaw style oral presentations
- Work with your group on the final lab write-up