Appendix 4. Reagent/Equipment List and Instructional Prep Sheets

Laboratory Week One: Using zebrafish as a developmental model system

*Three days, two days, and the night before class

- Prepare embryos and larvae at a variety of stages (ideally at least less than 24 hours, 24 hpf, and 48 hpf)
- clean petri plates
- 500 mL 1X E3 embryo medium (for 60X: 5 mM NaCl, 0.17 mM KCl, 10 mM HEPES, 0.33 mM $MgSO_4$, 0.33 mM $CaCl_2$, pH 7.8)
- plastic transfer pipets for removing dead or unfertilized embryos

*Day of Class

- Computer with internet access, Microsoft Office, USB port for camera
- stereomicroscopes
- at least one camera for the stereomicroscope (Celestron 44421)
- printer for captured images, if desired
- timer
- copy of developmental staging series images: https://www.swarthmore.edu/NatSci/sgilber1/DB_lab/Fish/fish_stage.html

Laboratory Week Two: Genotyping Zebrafish Part One

- 95°C heat block for 30-0.5mL tubes
- gloves
- freezer box
- Styrofoam ice bucket with lid and ice
- Scissors for parafilm
- small scissors for dissection (per table)
- Parafilm
- Eppendorf tube rack
- P1000, P200, P20, and P2 micropipets with the appropriate tips
- sterile 0.5 mL tubes
- squirt bottle of 70% ethanol
- kimwipes
- waste bag
- small pair of fine forceps
- masking tape
- fine point markers
- Blue dye tubes of 100 μL (1 per student)
- sterile deionized water
- 50mM NaOH, less than one week old
- petri plates (100 mm x 15 mm)
- 1X Tricaine in fish system water (for 25X stock solution: 153 □M ethyl 3-aminobenzoate methanosulfate (Sigma-Aldrich E10521))
- 1M Tris-HCl, pH 8
- Fish tanks
- Adult fish for genotyping, both mutants and wild-type as controls
- Net
- *Optional additional equipment: nanodrop to measure DNA concentration

Appendix 4. Reagent/Equipment List and Instructional Prep Sheets

Laboratory Week Three: Genotyping Zebrafish Part Two

- liquid nitrogen
- gloves
- Styrofoam ice bucket with lid and ice
- Sharps waste for razors
- small scissors for dissection
- sterile, or at least unused razor, for dissection
- eppendorf tube rack
- P1000, P200, P20, and P2 micropipets with the appropriate tips
- sterile 0.5 mL tubes
- squirt bottle of 70% ethanol
- kimwipes
- petri plates (100 mm x 15 mm)
- 1X Tricaine in fish system water (for 25X stock solution: 153 □M ethyl 3-aminobenzoate methanosulfate (Sigma-Aldrich E10521))
- waste bag
- small pair of fine forceps
- 2 fine point markers
- PCR tube rack
- PCR tubes
- Taq
- Sterile deionized water
- Fish tanks
- Adult fish from previous week
- Net
- 10 uM forward and reverse primers (ordered from Integrated DNA Technologies, diluted in sterile deionized water)
- genomic DNA from previous week
- 10 mM dNTPs (New England Biolabs Catalog # N0447)
- *Taq* DNA polymerase and buffer (New England Biolabs Catalog # M0273)
- Computer with internet access
- Thermocycler
- -80°C freezer

Laboratory Week Four: Genotyping Zebrafish Part Three

- P200, P20, and P2 micropipets with the appropriate tips
- 0.5 mL tubes
- eppendorf tube rack
- markers
- heat block set to 37°C
- agarose gel rigs
- power supply
- 1X TAE (for 50X: 2 M Tris, 1 M acetate, 50 mM EDTA, pH 8.6)

Appendix 4. Reagent/Equipment List and Instructional Prep Sheets

- PCRs from the previous week
- KpnI (New England Biolabs Catalog # R0142) with associated 10X CutSmart Buffer
- Sterile deionized water
- 100bp ladder (New England Biolabs Catalog # N3231)
- 10mg/mL ethidium bromide
- 2% agarose gels in 1X TAE with ethidium bromide
- Styrofoam ice bucket with lid and ice
- waste bag
- gloves
- UV transilluminator for imaging the gel
- Camera for imaging
- 6X loading dye (New England Biolabs Catalog # B7025)

Laboratory Week Five: Destroying Zebrafish Neuromasts with Copper Sulfate

*Six days before class

- Prepare embryos to be tested from each genotype. They should be 5 dpf for the day of class.

*Day of Class

- 20X Holtfreter's Buffer (120 mM NaCl, 1,8 mM $CaCl_2$, 13.4 mM KCl, and 200 mM HEPES)
- 10 mM copper sulfate
- parafilm
- scissors
- aluminium foil
- 1X E3 embryo medium (for 60X: 5 mM NaCl, 0.17 mM KCl, 10 mM HEPES, 0.33 mM $MgSO_4$, 0.33 mM $CaCl_2$, pH 7.8)
- 250 mL grad cylinders (must be very, very clean; rinse many times with di water)
- 10 mL pipets
- green roller pipet or pipet bulb
- P200 micropipets and associated yellow tips
- Marker
- 1 small petri plate (60 mm x 15 mm)
- 9 large petri plates (100 mm x 15 mm)
- 4 fish strainers (To make this, cut the pointed bottom off a 1.5 mL Eppendorf tube. Use a square of fine nylon mesh (approximately 2 cm^2, 150 micron mesh, brine shrimp or artemia net) to cover the bottom. Secure the mesh using a small rubber band.)
- 1 15mL conical tube (must be very, very clean; rinse many times with di water or use new ones)
- tweezers to hold the fish strainers
- paper towels
- masking tape
- 5 plastic transfer pipets
- deionized water

- *optional: 28.5°C incubator or room temperature incubator

*Two Days after Class

- 1.5 mL tubes
- marker
- Eppendorf tube rack
- Plastic transfer pipet
- 4% Paraformaldehyde/Phosphate Buffered Saline (for 10X PBS: 1.37 M NaCl, 27 mM KCl, 80 mM Na_2HPO_4, 20 mM $KHPO_4$, pH 7.4)
- refrigerator

Laboratory Week Six: Visualizing Neuromasts using Alkaline Phosphatase

- refrigerated larvae from last week
- 100 mL beaker for waste solutions
- P1000 micropipet and appropriate tips
- Eppendorf tube rack
- Waste bags
- PBS/0.3% Tween-20
- stereomicroscopes with ocular micrometer
- camera for microscope
- stage micrometer
- white porcelain spot plate with 12-wells
- plastic transfer pipets
- paraformaldehyde waste container
- staining buffer (50 mM $MgCl_2$, 100 mM NaCl, 100 mM Tris pH 9.5, 0.1% Tween-20)
- staining solution (must be made fresh, 2.25 µl NBT (Sigma-Aldrich Catalog # 11383213001) and 5.75 µl BCIP (Sigma-Aldrich Catalog # 11383221001) per mL staining buffer)

Laboratory Week Seven: Measuring Changes in Gene Expression (Part One: RNA Isolation)

- frozen tail fragments from week two
- P1000, P200, and P20 micropipets and appropriate tips
- Eppendorf tube rack
- Waste bag
- Qiagen RNeasy Mini Kit (Catalog # 74104)
- Gloves
- Styrofoam ice bucket with lid and ice
- Sterile 1.5 mL tubes
- Microcentrifuge
- 1 mL syringe with 22-25 gauge needle
- sharps waste for syringes
- waste beaker
- sterile RNase-free water
- 55°C heat block
- Styrofoam ice bucket with lid and ice

- -80°C freezer
- marker
- β-mercaptoethanol (14.3 M)

Laboratory Week Eight: Measuring Changes in Gene Expression (Part Two: RT-PCR)

- Styrofoam ice bucket with lid and ice
- Marker
- PCR tubes
- Thermocycler
- One Taq One-Step RT-PCR Kit (New England Biolabs Catalog # E5315)
- RNA from previous week
- 1:1 mixes of 10 μM forward and reverse primers (see lab protocol for sequences)
- P20 and P2 micropipets and appropriate tips
- Waste bag

Laboratory Week Nine: Measuring Changes in Gene Expression (Part Three: DNA Agarose Gel Electrophoresis)

- P20 micropipets with the appropriate tips
- agarose gel rigs
- power supply
- 1X TAE (for 50X: 2 M Tris, 1 M acetate, 50 mM EDTA, pH 8.6)
- RT-PCRs from the previous week
- 100bp ladder (New England Biolabs Catalog # N3231)
- 10mg/mL ethidium bromide
- 2% agarose gels in 1X TAE with ethidium bromide
- Styrofoam ice bucket with lid and ice
- waste bag
- gloves
- UV transilluminator for imaging the gel
- Camera for imaging and quantifying

Laboratory Week Ten: Mutation and Behavior

<u>*Six days before class</u>

- Prepare larvae to be tested from each genotype. They should be 5 dpf for the day of class.

<u>*Day of Class</u>

- Prepare one set of WT larvae so that they are already copper sulfate treated when class starts. Use the directions in lab five to do this. Students should have three samples of larvae: 1) *lin28a* mutants; 2) WT untreated larvae; and 3) WT copper sulfate treated larvae.
- Rheotaxis set up: In order to simplify the experiment in figure 1 and make it more affordable, we placed two 150 micron mesh barricades in a 2 L zebrafish tank. The tank had plastic tubing on either end to connect to a variable speed pump (Bio-Rad Catalog #1703644, purchased used from eBay), and the apparatus was filled with

fish system water. To better visualize the larvae, we placed the set up on piece of white paper.

- Cell phone camera
- Protractor
- Computer with Excel