

Instructor's Key for GloFish Protocol

Summary of Supplemental Materials

Supplemental Material 1	Animal training homework
Supplemental Material 2	GloFish Instructor's Key
Supplemental Material 3	Powerpoint introduction to GloFish
Supplemental Material 4	Introduction to GloFish laboratory
Supplemental Material 5	Worksheet for GloFish laboratory
Supplemental Material 6	GloFish laboratory homework
Supplemental Material 7	Rubric for grading worksheet/laboratory notebook

Time line

3 months-2 years before laboratory

Set up Parental fish in single pair matings and raise the resulting F₁ progeny to adulthood. Each clutch of F₁ progeny must be raised in a separate tank.

-Protocol for single pair matings:

<http://www.zfic.org/common%20techniques/mating.html>

-Protocol for raising fish from embryos to adulthood:

<http://www.zfic.org/common%20techniques/Raising%20baby%20fish.html> and

<http://www.zfic.org/common%20techniques/SOP.html>

-Protocol for labeling tanks:

<http://www.zfic.org/common%20techniques/tank%20labels.pdf>

-Protocol for assessing gender of adult fish:

<http://www.zfic.org/common%20techniques/Gender%20identification%20guide.pdf>

Week before the laboratory

Because this laboratory uses vertebrate animals, students need to complete any training required by the institution's Institutional Animal Care and Use Committee before starting. These requirements will vary from institution to institution. In our case, students complete three online training modules as a homework assignment, and then turn in the signed homework to document completion of this training (Supplemental Material 1).

Day of the laboratory

Move materials into the teaching laboratory:

1. Tanks containing F₁ progeny for analysis
2. Smaller tanks for sorting progeny into phenotype groups and counting
3. Several liters of fish water for filling smaller tanks
4. 4-5 nets for each tank of progeny
5. In class copies of the introduction to the laboratory (Supplemental Material 4)
6. Copies of the GloFish Worksheet for each student (Supplemental Material 5)
7. Copies of homework for each student (Supplemental Material 6)
8. If doing the gender part of the experiment, in class copies of the protocol for assessing gender of adult fish: <http://www.zfic.org/common%20techniques/Gender%20identification%20guide.pdf>

Example Outline for class period (this outlines assumes this is the first laboratory of the semester):

I. Introductions of class members-instructor, undergraduate TA(s) and students

II. Collect animal training homework (Supplemental Material 1). Students need to have this complete to work with the adult fish. If they do not have it completed, they need to go find a computer, come back, and finish as much of the lab as they can.

III. Go over Laboratory notebook expectations

A. Laboratory notebook rubric (Supplemental Material 7)

The laboratory notebook is what you write on in class to make sure you are recording everything needed about your experiment. It is much less formal than a laboratory report.

B. Keep laboratory notebook in 3 ring binder (optional but strongly encouraged)

1. Go through example notebook

2. Note that sections delineating each laboratory will make it easier to use and grade

3. Note that the laboratory worksheets can be incorporated with extra sheets, keeping everything from one experiment/laboratory together

C. Trying something new-laboratory notebook entry for this laboratory as a worksheet (Supplemental Material 5). Students cannot leave the lab (except for short breaks) until they show the instructor the completed worksheet. <-We use our own judgments on this one. We only do this if students are trying to rush through the laboratory. If we have this impression, we look at the students' laboratory notebooks when they think they are done and note places they should be more complete.

D. Lab notebooks will be collected at various times during the semester for grading-they should be kept up to date at all times

IV. Policy about lab handouts

A. Students will all get an electronic copy-they can print a copy for themselves if they wish

B. There will be "in class" copies of the laboratory handouts that they can use while they are in lab

V. Safety

A. Point out safety features of the laboratory

1. eye wash station

2. safety shower

3. first aid kit (on top of fridge)

4. sharps waste

5. glass waste

6. soap by sink-should wash hands with soap at the end of each lab

7. gloves not required to work with fish, but students should wear gloves if they have any injuries on their hands or any other concerns

B. There are no chemical safety concerns for this lab. Students must wash their hands with soap after every laboratory. It is especially important for this one, as there will be fish waste in the water.

- C. Students should wash any materials they used by rinsing them well in hot water and leaving them by the sink to dry. Everyone is responsible for washing their own dishes.
- VI. PowerPoint introduction to the laboratory (Supplemental Material 3)**
- VII. Handouts for today**
- A. Homework for GloFish laboratory (Supplemental Material 6)-due the following week at the start of lab
- B. Worksheet for the GloFish laboratory (Supplemental Material 5)
- C. Laboratory notebook rubric (Supplemental Material 7)
- VIII. In class materials for today**
- A. Introduction to laboratory for your reference (Supplemental Material 4)
- B. Guide to identifying males and females
(<http://www.zfic.org/common%20techniques/Gender%20identification%20guide.pdf>)
- IX. Discussion about how to work together to gather data for today (use whiteboard to record ideas and final plans)**
- A. Six tanks that need to be sorted
1. By phenotypes
 2. Number of fish with each phenotype
- B. 5 minutes, everyone go around and look at each tank
- C. Class discussion-we have hundreds of fish to sort-how do we do this efficiently and accurately?
- students told they have nets, extra tanks, extra water
 - We lead the students through the idea of efficiency and accuracy-we try to lead them to the idea that the fish in each tank should be scored at least twice to ensure accuracy (identify all phenotypes, and count how many fish have each phenotype). For example, this year the students came up with the plan that each small group (3-4 students formed a small group at each table) would sort one sibling group, and then each other small group would recount and confirm the initial sorting and counts.
- D. Class discussion
- We had every group sort two tanks by gender (one with a ~1:1 male:female ratio, and the other with a ratio very skewed towards one gender) so that all students learned how to identify males and females (ex: Figures 9 and 10, Tables 7 and 8, Supplemental Figures 1 and 5). This was good preparation for the last part of the laboratory, when the students set up their own single pair mating (see below).
- F. Demo on how to safely net fish
- one net/tank of fish to prevent spread of disease
 - cover net with hand to prevent fish from jumping out
 - move fish as short a distance as possible
- X. Send students out to sort fish (takes 1-2 hours for 6 tanks)**
- XI. Class discussion**
- A. Pool data for each cross on classroom whiteboard

- B.** If not all groups agree, then instructor can help with any discrepancies
 - wildtype males often have a yellow tint that can be confused with a Glo^{YFP} positive fish
 - orange and red fish can be hard to distinguish unless they are right next to each other
- C.** Class chooses one of the crosses to analyze together-go through all of the steps in the chi-square analysis, calling on students to help with each step
- D.** Students spend the rest of the laboratory working in their small groups to complete the chi-square analysis on the rest of their crosses
- XII.** At end lab, move fish back into their home tank

Two weeks later (or later in the semester)

Move materials into the teaching laboratory:

1. Tanks containing F₁ progeny
2. 1 mating tank for each student
3. Several liters of fish water for filling mating tanks (need ~1 L for each mating tank)
4. 4-5 nets for each tank of progeny
5. colored tape for labeling tanks
6. Sharpie markers for writing on tape
7. In class copies of the protocol for single pair matings:
<http://www.zfic.org/common%20techniques/mating.html>
8. In class copies of the protocol for labeling tanks:
<http://www.zfic.org/common%20techniques/tank%20labels.pdf>
9. In class copies of the protocol for assessing gender of adult fish:
<http://www.zfic.org/common%20techniques/Gender%20identification%20guide.pdf>

Example class outline (this will take < 1 hour, and so is typically done during a class period when we also start another laboratory)

- I.** Hand back graded GloFish homework (Supplemental Material 6): This homework includes their experimental design for testing one of the hypotheses from the GloFish laboratory
- II.** Instructor guided discussion of ideas generated from GloFish homework
 - A.** Students share ideas for hypothesis to be tested and experimental design
 - B.** For example, many students chose to test the hypothesis that Glo^{YFP} and Glo^{RFP} are incompletely dominant by crossing a red fish with a yellow fish to determine whether this cross yields orange progeny
- III.** Instructor briefly takes students through the process of correctly setting up a zebrafish mating
 - A.** Reminder about good practices for netting fish
 - B.** Go through protocol for setting up single pair matings
 - C.** Go through protocol for labeling tanks
- IV.** Each student sets up one single pair mating designed to test one of their hypotheses

Next day:

- Instructors put adult fish back in their home tanks
- Instructors collect eggs and raise these fish to adulthood
(<http://www.zfic.org/common%20techniques/Raising%20baby%20fish.html>) and
<http://www.zfic.org/common%20techniques/SOP.html>

Last day of class for the semester:

-students examine the progeny of their crosses to see if their hypotheses were correct

Pedagogy

Learning Objectives

1. Students begin to develop skills to efficiently and accurately gather data and work in a group
2. Students begin to learn the rules for doing research with vertebrate animals
3. Students gain hands on experience with the relationship between genotype and phenotype
4. Students learn how to form a hypothesis based on phenotypes present in F₁ progeny
5. Students learn how to test a hypothesis using chi-square analysis
6. Students learn how to interpret a P-value
7. Students begin learning how to design a genetics experiment

Summary of Assessments

1. Animal training homework (Supplemental Material 1)
2. GloFish laboratory worksheet (Supplemental Material 5)
3. GloFish homework (Supplemental Material 6)

Common Problems

1. Organizing to efficiently and effectively gather data

The number of fish that need to be counted and scored in this laboratory poses an organizational challenge. The first year that this laboratory was taught, the students were not given any guidance and each group spent a long time sorting each tank. Many students felt that this was “busy-work” that did not contribute to their learning. Towards solving this problem, this year we started with a group discussion during which the students generated a plan to efficiently collect their data. This turned out to be a good learning experience, as it established a cooperative atmosphere in the class and brought out important ideas such as making sure the data was accurate by having each sibling group scored and counted at least twice.

2. Difficulties identifying phenotypes

Wildtype males often have a yellowish cast that can make them similar in phenotype to fish carrying the Glo^{YFP} transgene. A good way to determine whether a fish is carrying a Glo transgene is to have a “fluorescent lantern” available to determine whether the fish is fluorescent (for instance, see <http://www.blacklight.com/items/UVPUVL4F>).

3. Difficulties calculating expected numbers for each phenotype group

Many students found calculating the expected number of progeny for each phenotype group in the trihybrid crosses containing both the Glo^{YFP} and Glo^{RFP} transgenes very challenging. Going through a example analysis or two in class was sufficient to solve this problem. There were two places that students especially needed guidance. The first was making sure they used good nomenclature for the phenotypes and matching genotypes. For example, it was important to first go through the hypotheses for each body color (red fish have Glo^{RFP} but not Glo^{YFP}, grey fish have neither transgene, etc.). In addition, it helped to

line up all of the calculations for each progeny group in an ordered array to prevent mathematical mistakes (see below for cross in Figure 8):

Phenotype	Fin phenotype	Yellow phenotype	Red Phenotype	Final fraction
grey, short finned	$\frac{1}{2}$ short	$\frac{1}{2}$ not yellow	$\frac{1}{2}$ not red	$\frac{1}{8}^{\text{th}}$ of progeny
grey, long finned	$\frac{1}{2}$ long	$\frac{1}{2}$ not yellow	$\frac{1}{2}$ not red	$\frac{1}{8}^{\text{th}}$ of progeny
yellow, short finned	$\frac{1}{2}$ short	$\frac{1}{2}$ yellow	$\frac{1}{2}$ not red	$\frac{1}{8}^{\text{th}}$ of progeny
yellow, long finned	$\frac{1}{2}$ long	$\frac{1}{2}$ yellow	$\frac{1}{2}$ not red	$\frac{1}{8}^{\text{th}}$ of progeny
orange, short finned	$\frac{1}{2}$ short	$\frac{1}{2}$ yellow	$\frac{1}{2}$ red	$\frac{1}{8}^{\text{th}}$ of progeny
orange, long finned	$\frac{1}{2}$ long	$\frac{1}{2}$ yellow	$\frac{1}{2}$ red	$\frac{1}{8}^{\text{th}}$ of progeny
red, short finned	$\frac{1}{2}$ short	$\frac{1}{2}$ not yellow	$\frac{1}{2}$ red	$\frac{1}{8}^{\text{th}}$ of progeny
red, long finned	$\frac{1}{2}$ long	$\frac{1}{2}$ not yellow	$\frac{1}{2}$ red	$\frac{1}{8}^{\text{th}}$ of progeny