ANIMAL USAGE FORM – TEACHING/TRAINING PROTOCOL

Version 3.0
Updated 24 July 2006

Download the Latest version of this form and applicable appendices at
http://www.research.umn.edu/subjects/animals/form.htm

Instructions

1. This form must be typewritten.
2. Fill out all of the questions on this form completely. (If there are questions about using the text form fields or checkboxes with this form, please contact the RSPP office 612-626-5654 or iacuc@umn.edu)
3. Fill out and attach the appropriate appendices required by responses in this application.
4. Complete the checklist that accompanies this form to assure all requirements for submission are completed so that review is not delayed.
5. Submit the following to the Research Subjects' Protection Programs Office (RSPP):
   a. The original application form and appendices;
   b. 12 copies of these forms (these may be copied double-sided); and
   c. 1 copy of the grant.

Mail to:
Research Subjects' Protection Programs
MMC 820
420 Delaware St. SE
Minneapolis, MN 55455-0392

On campus:
Research Subjects' Protection Programs
MMC 820
Minneapolis Campus

Or Deliver to our Office:
D-528 Mayo Memorial Building (map)

List of Appendices

Appendix A – Alternatives to Animals - Classified in Categories B or C
Appendix B – Protocol Specific Breeding
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Appendix N – Criteria for Euthanasia of Animals used in Research, Teaching & Display
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Appendix Q - Food Chain Review if animals are going to slaughter or into the human food chain

Useful Guidelines from the RAR Web site:

- ***RAR Housing and Husbandry Guidelines for Laboratory Animals***
- ***RAR Guidelines for the Use of Anesthetics, Analgesics and Tranquilizers in Laboratory Animals***
- ***RAR Euthanasia Guidelines***
- ***RAR Surgery Requirements***

DO NOT SUBMIT WITH APPLICATION

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Version 3.0
Updated 24 July 2006

IACUC Use Only					
IACUC Study #		Approved:		Approval Duration:	
IACUC Chair:			RAR Veterinarian:		

0. Project Identification and Signatures

0A.Type of Application: ☒**New Protocol** ☐**3-year Renewal of IACUC #**_____
(If this is a 3-year renewal, do not use language referring to the previous protocol or grant in this form.)

Anticipated Starting Date:	2/1/09

0B. Project Title: (Project title must match grant title. If different, also provide grant title) Title must have the course number in it.

Teaching Protocol: Genetics Laboratory (Biol 2202) and Developmental Biology (Biol 4361)

0C. Is this an Agricultural Teaching Project? (use of agricultural animals in non-biomedical research) ☐Yes. ☒ No.

0D. Instructor (must be faculty or academic professional administrative staff)

Name (Last name, First name MI):	**Liang, Jennifer O.**	
Mailing Address: 1035 Kirby Drive Duluth, MN 55812	**Phone Number:** 218-726-7681	
	Pager or Cell Phone Number: 218-355-8714	
Email: joliang@d.umn.edu	**U of M x.500 ID** (ex. smith001)**:** joliang	
Department: Biology	**Fax:** 218-726-8142	
Occupational Position: ☒Faculty ☐Staff		
Instructor Certification: If the IACUC approves my application, I agree to execute this work as described; request approval from the IACUC for changes; comply with the guidelines set forth by the IACUC and Research Animal Resources (RAR); follow Environmental Health and Safety guidelines; and be responsible for the supervision and work of my staff. If appropriate, this application accurately and completely reflects the animal use in the full grant application. The activities described in this study do not unnecessarily duplicate existing experiments.		
Original Signature	**Title of Instructor**	**Date**

Note: ***If PI is not a University of Minnesota faculty member, IACUC may notify you that additional signatures will be required.***

0E.Person preparing this document

Name: Jennifer O. Liang	**Phone number:** 218-726-7681	**Email:** joliang@d.umn.edu

0F. Source of Funding for Teaching, if applicable

Name of funding source:	None-teaching protocol

Grant: ☐Will be submitted.
☐Submitted.
☐Approved. If approved, what is the duration of approval: ☐5 years ☐Other:_____

If you are receiving external funds for this research, include an Appendix O for each source of funding.

0G. Teaching Personnel who will have animal contact (do not include students here, but all teaching staff and faculty, in addition to any outside consultants etc.)

Prior to approval being granted, everyone listed below must have attended an orientation session on animal use at the University of Minnesota (Schedule for sessions is at http://www.ahc.umn.edu/rar/seminars.html). Indicate the role each individual has in the project: co-investigator, technician, etc.

Name (Last Name, First Name MI)	**Role in Project** (i.e. Co-investigator, technician)	**Phone number**	**U of M x.500 ID** (ex. smith001)
Liang, Jennifer O.	**Coursemaster**	**218-726-7681**	**joliang**
Pung, Janel	**Teaching Assistant**	**218-726-6853**	**pungx008**
Gordon, Rebecca	**Teaching Assistant**	**218-726-7963**	**gord0207**
Hildebrandt, Lauren	**Teaching Assistant**	**218-726-8433**	**hild0213**
Eklund, Derek	**Teaching Assistant**	**218-726-6262**	**eklu0040**
Sloan, Jami	**Teaching Assistant**	**218-726-6125**	**joh04375**

0H. Personnel to receive correspondence from IACUC

The listed personnel will be included in e-mail and mail correspondence emanating from IACUC to the Principal Investigator. *(**Note:** Co-PIs listed above will receive only select correspondence.)*

Name (Last name, First name MI): Liang, Jennifer O.	
Mailing Address: 1035 Kirby Drive, Rm 207 Duluth, MN	**Phone Number:** 218-726-7681 **Fax:** 218-726-8142
Email: joliang@d.umn.edu	**U of M x.500 ID:** joliang

Name (Last name, First name MI):	
Mailing Address:	**Phone Number:** **Fax:**
Email:	**U of M x.500 ID:**

1. Specific Aims & Details of Animal Use

1A. What is the goal/specific aim of the course(s) and why is the use of animals necessary to achieve it? Syllabus must be attached.
Jargon should be avoided or explicitly explained (please define all acronyms).

Much of the excitement of being a scientist comes from discovering something new about how the world works. In this course, students will have the opportunity to carry out a simulated forward genetic screen using zebrafish, a relatively new model organism that has many advantages for teaching. Zebrafish embryos are easy to obtain in large numbers, even up to thousands of embryos a day. Because the embryos and egg shells are translucent, embryonic development can observed in vivo using simple dissecting microscopes. Finally and mutants and strains that express fluorescent proteins in specific tissues can be used to demonstrate principles of development and genetics.

1B. If this course is a continuation of an ongoing course, please state concisely if and how these goals differ from those described in the original IACUC application for the course.

The goals of this course have not changed, but the addition of my zebrafish expertise to the Department offers new opportunities for students to learn genetics and development using this powerful model system.

1C. Provide a complete and accurate description of what procedures will be performed on/with the animals. Answer in lay language or language understood by a person unfamiliar with your area of expertise (*define all acronyms*). Jargon should be avoided or explicitly explained. *Do not cut and paste from a syllabus or include language or explanations that are not relevant to animal use.*

Provide sufficient detail to allow evaluation by the IACUC. You are strongly encouraged to use a diagram or chart to explain complex designs. **(Use additional pages if needed)**

- *Clearly and separately describe each procedure or use of an animal. Describe what exactly will be done to the animal (include Appendix F if it involves a surgery), who will be doing it (student or faculty/staff. If both students and instructors will be involved in a given procedure or animal use, clearly describe who will be doing which parts. Include the frequency and time points over the course that each procedure or use will take place. Be certain to detail the pain classification of each animal group. This should correspond to the information you provided in the **Animal Request Table** (Section 1).*
- *If the course includes options for the students (i.e. they have the choice of doing one of two procedures etc.) make this clear in the description.*
- *Include how long the animals will be maintained. Include dose, route of administration and frequency of any drugs to be administered.*
- *Describe methods used in behavior studies (including use of noxious stimuli or other methods of positive or negative reinforcement).*
- *Surgery should be described here only as it relates to the study design. Surgical details should be provided in Appendix F.*

- *For animals used in agricultural projects, you may reference the study code number of the IACUC approved Standard Operating Procedures for the housing facility and husbandry, as applicable.*

Students, Teaching Assistants, and the Coursemaster will set up natural matings of adult fish throughout the semester. Note that adult fish can be set up in matings once or twice a week, so the same adult fish can be used many times during the semester. This is the only protocol that will be used, all experiments will be done on the embryos produced, which are too young to fall under IACUC oversight, as they do not yet have backbones.

Natural matings: see attachment at the end of this form for protocol and table explaining number of fish to be used.

2. Animal Contact

All live animal work conducted under teaching/classroom protocols must be supervised at all times by University faculty or staff listed in this Animal Usage Form. It is the Principal Investigator's responsibility to assure that all participants are properly trained in animal handling and the procedures conducted as part of this protocol. Please contact Dr. Ann Fitzpatrick, Office for Regulatory Affairs, 612-625-0499 if you require training, or assistance with training.

Prior to using live animals, all classroom participants should be instructed in the ethical use of animals in research, teaching or testing. **Please check the method you will use to accomplish this goal:**

☒ All participants will be required to review Part 1 of the tutorial:
http://www.iacuc.umn.edu/training/tutorial/viewTutorial.cfm?view=part1

☐ Request a member of the Research Subjects' Projection Program, IACUC or Office of Regulatory Affairs to make a brief presentation covering the ethical use of animals in research, teaching or testing.

3. Animal Genus, Numbers, and Classification by Stress Levels

Classification A: No pain, distress or use of pain-relieving drugs: Examples include post-mortem tissue harvest; and routine procedures causing only transitory discomfort such as venipuncture, injections, ear tagging, use of non-inflammatory adjuvants, etc.

Classification B: Pain/distress WITH appropriate analgesia/anesthesia/tranquilizers. Procedures involving accompanying pain or distress to the animals and for which the appropriate anesthetic (for surgery), analgesic (for inflammation or pain), or tranquilizing drug are used. **You must complete Appendix A.**

Classification C: Pain/distress WITHOUT analgesia/anesthesia/ tranquilizers. Procedures involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesics or tranquilizing drugs would adversely affect the procedures, results or interpretation. **You must complete Appendix A.**

Animal Request Table

Genus	Class A, B, or C (See above.)	Total # of animals to be used over 3 year period: Purchased (Or received from another institution)	Transferred**: From IACUC study code #*	Transferred**: # of Animals	Produced by in-house breeding*	Other (Specify: captured wildlife, observation)	Total
Danio rerio	A	180			900		1080

If more than one genus is listed, answer questions 5-7 of this form for each genus.

** Also include the IACUC Study Code Number from which the animals will be transferred (ex. 0202A12345 | 50). This form does not transfer the animals to this protocol once it is approved. **The Protocol Transfer Request Form (http://www.ahc.umn.edu/rar/transfer.pdf) *must* be completed and submitted to RAR.**

*Notes on breeding & transferring:

If you intend to breed animals for use by multiple researchers or multiple protocols, complete the *Breeding Protocol Form* to cover the breeding and continue completing this from to cover the experimental use of the animals.
If you will be transferring animals from a different IACUC protocol (including breeding protocols) to this experimental protocol include these animals in the "Transferred" column, not the "Produced by in-house breeding" column, along with the existing IACUC Study Code Number (ex. 0202A12345 | 50). The numbers proposed and justified in this application should only apply to the experimental animals.
If you will complete and submit a breeding protocol along with this application, fill in the number of animals that will be transferred from that breeding protocol to this IACUC study in the "Transferred" column and leave the "From IACUC study code #" column blank.
If you intend to breed animals for use in only this protocol, complete only this form listing the total number of bred animals in the "Produced by in-house breeding" column and include Appendix B.

Please call the RSPP Office at 612-626-5654 if you have any questions about how to complete either form.

4. Housing, *if applicable*

4A. Facility (building and room #) where animals are or will be housed: (such as RAR, Duluth Campus, Animal Science, Experimental Stations – specify, or other – specify)

Building	Room number	Campus
Swenson Science Building	105	Duluth
Swenson Science Building	66	Duluth

4B. Will animals be held outside of a centrally managed housing area for more than 12 hours?
(such as RAR, Duluth Campus, Animal Science, Experimental Stations – specify, or other – specify)

☐ **Yes.** Include Appendix D and fill in the table below.
☒ **No.**

Building	Room number	Campus

4C. If live animals will be removed from the above areas, please indicate the procedures to be performed, the location and name of contact person.

Procedure(s) to be performed:	
Building:	**Room:**
Name of contact:	**Phone number:**

4D. Will animals be transported through public places by anyone other than RAR?

☒Yes.
☐No.

If yes, complete the following:

From where to where will animals be transported?	From SSB Rm 66 to SSB Rm 105
Via what route will the animals be transported?	From Rm 66 to the research wing elevator (down 1/2 of a hallway), into the elevator to the 1st floor, and then out of the research wing and down another hallway to Rm 105
Who will transport the animals?	Dr. Liang or the Teaching Assistants
What equipment will be used to transport them?	A cart and plastic fish tanks
At what time(s) will transport occur?	During regular business hour
Contact RAR for appropriate transportation procedures at 612-624-6169	

5. Justification for Animal Species and Number Requested

5A. Describe the features of the species (e.g., anatomic, physiologic, genetic, etc) that make it desirable for this teaching/training. Contrast with other available models, if any.

Zebrafish embryos are easy to obtain in large numbers, even up to thousands of embryos a day. Because the embryos and egg shells are translucent, embryonic development can observed in vivo using simple dissecting microscopes. Finally and mutants and strains that express fluorescent proteins in specific tissues can be used to demonstrate principles of development and genetics.

5B. How are the number of animals requested justified for this teaching/training objective? (check all that apply and answer the subsequent questions):

☐ **Animal numbers determined by the number of students expected in the course. Explain your rationale for choosing the number of students per animal.**

☐ **Group sizes determined statistically. What statistical analysis was performed including the analysis employed and the power function?**

☒ **Group sizes based on quantity of harvested cells or amount of tissue required. Explain how much tissue is needed based on the number of experiments you will conduct and how much tissue you expect to obtain from each animal:** (Suggestion: "The study requires 50 experiments." is not sufficient.)

Six strains (WT, casanova; actin:GFP double mutants, cyclops, squint, one eyed pinhead, albino) of zebrafish will be used for these courses, giving students the opportunity to observe mutants with different developmental defects and different kinds of mutations (dominant, recessive, maternal zygotic, incompletely penetrant). Since the fish are used only for natural matings, the same fish will be used for both courses.

At least three large (10L) tanks are maintained at one time for every strain of fish. Every 10L tank can hold up to 60 adult fish, so at least 180 adult fish are maintatined of each strain at all times. This is important, as many factors (many unknown) affect the egg laying rate of the adult fish. Further, zebrafish do not have sex chromosomes, and it is not uncommon to get tanks that are almost all female or almost all male. Finally, adult fish can only be set up to mate once per week. Thus, having three adult tanks of every strain ensures that we will always have a laying population to generate embryos for the students to use in their experiments.

A new tank of fish is started for each strain approximately every 6 months, so that we always have fish in young adulthood, when they are most likely to produce lots of eggs.

☐ **Product Testing. If the number of animals needed is based on FDA guidelines, provide the citation from the regulations:**

☐ **Other - Elaborate, indicating criteria used to determine group size:**
(Suggestion: "This is the number used in the previous studies." is not sufficient. Statistical analyses should be available from prior studies.)

6. Potential Animal Pain and Distress

6A. What are the potential specific study-induced or related problems the animals might experience (i.e. health problems, pain, distress, complications, etc) OR any health problems due to the phenotype of the animal?

1. Describe the potential problems:

There should not be any affects from natural matings of the adult fish. In fact, regular mating the fish is good for their health. In particular, female fish that are not mated regularly tend to get "egg bound"- they get a build up of unlayed eggs that sometimes prevents theom from mating successfully.Occasionally, we get a sick fish in the fish facility, and these are removed from their home tank and either treated with appropriate medicines or euthanized to prevent the spread of any disease.

2. Do you expect these problems to occur?

☐ **Yes.** Answer 2a-c.
☒ **No. Explain the basis for this assessment:** (eg. prior experience, etc.)

See answer to #1 above

2a. How will pain and/or distress be monitored? Provide the specific clinical signs which will be monitored as well as the frequency of monitoring, including provisions for off hours. Please note that animals housed outside of centrally managed facilities must be monitored daily.

The adult fish are monitored twice a day during their feeding. Feeders look into every tank and remove and dead or sick fish, and then either treat or euthanize the sick fish.

2b. Will this monitoring include weekends and holidays? (In addition to routine RAR monitoring)

☒ **Yes.**
☐ **No.**

2c. Explain what steps will be taken to alleviate any pain, distress or discomfort the animals may experience. Provide the dose, route of administration, frequency, and type of analgesic drugs or tranquilizers to be administered. (Suggestion: Consider warming pads, fluids, soft bedding, etc.)

Not applicable

6B. Will cells, tissues, or body fluids be inoculated?

☐ **Yes.**
☒ **No. Continue to section 6.**

If yes, have they been screened for the presence of human or animal pathogens?

☐ **Yes.** Please provide documentation**.**
☐ **No.** RAR must be consulted to provide testing or to determine if there are special housing needs. Describe that consultation below:

7. Euthanasia/Disposition of Animals

7A. What will determine the natural endpoint(s) for using animals in this course? (i.e., time points, tumor size, etc.)

The fish are maintained as breeding stocks, and thus are kept until they are too old to mate and are exhibiting signs of old age. The life span of zebrafish is about two years. As fish are approaching this two year mark, we notice a decrease in their rate of reproduction, a decrease in their weight ("skinny fish"), and bends in their backbone. These fish are euthanized as below as a humane practice and because elderly fish are more likely to get diseases that could be passed on to other fish in the facility.

7B. Will the animals be euthanized at the end of the course?

☐ **Yes.**
☒ **No.**

If yes, specify method, agent and dosage and route of administration to be used for euthanasia for this species:
Euthanasia must be in accord with the methods approved by the AVMA Panel on Euthanasia. Note that the AVMA Panel does not allow cervical dislocation without anesthesia, unless scientifically justified. Please make sure to include the anesthetic regimen if proposing to use cervical dislocation. In addition, the AVMA panel on euthanasia does not allow dry ice as a source for carbon dioxide. If you choose to use carbon dioxide please confirm that you will use compressed carbon dioxide gas in cylinders.

If no, describe their final disposition:

Since the adult zebrafish are used only as mating stocks, they are very likely to be transferred to another protocol or used in a continuation of this protocol. They will be euthanized when they are getting old and can no longer be used in any of our research projects.

7C. Animals that are experiencing unrelieved pain or distress prior to the defined experimental endpoint must be humanely euthanized, unless doing so would interfere with, or compromise the scientific goals of the experiment. Do the guidelines interfere with your experimental objectives?

(Clinical signs of pain or distress that require euthanasia are listed in Appendix N)

☒ **No.** Initial and date that IACUC guidelines listed in Appendix N have been read and will be followed for early euthanasia.

PI Initials	**Date**

☐ **Yes.** Provide the criteria to be used by the PI to determine that euthanasia would be required prior to the end of the study AND provide scientific justification indicating why an earlier endpoint cannot be used:

7D. In the unexpected event that an animal meets these euthanasia criteria prior to the designated study endpoints, describe procedures to euthanize the animal: (provide agent, dosage, and route) ***Include who will be responsible for the euthanasia, if applicable.***

Fish will be euthanized by Dr. Liang

To euthanize, aquatic system water will be placed in a 2.75 L tank and ice added to make an ice-water bath. An insert will be placed into the bath to prevent fish from coming into direct contact with the ice. The fish will be netted within this insert in the water bath. The fish will be left in the ice water bath for 15-20 minutes, until the fish are dead, and then the carcasses will be disposed of according to our protocol.

This method of euthanasia is preferred as it does not expose laboratory members to any chamicals. The 2000 Report of the American Veterinary Medical Association Panel on Euthanasia does not approve this method for euthanisia of fish species in general, but does not address the specific case of tropical species, which have little or no ability to adapt to the cold. This deficiency was addressed in 2002 by the University of Washington IACUC committee: “Because tropical fish species, (i.e. zebrafish, medaka, and platyfish), have minimal to no physiologic adaptation mechanism for adjusting to cold (4°C) water, cooling to 4°C should be considered an acceptable method of euthanasia since the rapid decrease in temperature from 26°C (or higher) to 4°C induces rapid loss of consciousness and is lethal to these species.”

The full text of this report can be found at:
http://depts.washington.edu/compmed/iacuc/policies/fish_euthanasia.html.

Therefore, a cold water bath fulfills the requirements of the Panel on Euthanasia that a method of euthanasia cause rapid loss of consciousness, and minimize pain and distress.

Checklist for submitting a complete application

This checklist must be included as part of your application. Check all that pertain to your project. Where indicated complete the appropriate appendices and attach as part of your application.

☐ **Alternatives to animals classified in categories B or C - *Appendix A***

☐ **Breeding of animals - *Appendix B*** (see instructions in section 1 to determine if completing a separate breeding protocol is necessary)

☐ Use of Controlled substances – *Appendix C*

*****Important note regarding the use of non-pharmaceutical grade drugs*****

Investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute procedures. Non-pharmaceutical-grade chemical compounds should only be used after specific review and approval is granted by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings alone are not an adequate justification for using non-pharmaceutical grade compounds in animals. See http://www.aphis.usda.gov/ac/policy/policy3.pdf

☐ **Housing of animals outside of the primary housing area (> 12 hours) - *Appendix D***

☐ **Surgery – Please check: ☐Survival surgery. ☐Non-survival surgery. - *Appendix F***

☐ **Use of these specific agents in animals - *Appendix G***

☐ **Part I: Hazardous Chemicals**
☐ **Part II: Radiation**
☐ **Part III: Infectious agents and work with human blood and body fluids**
☐ **Part IV: Recombinant DNA including transgenic mice**

☐ **Immunization, antibody or ascites production, or collection of other body fluids - *Appendix H***

☐ **Pharmacologic/toxicologic studies - *Appendix I***

☐ **Dietary manipulations or fluid restriction - *Appendix J***

☐ **Conscious restraint for more than one hour - *Appendix K***

☐ **Free-ranging wildlife - *Appendix L***

☐ **Client-owned animals - *Appendix M* Client Consent Form**

☐ **Receiving external funds for this research - *Appendix O***

☐ **Animals are sent to slaughter or put into the human food chain – Appendix Q**

You have reached the end of this form. Please make sure that you have responded to every question on this application (even if your response is "not applicable") and that you have filled out all of the applicable appendices.

Strain	# purchased	# raised in house
WT from pet store	180	
casanova/ actin:GFP		180
squint		180
cyclops		180
one eyed pinhead		180
albino		180

Animal usage table for Teaching IACUC protocol from Dr. Liang.

Adult fish included in my research protocols (big time, other wildtype strains) may also be used in these courses when the fish are not being used for research.

Standard Operating Procedures-Aquatic Facility and Fish Maintenance Including raising fish to adulthood

Animal Housing
Liang laboratory aquatic zebrafish facility
University of Minnesota-Duluth
66 Swenson Science Building
1035 Kirby Drive
Duluth, Minnesota 55812

Emergency Contact: Jennifer Liang 218-355-8714 (cell phone)

Animals: Danio rerio (Zebrafish)
Housed: 66 Swenson Science Building
Room Temp: 76 F, Water Temp 82 F
Lights: 14 hour light: 10 hour dark, controlled by a digital timer

Research Personnel:

- Jennifer Liang (joliang@d.umn.edu)
- Po-nien Lu (boblutw@gmail.com)

Feeding
Frequency:
All the zebrafish should be fed 2 times daily.
AM feedings are done with Brine Shrimp and Paramecia
PM feedings are done with Flake Food and Paramecia
If there is food left over after 10 minutes from feeding the amount given was too much and should be corrected the following time.

Paramecium
Paramecia are very small organisms that are found in freshwater environments. They are only fed to babies 5 days old to 14 days old.

1. Filter a container full of mature paramecia culture through a cloth-the filtrate (the part that passes through the cloth) will be fed to the baby zebrafish. A mature culture will be about 1 week to 2 weeks old.

2. Put a few drops of the filtered culture in a petri dish and examine it under the dissecting microscope. You should see lots of paramecia in your field of vision, and very few other critters. If this is not what you see, through this batch away down the sink, and filter the next culture. If the next culture is also bad, come find Dr. Liang or someone else so we can order a fresh paramecia culture.

3. Turn off the water to all of the tanks with baby fish in them (they will all have a white sheet taped to the front of the tank).

4. Feed the baby fish. Look on the white sheet for the feeding directions-amount and kind of food. Typically, a large (10L) tank of baby fish in the 5-14 day old range will get 60 ml of paramecia culture twice a day.

5. When finished, you need to start a new batch of paramecia growing. First, scrub out the paramecia container you used with tap water and a brush.

4. Fill the container with RO (reverse osmosis) water and a 1/8 tsp of salt.

5. Add about 12 drops of Liquid Fry© along with 50mls of left over paramecia.

6. Put the lid on loosely and place container at the very end of the line of all the containers that need to be fed. Date the container.

7. Wait 15-30 minutes, and then turn the water to the tanks back on.

Brine Shrimp

Brine Shrimp are a bit less small organisms that are found in saltwater and are often referred to as Sea Monkeys in the back of comic books. They can be bought as eggs from various companies and then raised in the lab. Brine Shrimp are fed to older baby and adult zebrafish. Brine shrimp require ~24 hours in warm conditions (such as those in the fish facility) to mature and hatch. Do not feed lots of unhatched brine shrimp to the fish.

1. Remove air hose from oldest brine shrimp hatchery and let brine settle to the bottom.

2. Drain hatchery into a cloth to collect brine shrimp and get rid of salt water. We do this by placing a funnel into a large beaker (2 L or more) and then putting a white handkerchief into the funnel. The brine will stay on top of the cloth, and the salt water will flow through so that it can be discarded.

4. Rinse the brine with system water and resuspend to 1-2 liters with aquatic system water.

5. Clean out the hatchery using a brush and water only.

6. Fill the hatchery with aquatic system water and two (1/2 tsp) spoons of brine eggs and two spoons (1/4 tsp) of salt.

7. Replace the air hose. The brine will be ready to use the following day.

8. Turn off the water flowing to the tanks. For feeding, 1 dense pipette full (about 2 mls) should be fed to tank with 10 adults. 2 dense pipettes full (about 4 mls) should be fed to

tanks of 20 or more adults. For babies, feed the amount indicated on the white sheet of paper taped to the front of the baby tank. Typically, a large (10L) baby tank will get one pipet of brine shrimp twice a day.

9. Wait 15-30 minutes, and then turn the water to the tanks back on.

Flake Food
Flake food is a combination of Tertramin Flake©, OSI Flake©, and Freeze-dried bloodworms. The three types of flakes can be bought from many fish food companies. This mixture can be fed to baby or adult zebrafish. It has to be ground up into a powder before it is fed to the baby fish-we call this baby flake.

1. Mix 1/3 of Tetramin Flake©, 1/3 of OSI Flake© and 1/3 of Freeze-dried bloodworms together into a container.

2. Turn off water to tanks that you are going to feed.

3. Feed fish. Use 1/2 teaspoon to feed tanks of 10 or more adults and 1 tsp. to feed tanks of 20 or more adults. Babies are fed according to the directions posted on the white sheet taped to the tank. Typically, a baby tank will get 1/4-1/2 tsp. of baby flake during one feed.

4. Wait 15-30 minutes, and then turn the water to the tanks back on. It is really important not to feed too much flake food, as it messes up the filter system and the pH of the water and kills the biofilter. If you are feeding the correct amount, the food should all be gone in about 10 minutes. If there is still food left after this time, make sure to give the fish less flake food the next time you feed.

Cleaning and Maintenance:
Maintenance should be done on the recirculating system according to that recommended in the Aquatic Habitats® manual. Never use chemicals or cleaning agents to clean the fish room.

<u>Daily checks of the general system should be done by all workers</u>

- Aquarium covers should be on tight.
- Aquarium down spouts should be dripping into the tank
- Aquarium water should be running
- Inspect for any signs of leaks
- Doors to the facility should be closed at all times to maintain the room temperature
- A daily check of Nitrite should be done with the Nitrite Kit provided in the lab. Record readings on the Maintenance sheet provided in the fish room.

<u>Room cleaning</u>

The floor is mopped once a week and whenever there is a spill of water. Every two months, the floor is cleaned more thoroughly with warm water and brushes; the surfaces in the fish facility are wiped down to remove any accumulated dust and debris.

Tank changing

Adult fish: Tanks are changed whenever the fish cannot be easily seen through the front of the tank due to algae build-up. The identification tags are first moved to the new tank, which is filled at least half full with aquatic system water. The fish are then netted into the new tank, and the new tank replaces the old on the rack.

Baby fish: Zebrafish are not mature until they are 3 months old. Because they are small, netting baby fish can cause serious injury and even death. Therefore, they must be kept in the same tank for 2-3 months. This can cause algae build-up that makes it difficult to see into the front of the tank. Therefore, the baby fish should be checked through the lid of the tank. A white tag placed on the front of the tank specifies the correct amount of food.

Tank cleaning

Dirty tanks are first scrubbed with a stiff brush to remove the majority of the algae. They are then placed in a dilute bleach solution for 4-24 hours, washed by hand with hot water, and then stacked until they are thoroughly dry.

Fish Nets:

Nets are kept in the disinfectant net dip. If you are netting fish from more than one tank, every tank should have its own net-this prevents the spread of disease through the facility. To use a net, choose one of the appropriate size out of the net dip and rinse it thoroughly with tap water. It is then ready to use with the fish. When you are done with the net, replace it back into the net dip.

Fish Identification:

All fish in the facility are labeled with the stock number, date of birth, number of fish with in the stock and who's in charge. One should be mindful of this information and careful when putting fish into tanks. When setting up fish to breed, record stock name, tank and rack that the fish came from on the breeding chart. An individual fish can only be bred once per week.

Euthanasia:

If a fish is visibly sick, (lethargic, skinny, open wounds) it should be removed and euthanized. Record this information on the Fish Information sheets provided within the fish facility. To euthanize, put fish/system water into a large beaker or 2.75 L tank and add ice to make an ice-water bath. Net fish to be euthanized into one of the inserts to the crossing cages, and lower this into the ice water bath. Do not allow the fish to come into contact with the ice. Leave for 15-20 minutes, until the fish are dead and dispose of carcasses as described below.

Waste Disposal:

Any dead fish found in a tank should be removed immediately. All dead fish should be wrapped in a paper towel and put into the biohazard bag in the freezer until removal to the cooler in the RAR.

B. Hazard Identification and Risk of Exposure to the Hazards
The only hazards from this protocol come from potential pathogens in the fish water, the fish feces, and the fish themselves. The risk of exposure is very low. Before moving to Duluth, I ran a fish facility at Case Western Reserve University for six years. The water was tested for bacteria every six months, and no bacteria that could infect humans were ever detected.

C. Exposure Controls Specific to the Above Risk of Exposure
To reduce exposure to potential pathogens, laboratory members will wear nitrile or latex gloves and lab coats. Safety goggles will be available for any time there is danger of splashing. All laboratory will wash their hands with soap when they are finished working with the fish.

D. Waste Generated and Disposal Methods
No special waste procedures are required for the water that the fish have been kept in. Dead fish will be kept in a biohazard bag in the Liang laboratory freezer. When the bag is full, it will be taken to the cooler in the RAR for proper disposal.

E. Spill and Accident Response Procedures
No liquid waste with transgenes will be generated during this procedure, and so spill and accident response procedures are not applicable.

F. Notes
None

References:
This protocol is based on the one found in Westerfield, M. (2007) THE ZEBRAFISH BOOK, 5th Edition; A guide for the laboratory use of zebrafish (Danio rerio), Eugene, University of Oregon Press.

Zebrafish fertilization and embryo isolation

The following protocol outlines the basics on how to get embryos for the experiments. Make sure to identify the kinds of zebrafish that will be used in the experiment (mutant, wild-type, GFP, etc).

1. Take a net out of the net dip and rinse in tap water for a few seconds.

2. Net fish from your assigned tank into a smaller tank containing fish water (to more easily identify males and females). Zebrafish are jumpy, so it is good idea to have the smaller tank close by and to cover the net with your hand.

IMPORTANT-ALL OF OUR ADULT ZEBRAFISH LOOK PRETTY MUCH THE SAME. THEREFORE YOU MUST LABEL ALL TANKS WITH THE HOME TANK OF THE FISH YOU ARE USING. THIS LABEL WILL CONSIST OF THE GENOTYPE OF THE FISH (SUCH AS "AB"), THE TANK NUMBER AND THE TANK COLOR.

If you want to show that you are a TRUE zebrafish researcher, you will fold over one edge of the tape to make it easier to get off later. We use a lot of tape.

2. Prepare one additional small tank with fish water (make sure to label it). You will then use your net to sort the males and females into separate tanks. To identify the sex of the zebrafish, keep in mind that females tend to be larger and rounder with a silver-blue streak whereas males are somewhat yellowish and tend to be more active.
*Pictures of female/male fish-THIS IS MISSING-PLEASE TRY TO GET SOME GOOD PICTURES DURING CLASS USING LIANG LAB DIGITAL CAMERA

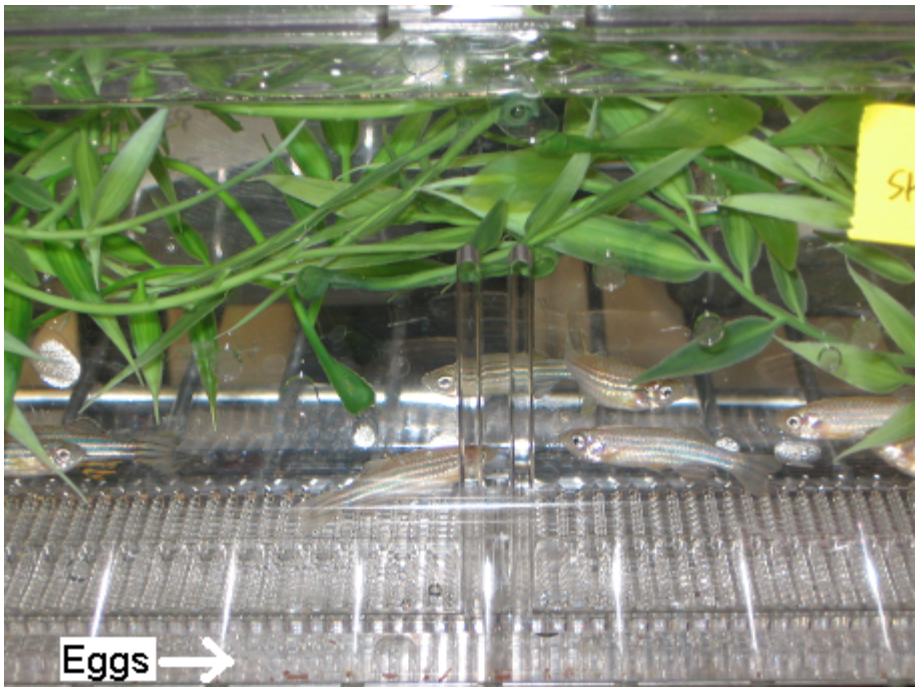
3. Determine how many single pair matings (one male, one female) you will be able to set up from your tank. Set up the appropriate number of crossing tanks. The crossing tanks consist of a top compartment with holes in the bottom for the eggs to fall through, a bottom reservoir, and a top. Fill these with fish water, some greenery, and label. Each crossing tank should also get a unique number (cross 1, cross 2, …).

4. Net one female and one male into the top compartment of the mating tank. Put the cover on, and place the mating tank on the cart. Leave the tank overnight and check in the next morning.

5. Net any unused fish back into the home tank. Return the net to the net dip.

NEXT DAY-WE WILL DO THIS

6. Look for eggs in the lower compartment, lift the tank and look from underneath if necessary. If there are eggs, turn the label vertically to indicate the presence of eggs. Obtain the lower part of another fertilization tank and fill it with water.



7. Carefully, move the upper compartment of the fertilization tank (together with the adult fish) to the second tank. The fish will be moving and splashing, be quick during this procedure for the fish not to dry out and dehydrate.
*Picture

8. Then, drain the water out of the initial tank filled with eggs (let them settle in the bottom if they are not already). Take the tank to a tap and gently and slowly pour the water out through one corner, making sure not to lose any eggs. Pour until all the remaining eggs and water cover one corner. Make sure to have a Petri-dish nearby.
*Picture

9. Pour the remaining eggs with sea water into a Petri-dish and label it.
Picture

10. Retransfer the parent adult zebrafish back into the first tank and place the label vertically.
*Picture

Syllabus and Schedule Spring 2009
Genetics Laboratory (Biol 2202)
12/4/08

Course objectives: Fundamental principles of genetics will be illustrated through classical genetic and molecular techniques including Mendelian genetics, the process of meiosis and recombination, forward genetic screens, complementation testing, reverse genetics using molecular techniques, and the analysis of genotypes and phenotypes. Further, this course will emphasize the presentation of scientific findings in written form and through oral presentations.

Instructor: Dr. Jennifer Liang, Coursemaster and Instructor for Section 1
Office: 252A Swenson Science Building
Phone: 726-7681
joliang@d.umn.edu

Dr. Liang's Office Hours: Tuesdays 9:00 a.m. – 11:00 a.m. or by appointment. If you would like to make an appointment, you must e-mail Dr. Liang with at least two potential times you are available. Dr. Liang would especially like to meet with students who have disabilities and are registered with Disability Resources (http://www.duluth.umn.edu/access/). It is University policy to provide, on a flexible and individualized basis, reasonable accommodations to students with disabilities that may affect their ability to participate in course activities or to meet course requirements. Students with disabilities are encouraged to contact Disability Resources to discuss their individual needs for accommodations.

Teaching Assistants (as yet to be assigned-these are the fall semester TAs):

Janel Pung, Sections 2 & 5
Office: 75 MWAH
Phone: 218-726-6853
pungx008@d.umn.edu

Rebecca Gordon
Office: LSci 339
Phone: 218-726-7963
gord0207@d.umn.edu

TA office hours will be announced in lab.

Place: Swenson Science Building, Rm 105

Times:
Section 1: Monday, 12:00 p.m.-3:50 p.m.
Section 2: Monday, 4:00 p.m.-7:50 p.m.
Section 3: Tuesday, 8:00 a.m.-11:50 p.m.
Section 4: Tuesday, 12:00 p.m.-3:50 p.m.
Section 5: Tuesday, 4:00 p.m.-7:50 p.m.

Prerequisites: BIOL 1011, BIOL 2201 (concurrent registration OK for BIOL 2201)

Reading: There is no textbook for this course, the readings will be in the form of handouts. You should have a three notebook with lined or graph paper to use for recording your laboratory notes and data.

Grading Scheme:

A	95-100%
A-	90-94.9%
B+	87-89.9%
B	83-86.9%
B-	80-82.9%
C+	77-79.9%
C	73-76.9%
C-	70-72.9%
D+	67-69.9%
D	60-66.9%
F	<60%

The following assignments will count toward your final grade:

Small (partial) laboratory reports	30%
Large (complete) laboratory report (s)	30%
Quizzes/Homework	30%
Laboratory Notebook	10%

Assignments:

Reading: Readings for each class must be completed before coming to class. This is to make sure that you are prepared to carry out each laboratory, are able to contribute to class discussions and group work, and are familiar with any safety precautions that must be taken.

Small laboratory reports: For the first several laboratories, we will learn how to write each section of a laboratory report (introduction, materials and methods, results, discussion) in turn.

Large laboratory report: For the last, or last several laboratories, students will write an entire laboratory report on the experiments they have done in class. These will be done in two drafts, with the first draft getting comments and the second draft getting a final grade for the assignment.

Quizzes and Homework: Quizzes and homework will be given periodically during the semester, and are designed to be a check on your learning of the material and your preparation for each class. Be aware that unannounced quizzes may be given at the beginning of lab periods to make sure that all have done the pre-lab reading.

Laboratory Notebook: Students will keep a detailed laboratory notebook on all experiments done in class. This notebook will be handed in for grading periodically during the semester.

Attendance and Participation: Students are required to attend every class period and come well prepared to discuss and carry out the laboratory that will be done that day. No late assignments will be accepted. In the case of illness, the missed assignment must be made up as soon as possible after returning to class. Students who will be absent during a class period must have a valid excuse, notify the appropriate instructor at least one week ahead of time, and complete any missed assignments in advance of the absence. Laboratories must be made up by arranging with Dr. Liang to attend one of the other laboratory sections. If this is not possible because of illness, make an appointment to meet with Dr. Liang.

Collaborative work: Discussion of the course material with other students is strongly encouraged. However, the written, graded assignments must be done independently. For example, laboratories will typically done in small groups, and students are free to discuss and help each other understand the materials and data in the laboratories. However, the laboratory reports must be written independently.

It is expected that all students uphold the University of Minnesota Student Code of Conduct, which can be found at http://www1.umn.edu/regents/policies/academic/Student_Conduct_Code.html and the Student Academic Integrity Policy, which can be found at http://www.d.umn.edu/assl/conduct/integrity/Academic_Integrity_Policy.htm. In particular, plagiarism from other students or published sources will not be tolerated. If you are in doubt about how to properly cite someone else's work, it is your responsibility to talk with Dr. Liang.

Extra Credit: Extra credit can be earned by attending a research seminar, and then submitting a one page paper to Dr. Liang. The paper should include the title and date of the talk, the name of the speaker, and a list of three things that you learned in enough detail so that they can be fully understood. You can attend up to two seminars during the semester. If they receive full credit, each paper will add an extra 0.5% to your final grade. These papers must be handed in by the last day of classes for spring semester (midnight on Friday, May 8). Other opportunities for extra credit may be announced as the semester progresses.

Tentative Schedule

Week	Date	Topic	Readings and due dates
1	**Tuesday January 20**	Extra lab for Tuesday sections- Fun?? with fish	Handout on setting up natural mating of zebrafish Complete web site training at (do all three sections): http://www.iacuc.umn.edu/training/tutorial/viewTutorial.cfm?view-part1
2	**Monday January 26 OR Tuesday January 27**	Laboratory 1 Tools, Microscopes, Safety Observation of zebrafish mutants	Laboratory 1 handout Complete web site training at (do all three sections): http://www.iacuc.umn.edu/training/tutorial/viewTutorial.cfm?view-part1
3	**Monday February 2 OR Tuesday February 3**	Laboratory 2 Modeling Mitosis and Meiosis	Laboratory 2 handout
4	**Monday February 9 OR Tuesday February 10**	Laboratory 3 Forward genetic F3 screen in zebrafish-part1	Laboratory 3 handout **Due:** Laboratory 2 Report-Introduction only
5	**Monday February 16 OR Tuesday February 17**	Laboratory 3 Forward genetic F3 screen-part2	Laboratory 3 handout
6	**Monday February 23 OR Tuesday February 24**	Laboratory 3 Forward genetic F3 screen-part3	Laboratory 3 handout
7	**Monday March 2 OR Tuesday March 3**	Laboratory 4 Analysis of linkage and crossing over in *Sordaria*	Laboratory 4 handout **Due:** Laboratory 3 Report-Materials and Methods only

8	**Monday March 9 OR Tuesday March 10**	Laboratory 5 C. elegans genetics and development	Laboratory 5 handout **Due:** Laboratory 4 Report-Results only
9	**March 16/17**	SPRING BREAK	NO CLASS
10	**Monday March 23 OR Tuesday March 24**	Laboratory 6 Mapping genes	Laboratory 6 handout **Due:** Laboratory 5 Report-Discussion only
11	**Monday March 30 OR Tuesday March 31**	Laboratory 7 Mendelian genetics in Drosophila melanogaster	Laboratory 7 handout **Due:** Laboratory 6 Report-Results only
12	**Monday April 6 OR Tuesday April 7**	Laboratory 7 continued Mendelian genetics in Drosophila melanogaster	Laboratory 7 handout
13	**Monday April 13 OR Tuesday April 14**	Laboratories 8 and 9 Reverse genetics using antisense morpholinos in fish and RNAi in C. elegans	Laboratory 7 handout
14	**Monday April 20 OR Tuesday April 21**	Continue Laboratories 8 and 9	Laboratory 8 and 9 handouts **Due:** First draft of web page project
15	**Monday April 27 OR Tuesday April 28**	Finish Laboratories 8 and 9 Laboratory 10 Making transgenic fish	Laboratory 10 handout **Due:** First draft of Laboratory 7 BIG Laboratory Report (all sections included)
16	**Monday May 4 OR Tuesday May 5**	Finish Laboratory 10 Wrap Up and Course Evaluations	**Due:** Final draft of web page project
FINAL	**Monday May 11 12:00 pm**		**Due:** Final draft of Laboratory 7 BIG Laboratory Report **Due:** All extra credit assignments

Principles of Developmental Biology 4361
11/17/08

Course Description: This course focuses on the mechanisms that control development of multicellular organisms. Topics will include morphogenesis, organogenesis, developmental genetics, cell-cell interactions, biochemical signaling pathways, and use of model organisms. It will emphasize using the scientific method, science writing, the experimental techniques and processes that are used to study developmental biology, and how to read and critically evaluate scientific literature.

Place:
Lecture: Chem 200, Tuesday/Thursday 8:00-8:50 am
Laboratory: SSB 105, 2 hrs 50 minutes/week
Laboratory sections:
Thursday, 09:00 A.M. - 11:50 A.M.
Thursday, 12:00 P.M. - 02:50 P.M.
Thursday, 03:00 P.M. - 05:50 P.M.
Friday, 09:00 A.M. - 11:50 A.M.

Prerequisites: Biol 2101, Biol 2201

Reading:
Scott F. Gilbert (2006) *Developmental Biology, 8th Edition*, Sinauer Associates, Inc.
Publishers Web Site for the book: http://www.sinauer.com/detail.php?id=2500

The course will also use handouts and other materials

Instructors:
Jennifer Liang (Coursemaster)
Office: 252A SSB
Phone: 726- 7681
E-mail: joliang@d.umn.edu

Dr. Tim Kroft (Guest speaker)
Office: 252B SSB
Phone
E-mail:

Dr. Pat Schoff (Guest speaker)
Office:
Phone:
E-mail:

Dr. Sigmund Degitz
Office: EPA
Phone:
E-mail:

Office hours: Dr. Liang's office hours are Tuesdays 9:00 a.m-11:00 a.m. or by appointment. If you would like to make an appointment, you must e-mail Dr. Liang with at least two potential times you are available.

Dr. Liang would especially like to meet with students who have disabilities and are registered with Disability Resources (http://www.duluth.umn.edu/access/). It is University policy to provide, on a flexible and individualized basis, reasonable accommodations to students with disabilities that may affect their ability to participate in course activities or to meet course requirements. Students with disabilities are encouraged to contact Disability Resources to discuss their individual needs for accommodations

Teaching Assistants:

Mr. Po-nien (Bob) Lu (lecture TA)
Office: 270 SSB
Phone:
E-mail: plu@d.umn.edu

Ms. Lauren Hildebrandt (laboratory TA)
Office:
Phone:
E-mail:

Mr. Derek Eklund
Office:
Phone:
E-mail:

.

Grading scheme:

A	95-100%
A-	90-94.9%
B+	87-89.9%
B	83-86.9%
B-	80-82.9%
C+	77-79.9%
C	73-76.9%
C-	70-72.9%
D+	67-69.9%
D	60-66.9%
F	<60%

The following assignments will count toward your final grade:

Exam I	15%
Exam II	15%
Exam III	15%
Homework/Quizzes (8-10)	15%
Mini-laboratory reports	15%
BIG laboratory report	10%
Web site project	5%
Laboratory Notebook	5%
Attendance	5%

Reading, writing and testing requirements:

Reading: Readings for each class will come from the textbook and supplemental handouts given in class. It is essential to read the assigned material **before** coming to class. A list of the assigned readings can be found on the class schedule.

Paper Discussions: During the semester we will read and discuss several primary research articles related to subjects covered in the textbook. This material WILL be covered on the exams.

Homework/Quizzes: Homework and Quizzes will be given periodically during the semester. These will contain questions that are similar to those that will be present on the exams, and are designed to help you test your knowledge of the material.

Exams: There will be three exams during the semester, each covering the material in the previous section of the course. Material from the textbook, supplemental handouts, class discussion and lectures, and research articles will all be covered in the exams. The exams will not be cumulative.

Final exam: Exam III will be held during the time set for the final exam. Like the first two exams, Exam III will not be comprehensive, but will cover the material in the last 1/3rd of the course. The final exam is scheduled for May 5, 8:30-11:30 am.

Mini-laboratory reports: For each of the experiments we do in the laboratory part of this course, students will write a partial laboratory report (for example, for one lab you might be assigned to write a background section, for the next, the results). The emphasis is going to be on building the quality of science writing skills.

BIG laboratory report: We will be conducting one student-designed multi week experiment. The goal of this is to generate publication quality data that will be used for a publication. If we are successful, all of you may gain authorship on a published manuscript! For this experiment, you will all write a complete laboratory report.

Laboratory notebook: For all experiments in the laboratory section of the course, students will keep a detailed and complete laboratory notebook. This will be handed in periodically during the semester for grading.

Web site project: Students will work in small groups to prepare one page of material to be included on the "Zebrafish in the Classroom" website (http://www.zfic.org).

Attendance: Attendance is required in all lectures and laboratories.

Collaborative work: Discussion of the course material with other students is strongly encouraged. However, the written, graded assignments must be done independently. For example, laboratories will typically done in small groups, and students are free to discuss and help each other understand the materials and data in the laboratories. However, the laboratory reports must be written independently.

It is expected that all students uphold the University of Minnesota Student Code of Conduct, which can be found at http://www1.umn.edu/regents/policies/academic/Student_Conduct_Code.html and the Student Academic Integrity Policy, which can be found at http://www.d.umn.edu/assl/conduct/integrity/Academic_Integrity_Policy.htm. In particular, plagiarism from other students or published sources will not be tolerated. If you are in doubt about how to properly cite someone else's work, it is your responsibility to talk with Dr. Liang.

Makeup work: No late assignments will be accepted. In the case of illness, the missed assignment must be made up as soon as possible after returning to class. Students who will be absent during a class period must have a valid excuse, notify the appropriate instructor at least one week ahead of time, and complete any missed assignments in advance of the absence. Laboratories must be made up by arranging with Dr. Liang to attend one of the other laboratory sections. If this is not possible because of illness, make an appointment to meet with Dr. Liang.

Extra Credit: Extra credit can be earned by attending a research seminar, and then submitting a one page paper to Dr. Liang. The paper should include the title and date of the talk, the name of the speaker, and a list of three things that you learned in enough detail so that they can be fully understood. You can attend up to two seminars during the semester. If they receive full credit, each paper will add an extra 0.5% to your final grade. These papers must be handed in by the end of the Final exam (May 5, 11:30 am). Other opportunities for extra credit may be announced as the semester progresses.

Biology 4361 **Spring 2009 Schedule**

Week	Date	Topic	Lecturer	Readings and due dates
1	**Tuesday January 20**	Morphogenesis and Differentiation	Liang	Chapter 1, pp. 8-23 (Topics: Primary germ layers, fate mapping, morphogenetic processes) Chapter 2, pp. 39-40 (rules of evidence) Chapter 3, pp. 53-67 (specification and differentiation)
	Thursday January 22	Morphogenesis and Differentiation Vertebrate Life Cycle	Liang	Chapter 2, pp. 25- 31 (Frog life cycle) Chapter 3, pp 67-74 (Morphogenesis and cell adhesion)
	Laboratory 1	Zebrafish intro (SMALL zebrafish experiment) Tools and Rules		Description: Staging zebrafish embryos Reading: Zebrafish lab handout Virtual staging experiment at http://www.zfic.org/virtual%20experiments/stage1StagingBackground.html
2	**Tuesday January 27**	Science Writing	Liang	Handout (Note taking, good writing practices, plagiarism,making a cv/resume, etc.)
	Thursday January 29	Morphogenesis and Differentiation Intro to Lab 2	Liang	Chapter 6, pp. 158-164 (Apoptosis, Notch Signaling)
	Laboratory 2	Dictyostelium Lab, day 1		Description: Preliminary experiment Reading: Dicty Lab Handout Chapter 2, p. 36-39
3	**Tuesday February 3**	Differential Gene Expression	Ling	Chapter 4, pp. 86-99 (Be familiar with the techniques that are covered) Chapter 5, pp. 101-116 (Review basics of gene structure and process of transcription, do not memorize transcription machinery components). Chapter 5, Figure 5.39 (Review different methods of regulation of protein expression and read parts of chapter needed to fill in gaps) Chapter 5, pp. 116-119 (methylation)

	Thursday February 5	Stem Cells Intro to Lab 3	Liang	Chapter 2. p. 44 (Totipotency in flatworms) Chapter 3, pp. 66-67 (Potency) Chapter 4, pp 80-86 (Cloning) Chapter 15, Sidelights and Spec. pp. 480-481 (hemoglobin switch) Chapter 15, 482-493 (Vasculogenesis and Angiogenesis) Chapter 21, pp. 684-692 (Adult stem cells- focus on Mesenchymal stem cells)
	Laboratory 3	Dicty Lab, day 2		
4	**Tuesday February 10**	*Paper Discussion*	Liang	Matunis paper
	Thursday February 12	Signaling	Liang	Chapter 6, focus on pp. 139-151, 155-158 (TGFβ and Smad), 157 (Sidelights and Speculations)
	Laboratory 4	Planaria Lab		Planaria Lab Handout
5	**Tuesday February 17**	Finish up and review Intro to BIG zebrafish experiment	Liang	No new reading
	Thursday February 19	**Exam I**		
	Laboratory 5	Day 1 of BIG zebrafish experiment		Description: Preliminary experiment Define hypothesis Design experiment Reading: Circadian review
6	**Tuesday February 24**	Fertilization Guest lecture by Dr. Tim Kroft	Kroft	Chapter 7
	Thursday February 26	Sea Urchin Development	Liang	Chapter 8, 210-229
	Laboratory 6	Sea Urchin Experiment		Description: Sea Urchin fertilization and early development Reading: Sea Urchin lab handout
7	**Tuesday March 3**	Early Development- Frog	Liang	Chapter 10 (focus on cell movements)
	Thursday March 5	Axis Determination- Frog	Liang	Chapter 10 (focus on signaling)
	Laboratory 7	Frog Lab?? or Day 2 of BIG zebrafish		TBA

		experiment		
8	**Tuesday March 10**	Zebrafish Development	Liang	
	Thursday March 12	Zebrafish Development	Liang	
	Laboratory 8	Day 2 of BIG zebrafish experiment or Frog lab		Description: First repeat of experiment
9	**Tuesday March 17**	SPRING BREAK		NO CLASS
	Thursday March 19	SPRING BREAK		NO CLASS
	NO LAB	SPRING BREAK		
10	**Tuesday March 24**	Hox genes	Liang	Chapter 11, pp. 358-364 Chapter 9, pp. 283-28
	Thursday March 26	**Exam II**		
	Laboratory 9	Day 3 of BIG zebrafish experiment		Description: Second repeat of experiment
11	**Tuesday March 31**	Limb development	Liang	Chapter 16
	Thursday April 2	Limb development *Paper Discussion*	Liang	Chapter 16/Sauders
	Laboratory 10	Day 4 of BIG zebrafish experiment		Description: Third repeat of experiment *or* Day 1/2 of whole mount in situ hybridization
12	**Tuesday April 7**	*Paper discussion*	Liang	Saunders
	Thursday April 9	Guest lecture by Dr. Pat Schoff	Schoff	TBA
	Laboratory 11	Day 5 of BIG zebrafish experiment		Description: Day 1/2 of whole mount in situ hybridization or Day 3 of whole mount in situ hybridization
13	**Tuesday April 14**	Plant development		
	Thursday April 16	Left/right asymmetry heart development	Liang	Chapter 14
	Laboratory 12	Day 6 of BIG zebrafish experiment		Description: Day 3 of whole mount in situ hybridization and/or pictures
14	**Tuesday April 21**	Somites	Liang	Chapter 14

	Thursday April 23	Differentiation of the neural tube	Liang	Chapter 12, pp. 373-385
	Laboratory 13	Day 7 of BIG zebrafish experiment		Description: Pictures and statistics Reading: Statistics handout
15	**Tuesday April 28**	Neural crest	Liang	Chapter 13, pp. 407-424
	Thursday April 30	Neural crest	Liang	
	Laboratory 14	Day 7 of BIG zebrafish experiment		Description: Finish up BIG experiment
16	**Tuesday May 5**	Discussion of BIG zebrafish experiment		
	Thursday May 7	Aging		No reading
	Laboratory 15	Chick heart experiment		Description: Observation and manipulation of chick heart Reading: Laboratory 15 handout
	May 13	**Exam III (during final exam time)**	8:00-9:55 am	**Extra credit papers due at final exam**

Office of Animal Welfare
University of Minnesota
April 2009

CHANGE IN PROTOCOL

This document may be used to request any change to an approved IACUC protocol. Minor changes will be reviewed by an RAR veterinarian; significant changes must be reviewed by the IACUC through either the Designated Member Review or Full Committee Review process. For guidance on Minor vs. Significant protocol changes, please view the Protocol Amendments vs. Minor Changes:

PI: Jennifer Liang	**Protocol #:** 0901A57321
Date: 10/8/10	**Species:** Zebrafish (Danio rerio)
PI E-mail: joliang@d.umn.edu	**PI Phone:** 218-726-7681 **Fax:** 218-726-8142

Please provide answers to the following questions (noting "not applicable" if your requested change does not include this facet of your protocol).

1. Provide a general summary of the currently approved animal activities on the protocol:
The protocol currently uses adult fish only for natural matings, and then all of the experiments are done with embryos or early larva that do not yet have vertebra (and so are not covered by the protocol). In addition, we raise zebrafish to adulthood.

2. Provide a general summary of the desired change in animal activities including the rationale/justification for the change:
I would like to add fin clips to the protocol. This procedure is analogous to tail clips in mice, except that the clipped fins of the zebrafish grow back. This procedure would enable us to carry out two very important techniques in the teaching laboratory. First, it would enable for my Genetics Laboratory (Biol 2202) students to learn how to make genomic DNA and PCR genotype. In addition, my Developmental Biology (Biol 4361) students would be able to use zebrafish fin as a model for studying regeneration. Again, I want to emphasize that the fish are not harmed by this protocol, and typically start swimming within one minute after return to the recovery tank.

3. If the change involves new experimental procedures, provide the rationale and a clear description of the new experiments and/or procedures:

1. Put on gloves

2. Rinse a small net with tap water and shake off excess water. Fill a 1L tank about half full of fish water.

3. Net the zebrafish you are going to fin clip into your 1L tank of water. Save this tank, it will also be your recovery tank.

4. Make sure all of your tools are in easy reach. You will need (1) the top of a petri dish, (2) a clean dissecting scissors, (3) a blunt forcep, (4) a tank of diluted Tricaine (there is one of these per table), (5) your fish and the tank of fish water, and (6) a fish net.

5. Using the net, move the fish from its tank and place it in the diluted tricaine solution. Watch the fish carefully, and as soon as movement slows or stops, move to the next step.

6. Net the fish out of the Tricaine and place in the Petri dish top.

7. Lift the tail up with the forcep, and then cut the tip of the tail fin off with the dissecting scissors.

8. Place fish in recovery beaker to wash away the tricaine solution. It is easiest just to gently lower the whole petri dish into the water. Watch the fish until it starts swimming again-this should take less than a minute. If your fish is not recovering well, use a transfer pipet to get some water moving past its gills.

4. Provide detail on any additional animals you will need as a part of this request, include group sizes and numbers justification as well as their pain classification:

We will carry out this procedure on approximately 100 fish per year. From an animal protocol at a previous university, I believe this is pain class A. The anaesthesia both prevents any pain and keeps the fish still during the procedure.

5. Provide details on potential study-induced or related adverse health conditions that might occur, if any, and plans to address them:

Occassionally, a fish that is old or already sick will die from the anesthesia. To prevent this, the fish are left in the Tricaine solution for only a very short period of time, and are monitored closely after they are moved to the recovery tank. There are no long term affects on the fish, as the fin grows back completely in about two weeks. Tricaine is already an approved method of euthanasia on my protocol.

6. List specific changes in anesthesia, analgesia, or euthanasia (type, dose, administration method, etc.):

anesthesia: short term immersion (1- 2 minutes) in 0.4% Tricaine (also called MS-222 and 3-amino benzoic acidethylester) dissolved in aquatic system water.

7. Any additional changes to the protocol? (Please review your original protocol to answer this question.)
No

8. List included appendices, if any: I have attached a paper that describes the use of zebrafish in the classroom to learn about regeneration.

You may submit the Change in Protocol request from the Principal Investigator's X500 email to IACUC@UMN.EDU If submitted via email, electronic submission of this form from the Principal Investigator's X.500 email address is considered legal documentation and confirmation of his/her agreement to execute all activities as described.

or Print, Sign, and submit paper copy.

	Assistant Professor	10/8/10
Original Signature of PI	Title of PI	Date

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