Problem Set #1 – Principles of Development
Zoology 470 – Spring 2009
20 Points Total

Problem Set Guidelines
1. Due date: This problem set is due by ***5 pm on Friday, February 20, 2009***. It may be submitted either in class or to the mailbox of Jeff Hardin, in the Zoology Research Building.

2. Sources: You may use any sources at your disposal to answer the following questions. Legitimate sources include classmates, knowledgeable friends and colleagues, written documents, and any other scientific resources you find useful. **If you work with other classmates on this problem set, we ask that you list the other students with whom you worked to answer these questions.** Although you may discuss these questions as part of a group, **you are expected to answer the questions as an individual.** If you believe that published references will help you answer these questions, you may cite those references. However, **citation of additional references is not required, nor is it expected.**

3. Answering the questions: This problem set is designed to be answered concisely. **Brief but complete answers should be written in the space provided.** It is acceptable to type your answers, but you must provide a hard copy of the document you generate. **Where you are asked for explanations, you must provide them to receive full credit.** If you find it helpful, feel free to include diagrams in your answers. You need only turn in your answers on pages 2 & 3. Necessary information: All of the information and techniques needed to answer the questions on p. 2-3 have been presented in class, or are to be found in Gilbert's *Developmental Biology*. This problem set requires you to learn about mollusks, whose development is described in Chapter 8 of Gilbert, but other sections of Gilbert may be useful for some questions. We will not cover mollusks in class further.

Problem Statement
Suppose the disruption of mud flats in the Mississippi basin after Hurricane Katrina resulted in the discovery of a new species of mud snail. The adult snails look similar to the mud snail, *Ilyanassa obsoleta*, a small gastropod that lives in mud flats along the Atlantic coast. A cursory examination of the embryos of the new species indicates that they have cell division patterns and produce polar lobes strikingly similar to those in *Ilynassa* embryos. You travel to Louisiana to study the new species. The questions on pp. 2-3 deal with your research.

Name:________________________________ Student Number:__________________

If you worked in a group, other collaborators: ____

1. *Classic concepts in developmental biology.* You perform classic embryology experiments to study how cells differentiate in the new species.
a. The polar lobe produced in the one-cell zygote of the new species is inherited by only one of the two cells in the 2-cell zygote. You wish to show that, as in other snails such as *Ilynassa obsoleta*, cleavage of the zygote produces two daughter cells that are no longer totipotent. **Describe <u>one</u> experiment** you could perform to demonstrate such lack of equivalence of the two cells in the zygote. **In your answer, be sure to clearly state the expected outcome.** (**4 points**)

Isolating the two cells of the 2-cell zygote, and then examining whether they can each make a complete larva would be such a test. In this case, each blastomere would generate an incomplete larva, in contrast to the situation in mammals and sea urchins. [Note: a nuclear transfer experiment would not show this. Just because a cell has the complete complement of DNA in its nucleus does NOT mean that it is capable of forming an entire embryo. Indeed, differentiated cells are genomically equivalent, but are clearly NOT totipotent (e.g., a muscle cells).]

b. The eyes in *Ilynassa* larvae are formed from micromeres, i.e., from the group of small cells formed at the 8-cell stage. You believe that eyes are formed by the identical cells in the new species. **Describe <u>one</u> experiment** that would show that your belief is correct. **In your answer, be sure to clearly state the expected outcome.** (**3 points**)

Joann Render generated a detailed fate map of Ilynassa using Lucifer yellow injection. I would perform the same set of lineage tracing experiments in the new species, and show that the same micromere cells for eyes as in Ilynassa.

c. Two micromeres, called 1a and 1c, give rise to cells that form eyes in *Ilynassa*. Hyla Sweet, then at the University of Texas, showed in 1998 that one of the other micromeres, 1b, is also capable of forming eyes. She believed that a macromere (one of the large cells at the 8-cell stage) called 1D induces 1a and 1c to form eyes normally, but that 1b is normally too far away. **Design <u>one</u> experiment** to test this hypothesis. **In your answer, be sure to clearly state the expected outcome.** (**4 points**)

This requires some sort of transplant experiment. I would transplant the 1b cell so that it is closer to 1D (or vice versa). Other options that would likely be acceptable: placing isolated 1D and 1b cells together, and seeing if the recombined blastomeres can generate eyes in vitro, etc.

2. *Genetics and Developmental Biology.* Later, you realize that some adults of the new species have right-hand ("dextral") coiling shells, but others have left-hand ("sinistral") coiling shells. You and a geneticist friend determine that **shell coiling is determined by a single gene, *S*, and that a <u>dominant</u>, <u>maternal effect</u> mutation causes right-handed coiling**, as in other species. [<u>Note</u>: S = dominant; s = recessive]

a. **Assuming that the genetic control of shell coiling works in <u>exactly</u> the same way as in other snails**, such as *Limnaea peregra*, complete the following table, which analyzes the expected results from a cross between snails of various genotypes. In your answer, **please indicate <u>the ratio</u> of genotypes expected, and the ratio of phenotypes expected** (**3 points**).

Cross	Shell coiling phenotype of mother	Genotype(s) of offspring and ratio of <u>genotypes</u> expected	Ratio of shell coiling <u>phenotype</u>(s) of offspring
Ss female x *Ss* male	Left-handed (sinistral)	*1:2:1* *SS:Ss:ss*	*100% right-hand coilers (because mom has one dominant allele)*

b. What was the *genotype* of the maternal grandmother snail (i.e., the **<u>mother of the female</u>** used for the cross)? **Explain your reasoning** (**2 points**)
*Mom was a lefty, so **her** mother did <u>not</u> have a dominant allele that confers right-handed coiling. Thus grandma was ss. [Note: full credit was given is the notation was reversed but the logic was correct, i.e., if s and S were transposed]*

3. Polar lobes may not contain the only important molecules passed on between cells in early snail embryos. David Lambert, working in Lisa Nagy's lab at the Univ. of Arizona, showed in 2002 that mRNA corresponding to the *Ilynassa decapentaplegic* (*dpp*) homologue (i.e., a gene encoding a protein of the BMP family of growth factors) associates with centrosomes in macromeres (i.e., the large cells at the 8-cell stage). [In some cases, localized mRNAs associate with some centrosomes but not others at particular cell divisions [Centrosomes are required for organizing microtubules correctly within eukaryotic cells.] After the macromeres form, *dpp* mRNA is found at the cortex, which lies just beneath the plasma membrane in early embryonic cells.

a. You have cloned the *dpp* equivalent (such related genes are called "homologues") in the new species, and you are repeating some of Lambert's experiments. **Name <u>one</u> technique** you could use to show where *dpp* mRNA localizes in 8-cell embryos of the new species (**1 point**).

<u>Technique</u>: *In situ hybridization*

b. The Nagy lab believes that *dpp* mRNA gets recruited to centrosomes by a dynein, but that its later localization depends on actin. **Describe <u>one</u> experiment** you could perform to show that the localization of *dpp* mRNA to the cortex is actin-dependent in the new species (**2 points**).
Once the dpp mRNA is recruited to centrosomes, I would treat the embryos with cytochalasin, which depolymerizes F-actin, and show that the subsequent localization to the cortex fails.

c. The Nagy lab is developing techniques to knock down the function of gene products in snails. They want to knock down the function of *dpp*, but they do not want to disrupt *dpp* mRNA localization. What technique would you recommend they use? (**1 point**)

<u>Technique</u>: *Morpholinos block translation without perturbing production of mRNA. We might also accept blocking antibodies introduced into the embryo (extracellularly) by microinjection, or, if a student knew something about dpp, a dominant-negative receptor experiment. RNAi, "knockouts", etc. would all perturb the RNA, so they are not correct.*