Problem Set #3 – Left/right (L/R) axis specification
Zoology 470 – Spring 2009
20 Points Total

Problem Set Guidelines
1. Due date: This problem set is due by ***5 pm on Monday, May 4, 2009***. **It must be submitted in class**, due to significant problems with submission of Problem Set 1 earlier in the semester. **Your problem set must be stapled**, again due to problems with Problem Set 1.

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. You need only turn in your answers on pages 1 & 2.** Necessary information: The following questions involve experimental and/or genetic manipulation of left/right axis specification in various vertebrates. All of the information and techniques needed to answer the questions on p. 1-2 have been presented in class, or are to be found in Gilbert's *Developmental Biology*, Chapter 11. **Complete answers may require you to recall information from previous units in this course, especially the question labeled "integrative" (question #2).**

Although much of this material is covered in your textbook, you may find the following paper helpful:

Kramer-Zucker AG, Olale F, Haycraft CJ, Yoder BK, Schier AF, Drummond IA. (2005). Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis. *Development* **132**:1907-21.

Name:_____ Student Number:_____

If you worked in a group, other collaborators:_____

1. After graduating with an "A" in Zoo 470 you proceed through medical school and are beginning your first internship in the emergency room. You are presented with a male patient who displays clear symptoms of acute appendicitis (the appendix, located near the waist, usually on the right side of the body, can become inflamed and must then be removed quickly before it can rupture and cause a severe peritoneal infection). A quick check of the patient's medical records reveals a history of sterility and recurrent bronchial infections. You place your stethoscope on the left side of his chest, and can
detect no heartbeat. After checking one more thing, you decide not to call in the heart failure unit and to proceed with the appendectomy.

a) What convinced you not to worry about heart failure? (**1 point**)

b) In beginning the appendectomy, where should you make your incision? **Explain your rationale briefly.** (**1 point**)

c) Further checking of the patient's family medical history reveals that his condition is hereditary, resulting from an autosomal recessive mutation. How can a mutation in a single gene cause the collection of symptoms this patient presents (not including the appendicitis!), and what is the primary defect likely to be? **Explain briefly.** (**3 points**)

2. **Integrative question**: The polycystic kidney disease gene, *pkd2*, is associated with left/right defects.
a. A naturally occurring mutation in the mouse *pkd2* gene does not exist. What technique do you predict was used to generate *pkd2* loss-of-function mutants? (**1 point**)

Technique used: _____

a. The *pkd2 (polycystin-2)* protein is a calcium-activated cation channel protein, i.e., it is thought to allow regulated entry of cations into cells. You believe that *pkd2* may regulate calcium levels within cells of the mouse node. It is possible to culture mouse embryos at the stage of development at which left/right axis specification is occurring. Assume that experiments that can be done in early embryos in a variety of species can be performed on such cultured mouse embryos. Design an experiment that would show that loss of *pkd2* function affects calcium levels within cells in or near the node (**3 points**)

3. You are a new student in Cliff Tabin's lab at Harvard, and you are learning how to overexpress proteins in chick embryos at the time when L/R/ axis specification occurs. Overexpression of soluble proteins can be performed by implanting a bead soaked in the soluble molecule, which then diffuses out of the bead to nearby tissues. Proteins that are normally found inside cells can be overexpressed by infecting cells with a genetically engineered virus that encodes the protein of interest. When cells are infected, the viral genome directs expression of the protein within the infected cell(s).

a. Predict what would happen in each of the following situations (**6 points**):

Experiment	**Expected effect on L/R axis**	**Reasoning**
Overexpress the chick nodal protein on the **right** side of the embryo		
Treat chick embryos with blocking antibodies against sonic hedgehog (shh)		

b. Of the two techniques described above for overexpressing protein in chicks, which technique would you use to perform the *pitx2* overexpression experiment listed in (a)? **Briefly state your reasoning** (**1 point**)

4. The *polaris* gene in mice is required for a process known as *intraflagellar transport* (IFT). IFT is required for formation of cilia, including both "9 + 2" and "9 + 0" cilia. Ian Drummond's lab examined effects of knockdown of zebrafish *polaris* on pitx2 expression.

a. Would you expect *pitx2* expression to be normal in zebrafish embryos knocked down for *polaris*? **Explain your answer** (**3 points**)

b. Drummond's lab also examined fluid flow associated with the ciliated structure that is the equivalent of the node in the mouse following *polaris* knockdown. What is this structure? (**1 point**)

Structure causing fluid flow: _____

c. Extra credit: What celestial object has the name Polaris? (**1 point**)