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**)

After checking the right side and finding a healthy heartbeat, you realize this patient has situs inversus totalis, i.e., complete mirror reversal of the L/R axis. Such patients are quite healthy, so there is nothing to worry about.

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

The problem statement says that the appendix is normally on the right side, we should make the incision on the **left** in this case.

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**)

The other symptoms of this patient indicate a defect involving primary cilia. A recessive mutation in a gene required for formation or function of these cilia could give al of these defects, due to defects in nodal cilia.

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: __"knockout"__

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**)

Assuming it's possible to introduce a calcium indicator, one could introduce the indicator in cells of the Node. In wild-type cells, we expect an elevation of calcium on the left side of the Node. In the *pkd2*mutant, elevation of calcium should be affected (i.e., it should be different from normal; likely a loss of elevation of calcium).

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	Disruption of normal L/R axial specification (we'll accept reversal, partial laterality, etc.)	Nodal is normally expressed on the **left** side of the Henson's node, leading to Pitx2 expression on the left. This experiment might be expected to lead to Pitx2 on the right side of the embryo.
Treat chick embryos with blocking antibodies against sonic hedgehog (shh)	Disruption of normal L/R axial specification.	Like nodal, shh normally acts on the left side to promote Pitx2 expression. Blocking its function will lead to reduced Pitx2 expression, and L/R defects.

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**)
Pitx2 is a transcription factor, so it is not released from cells (as opposed to shh, nodal, BMPs). Thus a bead is not the right approach, and we would use engineered viruses in this case.

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**)

We would expect knockdown for *polaris* expression to result in defects in *pitx2* expression, since nodal cilia are thought to initiate a cascade that leads to normal L/R axis specification (including left-sided *pitx2* expression). Indeed, the Drummond lab paper uses in situ hybridization to show that pitx2 mRNA is on both sides of *polaris* knockdown embryos.

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: ____Kupfer's vesicle_____

c. Extra credit: What celestial object has the name Polaris? (**1 point**) "Polaris" is another name for the North Star, the point around which the stars in the Northern Hemisphere appear to rotate throughout the year.