DEV Day 5 Interpretation Questions
(due BEFORE leaving lab on Thursday, May 5th and Friday, May 6th)

1. Interpret your data from observing the zebrafish embryos. This should include the answers to the questions:

   a. What stage embryos did you observe?

   b. Did you observe differences between untreated and LiCl treated embryos? If so, what differences?

   c. Of all the embryos you saw, can you conclude when the LiCL treatment has the most teratogenic affect on the embryos? What is the "window of teratogenicity" for LiCl (i.e. in what stage(s) does LiCl have a teratogenic effect)?

2. Interpret your Northern Blot data. This should include the answers to the questions:

   a. What is the size of the *zcyt1* mRNA transcript?

   b. What stages showed *zcyt1* expression and does expression increase or decrease over time? Support your conclusions by referring to specific evidence from your membrane and your film.

   c. Are your Northern results consistent with what you have observed under the microscope during stages 1-4? Explain your answer briefly.

3. Suppose you wanted to reuse your nylon membrane containing the 4 stages of zebrafish RNA and probe it with a nematode (worm) cytokeratin probe. After stripping the nylon membrane of the previous probe, you repeat the new hybridization the same as the first and get no results, including the positive control.

   a. Assuming the RNA is still intact on the filter, which of the following conditions of hybridization might you change, and why?
      i. 7% SDS
      ii. 50% Formamide
      iii. 5x SSC
      iv. 2% Blocking Reagent
      v. 50mM NaPhosphate Buffer
      vi. Incubation temperature of 50 degrees

   b. You will also need to change the washes slightly to optimize probe/target binding. Do you want to **increase** or **decrease** the stringency of your washes? How specifically can you do this?