

DEV Day 5 Interpretation Questions (and Answers)

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

a. What stage embryos did you observe?

They should have seen segmentation through hatching on Dev 5.

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

Most noticeably, people should see that there is no pigmentation, no eyes, and maybe also that the tails seem to be a bit shorter in the treated fish.

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)?

If the matings work on May 2nd and we are able to do the LiCl treatment, the students should see that treatment with LiCl has the most dramatic affect in cleavage through early blastula but has no teratogenic affect after gastrulation.

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

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

The *zcyt1* transcript is about 1.4 kb in size. Look at their blots to be sure their answer corresponds to their data!

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.

Ideally, students will get results that increase from stages 2-4. No one should get a transcript in stage 1 and most will not get anything in 2. Students should support their conclusions by noting the relative levels of total RNA on their membrane (1X, 2X, etc.) and taking this into account when drawing conclusions about expression levels.

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

Stage 1 is blastula and the cells have not yet been determined, so there are no cells producing cytokeratin yet. Stage 2 is gastrula and at the end of that, the cells are just becoming determined, so they may start to produce a bit of cytokeratin mRNA. Stages 3 and 4 there should have a lot of cytokeratin detected because the fish now has skin developing and is getting bigger!

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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

In order to have a successful Northern, one needs to consider how complementary the probe is to the mRNA. Since nematodes and fish aren't too closely related, we would expect that the hybridization conditions would need to be changed to encourage the binding of a probe which is < 100% complementary to the mRNA. In order to do that, we would need to increase the SSC (to have more Na⁺ ions around) and to decrease the temperature of the hyb (and/or decrease the Formamide), thereby encouraging the slightly mismatched interactions.

- 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?

During the washes, we would want to decrease the stringency. We could do this by using a higher concentration of SSC and a lower temperature for the washes than were used in the zebrafish Northern blot.