

DEV Day Four 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?
- b) What did you observe in these embryos that was expected and unexpected?
- c) Did you observe differences between untreated and LiCl treated embryos? If so, what differences?

Answers may vary from group to group, but there should be comparisons between the two samples, mostly through descriptions of phenotype. Also students should have labeled as many anatomical aspects of the embryos as possible (blood, eyes, etc) on their drawings.

2. Let's pretend that... Due to some confusion, during Day 3.5, three sets of students' blots underwent different forms of the protocol. One set of blots was crosslinked at a setting of 60,000 microjoules/cm², one set of blots was crosslinked at 120,000 microjoules/cm², and the last set of blots was crosslinked at 240,000 microjoules/cm². What would we expect to see when these blots are developed, and why?

60,000 microjoules/cm² is half of the amount of microjoules/cm² described in the protocol as allowing for good nucleic acid attachment. It could be expected that some of the RNA and DNA would not crosslink and then when the membrane is washed, so many of these pieces of nucleic acids may be washed off. This would mean that the probe will have less RNA to bind to, and there will be weaker signal when the blot is developed. 120,000 microjoules/cm² is the level used in the protocol, so we would expect to see the results described in the manual. 240,000 microjoules/cm² is twice the number of microjoules/cm² that is recommended to use, so we would expect over-crosslinking to occur. This means that the probe will have fewer places to bind to when we expose the membrane to the probe, because many of the target molecules will have covalent crosslinks between them and the membrane that will prevent target-to-probe annealing. The signal on the blots will therefore be weaker or non-existent.

3. Let's pretend that... During the preparation of the hybridization solution for today, formamide was not added. Most lab groups just added formamide to their hybridization solution to solve the problem. However, one group decided to continue the experiment without modification. Meanwhile, a second group decided that they would just increase the temperature of their water bath (instead of adding back formamide). What results might each group see when they develop their Northern Blot? Why?

The first group may get a lot of extra signal (background) on their Northern blot, because formamide normally increases stringency. Leaving out formamide but not compensating by raising the hybridization temperature means that you are lowering stringency a lot. Thus the probe may now be able to bind to targets on the blot with which the probe shares much less homology than it does with *z-cyt1*.

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The second group's probe will hybridize properly and specifically to the membrane as long as they increase the temperature of the water bath enough (but not too much). In this case, they would get the expected results. (However, this group now runs the risk of degrading their RNA by increasing the temperature of the water bath. If this were to occur, then the group could expect to not be able to see any or all of the bands that they were hoping to due to the degradation.)