DEV Day Two Interpretation Questions (and Answers)

1. Interpret your data, if possible, 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 will vary. The students may be observing blastula, gastrula-shield, straightening/prim and hatching/long pec stages. They also should be observing some stages treated with LiCl. At some stages LiCl makes a difference, and at others, it doesn't. The students should include drawings of any of the stages that they observed.

2. Interpret your data from visualizing your agarose gel. This should include the answers to the questions:

    a) The sizes of the 28S and 18S rRNAs are 4.7 kB and 1.9 kB, respectively. Can you see these bands on your gel? In which lanes?
    b) Are the bands distinct or fuzzy? What does it mean if they're fuzzy?
    c) Does the intensity of the bands fluctuate from one lane to another? If so or if not, why? Should the intensity fluctuate? What, if anything, are you going to do about intensity fluctuations for the gel for your Northern blot?

Answers will vary. Bands should be relatively distinct. Fuzzy bands or smears indicate degradation by RNases. The intensity of the RNA in these lanes shouldn't increase over the different stages because we are looking at rRNA, not mRNA, and rRNA is needed for all protein synthesis so should always be present in large amounts. If they have different intensities in the lanes, then that means that they prepped RNA from a non-constant number of embryos (perhaps because they took different volumes during the phenol/chloroform extractions). We will take this difference into account when analyzing the results of our Northern blot. (In the "real world," you would adjust for this difference by altering the volume of RNA you'd load on the gel.)

3. Give three examples of teratogens (other than LiCl, alcohol, and nicotine), and explain briefly why the alterations in development caused by teratogens are NOT heritable.

For teratogens, answers will vary. Teratogens generally fall into the categories of "chemicals," "viruses/bacteria," or (some forms of) ionizing radiation. NONE should cause DNA damage! Teratogens affect the amount of mRNA made (transcription), the stability or localization of that mRNA, the amount of protein made (translation), or the stability or localization of a protein. Unlike DNA changes, these effects cannot be passed on to an offspring, and thus are NOT heritable.

DEV Day Two Interpretation Questions (and Answers) (continued)

4. Why did we use a denaturing agarose gel today? If we did **not** run a denaturing gel today, what piece of information about *zcyt1* mRNA would we be unable to determine?

We used a denaturing gel because, unlike DNA, RNA is single-stranded and can form intra- and inter-molecular base pairing. These secondary structures affect the RNAs' migration down the gel; therefore the mobility of non-denatured RNA doesn't always correspond to the length of the chain. Therefore we denature to ensure linearity of the RNA so that the migration of the RNA is based on size only, and not on shape also. This allows us to accurately determine the molecular weight (size) of the *zcyt1* mRNA.