DEV Day 1 Interpretation Questions (and Answers)

1. Interpret your data by answering the following questions:

    a. What volume was saved from each sample for the first and second phenol/chloroform extractions? Did you succeed in saving "equal volumes" from each stage?

    Answers will vary; should be less than 450 μL for the first extraction and less than 350 μL for the second extraction.

    b. You started with 16 embryos for each stage. What is the final concentration of your RNA in terms of embryo equivalents of RNA/microliter?

    Sample calculation:
    Students are given aliquots of 500 μL containing 16 embryos. The students add 50 μL NaOAc, which will be in the aqueous layer. This gives a volume of 550 μL for the aqueous layer of the first extraction. They take some volume of that for the first extraction (eg 400 μL). The embryo equivalents would be:
    (400μL /550 μL)=x embryos /16 embryos
    ◊ (16*400)/550 = 11.64 embryo equivalents RNA for the first step
    They then take those embryos, and take a smaller amount of aqueous layer for the second step. The total aqueous layer is that from the 1st extraction, so 400 μL here. Say 300 μL of this layer are removed; there will be (300/400)=(x/11.64) = 8.73 embryo equivalents of RNA. Then divide this by 20 μL DEPC-treated water, and the answer is 0.437 embryo equivalents RNA/ μL.

2. DNA and RNA have similar structures, and are isolated using similar methods in both the RDM and DEV modules.

    a. Name two differences between DNA and RNA that make RNA more unstable/easily degradable.

    RNA contains the sugar ribose, which has a 2′ OH group that can attack the phosphodiester bond attaching the sugars together, leading to degradation. RNA is single-stranded.

    b. During the miniprep protocol in RDM, we isolated nucleic acids (both RNA and DNA) from the bacteria. What step did we take to get rid of the RNA during the procedure, and how did that work?

    We used TE + RNase which digested the RNA.

DEV Day 1 Interpretation Questions (and Answers) (continued)

c. During the RNA extraction procedure on DEV Day 1, we also wanted to separate RNA and DNA. Explain how the steps of the extraction help us to separate the DNA from the RNA.

The phenol is water-saturated and we also add sodium acetate at pH 4.0. This acidic condition protonates the RNA, making it hydrophilic (polar) so it stays in the aqueous layer. The DNA under these conditions is less polar and ends up at the interface. Hence, they're separated.

3. During PBC, we did a Western to detect our Bgal in different "fractions" from our purification. In the DEV module we will perform a Northern blot to look at a specific RNA of interest.

a. What type of RNA will we **see** most distinctly on our agarose gel prior to transfer, and why?

rRNA – it has 2 distinct sizes, 28S and 18S (unlike mRNA which has many different sizes), so we will see the rRNA as 2 bands.

b. What are the different "fractions" we'll be testing in our Northern?

Different stages of development (blastula, gastrula, straightening, hatching).

c. What is the gene we will be probing for (our "RNA of interest")?

zcyt1

d. If we did a Western blot on the same fractions (from which we had purified protein instead of RNA), would we necessarily detect our protein of interest in the same fractions as we saw our RNA of interest? Why or why not?

No. You will likely see a "delay" in the Western's results because proteins cannot be made until the mRNA is available. Also, eukaryotic mRNAs are not always translated immediately after translation. Therefore, perhaps if we saw results in lanes 1, 2, + 3 in the Northern, we might expect to detect the protein in lanes 2, 3, + 4 on a Western.