RDM Day 5 Interpretation Questions and Answers

1. Interpret your data. In that interpretation, make sure to discuss the following:

a) Which enzymes did you use to do your diagnostic digests on your minipreps? Why?
b) Do you think you used the optimal combination of enzymes? Justify your answer.
c) How many of your six experimental minipreps were correct?
d) How did you know/would you have known which minipreps were correct?

-- Which enzymes did you use to do your diagnostic digests on your minipreps? Why?

Answers will vary. Most students will write that they used XbaI and EcoRI because that will pop out the insert if there is one. Other students may write that they used SspI and XbaI because that will pop out the insert if there is one, and will allow one to distinguish between single and multiple inserts.

-- Do you think you used the optimal combination of enzymes? Justify your answer.

Answers will vary. If they used SspI and XbaI, they should say yes. If they used anything else, they should say no. They should say that they should have used SspI and XbaI, because these two enzymes will show whether or not they got an insert, and will distinguish between multiple inserts also.

--How many of your six experimental minipreps were correct?

Answers will vary.

--How did you know/would you have known which minipreps were correct?

Answers will vary. If the students used EcoRI and XbaI, then correct minipreps will have a 1.0 kb band and a 4.0 kb band. If the students used SspI and XbaI, then correct minipreps will have a 1.2 kb band and a 3.8 kb band.

RDM Day 5 Interpretation Questions and Answers (continued)

2. So far in 7.02, we have used two very popular "reporter genes." Reporter genes are genes that produce protein products that biologists can easily assay. Reporter genes are used in lots of different ways, but generally reporter genes are used when they are fused to parts of other genes using recombinant DNA technology. Such reporter gene fusions can be made in two ways – as "transcriptional fusions" or as "translational fusions." Transcriptional fusions are when reporter genes are placed under the control of promoters other than their natural promoters. Translational fusions are fusions of the open reading frame of the reporter gene to the open reading frame of another gene. Such fusions must be made such that the reading frames of the two genes are "in frame" with each other; when this occurs, a hybrid protein is produced from the gene fusion. Answer the following questions about reporter gene fusions:

a) In which module did we use a transcriptional fusion? What was the reporter gene? What was the promoter under whose control we put the reporter gene?

b) In which module did we use a translational fusion? What was the reporter gene? What was the open reading frame to which we fused the reporter gene?

c) Which of the fusion constructs (transcriptional, translational, both, or neither) could give you information about whether a promoter you are interested in is turned on or turned off?
We used a transcriptional fusion in RDM. We used the reporter gene GFP hooked up to a T7 RNA polymerase promoter.

We used a translational fusion in GEN. We used the reporter gene LacZ hooked up in frame to one of the ara genes (A, B, or C).

Both types of fusion constructs can be used to tell you whether the promoter that the reporter gene is under is turned on or turned off.

3. Now that you have found a construct that contains pET-GFP that you miniprepped from strain AG1111, you are going to transform strain BL21 with that plasmid. (That's what you'll be doing on Day 6.) Why do we do these two transformations, instead of either just transforming into AG1111, or just transforming into BL21? To address this question, answer the two following parts:

a) State one difference between the two strains that makes AG1111 better to use for the step that we used it for. Briefly explain why this difference makes AG1111 better at this step.

b) State one difference between the two strains that makes BL21 better to use for the step that we used it for. Briefly explain why this difference makes BL21 better at this step.

AG1111 has a high transformation efficiency (wherease BL21 does not). Thus AG1111 is good to use when you want to make large amounts of a plasmid that may be present in a very low concentration in your ligation mix.

BL21 expresses T7 RNA polymerase (whereas AG1111 does not). Thus BL21 is good to use when you want to express a protein that is encoded by a gene under control of a T7 promoter.