

RDM Day 5 Interpretation Questions

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?

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?

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.