Bi10 Problem Set #3

Questions regarding this prelab should be addressed to David at drmiller@caltech.edu.

1. (2pts) DNA and RNA share many physical properties, which make it hard to isolate one from the other. What process will prevent RNA contamination during the plasmid extraction?

2. (6pts)In *E. coli*, there are two types of DNA, genomic and plasmid. During the mini-prep process, how is the plasmid DNA separated from the genomic DNA (describe three crucial steps)?

3. (5pts) After the first wash with PE buffer, what will be left on the filter? Why?

4. (2pts) Why doesn't the linearized vector re-circularize during the ligation reaction?

5. (7pts) Describe three possible plasmid compositions expected, following the ligation procedure with pMAL vector and GFP insert DNA. What do you expect to see after EcoRI digestion in each case, in terms of number of DNA fragment(s) and size(s)?

6. (3pts) When the sizes of the insert and the vector DNAs are the same, two DNA fragments obtained after digestion at 3' and 5' flanking regions of the insert are not distinguishable on an agarose gel. How do you confirm that there is an insert?