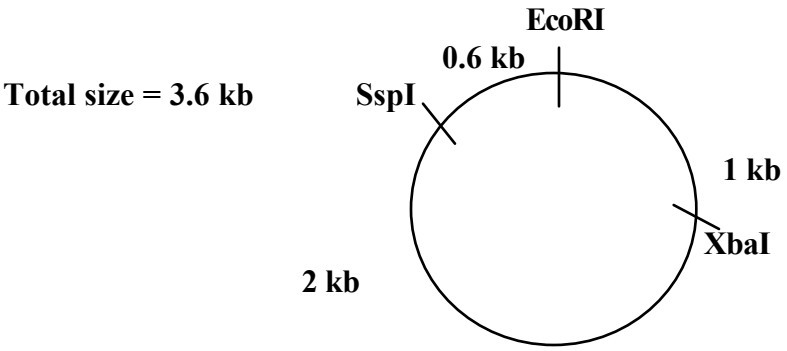
RDM Day One Interpretation Questions and Answers

1. Interpret your data. This interpretation should include answers to:
--Did you see what you expected to see on your gel?
--What were the sizes of the bands in each lane?
-- How did you figure out the sizes of the bands in each lane?
--What might each band represent?
-- What is enzyme X?

Answers will vary based on the quality of each group's gel. The sizes of bands in one lane may represent supercoiled DNA, nicked plasmid, uncut plasmid, and fragments that have been cut as would be expected by restriction enzymes. Enzyme X was EcoRI and XbaI.

2. Draw the restriction map of pUGFP that you determined from your data. Include the total size of the plasmid, the size of each fragment, and the location and identity of each restriction enzyme cut site.



3. Why do we want to insert the gene encoding GFP into pET? Why would leaving the gene encoding GFP in pUGFP not give us the experimental result (green glowing bacteria) we are seeking at the end of RDM?

pUGFP contains the GFP gene, but does not contain a promoter. We want to move the GFP gene from pUGFP into pET (which contains the T7 promoter) so that GFP can be expressed in the presence of T7 RNA polymerase. If we simply transformed pUGFP into bacteria and looked for fluorescence, we would not see any, because GFP would not be expressed.

4. Compare and contrast today's agarose gel electrophoresis with the SDS-PAGE we did in PBC by stating whether the two are SIMILAR or DIFFERENT based on the following properties of SDS-PAGE:

a. We used SDS-PAGE to separate molecules by size, such that the larger ones ran slower through the gel than the smaller ones. **SIMILAR**
b. We denatured the molecules we were going to run on our SDS-PAGE gel before we ran them. **DIFFERENT**
c. We used a non-specific dye that bound to all of the molecules that were run through our SDS-PAGE gel so that we could visualize them. **SIMILAR**