7.003 Spring 2022
Day 11 In-Lab Questions

1) Why are you performing a PCR reaction on your ligation samples today? Draw a diagram of what your final desired PCR product will look like.

2) What would have happened if you had tried to do PCR on your EcoRI-digested genomic DNA fragments without ligating them first? Explain.

3) For our plasmid recovery procedure, we digested the yeast genomic DNA with EcoRI restriction enzyme.

A) Approximately how often would you expect to see an EcoRI site within the yeast genome?

B) Ideally for our plasmid recovery procedure, we want there to be an EcoRI site in the yeast genome that is relatively close to the mTn3 transposon insertion site (e.g. within a couple kilobases or closer) but not too close. Why might it be bad for our plasmid recovery procedure if the EcoRI site in the genome is either too far away or too close to the transposon insertion site?

4) You have been unsuccessful in getting the 7.003 plasmid recovery technique to work for one of your αF-resistant mutants, so you want to try using a different restriction enzyme from EcoRI for the genomic DNA digest step. Your instructor suggests you use one of the following restriction enzymes below:

AbsI AgeI BamHI HindIII SspI

Which suggested restriction enzyme would be the best one to use (assume that you will keep the remainder of the plasmid recovery procedure exactly the same as the original 7.003 protocol)? Explain why you would not want to use the other enzymes for the genomic DNA digest.

(Hint: You can use SnapGene to find any restriction enzyme site locations in pRSQ2 and/or mTn3. You may also find it helpful to use the "Add primers…" in the Primers menu toolbar to add the locations of the lacZ and ori primers on your SnapGene .dna files.)

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