7.003 Spring 2022
Day 1 In-Lab Questions

1) In this class, we will be using the budding yeast *Saccharomyces cerevisiae* as a genetic model organism.

A) What is a model organism?

B) Give at least four general characteristics of a good genetic model organism for biological research. What are some additional characteristics specific to *S. cerevisiae* that makes them especially useful as a model organism to study in genetics?

2) You will be performing a mutagenesis screen in 7.003.

A) What is the "process of interest" we are studying in 7.003? Briefly explain what we will learn about our process of interest by performing the 7.003 mutagenesis screen.

B) What is the difference between a forward genetics screen and a reverse genetics screen? What are some advantages/disadvantages of doing each type of screen? Which type of screen are we doing in 7.003?

3) Draw a diagram of the mTn3-mutagenized genomic library plasmid. What is the purpose of each gene or DNA element on the plasmid?

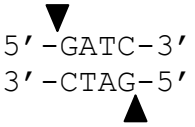
4) You are digesting your transposon plasmid library with NotI restriction endonuclease today. You will use this digestion reaction to transform yeast on Lab Day 2 to prepare a collection of transposon-mutagenized yeast colonies.

A) Why are you digesting the plasmid library? What will happen if you transform yeast without first digesting the plasmid?

B) How would your mutagenesis screen be affected if you digested the plasmid library with a restriction enzyme that cuts the plasmid once within the *ori* sequence?

5) To create the mTn3 transposon plasmid library pools, wild-type (WT) yeast genomic DNA (gDNA) was first partially digested with Sau3AI (recognition sequence below):

Sau3AI cut site:



Yeast gDNA digested fragments that were 2 – 4 kb in length were isolated, and these digested fragments were then cloned into plasmids using various recombinant DNA manipulation techniques such that each gDNA fragment was flanked by NotI sites at each end in the plasmid (see lab manual for NotI recognition sequence).

Once created, this WT yeast gDNA plasmid library was then exposed to the mTn3 transposon, and the transposon was allowed to insert randomly into the yeast gDNA on the plasmids. The end result was the mTn3 transposon plasmid library pools you used today.

A) Why do you think the initial digest of the yeast genomic DNA was done with a partial digest using Sau3AI (as opposed to a complete digest with Sau3AI, or a partial or complete digest with a restriction enzyme like EcoRI, etc)? (Hint: Think about how often you would expect Sau3AI to cut any random sequence of DNA.)

B) Why do you think NotI sites were used for flanking the gDNA fragment on the library plasmids (as opposed to another restriction enzyme, like Sau3AI or EcoRI)? (Hint: Think about how often you would expect NotI to cut any random sequence of DNA.)

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