Name: _____

7.003 Spring 2022
Problem Set #1 (47 points total)

This problem set has three questions total. You must answer all questions.

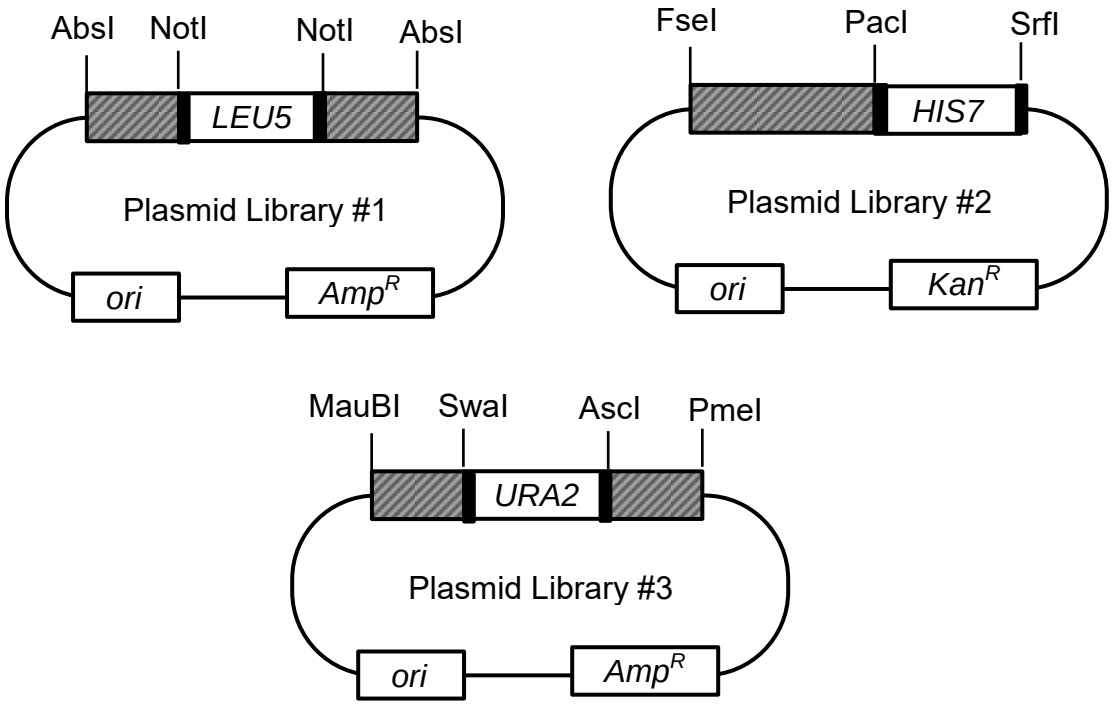
You may either write up your answers in a separate document, or you may write your answers directly into a copy of Pset #1 (digitally or on paper).

Question 1 (16 points total)
Because you're having so much fun in 7.003, you want to perform a similar transposon mutagenesis screen to find yeast mating pathway mutants in your UROP lab. However, your UROP lab has different yeast reagents compared to the 7.003 lab.

Your UROP advisor gives you yeast Strain A, with the following genotype:

Strain A genotype: *MAT***a** *his7 leu1 ura2*

Your lab also has available the following three transposon plasmid libraries shown below. Black bars indicate transposon terminal repeats. Gray shaded boxes represent yeast genomic DNA. Any unique restriction enzyme sites are indicated.



Your UROP advisor proposes three different possible transposon mutagenesis strategies described below.

- Transposon Mutagenesis Strategy #1: Digest Plasmid Library #1 with AbsI, transform into Strain A, and plate transformations on SC-Leu plates.

- Transposon Mutagenesis Strategy #2: Digest Plasmid Library #2 with FseI and PacI, transform into Strain A, and plate transformations on SC-His plates

- Transposon Mutagenesis Strategy #3: Digest Plasmid Library #3 with MauBI and AscI, transform into Strain A, and plate transformations on SC-Ura plates.

Question 1, continued

A) For each transposon mutagenesis strategy, fill in the table below to indicate whether anything will recombine/integrate into the Strain A yeast genome and what you will expect to see on the transformation plates (either no colonies, some distinct colonies, or a lawn of cells).

Transposon Mutagenesis Strategy	Will anything recombine into Strain A genome? (Yes/No)	Transformation plate result (No colonies, some colonies, or lawn)
#1		
#2		
#3		

B) Explain your answers to Part A for each mutagenesis strategy.

C) You realize that none of your advisor's mutagenesis strategies will work properly. Design your own transposon mutagenesis strategy using Strain A and one of the available transposon plasmid libraries above.

Plasmid Library (#1, #2, or #3) to use: _____

Restriction enzyme(s) to use: _____

Transformation plate to use: _____

Question 1, continued

Following your strategy from Part C, you have successfully obtained transformation plates with colonies of Strain A mutagenized with a transposon. You now want to quickly screen the transposon-mutagenized colonies to find those with mating pathway defects, Unfortunately, your UROP lab does not have any α-factor pheromone so you cannot test for α-factor resistance using α-factor plates like in 7.003.

D) Design an alternate replica-plating-based method you can use to easily and quickly screen your transposon-mutagenized colonies to find those mutants that are unable to mate. In your answer, describe any plates/reagents/strains you would use, what mutant phenotype you would be looking for, and how you would isolate your desired mutant colonies for further analysis. (Note that there are multiple correct answers to this question.)

From your mutagenesis screen in Part D, you isolate a mutant, Mut1, that is unable to mate. You identify the disrupted gene in Mut1 and find it encodes a protein involved in regulating histone modifications at the *HML* and *HMR* loci in the yeast genome.

E) How might mutating such a gene lead to the mating-defective phenotype in Mut1? Explain your reasoning.

Question 2 (16 points total)

You have performed a chemical mutagenesis screen in yeast Strain B (see genotype below) to look for mating-defective mutants. You decide to do some orthogonal assays on one of the mutants isolated in your screen, Mut2, to confirm its mutant phenotype. You perform a Shmoo Assay (similar to the 7.003 Shmoo Assay) to look at the following three yeast strains either untreated or treated with α-factor (six samples total). (Note that the "Δ" delta symbol indicates a gene deletion.)

Strain B (genotype = *MAT***a** *leu2 ura3*)
ste7Δ strain (genotype = *MAT***a** *his4 lys2 ste7*Δ)
Mut2

Unfortunately, you forgot to label your six sample tubes, so you are unsure which tube corresponds to which strain or treatment. Your advisor tells you not to worry though – assuming you set up your samples correctly, even though your tubes are unlabeled, you should still be able to determine whether or not Mut2 passed the Shmoo Assay.

A) State what you would expect to see for your six unlabeled tube samples in the Shmoo Assay if Mut2 is in fact a mating-defective mutant.

B) State what you would expect to see for your six unlabeled tube samples in the Shmoo Assay if Mut2 is <u>not</u> defective in the mating pathway.

Your Shmoo Assay results confirm that Mut2 is indeed a mating-defective mutant. Through sequencing studies, you eventually identify the mutated gene in Mut2 as the yeast gene *DIG2*. To verify your mutagenesis screen result, you now wish to create a yeast knockout strain where the *DIG2* gene is deleted.

C) Use SnapGene to design a <u>linear</u> DNA construct that you could directly transform into Strain B to delete the *DIG2* gene to generate your desired knockout strain. (Note that there are multiple correct answers to this question.) Instructions for obtaining the specific sequence of any yeast gene or genomic DNA region are provided at the end of this problem set. In your SnapGene file, label any notable features or DNA elements in your DNA construct. When submitting your Problem Set #1 on Canvas, please also upload this SnapGene file.

Question 2, continued

D) What plate would you use to select for successful transformants after transforming your DNA construct from Part C into Strain B?

E) Explain your design rationale for your DNA construct from Part C. What particular DNA elements or sequences did you include in your construct and why?

Having successfully created your *dig2Δ* knockout strain, you test its mating phenotype. You are surprised to find that your *dig2Δ* knockout strain has not apparent mating pathway defect and is still able to mate.

F) Assuming that the mutation in the *DIG2* gene was responsible for the mating-defective mutant phenotype in Mut2, what is the most likely reason why the Mut2 strain is unable to mate but the *dig2Δ* knockout strain is able to mate? Explain your reasoning.

G) What is the usual role of Dig2 in the yeast mating pathway? Explain why this would support your answer to Part F.

Question 3 (15 points total)

With the hopes of monetizing your newly acquired 7.003 yeast knowledge, you have started a biotech company that produces anti-fungal drugs against pathogenic yeast. Your company's first product, Drug X, showed initial promise as an anti-fungal agent, but recently, most pathogenic yeast strains have developed some resistance to Drug X. You plan to use *S. cerevisiae* as a model organism for studying what genes are involved in promoting resistance to Drug X in yeast. (Note that wild-type *S. cerevisiae* yeast are normally resistant to Drug X and can grow in its presence.)

You perform chemical mutagenesis on PPY295 yeast cells and obtain many YPD plates containing colonies of mutagenized PPY295 yeast.

A) Describe a screening strategy to easily screen your mutagenized PPY295 colonies for mutants that are sensitive to Drug X. In your answer, describe any plates/reagents/strains you would use, what mutant phenotype you would be looking for, and how you would isolate your desired mutant colonies for further analysis.

From your screen from Part A, you have isolated several mutants (Mut3, Mut4, and Mut5) that appear sensitive to Drug X. You wish to confirm and further characterize their mutant phenotype.

B) Design an orthogonal assay that will verify that Mut3, Mut4, and Mut5 are indeed sensitive to Drug X and also allow you to directly compare the strength of the mutant phenotype between the three mutants. In your answer, describe the overall assay setup and any plate(s) or reagent(s) you would use.

C) What would be the expected result of your assay from Part B if Mut3 was the most sensitive to Drug X (compared to Mut4 and Mut5)?

Question 3, continued

You identify the mutated gene in Mut3 as *UYG1*, an uncharacterized yeast gene which encodes a putative GTPase protein. You believe this GTPase protein is involved in a signaling cascade that is required for yeast to be resistant to Drug X.

D) Further investigation shows the *UYG1* gene in Mut3 is mutated within the putative GTP-binding site of the Uyg1 protein. One of your lab researchers suggests that this particular mutation in *UYG1* in Mut3 might act by eliminating the GTP hydrolysis activity of the Uyg1 protein. Do you agree? Why or why not? Explain your reasoning.

By pure coincidence, your arch-nemesis has started their own rival biotech company that is also studying Drug X, only working with mammalian cells instead of yeast. They have found an uncharacterized mouse gene called *MOUS3* with similar properties to *UYG1* – when the *MOUS3* gene is mutated in mouse cells, it causes the cells to become sensitive to Drug X. You wonder if *UYG1* and *MOUS3* might be functionally conserved genes since they produce similar mutant phenotypes in yeast and mouse cells, respectively.

E) Describe <u>two</u> different experiments/methods you could do to test and support your hypothesis that *UYG1* and *MOUS3* are functionally conserved. In your answer, include the expected results for each experiment if the two genes are in fact functionally conserved.

Instructions for finding yeast gene or genomic DNA sequences

1) Go to the Saccharomyces Genome Database (SGD) website at:

https://yeastgenome.org/

The SGD website contains annotated databases of all things yeast-related, including genomic DNA, gene, and protein sequences.

2) Go to the "Sequence" drop-down menu at the top of SGD homepage and click on "Gene/Sequence Resources."

3) Using the "1. Search a list of genes" option, enter the name of the gene you want the sequence of into the "Enter a list of names" window (e.g. STE5, HIS4, BAR1, etc).

4) The default setting will return just the coding sequence of the requested gene (i.e. from the ATG start codon to the stop codon of that gene's ORF). If you want the sequence of any of the flanking genomic DNA immediately upstream (5') or downstream (3') of that gene, enter the desired number of basepairs into the "Upstream:" and "Downstream:" textboxes accordingly. If you just want the coding sequence of the gene, you can leave these textboxes blank.

5) Leave the "Pick one or more strains:" option as "S. cerevisiae Reference Strain S288C" (this should be the default option selected).

6) Click on "Submit Form." This will open up a new results page.

7) On the results page, download the "Genomic DNA (.fsa)" file option (from the options listed near the top of the page).

8) This genomic_dna.fsa file contains the coding sequence of your requested gene (plus any upstream or downstream flanking DNA sequences you requested). This .fsa file can be opened in SnapGene and then saved as a .dna file that you can edit. Note that the .fsa file won't be annotated when you first open it in SnapGene, so you may find it helpful to add your own features/annotations to the SnapGene file to more easily visualize the DNA elements present.

7.003 Applied Molecular Biology Lab

Spring 2022