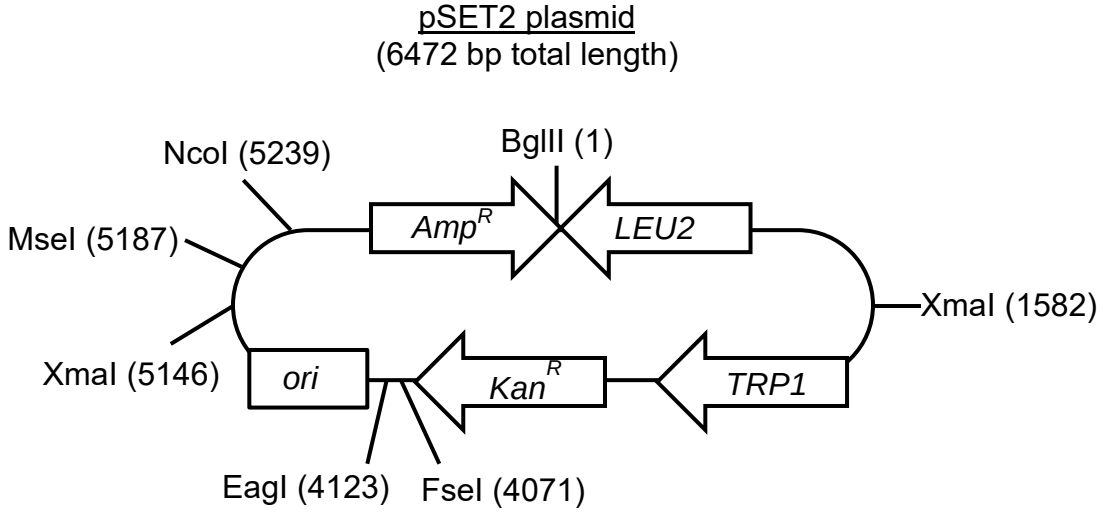
Name: _____

7.003 Spring 2022
Problem Set #2 (58 points total)

Note that for any “design/describe an experiment” type questions in this pset, if your answer involves a method that is not one of the established techniques previously covered in 7.003 lab or lecture, please be sure to fully describe the relevant experimental conditions, procedures, reagents, etc. of that method.

Question 1 (27 points total)

Because you're still having ridiculous amounts of fun in 7.003, you decide to repeat the entire transposon mutagenesis screen. You use mTn3 transposon and PPY295 yeast to isolate mutants defective in the yeast mating pathway, same as was done in 7.003. However, when you go to start the plasmid recovery procedure, the lab has run out of pRSQ2 plasmid. You find another available plasmid, pSET2, that you can use instead for the plasmid recovery process. A diagram of pSET2 plasmid is shown below. The basepair location of relevant restriction enzyme sites are listed in parenthesis.



You digest pSET2 with BglII and transform it into your mutants.

A) What plate should you use to select your transformants on?

B) Draw a diagram of what it will look like after BglII-digested pSET2 integrates into the mTn3 transposon. Label all genes/DNA elements in your diagram. (Note that you will be adding some primers to this diagram later in Part F, so make sure to draw this diagram large enough and leave yourself some space for adding the primers later!)

Question 1, continued

After you transform pSET2 into your mutants, you isolate the genomic DNA (gDNA) from the transformed mutants. You now need to digest the gDNA. For your plasmid recovery procedure, you want to have the option of doing either the inverse PCR or the alternate bacteria transformation method (as described in the Day 10 ILQ Question #2).

C) Of the restriction enzymes shown in pSET2, which enzyme would be the best to use for digesting your gDNA such that you can use either the inverse PCR or the alternate bacteria transformation method for your plasmid recovery procedure? Label this restriction enzyme site in your diagram from Part A.

D) Of the restriction enzymes in pSET2 that you did not pick for Part B, which one(s) would definitely not work at all for the plasmid recovery procedure (using either inverse PCR or the alternate bacteria transformation method)? Explain why not for each enzyme.

E) Of the restriction enzymes in pSET2 that you did not pick for Part B, which one(s) could in theory work for the plasmid recovery procedure (using either inverse PR or the alternate bacteria transformation method), but would not be optimal to use? Explain why each enzyme is not optimal to use.

You digest your gDNA using the restriction enzyme you picked in Part B and then ligate the digested fragments. You decide to use the inverse PCR method for the rest of your plasmid recovery procedure.

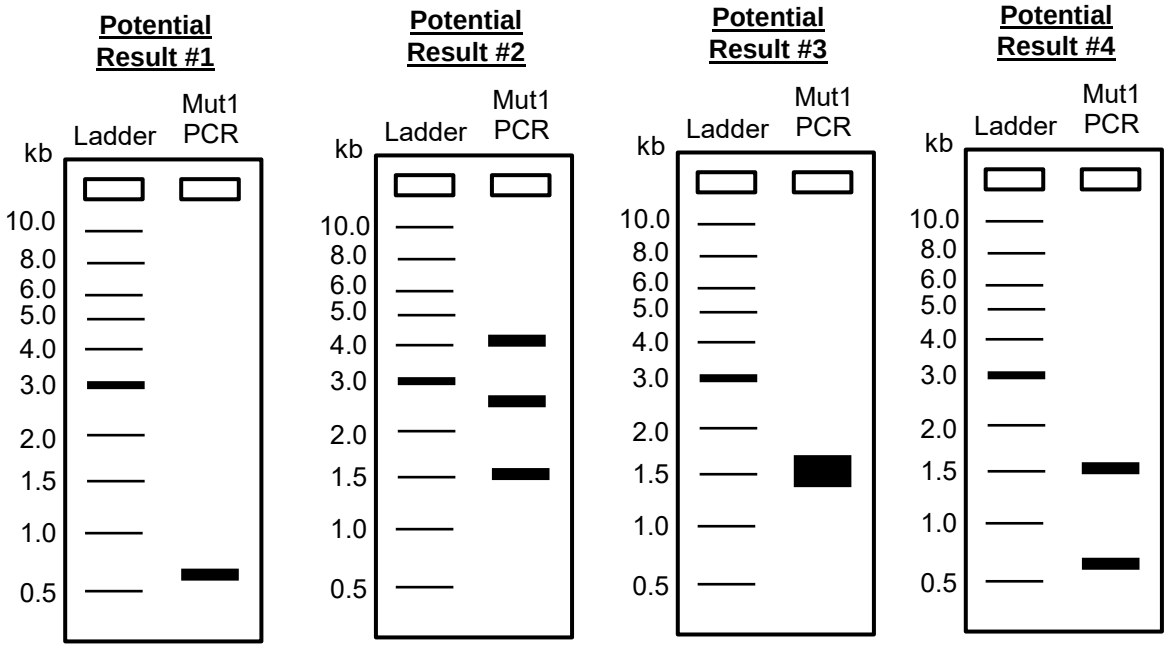
F) In the diagram you made in Part A, draw in two primers that you could use for your inverse PCR and label the two primers “PCR 1” and “PCR 2”. Keep in mind that your PCR primers have to work in the context of your specific plasmid recovery procedure. Draw the primers as arrows, with the base of the arrow representing the 5’ end and the arrowhead representing the 3’ end of the primer: 5’ → 3’. (Note there are multiple correct answers to this question.)

Question 1, continued

Using the primers you designed from Part E, you perform inverse PCR on your ligation products and run the PCR products on a gel. Your gel result for one of your mutants, Mut1, is shown on the right.

Your labmate, Copycat Cory, also performed the exact same plasmid recovery procedure on your Mut1 strain that you did, except for one change – Cory used a total volume of 15 μL for his ligation reaction while you used a total volume of 200 μL for your ligation reaction.

Cory runs his own Mut1 inverse PCR reaction on a gel to compare with your Mut1 PCR result. Four potential PCR gel results (#1 – #4) are shown below.



G) Which potential PCR gel result (#1 – #4) most likely corresponds to Cory's Mut1 PCR sample? Why? Explain your reasoning.

Question 1, continued
Satisfied with your inverse PCR results, you are ready to send your PCR products for Sanger sequencing.

H) Draw a diagram of what your desired inverse PCR product would look like. Label all genes/DNA elements in your diagram, as well as the locations corresponding to "PCR 1" primer and "PCR 2" primer (from Part F).

I) In the diagram you made in Part H above, draw a primer that you could use for your Sanger sequencing and label the primer “SS.” Keep in mind that your sequencing primer has to work in the context of your specific plasmid recovery procedure. Draw the primer as an arrow, with the base of the arrow representing the 5’ end and the arrowhead representing the 3’ end of the primer: 5’ → 3’. (Note there are multiple correct answers to this question.)

After receiving your Sanger sequencing results, you perform a BLAST analysis to try to identify the mutated genes in your mutant yeast strains. Your instructor suggests trying a range of word sizes between 12 and 32 bases in your BLAST search algorithm parameters.

J) Which BLAST search (word size = 12 bases or word size = 32 bases) would you expect to give you a greater number of matching results overall?

K) Which BLAST search (word size = 12 bases or word size = 32 bases) would you expect to give you more accurate results overall?

L) Explain your reasoning for your answers to Parts J and K.

Question 1, continued

You performed a blastn search on your Mut1 sequence results using the default website settings, but you did not get any informative gene name matches. Instead, the top 100 matches you got were for database entries consisting of the complete sequence of yeast Chromosome VII. While these matches are technically correct, they are not useful because they do not provide the actual yeast gene name in which the transposon inserted.

M) List <u>two</u> different ways you could modify your overall search approach or parameters on the NCBI BLAST site to increase the likelihood of getting matches for specific yeast genes.

Two of your labmates, Missy and Xavier, have also performed plasmid recovery on all of your mutant yeast strains, but they each used a different restriction enzyme for digesting their gDNA (and thus also designed different inverse PCR primers and sequencing primers as needed). Missy used MseI enzyme, and Xavier used XmaI enzyme.

N) Assuming that Missy and Xavier use the same overall BLAST search parameters, who would you predict on average to have <u>higher</u> expect values (e-values) for their BLAST search results? Why? Explain your reasoning.

Question 2 (13 points total)
You are studying a new uncharacterized yeast gene, *HSP7003*. Hsp7003 protein has sequence homology to known heat-shock proteins, so you want to see if *HSP7003* gene expression increases when yeast cells are undergoing a heat shock (e.g. incubated at 42^{o}C).

Following the general 7.003 protocols, you isolate RNA from yeast grown at 30^{o}C and at 42^{o}C, prepare cDNA, and then perform qPCR using primers that amplify a region of the *HSP7003* gene.

Four different sets of potential qPCR results are shown in the table below.

Potential qPCR results using *HSP7003* primers		
	C_t value for 30^{o}C cDNA sample	**C_t value for 42^{o}C cDNA sample**
Potential Result Set A	41.2	41.4
Potential Result Set B	30.7	25.5
Potential Result Set C	22.0	21.9
Potential Result Set D	20.4	26.8

A) State what you would conclude about the overall expression of the *HSP7003* gene at 30^{o}C and 42^{o}C for each potential result set.

Potential Result Set A:

Potential Result Set B:

Potential Result Set C:

Potential Result Set D:

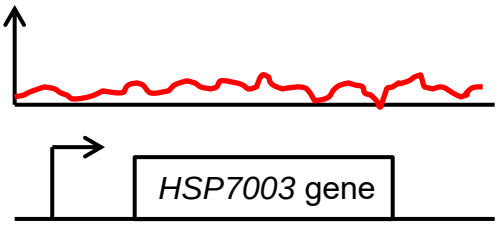
Question 2, continued

B) Suppose you were to have used less DNase I than instructed when isolating the RNA from your samples – how would you predict that would affect the C_t values of your final qPCR results? Explain your reasoning why.

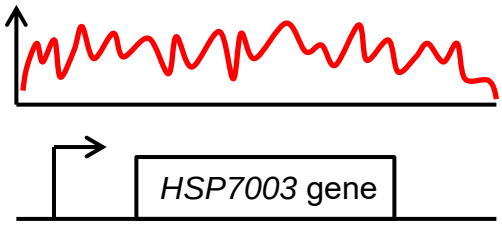
Your qPCR results ultimately show you that the *HSP7003* gene is actually highly expressed at the same level in yeast at both 30°C and 42°C.

C) Which sequencing profile result would you expect to see at the *HSP7003* genomic locus in yeast grown at 30°C if you were to perform an Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-Seq): Sequencing Profile #1 or #2 (shown below)? Explain your reasoning why. (Note that in each sequencing profile, the Y-axis indicates the number of sequence reads corresponding to that yeast genomic location.)

HSP7003 ATAC-Seq Profile #1



HSP7003 ATAC-Seq Profile #2



Question 2, continued

Because *HSP7003* RNA is always present at the same levels at both 30°C and 42°C, you perform a Western blot next to see if Hsp7003 protein levels change. Your Western blot experiments show that no Hsp7003 protein is detected at 30°C, but Hsp7003 protein levels greatly increase at 42°C. Your lab advisor suggests that this change in Hsp7003 protein levels could either be due to translational regulation (e.g. *HSP7003* mRNA is only translated at 42°C) or stability of the Hsp7003 protein (e.g. *HSP7003* mRNA is always translated at all temperatures, but the Hsp7003 protein product is rapidly degraded except at 42°C).

D) Design an experiment that would allow you to distinguish whether the changes in Hsp7003 protein levels are due to translational regulation or protein stability. In your answer, describe the expected results if the change in protein levels is due to translational regulation.

Question 3 (18 points total)

Who would have guessed – you found another new uncharacterized yeast gene to study called *TRF1*! Based on sequence homology, you believe that *TRF1* may encode a transcription factor that activates transcription. To test your theory, you obtain an engineered yeast strain, Strain R, that contains in its genome a *lacZ* reporter gene under the control of the *lac* promoter, which by itself is normally a very weak, inactive promoter. You plan to use CRISPR-Cas9 technology to bring the Trf1 protein to the *lac* promoter in Strain R and see if it can activate expression of the *lacZ* reporter gene.

A) Design a CRISPR-Cas9-based method to bring Trf1 protein to the *lac* promoter in Strain R. In your answer, describe any unique reagents/genes/DNA elements/etc that you would have to introduce into Strain R for your method to work.

Question 3, continued

From your CRISPR experiments, Trf1 appears to be a transcriptional activator that can turn on gene expression. Now you want to find out which are the genes in the yeast genome that Trf1 may regulate.

B) Describe two different experiments you could do to identify all the genes in the yeast genome that Trf1 may regulate. For each experiment, state what result would indicate whether or not a particular gene was likely regulated by Trf1.

Your experiments from Part B show that most of the genes activated by Trf1 are involved in cell cycle regulation. Aberrant regulation of the cell cycle is a common characteristic of tumor cells, so you're interested if there are any similar genes to *TRF1* in mammals.

You identify a *TRF1* homolog in mice and wish to completely knockout the *TRF1* gene from a mouse cell line so you can observe the phenotype of the *trf1Δ* cell lines in tissue culture.

C) Design a CRISPR-Cas9-based method to completely delete the *TRF1* gene in a mouse cell line. In your answer, describe any unique reagents/genes/DNA elements/etc you would have to introduce into your cells to create your *trf1Δ* knockout cell line.

Question 3, continued

You successfully create your *trf1Δ* knockout cell line and find that the *trf1Δ* cells divide much faster than wild-type cells. Excited that you may have come across a new potential tumor suppressor gene, you now want to create an entire *trf1Δ* knockout mouse to see if it has a higher rate of developing tumors. However, when you try to make your *trf1Δ* knockout mouse, you find that the mouse *TRF1* gene appears to be essential – all of the *trf1Δ* knockout mice you try to create die in the embryo stage.

To gain insight as to what cell tissues in the mouse embryo are the ones where Trf1 activity might be most critical, you want to see which cell types in the embryo express the *TRF1* gene.

D) Describe an experiment to determine which cell tissues or cell types of the mouse embryo express the *TRF1* gene. In your answer, describe any unique reagents/genes/DNA elements/etc you would use.

Your experiment from Part D shows that the *TRF1* gene is most highly transcribed in the epithelial cells of the mouse embryo. Since a regular *trf1Δ* knockout mouse was not viable, your lab advisor suggests you try making a conditional *trf1Δ* knockout mouse instead, where you delete the *TRF1* gene in a subset of cell tissues only after the mouse has already grown past the embryo stage.

E) Design an experiment to create a conditional *trf1Δ* knockout mouse where the *TRF1* gene will be deleted only in the mouse's epithelial (skin) cells after the mouse has already reached adulthood. In your answer, describe any unique reagents/genes/DNA elements/etc you would have to introduce into your mouse to create your *trf1Δ* conditional knockout mouse.

7.003 Applied Molecular Biology Lab

Spring 2022