BB2920-C14

Genetics Name:_____

Exam 1

Professor Farny

Instructions:

Do not open this exam until instructed to do so.

Write your name on the top of every page.

You will have 1 hour to complete the exam.

You may not leave the examination room during the exam.

Phones, tablets, computers, and any other electronic device are strictly prohibited. They must be completely out of sight for the entirety of the exam. You will not need a calculator.

Question:	possible points	points received
1	25	
2	10	
3	12	
4	8	
5	12	
6	18	
7	10	
8	5	
maximum total:	100	your score:

Question 1 (25 points)

You are a yeast geneticist. You are browsing through the yeast genome database, and you notice a region of the genome that has been labeled as a "hypothetical" gene, meaning that the database predicts a gene to be present in this region, but the expression of the gene has not been experimentally confirmed. You are intrigued, and you use your skill as a molecular geneticist to try and figure out the function of this new gene.

Below is the predicted map of the gene, which we will refer to as Gene X:

In order to investigate the function of this gene, you decide to make two mutants: mutant 1 is a 500-base pair deletion of the region upstream of and including the promoter region; mutant 2 is a nonsense mutation at the end of exon 2. You integrate these mutations into the yeast using homologous recombination.

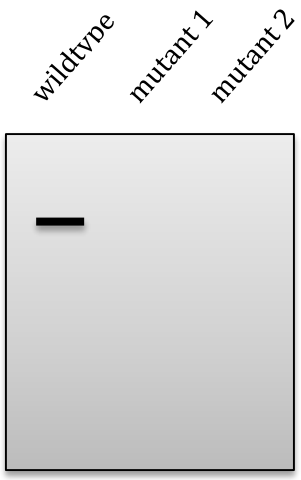
a) Clearly label the approximate location of your mutations on the map above.

b) How many introns does this gene contain?_____

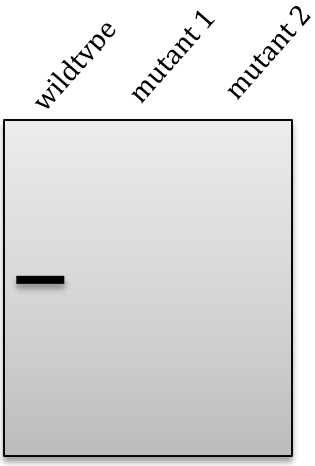
c) Label the 3'UTR with a bracket and star.

d) Below are a Southern blot, Northern blot and Western blot that you have performed to test your mutant yeast. The band locations for the wild-type DNA, RNA and protein (respectively) are shown. On each blot, draw the predicted location of each band for your two mutants (if one should be present), relative to the wild-type band. (NOTE for the Southern and Northern blots, the probe is location within exon 1. Assume the entire region shown is present the same genomic fragment after preparation of the DNA for Southern.)

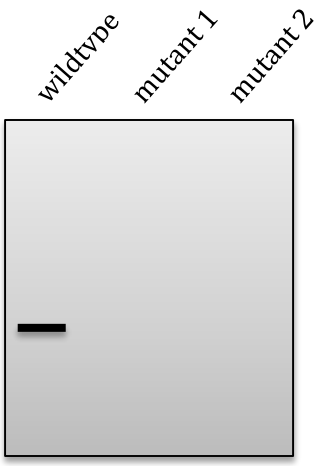
Southern blot, Gene X



Northern blot, Gene X mRNA



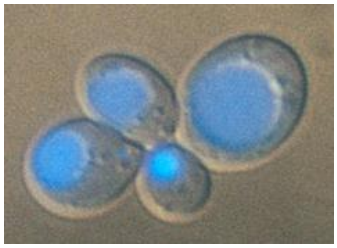
Western blot, Protein X



Question 1 continued

You next examine your mutant yeast to look for any abnormal phenotypes that might give you a hint as to the function of this gene. Wild type yeast have one large central vacuole, or storage cavity, in the center of the cell (shown in blue in the image below). You notice that some of your mutant cells are have very abnormal-looking, or completely missing vacuoles! To quantify this phenotype, you count approximately 250 of each type of yeast under the microscope and look at their vacuoles. Your data is shown below:

Yeast:	# of cells that have one large vacuole	# of cells that do NOT have a vacuole	# of cells with many small vacuoles instead of one large one
wildtype	242	0	0
mutant 1	0	252	0
mutant 2	0	0	244



You conclude from your data that Gene X is essential for the normal formation of vacuoles.

c) With respect to the formation of vacuoles, what is the best way to characterize the functional mutation displayed by mutant 1? (I'm looking for a "-morph!"). Explain your answer.

d) With respect to the formation of vacuoles, what is the best way to characterize the functional mutation displayed by mutant 2? (I'm looking for a "-morph!"). Explain your answer.

e) Is your study an example of forward genetics or reverse genetics? Explain.

Question 2 (10 points): Describe two important ways in which the process of gene expression

(DNA → RNA → protein) differs between prokaryotes and eukaryotes (there are several correct answers).

Question 3 (12 points): The Genetic Code is **1)unambiguous, 2)degenerate, 3)non-overlapping** and **4)universal**. Which one of these four features accounts for the following (no additional explanation required):

No single nucleotides is part of two different codons ____ _____

Silent mutations can exist within the coding region _____ _____

AUG always, and only, codes for methionine _____ _____

Human mRNA sequences can be translated in E. coli _____ _____

Question 4 (8 points): Which of the following sequences could NOT be cut by a typical restriction endonuclease? (circle one)

5’-GGATCC-3’ 5’-GTGCAG-3’ 5’-GCGCGC-3’ 5’-CTGCAG-3’ 5’-GAATTC-3’

Briefly explain your choice:

Question 5 (12 points): During DNA replication, the leading strand is created as one continuous DNA chain, whereas the lagging strand is created in short segments called Okazaki fragments.

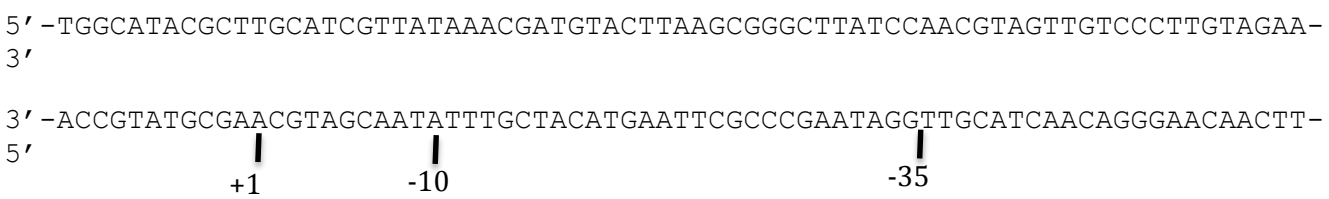
a) WHY is it necessary for the lagging strand to be made of Okazaki fragments?

b) What is the enzyme that “glues” the Okazaki fragments together? _____ _____

c) How do we refer to the sites in the genome where DNA replication begins? (i.e., what are they called?)

__ ___

Question 6 (18 points): Consider the following region of a gene:



a) Draw an arrow above the sequence to indicate the direction of transcription.

b) At the left end of each strand, indicate the template strand by labeling with a "T" and the coding strand by labeling with a "C".

c) Write the first six nucleotides of the RNA transcript (ensure your sequence is written 5' → 3'!!!)

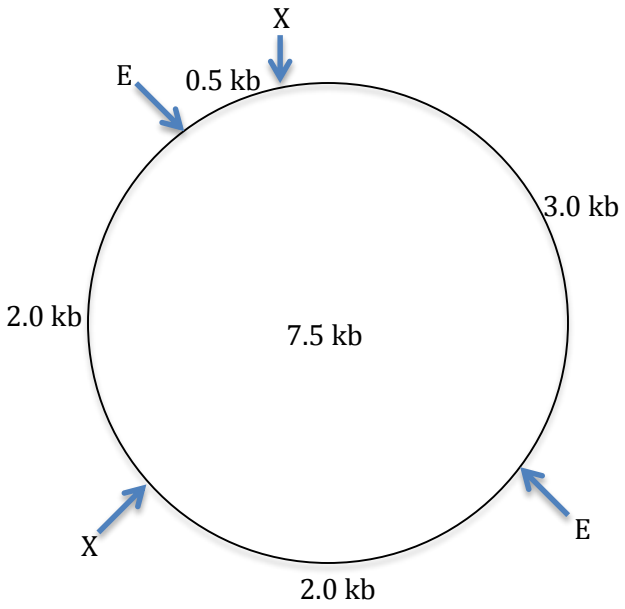
_____

d) Is this likely a prokaryotic or eukaryotic gene? How can you tell?

e) Which protein recognizes this promoter sequence? _____

f) Will this RNA be spliced? Why or why not?

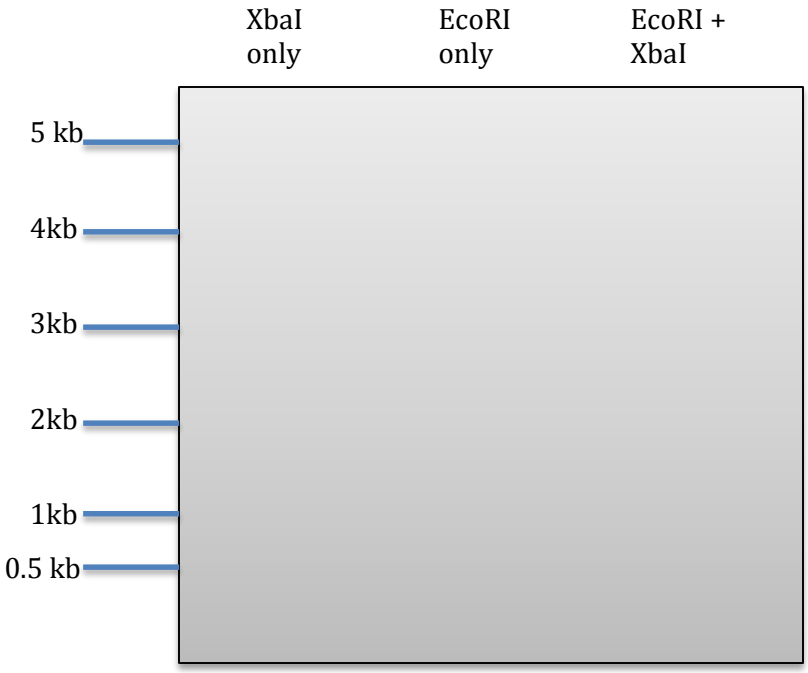
Question 7 (10 points): Consider the following plasmid map:



X = XbaI

E = EcoRI

On the gel below, draw in the location of the bands you would expect to see if you digested the plasmid with following restriction endonucleases: XbaI only, EcoRI only, or EcoRI+Xba1.



8) Briefly address the following short answer questions. 1-2 sentences should be sufficient.

a) What is a dideoxynucleotide? When is it used?

9) Explain the molecular reason why DNA migrates toward a positive charge during gel electrophoresis.