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.

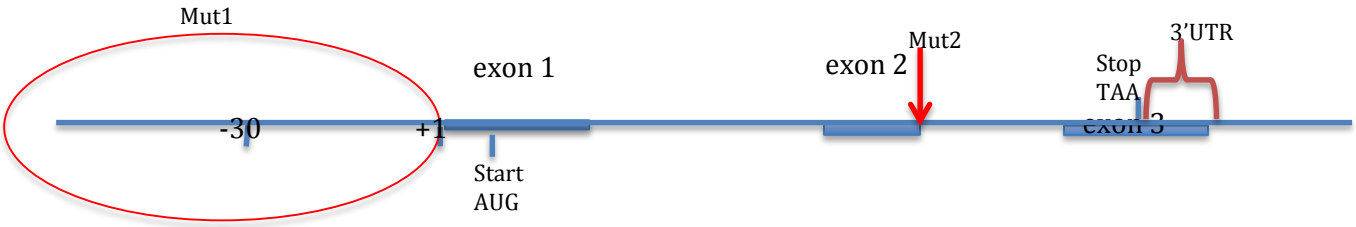
Exam 1 statistics (2014):

mean	82.5
median	84
high	100
low	50

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:



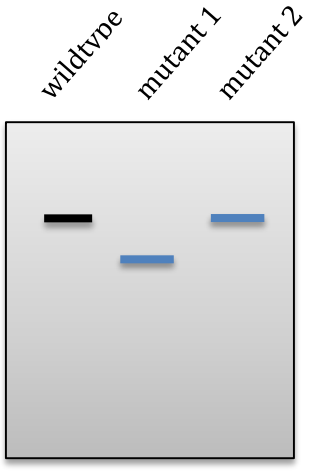
In order to investigate the function of this gene, you decide to make two mutants: mutant 1 is a 500-base pair deletion of the region upstream of and including the promoter region; mutant 2 is a nonsense mutation at the end of exon 2. You integrate these mutations into the yeast using homologous recombination.

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

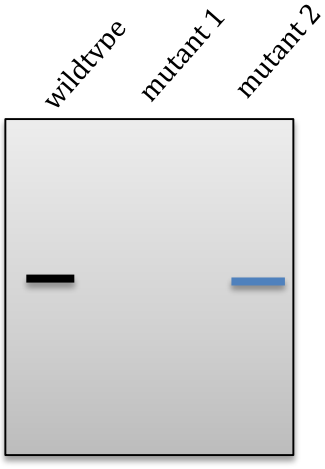
b) How many introns does this gene contain?_____2_____

c) 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.)

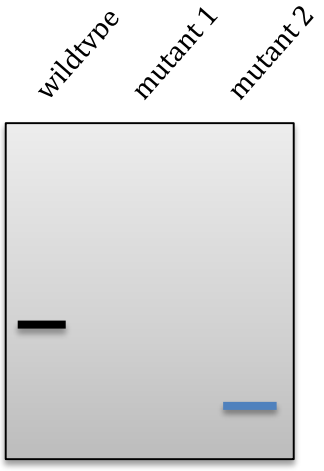
Southern blot, Gene X



Northern blot, Gene X mRNA



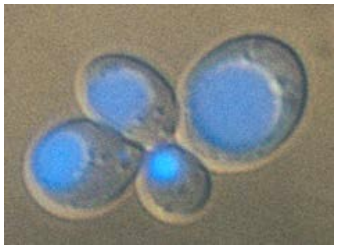
Western blot, Protein X



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	248	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.

amorph, because there is no activity (no vacuoles formed)

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.

Many possible answers accepted with valid explanation (hypomorph, hypermorph, neomorph and antimorph could all be argued) Amorph is NOT acceptable.

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

Reverse - started by looking at genome sequence (genotype) and then made mutations and looked for phenotypes (vacuoles).

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).

Many acceptable answers, including but not limited to:

Pro: no nuclei, Euk: have nuclei

Pro: circular chromosome, Euk: linear chromosome

Processing (splicing, capping, polyA) in euk but not pro

Promoter seqs are different (-35/-10 vs -30)

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 ___non-overlapping_____

Silent mutations can exist within the coding region ____degenerate_____

AUG always, and only, codes for methionine _____unambiguous____

Human mRNA sequences can be translated in E. coli _____universal_____

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:

This sequence is not a palindrome (you did not have to use the word palindrome, but you needed to indicate it's a different sequence on the 5' and 3' strands or something equivalent)

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?

DNA can ONLY be synthesized 5'-3'. In order for this to occur on the lagging strand, the polymerase

must wait until some DNA is "unzipped", then start close to the replication fork and work back toward the origin

b) What is the enzyme that "glues" the Okazaki fragments together? _____ligase_____

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

__origin of replication ("origin" or ORI are ok)____

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

```
5′-TGGCATACGCTTGCATCGTTATAAACGATGTACTTAAGCGGGCTTATCCAACGTAGTTGTCCCTTGTAGAA-
3′

3′-ACCGTATGCGAACGTAGCAATATTTGCTACATGAATTCGCCCGAATAGGTTGCATCAACAGGGAACAACTT-
5′
              |          |                                  |
             +1         -10                                -35
```

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

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". T=top, C=bottom

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

____AAGCGU_____

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

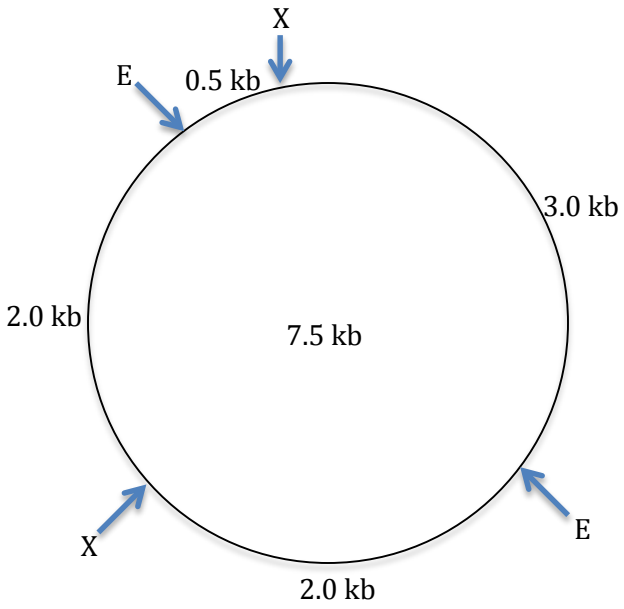
prokaryotic: promoter has two sequences at -35 and -10

e) Which protein recognizes this promoter sequence? _____Sigma 70_____

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

no, prokaryotic RNAs do not have introns

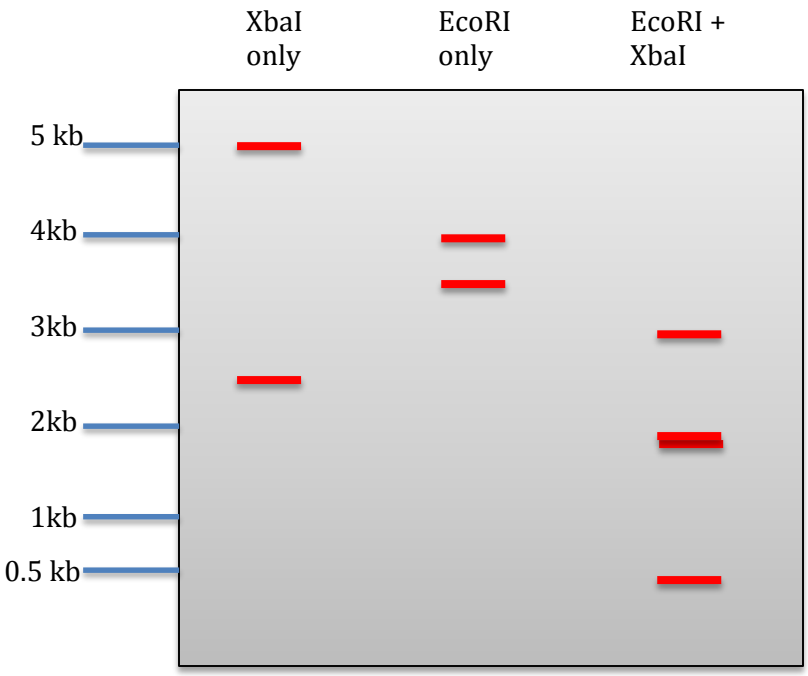
Question 7 (10 points): Consider the following plasmid map:



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?

a ddNTP is a nucleotide that is missing the 3'OH group. Without a 3'OH, no phosphodiester bond can be formed with an adjacent nucleotide. It is used in DNA sequencing to mark the location of specific nucleotides.

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

DNA contains a phosphate backbone which is negatively charged. The negatively charged DNA is attracted to the positive charge applied to the gel.