Solutions to 7.014 Quiz II
4/8/11

Class Ave = 65
Standard Dev = 16.5

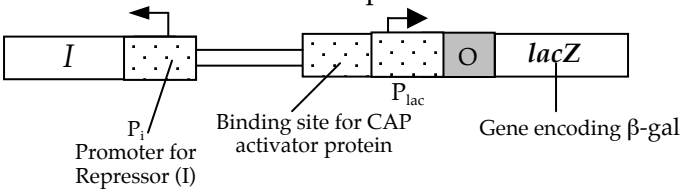
Approximate grade	Range	%
A	80 - 100	21%
B	63 - 79	31%
C	47 - 62	35%
D	31– 46	12%
F	0 – 30	1%

Initials_____

Initials_____

Question 1 (22 points)

You design a summer class where you recreate experiments studying the *lac* operon in *E. coli* (see schematic below). In your experiments, the activity of the enzyme β-galactosidase (β-gal) is measured by including X-gal in the growth media. X-gal is a lactose analog that turns blue when broken down by β-gal. X-gal DOES NOT act as an inducer of the lac operon.



a) Do you expect that X-gal binds to the enzyme β -galactosidase? Why or why not.
Yes, If the enzyme β –galactosidase has the ability to break down X-gal, then X-gal must bind to β –galactosidase.

b) Do you expect that X-gal binds to the *lac* repressor protein? Why or why not.
No, If X-gal does not act as an inducer, then it is not binding to the repressor protein.

c) As seen in the tables below, there is no expression of β-gal in the presence of glucose in wild-type cells. Briefly explain why this would benefit the cell.
The preferred energy source for cells is glucose as the enzymes needed to break down glucose are already present. If glucose is present, it is a waste of energy to make the enzymes needed to break down lactose.

After mutagenesis you find 7 mutants that never turn blue as shown in the table below. *Note each mutant has a single loss-of-function mutation.

Cell Type	**Media**			
	+ glucose No lactose + X-gal	**+ glucose + lactose + X-gal**	**No glucose No lactose + X-gal**	**No glucose + lactose + X-gal**
Wild type	White colonies	White colonies	Pale blue colonies	Dark blue colonies
Mutants 1-7	White colonies	White colonies	White colonies	White colonies

d) A single loss-of-function mutation in which component or components (I, P_i, CAP binding site, P_{lac}, O, *lacZ*) could produce the phenotype seen in these mutants? List all that apply.
CAP binding site, P_{lac}, lacZ

You also find three mutants with the following phenotype. *Note that each mutant has a single loss-of-function mutation.

Cell Type	**Media**			
	+ glucose No lactose + X-gal	**+ glucose + lactose + X-gal**	**No glucose No lactose + X-gal**	**No glucose + lactose + X-gal**
Wild type	White colonies	White colonies	Pale blue colonies	Dark blue colonies
Mutants	White colonies	White colonies	Dark blue colonies	Dark blue colonies

e) A loss-of-function mutation in which component or components (I, P_i, CAP binding site, P_{lac}, O, *lacZ*) could produce the phenotype seen in these mutants?
I, P_i, O

Question 1, continued

f) You find a different mutant, mutant X, which produces β-galactosidase in the presence of glucose. You find that this mutation is in the promoter, P_{lac}.

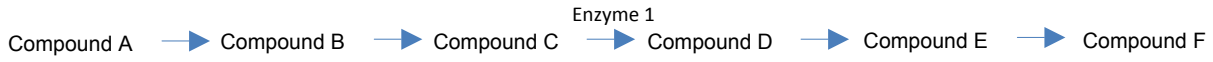
- Which of the following promoter sequences (Promoter 1, 2, or 3) would you expect to find in this constitutive mutant?

-35 region	-10 region	
TTGACAT----------	----------TATAAT	Promoter Consensus sequence
TTTACAT----------	----------TATGTT	Sequence of wild-type P_{lac}
CTGACGT----------	----------TACTGT	Promoter 1
TTGATAT----------	----------TATAAT	Promoter 2
TTTACGT----------	----------TATGTT	Promoter 3

- Explain why you made this choice.
The expression is constitutive in the presence of glucose, which means that the activator protein that binds to the CAP site is not needed. This implies that the mutation has created a stronger promoter. A strong promoter is one that is like the consensus sequence.

Question 2 (10 points)

Assume that the following pathway shows all of the intermediates and all the steps involved in the synthesis of Compound F in yeast cells.



You isolate 11 mutants defective in the pathway shown above. Each mutant has a recessive phenotype and carries only a single mutation.
a) You do a complementation test with these mutants and assign each to a complementation group.

- What is the maximum number of complementation groups you could have represented by these 11 mutants?
There are 5 independent enzymes for this pathway, each encoded by a unique gene. The maximum number of complementation groups would therefore be 5.

- What is the minimum number of complementation groups you could have represented by these 11 mutants?
The minimum number of complementation groups you could have would be 1, where each mutant has a mutation in the same gene.

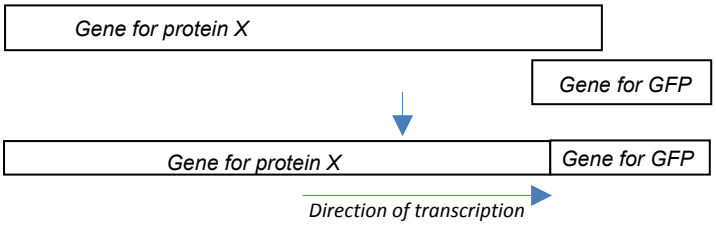
b) One of your mutants is homozygous for a mutation in the gene encoding enzyme 1 and thus can not make enzyme 1. This mutant accumulates Compound C. Label the arrow in the diagram above with enzyme 1.
Enzyme 1 performs the step: Compound C → Compound D

c) A diploid cell homozygous for a mutation in enzyme 1 and homozygous for a mutation in enzyme 3 also accumulates Compound C. What does this indicate about enzyme 3?
Because the double mutant (a cell missing both enzyme 1 and enzyme 3) looks like a cell that is missing only enzyme 1, you know that enzyme 3 acts at a step after enzyme 1.

Initials_____

Question 3 (28 points)

You have identified a human protein, protein X. You plan to ligate the DNA encoding GFP (green fluorescent protein) to the DNA encoding protein X (see diagram below). The resulting fusion protein will allow you to visualize the localization of protein X.



Below is the part of the cDNA sequence that encodes the C terminus of protein X. The sequence encoding the stop codon is shown in bold. The bars above the sequence show the restriction enzyme recognition sites.

```
              BamHI        KpnI     EcoRI
5′   … TCAAGAGGATCCCCGCGGTACCGAATTCCATGTTATAGCAAGCTCGGAATTAACCCTCAC   3′
     ---------+---------+---------+---------+---------+---------+
3′   … AGTTCTCCTAGGGGCGCCATGGCTTAAGGTACAATATCGTTCGAGCCTTAATTGGGAGTG   5′
```

Below is the part of the cDNA sequence that encodes the N terminus of GFP. Your cloning should attach the GFP sequence such that it is in the same reading frame as the one established for protein X, so a start codon is not needed. The bars above the sequence show the restriction enzyme recognition sites.

```
                  KpnI      BamHI     EcoRI
5′ TCTAGAGGTACCGGGATCCGGAATTCCC GTG CCA AGC GGC … 3′
3′ AGATCTCCATGGCCCTAGGCCTTAAGGG CAC GGT TCG CCG … 5′
```

The recognition sequences and the cleavage sites (indicated by /) for each enzyme are given below.

```
EcoRI:
5′ G/AATT C 3′
3′ C TTAA/G 5′

KpnI:
5′ G/GTAC C 3′
3′ C CATG/G 5′

BamHI:
5′ G/GATC C 3′
3′ C CTAG/G 5′
```

a) To ligate these two pieces of DNA together with the goal of making a fusion protein, which enzyme or enzymes can you use to cut the DNA encoding protein X. List all that apply.
KpnI

b) To ligate these two pieces of DNA together with the goal of making a fusion protein, which enzyme or enzymes can you use to cut the DNA encoding GFP. List all that apply.
KpnI

Question 3, continued

You successfully create a DNA fragment that encodes the fusion protein X-GFP. You plan to clone this fragment into a vector that will allow you to express protein X-GFP in yeast cells and study the localization pattern.

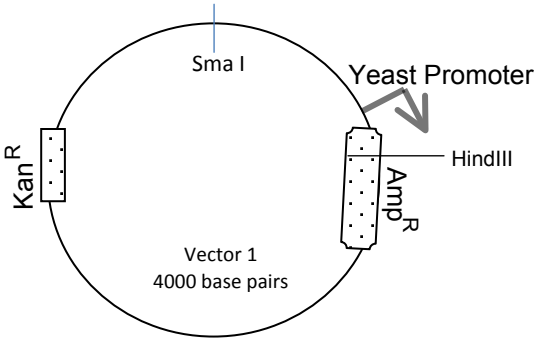
Your plan is to:

1) Cut an appropriate vector and the DNA fragment with *Hind*III.
2) Ligate the cut vector and the fragment together.
3) Transform *E. coli* cells with the ligation mix.
4) Select for *E. coli* cells that have a plasmid.
5) Identify the *E. coli* cells carrying a plasmid containing the protein X-GFP fusion gene by screening. *__Note,__ you **DO NOT** want to express protein X-GFP in *E. coli*.
6) Obtain large amounts of the plasmid containing the protein X-GFP fusion gene from a large number of *E. coli* cells.
7) Transform yeast cells with the plasmid containing the protein X-GFP fusion gene.
8) Screen the yeast cells for those that make green fluorescent protein.

The following is a partial schematic of vector 1 that will allow you to complete the plan outlined above.

The Kan^R gene confers resistance to the drug kanamycin.

The Amp^R gene confers resistance to the drug ampicillin.



c) To allow selection for *E. coli* cells that have a plasmid (step 4), and screening of the *E. coli* cells for protein X-GFP fusion (step 5), you will transform a particular strain of *E. coli* cells. What will be the phenotype of this strain prior to transformation?
The strain should be sensitive to both ampicillin and kanamycin.

d) To allow selection for *E. coli* cells that have either vector 1 or a recombinant plasmid, you will plate the transformation mix on media that contains which the following drugs?

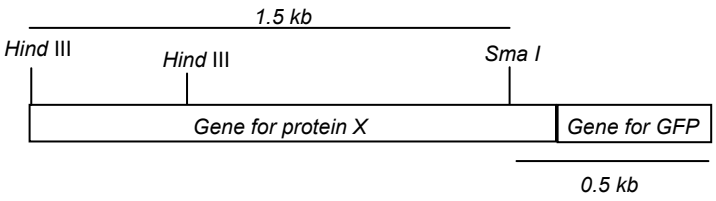
Ampicillin | Kanamycin | Both ampicillin and kanamycin | Neither ampicillin or kanamycin

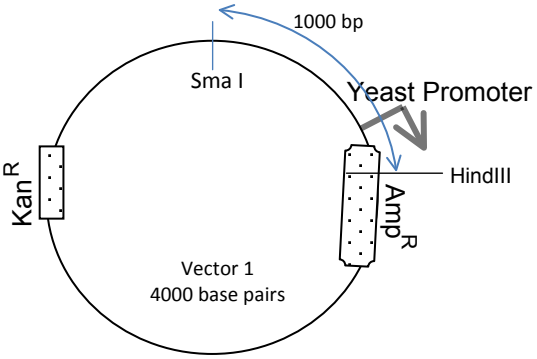
e) After selection you need to identify cells that carry a recombinant plasmid. Briefly explain how you would distinguish the colonies that contain cells with a recombinant plasmid from the colonies that contain cells with the original vector 1.
You would take the plate that has the kanamycin resistant colonies, and replica plate all of those colonies onto a plate that contains both ampicillin and kanamycin. The cells that carry a recombinant plasmid will die on the plate with ampicillin and kanamycin.

Initials_____

Question 3, continued

Because the protein X-GFP fusion gene was inserted into vector 1 as a Hind III fragment (See diagram below), you obtain two different recombinant plasmids.





f) Only one of these recombinant plasmids is the one that you want. To identify the correct plasmid, you cut both with restriction enzyme(s) and separate the resulting fragments by gel electrophoresis.

- What restriction enzyme or enzymes would you use to identify the correct plasmid?
  Sma I

- Given your answer above, what sized DNA fragments should you see from this restriction enzyme digestion of the correct plasmid?
  You would see two bands, one 2.5 kb and the other 3.5 kb.

g) In addition to the features indicated on the schematic, what additional DNA sequence or sequences must be present on Vector 1 to allow the completion of steps 1-8? Circle from the list below **all** that apply:

E. coli promoter adjacent to the cloning site	Yeast selectable marker
Human promoter for gene X adjacent to the cloning site	Operator region
Human origin of replication	Yeast origin of replication
E. coli origin of replication	LacZ gene

Initials_____

Question 4 (24 points)

You continue your study of cats. You are studying two traits, fur length and eye color. You cross two pure-breeding cats:

Parent 1: short fur and blue eyes X Parent 2: long fur and yellow eyes

⇓

F1: all long fur with blue eyes

- Use the upper case letters for the alleles associated with the dominant phenotype and the lower case letters for the alleles associated with the recessive phenotypes.
- Use the letters F or f for the fur alleles.
- Use letters E or e for the eye color alleles.

a) What is the genotype of the Parent 1? *ffEE*

b) What is the genotype of the Parent 2? *FFee*

c) What is the genotype of the F1 kittens? *FfEe*

d) You then make a series of crosses between different F1 cats:

F1 cat: long fur and blue eyes X F1 cat: long fur and blue eyes

You obtain 320 kittens from this cross. If the fur and eye color loci are unlinked complete the table below.

Phenotype	**Expected <u>number</u> of offspring with this phenotype**
long fur with blue eyes	*180*
short fur and blue eyes	*60*
long fur and yellow eyes	*60*
short fur and yellow eyes	*20*

e) You study a third trait, tail length. Tail length is a single gene trait with a simple dominant/recessive mode of inheritance. You cross two true-breeding parents to get all long tailed, blue-eyed cats.
You do a series of test crosses with F1 cats where:
TtEe (long tailed, blue eyed cats) X ttee (short tailed, yellow-eyed cats)

F2:

long tailed and yellow-eyed	147
short tailed and yellow-eyed	44
short tailed and blue-eyed	153
long tailed and blue-eyed	56
	400 total

- What were the phenotypes and genotypes of the two parental cats (P generation)?
  P1 = TTee long-tailed, yellow-eyed cats
  P2 = ttEE short-tailed, blue-eyed cats

- What is the recombination frequency between the tail size and eye color loci?
  = 44 + 56/100
  = 100/400 or 25%

Initials_____

Question 5 (16points)

Sex in cats is determined by the presence of the sex chromosomes, X and Y, as follows:

XX	female
X__ (only one X, no Y)	female
XXX	female
XY	male
XXY	male
__Y (only a Y, no X)	dead

Extra X chromosomes aren't as serious as other chromosome number abnormalities, because during embryonic development, only one X chromosome in each cell remains "active", *i.e.*, the genes on the chromosome are transcribed and translated. Coat color in cats is an X-linked trait, with alleles for black fur (X^B) and orange fur (X^O). However, when a cat has two different alleles of the color gene some of the cells will turn off the X^B chromosome, while others will turn off the X^O chromosome. This will lead to patches of skin where black fur will be produced, while other patches will produce orange fur. This patchy coat color is called **calico**. Thus you see:

$X^B\_$ or X^BX^B or $X^BX^BX^B$ or X^BX^BY or X^BY	black
$X^O\_$ or X^OX^O or $X^OX^OX^O$ or X^OX^OY or X^OY	orange
X^OX^B or $X^OX^BX^O$ or $X^OX^BX^O$ or X^OX^BY	calico

A cat with the genotype X^BX^B or X^B Y will have a black fur, while X^OX^O and X^OY cats will have orange fur. However, when a cat has two different alleles of the color gene (X^BX^O or X^BX^OY), some of the cells will turn off the X^B chromosome, while others will turn off the X^O chromosome. This will lead to patches of skin where black fur will be produced, while other patches will produce orange fur. This patchy coat color is called **calico**.

To investigate meiosis and non-disjunction (the failure of chromosomes or chromatids to separate) you use the two alleles of the coat color marker. You carry out the following cross:

X^BX^O x X^OY

Calico female Orange male

a) If meiosis is normal in both parents, what possible offspring would you expect? Give the ratios of the genotypes and respective phenotypes in terms of coat color and sex.

Genotypes of expected offspring	Percent of offspring with this genotype	Phenotype	
		Color	Sex
X^BX^O	*25%*	*calico*	*female*
X^OX^O	*25%*	*orange*	*female*
X^BY	*25%*	*black*	*male*
X^OY	*25%*	*orange*	*male*

b) Now assume that non-disjunction always occurs between the X chromosomes during meiosis I in the female, but meiosis II is normal. Assume that meiosis in the male is always normal. What offspring would you expect? Give the ratios of <u>all</u> expected genotypes and respective phenotypes in terms of color and sex.

Genotypes of expected offspring	Percent of offspring conceived with this genotype	Phenotype	
		Color	Sex
$X^BX^OX^O$	*25%*	*calico*	*female*
X^BX^OY	*25%*	*calico*	*male*
X^O	*25%*	*orange*	*female*
Y	*25%*	*dead*	