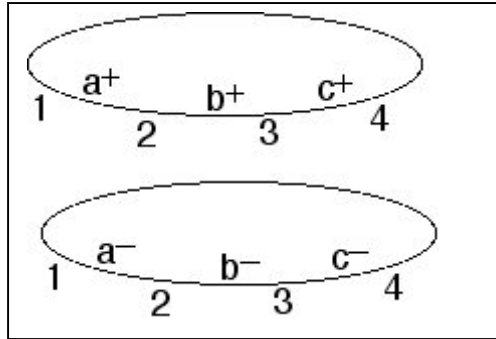


Graders: 1=KG, 2=CA, 3=KK, 4=CM, 5=KK, 6=CL, 7=VH

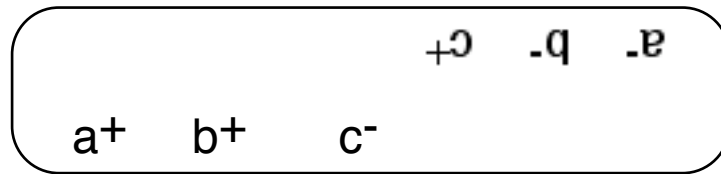
Gene Action  
Exam 1 2008

Name:

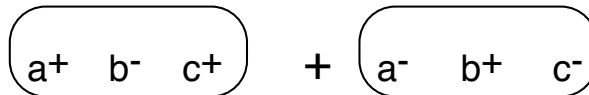
1. (12 pts) Imagine two circular double-stranded DNA molecules, as shown below. The sequences are closely enough related that recombination can occur anywhere in region 1-4.  $a^+$  represents the wild-type and  $a^-$  represents a mutation at site a, etc.



a. Draw the structure of the molecule(s) resulting from a single recombination event at site 3:

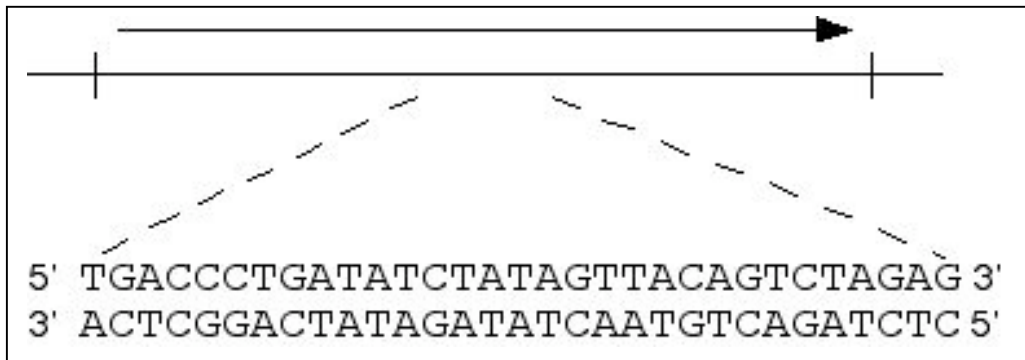


b. Draw the structure of the molecule(s) resulting from two recombination events, one at site 2 and one at site 3:

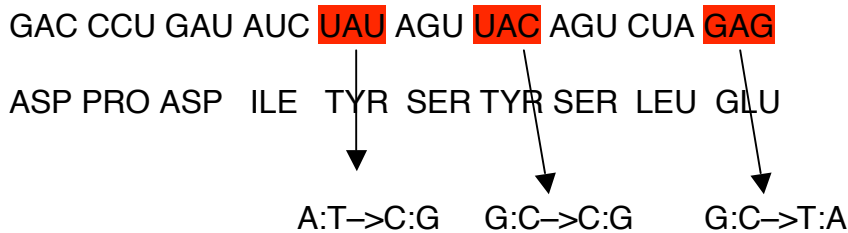


Graders: 1=KG, 2=CA, 3=KK, 4=CM, 5=KK, 6=CL, 7=VH

2. (16 pts) Imagine that the following DNA sequence comes from the middle of the wild-type *lacI* gene, as indicated (the arrow shows the direction of transcription and translation of the gene).



a. What amino acid sequence does this DNA sequence encode? (Diagram the codons and the corresponding amino acid sequence below.)

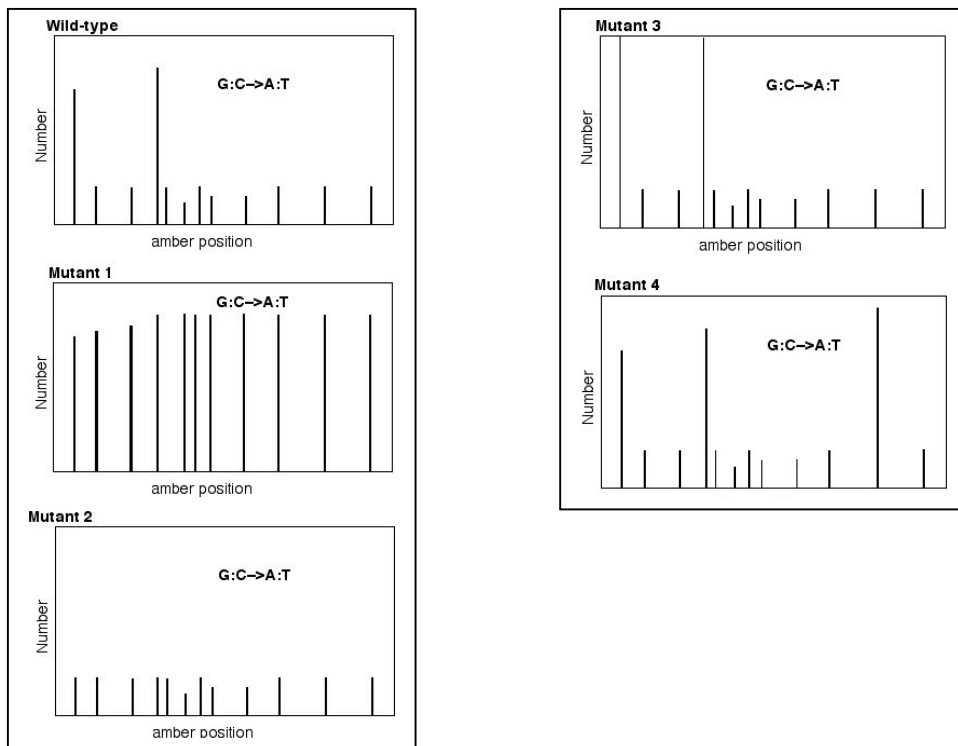


b. On your sequence above, circle the codons which can be converted into amber stop codons (UAG) by single base pair changes in the DNA, and write the changes necessary below each.

		Second Position					
		U	C	A	G		
First Position (5' end)	U	UUU ] Phe UUC ] UUA ] Leu UUG ]	UCU ] Ser UCC ] UCA ] UCG ]	UAU ] Tyr UAC ] UAA Stop UAG Stop	UGU ] Cys UGC ] UGA Stop UGG Trp	U C A G	
	C	CUU ] Leu CUC ] CUA ] CUG ]	CCU ] Pro CCC ] CCA ] CCG ]	CAU ] His CAC ] CAA ] Gln CAG ]	CGU ] Arg CGC ] CGA ] CGG ]	U C A G	
	A	AUU ] Ile AUC ] AUA ] Met AUG ]	ACU ] Thr ACC ] ACA ] ACG ]	AAU ] Asn AAC ] AAA ] Lys AAG ]	AGU ] Ser AGC ] AGA ] Arg AGG ]	U C A G	
	G	GUU ] Val GUC ] GUA ] GUG ]	GCU ] Ala GCC ] GCA ] GCG ]	GAU ] Asp GAC ] GAA ] Glu GAG ]	GGG ] Gly GGC ] GGA ] GGG ]	U C A G	

Graders: 1=KG, 2=CA, 3=KK, 4=CM, 5=KK, 6=CL, 7=VH

**3. (16 pts)** Shown below are hypothetical *lacI* G:C→A:T “fingerprinting” profiles for wild-type *E. coli* and four unknown *E. coli* mutants.



Six mutations (A-F) affecting the processing of cytosine deamination products are given below.

- A=uracil N-glycosylase negative (*ung*<sup>-</sup>)
- B= DNA cytosine methylase negative (*dcm*<sup>-</sup>)
- C=Very short patch repair negative (*vsp*<sup>-</sup>)
- D= Overactive cytosine methylase that methylates all cytosines in *lacI*
- E=Mutation in *lacI* creating one new cytosine methylation site
- F= Mutation in *lacI* eliminating one cytosine methylation site

In the space next to each mutant profile number below (1-4), write the letters of all of the mutations (A-F) which are compatible with the corresponding fingerprinting profile. If none of the mutations are compatible with a profile, write “none”.

**Mutant 1 profile: A, D (4 pts).** [A, D, X where X is C, D, E or F (3 pts). A (2 pts). D (2 pts). A and X where X is not D (2 pts). D and Y where Y is not A (2 pts).]

**Mutant 2 profile: B (4 pts).** [B and X where X is not B (3 pts).]

**Mutant 3 profile: C (4 pts).** [C and X where X is not C (3 pts).]

**Mutant 4 profile: E (4 pts).** [E and X where X is not E (3 pts).]

Graders: 1=KG, 2=CA , 3=KK, 4=CM, 5=KK, 6=CL, 7=VH

**4. (10 pts).** As described in the text, *E. coli* Dam<sup>-</sup> mutants are killed at much lower concentrations of 2-aminopurine than are Dam<sup>+</sup> wild-type cells. Double mutants carrying mutations in both *dam* and a mismatch repair gene (*mutS*, *mutH* or *mutL*) are as resistant to killing by 2-aminopurine as wild-type. Why are Dam<sup>-</sup> mutants hypersensitive to killing by 2-aminopurine? (Assume all mutations completely eliminate functions of the genes they affect.) (<50 words)

Mutant	Spont mutation freq	2-amino purine response
wt	10 <sup>-10</sup>	lives
dam <sup>-</sup>	10 <sup>-9</sup>	dies
dam <sup>-</sup> mutS <sup>-</sup>	10 <sup>-9</sup>	lives

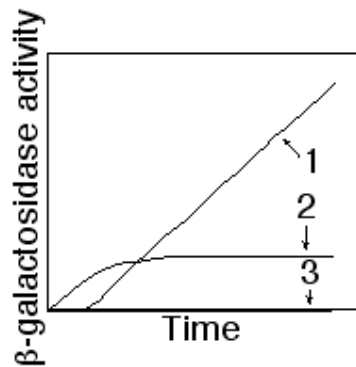
Hint: Consider the DNA processing steps carried out by the different functions involved in mismatch repair

**Mismatch repair acts on 2-AP-containing base pairs. Without Dam methylation, the cell can't distinguish which DNA strand is new and tries to repair both strands. If two 2-APs are incorporated close to each other, repairing mismatches on opposite strands can lead to lethal double-strand DNA breaks.**

Graders: 1=KG, 2=CA, 3=KK, 4=CM, 5=KK, 6=CL, 7=VH

**5. (16 pts)** The "Pajama" experiment first demonstrated the fact that *E. coli lac* operon regulation follows a negative logic, with a repressor of operon expression inactivated in the presence of lactose. The experiment consisted of measuring  $\beta$ -galactosidase enzymatic activity (a measure of  $\beta$ -galactosidase synthesis) in recipient mutant cells following Hfr conjugal transfer into the cells of a second *lac* region. The analysis utilized a regulatory mutation leading to constitutive expression of beta-galactosidase ( $lacI^C$ ).

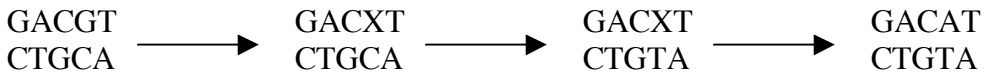
The table at the bottom of the page lists a series of potential donor and recipient strains and shows the hypothetical nature of *lac* regulation (positive or negative) in each case. The figure immediately below shows a series of lines representing  $\beta$ -galactosidase synthesis in different recipient cells grown in media lacking inducer after conjugation with different Hfr donors. Predict which line would most closely represent the pattern expected for each combination shown in the table based on the assumption of negative or positive "logic" of regulation. (Write one number (1 – 3) in the "Predicted relationship" column of the table.



Regulation	Hfr donor	F <sup>-</sup> recipient	Predicted relationship
positive	$lacI^C lacZ^-$	$lacI^+ lacZ^+$	<b>1 (2 pts)</b>
positive	$lacI^+ lacZ^+$	$lacI^+ lacZ^+$	<b>3 (2 pts)</b>
positive	$lacI^+ lacZ^+$	$lacI^C lacZ^-$	<b>1 (2 pts)</b>
positive	$lacI^C lacZ^+$	$lacI^+ lacZ^+$	<b>1 (2 pts)</b>
negative	$lacI^+ lacZ^+$	$lacI^C lacZ^-$	<b>2 (2 pts)</b>
negative	$lacI^C lacZ^+$	$lacI^C lacZ^-$	<b>1 (2 pts)</b>
negative	$lacI^C lacZ^-$	$lacI^C lacZ^-$	<b>3 (2 pts)</b>
negative	$lacI^+ lacZ^-$	$lacI^+ lacZ^+$	<b>3 (2 pts)</b>

Graders: 1=KG, 2=CA, 3=KK, 4=CM, 5=KK, 6=CL, 7=VH

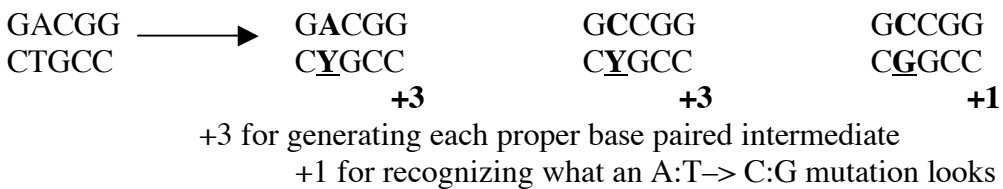
**6. (14 pts)** Imagine a base X that behaves identically to guanine except that it isomerizes more frequently to a form that base pairs with T rather than C (although base X exists **predominantly** in the form base pairing with C). Exposure of cells to X increases the frequency of G:C → A:T mutations. The increase is thought to be a consequence of the events diagrammed below. In the diagram each arrow corresponds to a round of replication and “X” represents the hypothetical new base (Note: the diagram immediately below shows only the DNA duplex that eventually becomes mutant.)



Imagine a second base Y that behaves identically to thymine except that it isomerizes more frequently to a form that base pairs with C rather than A (although base Y exists predominantly in the form base pairing with A). Exposure of cells to base Y increases the frequency of both A:T → C:G and G:C → T:A mutations.

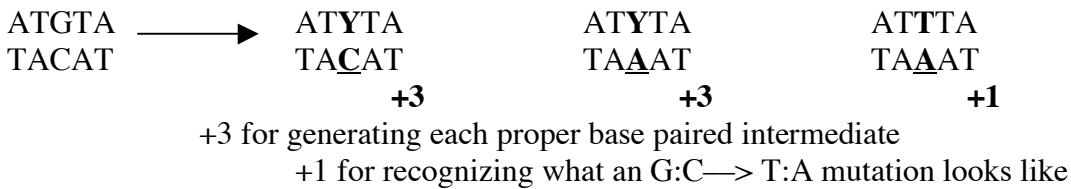
**7 Points**

a. Starting with the DNA duplex shown below, draw a diagram analogous to that above showing how Y treatment could generate an A:T → C:G mutation.



**7 Points**

b. Starting with the DNA duplex shown below, draw a diagram analogous to that above showing how Y treatment could generate a G:C → T:A mutation.



Graders: 1=KG, 2=CA, 3=KK, 4=CM, 5=KK, 6=CL, 7=VH

**7. (16 pts.)** You isolate four potential mutants (mutants a-d) with different growth properties on different media (shown below). **A.** Which of the following seven genotypes are compatible with the results? (List all compatible genotypes (1-7) for a, b, c and d below. Each number corresponds to one complete combination of genes in a cell. Remember that melibiose acts as an inducer of *lac* operon transcription.)

- 1 = *lacA*<sup>-</sup> (*lacI*<sup>+</sup> *lacZ*<sup>+</sup> *lacY*<sup>+</sup>)
- 2 = *lacI*<sup>-</sup> (*lacZ*<sup>+</sup> *lacY*<sup>+</sup> *lacA*<sup>+</sup>)
- 3 = *lacZ*<sup>-</sup> (*lacI*<sup>+</sup> *lacY*<sup>+</sup> *lacA*<sup>+</sup>)
- 4 = *lacY*<sup>-</sup> (*lacI*<sup>+</sup> *lacZ*<sup>+</sup> *lacA*<sup>+</sup>)
- 5 = *lac* deletion (*lacI*<sup>-</sup> *lacZ*<sup>-</sup> *lacY*<sup>-</sup> *lacA*<sup>-</sup>)
- 6 = *lac*<sup>+</sup> (*lacI*<sup>+</sup> *lacZ*<sup>+</sup> *lacY*<sup>+</sup> *lacA*<sup>+</sup>)
- 7 = *cya*<sup>-</sup> (=unable to synthesize cAMP) (*lacI*<sup>+</sup> *lacZ*<sup>+</sup> *lacY*<sup>+</sup> *lacA*<sup>+</sup>)

	Minimal lactose	Minimal melibiose (42°)	MacConkey lactose	L agar +IPTG +Xgal
a.	no growth	growth	white	white
b.	no growth	no growth	white	white
c.	growth	growth	red	blue
d.	no growth	no growth	white	blue

a: **3**

b: **5 7**

c: **1 2 6**

d: **4**

**10 points maximum, -1 pt for each number missing and each wrong number, to a minimum of 3 points.**

**B.** Which of the mutants (1-7) would you predict would form papillae on MacConkey lactose medium? (List the appropriate numbers. Assume mutations are single base pair changes unless stated otherwise.)

**3 (+2pts)**

**4 (+2pts)**

**7 (+1pt)**

**+1 point if no other numbers were written.**

**If “a” and “d” were written instead of “3” and “4”, they get 2 points instead of 4.**

**6 points maximum.**