NAME:

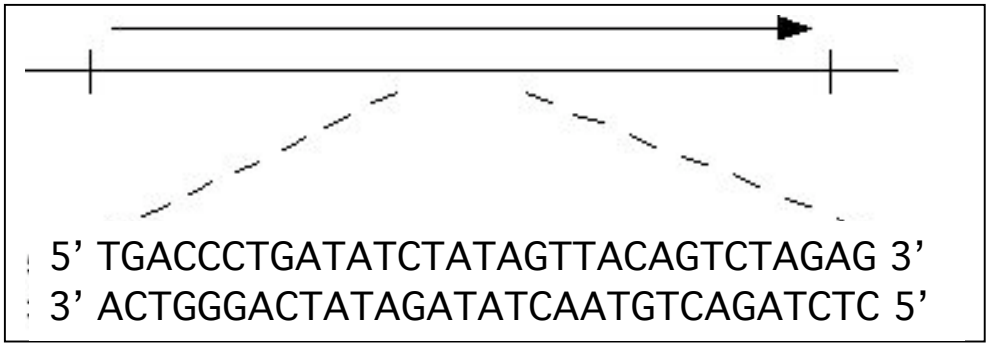
Gene Action Exam 1 Winter, 2009

1. (10 pts). Which "sense" codons (in DNA) can mutate in a single step by G:C–>T:A changes to yield UGA stop codons? (Circle the DNA codons on the chart below.)

Second Position

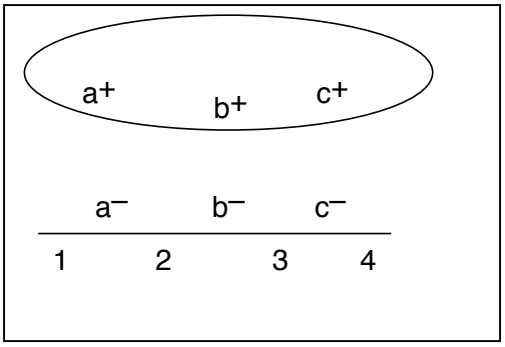
First Position (5' end)	U	C	A	G	Third Position (3' end)
U	UUU Phe UUC UUA Leu UUG	UCU UCC UCA Ser UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G
C	CUU CUC CUA Leu CUG	CCU CCC CCA Pro CCG	CAU His CAC CAA Gln CAG	CGU CGC CGA Arg CGG	U C A G
A	AUU AUC AUA Ile AUG Met	ACU ACC ACA Thr ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
G	GUU GUC GUA Val GUG	GCU GCC GCA Ala GCG	GAU Asp GAC GAA Glu GAG	GGG GGC GGA Gly GGG	U C A G

2. (14 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). What amino acid sequence does this DNA sequence encode? (Diagram the codons and the corresponding amino acid sequence below.)



Asp-Pro-Asp-Ile-Tyr-Ser-Tyr-Ser-Leu-Glu

3. (15 pts) Imagine two double-stranded DNA molecules, one linear and one circular, 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.

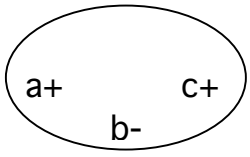


a. Draw the structure of the molecule(s) resulting from a single recombination event at site 2:
5 pts

a- b+ c+ a+ b- c-

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

a- b+ c-



c. Draw the structure of the molecule(s) resulting from three recombination events, at sites 1, 3 and 4:
5 pts

a+ b+ c- a- b- c+

4. (14 pts) A spontaneous frameshift hotspot is found in a gene at a site where there is a repeat of five base pairs as follows (note that this sequence is from the **wild-type** gene):

GCGACGTAACGTAGCG
CGCTGCATT GCATCGC

a. By analogy to the lacI frameshift hotspot, predict the likely sequence or sequences of the **mutant** gene(s) at this site (Draw the sequence(s) below):

GCGACGTAACGTAACGTAGCG
CGCTGCATTGCATT GCATCGC

+

GCGACGTAGCG
CGCTGCATCGC

b. For six mutant colonies, you examine the rate at which the mutations spontaneously revert (=mutate back) to the wild-type sequence. Three of the six revert at a high frequency, while the other three revert at a low frequency. Explain why there are two reversion frequencies. (≤30 words)

The three-repeat variant (top) can revert to wild-type by repeat-mediated polymerase slippage, but the deletion variant (bottom) cannot revert to wild-type by a similar mechanism since there is only one of the repeat sequences

5. (16 pts.) Imagine that a bacterium was discovered that used uracil in place of thymine in its DNA but that is otherwise as similar as possible to *E. coli.* (Hint: Think about what functions would have to be different from *E. coli* in such a bacterium.)

a. Predict the most frequent spontaneous base pair change expected in the hypothetical bacterium.

G:C —> A:U

b. How would you expect the spontaneous mutation rate of this hypothetical bacterium would compare to "normal" *E. coli*. (same, increased or decreased) Explain your choice (≤ 30 words).

Increased. The cell would not be able to distinguish the cytosine deamination product (uracil) from the normal base in DNA and selectively excise it using uracil N-glycosidase.

c. Imagine that, like normal *E. coli*, the hypothetical bacterium contains some 5-methyl cytosine in addition to cytosine in its DNA. Unexpectedly, the spontaneous mutation rate at these 5-methyl cytosine residues is much lower than at cytosine residues. How might you explain these mutational "cold spots"? (≤ 30 words)

Since the spontaneous deamination product of 5-methyl cytosine (thymine) would be unique in DNA, it could be distinguished and excised, e.g., by a thymine N-glycosidase-function.

6. (13 pts.) . Several strains of *E. coli* are analyzed to determine their mutation frequencies. The frequencies are shown below, and are found for mutations of a number of different genes.

	Mutant	Mutation frequency
1	wild-type	10^{-9}
2	proofreading-minus	10^{-5}
3	mismatch repair-minus	10^{-8}
4	proofreading-minus and mismatch repair-minus	2×10^{-5}

a. The naive expectation based on the mutation frequencies of the wild-type and proofreading-minus and mismatch repair-minus single mutants (rows 1-3 in the table above) was that the mutation frequency of the proofreading-minus and mismatch repair-minus double mutant would be 10^{-4}. Why was this expected? (≤ 30 words)

The two systems act on the same types of mutations (i.e., point mutations introduced during replication), so you would expect their effects to be multiplicative in the double mutant. (To do the math: 10^4-fold increase in proofreading minus x 10^1-fold increase in mismatch-minus = 10^5 increase in double mutant; 10^{-9} wild-type frequency x 10^5 increased frequency = 10^{-4} expected frequency.)

b. How could you account for the fact that the mutation frequency observed (2×10^{-5}) (row 4) was considerably lower than 10^{-4}? (≤ 30 words)

The 10,00-fold increase in mutation rate by proofreading-minus mutants saturates the ability of the mismatch system to make corrections. Deleting the mismatch system thus has little further effect.

7. (18 pts). A series of complementation assays is performed in which F'*lac* factors are conjugated into broth cultures of *lac* mutant strains. The cells are then spread onto selective indicator media and incubated. In every case, the *E. coli* F' donor strains are auxotrophic for proline and tryptophan biosynthesis, while the *lac* mutant recipient strains are prototrophic and streptomycin (Sr) resistant. (The donor strains are all Sr sensitive.) In addition to the specific *lac* genes indicated below, each F'*lac* factor also carries a gene conferring resistance to tetracycline (Tc). (The recipient strains themselves are Tc sensitive.) (go to next page)

For each combination of donor, recipient, and medium listed in the table below, describe the appearance of the final result you would expect to obtain. Give your answer in terms of "growth", "no growth", "blue", "red", "white (or colorless)", etc., as appropriate in each case (be as specific as possible). Assume that the strains are wild-type for any *lac* genes not specifically listed in the table. The first cross has been completed as an example.

F'*lac* donor	F- recipient	Medium used *	Expected result after incubation
lac+	Δ*lac*	LB + Xgal + IPTG + Sr + Tc	**Growth of blue colonies**
lac+	Δ*lac*	TZ-Lac + Sr + Tc	**Growth of white colonies**
lac+	Δ*lac*	MinMeli + Sr + Tc (42°C)	**Growth of (white) colonies**
lacI- lacZ-	*lacZ- lacY-*	TZ-Lac + Sr + Tc	**Growth of red colonies**
lacI- lacZ-	*lacZ- lacY-*	MinMeli + Tc (42°C)	**Growth of (white) colonies**
lacI- lacZ-	*lacZ- lacY-*	LB + Xgal + IPTG + Sr + Tc	**Growth of white colonies**
lacZ- lacY-	*lacI-*	MacLac + Sr + Tc	**Growth of red colonies**
lac+	*lacI-*	MinPG + Sr	**Growth of a (white) lawn**
lac+	*lacI-*	MinPG + Tc	**No growth**
lacI-	Δ*lac*	TZ-Lac + Sr + Tc	**Growth of white colonies**
lacI-	*lacO-*	MacLac + Sr + Tc	**Growth of red colonies**
lacI- lacO-	*lacY-*	MacLac + Sr + Tc	**Growth of red colonies**
lacI- lacO- lacY-	*lacY-*	LB + Xgal + Sr + Tc	**Growth of blue colonies**

* Abbreviations: TZ-Lac = tetrazolium lactose, MinMeli = minimal melibiose, MacLac = MacConkey lactose, MinPG = minimal phenylgalactoside, Sr = streptomycin, Tc = tetracycline