Exam 1 **Name _____**
Mcbio 316

Q1. Mutants defective for the proofreading function of DNA polymerase III typically form small, unhealthy looking colonies on rich medium.

(2) Why?

- **Such mutations in the *dnaQ* gene (called *mutD*) produce a "mutator" phenotype. Because they are unable to proofread errors that occur during DNA replication, such strains accumulate mutations at a high frequency. The resulting large number of "lethal mutations" that arise during cell division slows the growth of the colonies.**
- **The mutation frequency is highest when the cells are growing on rich medium which allows the cells to grow more rapidly than the resulting errors can be corrected by other repair systems.**

Q2. The DNA and corresponding amino acid sequence of a portion of the wild-type *trpA* gene is shown below. The DNA sequence of three different *trpA* mutations is shown directly below the corresponding region in the wild-type sequence. [The DNA sequence of the coding strand is shown -- i.e., TTG = UUG => Leu.]

209	210	211	212	213	214	215	216	217	218	219	220	221
TTG Leu	CAG Gln	GGA Gly	TTT Phe	GGT Gly	ATT Ile	TCC Ser	GCC Ala	CCG Pro	GAT Asp	CAG Gln	GTA Val	AAA Lys

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TAG **Amber**	CAG	GGA	TTT	GGT	ATT	TCC	GCC	CCG	GAT	CAG	GTA	AAA

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TTG Leu	CAG Gln	GGA Gly	TTT Phe	GGT Gly	GTT **Val**	TCC Ser	GCC Ala	CCG Pro	GAT Asp	CAG Gln	GTA Val	AAA Lys

Δ

TTG Leu	CAG Gln	GGA Gly	TTT Phe	GGT Gly	ATT Ile	TCC Ser	GCC Ala	CCG Pro	ATC **Ile**	AGG **Arg**	TAA **Ochre**	AA-

(6) Using the attached codon table, show the amino acid sequence for the protein made in each of the three *trpA* mutants. **See table above.**

(6) What is the type of mutation in each of the three *trpA* mutants?

- **Mutant #1 = Nonsense mutation, amber stop codon prematurely terminates protein synthesis.**
- **Mutant #2 = Missense mutation, inserts Val instead of Ile at position 214**
- **Mutant #3 = "-1" Frameshift mutation, shifts reading frame resulting in incorrect amino acids after position 218 and results in premature termination due to an "out of frame" nonsense codon.**

Q3. The reversion frequency of four different *Salmonella typhimurium his* mutants was assayed as shown below.

Mutant	Number of Revertants /10^8 Cells			
	ICR-191	EMS	Spontaneous	Q
his-1	0	0	0	0
his-2	1926	18	20	22
his-3	17	859	18	367
his-4	8	9	9	6

(4) What is the most likely type of mutation present in each mutant? [Explain your logic.]

his-1 **Probably a deletion because it doesn't revert**

his-2 **Probably a frameshift mutation because reversion is increased by the intercalating agent ICR-191**

his-3 **Probably a base substitution mutation because reversion is increased by the alkylating agent EMS**

his-4 **Probably an insertion mutation because reverts at a low frequency, but the frequency of reversion is not stimulated by mutagens**

(4) Is compound Q a mutagen? If so, what can you say about its specificity?

Q seems to be a mutagen because it causes a dramatic increase in the reversion frequency of *his-3*. (Although not as powerful of a mutagen as EMS.) Because it increases the reversion frequency of *his-3* which is likely to be a base substitution mutation (as described above) but does not increase the reversion frequency of *his-2* which is likely to be a frameshift mutation, Q probably causes base substitution mutations.

Q4. Suppressor mutations that restore functional interactions within a mutant protein may suppress some alleles of the protein much better that others. Such mutations are said to be allele-specific.

(4) What does allele-specific mean?

Allele-specific means that when one particular nucleotide or codon is at a specific site in a gene, another particular nucleotide or codon must be at a precise second site in that gene product or in a gene product it interacts with to produce a functional phenotype.

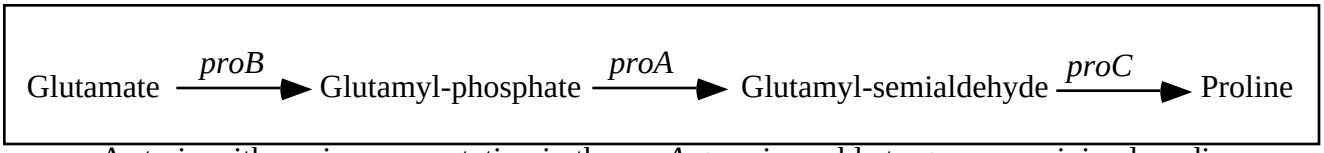
(2) What does it tell you if a suppressor is allele-specific?

If a suppressor is allele specific it indicates that the nucleotide or codon at the supressor site directly interacts with the nucleotide or codon at the site of the original mutation.

(4) Describe how intragenic suppressors that are not allele specific commonly restore function of a protein.

They usually work by making the protein more stable or by causing a general increase in activity of the protein.

Q5. The pathway of proline biosynthesis is shown below:



A strain with a missense mutation in the *proA* gene is unable to grow on minimal medium without proline. You isolate Pro^+ revertants by selecting for prototrophs.

(2) What medium would you use to select for Pro^+ prototrophs?

Minimal medium with glucose as a carbon source and no proline.

(12) List 6 different potential types of revertants that may arise and describe how each type of reversion would restore the Pro^+ phenotype. [That is, how would the revertant affect the structure or function of the *proA* gene.]

Some examples include the following:

(i) A missense suppressor tRNA which would occasionally misread the *proA* mRNA and produce a functional protein.

(ii) An allele specific, intragenic interaction suppressor with a mutation at a second site in the *proA* gene that restores the structure and function of the ProA protein.

(iii) A non-allele specific, intragenic suppressor with a mutation at a second site in the *proA* gene that increases the stability or activity of the ProA protein.

(iv) An allele specific, interaction suppressor in a protein that interacts with the ProA protein that restores the structure and function of the ProA protein.

(v) A bypass suppressor that produces glutamyl-semialdehyde via another pathway (for example, via the Arg pathway as discussed in class).

(vi) An overexpression suppressor that increases the amount of the mutant ProA protein to sufficient levels that it allows cell growth.

(vii) A true revertant that restores the wild-type *proA* DNA sequence.

Q6. The *aceA* gene product is required to grow on acetate as a sole carbon source. Temperature sensitive (Ts) mutants that affect this gene were desired, but it is not possible to directly select for *aceA* mutants. Therefore, Ts revertants of an *aceA* mutant were isolated.

(4) How would you obtain the Ts revertants? **Note: the original mutation is not Ts --you are looking for revertants that have a Ts phenotype!**

(i) Select for Ace$^+$ revertants by plating on minimal acetate plates at 30°C.

(ii) Replicate the colonies onto minimal acetate plates incubated at 30°C and minimal acetate plates incubated at 42°C.

(iii) Pick any colonies that grow on minimal acetate plates at 30°C but not 42°C and recheck their phenotype. Note an essential control is to make sure these are not simply temperature sensitive lethal mutations by checking for growth on some other carbon source at 42°C.

(4) Why is this approach easier than simply screening for *aceA* (Ts) mutants?

Intragenic pseudorevertants are often temperature sensitive. Since there is a direct selection for revertants, it is possible to obtain the revertants without heavy mutagenesis, so the cells are not likely to have secondary mutations (which might have an unrelated temperature sensitive lethal phenotype). Thus, by looking for revertants first then scoring for temperature sensitivity, <u>many</u> fewer colonies will need to be screened to find the desired mutants.

(4) The Ts revertants were grown at a permissive temperature, then isocitrate lyase activity (the *aceA* gene product) was assayed at a non-permissive temperature. The cells retained high levels of isocitrate lyase activity. Suggest a potential explanations for this result?

I only required one reasonable answer. The best answer is that the mutation is temperature sensitive for synthesis (Tss), but once made it remains active at the nonpermissive temperature.

Q7. Phage P1 efficiently infects and lyses both *galE* and *galU* mutants of *Salmonella typhimurium* , but not *gal*$^+$ strains. When a culture of a *galE62* mutant is infected with P1, most of the cells are killed but about 1 in 10^7 of the *S. typhimurium* cells is a Gal$^+$ revertant that is resistant to P1. Similarly, when a culture of a *galU14* mutant is infected with P1, about 1 in 10^7 of the *S. typhimurium* cells is a Gal$^+$ revertant that is resistant to P1. When a culture of a *galE62 galU14* double mutant is infected with P1, the reversion frequency is somewhat less — about 1 in 10^8 of the *S. typhimurium* cells is a Gal$^+$ revertant that is resistant to P1.

(4) If the reversion of the *galE62* and *galU14* mutations were independent events, what would be the predicted reversion frequency of the double mutant?

$10^{-7} \times 10^{-7} = 10^{-14}$

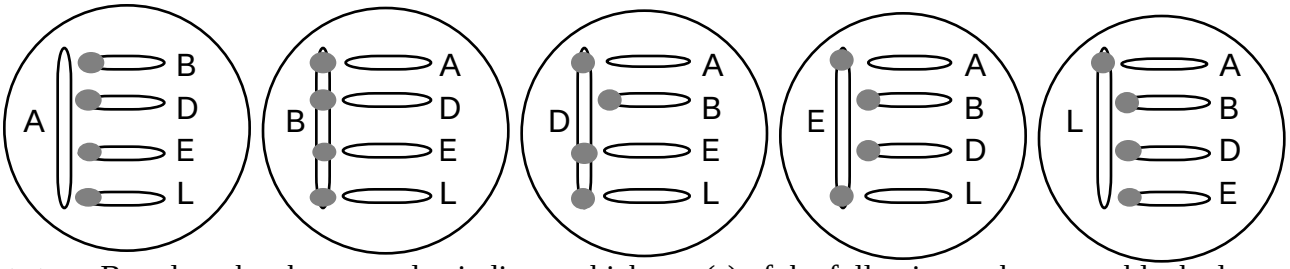
(4) The frequency of reversion of both the *galE62* and *galU14* mutants is greatly increased by the mutagen ICR-191 but not alkylating agents. What does this suggest about the nature of these mutations? Explain your answer.

Both mutants are probably due to frameshift mutations because ICR-191 is an intercalating agent that increases the frequency of +1 and -1 frameshift mutations.

(4) Given the above results, what is a likely reason that the frequency of double revertants is much higher than expected for two independent events?

Reversion of the double mutants is probably due to formation of a <u>frameshift suppressor</u> tRNA which can suppress the independent frameshift mutations in the two different *gal* genes.

Q8. Five different aromatic amino acid auxotrophs were isolated. To determine if they affect different steps of the aromatic amino acid biosynthesis pathway, each of these mutants was tested for the ability to crossfeed the other mutants. The results are shown in the figure below. [The shaded arrows indicate growth.]



(10) Based on the above results, indicate which step(s) of the following pathway are blocked in each of the five mutants and explain your logic below the figure. [Each arrow indicates an enzymatic step.]

PEP + E-4-P ⟶⟶ DHQ ⟶ DHS ⟶ S ⟶ SP ⟶⟶ Chorismate

aroA **after SP (not fed by any other mutants in pathway), can't say which of the two enzymatic steps**

aroB **before DHQ (fed by all mutants later in pathway), can't say which of the two enzymatic steps**

aroD **between DHQ and DHS**

aroE **between DHS and S**

aroL **between S and SP**

(2) If another Aro$^-$ mutant was isolated that <u>behaved the same</u> as the *aroB* mutant <u>in crossfeeding experiments,</u> describe a genetic test you could do to determine if both mutations affect the same enzymatic step. [No DNA sequencing or enzyme assays allowed.]

Note that if the mutations behave the same in crossfeeding experiments, doing additional crossfeeding experiments would not be a very good approach. Assuming that both mutations were recessive, then the best test would be an *in vivo* complementation test with two copies of the genes, one copy with a mutation in *aroB* and one copy with a mutation in the new Aro$^-$ mutant. This test must come with controls for each mutation against itself.

(2) What results would you would obtain if the two mutations affected different enzymatic steps.

If the two mutations affected two different steps (for example, the one affected each arrow in the synthesis of DHQ), then the complementation test described above would allow growth without aromatic amino acids.

Q9. Complementation tests were done on five mutants of phage T4 by co-infecting a *sup^o* strain of *E. coli* with two mutant phage. The results are shown below.

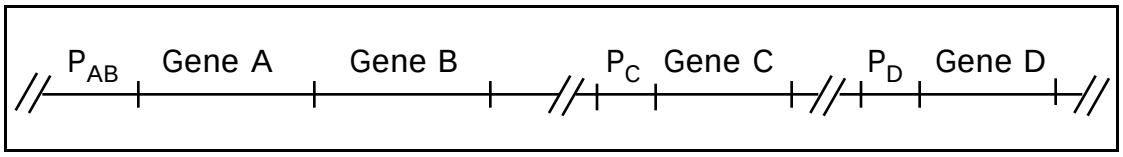
	1	2	3	4	5
1	−	−	−	+	+
2		−	+	+	+
3			−	+	+
4				−	+
5					−

(4) How many complementation groups do these mutants represent? [Explain your logic.]

Four complementation groups:

Mutant 1 and either (2 or 3)
Mutant 3 (if above is 1 and 2) or 2 (if above is 1 and 3)
Mutant 4
Mutant 5

Mutants 4 and 5 are clearly in separate complementation groups. The logic for the first two complementations groups is described below. In addition, mutant 2 can complement mutant 3 suggesting that they are in separate complementation groups. But, the surprising result is that mutant 1 cannot complement either mutant 2 or mutant 3. The simplest explanation for this result is that mutant 1 and mutant 2 or 3 are in the same complementation group but mutant 1 has a negative dominant effect on the other mutant.
For example, imagine that gene A and gene B are in an operon -- mutant 1 could be an amber mutation in gene A and mutant 3 a missense mutation in gene A -- if mutant 2 was in gene B then mutant 1 would be phenotypically $A^- B^-$ while mutant 3 would be $A^- B^+$. (See figure below.)



Q10. Three conditional mutants of phage T4 were isolated. One is cold sensitive (Cs), one is temperature sensitive (Ts), and one is an amber mutant (Am). The ability of these mutants to infect and lyse two different *E. coli* strains is shown in the table below.

Phage	Strain EC1			Strain EC2		
	25°C	30°C	42°C	25°C	30°C	42°C
Cs	−	+	+	−	+	+
Ts	+	+	−	+	+	−
Am	−	−	−	+	+	+

(6) Fill in the following table to show the expected phenotype if the bacteria were infected with the double mutant phage. [i.e. Each cell is infected with a single phage particle that has two mutations.]

Phage	Strain EC1			Strain EC2		
	25°C	30°C	42°C	25°C	30°C	42°C
Cs Ts	−	+	−	−	+	−
Cs Am	−	−	−	−	+	+
Ts Am	−	−	−	+	+	−

Note that these results strongly suggest that strain EC2 has an amber suppressor!

(6) When the bacteria were co-infected with two different mutant phage, the following results were obtained. [i.e. Each cell is infected with two phage particles.]

Phages		Strain EC1			Strain EC2		
#1	#2	25°C	30°C	42°C	25°C	30°C	42°C
Cs	Ts	−	+	−	+	+	+
Cs	Am	−	−	−	+	+	+
Ts	Am	−	−	−	+	+	+

Suggest a likely explanation for these results.

Strain EC2 has an amber suppressor.

When it is suppressed, the Am phage complements the Ts phage but not the Cs phage.