Mcbio 316 - Fall 1996 **ANSWER KEY**

Exam 1

Q1. A frameshift mutation in the *arc* gene results in premature termination of the encoded protein at an amber codon. However, the mutant is not suppressed by an amber suppressor.

(5) Explain why.
Downstream of the frameshift mutation translation occurs in the wrong reading frame. Thus even if premature termination is prevented by suppression of the "out-of-frame" nonsense codon, translation in the wrong reading frame will produce multiple amino acid changes, resulting in an inactive protein.
Note - this question specifically asked about the effect of an amber suppressor on a frameshift mutation, not how amber suppressors work or how frameshift suppressors work.

Q2. It is not possible to obtain true revertants of a deletion mutation in a haploid organism. However, it is sometimes possible to obtain second site revertants of a deletion mutation.

(5) a. What type of second site reversion event is most likely? [Explain.]
The most likely type of reversion event is a bypass suppressor because it completely avoids the need for the gene product.
Note - failure to obtain true revertants indicates that the deletion mutation described is not a simple frameshift mutation. Thus, your answer should explain how a large deletion can revert. It is true that in some very rare cases an insertion mutation could compensate for the deletion, but this is less likely than a bypass suppressor. Furthermore, if you suggested that an insertion mutation could compensate, you would need to describe (i) how it would compensate, and (ii) where the insertion of DNA might come from.

(5) b. Would this second site mutation be dominant or recessive? [Explain.]
A bypass suppressor is an altered/gain of function mutation and thus would probably be dominant to all types of loss of function mutations in the gene and the wild-type gene. (In the case of the wild-type gene, both pathways would probably function simultaneously. Sometimes such mutations are said to be "co-dominant".)
Note - by defination the suppressor is dominant to the original mutation. Therefore, in such cases dominance refers to the wild-type allele (or a different mutant allele if indicated).

Q3. Wild-type *E. coli* is unable to grow on the fatty acid decanoate (Dec^-). A Luria-Delbruck fluctuation test was done by plating 10^8 cells from 10 independent cultures onto decanoate plates, and at the same time plating 10^8 cells from a single culture onto 10 decanoate plates. The results obtained are shown below.

Independent cultures (Culture #)	Number of Colonies	Culture 11 (Plate #)	Number of Colonies
1	22	1	17
2	18	2	24
3	19	3	23
4	24	4	26
5	20	5	20
6	23	6	22
7	21	7	21
8	22	8	24
9	21	9	20
10	17	10	19

(5) Based upon these results, would you conclude that the mutation to Dec^+ is random or adaptive? [Explain your answer.]

The variance between the number of Dec^+ colonies from individual cultures equals that seen with multiple samples of a single culture. These results suggest that the phenotype is not likely to be due to a random, spontaneous mutation. (A random mutation which would show much greater variance between individual cultures.) The simplest interpretation of these results is that the mutation to Dec^+ is an example of "adaptive mutagenesis".
Note - your answer must describe the comparison of the variance between cultures #1-10 vs the multiple platings of culture #11. When given a problem like this, you need to evaluate the data given, do not simply say that it must be random mutagenesis because I said in lecture that mutagenesis is random!

Q4. Resistance to the toxic proline analog Azetidine-2-carboxylic acid can occur in two ways: (i) specific missense mutations in the *proB* gene (the first step in proline biosynthesis) which make it insensitive to feedback inhibition; and (ii) mutations that inactivate the *putP* gene (the permease which transports proline into the cell).

(5) a. Which class of mutants would you expect to be more common and why?
The *putP* mutations would be most common because a null mutation anywhere within the gene could inactivate the protein. Only a limited number of sites in *proB* would be likely to produce a functional protein which is insensitive to feedback inhibition.

(5) b. Indicate whether each of the two types of mutation is dominant or recessive to the wild-type allele of that gene. [Explain your logic.]
A *putP* null mutation is a simple loss of function mutation which would be recessive to the wild-type allele. That is, $putP^-/putP^+$ would be able to transport both proline and Azetidine-2-carboxylic acid into the cell, resulting in sensitivity to the proline analog.

A *proB* mutant insensitive to feedback inhibition ("*proB") would be dominant to wild-type. That is, $proB^*/proB^+$ would have one copy of the gene which is insensitive to feedback inhibition, allowing accumulation of a high intracellular proline concentration and thus resulting in resistance to the proline analog.**

Q5. Two new tryptophan auxotrophs were isolated. The mutant *trp-1111* excretes anthranilate and the mutant *trp-2222* excretes indole. Both of these mutants can cross-feed a known *trpE* mutant.

(5) a. How could you determine the order of genes in this pathway using cross-feeding tests? [Explain and give an example.]
From the results described you already know that both mutants crossfeed *trpE*.
Determine whether *trp-1111* and *trp-2222* can crossfeed each other:
- **if excretion of anthranilate by *trp-1111* allows growth of trp-2222, then *trp-2222* must be an earlier step in the pathway;**
- **alternatively, if excretion of indole by *trp-2222* allows growth of *trp-1111*, then *trp-1111* must be an earlier step in the pathway.**
- **If neither mutant excretes an intermediate that allows growth of the other, then they probably affect the same step of the pathway. An alternative explanation for this third possibility would be that one of the mutants is blocked for a step immediately before or after the step that is defective in the second mutant, but the intermediate is not diffusible. However, neither of these answers fits the data because the question explicitly states that both mutants excrete a different intermediate.**

(5) b. How could you determine the order of genes in this pathway using epistasis analysis?
Construct the double mutant (*trp-1111 trp-2222*). If the double mutant excretes anthranilate then *trp-1111* must be an earlier step in the pathway. If the double mutant excretes indole then *trp-2222* must be an earlier step in the pathway.

Q6. Four mutants of phage P22 were isolated. Three of the mutants (P22-1, P22-2, and P22-3) have missense mutations, and one of the mutants (P22-4) has an amber mutation. Complementation tests between these mutants gave the following results. [+ indicates growth of P22; − indicates no growth of P22].

	P22-1	P22-2	P22-3	P22-4
P22-1	–	+	+	+
P22-2		–	+	–
P22-3			–	–
P22-4				–

(5) a. How would you test for complementation between two mutations? [How would you test the mutants in the experiment described?]
Coinfect with two different phage. Score for lysis.

(2) b. How many complementation groups are there?
3 or 4 ***(Not simply 3 and not simply 4 !)***

(4) c. List the mutants in each complementation group.
Group #1 = P22-1
Group #2 = P22-2
Group #3 = P22-3
Possibly Group #4 = P22-4 (see comments in 6d)

(5) d. Suggest a likely explanation for any unusual complementation results.
P22-4 cannot complement either P22-2 or P22-3 which are in different complementation groups, therefore it is probably dominant. Because P22-4 has an amber mutation, this may be due to a cis-dominant, polar mutation on a downstream gene or genes.

Q7. Jarvick and Botstein used suppressor analysis to identify protein-protein interactions required for assembly of phage P22. Beginning with a temperature sensitive (TS) mutant that prevents growth of P22 at 42°C, they selected for second site suppressors that allow growth of P22. When the second site suppressors were backcrossed onto wild-type P22, some of them had a cold sensitive phenotype. These results are shown in the table below.

Strain	Mutations present:		Growth	
	Original TS	Suppressor	30°C	42°C
Wild-type	no	no	+	+
Original mutant	yes	no	+	–
Revertant	yes	yes	+	+
Backcross	no	yes	–	+

(6) a. Propose a likely explanation for the phenotype of the revertant and backcrossed suppressor mutant.

The revertant suppresses the temperature sensitive phenotype of the original mutation. When both the revertant and original mutant allele are together then the phenotype is wild-type at both temperatures, suggesting that the two mutations compensate for the conditional phenotype. When the revertant is combined with the wild-type original allele (in the backcross), then the revertant has a cold sensitive phenotype. These results suggest that the revertant allele specifically interacts with the original mutant allele.

(6) b. What is the most likely type of suppressor mutation obtained? [Explain your rationale]

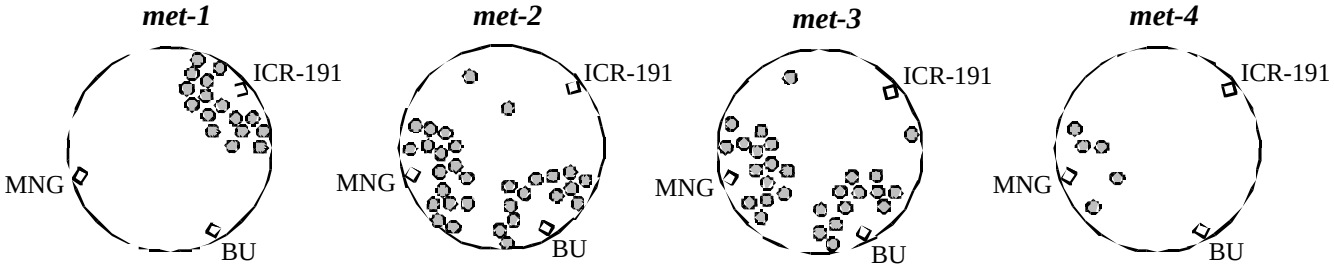
An interaction suppressor based upon the failure to interact with the wild-type allele (suggesting it may be allele specific).

Note - it is extremely unlikely that a missense suppressor would give the cold-sensitive phenotype because due to the low efficiency of missense suppressors most of the protein produced would not be suppressed.

Q8. Four Met$^-$ mutants of *E. coli* were isolated. About 10^9 cells of each of these mutant strains was spread onto three types of plates: minimal medium plates (MM), minimal medium + methionine plates (MM+Met), and rich medium plates (LB). The plates were incubated overnight at several growth temperatures, then the number of colonies on each plate were counted. The results are shown in the table below. [TNTC indicates the number of colonies was too numerous to count.]

Mutant	30°C			37°C			42°C		
	MM	MM+Met	LB	MM	MM+Met	LB	MM	MM+Met	LB
met-1	3	TNTC	TNTC	2	TNTC	TNTC	2	TNTC	TNTC
met-2	23	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
met-3	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	16	TNTC	TNTC
met-4	0	TNTC	TNTC	TNTC	TNTC	TNTC	0	TNTC	TNTC

Next, about 10^9 cells of each of the four Met$^-$ mutants were spread on a minimal medium plate and a crystal of several mutagens was placed on the medium at different places near the edge of the plate. The three mutagens used were: ICR-191, an intercalating agent; Bromouracil (BU), a base analog; and nitrosoguanidine (MNG), an alkylating agent. The plates were incubated at the nonpermissive temperature. Growth is indicated by shaded circles in the figures below.



(12) a. Suggest a likely explanation why each of the four mutants forms colonies efficiently under some growth conditions but not others. [Your answer should account for the results observed for each of the mutants in each of the growth conditions shown *in the table*.]

- **All four mutants are methionine auxotrophs because they fail to grow on minimal medium but grow on minimal medium + methionine and on rich medium which contains methionine.**

- **Mutant 1 is not due to a conditional mutation (it is probably a null mutation).**
- **Mutant 2 is cold sensitive indicating it has probably a missense mutation.**
- **Mutant 3 is temperature sensitive indicating it has probably a missense mutation.**
- **Mutant 4 probably has both a cold-sensitive and temperature sensitive mutation because it cannot grow at either temperature extreme but it can grow at 37°C.**

(8) b. Based upon these results, what is the most likely type of mutation in each of the four mutants? [Explain your logic. Your conclusion should take into account both the data in the table and the data in the figure.]

- **Mutant 1 is due to a frameshift mutation because reversion is stimulated by an intercalating agent.**
- **Mutant 2 is probably a base substitution mutation because reversion is stimulated by the base analog and alkylating agent, and because it is a CS mutation.**
- **Mutant 3 is probably a base substitution mutation because reversion is stimulated by the base analog and alkylating agent, and because it is a TS mutation.**
- **Mutant 4 is probably a double mutant because it shows essentially no spontaneous reversion, but it can be reverted by a powerful mutagen (MNG). An alternative explanation could be that this mutant is due to a deletion mutation and MNG mutagenesis yields a rare bypass suppressor that cannot function at 30°C or 42°C. (The second idea seems much less likely. How could you distinguish these two possibilities?)**

(4) c. Is this a selection, a screen, or an enrichment? [Explain.]

A selection because <u>only</u> cells with a Met^+ phenotype can grow on minimal medium without methionine.
Note - some people answered screen because some of the cells were plated on non-selective media. Note that in addition to being non-selective, the LB and MM+Met media didn't provide a screen or enrichment for mutants either.

(4) d. Why are there no colonies growing close to the mutagen in the figure? [Explain the reason.]
High doses of mutagens are lethal because they may cause mutations in essential genes and because they often damage other cellular macromolecules as well as DNA.

(4) e. How would you determine if these four mutations affect the same gene or different genes?
Complementation tests with two copies of each DNA fragment (each copy bearing a different mutation) in a single cell.
If the mutations are in the same gene, they will not complement so the cell will still have a mutant phenotype.
If the mutations are in different genes (and they are recessive), they will complement so the cell will have a wild-type phenotype.