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.]

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.
<table>
<thead>
<tr>
<th>Mutant</th>
<th>Number of Revertants /10^8 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICR-191</td>
</tr>
<tr>
<td>his-1</td>
<td>0</td>
</tr>
<tr>
<td>his-2</td>
<td>1926</td>
</tr>
<tr>
<td>his-3</td>
<td>17</td>
</tr>
<tr>
<td>his-4</td>
<td>8</td>
</tr>
</tbody>
</table>

(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.  Suppressors 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 suppressor 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:

<table>
<thead>
<tr>
<th>Glutamate</th>
<th>proB</th>
<th>Glutamyl-phosphate</th>
<th>proA</th>
<th>Glutamyl-semialdehyde</th>
<th>proC</th>
<th>Proline</th>
</tr>
</thead>
</table>

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\(^\circ\)C.

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

(iii) Pick any colonies that grow on minimal acetate plates at 30\(^\circ\)C but not 42\(^\circ\)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\(^\circ\)C.

(4) Why is this approach easier than simply screening for ace\(^A\) (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, many 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 ace\(^A\) gene product) was assayed at a non-permissive temperature. The cells retained high levels of isocitrate lyase activity. Suggest a potential explanation 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 gal\(^E\) and gal\(^U\) mutants of Salmonella typhimurium, but not gal\(^+\) strains. When a culture of a gal\(^E62\) 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 gal\(^U14\) 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 gal\(^E62\) gal\(^U14\) 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 gal\(^E62\) and gal\(^U14\) mutations were independent events, what would be the predicted reversion frequency of the double mutant?

10\(^{-7}\) x 10\(^{-7}\) = 10\(^{-14}\)

(4) The frequency of reversion of both the gal\(^E62\) and gal\(^U14\) 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 frameshift suppressor 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.]

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 Ara^- mutant was isolated that behaved the same as the araB mutant in crossfeeding experiments, 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 araB and one copy with a mutation in the new Ara^- mutant. This test must come with controls for each mutation against itself.
(2) What results would you 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 sup⁰ strain of E. coli with two mutant phage. The results are shown below.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>−</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−</td>
</tr>
</tbody>
</table>

(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.

<table>
<thead>
<tr>
<th>Phage</th>
<th>Strain EC1</th>
<th>Strain EC2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Cs</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Ts</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Am</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

(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.]

<table>
<thead>
<tr>
<th>Phage</th>
<th>Strain EC1</th>
<th>Strain EC2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Cs Ts</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Cs Am</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Ts Am</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*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.]

<table>
<thead>
<tr>
<th>Phages</th>
<th>Strain EC1</th>
<th>Strain EC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>25°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Cs</td>
<td>Ts</td>
<td>−</td>
</tr>
<tr>
<td>Cs</td>
<td>Am</td>
<td>−</td>
</tr>
<tr>
<td>Ts</td>
<td>Am</td>
<td>−</td>
</tr>
</tbody>
</table>

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.