1. A wild-type, prototrophic *Bacillus subtilis* strain was mutagenized with the intercalating agent ICR-191. [copy#2 Shigella mutagenized with 5-BU, copy #3 Citrobacter mutagenized with UV]

a. Describe an approach you could use to easily determine that mutagenesis was random. [i.e. How could you demonstrate that you obtained mutations in a variety of genes?]

   ANSWER: Replica plate from rich medium to minimal medium to screen for auxotrophic mutations. Because this would identify mutations in any gene which encodes a product required for any biosynthetic pathway that is not required in rich medium (including amino acids, purines, pyrimidines, vitamins, etc), auxotrophic mutations provide a very large target. If the mutations are random, a variety of types of auxotrophs should be obtained.

b. How could you determine the mutation rate? [copy #2 switch b and c]

   ANSWER: Do a fluctuation test and use the Poisson distribution to calculate the number of mutations per cell division.

c. How could you determine the mutant frequency?

   ANSWER: Simply count the number of mutants and divide by the number of total cells in a culture.

d. What is the difference between mutation rate and mutant frequency?

   ANSWER: Because it is based upon multiple populations, mutation rate defines the probability that a cell will acquire a mutation per cell division, independent of when the mutation occurs during growth of the culture. In contrast, mutant frequency may vary greatly from culture to culture depending upon when the mutation arose during the growth of each culture.

2. When grown on MacConkey-Lactose indicator plates, Lac⁺ bacteria form red colonies and Lac⁻ bacteria form white colonies.

a. How could you most easily find strains with a mutator phenotype using MacConkey-Lactose plates? Explain your answer. [copy #2 TTC reverse colors; copy #3 Xgal blue]

   ANSWER: "Mutator phenotype" indicates that the desired mutant strains exhibit an enhanced spontaneous rate of mutagenesis due to lesions in one or more of the DNA repair genes. The key to this question is realising that the Lac⁻ colonies will appear white on the MacConkey lactose medium thus allowing rare Lac⁺ revertants to be easily identified as red papillae or sectors within a colony. If a strain exhibits a high level of spontaneous mutation (e.g. a mutator phenotype), it will accumulate mutations after beginning to grow on the medium. If a reversion occurs early in the life of the colony,
the daughter cells within the colony will be numerous and form a sector, if the reversion occurs later, fewer daughter cells will arise before the colony stops growing and so a small spot or papillus arises. This idea is analogous to the "jackpot" theory that was discussed with the Luria-Delbruck fluctuation test.

For copy #2 the answer would demand looking for red Lac` colonies on TTC plates; for copy #3 the answer would demand looking for blue Lac` colonies on Xgal plates. Note difference between screen for mutants vs screen for revertants depends upon what type of differential color is available – it is much easier to see one red or blue colony in a lawn of white cells than to see one white colony in a lawn of red or blue cells.

b. Is this a selection, screen, or enrichment?

**ANSWER:** Screen

(10) **3.** Wild-type *Salmonella* grows on minimal medium plates without addition of fatty acids. Fatty acid auxotrophs are normally rare – typically about $10^{-6}$ fatty acid auxotrophs are found in a population of bacteria. [Copy #2 lipoic acid $10^7$; copy #3 acetate $10^5$]

a. How would you distinguish fatty acid auxotrophs from wild-type prototrophic cells? [Specify the phenotype of both types of cells.]

**ANSWER:** Replica plate on minimal medium + or - fatty acids. Wild-type cells will grow with or without fatty acids. Fatty acid auxotrophs will not be able to grow without fatty acids in this screen.

b. Draw a diagram showing how you would do a penicillin enrichment for fatty acid auxotrophs. [Indicate the conditions and medium you would use and show the relative proportion of auxotrophs in the population at each step.]

**ANSWER:** The basic penicillin enrichment approach is shown in the following figure, with aux = fatty acid auxotrophic mutation, and supplement = addition of fatty acids. Each cycle of penicillin enrichment should result in approximately 100-fold enrichment for the mutant cells relative to the wild-type cells. [Note that the number of cycles required will depend upon the initial mutant frequency expected.]
c. How would you avoid isolation of other types of auxotrophs?

ANSWER: Any other auxotrophs that accumulated would not be killed during the minimal medium + penicillin step, but they would not be able to grow in minimal medium with fatty acids. (This is a reason why you wouldn't want to use rich medium for outgrowth between penicillin enrichment steps.)

d. How would you avoid isolation of siblings?
ANSWER: A major problem with this method is that each enrichment culture is likely to contain siblings. This problem can be avoided by doing multiple, independent enrichments and only choosing one colony from each enrichment. (As described in the text, a second problem may be that lysis of cells in the cultures with penicillin could feed any penicillin auxotrophs, allowing their growth and subsequent killing of the desired auxotrophs if the enrichment is not done properly. However, this is not the question asked here.)

4. RecBCD catalyzes recombination between a linear DNA substrate and the circular bacterial chromosome. RecFOR catalyzes recombination between two circular DNA substrates, but does not efficiently catalyze recombination between a linear DNA substrate and the bacterial chromosome. Loss of function mutations in the \( \text{recBC} \) genes results in about 1000x decrease in recombination frequency between a linear DNA substrate and the bacterial chromosome. It is possible to isolate suppressors that increase the recombination frequency of a \( \text{recBC} \) missense mutant.

   a. In the table below, list 4 different types of suppressors that might be obtained. [Note any properties of the primary mutation required for the proposed suppressor.]

   ANSWER: A few examples are shown in the following table.

   b. In the table below, briefly indicate how each type of suppressor would restore the Rec\(^+\) phenotype. Indicate whether each type of suppressor might be allele specific.

   c. In the table below, indicate whether each of the suppressors would be dominant or recessive, to the wild-type allele. [If the suppressors is neither dominant or recessive to the wild-type allele, indicate “neither”.

<table>
<thead>
<tr>
<th>Type of suppressor</th>
<th>How does it increase recombination?</th>
<th>Dominant or recessive</th>
</tr>
</thead>
<tbody>
<tr>
<td>i Bypass (Intergenic suppression)</td>
<td>Turns on RecFOR pathway</td>
<td>Dominant</td>
</tr>
<tr>
<td>ii Missense sup tRNA (Intergenic suppression)</td>
<td>Inserts permissible amino acid</td>
<td>Dominant</td>
</tr>
<tr>
<td>iii 2(^{nd}) change in same codon (Intragenic suppression)</td>
<td>Substitution of permissive amino acid</td>
<td>Neither</td>
</tr>
<tr>
<td>iv 2(^{nd}) change elsewhere in protein – intragenic interaction suppressor</td>
<td>Restores folding / function</td>
<td>Neither</td>
</tr>
</tbody>
</table>

d. Which (if any) of these suppressors might be allele specific? How could you test for allele specificity of suppressor mutations?
ANSWER: The interaction suppressor might be allele specific. To test for allele specificity, first backcross against the wild-type and determine if the 2nd mutation can function with the wild-type amino acid in the first position. If so, test for suppression of multiple other alleles.

(10) 5. A Salmonella strain is auxotrophic for both histidine and cysteine due to a mutation in the hisC gene and a mutation in the cysA gene. His\(^+\) revertants are found at a frequency of 1 per 10\(^6\) cells. Cys\(^+\) revertants are found with a frequency of 1 per 10\(^7\) cells. [copy#1 leucine \(10^{-6}\) and tryptophan \(10^{-6}\); copy #3 proline \(10^{-5}\) and threonine \(10^{-6}\)]

a. How would you obtain His\(^+\) revertants only or Cys\(^+\) revertants only? [What kind of medium would you plate the cells on.]

ANSWER: For His\(^+\) revertants, plate the cells on minimal medium with cysteine. For Cys\(^+\) revertants, plate the cells on minimal medium with histidine. [Other copies of exam substitute different auxotrophic requirements.]

b. Is the method you proposed a selection, a screen, or an enrichment? Briefly explain your answer.

ANSWER: Selection (parental cells cannot grow, revertants can form colonies)

c. What frequency would you expect to find revertants that are both His\(^+\) and Cys\(^+\)?

ANSWER: Simply multiply the frequency of each independent event: \(10^{-5} \times 10^{-5} = 10^{-11}\) [Copy #1 \(10^{-12}\); copy #3 \(10^{-11}\)]

d. How could you directly select for such double revertants?

ANSWER: Plate the cells on minimal medium without histidine or cysteine.

e. His\(^+\) Cys\(^+\) revertants are actually found at a frequency of 1 per 10\(^8\) cells. Suggest two different explanations for this result.

ANSWER: The observed frequency is much higher than predicted for independent events, suggesting that certain types of mutations can simultaneously affect both processes. This could occur in several ways. For example, the two pathways may share a common intermediate, genes for the independent pathways may lie in the same operon, the two pathways may share a common regulator, or two mutations may be suppressed by a common mechanism.

(10) 5. Under conditions that favor lytic growth, phage \(\lambda\) will infect and kill \(E.\ coli\) cells. Phage \(\lambda\) uses the LamB protein as its receptor. Hence, phage \(\lambda\) will only infect \(E.\ coli\) cells that make functional LamB protein. The lamB gene is located in an operon along with the malK gene which
is also required for cells to use maltose as a carbon source. The direction of transcription is from \textit{malK} to \textit{lamB} as shown by the arrows in the figure below. The \textit{malK-lamB} operon is activated by the product of the \textit{malT} gene which is located at a distant position on the chromosome.

![Operon diagram]

Phage $\lambda^R$ mutants were isolated by selecting for \textit{E. coli} colonies that grow on LB plates spread with phage $\lambda$. The $\lambda^R$ mutants included point mutants in each of these three genes.

a. How could mutants in \textit{malT}, \textit{malK}, and \textit{lamB} cause the lambda-resistant phenotype?

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{malT}</td>
<td>Any mutation that inactivates \textit{malT} (missense, nonsense, frameshift, etc) will prevent formation of active MalT protein so that the \textit{malK-lamB} operon cannot be activated.</td>
</tr>
<tr>
<td>\textit{malK}</td>
<td>A mutation that leads to a non-sense codon in \textit{malK} can lead to polarity on expression on the \textit{lamB} gene. Thus the LamB protein is not made in an amount that allows $\lambda$ to infect and kill.</td>
</tr>
<tr>
<td>\textit{lamB}</td>
<td>Any mutation that inactivates the LamB protein will make the cells resistant to infection by $\lambda$.</td>
</tr>
</tbody>
</table>

b. What is the predicted maltose-utilization phenotypes of each of these mutants? Briefly explain your answer.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{malT} ($\lambda^R$)</td>
<td>Cannot use MD because no expression of MalK and LamB required for growth</td>
</tr>
<tr>
<td>\textit{malK} ($\lambda^R$)</td>
<td>Cannot use MD because MalK required and LamB is missing.</td>
</tr>
<tr>
<td>\textit{lamB} ($\lambda^R$)</td>
<td>Cannot use MD because LamB not active.</td>
</tr>
</tbody>
</table>
c. Would you expect any of the mutants to be suppressed by \( supF \)? (\( supF \) is an amber suppressor that inserts tyrosine at amber codons.) If so, explain the mechanism for each case.

**ANSWER:** In principle, any of the genes could have mutations that could be suppressed since the gene products are proteins. One would need a mutation that leads to an amber codon and suppression would require that Tyr is an acceptable amino acid at the particular position in the protein.

(10) 6. It is possible to isolate revertants of \( malT, malK, \) or \( lamB \) mutants that can utilize maltodextran. Three mutants were isolated from a strain that contained both a \( lamB \) mutation and an amber mutation in the \( argE \) gene that results in arginine auxotrophy. The results for three independent revertants of the \( lamB \ argE(\text{Am}) \) strain are shown below.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity to phage ( \lambda )</th>
<th>Growth without arginine</th>
<th>Growth on maltose as carbon source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revertant 1</td>
<td>sensitive</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Revertant 2</td>
<td>sensitive</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Revertant 3</td>
<td>resistant</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

a. From the results in the table above, what is the probable mechanism for each revertant?

<table>
<thead>
<tr>
<th>Revertant 1</th>
<th><strong>Amber suppressor (because it simultaneously restores function of both mutations including the ( argE(\text{Am}) ) mutation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Revertant 1</td>
<td><strong>True reversion of ( lamB ) gene because it restores complete function of ( lamB ) but does not affect function of ( argE ) mutation</strong></td>
</tr>
<tr>
<td>Revertant 1</td>
<td><strong>Second site pseudorevertant of ( lamB ) that restores one function of LamB gene product but not the other.</strong></td>
</tr>
</tbody>
</table>

b. Does the data provide specific information for the mutation that caused the original \( lamB \) phenotype?

**ANSWER:** The data indicates that the \( lamB \) mutation is due to an amber stop codon.
7. Farabaugh et al. measured the frequency of spontaneous mutations at different sites in the *lacI* gene of *E. coli*. Of 140 spontaneous mutations obtained, 37 were deletions. Of these deletions, 18 removed a specific 4 bp sequence. The DNA sequence of the region surrounding this deletion hot spot is shown below. Which base pairs are most likely to be deleted and why?

```
TCGGCGCGTCTGCGTC TGGC TGGC TGGC ATAAA
AGCCGCGCAGACGCAG ACCG ACCG ACCG TATTT
```

**ANSWER.** The deletion hot spot was most likely due to strand slippage between the three, direct four bp repeats in the *lacI* gene. Although there are other four bp repeats in this sequence, only the TGGC sequence is repeated three times, hence it is the most likely deletion hot spot. In addition, a deletion resulting between any adjacent pairs of this sequence would remove a specific 4 bp sequence.

8. The *Bacillus subtilis yqfI* gene encodes a protein which catalyzes recombination between circular DNA molecules. Epistasis analysis was done between *yqfI* and other mutations known to affect recombination. The results are shown below. In the last column of the table, indicate the interpretation of the epistasis tests for the double mutants. [i.e. Based upon these results, which genes are likely to affect separate recombination pathways?]

<table>
<thead>
<tr>
<th>mutations</th>
<th>recombination frequency</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>yqfI</em></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td><em>recA</em></td>
<td>0.000001</td>
<td></td>
</tr>
<tr>
<td><em>yqfI recA</em></td>
<td>0.000001</td>
<td><em>recA affects master pathway</em></td>
</tr>
<tr>
<td><em>recF</em></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td><em>yqfI recF</em></td>
<td>0.04</td>
<td><em>recF affects same pathway as yqfI</em></td>
</tr>
<tr>
<td><em>recR</em></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td><em>yqfI recR</em></td>
<td>0.04</td>
<td><em>recR affects same pathway as yqfI</em></td>
</tr>
<tr>
<td><em>recL</em></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td><em>yqfI recL</em></td>
<td>0.04</td>
<td><em>recL affects same pathway as yqfI</em></td>
</tr>
<tr>
<td><em>addA</em></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td><em>yqfI addA</em></td>
<td>0.004</td>
<td><em>addA affects different pathway than yqfI</em></td>
</tr>
<tr>
<td>Gene</td>
<td>Frequency</td>
<td>Notes</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>addB</td>
<td>0.04</td>
<td>addB affects different pathway</td>
</tr>
<tr>
<td>yqfI addB</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>recH</td>
<td>0.04</td>
<td>recH affects different pathway</td>
</tr>
<tr>
<td>yqfI recH</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>recU</td>
<td>0.04</td>
<td>recU affects same pathway as yqfI</td>
</tr>
<tr>
<td>yqfI recU</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

(15) **9.** Based upon proflavin-induced mutants in the rII locus of phage T4 and pseudo-revertants (suppressors) of these original mutants, Crick et al. deduced that the genetic code must be a multiple of three nucleotides.

Wild-type T4 phage produce smooth plaques (w) on *E. coli* B, but T4 rII mutants form rough plaques (r) on *E. coli* B). A partial sequence of the rII region of phage T4 is shown below. Mutants (designated as FC followed by a number) are shown below the sequence. Some mutants, such as FC0 and FC41 insert a base while others such as FC1 and FC9 delete a base.

```
5' C C A A A A C G A A G A A G C T G A A A T T G T T A A A C
   +A -G -A +A
```

FC41 FC1 FC9 FC0

The phenotype of phage with combinations of mutants is shown in the following table. "r" means that the phage makes rough plaques. "w" indicates that the phage makes wild-type plaques.

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>Plaques on <em>E. coli</em> B</th>
<th>Reason for plaque phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC41</td>
<td>r</td>
<td></td>
</tr>
<tr>
<td>FC0</td>
<td>r</td>
<td></td>
</tr>
<tr>
<td>FC1</td>
<td>r</td>
<td></td>
</tr>
<tr>
<td>FC9</td>
<td>r</td>
<td></td>
</tr>
<tr>
<td>FC41 FC1</td>
<td>w</td>
<td>The + and – restore the correct reading frame</td>
</tr>
<tr>
<td>FC0 FC1</td>
<td>w</td>
<td>The + and – restore the correct reading frame. Note-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>the mRNA sequence in region between th frameshifts</td>
</tr>
</tbody>
</table>
would have at least six amino acid residues that are wrong. This indicates that the amino acid residues in this region of the protein are not essential for function.

<table>
<thead>
<tr>
<th>Combination</th>
<th>Result</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC0 FC9</td>
<td>w</td>
<td>The + and – restore the correct reading frame.</td>
</tr>
<tr>
<td>FC41 FC0</td>
<td>r</td>
<td>Two + framshifts do not restore the proper reading frame.</td>
</tr>
<tr>
<td>FC41 FC9</td>
<td>r</td>
<td>This is a + and a – that restores the proper reading frame but not function. This means that there is a nonsense codon in the correct reading frame (UGA) or that an amino acid residue that does not allow protein function in present. The latter is unlikely because the experiment with the FC0 / FC1 combination showed that this region of the protein is not essential for function.</td>
</tr>
<tr>
<td>FC1 FC9</td>
<td>r</td>
<td>Two – framshifts do not restore the correct reading frame.</td>
</tr>
</tbody>
</table>

a. In the last column of the table above, explain the reason why each combination produces “w” or “r” plaques. Include an explanation that explains the phenotype at the level of messenger RNA translation. [A genetic code table is included at the back of the exam.]

b. Brenner isolated a mutant of *E. coli* that allowed the FC41 and FC9 combination to make wild-type plaques on *E. coli* K-12(λ). What is the most likely explanation for this result?

**ANSWER:** The most likely explanation is that Brenner’s strain had a UGA suppressor that suppressed the UGA codon in the mRNA between the FC41 and FC9 mutations. It is very unlikely that an amino acid that disrupts function occurs in the region between the mutations because this region is not necessary for protein function.

c. What does this work suggest about the amino acid sequence of this region of the rII B gene?

**ANSWER:** As stated above, this region of the protein is not essential for function.