1. Predict the requested outcomes for the following experimental protocol: *E. coli* is grown in minimal medium containing both the radioactive isotopes $^{35}S$ and $^{32}P$, collected on a filter, washed and then re-suspended in fresh medium. When the new culture reaches mid-log phase, phage T2 is added and within a few hours the culture has cleared, meaning almost all the bacterial cells have lysed to release the progeny phage.

(You may include any 'qualifying' statements in your answer you feel are necessary.)

a) What macromolecules/components would be radioactively labeled in the bacteria? $^{35}S$ will label any proteins made, $^{32}P$ any new DNA and RNA

b) What macromolecules/components would be radioactively labeled in the progeny phage? Primarily $^{32}P$ DNA since they will degrade the labeled host DNA and re-incorporate the label. There may be some $^{35}S$ if there is turnover of labeled protein.

c) If the progeny phage were now used to infect un-labeled *E. coli* grown on rich medium, what label(s) would be found in the bacteria before lysis and in the progeny phage? $^{32}P$ before lysis and in the progeny phage; not all DNA strands would have to be labeled.

d) Although both $^{35}S$ and $^{32}P$ are beta emitters they emit at different energies that can be detected by modern scintillation counters. Would the duel label procedure described here improve on the method used by Hershey and Chase? Why or why not? No, it might save a bit on the time needed to do the experiments, but using labels one at a time should provide the same answer as a mix of both.

2. The chromosomes found in pro- and eu- karyotic organisms have some basic differences. Name at least two that have already been covered in class and describe how DNA replication accommodates the difference(s).

*Circular (Prok) vs linear (Euk)*  While both have origins for replication and proceed 5' to 3' in both directions, the ends in the much larger eukaryotic chromosomes cause a problem. Since an RNA primer is used to initiate replication, when that primer is removed, the ends will lose bases. Eukaryotes use telomerase, with an RNA guide strand to restore the ends.

Prokaryotic chromosomes do not have centromeres, the Polymerase (III) is attached to the cell membrane to separate the chromosomes to daughter cells via fission vs having spindle fibers that pull them to opposite poles.

Prokaryotic chromosomes do not include histones so do not have to remove/rebuild them during replication. This may account for needing more DNA polymerases in eukaryotes.
3. Although selenocysteine was identified as a component of selenoproteins in the 1950s, it was almost 30 years before the mechanism by which it is incorporated was discovered.

What is the mechanism of incorporation and why do you think it took so long to discover.

Selenocystine is incorporated using a normal ‘stop’ codon (UGA) when that codon appears in a specific context. On a special tRNA coded by selC in E. coli that initially carries serine (activated by sel D), the serine is modified by enzymatically adding a selenium (selA) that with a special ‘alternate’ translation factor (selB). In eukaryotes there is a SECIS RNA component that participates in inserting the selenocysteine. The most likely reason for the late discovery of the mechanism along with the relative rarity, is that there are many other modifications such as hydroxy proline that occur post translation.

4. Ames tester strains TA98 and TA1538 show 70X and 80X rate of his+ colonies over the background rate when treated with ethidium bromide (ETBR) while strains TA97, 100 102 and 1535 show little increase over the background rate. How are these observations explained?

Ethidium bromide is intercalates into the DNA double helix, and therefore it is a frameshift inducing mutagen. Thus the initial his- mutant in these strains is also a frameshift. The other strains detect transitions or transversions.

5. Strains of E. coli have been identified in which UGA nonsense mutations are suppressed by the insertion of Tryptophan, but none have been reported for which Tryptophan suppresses UAG nonsense mutations. Propose reason(s) this may be true.

The UGG anticodon will be ACC since a C in the third pairing position only pairs with G. If the second C should be replaced by U via mutation, according to Wobble, the ACU anticodon would pair with both UGG and UGA allowing tryptophan to be inserted for UGA. There must be only one trp tRNA since the same change in the second C (anticodon now AUC) appears to mean that there is no tRNA to insert Try at UGG codons.

6. A large number of point mutations that disrupt the function of the CFTR (cystic fibrosis membrane regulator) gene have been found, many with different modes of action. The 1480 amino acid protein is made from a gene over 250,000 bp long with 24 exons. The C-terminus provides a membrane anchor and there are 12 membrane spanning regions. Many mutant alleles are known. Describe as many unique mechanisms as possible that would explain why different point mutations can result in non-functional CFTR.

Changes in splice signals; changes in a transmembrane spanning sequence, change in the signal sequence, frameshifts, loss of initial AUG or poly A tial signal, amino acid substitutions in a binding site, nonsense mutations etc.
7. 94% of mice homozygous for an asp to ala mutation in position 400 of POLD protein (polymerase D) died of a variety of forms of cancer by 10 months of age versus less than 4% if no or 1 copies were present. Propose a function for this protein that would explain these results; be as specific as possible.

One possibility: It is involved with the proof reading function meaning mismatch repair is defective, so that many mutations are incorporated at each replication.

8. In an early study with Aspergillus, a filamentous fungus that unlike Neurospora can synthesize biotin, a series of biotin auxotrophs were isolated. Mutants were tested on the ability to grow on media supplemented with prospective precursors K, T, A and P as shown in the table below.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Biotin</th>
<th>K</th>
<th>T</th>
<th>A</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>73</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>81</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

a) Based on this information set up a prospective pathway for biotin synthesis, showing where each mutant potentially blocks.

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 P 12 K 81/73 (A/T) 41 Biotin
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b) Since complete resolution was not possible, extracts were made for each strain grown on limiting amounts of biotin and used to test a series of biotin auxotrophs created in E. coli. One of these E. coli bio mutants grew on extracts from strain 41 that contained compound T. Does this help determine the pathway? If so, show how.

Strain 41 accumulates T, suggesting it is the compound just before biotin

c) Propose a rapid method for isolating biotin auxotrophs in E. coli that would not work in the filamentous fungi.

Penicillin counterselection will kill dividing bacteria so that biotin auxotrophs that can’t divide on minimal will survive and can be rescued by plating on biotin medium. This does not work on yeast.

9. A conjugation experiment starting with 4 different HFR strains was conducted where each pair was allowed to mate for 30 minutes with the same strain of F- females. After disruption males were eliminated and donor markers present in the exconjugants were evaluated. No attempt was made to determine the timing or level of donor markers present, so the donor genes found are just listed in alphabetic order.
HFR Donor genes in F- strain

<table>
<thead>
<tr>
<th>HFR strain</th>
<th>Donor alleles recovered in F-</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G, I, R</td>
</tr>
<tr>
<td>2</td>
<td>E, N, R</td>
</tr>
<tr>
<td>3</td>
<td>N, O, T</td>
</tr>
<tr>
<td>4</td>
<td>G, H, T</td>
</tr>
</tbody>
</table>

a) make a map showing relative order of the genes and HFR insertion sites.

R I G H T O N E in a circle with the HFR at either end of each set of genes as long as it is consistent.

b) Could the same marker gene be used to eliminate males in all 4 crosses? Explain.

No: Any marker would have to be within one set of markers so would transfer to recipients in at least one cross.

10. Tell whether β-galactosidase, lac permease, and TAase would not be made (N), be constitutive (C) or be regulated (R) in the following partial diploids in *E. coli*. FS is a frameshift.

<table>
<thead>
<tr>
<th>Strain Genotypes</th>
<th>Z</th>
<th>Y</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. p I p O Z Y\text{FS} A / p I p O Z Y^- A</td>
<td>R</td>
<td>N</td>
<td>R</td>
</tr>
<tr>
<td>2. p I S p O Z^- Y A / p I p O Z Y^- A</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>4. p I p O Z\text{FS} Y A / p I p O Z Y A^-</td>
<td>R</td>
<td>R</td>
<td>N</td>
</tr>
</tbody>
</table>

Which strains, if any, would show induction if IPTG was added

1, 3, 4

11. Muller relied on *C*, *l*, and *B*, three ‘genes’ on the X chromosome of Drosophila to prove that X-rays cause mutations. What is the reason for including of each of these markers and how did the experiment provide proof that X-rays cause mutations.

*C* is a ‘crossover suppressor, included so that recombination will not create new gene combinations (It is actually a very large inversion)

*l* is a recessive lethal so that an XY ‘son’ will not survive

*B* is a Bar eye mutation that narrows the eye in heterozygous females so that female progeny from a cross to an irradiated male can be identified.

If the irradiated X from the sperm had a different recessive lethal, the Wide Bar females would have no sons.