1. Consider both aspects of control (negative and positive) of the lac operon. Is enzyme production by each of the following genotypes constitutive, uninducible or inducible? (Give separate answers for β-galactosidase and permease). Cap refers to no cap protein, i^s refers to super repressor and o^c refers to constitutive operator.

(a) cap^-i^+p^+o^c^+y^+ β-galactosidase constitutive permease constitutive

(b) cap^-i^+p^+o^c^-y^-/cap^+i^-p^+o^z^+y^+ inducible inducible

(c) cap^+i^-p^+o^c^-y^-/cap^+i^-p^+o^z^-y^- constitutive uninducible

*I accepted two answers for part (a): without a cap protein, induction will not occur. However, often this regulation is leaky, and a small amount of synthesis still occurs. However, this synthesis would be constitutive.

2. A short DNA fragment with the sequenced 3′CTGATAAGGCTTTG5′ is used as a template for sequencing by the dideoxynucleotide methods. Each of the four reaction vessels contains labeled deoxynucleotide triphosphates plus a small amount of either ddATP, ddCTP, ddTTP, or ddGTP.

a) Why is ddCTP used in one of these reactions?

ddCTP lacks a 3′-hydroxyl and therefore is a chain-terminator for DNA synthesis. Including it in one of these reactions allows for the generation of a series of DNA fragments, each of which stop at the place a dCTP could be incorporated in the new strand of DNA.

b) What is the sequence of the DNA molecules that will be generated in the ddCTP vessel? Assume that the primer for this reaction is 5′GAC3′.

5′-GACTATTddC
5′-GACTATTCdC
5′-GACTATTCGGAAAddC
5′-GACTATTCGGAAAC
3. A codon that specifies the amino acid Gly undergoes a single-base substitution on the template strand to become a nonsense mutation. Remember that mutations occur at the DNA level.

a) Is this mutation a transition or a transversion?

<table>
<thead>
<tr>
<th>Codons for Gly:</th>
<th>GGU</th>
<th>Sequences of stop codons:</th>
<th>UAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GGC</td>
<td>UAG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GGA</td>
<td>UGA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

At RNA level: GGA → UGA

At DNA level (template strand): CCT → ACT

Therefore, the mutation is a C → A (a pyrimidine to a purine) which is a transversion.

b) At which position of the codon does the mutation occur?

The mutation occurs within the 1st position of the codon

4. The non-template strand at the beginning of a coding region reads

5′-ATGCATCCGGGCTCATTAGTCT-3′

Two mutations are studied. Mutation X as an insertion of a G immediately after the underlined G and mutation Y has a deletion of the bold A. What is the amino acid sequence of each of the following:

a) the wild type polypeptide

met-his-pro-gly-ser-leu-val

b) the polypeptide in mutant X

ATG GCA TCC GGG CTC ATT AGT CT
met-ala-ser-gly-leu-ileu-ser

c) the polypeptide in mutant Y

ATG CAT CCG GGC TCT TAG TCT
met-his-pro-gly-ser-stop codon
5. Your colleagues discover a new mutagen. You learn that the mutagen acts by changing the base-pairing properties of a base, such that its base-pairing properties are altered. In order to determine the specificity of the alteration, you examine the amino acid changes that take place after mutagenesis. Three mutations caused by this mutagen are shown below.

Mutant 1: Met – His – Met – Gln – Arg
Wild type: Met – His – Ile – Gln – Arg

Mutant 2: Ala – Tyr – Ser – Lys
Wild type: Ala – Tyr – Asn – Lys

Mutant 3: Arg – Ser – Leu – Trp – Tyr – Phe – Ser
Wild type: Arg – Ser – Leu

What single nucleotide change at the DNA level can account for these three mutant sequences?

Mutant 1: AUA → AUG
Mutant 2: AAC → AGC
Mutant 3: UGA → UGG

In all three cases, it’s an A → G mutation at the RNA level, which is a T → C mutation at the DNA level (template strand).

6. The normal sequence of genes on chromosome 2 in Drosophila is ABC*DEFGHI. This strain is crossed against a Drosophila strain in which an inversion has occurred on chromosome 2, resulting in abc*dgfehi as the order of genes. (* represents the centromere)

a) Draw these two chromosomes as they would pair during meiosis I.

See Fig. 22 in Chapter 15. This shows how the inversion chromosome matches up with DNA sequence on the non-inversion chromosome.
b) What are the products of recombination if a cross-over occurs between genes E & F on non-sister chromatids 2 and 3? Show your reasoning.

7. You transform a mouse with DNA containing a neomycin resistance gene. Progeny from this mouse are mated with wild type mice. What proportion of their pups will be resistant to neomycin if the neomycin-resistance gene was inserted into a single site on both chromosome 2 and chromosome 5?

\[ \text{neoR}^1/-, \text{neoR}^2/-, X/-, -/- \]

1) For chromosome 2 (carrying \( \text{neoR}^1 \)), the transgenic pup can provide the chromosome containing the neomycin gene or the normal chromosome to it’s progeny – each at equal frequency – so the chance of passing this on is 50%.

2) For each of these classes, 50% will also receive the chromosome 5 that contains the neomycin transgene and 50% will receive the normal chromosome 5. Therefore, half of the gametes that received a normal chromosome 2 will receive a copy of chromosome 5 containing the neomycin gene (this is also true for the gametes that received chromosome 2 containing the transgene).

Therefore, the proportion of pups that will be resistant to neomycin is 50% + 25% = 75%.
8. Your lab is studying the pathway for biotin synthesis in Neurospora and you have an auxotrophic mutant that cannot grow without the addition of biotin to the media. One of your students has isolated a revertant that can grow on minimal media. You cross this revertant with a wild type strain and examine the progeny.

a) What proportion of the progeny will be able to grow in the absence of biotin if the revertant is caused by a suppressor mutation in a gene on a separate chromosome?

bio-, sup- X bio+, sup+

You will get four classes of progeny, since the two genes are on separate chromosomes:

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Classes</th>
<th>percent of progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>bio-, sup-</td>
<td>parental</td>
<td>25%</td>
</tr>
<tr>
<td>bio+, sup+</td>
<td>parental</td>
<td>25%</td>
</tr>
<tr>
<td>bio-, sup+</td>
<td>recombinant</td>
<td>25%</td>
</tr>
<tr>
<td>bio+, sup-</td>
<td>recombinant</td>
<td>25%</td>
</tr>
</tbody>
</table>

Of these, only the bio-, sup+ genotype will not be able to grow in the absence of biotin. Therefore, 75% of the progeny will be able to grow on minimal media.

b) What proportion of the progeny will be able to grow in the absence of biotin if the revertant is caused by a suppressor mutation in a gene located 8 map units away from the original mutant gene?

The cross and the classes of progeny are the same as in (a). However, since the genes are linked, the percent of progeny in each class will differ. You will have many more individuals in the parental classes and fewer in the recombinant classes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Classes</th>
<th>percent of progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>bio-, sup-</td>
<td>parental</td>
<td>46%</td>
</tr>
<tr>
<td>bio+, sup+</td>
<td>parental</td>
<td>46%</td>
</tr>
<tr>
<td>bio-, sup+</td>
<td>recombinant</td>
<td>4%</td>
</tr>
<tr>
<td>bio+, sup-</td>
<td>recombinant</td>
<td>4%</td>
</tr>
</tbody>
</table>

Therefore, 96% of the progeny will grow on minimal media.

9. Who was Cumulina? (only a short answer required)

Cumulina was the first mouse to be cloned. This was done using cumulus cell nuclei implanted into enucleated eggs and implanted in pseudopregnant female mice.