Question 1

Shown below are the chromosomes for a diploid organism after the organism has completed S phase. The letters represent alleles of genes.

(a) Give two pairs of genes that are tightly linked.

\[
\text{pair 1: } A\_ & D\_ \quad \text{pair 2: } L\_ & G\_
\]

(b) Give two pairs of genes that are unlinked.

\[
\begin{align*}
\text{pair 1: } A\_ & L\_ \\
\text{pair 2: } A\_ & G\_
\end{align*}
\]

\[
\begin{align*}
\text{pair 3: } A\_ & F \\
\text{pair 4: } D\_ & G
\end{align*}
\]

\[
\begin{align*}
\text{pair 5: } D\_ & F \\
\text{pair 6: } D\_ & L
\end{align*}
\]

\[
\begin{align*}
\text{pair 7: } G\_ & F \\
\text{pair 7: } L\_ & F
\end{align*}
\]

(c) For which alleles is this organism homozygous? Use the allele names to indicate your choice(s).

Homozygous for D and F.

(d) If a cell with these chromosomes went through meiosis, indicate the possible genotypes of the gametes for only the A/a and L/l genes:

\[
\begin{align*}
\text{gamete 1: } & AL \\
\text{gamete 2: } & al
\end{align*}
\]

\[
\begin{align*}
\text{gamete 3: } & Al \\
\text{gamete 4: } & aL
\end{align*}
\]
Question 2

You are studying the mitotic cell cycle in a diploid organism and think that it would be helpful if you could synchronize your cells so that they are all undergoing the same steps of the cell cycle at the same time. In order to do this you decide to make use of certain chemicals.

(a) Nocodazole is a compound that results in the disassembly of the mitotic spindle, which plays a role in separation of sister chromatids.

(i) If you use this compound on your cells, when during the cell cycle would you expect your cells to arrest? Justify your answer.

*The drug prevents the separation of sister chromatids. The process of sister chromatid separation occurs during mitosis, so we would expect that the cell would arrest during mitosis or late G2.*

(ii) What would be the expected ploidy (1n, 2n, 4n etc) of your cells at this stage?

*The ploidy would be 4n. By this time in the cell cycle, the chromosomes have been duplicated.*

(b) Aphidicolin is another compound that you decide to try. Aphidicolin inhibits DNA polymerase.

(i) If you use this compound on your cells, when during the cell cycle would you expect your cells to arrest? Briefly explain your answer.

*The drug inhibits DNA polymerase, which is essential for DNA replication during S phase. Most likely, the cell will not enter S phase because DNA polymerase is inactivated, therefore the cell would arrest in G1. Alternatively, the cell might enter S phase and then stop before replication begins.*

(ii) What would be the expected ploidy (1n, 2n, 4n etc) of your cells at this stage?

*Whether the cell is in G1 or S phase, without DNA polymerase, the DNA will not be replicated, so the ploidy of the cells will be 2n.*

(c) Which process, mitosis or meiosis, results in daughter cells that are not identical to the parental cell? Provide one example of how the daughter cells different from the parental cell.

*Meiosis is the process that results in daughter cells that are not identical to the parental cell. The daughter cell is different from the parental cell in two ways: 1. has only 1n DNA instead of 2n 2. the genetic makeup of the daughter cell will be different due to recombination.*
Question 2 continued

(d) Now you want to examine the effects of the estrogen-mimicking compound bisphenol A (BPA) on meiosis in females. BPA is a compound found in some plastics and has been implicated in causing aneuploidy (incorrect number of chromosomes) during the formation of gametes. You want to determine whether BPA causes nondisjunction during meiosis II. Starting with the given cell below (that is diploid for a single chromosome), draw out the gametes you would expect to form if non-disjunction occurs during meiosis II in one of the cells. The circles represent the nucleus of the cell.

![Diagram of meiosis with DNA replication and meiosis stages]

Question 3

You are studying genes that control the physical appearance of ants. You have Small Red ants and Large White ants, both of which are true-breeding. You perform the following cross:

\[
\text{P} \quad \text{Large White ants} \times \text{Small Red ants} \\
\downarrow \\
\text{F1} \quad \text{All Large Red ants}
\]

(a) Are either one of your parental strains homozygous for the alleles that cause both of the recessive phenotypes? If so, indicate which parental strain.

*Neither of the parental strains is homozygous for the alleles that cause the recessive phenotypes (white and small)*
(b) Using \(L/l\) for the alleles that cause the dominant/recessive size phenotypes and \(R/r\) for the alleles causing the dominant/recessive color phenotypes, write the genotypes of the parental and F1 generations from part (a).

\[
P1 \text{ (Large White)} \quad LLrr \\
P2 \text{ (Small Red)} \quad llRR \\
F1 \text{ (Large Red)} \quad LlRr
\]

(e) Next, you want to determine if the genes for size and color are linked or unlinked. You cross one of the Large Red ants from the F1 generation in part (a) with a double homozygous recessive ant (i.e. – true-breeding for both recessive phenotypes). You get the following results from your cross:

\[
\begin{array}{c|c}
F1 & \\
\hline
\text{Small Red ants} & 230 \\
\text{Large White ants} & 241 \\
\end{array}
\]

Are the genes for size and color linked or unlinked? Use the data above to justify your answer.

The genes for size and color are linked. This is apparent from the 1:1 ratio of the F1 generation and the lack of the double recessive (Small White ants) phenotype and the double dominant (Large Red). Neither of these two phenotypic classes appear because neither of the parental strains in part (a) have the allele combinations to produce these phenotypes together. If the genes were unlinked, the ratio would also be 1:1:1:1.

You are also interested studying the texture of the ant exoskeleton. You have noticed two different texture phenotypes: smooth and rough. You want to know the relationship between the genes for color, size and texture, so you perform the following cross with true-breeding strains:

\[
P \quad \text{Small Red Smooth} \times \text{Large White Rough} \\
F1 \quad 1. \text{Small Red Smooth} \quad 400 \\
\quad 2. \text{Large White Rough} \quad 397 \\
\quad 3. \text{Small Red Rough} \quad 97 \\
\quad 4. \text{Large White Smooth} \quad 106 \\
\quad 5. \text{Small White Smooth} \quad 1 \\
\quad 6. \text{Large Red Rough} \quad 4 \\
\quad 7. \text{Small White Rough} \quad 22 \\
\quad 8. \text{Large Red Smooth} \quad 25 \\
\text{total number of offspring} = 1052
\]

(d) Which of the above phenotypic classes are the non-parental classes for the size and color phenotypes? Use the numbers to indicate your choices.

#’s 5, 6, 7 and 8. The parental strains are “Small Red” and “Large White”. Therefore, the non-parental classes in the F1 generation would be anything that is “Large Red” or “Small White”.
Question 3 continued

(e) Write down the equation you would use to determine the recombination frequency for the size and texture phenotype. Make sure to specify which classes you included in your equation either by writing the phenotype of the class or writing the number of offspring produced by that class.

To determine RF, you need to add up all of the non-parental classes for the phenotypes of interest and divide those by the total number of offspring. For size and texture, the parental phenotypes are “Small Smooth” and “Large Rough”. Therefore, the non-parental classes are any classes that are “Small Rough” or “Large Smooth”.

\[
\frac{106 + 97 + 22 + 25}{1052} \times 100 = 25\%
\]

\[
\frac{\text{SmallRedRough} + \text{LargeWhiteSmooth} + \text{SmallWhiteRough} + \text{LargeRedSmooth}}{\text{AllClasses}} \times 100 = RF
\]

Question 4

You were interested in the concept of the methylation/restriction system that Professor Walker described in class, which bacteria use a self-defense mechanism. Remembering that EcoRI is a restriction endonuclease isolated from the bacteria *Escherichia coli* (*E. coli*), you want to clone the gene that encodes for the enzyme that methylates the DNA sequence that EcoRI recognizes, called the EcoRI-methylase. This methylation prevents the *E. coli* DNA from being cut by EcoRI.

(a) You start your search by creating a genomic DNA (gDNA) library from the strain *E. coli* RY13, which has an intact methylation/restriction system. After isolating *E. coli* RY13 DNA, you need to cleave the gDNA to put the DNA fragments into a plasmid. You decide to use the enzyme BamHI.

(i) Why did you not use EcoRI?

*The DNA you isolated is from the *E. coli* RY13 that has the intact methylation system. Therefore, the DNA will be methylated and EcoRI will not cut that DNA.*

(ii) Below is the first half of the sequence that BamHI recognizes. Fill in the last three nucleotides:

5’ – G G A ___ T ___ C ___ C ___ – 3’

3’ – C C T ___ A ___ G ___ G ___ – 5’
Question 4 continued

(b) You plan to clone the EcoRI-methylase gene by complementation using a strain of *E. coli* RY13 that does not have functional copies of the EcoRI-methylase or the EcoRI endonuclease. Your experiment to identify the EcoRI-methylase is outlined below:

1. transform the library plasmids into *E. coli* RY13
2. select for bacteria with the library plasmids
3. transform a second plasmid that can express the EcoRI endonuclease into the *E. coli* RY13 bacteria that have library plasmids
4. select for bacteria that have both the library and EcoRI-containing plasmids
5. identify those bacteria that can grow in the presence of the EcoRI endonuclease
6. isolate the library plasmid from the colonies identified in step 4

(i) Your library plasmid has a copy of the *ampicillin* resistance gene, AmpR. You select for bacteria in step 2 on LB + *ampicillin* media. What is the purpose of this selection?

The purpose of the selection is to identify bacteria that have taken up your plasmid.

(ii) Should your plasmid that expresses the EcoRI endonuclease (step 3) also have a gene that encodes for *ampicillin* resistance? Explain your answer.

No. You need a selection marker on your second plasmid, but you would not want it to be the same marker as your first plasmid. If you used the same marker, you would never know if your bacteria had the second plasmid. You could only be sure that the bacteria had the first plasmid. Therefore, you would want a second selection marker.

*ALTERNATIVE* partial credit: yes – every plasmid needs a selection marker.

(d) You want to amplify the gDNA sequence from each of the plasmids using the Polymerase Chain Reaction (PCR). You only design one set of primers to amplify the gDNA from each of the library plasmids. Choose which two primers from the diagram below. Direction of the arrows indicate direction of synthesis.

<table>
<thead>
<tr>
<th>Primer 1</th>
<th>Primer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>
(e) You want to insert your amplified DNA into a plasmid that will express the EcoRI-methylase in a eukaryote. Below is a diagram of your starting plasmid and the plasmid into which you will move the sequence. Arrows represent bacterial or eukaryotic promoters.

(i) Plasmid A does not have a promoter, yet you got colonies. This suggests that the gDNA fragment has both the coding sequence and the promoter for the EcoRI-methylase gene. If you had used a cDNA library instead of gDNA library, would your cDNA fragment have both the coding sequence and the promoter? Briefly explain your answer.

No, the cDNA fragment would not have both the coding sequence and the promoter. The cDNA fragment would only have the coding sequence. This is because cDNA is created by using an mRNA template and the enzyme reverse transcriptase. The mRNA template does not contain the promoter sequences because mRNA is a product of transcription. Transcription begins after the promoter.

(ii) The hash marks on Plasmid A represent the region of the gDNA that contains the coding sequence for the EcoRI-methylase. From the available Restriction Endonuclease sites, choose the best enzyme(s) to transfer the gene from plasmid A to plasmid B.

NotI and XbaI
partial credit for XbaI alone or KpnI/XbaI or NotI/PstI
Question 5

(a) Indicate which type of immunological response is being described by circling one appropriate answer:

(i) B cells, T\textsubscript{H} cells, Antibodies, MHC II  
   Innate  Cellular  Humoral

(ii) Phagocytes, Mucous Membranes, Skin  
    Innate  Cellular  Humoral

(iii) MHC I, T\textsubscript{C} cells and T\textsubscript{H} Cells  
    Innate  Cellular  Humoral

(iv) destroys pathogens by coating with antibodies  
    Innate  Cellular  Humoral

(v) destroys altered, self cells  
    Innate  Cellular  Humoral

(b) Bob and Jane have one child. Are the antibodies that are produced by their child most likely (circle the appropriate answer):

(i) identical to Jane
(ii) identical to Bob
(iii) a mixture of Jane and Bob
(iv) different from Jane and Bob

(c) Justify your above choice.

Bob and Jane’s child will inherit one copy of Jane’s original antibody genes (i.e. – not re-arranged) and one copy of Bob’s original antibody genes. So, the child will have the same alleles. Due to the process of DNA re-arrangement, however, the child will produce mature antibody genes that are different from Jane and Bob.
Question 5 continued

(e) It is hundreds of years in the future and you are working on a space cruiser that makes contact with other life forms and you trade information with them about the biology of their planet for information about Earth biology. One of the recent life forms you have met, the Mitians, has an immune system that shares some properties with the human immune system.

You discover that the Mitians have antibodies similar to our own. While analyzing the antibodies, you discover that their antibodies are not identical to our own. The Mitians antibodies are missing light chains:

Based on your knowledge of how human antibodies interact with antigens, do you think that the Mitian antibodies have the capability to interact with as many, antigens as human antibodies, more antigens or fewer antigens? **Circle your answer and justify your choice.**

- as many
- more
- fewer

The Mitians do not have a light chain peptide and therefore they do not have a light chain gene. Because the antigen recognition site is created by both the light chain and the heavy chain, the Mitians will have less diversity overall without the light chain gene.