1. You are fascinated by Professor Chisholm’s discussion of Biosphere 2 and how the respiration of microorganisms in the rich soil led to a decrease in available oxygen. You know that the increased CO\(_2\) reacted with the concrete, but you wonder if plants were able to take up CO\(_2\) more quickly, could they replace the necessary O\(_2\) in the atmosphere of Biosphere 2? To test this hypothesis, you want to use your new skills in recombinant DNA technology to engineer a plant that has an increased CO\(_2\) uptake.

You know of one plant, called Plant X, that naturally has increased CO\(_2\) uptake. You want to clone the gene that allows this plant to take in more CO\(_2\) and put this gene into many other plants.

(a) Based on our previous discussions about evolution, do you think that Plant X is more likely to have an additional gene(s) in its genome that allows that plant to take in more CO\(_2\) or is it more likely that Plant X has a different allele of a particular gene that all plants have? Explain your choice.

(b) You decide to make a genomic DNA (gDNA) library from Plant X. Below are statements that might describe a type of DNA library. Indicate if the statement describes a gDNA library, a cDNA library, both types of libraries or neither by circling the appropriate word.

(i) the library is “kept” in bacteria

   gDNA  cDNA  both  neither

(ii) making the library requires they enzyme reverse transcriptase

   gDNA  cDNA  both  neither

(iii) the Plant X DNA is inserted into a plasmid

   gDNA  cDNA  both  neither

(iv) the library contains all possible DNA sequences

   gDNA  cDNA  both  neither
(v) Plant X DNA fragment sizes are different due to restriction enzyme sites in the genome

<table>
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<tr>
<th>gDNA</th>
<th>cDNA</th>
<th>both</th>
<th>neither</th>
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(vi) Plant X DNA fragment sizes are different due to gene length

<table>
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<tr>
<th>gDNA</th>
<th>cDNA</th>
<th>both</th>
<th>neither</th>
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(vii) Plant X DNA fragment sizes are identical

<table>
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<tr>
<th>gDNA</th>
<th>cDNA</th>
<th>both</th>
<th>neither</th>
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(c) You decided to identify the gene responsible for the increased CO$_2$ uptake in Plant X by transforming your gDNA library into autotrophic bacteria and monitoring the bacteria for increased CO$_2$ uptake. Below is an example of a plasmid from your library.

![Plasmid Diagram]

A lab mate suggests that the bacteria might not be able to express genes from the eukaryotic plant. Identify two possible problems with the expression of eukaryotic genes in a prokaryotic organism.

(d) Because of the problems identified above, you start over and make a cDNA library from Plant X, which you transform into the autotrophic bacteria. You identify several bacteria that have increased CO$_2$ uptake! You isolate the library plasmid from these bacteria and now you want to use PCR to amplify the cDNA fragments in the plasmids. Below is a representation of the sequence you want to amplify. Design primers that are 10 nt in length and would be suitable to amplify the cDNA. Make sure to label the 5’ and 3’ ends.

Primer 1: 5’ CATCGATCATTAGC [cDNA Fragment] CGAGCCATCAGC 3’
Primer 2: 3’ GTAGCTAGTAATCG [cDNA Fragment] GCACGGTAGTCG 5’
(f) Now that you have amplified the cDNA fragments, you use them as probes to identify related sequences in a genomic DNA library from another plant, Plant Y (“cloning by hybridization”). Several of your cDNA “probes” hybridize to fragments of genomic DNA from Plant Y! You want to determine the differences between the cDNA fragments of Plant X and the genomic fragments from Plant Y.

(i) What is the name of the technique that you would use?

(ii) Which reagents would you need? Circle all that apply.

<table>
<thead>
<tr>
<th>Primer</th>
<th>dNTPs</th>
<th>RNA polymerase</th>
<th>fluorescent ddNTPs</th>
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<tbody>
<tr>
<td>EcoRI</td>
<td>DNA polymerase</td>
<td>NTPs</td>
<td>ligase</td>
</tr>
<tr>
<td>Plant X cDNA fragment</td>
<td>Plant Y genomic DNA fragment</td>
<td>reverse transcriptase</td>
<td>random plasmid DNA</td>
</tr>
</tbody>
</table>

(g) Finally, you want to move one of the cDNA fragments from the plasmid it is currently in to a new plasmid. Both the plasmid containing the cDNA fragment and the new plasmid are depicted below. Included in the pictures are restriction enzyme sites that are available on the plasmid. Select the **best possible** enzyme(s) that would allow you to cut both plasmids and move the cDNA fragment to the new plasmid. Justify your choice.

**Enzyme(s):**
2. You want to create a reaction similar to PCR that allows you to create many copies of protein instead of DNA. Think about the reagents necessary for PCR and list the analogous reagents you would need for in vitro protein amplification.

3. As it turns out, Jurassic Park is not just a movie, but a real-life park that has been set up off the Massachusetts coast. Thus far, no human has been allowed to visit Jurassic Park, but the owners of the park would like to open it to the public. Before they let people in, they want to know how the dinosaurs’ immune systems work so they can monitor their reaction to any microbes that humans may bring in with them. As a prominent immunologist, you and your team have been asked to take on the project.

You suspect that dinosaurs have a specific immune response similar to that of humans. First you study their blood and note that they have both red and white blood cells. You are able to isolate B lymphocytes (B cells) and use them to make hybridomas (“Research Method” on pg 377 of the 7th edition of Purves).

a) You culture your hybridomas (each made from a single B cell) and isolate the antibody secreted by these cells. You do several experiments digesting the antibodies with a protease and thus determine that the structure of the dinosaur antibodies is analogous to the structure of human antibodies. Label the following parts on the diagram of the antibody below:

i) light chain
ii) heavy chain
iii) antigen binding site
iv) variable region (label on heavy chain only)
v) constant region (label on heavy chain only)
vi) if this were a secreted antibody, the area which interacts with phagocytes
vii) if this were a membrane-bound antibody, the transmembrane domain
viii) How are the heavy and light chains held together?

b) Antibody diversity in the mammalian immune system is generated by the rearrangement of the chromosomal DNA containing the genes for antibodies (in B cells) and T cell receptors (in T cells). You think that dinosaurs use a similar mechanism. To test your hypothesis, you try the following experiment: You isolate the region of genomic DNA (gDNA) that encodes the heavy chain of the antibodies from your hybridoma and from dinosaur skin cells. You mix the two types of gDNA in a tube and then heat the tube to 95°C to denature the strands. You allow the contents to slowly cool down, allowing the DNA strands to re-anneal. Finally, you use an electron microscope to visualize the products of your experiment. In the boxes below draw what you would see if one DNA strand from the hybridoma re-annealed with one DNA strand from the skin cell.

i) Draw what you would expect if dinosaurs do use DNA rearrangement to generate diversity. Label the strand that came from the hybridoma.

ii) Draw what you would expect if dinosaurs do NOT use DNA rearrangement to generate diversity. Label the strand that came from the hybridoma.

iii) You were right! Dinosaurs do use DNA rearrangement to generate antibody diversity. What are two other ways that human B cells generate antibody diversity?

1. 

2. 
4. Next you isolate both B and T cells from dinosaurs and postulate that they have humoral and cellular responses similar to humans. To test this, you inject one of your dinosaurs (which you give the nickname Walkersaurus) with the influenza virus. Four days later, you inject another of your dinosaurs (which you give the nickname Chisholmator) with the same virus. Note: four days is long enough for Walkersaurus to mount a sufficient immune response to influenza.

a) You purify antibodies from Walkersaurus’s blood and inject them into Chisholmator. Assuming that your hypothesis is correct, Chisholmator’s immune system will eventually recognize the antibodies as foreign, but before that happens, would these antibodies help Chisholmator to rid himself of the virus? Why or why not?

b) Next you purify the anti-influenza helper T cells and anti-influenza killer T cells from Walkersaurus and inject them into Chisholmator. Again, Chisholmator’s immune system will eventually recognize the cells as foreign, but before that happens, will the T cells help Chisholmator rid himself of the virus? Why or why not?

c) If Walkersaurus and Chisholmator were identical clones would you expect Walkersaurus’s T cells to help Chisholmator? Why or why not?
Your final question concerning the specific immune response of dinosaurs is about immunological memory. You infect Weinborg with the polio virus and monitored the production of antibodies. You administer a second dose of polio virus to Weinborg and again monitor the production of antibodies. Below is your data:

d) Do dinosaurs possess immunological memory like humans do? Why or why not?