Problem 1: DNA Forms and DNA Purification (13 points)

a) (3 pts) Label the following forms of DNA as either A, B, or Z. Explain at least three properties that differentiate each form of DNA and what these properties are for the three forms.

![DNA forms](image)

(1)  (2)  (3)

b) (2pts) DNA can be purified via anion exchange chromatography. Explain how DNA can be purified via this method.

c) (5 pts) What are the pKa’s of structural elements of DNA? What will be the protonation state of DNA structural elements at physiological pH?

d) (3 pts) If the column were kept at a pH 2, how would this affect DNA purification via anion exchange chromatography?

Problem 2: Tools of Gene Exploration (25 points)

a) Please describe a cycle of PCR (polymerase chain reaction). Explain the conditions and the consequences of each step. If PCR cycles to temperatures that denature proteins, how is DNA eventually synthesized? What is one shortcoming of this solution? Please describe a use for PCR. (6 points)

b) Details of Primers and Probes -- Gene amplification, genome modifications, cleavages, sticky end fusions… these are all invaluable tools that allow us to both investigate systems already in existence as well as further probe questions and biological concepts via modifications to already existing systems. These tools are robust and function across a wide array of species and conditions, but they do carry constraints and require rigorous optimization and design.
i) Please describe the selection criteria for DNA primers and those for oligo probes. (10 points)

ii) Restriction enzymes: Please explain what comprises “compatible cohesive ends”. Name and draw an endonuclease that can produce compatible cohesive ends with SacI? (2 points)

iii) Which is the best of the primers listed below? Please explain the advantages/disadvantages of each pair. Please calculate the Tm of the best primer. (7 points)

Primer 1: 5’-GATC-3’
Primer 2: 5’—GAGCCGTATGGGATACGGCAC—3’
Primer 3: 5’-GATCCTAGATTTGATCGGC-3’
Primer 4: 5’ -GCGTCAGCATCATACTACTT-3’
Primer 5: 5’-ATCAGTCAGATTATCATAACA- 3’

Problem 3: Recombinant DNA technology and the manipulation of eukaryotic genes (20 points)

For your thesis, you’ve decided to investigate the function of a human protein, HPro1. You decide you want to first express and purify the protein from E. coli for in vitro studies.

a. (5 points) You decide to use PCR to get the DNA encoding HPro1. Why can’t you use genomic DNA as PCR template to obtain HPro1 DNA for expression in bacteria? Describe another method you can use to get the DNA from human cells that encodes HPro1 suitable for expression in bacteria.

b. (5 points) You now have your DNA for HPro1 with an added HindIII restriction site on the 5’-end and an EcoRI site on the 3’-end via PCR. You want to use restriction digestion and ligation to put your gene into the pUC18 expression vector. Explain how the process of forming the recombinant DNA using these two parts works, and how you can use blue-white screening to visualize which of your bacterial colonies contain your insert.

c. (5 points) After studying HPro1 in vitro, you want to know how its function affects the cell. You decide to transiently knock down the expression of HPro1 in cultured human cells using RNA interference. Briefly explain how RNA interference works.

d. (5 points) Mutant HPro1 causes disease. To study this process, you want to create a human cell line in culture that expresses a mutant form of HPro1 instead of wild type HPro1. Briefly explain one way to edit the genome so that mutant HPro1 is expressed.

Problem 4: Lipids (27 points)

Lipid bilayers are essential to cellular function; the following questions will hopefully give you an appreciation for their importance and function.
a) What factors are considered for solvation and consequently what drives the process of lipid bilayer formation? (4 pts)

b) Name and draw the three common types of membrane lipids. (3 pts)

c) Rationalize the validity of the statement: lipids are symmetric and "static". (3 pts)

Hydrophobic plots (as shown above) reveal structural information on protein domains.

d) What is the most likely secondary structure and domain for the above hydrophobic plot, and give a relevant biological member of the protein family. (3 pts)

The structure of the lipid membrane is important toward cellular function in maintaining a concentration gradient of ions/molecules.

e) Rank and rationalize the order (highest to lowest) of the permeability for the following ions/molecules to traverse across the lipid bilayer: (3 pts)

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\text{Glucose, Na}^+, \text{H}_2\text{O, CO}_2
\]

The structural importance of lipid membranes, however, requires some reduction in rigidity to accommodate molecules.

f) What is a common way bacteria regulate fluidity of their membranes? In animal cells? Give an example of why lipid fluidity would be important. (3 pts)

g) Discuss what lipid rafts are and why they may be desirable for proteins. (3 pts)

h) How many sodium ions are needed to provide the free energy to transport a molecule of glutamic acid from a concentration of 0.1 mM outside the cell to 20 mM inside the cell?
Assume, for the purpose of this problem, a temperature of 37 °C, a resting membrane potential of -70 mV, and each sodium ion releases 3.3 kcal/mol. (5 pts)

Problem 5: Enzymes (15 points)

a) Explain the effect that enzymes have on the following properties of a reaction:
   i. Activation energy (1 pt)
   ii. Rate (1pt)
   iii. ΔG of the reaction (1pt)

b) What are some mechanisms by which enzymes exert the above effects on reactions? (2pts)

c) Explain the effect of the following on an enzyme. Explain why this is significant with regards to biological systems.
   i. Temperature (2pts)
   ii. pH (2pts)

d) Draw a graph to represent the rate of a reaction as the enzyme concentration increases, given a high substrate concentration and an ideal pH/temperature. Explain the trend. (3 pts)

e) Draw a graph to represent the rate of a reaction as the substrate concentration increases, given a constant enzyme concentration. Explain the trend. (3 pts)