Bi/Ch110 Problem Set 2 Solutions

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.

1: B DNA
2: A DNA
3: Z DNA

Shape, rise per base pair, helix diameter, screw sense, glycosidic bond, base pairs per turn of helix, pitch per turn of helix, tilt of base pairs from perpendicular to helix axis (Stryer Pg. 112).

0.5 pt/correct labeling of 1, 2, 3
0.5 pt/property

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

Anion-exchange chromatography is a process that separates substances based on their charges using an ion-exchange resin containing positively charged groups. Anion exchange resins will bind to negatively charged molecules, displacing the counter-ion.

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?
Bases and phosphates:
0.5pt/bases and phosphate pKa’s (=2.5pts)
If pKa > pH: protonated
If pKa < pH: deprotonated
0.5pt/protonation state for bases and phosphate (=2.5pts)

DNA pKa’s:
T: 9.9
A: 3.5
C: 4.2
G: 9.2 and 2.1
Phosphate: 2.2, 7.2, and 12.3

Physiological pH ~ 7
T: protonated (left structure)
A: deprotonated (left structure)
C: deprotonated (left structure)
G: partially protonated (middle structure)
Phosphate: partially protonated (since pKa is very close to physiological pH phosphate will occupy the middle two structures)

d) (3 pts) If the column were kept at a pH 2, how would this affect DNA purification via anion exchange chromatography?
If the column were kept at pH 2, all of the bases and phosphate groups would be protonated and DNA would no longer be negatively charged. Because anion exchange relies on interaction with
negatively charged elements, DNA would no longer bind to the column and thus could not be purified in this way at this pH.

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)

Polymerase chain reaction - PCR

A cycle consists of three steps: DNA double strand separation via heat with the addition of primers (1), the hybridization of primers via cool to allow annealing (1), and the extension of primers by DNA synthesis via moderate temperature (1).

DNA is synthesized via Taq polymerase isolated from a thermophilic bacterium. It’s optimum temperature for activity is much higher than other polymerases, with optimum activity between 70 and 80°C, and a half-life of > 2 hrs at 95°C. (1) One of Taq’s drawbacks, however, is that it lacks 3’ to 5’ exonuclease proofreading activity. This means relatively low replication fidelity and a much higher incidence of mutations. (1)

3 Uses for PCR: Broadly the amplification of DNA from minute quantities to levels that permit us to perform analyses -- the verify, for instance, whether we have cultured the correct bacterium or to determine the source of biological material left behind at a crime scene. (1)

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)

1) Primers: Primers should flank the DNA you want to amplify (1) Tm--melting temperature compatibility for primers (1)-- the range for each as we want to avoid secondary annealing with too high a melting temperature. The GC content of a sequence gives a good indication of primer tm. Primer length: 18-22 bp. (1) This length is long enough for adequate specificity and short enough for primers to bind easily to the template at the annealing temperature. GC content should be 40-60%. No primer secondary structure, dimers, or repeats. (1)

2) Probes: conditions: they are sensitive (1) (they do not have any internal secondary structure nor do they bind to other identical probes on the array) (1) specific (1), ie it does not cross-hybridize, and isothermal (1)
Other factors: should not overlap repeat regions, melting point should be in desired range, should fall within the 3’ portion of a gene, avoid lots of TTTTT’s since a poly T primer is complementary to the polyadenylation site at the 3’ end of mRNA (would cause cross-hybridization), avoid TATATA as this would also interact with the TATA repeat to cause hybridization. (1 point for any of these)

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)

Compatible cohesive ends are produced when you have two separate enzymes that recognize very similar sequences, and cut so that the overhang produced by one can hydrogen-bond with the overhang produced by the other. (1 point)

(½ point for answer, ½ for drawing)

<table>
<thead>
<tr>
<th>SacI * (GAGCT/C)</th>
<th>BanII (GAGCT/C), BsiHKAI, Bsp1286I (GAGCT/C)</th>
</tr>
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</table>

Sac I
5’…GAGCTC…3’
3’…CGAGT…5’
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)

5’-GATC-3’

5’—GAGCCGTATGGGATACGGCAC—3’

5’-GATCCTAGATTTGATCGGC-3’

5’-GCGTCAGCATCATACTTT-3’

5’-ATCACGTACATATCATAAACA- 3’

5’-GATC-3’ --- far too short! The probability of additional binding outside the flanking sequence is extremely high (1 point)

5’—GAGCCGTATGGGATACGGCAC—3’ --- sequence is complementary to itself- it will preferentially form a hairpin structure and thus a much lower yield of desired sequence (1 point)

5’-GATCCTAGATTTGATCGGC-3’ ---best option (1 point) -- optimal length for specificity and ideal annealing and separation. Additionally, the GC content of the entire primer is optimum, and more specifically the GC content is localized to the 3’ end. Since G/C form 3 hydrogen bonds with the template, it makes the primer/template complex stable. This is important for DNA polymerase to efficiently add nucleotides to the 3’ OH of the primer. This will hybridize particularly well to the template. (1point)
5’-GCGCCAGCATCATAATACTT-3’ -- GC content localized to 5’, which improves initial section hybridization, but the low stability at the 3’ end, makes for a less efficient addition of nucleotides to the growing strand. (1 point)

5’-ATCATTTCAGATTACATAACA- 3’ -- low total GC content (1 point)

5’-GATCCTAGATTGATCGCGC-3’ Tm calculation:

Acceptable answers (depending on resource/work cited):

½ if only the answer from one online calculator with no work, explanation, or discussion of assumptions

Tm = 63 (online calc) NEB ** assumes Q5 polymerase
Tm = 58.29 (online calc) applied biosystems
Tm = 55.3 (online calc) thermo-fischer ** assumes taq polymerase (if not taq, same as NEB)

50% GC content, length 20
Tm= 64.9 +41*(yG+zC-16.4)/(wA+xT+yG+zC)

Tm = 64.9 + 41(5+5 -16.4)/20
Tm = 51.78

*equation assumes that the annealing occurs under the standard conditions of 50 nM primer, 50 mM Na+, and pH 7.0

Or another simplistic calculation:

T m = 4(G + C) + 2(A + T) °C
Tm = 4(10) + 2(10) = 60 C

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.

KEY

Problem X: Recombinant DNA technology and the manipulation of eukaryotic genes (18 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.

DNA in the human genome contains introns and exons. When a gene is expressed in human cells, the mRNA is processed so that the introns are cut out and the exons remain to form the protein coding sequence. This splicing machinery is not present in bacteria, so sequences straight from the genome will still have introns when expressed in bacteria. A way to get around this is to first make a complementary DNA (cDNA) library by using reverse transcriptase and a primer specific to the polyA tail of mRNA to turn mRNAs into DNAs. These mRNA → DNAs are already spliced and are therefore 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.
The vector (pUC18) and the PCR product insert would both be cut with HindIII and EcoRI to produce sticky/cohesive ends. The two pieces of DNA can be mixed, and the ends that are complementary will anneal. DNA ligase will then join the ends together to create one piece of DNA plasmid. This can be transformed into bacteria, and the bacteria can be spread on a plate with the appropriate antibiotic and X-gal. Because the site in pUC18 that you cut into with HindIII and EcoRI was in the middle of a gene encoding β-galactosidase, plasmids with insert will no longer have active β-galactosidase. β-galactosidase converts X-gal from colorless into a blue dye. Therefore, colonies that are white have insert, while colonies that are blue do not.

c.) (4 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.

In RNA interference, double stranded RNA in the cell is cleaved by the enzyme Dicer into ~21 base pair fragments. These fragments are taken up by the RNA-induced silencing complex (RISC), which cleaves one of the strands (the “passenger strand”) leaving a section of unpaired RNA on the other strand (the “guide strand”). When the loaded RISC complex binds to an mRNA that is complementary to the guide strand, that mRNA is cleaved and thereby the expression level of the protein encoded by that mRNA is reduced. Therefore, by introducing siRNA specific to HPro1, you can reduce its expression.

d.) (4 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.

There are a variety of correct answers. Briefly:

- Homologous recombination: add a copy of HPro1 with the mutation to the cell with flanking DNA regions that are identical to those in the cell. A percentage of the cells will use homologous recombination to exchange the WT HPro1 with the mutant HPro1, and cells with both copies exchanged can be selected for.
- Zinc-finger nucleases/TALENs: ZFNs or TALENs are made to bind to a specific sequence of DNA. They are fused to a nuclease, which cuts the DNA when the ZNF or TALEN binds. If two of these are used to cut complementary strands, a double strand break can be introduced. If there is mutant template DNA in the cell also, the repair machinery can use this mutant DNA to “repair” the break and change the genome.
- CRISPR: Similarly to ZFN/TALENs, CRISPR can be used as a way to introduce a double strand break at a specific sequence which can then be changed by using a new DNA template to “repair” the break. CRISPR does this by using specific RNA templates complementary to HPro1 that are bound to Cas9, which then cuts the DNA.
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)

The factors taken into consideration when considering whether a solute will dissolve are: (1) stabilizing energy associated with solvent molecules interacting (as we break these interactions when a solute “dissolves”), (2) energy associated with solute-solvent interactions, and (3) energy associated with solute-solute interactions.

In the cases of lipid bilayers, the hydrophilic head favorably interacts with the solvent while the hydrophobic tail will favorably cluster together. Thus, the main driving force in lipid bilayer formation is the hydrophobic effect, which shields unfavorable hydrophobic-solvent interactions.

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

The three common types of membrane lipids are:

(1) Phospholipids
   a. Phosphoglycerides consist of a glycerol backbone, two fatty acid chains, and a phosphorylated alcohol

   ![Phospholipid structure]

   b. Sphingomyelin contains a sphingosine backbone instead of glycerol

   ![Sphingomyelin structure]

(2) Glycolipids
   a. Sugar-containing lipids derived from sphingosine.
(3) Cholesterol
   a. Modulate membrane fluidity constructed from a steroid scaffold.

C) Rationalize the validity of the statement: lipids are symmetric and “static”. (3pts)

The statement is false: lipids are asymmetric owing to (1) where the lipids are made and (2) their initial orientation. This has important biological consequences on cell signaling following apoptosis, by which lipids normally present on the interior of the cell are exposed to the outside. Furthermore, the exterior of the lipid bilayer is often highly glycosylated, due to the exposure to sugars.

Lipids are not static; lipids diffuse rapidly in the plane of the membrane unless restricted by special interactions. Rotation of lipids (or flip-flop) is possible, but very slow.

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. (3pts)
Alpha helices are common of transmembrane proteins. Beta sheets, on the other hand, require multiple sheets to bond to each other to form Beta barrels. In the case above, it is a 7 transmembrane alpha helical domain, which are common of signaling related pathways.

Ex: GPCRs (G protein-coupled receptors)

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)

Glucose, Na⁺, H₂O, CO₂

CO₂ > H₂O > Glucose > Na⁺

The permeability of an ion/molecule is related to two factors (1) size and (2) electronics.

Compounds that are small and nonpolar will diffuse the best, therefore putting CO₂ at the fastest rate.

Water, while containing a dipole moment, is small and prevalent enough to traverse the lipid membrane more easily than the other remaining choices (10⁻² cm s⁻¹). (There are, however, aquaporins that help aid this process).

Glucose, which is mainly hydrophobic, is a lot larger and therefore has a lower permeability. It often requires its own associated protein transporter to cross the lipid membrane (10⁻⁸ cm s⁻¹).

Finally the small, charged ion will travel the slowest due to the unfavorable interactions with the interior of the lipid membrane. Therefore, Na⁺ almost always require a transport channel to traverse the lipid membrane at an appreciable rate.

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)

The most common way that bacteria regulate the fluidity of their lipid layer is by varying the number of double bonds and length of their fatty acids. The cis double bond produces a bend in the hydrocarbon chain that interferes with the highly ordered packing of fatty acid chains. Shorter hydrocarbon chains interact less strongly than longer fatty acids due to a reduction in Van Der Waals forces and consequently have a similar affect on membrane fluidity.
In animals, cholesterol is the key regulator of membrane fluidity, inserting into bilayers with its long axis perpendicular to the plane of the membrane, and the hydroxyl group interacts with the nearby phospholipid head groups. Consequently, the regular phospholipid interaction is disrupted.

At low temperatures, cholesterol increases membrane fluidity preventing freezing, and at higher temperatures cholesterol decreases membrane fluidity to maintain structure.

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

Cholesterol can form specific complexes with lipids that contain the sphingosine backbone. These complexes concentrate into small highly dynamic regions (lipid rafts), allowing proteins that participate in signal transduction pathways to concentrate appropriately for function.

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)

At pH 7 (physiological pH), glutamic acid carries a net negative charge of 1.

Thus, the problem is two fold:
- moving against a concentration gradient
- moving against an electrostatic gradient

\[
\Delta G = (R)(T) \times \ln(20/0.1) + (z)(F)(V_m)
\]
\[
= [(2)(310) \times \ln(200)] + [(-1)(23,062)(-0.070)]
\]
\[
= (620) \times (5.3) + 1614
\]
\[
= 3286 + 1614
\]
\[
= 4900 \text{ or } 4.9 \text{ kcal/mole}
\]

Because sodium ions release only 3.3 kcal/mol, at least 2 Na\(^+\) are needed to cotransport one molecule of glutamic acid.

**Problem 5: Enzymes (15 points)**

a) Explain the effect that enzymes have on the following properties of a reaction:
   i. Activation energy (1pt)
      Enzymes lower the activation energy of a reaction, allowing for it to proceed more readily than without the catalyst. (1 pt)
   ii. Rate (1pt)
      The rate of the reaction increases as a result of the lowered activation energy. (1 pt)
iii. $\Delta G$ of the reaction (1pt)

The $\Delta G$ of the reaction does not change for the reaction, even though the activation energy decreases and the rate of the reaction increases. (1 pt)

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

Some enzymes destabilize bonds within the substrate, which lowers the activation energy. Conformation changes that occur in the enzyme when the substrate is near can stabilize the substrate in the transition state, which lowers the activation energy. Covalent bonding to various side chains or cofactors of the enzymes can also lower the energy of the transition state. Overall, these mechanisms contribute to the increased reaction rate because of the lower energy transition states induced. (2 pts for any two mechanisms)

c) Explain the effect of the following on an enzyme. Explain why this is significant with regards to biological systems.

i. Temperature (2pts)

Enzymes tend to denature at higher temperatures, though they increase in activity up to a certain point. This is biologically relevant because a fever can cause denaturation of enzymes, which can decrease their activity in the body in the case of infection. (2 pts)

ii. pH (2pts)

Most enzymes have an optimal pH range because deviations can cause denaturation of the enzyme. In the digestive tract, for example, the optimal activity is at pH 2 for pepsin in the stomach. Large deviations in pH from acidemia, for example, could severely inhibit the activity of certain enzymes in the body. (2 pts)

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)

Given the high substrate concentration and ideal pH and temperature, the rate of reaction is proportional to the enzyme concentration, as adding more enzyme to the system will catalyze a proportional number of reactions. (2 points for graph, 1 pt for explanation)

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)

Initially, the rate of the reaction will increase proportional until the enzyme is saturated with the substrate. After this point, the increased concentration of substrate will not affect the rate because there will be no available enzyme to react with the excess substrate. (2 points for graph, 1 pt for explanation)