**Question 1**

You are an avid Diet Pepsi fan. You drink it for breakfast, lunch, and dinner; you tell strangers on the T about its value as a home remedy for wrinkles and warts. You are concerned however, because on the side of the can it says, "Phenylketonurics: product contains phenylalanine." One night, after drinking several liters of Diet Pepsi and being wired enough to see through time, you have an epiphany. You dream of a day when all will be able to enjoy Diet Pepsi as much as you do and decide to dedicate the rest of your life to studying and curing phenylketonuria (PKU). You clear off some room on your mantle where your Nobel Prize will undoubtedly soon sit, and you begin...

You first scour the literature to find out what work has already been done for you. You find out that PKU is 100% penetrant and is characterized by excess levels of the amino acid phenylalanine. This high concentration of phenylalanine damages the developing central nervous system in early childhood, leading to mental retardation.

Remembering fondly your 7.012 lectures, you know that in order to do science, you need data, so you go out and collect some pedigrees of families with high incidence of PKU. Presented here is a typical pedigree.

![Pedigree Diagram]

a) Which mode of inheritance does this disease exhibit? (Circle one.)

- Autosomal dominant
- Autosomal recessive
- Y-linked
- X-linked dominant
- X-linked recessive

b) What is the probability that person C is a carrier of the mutant allele? Show your work. Circle your answer.

\[
m/+ \times m/+ \rightarrow 1/4 = m/m, 3/4 = \text{unaffected}, 2 \text{ of which are } m/+ \text{ or carriers. SO: } \frac{2}{3}
\]

c) If person A and person B had a third child, what is the probability that this child would have the disease?

\[
\frac{1}{4}
\]

d) You collect data from a statistically significant number of families that have children with PKU. Your data shows, on average, that 1 in 3 children have the disease. Give one reason for why you might see this 1 in 3 ratio.

*Didn’t count families where no m/m children resulted. That is, two heterozygous parents → no affected children - These families would not have been included in your data.*

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1. Epiphany: a moment of intense self-awareness and awakening. Used to great effect by the Irish author James Joyce in works such as *The Dubliners* and *Ulysses*. 
PKU is a genetic disease. In order to understand the disease better, you decide to clone the gene responsible for PKU, which you call pah. Once you clone the wild type pah gene, you can use information from its sequence to clone and amplify mutant forms of pah from different PKU patients. Using the cloned pah genes, you will express and characterize wild type and mutant forms of the PAH protein.

To clone the pah gene, you must first make a library of human cDNAs. For your initial experiments, you need to clone human cDNAs into a plasmid that contains a human promoter, bacterial origin of replication (ORI) and the gene for Ampicillin resistance ($\text{Amp}^R$). Your cloned cDNAs can be replicated in bacteria and can be expressed in human cells. The final product will look like that diagrammed below.

a) Order the following steps (1-6) to describe the process of creating a cDNA library.

1___ Isolate mRNA from wildtype human cells.
2___ Use reverse transcriptase to make double stranded DNA.
3___ Add human cDNAs to linearized plasmid with blunt ends and ligate.
4___ Transform bacteria.
5___ Select on ampicillin plates.
6___ Isolate the pcDNA plasmids with human cDNA inserts from the bacterial colonies.
You are successful in producing a cDNA library from human cells. You plan to use this cDNA library to clone the wild type pah gene by complementation. You hope your experiments will help explain the high cellular levels of phenylalanine characteristic of PKU patients.

b) Circle the one method that would allow cloning the pah gene by complementation.

- Prepare your cDNA library from an unaffected individual. Use that library to transform cells from a PKU patient. Plate the transformed cells on medium that allows the growth of cells that accumulate phenylalanine. Purify plasmids from the cells that accumulate phenylalanine.

- Prepare your cDNA library from a PKU patient. Use that library to transform cells from a PKU patient. Plate the transformed cells on medium that allows the growth of cells that accumulate phenylalanine. Purify plasmids from the cells that accumulate phenylalanine.

- Prepare your cDNA library from a PKU patient. Use that library to transform cells from an unaffected individual. Plate the transformed cells on medium that allows the growth of cells that accumulate phenylalanine. Purify plasmids from the cells that accumulate phenylalanine.

- Prepare your cDNA library from an unaffected individual. Use that library to transform cells from an unaffected individual. Plate the transformed cells on medium that allows the growth of cells that accumulate phenylalanine. Purify plasmids from the cells that accumulate phenylalanine.

- Prepare your cDNA library from an unaffected individual. Use that library to transform cells from a PKU patient. Plate the transformed cells on medium that kills cells that accumulate phenylalanine. Purify plasmids from the cells growing on the selective plate.

- Prepare your cDNA library from an unaffected individual. Use that library to transform cells from an unaffected individual. Plate the transformed cells on rich medium. Radioactively label the pah gene as a DNA probe and use it to hybridize to the cells growing on the selective plate.
After successfully cloning the wild type *pah* cDNA, you design primers for amplifying a coding region of *pah* using PCR. You perform PCR using template from a wild type individual and from two PKU patients from different families. The following is a stained agarose gel showing the products from each of your individual PCR reactions.

<table>
<thead>
<tr>
<th>Lane 1</th>
<th>Lane 2</th>
<th>Lane 3</th>
<th>Lane 4</th>
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</thead>
<tbody>
<tr>
<td>Wild type Individual</td>
<td>PKU Patient #1</td>
<td>PKU Patient #2</td>
<td>Empty lane</td>
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<table>
<thead>
<tr>
<th>Molecular Weight Markers</th>
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<tr>
<td>8 kb</td>
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**c)** Given that the region amplified contains the mutation causing PKU, for each of the PKU patients, what is the most probable type of mutation? Choose from the following possibilities: big deletion, big insertion, or point mutation.

- PKU Patient #1: big deletion
- PKU Patient #2: point mutation

**d)** In Lane 4, the “empty lane” of the agarose gel above, draw in the band(s) that you might expect from a PCR of PKU Patient #1’s unaffected mother’s DNA.

To further study the genetic cause of PKU, you decide to analyze disease from more families. You obtain the following data with two patients from different families:

- Both patients have low levels of PAH protein present in their cells.
- One patient (#3) has wild type levels of *pah* mRNA in her cells.
- The other patient (#4) has little or no *pah* mRNA in his cells.

**e)** For each of the PKU patients, what reasons are sufficient and plausible to explain the cause of the disease? Choose all that apply from the reasons below (i-v).

- PKU Patient #3: iii
- PKU Patient #4: i, ii, v

- i) the mutation is in the promoter of the *pah* gene
- ii) the mutation causes the mRNA to be less stable
- iii) the mutant enzyme is degraded much more rapidly than wild type
- iv) a repressor for the *pah* gene is deleted
- v) an activator for the *pah* gene is deleted
Now that you have identified some of the genetic defects associated with PKU, you wish to study the PAH enzyme’s structure and function. To proceed, you would like to express large quantities of wild type and mutant PAH proteins in bacteria.

f) Why is it necessary to use bacterial plasmids containing pah cDNA as opposed to pah genomic DNA in order to express the PAH enzyme?

You wish to directionally clone wild type and mutant pah cDNAs from plasmids described in 2 a) (also shown below), each into a plasmid with a bacterial promoter (pcBact) shown below.

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<table>
<thead>
<tr>
<th>Restriction Enzyme Recognition Sites</th>
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<tbody>
<tr>
<td><strong>Bam HI</strong></td>
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Fill in the blanks to ensure that your cloning will allow for all plasmids to properly express the PAH enzyme.

g) What enzyme(s) would you use to cut out the pah cDNA out of pcDNA? ___Bam HI and Xho I__

h) What enzyme(s) would you use to cut pcBact? _Bgl II and Xho I__

i) Following ligation of the pah cDNA insert into pcBact, what enzyme(s) could you use to cut the pah cDNA insert out of pcBact? _______Eco RI_______
**Question 3**

Interested in why the Red Sox perform so poorly, you analyze blood samples of current Red Sox players. You discover a unique protein found only in Red Sox players that may explain their lack luster performance. To fully characterize this protein would require constant drawing of liters of blood from the players, (hardly unlikely to improve their performance), so you decide to express this protein you name “YksSk” in bacteria.

You screen a cDNA library for YksSk by hybridization, and you identify a positive clone. You want to obtain sequence of the insert. You know the sequence of the plasmid DNA adjacent to your cDNA insert (shown below).

5’ TCCAGGTCAGTCG 3’
3’ AGGTCCAGTCAG 5’

**9000 bp cDNA insert**

GCGCCGTTAAT 3’
CGCGGCAATTA 5’

a) In order to sequence this insert, you use the following primers.

Primer 1: 5’ TCAGTC 3’
Primer 2: 5’ CGGCGC 3’


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<thead>
<tr>
<th>ddCTP</th>
<th>ddATP</th>
<th>ddTTP</th>
<th>ddGTP</th>
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Sequence using Primer 1

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Sequence using Primer 2

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a) Which of the following shows the ends of your insert, as determined from the above gels?

5’ GAATGGTAATGCTCCAG..........................CCAATAATGCTATCGTA 3’
5’ GAATGGTAATGCTCCAG..........................ATGCTATCGTAATAACC 3’
5’ GACCTCGTAATGGTAAG..........................CCAATAATGCTATCGTA 3’
5’ ATGCTATCGTAATAACC..........................GACCTCGTAATGGTAAG 3’
5’ GAATGGTAATGCTCCAG..........................TACGATAGCATTATTGG 3’
You name your plasmid with the ynksks insert, pYS. In order to express the protein in bacteria, you must sublone ynksSk into pUC19 (pronounced "puck"), that has a bacterial promoter.

You digest pYS and pUC19 with BglII, run the reactions on an agarose gel. After staining for DNA you see the image below.

b) Which fragments would you purify to mix for ligation? Circle the correct fragments in the gel above.

c) You transform cells with your ligation mix. What must you add to the petri plate medium to isolate only bacteria that have taken up a plasmid?

AMPICILLIN
You pick three colonies (X, Y, and Z), isolate their plasmid DNA, and perform a restriction analysis. The stained gel is shown below.

<table>
<thead>
<tr>
<th>MW size Standards (kb)</th>
<th>Plasmid from Colony X</th>
<th>Plasmid from Colony Y</th>
<th>Plasmid from Colony Z</th>
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<tbody>
<tr>
<td></td>
<td>Hind III</td>
<td>Hind III</td>
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<td>Sma I</td>
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d) Indicate the plasmid contained in each colony. Choose from the plasmids shown at the bottom of this page, (p1, p2, or p3).

Colony X contains __p1__ Colony Y contains __p3__ Colony Z contains __p2__

e) Which of the plasmids below could you find by "cloning by hybridization" using the YksSk DNA as a probe?

p1 and p3

f) Which plasmid would you use to purify the YksSk protein? _____p3_____
For protein purification purposes, you would like to add four histidines to the N terminus of YksSk, as these histidines will tightly bind nickel, allowing you to separate YksSk from bacterial proteins.

**g) Given the following coding ends in the YksSk gene, which two PCR primers would you choose to amplify the YksSK gene and add 4 histidines on the N terminus of YksSk? (Note: The codon for histidine is CAU.)**

YksSk gene

5’ ATG CGG ATA CGT .......... CGG CTT CCC TAA 3’
3’ TAC GCC TAT GCA .......... GCC GAA GGG ATT 5’

Circle one primer from each column for PCR amplification.

<table>
<thead>
<tr>
<th>Primer A</th>
<th>Primer B</th>
</tr>
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<tbody>
<tr>
<td>5’ ATG CGG ATA CGT 3’</td>
<td>CGG CTT TAA GTA GTA GTA GTA TAA 3’</td>
</tr>
<tr>
<td>ACG CAT CAT CAT TAT CCG CAT 3’</td>
<td>5’ CGG CTT CCC TAA 3’</td>
</tr>
<tr>
<td>ATG CAT CAT CAT ATG CGG ATA 3’</td>
<td>5’ TTA GGG AAG CCG 3’</td>
</tr>
<tr>
<td>5’ ACG TAT CCG CAT 3’</td>
<td>TTA ATG ATG ATG GGG AAG CCG 3’</td>
</tr>
</tbody>
</table>

h) You choose your primers and add them to your PCR tube, as well as some YksSk DNA template and Taq polymerase. You set up the PCR machine to perform 30 rounds of amplification. After running your reaction on an agarose gel and staining the gel for DNA, you see... nothing! Your lab partner, who was watching you do all this, smirks and points out your big mistake. What was it?

**You forgot to add nucleotides (NO Nukes!!!!!!)**
Question 4

a) Upon stimulation to the -50 mV threshold, the mutant 1 neuron is unable to further depolarize.

\[ \text{Na}^+ \text{ influx} \quad \text{Na}^+ \text{ efflux} \quad \text{K}^+ \text{ influx} \quad \text{K}^+ \text{ efflux} \quad \text{Ca}^{2+} \text{ influx} \quad \text{Ca}^{2+} \text{ efflux} \quad \text{Vesicle fusion} \quad \text{myelin} \]

d) Upon stimulation by a neurotransmitter, post-synaptic mutant 4 neuron, at resting potential, fails to depolarize.

\[ \text{Na}^+ \text{ influx} \quad \text{Na}^+ \text{ efflux} \quad \text{K}^+ \text{ influx} \quad \text{K}^+ \text{ efflux} \quad \text{Ca}^{2+} \text{ influx} \quad \text{Ca}^{2+} \text{ efflux} \quad \text{Vesicle fusion} \quad \text{myelin} \]

e) With a harmful stimulus, animals typically learn to respond more vigorously not only to that stimulus, but also to other stimuli, even harmless ones associated with it. This process is called...

\[ \text{Habituation} \quad \text{Sensitization} \quad \text{Recapitulation} \quad \text{Myelinization} \quad \text{Inhibition} \]

Question 6

a) Upon stimulation to the -50 mV threshold, the mutant 1 neuron is unable to further depolarize.

\[ \text{Na}^+ \text{ influx} \quad \text{Na}^+ \text{ efflux} \quad \text{K}^+ \text{ influx} \quad \text{K}^+ \text{ efflux} \quad \text{Ca}^{2+} \text{ influx} \quad \text{Ca}^{2+} \text{ efflux} \quad \text{Vesicle fusion} \quad \text{myelin} \]

b) Upon stimulation beyond the threshold, mutant 2 depolarizes completely but fails to repolarize.

\[ \text{Na}^+ \text{ influx} \quad \text{Na}^+ \text{ efflux} \quad \text{K}^+ \text{ influx} \quad \text{K}^+ \text{ efflux} \quad \text{Ca}^{2+} \text{ influx} \quad \text{Ca}^{2+} \text{ efflux} \quad \text{Vesicle fusion} \quad \text{myelin} \]

c) Upon arrival of an action potential to the nerve terminal, neurotransmitters fail to release in mutant 3.

\[ \text{Na}^+ \text{ influx} \quad \text{Na}^+ \text{ efflux} \quad \text{K}^+ \text{ influx} \quad \text{K}^+ \text{ efflux} \quad \text{Ca}^{2+} \text{ influx} \quad \text{Ca}^{2+} \text{ efflux} \quad \text{Vesicle fusion} \quad \text{myelin} \]

d) Upon stimulation by a neurotransmitter, post-synaptic mutant 4 neuron, at resting potential, fails to depolarize.

\[ \text{Na}^+ \text{ influx} \quad \text{Na}^+ \text{ efflux} \quad \text{K}^+ \text{ influx} \quad \text{K}^+ \text{ efflux} \quad \text{Ca}^{2+} \text{ influx} \quad \text{Ca}^{2+} \text{ efflux} \quad \text{Vesicle fusion} \quad \text{myelin} \]

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