7.014 Problem Set 4

Answers to this problem set are to be turned in. Problem sets will not be accepted late. Solutions will be posted on the web.

Question 1

Shown below is a fragment of the sequence of a hypothetical bacterial gene. This gene encodes production of CHWDWN, protein essential for metabolizing sugar yummose. The transcription begins (and includes) the G/C base pair in bold and proceeds to the right.

5’ …TTCGAGCTCTCGTGCAGATACGCGATGATATTAGTGGTAATATGGGGATGCACT…3’
3’ …AAGCTCGAGAGCAGCAGCATCGCTACTATAATCACCATTATACCCCTACGTGA…5’

a) Give the sequence of the mRNA transcribed from this gene and indicate the 5’ and 3’ ends of the mRNA.

b) Give the sequence of the peptide that will be translated from this mRNA. Label the amino and carboxy termini of the peptide.

c) You study two different mutants, Mutant A and Mutant B.

i) In the DNA sequence for Mutant A, you find the insertion of a G/C base pair between positions 22 and 23 (position of insertion is indicated by an arrow):

5’ …TTCGAGCTCTCGTGCAGATACGCGATGATATTAGTGGTAATATGGGGATGCACT…3’
3’ …AAGCTCGAGAGCAGCAGCATCGCTACTATAATCACCATTATACCCCTACGTGA…5’

Give the sequence of the new peptide produced by mutant A. Label the amino and carboxy termini of the peptide.

ii) In the DNA sequence for Mutant B, two consecutive G/C base pairs are inserted between positions 22 and 23 (position of insertion is indicated by an arrow on the figure above). Give the sequence of the new peptide produced by mutant B. Label the amino and carboxy termini of the peptide.
Question 1, continued

d) One of these two mutants is fully functional, while the other is not. Which mutant peptide do you predict is functional and which one is not? Why?

Question 2

You are fascinated by CHWDWN, and decide to continue your research over the summer. A graduate student in your lab has developed a collection of strains of bacteria containing different mutant tRNAs.

a) In wild-type cells, what is the anticodon on the tRNA charged with trp? Indicate 5' and 3'.

b) In strain X, the 5' nucleotide of the anticodon on the trp tRNA is changed to a G, and no wild-type trp tRNA is present.
   i. Would you expect CHWDWN polypeptide production in X to be affected? If yes, explain how it would be affected. If no, explain why not.

   ii. What proteins other than CHWDWN would you expect to be affected? Why?

   iii. Would you expect strain X to grow on media containing yummose as the only carbon source? If yes, how strong would you expect that growth to be with respect to the wild-type strain? If no, explain why you expect no growth.

c) In strain Z, the tRNA with the anticodon for trp found in wild-type cells is actually charged with amino acid gln, and no wild-type trp tRNA is present.
   i. Would you expect CHWDWN polypeptide production in Z to be affected? If yes, explain how it would be affected. If no, explain why not.

   ii. What proteins other than CHWDWN would you expect to be affected? Why?
Question 2, continued

iii. Would you expect strain Z to grow on media containing yummose as the only carbon source? If yes, how strong would you expect that growth to be with respect to the wild-type strain? If no, explain why you expect no growth.

d) You find that the protein sequence of CHWDWN is highly conserved (~80%) in humans. Excited, you acquire DNA fragments encoding bacteria and human CHWDWN proteins. You

1. combine both samples into one test tube
2. briefly treat the sample in the test tube with heat
3. let the sample cool
4. examine the contents of the test tube in electron microscope.

You find that you have three types of complexes in your sample:

You reason that two of the types are the original double stranded bacteria and human DNA, and that the third was made when a strand of the human DNA base paired with a strand of bacteria DNA.

On the figure above, identify each complex. For the bacteria-human hybrid, indicate which strand is bacterial, and which is human.

Briefly justify your choices.
Question 3

As a UROP student, you are working in bacteria on the metabolism of the monosaccharide, theose. Metabolism of theose requires the enzymes X, Y, and Z. The genes encoding these enzymes are part of one operon, and the product of gene N (N protein) regulates the transcription of these three genes. In a normal cell, protein A is always produced. A diagram of this operon is shown below.

\[ \text{A} \quad \text{N} \quad \text{O} \quad \text{X} \quad \text{Y} \quad \text{Z} \]

\[ P_N \quad P_{XYZ} \]

a) Give a brief definition of an operon.

b) In a cell where there is a high level of N protein, you detect no transcription of genes X, Y, and Z. You conclude that…

The N protein is (circle one) a repressor. an activator.

c) In further study, you discover that

- transcription of the N gene is controlled by protein A (protein A is the product of gene A shown in the diagram), and
- theose binds to the N protein.

You examine the transcription of genes N, X, Y, and Z in cells where gene A is normal (A\(^+\)) and where gene A is not functional (A\(^-\)) and in the presence (+) and absence (-) of theose. The data is shown below.

<table>
<thead>
<tr>
<th>gene A</th>
<th>theose</th>
<th>Transcription of gene N</th>
<th>Transcription of genes X, Y, and Z</th>
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</thead>
<tbody>
<tr>
<td>A(^-)</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>A(^+)</td>
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<td>A(^+)</td>
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i) Given the results above, what does protein A do?

ii) Given the results above, what does theose do?
d) You construct the following diploids containing two copies of the *theose* operon. Some of the genes or control regions are mutated where (−) = complete loss of function and (+) = normal. Predict whether you would see transcription of genes X, Y, and Z for each diploid below.

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<tr>
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<tr>
<td>( \frac{P_A^+ A^+ N^+ P_N^+ P_{XYZ}^+ O^+}{P_A^+ A^+ N^+ P_N^+ P_{XYZ}^+ O^-} )</td>
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<tr>
<td>( \frac{P_A^+ A^+ N^- P_N^+ P_{XYZ}^+ O^+}{P_A^+ A^+ N^- P_N^+ P_{XYZ}^+ O^-} )</td>
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<td>( \frac{P_A^+ A^+ N^+ P_N^- P_{XYZ}^+ O^+}{P_A^+ A^+ N^- P_N^+ P_{XYZ}^+ O^-} )</td>
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<td>( \frac{P_A^+ A^+ N^+ P_N^- P_{XYZ}^- O^+}{P_A^+ A^+ N^- P_N^+ P_{XYZ}^- O^-} )</td>
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<tr>
<td>( \frac{P_A^- A^+ N^+ P_N^+ P_{XYZ}^- O^-}{P_A^+ A^+ N^- P_N^+ P_{XYZ}^- O^-} )</td>
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The Genetic Code

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**Question 4**

Recall (from problem set 1) that sickle-cell anemia is a disease that results from the presence of abnormal hemoglobin (HbS) in the red blood cells. In order to have the disease, a person needs to have only HbS hemoglobin. Recall also that a carrier of the disease is a person who has both HbS and HbA (wild-type hemoglobin) in their red blood cells.

Suppose a colleague at your lab created some human stem cell lines from adult human carriers of sickle cell disease. As it happened, she left a dish with some of these cells in a hood where you were performing your UV mutagenesis experiments on yeast.

Later she told you that, strangely, when she coaxed those cells to differentiate into red blood cells (RBCs) and placed these new RBCs into low O₂ environment, some cells assumed rigid sickle-like shapes.

a) Do you think that appearance of the sickle-like cells is related to your colleague leaving the dish in the hood? If yes, how? If no, what caused this phenomenon?

b) Is the DNA in the sickle-like cells different from that in the normal-shape cells? If so, how is it different? If not, explain why not.

c) Is the protein content of the sickle-like cells different from that in the normal-shape cells? If so, how is it different? If not, explain why not.

d) Are your answers to parts b and c related? If yes, how are they related? If no, explain why they are not related.
STRUCTURES OF AMINO ACIDS
at pH 7.0

ALANINE (ala)
arginine (arg)
asparagine (asN)
aspartic acid (asp)
cysteine (cys)
glutamic acid (glu)
glutamine (glN)
glycine (gly)
histidine (his)
lysine (lys)
methionine (met)
phenylalanine (phe)
proline (pro)
serine (ser)
threonine (thr)
tryptophan (trp)
tyrosine (tyr)
valine (val)