Solutions to 7.014 Quiz II

Class Average = 79
Median = 82

Grade	Range	%
A	90 - 100	27
B	75 - 89	37
C	59 – 74	25
D	41 – 58	7
F	0 – 40	2

Name:_____ TA:_____

Question 1 (29 points)

Barney is an alien. On his ship, hidden in the Stata building, you find alien bacteria that metabolize wood. You call this species *A. termiticus*, and call your original strain BLT (for "Barney's little termiticus").

You subject a sample of BLT to mutagens, and isolate a new strain that no longer metabolizes wood. You conclude that you have succeeded in disrupting at least one gene necessary to metabolize wood. You call the mutant strain M.

You mix a sample of M with a sample of heat-killed wild type BLT, and the resulting strain metabolizes wood. You summarize your data in the following table:

Strains	Metabolizes wood?
BLT	Yes
Heat-killed BLT	No
M	No
Heat-killed BLT +M	Yes

a) Did the content of any of the BLT or M cells change in the experiment? If yes, which cells underwent the change, and what change occured? If not, explain why there was no change.

Some cells of type M underwent the change. They acquired some of the genetic material from the heat-killed BLT cells. In particular, some part of the genetic material they acquired encoded for the gene that was mutated in M. Having received this gene, M cells now behave like the wild-type BLT cells because they can now produce the agent (like the Earth proteins) that restores the wood metabolism pathway.

You plan to characterize the alien genetic material. You start by breaking some *A. termiticus* cells open to determine their molecular composition. You find that they contain various small molecules, carbohydrates, lipids, and two other macromolecules, A and B.

In order to determine which macromolecule is the carrier of genetic information, you repeat your previous experiment, but include test tubes where you treat the sample of the heat-killed BLT with either an agent that destroys macromolecule A (A-ase) or macromolecule B (B-ase). You find the following results (including the repeat of your previous experiment in the first 4 lines):

Strains and agents	Metabolizes wood?
BLT	Yes
Heat-killed BLT	No
M	No
Heat-killed BLT +M	Yes
A-ase treated heat-killed BLT +M	Yes
B-ase treated heat-killed BLT +M	No

b) Which molecule is the carrier of genetic information in *A. termiticus*? Justify your answer.

B is the carrier of genetic information in the alien organism. This is because when the BLT cells are treated with B-ase, no B remains in the sample, and, as a result, no transformation occurs. On the other hand, treatment with A-ase has no bearing on the transformation ability of the heat-killed BLT cells. We, therefore, must conclude that B is the carrier of genetic information.

Name:_____ TA:_____

Question 1, continued

Next, you set out to determine the structure of the alien genetic material molecule. You first determine that it has six types of bases that you name S, V, W, X, Y, Z. You further determine that the alien cell's content of S is the same as its content of each of X and Z; and that the content of V is the same as its content of each of W and Y.

When you determine the structure of this molecule by X-ray crystallography, you are not surprised to find that the molecule consists of 3 interacting strands.

c) What base interaction combinations do you expect for this molecule?
Based on the data, we expect S on one strand to interact with X on another strand and Z on the third strand. We would also expect V on one strand to interact with W on another strand and Y on the third strand.

You want to investigate the mechanism of replication of the alien genetic material. You decide to repeat the Meselson-Stahl experiment. Recall that labeled strands are "heavy" (low in the gradient) and unlabeled strands are "light" (high in the gradient). Also recall that in the experiment, the culture is grown on heavy Nitrogen (^{15}N), and is switched to light Nitrogen (^{14}N) at time=0.

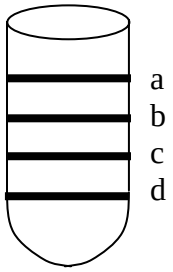
Before proceeding you define three possible models of alien replication:

- conservative, in which, after replication, old strands stay together, and new molecules are made entirely of new strands;
- semi-conservative, in which, after replication, each molecule has one old strand; and
- asymmetric, in which replication creates a molecule with one new strand, and a molecule with two new strands.

Each column in the table below reflects the predictions one of these models makes about the outcome of the experiment. The outcomes are described using symbols a-d to indicate the levels on the gradient as depicted in the figure on the right.

d) Label each column with the name of the appropriate model.

# cycles of replication	*Asymmetric*	*Conservative*	*Semi-conservative*
0	d	d	d
1	b, c	a, d	b
2	a, b, c	a, d	a, b
3	a, b, c	a, d	a, b



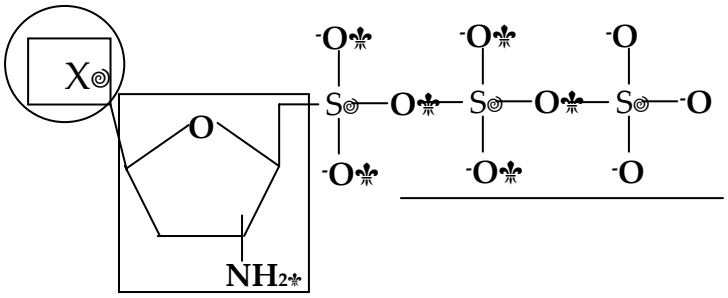
You determine that each *A. termiticus* mother cell completes one round of genetic material replication, but gives rise to three daughter cells.

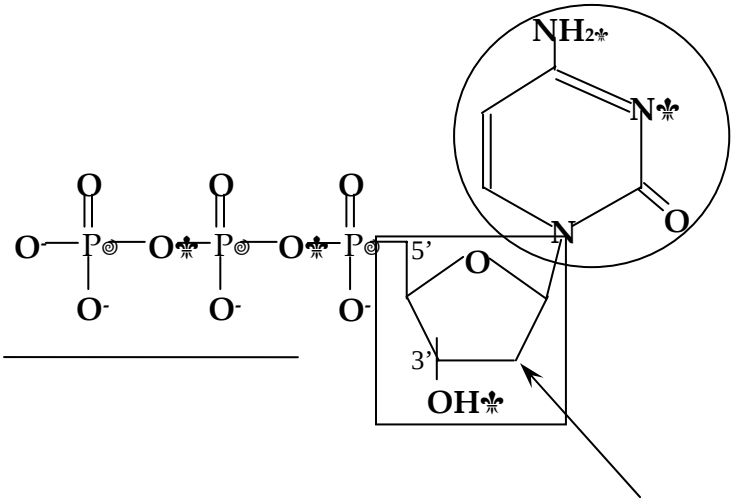
e) Which of the above models is not consistent with this data? Why?
In order to create three daughter cells, replication must produce three molecules of genetic material per cycle. Asymmetric model predicts creation of only two molecules of genetic material in each replication cycle. Therefore, asymmetric model is not consistent with data.

Name:_____ TA:_____

Question 1, continued

Below are the structures of an alien nucleotide X and the earth DNA nucleotide cytosine (C).





f) For both structures,

i. box the sugar

ii. circle the base

iii. underline the part of the structure used to power the addition of the nucleotide onto the growing chain.

g) For the DNA nucleotide,

i. label the 5′ end

ii. label the 3′ end

iii. draw an arrow to the part of the molecule that identifies it as a nucleotide used in DNA rather than in RNA.

Name:_____ TA:_____

Question 2 (30 points)

a) Transcription

i. is the process that transfers information from __*DNA*____ to ____*RNA*__.

ii. in eukaryotic organisms, transcription occurs in the

(Nucleus) Ribosome Membrane

b) Translation

i. is the process that transfers information from ____*RNA*__ to __*protein*__.

ii. in eukaryotic organisms, translation occurs in the

Nucleus (Ribosome) Membrane

The following sequence of DNA encodes a hypothetical polypeptide called Playdo in a hypothetical bacteria *E. hypotheticus*. Transcription starts at and includes the C/G base pair in bold. The underlined T/A base pair indicates the terminator.

5′-TT**C**CCCTATGGATGGTCATCTACGATGCCCCCATCACTAAAGCTTG-3′
3′-AA**G**GGGATACCTACCAGTAGATGCTACGGGGGTAGTGATTTCGAAC-5′

c) What are the first 6 bases of the transcribed RNA? Be sure to label the 5′ and 3′ ends.

5′-CCCCUA-3′

d) What are the first 3 amino acids of the subsequent polypeptide? Be sure to label the N- and C- termini.

N-Met-Asp-Gly-C

e) How many total amino acids are encoded in this polypeptide?
The gene encodes 10 amino acids. The 11th in-frame codon is the stop codon UAA.

You identify a strain of bacteria containing a mutant tRNA that is capable of adding a tryptophan residue when it recognizes the codon UAG in the mRNA.

f) What is the sequence of the anticodon of the mutant tRNA? Be sure to label the 5′ and 3′ ends.
3′-AUC-5′ or 5′-CUA-3′

Name:_____ TA:_____

Question 2, continued

g) The Playdo polypeptide would be longer / the same length / shorter in the presence of the mutant tRNA.

Why?

The length of Playdo would be the same in the presense of the mutant tRNA because the Playdo gene sequence does not include the TAG, so the mutant tRNA would never be used in translating Playdo

You isolate a mutant bacteria with the Playdo gene sequence below. The bold, boxed G/C base pair is the site of the only difference between wild-type and mutant Playdo—a substitution of a G/C base pair for a A/T base pair. As before, the bold C/G base pair indicates the start of transcription, and the underlined T/A base pair indicates the terminator.

5′-TT**C**CCCTATGG**G**TGGTCATCTACGATGCCCCCATCACTAAAGCTTG-3′
3′-AA**G**GGGATACC**C**ACCAGTAGATGCTACGGGGGTAGTGATTTCGAAC-5′

h) What is the effect of this substitution on the peptide?

The second amino acid in the peptide, Asp, is changed to Gly in this mutant variant.

i) Do you expect this peptide to have the same function as the wild-type bacterial peptide? Why or why not?

We would not expect the mutant Playdo to function like the wild-type peptide. This is because the mutant peptide now has Gly in place of Asp. Asp is a charged amino acid, while Gly is not charged. In addition, Asp is significantly larger than Gly, which has the smallest side chain of all the amino acids. This substitution is likely to affect tertiary structure of Playdo, and, therefore, to alter its function.

Intrigued by Playdo, you search for a similar protein in mice by looking for similar DNA sequences in the mouse genome. You find a gene that matches bacterial Playdo almost perfectly but contains a 36 DNA base pair insertion in the center of it.

When you purify the Playdo polypetide from mouse cells you are shocked to learn that mouse Playdo is the same length in amino acids as bacterial Playdo.

j) Explain how is it possible that mouse Playdo and bacterial Playdo are the same polypeptide length even though they have substantially different gene lengths.

Mouse genes have introns—regions of DNA within the coding sequence of a gene that do not get translated. These regions are spliced out of the initial transcript when mRNA is prepared. The 36 extra base pairs in the mouse gene are such an intron.

k) Do you expect bacterial and mouse Playdo to have the same promoter and terminator sequences? Why or why not?

The promoter and terminator sequences belong to a language written in DNA that is "read" by the transcription machinery. This language is not universal, but is species-specific. Therefore, we would not expect bacteria and mouse to have the same promoter and terminator sequences.

Name:_____ TA:_____

Question 3 (25 points)

a) Which of the following could alter gene regulation (circle all that apply)

i. Deleting a promoter

ii. Moving a yeast culture to a new food source

iii. Raising the temperature of a bacterial culture

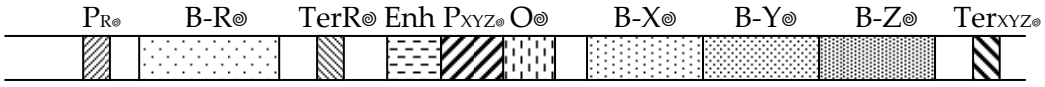
iv. Mutating a repressor gene, such that the resulting protein no longer functions

The B operon contains the genes involved in the breakdown of sugar B in the bacteria *E. fake.* Sugar A is the preferred sugar in *E. fake*. In the absence of sugar A, *E. fake* can also use sugar B. The B operon is subject to both negative and positive regulation.

b) Indicate with a Low or a High the expected level of B operon expression when *E. fake* cells are grown in the presence (+) or absence (-) of the sugars A and/or B.

B operon expression	Sugar A only	Sugar B only
Low	-	-
High	-	+
Low	+	-
Low	+	+

Below is the diagram of the B operon. B-R encodes B Repressor, the repressor of the B-XYZ genes. Ter sequences denote transcription terminators. P sequences denote promoters. O denotes an operator, and Enh—an enhancer. Recall that the B operon is subject to both negative and positive regulation.



c) How many in frame translation stop signals (stop codons) are present in the mRNA transcript originating with P_{XYZ}? *3*

d) To which element does the B Repressor protein bind? *Operator (O)*

e) What is the effect of the B Repressor binding on

i. transcription of B-XYZ?

decrease no change increase

ii. translation of any B-XYZ transcripts already made?

decrease no change increase

Name:_____ TA:_____

Question 3, continued

You have isolated three loss of function mutants in the B operon.

f) For each mutant in the table below, for each condition, indicate (with a Yes or a No) whether the repressor protein and the activator protein are each bound to their respective binding sites, and what the resulting level of expression (None, Low, or High) of the B operon is. Data for the wild-type strain is filled in for you.

Strain	Mutation in	Sugar A only			Sugar B only			Sugars A and B		
		Repressor	Activator	Expression	Repressor	Activator	Expression	Repressor	Activator	Expression
WT		Yes	No	None	No	Yes	High	No	No	Low
M1	B-R	*No*	*No*	*Low*	*No*	*Yes*	*High*	*No*	*No*	*Low*
M2	Enh	*Yes*	*No*	*None*	*No*	*No*	*Low*	*No*	*No*	*Low*
M3	P_{XYZ}	*Yes*	*No*	*None*	*No*	*Yes*	*None*	*No*	*No*	*None*

Question 4 (16 points)

You hope to understand the lysine biosynthesis pathway, so you decide to look for mutants that can not survive without supplementation with the amino acid lysine.

You mutagenize some bacteria, and plate them on rich media. You then replica plate from your original plate onto three plates: one containing complete media, one containing minimal media, and one containing minimal media plus lysine.

By comparing the minimal and the +lysine plates, you identify six colonies that are lysine auxotrophs, that is, they require lysine from the media to grow.

Below are the results of the complementation test, where + means growth and – means no growth.

Mutant	lys1	lys2	lys3	lys4	lys5	lys6	wild-type
lys1	–	+	+	–	+	–	+
lys2		–	–	+	+	–	+
lys3			–	+	+	–	+
lys4				–	+	–	+
lys5					–	–	+
lys6						–	–
wild-type							+

a) For each mutant, circle whether the mutation is dominant or recessive.

lys1	dominant	recessive
lys2	dominant	recessive
lys3	dominant	recessive
lys4	dominant	recessive
lys5	dominant	recessive
lys6	dominant	recessive

Name:_____ TA:_____

Question 4, continued

b) Place the recessive mutants into complementation groups.
(1, 4) (2, 3) (5)

c) How many genes (at least) must there be in the lysine biosynthesis pathway?
Because there are three complementation groups, there must be at least three genes in the lysine biosynthesis pathway.

You find some lysine precursors (X, Y, and Z) that, when added to the media, allow the growth of some mutants. You try growing several mutants on minimal media with X, Y, or Z added, and get the following results. + means growth and – means no growth.

	Precursor supplement		
Mutant	X	Y	Z
lys1	–	+	–
lys2	+	+	–
lys5	–	–	–
wild-type	+	+	+

d) Circle the pathway(s) consistent with data.

$$Y \xrightarrow{lys2} X \xrightarrow{lys1} Z \xrightarrow{lys5} lysine$$

$$Y \xrightarrow{lys5} X \xrightarrow{lys1} Z \xrightarrow{lys2} lysine$$

$$X \xrightarrow{lys1} Z \xrightarrow{lys5} Y \xrightarrow{lys2} lysine$$

$$Z \xrightarrow{lys2} X \xrightarrow{lys1} Y \xrightarrow{lys5} lysine$$

$$Y \xrightarrow{lys5} Z \xrightarrow{lys2} X \xrightarrow{lys1} lysine$$

$$X \xrightarrow{lys2} Y \xrightarrow{lys1} Z \xrightarrow{lys5} lysine$$

$$Z \xrightarrow{lys5} Y \xrightarrow{lys1} X \xrightarrow{lys2} lysine$$