Quiz 2 on Wednesday 4/3 from 11-noon. Bring IDs to exam.

Last Name from A through S (Alwani through Sutherland) in Walker

Last name from T through Z (Tenenbein through Zhugralin) in 10-250

Review Session 4/1 from 7-9 pm in 10-250
Tutoring Session 4/2 from 4-6 pm in 26-302

Tutors (HELPFUL PEOPLE)

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More helpful people

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<tr>
<td>Claudette Gardel</td>
<td>12-1pm Monday 1-2 pm Wednesday 68-120d</td>
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<td>Matthew Lahaie</td>
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<td>Kevin Lai</td>
<td>Mon 4-5 E17-524</td>
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<td>Isaac Manke</td>
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<td>Roberto Rodriguez</td>
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<td>Luke Tomycz</td>
<td>Mon 9 pm Lobdell dining room (student center)</td>
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<tr>
<td>Sunny Wong</td>
<td>Tues 7-8 pm 26-310</td>
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Question 1

a) Indicate whether each of the following statements is true or false. If false, correct the statement or provide a brief explanation for why it is false.

i) DNA replication is initiated at promoter sequences in the DNA.

ii) RNA polymerase requires primers to initiate RNA synthesis.

iii) Okazaki fragments are the short fragments of DNA that are produced on the leading strand at the DNA replication fork.

iv) The 5' to 3' direction of DNA synthesis implies that deoxyribonucleotides are added to the 5' OH group on the growing strand.

v) Transcription is terminated at stop codons in the mRNA.

b) Shown below is the DNA sequence of a gene from a virus that encodes a short viral peptide. Also shown is the sequence of the mRNA synthesized from this gene.

**genomic DNA sequence:**

```
5'–AGCTCATGTGCGAGTCCTGACGCTGACTAGG–3'
3'–TCGAGTACACGCTCAGGACTGCGACTGATCC–5'
```

**mature mRNA sequence (G* = G cap):**

```
5'–G*UCAUGUGCGAACGCUGACUAGGAAAAA…–3'
```

i) In the genomic DNA sequence shown above, draw a box around each of the two exons in the gene.

ii) In the mRNA above, some nucleotides are present that are not coded for in the genomic DNA sequence. Name the two processes that have occurred to add these nucleotides to the mRNA.

iii) How many amino acids are in the viral peptide encoded by this gene? _______

iv) Is this virus more likely to replicate in prokaryotic or eukaryotic cells? Briefly explain your reasoning.
Question 2

The term "central dogma" refers to the flow of biological information from DNA to RNA to protein.

![DNA to RNA to Protein Diagram]

a) i) In the spaces below, indicate the process that corresponds to each arrow.

1. __________  2. __________  3. __________

   ii) Name the initiation site for each processes, and on which molecule this site exists.

   1. 
   2. 
   3. 

   iii) What cellular machinery carries out each process?

   1. __________  2. __________  3. __________

b) What is a gene? Please answer in one sentence. The first sentence written will be considered as your answer.

c) The fidelity of the process of DNA replication is very important. Occasionally, however, DNA polymerase will make a mistake while replicating DNA. Name one process that helps prevent changes in the nucleotide sequence of DNA.
Question 2 continued

d) Many environmental factors, such as ultraviolet light, are mutagenic and can introduce changes (lesions) in the DNA. When DNA polymerase encounters these lesions, it usually pauses at the lesion until the abnormality is resolved. In *Escherichia coli*, however, if there is irreparable levels of DNA damage, cells will signal DNA polymerase to randomly incorporate any nucleotide opposite the sites of the lesions.

i) What would happen to the cell if DNA replication did not occur?

ii) What result would this error-prone replication have on the daughter DNA?

iii) Why might a cell choose to use error-prone replication to resolve situations where there was a large amount of DNA damage?

e) Many antibiotics are compounds that interfere with the transfer of genetic information from RNA to protein.

i) Streptomycin is a compound that affects the small ribosomal subunit in prokaryotes. Streptomycin interferes with the binding of all Methionine-tRNAs to ribosomes. What two specific effects will streptomycin have on protein synthesis in prokaryotes?

ii) Puromycin is an antibiotic that has an effect on both prokaryotes and eukaryotes. Puromycin, which is structurally similar to the aminoacyl terminus of an aminoacyl-tRNA (see diagram), inhibits protein synthesis by releasing nascent polypeptide chains before their synthesis is completed.

R represents the side group of the amino acid
R’ is the remainder of the tRNA

Explain how puromycin can effect this result on growing polypeptide chains and why the peptide chain is released.
Question 3

A.
The primer shown below is used to sequence the following template DNA.

primer: template DNA:

5′–ACTGAC–3′  5′–ACCACTAACGTCAGT–3′

Draw the resulting DNA fragments that would be produced from each of the 4 sequencing reactions at the correct position (length in nucleotides) as they would appear on the diagram of the sequencing gel below.

B.
Polio has been practically eliminated from the American population, however, in countries where people have little or no access to vaccinations, it is still prevalent. As a biologist with a global vision, you seek to create a transgenic banana that produces the protein used in the vaccine against polio. By consuming these bananas, individuals will develop immunity against the disease. The gene for this protein has already been cloned into a plasmid with a kanamycin-resistance gene (pKR-polio). You need to attach to the gene a banana-specific promoter and DNA sequences that will allow the gene to be incorporated into banana DNA. These sequences are contained in the pBAN plasmid, which carries a gene for ampicillin resistance. Maps of these two plasmids are shown on the next page, including important restriction sites and distances (in base pairs) between the sites.
Question 3 continued

Banana specific promoter

BamHI

EcoRI

120

1120

BglII

start codon

BamHI

stop codon

BglII

gene for polio antigen

ori

EcoRI

kan gene

amp gene

banana insertion sequences
which allow DNA to integrate into
banana genome

pBAN
8900 bp

pKR-polio
4500 bp

BglII:

5'...A G A T C T...3'

3'...T C T A G A...5'

BamHI:

5'...G G A T C C...3'

3'...C C T A G G...5'

EcoRI:

5'...G A A T T C...3'

3'...C T T A A G...5'

a) An end generated by digestion with BamHI can be ligated to an end generated by digestion with BglII. Why is this possible?

b) You want to insert the gene encoding the polio antigen into pBAN. Devise a strategy to accomplish this. Identify the enzyme(s) you would use to cut pBAN, the enzyme(s) you would use to cut pKR-polio, and the steps necessary to generate the intact plasmid.

c) You next transform E. coli with the plasmids you have made. You grow the transformed cells on media containing (circle one):

ampicillin

both ampicillin and kanamycin

ampicillin and kanamycin

neither ampicillin nor kanamycin

Why?
d) You isolate plasmid DNA from three colonies that pass your antibiotic resistance test. You digest the DNA with the restriction enzyme *Eco*RI. You size separate the resulting fragments from each plasmid on an agarose gel. You find the following results. DNA fragment sizes are indicated to the left.

![Agarose gel image]

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**d)** i) Draw plasmids associated with the colonies 1, 2, and 3. Indicate all relevant features such as the promoter, the origin of replication, the genes for ampicillin resistance and the polio antigen.

ii) Which of the three plasmids would allow synthesis of the protein in a banana? Explain your reasoning.
**Question 4**

Consider the following signal transduction pathway involved in smelling pumpkin pie.

1) A pie odorant molecule (π), one of the many aromatic compounds present in pumpkin pie, binds to a specific G protein-coupled olfactory receptor in an olfactory cell in your nose.

2) The receptor-odorant complex activates a G protein, which displaces GDP and then binds to a molecule of GTP.

3) The α subunit of the G protein dissociates and activates adenylate cyclase, which catalyzes the production of cAMP from ATP.

4) cAMP binds to a sodium channel, opening it and allowing Na⁺ to enter the cell. This creates a nerve stimulus, which travels to the brain. Your brain integrates the π odorant signal with signals from other olfactory receptors in your nose. The brain interprets the signal as the odor of pumpkin pie. You think, “Mmm...pie.”

a) How does binding of the π odorant molecule stimulate the receptor protein and how does this activate the G protein?

b) Although it might be nice to have the sensation of smelling pie continuously, once the π odorants are gone, signaling stops. How is the signaling pathway turned off? Discuss why once the G protein is activated, it does not continuously activate adenylate cyclase. What chemical reaction is involved in this process?

c) List two advantages to having multiple steps in a signal transduction pathway.

d) A turkey odorant molecule comes in contact with the π odorant receptor. Would this have an effect on the nerve stimulus being sent from this olfactory cell? Explain your answer.
**Question 5**

You are studying a family with hemophilia, a sex-linked recessive disease, caused by mutations in the Factor VIII gene. The Factor VIII gene contains 35 exons. The complete sequence and exon/intron structure of this gene are known. The start codon is in exon 3; the stop codon is in exon 34.

A partial restriction map and a diagram showing the location of exons 12, 13 and 14 is shown below. You synthesize two PCR primers, which anneal to sequences located within exons 12 and 14, as shown.

Wild-type(normal) Allele:

![Restriction Map](image)

Using these PCR primers, you amplify DNA from a normal male and his hemophiliac brother. You determine the restriction map for the PCR product from these two individuals, shown below:

**PCR-amplified DNA fragment from normal male:**

![Restriction Map](image)

**PCR-amplified DNA fragment from hemophiliac brother:**

![Restriction Map](image)

a) Briefly describe the likely DNA alteration in the hemophiliac.
Question 5 continued

b) Shown below is a pedigree for the family:

KEY: □ = phenotypically normal male
■ = hemophiliac male
○ = phenotypically normal female
◇ = hemophiliac female

You prepare DNA from some of the family members (individuals #1 - #6) and amplify the region of DNA shown above using your PCR primers. You then digest the resulting PCR product to completion with the restriction enzyme HindIII. You separate the fragments by gel electrophoresis. On the gel below, indicate the DNA fragment sizes that you would expect in each of the six individuals in the above family. Please draw the fragments as lines in the picture below and adjacent to each DNA fragment in the gel indicate their length in base pairs.

Individual's PCR-amplified product, digested with HindIII

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ANSWERS

Question 1

a) i) FALSE. DNA replication is initiated at the origin of replication. RNA polymerases bind to promoter sequences to initiate transcription.

ii) FALSE. RNA polymerase does not require primers to initiate RNA synthesis. DNA polymerase requires primers to initiate DNA replication.

iii) FALSE. Okazaki fragments are made on the lagging strand at the replication fork.

iv) FALSE. DNA synthesis occurs by addition of dNTPs to the 3' OH group of the nucleotide at the end of the growing strand.

v) FALSE. Transcription terminates at the transcription termination sites in the DNA. Translation terminates at stop codons in the mRNA.

b) genomic DNA sequence:

\[
5'\text{-AGCTCATGTGCGAGTCCTGACGCTGACTAGG}-3' \\
3'\text{-TCGAGTACACGCTCAGGACTGCGACTGATCC}-5'
\]

mature mRNA sequence (G* = G cap):

\[
5'\text{-G*UCAUGUGCGAACGCUGACUAGGAAAAAAAAAAAAA...3'}
\]

i) see DNA sequence above

ii) 1) 5' capping
    2) 3' polyadenylation

iii) There are four amino acids in this viral peptide:
    \[\text{NH}_3^+\text{-met-cys-glu-arg-COO^-}\]

iv) In eukaryotic cells because the RNA processing and splicing machinery is only present in eukaryotes.

Question 2

The term "central dogma" refers to the flow of biological information from DNA to RNA to protein.

\[
\text{DNA} \rightarrow^2 \text{RNA} \rightarrow^3 \text{Protein}
\]

a) i) In the spaces below, indicate the process that corresponds to each arrow.

1. replication  2. transcription,  3. translation
Question 2 continued

ii) Name the initiation site for each process, and on which molecule this site exists.

1. Origin of replication, DNA
2. Promoter, DNA
3. Start codon (first AUG), mRNA

iii) What cellular machinery carries out each process?

1. DNA polymerase  
2. RNA polymerase  
3. ribosome

b) What is a gene? Please answer in one sentence. The first sentence written will be considered as your answer. A gene is a segment of DNA containing information for directing the synthesis of a protein (or RNA).

c) Name one process that helps prevent changes in the nucleotide sequence of DNA. Proofreading during replication, cellular error repair processes

d) i) What would happen to the cell if DNA replication did not occur? No cell proliferation, Cell death.

ii) What result would this error-prone replication have on the daughter DNA? Many mutations.

iii) Why might a cell choose to use error-prone replication to resolve situations where there was a large amount of DNA damage?

Cells that can’t complete DNA replication will die. In the event of a large quantity of DNA damage, the cellular repair processes are unable to deal with all the damage, so the cell’s recourse is error-prone replication. This can be seen as a last ditch effort to replicate the genome and stave off cell death; better to live with mutations than not live at all.

e) i) What two specific effects will streptomycin have on protein synthesis in prokaryotes?

Streptomycin will prevent the correct initiation of protein synthesis since it prevents association of the met-tRNA with the ribosome. Streptomycin will also lead to inaccurate translation (insertion of incorrect amino acids) in those proteins that were in the process of being translated.

ii) Explain how puromycin can effect this result on growing polypeptide chains and why the peptide chain is released.

Puromycin functions by entering the A site of the ribosome. Here, because puromycin is structurally similar to the aminoacyl-tRNA, it can participate in formation of a peptide bond with the nascent polypeptide chain. Puromycin causes peptide release from the ribosome because there is no t-RNA anticodon to link the mRNA to the peptide chain.

Question 3

A. primer: 5'–ACTGAC–3'  
template DNA: 5'–ACCACTAAGCTCAGT–3'

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a) An end generated by digestion with BamHI can be ligated to an end generated by digestion with BglII. Why is this possible?
Digestion with BglII or BamHI produce the same overhangs, or sticky ends. Thus the ends produced by cutting with BglII are complementary to the ends produced with BamHI, base pairing and ligation can occur.

b) You want to insert the gene encoding the polio antigen into pBAN. Devise a strategy to accomplish this. Identify the enzyme(s) you would use to cut pBAN, the enzyme(s) you would use to cut pKR-polio, and the steps necessary to generate the intact plasmid.
Cut pBAN with BamHI to linearize.
Cut pKR-polio with BglII, this gives a 1400 bp fragment containing the gene for the polio antigen and a 3100 bp fragment.
Size select the DNA from the pKR-polio plasmid to obtain the 1400 bp fragment.
Ligate the 1400 bp fragment together with the cut pBAN plasmid.

c) You next transform *E. coli* with the plasmids you have made. You grow the transformed cells on media containing (circle one):

- ampicillin
- kanamycin
- both ampicillin and kanamycin
- neither ampicillin nor kanamycin

Why? The media should contain ampicillin only, because the plasmid with the promoter and banana insertion sequences has a gene for ampicillin resistance.

d) i) Draw plasmids 1, 2, and 3, indicating all relevant features such as the promoter, the origin of replication, the genes for ampicillin resistance and the polio antigen, restriction sites and distances (in base pairs) between the sites.

B.

ii) Which of the three plasmids would allow synthesis of the protein in a banana? Explain your reasoning.

*Only plasmid two would allow synthesis of functional protein.*

The polio antigen gene must be correctly oriented with respect to the promoter.
**Question 4**

a) How does binding of the π odorant molecule stimulate the receptor protein and how does this activate the G protein?

*The binding of a π odorant molecule to the extracellular domain of the receptor changes the conformation of the protein. This allows the intracellular domain of the receptor to bind the G protein heterotrimer. This binding induces the α subunit to exchange GDP for GTP, activating the α subunit and releasing it from the βγ subunit.*

b) Although it might be nice to have the sensation of smelling pie continuously, once the π odorants are gone, signaling stops. How is the signaling pathway turned off? Discuss why once the G protein is activated, it does not continuously activate adenylate cyclase. What chemical reaction is involved in this process?

*The α subunit of the G protein has an intrinsic GTPase activity. This activity converts GTP bound to the α subunit to GDP. Since the GTP is required for the α subunit to be active, the conversion of GTP to GDP deactivates the α subunit of the G protein. The inactive α subunit then can rejoin the βγ subunit to await another signal from the receptor.*

c) List two advantages to having multiple steps in a signal transduction pathway.

*Multiple steps in the pathway can provide a large amplification of the signal. It also allows multiple points of regulation. In addition, if several different receptors work through the same transduction pathway, integration of different inputs is possible.*

d) A turkey odorant molecule comes in contact with the π odorant receptor. Would this have an effect on the nerve stimulus being sent from this olfactory cell? Explain your answer.

*No, a turkey odorant molecule would have no effect on the stimulus from this cell. The π odorant receptor specifically recognizes the π odorant. However, the turkey odorant molecule would affect olfactory cells specialized for turkey.*

**Question 5**

a) Briefly describe the likely DNA alteration in the hemophiliac.

*There is a deletion of 330 bp in the DNA, including exon 13 and possibly part of exon 14, which also removes a Hind III site and an Eagl site.*

b) Individual’s PCR-amplified product, digested with HindIII

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