

FUNDAMENTALS OF BIOMEDICAL SCIENCES**FALL 2008: INTD 5000****MOLECULAR BIOLOGY MODULE EXAMS AND TAKE-HOMES**

Question 1 (15 points)

- a. **Is the following statement TRUE or FALSE?** (1 point)

Linear DNA in solution will form positive supercoils if turns are added to the helix.

- b. **Is the following statement TRUE or FALSE?** (1 point)

In B-form DNA the two DNA strands are anti-parallel, whereas the two DNA strands in Z-form DNA are parallel.

- c. **Is the following statement TRUE or FALSE?** (1 point)

In eukaryotic cells, the binding of some proteins to DNA can be enhanced by DNA methylation.

- d. **Is the following statement TRUE or FALSE?** (1 point)

All ARS elements in the yeast *Saccharomyces cerevisiae* are true origins of replication in the chromosome.

- e. **Will the structure of a double-stranded nucleic acid in which one strand is DNA and the complementary strand is RNA be A form, B form or Z form?** (1 point)

- f. **Give a concise (1 - 2 sentence) definition of a "CpG island".** (1 point)

- g. **Linear double-stranded DNA containing a spectinomycin-resistance gene can be introduced into *E. coli* by electroporation (artificial transformation). Which of the following is the most likely "fate" of the DNA that gets into *E. coli*? Circle the correct answer.** (1 point)

- i. The DNA will be degraded.
- ii. The DNA will replicate as a spectinomycin-resistant plasmid in *E. coli*.
- iii. The DNA will undergo a reciprocal (double) recombination with the host genome and generate spectinomycin resistant colonies.

Question 1 (continued)

- h. List two ways in which an origin of replication from the yeast *Saccharomyces cerevisiae* differs from a typical origin in a higher eukaryotic cell. (2 points)

_____ *S. cerevisiae* _____

_____ Higher Eukaryote _____

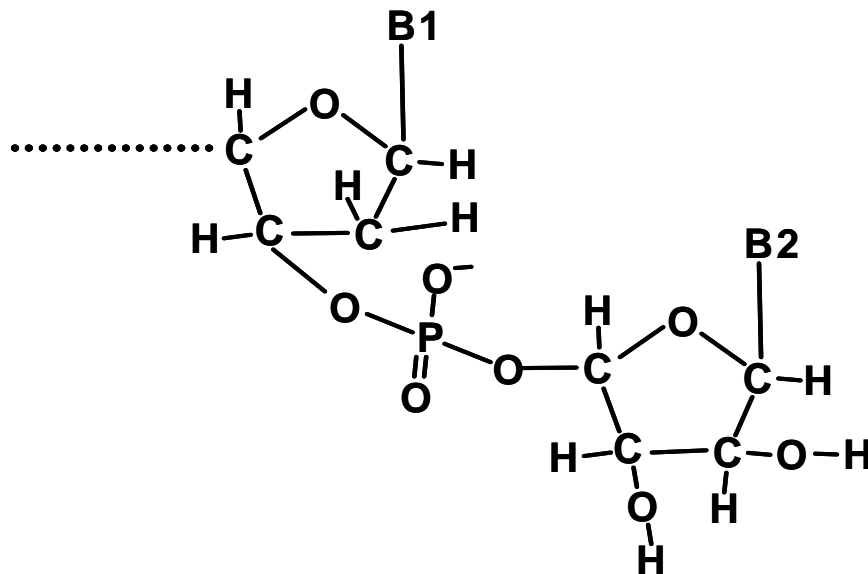
1.

2.

- i. What enzymatic function does DNA Polymerase I from *E. coli* have that is also known as its “proofreading” activity? (1 point)

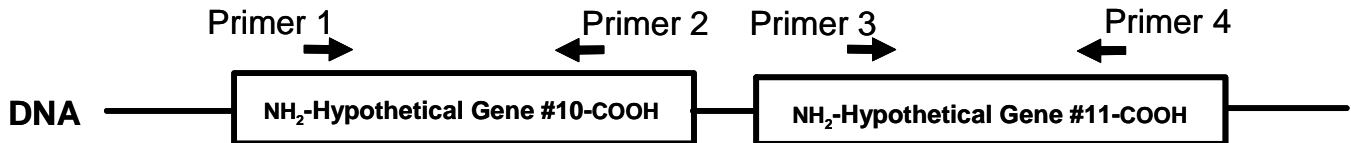
- j. DNA polymerase alpha makes primers for the major eukaryotic DNA polymerases, delta and epsilon. DnaG is the protein that makes the primer for DNA polymerase III in prokaryotic cells. What is the major difference between the primers made by these two enzymes? Note: A difference in the length of the primers is not the answer. (2 points)

- k. List (or circle) the two things that are wrong with this representation of the sugar-phosphate backbone of DNA. The bases are indicated by B1 and B2. (3 points)



Question 2 (4 points)

- a. From sequence analysis, the *E. coli* genome encodes two hypothetical genes, called #10 and #11, that are immediately adjacent to one another and are in the same orientation. To determine whether or not these two genes are co-transcribed (part of a poly-cistronic RNA), *E. coli* RNA was isolated and used as a substrate in Reverse Transcriptase PCR (RT-PCR). The position of the primers used are shown in the diagram below. The reaction with primer 1 and primer 2 gave a PCR product of the expected size as did the sample with primers 3 and 4. However, the sample with primers 1 and 4 gave no PCR product at all. **Based upon these data, are genes #10 and #11 co-transcribed (part of a poly-cistronic RNA)? Explain your reasoning in one sentence.** (1 point)



- b. In class, we discussed several mechanisms used to prevent inappropriate re-initiation of DNA replication at OriC in *E. coli*. One mechanism proposed that a cluster of five *dnaA* binding sites near, but not in, OriC can serve as a “sink” to bind extra *dnaA* proteins and thus help prevent early re-initiation. **Describe, in general terms, the experiment you would do to show that these five *dnaA* binding sites are indeed important for preventing early re-initiation at OriC. Be sure to include the results you expect.** You may assume you have any reagents necessary for the experiment. A 3 – 4 sentence answer should be sufficient. (3 points)

Question 3 (7 points)

Your dissertation advisor, Dr. Dina Binder recently discovered a new bacterium which she called *Big Troublis* (*Bt*) because this bacterium is resistant to every antibiotic that she tested. In addition, she showed that the multiple antibiotic resistance genes in this bacterium were encoded on a large plasmid. Using appropriate containment conditions, Dr. Binder then demonstrated that this plasmid can be transferred via conjugation from *B. troublis* into *E. coli*. Because of the potential importance of this organism and plasmid in spreading antibiotic resistance to disease-causing bacteria, Dr. Binder has asked you to begin to study the mechanism by which this plasmid transfers from one bacterium to another.

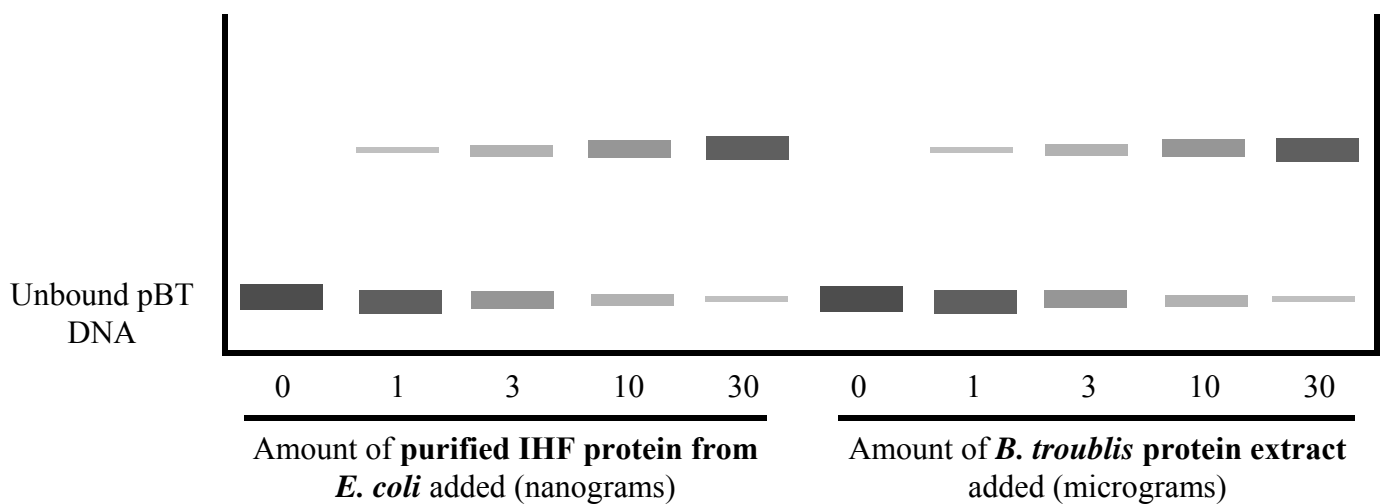
- a. **Sketch and/or describe the steps involved in conjugation between *Big troublis* and *E. coli*. Assume the same mechanism is used as when two *E. coli* cells mate. Be sure to include the function(s) of *oriT* in conjugation and name the key protein (or protein complex) involved in *oriT* function.**

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Question 3 (continued)

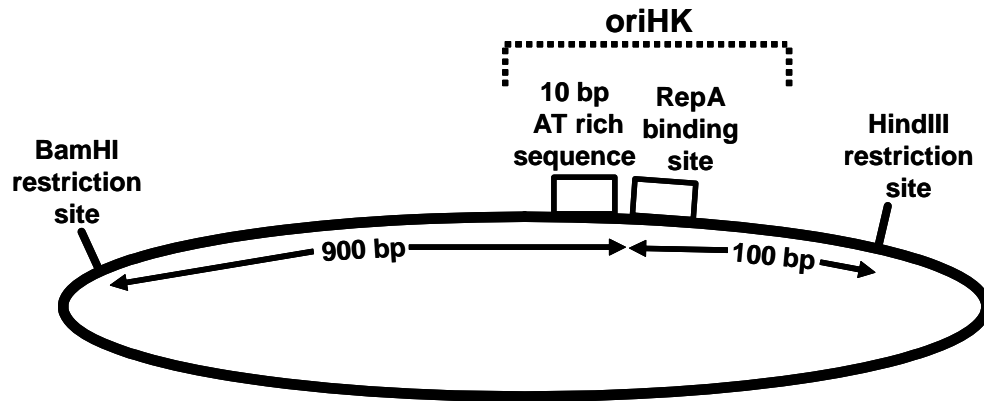
Of course, Dr. Binder has had the *B. troublis* plasmid sequenced and so you look for a stretch of DNA whose sequence is similar to the oriT sequence of the *E. coli* F-plasmid. You quickly identify a 250 bp segment whose sequence is similar, but not identical, to the F-plasmid oriT. Included in the 250 bp is a 12 bp sequence that matches the consensus binding site for *E. coli* IHF at 10/12 bases (= “near-IHF-consensus”). To help with your future experiments, you clone the 250 bp *B. troublis* sequence into an *E. coli* vector. You call this plasmid, with the presumptive *B. troublis* oriT, pBT.

- b. To begin to analyze the mechanism by which this plasmid transfers, you decide to see if *B. troublis* has an Integration Host Factor (IHF) similar to the *E. coli* IHF that binds the 12 bp IHF-consensus sequence in the oriT region of the F-plasmid. Since the genomic sequence of *B. troublis* is not available, you decide to use a biochemical approach. You therefore collaborate with a new postdoctoral fellow in the lab who is an expert at running gel mobility shift assays. The postdoc does several gel mobility shift assays for you with ^{32}P -labeled plasmid pBT as the DNA and the protein preparations shown below (purified *E. coli* IHF protein and a total protein extract from *B. troublis*). A few days later the postdoc comes to you and tells you that he has shown definitively that *B. troublis* has IHF. After looking at the data (below), you quietly inform him that he may be wrong. Explain your reasoning in one or two sentences.
- c. How would you alter or expand the gel mobility shift experiment in order to get a more definitive answer to the question of whether or not the *B. troublis* protein is binding to the 12 bp “near-IHF-consensus” sequence in pBT? Your answer can be given in broad terms and should only take one or two sentences.



Question 4 (6 points)

- a. Your advisor, Dr. Harry Knuckles, has been studying replication of the bacterial plasmid pHK200 for several years. He has shown that initiation of replication from a pHK200 double stranded DNA template requires a small origin, *oriHK*, and a small protein he calls repA. Furthermore, he has found that repA binds to a specific sequence in *oriHK*. Based upon your knowledge of previous experiments with *oriC* and *dnaA* from *E. coli*, he asks you to design an experiment to show that repA opens up (or melts) double stranded DNA at a particular position. Starting with the DNA template shown below, briefly describe the experimental protocol you would use to address Dr. Harry Knuckles' question. Show the results you would expect if the DNA is opened up in the 10 bp AT-rich region when repA is bound.

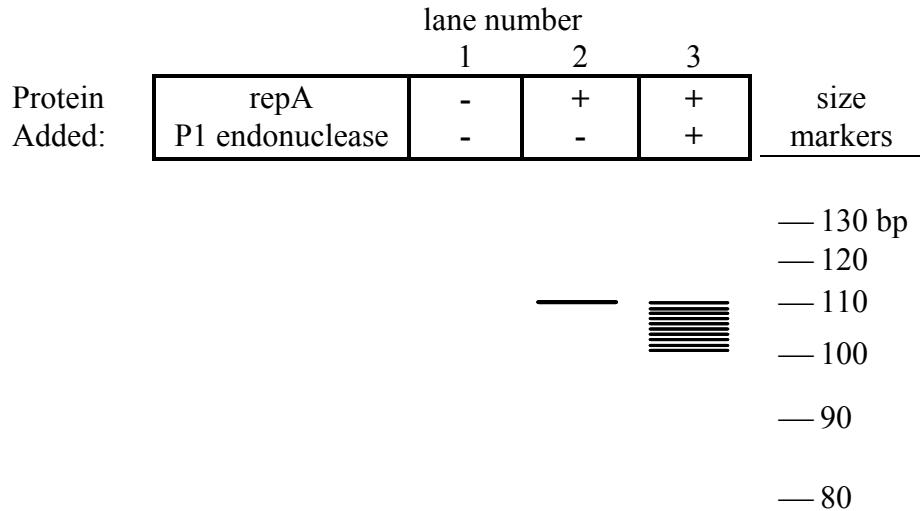


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Question 4 (continued)

- b. You did your proposed experiment and got the following results when you used the restriction endonuclease *Hind*III and ran the reaction products on a denaturing gel (any DNA fragments larger than 130 bp or smaller than 80 bp are not visualized on this gel):



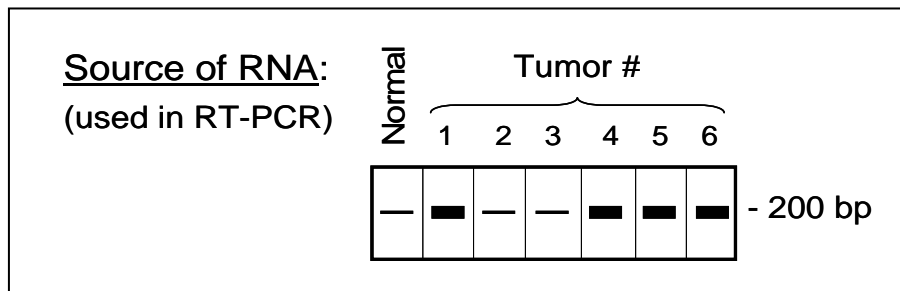
What conclusion(s) would you draw from a careful and complete evaluation of these data? A two to three sentence answer is sufficient.

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Question 5 (14 points)

In the September 15th issue of the journal, Cancer Research, Drs. Wei-Zin Wei and colleagues described a novel therapy for the treatment of Her-2/neu-expressing breast cancers. They tested this new therapy in mice and saw very promising results. Their basic approach was to immunize the mice with a DNA-based vaccine such that the mice would make an immune response to the Her-2/neu protein. In the immunized mice, this immune response killed the tumor cells, prolonging survival of those animals. [Note: you do not need to understand the details of this new therapy in order to answer the questions below.]

You are very interested in determining which human tumors might be susceptible to this type of therapy. So, you contact Dr. Wei and she is happy to collaborate with you. She sends you RNA, DNA, and protein from 6 different human breast tumor lines and from normal breast tissue as well. Using the same amount of RNA from each sample, you perform an RT-PCR (reverse transcriptase coupled polymerase chain reaction) using primers specific for the Her-2/neu oncogene. You electrophorese the PCR products on an agarose gel and stain them; the results are diagrammed below:

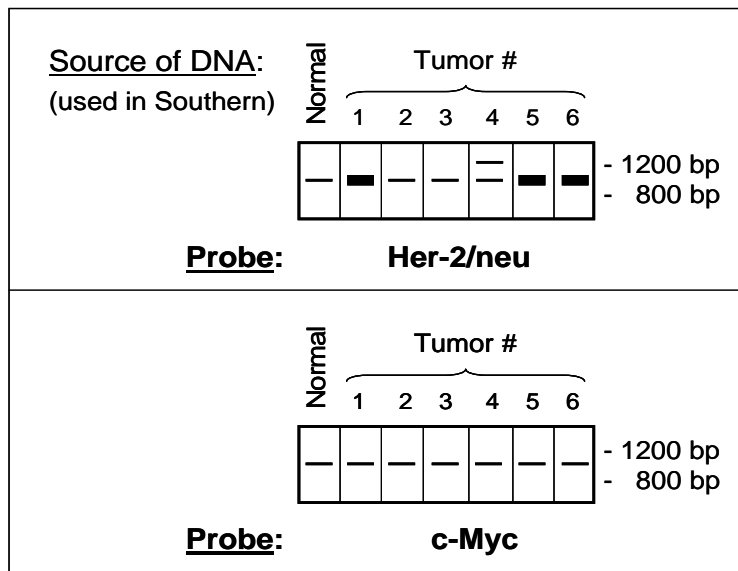


- Which of the tumors are over-expressing Her-2/neu mRNA? Explain how you know this in 1 sentence (or less).
- From this method, can you accurately quantitate the level of over-expression, compared to the normal tissue (yes or no)? If not, what method would you use instead?
- From this method, can you determine the size of the mRNA encoding Her-2/neu (yes or no)? If not, what method would you use instead?

Question 5 (continued)

- d. From this method, can you quantitate the level of **protein** expression (yes or no)? If not, what method would you use instead?

You would like to know more about the regulation of the Her-2/neu oncogene in these breast tumors, so you perform Southern blots using DNA from each of the samples. You hybridize the Southern blots independently with either a probe for Her-2/neu or a probe for c-Myc; the results are diagrammed below:



- e. Which of the tumors contain DNA amplifications of the Her-2/neu gene? Explain how you know this (answer in 1 sentence or less).

- f. For those tumors in which DNA amplification did occur, note which of the following would be consistent with amplification - put YES or NO as shown in the one example given.

	YES or NO
Increased growth of those tumor cells in vitro	YES
Double-minute chromosomes on FISH analysis with the Her-2/neu probe	
Resistance to methotrexate when the tumors are grown in vitro	
DNA microarrays would shown increased copy number of sequences near the Her-2/neu gene (on chromosome 17)	

Question 5 (continued)

- g. What is the most likely molecular explanation for tumor #4? EXPLAIN YOUR ANSWER.**
You must be able to account for both the DNA data (Southern blot) and the RNA data (RT-PCR).

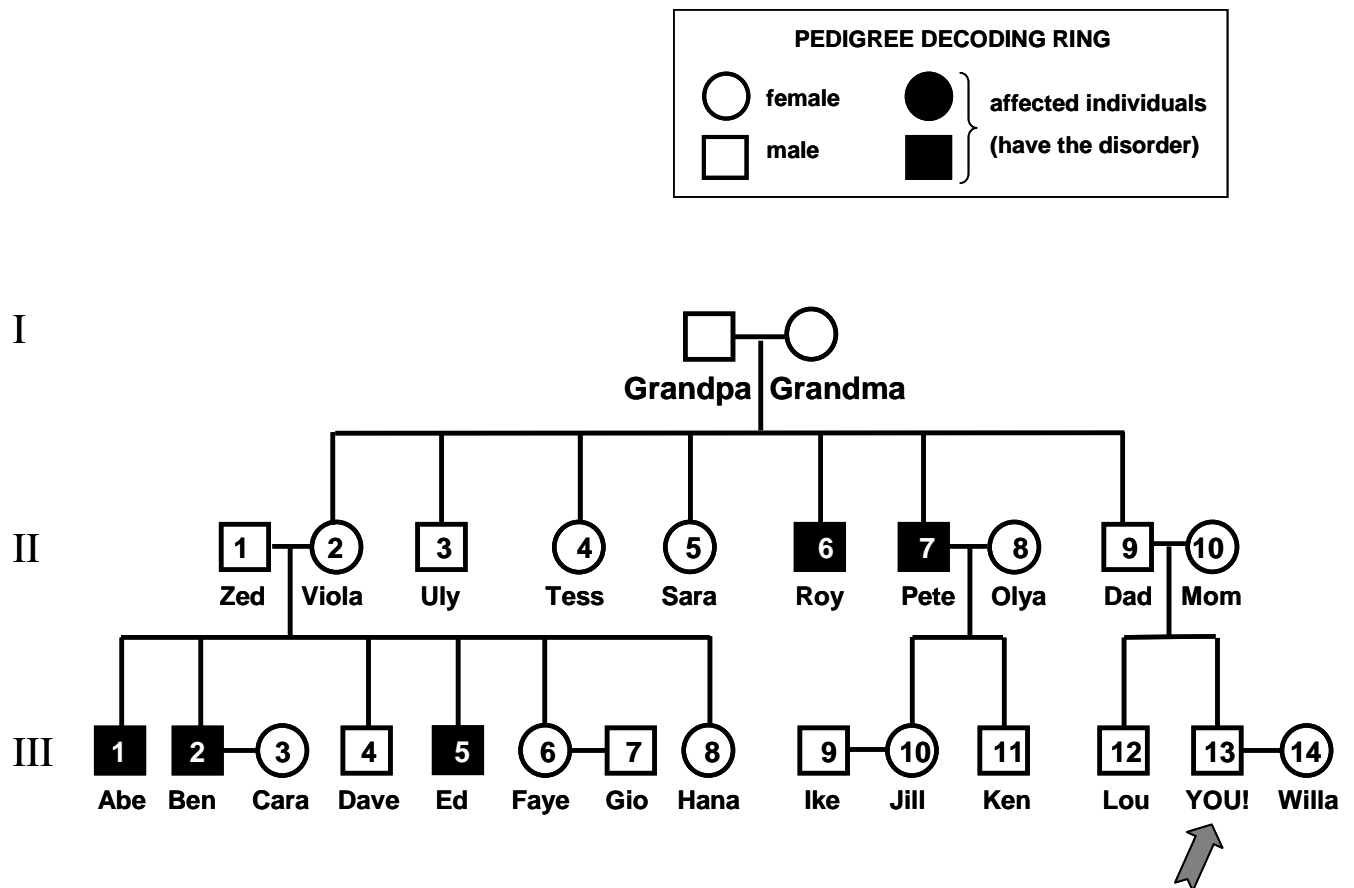
You are growing these tumors in your lab and everything is going well, until you take a long weekend trip to South Padre Island. You are having such a great time that you forget to come back on Sunday to split your cells. Unfortunately, they become contaminated and your mentor is very upset with you. She suggests that you find out whether your contaminant is prokaryotic (bacteria) or eukaryotic (mold) so that you can try to treat it and recover your tumor cell lines. You look at several different characteristics to determine whether the contaminant is eukaryotic or prokaryotic.

- h. For each statement below, note whether it would be seen only in eukaryotes (E), only in prokaryotes (P), or in both eukaryotes and prokaryotes (both). One answer is shown.**

- E **The cells have nuclei and intracellular organelles**
- Transcription and translation are coupled**
- Transposable elements are found in the genome**
- Most of the mRNA molecules have a poly(A) sequence at the 3' end**
- Most genes are organized in operons so the mRNAs are polycistronic**
- RNA splicing is required to remove introns from most mRNAs**
- Typically, there is one circular chromosome**

Question 6 (20 points)

You are very interested in doing your dissertation with Dr. A. Cula, a distinguished scientist from Transylvania University who has been studying a rare bleeding disorder that occurs in only 200 people world-wide. He has identified a family with several affected members and provides you with the pedigree below. To your surprise, you discover that this is a pedigree of your family! You knew that your cousins, Abe, Ben, and Ed all had a bleeding disorder and bruised very easily, but you didn't realize that their case was of interest to anyone else. Clearly, you are excited to take on this project as it is so relevant to you.



- a. They have already done mapping studies with another family and shown that this gene is X-linked. In your family (above), is this bleeding disorder more likely due to a mutation that is dominant or recessive? Explain your reasoning and be very specific.

Question 6 (continued)

- b. **List all of the members of your family who are obligate heterozygotes.** [This means that you know from the pattern of inheritance shown in the pedigree that they must be heterozygous (not hemizygous and not homozygous) at this locus.] Obligate heterozygote – mutant / +. NOTE: this is a very RARE disorder, so you can assume that anyone who marries into the family will carry only the normal (not mutant) allele. Explain your answer.
- c. **Congratulations - you and Willa are expecting a baby boy! Based on the pedigree and your answer to part a., what is the probability that your unborn son will be affected with the bleeding disorder? Circle the best answer below:**

0% 25% 33% 50% 75% 100%

Explain your reasoning:

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Question 6 (continued)

Your cousin Faye (III-6) and her husband are also expecting a baby boy. Faye is worried as she knows that she might carry the mutant allele and pass the bleeding disorder on to her son. You offer to perform a SNP analysis across the relevant region of the X chromosome. She is thrilled and even gives you DNA from her fetus (since she had routine amniocentesis performed). The data are shown in the table below:

Generation	II		III						IV	
Individual	Zed	Viola	Abe	Ben	Dave	Ed	Faye	Gio	Hana	fetus
SNP1	c	a,b	b	b	a	b	b,c	d	a,c	c
SNP2	e	e,f	f	f	e	f	e,f	g	e	e
SNP3	h	h,j	j	j	h	j	h,j	h	h	h

- d. Based on your SNP analysis, you conclude several things. Circle all correct answers below. Note: one correct answer is already circled for you as an example.

The unborn child (fetus) is indeed a male

Faye does NOT carry the mutant allele

The fetus does NOT carry the mutant allele

Faye, Gio, and the baby will all be normal and will not have a bleeding disorder

- e. You decide to generate a mouse model of this bleeding disorder. You name the affected gene X-clot and clone both the normal and mutant alleles. Which basic approach would you take and why? [You do not need to describe methodological details, but you do have to explain WHY you chose this particular approach and why you think it will work.]


Question 6 (continued)

- f. **You discover that the bleeding disorder is caused by defective platelets in the blood. They fail to produce X-CLOT, the protein that is required for clotting. Using your mouse model, you decide to try to correct this defect. What basic approach would you take to replace the missing protein in platelets in vivo (in a mouse)? Do not describe methodological details, but do provide enough information so that I know you understand the method that you propose to use. Your method should result in expression of the clotting protein **ONLY** in platelets, not in every cell of the mouse.**

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Question 7 (16 points)

For each of the statements below, note whether it is "true" or "false".
If the statement is "false", correct it so that it will be "true".

Statement	True or False?
Insertion of transposable elements often results in a small duplication at the target site due to a staggered double-stranded cut followed by replication.	
The CRE-loxP recombination system derives from a bacteriophage and can be used to generate <u>conditional</u> knockout mice.	
A CNV (copy number variant) is a type of SNP (single nucleotide polymorphism).	
Ty elements in yeast transpose through an RNA intermediate and they express an enzyme with reverse transcriptase activity.	
Antisense DNAs can decrease translation of specific proteins by hybridizing to the homologous mRNA molecules.	
A DNA hairpin structure is found during V, D, J rearrangement and also during the transposition of some prokaryotic transposons.	
Transfection of mammalian cells with a DNA construct typically results in a targeted insertion by recombination with the endogenous gene.	
<p>The DNA structure shown would be seen in the DNA of a sperm cell but is transcriptionally silent.</p>  <p>The diagram shows a DNA construct within a rectangular box. On the left, there is a white rectangular region labeled V_H above it, followed by a smaller black rectangular region labeled $D_H J_H$ above it. To the right of these is a larger black rectangular region labeled C_H above it. Horizontal lines extend from the left and right sides of the box, representing the DNA strand.</p>	

HAVE YOU CORRECTED ALL OF THE FALSE STATEMENTS?

Question 8 (5 points)

- a. The following list has five examples of DNA sequence changes. Which are “indel” mutations? **Circle the one correct answer from the choices (1 – 5) below the list.** (1 point)

- A. AGTCG → AGTCG
- B. AGTCG → AGGCG
- C. AGTCG → ATCG
- D. AGTCG → AGTCCG
- E. AGTCG → AGCTG

1. A 2. C & D 3. C, D & E 4. A, D & E 5. All of the above

- b. Give **TWO** examples of endogenous sources of DNA damage. (1 point)

- 1.
- 2.

- c. Define a **nonsense mutation**. (1 point).

- d. List **TWO** examples of cross-linking agents that cross-link DNA. (1 point)

- 1.
- 2.

- e. Give **TWO** conditions that will result in an increased level of spontaneous depurination. (1 point)

- 1.
- 2.

Question 9 (5 points)

a. Which of the following is/are features common to DNA polymerases involved in translesion synthesis? (1 point)

- A. processive
- B. distributive
- C. very large proteins
- D. 3' to 5' proof-reading activity
- E. infidelity on undamaged DNA template

1. A

2. B & D

3. C, D & E

4. B & E

5. All of the above

b. Give **ONE** example of an enzyme involved in DNA repair by direct reversal **AND** the type of DNA damage that it acts on. (2 points)

c. The first enzyme involved in Base Excision Repair is a DNA glycosylase. There are many DNA glycosylases but they are generally classified by the presence or absence of AP-lyase activity. In the absence of AP-lyase activity, AP-endonuclease is utilized. In one sentence, describe what each of these enzymes do to the DNA and where do they do it (5' or 3') in relation to the AP site. (2 points)

AP-lyase:

AP-endonuclease:

Question 10 (8 points)

- a. Xeroderma pigmentosa (XP) and Cockayne syndrome (CS) are both human diseases that result from mutations in genes involved in Nucleotide Excision Repair. However, patients with XP are predisposed to get cancer, whereas CS patients do not. Please explain why there is a difference. (4 points)
- b. UV exposure is most commonly thought to cause pyrimidine dimers. As it is a form of irradiation, it is also known to cause oxidative stress and DNA breaks. If you wanted to look at the amount of DNA breaks that are present in the cell following UV exposure, what sort of method might you use? **Naming the technique/approach is sufficient.** (1 point) -

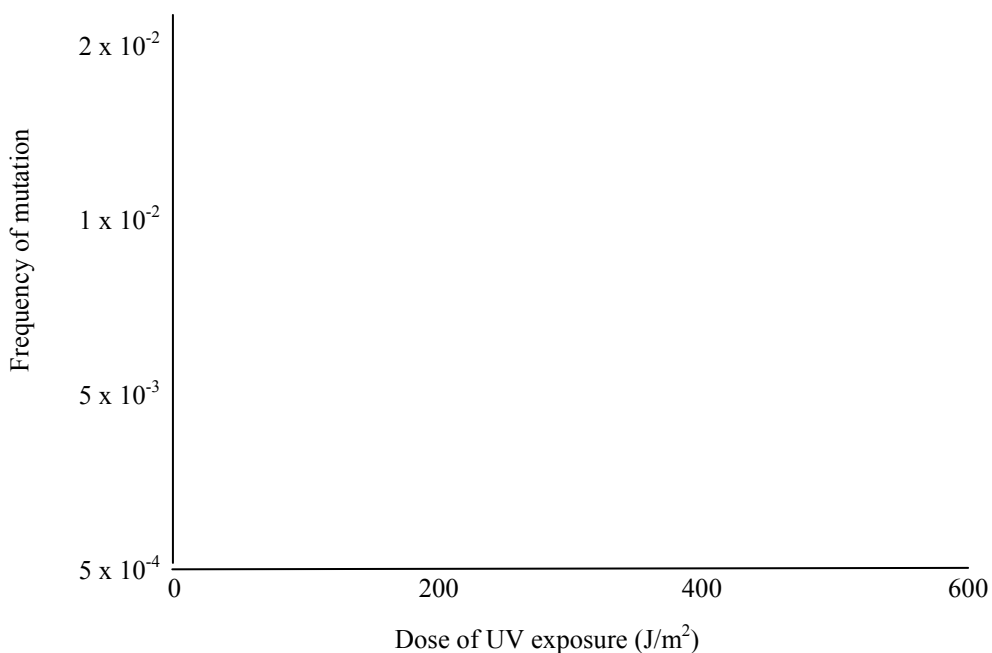
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Question 10 (continued)

- c. Using cells from normal, XP and CS patients, you expose them to 3 different doses of UV light (200, 400 and 600 J/m²). Draw a graph of the **relative frequency of mutation** you would expect to find in each of these cells. *Do not worry about getting the mutation-frequency/dose exactly right; I am most interested in the general trend of how each cell line responds compared to one another.*

Use the following lines for each genotype and start your line from 0 J/m²

WT _____
XP _____+_____
CS _____○_____



Question 1 (10 points)

Indicate whether the following statements are true (T) or false (F). Each of these ten statements refers to prokaryotic transcription. Each question is worth one point.

- a. _____ The repressor (LacI) of the *lac* operon binds to its DNA target only when glucose is present.
- b. _____ CAP/cAMP bound to the *lac* promoter region allows RNA polymerase to more readily bind the promoter to form a “closed” promoter complex.
- c. _____ Sigma (σ) factors remain bound to RNA polymerase until the ends of operons are reached and the polymerase leaves the DNA template.
- d. _____ Small regulatory RNAs can control either the transcription of operons or the translation of mRNA.
- e. _____ Riboswitches are mRNA elements that can form alternative structures to regulate transcription or translation.
- f. _____ Heparin can release RNA polymerase from “closed” RNA polymerase/DNA complexes.
- g. _____ “Two component” systems refer to regulatory devices that control operon expression by the simultaneous use of repressors and gene activator proteins.
- h. _____ The attenuator site on the tryptophan biosynthetic operon forms an “intrinsic terminator” when tryptophan is limiting.
- i. _____ Nus proteins will only bind to RNA polymerase if sigma factor is also bound.
- j. _____ Operons that are controlled solely by repressors can display constitutive expression following mutations in either their repressors or operators.

Question 2 (9 points)

- a. **Transcription from a particular operon is regulated by a positive activator protein (X). Suppose two separate mutations (A and B) were isolated in the promoter of this operon. In order to determine if either mutation (A or B) is in the target sequence for protein “X”, strains were made in which the wildtype promoter, the mutant A promoter or the mutant B promoter were each placed in X⁺ and X⁻ strains . The activity level of each mutant promoter was then compared to a wildtype promoter in both wildtype and activator minus strains. The following results were obtained:**

Promoter	Activity (arbitrary units)	
	Activator + (X ⁺)	Activator – (X ⁻)
Wildtype	1000	100
Mutant A	100	8
Mutant B	100	90

Which one of the two mutations (A or B) is likely to have affected the target sequence for the activator protein? _____ (2 points)

- b. **List the 3 types of evidence that have been used to define the promoter regions of operons. (2 points)**

1. _____
2. _____
3. _____

Choose the one best answer for each of the following five questions, which refer to prokaryotic transcription. Each question is worth 1 point.

- c. _____ The two component system that activates nitrogen assimilation (e.g., *glnA* [glutamine synthetase] operon transcription):
1. induces *glnA* transcription when glutamine levels are high.
 2. relies on an adenylyltransferase (GlnE) to modify the system's histidine protein kinase (NtrB).
 3. is regulated by a modified GlnA protein (glutamine synthetase) functioning as a gene activator protein.
 4. relies on repression by NtrC-PO₄.
 5. uses a uridylyltransferase (UTase/GlnD) to detect nitrogen deficiency.

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Question 2 (continued)

d. _____ A loss of function mutation (null) mutation in the sequence of an operator site:

1. allows RNA polymerase to initiate transcription of the operon that it controls without the need for sigma subunits.
2. can be masked/suppressed by mutations that inactivate the repressor protein that targets it.
3. will only alter the transcription of the operon to which it is physically joined .
4. will lead to premature termination of the RNA transcript from the operon that it controls.
5. restricts the binding of gene activator proteins to the site.

e. _____ Amino acid biosynthetic operons (e.g., *trp*):

1. are regulated by the translation of their “leader peptides”.
2. are activated by CAP/cAMP.
3. are maximally transcribed only when the amino acids that their gene products can synthesize are abundant.
4. normally encode several “anti-sense” RNAs.
5. are primarily controlled at the point of RNA polymerase binding to their promoters.

f. _____ Glucose repression of *lac* and other catabolic operons:

1. refers to the inhibition of glucose utilization operons by lactose and other sugars.
2. uses a repressor similar to that which controls *lac*.
3. leads to premature termination of initiated RNA transcripts.
4. occurs when cAMP levels are low.
5. involves repression of operons with strong consensus promoters.

g. _____ RNA polymerase bound to a promoter in a “closed complex”:

1. has released its sigma factor.
2. rests on one “face” of the DNA template.
3. has unwound the duplex DNA of the promoter.
4. is bound to the operator region of the promoter.
5. is stabilized by “Nus” protein.

Question 3 (14 points)

Indicate whether the following six statements are true (T) or false (F). Each of these statements refers to eukaryotic transcription. Each question is worth one point.

- a. _____ RNA polymerase II only synthesizes mRNA.
- b. _____ The mediator complex is generally required for transcriptional initiation by RNA polymerase II.
- c. _____ Locus control regions are comprised of enhancer, silencer and insulator elements and direct transcriptional regulation of a single target gene.
- d. _____ Transcriptional regulatory networks are likely to have evolved by gene duplication.
- e. _____ The C-terminal domain of RNA polymerase II plays an important role in transcriptional initiation, elongation, CAPing, splicing and polyadenylation.
- f. _____ Transcriptional activation and repression domains are modular and can target multiple components of the general transcription machinery.
- g. You have identified a novel hormone receptor which, in the presence of ligand, functions as a strong transcriptional activator of target genes. Interestingly, you observe that in the absence of ligand, this receptor is a strong repressor of the same target genes. **Briefly describe a mechanism by which the hormone receptor can be an activator with ligand but a repressor without ligand.** (3 points)
- h. Suppose you expose a human skin fibroblast cell line to epidermal growth factor, a very strong mitogen, and observe increased protein synthesis and rapid cellular proliferation. **Briefly describe the series of events that cause increased transcription of several key components of the protein synthesis apparatus.** (2 points)
- i. **What two key events are required for RNA polymerase II to clear the transcriptional pre-initiation complex and the subsequent pausing step in order to proceed with transcriptional elongation? Be specific.** (3 points)

Question 4 (7 points)

Suppose you have recently cloned the promoter of a human gene of interest (*YFG1*). A preliminary bioinformatics analysis indicates that there are DNA-binding sites for four known transcriptional regulators (listed below). In order to prove that the regulatory elements are functional, you decide to carry out a deletion analysis using the *LACZ* reporter constructs shown below in a liver cell line.

SA= strong activator; increases transcription 10-fold

WA= weak activator; increases transcription 2-fold

SR= strong repressor; reduces transcription 10-fold

WR= weak repressor; reduces transcription 2-fold

- a. Assuming, that *YFG1* expression is controlled only by the regulators listed above and that all of these regulators are active in the liver cell line being used, **indicate (numerically) the levels of β -galactosidase activity that you would expect to observe using the deletion constructs shown below. In the right-hand column also indicate your reasoning** (how you decided on each β -galactosidase activity value). (5 points)

	β -galactosidase activity	Reasoning
	50	XXX
	50	XXX

- b. The expression of *YFG1* is the same in the gene with all four regulatory sites (upper-most construct) and in the gene with no regulatory sites (lower-most construct). **Why should the *YFG1* gene have such a complex regulatory region if you can get the same level of transcription without the entire upstream region?** (1 point)

- c. **What technique would you use to identify the complete set of binding sites in the entire yeast genome for one of the transcriptional regulators described in part (a) above?** (1 point)

Question 5 (8 points)

Indicate whether the following three statements are true (T) or false (F). Each question is worth one point.

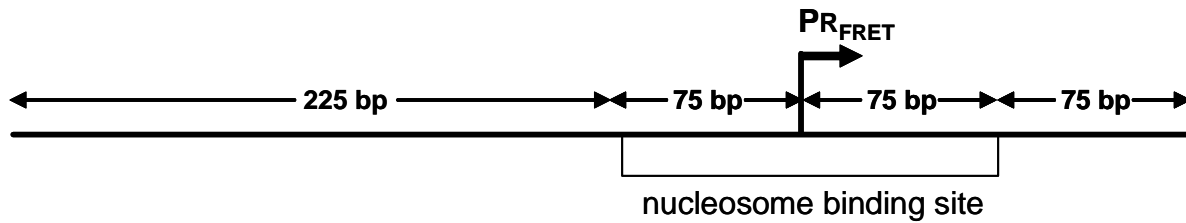
- a. _____ The major interaction of the histone octamer with DNA is via major groove contacts in the DNA.
- b. _____ The histone octamer consists of one H2A/H2B tetramer and two H3/H4 dimers.
- c. _____ Protein-protein interactions are important for X-chromosome inactivation.
- d. **Chromatin remodelers, such as swi/snf, are multi-subunit proteins. The key catalytic subunit in these complexes is:** Circle the correct answer. (1 point)
- i) a deacetylase. ii) a methylase. iii) a helicase.
- iv) an ATPase. v) a topoisomerase.
- e. **C-methylation plays an important role in genomic imprinting by leading to alterations in transcription. List one of the two mechanisms discussed in class by which C-methylation can alter transcription.** (1 point)
- f. **After the Xist-RNA/protein complex binds to its own X chromosome, what is the next key event, initiated by the Xist-RNA/protein complex, in X chromosome inactivation?** (1 point)
- g. **300 nm loops/domains are a “higher level” of chromatin compaction that can play a role in transcriptional regulation in response to an external signal (e.g., IFN- γ). In one or two sentences, describe how this might occur. I am not looking for specific protein names.** (1 pt)
- h. **During transcription, it is important for nucleosomes to be put back onto the DNA in the ORF behind the transcribing RNA Pol II. Why?** A one or two sentence answer should be sufficient. (1 point)

Question 6 (7 points)

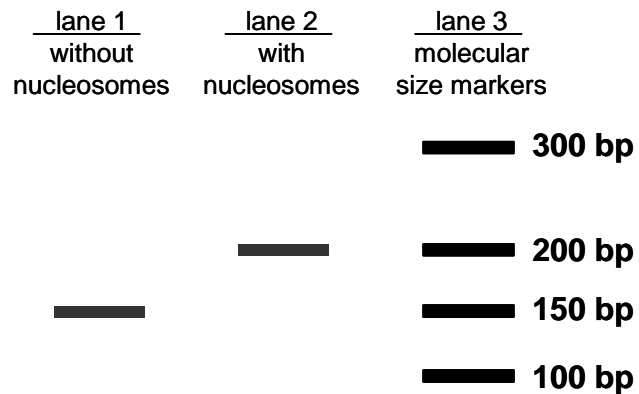
- a. In class, we discussed Lorch and Kornberg's *in vitro* experiment which showed that nucleosomes inhibited the initiation of transcription by eukaryotic RNA polymerase II from the adenovirus major late promoter, P_{Ad}. **Briefly sketch or describe the experiment they did and the results they obtained.** Be sure to include appropriate control(s). (Do not describe the experiment testing the effect of nucleosomes on elongation.)
- b. Your advisor, Dr. Details Count, recently cloned a gene called "fretfulase" from a graduate student. He has mapped the transcription start site (P_{R_{FRET}}) and has shown that the "fretfulase" gene is always transcribed at high levels in graduate students. He wants you to determine whether or not nucleosomes block the initiation of transcription at the "fretfulase" promoter. Therefore, you make the DNA construct shown on the next page and repeat the Lorch and Kornberg experiment (which you described in part a) except that you used your DNA as the template for transcription. **The results of this experiment are diagrammed on the next page. What conclusion(s) would you draw from these data?** A two or three sentence answer should suffice.

Question 6 (continued)

DNA construct that you used as template in an *in vitro* transcription assay.



RESULTS of your *in vitro* transcription assay using the construct above, with and without nucleosomes. Shown is an autoradiograph of the RNA products of the indicated reactions after electrophoresis.



Question 7 (7 points)

The *Pho5* gene from the yeast *Saccharomyces cerevisiae* is repressed (not transcribed) in media with high concentrations of phosphate and is induced (transcribed) in media containing low concentrations of phosphate. Grunstein and his colleagues used histone H4 depletion experiments to demonstrate that nucleosomes are involved in repression of the *Pho5* gene *in vivo*.

- a. The amino terminal "tail" of histone H4 has been postulated to play an important role in the regulation of transcription. **Briefly describe or sketch an experiment you would propose to do in order to test the hypothesis that the amino terminal tail of histone H4 plays a role in the regulation of *Pho5* transcription of *in vivo*. Be sure to indicate what your "wild type" control will be. You do not need to indicate your expected results.**
- b. Grunstein and his colleagues have actually tested the role of the amino terminal "tail" of histone H4 on *Pho5* transcription *in vivo*. They found that under low phosphate conditions, the *Pho5* gene was still repressed when the "tail-less" H4 was the only histone H4 present. (When normal histone H4 is present, *Pho5* is induced under low phosphate conditions). **Based upon what is known about chromatin and histone tails, is this the result you would have predicted? Explain your reasoning in one or two sentences.**

Question 8 (19 points)

Indicate whether the following four statements are true (T) or false (F). Each question is worth one point.

a. _____ Yeast and humans have the same proportion of genes regulated by alternative splicing.

b. _____ Alternative splicing takes place only in the 3' UTR.

c. _____ Capping is important for the splicing of the last intron.

d. _____ The function of U2AF is to recognize the branch point.

e. Which **one** of the following statements is **TRUE**? (1 point)

() rRNA processing does not require the spliceosome.

() The spliceosome contains only non coding RNAs.

() Trans-splicing is very common among insects.

() The branch point is part of the 5' splice site.

() Exon enhancers are normally recognized by eIF4E.

f. **Describe how positive regulators of splicing, such as SR proteins, work.** (3 points)

g. **Draw the A complex (splicing). Include the factors and the sequences involved.** (2 points).

Question 8 (continued)

- h. The gene BRAC1 has two alternative splicing variants with distinct 5'UTRs. Isoform A has a 100 nucleotide 5' UTR while isoform B has a 800 nucleotide 5' UTR. Although the overall amount of BRAC1 mRNA is the same in fibroblasts and in brain cells, in fibroblasts 80% of BRAC1 mRNAs are isoform A while in brain cells 80% of BRAC1 mRNAs are isoform B. Interestingly, the amount of BRAC1 protein made in fibroblasts is high while the amount of BRAC1 protein produced in brain cells is low. **Based on what you learned in class, describe a mechanism that would explain why the two isoforms give rise to different amounts of protein.** A two or three sentence answer should be sufficient. (3 points).
- i. **From transcription till translation, briefly describe four of the cellular events that take place during the “life” of a eukaryotic mRNA.** (2 points)
- j. **Define at least three functions of the poly A tail.** (2 points)
- k. **What is the function of the exosome and what is the key to its broad specificity?** (2 points)

Question 9 (7 points)

The translational machinery in eukaryotes is different from that in prokaryotes. One of the major differences is that monocistronic translation is the mechanism in eukaryotes and polycistronic translation is often used in prokaryotes.

- a. **Briefly define monocistronic and polycistronic.** (2 points).

Monocistronic -

Polycistronic -

- b. **Based upon what is known about the mechanism of efficient translation initiation in eukaryotes, please explain why transcription and translation in eukaryotes cannot be coupled.** Saying that transcription occurs in the nucleus and translation occurs in the cytoplasm is not the answer to this question. (2 points)

In a eukaryotic cell lysate, the polynucleotide 5'-AUGAAAUUUCUU-3' directs the synthesis of Met-Lys-Phe-Leu. In the presence of Exomycin, a new antibiotic made by Fluhardy Pharmaceuticals, this polymer directs synthesis of Met-Lys-Arg-Leu.

- c. **What type of translational error was generated by the treatment with Exomycin?** (1 point)
- d. **Which one of the following deductions can you make about Exomycin? Circle the correct answer and explain your reasoning in one sentence.** (2 points)
- A. It prevents formation of the 80S initiation complex, which contains the initiation tRNA and both ribosomal subunits.
 - B. It inhibits binding of aminoacyl-tRNA to the A-site in the ribosome.
 - C. It affects aminoacyl-tRNA synthetase function by enhancing the probability that this enzyme will make an error.
 - D. It blocks translocation of peptidyl-tRNA from the A-site to the P-site of the ribosome.
 - E. It interferes with chain termination and release of the peptide.

Question 11 (6 points)

siRNA and shRNA are powerful tools to test the functional role of proteins in mammalian cells.

- a. **Which one of the statements listed below is NOT correct? Why?** (2 points)
- A. One can knock out a particular gene using an siRNA approach.
 - B. siRNA matches its target mRNA perfectly by base-pairing.
 - C. siRNA does not integrate into the chromosome.
 - D. We can detect the effect of siRNA by western blot analysis.
- b. **Describe two advantages that shRNA has over siRNA when trying to reduce the expression of a particular gene product.** (2 points)
- 1.
 - 2.
- c. **Which one of the statements listed below is NOT correct? Why?** (2 point)
- A. Both siRNA and miRNA can down-regulate protein expression in mammalian cells.
 - B. Both siRNA and miRNA can cause mRNA degradation.
 - C. siRNA blocks the translation process.
 - D. miRNA is generated from an endogenous source.

Gene therapy take-home test question: Attendance at lectures M35 and M36 is required

These questions are worth up to 1.5 points of your final INTD5000 grade! To receive the full 1.5 points, you must attend both lectures (M35 and M36; 8:30 – 10:45 on Monday, December 15th), complete the answers correctly, and turn in this portion of the exam by 5:00 PM on Thursday, December 18th.

Please bring your answer (with a copy of your selected article) to my office – room 4.013V by 5:00 on Thursday. If you have any questions concerning this assignment, please email me at kraig@uthscsa.edu or call me at 567-3818 (work) or 697-8185 (home). **Thanks, good luck, and have fun! Ellen Kraig**

The rules for this take-home exam question:

You may consult your notes, books, papers, or any other inanimate source of published reference materials.

However, you may **NOT** get help from any other student or living person of any kind. You must work independently. You may also **NOT** provide help to any other student in the course; this will be considered academic misconduct. You should **NOT** discuss this assignment with any other person (except Dr. Kraig) at any time! If you have any questions regarding these rules, please contact Dr. Kraig. Thanks.

The assignment:

- Find a paper that describes the use of gene therapy to treat a disease or disorder that is of interest to you.**

The gene therapy can be in humans or in an animal, but must involve an intact organism, not just a tissue culture cell.

Please choose a paper that was published in a peer-reviewed journal (ask me if you don't know what this is) in either 2007 or 2008. This should be a primary scientific paper, not a review article. **Also, this paper must NOT be one of the ones that I presented in class - find a new one - have some fun!!!** If you have any question about whether the paper you have chosen satisfies these criteria, please email or call Dr. Kraig.

- Based on your paper, answer the questions below and carefully follow the instructions.**

- What is the disease/disorder being targeted by the gene therapy in your chosen paper?

- Why did you choose this paper? In other words, explain to me why it was interesting to you (1-3 sentences).

- In your chosen paper, what species was treated by gene therapy?

_____ (put the name of the species please).

[If it was only used to “treat” tissue culture cells, you must pick another paper]

- Is the gene therapy being used *in vivo* or *ex vivo*? _____

NAME _____

Highlight (mark) the section of text in the paper that answered this question for you and explain in the space below how this section tells you whether it's *in vivo* or *ex vivo*. Please be VERY specific in your answer (so that I know that you actually read the paper!

- e. What is the vector system and/or delivery system being used? _____

Highlight (mark) the section of text in the paper that answered this question for you.

- f. In 2-3 sentences, please explain why you think that they chose this vector and/or delivery system for this particular gene therapy. Again, please be as specific as possible.
- g. Given what you know about the vector and/or delivery system used, list one possible side effect (bad outcome) that may occur in the animals or humans treated with this gene therapy.