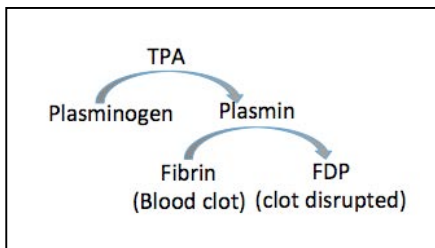


7.013 Problem Set 4- 2018

Question 1

Cardiovascular disorders such as heart attack and stroke are among the top ten causes of death in the western hemisphere. Stroke patients, under certain circumstances, have better outcomes when treated with tissue plasminogen activator (TPA).

a) TPA is a **serine protease** that catalyzes the **hydrolysis** of plasminogen to plasmin. Plasmin is an essential enzyme for clot breakdown (hydrolysis of fibrin protein to Fibrinogen degradation product (FDP)).



- i. How would you categorize the TPA catalyzed reaction: **exergonic or endergonic**?
- ii. Which covalent bond in plasminogen does TPA hydrolyze: **ester bond/ peptide bond/ glycosidic bond/ phosphodiester bond**?

b) In humans, the *PLAT* gene encodes the TPA protein.

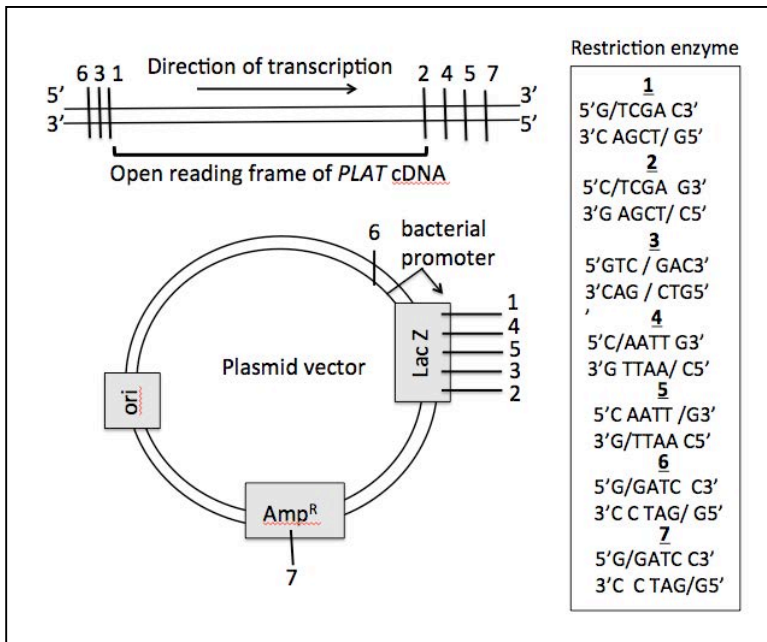
- i. Would you expect all the cell types in the body to have the *PLAT* gene? **Why or why not**?
- ii. The *PLAT* gene is expressed only in endothelial cells that line the blood vessels. How might *PLAT* gene expression be regulated in the endothelial cells? Propose **one** explanation.

c) Considering the therapeutic potential of TPA, you want to produce a large amount of recombinant human TPA (rTPA) in bacterial cells using the recombinant technologies that you have just learned about in 7.013.

- i. You suggest cloning the *PLAT* gene in bacteria to express it and isolate PLAT protein. Your UROP supervisor suggests that you instead clone the *PLAT* cDNA. Which strategy is better? **Explain** why.
- ii. Which human cell types would you use for preparation of human PLAT cDNA and **why**?
- iii. Give the sequence of the 5 bases long primer that you would use to make single-stranded *PLAT* cDNA and label its 5' and 3' ends. How can you be sure that this primer will base pair with the mRNA?

Question 2

You generate *PLAT* cDNA and clone it into the plasmid diagrammed below.



Note: Both the *PLAT* cDNA and plasmid vector have recognition sites for restriction enzymes 1-7. A slash (/) represents the cut site for each restriction enzyme. The plasmid has a bacterial origin of replication (*ori*), an ampicillin resistance gene (*Amp^R*) that degrades ampicillin antibiotic. It also has *LacZ* gene that encodes the β -gal enzyme, which converts white X-gal substrate into a blue product.

a) The table below shows different restriction enzyme pairs that you can use to digest the *PLAT* cDNA and clone it in the bacterial plasmid. Complete the table below for each restriction enzyme pair.

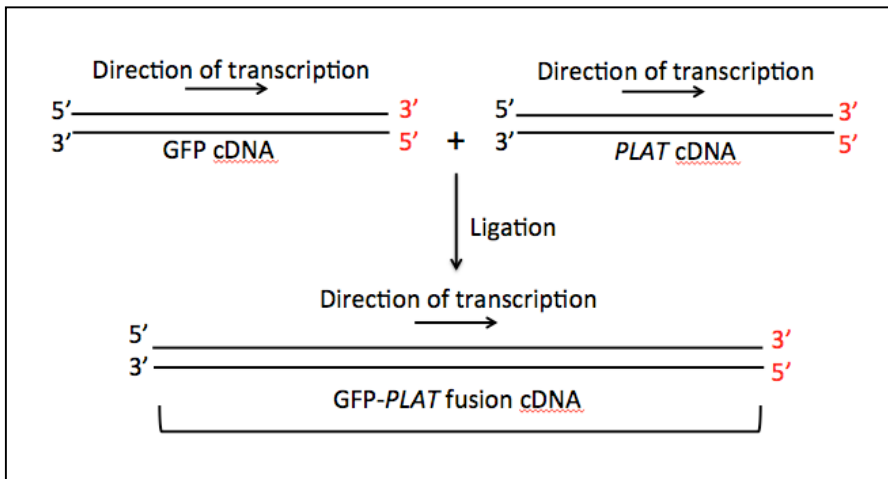
Restriction enzyme pair used to cut the <i>PLAT</i> cDNA	Will you be able to....		
	Column 1: Clone the digested <i>PLAT</i> cDNA into the plasmid: Yes/ No?	Column 2: Amplify the recombinant plasmid in the ampicillin sensitive bacterial cells: Yes/ No?	Column 3: Express the <i>PLAT</i> cDNA in the bacterial cells: Yes/ No/ maybe?
1 & 5			
1 & 2			
2 & 6			
3 & 7			

b) If you selected “maybe” for any enzyme pair in Column 3 in the table above, please provide an **explanation** for your selection.

c) After transformation, you grow the bacterial cells. Give the sequence of the steps you would use to find the bacterial cells that are transformed with the recombinant plasmid that has the *PLAT* cDNA insert?

Question 3

Your fellow classmate plans to construct a GFP-PLAT fusion protein. **Note:** GFP stands for the Green fluorescent protein.



The following is the partial cDNA sequence encoding the C- terminus of the GFP gene. **Note:** The DNA corresponding to the stop codon is bold and underlined. The sequence specifically recognized by each restriction enzyme is shown in gray. Each codon is separated from the next by a space.

GFP:

					<u>1</u>		<u>2</u>					
5'	AAG	AAA	ATT	GCC	GCG	AAT	TCC	CCC	TCG	AGC	ATG	<u>TAG</u> 3'
3'	TTC	TTT	TAA	CGG	CGC	TTA	AGG	GGG	AGC	TCG	TAC	ATC5'

The following is the partial cDNA sequence encoding the N- terminus of the PLAT gene. **Note:** The DNA corresponding to the start codon is bold and underlined. The recognition sequence for each restriction enzyme is shown in gray. Each codon is separated from the next by a space.

PLAT:

		<u>1</u>		<u>3</u>		<u>4</u>				
5'	G	GAA	TTC	GCA	ATT	GGA	GGG	TCG	ACC	<u>ATG</u> CCA..... 3'
3'	C	CTT	AAG	CGT	TAA	CCT	CCC	AGC	TGG	TAC GGT.....5'

The recognition sequences and the cleavage sites (indicated by /) for each enzyme are given below.

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
5'G/AATT C3'	5'C/TCGA G3'	5'C/AATT G3'	5'G/TCGA C3'
3'C TTAA/G5'	3'G AGCT/C5'	3'G TTAA/ C5'	3'C AGCT/G5'

a) To make a GFP-PLAT fusion gene that expresses the GFP-TPA fusion protein, which restriction enzyme would you use to cut the...

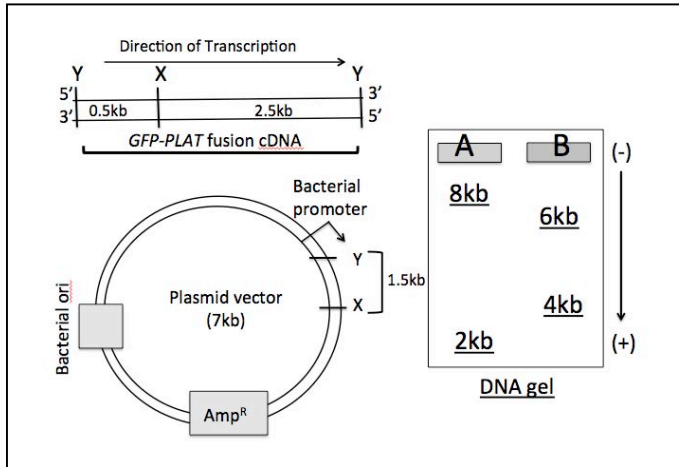
i. C terminus of the GFP gene? _____

ii. N- terminus of the PLAT gene? _____

Question 3 continued

This GFP-PLAT fusion cDNA has restriction enzyme Y sites at each end. Your friend digests both the GFP-PLAT fusion gene and the plasmid vector diagrammed below with the restriction enzyme Y. She incubates the restriction enzyme Y digested DNA fragments with DNA ligase, transforms the ampicillin-sensitive bacterial cells with the ligation mix and grows them on ampicillin containing plates. She decides to further analyze two bacterial colonies (Colony A and Colony B) both of which have the recombinant plasmid with the GFP-PLAT cDNA insert. **Note:** The plasmid has recognition sites for restriction enzymes Y and X.

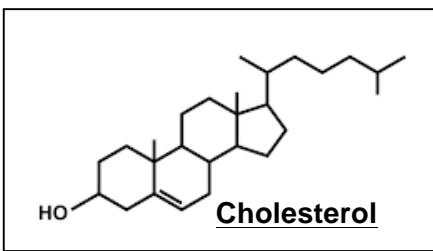
To further examine the structure of the recombinant plasmid, your friend isolates the recombinant plasmid from Colony A and Colony B and digests it with restriction enzyme "X". She then resolves the sizes of the digested DNA fragments on a DNA gel and gets the profile shown below.



b) Which cell population will express the GFP-PLAT fusion cDNA: Colony A or Colony B and why?

Question 4

An increase in cholesterol and low-density lipoprotein (LDL) levels is associated with heart attacks and stroke. Cholesterol and cholesterol esters are packaged into LDL and transported throughout the circulation. When the LDL packaged cholesterol complex reaches liver cells, it binds to the cell surface LDL-receptors (LDL-R). The LDL bound LDL-R complex is endocytosed and further processed.

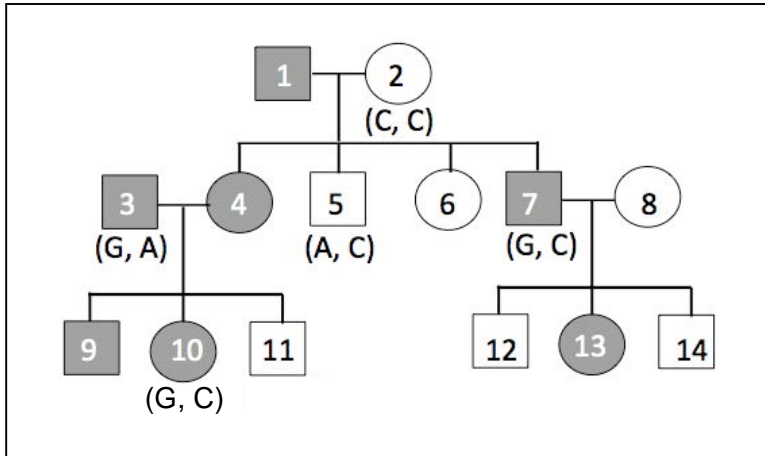


a) Why must cholesterol and cholesterol esters be packaged in LDL particles for transport?

b) Give a location in the cell where cholesterol can be found.

Question 4 continued

Familial hypercholesterolemia (FH) is an inherited genetic disorder that leads to aggressive and premature cardiac diseases such as a heart attack or stroke. The following is a pedigree for FH that is caused by a mutation in the *LDL-R* gene. **Note:** All affected individuals are shaded. Also listed are the SNPs for some individuals.



c) Assuming complete linkage between the *LDL-R* gene and the given SNP, list the **mode of inheritance** of FH.

d) Give the SNP profile(s) for each of the following individuals and **circle** the SNP that is associated with FH.

1: _____

4: _____

11: _____

e) You were introduced to **DNA and mRNA** expression microarrays.

- i. Which microarray (**DNA / mRNA expression microarrays**) would you use for SNP analysis and **why**?
- ii. Which microarray (**DNA / mRNA expression microarrays**) would you use to analyze the rate of transcription of *LDL-R* gene?

f) You alleviate the symptoms of this disease in Individual #10 by using **somatic cell gene therapy**. Following treatment, this patient has a child with a normal healthy male. Would their child have the disease? **Why or why not?**

g) You fellow classmate wants to use the **Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)** and CRISPR associated endonuclease (Cas9) technologies to create an FH mouse model.

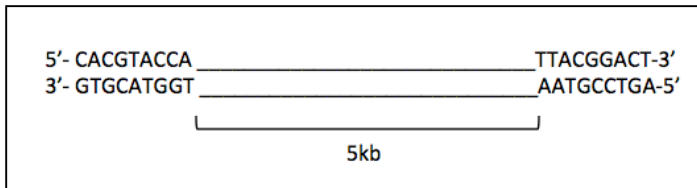
- i. Which gene sequence does the guide RNA recognizes?
- ii. Propose one mechanism by which the Cas9-exonuclease generates mutations in the target gene to produce an FH mouse model.

Question 5

You are studying a disease that shows an **autosomal recessive mode of inheritance** and is associated with a mutation in the *LRP* gene, which encodes LDL-R related protein (LRP). You clone a 5.0kb linear double stranded DNA fragment that includes the allele of the *LRP* gene associated with the wild-type phenotype (Fragment A) into a plasmid. You also clone the 5.0kb linear double stranded DNA fragment that includes the allele of the *LRP* gene associated with the disease phenotype (Fragment B).

You know that Fragment B differs from Fragment A by a **single base pair insertion mutation**.

The DNA sequence that flanks (at the two ends) Fragment A and Fragment B is given below.

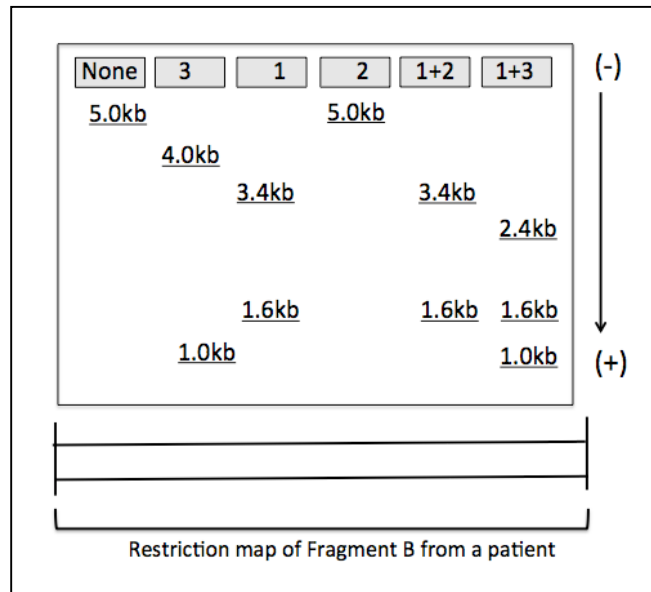
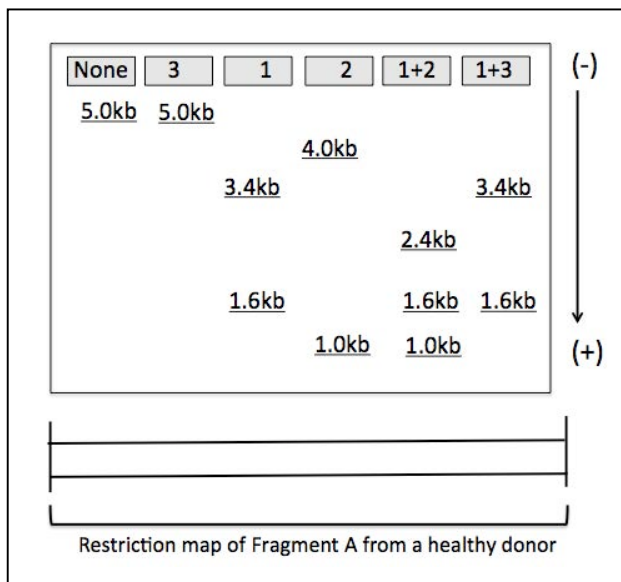


a) You use the polymerase chain reaction (PCR) to amplify Fragments A and B. Design the primers (each 5 nucleotides long) that you would use to amplify both strands of the 5.0kb long DNA fragments and label their 5' and the 3' ends.

- i.** **Forward primer** to make the top strand: 5'- _____ -3'
- ii.** **Reverse primer** to make the bottom strand: 5'- _____ 3'

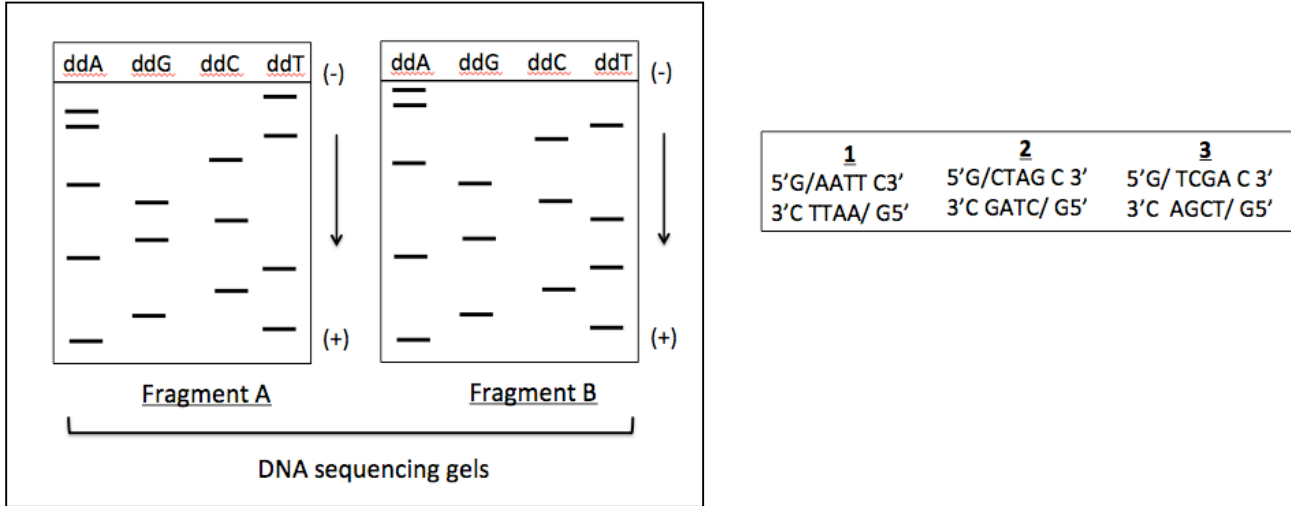
b) You PCR amplify Fragment A and Fragment B and digest them separately with a series of restriction enzymes. You resolve the restriction digested DNA fragments on two separate DNA gels and obtain the profiles below. **Note:** The size of each digested DNA fragment is written in kilobases (kb).

Based on the restriction pattern shown below, draw the restriction map of Fragments A & B. Indicate the restriction sites that are present and the distance (in kb) between the sites.



Question 5 continued

Using the fluorescence di- deoxy sequencing method, you sequence both Fragment A and Fragment B by using the **template DNA strand for sequencing**. The profiles below show the base sequence corresponding to the first 15 bases in the coding/ mRNA like strands of Fragment A (in schematic A) and Fragment B (in schematic B). **Note:** *The sequence specifically recognized by restriction enzymes 1, 2 and 3 is given below and the site at which they cut is shown by a slash (/).*



c) Write the sequence of the first 15 base pairs of the double helical DNA Fragments A and B and label their 5' and 3' ends.

- **Fragment A:**

- **Fragment B:**

d) **Circle** the recognition site for the relevant restriction enzymes (1/2/3) on Fragment A and fragment B in part (c) above.

e) Write the amino acid sequence encoded by the coding sequence shown in schematic A and schematic B for Fragment A and Fragment B. Label the N and the C ends. **Note:** *A codon chart is provided on the last page of this problem set.*

- **Amino acid sequence for Fragment A:** N-_____ -C

- **Amino acid sequence for Fragment B:** N--_____ -C

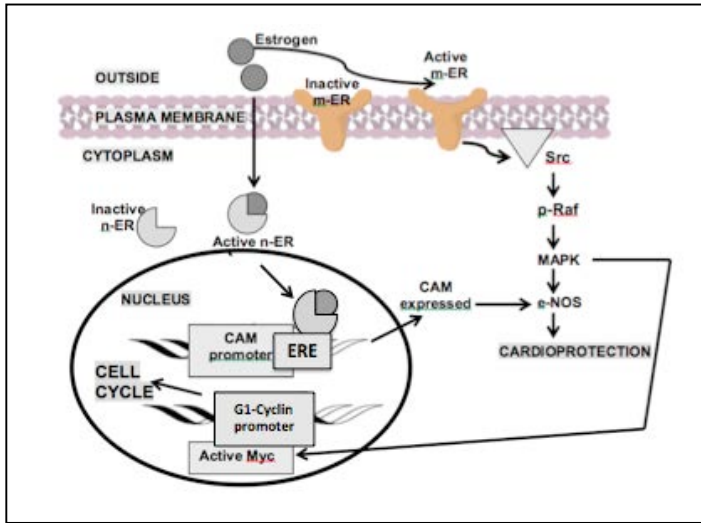
f) On sequence that you gave in part (c), box the base pair in Fragment B that accounts for the point mutation that results in the disease phenotype.

g) Identify the mutation that you circled in part (f) as **nonsense/ missense/ silent or frame shift**.

Question 6

It has been observed that the incidence of cardiovascular disorders in pre-menopausal females is significantly less than the age matched males. This is attributed to cardio-protective effects of estrogen hormone.

Estrogen hormone based signaling involves several receptors which can be classified as INTRACELLULAR receptors (n-ERs) and CELL MEMBRANE BOUND receptors (m-ERs). The pathway and its steps are outlined below.



Step 1: Estrogen mediates its cardio-protective effects by binding to and activating the membrane estrogen receptor (m-ER).

Step 2: The activated m-ER activates Src kinase, which activates the Raf-MAPK kinase cascade. This results in the phosphorylation and activation of the eNOS enzyme, which provides cardio-protection.

Step 3: Active MAPK also activates Myc, which promotes cell cycle progression by triggering G1-Cyclin expression.

Step 4: Estrogen also binds to and activates the nuclear estrogen receptor (n-ER). The activated n-ER translocates to the nucleus and binds to a specific sequence called estrogen response

element (ERE) on the DNA and acts as a transcription factor.

Step 5: The n-ER – ERE binding upregulates the transcription of CAM gene, which further provides cardio-protection.

Consider the following **mutations** in different components of the estrogen-signaling pathway.

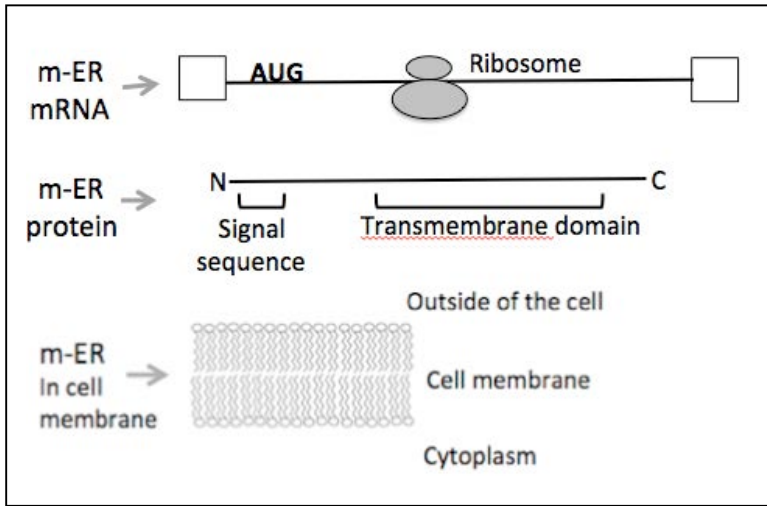
- **Mutant #1:** The n-ER undergoes a loss-of-function mutation.
- **Mutant #2:** eNOS shows a deletion of the MAPK binding site.
- **Mutant #3:** MAPK is constitutively active.
- **Mutant #4:** SRC undergoes a gain-of function mutation
- **Mutant #5:** Promoter region of myc gene is heavily methylated

Complete the table for each of the following **homozygous mutations** in the **presence** of **estrogen**.

Mutants	m-ER activated (Yes/ No)?	CAM gene expressed (Yes/ No)?	Cardio-protection compared with wild-type (same/ more/ less/ none)?	G1-cyclin expressed (Yes/ No)?	Cell division (Yes/ No)?
#1 & #2					
#3 & #4					
#4 & #5					

Question 7

The m-ER protein is shown as part of the schematic below. The start codon on the m-ER mRNA is shown.



a) Label the 5' and 3' ends of the mRNA that codes for m-ER.

b) On the **m-ER mRNA**, show the approximate location of the codons corresponding to the signal sequence by drawing a box.

c) Which part of the **m-ER protein** is cleaved off before it is trafficked to the cell membrane?

d) List the parts of the m-ER protein that is **ABSENT** in the n-ER protein.

e) Draw the **orientation of m-ER protein** in the cell membrane and label its amino (-NH₂) terminus and carboxyl (-COOH) terminus.

CODON CHART

	U	C	A	G	
U	UUU phe UUC phe UUA leu UUG leu	UCU ser UCC ser UCA ser UCG ser	UAU tyr UAC tyr UAA STOP UAG STOP	UGU cys UGC cys UGA STOP UGG trp	U C A G
C	CUU leu CUC leu CUA leu CUG leu	CCU pro CCC pro CCA pro CCG pro	CAU his CAC his CAA gln CAG gln	CGU arg CGC arg CGA arg CGG arg	U C A G
A	AUU ile AUC ile AUA ile AUG met	ACU thr ACC thr ACA thr ACG thr	AAU asn AAC asn AAA lys AAG lys	AGU ser AGC ser AGA arg AGG arg	U C A G
G	GUU val GUC val GUA val GUG val	GCU ala GCC ala GCA ala GCG ala	GAU asp GAC asp GAA glu GAG glu	GGU gly GGC gly GGA gly GGG gly	U C A G

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