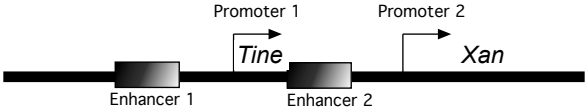
Name _____KEY March 22, 2009_____

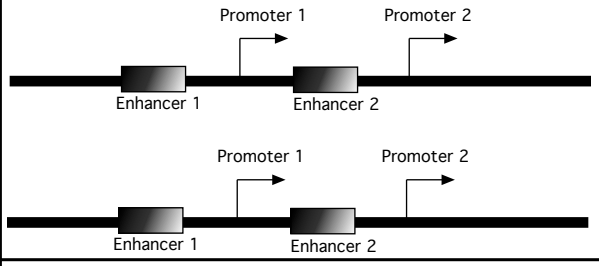
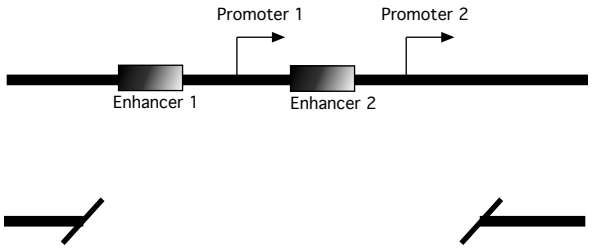
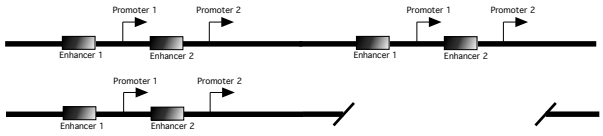
Essays

1. ***An animal normally has two copies of the Xan gene.*** The protein made by this gene helps the animal clear petroleum-based pollutants from its body.

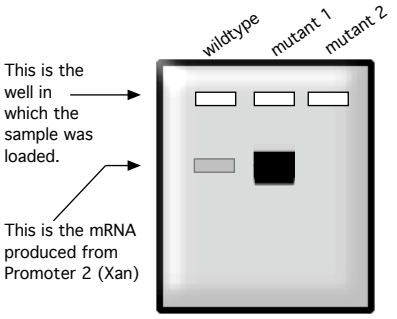


What we know about the *Xan* gene is shown in the cartoon above. We know that Enhancer 2 and Promoter 2 work together to express the *Xan* gene. Also shown is a bit of information about the neighboring gene. It is called *Tine* (pronounced Tiny). We know that Enhancer 1 and Promoter 1 work together to express *Tine*.

You are going to analyze a collection of mutant animals and compare them to the wild type animals.

Wild type diploid animals have two copies of both genes and will be represented like this--->	
Mutant 1 is an animal in which one copy of the genes have been deleted. It is represented like this ---> It is heterozygous for the deletion. The bottom chromosome has had both genes deleted.	
In Mutant 2, the deletion is homozygous. All copies of the genes have been removed.	
In Mutant 3, both genes have been duplicated. The top chromosome has 2 copies of the genes and the bottom chromosome has 2 copies of the genes. Therefore the animal has 4 copies of both genes. Mutant 3 will be represented like this-->	
In Mutant 4, one chromosome carries the duplication of the genes while the other chromosome carries only one copy of the gene. Therefore it carries 3 copies of *Tine* and 3 copies of *Xan*. Mutant 4 will be represented like this -->	

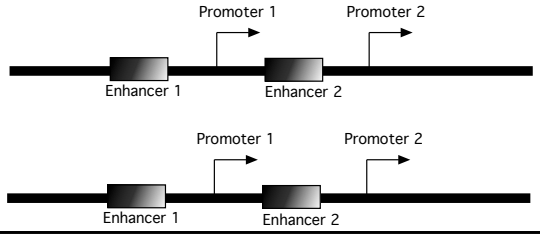
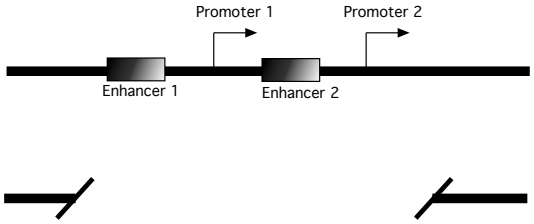
In this experiment, you will be measuring the expression level (amount of mRNA produced) from *Xan*. You never measure the amount produced by *Tine*. You will be shown a gel that shows the relative expression level from Promoter 2 (*Xan*) from wild type and mutant animals. The gel will look somewhat like this -->



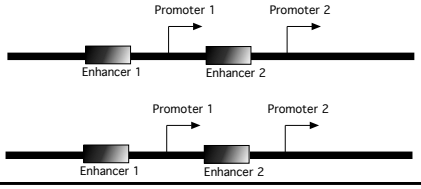
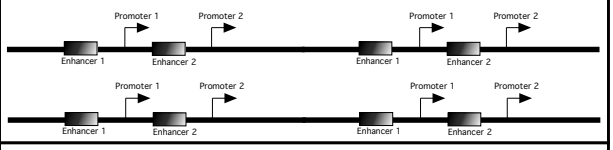
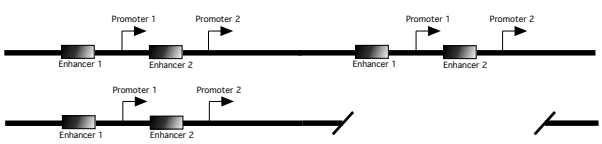
I have also given you a histogram that displays the level of *Xan* expression in wild type and mutant animals.

Here are the results of the study.

Experiment 1 compares the expression level from Promoter 2 (*Xan*) in wild type and mutant animals.

Wild type		Data showing expression from gene 2 
Mutant 1		
Mutant 2	 This one is homozygous for the deletion.	Mutant 2 does not produce any detectable *Xan* mRNA.

Experiment 2 compares the expression level from Promoter 2 (*Xan*) in wild type and mutant animals.

Wild type		Data showing expression from gene 2 
Mutant 3		
Mutant 4		

Name _____

1A) What controls are missing from this experiment? Tell me which control is most important and tell me why it is important.

**Answer:

best - Internal control. Probe for another message. It's abundance should not change.

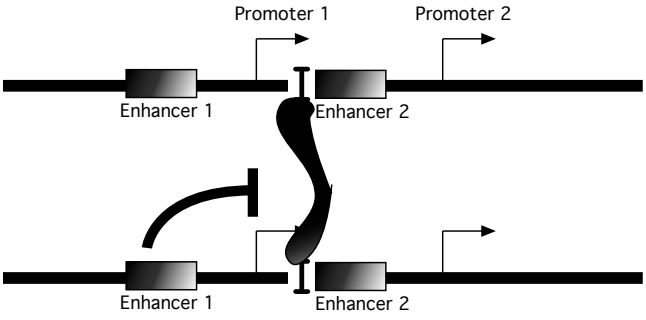
2nd best - Loading control. In some way show that the same amount of cellular RNA has been loaded into each lane. This could be with a spectrophometer or by staining for total RNA in the gel.

**

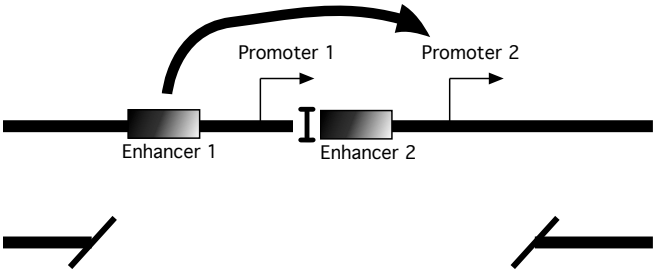
1B) Assume that the data that I have given you accurately describes the expression level of the Xan gene (gene 2). Explain how a deletion could lead to increased expression from the remaining wild type gene.

**Answer:

The deletion has removed an insulator.



When the insulator is gone the protein that binds the insulator cannot prevent Enhancer 1 from acting on Promoter 2.



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Name _____

2. ***You have developed an antibody that is specific for methylated cytosines. It does not recognize anything else.*** You are working with two cell lines called cell line A and cell line B. Cell line B was made from cell line A. Here is the process: cell line A is treated with a chemical that causes it to behave like a cancer cell (loss of growth control). Thus, cell line B is a "cancerous" form of cell line A.

You would like to understand how cell line A and B differ.

You grow cell lines A and B in separate petri dishes. You take 20,000 cells of each one and purify the genomic DNA from the cells. This is pure genomic DNA with no contaminants. You shear this DNA into 250 bp fragments.

To tubes containing sheared genomic DNA *A* and sheared genomic DNA *B* you add the anti-methylated cytosine antibody. You then add protein A to each tube. Protein A will bind the antibody and can be pelleted by centrifugation. You centrifuge these material and isolate the pellet. At this stage you have Pellet A and Pellet B (A is from genomic A and B is from genomic B).

In the exam I will ask you to accomplish a specific goal with this material. You should try to anticipate how these pellets could be used to accomplish specific goals. What techniques will you need? You should consider multiple solutions to these questions.

Question. You suspect that the Xan gene is involved in the change from normal cell to cancerous cell (A-->B). How could you use the A & B pellets to test this idea? Describe the technique that you would use in a much detail as you can. If you so desire, I will provide you with cloned Xan DNA that you can use as a tool (some answers to this question require this but other answers do not). Describe how you would interpret the results.

**Answer:

Multiple answers are possible. The simpliest is to run the pellels in a gel and do a Southern blot. Then they should used cloned Xan DNA to probe the blot. Increased intensity equals increased CpG methylation.

Increased CpG methylation is associated with reduced gene expression (They don't have to say this since it was part of the previous exam.).

If B has more then one would suspect that the treatment resulted in increased methylation of Xan DNA which leads to decreased expression. If A has more then one would assume the opposite.

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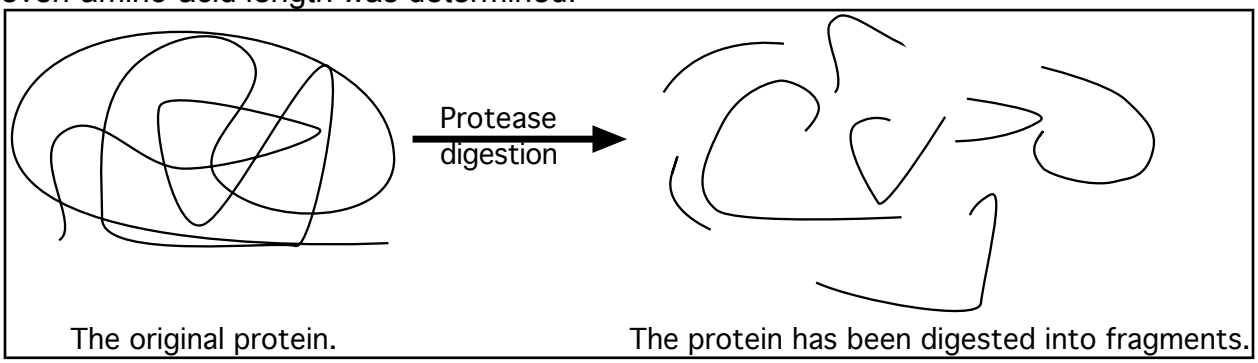
Name _____

3. ***You are studying a protein. To learn more about the protein, you need to have a clone of the gene that encodes the protein.*** You choose to clone this gene by PCR. To do so, you first determine the sequence of fragments of the protein. The entire protein is 1000 amino acids long. The technology available at the time of protein sequencing allows you to sequence only small peptides. So, the protein was digested into small pieces. For each piece, the sequence of a seven-amino-acid length was determined.



You obtained 9 peptide sequences. They are:

FLSYCWP	PHQRIMT	MNKVADE
MCPCFIE	HEDKFYC	HEDWKFM
DNKMYFH	WPQHHRR	RR

Unfortunately, you do not have any more information about the sequence of the protein or the gene.

3A) Please write down the sequence of the two best primers that you would use to PCR amplify a portion of the gene.

**Answer

FLSYCWP 2*6*6*2*2*1*4 = 1152	PHQRIMT 4*2*2*6*3*1*4 = 1152	MNKVADE 1*2*2*4*4*2*2 = 256
MCPCFIE 1*2*4*2*2*3*2 = 192	HEDKFYC 2*2*2*2*2*2*2 = 128	**HEDWKFM 2*2*2*1*2*2*1 = 32**
DNKMYFH 2*2*2*1*2*2*2 = 64	WPQHHRR 1*4*2*2*2*6*6 = 1152	RR 6*6 = 36

The two best ones are derived from
DNKMYFH and HEDWKFM

PRIMER 1

```
D  N  K  M  Y  F  H
THE DEGENERATE PRIMER DESIGNED FROM THE ABOVE PEPTIDE IS
GATAATAAAATGTATTTTCAT
  C  C  G     C  C  C

THE 5' END OF THIS PRIMER IS ON THE LEFT.

HERE IS THE ANSWER WRITTEN USING AMBIGUOUS BASES
GAYAAYAARATGTAYTTYCAY
THE STUDENTS DO NOT HAVE TO ANSWER USING AMBIGUOUS BASES.
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PRIMER 2 SELECTION
H  E  D  W  K  F  M
A DEGENERATE SEQUENCE THAT COULD ENCODE THE ABOVE PEPTIDE IS
CATGAAGATTGGAAATTTATG
  C  G  C     G  C

HERE IT IS WRITTEN WITH AMBIGUOUS BASES
CAYGARGAYTGGAARTTYATG

OK, BUT ONE OF THESE MUST BE REVERSE COMPLEMENTED IN ORDER TO HAVE ANY
CHANCE OF POINTING AT THE OTHER PRIMER.
THE DEGENERATE PRIMER DESIGNED FROM THE ABOVE PEPTIDE IS
CATAAATTTCCAATCTTCATG
   G  C     G  C  G

THE 5' END OF THIS PRIMER IS ON THE LEFT.

HERE IS THE ANSWER WRITTEN USING AMBIGUOUS BASES
CATRAAYTTCCARTCYTCRTG
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Name _____

3B) Why can you not be certain that these primers will work?

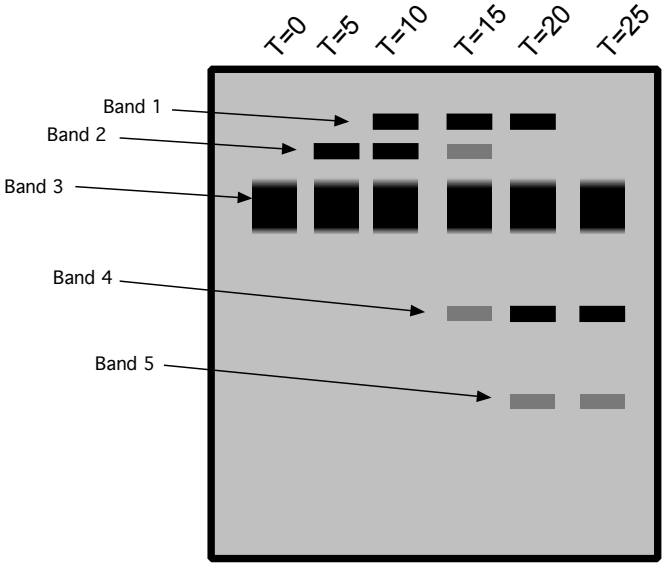
**The most important reason is that you cannot be certain that you have downstream primer that points towards the upstream primer. **

The less important answer is the size of the PCR product.

3C) Why is it better to perform the PCR amplification on cDNA rather than on genomic DNA?

introns

4. ***This is an in vitro splicing reaction.*** Samples are taken from the reaction and loaded on a polyacrylamide gel. The precursor mRNA (Band 3) used in the reaction is radiolabeled. Darker means that the RNA producing this band is more abundant. The precursor produces an extremely broad band because it is very abundant.



Choices in random order are
Exon 1 spliced to Exon 2
Exon 1
Lariat form of Exon 1
Exon 2 joined to the lariat form of the intron
Exon 2 joined to the non-lariat (linear) form of the intron
Lariat form of Exon 2
Exon 2
Intron in lariat form
Intron in non-lariat form (linear)

Name _____

Please describe the likely identity of each band. I say likely because to be 100% certainty would require additional experiments. Your choices are limited to those in the table above. Please write one or two lines that clearly explain the rationale (reason) that you have made each identification.

**Answer

Best answers are

Band 1 Intron in lariat form.

Lariats have aberrant mobility on gels and can often appear to be longer than the precursor (which in fact is longer). This band has an "apparent" size that is greater than the size of the precursor. Because it is produced from intron-lariat-exon2 it must appear after lariat-intron-exon2.

Because the free lariat intron is produced from the exon2-lariat form it should persist longer than the exon2-intron lariat species.

Band 2 Exon 2 joined to the intron in lariat form.

Lariats have aberrant mobility on gels and can often appear to be longer than the precursor (which in fact is longer). This band has an "apparent" size that is greater than the precursor. This one fades out before band 1. This is sensible if band 2 is a precursor to band 1. The fact that the free lariat is bigger is not relevant because this is not a linear molecule. This lariat form should appear before free-lariat-intron because it is produced from lariat-intron-exon2.

Band 4 Exon 1 spliced to Exon 2

One band must be the final splicing product. This is the most likely choice since we also expect to see a smaller band that is just exon 1 or the degraded form of the lariat intron.

Band 5 Most likely it is a degraded form of the lariat intron. Second most likely is that it is free Exon1.

Worth 80% If they say that this one it is Exon1.
Exon 1 is a possibility because often in vitro splicing reactions produce exon 1 that has not yet been spliced to exon 2. This species should be smaller than both the precursor and smaller than the fully spliced product.

However, there is evidence against this conclusion as well. This evidence makes the second answer a bit more probable. This negative evidence is that for every molecule of free exon 1 that we have we should also have a molecule of exon 2-lariat-intron. But we do not. It might still be free exon 1 but in this case it would mean that the exon 2-lariat-intron has degraded and is not producing a visible band.

Worth 100% Therefore, the absence of Exon 2-lariat makes it likely that this band is something else. Notice that it exists when all of the intron-lariat has dissappeared. Therefore it is likely that this band is degraded intron-lariat.

**