

## 2006 7.012 Problem Set 7 KEY

\*\* Due before 5 PM on **FRIDAY**, December 8, 2006. \*\*

Turn answers in to the box outside of 68-120.

PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

- 1.** Because producing effective HIV vaccines is problematic, the most common treatments for HIV infections are currently are anti-viral drugs.
- (a) Why do you think that there are no serious side effects to humans being treated for HIV by drugs that target the enzyme reverse transcriptase?

**Reverse transcriptase is specific to HIV; humans don't have the reverse transcriptase enzyme.**

- (b) The drugs used to treat AIDS that are protease inhibitors need to be specific for HIV proteases, and cannot inhibit proteases in general. Why do you think this is?

**We need proteases to degrade proteins on a regular basis in our own cells, so we don't want to target all proteases with the drug. By doing so, we would be inhibiting proteases that are essential for the host's cell survival.**

- (c) You are attempting to come up with new ideas for HIV drugs. As a start you'd like to try to develop a drug you call anti-CD4, which would inhibit the function of CD4 proteins on the surface of helper T cells. Why would anti-CD4 be useful for preventing HIV infection?

**The HIV virus binds to the CD4 protein on the surface of T<sub>H</sub> cells so by inhibiting the ability of CD4 to bind molecules, you prevent its binding to HIV and thus HIV's ability to enter human T cells.**

- (d) What is the most major disadvantage of anti-CD4 treatment?

**The ability of CD4 to recognize and bind MHCII molecules is critical for the human immune system and so, by disrupting CD4 activity, this treatment has a negative effect on the patient's immune system.**

- (e) HIV viral particles cannot dock onto hamster cells, although it can dock onto human cells. You want to study HIV entry into host cells in lab using hamster cells. How do you think you might genetically manipulate hamster cells such that HIV viral particles can dock onto them?

**You could genetically manipulate hamster cells so that they contain the human CD4 gene and thereby express the human CD4 protein on the cell surface.**

**2.** You are working in a lab that is trying to perform stem cell therapy on mice with Parkinson's Disease by inducing stem cells to become nerve cells *in vitro*, and then delivering these nerve cells to the diseased mouse's brain. The mouse strain you are trying to treat has a form of Parkinson's Disease that is autosomal recessive and is caused by loss-of-function in the PARK2 gene.

**(a)** You initially decide that you want to do the stem cell treatment with stem cells harvested from a wild-type mouse. What is a potential problem with treating the diseased mouse with stem cells taken from another mouse that is not related at all to the diseased mouse?

**The mouse receiving the transplant will recognize the transplanted cells as non-self and mount an immune response against these cells.**

**(b)** You decide to try the kind of therapy discussed in part **(a)** anyway, regardless of the problem listed above. You decide that you will do the therapy using adult stem cells harvested from a wild-type mouse. What is one major problem with trying to use **adult** stem cells specifically for this therapy?

**Adult stem cells have a more limited differentiation potential than embryonic stem cells and so they may not appropriately generate new nerve cells in the host mouse. Adult stem cells are also very rare, and hard to isolate from the adult.**

**(c)** You change your mind, and decide that you want to work with ES cells that are genetically identical to those of the diseased mouse, due to the problems listed in part **(a)** and **(b)**. Why can't you directly isolate ES cells from the diseased mouse?

**Embryonic stem cells are derived from the inner cell mass of a developing blastocyst. Since the diseased mouse is a fully developed adult, ES cells do not exist in this adult.**

**(d)** You decide to use Somatic Cell Nuclear Transfer in order to derive ES cells that are genetically identical to the diseased mouse. List the steps of the procedure you would need to do to accomplish this.

- 1. Remove nucleus from an unfertilized mouse egg**
- 2. Remove a diploid nucleus from a cell from the adult diseased mouse**
- 3. Put the diploid nucleus into the enucleated egg**
- 4. Allow the newly nucleated egg to grow up to the blastocyst stage**
- 5. Remove ES cells from the inner cell mass of the developing blastocyst**
- 6. In culture, induce the ES cells to form neurons**

**(e)** Before you induce the ES cells you derived in part **(d)** to become nerve cells, what must you do to the ES cells to make the stem cell treatment actually beneficial to the diseased mouse? (Hint: re-read the introduction to this question.)

You first have to give a wild-type copy of the PARK2 gene to the ES cells.

(f) You do your treatment described in parts (c) – (e) on a female mouse, and the treatment is successful. Your mouse patient has now received the stem cells and has been relieved of its symptoms. For each cell in the treated mouse described below, state how many total alleles of the PARK2 gene are in that cell, and how many are wild-type versus mutant:

-- a nerve cell originally present in the mouse:

**2 alleles; both mutant.** Since this form of Parkinson's is an autosomal recessive disease the cells of the brain that are affected must carry a mutation in both of their alleles.

-- a nerve cell given to the mouse during the treatment:

This depends on whether you have simply given the mouse an extra copy of wild-type PARK2 (which would integrate into the mouse genome at a random location) or whether you have targeted the mouse's mutant copy of PARK2 with a wild-type copy of PARK2. If you did the former, the answer would be: **3, two mutant, one wild-type.** OR if you did the latter, the answer would be: **2, one mutant, one wild-type.**

-- an egg cell from the mouse:

**1 allele; mutant.** The mouse affected with Parkinson's would be of the genotype PARK2<sup>-</sup>/PARK2<sup>-</sup> so each of its egg cells would contain one mutant allele of PARK2.

**3.** Organismal cloning proves that an adult cell's nucleus contains all of the genetic material necessary to generate every cell type in an organism.

(a) All nucleated cells in the body of an adult human female contain the same DNA except for which three cell types?

**B cells and T cells** (white blood cells) – they undergo chromosomal rearrangements. The B cells rearrange the antibody gene and the T cells rearrange the T cell receptor gene.

**Egg cells** – they are haploid

**Red blood cells** – they lose their DNA during erythrocyte development

(b) Could you generate a mouse that was born if you created that mouse by doing organismal cloning, and if the adult cell you began with was a mature B cell? If yes, then predict what the phenotype would be of the organism as it develops from a newborn to an adult mouse. If no, explain why not.

**Yes.** However each mature B cell has rearranged its antibody gene such that it only has the capability of making one kind of antibody. Thus, when the organism develops, it will

not have an immune system that develops properly. Instead, it will only be capable of making antibody against one foreign protein. **This means that the animal will be immunocompromised and will most likely die of an infection.**

(c) Could you generate a mouse that was born if you created that mouse by doing organismal cloning, and if the adult cell you began with was a red blood cell? If yes, then predict what the phenotype would be of the organism as it develops from a newborn to an adult mouse. If no, explain why not.

**No. Red blood cells have no nucleus and so do not contain the organism's genome.**

(d) Red blood cells (RBCs) are generated from precursor RBCs whenever needed. The body is capable of sensing when more RBCs must be generated, and produces a hormone that stimulates RBC production. What is the body sensing that allows it to know when more RBCs are necessary?

**The body is sensing low oxygen levels in tissues.**

(e) What is the hormone that stimulates RBC formation, and what type of macromolecule is this hormone?

**Erythropoietin (Epo) is the hormone. It's a protein.**

(f) In what cell type is this hormone produced?

**Erythropoietin is produced and released by kidney cells.**

(g) In what cell type is the receptor protein for this hormone produced?

**Erythrocyte (RBC) precursor cells receive the signal for Epo.**

(h) What is the enzymatic activity of this receptor protein and what is its substrate?

**The receptor is a kinase that phosphorylates itself and thereby activates itself.**

(i) Patients with kidney disease/failure are typically anemic. Why do you think that is?

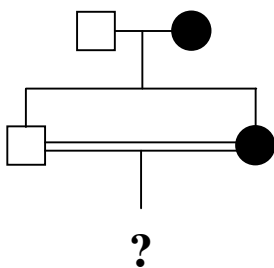
**Anemia is a drop in the number of red blood cells in a person's circulation. Since the kidney cells produce erythropoietin to signal the need for more red blood cells to be produced, a kidney disease or failure that keeps the kidney from carrying out this function would lead to low numbers of red blood cells.**

(j) What is the most common way of treating the anemia of these patients?

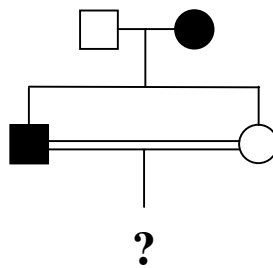
The most common way to treat this type of anemia is to give the patient recombinant erythropoietin to stimulate red blood cell production.

4. Throughout the course of the semester, we have learned about many different patterns of inheritance. In the unit on Mendelian inheritance, we learned about **autosomal** inheritance and **X-linked** inheritance. In the unit on Development, we learned about **maternal effect** inheritance. In the lecture on Molecular Evolution, we will discuss **Y-linked** inheritance and **mitochondrial** inheritance. Below are 5 pedigrees of mouse matings. For each pedigree below, assume that the mode of inheritance listed is the mode of inheritance for the trait. Assume for all pedigrees that the trait indicated by shaded circles and squares is recessive. Your task is to state how many mice in the next generation would be shaded versus not shaded, given each stated mode of inheritance. Assume that the next generation of mice contains 100 mice, 50 female and 50 male.

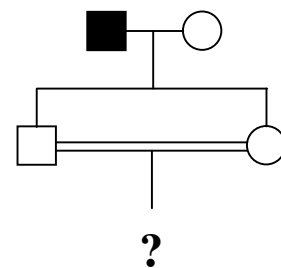
autosomal:



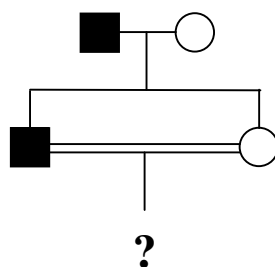
X-linked:



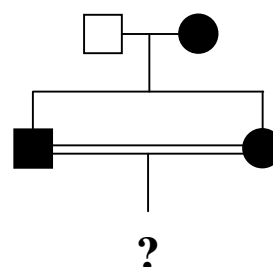
maternal effect:  
(the gene is on an autosome)



Y-linked:



mitochondrial:



|           | Number of shaded females in next generation | Number of unshaded females in next generation | Number of shaded males in next generation | Number of unshaded males in next generation |
|-----------|---|---|---|---|
| autosomal | 25  | 25  | 25  | 25  |
| X-linked  | 25  | 25  | 25  | 25  |

| maternal effect | Inconclusive (see below) | Inconclusive (see below) | Inconclusive (see below) | Inconclusive (see below) |
|-----------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Y-linked        | 0                        | 50                       | 50                       | 0                        |
| mitochondrial   | 50                       | 0                        | 50                       | 0                        |

**Autosomal inheritance** – The dad of the “? generation” must be heterozygous (Aa) since one of his parents carries two mutant alleles. The mom of the “? generation” must be homozygous recessive, as she displays the trait. Crossing a heterozygous individual with a homozygous recessive individual means that 50% of the offspring are heterozygous for this trait and 50% are homozygous recessive. This form of inheritance affects both males and females equally.

**X-linked inheritance** – We know that the grandma is affected and so she must be homozygous for the mutation, while the dad carries the wild type allele on his X chromosome. The father of the “? generation” receives his X chromosome from his mom and so is affected and a Y from his father. The mother of the “? generation” is heterozygous for this trait, receiving the wild type allele from her dad and the mutant allele from her mom. Given this mating of an  $X^A X^a$  mom to an  $X^a Y$  dad, the children will be 25%  $X^a Y$ , 25%  $X^A Y$ , 25%  $X^A X^a$ , and 25%  $X^a X^a$ .

**Maternal affect inheritance** – Since the parents of the “? generation” are not affected, the grandmother must either be Aa or AA. However we do not know which one she is. The grandfather is affected, so his mom must have been aa. So he must have an “a” allele himself. He can therefore either be aa or Aa. However we do not know which one he is. Given that grandma can be AA or Aa and grandpa can be aa or Aa, the mother of the “? generation” can be AA, Aa, or aa. Therefore you cannot predict what the offspring will be. If the mother of the “? generation” is AA or Aa, she will have no affected children, so the numbers would be 0, 50, 0, 50. If the mother of the “? generation” is aa, she will have all affected children, so the numbers would be 50, 0, 50, 0.

**Y-linked inheritance** – We know that the dad must carry the mutation on his Y chromosome and we also know that only the male children will be affected by this trait. Since the only Y chromosome in this pedigree is mutated, all of the male children will be affected. The females do not have the gene that confers this trait and thus cannot have the trait.

**Mitochondrial inheritance** – Mitochondria are only inherited from the mother and since the mother of the “? generation” is affected, all of her children, male or female, will also be affected.

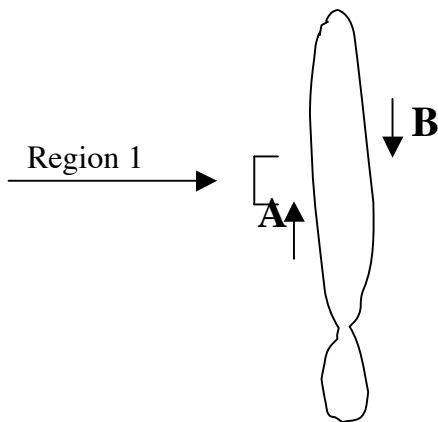
**5.** During the last week of class, we will be discussing molecular medicine and the future of biology. To introduce you to just one of the many interesting topics relating to molecular medicine and the future of biology, we ask that you read a report from the US Government Accountability Office. The report is published at the website <http://www.gao.gov/new.items/d06977t.pdf>

Go to this website and print out pages 1-9 of the PDF (which includes 2 coversheets and pages 1-7 of the GAO article). Read this article and respond to it below in 8 sentences or less. By respond to the article, we DO NOT mean summarize the article, but rather we want to hear your reaction to this report. For instance, you could tell us something you found interesting or surprising about this article, or something this article made you think about. Write your response (handwritten) in the space below.

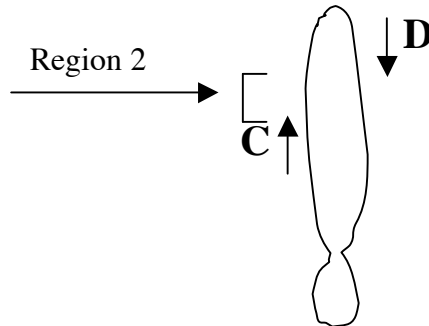
**All reasonable answers were accepted.**

**6.** You are a human geneticist studying cancer. You have four cell types that have been derived from four different tumors (Cell Types Q, R, S, and T, which are each from a different patient with a different type of cancer). You have designed a PCR-based assay to detect large chromosomal abnormalities such as deletions, duplications, inversions, and translocations. It turns out that each of the cancerous cell types has a different one of these abnormalities affecting either one or both of the following chromosomal regions (Regions 1 & 2). In each cell type, this chromosomal abnormality contributes to the development of the cancer in these cells. In the diagram below, the small arrows indicate PCR primers you will be using in your assay. Note that Regions 1 and 2 are not the same size (i.e. they are not drawn to scale in the drawing).

**Schematic of Chromosome 3**



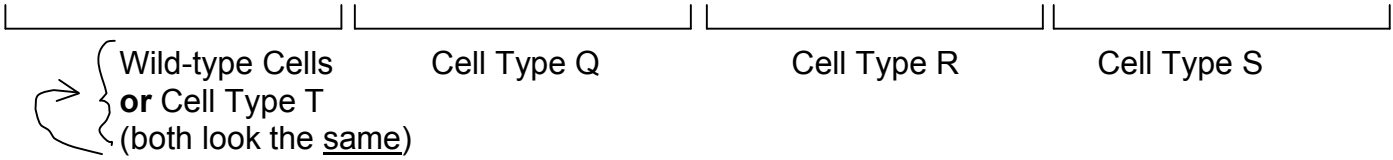
**Schematic of Chromosome 7**



You do PCR using four different pairs of primers (in four separate reactions) on each of the four cell lines, and wild-type cells. The primers used are listed at the top of each lane in the gel.

Name: \_\_\_\_\_ KEY \_\_\_\_\_

A&B A&D B&C C&D    A&B A&D B&C C&D    A&B A&D B&C C&D    A&B A&D B&C C&D



(a) State which type of chromosomal abnormality is present in each cell type, and whether you think it is present in a heterozygous or homozygous state. If you cannot conclude, write “*inconclusive*.”

|             | Type of rearrangement | Heterozygous or homozygous |
|-------------|-----------------------|----------------------------|
| Cell Type Q | Translocation         | Heterozygous               |
| Cell Type R | Deletion              | Homozygous                 |
| Cell Type S | Duplication           | Heterozygous               |
| Cell Type T | Inversion             | Inconclusive               |

Cell Type Q must have a translocation, because now you can get exponential amplification of DNA using one primer that is to chromosome 3 and another primer that is to chromosome 7. The translocation must be heterozygous because you still get amplification from the two primers that face each other on chromosome 3 and the two primers that face each other on chromosome 7.

Cell Type R must have a deletion because chromosome 3 has gotten shorter, as evidenced by the fact that the distance between primers A and B has decreased in this cell type. The deletion must be homozygous because there is not a second band in the A&B lane at the usual size.

Cell Type S must have a duplication because chromosome 3 has gotten longer, as evidenced by the fact that the distance between primers C and D has increased in this cell type. The

**duplication must be heterozygous because there is a second band in the C&D lane at the usual size.**

**Cell Type T must have an inversion. You cannot tell which chromosome has had a piece inverted, because the length of each chromosome is the same as the wild-type chromosomes. You can also not tell whether both homologs have had a segment inverted or not, because the overall length of the chromosomes is the same after the inversion as before the inversion.**

**(b) Do you think that each of the following chromosomal abnormalities is more likely to cause cancer by affecting an oncogene or a tumor suppressor gene?**

-- Duplication

**This is more likely to cause cancer if it affects an oncogene. This duplication would cause you to have more copies of a gene that encodes proteins that promote the cell cycle.**

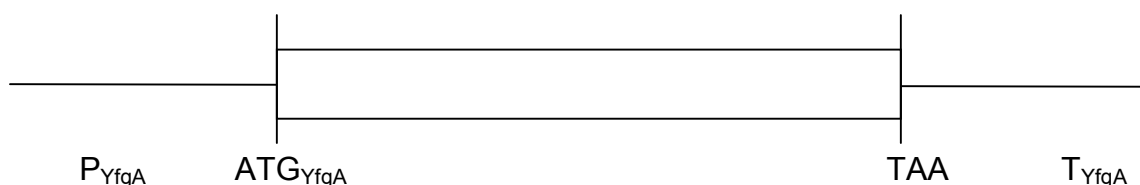
-- Deletion

**This is more likely to cause cancer if it affects a tumor suppressor gene. This deletion would cause you to have a loss of function mutation and thus the affected gene will make fewer or no proteins that are needed for inhibition of the cell cycle.**

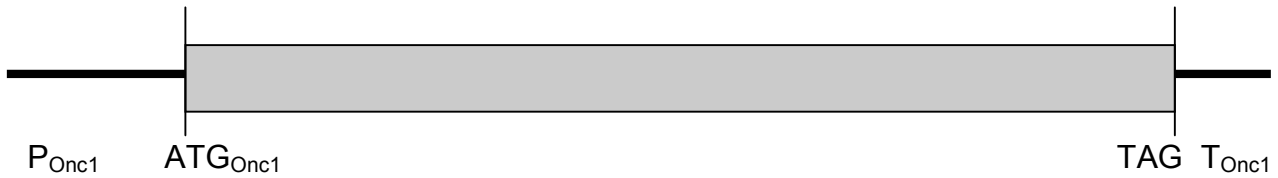
We have discussed two different types of translocations that can affect oncogenes and cause cancer. One type (such as the translocation that places the myc open reading frame under control of the antibody gene promoter) is called a “transcriptional fusion.” The other type (such as the translocation that fuses the two open reading frames of Bcr and Abl in chronic myelogenous leukemia) is called a “translational fusion.”

Assume you are studying an oncogene called Onc1. This oncogene can be activated by translocation in two ways. One translocation produces a transcriptional fusion of the gene YfgA to the gene Onc1; YfgA has a very strong promoter. This translocation causes the regulatory region (“P<sub>YfgA</sub>”) that lies upstream of the YfgA open reading frame to be fused to the Onc1 coding sequence and terminator. The other translocation produces a translational fusion of the gene YfgA to the gene Onc1. This translocation causes almost the entire YfgA gene (beginning with its promoter and ending right before its stop codon) to be fused directly to a portion of the Onc1 gene (from the start codon through the terminator).

The gene for YfgA looks like this: (T = transcriptional terminator)



The gene for Onc1 looks like this:



The gene for YfgA produces a protein that looks like this:

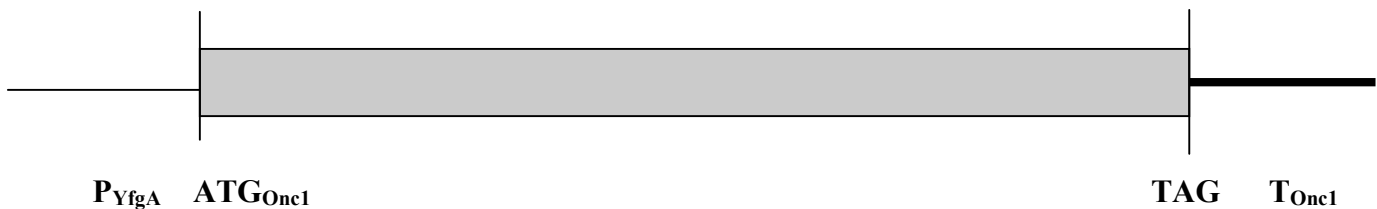
N terminus



The gene for Onc1 produces a protein that looks like this:

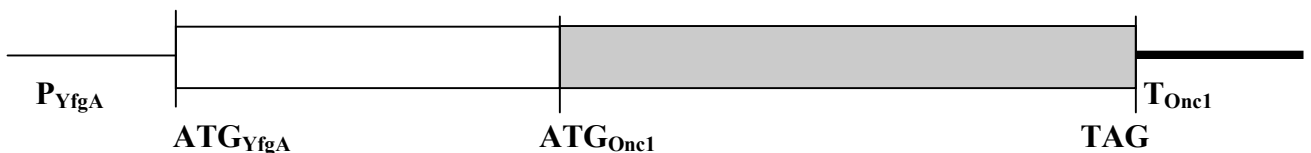


(c) Based on the diagrams above, draw a schematic of the transcriptional fusion gene that is produced by one type of translocation. Label all parts that are labeled in the original diagrams.



**This translocation causes the regulatory region (“P<sub>YfgA</sub>”) that lies upstream of the YfgA open reading frame to be fused to the Onc1 coding sequence and terminator.**

(d) Based on the diagrams above, draw a schematic of the translational fusion gene that is produced by one type of translocation. Label all parts that are labeled in the original diagrams.



Note, this image is not drawn to scale with the other images.

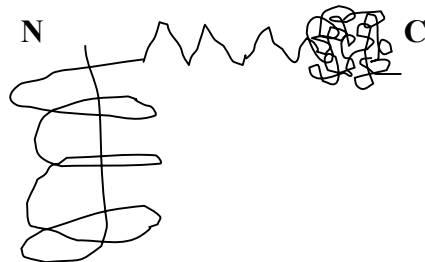
This translocation causes almost the entire YfgA gene (beginning with its promoter and ending right before its stop codon) to be fused directly to a portion of the Onc1 gene (from the start codon through the terminator).

(e) Based on the diagrams above, draw a schematic of the transcriptional fusion protein that is produced by one type of translocation. Label the N and C termini.



The transcriptional fusion puts the Onc1 gene under a strong promoter so it will be expressed more often, but its protein structure is unaffected.

(f) Based on the diagrams above, draw a schematic of the translational fusion protein that is produced by one type of translocation. Label the N and C termini.



The YfgA open reading frame precedes the Onc1 open reading frame (with no stop codon in between) and so the YfgA protein must be produced first, followed by it being covalently bound to the Onc1 protein. One continuous chimeric protein will result.