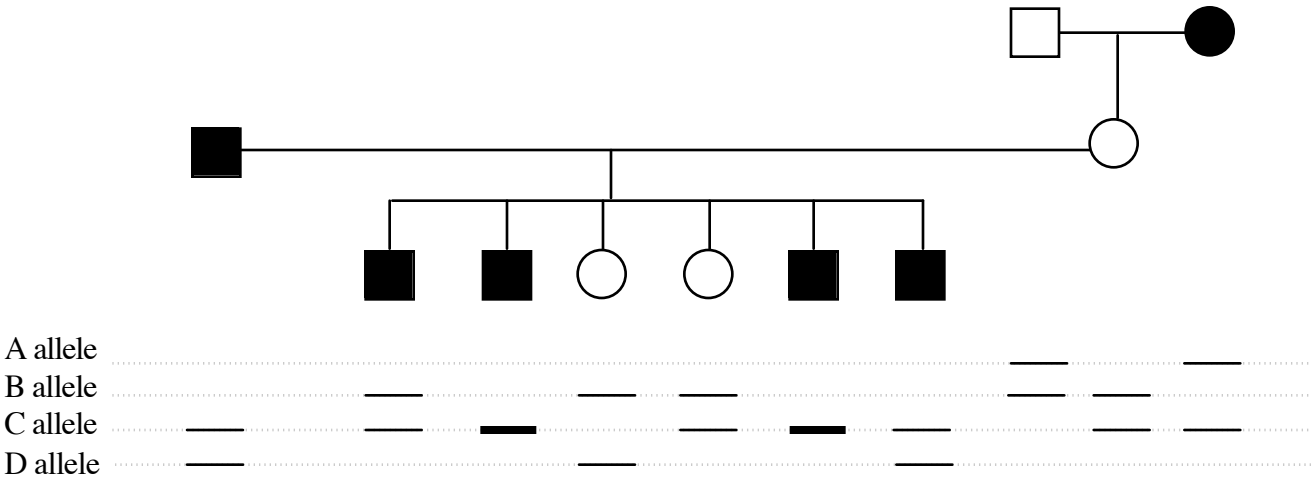
2005 7.03 Problem Set 7

NO DUE DATE. This problem set is to provide practice on concepts from lectures 30-36.

1. The following pedigree shows the inheritance of an autosomal recessive trait in a specific family. This trait is caused by a specific allele "g" at the G/g locus. You have some reason to suspect that the G/g locus is linked to an SSR on chromosome 6 called SSR41. You obtain blood samples from each member of the family, and perform a PCR reaction on the DNA of each individual that allows for the genotyping of SSR41. The results of the PCR reactions are shown below each family member in the pedigree, in a schematic of an agarose gel in which you have loaded the PCR reactions from each family member into a separate well in the gel.



paternally inherited allele at SSR41						
maternally inherited allele at SSR41						

paternally inherited allele at G/g locus						
maternally inherited allele at G/g locus						

(a) Fill in the tables above to indicate which alleles have been passed on to each child from their mother and father.

(b) Whose alleles (the mother's or the father's or both) should you follow to calculate the LOD score for the linkage of the SSR to the G/g locus?

(c) Draw all possible phases for the parent(s) you listed in part **(b)**.

(d) For each phase you drew you drew in part **(c)**, state how many children are recombinants and how many children are parentals given that phase.

(e) Calculate the LOD score for this family at theta = 0.04 for the linkage of the SSR to the G/g locus.

(f) At what theta value would you achieve the maximal LOD score for this family, knowing everything you know about them?

(g) What is the LOD score value for the theta value you listed in part **(f)**?

(h) If you had never seen the genotyping results for this family, and only had their pedigree available, what would have been the theoretical maximum LOD score value that you could have ever calculated for this family? (**Hint:** Start by thinking about which theta value could give you the maximum possible LOD score.)

(i) If you had never seen the genotyping results for this family, and only had their pedigree available, what is the minimum number of kids that the family would have had to have contained in order to reach a theoretical maximum LOD score that is > 3?

2. A tumor results when a cell in the body loses control over cell growth and division such that the cell divides many times, forming a ball of cells. Cancer can be extremely harmful to the organism when these balls of cells either physically interfere with function of an essential organ, or begin to steal the nutrients away from cells of essential organs. Cells become capable of growing and dividing inappropriately when they have accumulated multiple mutations in genes (such as oncogenes and tumor suppressor genes) whose normal functions are to control cell growth and division (i.e. to control the cell cycle).

(a) Why is the notion of there being "a cure for cancer" unreasonable?

(b) What is the wild-type function of an oncogene?

(c) What phenotype may result if an oncogene gets mutated so that it becomes over-active?

(d) Would an over-active allele of an oncogene cause a dominant or a recessive phenotype?

(e) Would an over-active allele of an oncogene be the result of a loss-of-function mutation or a gain-of-function mutation?

(f) Could a mutation in an oncogene (that caused the gene to become over-active) have occurred in the cis regulatory regions of the gene? If so, give an example of how a change in the cis regulatory regions of an oncogene could lead to an over-active mutant allele of an oncogene.

(g) What is the wild-type function of a tumor-suppressor gene?

(h) What phenotype may result if a tumor-suppressor gene is mutated so that it no longer functions?

(i) Would the inactivation of a tumor suppressor gene cause a dominant or a recessive phenotype?

(j) Would the inactivation of a tumor suppressor gene be the result of a loss-of-function mutation or a gain-of-function mutation?

(k) Could a mutation in a tumor suppressor gene (that caused the gene to become inactive) have occurred in the coding region of the gene? If so, give an example of how a change in the coding region of a tumor suppressor gene could lead to an inactive mutant allele of a tumor suppressor gene.

(l) Could a mutation in a tumor suppressor gene (that caused the gene to become inactive) have occurred in the cis regulatory regions of the gene? If so, give an example of how a change in the cis regulatory regions of a tumor suppressor gene could lead to an inactive mutant allele of a tumor suppressor gene.

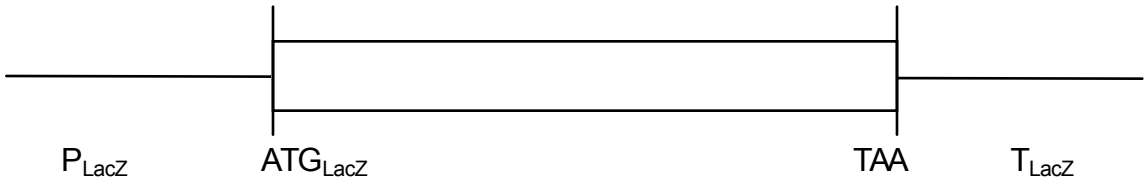
3. You are studying a yeast gene (Act1) which encodes a transcriptional activator protein; Act1 activates the Yst1 gene, which encodes a yeast enzyme that helps yeast cells deal with high salt conditions. Act1 is normally transcribed only when yeast cells are grown in high salt concentrations. You create two different DNA constructs that will allow you to visualize when and/or where Act1 is expressed in cells. Each DNA construct is a fusion of part of the Act1 gene to part of the *E. coli* LacZ gene. You make these two fusion constructs because you want to visualize when and/or where Act1 is expressed in yeast cells, but you don't have a good assay for measuring the presence or activity of Act1 protein. You do, however, have a good assay for measuring the presence and activity of *E. coli* beta-galactosidase, because you know that this enzyme cleaves X-gal and releases a blue-colored compound.

The first DNA construct you make is called an "Act1-LacZ transcriptional fusion." To make this construct, you fuse the cis regulatory region ("P_{Act1}") that lies upstream of the Act1 open reading frame to the LacZ coding sequence and terminator. You then place this hybrid gene on a yeast plasmid.

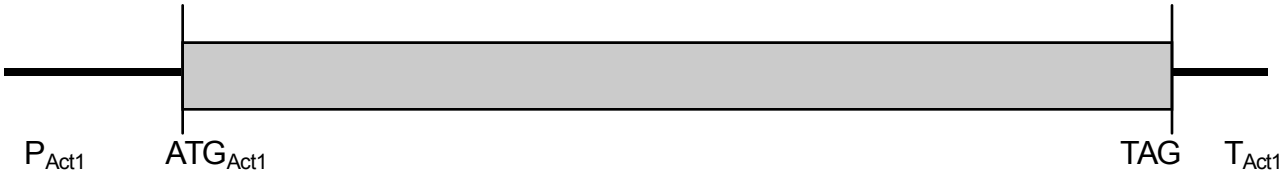
The second DNA construct you make is called an "Act1-LacZ translational fusion." To make this construct, you fuse almost the entire Act1 gene (beginning with its promoter and ending right before its stop codon) directly upstream of a portion of the LacZ gene (from the start codon through the terminator). You then place this hybrid gene on a yeast plasmid.

The beta-galactosidase enzyme (which is encoded by the lacZ gene) is found in the cytoplasm of *E. coli* bacterial cells. When beta-galactosidase is expressed in yeast cells, it is also found in the cytoplasm. The transcriptional activator protein Act1 is found in the nucleus of yeast cells.

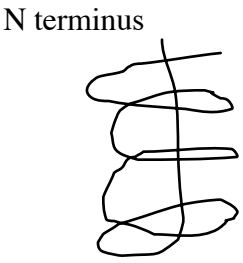
The gene for beta-galactosidase (which is *lacZ*) looks like this: (T = transcription terminator)



The gene for Act1 looks like this:



The gene for beta-galactosidase produces a protein that looks like this:



The gene for Act1 produces a protein that looks like this:

(a) Why does it make sense that the transcriptional activator protein Act1 is found in the nucleus of yeast cells?

(b) What might be the functions of the two different protein domains possessed by the Act1 protein?

(c) Based on the diagrams above, draw a schematic of the DNA construct that would result when you made the transcriptional Act1-LacZ fusion.

(d) Based on the diagrams above, draw a schematic of the DNA construct that would result when you made the translational Act1-LacZ fusion.

(e) Under what cellular conditions is Act1 normally transcribed and translated?

(f) Under what cellular conditions is beta-galactosidase normally transcribed and translated?

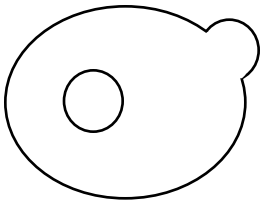
(g) Under what conditions would the protein be made that is produced from the transcriptional Act1-LacZ fusion?

(h) Under what conditions would the protein be made that is produced from the translational Act1-LacZ fusion?

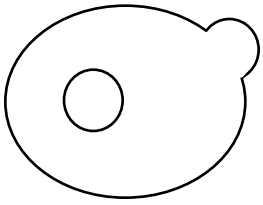
(i) Based on the diagrams above, draw the protein that would be produced from the transcriptional Act1-LacZ fusion.

(j) Based on the diagrams above, draw the protein that would be produced from the translational Act1-LacZ fusion.

(k) Below is drawn a budding yeast cell with a nucleus inside the cell. Shade in where the beta-galactosidase enzyme would be found in a yeast cell expressing the transcriptional Act1-LacZ fusion. (To answer parts **(k)** and **(l)**, you must know that most signals used by the cell to direct intracellular protein localization are found at the very N terminus of the protein.)



(l) Below is drawn a budding yeast cell with a nucleus inside the cell. Shade in where the beta-galactosidase enzyme would be found in a yeast cell expressing the translational Act1-LacZ fusion.



(m) Say that you had a haploid yeast strain that had the endogenous chromosomal copy of Act1 deleted. If you transformed the act1^{-} haploid yeast strain with the transcriptional Act1-LacZ fusion plasmid, would the strain be able to cleave the compound X-gal?

(n) If you transformed the act1^{-} haploid yeast strain with the transcriptional Act1-LacZ fusion plasmid, would the strain be able to induce Yst1 gene expression?

(o) If you transformed the act1^{-} haploid yeast strain with the translational Act1-LacZ fusion plasmid, would the strain be able to cleave the compound X-gal?

(p) If you transformed the act1^{-} haploid yeast strain with the translational Act1-LacZ fusion plasmid, would the strain be able to induce Yst1 gene expression?