7.03 PROBLEM SET 5

BASED ON LECTURES 20-25
DUE BEFORE 5PM ON WEDNESDAY, NOVEMBER 15
SUBMIT ANSWERS DURING RECITATION OR PLACE IN BOX OUTSIDE OF THE BIOLOGY EDUCATION OFFICE

1) Genetic pathways in eukaryotes often are investigated using gene fusions. This approach could be used in yeast to study regulation of a detoxification gene, DTX1. This gene encodes an enzyme that neutralizes benzene, which is a known carcinogen. To investigate DTX1 regulation, you made a gene fusion (P_{DTX1} - LacZ) that consists of the cis regulatory region of DTX1 and the coding region of LacZ. When integrated into the yeast genome, this gene fusion shows wild-type expression. In addition, you isolated two recessive loss-of-function mutations, dtx2- and dtx3-, which show un-inducible gene fusion expression. Your analysis shows that the dtx2- and dtx3- mutations reside in different genes, are not linked to each other, and are not linked to the gene fusion.

a) Diagram three possible models that illustrate the wild-type regulatory relationships among DTX2 and DTX3 and P_{DTX1} - LacZ.

b) You have access to a dominant allele of the DTX2 gene, dtx2-^{D}, which causes constitutive expression of P_{DTX1} - LacZ. Describe the experiment you would perform to distinguish between two of the three models diagrammed in **part (a)**. Show all crosses, the resulting tetrads, and how each result should be interpreted.

c) Now you want to investigate the mechanism by which DTX2 and DTX3 act in the pathway. To determine if DTX2 and DTX3 can bind to each other, you decide to perform a yeast-two-hybrid assay. Which cis-regulatory regions would you put upstream of LacZ?

Suppose you engineer the protein fusion genes DTX2 : AD and DTX3 : DB on a selectable plasmid. What results from the yeast-two-hybrid assay would show that DTX2 and DTX3 interact/bind directly to each other? Complete the chart below and include the necessary controls.

Assay	Yeast strain	Reporter gene expression (expected)
Control 1		None
Control 2	DTX2 : AD /	
Control 3	DTX3 : DB /	
Experiment 1 (assume no direct interaction)		
Experiment 2 (assume direct interaction)		

d) What if one of the controls listed above shows reporter gene expression? For each control, suggest a possibility as to what is occurring in the cell if reporter gene expression is observed.

Control 1 ⟶

Control 2 ⟶

Control 3 ⟶

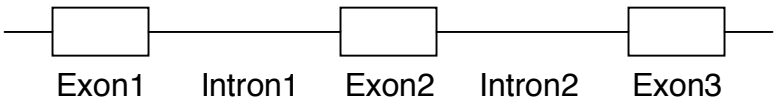
e) Based on the results from the yeast-two-hybrid assay, what can be concluded about your models in **part a**?

2) Genes in the body that suppress the development of cancer are known as tumor suppressor genes. Due to its role in the repair of double-strand breaks in DNA, REP1 is regarded as a tumor suppressor gene. Individuals with loss-of-function mutations in both REP1 alleles show a much greater risk for developing some cancers. After brainstorming with colleagues, it is decided that the role of REP1 in cancer development could be investigated effectively using genetically modified mice.

a) In order to obtain an effective mouse model to study the role of REP1 in cancer development, what genotype would you generate? Explain.

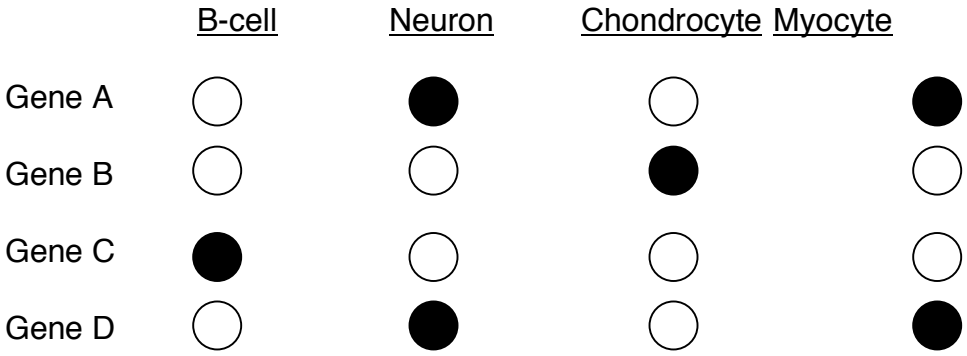
b) Would pronuclear injection or gene targeting techniques be required to construct the desired mouse model? Explain.

c) Exon 2 is essential for the tumor-suppressor activity of the REP1 gene (illustrated below). Draw the DNA construct you would use to modify the mouse genome. How would it integrate into the genome? How would you detect its integration?



A number of genes, such as gene **X**, are involved in the normal regulation of cell growth. Oncogenes are mutated versions (alleles) of these genes that promote unregulated cell growth and lead to cancer. In one type of cancer, B-cell lymphoma, gene **X** is highly expressed. You hypothesize that the over-expression of gene **X** is the causative mutation of B-cell lymphoma.

The expression data illustrated below were generated using microarray analysis (dark circles signify unusually high expression and clear circles signify no expression).



d) Given these results, propose a strategy to generate a mouse model to investigate the role of gene **X** in B-cell lymphoma. Be sure to include the genotype you would create, whether pronuclear injection or gene targeting methods would be used, and how gene **X** would be expressed in B-cells.

3) PhiP and IQ are heterocyclic amines that are mammary gland carcinogens in mice. Both of these chemicals are present in certain food products such as cooked meats. To better understand the biology behind the carcinogenic properties of PhiP and IQ, we would like to identify genes that protect cells from their toxicity.

Wildtype yeast grow at a reduced rate in the presence of 50 mM PhiP but arrest completely in the presence of 100 mM PhiP.

a) Using the yeast *Saccharomyces cerevisiae*, design a screen to isolate mutants that are hypersensitive to PhiP. Be as specific as possible.

From this screen you identify two mutants that are hypersensitive to PhiP. You name these mutants Mut1 and Mut2. You find that Mut1 and Mut2 both confer a recessive mutant phenotype.

b) Design an experiment to determine if Mut1 and Mut2 are alleles of the same gene. Be as specific as possible.

You determine that Mut1 and Mut2 define two different genes. Furthermore, you have mapped and cloned Mut1. Now that you know the sequence of the MUT1 gene you decide to make a gene fusion consisting of the cis regulatory region of MUT1 ligated to the LacZ coding sequence. A single copy of this gene fusion (P_{MUT1}- LacZ) then is incorporated into Chromosome III of the yeast genome. You perform the following experiments and test for ß-galactosidase activity:

Genotype	no PhiP	25 mM PhiP
P_{MUT1}- LacZ	-	+
Mut1; P_{MUT1}- LacZ	-	+
Mut2; P_{MUT1}- LacZ	-	-

c) What do these results tell you about Mut1 and Mut2? Why is this experiment performed with 25mM PhiP and not 50 mM PhiP?

You want to identify a mutant that constitutively expresses P_{MUT1}- LacZ. You mutagenize your P_{MUT1}- LacZ haploid strain and look for ß-galactosidase activity in the absence of PhiP. You identify a mutant that you call Mut3.

Genotype	no PhiP	50 mM PhiP
P_{MUT1}- LacZ	-	+
Mut3; P_{MUT1}- LacZ	+	+

You cross this mutant to a wildtype strain (no P_{MUT1}- LacZ) and do the following experiments:

Genotype	no PhiP	50 mM PhiP
P_{MUT1}- LacZ/ +	-	+
Mut3/+; P_{MUT1}- LacZ/+	-	+

d) What conclusions can you draw from the results of the above experiments? Be as specific as possible.

e) Given the available data, draw the two most likely pathways that illustrate the involvement of PhiP, Mut2, and Mut3 in the regulation of MUT1. Assume the regulatory genes operate in series.

You cross the Mut2 strain to the Mut3 strain (both containing the P_{MUT1}- LacZ reporter), sporulate the diploid, and then measure the ß-galactosidase activity of the resulting spores grown on three different types of media. You examine 50 tetrads and observe the following:

# of tetrads	no PhiP	25 mM PhiP	50 mM PhiP
49	+	+	+
	+	+	+
	-	-	Arrest
	-	-	Arrest
1	+	+	+
	+	+	+
	-	-	Arrest
	-	+	+

f) Based on these data, what can you conclude about the relative positions of MUT2 and MUT3 in the pathway? Be as specific as possible.

g) Which of the two models drawn in **part e** must be correct?