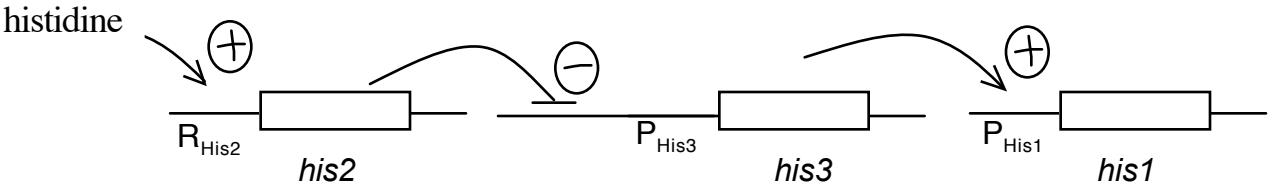
2005 7.03 Problem Set 5

Due before 5 PM on WEDNESDAY, November 16, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. You are studying the regulation of a yeast gene (His1), which is necessary for synthesis of the amino acid histidine. To begin your analysis of the regulation of His1, you fuse the cis regulatory region ("P_{His1}") that lies upstream of the His1 open reading frame to the LacZ coding sequence. You then place this hybrid gene on a yeast plasmid. This reporter gene construct behaves the way you expected based on the pathway for the regulation of His1, which is as follows:



Keep in mind that this model is a genetic pathway that should not be interpreted as a molecular model.

You monitor the expression of the *his1* gene using your reporter gene construct in order to perform a genetic screen looking for mutants that do not properly regulate expression of *his1*. In your screen, you isolate a series of haploid mutant strains that either show constitutive or uninducible expression of *his1*. You identify the genes that are mutated in the mutants you find, and discover that you have identified new alleles of two genes, *his2* (which lies on chromosome #1), and *his3* (which lies on chromosome #5).

In your screen, you isolate five strains, each of which contains one of the following single mutations:

his2a, which is in the **coding region** of *his2*. This mutation gives a recessive phenotype.
his2b, which is in the **coding region** of *his2*. This mutation gives a constitutive phenotype.
his3c, which is in the **coding region** of *his3*. This mutation gives a constitutive phenotype.
his3d, which is in the **coding region** of *his3*. This mutation gives a recessive phenotype.
R_{His2}^{-}, which is a deletion in the **cis regulatory region** in front of *his2.*

(a) Is *his2a* cis-acting or trans-acting with respect to *his1*?

(b) Is R_{His2} cis-acting or trans-acting with respect to *his1*?

(c) What is the phenotype of a *his2a his3d* double mutant with respect to expression of *his1*?

(d) Would the *his3c* mutation give a dominant or recessive phenotype with respect to expression of *his1*?

(e) What type(s) of mutation might *his2b* be with respect to *his1*? (Your choices are: repressor⁻, activator⁻, promoter⁻, UAS⁻, URS⁻, dominant negative repressor, dominant negative activator, super-repressor, super-activator.)

(f) You cross a *his3d* haploid mutant strain to a *his2a* haploid mutant strain. What is the phenotype of the resulting diploid with respect to expression of *his1*?

(g) You induce sporulation of the diploid from part **(f)**. You analyze 90 tetrads. Three distinct tetrad types are obtained. Below, fill in each blank with the phenotype of each of the spores that is not provided to you.

Type 1:	**Type 2:**	**Type 3:**
regulated (wt)	_____	constitutive
_____	_____	_____
_____	_____	_____
constitutive	_____	_____

(h) How many “Type 3” tetrads would you have most likely observed?

2. You are studying the metabolism of a sugar called struliose by yeast cells. (Note that yeast will use struliose even when glucose is present.) You have already isolated one gene that is necessary for the use of struliose as a carbon source. This gene is induced whenever struliose is present. You want to do a genetic procedure (i.e. a screen or selection) to look for more genes involved in struliose metabolism, and you have two reagents that could help you do this. One reagent is a reporter gene that you have created by attaching the promoter region of the known struliose-utilization gene to the open reading frame for *E.coli lacZ*. The other reagent is a form of struliose (called toxo-struliose) that can be metabolized in the same way as struliose, but when it is metabolized, it creates a byproduct that is toxic to yeast cells. You have a collection of thousands of haploid yeast, and each yeast is mutant in a different gene. However, you don't know which of these yeast are mutant in "struliose metabolism" genes (versus which yeast are mutant in any of the other genes in the yeast genome that have nothing to do with struliose metabolism).

(a) Outline a genetic procedure that you would do to find more genes involved in struliose metabolism. In your procedure, use the reporter gene (but not toxo-struliose). To outline your procedure, include: **i)** the type(s) of growth medium you would plate your yeast mutants on (i.e. what would have to be added to a basic growth medium that contains everything necessary for yeast to grow except a carbon source), **ii)** how you would identify the yeast mutants you are looking for (i.e. what would mutants and non-mutants look like on each type of growth medium), and **iii)** whether this method is a screen or a selection.

i)

ii)

iii)

(b) Outline a genetic procedure that you would do to find more genes involved in struliose metabolism. In your procedure, use toxo-struliose (but not the reporter gene).

i)

ii)

iii)

3. You have a true-breeding mouse that displays the phenotype of big feet. This phenotype is caused by a specific allele of the "FT1" gene called FT1*. You isolate the FT1 gene from this mutant mouse, and inject it into a fertilized egg produced by the mating of two wild-type mice. You then transfer this injected fertilized egg into a pseudopregnant mouse. The mouse that is born has big feet.

(a) What specific conclusion can you draw regarding FT1* from this experiment?

(b) Which breeding experiment could you have done to reach the same conclusion that you reached from part **(a)**?

You make a transgenic mouse that is transgenic for a gene that is involved in determining petal color in petunias. This mouse has no detectable mutant phenotype. You then mate two transgenic mice together to generate a mouse that has two copies of the same transgene. These TG+/TG+ mice now have a phenotype of slow movement. You hypothesize that this slow movement is caused either:
-- by the presence of two copies of the petunia transgene (for unknown reasons)
-- because each of the transgenes disrupted one copy of the "Dext" gene, a gene that is important for mouse motor skills

The scenario in this question asks a biological question that can be addressed by creating genetically engineered mice. When creating engineered mice, the following 8 steps need to be considered. **For each mouse you make**, please state:

i) whether you are using pronuclear injection **or** gene targeting techniques
ii) what **DNA** you would introduce into the mouse cells (also draw the DNA)
iii) whether you would put the DNA into a fertilized egg **or** ES cells
iv) what is the **genotype** of the fertilized egg or the ES cells you would start with
v) **where** in the mouse genome the DNA you introduced would integrate
vi) whether creating the mouse should involve the generation of a chimera **or** not
vii) which **additional breeding** steps you would do to make the mouse you wanted
viii) **two possible** phenotypic results you could get from the newly made mice, **and** the corresponding conclusions you would make for each result

Create a genetically modified mouse to distinguish between your two hypotheses if:

(c) You can use the TG+ DNA, but not the “Dext” gene.

i)

ii)

iii)

iv)

v)

vi)

vii)

viii)

(d) You can use the “Dext” gene, but not the TG+ DNA.

i)

ii)

iii)

iv)

v)

vi)

vii)

viii)