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7.03 Problem Set 4
Due Monday, April 6, 2015 by 3 PM

1. You recently discovered that *E.coli* synthesize a previously unidentified compound *fictitious* (fic) to supplement its availability in the environment. Fic synthesis depends upon enzymes coded for by the Fic operon, and *E.coli* that lack a Fic operon are unable to grow on media without fic.

   a) Would you expect the fic compound to be a positive or negative regulator of the Fic operon? Explain your reasoning briefly.

   You insert LacZ directly downstream of the Fic operon promoter to study its regulation.

   b) Indicate what LacZ activity you would expect for a WT (Wild Type) strain of *E.coli* and label each of the mutants (*f1-6*) as uninducible or constitutive.

	LacZ Activity		
	Low fic	High fic	Interpretation
WT			
f1	-	-	
f2	+	+	
f3	-	-	
f4	+	+	
f5	+	+	
f6	+	+	

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c) Based on the data below state whether each Fic operon mutant (*f1-6*) is dominant or recessive.

	LacZ activity		
	Low fic	High fic	Interpretation
f1/F'f1+	WT	WT	
f2/F'f2+	WT	WT	
f3/F'f3+	-	-	
f4/F'f4+	WT	WT	
f5/F'f5+	+	+	
f6/F'f6+	WT	WT	

d) Based on the tests in the table below, classify mutants *f1* and *f5* as either cis-acting or trans-acting. Explain your answer briefly.

	LacZ Activity	
	Low fic	High fic
LacZ-	-	-
LacZ-/F'LacZ+	WT	WT
f1 LacZ+/F'f1+LacZ-	-	-
f1 LacZ-/F'f1+LacZ+	WT	WT
f5 LacZ+/F'f5+LacZ-	+	+
f5 LacZ-/F'f5+LacZ+	WT	WT

e) Classify the *f1* and *f5* mutations, choosing from: repressor-, activator-, promoter-, operator-, super activator, super repressor.

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f) Through a number of experiments you find that the mutant *f3* codes for an altered version of the Fic repressor that constitutively binds the Fic operator. Look back at the experiment in (c) where you observed the phenotype of bacteria that were *f3*/F'f3+. Given that the Fic repressor must form a hexamer (complex of six) in order to bind to the Fic operator, what proportion of the repressor complexes in these bacteria are expected to be WT?

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2. *Pseudomonas synringae* is a species of bacteria found on the surface of many plants. These bacteria have on their cell membrane Ina proteins (Ice-nucleation activators) that are able to nucleate the formation of ice, and thus cause water to freeze at higher temperatures than normal. You are really interested in understanding how the expression of these proteins is regulated, as they are the source of significant frost damage for many essential crops.

   You discover that Ina proteins are only expressed at temperatures of 4°C or below, and in an attempt to understand this regulatory mechanism you clone the LacZ gene downstream of the coding sequence of one such Ina protein – Ina1. Using X-gal to look at Ina operon gene expression, you observe the following results:

Genotype	**10°C**	**0°C**
WT	-	+
Ina1-	-	+
Ina2-	+	+
Ina3-	-	-
Ina4-	+	+
Ina2-/F'Ina2+	-	+
Ina3-/F'Ina3+	-	-
Ina4-/F'Ina4+	+	+

   You also perform cis/trans tests and obtain the following,

Genotype	**10°C**	**0°C**
Ina2-LacZ+/F'Ina2+LacZ-	-	+
Ina2-LacZ-/F'Ina2+LacZ+	-	+
Ina3-LacZ+/F'Ina3+LacZ-	-	-
Ina3-LacZ-/F'Ina3+LacZ+	-	-
Ina4-LacZ+/F'Ina4+LacZ-	+	+
Ina4-LacZ-/F'Ina4+LacZ+	+	+

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a) For each mutant (Ina2-4), summarize the information you have obtained (constitutive/inducible/uninducible, dominant/recessive and cis/trans acting) from your experiments.

b) Based on these results which of the following statements about wild type Ina4 can you **exclude** as possibilities (You can choose none, one or multiple statements):

i) It codes for a repressor of the Ina operon
ii) It codes for an activator of the Ina operon
iii) It is an operator for the Ina operon
iv) It is a promoter for the Ina operon

c) You discover a way to directly measure the activity of Ina proteins without using LacZ. You are able to assay for their ability to nucleate ice formation at various temperatures. This makes it possible to study the regulation of factors that may not be controlled by the same promoter as the Ina operon. You suspect that WT Ina2 might be a promoter for either Ina3 or Ina4. Using your new assay system and the tests we have learned about in class, design an experiment that will allow you to test this hypothesis and briefly explain why you chose this experiment.

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d) You find that Ina2 is the promoter for Ina3 and that the Ina2 mutation is a loss of function. What does this tell you about wild type Ina3 function in the regulation of the Ina operon? What might the Ina3 mutation be causing?

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3. Biotin is a cofactor required for many essential processes, including cell growth. As such, you want to study the regulation of its synthesis in yeast. In wild-type yeast, biotin is synthesized from the amino acid alanine and pimeloyl-CoA, and can grow in media lacking it. In bio2- yeast, the BIO2 gene (biotin synthase) is mutated, and these mutants cannot grow on media lacking biotin.

You generate a reporter plasmid P_{BIO2}::LacZ and identify several mutants that change the reporter's expression:

	ß-galactosidase activity (P_{BIO2}::LacZ)	
Genotype	**+ biotin**	**- biotin**
Wild type	white	blue
b1-	white	white
b1-/B1+	white	blue
b3-	blue	blue
b3-/B3+	white	blue
b4-	blue	blue
B4-/B4+	white	blue

a) What is the effect (positive or negative) of each of the following on BIO2 expression?

Biotin

B1

B3

B4

b) Why would you expect the presence or absence of biotin to regulate the activity of BIO2? Please explain **briefly**.

The enzyme encoded by ALT1 synthesizes alanine in yeast. When you clone a very strong promoter upstream of the normal ALT1 gene in haploid yeast with your reporter plasmid, you find that BIO2 is now constitutively expressed (blue even in the presence of biotin).
c) Why might you expect that an excess of alanine leads to constitutive expression of BIO2?

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d) Conducting an epistasis analysis, you infer that the phenotype of the b1- b4- mutant is:

	ß-galactosidase activity (P_{BIO2}::LacZ)	
genotype	**+ biotin**	**- biotin**
b1- b4-	white	white

Order biotin, BIO2, B1, and B4 in a linear pathway, with pointy and blunt arrows connecting them.

e) Can you perform an epistasis test for the b3- and b4- mutants? Explain.

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4. You are studying regulation of the yeast enzyme proline synthetase (PS), which is encoded by the PRO1 gene. You have isolated two mutants that give altered PS activity, designated pro2– (reduced PS activity) and pro3- (increased PS activity). You determine that these mutants result from mutations in separate genes from PRO1. Mating of either pro2– or pro3– haploids to wild type produces heterozygous diploids that show normal amounts of PS expression. When you cross either a pro2– or pro3– haploid to a pro1– strain the resulting diploids show normal expression of PS.

(a) From these experiments classify the pro2– and pro3– mutations in terms of their basic genetic properties explaining the rationale behind your conclusions. In particular, identify if these mutations are dominant or recessive, act in cis or in trans, and are constitutive, inducible, or uninducible. Based on these properties make a proposal for the types of regulatory functions affected by the pro2– and pro3– mutations.

(b) Diagram two different linear models and one parallel model that could explain the effect of the pro2– and pro3– mutations on the regulation of PRO1.

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The PRO1 gene shows a rather complex regulation in response to different amino acids. When glutamate (glu) is added to the medium, the amount of PS expression is increased, although when proline (pro) is added to the medium, the amount of PS expression is diminished. When both glutamate and proline are added to the medium, there is no change in expression. The effects of different mutants on the response to glu and pro are shown below.

Units of PS activity

	–	+glu	+pro	+glu&pro
wild type	100	150	50	100
pro1–	0	0	0	0
pro2–	75	75	25	25
pro3–	125	175	125	175

(c) Which of the models from part (b) best fits these experimental results? Diagram a complete model for the regulation of PRO1 that includes the effects of glu and pro.

(d) Based on your model for part (c) how would you expect a pro2– pro3– double mutant to behave?

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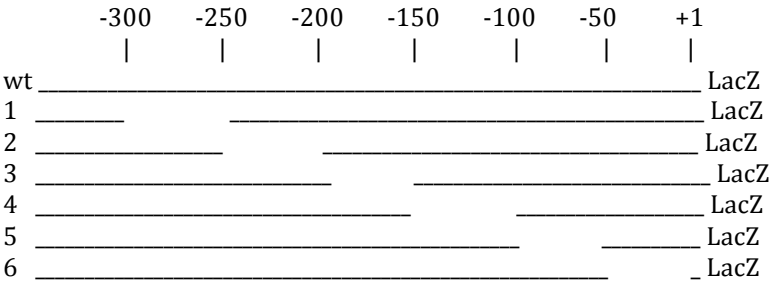
Next, you decide to examine the promoter for the PRO1 gene. To do this, you first fuse the promoter region to the coding sequence for LacZ and then place this reporter gene on an appropriate yeast plasmid. As you might expect, cells carrying the reporter gene express activity under the same conditions that PS is expressed in wild type cells, meaning that the promoter region you have selected contains all of the necessary cis-regulatory sequences. The figure below shows the effect of different 50 bp deletions in the promoter region on the amount of ß-galactosidase activity expressed by the reporter gene.



	Units of ß-gal		
	–	+glu	+pro
wt	100	150	50
1	100	150	50
2	50	50	25
3	100	150	50
4	100	150	50
5	0	0	0
6	0	0	0

(e) Describe the cis-acting elements in the PRO1 promoter that can be identified from this experiment, giving both their position and as much of their function.

(f) How many units of ß-galactosidase would you expect to be expressed from deletion 2 in a pro2– mutant without glutamate or proline? How many units of ß-galactosidase would you expect to be expressed from deletion 6 in a pro2– mutant without glutamate or proline? Explain **briefly**.