7.03 Problem Set 5

Due before 5 PM on Thursday, November 13

Hand in answers in recitation section or in the box outside the class

1. You are studying regulation of the yeast enzyme glutamine synthetase (GS), which is encoded by the GLN1 gene. You have isolated two mutants, designated gln2$^-$ and gln3$^-$, that give decreased GS activity. Mating of either gln2$^-$ or gln3$^-$ haploids to wild type produces heterozygous diploids that show normal amounts of GS expression. When you cross either a gln2$^-$ or gln3$^-$ haploid to a gln1$^-$ strain the resulting diploids show normal expression of GS.

(a) From these experiments classify the gln2$^-$ and gln3$^-$ mutations in terms of their basic genetic properties explaining the rationale behind your conclusions. Based on these properties make a proposal for the types of regulatory functions affected by the gln2$^-$ and gln3$^-$ mutations.

(b) Diagram two different linear models and one parallel model that could explain the effect of the gln2$^-$ and gln3$^-$ mutations on the regulation of GLN1.

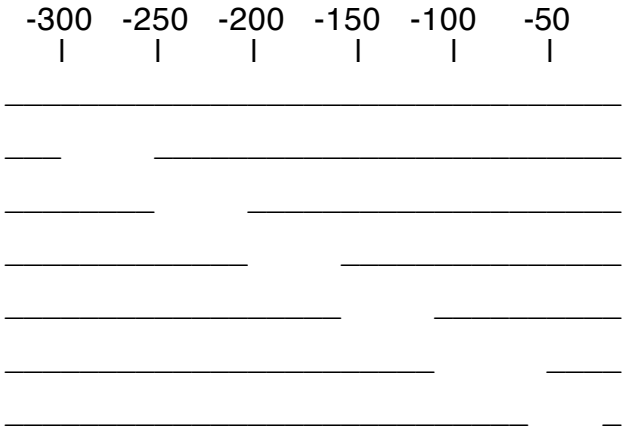
The GLN1 gene shows a rather complex regulation in response to different amino acids. When either glutamate (glu) or glutamine (gln) is added to the medium, the amount of GS expression diminished and when both glutamate *and* glutamine are added to the medium GS expression is shut off completely. The effects of different mutants on the response to glu and gln are shown below.

	Units of GS activity			
	–	+ glu	+ gln	+glu& gln
wild type	100	50	50	0
gln1$^-$	0	0	0	0
gln2$^-$	50	50	0	0
gln3$^-$	50	0	50	0

(c) Which of the models from part (b) best fits these experimental results? Diagram a complete model for the regulation of GLN1 that includes the effects of glu and gln.

(d) Based on your model for part (c) how would you expect a gln2$^-$ gln3$^-$ double mutant to behave?

Next, you decide to evaluate the promoter for the GLN1 gene. To do this you first fuse the promoter region to the LacZ coding sequence and then place this hybrid gene on an appropriate yeast plasmid. You find that cells carrying the hybrid gene express activity under the same conditions that GS is expressed in wild type cells, meaning that the promoter region you have selected contains all of the necessary cis-acting sequences for normal regulation. 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.

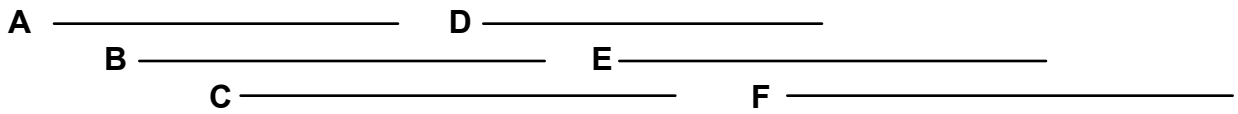


	Units of ß-galactosidase		
	–	+ glu	+ gln
wt	100	50	50
1	50	50	0
2	100	50	50
3	50	0	50
4	50	0	50
5	100	50	50
6	0	0	0

(e) Describe the cis-acting elements in the GLN1 promoter that are evident from these experiments, giving both their position and as much of their function as you can deduce.

(f) How many units of ß-galactiosidase would you expect to be expressed from deletion 1 in a gln2⁻ mutant? How many units of ß-galactiosidase would you expect to be expressed from deletion 4 in a gln2⁻ mutant?

2. You obtain 6 BACs (of **known** order, as shown below) and 7 STSs (of **unknown** order) that derive from a region of mouse chromosome 16 whose genomic sequence has not yet been finished.



By PCR (using 20-bp primers at either end of each STS), you test each of the 6 BACs for the presence (+) or absence (-) of each of the 7 STSs. You obtain the following results:

	STSs						
BACs	1	2	3	4	5	6	7
A	+	-	+	-	-	-	+
B	+	+	-	-	+	-	+
C	+	+	-	+	+	-	-
D	-	+	-	+	+	-	-
E	+	-	-	+	+	-	-
F	+	-	-	-	+	+	-

(a) Construct a physical map of this region that shows the order of the STSs, and the placement of the STSs within the BACs. (Hint: Consider the possibility that one or two of the STSs are present twice in the region spanned by the 6 BACs.) Are there any ambiguities within your map? If so, describe them.

(b) You determine the DNA sequences of PCR products obtained at STS5 using BACs B, C, D, E, and F as PCR templates. The DNA sequences of PCR products obtained using BACs B and C are identical, but they differ by several nucleotide substitutions from the DNA sequences of PCR products obtained using BACs E and F. Oddly, PCR product obtained using BAC D appears to be a mixture of two sequences – one corresponding to the BAC B/ BAC C sequence and the other corresponding to the BAC E/ BAC F sequence. Briefly account for these findings, and explain how a single PCR assay could amplify two different sequences.

(c) Do the findings presented in part (b) confirm, refine, or refute your answers to part (a)? Briefly explain your answer.

(d) Would you expect the DNA sequence of PCR product obtained at STS5 using mouse genomic DNA as template to more closely resemble that obtained using BAC B, BAC D, or BAC F? Briefly explain your answer.

(e) Is there a second STS at which you would like to sequence PCR products obtained using BACs as templates? If so, which BACs would you test in this way, and what sequencing results might you predict for each of the BACs tested?

(f) How would you use the sequence information presented in part (b) to design two new STSs (with new PCR primer pairs) to replace STS5? (Call the new ones STS51 and STS52. STS51 should be present (+) in BAC B, and STS52 should be present (+) in BAC F.)

(g) Would you expect STS51 and STS52 to be present in BAC D? In mouse genomic DNA?

3. Many mouse genes are "tissue-specific," that is, they are present throughout the body but are expressed in only one of the animal's many tissue types. (Other mouse genes are expressed throughout the body, or in multiple tissues.) Geneticists can study the regulation of a mouse gene by fusing the gene's promoter region to the LacZ coding sequence and injecting the construct to create a transgenic mouse. Fusion of the mouse amylase promoter to LacZ yielded a $P_{amylase}$-LacZ construct.

(a) Would microinjection of the $P_{amylase}$-LacZ construct into the male pronucleus of a fertilized egg likely result in integration of the construct into the amylase gene? Briefly explain your answer.

(b) Mice heterozygous for the resulting $P_{amylase}$-LacZ transgene displayed LacZ expression exclusively in the pancreas. Would you expect homozygotes for the transgene to also display LacZ expression in the pancreas? Elsewhere? Briefly explain your answer.

(c) You are surprised to observe that mice homozygous for the transgene insertion display a serious heart defect. (Heterozygotes have normal hearts.) Suggest a possible explanation.

(d) Propose an experimental test of your hypothesis from part (c) using gene targeting in mice. Draw a labeled diagram of the targeting construct that you would use, and outline any breeding experiments required to test your hypothesis.

(e) Propose how you might use LacZ in a gene targeting experiment in mice to test whether the amylase gene is expressed exclusively in the pancreas. Draw a labeled diagram of the targeting construct that you would use, and outline any breeding experiments required to test your hypothesis.