

Name: _____

1. You are studying how yeast cells grow on sucrose and you find that both sucrose and glucose regulate expression of the *SUC1* gene, which encodes an enzyme for sucrose breakdown. *SUC1* is not expressed in cells grown without sucrose, but is induced when sucrose is added to the growth medium. In cells grown in medium that contains both sucrose and glucose, *SUC1* is not expressed. You have isolated mutations in three different genes that alter *SUC1* regulation, called **A⁻**, **B⁻** and **C⁻**. All three mutations are recessive and none of the mutations are linked to one another or to *SUC1*. *SUC1* expression in wild type yeast and each of the three mutants are shown below.

	<u><i>SUC1</i> expression</u>		
	- sucrose	+ sucrose	+ sucrose & glucose
Wild type	-	+	-
A⁻	+	+	-
B⁻	-	-	-
C⁻	-	+	+

(a 6 pts.) For each of the three genes, state whether it affects regulation by sucrose or glucose and whether it is a positive activator or a negative regulator.

Gene **A**

Sucrose - negative regulator

Gene **B**

Sucrose - positive regulator

Gene **C**

Glucose - negative regulator

Name: _____

You cross an **A⁻** mutant to a **B⁻** mutant. After tetrads are dissected and evaluated for *SUC1* expression in either the presence or absence of sucrose, (no glucose is present in this experiment) the following tetrad types are observed.

<u>Type 1</u>	<u>Type 2</u>	<u>Type 3</u>
constitutive	constitutive	constitutive
constitutive	constitutive	constitutive
regulated	regulated	uninducible
uninducible	regulated	uninducible

(b 8 pts.) What is the phenotype of the **A⁻ B⁻** double mutant? Explain how you arrived at your answer.

Based on the spore phenotypes, the Type 2 tetrads must be the NPD tetrads. The spores within the NPD tetrad show either a regulated or constitutive phenotype. The regulated phenotype corresponds to an A⁺ B⁺ genotype. Thus, the spores showing the constitutive phenotype must have the double mutant genotype (A⁻ B⁻).

(c 10 pts.) Draw a model showing the interactions between the different regulatory factors encoded by **A** and **B**. Be sure to include the *SUC1* gene and to indicate where and how sucrose acts.



Sucrose and gene B are net activators of *SUC1* expression, while gene A is a net negative regulator.

Name: _____

Next, you construct a set of deletions within the promoter region of the *SUC1* gene (+1 indicates the first transcribed nucleotide). The ability of each of these deletions to express *SUC1* in cells grown on different sugars is shown below.

	-300	-250	-200	-150	-100	-50	+1	- sucrose	+ sucrose	+ sucrose & glucose
1)	_____							-	+	-
2)	_____		_____					-	+	+
3)	_____			_____				-	-	-
4)	_____				_____			-	+	-
5)	_____					_____		-	+	-
6)	_____						_____	-	-	-

(d 5 pts.) The DNA sequence of gene **C** reveals that this gene is likely to encode a DNA-binding protein. Assuming that the product of gene **C** binds to the promoter region of the *SUC1* gene, where is it most likely to bind? Explain your reasoning.

The deletion 2 mutant shows *SUC1* expression in the presence of glucose. This suggests that the nucleotide region -250 to -200 is the site where the *Gene C* regulatory protein binds.

In addition, the deletion 2 mutant and the *Gene C* mutant exhibit the identical phenotype, which suggests that the deleted -250 to -200 region is the binding site for the *Gene C* regulatory product.

(e 5 pts.) In general, upstream activation sequences function normally regardless of their distance from the start of transcription. Which of the deletion mutants shown above show this to be true for the upstream activation sequence that responds to sucrose activation?

The results above show that the UAS responsive to sucrose lies in the -200 to -150 region. Deletion mutants 4 & 5 show wild-type regulation and reduce the distance between the UAS and promoter and support the hypothesis that a UAS can function normally despite its distance from the transcription start site.

Name: _____

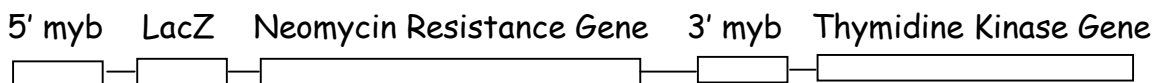
2. (a 12 pts.) Recently your lab has become interested in the function of a mouse gene called *myb*. To determine when and where *myb* is expressed during development you decide to construct a reporter for *myb* expression that can be examined in developing mice. Describe the basic procedure that you would use to produce a useful reporter for *myb* expression. For your answer include such specifics as cell type(s), a diagram of the DNA construct, the site of integration, and any additional breeding steps needed to obtain mice useful for your study.

A transgenic approach or knock-in approach would be effective.

For the transgenic approach, a gene fusion consisting of the *myb* promoter ligated to the LacZ or GFP coding region (P_{myb} - LacZ or P_{myb} - GFP) could be introduced into a wild-type fertilized egg by micro-injection. This transgene would insert randomly into the genome. No additional breeding would be required. This transgenic mouse would serve as an effective model to monitor the spatial and temporal pattern of *myb* gene expression during development.

For the knock-in approach, the endogenous *myb* gene could be replaced with a reporter gene such as lacZ or GFP under control of the *myb* promoter.

The DNA construct, which would replace the endogenous *myb* gene through homologous recombination, would contain the LacZ gene and an anti-biotic resistance gene. DNA homologous to the 5' and 3' regions of the *myb* gene would border these two genes. In addition, a gene encoding thymidine kinase would be placed outside of the homologous 5' and 3' regions of the *myb* gene.



After selecting for homologous recombination of the construct into wild-type embryonic stem cells in culture, the knock-in cells would be introduced into an embryo, which would be injected into the uterus.

The chimeric offspring would be mated with wild-type mice in order to yield heterozygous mice (KI/+). These heterozygous mice would serve as effective models to monitor the spatial and temporal pattern of *myb* expression during development.

(b 8 pts.) From the procedure above you obtain two different lines of mice carrying integrated *myb* reporter constructs. You cross heterozygous mice from Line 1 to one another and score how many of the resulting progeny carry the reporter construct and how many don't. You repeat the procedure for Line 2. The results of these two crosses are shown below.

	Contain Reporter	Do not contain Reporter
Progeny from Line 1 heterozygotes:	74	24
Progeny from Line 2 heterozygotes:	41	23

Provide an explanation for this data, keeping in mind that you did the same number of crosses for each line, but obtained fewer progeny overall from the Line 2 crosses.

The absence of homozygous recessives in line 2 suggests that the reporter gene fusion inserted into an essential gene resulting in a loss of function mutation. The absence of a functional allele of this essential gene in homozygous recessives is lethal.

An alternative explanation is that two copies of the reporter gene fusion in homozygous recessives somehow are lethal due to some sort of toxicity.

Name: _____

(c 10 pts.) With Line 1, you design a screen looking for regulators of *myb* expression. After mutagenesis with a chemical mutagen, you find a mutant mouse (M) that has increased expression of your construct. The regulatory mutant M is genetically recessive and after extensive mapping experiments, you narrow down the location of the regulatory mutation to a region that contains two genes, TF1 and Db. Describe how you would construct a useful mouse model to test the idea that Db is a regulator of *myb* expression. For your answer include the cell type(s) you would target, a diagram of the DNA construct, the site of integration, and any additional breeding steps needed to get mice useful for your study.

A knockout or transgenic could be used.

For the knockout approach, the construct illustrated below would homologously recombine into the Db gene of wild-type embryonic stem cells.



After producing chimeric mice, they would be crossed with wild-type mice to generate heterozygous offspring (KO/+). The resulting heterozygous offspring would be mated among themselves to yield homozygous knockout mice (KO/KO). The homozygous knockout could be used to determine if Db is a regulator of *myb*.

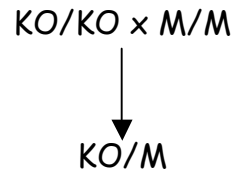
For the transgenic approach, a wild-type Db allele would be injected into a MM fertilized egg through micro-injection. The wild-type Db allele would insert randomly into the MM mutant genome. No additional breeding steps would be required.

The phenotype of the transgenic mouse would enable the role of Db in *myb* expression to be determined.

Name: _____

(d 8 pts.) Using any mice generated in parts a-c, describe how you would carry out breeding experiments to test whether the mutation M is an allele of the Db gene. For your answer, include possible results and how you would interpret them.

The following cross could be performed -



and the phenotype of the KO/M mice would be examined.

If no complementation occurs, then M is an allele of the Db gene.

If complementation occurs, then M is an allele of a gene other than Db.

Another cross could be performed -



If the progeny show a wild-type phenotype, then M is an allele of the Db gene.

3. (a 6 pts.) Consider a blood antigen called D. The allele for this antigen is dominant (i.e. individuals who are either D/d or D/D will express the antigen). If 84% of a population in Hardy-Weinberg equilibrium expresses the D antigen, what percent of the population are heterozygous for the D allele?

$$f(D/D) + f(D/d) = .84; \text{ Since } f(D/D) + f(D/d) + f(d/d) = 1, f(d/d) = 1 - .84 = .16$$

$$f(d/d) = .16, \text{ so the frequency of } d = .4$$

$$D + d = 1, \text{ so the frequency of } D = 1 - .4 = .6$$

$$f(D/d) = 2 (D) (d) = 2 \times .6 \times .4 = .48$$

(b 6 pts.) The population in part (a) mixes in equal numbers with a population in which all of the individuals express the blood antigen. After one generation of random interbreeding between the two populations what percent of the population will express the antigen?

	<u>f(D)</u>	<u>f(d)</u>
Population 1	.6	.4
Population 2	1	~0, so $d^2 = 0$

$$\begin{aligned} \text{Mixed Population: } D_{\text{new}} &= (.5) f(D_{\text{population 1}}) + (.5) f(D_{\text{population 2}}) \\ &= (.5) .6 + (.5) 1 \\ &= .8 \end{aligned}$$

$$\text{Mixed Population: } D + d = 1, \text{ so } d = .2$$

$$\begin{aligned} f(\text{individuals that express antigen}) &= f(D/D) + f(D/d) \\ &= .64 + .32 = .96 \end{aligned}$$

Name: _____

(c 9 pts.) You find an isolated population in which an unusual allele for the blood antigen (called d^*) is quite prevalent. By analyzing the DNA from this population you find that 18% of the population has genotype D/d^* , but none of the thousands of individuals examined has genotype d^*/d^* . Explain this result by assuming that d^* is a balanced polymorphism. For your answer calculate parameters h (the heterozygous advantage for d^*) and S (the selective disadvantage of d^*/d^*).

$$d^* = \frac{1}{2} f(D/d^*) + f(d^*/d^*); \text{ Since } f(d^*/d^*) = 0, d^* = \frac{1}{2} f(D/d^*)$$

$$d^* = \frac{1}{2} f(D/d^*) = 1/2 (.18) = .09$$

$$\text{OR } f(D/D) = .82 = D^2, \text{ so } D = .905 \text{ and } d^* = 1 - .905 = .095$$

$$Sq = h; \quad S=1, \text{ so } q = h = .09 \text{ or } .095$$

(d 9 pts.) Consider a population in which 1% of matings are between second cousins, yet all other matings are between unrelated individuals (second cousins have great grandparents in common). If half of the individuals with a recessive trait have parents that are second cousins, what is the (approximate) allele frequency for the trait.

The inbreeding coefficient for second cousins is $1/64$ ($F = 1/64$).

$$f(a/a) = .01 (Fq) + .99 (q^2)$$

$$\frac{1}{2} f(a/a) = .01 (Fq)$$

$$.02 (Fq) = .01 (Fq) + .99 (q^2)$$

$$.99 q = .01 (1/64)$$

$$q = 1.58 \times 10^{-4}$$