

GENE REGULATION**Example Problem**

You are studying the regulation of a yeast enzyme Cal1, whose activity you can measure directly. Expression of the gene encoding Cal1 is inhibited by the presence of calcium ions. You perform a genetic screen looking for mutants that do not properly regulate expression of this enzyme. In this screen, you isolate a mutant that you call "Cal2-." You analyze the following strains, with the following results:

		Units of Cal1 enzyme activity	
		- Ca	+Ca
Cal1+	Cal2+	200	3
Cal1-	Cal2+	0	0
Cal1+	Cal2-	3	3
Cal1-	Cal2+ / Cal1+ Cal2+	200	3
Cal1+	Cal2- / Cal1+ Cal2+	200	3

Sporulation of diploids of the genotype Cal1- Cal2+ / Cal1+ Cal2- yields only one type of tetrad. All 40 tetrads that you analyze look as follows. Each column below contains the four colonies that grew up from each tetrad you have analyzed. Dark colored circles would indicate yeast colonies that are strongly expressing Cal1.

- Ca	+Ca
○	○
○	○
○	○
○	○

- (a) Categorize this type of tetrad as PD, NPD, or TT.
- (b) Can you conclude whether the Cal1 and Cal2 loci are linked? **If you can conclude**, state whether they are tightly linked, loosely linked, or unlinked.
- (c) Classify Cal2- as constitutive or uninducible.
- (d) Can you conclude if Cal2- is dominant or recessive? Why or why not? **If you can conclude**, state whether Cal2- is dominant or recessive.
- (e) Can you conclude if Cal2- is cis-acting or trans-acting? Why or why not? **If you can conclude**, state whether Cal2- is cis-acting or trans-acting.
- (f) List **all** of the possible kinds of mutations that Cal2- could be with respect to Cal1 (your choices are: repressor -, activator -, UAS-, URS-, super activator, super repressor, dominant negative repressor, dominant negative activator).

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- (g) State the genotype of one strain that you could make to distinguish between all of the models that you listed in part (f) for the kind of mutation that Cal2– is. **For each model**, state what phenotype (uninducible, constitutive, OR regulated normally) that strain would display.
- (h) The strain that you described in part (g) actually allows you to perform two different genetic tests at once, using only one strain. What are the two genetic tests that you simultaneously performed using the strain from part (g)?

GENETIC ENGINEERING OF MICE

Example Problem

You have a **true-breeding mutant mouse** that displays the phenotype of blue fur color. You determine that this phenotype is caused by a specific allele of the “GO3” gene called GO3*. You isolate the GO3 gene from this **blue mutant mouse**, and inject it into a fertilized egg produced by the mating of two grey wild-type mice. You then transfer this injected fertilized egg into a pseudopregnant mouse. You analyze the transgenic mouse that is born.

Your friend performs the same experiment with GO3* to create his own transgenic mouse.

Rationalize each of the following scenarios.

- (a) **Scenario #1:** All of the transgenic mice have wild type grey fur color.

What new strategy could you possibly use to create a mouse with blue fur color?

- (b) **Scenario #2:** Mice that are homozygous for your transgene insertion have a severe heart condition while your friend’s mice do not.
- (c) **Scenario #3:** Cells isolated from your friend’s mouse express twice as much GO3* mRNA transcript as cells isolated from your mouse.

Example Problem

Many mouse genes are expressed in a “tissue-specific” manner; that is, the genes themselves are present in all cells in the body, but the genes are expressed (transcribed and translated) in only one of the animal’s many tissue types.

(a) Devise a mouse model (using pronuclear injection) to determine what tissues the mouse amylase gene is expressed.

- What DNA construct would you use?
- Add DNA to fertilized egg or ES cell?
- Genotype of fertilized egg or ES cell?
- Where does construct integrate?
- Chimera generated?
- Additional breeding required?
- How would you analyze the phenotype of the mouse?

(b) Would microinjection of your construct into the male pronucleus of a fertilized egg likely result in integration of the construct into the copy of the amylase gene present in the male pronucleus? Briefly explain your answer.

(c) You make one strain of mice that are heterozygous for your transgene. These mice display LacZ expression exclusively in the pancreas. Would you expect homozygotes for the transgene to also display LacZ expression in the pancreas? Elsewhere?

(d) 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.

- What DNA construct would you use?
- How would you analyze the phenotype of the mouse?

Population Genetics Review Questions:

1. What equations can you use if the population is NOT in Hardy-Weinberg equilibrium?

2. Assume you have a Hardy-Weinberg population of individuals with a constant mutation rate of 10^{-5} . The population is made up of individuals with alleles for short height and tall height. Normally, the short individuals have a harder time reaching food out of cupboards and have a 60% chance of surviving starvation during childhood.

a) If the allele for short height is rare **autosomal recessive**, calculate the short height allele frequency. Show the steady-state equation you would use.

b) If the allele for short height is rare **autosomal dominant**, calculate the short height allele frequency. Show the steady-state equation you would use.

c) If the allele for short height is rare **X-linked recessive**, calculate the short height allele frequency. Show the steady-state equation you would use.

d) Take the same population from 2. A group of man-eating mountain lions are introduced into the environment and the short elderly individuals have a 25% chance of being eaten by these predators.

If the allele for short height is rare **autosomal recessive**, calculate the short height allele frequency. Show the steady-state equation you would use.

e) Take the same population from part 2. Suppose a strain of flesh-eating bacteria is introduced into the environment, but this strain of flesh-eating bacteria is sensitive to a protein excreted by cells on the surface of the skin of individuals who are heterozygous for the short height allele. These heterozygotes have 95% better chance of surviving an infection by the flesh-eating bacteria.

If the allele for short height is rare **autosomal recessive**, calculate the short height allele frequency. Show the steady-state equation you would use.

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3. Assume you have a population in Hardy-Weinberg equilibrium. Suddenly, some inbreeding begins to take place among members of the population. How does inbreeding affect allele frequency, in the following situations? (increase, decrease, or stay the same)

a) For an autosomal recessive allele, with fitness = 1.0? _____

b) For an autosomal recessive allele, with fitness = 0? _____

c) For an autosomal dominant allele, with fitness = 0.1? _____

4. Draw a pedigree below that shows a mating between two relatives that would correspond to an inbreeding coefficient that equals 0.00390625.

5. A ship carrying 7,000 passengers is about to land on an island that has 33,000 occupants. Each of these two populations is at Hardy-Weinberg equilibrium before the ship's landing, and each population contains an equal number of males and females. Of the 7,000 ship passengers, only 21 are displaying the X-linked recessive trait "huge toes" (and all 21 are male). Of the 33,000 island occupants, only 6 have huge toes (and all 6 are male). When answering the following parts, show all of your calculations.

(a) On the ship before landing, what is your best estimate of the allele frequency for the allele that causes huge toes?

(b) If you select a female child at random from the island (before the ship lands), what would the probability be that she is a carrier of the allele for huge toes?

(c) Now the ship lands on the island, and the passengers and island occupants mate together randomly to produce the next generation "G2". What is your best estimate of the allele frequency for the allele that causes huge toes in generation "G2"?

(d) If you select one child from generation "G2" at random, what would the total probability be that it has huge toes? (Take both males and females into account.)

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