

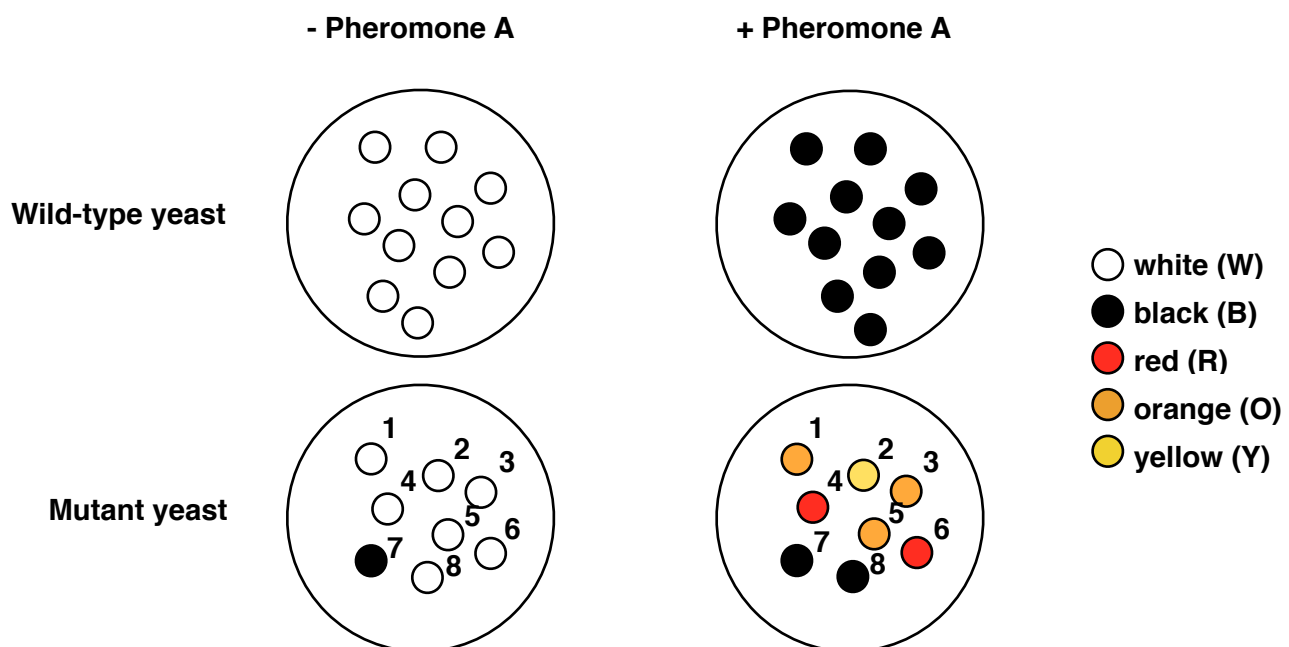
Bio393: Genetic Analysis
Problem Set #4
Due on Friday, February 9, 3 PM

Name: _____

Question 1:

Your lab studies how yeast respond to different chemical signals (pheromones). You work with a newly isolated haploid strain. When you grow colonies of this yeast on a petri plate, the colonies are white. If you grow the bacteria on a petri plate with Pheromone A, the colonies are black!

You want to understand how this response works and decide to perform a mutant screen. You mutagenize the yeast and plate all of the resulting mutants on petri dishes with rich media but no Pheromone A. You then replica plate the mutants onto petri dishes with rich media plus Pheromone A. Below are some of your results:



	WT	mut1	mut2	mut3	mut4	mut5	mut6	mut7	mut8
no pheromone	W	W	W	W	W	W	W	B	W
pheromone A	B	O	Y	O	R	O	R	B	B

(a) You know that you mutagenized 1000 cells, but when you plated the mutants onto rich media without Pheromone A, only 800 colonies grew. Why did 20% of the cells die?

(b) Of the colonies that grew, you isolated seven mutants (labeled 1-7 above). Why is colony 8 not interesting to you?

You perform complementation tests among the eight isolated mutants and grow the diploid strains in the presence of pheromone A. You get the following results:

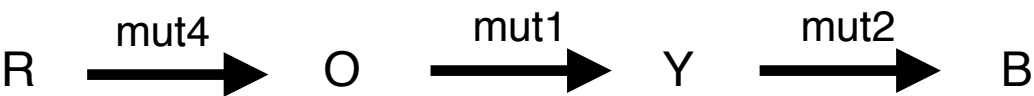
	WT	mut1	mut2	mut3	mut4	mut5	mut6	mut7	mut8
WT	B	B	B	B	B	B	B	B	B
mut1		O	B	O	B	O	B	B	B
mut2			Y	B	B	B	B	B	B
mut3				O	B	O	B	B	B
mut4					R	B	R	B	B
mut5						O	B	B	B
mut6							R	B	B
mut7								B	B
mut8									B

(c) Can you tell which of these mutations confers a dominant phenotype?

(d) How many genes are involved in the black pigment biosynthetic pathway?

(e) You decide to use your mutants to order the genes in the black pigment biosynthetic pathway. You do not know the intermediates involved, but you can tell when a particular intermediate builds up because of the color of the colony. In order to build the pathway, you need to make double mutants. Using two representative mutations in different genes, show how you would build a double mutant strain. For this exercise, assume that each gene is unlinked from each other and from the mating locus.

(f) You construct double mutant cells, where each mutation is in a different gene (e.g. mut1 mut2 is mutated for both the mut1 and mut2 genes). You then grow each of your double mutants in the presence of Pheromone A. Given the pathway below, fill in the chart for the single and double mutant phenotypes in the presence of Pheromone A.



	+ Pheromone A
WT	
mut1	
mut2	
mut4	
mut1 mut2	
mut1 mut4	
mut2 mut4	

Question 2:
 Ever since childhood, you have often wondered how do fish control their depth in the water. You know that the swim bladder can inflate or deflate to move the fish up or down in the water, but how is the swim bladder made? After Bio393, you join a zebrafish lab to do a mutant hunt for swim bladder defective mutants. You identify mutant fish that are either floaters or sinkers (bladder (bl) mutants). Floaters have defective swim bladders that are constitutively inflated, while sinkers have swim bladders that are constitutively deflated.

(a) Did you perform a screen or a selection? Describe the logic behind your answer.

(b) The mutant fish fall (or maybe sink - ha!) into three complementation groups (bl1 through bl3) with one additional mutant that has a dominant hypermorphic phenotype (bl4). You want to figure out the swim bladder regulatory pathway, so you make double mutants to measure genetic interactions. The phenotypes are below:

Genotype	Phenotype
bl1	partially sinks
bl2	floats
bl3	floats
bl4	sinks
bl1 bl2	floats
bl1 bl3	floats
bl1 bl4	sinks
bl2 bl3	floats
bl2 bl4	sinks
bl3 bl4	floats

Draw out the gene regulatory pathway for swim bladder inflation.

(c) The bl1 mutant only partially sinks. Propose two explanations for this mutant phenotype.

Question 3:

You are a beginning faculty member and have decided to genetically dissect the process of sex determination in the Mediterranean fruit fly (medfly) *Ceratitis capitata*, reasoning that such knowledge might allow development of control procedures for this invasive crop pest. Also, it might bring you much needed funding and respect. Fortunately, you met a researcher who already has sex determination mutants but has not studied them. You decide to collaborate.

(a) In case the collaboration goes sour, describe how you would isolate a heat-sensitive mutant that when homozygous causes males to develop as females? This mutation would have no effect on females. Also, remember there are no balancers in medflies.

You collaborator has three mutants (fem, del1, and del2) that all causes males to develop as females. You would like to know if they have mutations in the same gene. fem is temperature-sensitive; del1 is cold-sensitive; and del2 has a recessive phenotype that is not cold or temperature-sensitive.

(b) If del1 and del2 have mutations in the same gene and fem complements both del1 and del2, describe the conditions under which you would do the complementation tests and the results that would be expected.

Another mutant called lx has a dominant phenotype that causes males to develop as intersexes (a mix of male and female).

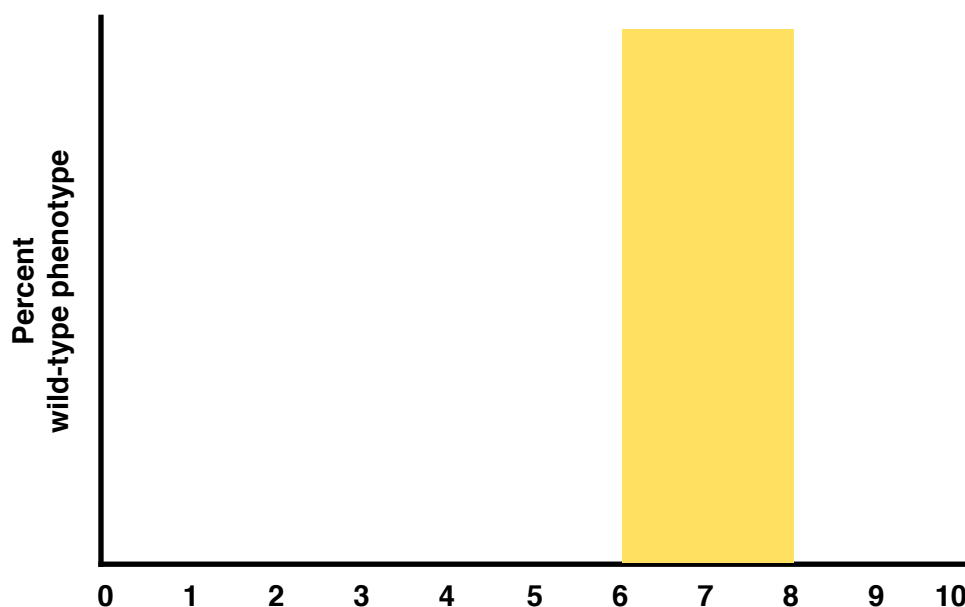
(c) Using the mutagen EMS, how can you determine if the mutated locus is haploinsufficient or a gain-of-function?

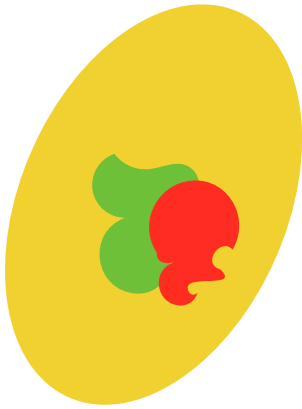
Question 5:

Draw a cross to differentiate between maternal-effect inheritance and cytoplasmic inheritance? Hint: think about multiple generations.

Question 6:

Using a temperature-sensitive allele, you perform upshift and downshift experiments over the course of ten hours with a shift every hour. After the ten hours is complete, you measure the penetrance of the mutant phenotype. You find that the temperature-sensitive period is between six and eight hours. Please draw the upshift (solid line) and downshift (dotted line) on the graph below.



**Question 7:**

In *Drosophila*, you can generate twin spots using cell-specific markers. In the example below, red ommatidia are homozygous for the mCherry gene, green ommatidia are homozygous for the GFP gene, and yellow ommatidia are heterozygous for mCherry and GFP. Draw out the diploid homologous chromosomes with centromeres demarcated as open and closed circles and locations of the GFP and mCherry insertions that would lead to this mitotic recombination result.

Question 8:

A developmental geneticist at the University of Toronto identified four different promoters that drive expression of any gene in different parts of an isopod. She sends you the promoter sequences for expression in carapace, legs, antennae, and the whole animal. She also helps you to make transgenic isopods. You drive expression of the wild-type *red* gene, which when mutated makes the red color phenotype, using all four promoters.

Describe the experiment (strains, promoters, etc.) that will determine where the function of the *red* gene is required in the animal using these reagents and any mutant or wild-type strains. Assume that the *red* mutant phenotype is recessive.