Bio393: Genetic Analysis **Name:**_____
Problem Set #2
Due on Friday, April 24, 2 PM

Question 1:
You isolate ten new mutant yeast strains that are defective in synthesis of leucine, an amino acid. These Leu- mutants (numbered 1-10) were all isolated in a strain of mating type a (MAT a). *S. cerevisiae* yeast are either mating type a or α. As it turns out, your high school classmate, now at the University of Chicago, has independently isolated ten yeast Leu- mutants (numbered 11-20) in a strain of mating type α (MAT α). You and your ex-classmate decide to combine your resources and determine how many different genes are represented by your 20 mutant strains. You cross each of the MAT a strains to each of the MAT α strains. Your experimental observations are shown in the table below, where an empty square indicates that the diploid did not grow on minimal medium and a filled square indicates that the diploid did grow on minimal medium.

a/α	1	2	3	4	5	6	7	8	9	10
11	■	■		■	■		■	■	■	■
12	■	■	■	■			■	■	■	■
13	■	■	■	■	■		■	■	■	
14	■	■	■	■	■		■	■	■	
15	■	■	■	■			■	■	■	■
16			■		■		■		■	■
17	■	■		■	■		■	■	■	■
18	■	■	■	■	■			■		■
19										
20	■	■		■	■		■	■	■	■

(a) What property do mutants 6 and 19 share?

(b) Which mutations do you know to be in the same gene?

(c) Could mutations 6 and 10 be in the same gene?

(d) Based on this experiment, what is the minimum number of genes required for leucine synthesis?

(e) Based on this experiment, what is the maximum number of genes required for leucine synthesis?

Question 2:
One way to isolate nonsense suppressor mutations in tRNA genes is to select for the simultaneous reversion of nonsense mutations in two different genes. This selection works because it is extremely unusual to get back mutations in two different genes at the same time. The yeast *HIS1* and *HIS2* genes are required for histidine synthesis and strains harboring mutations in either gene will not grow unless histidine is provided in the growth medium.

(a) If you wanted to isolate nonsense suppressor mutations, explain why it would be a bad idea to start with a strain that has an amber mutation (TAG) in *HIS1* and an ochre mutation (TAA) in *HIS2*.

Instead of starting with a double mutant, you start with a strain containing an amber mutation in just *HIS1*. After mutagenesis with EMS, you select his+ revertants by their ability to grow on medium without histidine. In this case, it is necessary to consider the possibility of a back mutation in *HIS1* as well as extragenic suppressor mutations in tRNA genes.

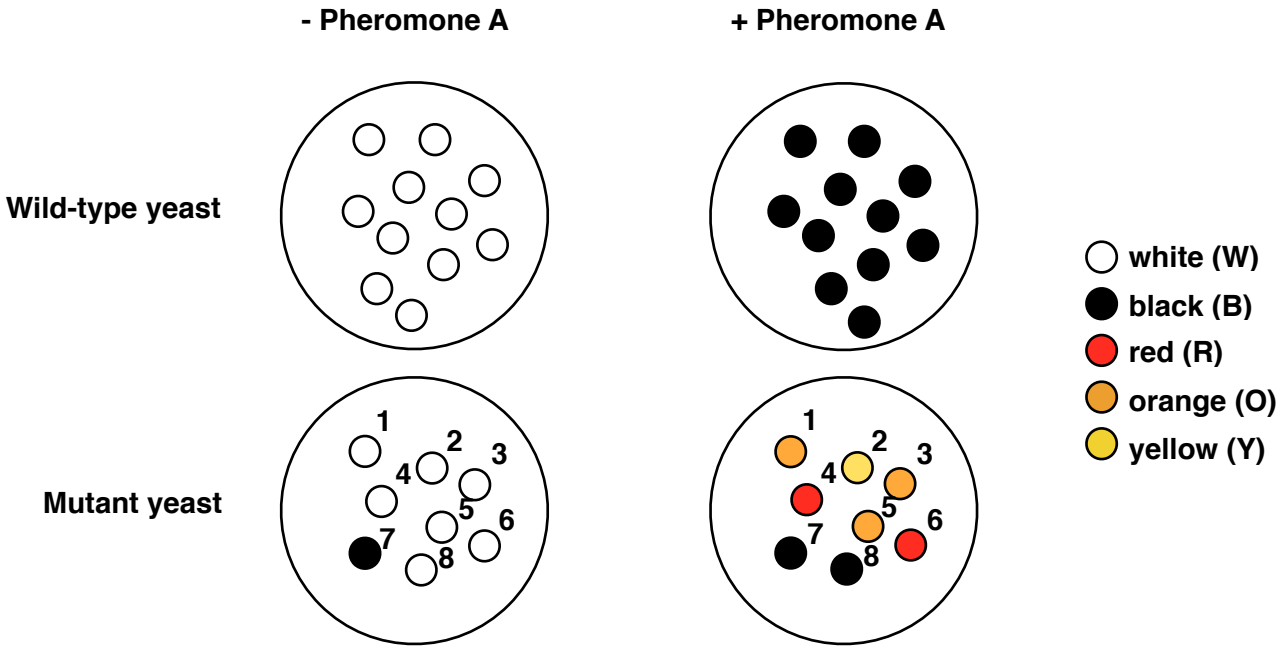
(b) Explain why it would be very unlikely in this case to acquire an intragenic suppressor mutation in *HIS1*.

Question 3:
(a) Assume that hairy toes are inherited as a recessive trait with 80% penetrance in humans. A couple decide to have a baby but worry about the stigma of hairy toes. This malady affected both of their fathers. What is the probability that their first child will have hairy toes?

(b) After taking a genetics class, the mother decides that the risk of a hairy-toed baby is worth it. She believes that the trait has variable expressivity. How would she be able to tell?

Question 4:
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:

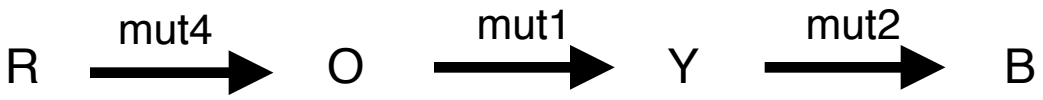
	WT	mut1	mut2	mut3	mut4	mut5	mut6	mut7	mut8
WT	B	B	B	B	B	B	B	B	B
mut1		O	B	O	B	O	B	B	B
mut2			Y	B	B	B	B	B	B
mut3				O	B	O	B	B	B
mut4					R	B	R	B	B
mut5						O	B	B	B
mut6							R	B	B
mut7								B	B
mut8									B

(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	Black
mut1	
mut2	
mut4	
mut1 mut2	
mut1 mut4	
mut2 mut4	

Question 5:
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.

(d) What type of screen would you perform to isolate mutants to test between the two models proposed in part (c)? Describe the crosses to separate these two possibilities.