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? *dominant phenotype*

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

[1,2,4,8,16] [3,11,17,20] [5,12,15] [7,9,18] [10,13,14]

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

(d) Based on this experiment, what is the minimum number of genes required for leucine synthesis?
Five
(e) Based on this experiment, what is the maximum number of genes required for leucine synthesis?
Seven - five that have a recessive phenotype and two that have a dominant phenotype

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*.

You would need nonsense suppressors for both the amber and ochre stop codons. Those two suppressors are encoded by different genes. Therefore, it would be very difficult to identify mutations in both genes in one selection.

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*.
The amber mutation in HIS1 causes an early stop in the coding sequence. Intragenic suppressors act by eliminating or reducing the effect of the first mutation in the gene. It is difficult to envision how the effects of a truncated protein could be alleviated by changing additional amino acids in the same gene.

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?

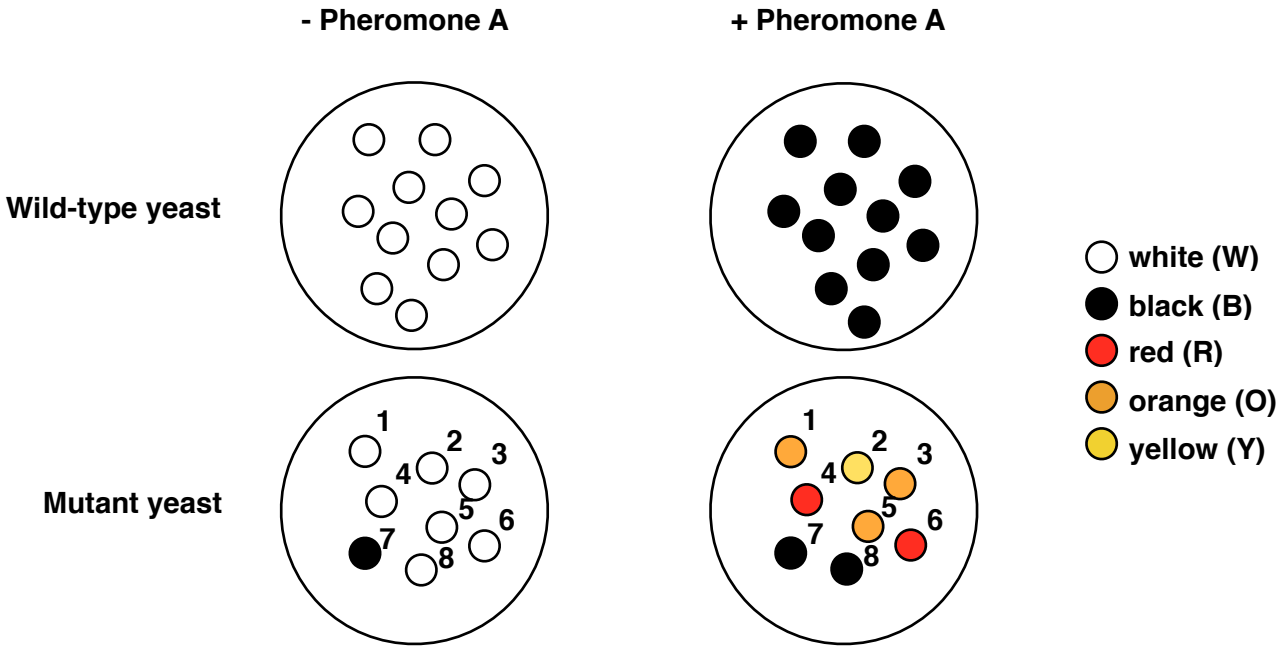
*Because both the man and woman had fathers with hairy toes, they must be carriers for this trait. Therefore, they have a 25% chance that their child with be homozygous for the allele that causes the recessive hairy toe trait. Because the penetrance is 80%, even if the child inherits both hairy toe alleles, he/she might not have hairy toes. 0.25 * 0.80 = 0.2 or 20% chance of having 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?

She would see that her hairy-toed baby has more or less severe hairy toes that her father or father-in-law. Of course, she would likely hope that her baby has a less severe hairy toe phenotype.

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?

Mutagenesis induces random mutations throughout the genome. Those cells likely had mutations in essential genes and could not grow.

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

Colony 8 has the wild-type phenotype. We are only interested in mutants.

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?

Mutant 7 has a dominant black pigment phenotype.

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

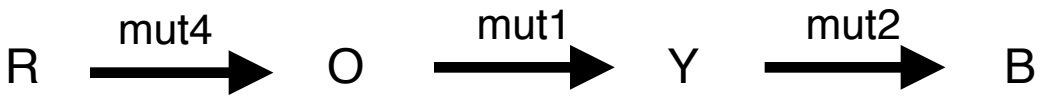
[1,3,5] [2] [4,6]

(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.

You have to build the strains without knowing the genetic interactions and then confirm the presence of the two mutants using complementation.

For example, let's build the mut1 mut2 double mutant. Cross mut1 haploid cells to mut2 haploid cells to get heterozygotes for both. In the spores generated from meiosis of the diploid cell, some tetrads (or groups of four spores) will have four different genotypic classes (tetratypes for aficionados). The four different genotypic classes will be observed because one spore will have the wild-type phenotype and the other three will be two single mutants one double mutant. Because you do not know which one is a single or a double mutant, you grow up cultures of each mutant spore to get a population of haploids. Mate each of these haploid mutants to both mut1 and mut2 single mutants. Double mutants should fail to complement the mutant phenotype of both mut1 and mut2.

(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	*orange*
mut2	*yellow*
mut4	*red*
mut1 mut2	*orange*
mut1 mut4	*red*
mut2 mut4	*red*

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.

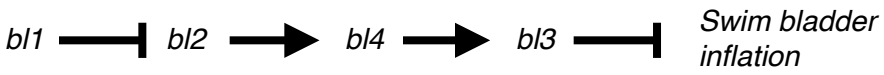
(a) Did you perform a screen or a selection? Describe the logic behind your answer.

In principle, you could perform either, but a selection would be easier. After mutagensis and crossing to generate homozygous mutant animals, you can collect mutants that are found at the top of the tank (floaters) and those mutants that are always at the bottom of the tank (sinkers). You do not have to go through all of the other wild-type animals that are swimming up and down in the water.

(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.

(1) The bl1 mutant could be a hypomorph.
(2) The bl1 mutant could be redundant with some other gene.
(3) The bl1 mutant phenotype could have variable expressivity. Because I didn't say that it was a strain, you don't know. If the bl1 mutant stock had some partial sinkers and some complete sinkers then it could be variable expressivity.

(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.

For the sake of clarity, let's assume you have a strain of bl1 and that the mutant phenotype is partially sinking for every mutant animal.

A non-complementation screen for bl1 null mutants might differentiate (1) and (2). Null alleles of bl1 would have a full sinker phenotype and would enhance the bl1 sinker phenotype as heterozygotes. If no strong sinker mutants are identified then it might be a redundant pathway because in the F1 all animals will be heterozygous for the enhancer mutant and the bl1-1 mutation. In other words, you might already be looking at the null phenotype of bl1 in the bl1-1 mutation. New alleles can not get more severe.