Bio393: Genetic Analysis **Name:**_____
Problem Set #2
Due on Friday, January 19, 3 PM, Cook 3125

Question 1:
You perform a selection for mutant *Arabidopsis* plants that can grow in the presence of high salt. You get four mutants (m1-m4). To determine how many genes are mutated, you perform

	m1	m2	m3	m4
m1	Alive	Dead	Alive	Dead
m2	Dead	Alive	Dead	Alive
m3	Alive	Dead	Alive	Dead
m4	Dead	Alive	Dead	Alive

(a) You were lucky that your results were interpretable. What should you have done first?

(b) How many genes are there?

Question 2:
PhiP and IQ are heterocyclic amines that are mammary gland carcinogens in mice. Both of these chemicals are present in certain food products such as cooked meats. To better understand the biology behind the carcinogenic properties of PhiP and IQ, we would like to identify genes that protect cells from their toxicity.

Wild-type *Saccharomyces cerevisiae* yeast grow at a reduced rate in the presence of 50 mM PhiP but arrest completely in the presence of 100 mM PhiP.

Using yeast, design a screen to isolate mutants that are hypersensitive to PhiP. Be as specific as possible.

Question 3:
The roundworm nematode *Caenorhabditis elegans* is a powerful and tractable model to understand how parasitic roundworms become resistant to anthelmintic (anti-nematode) compounds. Design a screen to identify strains that are resistant to albendazole and have mutant alleles on chromosome III. Resistant mutants will survive when grown on plates containing albendazole. You have albendazole and normal worm plates for propagating *C. elegans*, the mutagen EMS, the laboratory wild-type strain N2, and triple mutant strain *bli-3; lin-1; unc-32*. The triple mutant strain is true-breeding for mutant alleles that each confer recessive phenotypes. *bli-3* is located on chromosome I and causes a recessive Blistered phenotype. *lin-1* is located on chromosome II and causes a recessive Multivulva phenotype. *unc-32* is on chromosome III and causes a recessive Uncoordinated phentoype. All three mutant phenotypes can be observed in the same mutant worm.

Question 4:
Billy Bob wants to study the function of the yeast *URA5* gene, which encodes an enzyme that catalyzes a step in uracil biosynthesis. To begin his study, he plans to design an experiment to look for mutations in the *URA5* gene that affect its function. He knows that having non-functional *URA5* gene allows for growth of yeast on medium containing 5-fluoro-orotic acid (5-FOA) (because wild-type cells catalyze a reaction that turns 5-FOA into a toxin that kills the cell). For his screen, he used the following protocol:

1. Mutagenize wild-type yeast with appropriate treatment.
2. Spread mutagenized cells on 10 plates (Set #1).
3. Let the cells grow into well-separated colonies.
4. Replica plate colonies onto new plates (Set #2) to detect ura5 mutant strains.

(a) Which type of plate did Billy Bob use for set #1? for set #2? Explain your answer.

(b) What phenotype is expected for *ura5* mutant strains?

Three days later, when he looks at the second set of plates, Billy Bob is crestfallen to realize that he sees NO colonies that fit his criteria for potential *ura5* mutant strains.

(c) Explain to him why he might not have found any strains of interest, even if the mutagenesis in Step 1 worked.

Following your thoughtful explanation, Billy Bob decides to try to use selection to find strains with mutations in the *URA5* gene. He once again mutagenizes wild-type cells and then plates cells on a set of 10 plates.

(d) What type of plates did Billy Bob use for his selection and why?

(e) What phenotype does he expect to see for *ura5* mutant strains?

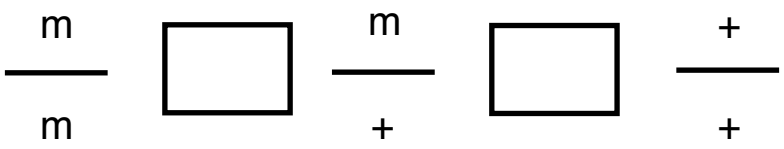
Billy Bob is ecstatic to find that his selection has worked--he finds strains with the appropriate phenotype. However, his enthusiasm is a bit damped when he realizes that he cannot yet be SURE that these strains have mutations in the *URA5* gene.

(f) Why can't he be sure that these strains have *ura5* mutations?

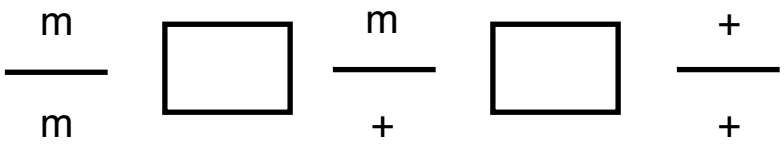
Question 5:
For the following questions, write the phenotypic relationships that would show the mutation effect in the boxes.

Use > or < symbols to denote when mutant phenotypes will be worse or better (or = equal).

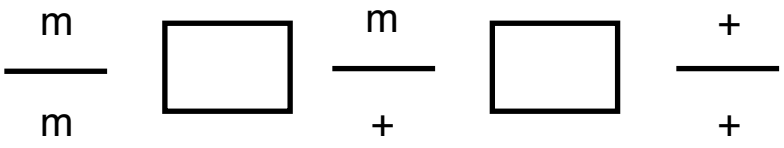
(a) Hypermorph (increase in wild-type function)



(b) Neomorph (altered function)



(c) Hypomorph (partial loss of gene function)



Question 6:
You are interested in motivated behaviors and have experience with the fruit fly *Drosophila*. You design a phenotype assay where you shoot the flies with a laser and observe how quickly they fly away from the heat stimulus.

(a) Draw out the genotypes and crosses for how you would generate flies with mutations on chromosome II to assay in your new system. You have a wild-type stock, a strain with the genotype Sk/Cyo, and a bottle of EMS. Sk causes a dominant streak phenotype, and CyO is a second chromosome balancer with a dominant curly wing phenotype.

(b) You identify 10 mutant strains in your screen. Four have dominant phenotypes, and six mutants have recessive phenotypes. All six mutants are defective in the same gene. You rudimentarily map the mutant alleles so that you can use different deficiencies (Df) and duplications (Dp) to test the genetic effects of the individual mutant alleles using dosage. Remember that deficiencies have one fewer copy of the wild-type allele of the gene mutated from the screen, and duplications have one more copy of the wild-type allele of the gene mutated from the screen. Please use the following tables to describe how do the mutations affect gene function.

Mutant #1 (m1)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m1/+	2 sec
m1/m1	4 sec
m1/Df	4 sec
m1/Dp	1 sec
+/Df	0.01 sec
+/Dp	0.01 sec

Mutant #2 (m2)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m2/+	~10 sec
m2/m2	~10 sec
m2/Df	~10 sec
m2/Dp	0.01 sec
+/Df	~10 sec
+/Dp	0.01 sec

Mutant #3 (m3)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m3/+	6 sec
m3/m3	12 sec
m3/Df	4 sec
m3/Dp	10 sec
+/Df	0.01 sec
+/Dp	4 sec

Mutant #4 (m4)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m4/+	5 sec
m4/m4	5 sec
m4/Df	5 sec
m4/Dp	5 sec
+/Df	0.01 sec
+/Dp	0.01 sec

For mutants #5-10, you perform similar tests. Please write out the results as an allelic series (*e.g.* m5>m10.

Mutants #5-10 (m5-m10)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m5/+	0.01 sec
m6/+	0.01 sec
m7/+	0.01 sec
m8/+	0.01 sec
m9/+	0.01 sec
m10/+	0.01 sec
m5/m5	~10 sec
m6/m6	~10 sec
m7/m7	4 sec
m8/m8	3 sec
m9/m9	~10 sec
m10/m10	3 sec

Mutants #5-10 (m5-m10)	
Genotype	**Phenotype**
Df/Df	~10 sec
m5/Df	~10 sec
m6/Df	~10 sec
m7/Df	~10 sec
m8/Df	8 sec
m9/Df	~10 sec
m10/Df	~10 sec
m5/m8	8 sec
m6/m8	8 sec
m7/m8	6 sec
m8/m8	3 sec
m9/m8	8 sec
m10/m8	4 sec

Question 7:
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?