**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 complementation tests and get the following results when plants are grown on high salt.

| | 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?

***Check whether the mutant phenotypes are dominant or recessive. If the phenotype is dominant, then you might be confused about a failure to complement.***

**(b)** How many genes are there?

***2***

**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.

*Mutagenize wild-type yeast and plate many cells on complete media lacking PhiP. After colonies have grown up, replica plate the cells onto a complete media plate with 50 mM PhiP. Compare the colonies that grow on the complete media plates with and without 50 mM PhiP. Colonies that fail to grow on 50 mM PhiP are hypersensitive to the compound.*

**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 with recessive albendazole resistance 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 phenotype. All three mutant phenotypes can be observed in the same mutant worm.

*Use EMS to mutagenize +/+ males then cross to bli-3; lin-1; unc-32 hermaphrodites. The cross progeny will not be blistered, multivulva, or uncoordinated because those mutations cause recessive phenotypes. These animals should be grown on normal plates. If the mutation is on the third chromosome, the genotype is:*

$$\frac{\textit{unc-32} \quad +}{+ \quad \textit{mut}}$$

*When this hermaphrodite is allowed to cross with itself (self) on albendazole plates. Only homozygous m/m mutants will survive. If the mutation is linked to unc-32, then you will never (or rarely) see uncoordinated animals that are resistant to albendazole. If the mutation is linked to other chromosomes, then you will see 25% of the albendazole resistant progeny are also uncoordinated.*

**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. 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.
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.
*He performed a genetic screen for mutants that require uracil to survive. For set #1, he used complete media plates so that all cells (non-mutant and mutant) grow. For set #2, he used complete media lacking uracil.*

**(b)** What phenotype is expected for *ura5* mutant strains?
*Mutants in ura5 require uracil to grow. Therefore, they would grow on set #1 plates but fail to grow on set #2 plates.*

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 criterion 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.
*Three reasons: (1) He might not have screened enough cells. Mutagenesis is a random process. In a perfect screen, one needs to score enough cells so that every gene in the genome has been mutated. Oftentimes, this level of screening is not possible or practical. (2) Mutants that require uracil for survival might also be inviable when grown on complete media. In other words, these mutants might be lethal regardless of uracil status. (3) Some functions have multiple genes that act redundantly. One would need to mutate two (or more) redundant genes to see a uracil requirement phenotype. Option #1 is the most likely for this phenotype.*

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?
*He used plates with complete media and 5-FOA. In this way, wild-type cells will die because they will turn 5-FOA into a toxin that will kill the cell. Mutant cells that require uracil will not process 5-FOA and will survive on those plates.*

**(e)** What phenotype does he expect to see for *ura5* mutant strains?
*Survival on 5-FOA plates*

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?
*These strains could have mutations in other ura genes not ura5.*

**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)

$$\frac{m}{m} \quad \boxed{>} \quad \frac{m}{+} \quad \boxed{>} \quad \frac{+}{+}$$

**(b)** Neomorph (altered function)

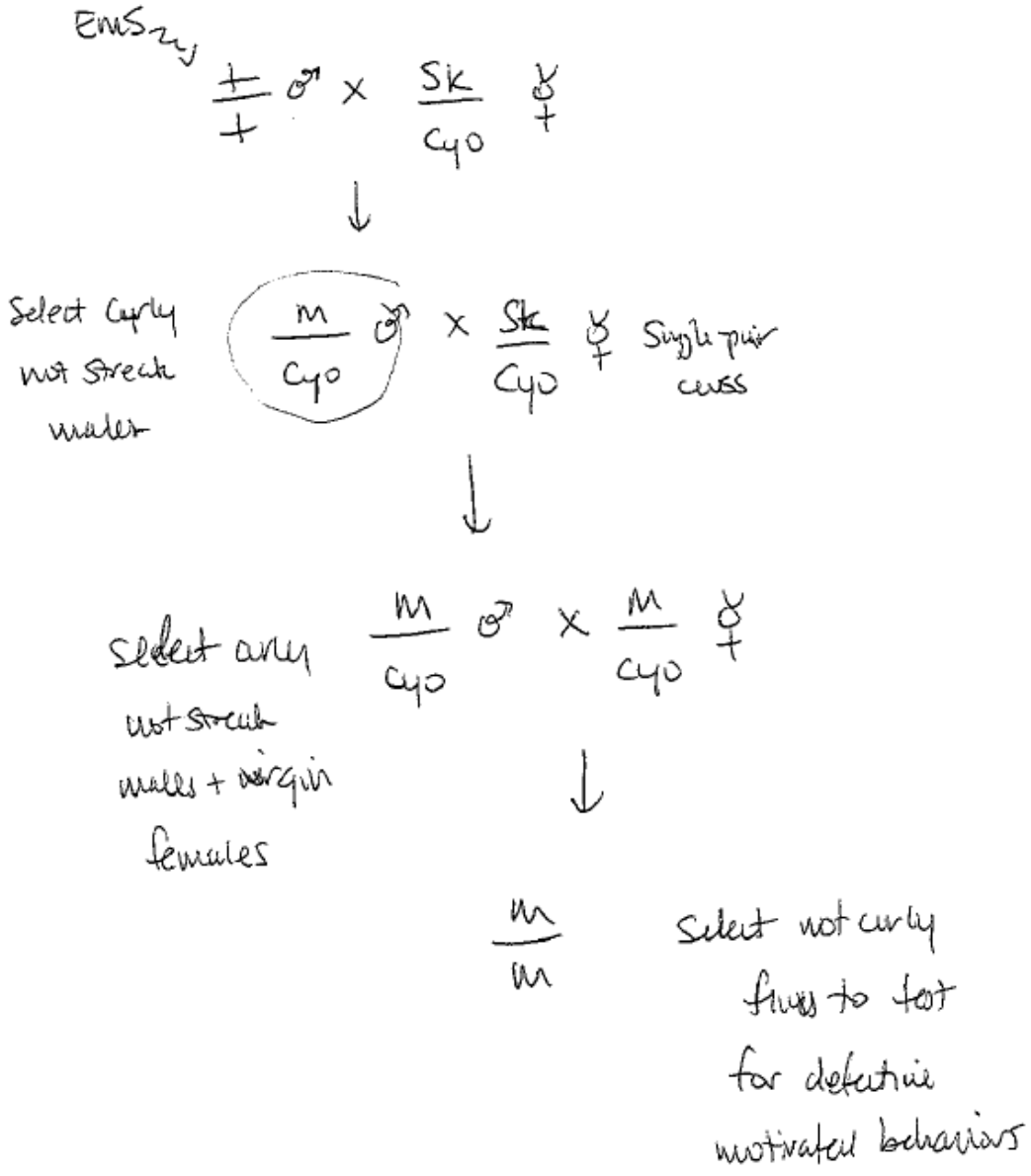
$$\frac{m}{m} \quad \boxed{= \text{ or } >} \quad \frac{m}{+} \quad \boxed{>} \quad \frac{+}{+}$$

**(c)** Hypomorph (partial loss of gene function)

$$\frac{m}{m} \quad \boxed{>} \quad \frac{m}{+} \quad \boxed{=} \quad \frac{+}{+}$$

**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 is on chromosome two. 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 with recessive phenotypes 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 |

*m1 is a dominant negative (antimorph) because its mutant phenotype gets less mutant with wild-type copies of the gene.*

| **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 |

*m2 is a haploinsufficient locus. It has a dominant phenotype observed in the deletion heterozygote.*

| **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 |

*m3 is a hypermorph causing an increase in wild-type gene function. Its mutant phenotype becomes worse with more wild-type or mutant copies of the locus.*

| **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 |

*m4 is a neomorph causing an altered gene function. Its mutant phenotype is not modified by extra or fewer wild-type gene copies.*

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 |

*All mutants cause a recessive phenotype. m5, m6, and m9 cause a complete loss of gene function because their mutant phenotypes do not get worse when combined with a deficiency. m7, m8, and m9 cause a partial loss of function or they are hypomorphs. m8 is the least mutant because its mutant phenotype retains the most wild-type function when combined with a deficiency. The homozygous m7 phenotype is more severe than the m10 phenotype. The allelic series order is:*

*m6 = m9 = m5 > m7 > m10 > m8*

**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? *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*