Bio393: Genetic Analysis **Name:**_____
Midterm (8 pages, 100 points)

Question 1 (9 points):
You are interested in optimizing corn ear size in *Zea mays* so you perform a screen for larger ears. A rare mutant is identified with large ears. Upon backcrossing and selfing to re-isolate the mutant phenotype, you find that only 1/16 of the offspring have large ears of corn.

(a, 3 points): Propose a model for why only 1/16 of the offspring have larger ears.

Unfortunately, the larger ears are heavier and make the corn stalks bend and break more easily. Your friend has a strain with more stout stalks that could stabilize the heavier ears. His strain has normal size ears. You cross his strain with your strain and then self the resulting cross progeny. Only two plants out of 128 have big ears and more stout stalks.

(b, 6 points) Write out the cross and propose a model for the phenotypic ratio that you observe.

Question 2 (5 points):
Using linkage mapping, you determined the genetic distance between A and B is 100 cM. What is the minimum number of intermediate markers between A and B that you would need to make this estimate possible? Please explain your reasoning.

Question 3 (8 points):
Over the past summer, you mentored a high school student in the lab. He worked hard but did not take any notes or keep a lab notebook. You had him look for suppressors of a mutant phenotype caused by a point mutation resulting in a null phenotype (not necessarily a stop codon mutation).

(a) Propose a cross to differentiate revertants (or back suppressors) from extragenic suppressors and how the outcomes of the cross would show either option.

(b) Is it possible that he isolated an intragenic suppressor? Explain why or why not.

Question 4 (14 points):
Your roommate does not believe all this mumbo jumbo about genetics. He says that there is no way for you to predict the phenotypes of offspring from the following cross.

$$\frac{x-\ \ y-\ \ z-}{+\ \ +\ \ +} \times \frac{x-\ \ y-\ \ z-}{x-\ \ y-\ \ z-}$$

You look up on wikipedia that the x, y, and z genes are all linked. Also, x is 30 map units from y, and y is 10 map units from z. The gene order is x, y, z. Fill out the table below for the numbers of offspring with each phenotype (Total offspring = 1000).

Phenotype	Number of offspring
x y z	
+ + +	
x y +	
+ + z	
x + +	
+ y z	
x + z	
+ y +	

Question 5 (36 points):
RNA interference (RNAi) is a conserved endogenous process found in most organisms. You would like to understand the genes that control this amazing process, so you turn to the obligate intracellular parasite *Toxoplasma gondii* because it readily and strongly induces RNAi. You have a variety of constructs that induce RNAi to cause 100% penetrant mutant phenotypes. One such example is the gene *let-1* that causes 100% lethality in the wild-type strain when inhibited by RNAi.

(a, 10 points) Describe and write out a selection that will allow you to identify mutations in genes that promote RNAi. *T. gondii* does not have balancer chromosomes, can self cross, and (for our purposes) is diploid. You can use a mutagen.

(b, 6 points) You would also like to identify mutations in genes that inhibit RNAi. Your collaborator sends you two RNAi constructs. One construct when applied to wild-type cells causes a weakly penetrant coloration defect where ~5% of the cells are less opaque than the wild type, and the other causes a weakly penetrant disorganized movement defect where ~10% of the cells move weirdly. Both mutant phenotypes do not affect the ability of the *T. gondii* cells to grow or reproduce. Using these two RNAi constructs, describe and write out a screen to identify negative regulators of RNAi.

(c, 2 points) Why do you need to use two RNAi constructs in the screen in part (b)?

(d, 8 points) From your selection in part (a), you identify two complementation groups. Describe the cross, genotypes, and phenotypic results for two mutations that complement each other (mut1 and mut2) and then two mutations that fail to complement each other (mut1 and mut3).

(e, 10 points) You have two mutants in separate genes that promote RNAi (*rde-1* and *rde-2*; rde = RNAi defective) and two mutants in separate genes that inhibit RNAi (*eri-1* and *eri-2*; eri = enhanced RNAi). Using an RNAi construct with intermediate penetrance, you can measure enhancement or suppression of RNAi by these different mutant genes. You obtain the following results from single and double mutants. Please draw out a linear pathway for RNAi responsiveness in *T. gondii*. Note any ambiguities.

Genotype	Percent mutant after RNAi
+	50
rde-1	0
rde-2	0
eri-1	100
eri-2	100
rde-1; eri-1	0
rde-2; eri-1	0
rde-1; eri-2	100
rde-2; eri-2	100

Question 6 (15 points):
Topoisomerases are essential for proper DNA replication, transcription, and chromosome segregation. In *Drosophila melanogaster*, topoisomerase II (encoded by the *Top2* gene) is required for proper development to adulthood. Animals that are homozygous for a *Top2* null allele die during early larval development. You would like to get additional alleles of *Top2*.

Describe a mutagenesis screen and crosses to identify *Top2* but not mutations in other genes that cause larval lethality. You have a bottle of EMS and three fly stocks: the wild type, *Sp/Cyo* (Sternal plural dominant mutant with the Curly of Oster balancer chromosome), and a *Top2(Δ)/In(2LR)Gla* stock (*Top2(Δ)* is a null allele of *Top2* balanced by the *In(2LR)Gla* balancer chromosome that causes recessive lethality and a dominant increase in eye pigmentation). Note, *Cyo/In(2LR)Gla* flies are viable.

Question 7 (13 pts):
You are interested in understanding the genetics of congenital heart defects, so you perform an ENU-mutant screen in mice. After lots of work and heavy mutagenesis, you identify a mutant with a recessive heart defect and generate a pure-breeding stock. You notice that this stock also has a patchy hair loss phenotype, and your classmate (a self-purported mouse expert) points out that the stock is more aggressive than the parental stock.

You decide to cross wild-type parental males to your mutant stock. All of the resulting male offspring from that cross are patchy, but the mice have normal hearts and are less aggressive. After crossing siblings from that cross, you can identify males and females with heart defects and aggressiveness (but no patchiness) and use them to establish a stock that lacks patchy but has heart defects and aggressiveness.

(a, 6 points) Write out the cross and genotypes along with a brief explanation of why you performed this cross.

(b, 7 points) Provide a brief explanation for whether (or not) the same gene could be causing all three abnormal traits (heart defects, patchiness, and aggressiveness).

Please fill out the post-midterm survey at bio393.andersenlab.org