Bio393: Biomedical Genetics **Name:**_____
Extra Problems (Enhancer/Suppressor)

Question 1:
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).

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

Extragenic suppressors are often unlinked from the original mutant allele.

Crosses to a wild-type strain will generate heterozygotes for the suppressor and the original mutant allele. After crossing these heterozygotes, you would get mutant individuals in the next generation because three-sixteenths will be mutant without the suppressor allele being homozygous.

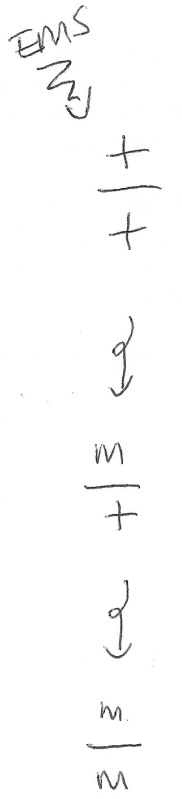
Or, you could cross to the mutant allele. In the next generation, revertants would still express the wild-type phenotype whereas suppressors with a recessive phenotype will look mutant.

Question 2:
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) 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.

Mutations in genes that promote RNAi would be RNAi deficient or RNAi resistant. We have the let-1 RNAi construct that causes 100% lethality in the wild-type strain. This situation sets up a system where we can use selection to identity a large number of RNAi deficient mutants using a lethal RNAi like let-1. If we RNAi let-1, then all animals will die except for mutants that fail to do RNAi because they have mutations in genes that promote RNAi.

Perform the following mutagenesis and in the last generation raise all of the offspring after exposure to let-1 RNAi. All heterozygotes and wild-type individuals in that generation will die. Only RNAi deficient mutants will survive.



(b) 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.

You can use the same mutagenesis protocol and crosses as (a), but you will expose the last generation of animals to both of the new RNAi constructs instead of let-1. We want genes that normally inhibit RNAi, so mutants in those genes will be RNAi sensitive. We can identify them in the last generation because they will have increased levels of the opaque mutant phenotype AND increased levels of weird cell movement.

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

We need to two RNAi constructs because if we use just one we might identify mutants that enhance just the opaqueness OR the weird cell movement RNAi phenotypes and not generally inhibit RNAi.

(d) 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).

We will cross the two mutants to get a heterozygote for both.

If mut1 and mut2 complement, then we would get

mut1 mut2+
mut1+ mut2

These individuals would not be mutant because they are deficient in different functions. Therefore, they would die in the presence of the let-1 RNAi construct.

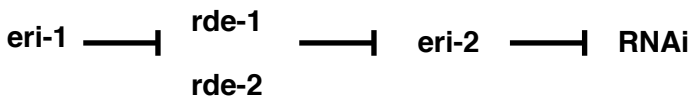
If mut1 and mut3 fail to complement, then we would get

mut1
mut3

These individuals would be mutant because they are deficient in the same function. Therefore, they would survive in the presence of the let-1 RNAi construct.

(e) 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



We do not know the order of rde-1 and rde-2 because they have the same mutant phenotype.