Bio393: Genetic Analysis   **Name:**_____
Problem Set #3
Due on Friday, January 26, 3 PM

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.

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.

(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.

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

(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).

(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