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The CRISPR/Cas9 system has been an important discovery for medicine, biotechnology, and agriculture. This genetics method allows for scientists to target DNA in living cells in order to alter the genetic code. The Cas9 protein first binds a piece of RNA (called the guide RNA) that has a programmed sequence. Cas9 will then search all the DNA in a cell for the matching sequence of DNA, bind it, and then create a double stranded break. The reason why this system is exciting for molecular biologists is because cells have repair systems to fix broken DNA. One of the special types of CRISPR methods is called a “gene drive.” This technique allows a chosen genetic sequence to be moved into a population without using Mendelian genetics. One of the main reasons for studying gene drives is that they can control populations such as insects (and many insect species carry diseases like malaria). The Finnigan Lab has created an artificial gene drive to study in yeast cells. One recent discovery (2017) was that viruses evolved “anti-CRISPR” proteins to combat bacterial CRISPR proteins like Cas9. The lab has been studying the AcrIIA2 and AcrIIA4 proteins that bind and inhibit Cas9. My project involved creating mutations (using PCR) in the amino acid sequence of these anti-CRISPR proteins and also mutations in the Cas9 protein. After changing the DNA sequence, I cloned the mutant versions into plasmids to express in cells. Finally, I tested to see whether the mutation would change the protein function and inhibit Cas9. The lab uses both haploid yeast and diploid yeast cells to test CRISPR editing. Therefore, I tested to see how these mutant proteins changed DNA editing. A second part of my project involved testing small protein sequences on Cas9 that cause trafficking to the nucleus. These are called nuclear localization signals (NLSs). Because Cas9 evolved in bacteria (which do not have a nucleus), scientists must first add the NLS to Cas9 so the cell can bring the protein into the nucleus and to the DNA of the genome. I have started working on creating mutated versions of NLS sequences to test how they will change CRISPR effects in yeast cells. These projects will help our understanding of how to control gene drives in other creatures someday.