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The CRISPR Cas9 system is a new and powerful gene editing technology. CRISPR evolved in bacteria as a primitive defense system. This method utilizes the Cas9 enzyme to create a double stranded break in DNA. In order to allow this double stranded break a guide RNA (gRNA) must be added that has the complementary sequence of DNA at the target location. Finally, the target DNA must contain a specific sequence called a PAM (protospacer adjacent motif). In this way, the CRISPR system is programmable. It is extremely accurate, inexpensive, and allows for a wide range of utilization. It is currently being used in agriculture, molecular biology research, and medical applications. The Finnigan Lab uses a model organism (budding yeast) to test different aspects of this gene editing technology. Yeast are a eukaryotic organism and can exist in both haploid and diploid states (and undergo both mitosis and meiosis). The most popular CRISPR editing system uses the type II S. pyogenes Cas9 nuclease. This protein has a specific requirement for binding and cutting sections of DNA. However, for some genomes, this might present limitations or problems. Therefore, researchers have been interested in testing other types of Cas9 enzymes or related enzymes from other species of bacteria. One of these is the type V Cas12a (also known as Cpf1) nuclease. It has a number of differences compared to Cas9. Cas12a requires a PAM sequence (TTTV) on the 5' end of the target DNA while Cas9 requires a PAM sequence (NGG) on the 3' end. Also, Cas12a has a unique ability to have multiple gRNA sequences included within the same initial RNA fragment (allowing the targeting of numerous DNA positions). Finally, Cas12a creates a staggered double stranded break while Cas9 creates a blunt ended break. My research project in the lab focused on cloning the F. novicida Cas12a gene into the yeast genome and testing the expression of different gRNA plasmids. The Cas12a guide requires a repeated sequence surrounding the intended target sequence and it does not include a tracer RNA sequence (which is required for Cas9). Previous studies have tested different lengths of this repeated guide sequence. I cloned a gRNA for Cas12a that included either a 19 base pair or 36 base pair repeated sequence. I used haploid yeast cells to target the ADE2 gene which is required for the biosynthesis of adenine. If the adenine pathway is deleted by removing this gene, then a red byproduct builds up in cells and the yeast turn a red color. I tested expression of Cas12a in wild-type yeast cells and included the gRNA plasmid to target the ADE2 sequence and also added a PCR fragment to delete the entire ADE2 gene. Cas12a added a double stranded break into the chromosome and the homology directed repair system used the PCR fragment to fix the broken DNA and deleted the ADE2 gene. The result was that some of the yeast colonies turned red and I quantified how efficient this was for different guide RNA sequences. My findings show that the 19 base pair repeated sequence is much more efficient for genome editing with FnCas12a in yeast. The lab is interested in testing other mutations to the RNA sequence or to the nuclease enzyme to determine the most efficient combination for editing in future experiments.