

CRISPR/Cas biotechnology was discovered in 2012—it was part of the bacterial immune system used to fight off infecting viruses. This technology has been transformed into a gene editing system by scientists at UC Berkeley and has been used to change the DNA of all types of microbes, plants, animals, and even human cells. Briefly, the Cas9 enzyme (or other protein types) binds to a piece of RNA (guide) and the protein/RNA complex searches for the matching nucleotide sequence in the genome of a living cell. Once it gets there, it cleaves the DNA. Breaks in chromosomes are not tolerated in living cells and therefore life has evolved repair systems to fix DNA breaks. Using these natural repair mechanisms and by adding in artificial DNA, researchers can include new sequences or genes into the genome. The Finnigan Lab uses CRISPR/Cas technology to create new yeast strains and to also study genome editing and gene drives. One of the primary uses for CRISPR in most labs today is to be able to create a mutation or new protein sequence in a cell or organism of study. However, in most cases this means first creating a brand new RNA fragment to target Cas9 to the new gene and also delivering both the Cas9 and RNA into the cell. These steps can be costly and time consuming when repeated many times for the same genes being targeted. My project was to create a collection of yeast strains that already had both the Cas9 and RNA fragment included. The goal was to target common DNA sequences that are often used in molecular biology labs on DNA plasmids (like marker genes, biochemical tags, or fluorescent proteins). Once created, this system could help generate more plasmids for research at a much lower cost. I was successful in creating a set of 9 yeast strains that included both Cas9 nuclease and a chosen RNA guide. Both of these were placed in the genome, which was challenging. The initial experiments to target plasmid DNA in yeast were not successful and will require changing the method in future trials. However, creating these strains was still very useful to the lab because other projects have now used the design of having both a nuclease gene and the guide RNA expression gene at the same place in the genome. This research project demonstrated the challenges of creating new technology in the lab and also showed that one project's failures can be successes too in other ways.