Introduction:

Both selectable and counter-selectable markers are useful tools for genome engineering. Selectable markers are mainly used for plasmid maintenance, engineered conjugation and genome manipulation. On the other hand, counter-selectable markers can aid in scar-less gene deletion, plasmid curing, and engineering double-crossovers. Since selectable and counter-selectable markers both have practical applications that can be of great utility, it is desirable to optimize dual-selectable markers, which can serve as both selectable and counter-selectable markers. Like counter-selectable markers, dual selectable markers also suffer from high counter-selection escape; this is one of the primary complications of finding suitable dual selectable markers.

Methods:

In this project we will aim towards the development of a selection marker for automated genome engineering. We will be working with the enzyme thymidine kinase (tdk) and demonstrate whether it can be used for coselection Multiplex Automated Genome Engineering (coS-MAGE). We will be alternating between selections that utilize either azidothymidine (AZT) or 5-Fluorodeoxyuridine (FDU) and thymidine through the cycles and determining where the break out point lies.

Results and Future Directions:

An effective dual-selectable marker is one that can survive for at least 10 cycles without breaking down. Our marker managed to survive and maintain stability throughout 10 cycles at three different concentrations (0.2μM, 0.5μM, and 2.0μM). The next steps in our project are verification and segment integration. If successful, tdk will serve as a useful tool for genome engineering applications and prospective synthetic biology research.