

## Engineering Plasmid Performance in *Yarrowia lipolytica*

\*Bailey, M<sup>1</sup>., Spagnuolo, M<sup>2</sup>., Blenner, M<sup>3</sup>.

<sup>1</sup>Research Associate, Chemical and Biomolecular Engineering Department, Clemson University;

<sup>2</sup>Graduate Student, Chemical and Biomolecular Engineering Department, Clemson University;

<sup>3</sup>Professor, Chemical and Biomolecular Engineering Department, Clemson University

[mbaile6@g.clemson.edu](mailto:mbaile6@g.clemson.edu)

**Key Words:** Episomal plasmid, Plasmid stability, Ori sequence, Cen sequence, ARS sequence

**Abstract:** The main goal of this project is to determine an optimal plasmid sequence that will give the strongest, most uniform gene expression. In other words, we aimed to increase plasmid stability on the yeast *Yarrowia lipolytica* by engineering a better means of plasmid replication and dissemination. First, native autonomously replicating sequence (ARS) were identified by our collaborators and isolated by using PCR. The ARS sequence was characterized by performing serial truncations. The sequences were then placed into a plasmid containing the green fluorescence protein reporter gene through SLIC and then transformed into yeast. Once visible colonies were present, cells were normalized and grown in selective media for 24 and 48 hours. Finally, flow cytometry was used to sort the cells based on fluorescence strength. The same procedure was then repeated with systematic truncations of the longest ARS sequence, or ARS68; this was done to determine the significance of the base pairs located after the Cen sequence. The ARS sequence that contained the truncated Cen showed a larger increase in the percent of cells fluorescing at 24 hours, but returned to values matching its peers after 48 hours. Next, the additional Ori sequence on the plasmid was removed to test if two Ori sequences were necessary. When flow cytometry was performed, there was no significant change in the percent of cells fluorescing or mean fluorescence indicating the extra Ori sequence did not have a significant effect. The next step was to perform a mitotic stability study to quantify a percent loss of plasmid per generation. By growing the strains on selective and nonselective media, it was shown that despite the apparent improvements seen in the GFP study, none of the ARS-derived sequences performed any better or worse than the existing standard Ori and Cen sequences. Finally, the GFP gene was replaced with a more functional gene that produces PHA to test the practical application of this work. Future work for this project would include exploring the location dependency of the sequences and looking upstream and downstream of the ARS sequences.