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Testing SSA4:ADE3 Reporters for MCS Screening

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In eukaryotic cells, after transcription, mRNA is escorted from the nucleus to the cytoplasm to be translated. This process, called mRNA export, is essential for gene expression. However, when the cell exists in stressful conditions, mRNA export becomes regulated, and only select transcripts, including the stress-responsive SSA4 mRNA, can be exported. This project aims to uncover the mechanism of selective SSA4 mRNA export by generating a reporter that enables phenotypically visible expression under stressful conditions. Specifically, the ADE3 ORF was placed under the regulatory sequence of SSA4, which was anticipated to induce a red color for colonies only following stress. Using these reporters, mutant yeast strains were heat shocked to observe if there was a color change from white to red, indicating successful induction and export of the SSA4 mRNA. We have found that different media conditions reduce background expression of the SSA4 reporter, but do not allow stress-induced expression. From this, a CRISPR-based approach was adopted to reduce leaky expression and allow for direct integration of the SSA4 reporter plasmids. This semester, a series of integrated strains were successfully generated, and future studies will be aimed at testing these strains. Once conditions are found using the mutant yeast strains with the integrated reporters, where expression is enabled only when the yeast is under stress, a Multicopy Suppression Screen can be conducted to identify factors involved in selective SSA4 export.

Key Words: mRNA export, SSA4, selective mRNA export, condition testing, CRISPR, Multicopy Suppression Screen