Developing a flow cytometry based viability assay for post-oxidative stress survival in yeast species

Introduction

Yeasts display an acquired stress resistance (ASR) response where a mild dose of primary stress helps the cells to survive a secondary dose of severe stress. The mild primary stress may be the same or different than the secondary severe stress. While the paper focuses on genes necessary for ASR in surviving oxidative secondary stress, our lab is interested in understanding ASR traits in evolutionary related yeasts species. Specifically, previous work in the lab has identified an increased magnitude of ASR response in C. glabrata when exposed to phosphate starvation as a primary stress and hydrogen peroxide treatment as a secondary stress. This increased magnitude of ASR is not seen in the closely related S. cerevisiae. To understand the evolutionary trajectory of ASR, the magnitude of ASR in other related species need to be accessed and compared. The post-oxidative stress survival rate in ASR is traditionally accessed using the gold standard colony forming units (CFU) assay. CFU assay can be labor intensive and the small sample plated may induce variance in the output. While this paper mentioned a flow-cytometry based validation that was used for validating post-oxidative survival, the data analysis and method for quantifying survival rate remains elusive. My project aims to develop a flow cytometry based high-throughput viability assay using yeast viability dyes. The data generated is mostly flow cytometry based, which are FSC files. In this course project, I hope to build a pipeline for processing and analyzing flow cytometry based data and use R to construct informative figures that help in evaluating the results. An example of the figure I want to generate is the ridge plot below, where intensity of multiple samples can be easily compared.

Materials and Methods

- Post-oxidative stress yeast cells were collected and stained with FUN-1 or Fungalite dyes. (See ELN on specific collection protocol).
- Stained cells were run through Attune flow cytometry. Settings in ELN.
- Data collected are FCS files, with one file for each experimental condition.
- To generate figures, need to first shorten FCS file names by removing common parts and keep the unique parts.
- Then, use meta data to link each specie's data to meta data.
- Use "flow core" package to read FCS files.
- Generate graph using ggplot2, ggcyto, and ggridges.
- Adjust graph axes and change order of experimental conditions as needed.

Figure to reproduce

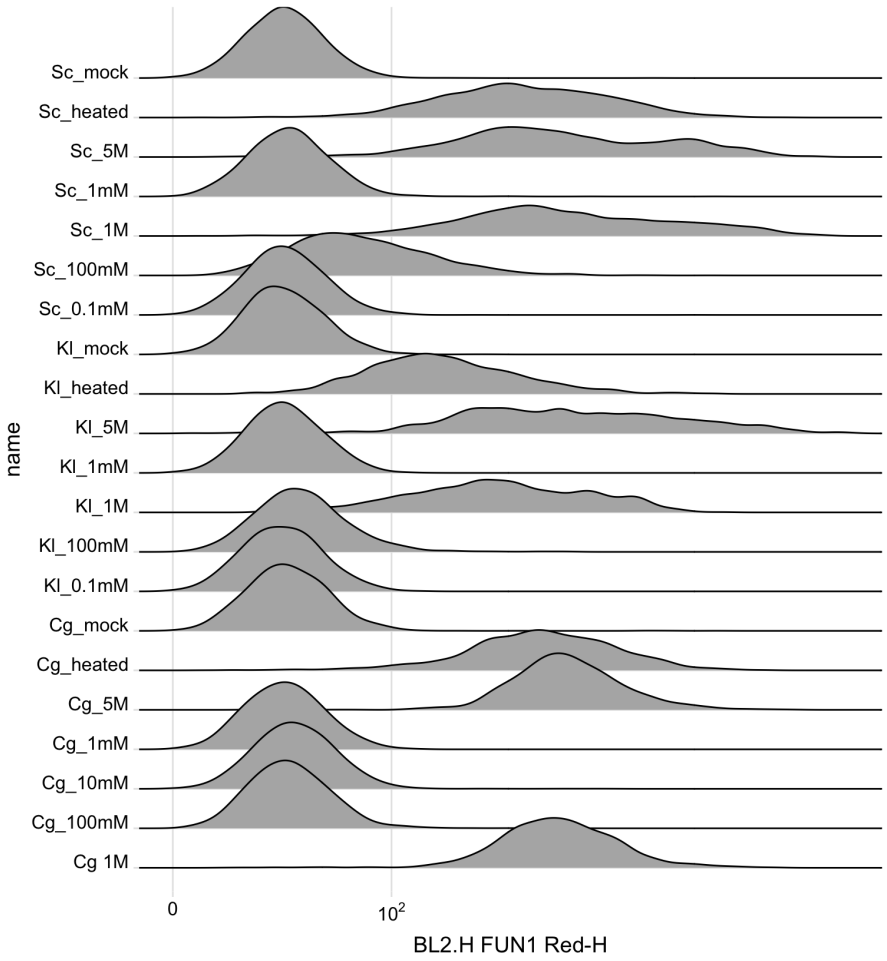

Figure 1: image

Resluts

TBD

Discussion

TBD

Conclusion

TBD