Introduction

- Copy number variants (CNVs) are duplications or deletions of genomic segments representing an important form of structural variation.
- CNVs:
  - range from 8-25 kilobases per generation in humans
  - play a part in dietary and brain development throughout our evolution
  - affect mRNA and protein levels that can change cell physiology
  - contribute to a range of human diseases, including autism, Crohn’s disease, and cancer
  - are frequently observed in experimentally evolving populations of microbes
  - form in a highly variable behavior, between 10^-10 and 3.4 x 10^6 amplifications per cell per division
- The budding yeast Saccharomyces cerevisiae is an ideal model organism to study these genome changes in response to selective pressure simulating adaptive evolution.
- The inability to measure the rate of both spontaneous and selective formation of CNVs makes studying these variants difficult.
- We have identified a deletion event for the general amino acid permease, GAP1.
- **Objective: Develop a reporter of GAP1 deletion**

GAP1 excision through homologous recombination can form a self-replicating functional extrachromosomal element

![Diagram of GAP1 excision](Image)

Intrachromosomal recombination excises the GAP1 gene from the endogenous genome. An extrachromosomal element forms and retains its function, replicating independently. This deletion event was characterized in nitrogen-limited environment and could indicate a cell’s increased fitness in a nutrient-limited environment. Figure adapted from 1.

Questions

1. What is the spontaneous rate of GAP1 deletion?
2. What factors affect the rate of GAP1 deletion?

Methods

- **TEF**
- **YKRC11**
- **GAP1**
- **YKRC12**
- **G418**

GAP1 Excision Event

- We employed plasmid cloning methods to create a genetic construct, inserting the GAP1 cassette between the highly constitutive TEF promoter and the G418 resistance gene.
- The large decrease in genomic distance after excision likely confers drug resistance.
- Therefore, we expect cells containing this construct and exposed to G418 to survive only if the recombination event occurred.

Conclusions & future directions

- The construct’s viability will be confirmed when introduced into the S. cerevisiae genome.
- Inoculation of yeast strain containing the GAP1 model construct in non-selective media
- Extended growth of population in selective or non-selective media
- Measurement of cell/volume using counter counter and plating on YPD media with G418
- Observed colony growth gives the rate of GAP1 deletion as the number of mutants per million cells
- A Luria-Delbruck fluctuation assay will quantitatively measure the deletion rate after verifying the construct’s ability to identify GAP1 deletion mutants.

Acknowledgements

Thank you to Stephanie Lauer & the Gresham Lab for their continued advice and support throughout this project.

Literature Cited