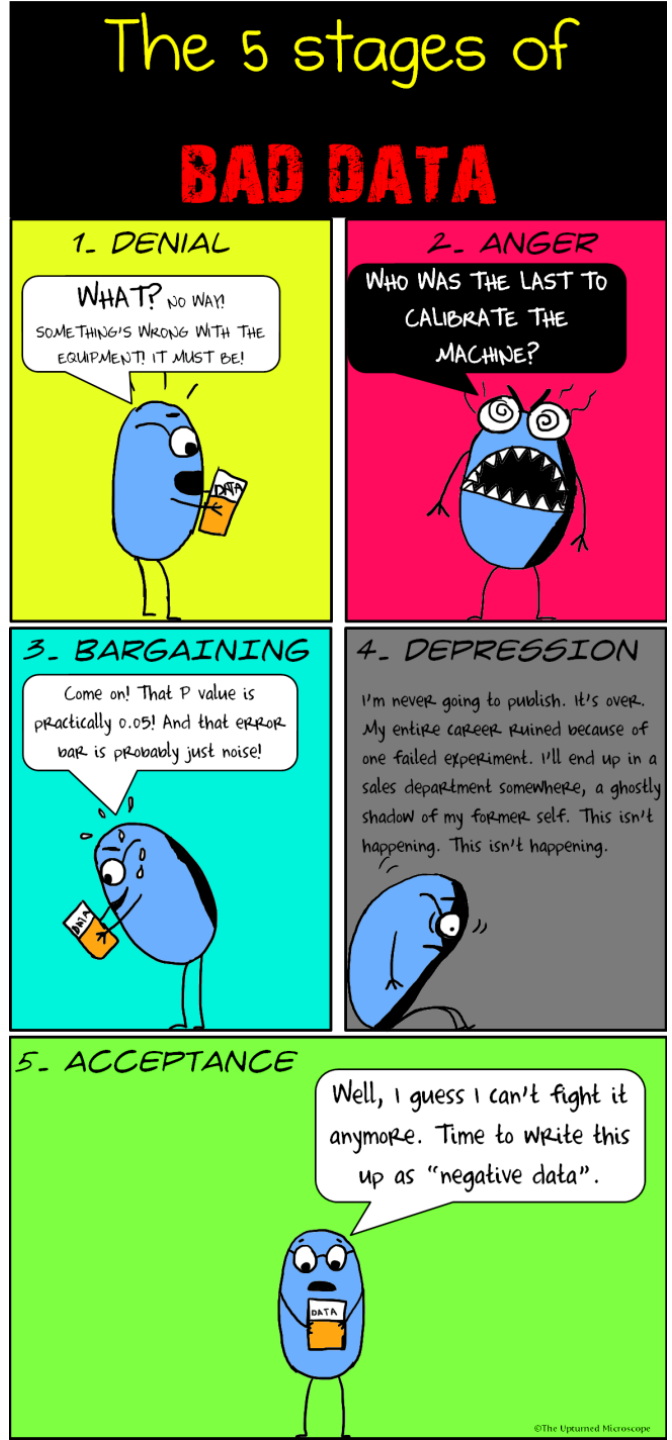
M2D6: Analyze expression data and prepare metal uptake experiment

- Prelab
- Perform metal uptake experiment and prepare samples for ICP-OES

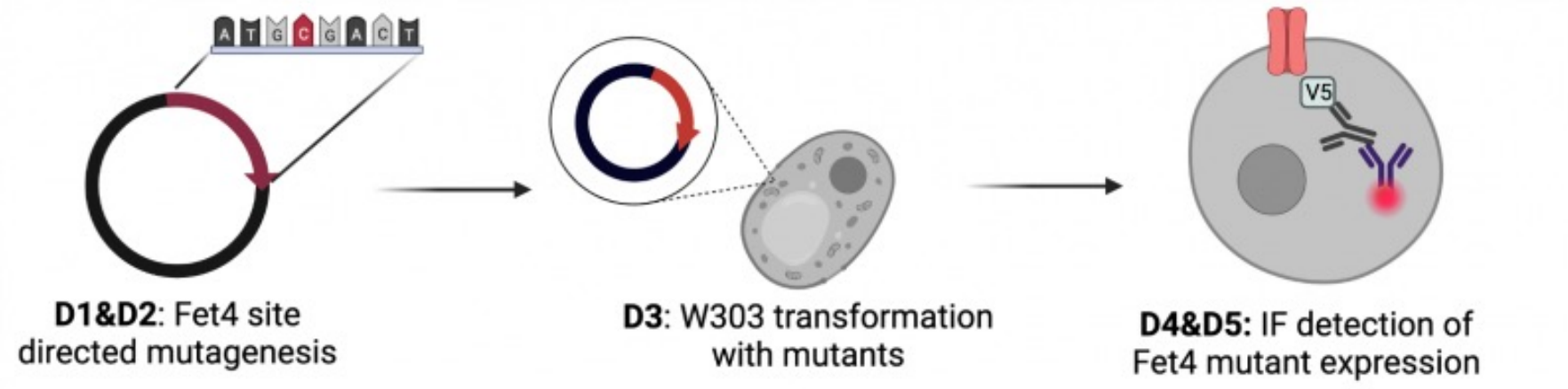


Overview of Mod 2 experiments

Last lab:

This lab:

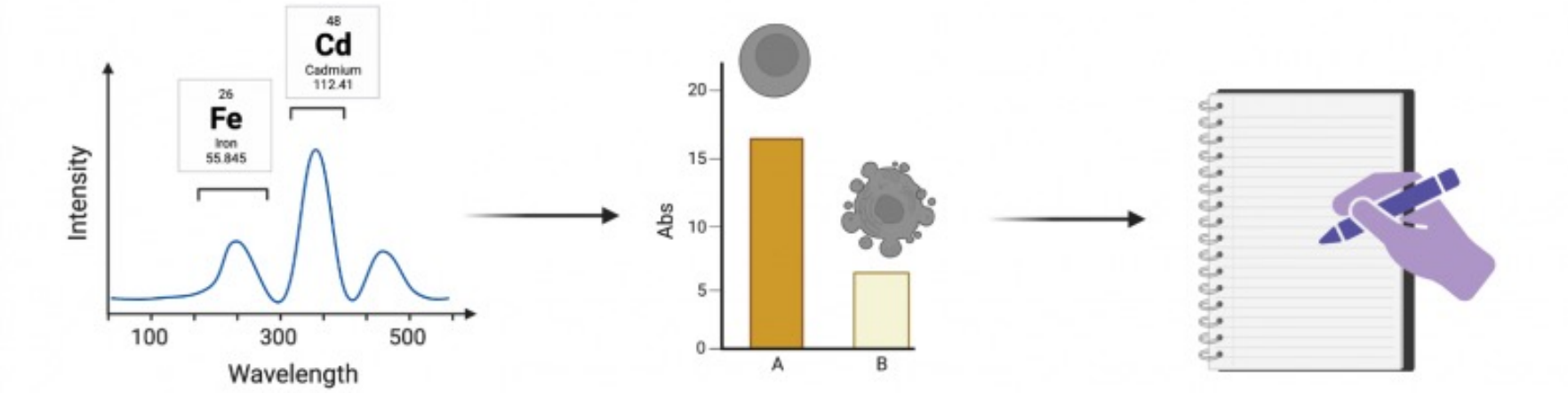
Next lab:



D1&D2: Fet4 site directed mutagenesis

D3: W303 transformation with mutants

D4&D5: IF detection of Fet4 mutant expression

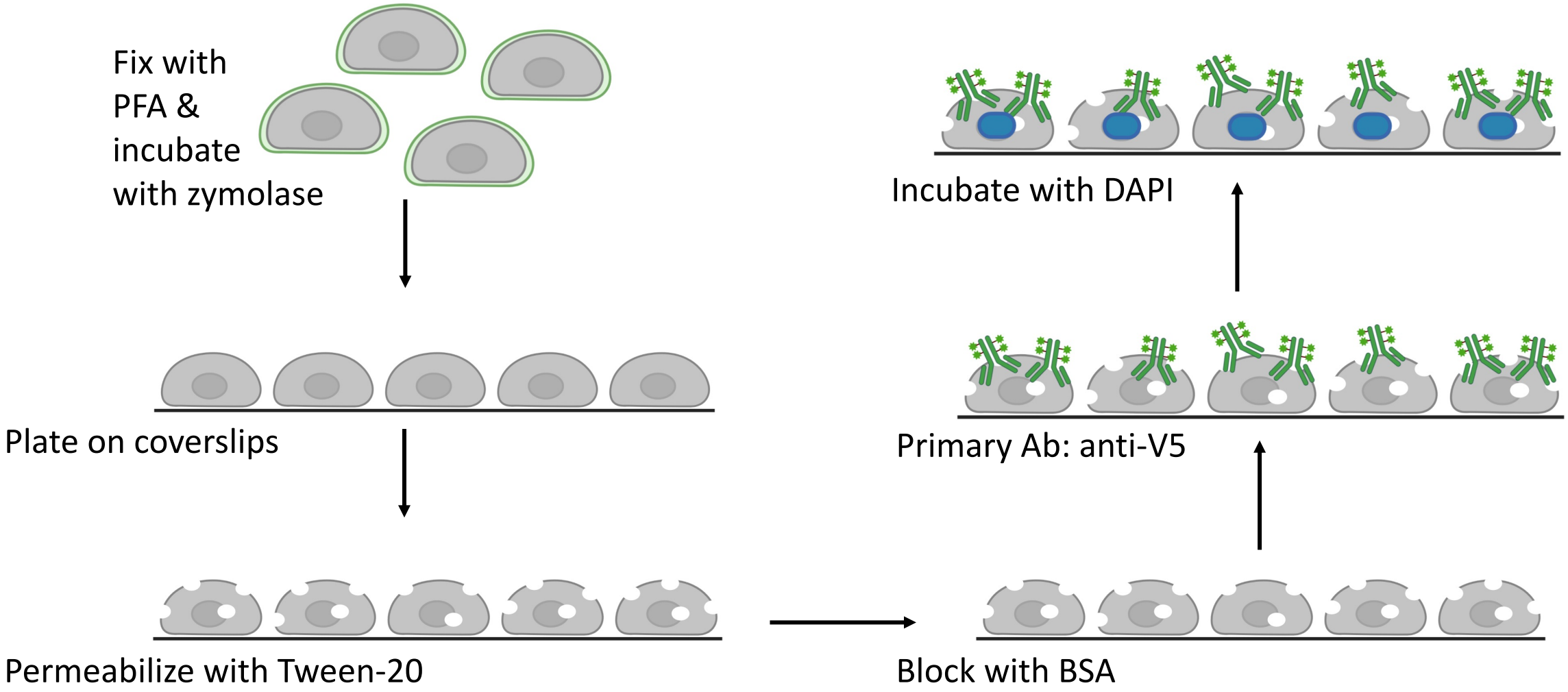


D6: ICP-OES analysis of heavy metal uptake

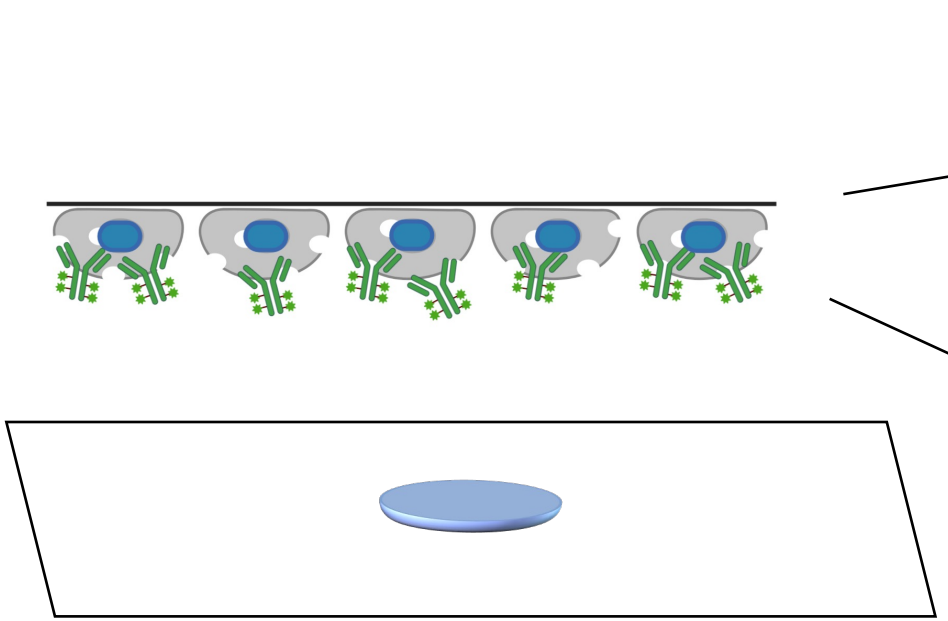
D7: Determine tolerance of mutant W303 to metal

D8: Statistics and Wrap-Up

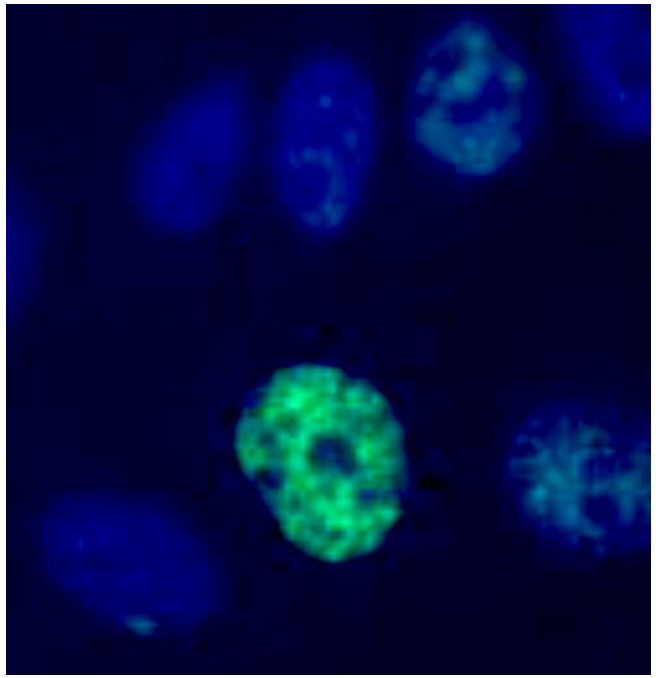
Using immunofluorescence (IF) in yeast: steps in protocol



Finish IF by mounting coverslips on slides



Mount coverslip on glass slide
with mounting media



Blue= DAPI
Green= antibody staining

What does your IF data look like?

The Good

- You have fluorescently stained cells!

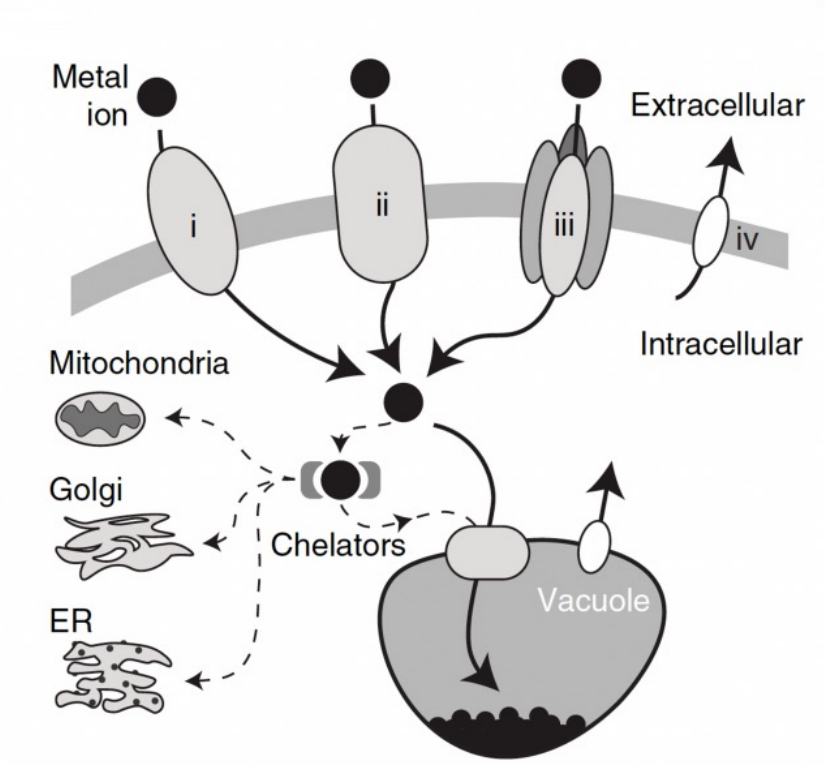
The Bad

- The adherent yeast loosened during the staining process and are currently floating in the mounting media

The Ugly

Uptake experiment overview

- Examine OD_{600} for your Fet4_mutant culture
- Dilute your culture to achieve 8ml of culture at ~1.0 OD_{600}
- Spike your yeast culture with 100uM metal
- Incubate for 2.5 hours
- Remove your yeast through centrifugation
- Digest material in media with nitric acid
- Filter the digested media to remove particulates
- Profit



For today:

1. Set up metal uptake experiment
2. Play around with ImageJ as an image tool
3. Work on homework
4. Process metal samples for ICP-OES

For M2D7

1. Create a research overview schematic
   - Visualizes key components of the project
   - Not an experimental schematic on a larger scale
2. Work on questions for discussion
   - First follow up experiment is to determine mutant expression!
   - Please propose 2 additional experiments for your discussion section