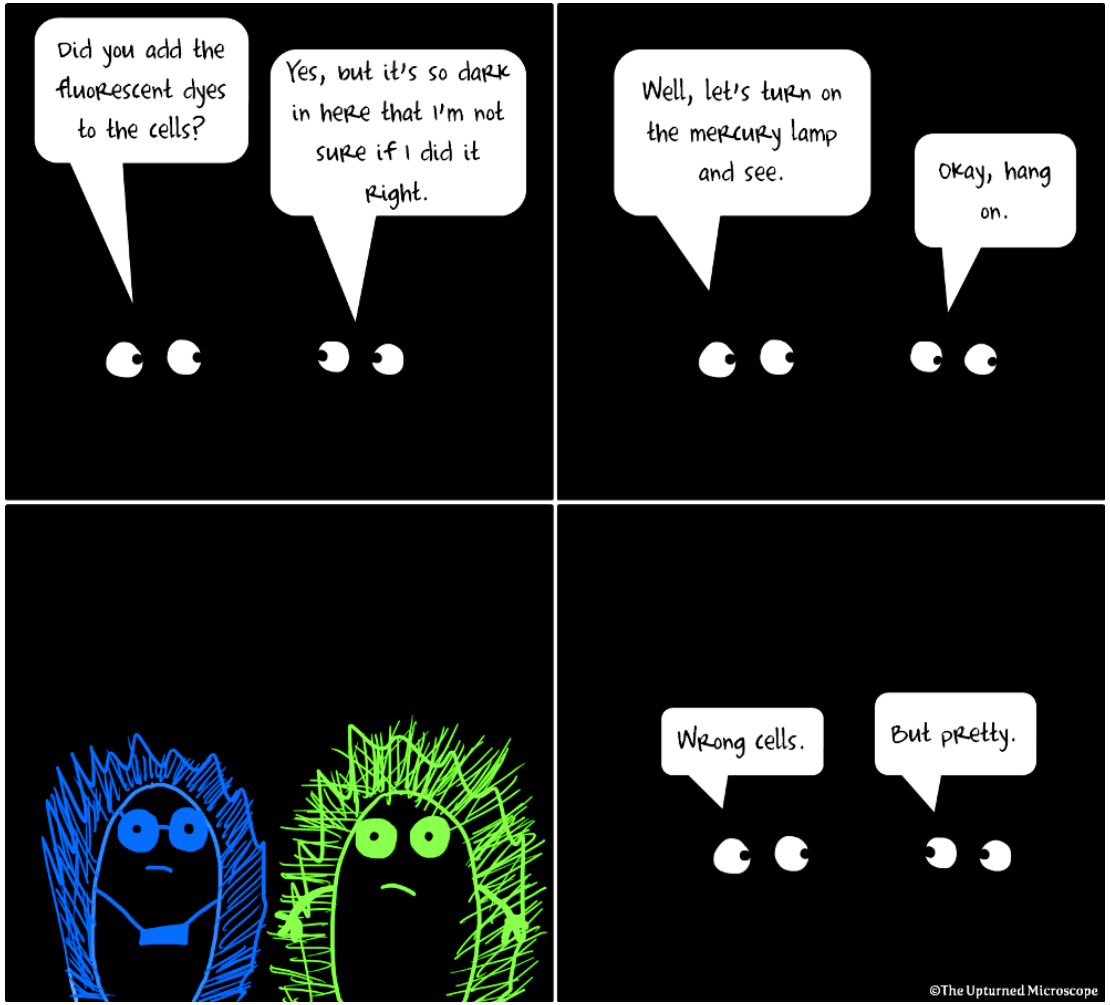
M2D5: Immunofluorescence staining

1. Prelab
2. Fix yeast
3. Antibody staining of Fet4_mutant

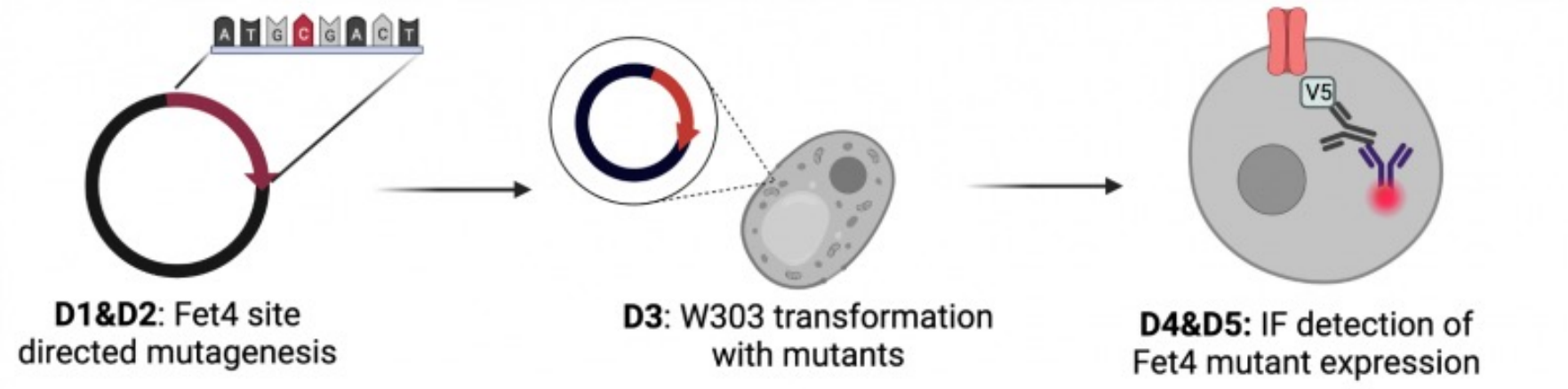


Overview of Mod 2 experiments

Last lab:

This lab:

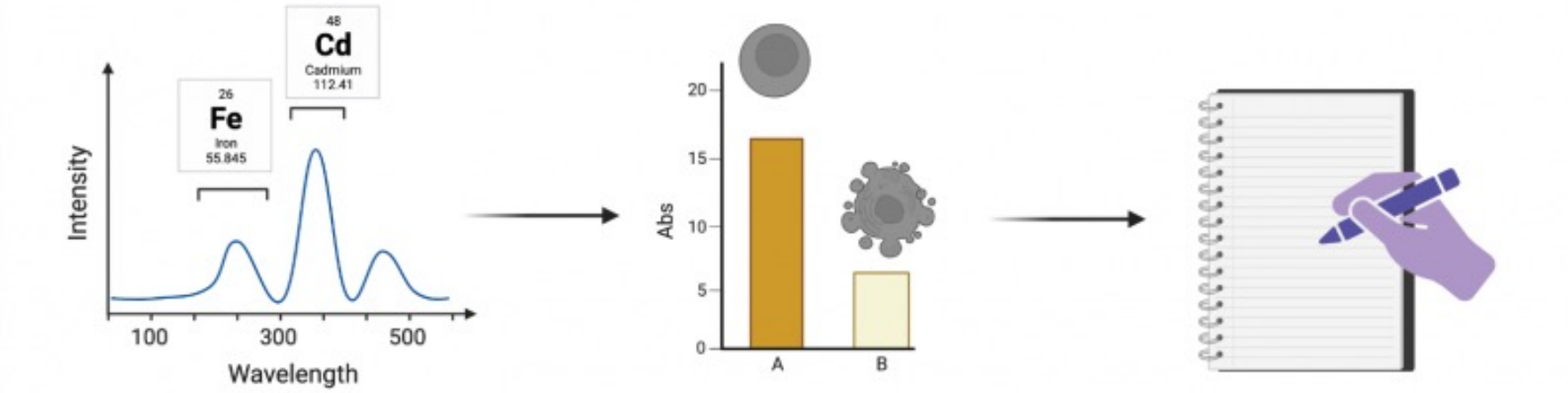
Next lab:



D1&D2: Fet4 site directed mutagenesis

D3: W303 transformation with mutants

D4&D5: IF detection of Fet4 mutant expression



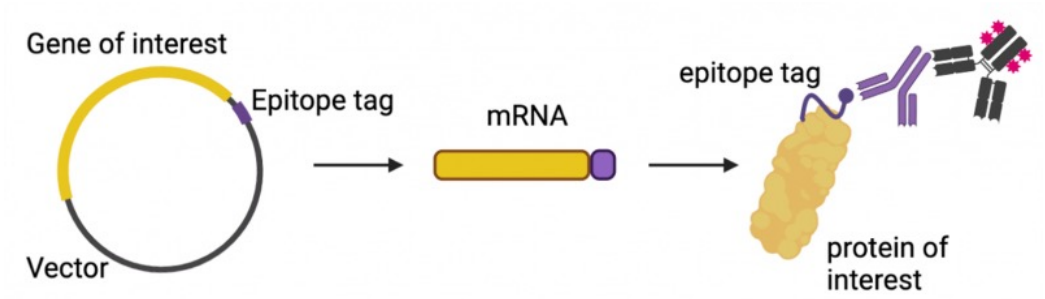
D6: ICP-OES analysis of heavy metal uptake

D7: Determine tolerance of mutant W303 to metal

D8: Statistics and Wrap-Up

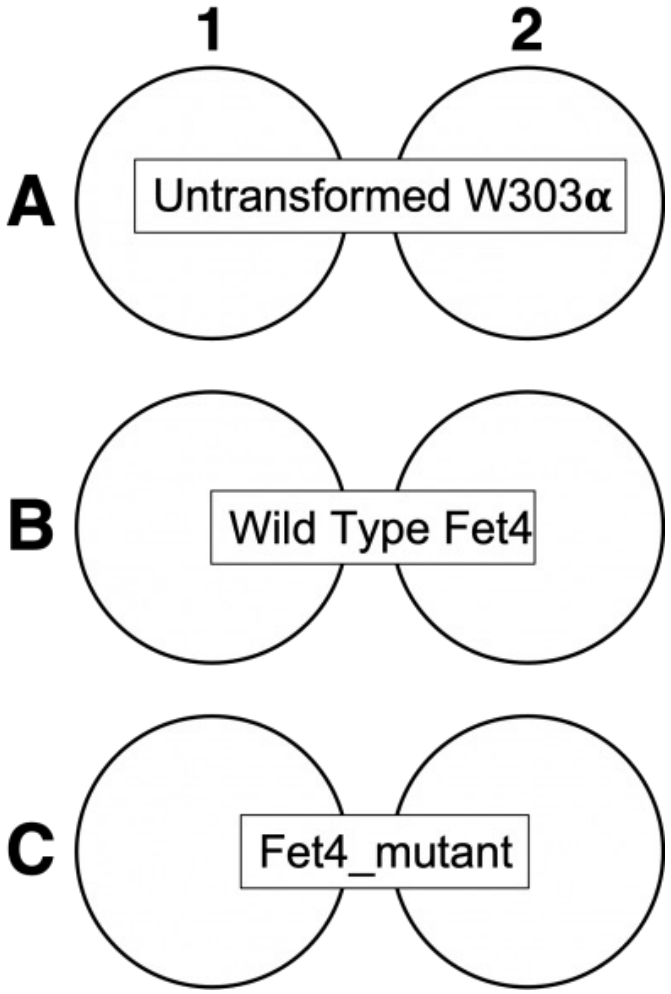
Using immunofluorescence: Expression of Fet4_mutant in yeast

- Yeast cells transformed to express Fet4_mutant protein

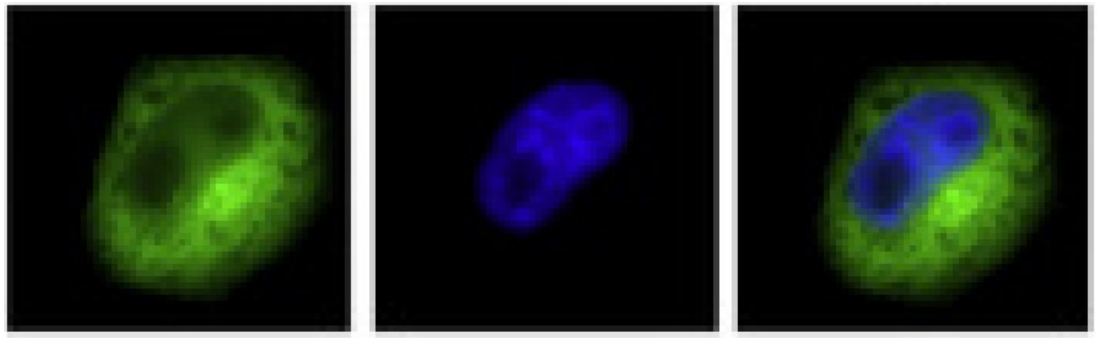


- Why untransformed cells?

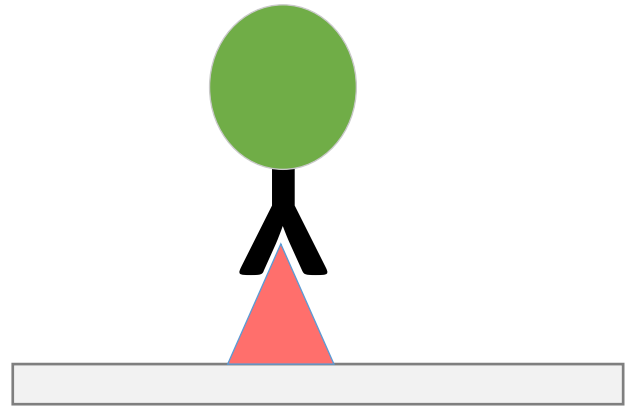
- Why wild-type Fet4?



Using immunofluorescence: Identification of Fet4_mutant expression

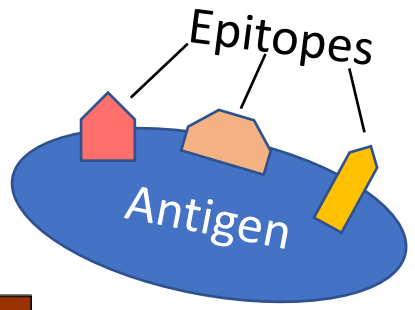


V5 DAPI Merge

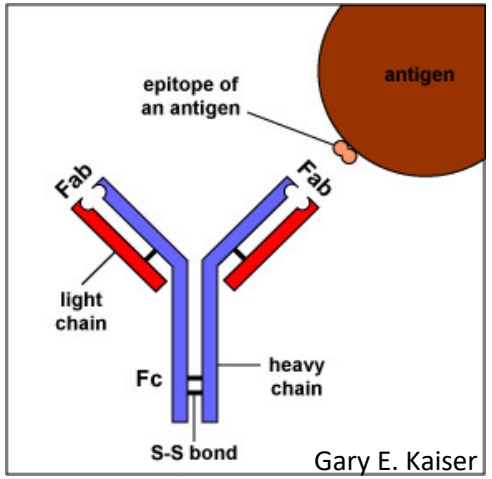


protein of interest	V5
primary antibody	mouse anti-V5
secondary antibody	none
Fluorophore (conjugated to secondary antibody) exc./ em. wavelengths	488/525 nm

Considerations for using antibodies in the lab

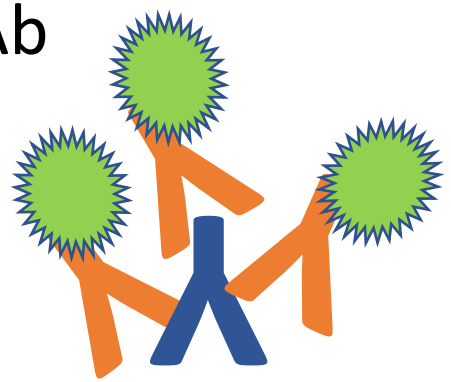


- Antibodies bind to specific epitopes on antigens
  - Antigens may have multiple epitopes



- Primary antibody recognizes the antigen
  - Specific protein sequence
  - Specific conformation of protein
  - Specific state of protein (i.e. phosphorylation)

- Secondary Ab recognizes the species of the primary Ab
  - Often conjugated to tag for visualization
    - Enzyme or fluorophore
  - Amplifies signal through multiple bindings
  - Consider sample species when choosing antibodies!



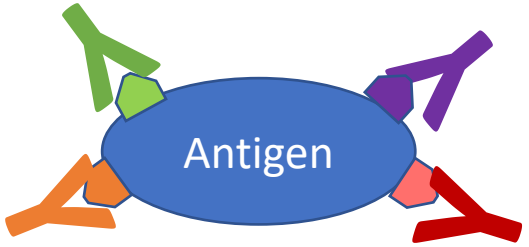
Secondary antibody conjugated to a fluorophore

Primary antibody

Polyclonal vs. monoclonal antibodies

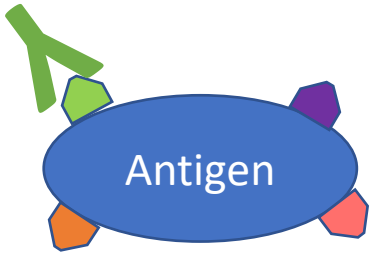
Polyclonal

- How it's made: animal (often rabbit) immunized with antigen of interest then antibodies collected from blood sera and affinity purified
- Advantages:
  - Less expensive and faster to produce than monoclonal
  - Multiple antibodies in one polyclonal mixture can increase antigen recognition by binding multiple epitopes
    - Especially useful for proteins with low expression
- Disadvantages:
  - Variability from lot to lot

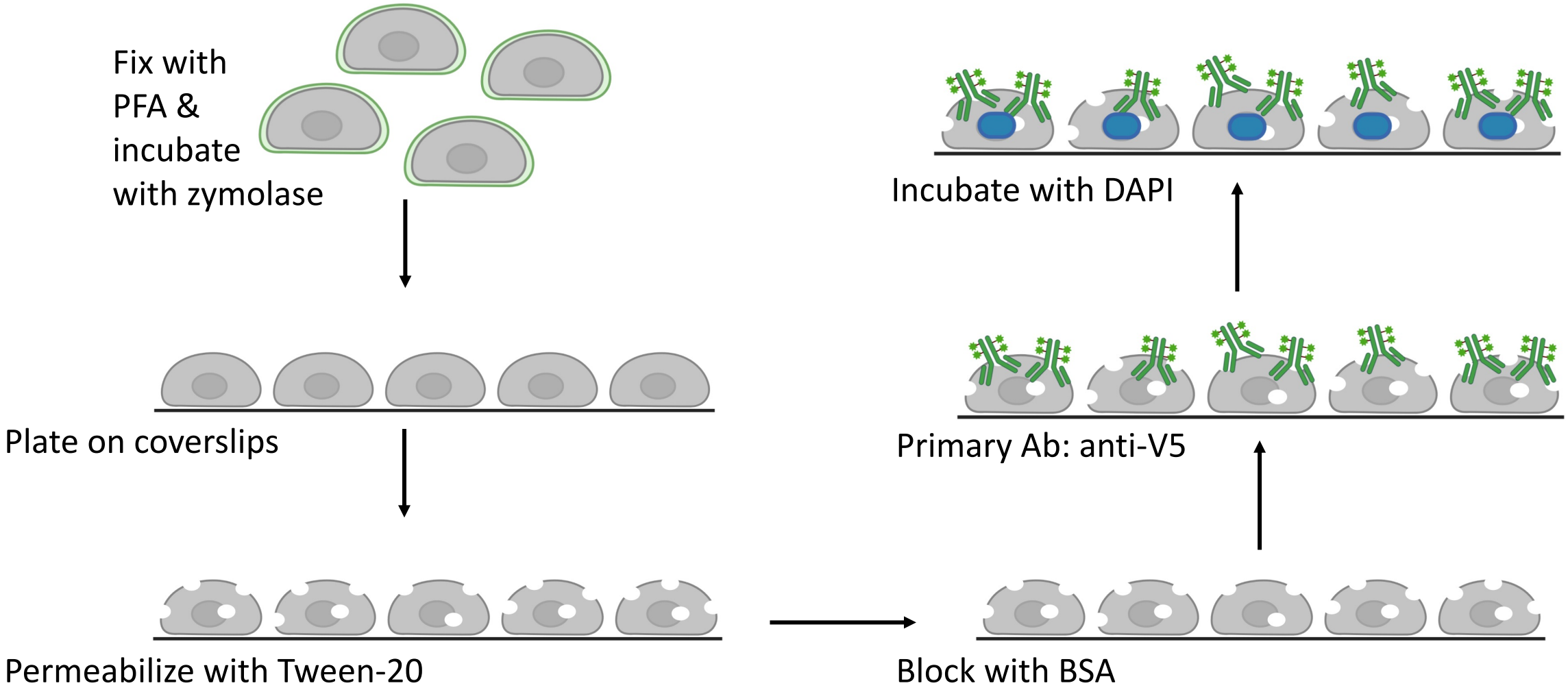


Monoclonal

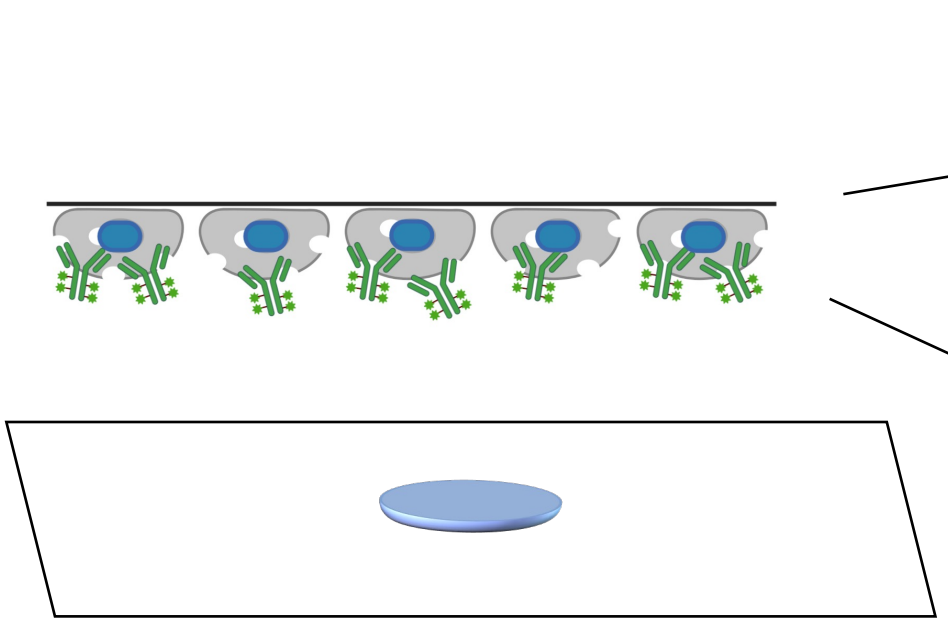
- How it's made: animal (usually mouse) immunized with antigen of interest then B cells from spleen are harvested and fused with myeloma cells to create hybridoma cell line that will continually produce single antibody clone
- Advantages:
  - Very consistent
  - Binds single epitope (can also be disadvantage)
- Disadvantages:
  - More expensive and requires animal sacrifice



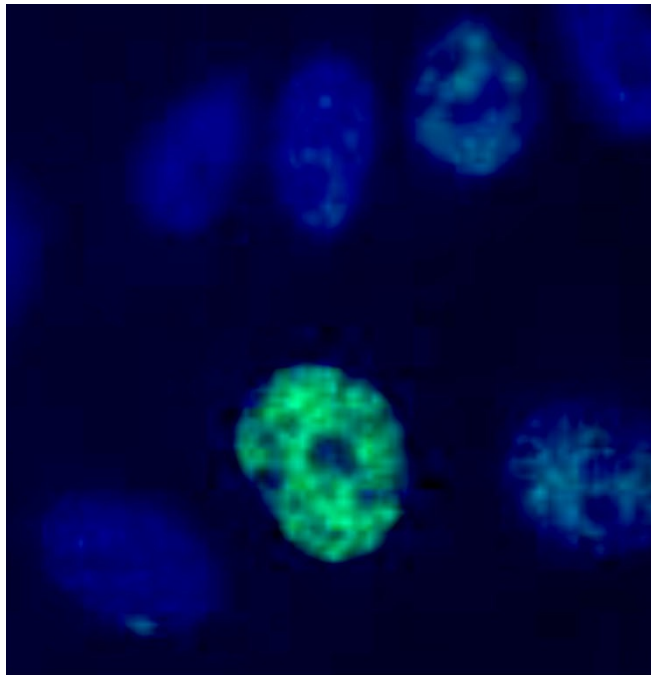
Using immunofluorescence (IF) in yeast: steps in protocol



Finish IF by mounting coverslips on slides



Mount coverslip on glass slide
with mounting media



Blue= DAPI
Green= antibody staining

For today:

1. Fix yeast samples
2. Perform IF
    1. Downtime: Look at new alignments on Dropbox that show the mutations
3. Mount coverslips on slides for imaging

For M2D6

1. Write methods for M2D2-M2D5

*** Individual Assignment ***