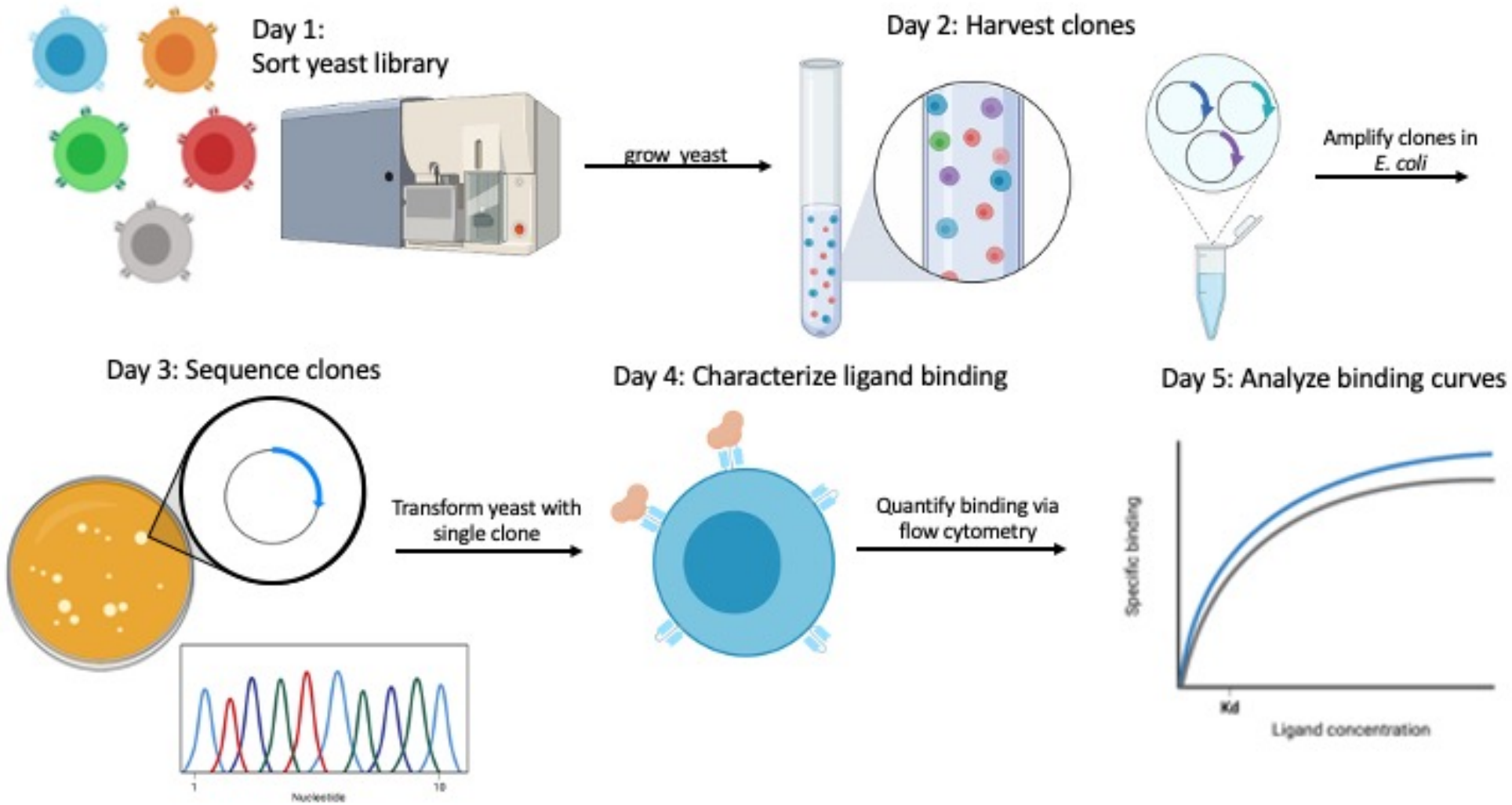
M3D4:
Characterize clone by titration using flow cytometry

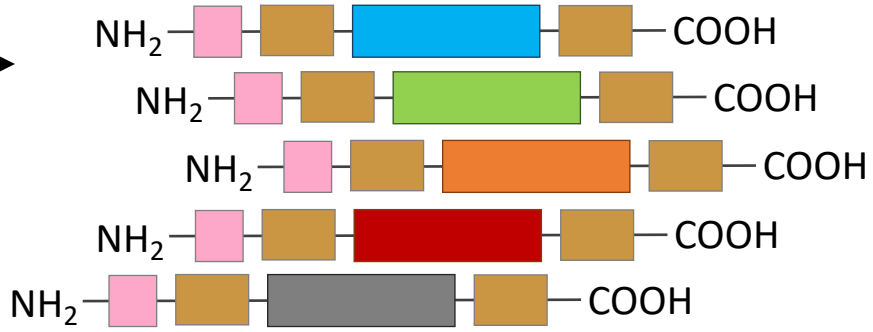
1. Prepare titration for K_d calculations
2. Quantify lysozyme binding to clone
3. Begin data analysis

Overview of Mod3 experiments

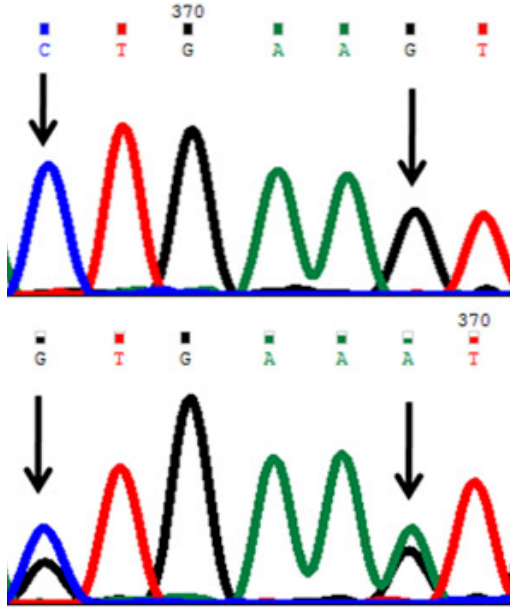


Let’s review our progress…

scFv library screened and clones isolated



isolated clones sequenced and mutations identified

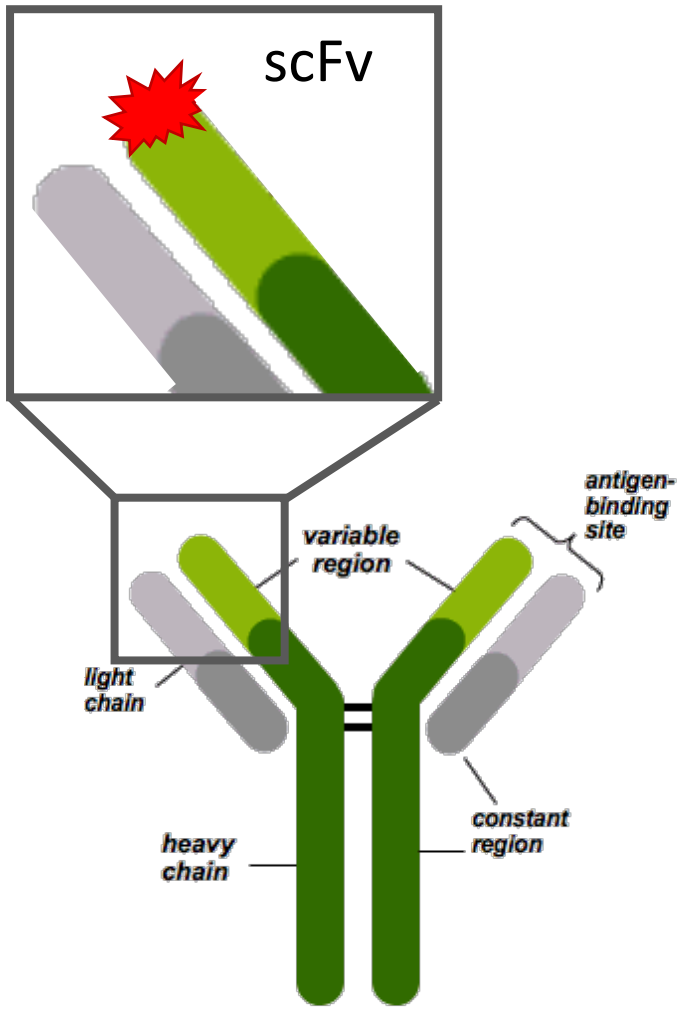


What mutation(s) occurred in the isolated clones?

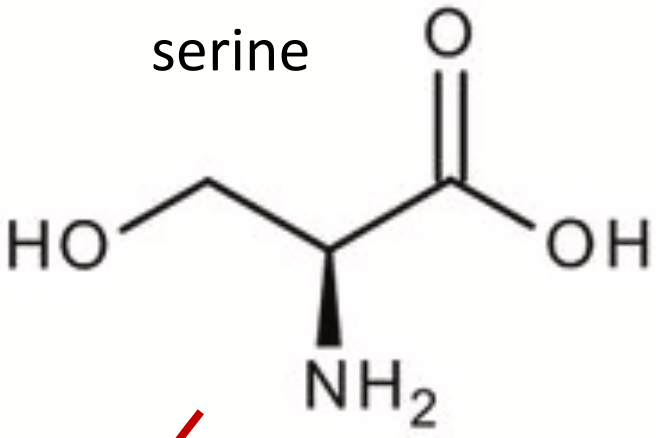
Did the mutation(s) result in an amino acid substitution?

If yes, how might this substitution influence lysozyme binding?

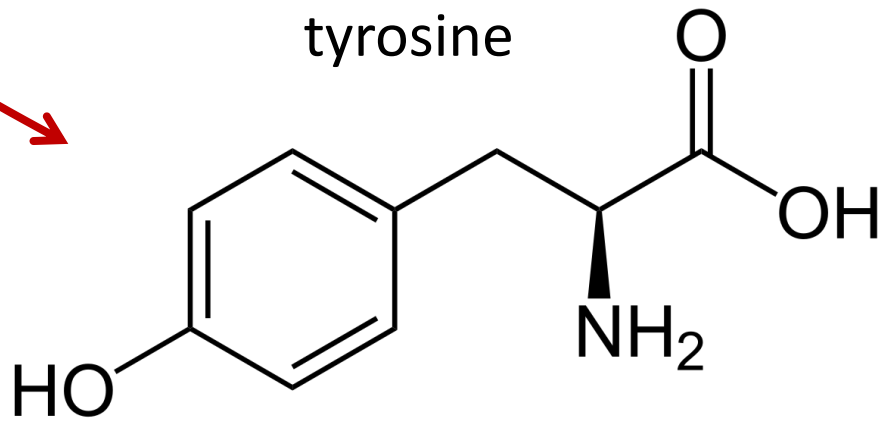
What mutation(s) occurred in your clones?



serine



tyrosine



How might this substitution influence scFv clone binding to antigen (lysozyme)?

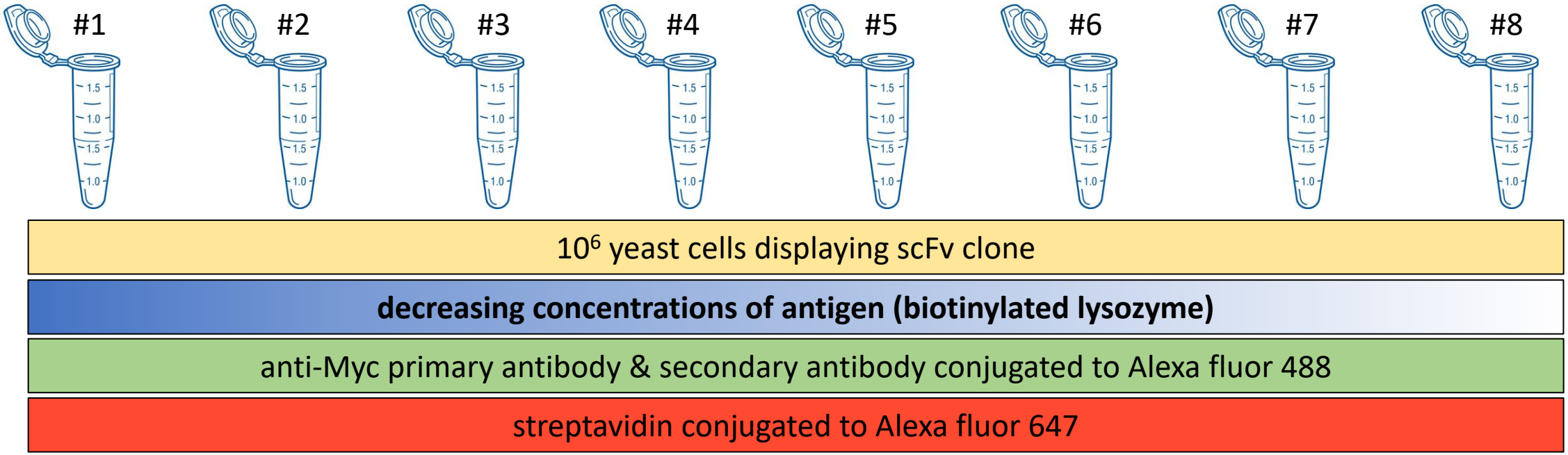
How will we determine K_d of isolated clones?

- Titration curves used to measure the signal that is proportional to the concentration of bound antigen, this is plotted against the total concentration of antigen and provides the apparent, or estimated, K_d

To prepare samples for a titration curve, the amount of what should be constant in each tube?

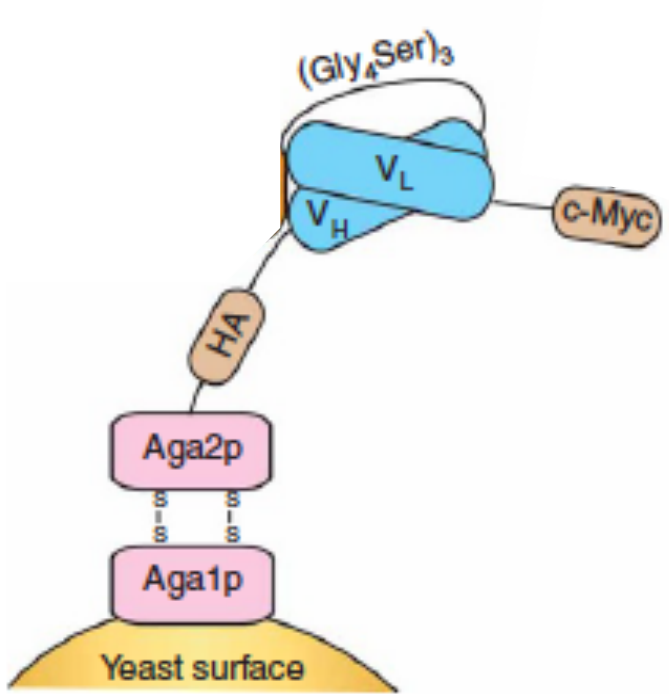
What is the variable that is different between the tubes?

Sample preparation for titration curve



- Antigen range ideally spans two orders of magnitude above / below estimated K_d
- Titrations must be at equilibrium prior to measuring fluorescence

How will you validate / interpret titration experiment?



Negative controls:

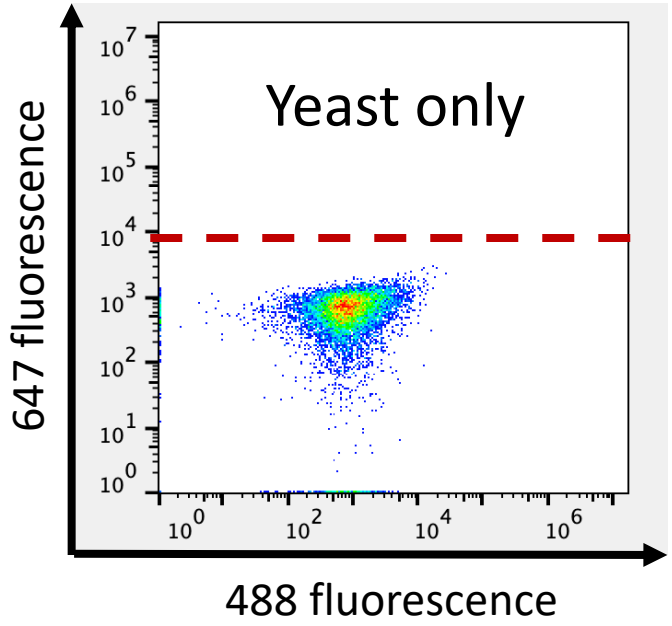
- No lysozyme (antigen), no fluorescent markers
- No lysozyme (antigen), only secondary antibody (Alexa fluor 488)

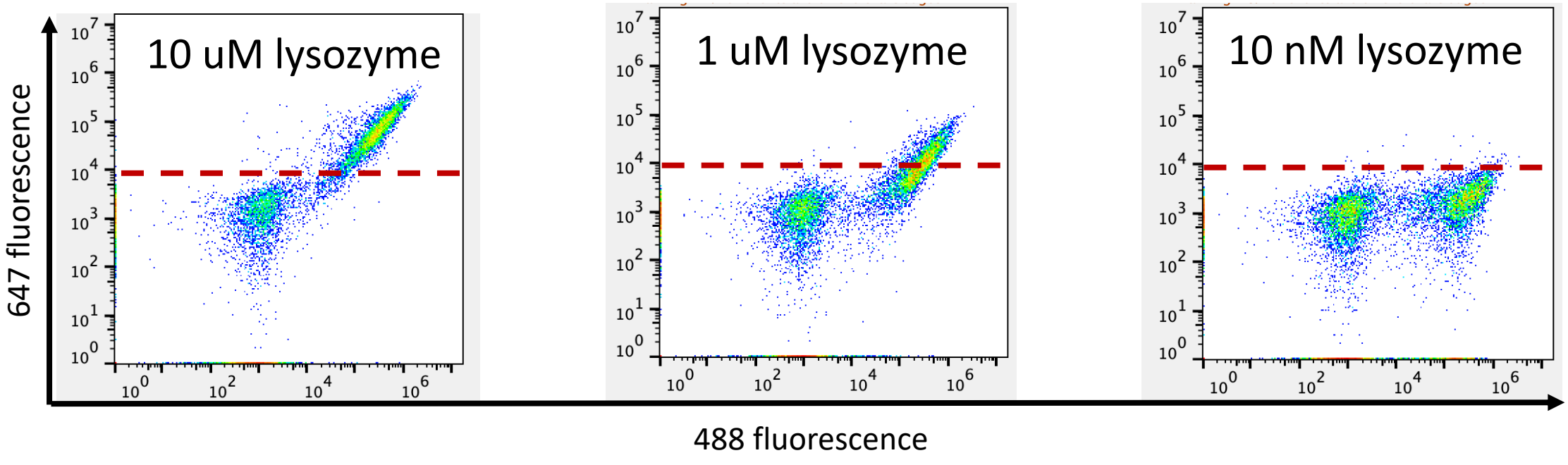
Positive controls:

- Primary antibody (anti-Myc) and secondary antibody (Alexa fluor 488)
- Lysozyme w/ marker (Alexa fluor 647) at known binding concentration

Flow cytometry used to quantify binding of lysozyme to scFv clones

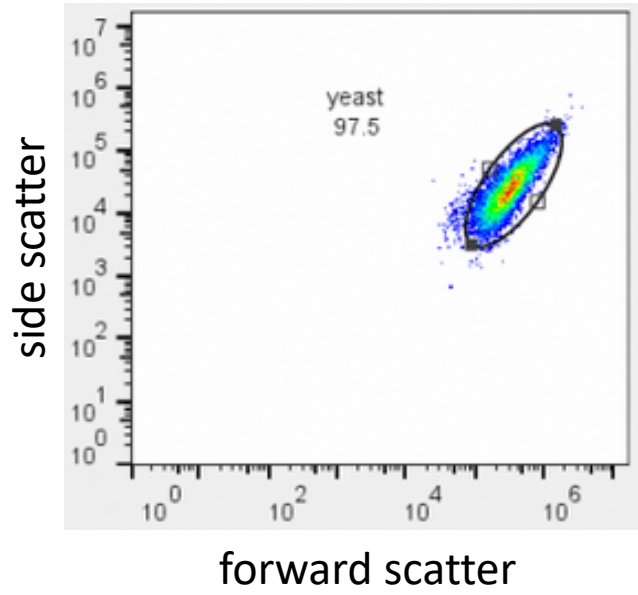
- Higher concentrations of lysozyme show more binding via increase in cells expressing signal in 647 channel

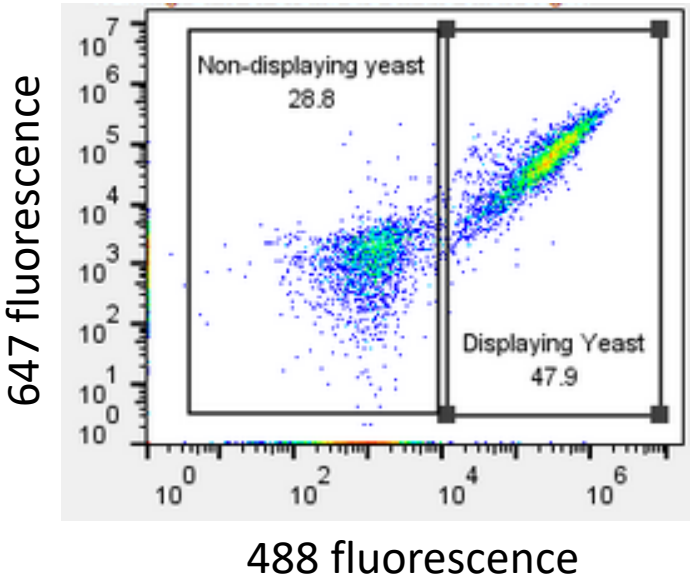




FlowJo used for data analysis

1. Define background fluorescence using unlabeled yeast cells
   - Side scatter = measure of cell complexity
   - Forward scatter = measure of cell size
2. Correct for yeast that are not displaying scFv clone
3. Determine Median Fluorescence Intensity (MFI) for yeast cells that are displaying scFv clone
4. Confirm gates are correct using controls
5. Plot titration curves in Excel





For today…

- Read through M3D4 wiki protocol and begin M3D5 data analysis!
- Watch flow cytometry video (https://www.youtube.com/watch?v=5IdYFgYb9ls)

For M3D5…

- Complete with your co-investigator; outline Background & Approach section of Mini-report.