Research article due Monday, Apr 20 at 10p

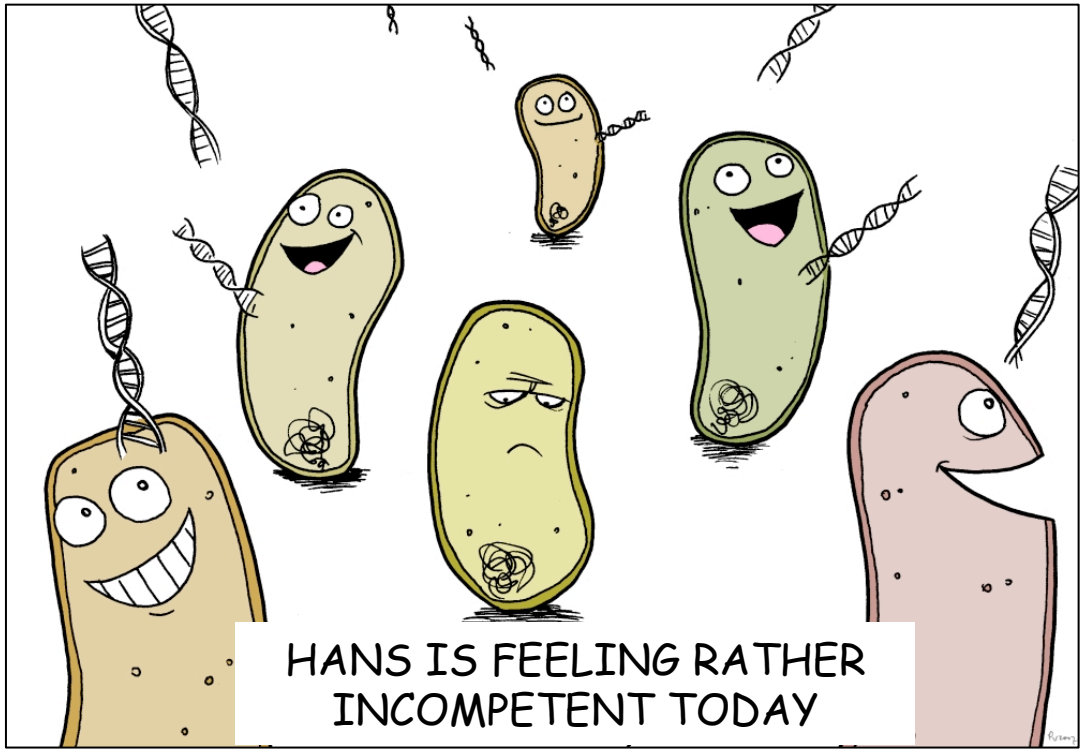
- Weekend office hours (https://mit.zoom.us/j/95904320728)
  - Becky: Saturday, 12 – 2p
  - Leslie: Saturday, 2 – 4p
  - Noreen: Sunday, 12 – 2p
  - Ernest & Noreen: 2 – 4p
- Email with Zoom link will be sent on Friday
- Also posted on FYI tab

M3D2:
Harvest candidate clones and prepare for sequencing

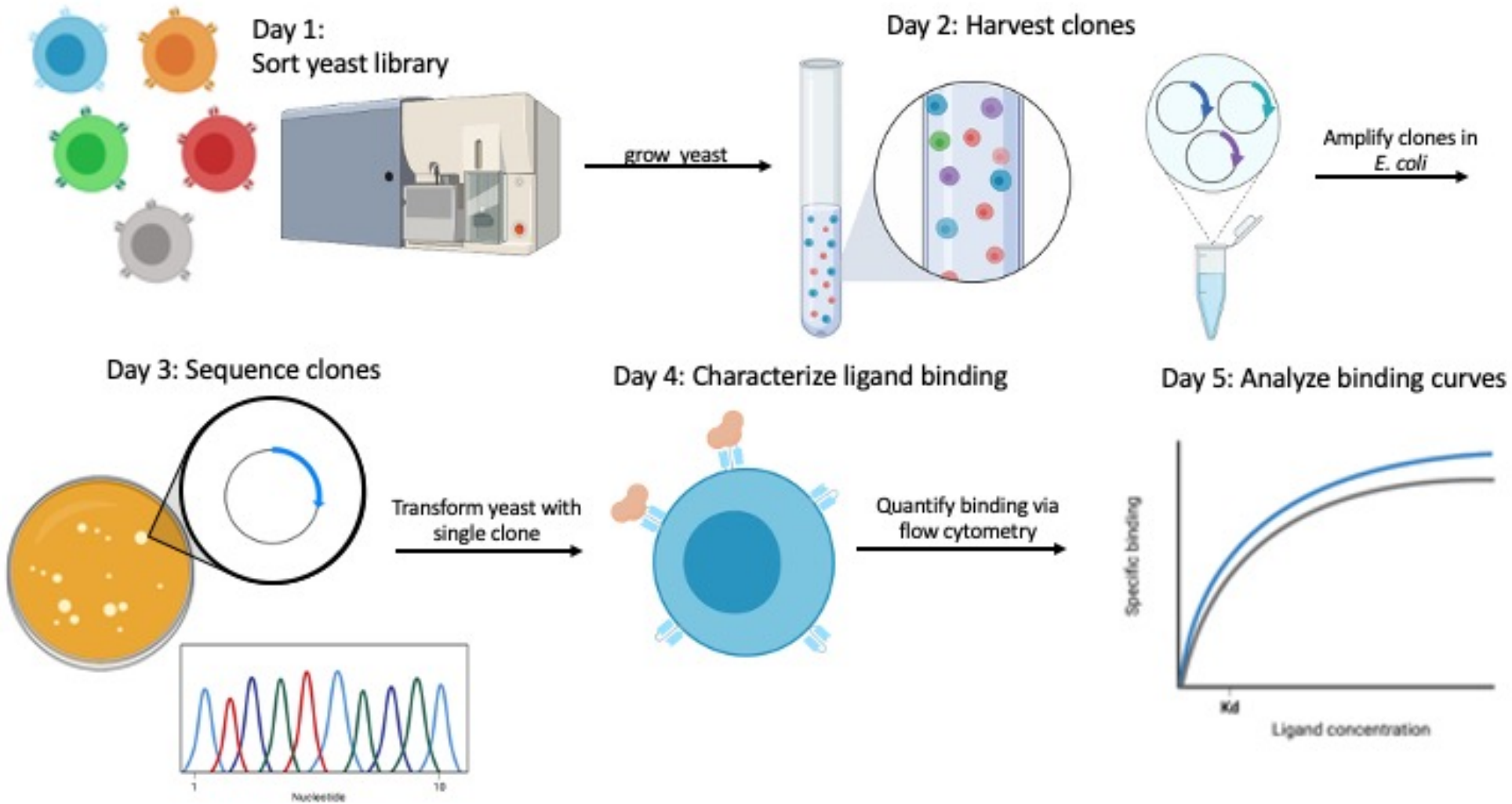
1. Isolate clones from yeast
2. Transform clones into *E. coli*

   (incubate ~18 hours)

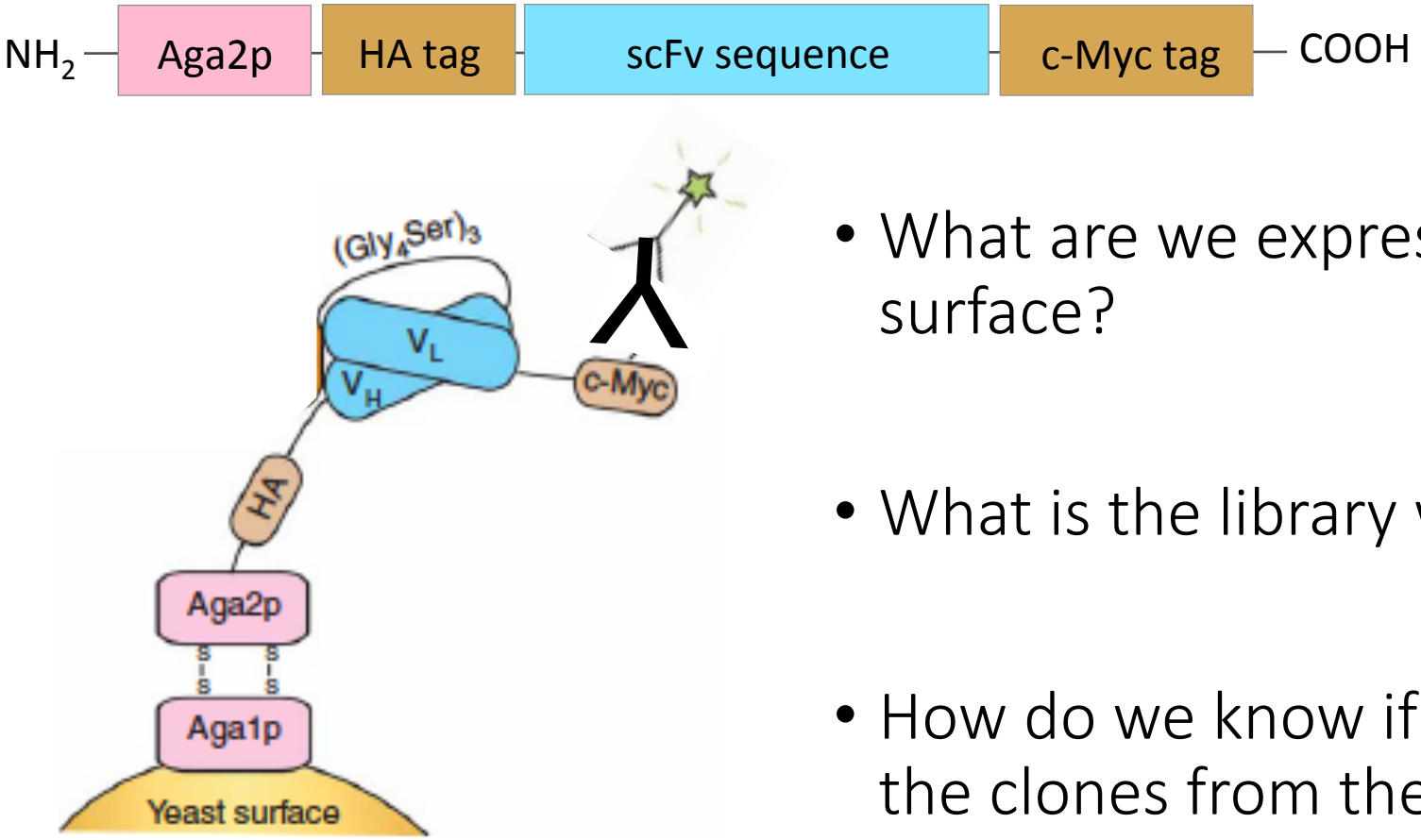
1. Purify clones from *E. coli*



Overview of Mod3 experiments

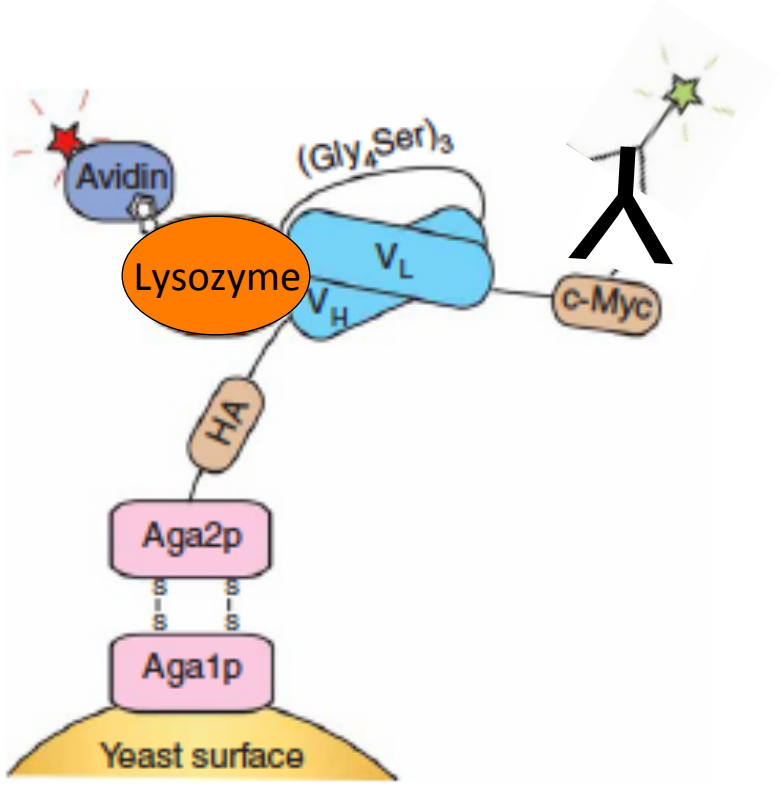


What is yeast display?



- What are we expressing on the yeast surface?
- What is the library we are screening?
- How do we know if the yeast are displaying the clones from the library?

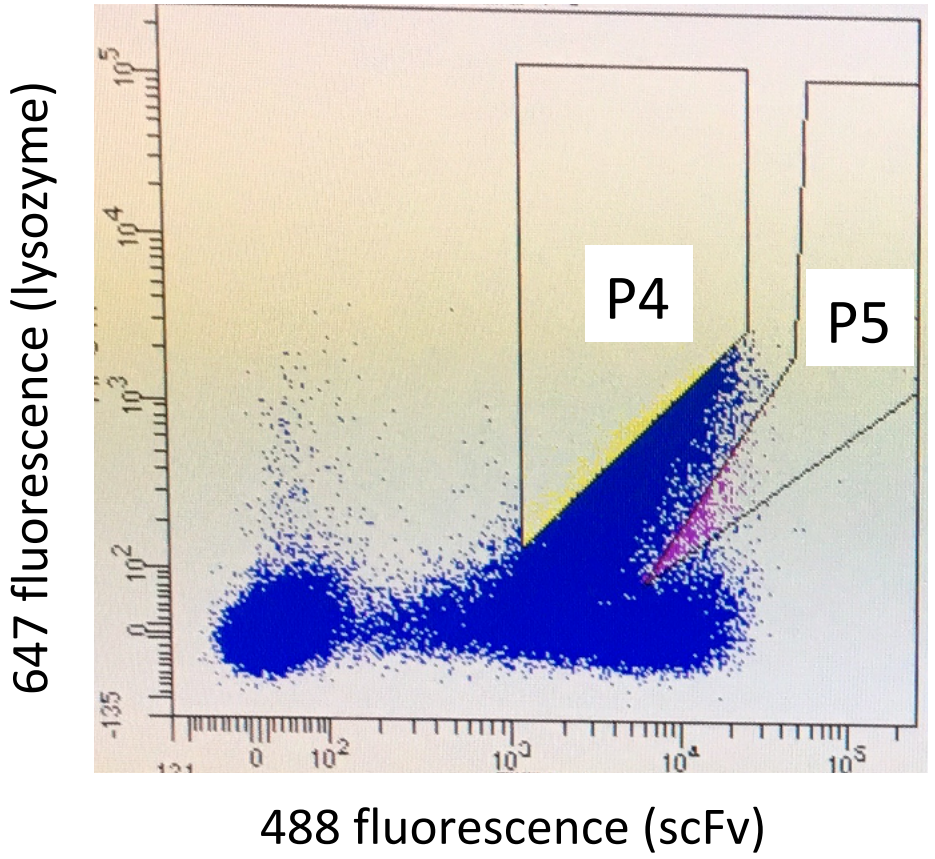
Why are we using yeast display?



- What is the antigen for the scFv in your experiment?
- How do we know if the antigen is bound to the scFv displayed on the yeast surface?

How did we screen our scFv library?

- What features / characteristics were used to sort the cells?
- How are gates used to define which cells are sorted / collected?
- How does FACS sort cells?



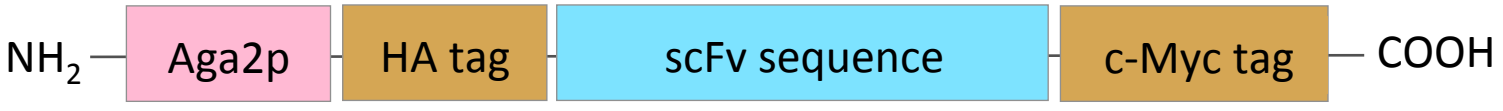
What is your experiment?

- Background: scFv sequence specific to lysozyme was cloned into yeast display plasmid and then error-prone PCR was used to randomly mutate the sequence

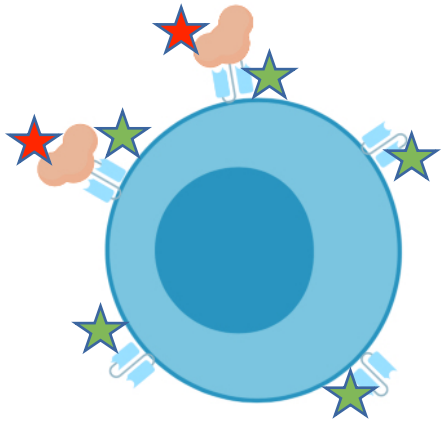
- Goals:

1. Identify lysozyme-specific scFv sequences that might bind lysozyme better
2. Characterize binding properties of mutated lysozyme-specific scFv antibodies

Workflow for isolating scFv clones

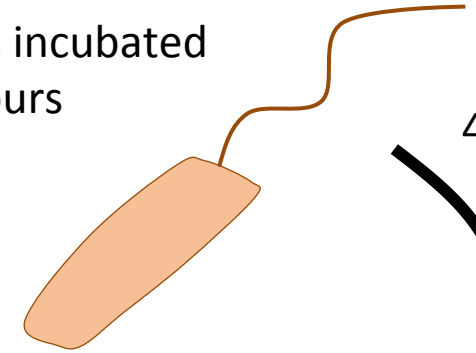


2. scFv clones isolated from yeast cells



1. sorted yeast cells recovered at 30 C for 3 days

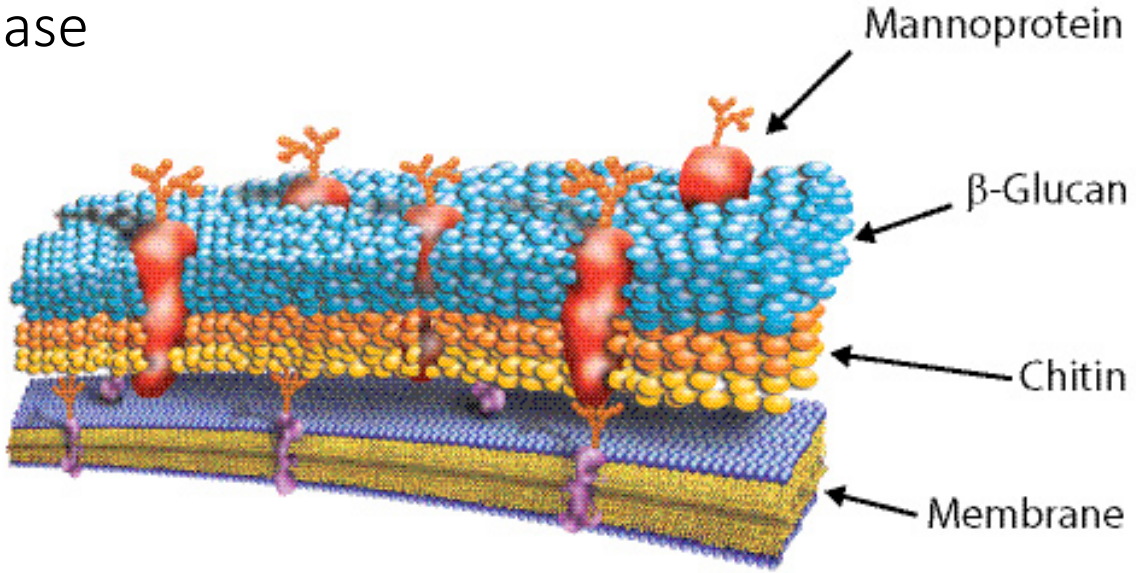
3. scFv clones transformed into *E. coli* cells and transformants incubated ~18 hours



4. scFv clones isolated from *E. coli* cells

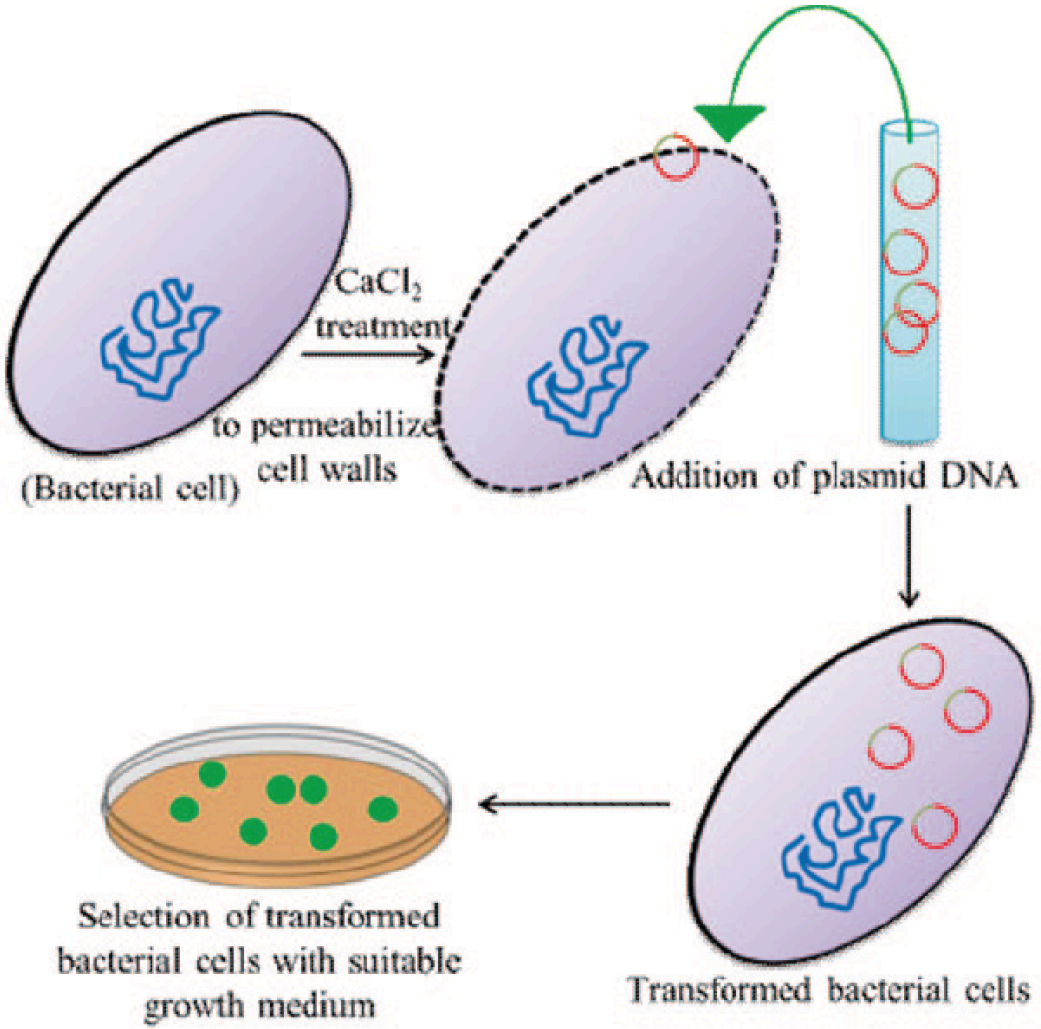
Yeast wall composed of sugars, proteins, lipids

- Proteins linked to mannon-oligo-saccharide (mannoprotein complex)
- Layers of polysaccarides (β-glucan and chitin) surround cell membrane
- Yeast wall complex disrupted using Zymolase
  - β-1,3-glucan laminaripentao-hydrolase
  - β-1,3-glucanase
- DNA purification completed via alkaline lysis

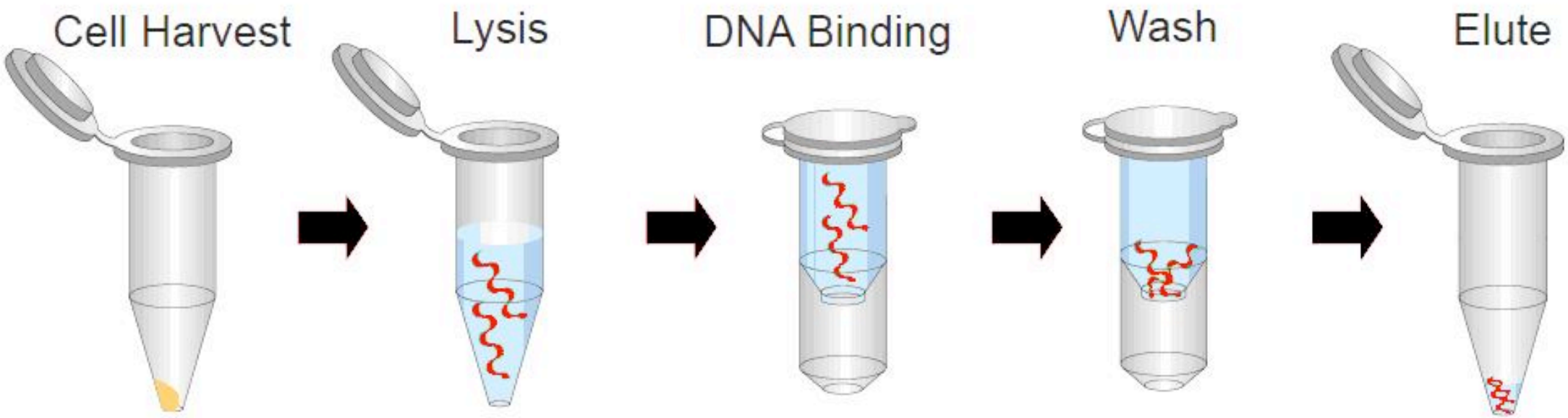


Transformation used to move DNA into *E. coli*

- *E. coli* cells treated with $CaCl_2$ to promote competency
- Heat shock used to permeabilize cell membrane
- Cells incubated in rich media for recovery, then plated for selection

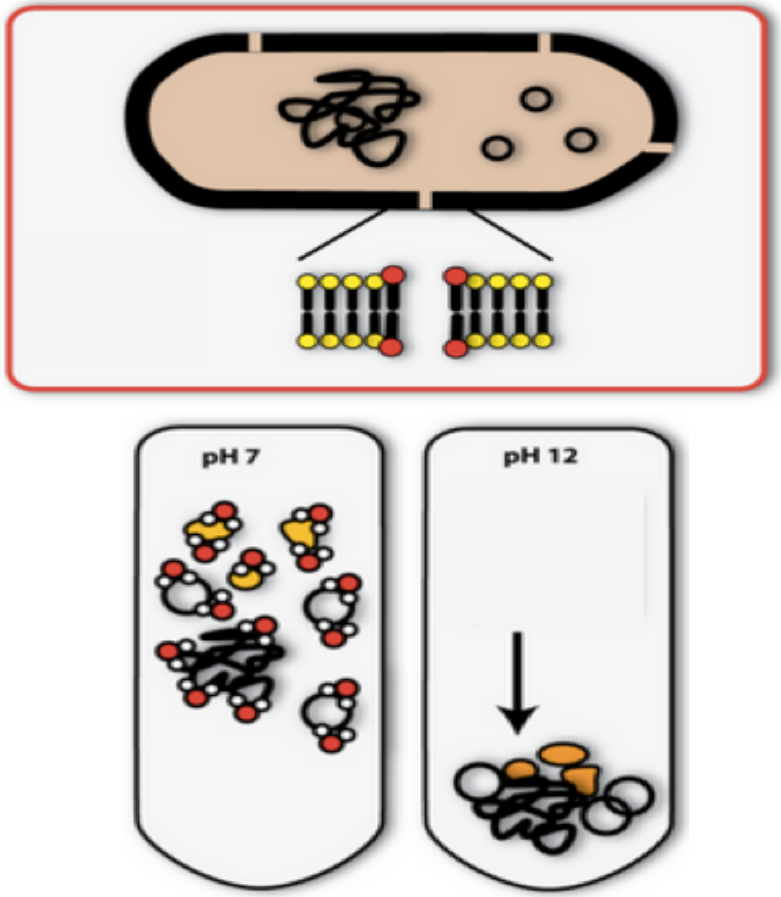


Alkaline lysis used to isolate DNA from cell lysate



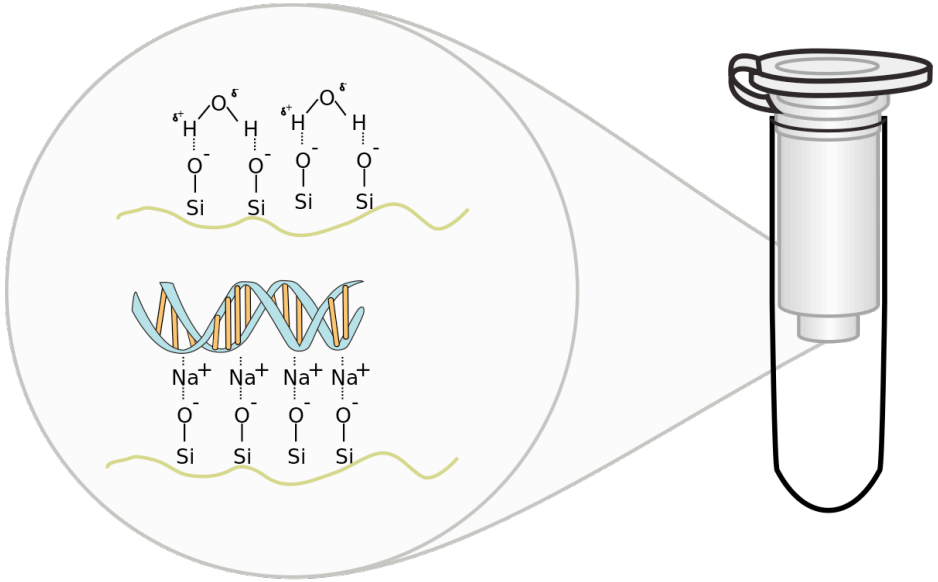
Alkaline lysis: prepare and lyse cells

- Cells resuspended with Buffer P1
  - Tris / Ethylenediaminetetraacetic acid (EDTA)
  - RNAse
- Cells lysed with Buffer P2
  - Sodium dodecyl sulfate (SDS)
  - Sodium hydroxide (NaOH)



Alkaline lysis: neutralize cell lysate

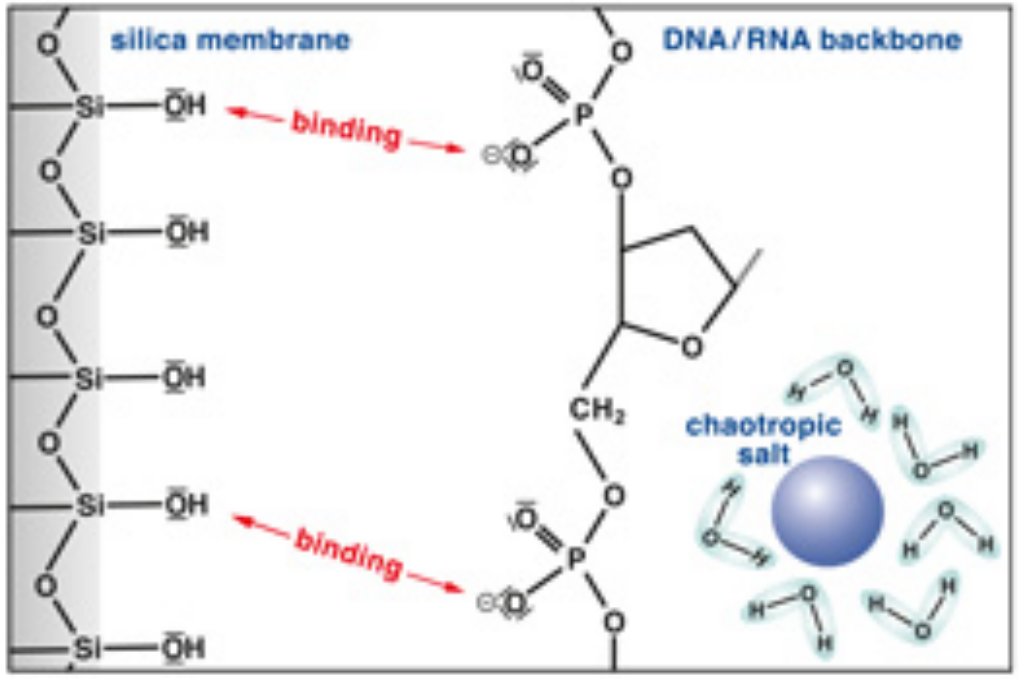
- Cell lysate neutralized with Buffer N3
  - Acetic acid / Potassium acetate
  - Guanidine hydrochloride (chaotropic salt)

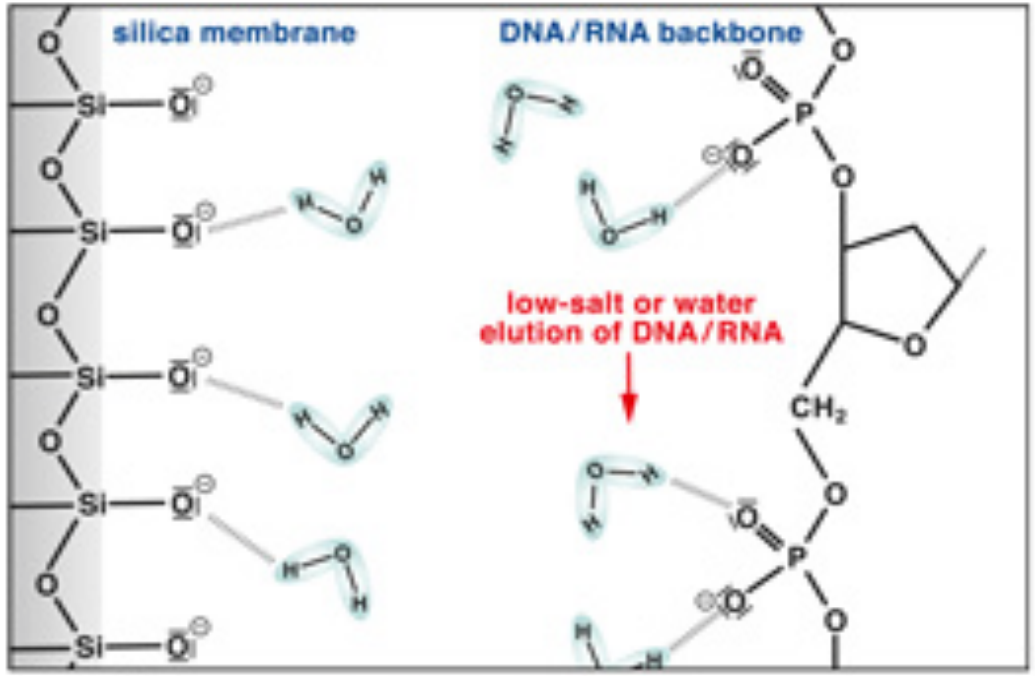


- After DNA bound to column, wash steps remove contaminants
  - Buffer PB: isopropanol and Guanidine hydrochloride
  - Buffer PE: ethanol and Tris-HCl

Alkaline lysis: elute DNA

- DNA eluted from column with H_20, pH = 8





= water molecule

For today…

- Read through wiki information!
- Discuss potential research topics with your lab partner / co-investigator

For M3D2…

- Complete with your co-investigator; discuss potential research topics and consider which research question to pursue
  - Review the prompts on the wiki
  - Summarize your potential idea in 1-2 paragraphs