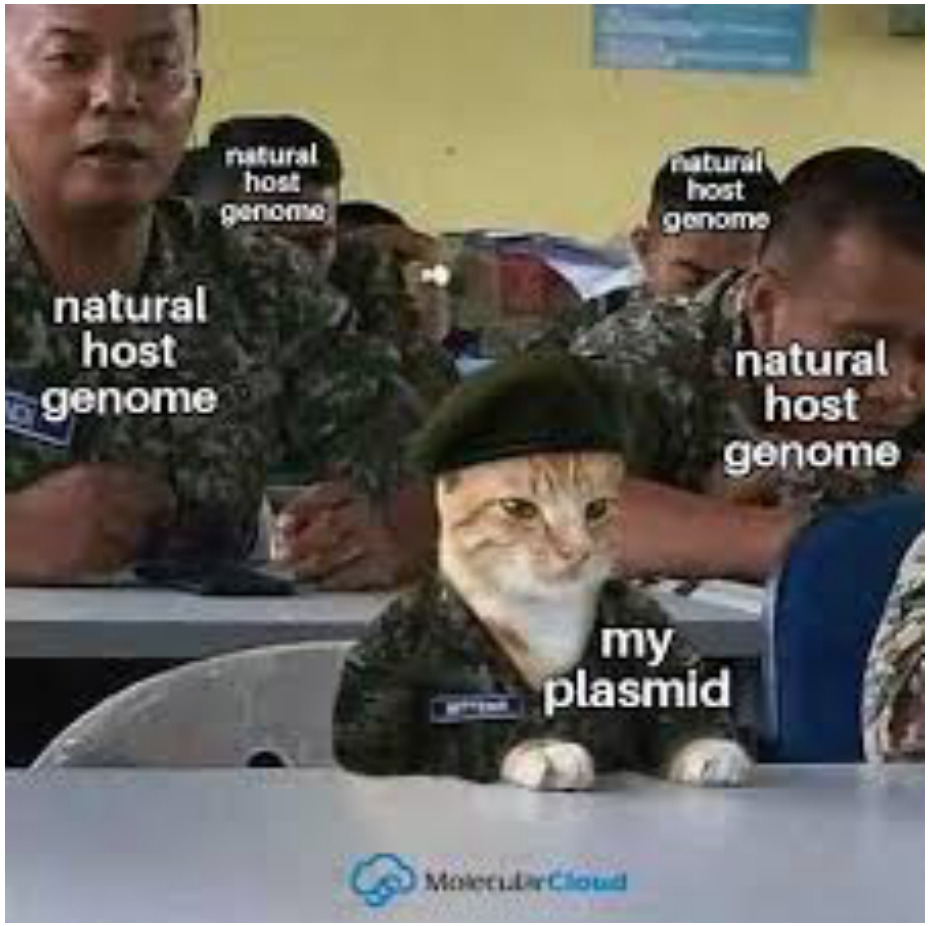
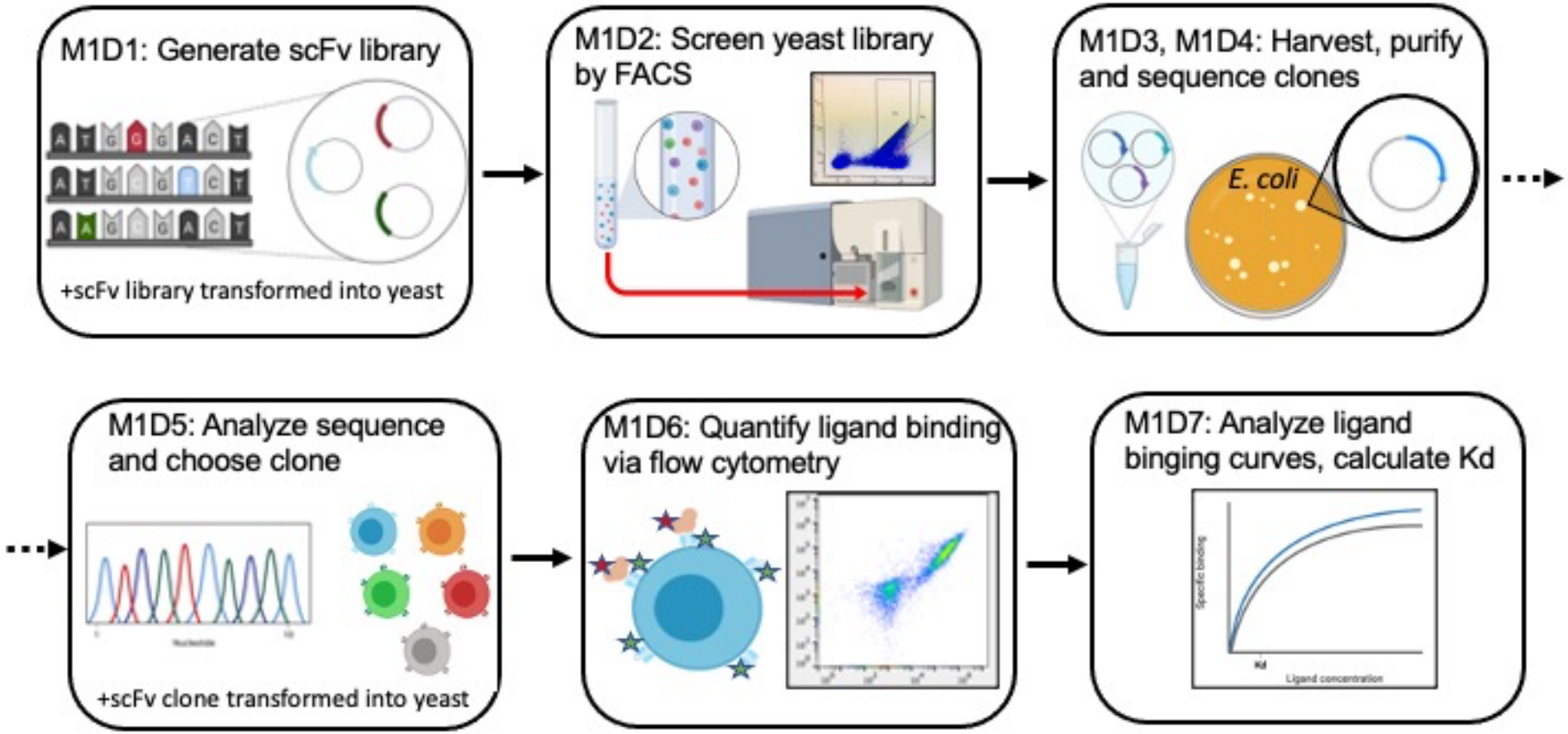
M1D3: Harvest candidate clone plasmids from library

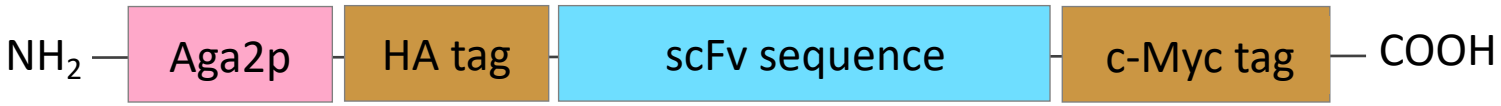
1. Comm Lab
2. Prelab discussion (short)
3. Isolate clones from yeast
4. Transform clones into *E. coli*

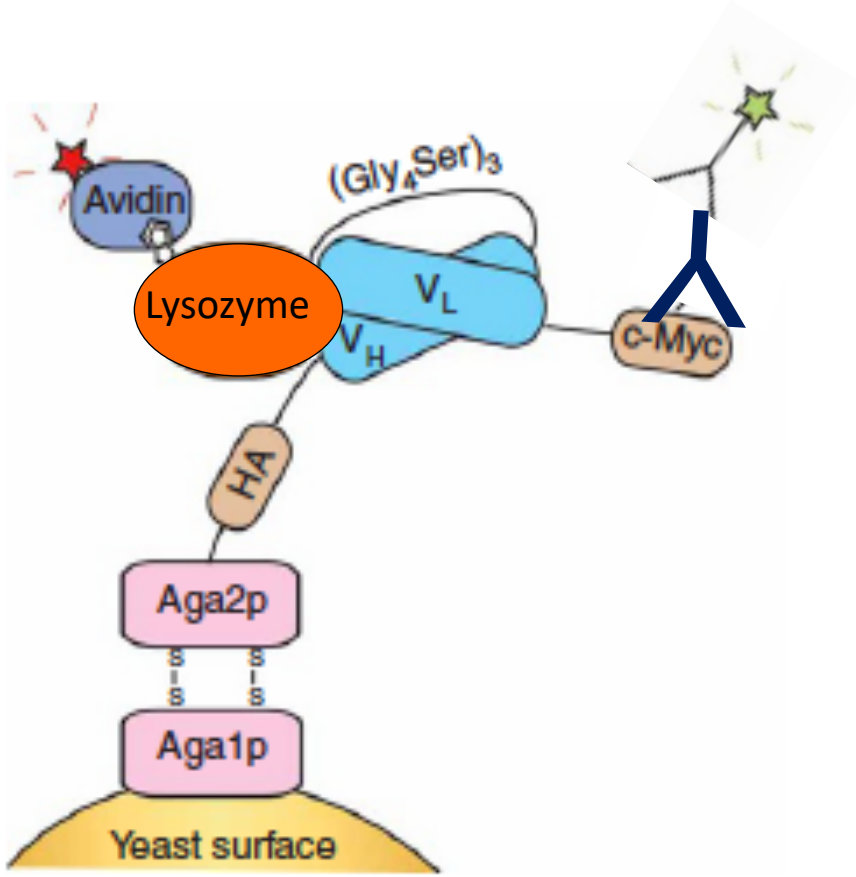


Overview of Mod1 experiments



How are we using yeast display to engineer antibodies?



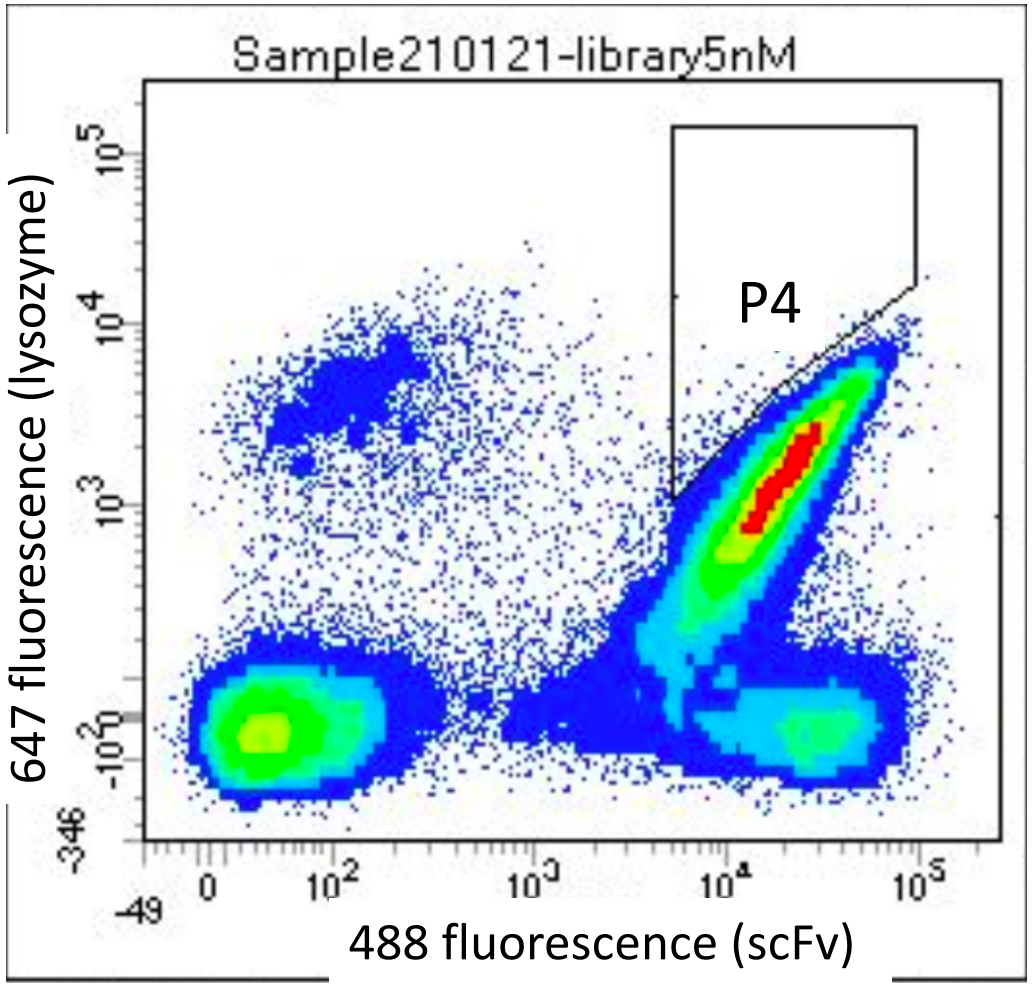


- What are we expressing on the yeast surface?
- What is the library we are screening?
- How do we know if the yeast are displaying the clones from the library?
- How will we know our antigen has bound?

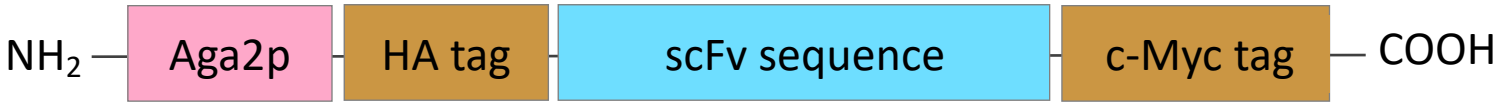
How did we screen our scFv library?

- What characteristics were used to sort the cells?

- How are gates used to define which cells are sorted / collected?

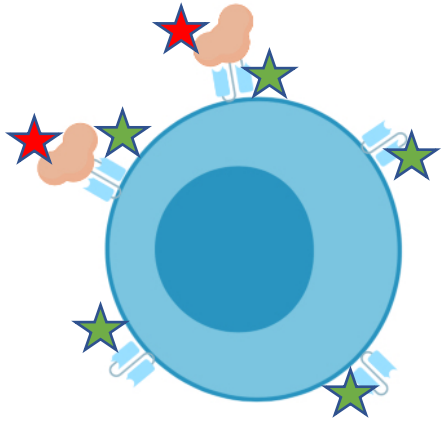


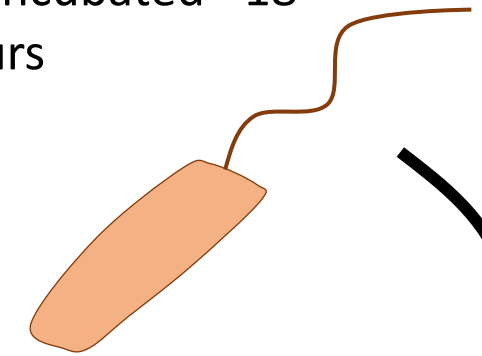
Workflow for isolating scFv clones



2. scFv clones isolated from yeast cells

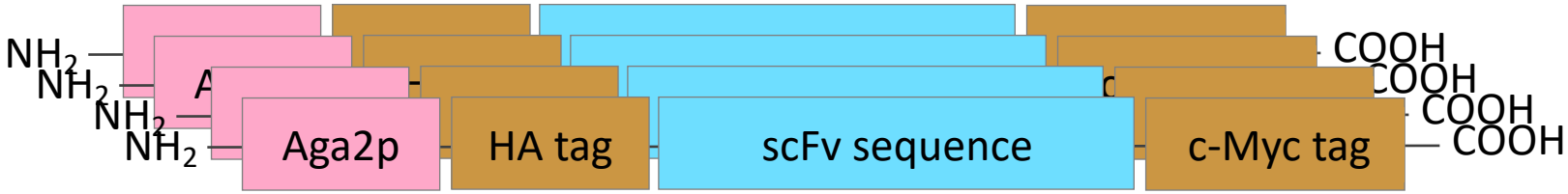
3. scFv clones transformed into *E. coli* cells and selected transformants incubated ~18 hours





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

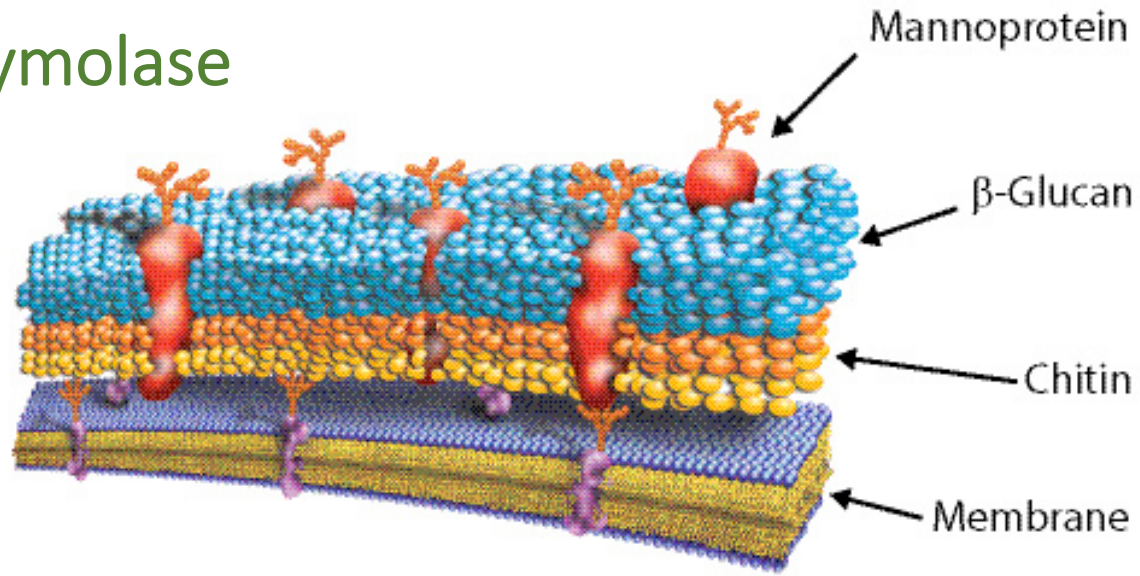
1. sorted yeast cells recovered at 30 C for 3 days



Yeast cell wall is a complex fortress

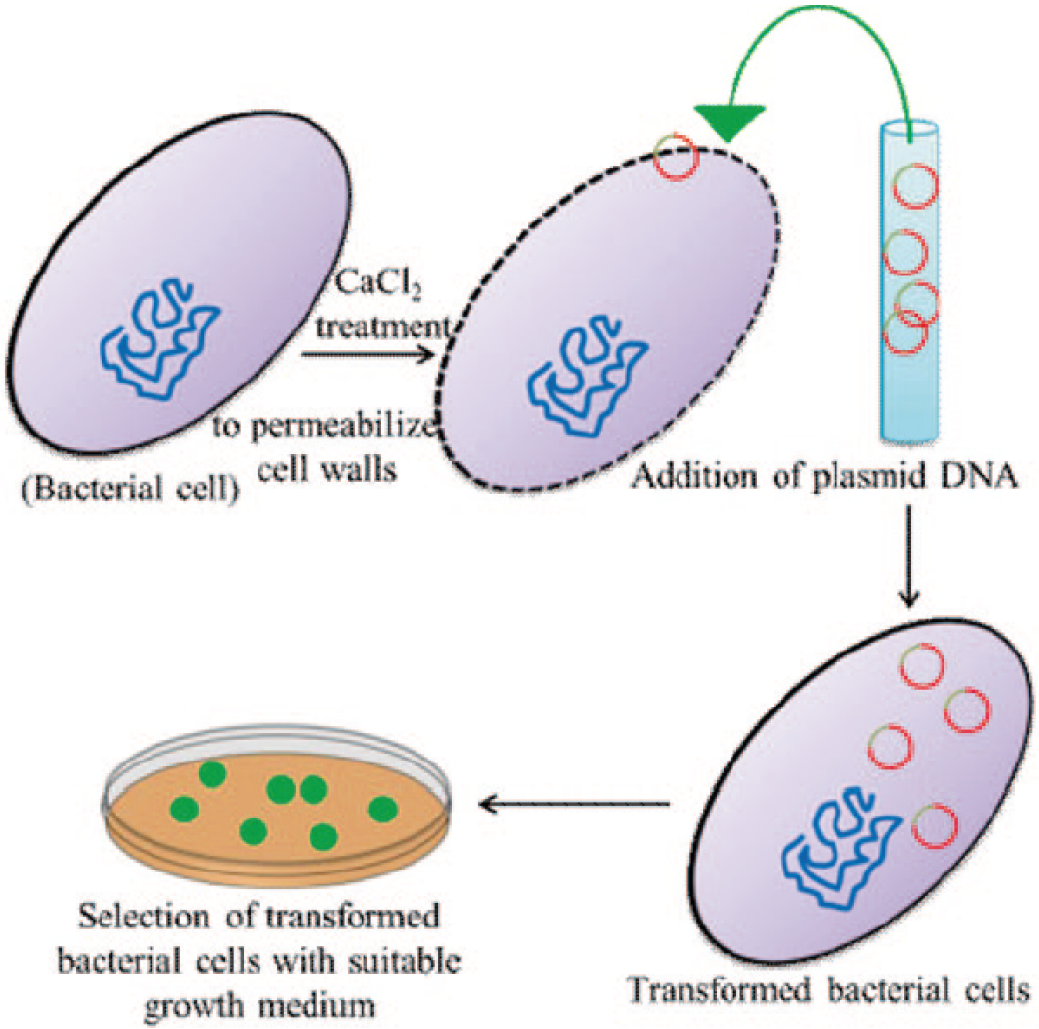
Comprised of sugars, proteins, and lipids

- Proteins linked to mannon-oligo-saccharide (mannoprotein complex)
- Layers of polysaccarides (β-glucan and chitin) surround cell membrane
- Yeast wall complex disrupted using Zymolase
- DNA purification completed via alkaline lysis to prepare for bacterial transformation

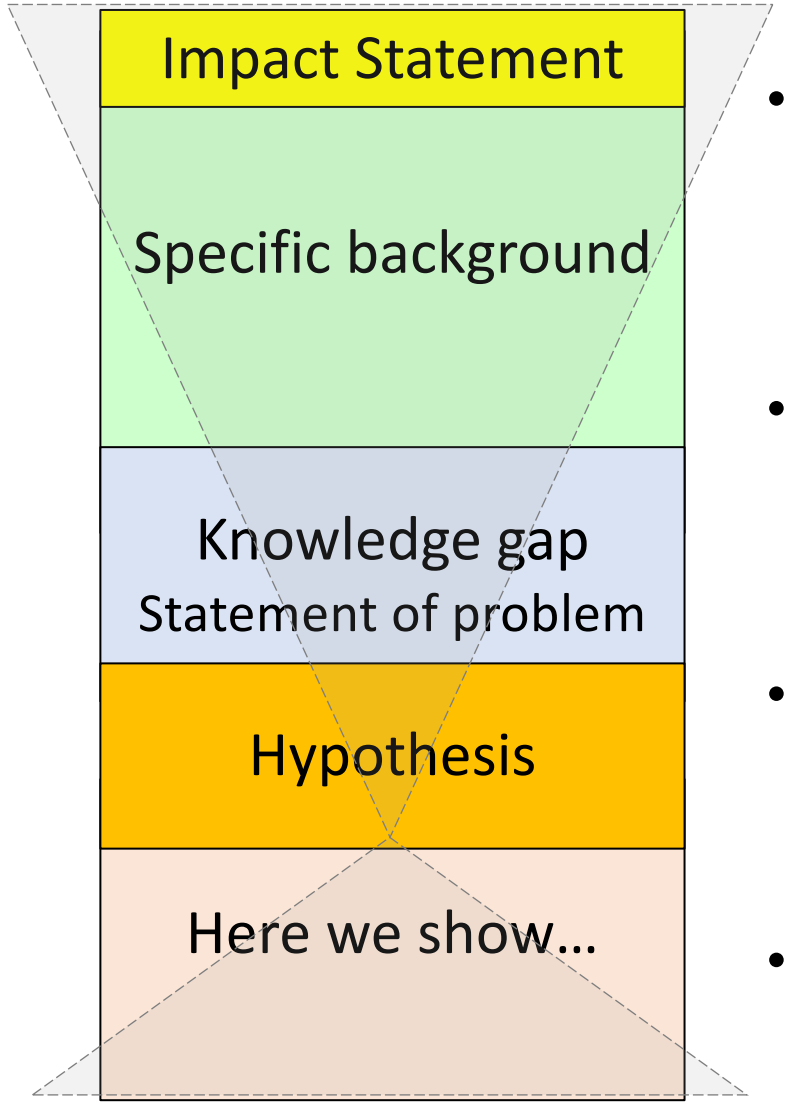


Transformation moves DNA into *E. coli*

- *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



What goes into a background/motivation section?



- Your research is anchored in a general topic. Motivate the topic in a way that draws your audience into your study.
- Specific background connects your project with the general background.
  - Minimum essential information
  - References current work in the field
  - Introduce specific technologies necessary for understanding the project
- Clearly state the unknown or problem your research is addressing.
  - The question you address is clearly articulated, connected to the background, and has appropriate scope for the project
  - Give evidence of incompleteness of current understanding, therefore motivating the investigation
- The hypothesis specifically addresses the problem and has a measurable outcome.
  - Draft a hypothesis (this does not need to be the hypothesis you use in your data summary)
- A preview of your findings and their implications
  - Light on Methods
  - include a space holder for this section in your homework

How should you introduce your story?

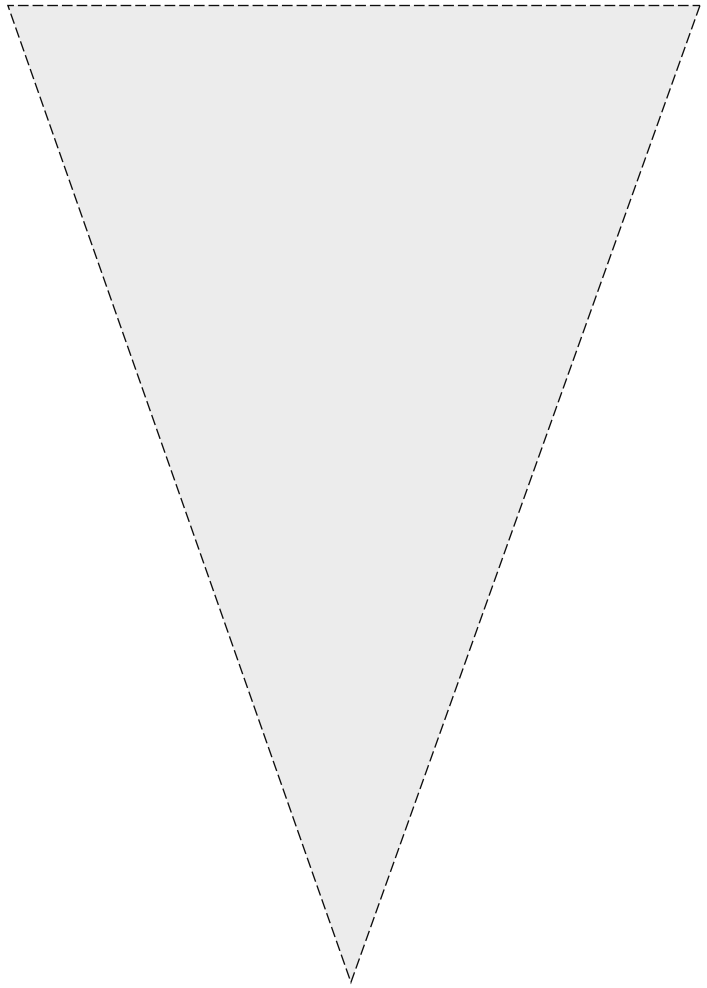
1st paragraph: what is the big picture / problem?

2nd paragraph: what is currently known / what should the reader know to understand your work?

3rd (or 4th) paragraph: what is your research question?

4th (or 3rd) paragraph: how will you address your question?

5th paragraph: here we show…



Notes on methods section…

Include enough information to replicate the experiment

- Cite manufacturer for supplies / equipment (Company)
- Be **concise and clear** in your description

Use subsections with descriptive titles

- Put in **logical order**, rather than chronological order
- Begin with topic sentence to introduce purpose / goal of each experimental procedure

Use clear and concise full sentences

- NO tables or lists, all information should be provided in **full sentences** and paragraphs
- Write in passive voice and use past tense

Use the most flexible units

- Write **concentrations** (when known) rather than volumes

Eliminate 20.109 specific language and obvious details

- Example “labeled Row A, Row B…”
- Do not include details about tubes and water!
- Assume reader has some biology experience
- Include parts of the protocol that the teaching faculty completed, but do not say “completed by teaching faculty.”

How can you improve this example?

The cells were grown in media. Cell number was estimated and yeast

were prepared. Cells were spun, washed and resuspended in cold PBSA

[Phosphate buffered saline with 1ul albumin (Sigma)] for use later.

How can you improve this example?

What cells?

What constitutes the media?

Under what conditions?

How

The cells were grown in media. Cell number was estimated and yeast

Specifically, why was this done?

What does this mean?

Colloquial…use more scientific language. Also, include details.

were prepared. Cells were spun, washed and resuspended in cold PBSA

Use the most flexible units

What would be more informative?

[Phosphate buffered saline with 1ul albumin (Sigma)] for use later.

Edited example…

Library expression, labeling and screening

The EBY100 yeast expressing scFv clones were grown in SGCAA [sterilized, 2% Galactose, 0.67% Yeast Nitrogen Base, 0.5% Casamino Acids, 0.54% Na2HPO4, 0.86% NaH2PO4 (Sigma)] overnight at 20°C shaking. Cell number was estimated by optical density at 600nm and $1x10^7$ yeast were prepared for each condition. Cells were pelleted at 7,500g for 30sec, washed with 1mL cold PBSA [Phosphate buffered saline with 0.1% albumin (Sigma)] and resuspended in a solution of 5nM of biotinylated lysozyme (Sigma) in PBSA for 60min at room temp, rocking.

Other useful information for methods

- **FACS Machine details:** "cells analyzed and sorted on BD FACS Aria II with assistance of Koch Institute Flow Core staff."

- **Flow Cytometer details:** "cells analyzed on BD Accuri C6 flow cytometer"

For today…

- Work through wiki
- Use Comm Lab advice to update figure if desired
  - Resubmit updated figure for grading by 10pm tonight

For M1D4

- Write 4-5 topic sentences to outline the Background and Motivation section of the Data Summary
  - Include references!
- Write M1D2-M1D3 Methods **as a team**
- Make appointment/visit BE Comm lab