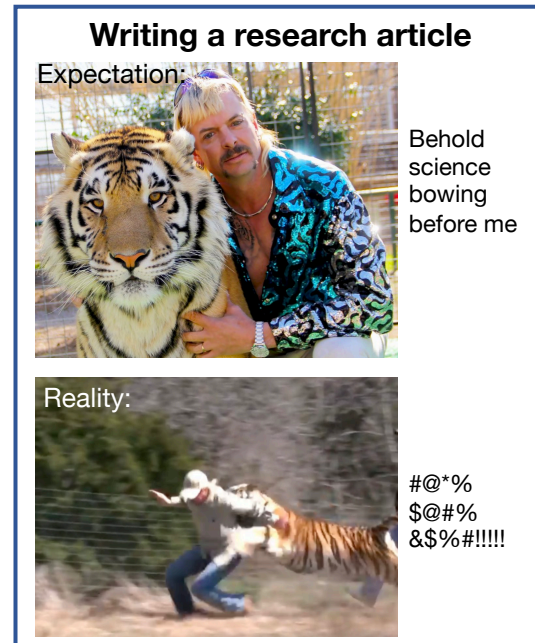


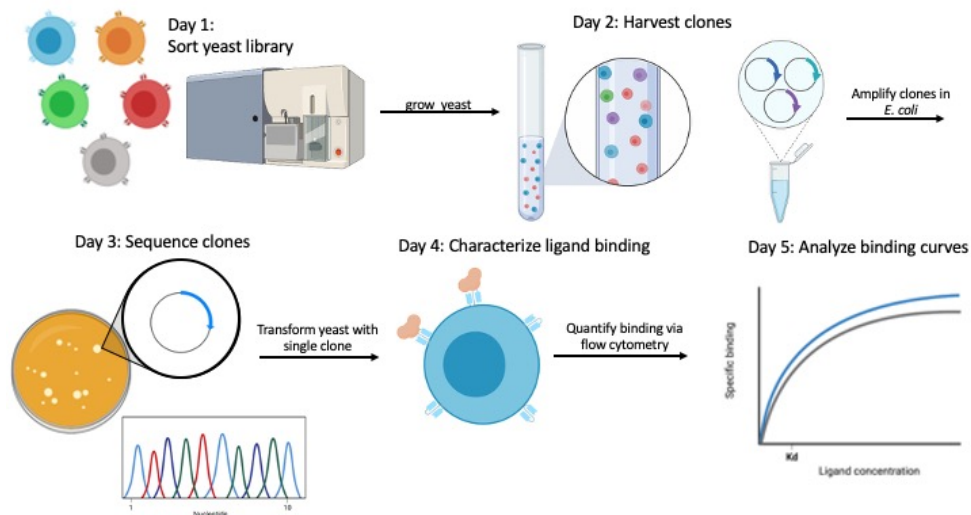
M3D3:

Identify clones to characterize

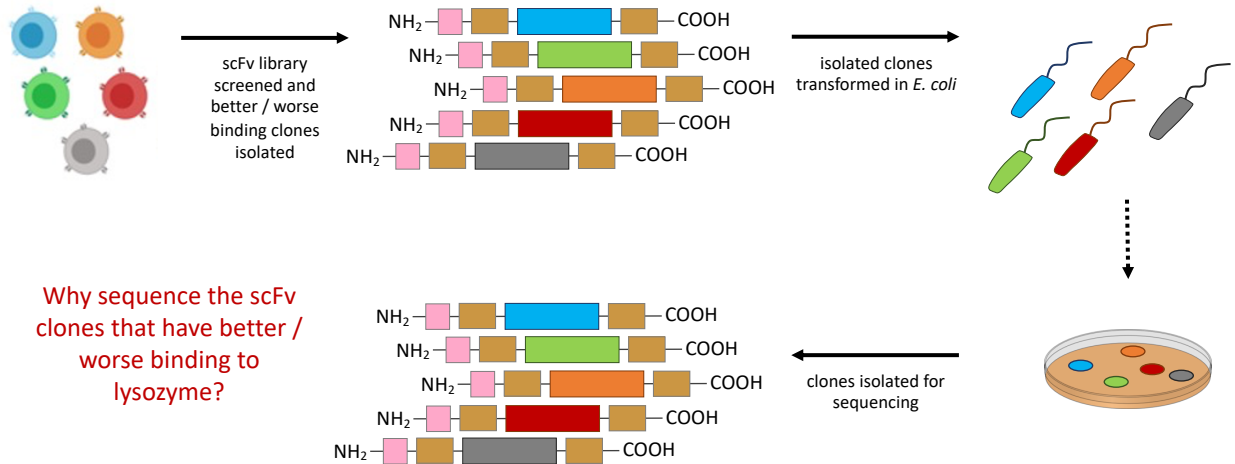
1. Determine sequences of scFv clones
2. Discuss and develop research proposal ideas



Overview of Mod3 experiments

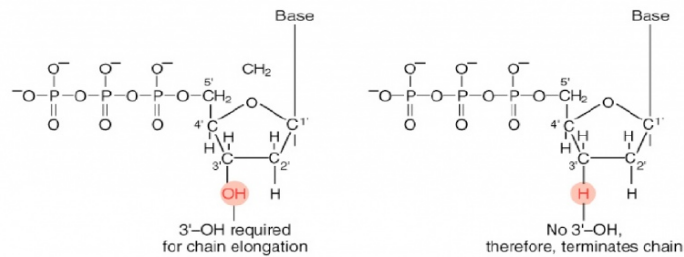


How did we get to M3D3?

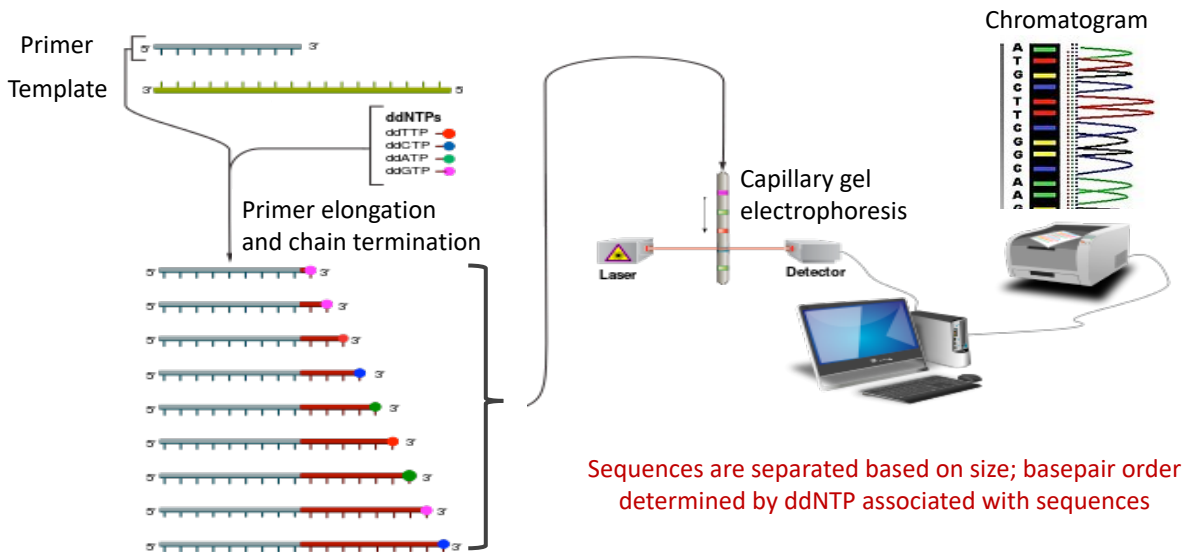


Sanger sequencing used to identify mutations in scFv clones

- Di-deoxynucleotides terminate sequence elongation
- 3' hydroxy is lacking which prevents addition of subsequent base (required for nucleophilic attack at 5' phosphate)



Sequence determined from chain termination products



Sequencing alignments will be used to identify mutations in scFv clones

- Use SnapGene or Benchling to compare clone sequence to parental sequence
 - Parental sequence = scFv used to generate library we screened
- First, identify basepair changes in the sequence
- Then determine if basepair change result in amino acid substitutions

| | Match | | | | |
|----------|-------|----------|---|---|-----|
| Parental | A | C | T | C | G |
| Mutant | A | T | T | C | - |
| | | Mismatch | | | Gap |

For today...

- Identify mutations in scFv clone sequences
- Watch Sanger sequencing video
https://www.youtube.com/watch?v=-QIMkQ4E_wE
- Discuss potential proposal topics with your research colleagues

For M3D4...

- Complete with your co-investigator; using the feedback from the peer discussions, begin to refine your research proposal idea by creating a project overview
 - See prompts on wiki for what information / details to include

Research proposal discussions

First Round:

- Talk about your proposal ideas with your co-investigator to hash out details/plans
 - **Be back in main class Zoom by 3:40pm**

Second Round:

- Each person will discuss research proposal ideas with another research colleague (peers from other teams)
- Discussion will occur in two different Zoom breakout groups
 - First meeting with start at 3:40p, second at 4p
 - Instructor will assign random breakout groups