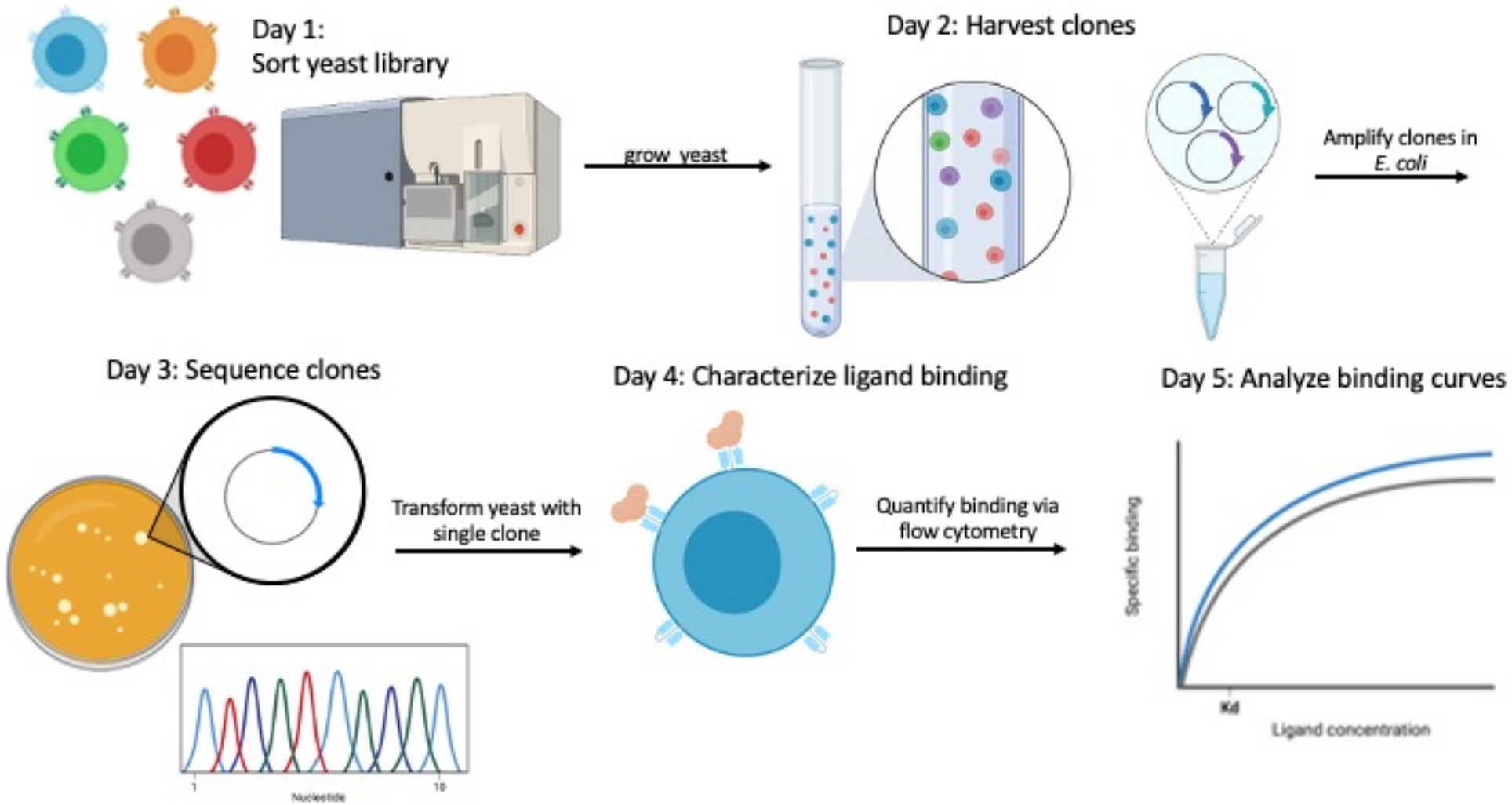
M3D3:
Identify clones to characterize

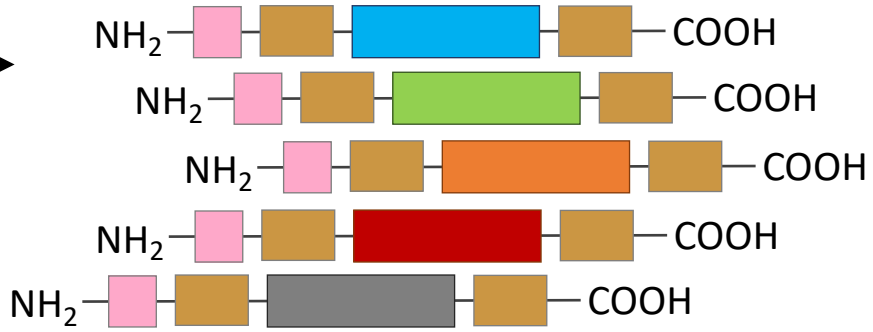
1. Determine sequences of scFv clones
2. Discuss and develop Research proposal ideas

Overview of Mod3 experiments

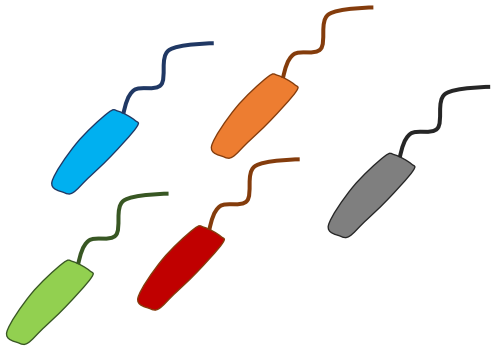


Let's review our progress…

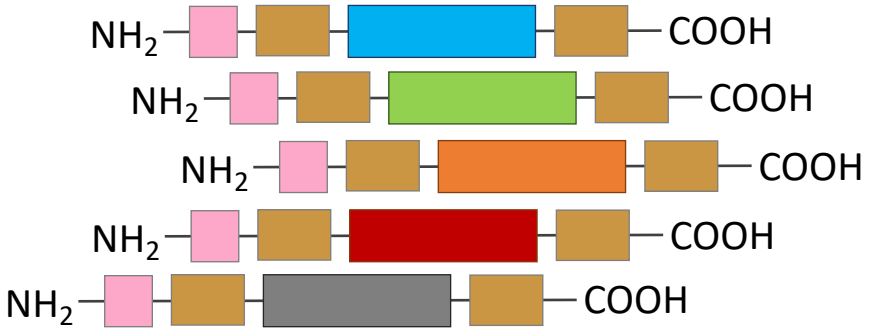
scFv library screened and better / worse binding clones isolated



isolated clones transformed in *E. coli*



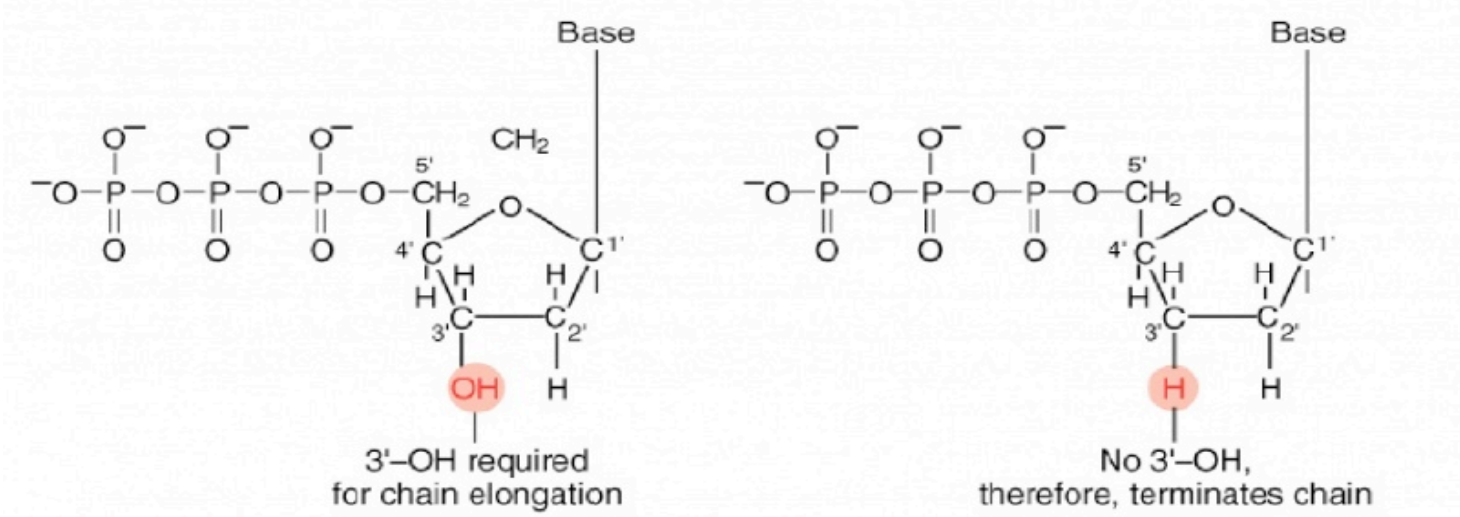
clones isolated for sequencing



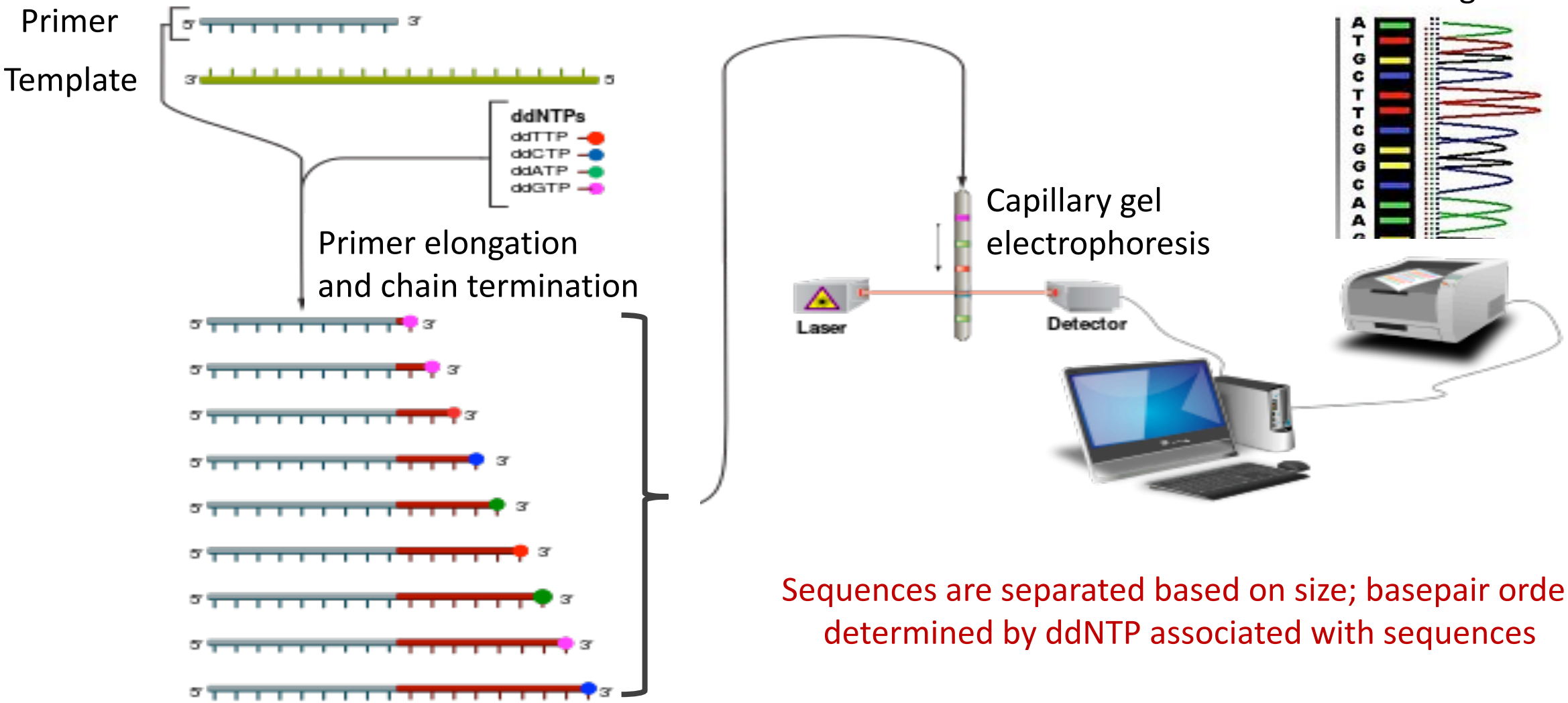
Why sequence the scFv clones that have better / worse binding to lysozyme?

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)



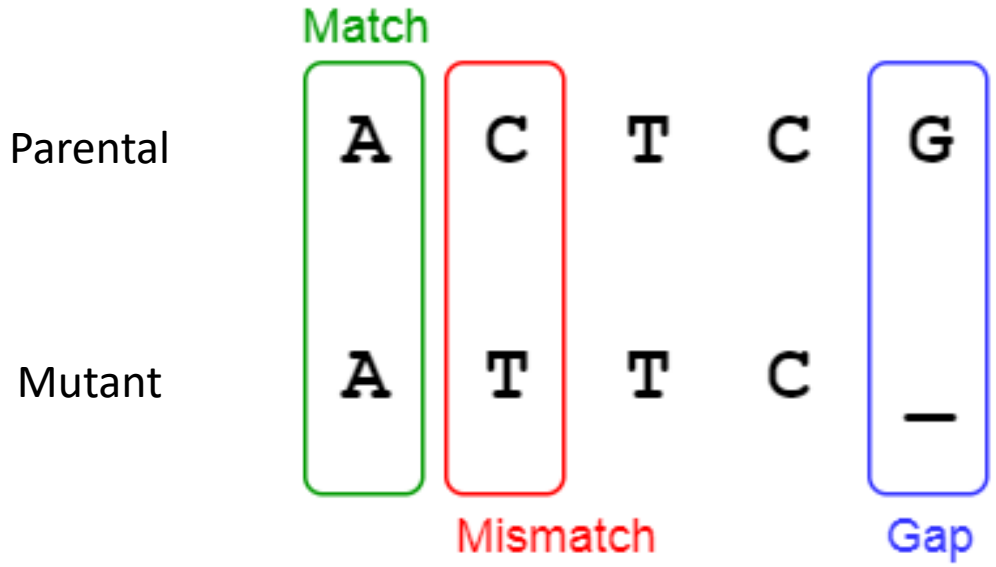
Sequence determined from chain termination products



Sequences are separated based on size; basepair order determined by ddNTP associated with sequences

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



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

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