M1D1: Generate scFv library

1. Prelab discussion
2. *In silico* mutagenesis PCR
3. *In silico* yeast transformation
4. Orientation quiz @ 3:30pm

We couldn't afford one of those cool PCR robots,
so we just got an undergrad and a cardboard box.

Major assignments for Mod1

- Data summary (15%)
  - In a team
  - Draft due 3/24, final revision due 4/4
  - Format: Bullet points, .PPTX
- Mini-presentation (5%)
  - Individual, submit video via gmail
  - Due 3/28 by 10pm
- Lab quizzes (5% collectively)
  - In a team
  - Due 4pm day of lab, submit on Stellar
- Notebook (5% collectively)
  - Due 3/17 at 10pm, graded by Jeff
- Blog (part of 5% Participation)
  - Due 3/29 at 10pm

Experimental goals for Mod1

Overall research goal: Identify and characterize an antibody fragment (scFv) that shows improved binding to the antigen, lysozyme.

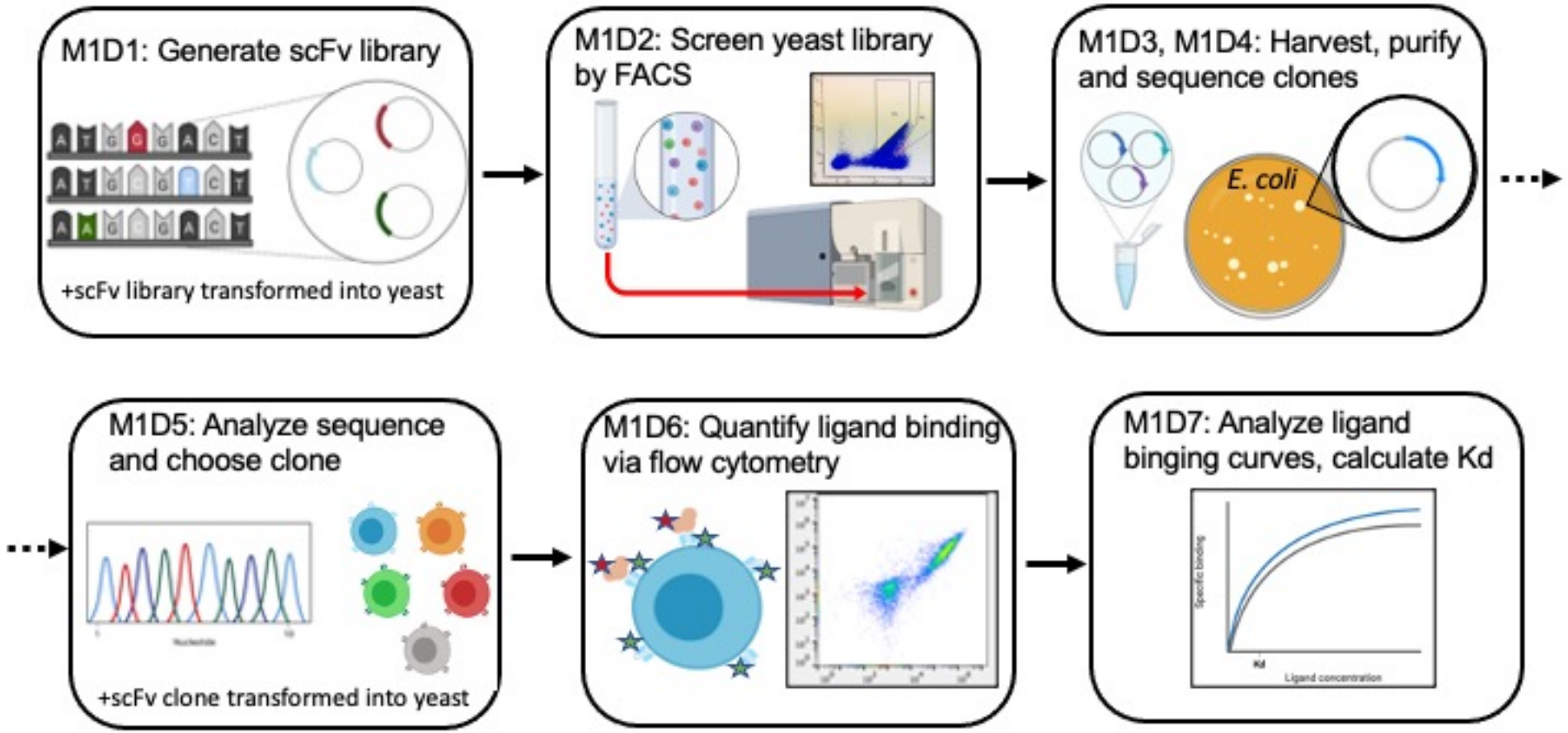
1. Using a parental clone of a **single chain variable fragment (scFv)** known to bind lysozyme, generate a library of mutant scFv clones

2. Screen that library to identify lysozyme-specific scFv sequences that might bind lysozyme better

3. Characterize binding properties of mutated lysozyme-specific scFv antibodies

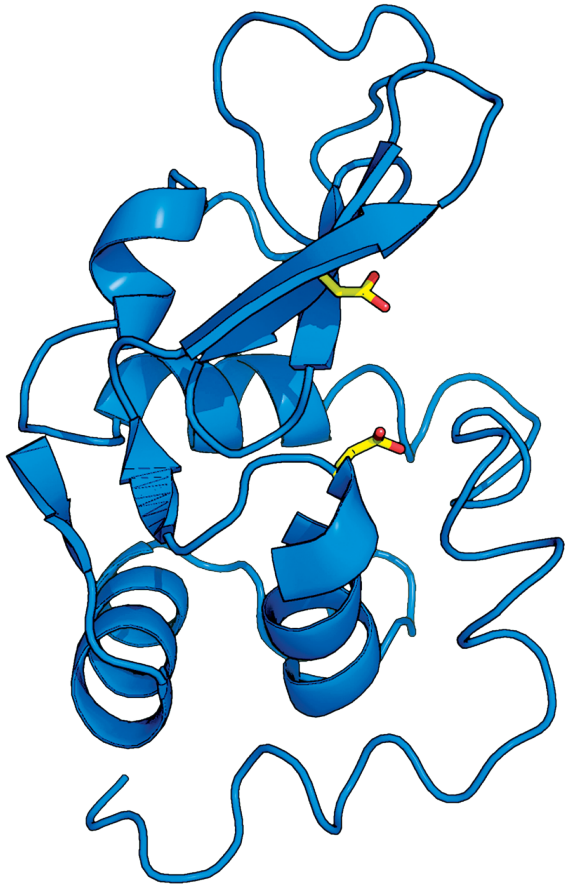
Overview of Mod1 experiments

Research goal: Identify and characterize an antibody fragment (scFv) that shows improved binding to the antigen, lysozyme.

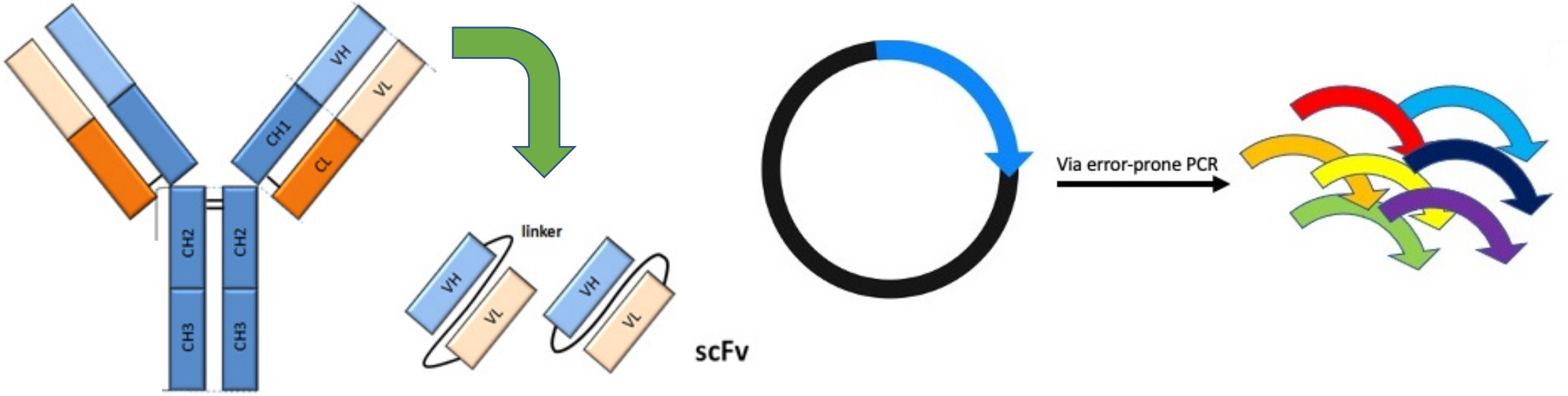


Lysozyme is the target for the scFv you will study

- Antimicrobial enzyme produced by animals
  - Part of the innate immune system, present in tears and mucosal membranes
- Catalyzes the breakdown of bacterial cell membranes
  - Glycoside hydrolase that hydrolyzes 1, 4-beta linkages in peptidoglycan

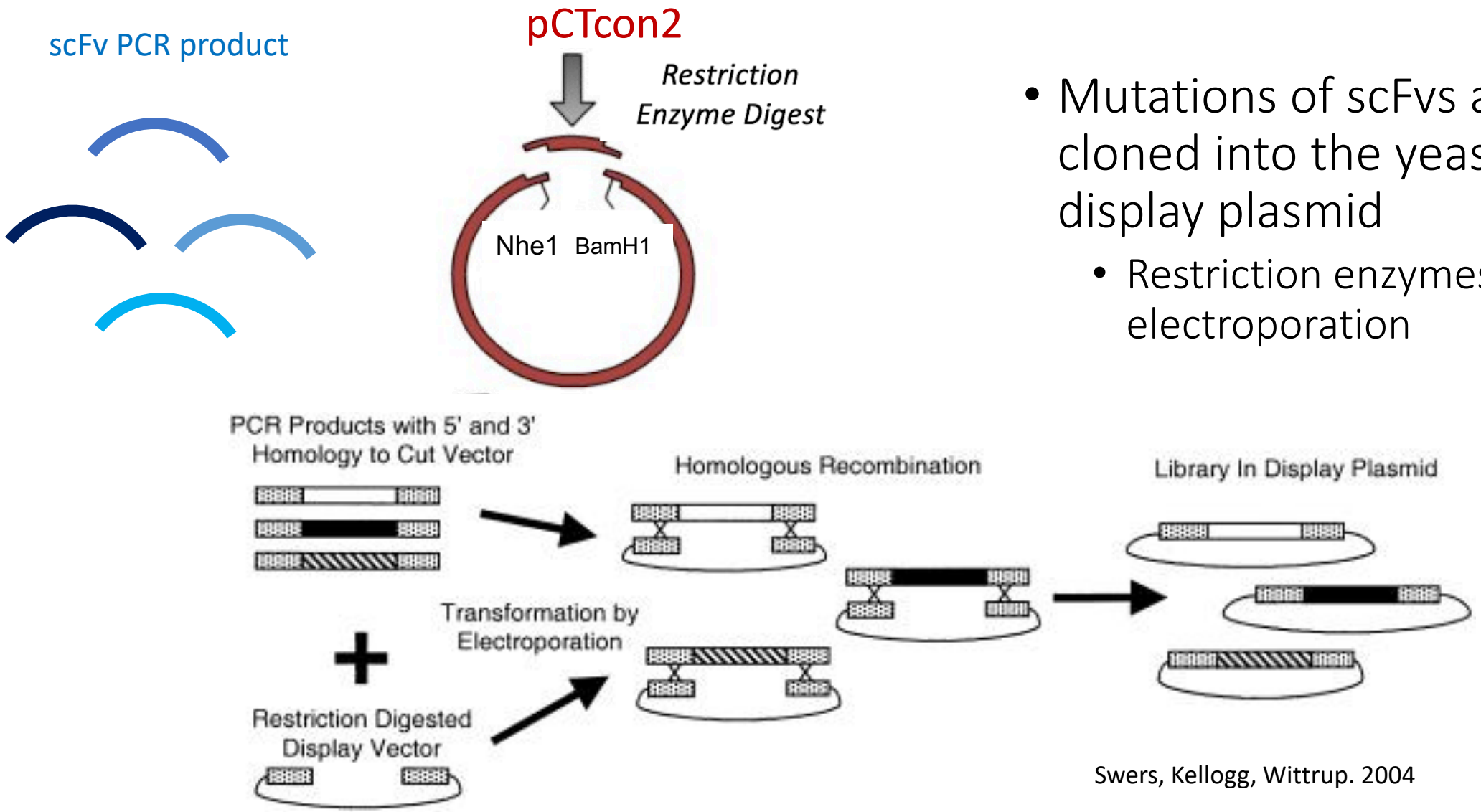


Engineering antibodies: Generating the library



- scFv sequence for antibody of interest is mutated in effort to improve affinity or specificity for target
  - Error prone PCR

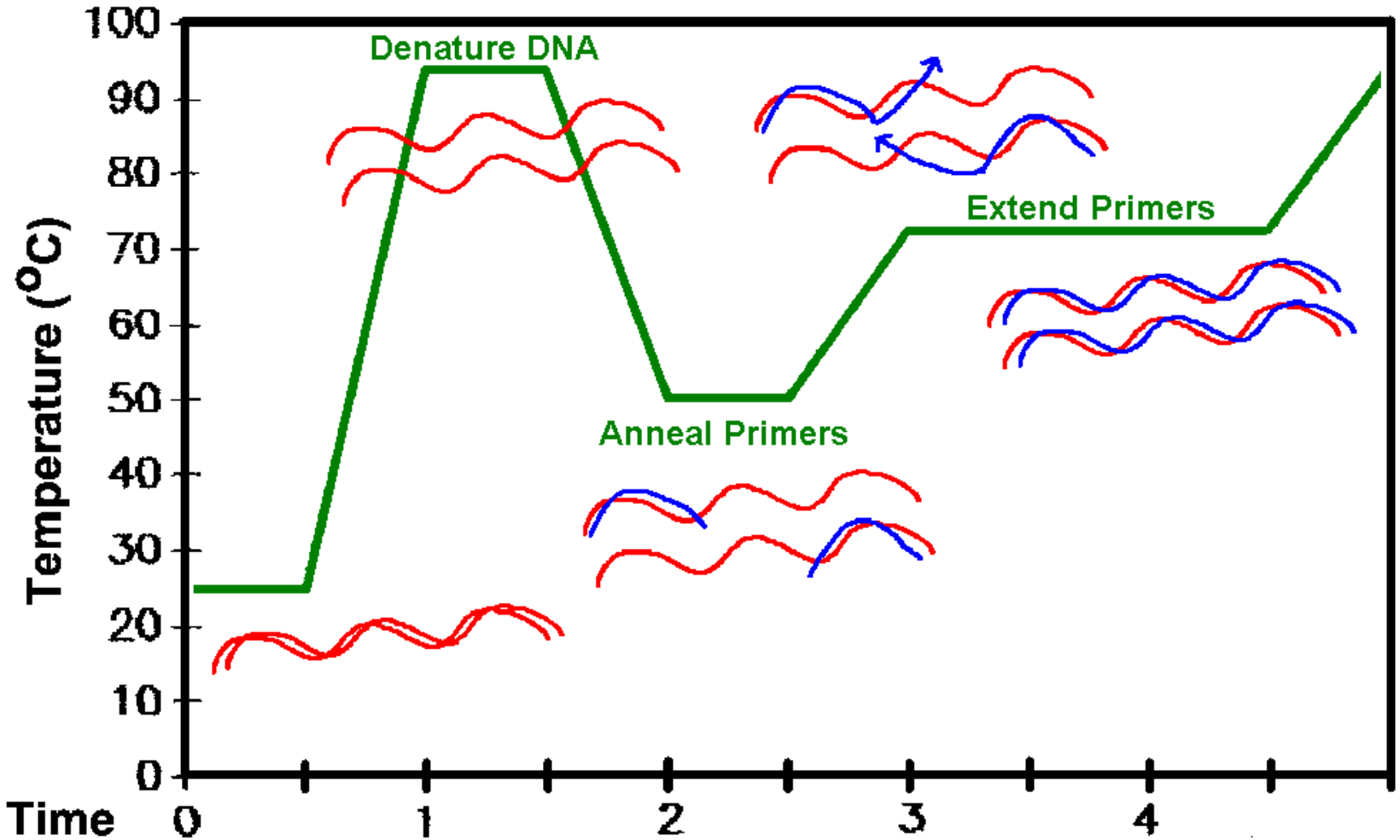
Engineering antibodies: Generating the library



- Mutations of scFvs are cloned into the yeast display plasmid
  - Restriction enzymes and electroporation

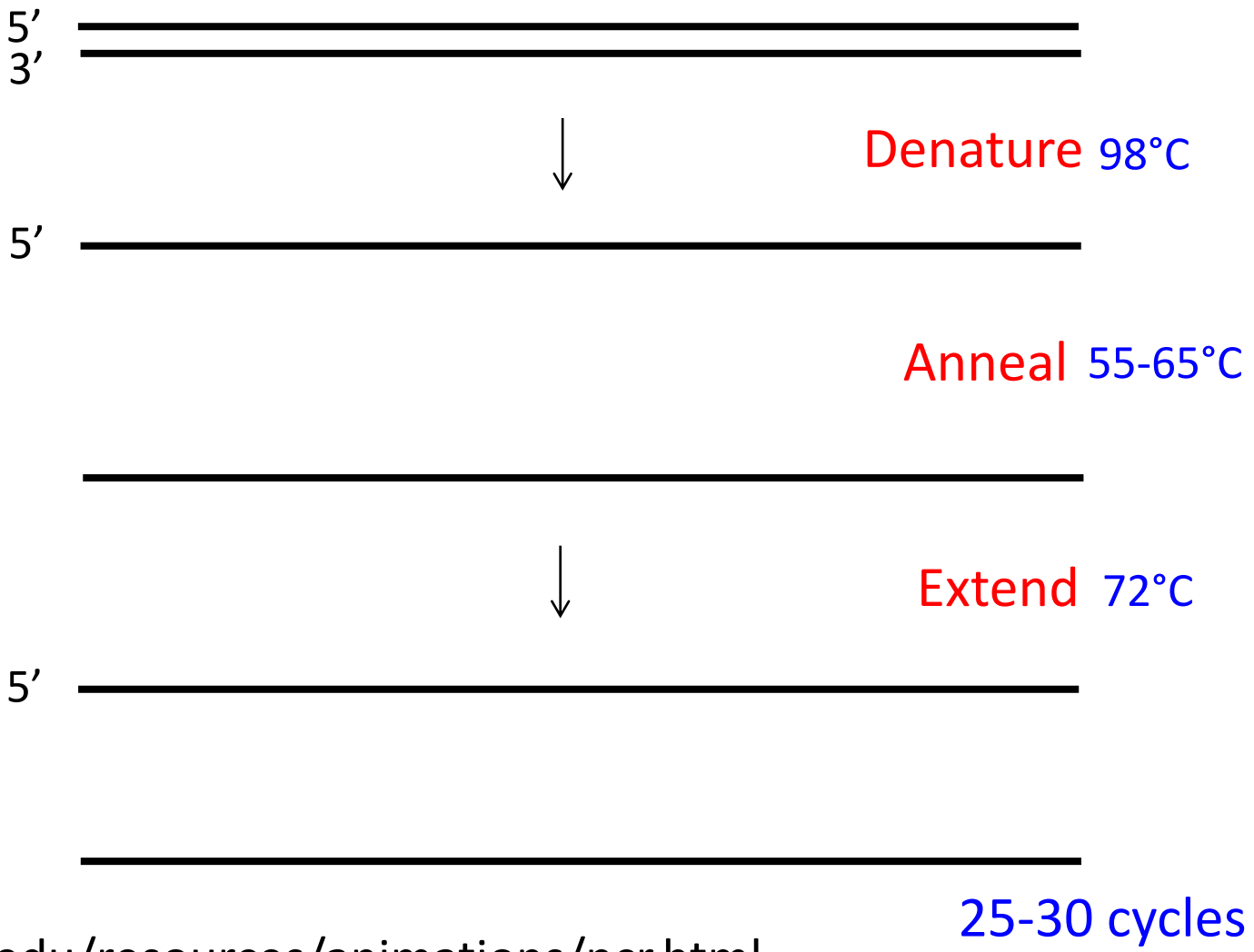
Swers, Kellogg, Wittrup. 2004

DNA sequence amplification: Polymerase Chain Reaction



Amplification– PCR reagents and conditions

Reagents

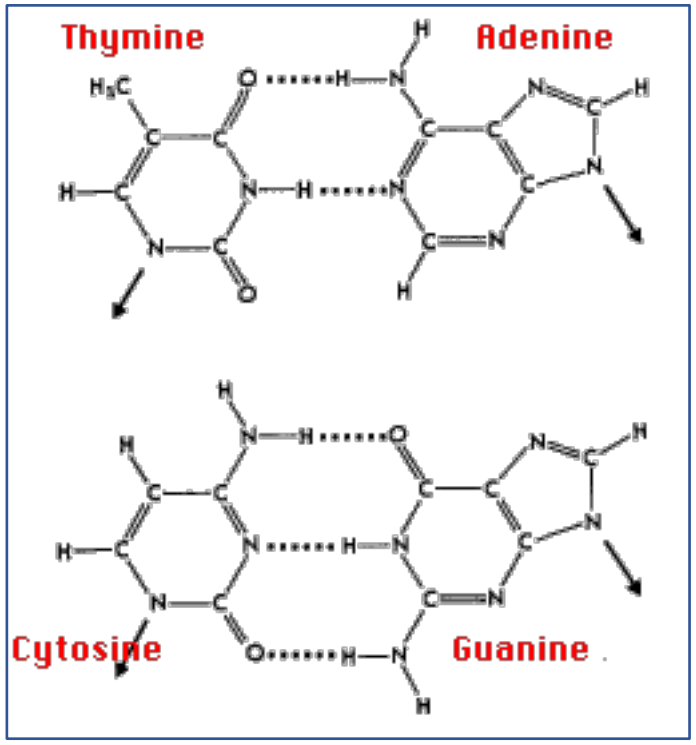


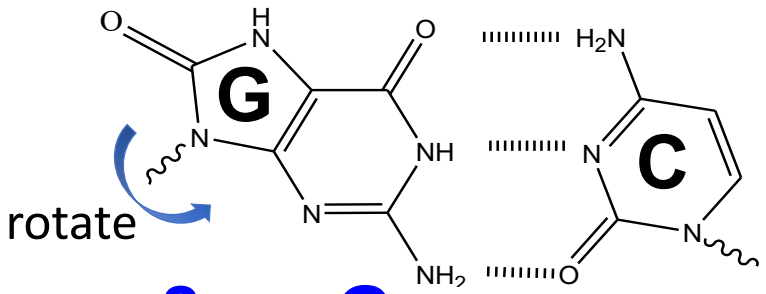
Additional PCR animation: https://dnalc.cshl.edu/resources/animations/pcr.html

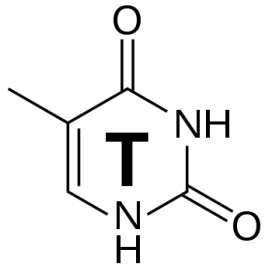
Error-prone PCR using 8-oxo-dGTP

dNTPs (deoxy-nucleoside triphosphate)

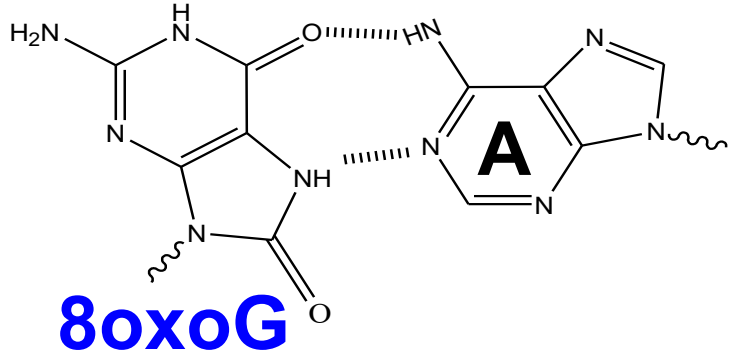
- dTTP
- dATP
- dCTP
- dGTP







G now looks like T

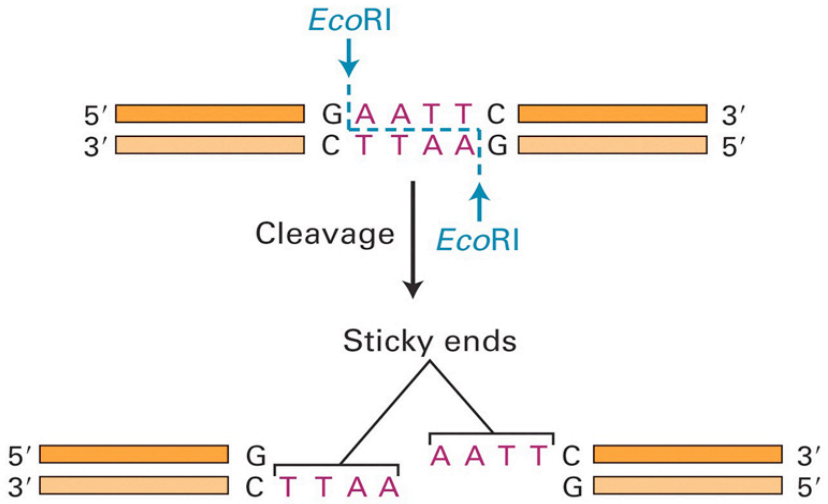


Error-prone PCR generates scFv mutants

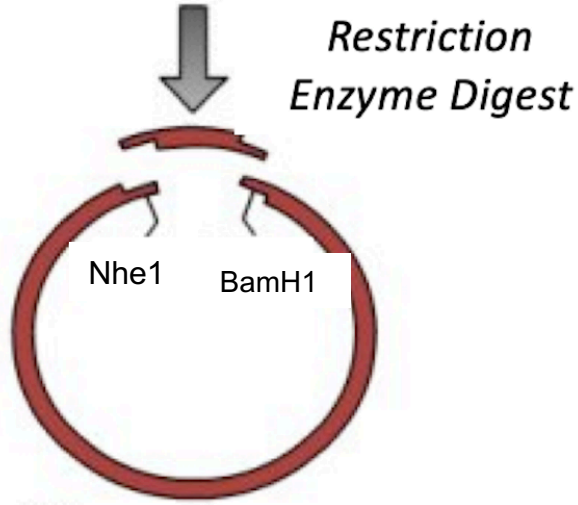
Gel purify and amplify those mutants for further cloning

Insert mutated scFvs into new plasmid backbone for yeast expression

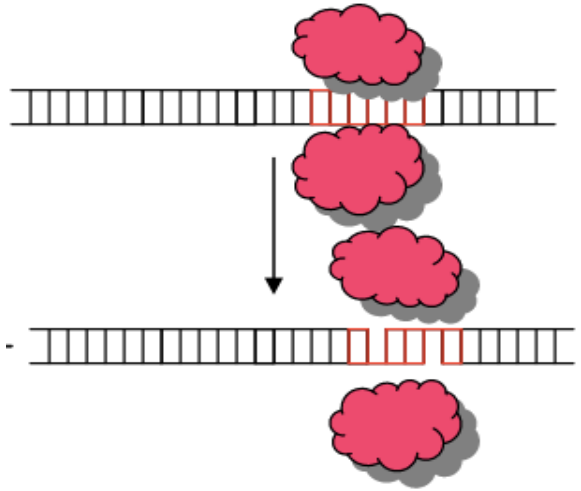
Restriction enzyme digestion prepares plasmid backbone for transformation



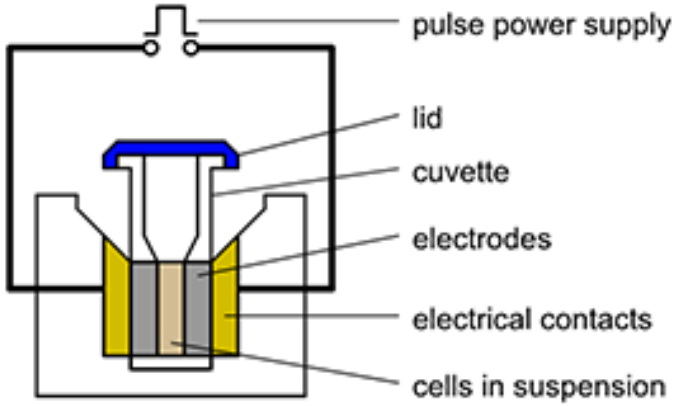
pCTcon2 vector



- many restriction enzymes function as homodimers
- binds palindromic sequences
- cleaves backbone



Transformation of DNA into yeast: Electroporation



Electrical pulse creates
temporary pore in membrane

scFv mutant

Yeast cell

Digested
backbone

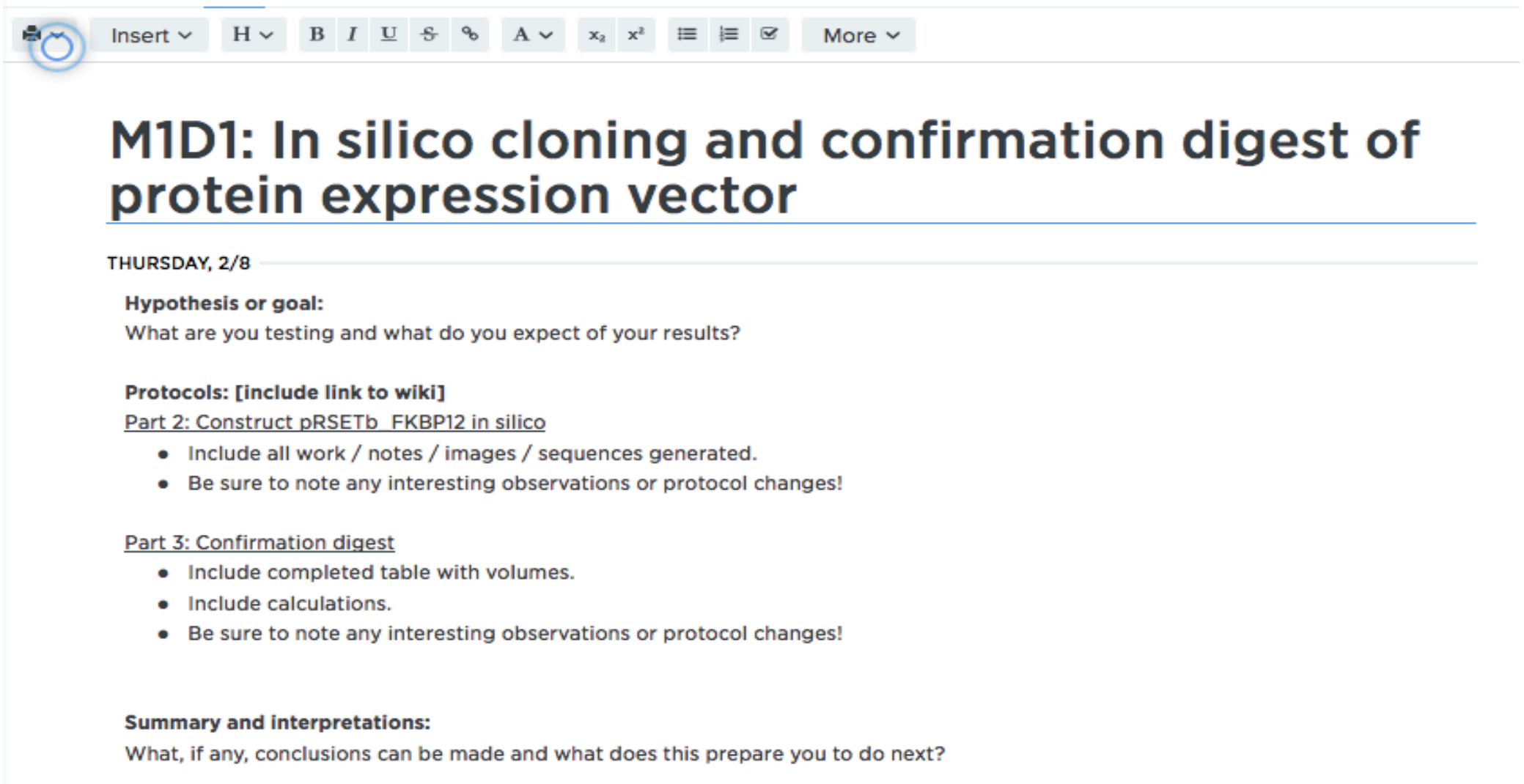
What should go in your notebook?

Laboratory notebook entry component:	Points: Complete	Partial	Missing
Date of experiment (include Module#/Day#) and Title for experiment	1	0.5	0
Hypothesis or goal / purpose	2	1	0
Protocols (link to appropriate wiki sections)	1	0.5	0
Answering questions embedded in wiki sections	5	3	0
Observations from demonstrations and video tutorials	3	2	0
*Visual details			
*Qualitative information			
*Raw data			
Data analysis	3	2	0
*Calculations			
*Graphs and Tables			
Summary and interpretation of data	3	2	0
*What did you learn?			
*How does this information fit into the larger scope of the project?			
Information is clear	2	1	0
All days represented	5	3	0
OVERALL /25			

Note:

* This rubric is used to grade the notebook at the end of each module
* Notebook check ins are for participation and due by 10pm after each lab

How should you format your notebook?



For Today:

- Work through the wiki exercises with your partner
- Be in breakout room at 3:30pm to take orientation quiz
  - Turn in completed quiz to Stellar by 4pm

For M1D2:

- Create a Benchling template to use for future notebook entries
- Read a paper linked on the wiki and prepare for an in class discussion

Yeast surface display for screening combinatorial polypeptide libraries

Eric T. Boder and K. Dane Wittrup*