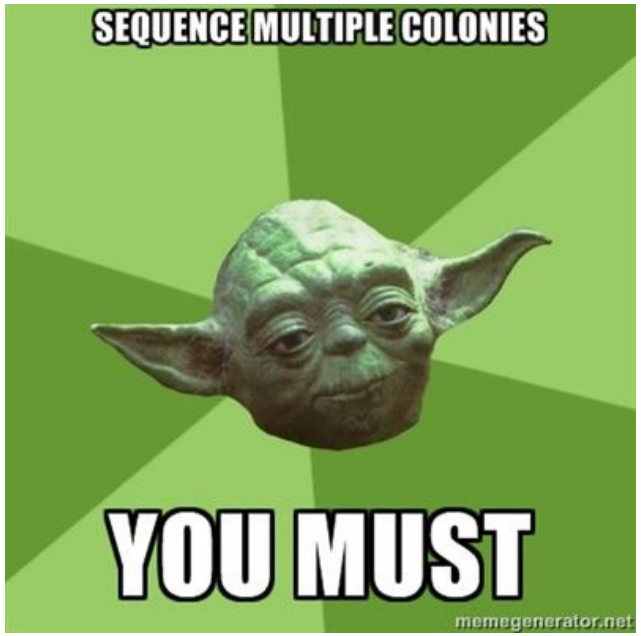
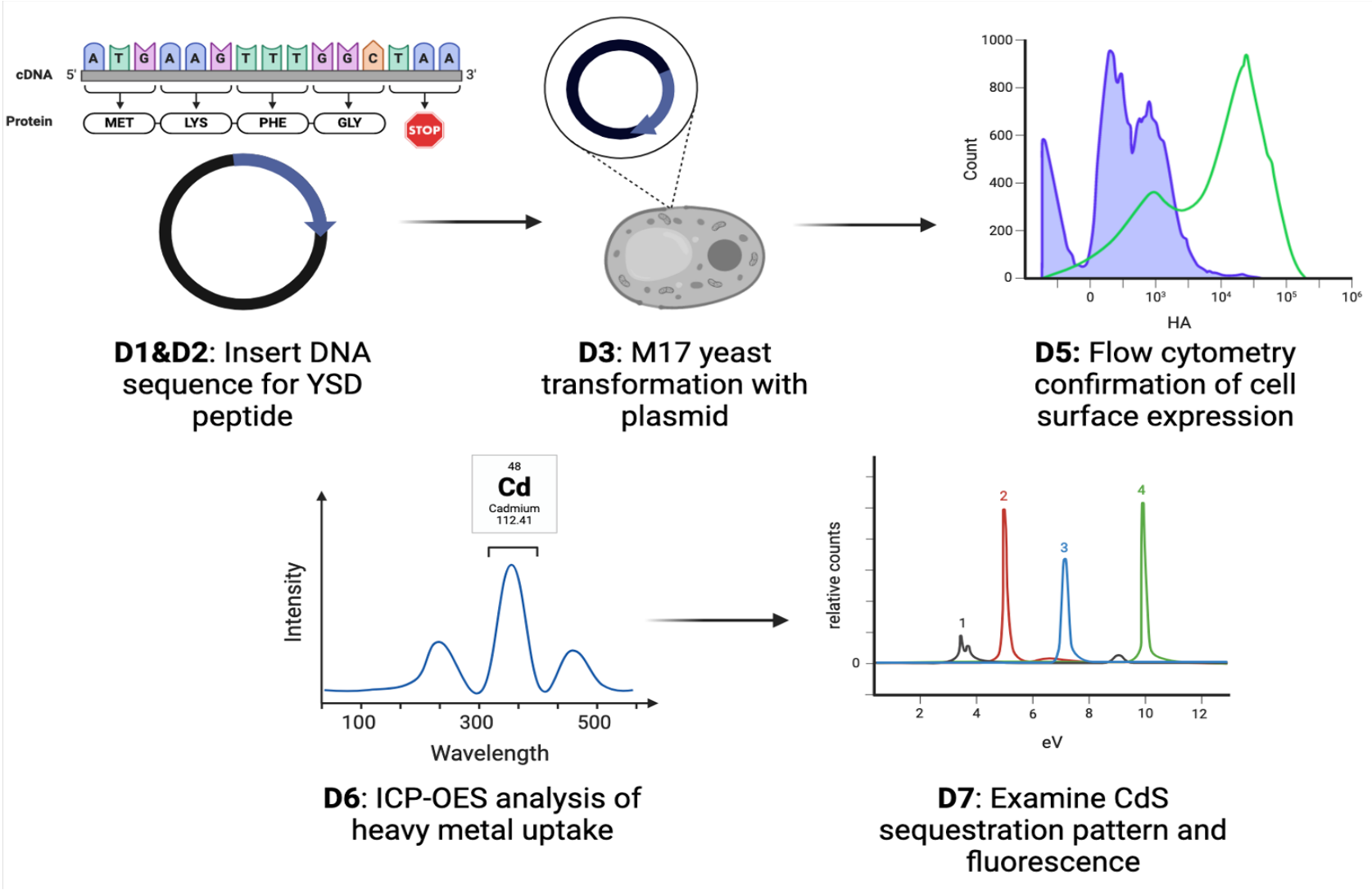
M2D3:
Sequence clones and transform into yeast cells

1. Prelab discussion
2. Isolate peptide_pCTON2
3. Transform peptide_pCTON2 into yeast cells
4. Prepare peptide_pCTON2 for sequencing



Overview of Mod 2 experiments:



D1&D2: Insert DNA sequence for YSD peptide

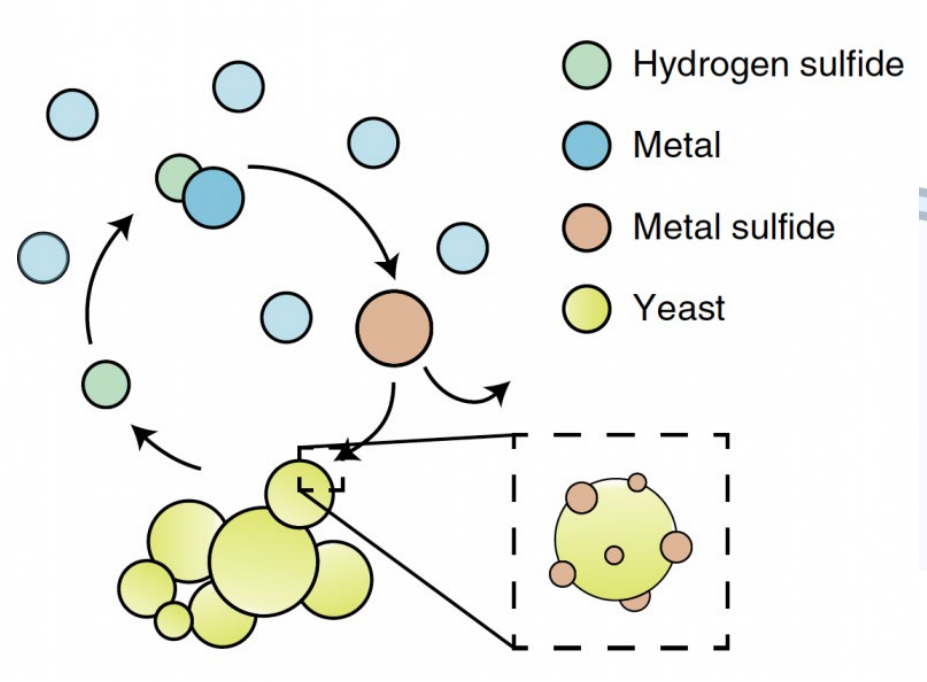
D3: M17 yeast transformation with plasmid

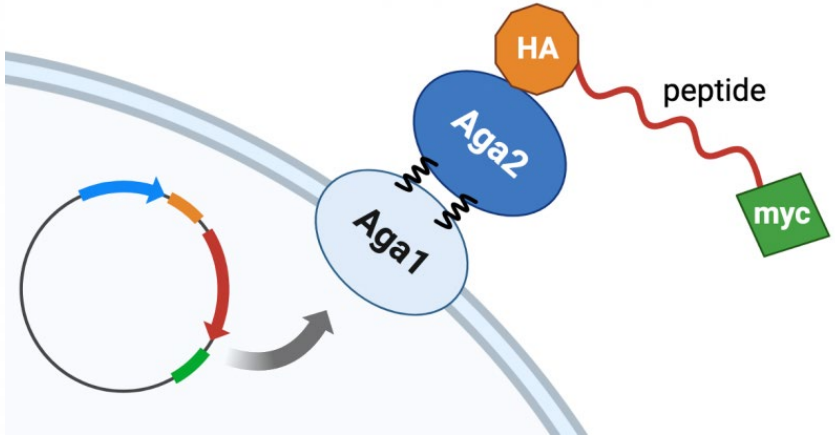
D5: Flow cytometry confirmation of cell surface expression

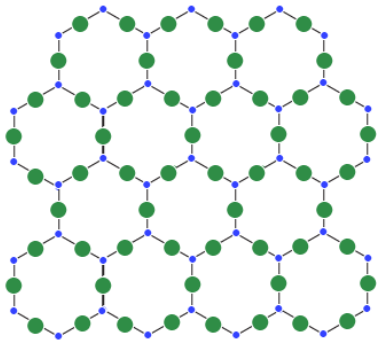
D6: ICP-OES analysis of heavy metal uptake

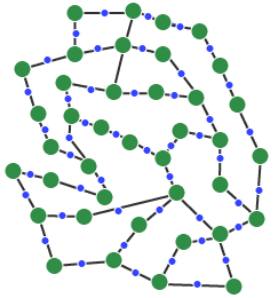
D7: Examine CdS sequestration pattern and fluorescence

Capturing and reusing cadmium



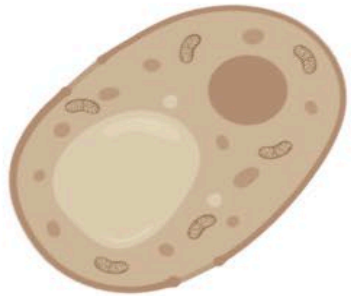




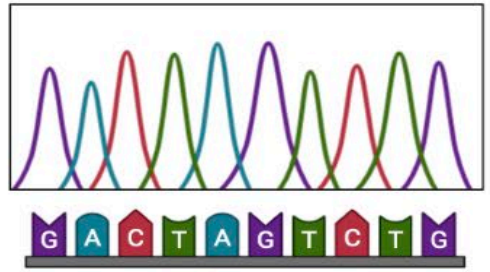


What are the tasks for today?

isolate peptide_pCTON2
from bacterial cells



Task 1: transform peptide_pCTON2 into yeast cells

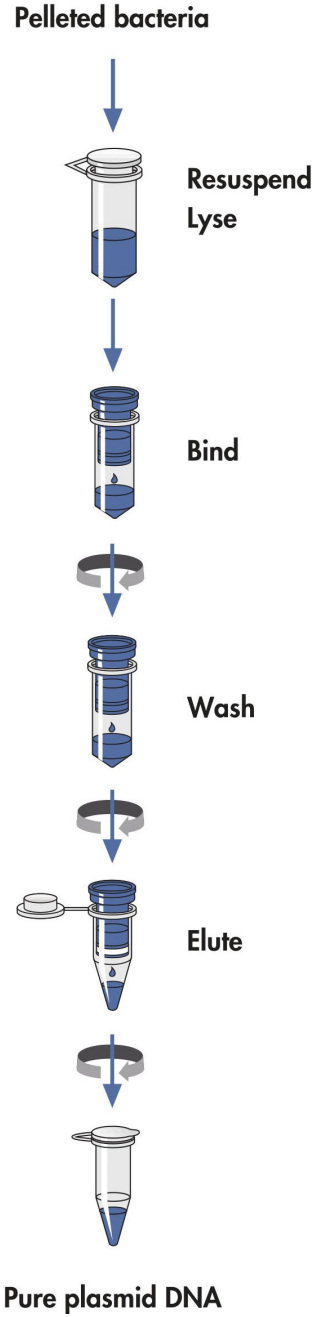


Task 2: prepare peptide_pCTON2 for sequencing

Why transform peptide_pCTON2 plasmid into *E. coli* and then into *S. cerevisiae*?

Isolate peptide_pCTON2 plasmid from bacterial cells

- How is genomic DNA separated from plasmid DNA using a commercial miniprep kit?
- Guanidine hydrochloride is a chaotropic salt that aids in isolation of plasmid DNA
  - Denatures proteins / enzymes, including DNAse
  - Disrupts hydrogen bonds formed between water and DNA to facilitate binding to silica-based column
  - **Must be collected in separate waste stream!!**



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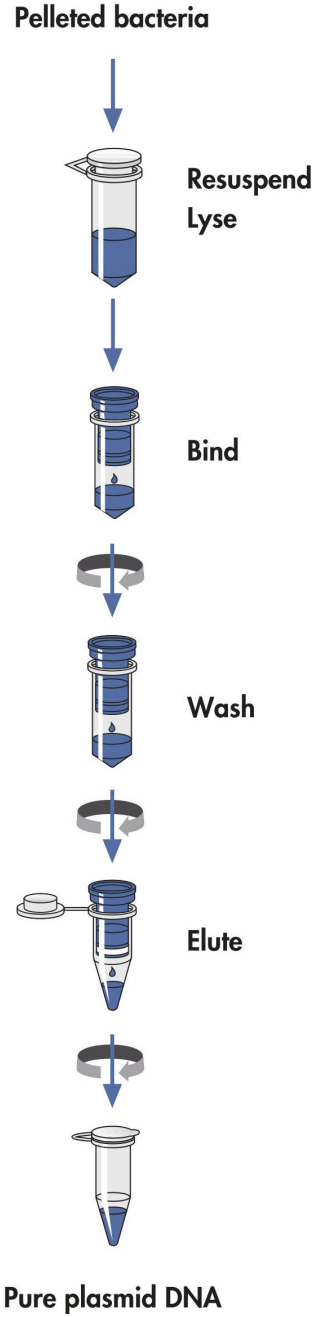


Task 1: Transform peptide_pCTON2 plasmid into △Met17 yeast cells

- Mechanism used to transform yeast cells not well understood

What is in this kit and how does it work?

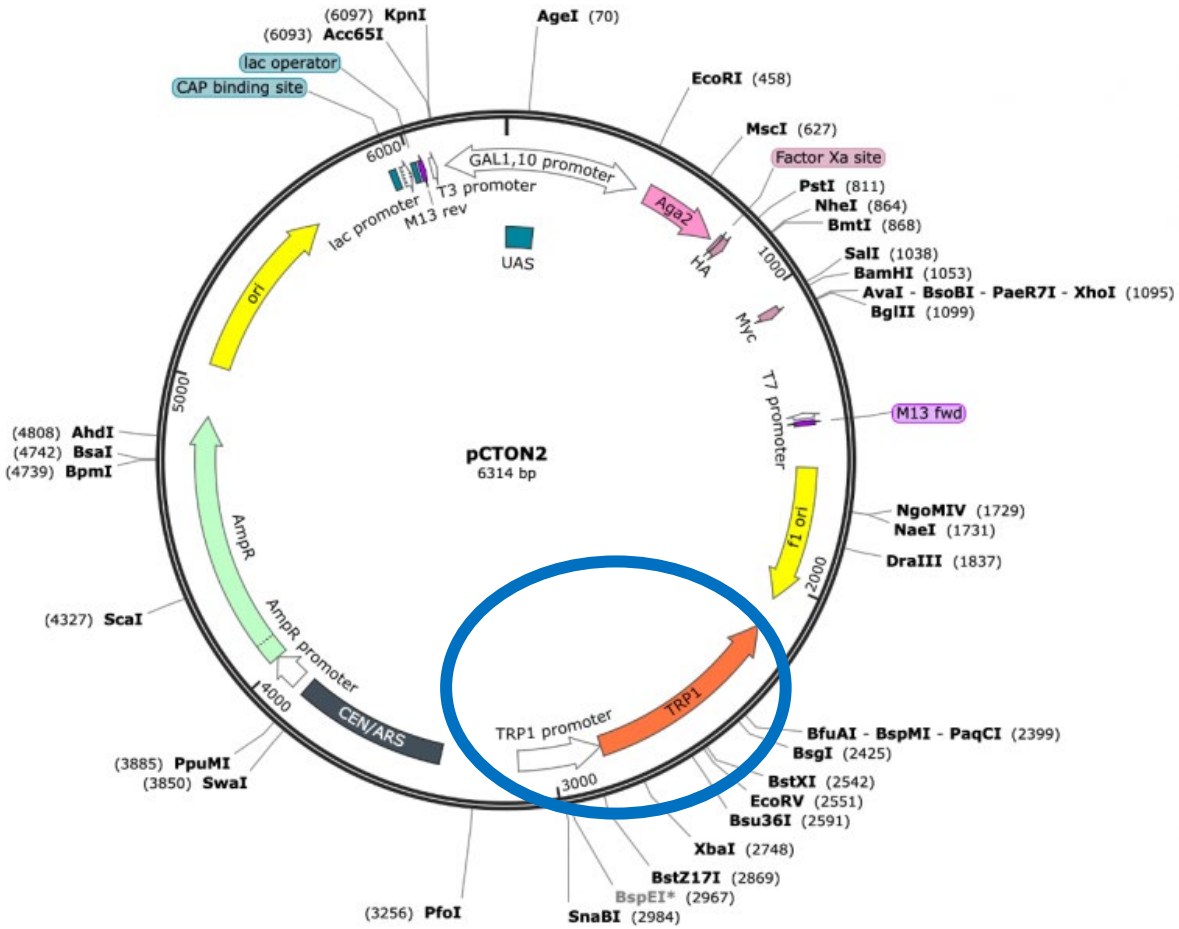
  - *"…procedure utilized in this kit is designed, in some ways, similar to the lithium cation based method…mechanism probably involves some metabolic pathways that we do not fully understand."*

- Hypothesized that incubation with positively-charged lithium cations neutralize charges on the yeast cell membrane

Dropout media used to select for yeast cells that carry peptide_pCTON2 plasmid

- △Met17 yeast cells engineered such that gene needed to endogenously generate tryptophan was removed / mutated

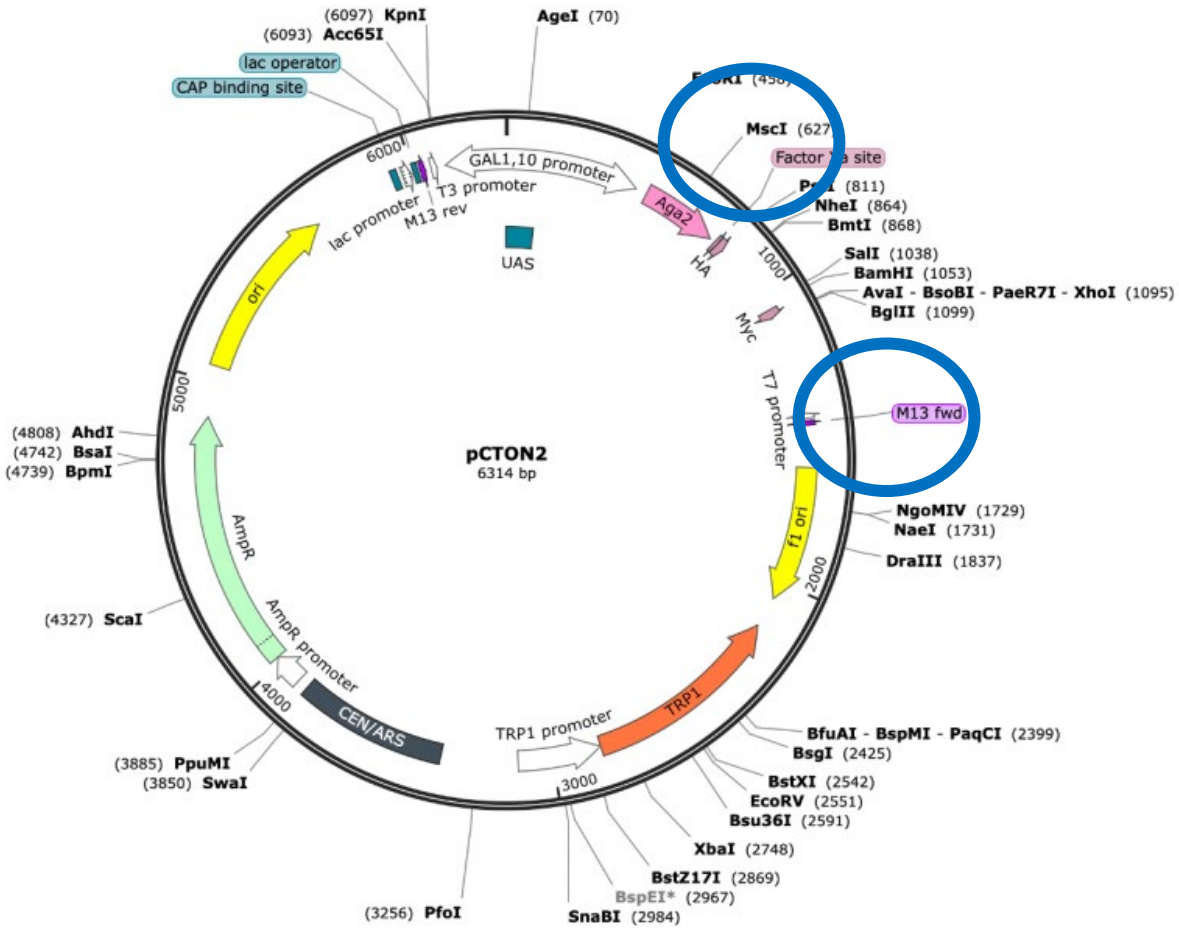
- Cells must acquire tryptophan from the environment (growth media) or be equipped to generate tryptophan from exogenous DNA (plasmid)



Task 2: Prepare peptide_pCTON2 plasmid for sequencing

- Reactions prepared by combining isolated peptide_pCTON2 plasmid and sequencing primers
  - One primer per reaction!
- Primers were designed to amplify across peptide insert

- Why do we sequence with a forward and reverse primer?



For today…

- Prepare sequencing reactions during transformation incubation time

For M2D4…

- Prepare draft slide for Journal article presentation
  - Use data figure from article to draft 1-2 slides that highlights the information
  - Include the script for how you would describe the information presented on the slide(s)