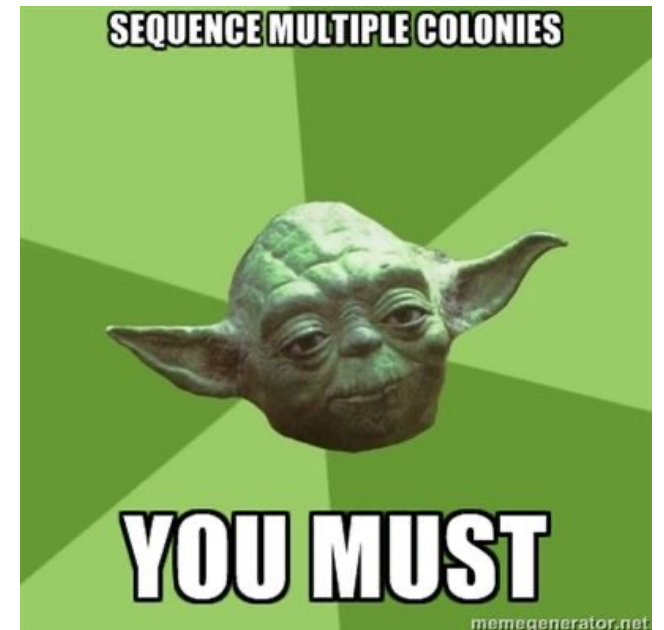


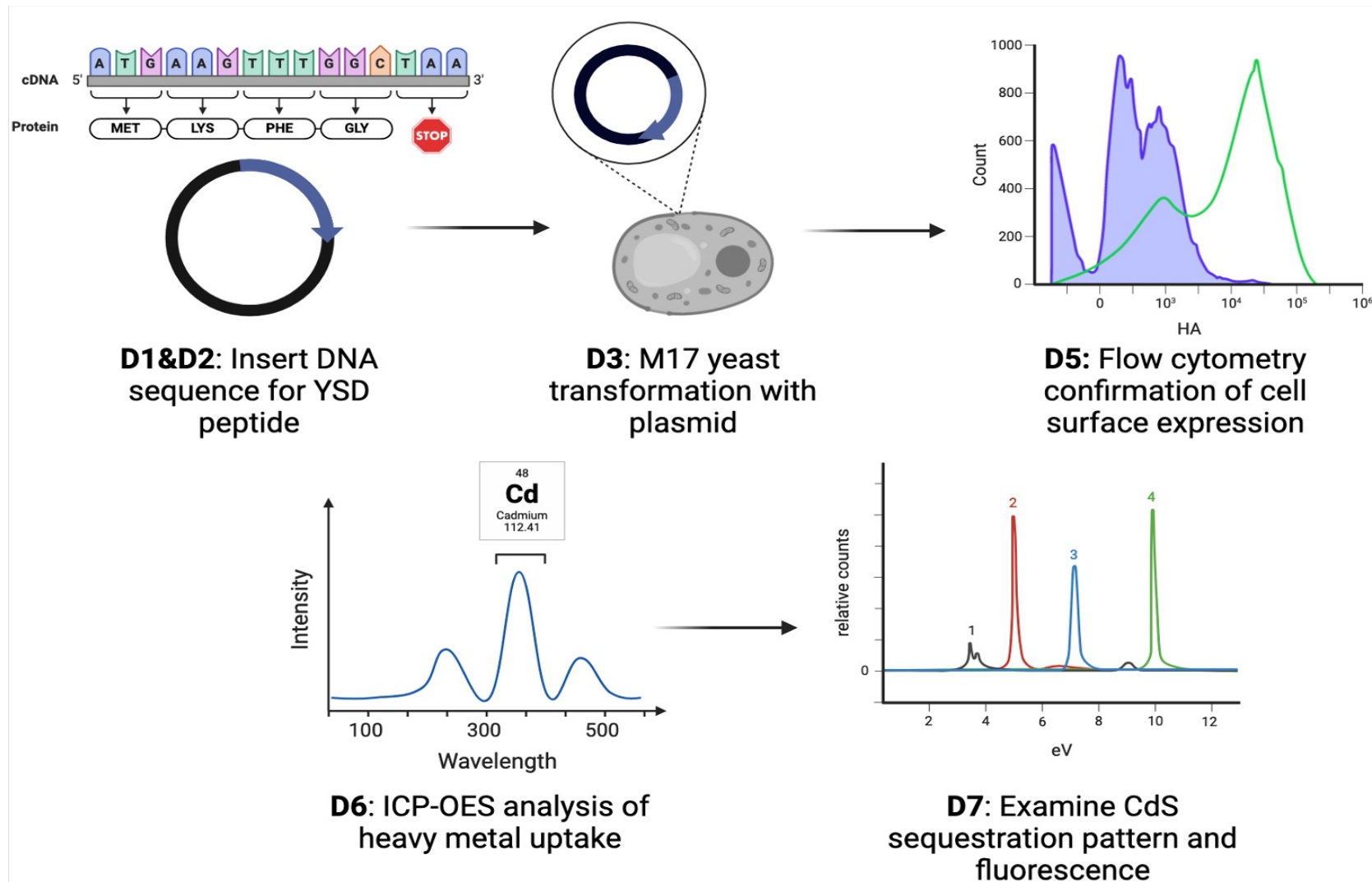
M2D3:

Sequence clones and transform into yeast cells

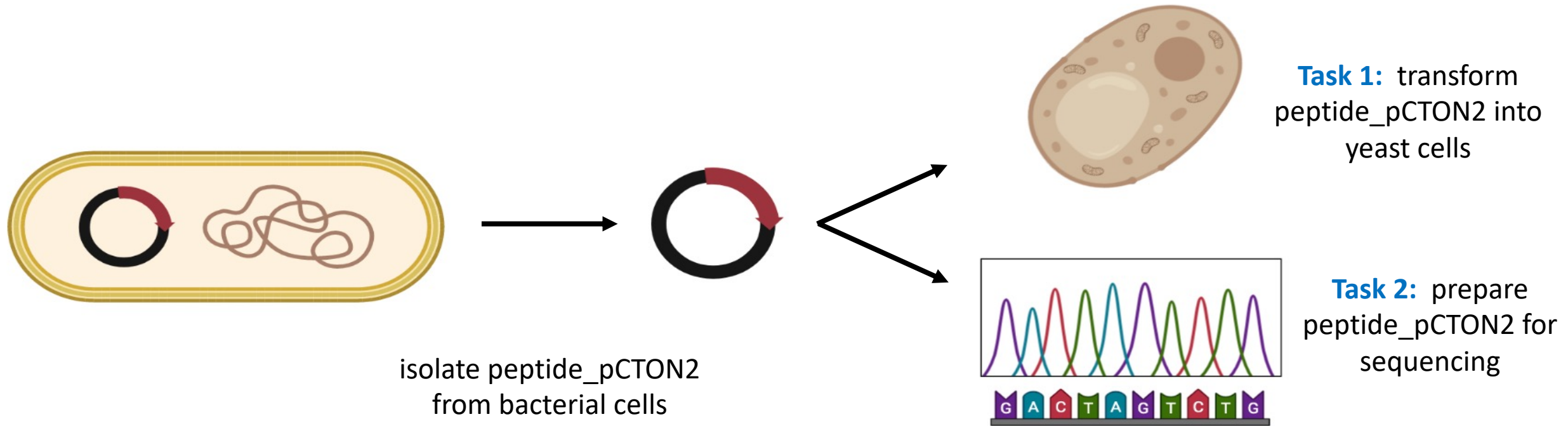
1. Prelab discussion
2. Isolate peptide_pCTON2
3. Transform peptide_pCTON2 into yeast cells
4. Prepare peptide_pCTON2 for sequencing



Overview of Mod 2 experiments:



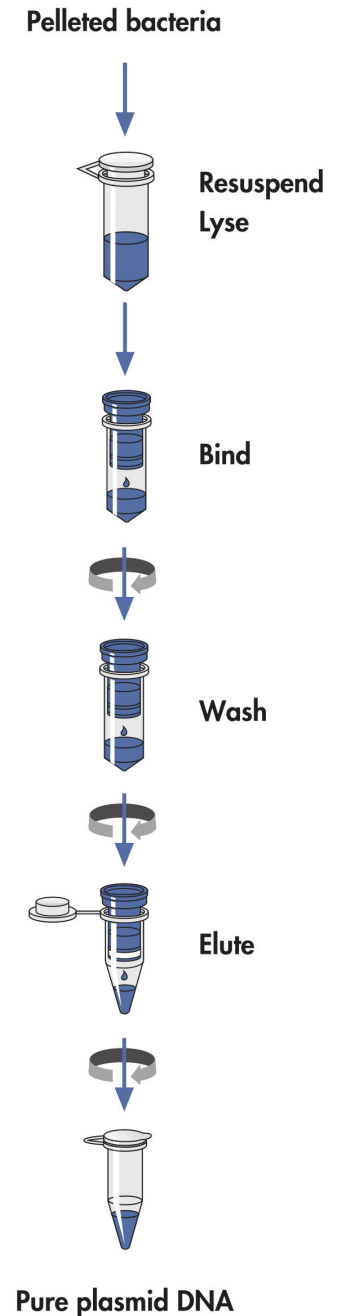
What are the tasks for today?



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!!



Task 1: Transform peptide_pCTON2 plasmid into Δ Met17 yeast cells

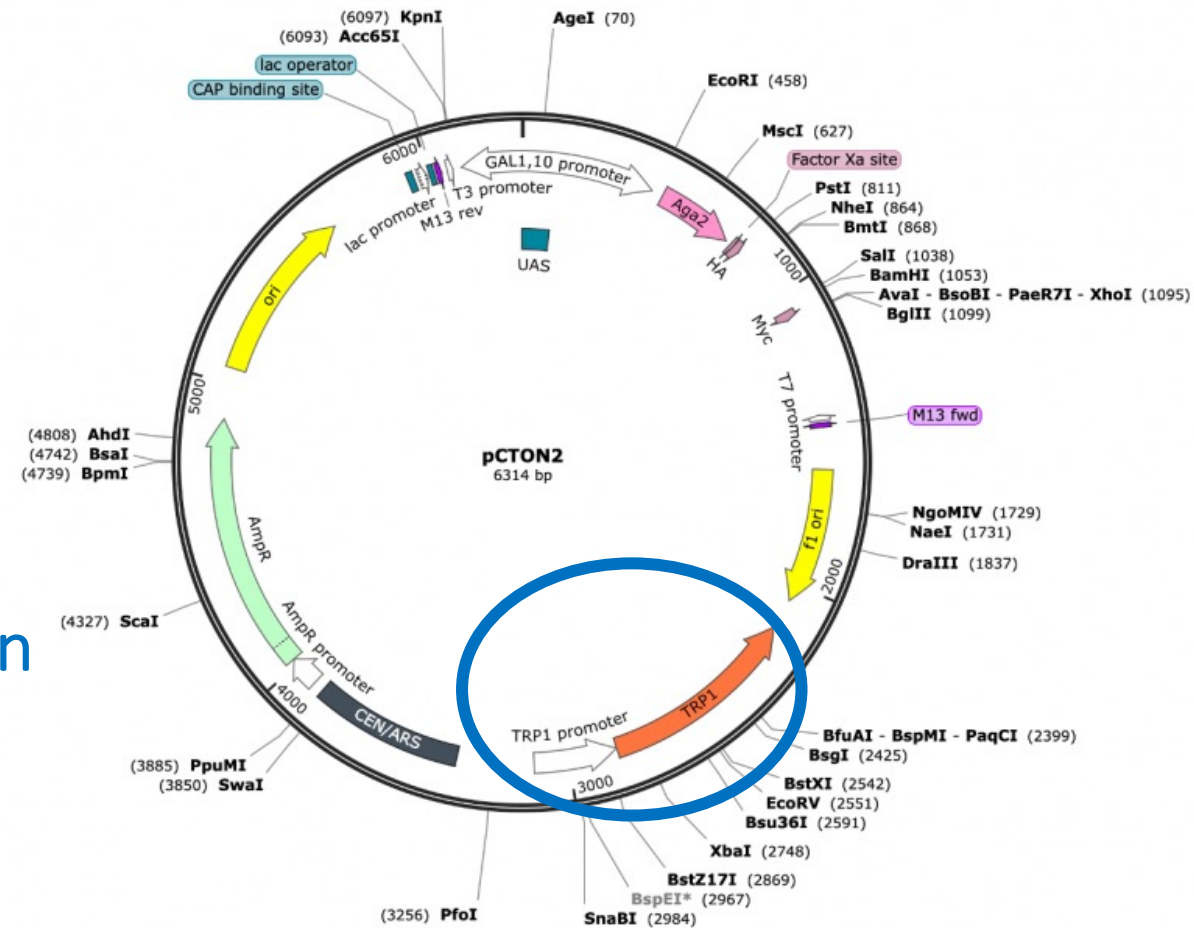
- Mechanism used to transform yeast cells not well understood

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

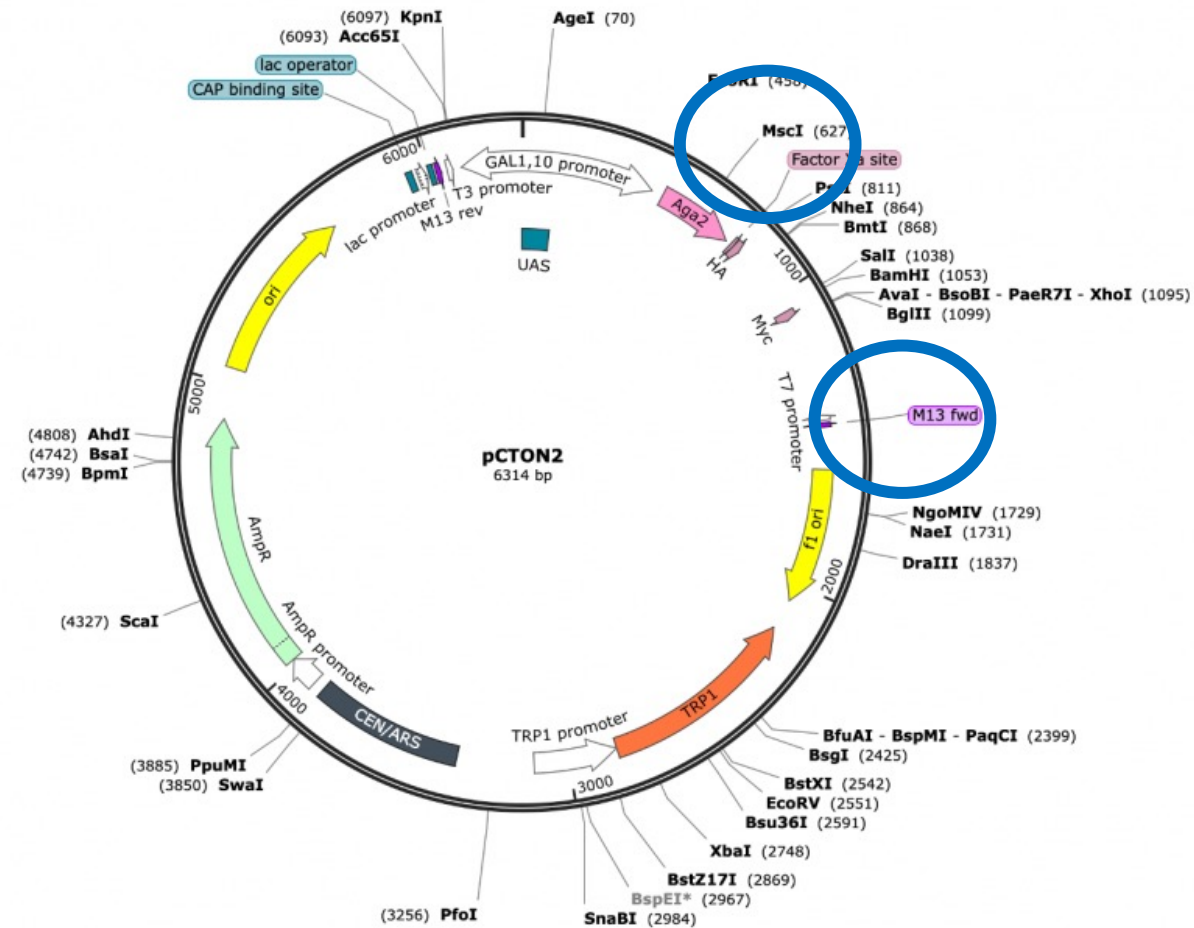
Dropout media used to select for yeast cells that carry peptide_pCTON2 plasmid

- Δ 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)