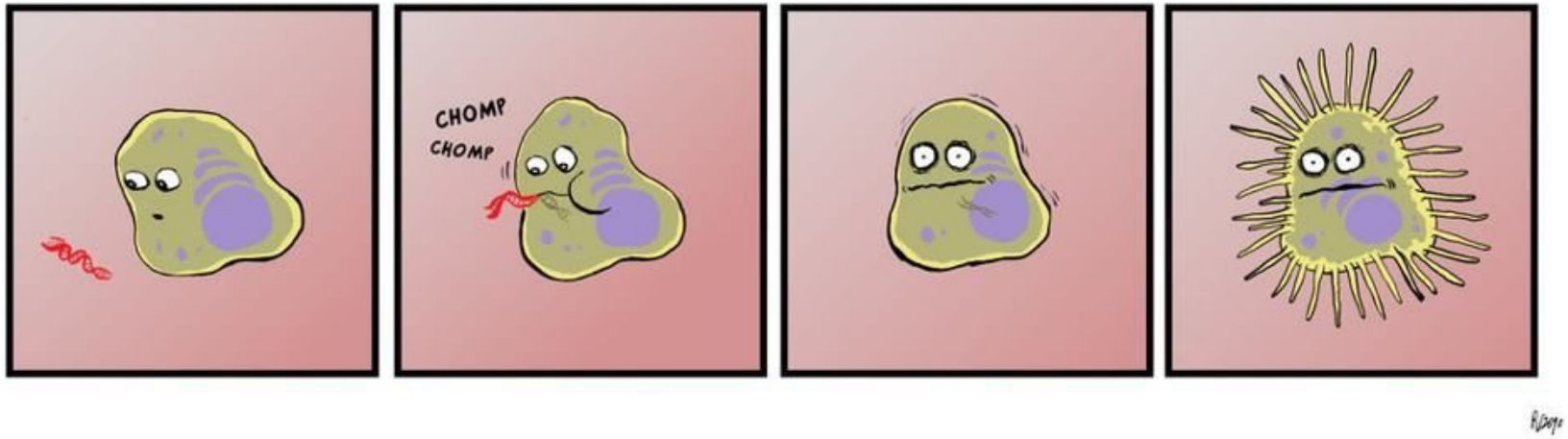
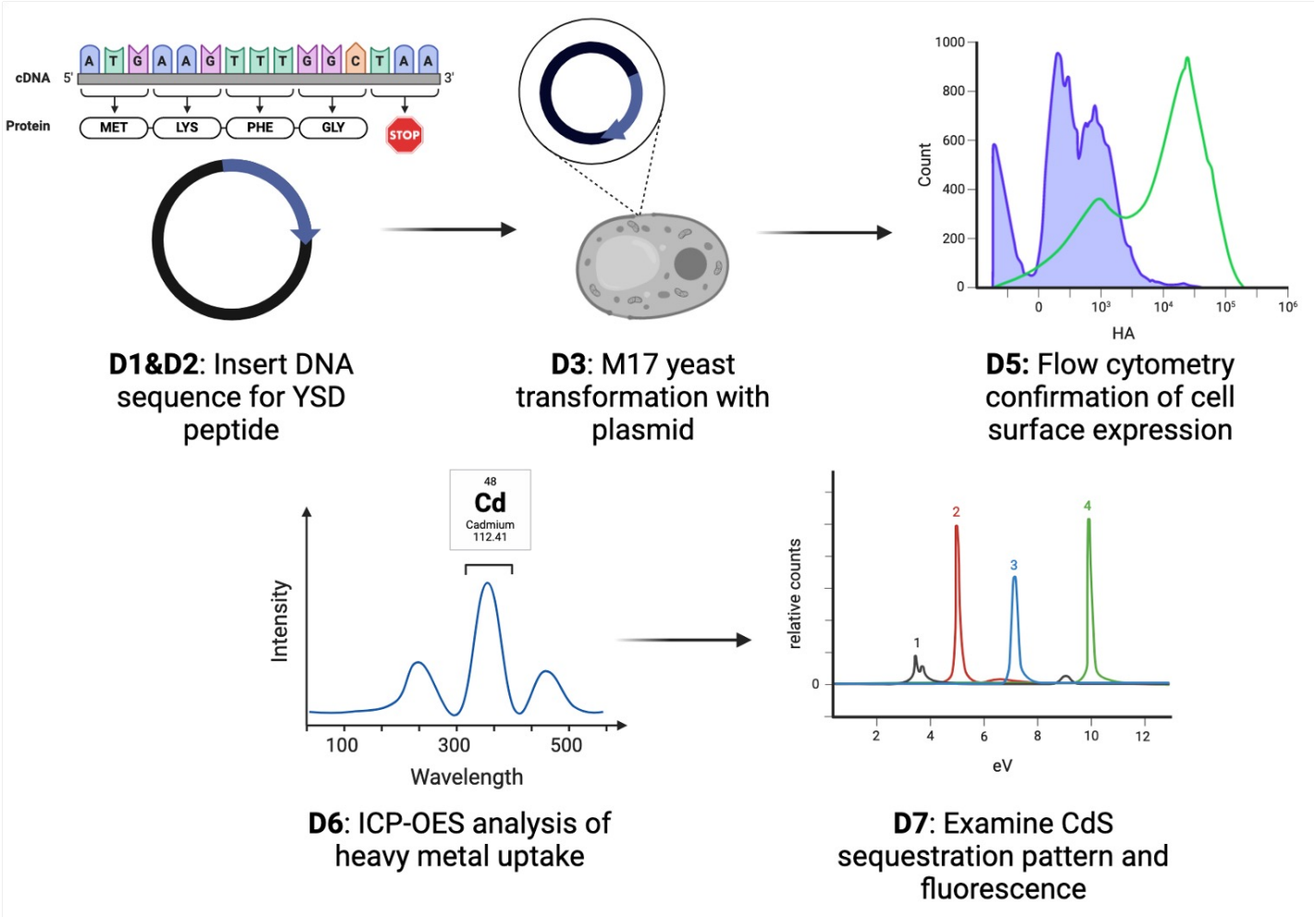
M2D2:
Clone cell surface peptide display plasmid

1. Prelab discussion
2. Use primers to insert peptide sequence into expression vector



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

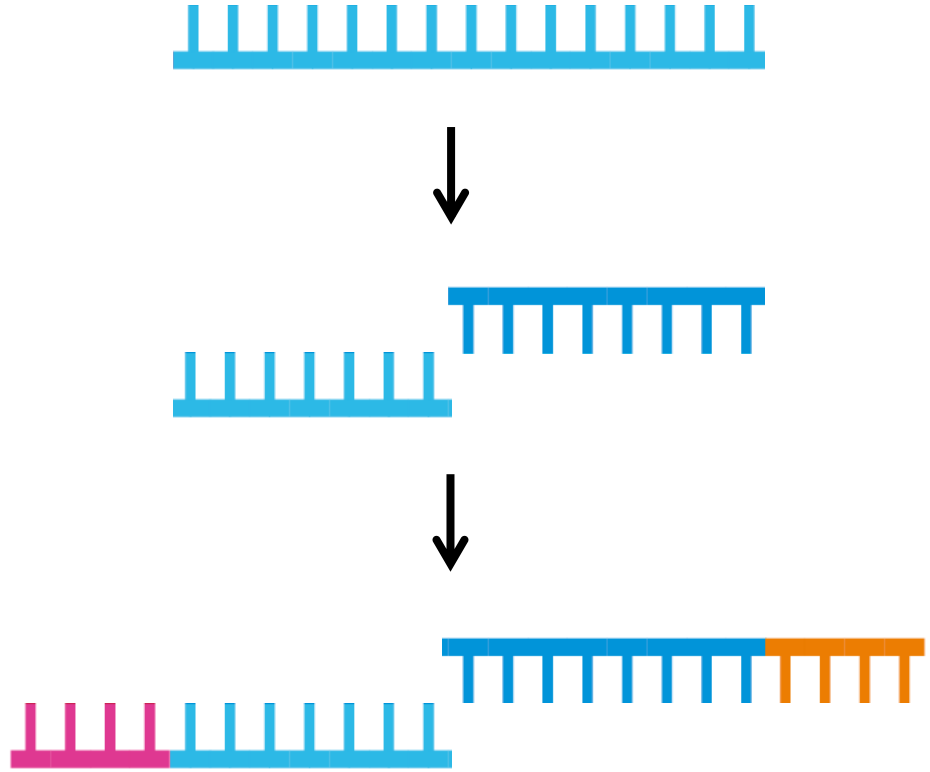
D6: ICP-OES analysis of heavy metal uptake

D7: Examine CdS sequestration pattern and fluorescence

How were your primer sequences modified?

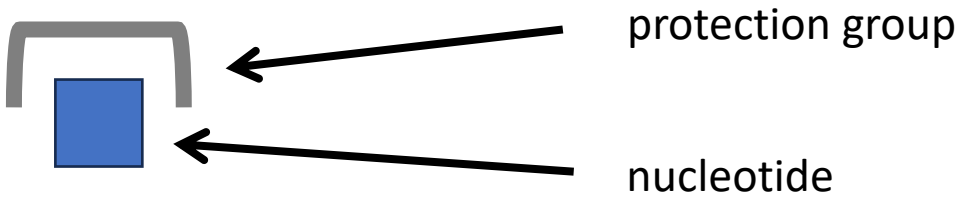
Forward and reverse primers necessary for cloning strategy

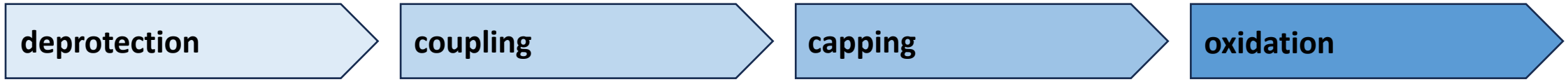
1. Your sequence was split and reverse compliment used for to generate reverse primer
2. Linker sequences added to C-termini that will anneal primers to the template

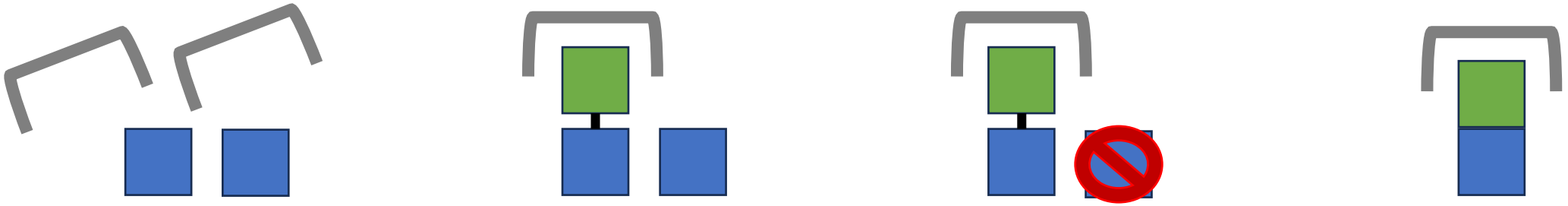


How were your primers synthesized?

- Process uses phosphoramidite monomers



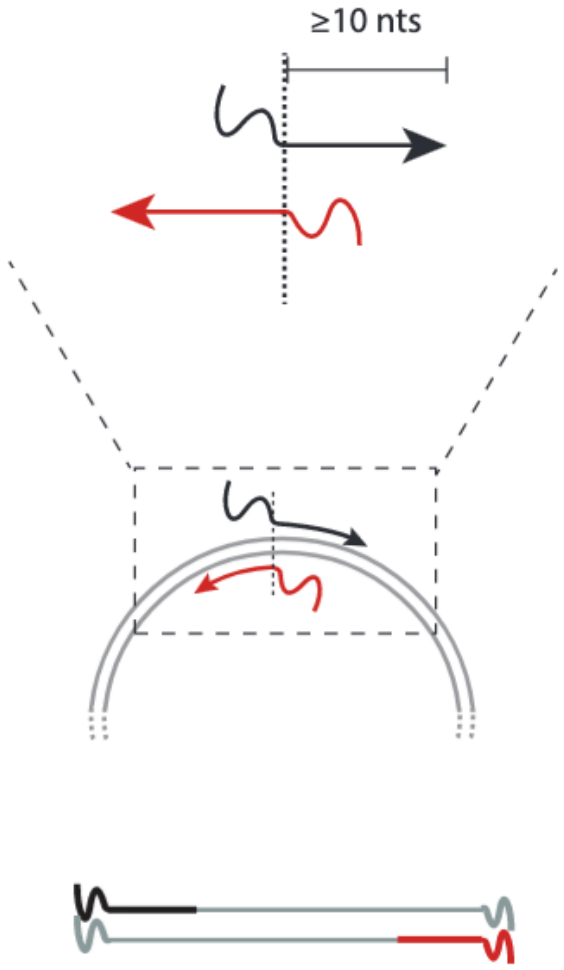




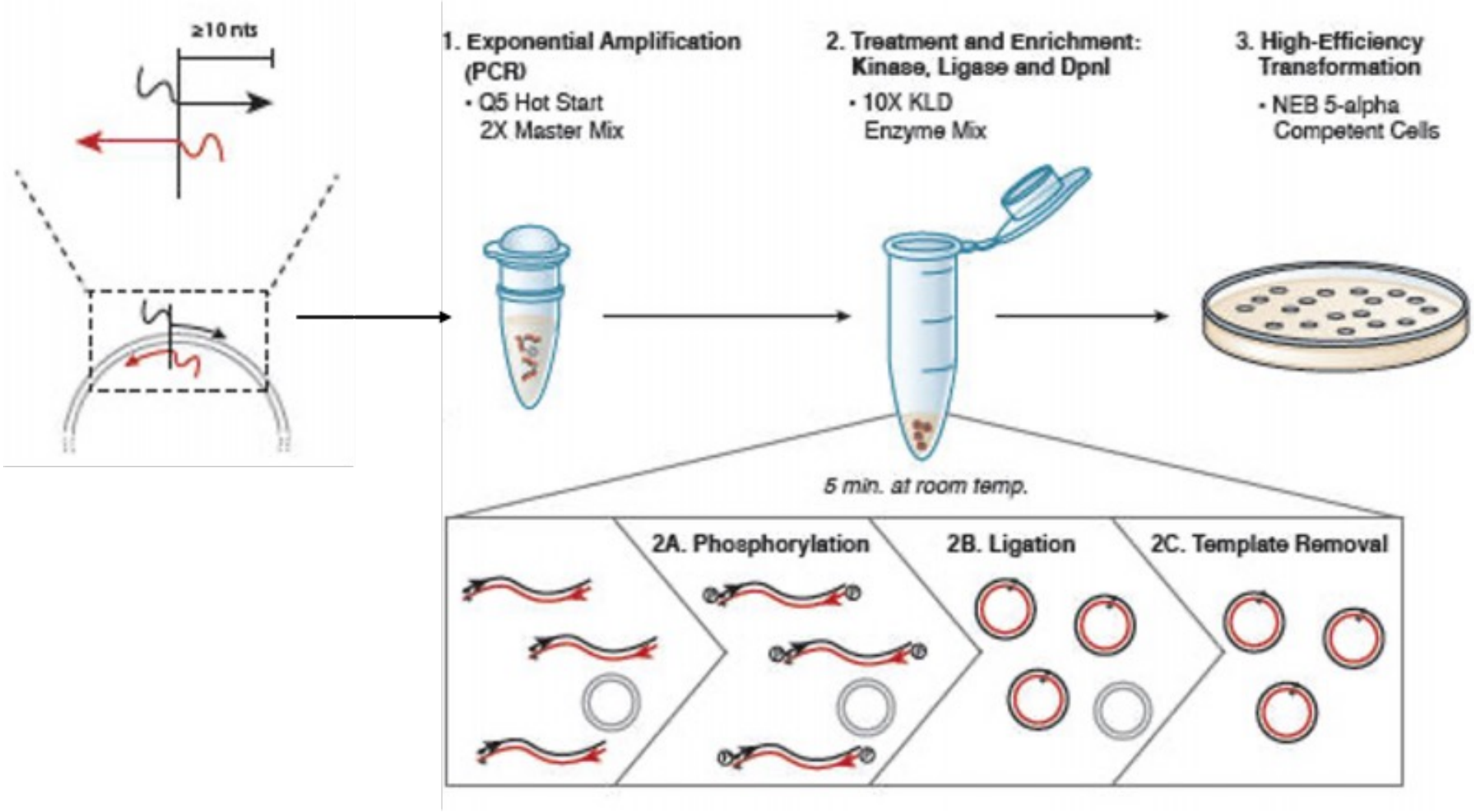
protection group removed | incoming nucleotide linked | cap added | nucleotide link stabilized

Amplification used to insert primer sequence

- Primer ‘squiggles’ encode your peptide sequence that will be inserted into the YSD expression vector
- Primer ‘arrows’ encode linker sequences that enable primers to anneal to the YSD expression vector template
- Amplification proceeds from each primer and continues around the expression vector template
- Product is two linear fragments of complementary DNA
- How does this result in your expression plasmid?



Expression plasmid generated from amplification products



For today…

- Use extra time to work on Data summary!
- Reminder: office hours offered on Saturday, March 16 from 10-5p
  - Use Instructor Zoom links for access

For M2D2…

- Choose key figures from your selected Journal article that can be used to present the author's conclusion(s) as a cohesive story
- Email Becky (rcmeyer@mit.edu) to request presentation date