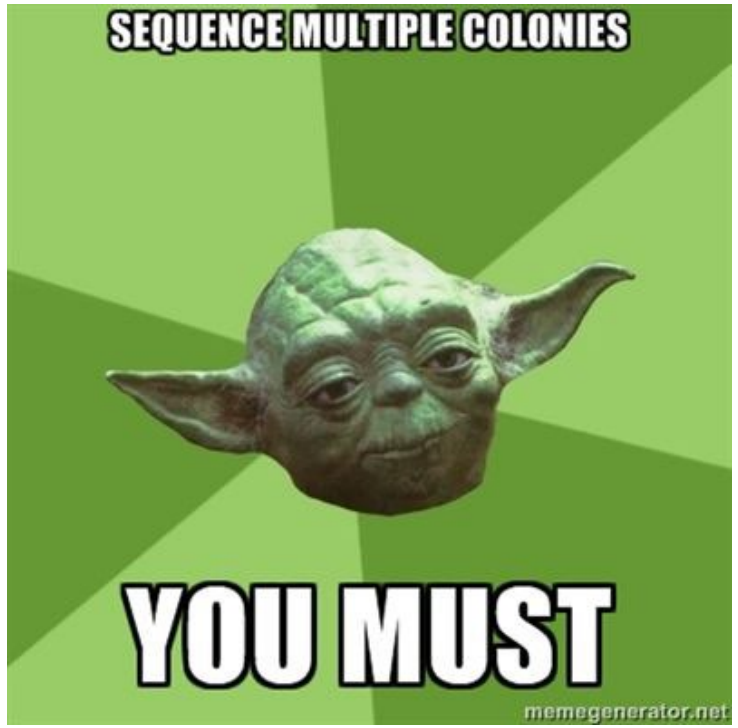
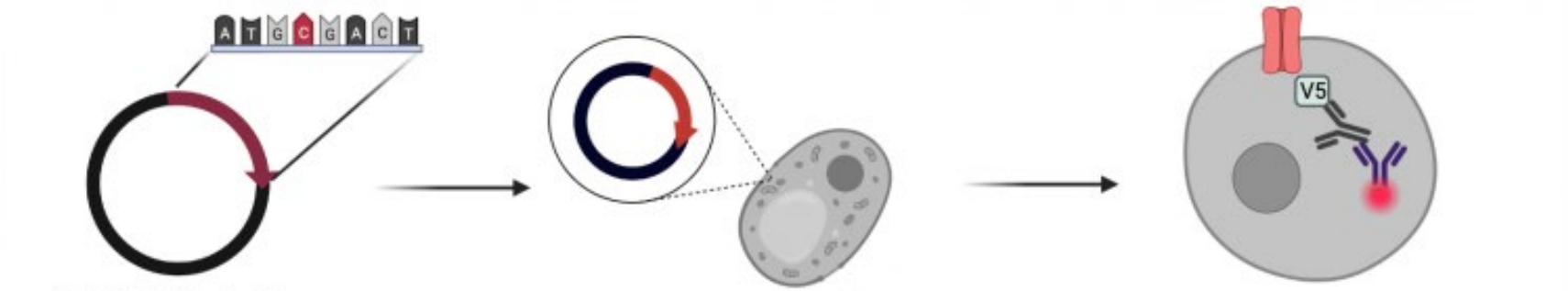
M2D3:
Sequence clones and transform into yeast cells

1. Prelab discussion
2. Isolate FET4_mutant plasmid
3. Transform FET4_mutant plasmid into yeast cells
4. Prepare FET4_mutant plasmid for sequencing



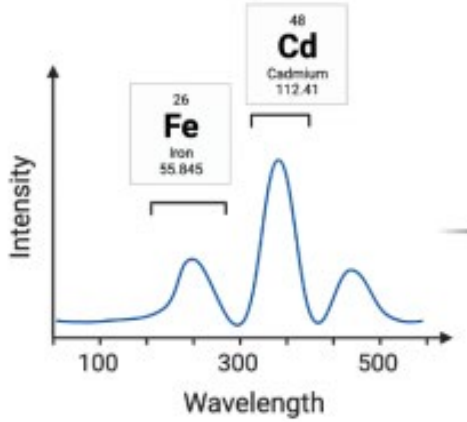
Overview of Mod 2 experiments:



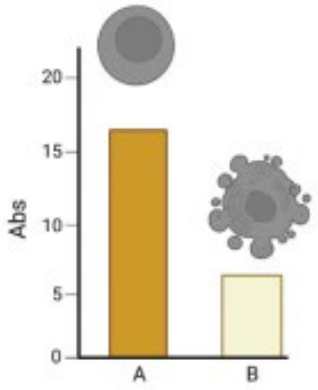
D1&D2: Fet4 site directed mutagenesis

D3: W303 transformation with mutants

D4&D5: IF detection of Fet4 mutant expression



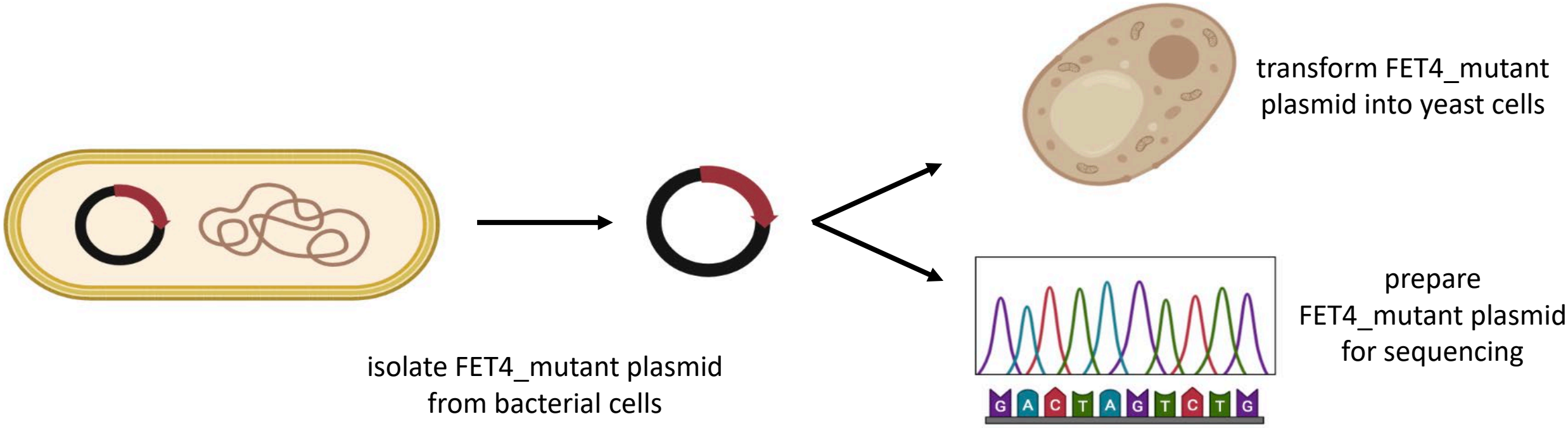
D6: ICP-OES analysis of heavy metal uptake



D7: Determine tolerance of mutant W303 to metal

D8: Statistics and Wrap-Up

What are the tasks for today?



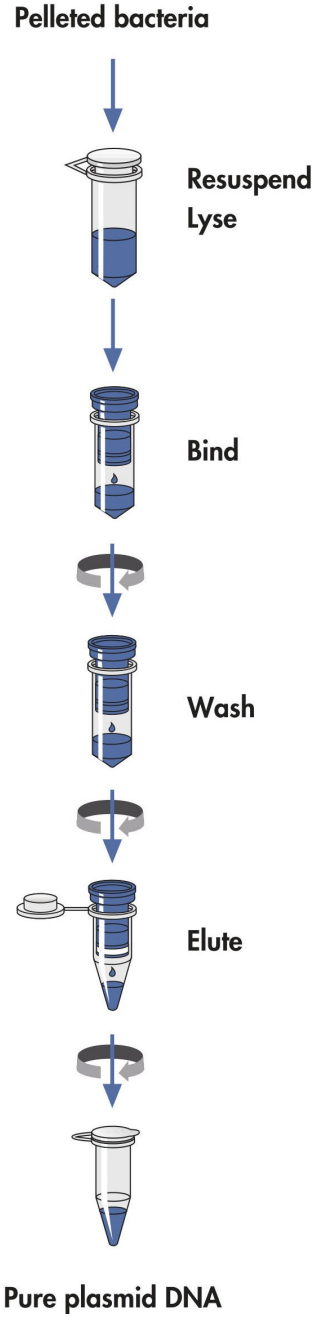
Why transform FET4_mutant plasmid into *E. coli* and then into *S. cerevisiae*?

Isolate FET4_mutant 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!!**



Transform FET4_mutant plasmid into yeast cells

- Mechanism used to transform yeast cells not well understood

What is in this kit and how does it work?

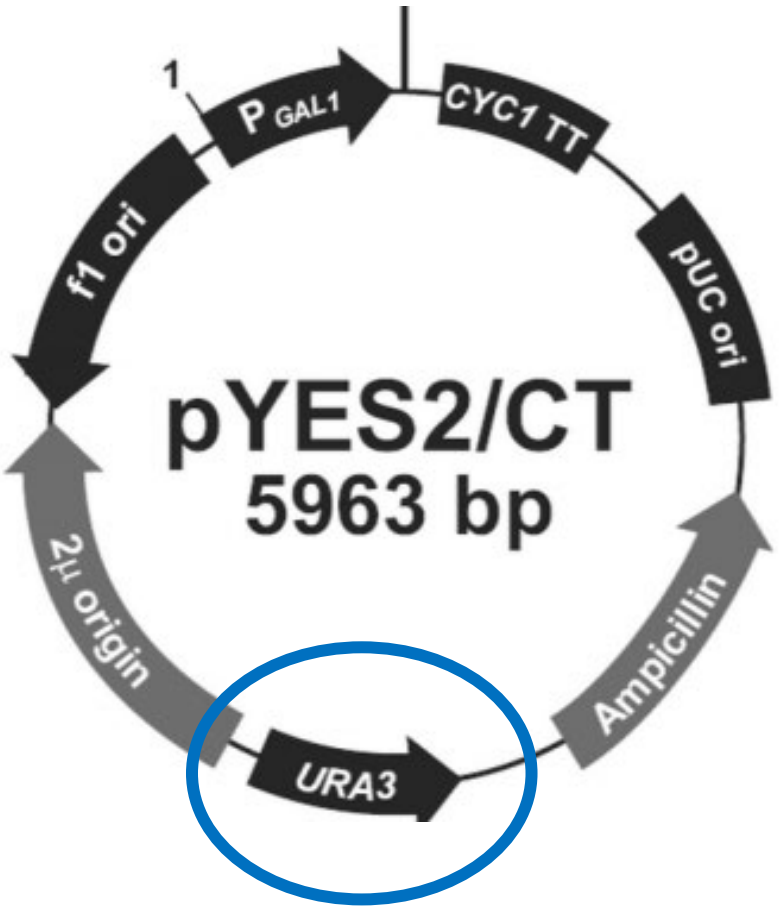
  - *"…procedure utilized in this kit is designed, in some ways, similar to the lithium cation based method…mechanism probably involves some metabolic pathways that we do not fully understand."*

- 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 FET4_mutant plasmid

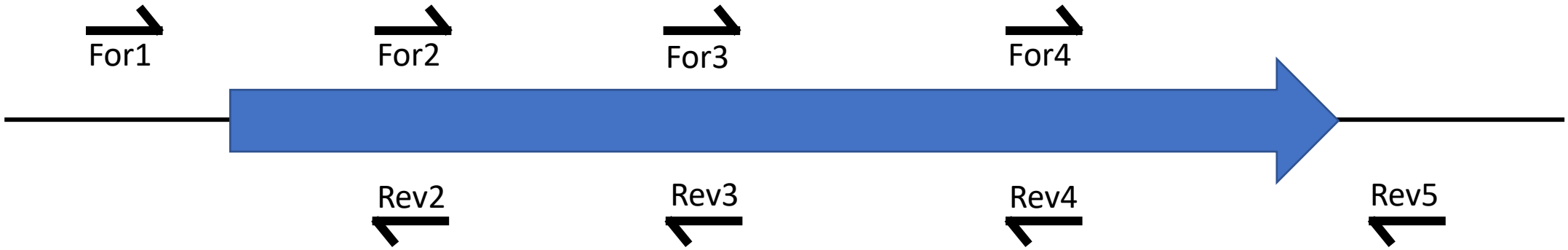
- W303a yeast cells engineered such that gene needed to endogenously generate uracil were removed / mutated

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



Prepare FET4_mutant plasmid for sequencing

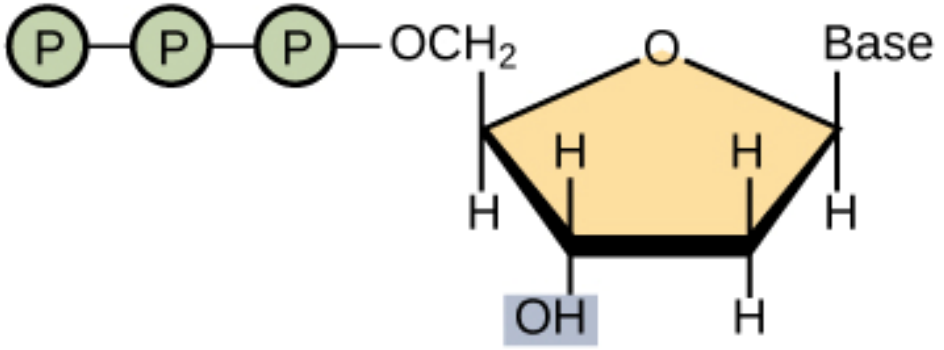
- Reactions prepared by combining isolated FET4_mutant plasmid and sequencing primers
  - One primer per reaction
- Primers were designed to amplify across FET4 insert
  - Primer pair used for sequencing will be determined by location of your mutation!



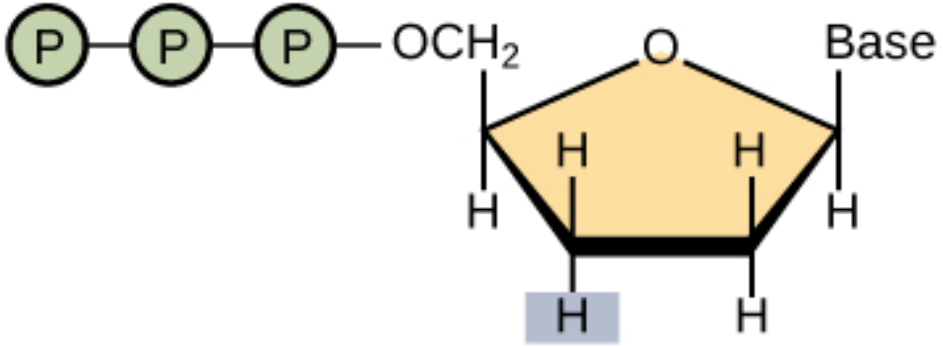
Modified bases used in sequencing reactions

- DNA polymerase acts at 3’ OH of growing DNA strand to create phosphodiester linkage with 5’ P of incoming nucleotide

- Dideoxynucleotides lack OH group needed to extend sequence
  - Causes growing DNA strand to terminate

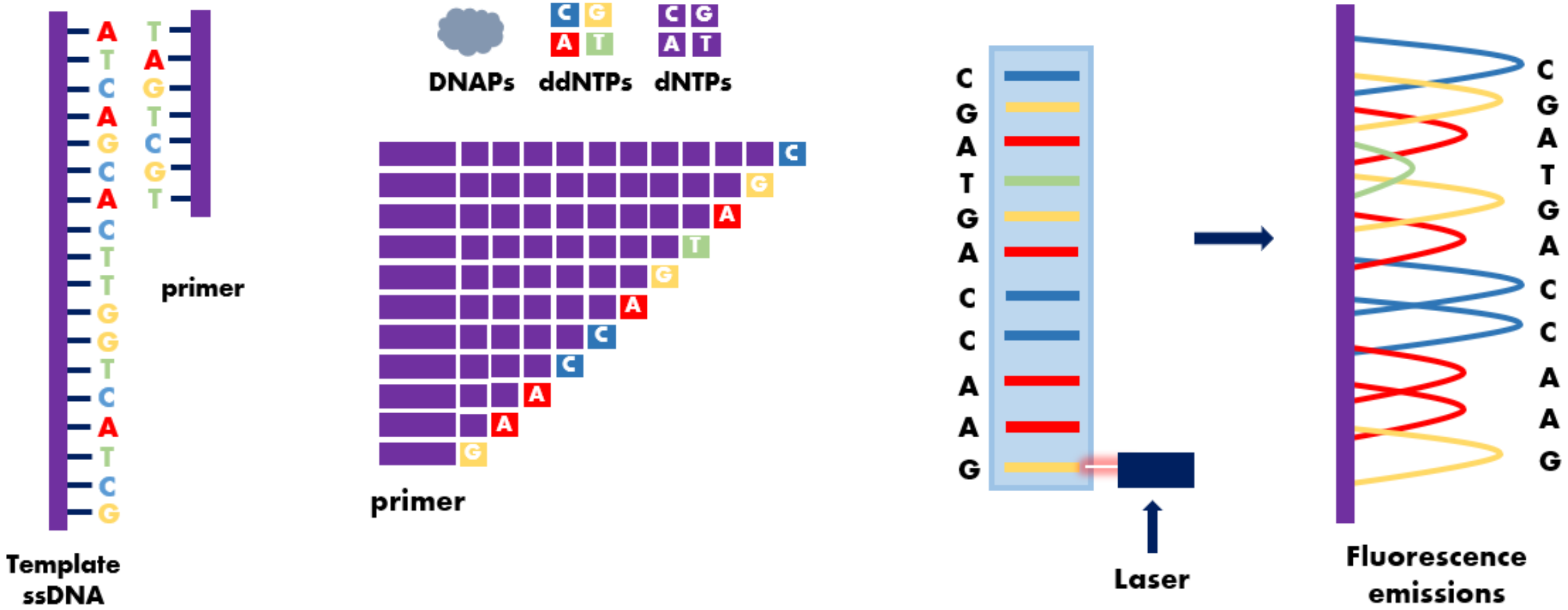


Deoxynucleotide (dNTP)



Dideoxynucleotide (ddNTP)

How is sequence determined using DDNTPs?



For today…

1) Mini prep 2 mutant colonies
2) Use isolated plasmids for
    1) Sequencing reactions
    2) Transformation of your W303a yeast

For M2D4…

- Prepare draft slide for Journal article presentation
    - Use data figure from article to draft 1-2 slides that highlight the conclusion
        - If a figure has abillion panels, pick the most important ones
    - Include the script for how you would describe the information presented on the slide(s)