M2D1:
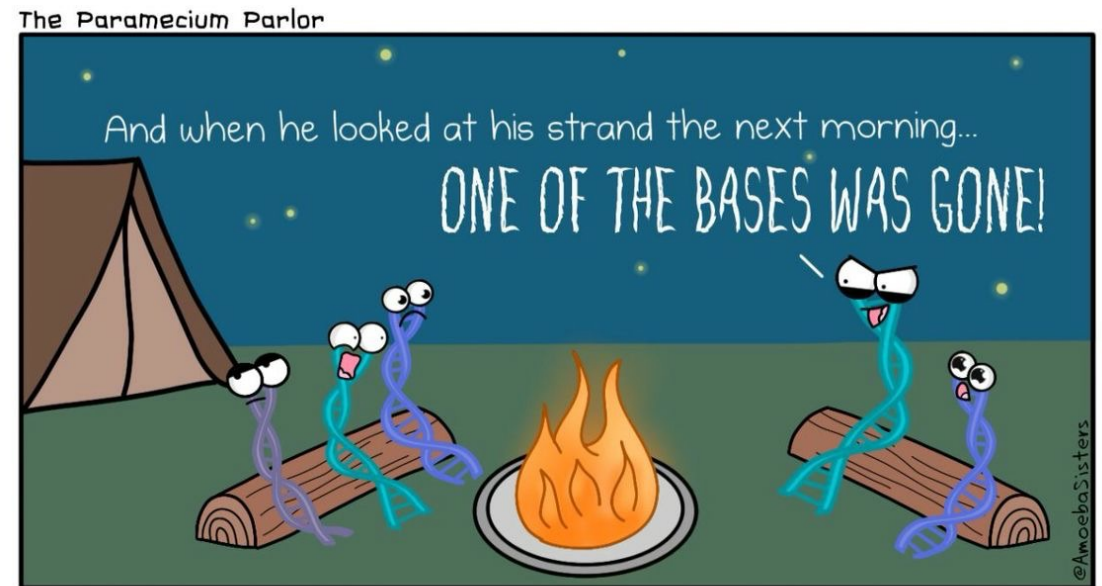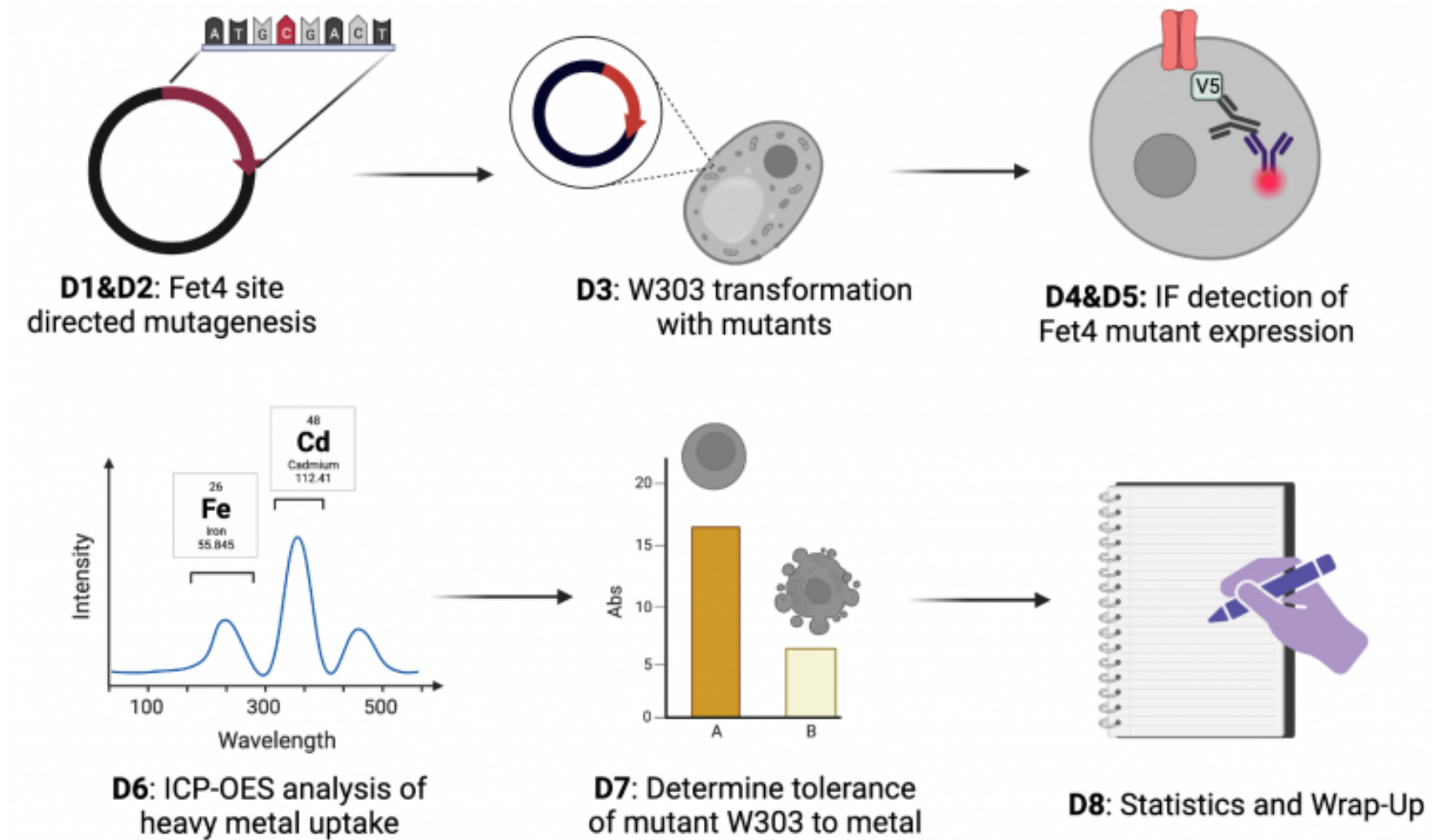## Determine mutagenesis strategy

1. Prelab discussion
2. Review literature and identify point mutation site
3. Design mutagenesis primer


The Paramecium Parlor

And when he looked at his strand the next morning...
ONE OF THE BASES WAS GONE!

Sharing mutation stories was a DNA camping tradition.

@AmoebaSisters

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

FET4 functions as a Fe^{2+} transporter protein

- Integral membrane protein located in plasma membrane

- Regulated by environmental iron levels

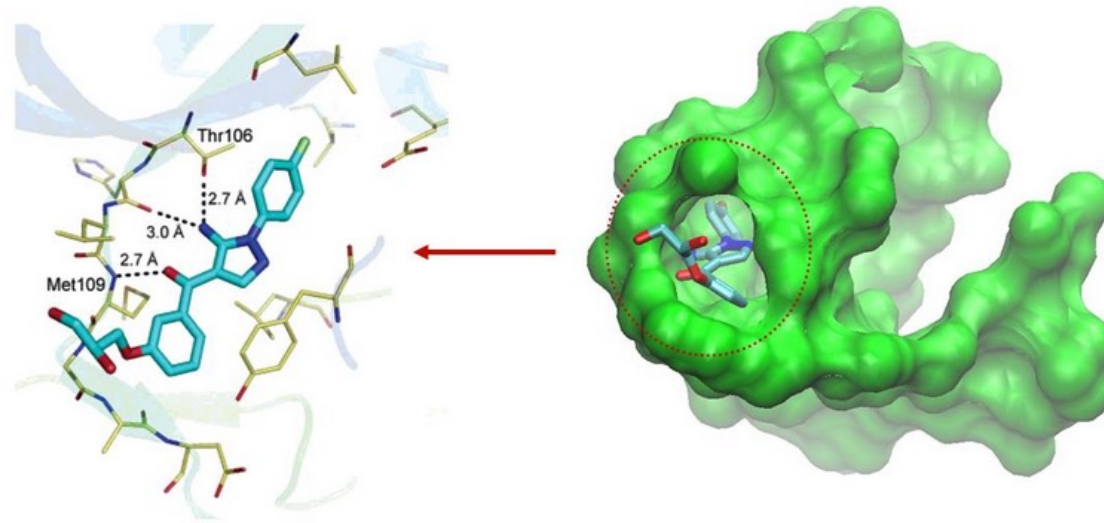- Residues important in Fe^{2+} binding located in transmembrane domain
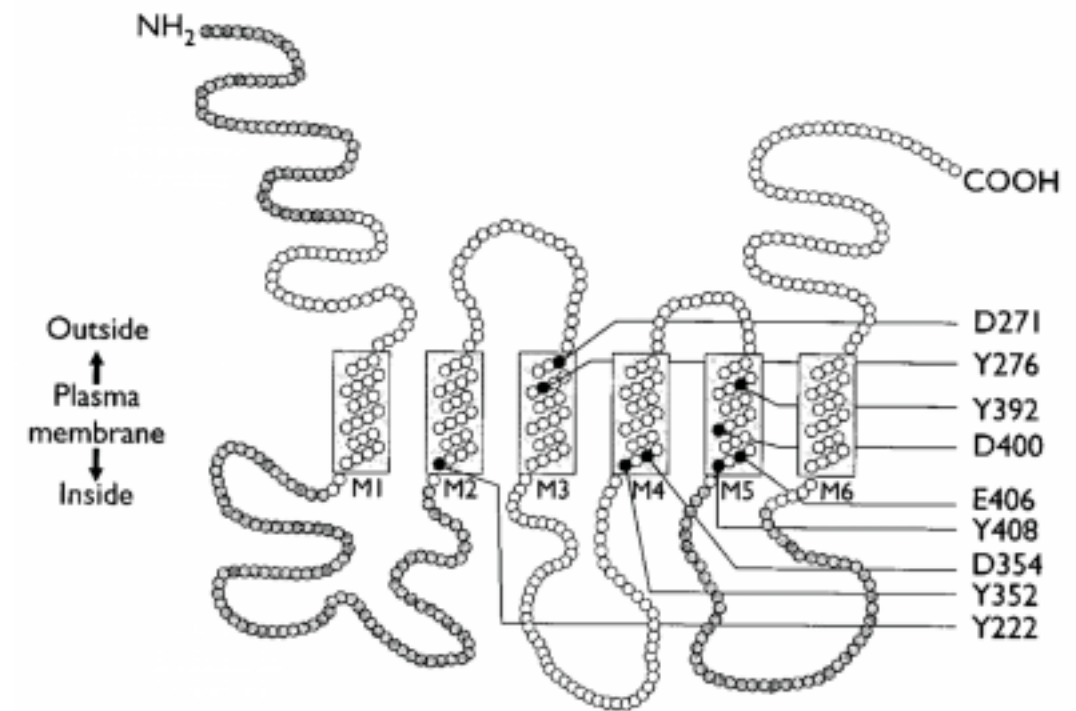
What is your research goal?

- Genetically modify the FET4 Fe^{2+} iron transporter to preferentially take-up cadmium

What is your experimental approach?

- Incorporate point mutations into FET4

- Analyze data from class to assess ~14 unique mutations

How do point mutations alter binding?

Change in single protein residue can:

- Alter size / shape of the binding pocket
- Alter charge of the binding pocket
- Collapse the binding pocket
- Eliminate / promote access to binding pocket

How will you generate point mutations?

- Gene sequence for FET4 cloned into expression vector

- Primers used to amplify FET4-containing expression vector
  - Forward primer incorporates sequence that contains wanted point mutation

- Amplification reaction results in linear PCR product that contains point mutation

- Linear PCR product ligated to generate mutated FET4-containing expression vector

≥10 nts

Design Strategy

PCR Product

Final Product
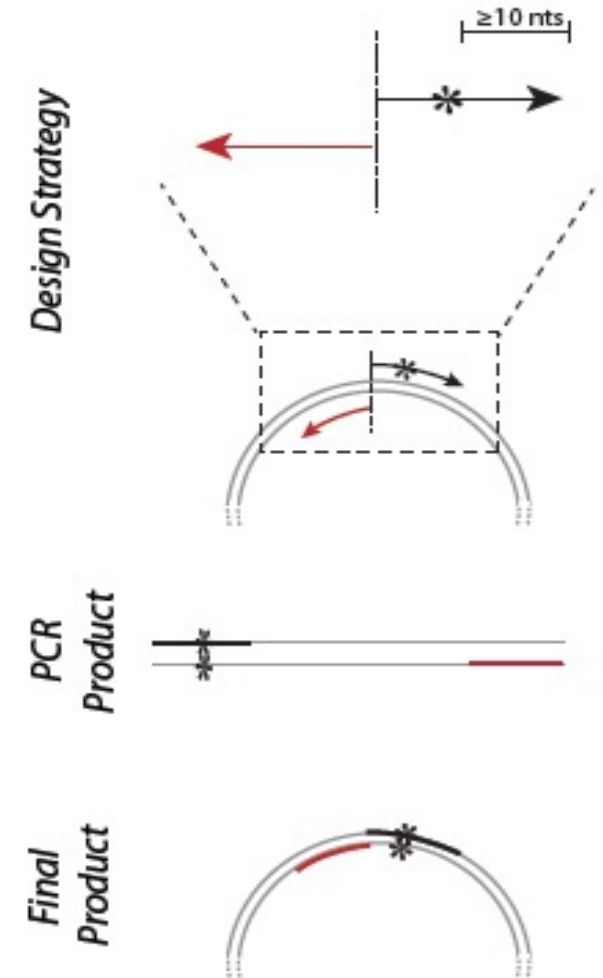
How will you design your primers?

- Wanted mutation must be in the center of the forward primer
  - Ensures primer is able to bind sequence located upstream and downstream of mutation site
- Forward and reverse primers must 'face away' in opposite directions

- Primers should: be 25-45 bp, contain 40% G/C bps, have melting temperature <78C, and terminate in G/C
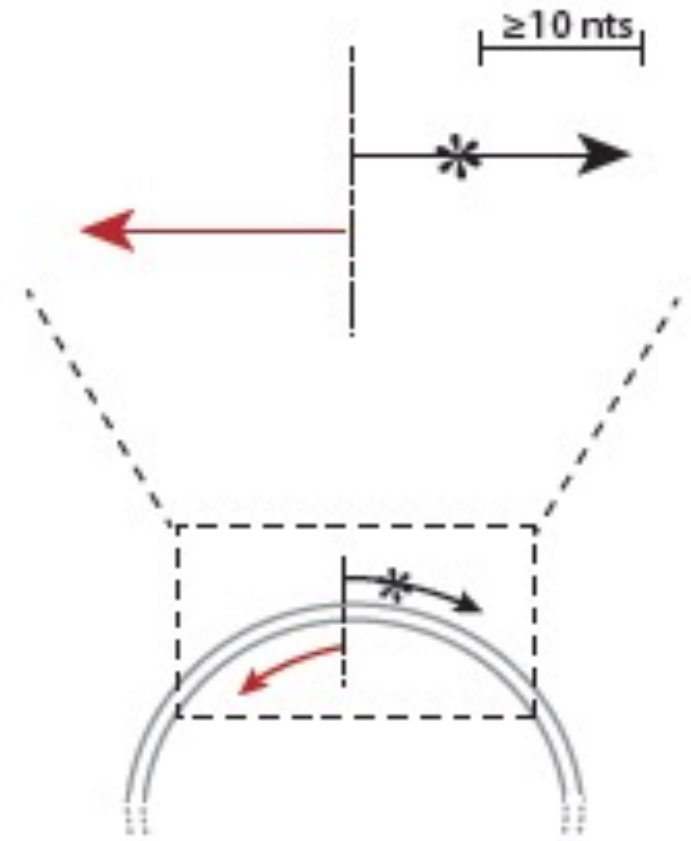
For today…

- Primers must be completed / submitted by 5p!

For M2D2…

- Reserve article for Journal article presentation and submit summary that highlights why you think the research is interesting