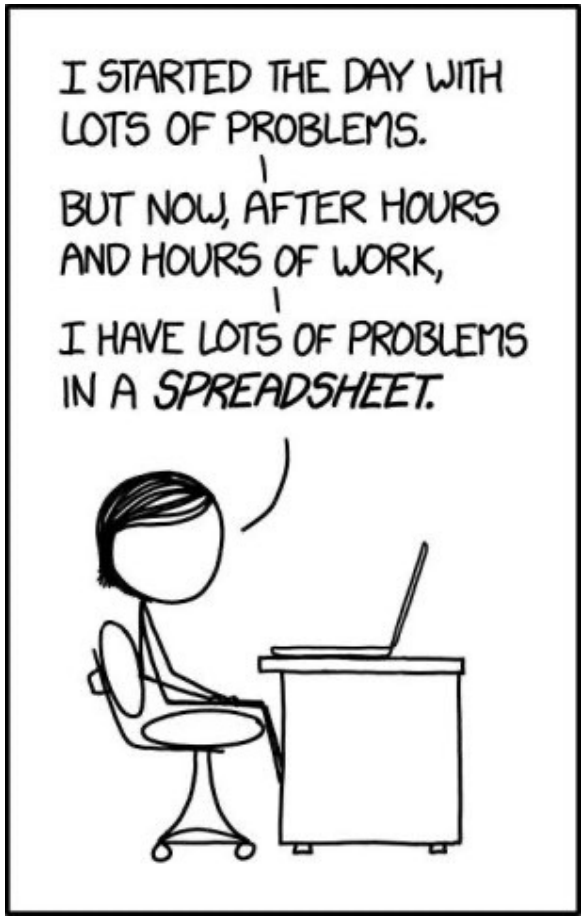
M2D7: Analyze ICP-OES data and examine yeast tolerance to metal

- Prelab
  - Review Mod2 project experiments
- Examine and compile ICP-OES data
- Perform metal tolerance test using Fet4_mutant yeast

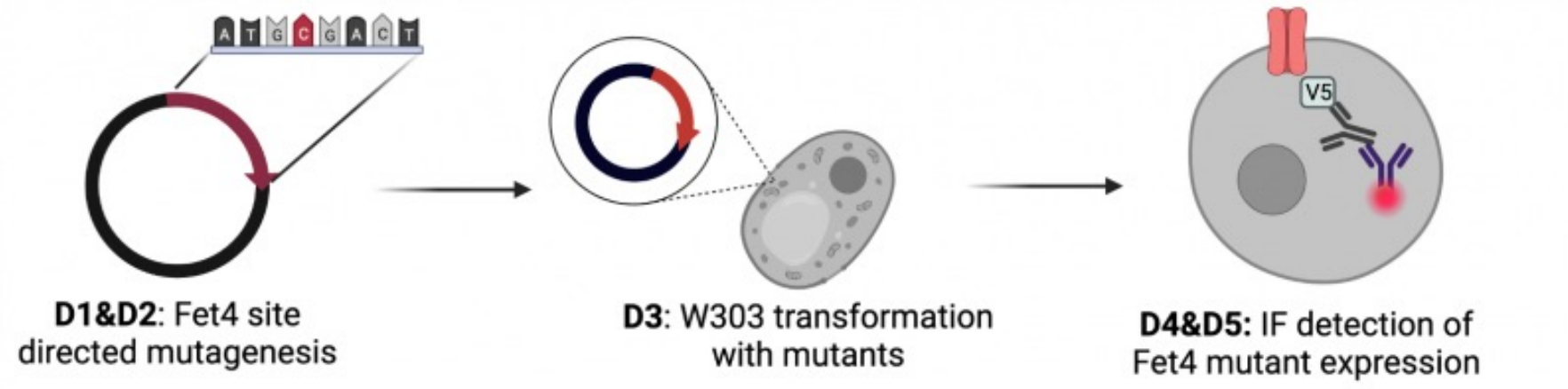


Overview of Mod 2 experiments

Last lab:

This lab:

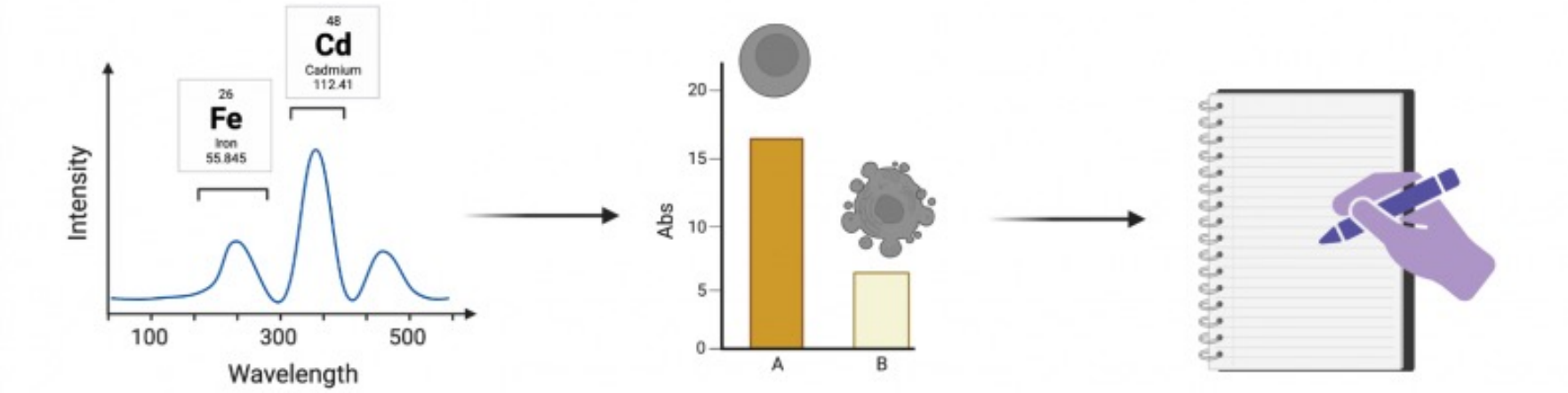
Next lab:



D1&D2: Fet4 site directed mutagenesis

D3: W303 transformation with mutants

D4&D5: IF detection of Fet4 mutant expression



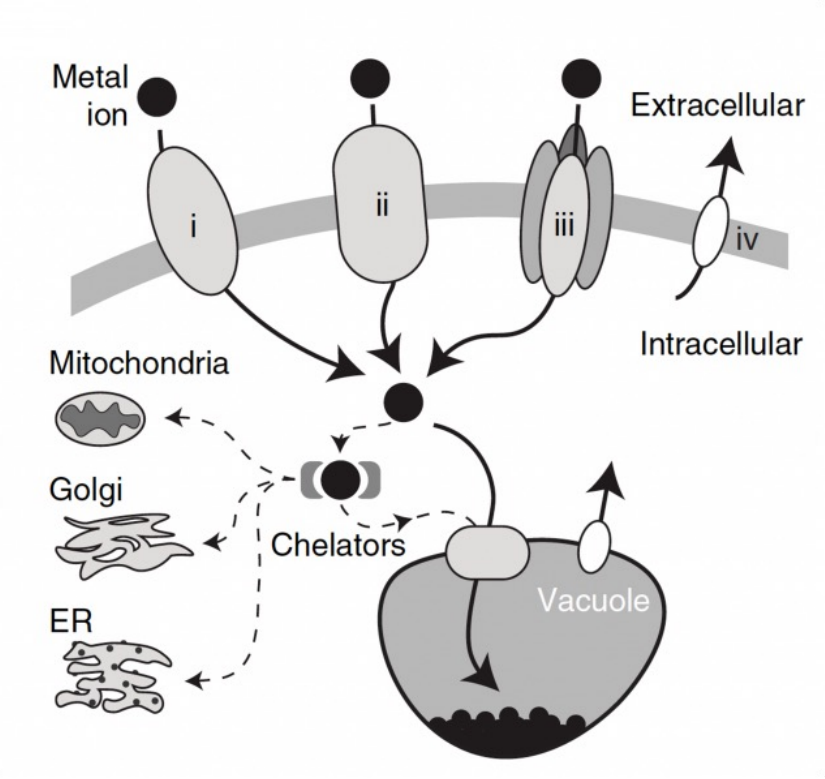
D6: ICP-OES analysis of heavy metal uptake

D7: Determine tolerance of mutant W303 to metal

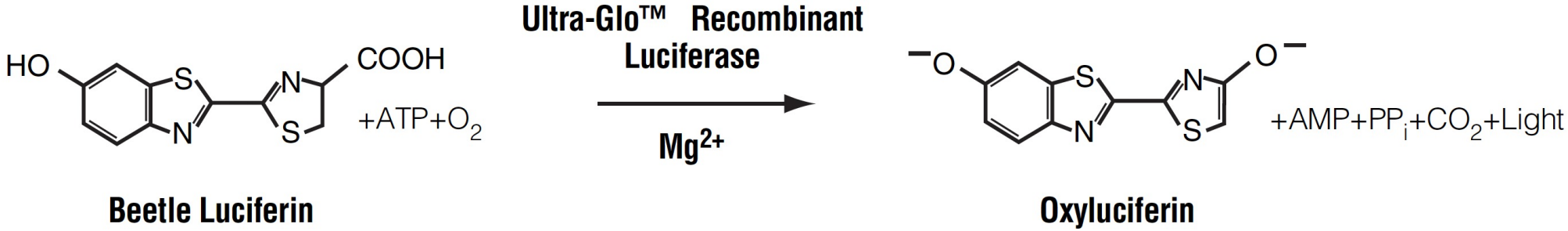
D8: Statistics and Wrap-Up

Metal tolerance experiment overview

- Examine OD_{600} for your Fet4_mutant culture
- Dilute your culture to achieve 5ml of culture at ~1.0 OD_{600}
- Spike your yeast culture with 100uM Cadmium
- Incubate for 2.5 hours
- Recheck OD_{600}
- Add cell suspension and BacTiter-Glo reagent to 96 well plate
- Read luminescence on platereader upstairs



What is the BacTiter-Glo assay measuring?

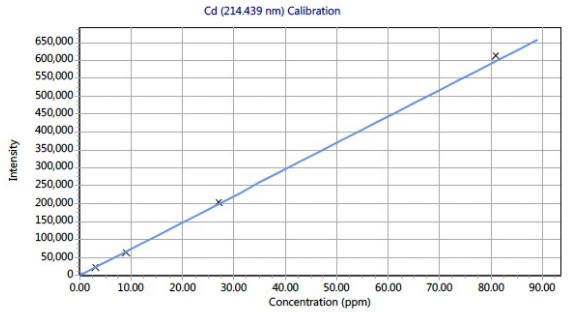


- Generation of bioluminescence
- The luciferase enzyme oxidizes luciferin substrate
  - utilizes Mg^{2+} and ATP
- Light is produced as part of the reaction

Measured bioluminescence has a linear relationship with ATP
ATP production is indicative of a live, metabolically active cell

ICP-OES calibration data (found in pdf)

Cd (214.439 nm)
Intensity = 7379.58566373 * Concentration + 13.00393963
Correlation coefficient: 0.99997



Standards	Intensity	Method Concentration	Calculated Concentration	% Error
Blank	9.41	0.00	0.00	N/A
Standard 1	22998.83	3.00	3.11	3.83
Standard 2	63918.04	9.00	8.66	3.78
Standard 3	205044.11	27.00	27.78	2.90
Standard 4	614049.08	81.00	83.21	2.73

- each wavelength has a calibration curve established using the known standards we generated
- Standards= 0ppm, 3ppm, 9ppm, 27ppm, 81ppm

ICP-OES sample data (in pdf and csv file)

- Each team is a sample
- Each class has a control
  - Untransformed (WT)
  - Fet4 overexpression(Fet4)
- concentration is calculated in parts per million (ppm)
  - based on peak intensity at the listed wavelength and calibration curve

Sample Name: Sample 17

Date: 4/18/2023 11:22:03 AM Rack:Tube: 2:22

Weight (g): 1 Volume (mL): 1 Dilution: 1

Analyte Results

Label	Solution Concentration	Unit	SD	%RSD	Intensity	Calculated Concentration
Cd (214.439 nm)	12.58	ppm	0.06	0.49	92819.61	12.58 (ppm)
Cd (219.463 nm)	12.15	ppm	0.56	4.57	97.35	12.15 (ppm)
Cd (223.986 nm)	11.31	ppm	0.75	6.67	63.52	11.31 (ppm)
Cd (226.502 nm)	12.12	ppm	0.07	0.57	175365.66	12.12 (ppm)
Cd (226.742 nm)	10.62	ppm	0.70	6.59	95.29	10.62 (ppm)
Cd (228.802 nm)	12.15	ppm	0.10	0.80	52154.44	12.15 (ppm)
Cd (230.662 nm)	11.68	ppm	0.07	0.63	198.56	11.68 (ppm)
Cd (231.275 nm)	11.81	ppm	0.77	6.55	221.83	11.81 (ppm)

Replicates Concentration

Label	Replicate 1	Replicate 2	Replicate 3	Units
Cd (214.439 nm)	12.51	12.64	12.58	ppm
Cd (219.463 nm)	12.77	11.99	11.69	ppm
Cd (223.986 nm)	12.13	11.14	10.65	ppm
Cd (226.502 nm)	12.12	12.05	12.19	ppm
Cd (226.742 nm)	9.89	11.29	10.68	ppm

Mod2 project review (AKA: what are we doing again?

- Research goal:

- How does your mutagenesis design fit into this research goal?

Mod2 project review (AKA: what are we doing again?)

- How do your experiments fit into this goal?
  - Sequencing alignment:
  - ICP-OES:
  - BacTiter-Glo assay:

For today:

1. Set up metal tolerance experiment
2. Examine ICP-OES data during incubation
3. Collect data on metal tolerance (last data for the module!)

For M2D8

- Draft an outline of the Research Article discussion using the prompts on the homework section of the wiki and questions you answered for M2D7