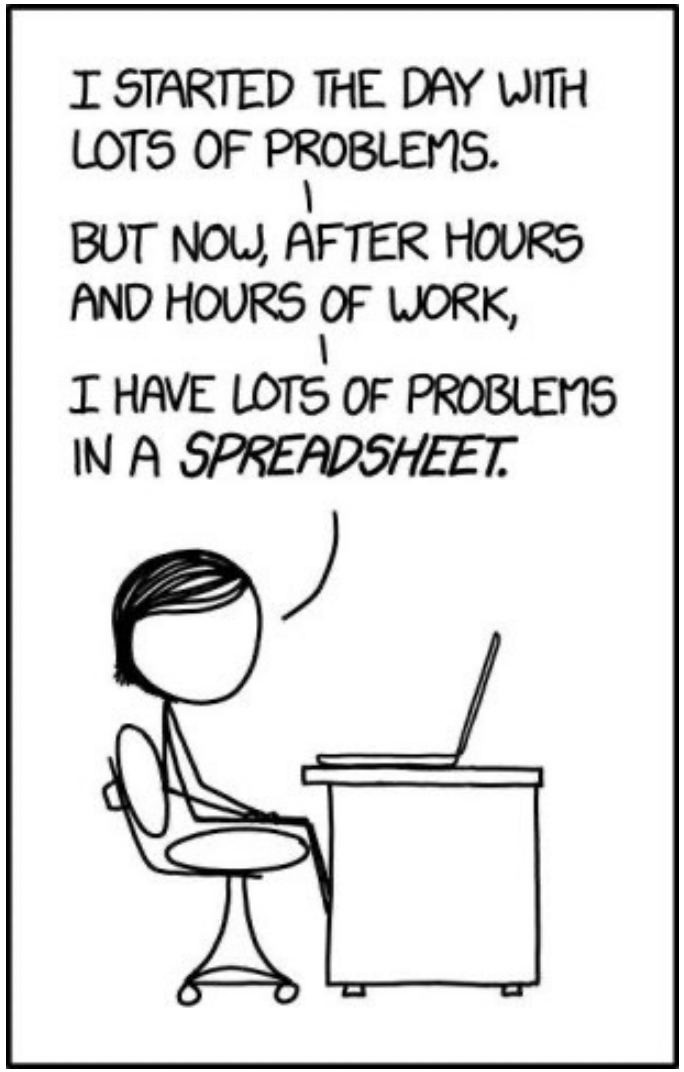
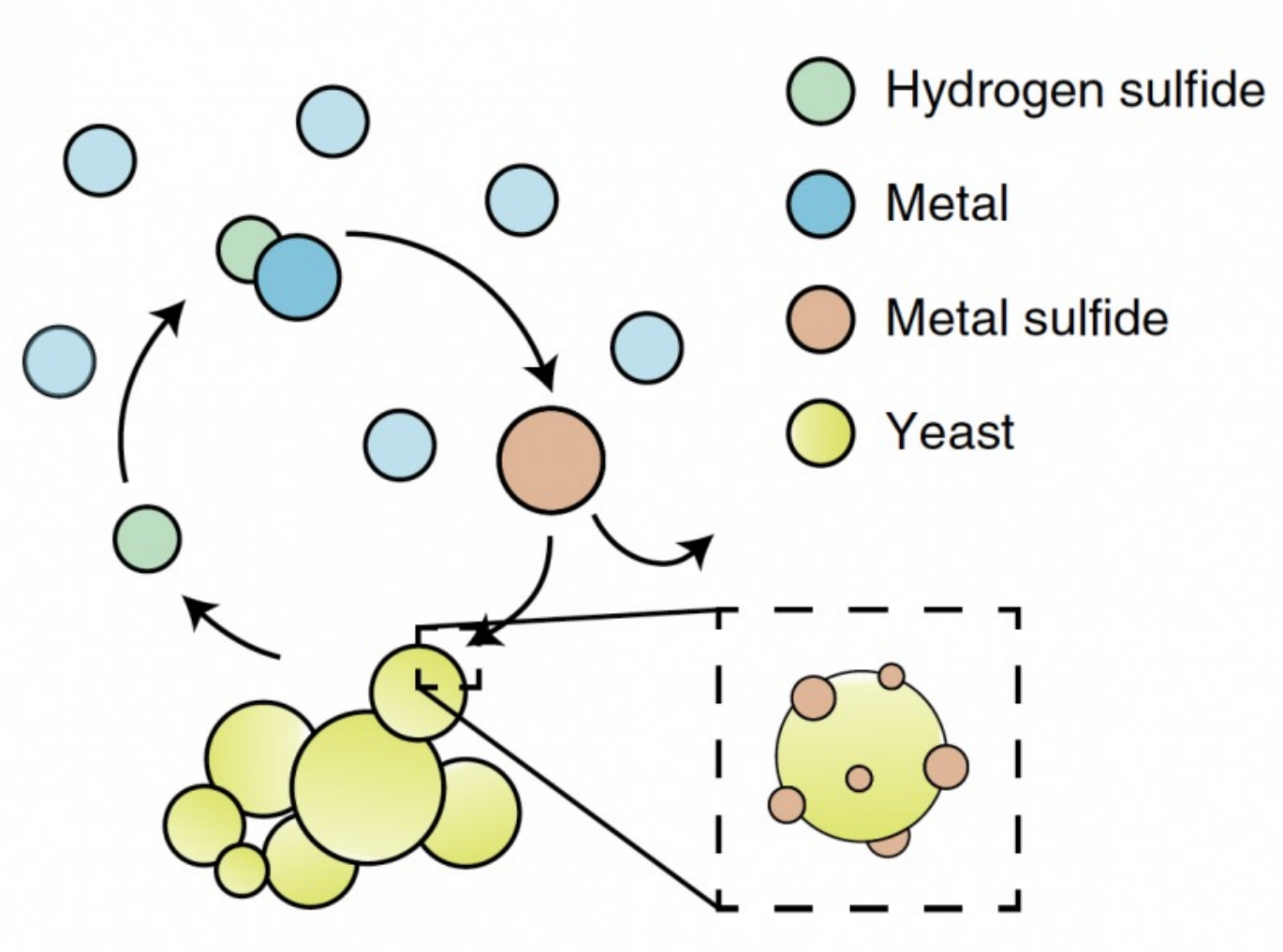
M2D6: Quantify cadmium removal from the media

- Prelab
  - Review

- Examine ICP-OES data

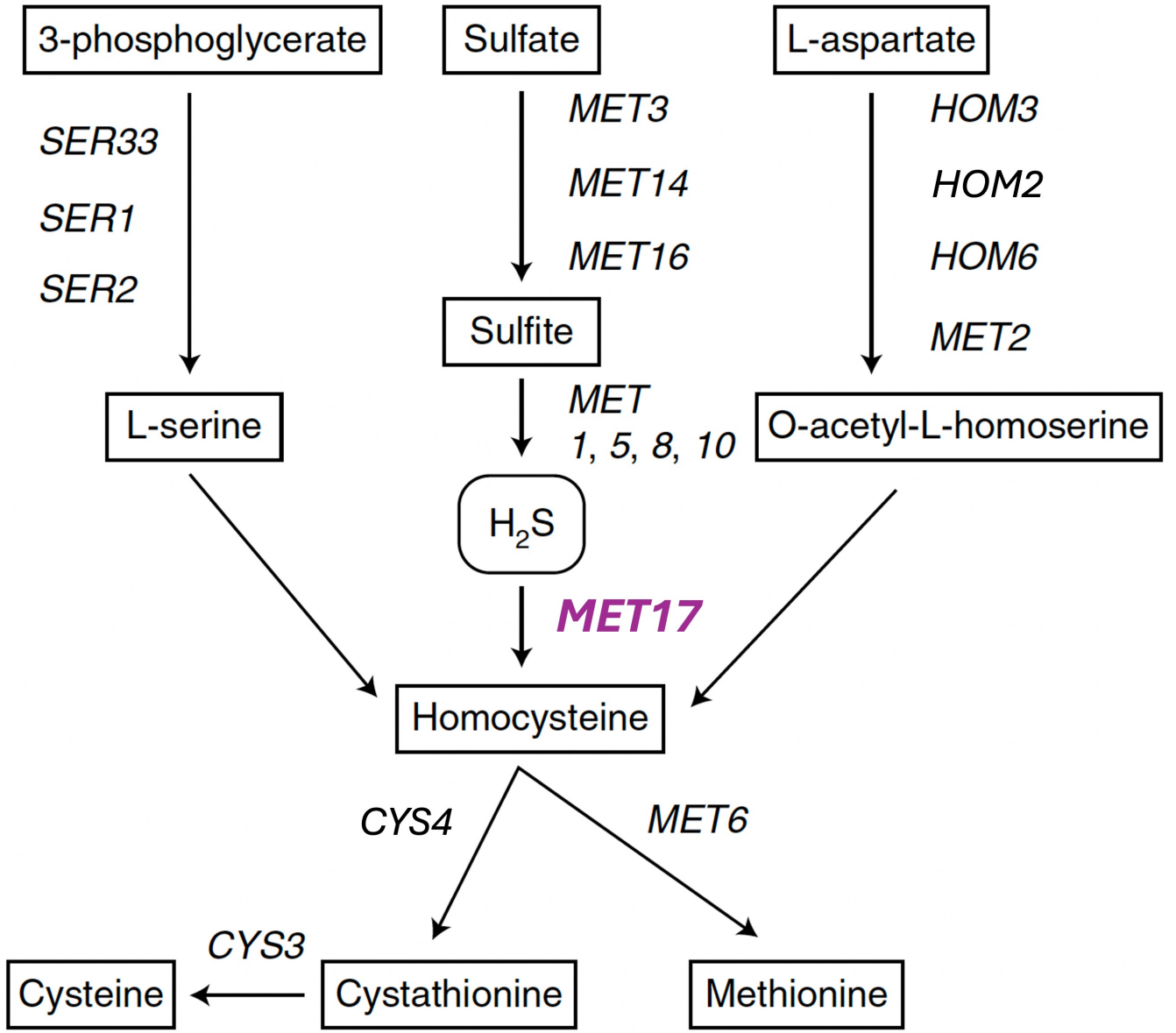


Overall concept: capturing cadmium from media environment



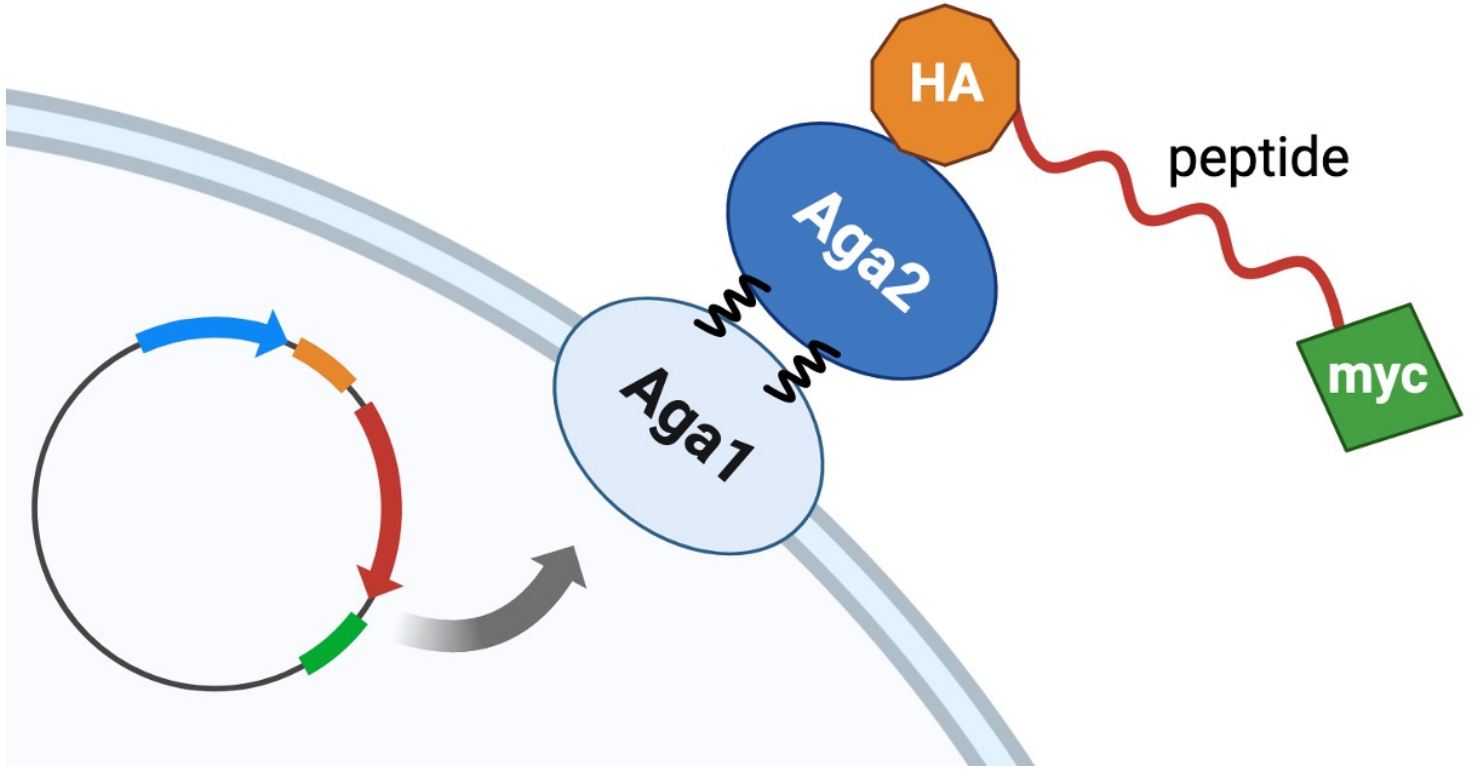
How are yeast programed to produce hydrogen sulfide?

- Metabolic engineering of W303α strain of yeast



Yeast cell surface display of designed peptide

- Design a peptide to capture precipitating cadmium sulfide
- Peptide fused to Aga2 and two tags
- Aga2 anchors peptide at the cell surface
- Tags allow detection of peptide at surface

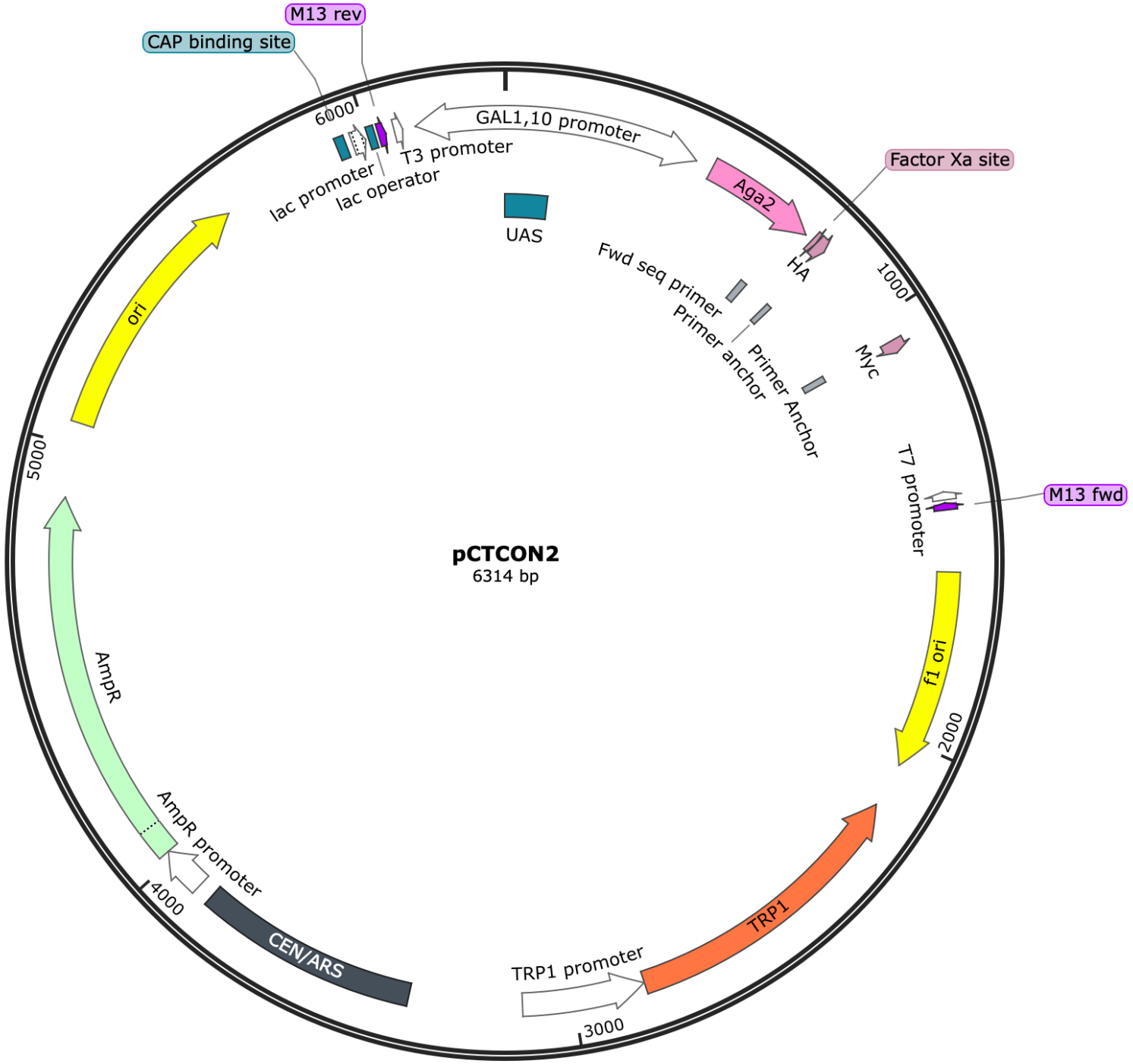


Mutagenesis cloning

Sequence insertion into expression plasmid

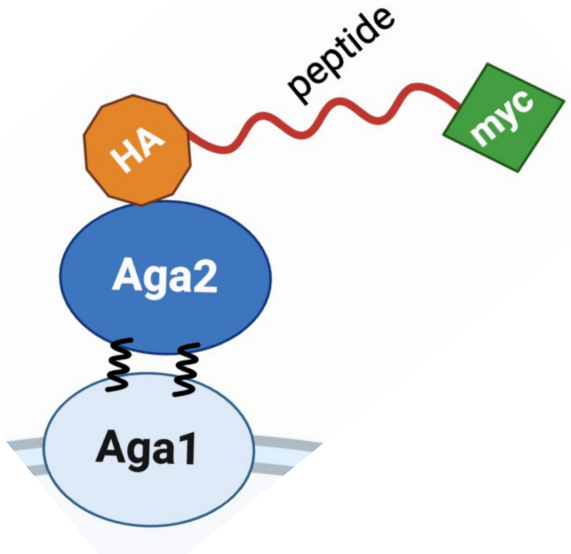
pCTCON2 plasmid

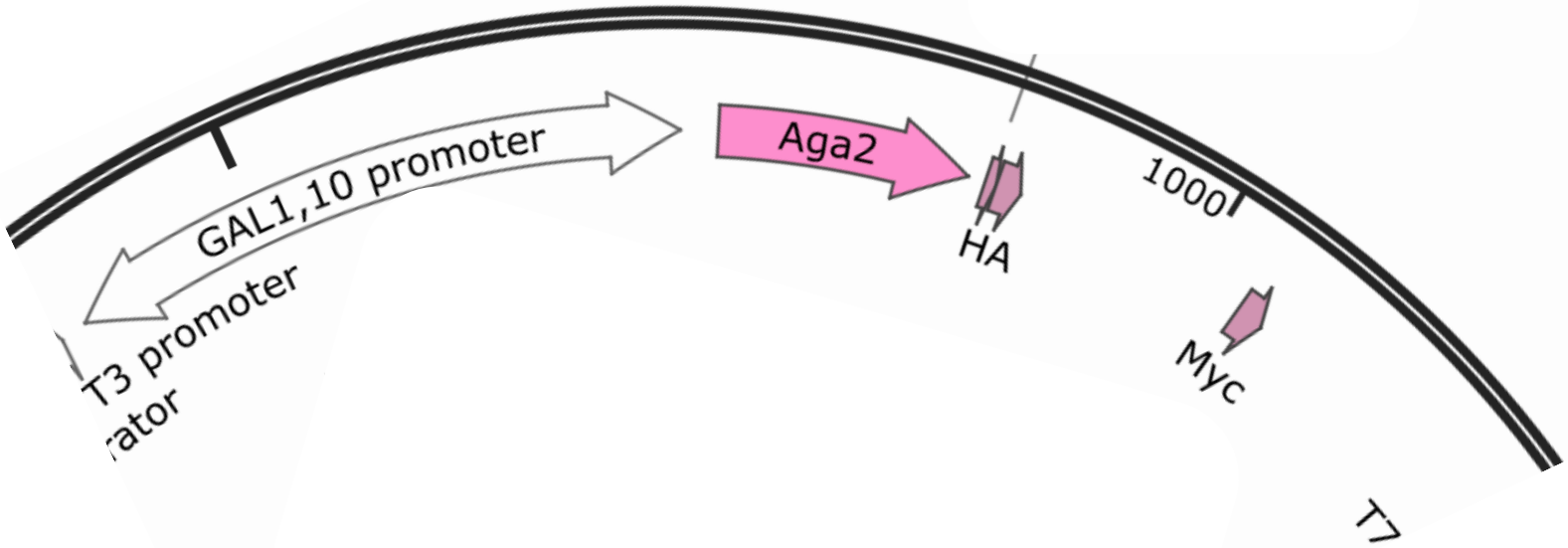
- Different components
- Where does insert go?
- How do the sequencing primers work?



Close up on cloning site of the plasmid

- GAL promoter
- Aga2
- HA
- Myc





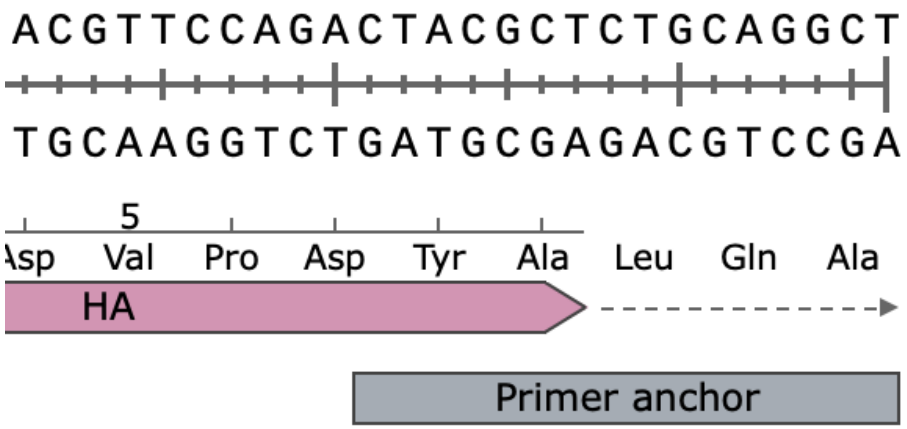
How do you cloning primers insert the sequence?

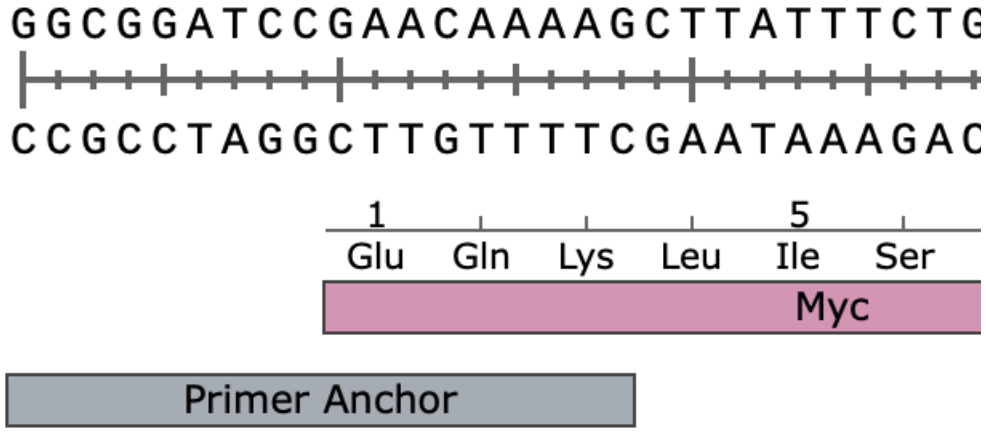
TR orange	DCCDCC	GAC TGT TGC GAT TGT TGC
Fwd Primer		GATTGTTGCggcggatccgaacaaaag
Rev Primer		GCAACAGTCagcctgcagagcgtag

Reverse complement of
Reverse primer

Forward primer

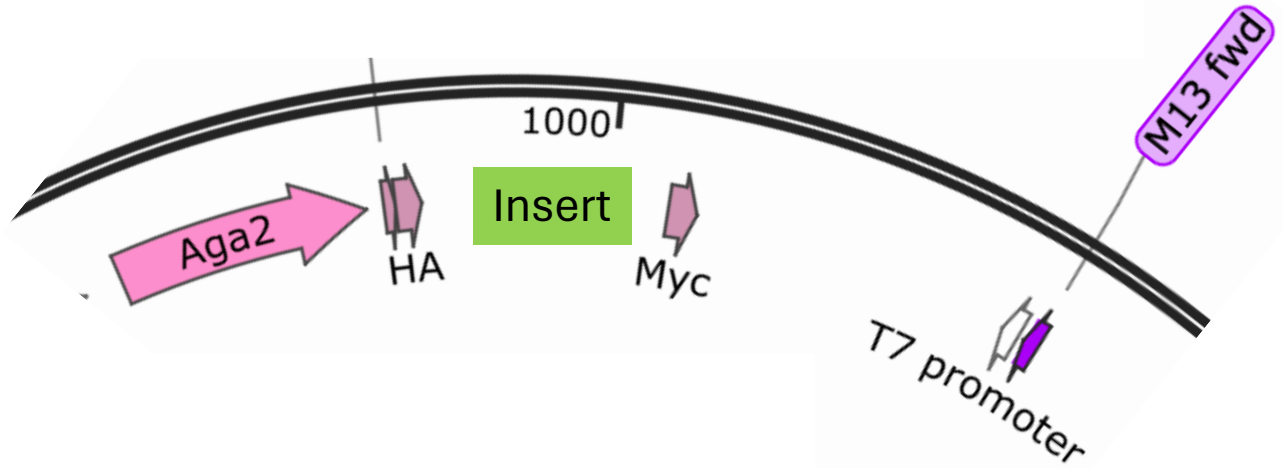
5’ –ctacgctctgcaggctGACTGTTGC GATTGTTGCggcggatccgaacaaaag – 3’

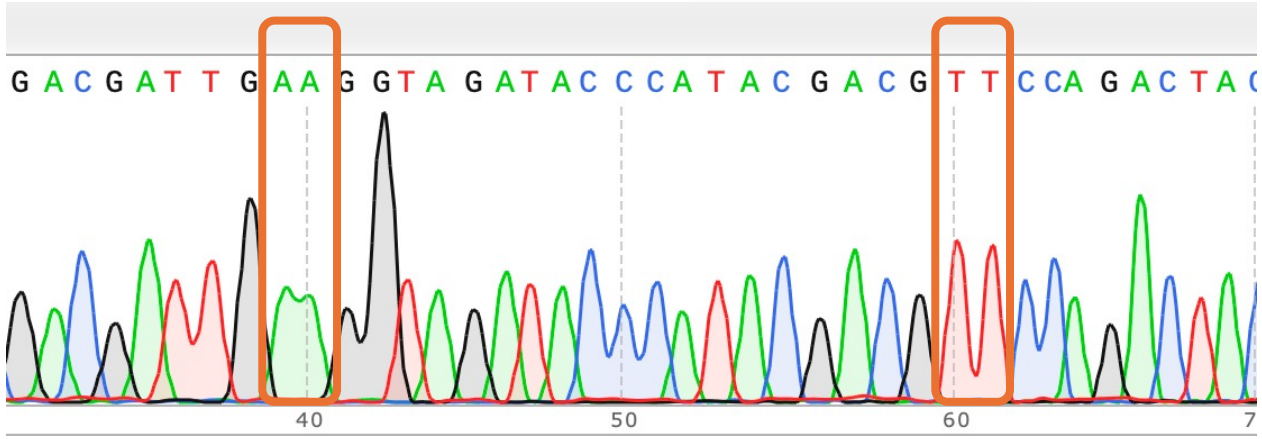




Sequencing to create alignment figure

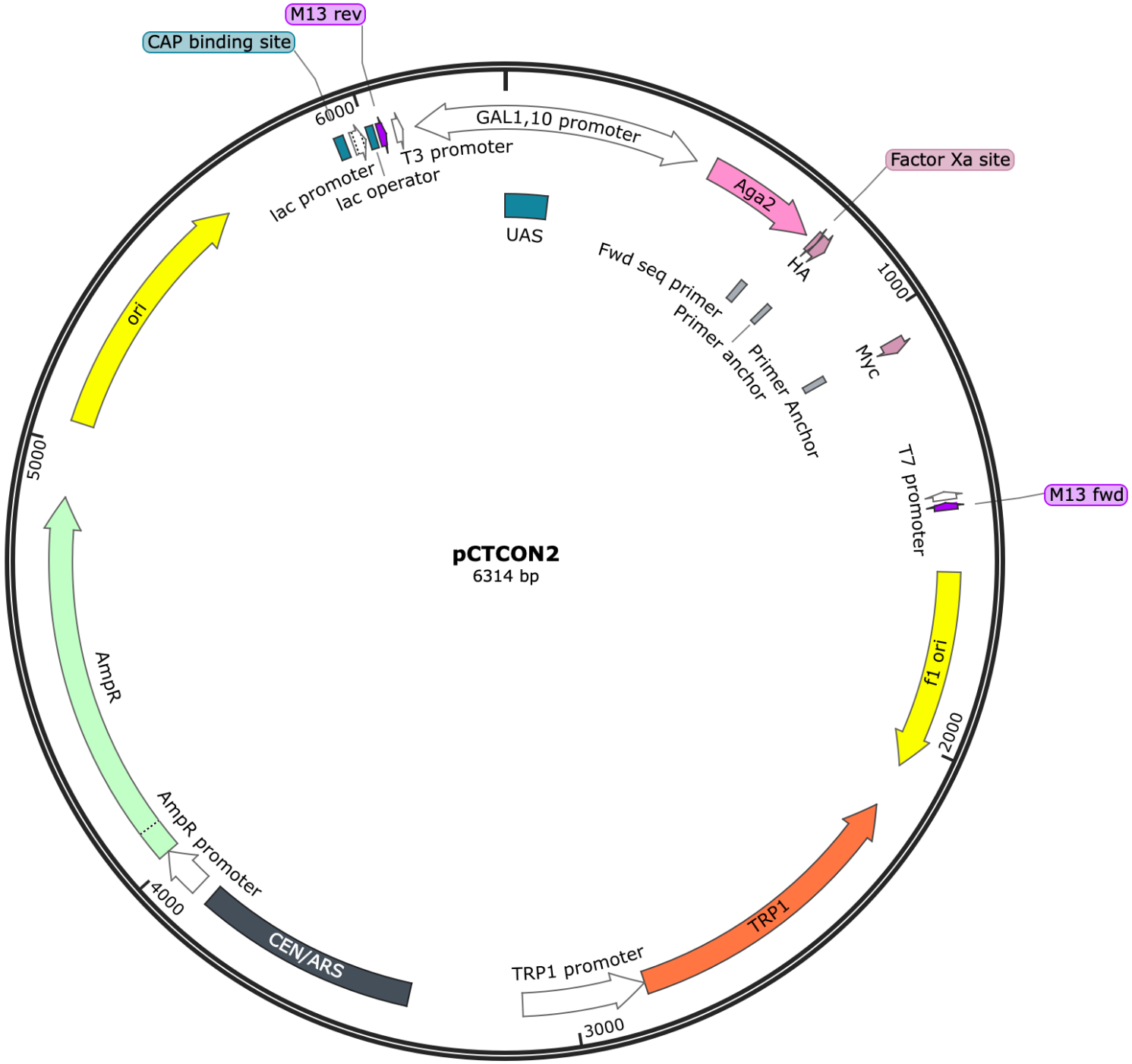
- confirm correct insertion
- Why forward and reverse primers?





Induction of YSD peptide

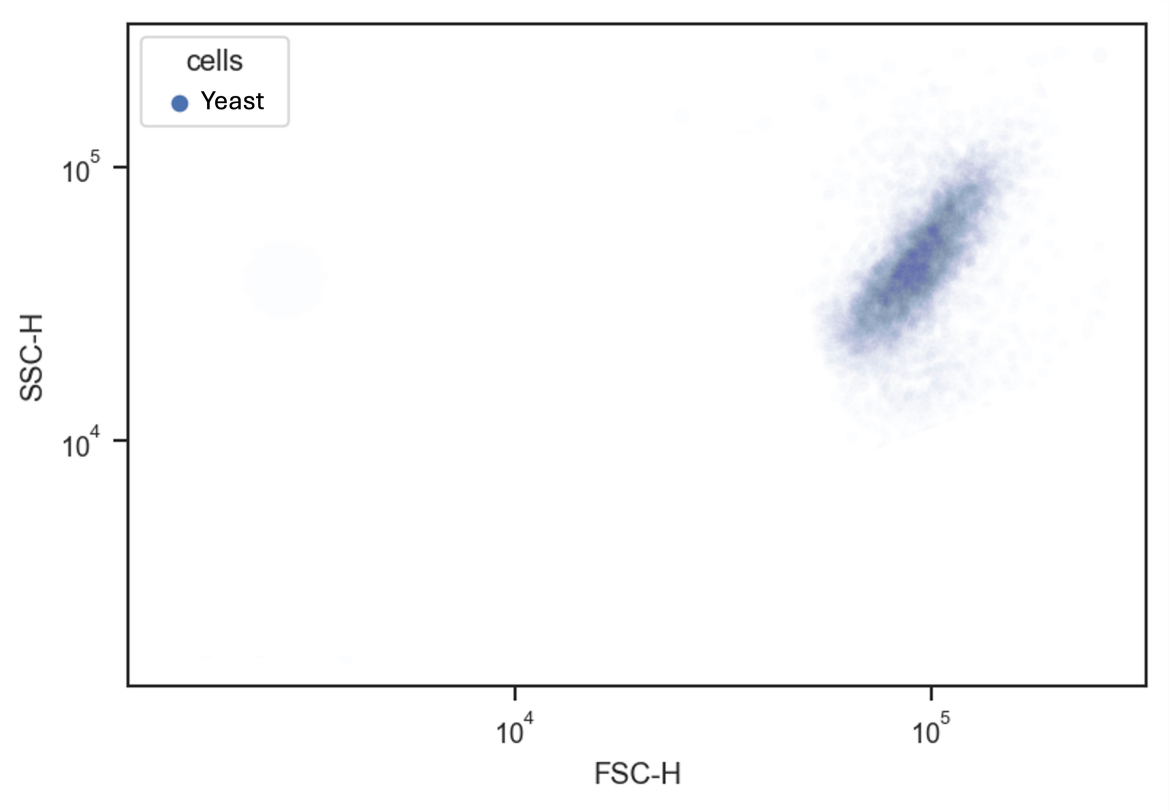
- How is plasmid kept in transformed yeast?
- How is peptide expressed?

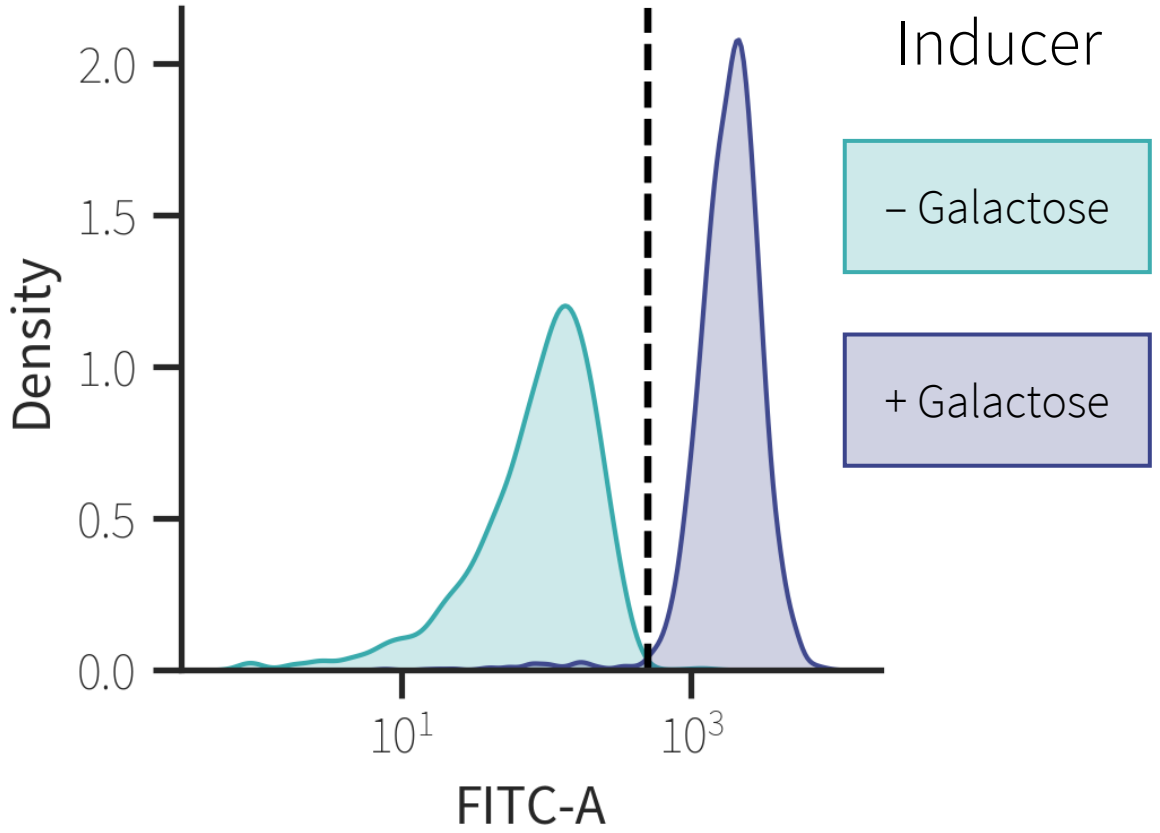


Flow cytometry

Confirm peptide expression

Example flow cytometry data





- What can we learn/confirm with these graphs? (3 things)
- What can go wrong…

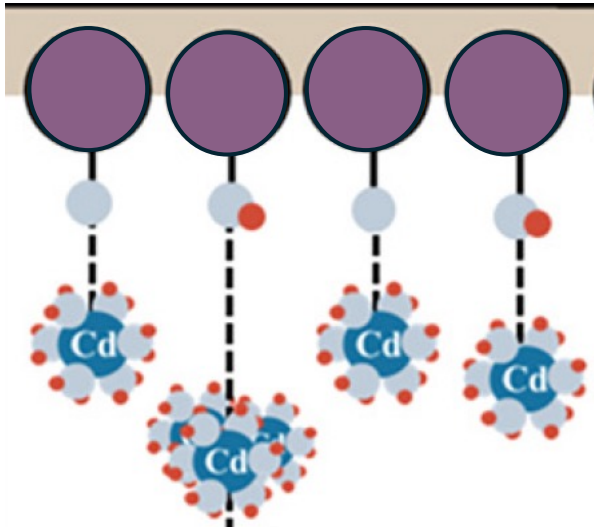
Nucleation experiments

Do yeast capture cadmium?

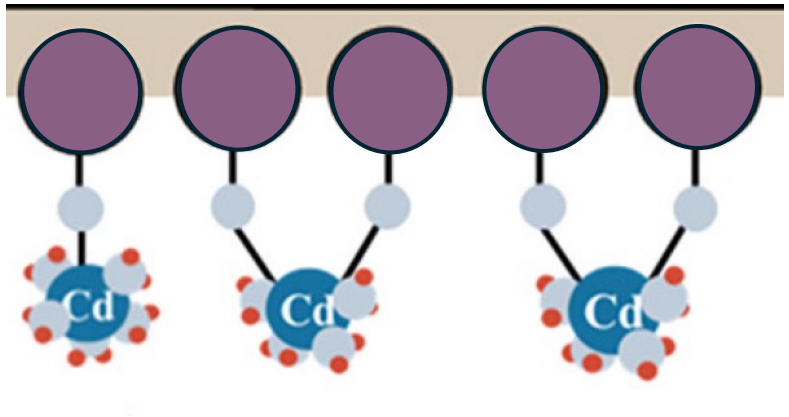
Is cadmium sulfide captured in a usable form?

Cadmium nucleation to cell surface peptide

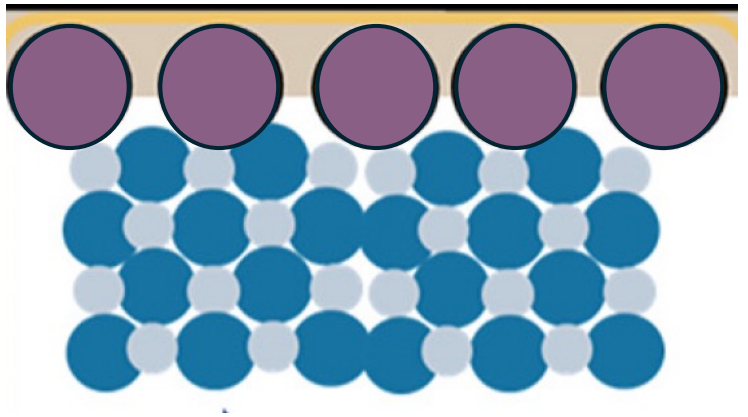
cell surface peptide



- H_2S interacts with $Cd(NO_3)_2$ to form CdS which will start to precipitate out of solution
- That CdS interacts with cell surface peptides as it precipitates



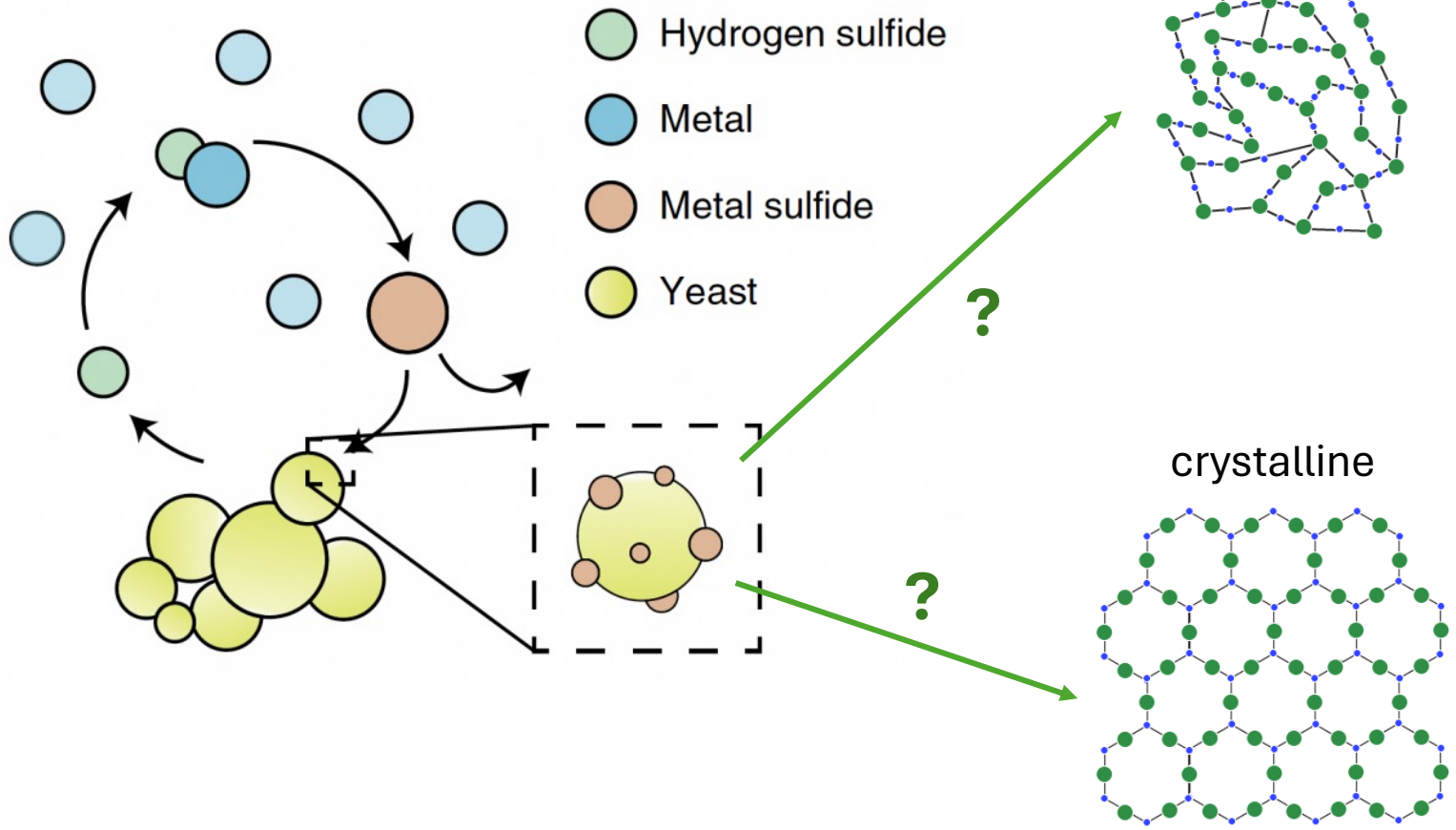
- CdS starts forms bonds with amino acids that it has an affinity for and begins to collect on the cell surface peptide

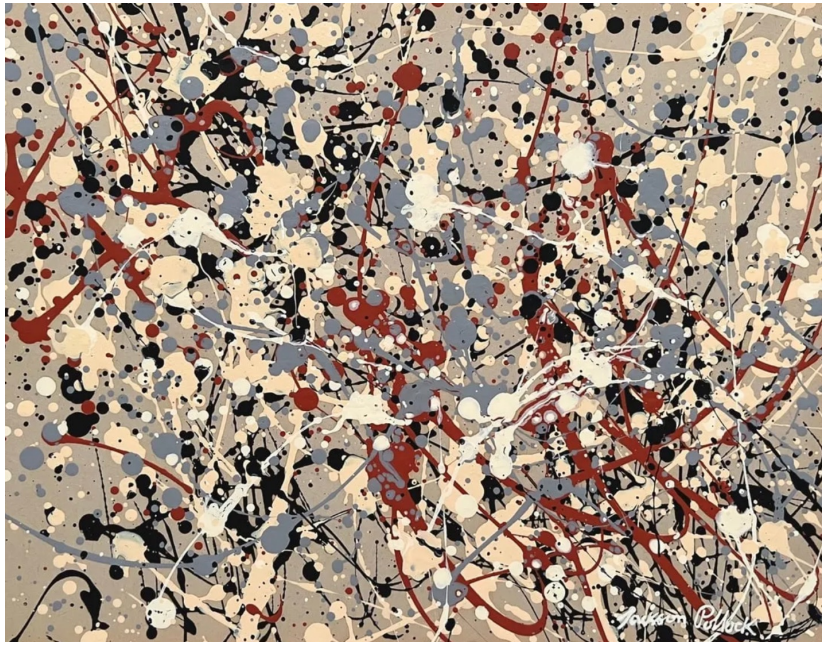


- If different parameters of the precipitation are effectively controlled, the CdS will nucleate as highly ordered molecules

- How can these parameters be controlled?

Cadmium nucleation to cell surface peptide





Jackson Pollock

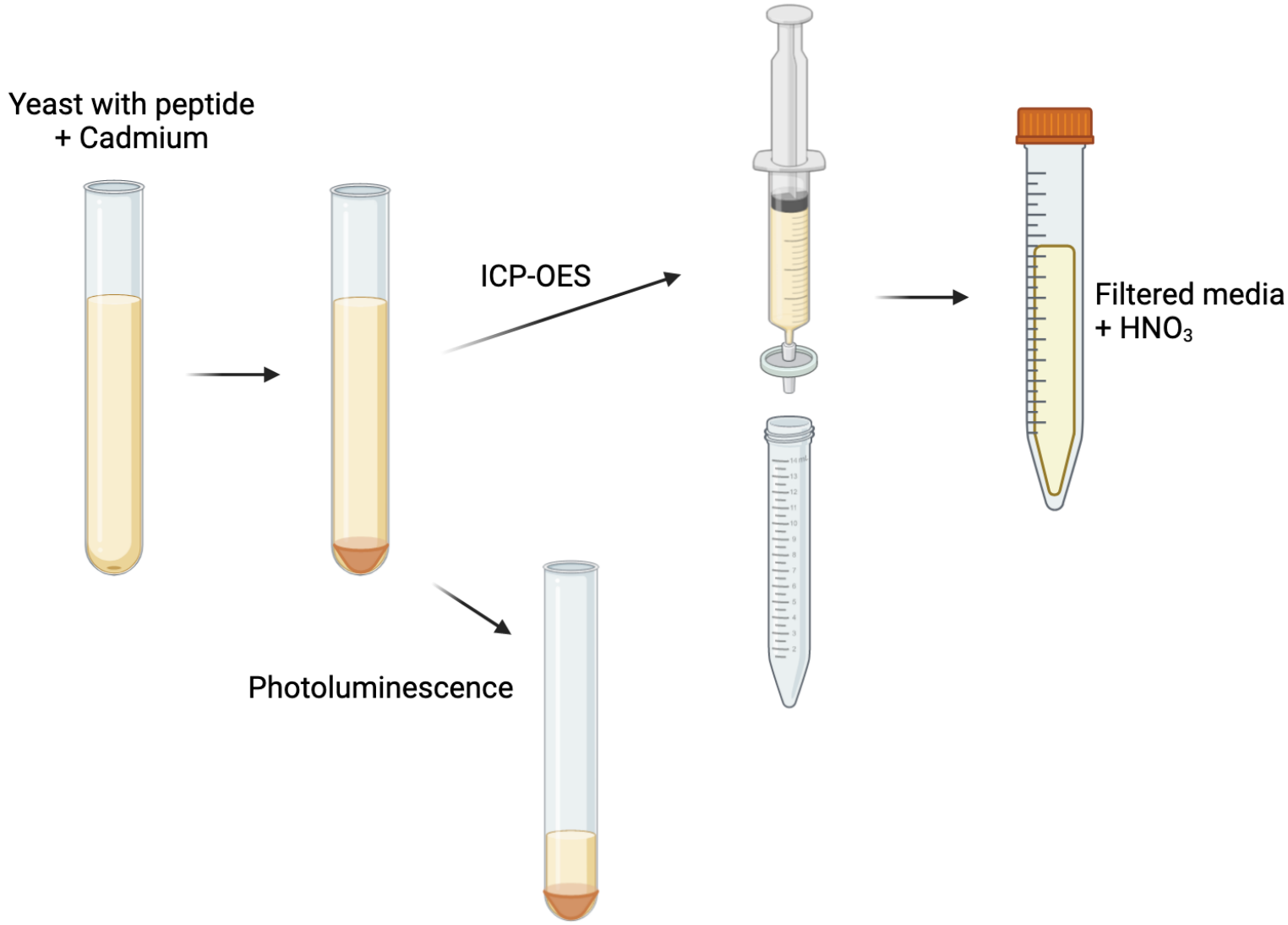
Georges Seurat

- control rate of nucleation to promote more structure

What am I calling the “nucleation experiments”?

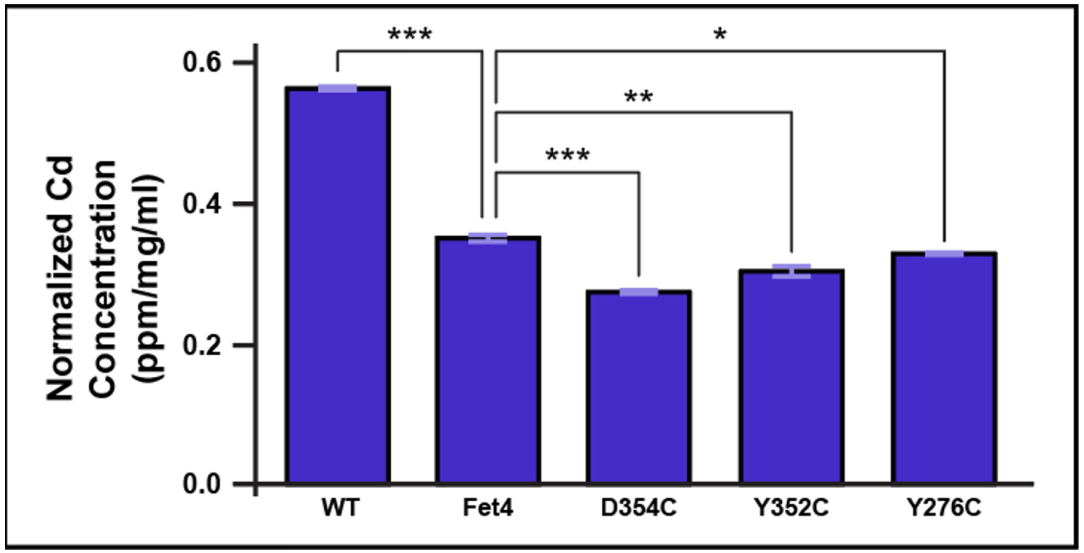
- ICP-OES
  - Use spectroscopy to measure concentration of cadmium remaining in media
- Yeast microscopy
  - Visualize cadmium on the yeast
    - (if it has collected in structured semi-conductor quantum dots, the cadmium will fluoresce)
- Microscopy
  - Fluorescent or TEM (stay tuned)

Experimental set up for nucleation experiments



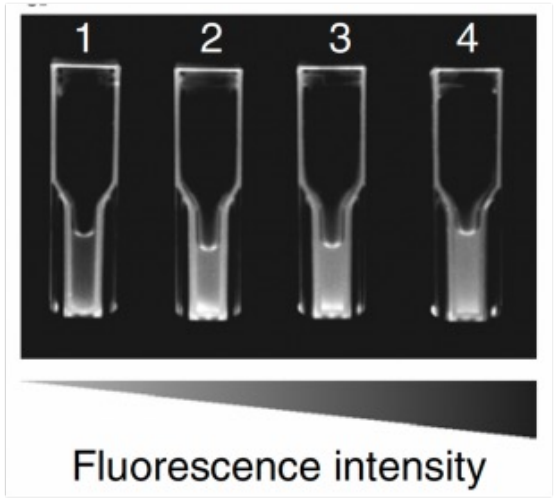
ICP-OES

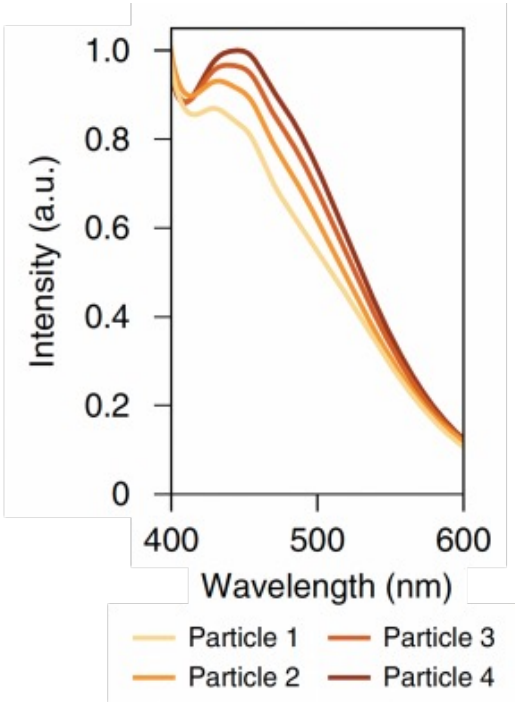
- Overview
- What is measured
- What data do we get
- How does it relate to big picture
- Alternatives



Fluorescence spectroscopy (AKA Fluorimetry)

- Overview
- What is measured
- What data do we get
- How does it relate to big picture
- Alternatives



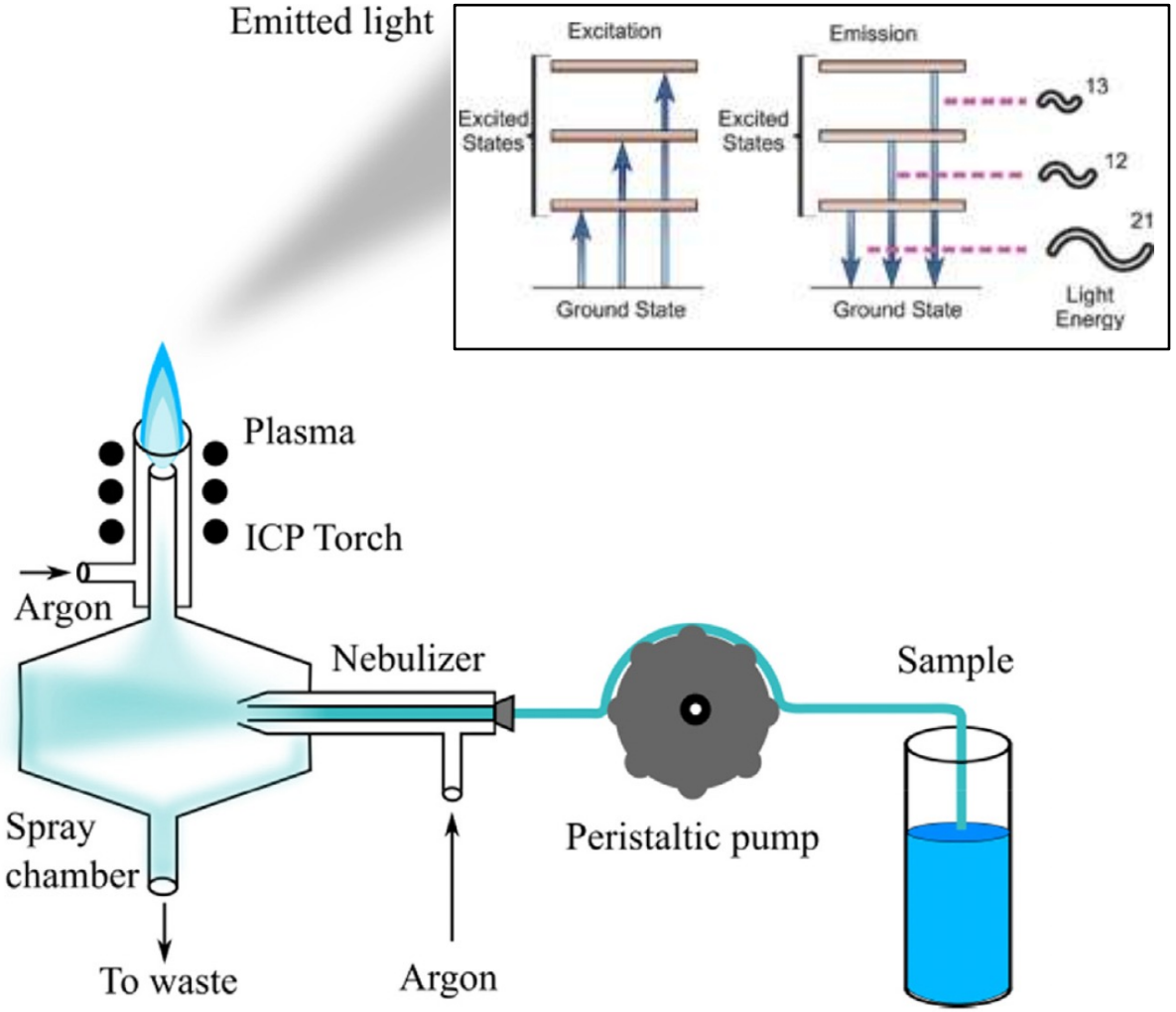


ICP-OES

Candy break?

Inductively coupled plasma– optical emission spectroscopy (ICP-OES)

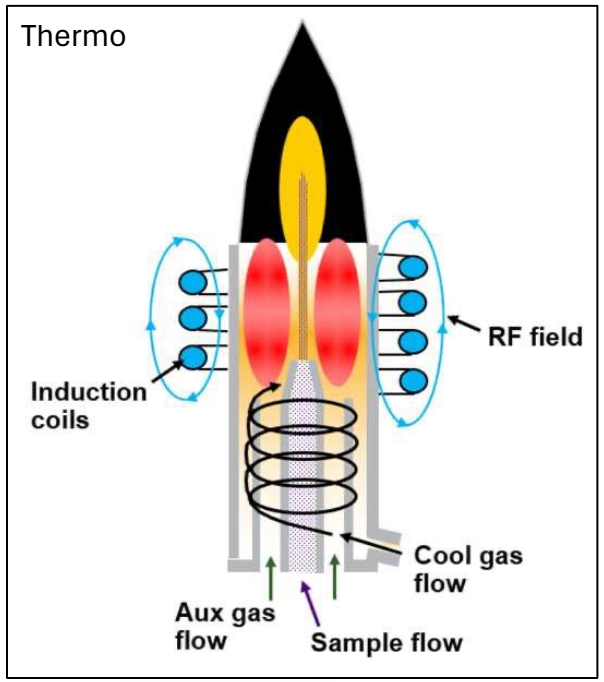
- also known as ICP-AES (atomic emission spectroscopy)
- Samples are digested with nitric acid and pumped into a nebulizer where it mixes with argon and creates an aerosol in the spray chamber
- The spray enters a central chamber with radiofrequency-induced electromagnetic field of argon plasma where the sample is vaporized, atomized, and ionized
- The plasma energy excites electrons which emit photons at specific wavelengths unique to each element as the electrons fall back to an unexcited state
- This photon emission data is collected to identify composition and relative concentration of elements in the sample

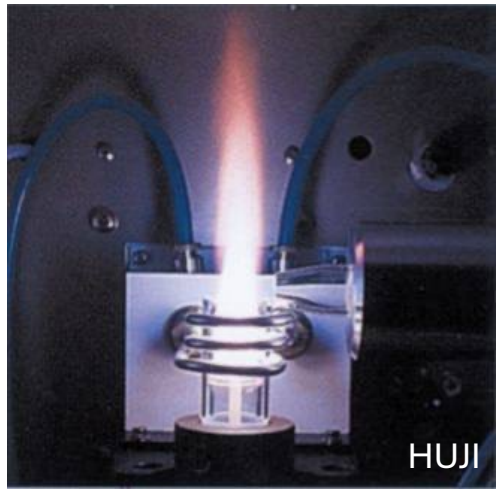


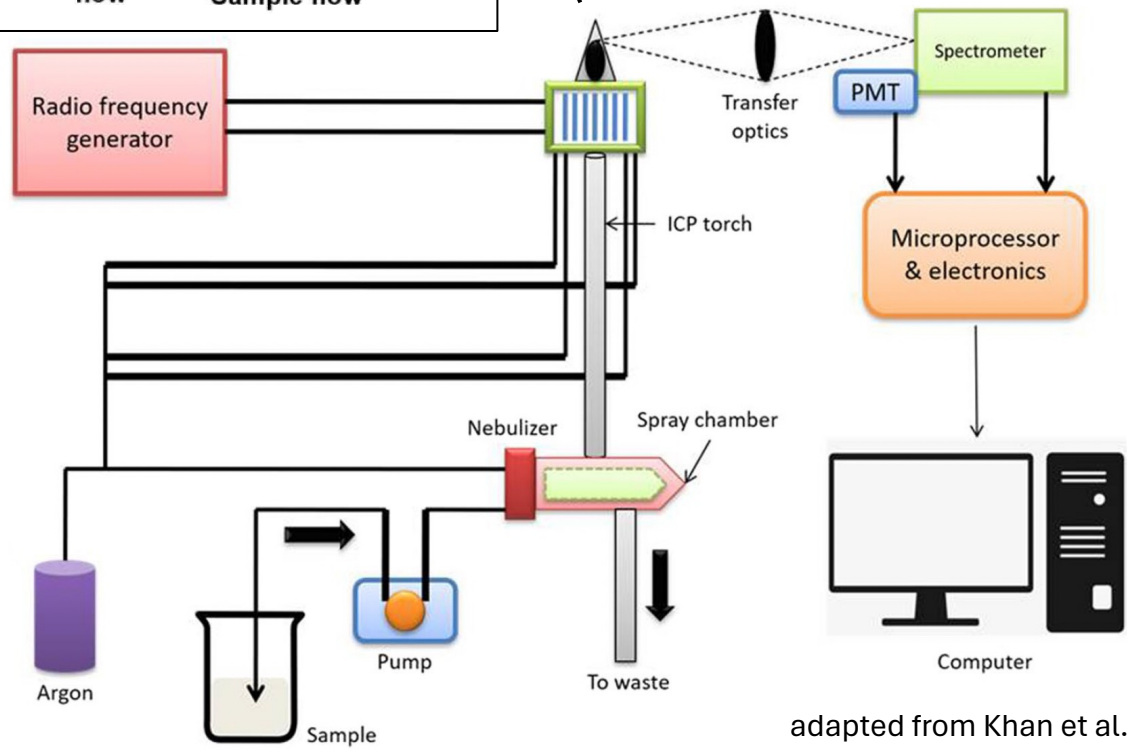
adapted from Cherevko. 2017

ICP-OES: the torch

- Inductively coupled plasma torch is created when:
  - Argon gas is pumped into a quartz tube encircled by induction coils
  - A radiofrequency generation powers these induction coils to create strong variable electromagnetic field
  - This produces a plasma torch which typically burns at ~ 6,000K
    - Same temperature as the surface of the sun



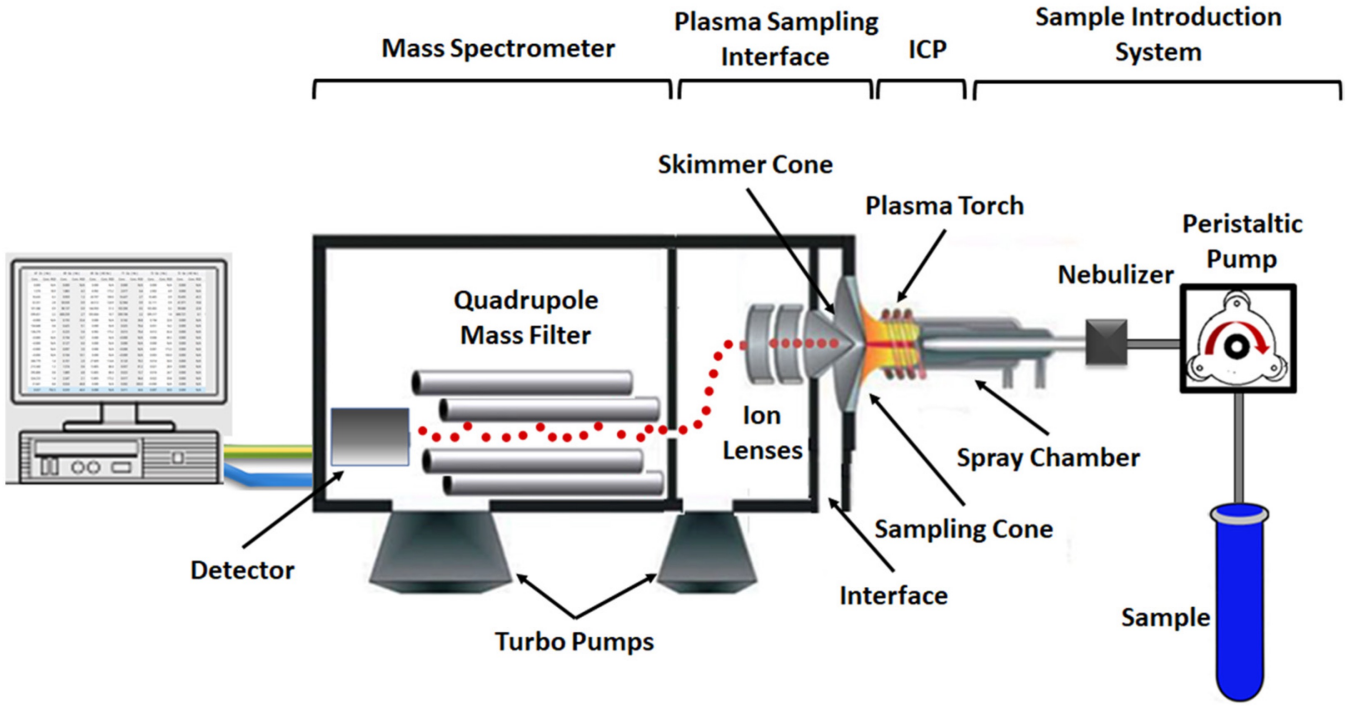




adapted from Khan et al. 2022

ICP-Mass Spectrometry (ICP-MS)

- Samples are digested with nitric acid and diluted with ultra-pure water
- The beginning process is the same: sample is pumped through a nebulizer to form a spray which passes into the plasma torch to ionize the samples
- The ionized samples are passed through a skimmer cone to focus the ions into a beam which enters the vacuum of the quadrupole mass analyzer
- Ions are separated based on their mass/charge ratio and strike the detector resulting in a signal pulse that can be measured



Mazarakioti et al, 2022

Ideal spectrometry analysis approach depends on experimental parameters

ICP-OES

- Sample volume: 5ml
- LOD: ppb
- 50+ metals in single sample
  - Spectral interference between elements
- More tolerant of high concentration of dissolved solids in sample

ICP-MS

- Sample volume: 2ml
- LOD: ppt
- 50+ metals in single sample
  - Mass interference by metals with same isotopic mass or are doubly charged
- Lighter elements are more difficult to detect

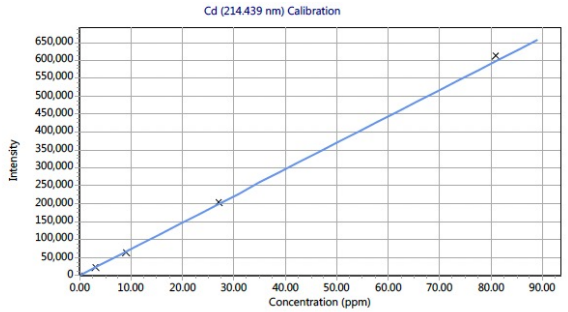
ICP-OES practice data

- Previously tried experiments to create a heavy metal sink in yeast
- Expressed a low affinity iron transporter (Fet4) and made mutations to try and increase cadmium uptake via Fet4
- Used ICP-OES to identify changes in cadmium in media following incubation

Sample Number	**Sample Name**
1	Metal only
2	UT-TR
3	Fet4-TR
4	UT-WF
5	Fet4-WF
6	TR Red
7	TR Orange
8	TR Yellow
9	TR Green
10	TR Blue
11	TR Pink
12	TR Purple
13	WF Red
14	WF Orange
15	WF Yellow
16	WF Green
17	WF Blue
18	WF Pink
19	WF Purple

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)

Sample Name: Sample 17

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

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

- Concentration is calculated in parts per million (ppm)
  - based on peak intensity at the listed wavelength and calibration curve
- Each overall concentration is calculated by processing 3 replicates

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

ICP-OES samples (also in CSV file)

- Each team is a sample
- Controls:

  UT: Untransformed ΔM17 yeast

  EV: ΔM17 yeast transformed with pCTCON2, but not induced

  6xG: ΔM17 yeast expressing a glycine hexapeptide

  2xGCC: ΔM17 yeast expressing –GCCGCC- peptide

Tube #	Tube Name / Description
1	Standard 1 (0 ppm Cd)
2	Standard 2 (3 ppm Cd)
3	Standard 2 (9 ppm Cd)
4	Standard 4 (27 ppm Cd)
5	Standard 5 (81 ppm Cd)
6	Sample 1 (Media + 100uM $Cd(NO_3)_2$)
7	Sample 2 (UT)
8	Sample 3 (EV)
9	Sample 4 (6x G)
10	Sample 5 (2x GCC)
11	Sample 6 (TR Orange)
12	Sample 7 (TR Yellow)
13	Sample 8 (TR Green)
14	Sample 9 (TR Blue)
15	Sample 10 (TR Pink)
16	Sample 11 (WF Red)
17	Sample 12 (WF Orange)
18	Sample 13 (WF Yellow)
19	Sample 14 (WF Green)
20	Sample 15 (WF Blue)
21	Sample 16 (WF Teal)
22	Sample 17 (WF Pink)
23	Sample 18 (WF Purple)

Homework

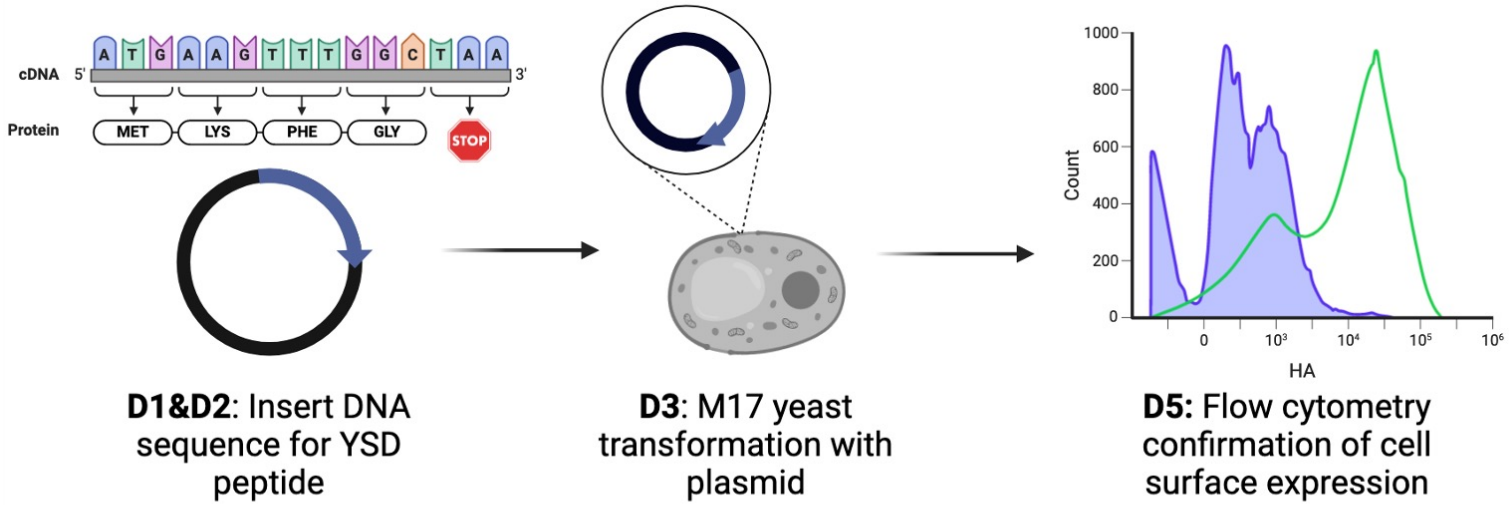
Create an Overview Schematic and
answer questions for the Discussion

Overview schematics

- Give an overview of the project as a whole
  - Visually represent key concepts/approaches of the project
  - Not much focus on technical details (unlike the experimental schematic)
  - Builds on skills of developing an experimental schematic

- Because it is a figure
  - Include a figure title and caption

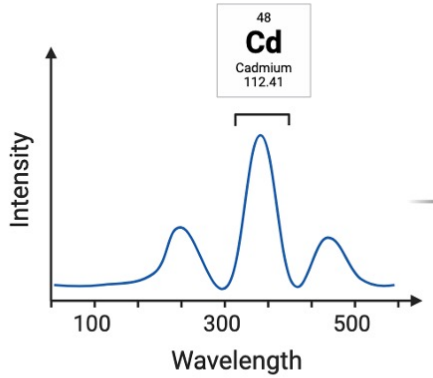
Mod2 Overview (AKA a deliberately terrible overview schematic)



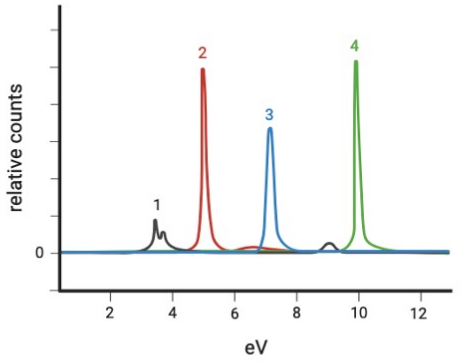
D1&D2: Insert DNA sequence for YSD peptide

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