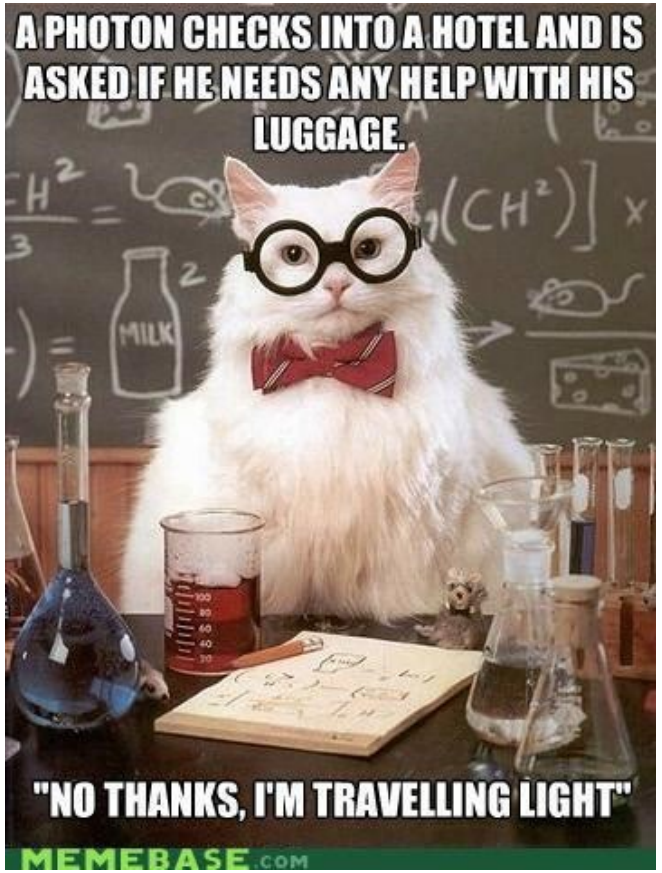
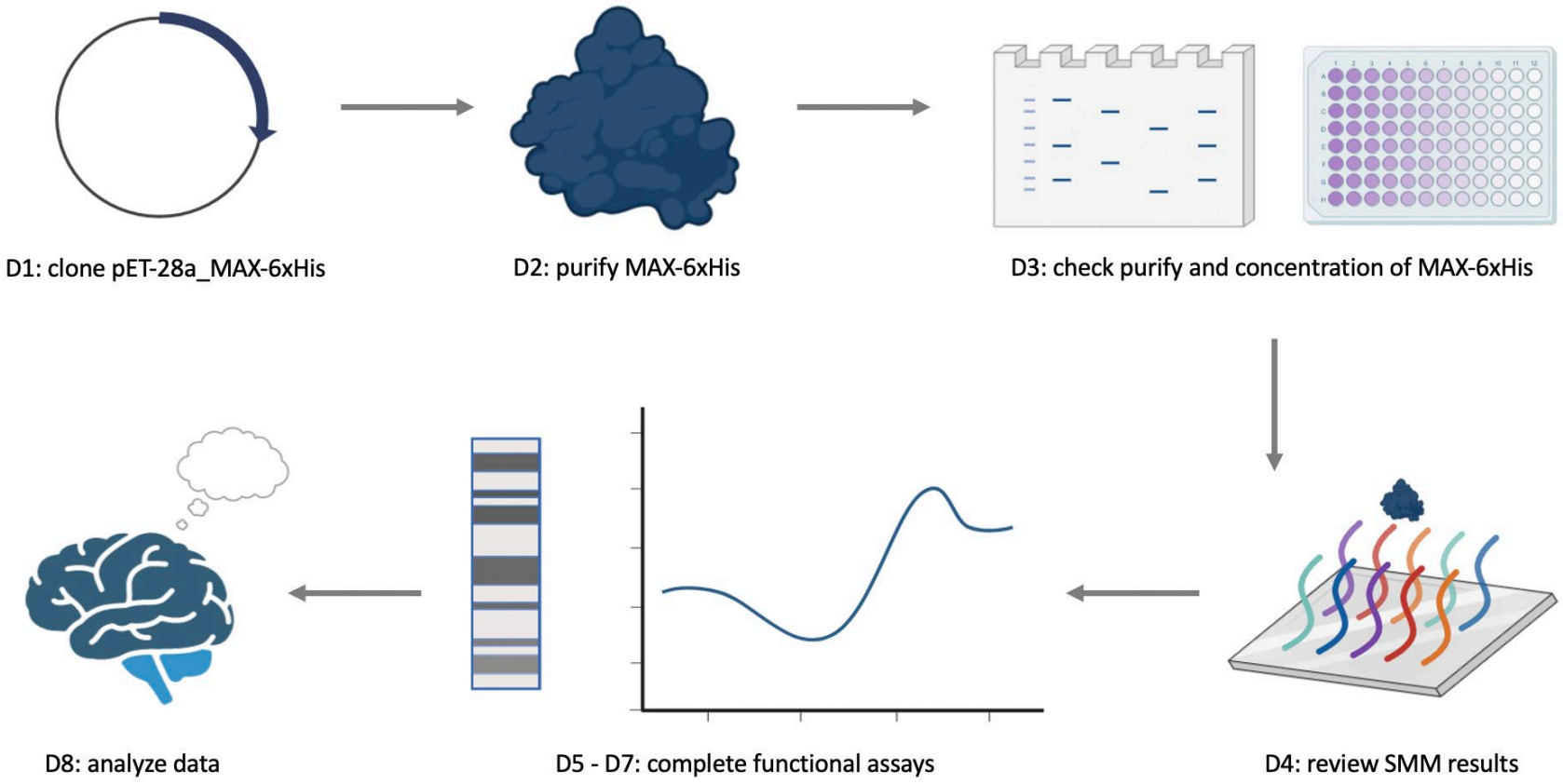
M1D5: Setup differential scanning fluorimetry (DSF) experiment

1. Comm Lab workshop
2. Prepare samples for DSF
3. Seed cells for EMSA



Overview of Mod 1 experiments:



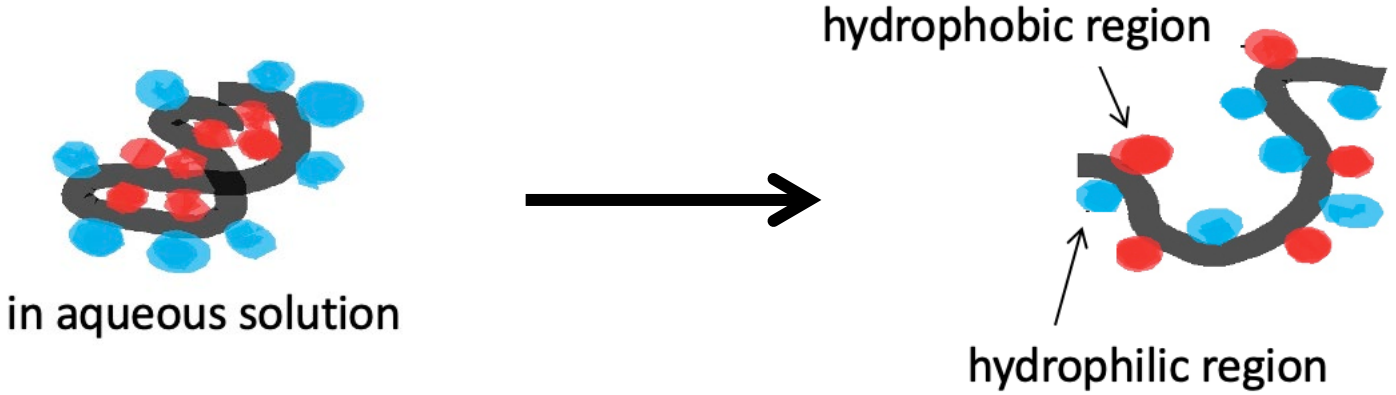
Workflow for secondary assays

	M1D5	M1D6	M1D7	M1D8
DSF	prepare samples and setup assay run DSF experiment	plot data to identify shifts in melting temperature		apply statistics to data interpret results
EMSA	seed cells	extract nuclear proteins	complete electrophoresis and transfer nuclear proteins onto membrane	image EMSA experiment to assess binding interpret results

What are we testing with each experiment?

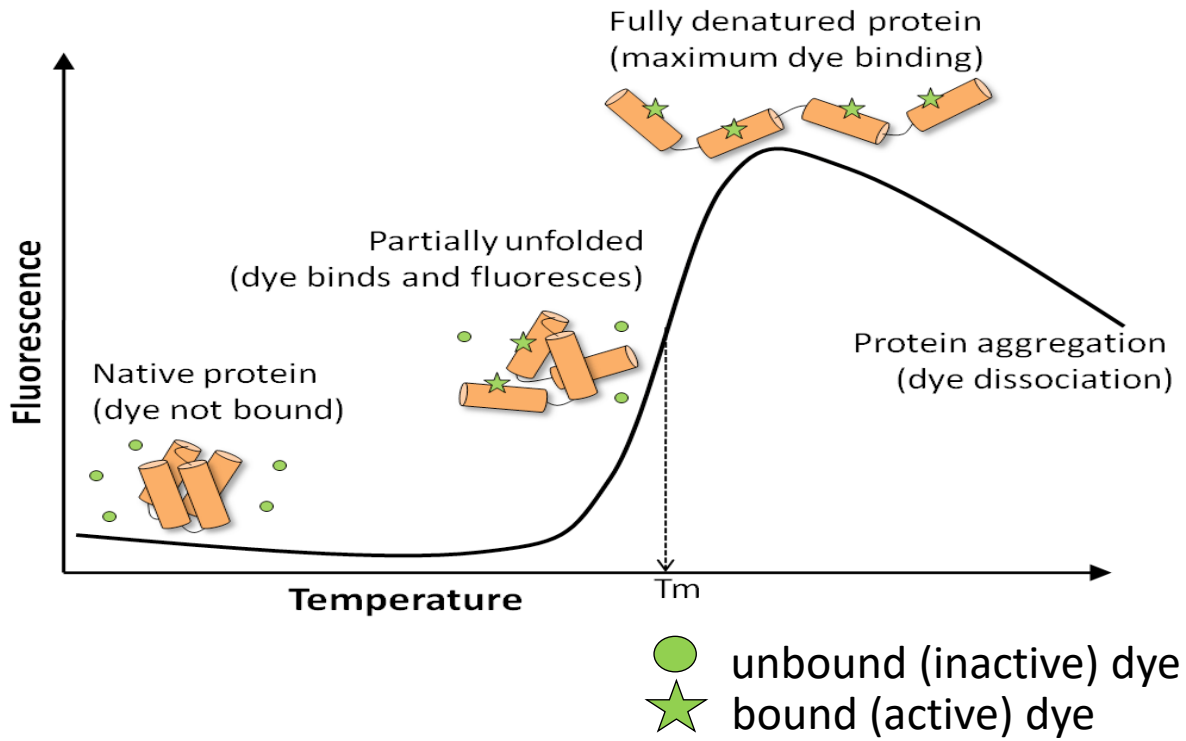
Task 1: setup DSF experiment

- Probe protein folding by adding a SYPRO Orange dye that interacts with hydrophobic regions of proteins
- If protein is folded, dye is unable to access hydrophobic residues and is inactive (fluorescence quenched in aqueous solution)
- As protein unfolds, dye binds hydrophobic residues and emits fluorescent signal



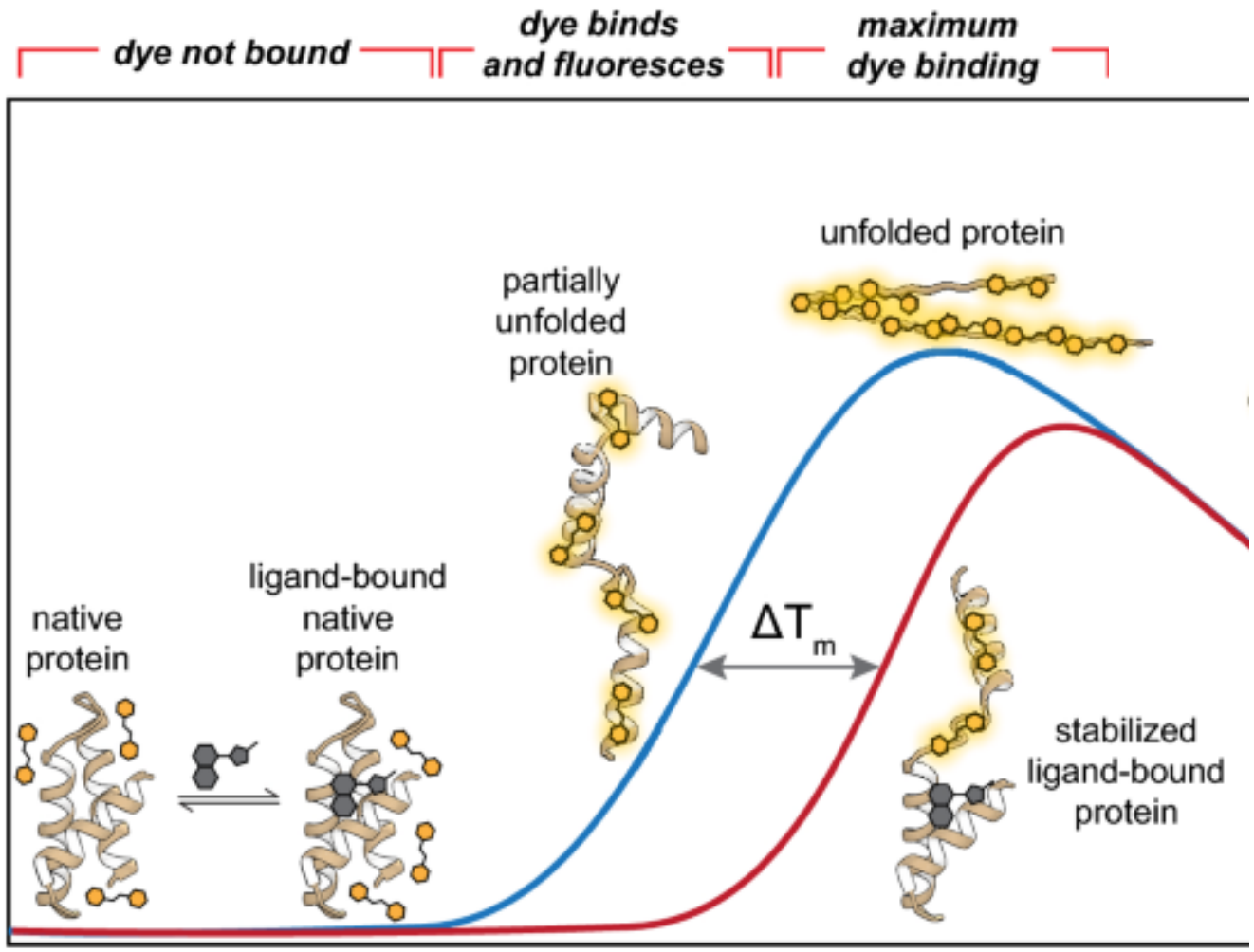
DSF detects protein unfolding / melting

- As protein unfolds with temperature increases, SYPRO Orange increasingly binds to hydrophobic regions
- Can calculate a melting temperature (T_M) where 50% of the protein is denatured from quantifying the increase in fluorescent signal



Small molecule binding can alter protein unfolding

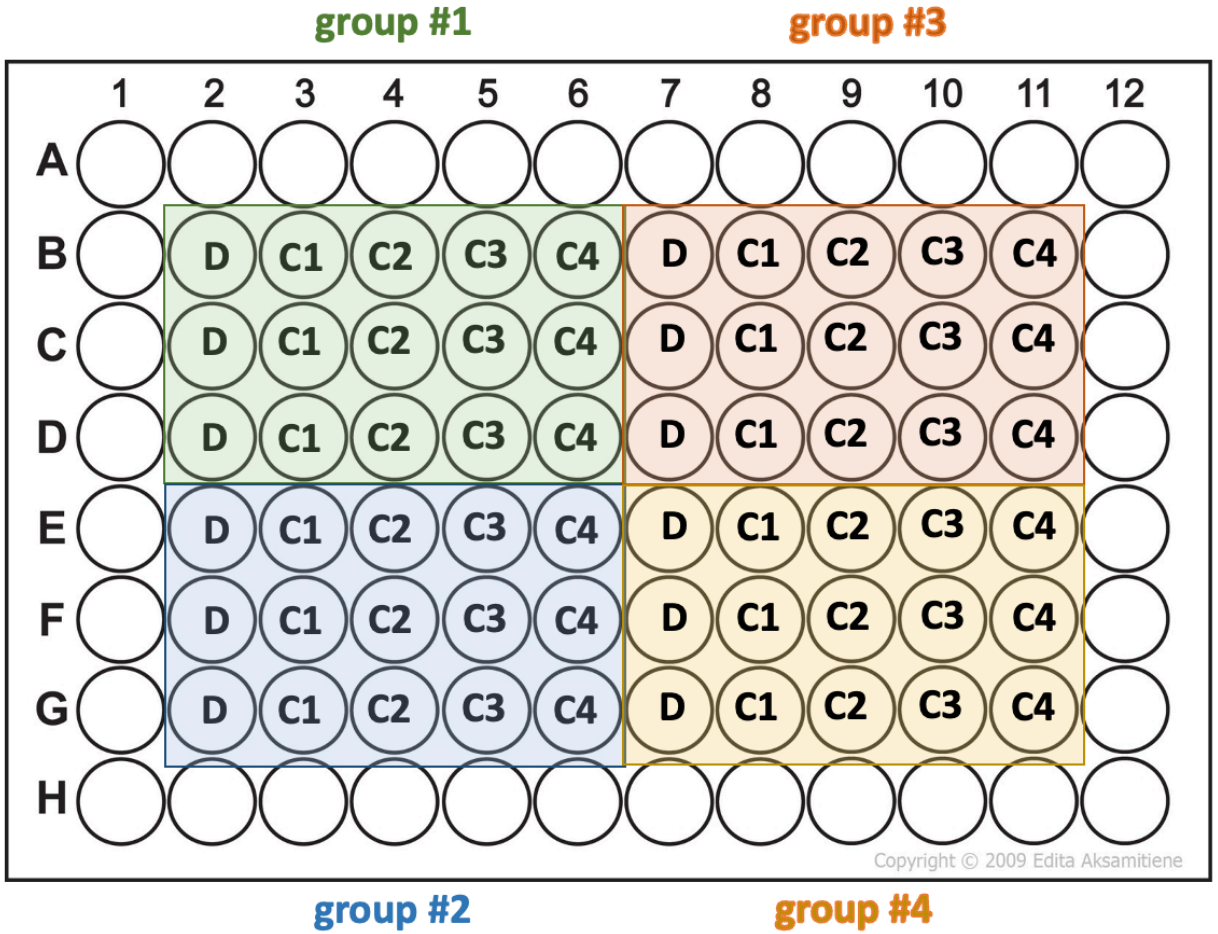
- Small molecule binding can stabilize protein structure
  - Slows protein unfolding / melting
  - Causes shift in melting temperature



Multiple teams will use same plate for DSF setup

- Four teams per plate
- Samples:
  - One DMSO
  - Four small molecules
- All samples tested in triplicate

D = DMSO
C1 = compound 1
C2 = compound 2
C3 = compound 3
C4 = compound 4



Task 2: seed cells for EMSA experiment

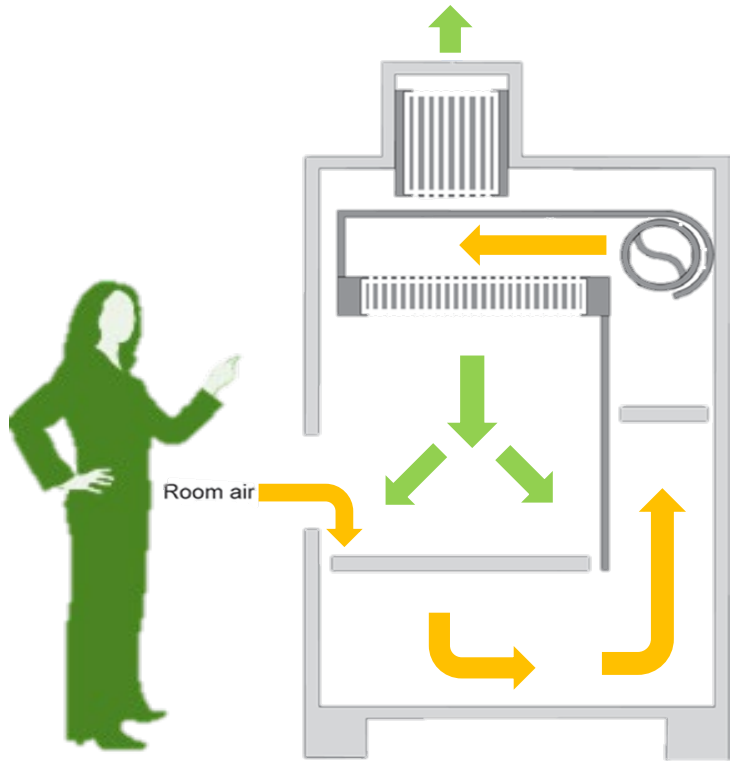
- HeLa cells will be used to test Myc:MAX binding in presence of small molecules
  - First immortal human cells to be grown in culture
  - Isolated from cervical carcinoma patient

Henrietta Lacks

- Myc:MAX dimers bind specific DNA sequence in promoters to drive transcription
  - Myc has low affinity for DNA sequence when not dimerized with MAX

Best practices for cell culture

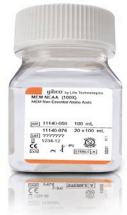
- 70% ethanol everything:
  - Wipe cabinet before and after use
  - Wipe everything that enters the cabinet
  - Do not spray cells with EtOH
- Do not disturb air flow:
  - Do not block grille or slots
  - Minimize side-to-side arm movements
  - Work > 6” away from sash
  - Leave blower *on always*
- Do not talk into incubator!
- Only open sterile media in hood



Cell culture growth conditions

- DMEM (Dulbecco's Modified Eagle Media)

- FBS (fetal bovine serum)



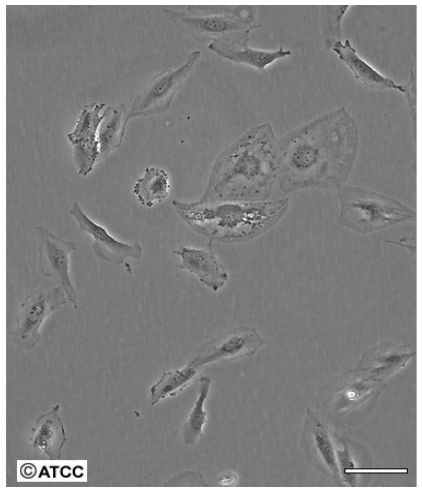
- Antibiotics: penicillin and streptomycin

Which is food / non-food? Which is defined / undefined?

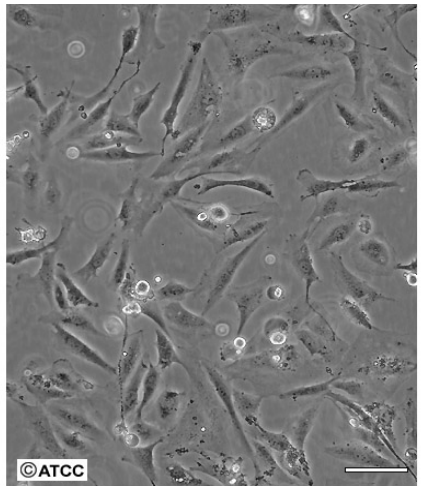
Cell culture terminology

- Confluence
- Adherent / Non-adherent
- Splitting / Passaging
- Seeding

Low Density



High Density



Preparing cells for small molecule treatment

- Specific number of cells will be seeded into a culture dish for small molecule treatment prior to EMSA experiment
  - Trypan blue stain used to differentiate between live and dead cells
  - Cells counted using hemocytometer

1. Count cells in each of four corner quadrants

2. Calculate # cells / mL = 10,000 x average of 4 corners

For today…

- Teams will be divided between two parts:
  - Orange, Green, Pink, Teal start with DSF setup
  - Red, Yellow, Blue, Purple start with cell culture work
- Keep notes to track progress for each experiment!!
- You may resubmit M1D5 Title&Caption by 10 PM today for updated feedback

For M1D6…

- Craft experimental schematic for protein purification procedure
- Submit summary of Comm Lab appointment

Notes on experimental schematics…

How to bake bread using dry active yeast

In a large mixing bowl, sift 500 g of King Authur's Flour, 2 tsp active dry yeast, 1 tsp kosher salt

Add 1 cup of water. Use a rubber spatula to mix until a shaggy dough is formed

Lightly dust hands with flour. Knead dough by pulling, folding and pushing the dough onto itself

Knead until a wet, but not sticky dough is formed, roughly 10 minutes

Transfer into a lightly oiled bowl and cover with a damp teacloth. Allow dough to rise until doubled in size, about 1 hour

Punch out the air in the dough, fold the dough into itself, cut the dough in half and shape into two loaves.

Let rest for 1 hour. Bake at 400F until golden brown

Notes on experimental schematics…

How to bake bread using dry active yeast

In a large mixing bowl, sift ~~500 g~~ of King ~~Author~~'s Flour, ~~2 tsp~~ active dry yeast, ~~1 tsp~~ kosher salt

1 2

Add ~~1 cup~~ of water. Use a ~~rubber spatula~~ to mix until a shaggy dough is formed

2 2

Lightly dust hands with flour. ~~Knead dough by pulling, folding and pushing the~~ dough onto itself

3

Knead until a wet, but not sticky dough is formed, roughly 10 minutes

Transfer into a lightly oiled bowl and cover with a damp teacloth. Allow dough to rise until doubled in size, ~~about 1 hour~~

2

Punch out the air in the dough, fold the dough into itself, cut the dough in half and shape into two loaves.

Let rest for 1 hour. Bake at ~~400F~~ until golden brown

2

Notes on experimental schematics…

Combine dry ingredients with water and knead into a dough

Allow dough to proof

Deflate the dough, then shape into two loaves and proof

The dough is baked into bread

Figure 1: Using Dry Active Yeast to Reduce Bread Baking Time. Dry Active Yeast is a novel ingredient that greatly reduces time required to bake bread. (A) Dry active yeast, flour, and salt are combined with water to form a shaggy dough. (B) The dough is allowed to rest until doubled in size . (C) The dough is deflated, then reformed and allowed to double in size. (D) The dough is baked until golden brown.

What should be in the Title and Caption?

Title: State what is shown / represented in the schematic

Caption:

- Explain the flow of information using concise / clear language
- Expand on text shown in figure labels to eliminate excess wordiness / clutter from the figure
- Define all abbreviations / jargon / labels / symbols