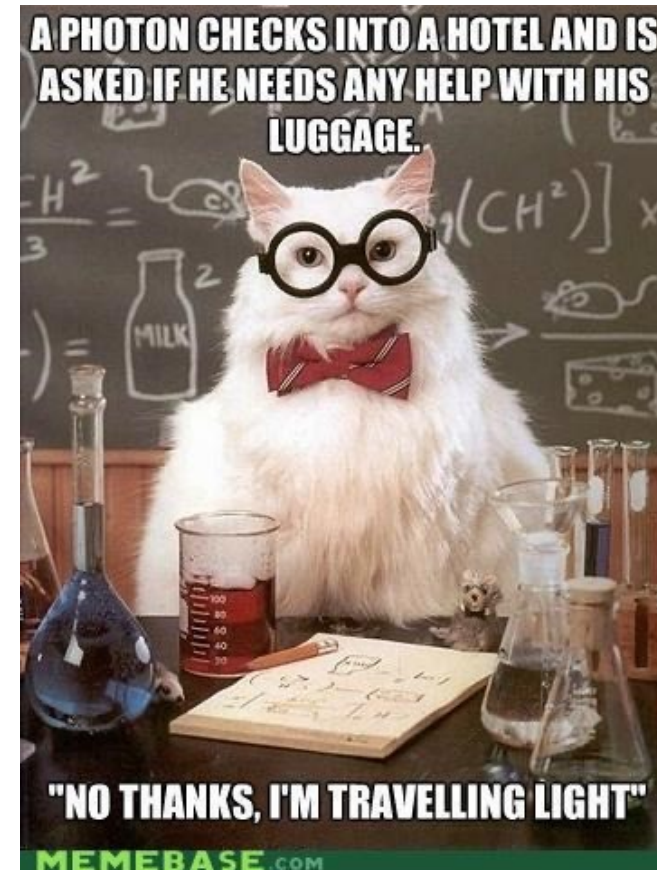
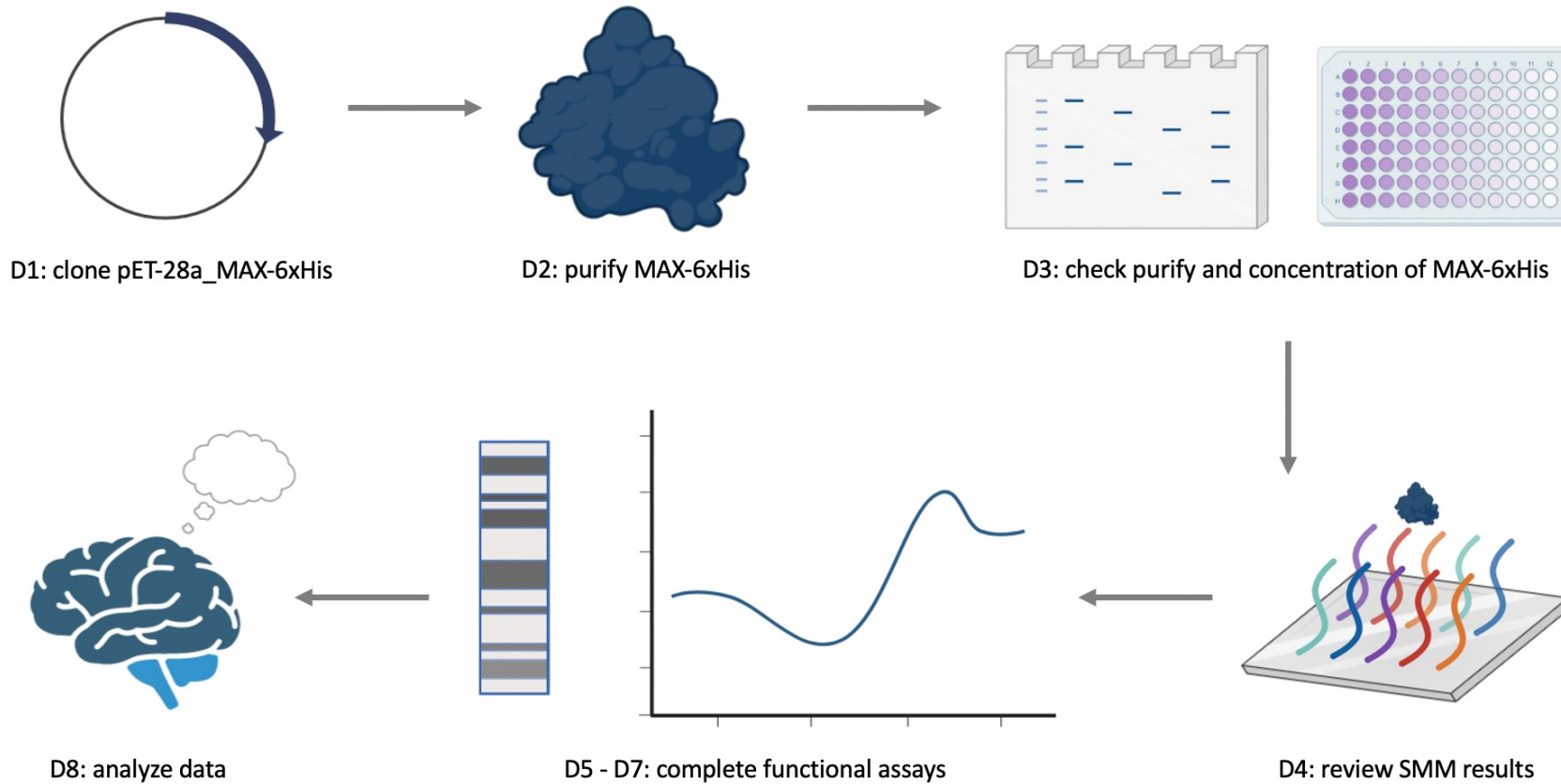


# M1D5: Setup differential scanning fluorimetry (DSF) experiment

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



# Overview of Mod 1 experiments:



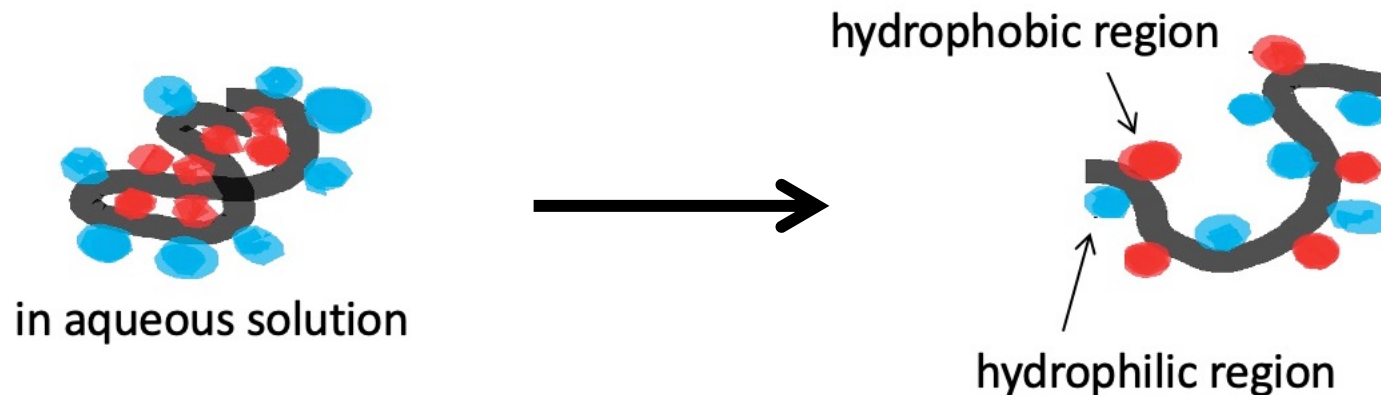
# Workflow for secondary assays

	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?

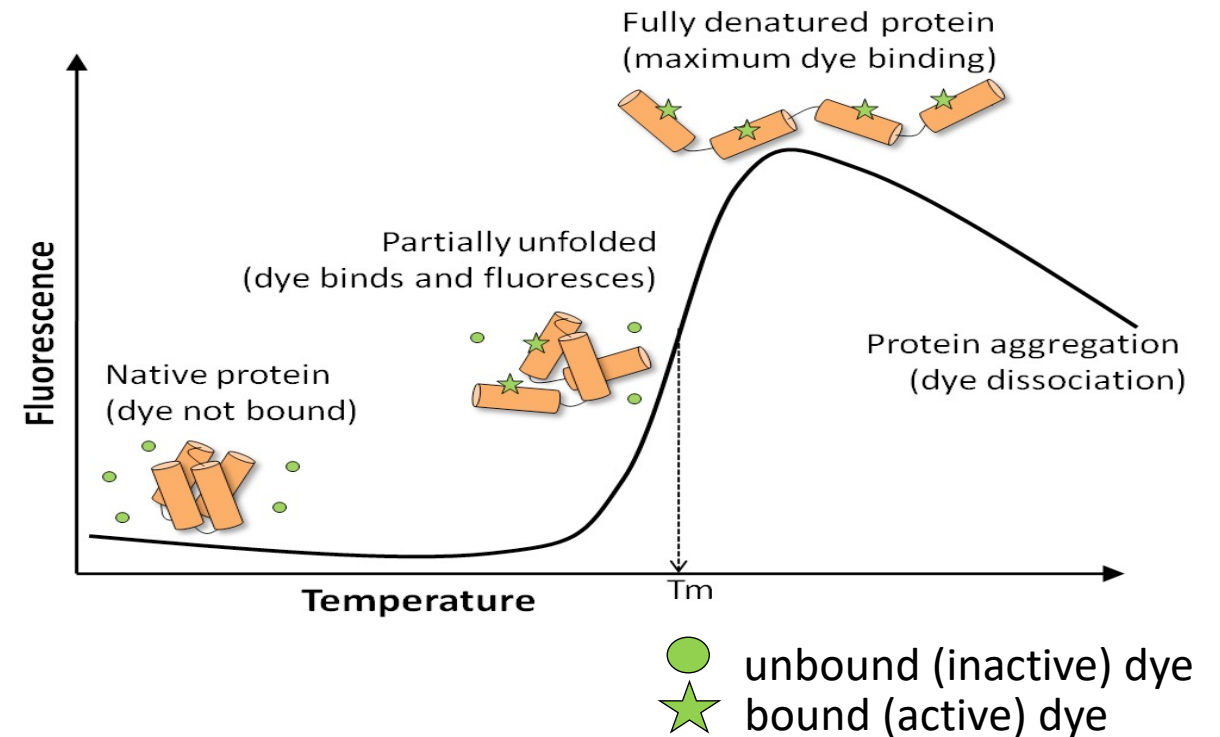
# Task 1: setup DSF experiment

- 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



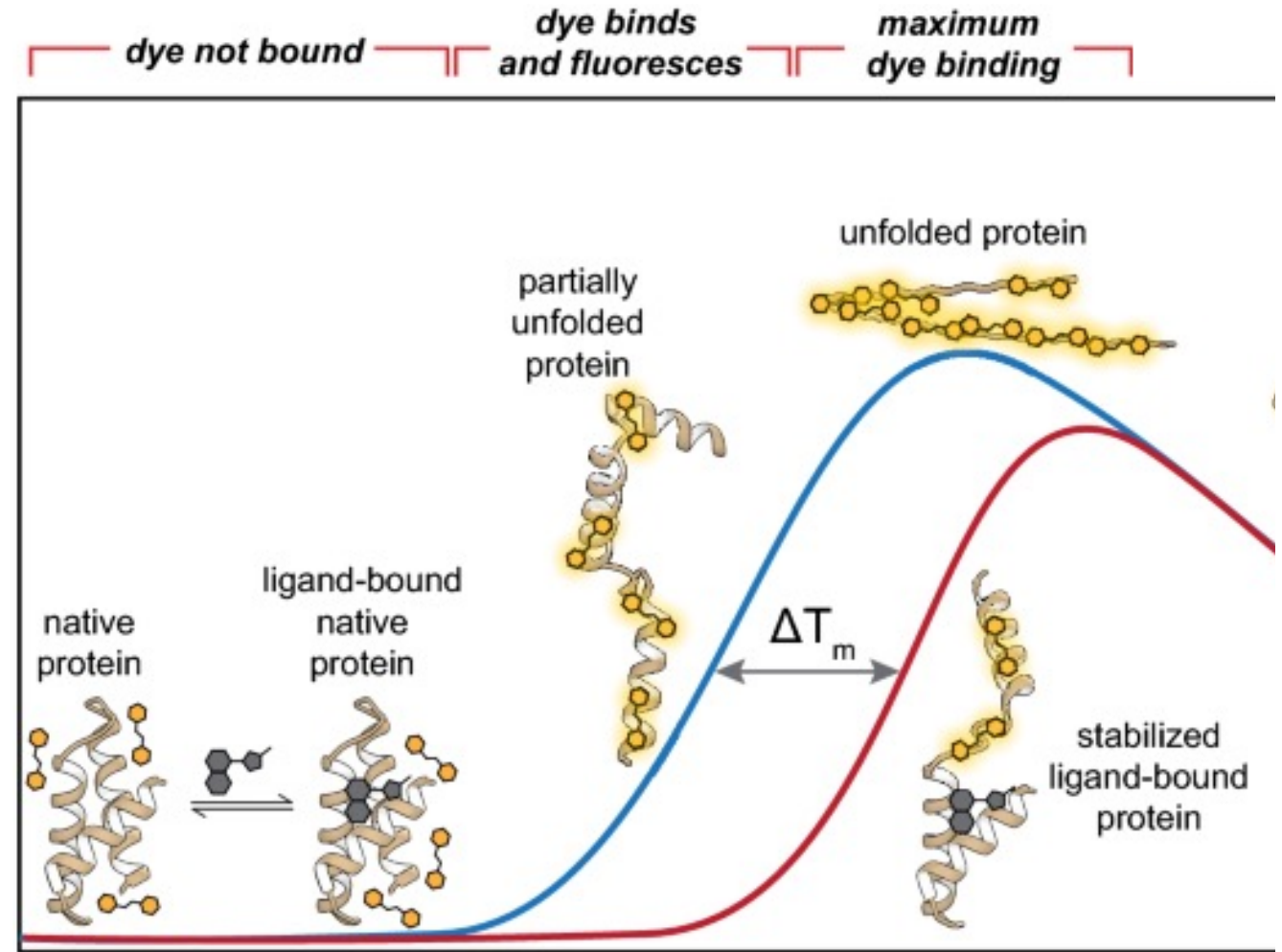
# DSF detects protein unfolding / melting

- 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

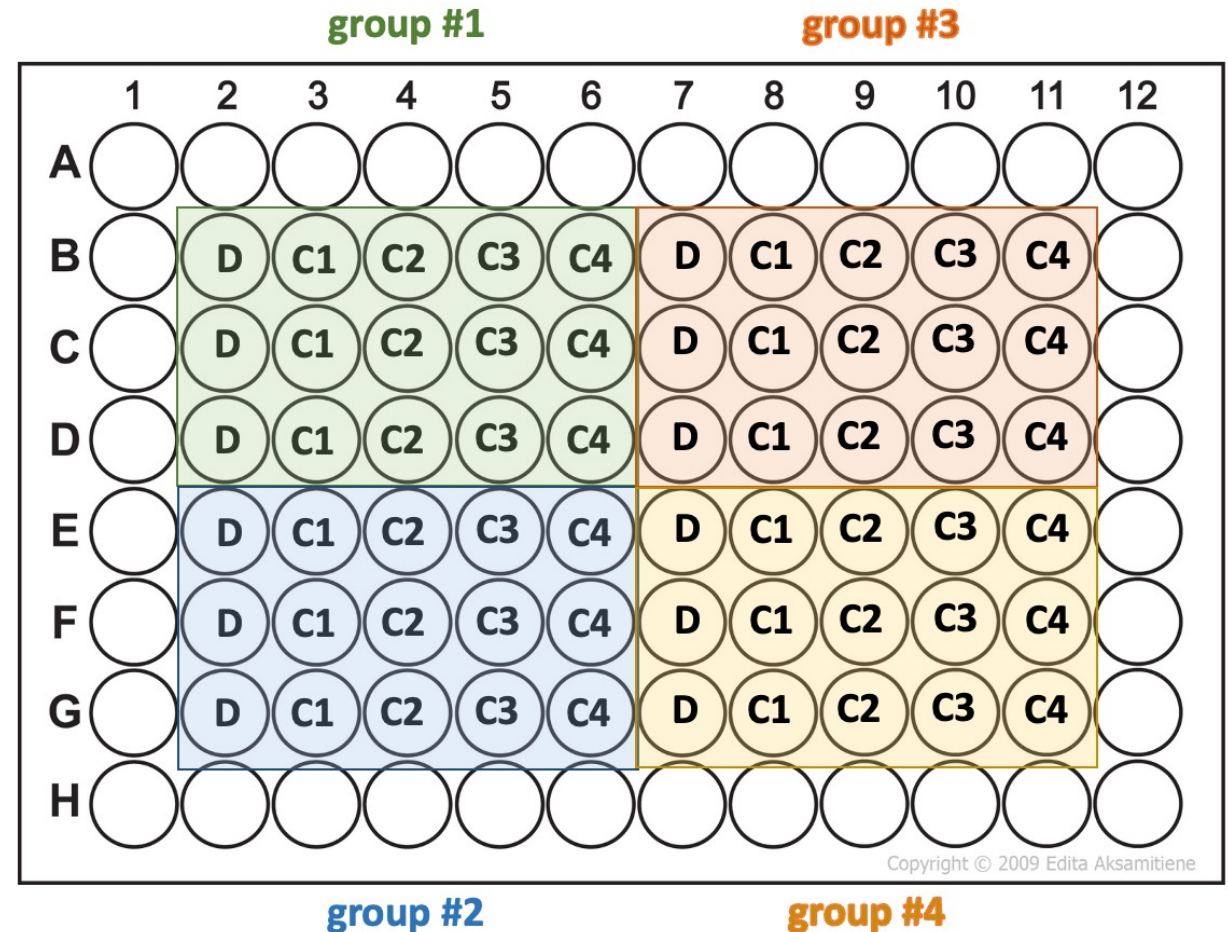




# Multiple teams will use same plate for DSF setup

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

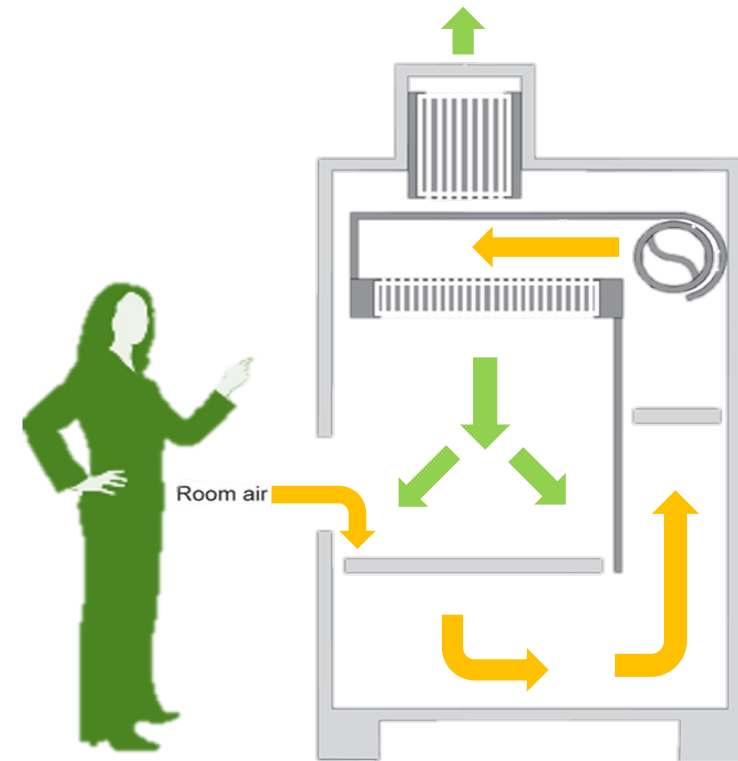


Henrietta Lacks



# 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)



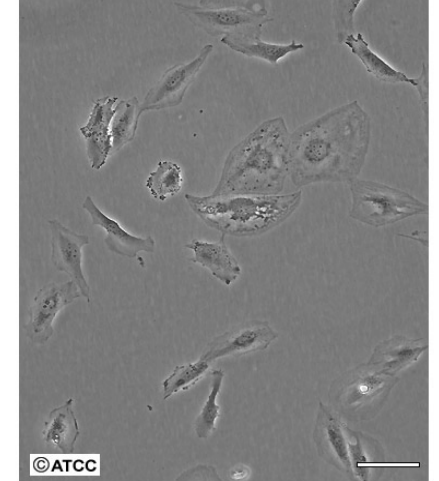
- Antibiotics: penicillin and streptomycin

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

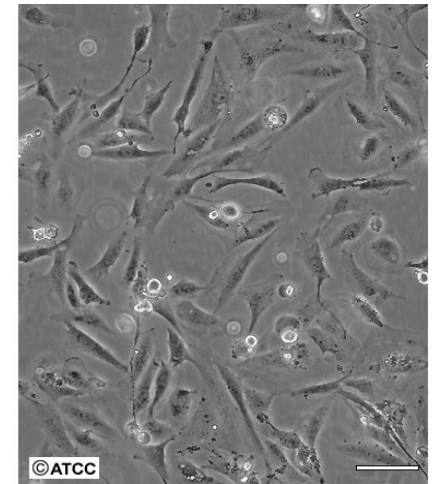
# Cell culture terminology

- 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 =  
 $10,000 \times \text{average of 4 corners}$

## For today...

- Teams will be divided between two parts:
  - Orange, Yellow, Green start with DSF setup
  - Blue and Pink start with cell culture work
- Keep notes to track progress for each experiment!!

## For M1D6...

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

# Notes on experimental schematics...

## How to cross-stitch.



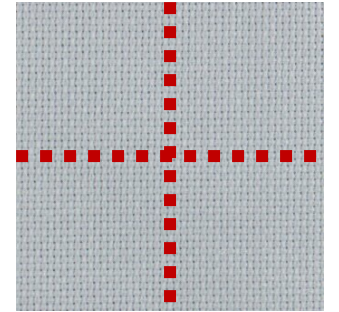
search Etsy for pattern



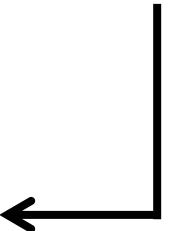
gather thread colors from my collection



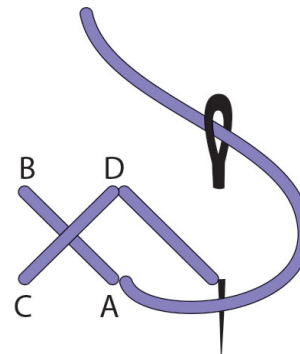
buy needed thread colors from Michael's



prepare aida fabric by finding the center



secure fabric in embroidery hoop



follow the pattern to create a picture using x-stitches



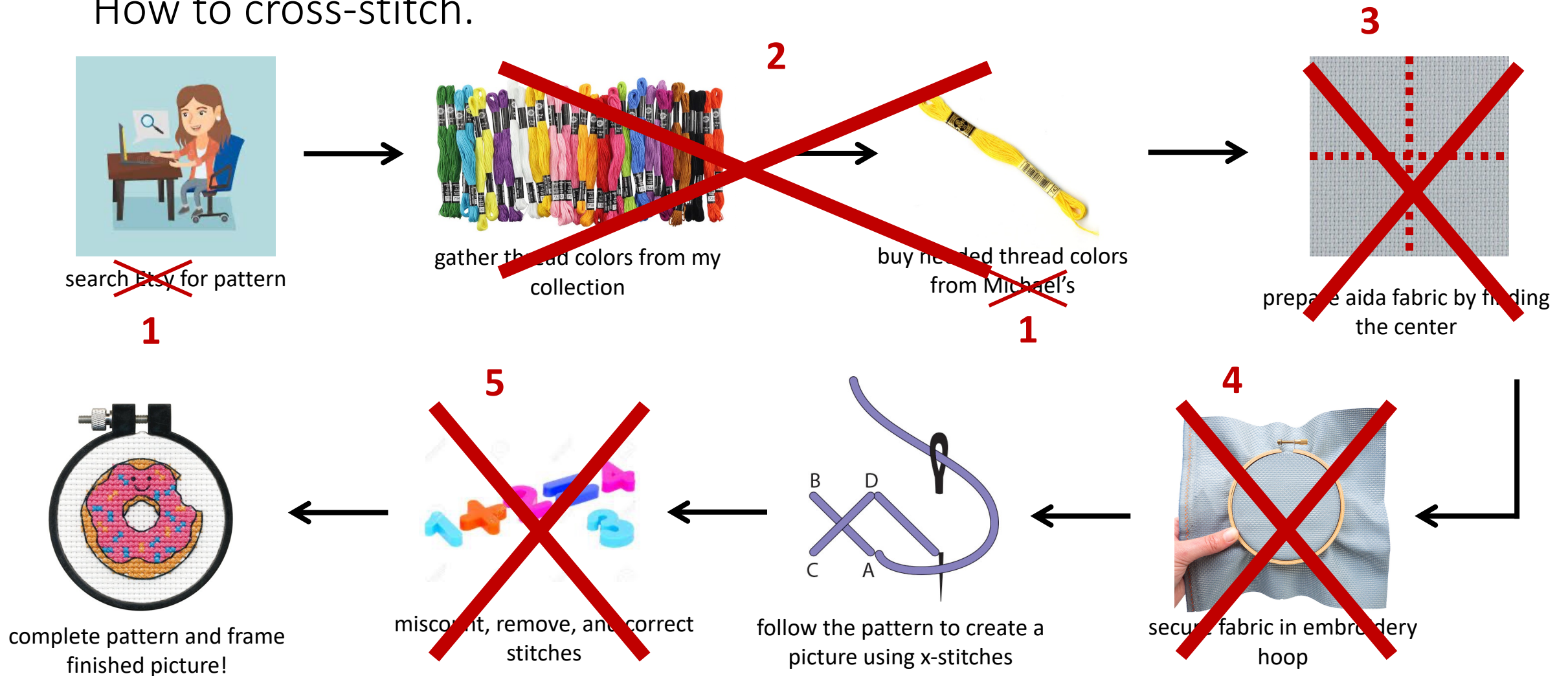
miscount, remove, and correct stitches



complete pattern and frame finished picture!

# Notes on experimental schematics...

How to cross-stitch.





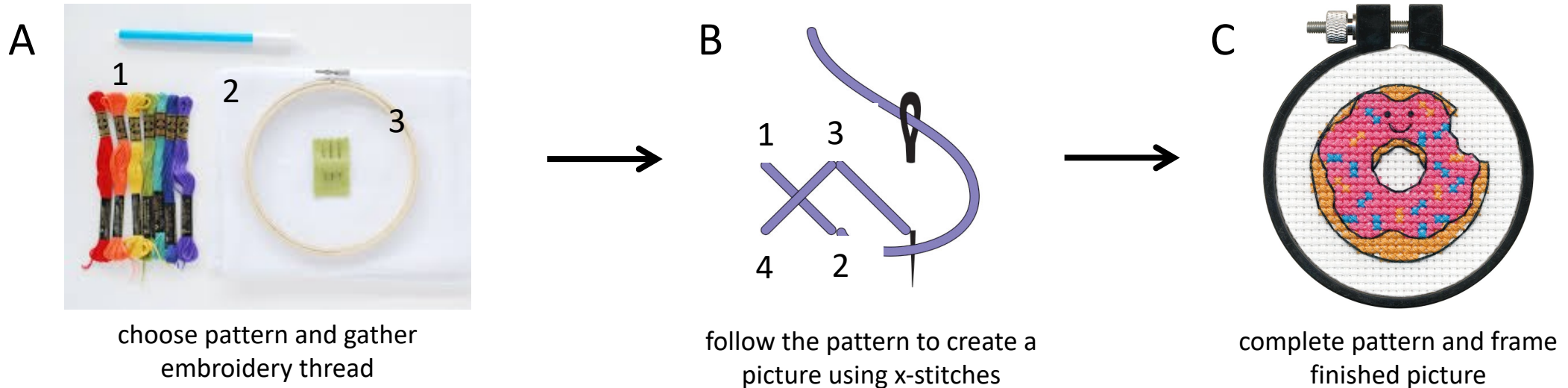
# What should be in the Title and Caption?

**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

# Notes on experimental schematics...



**Figure 1: Noreen uses cross-stitching to embroider pictures.** Cross-stitching is a method of embroidery that is used to create pictures from x-shaped stitches. (A) The supplies for cross-stitching include: 1. embroidery thread, 2. aida fabric, and 3. a hoop. (B) The x-shaped stitches are completed by using the strokes in the order indicated by the numbers. (C) ...