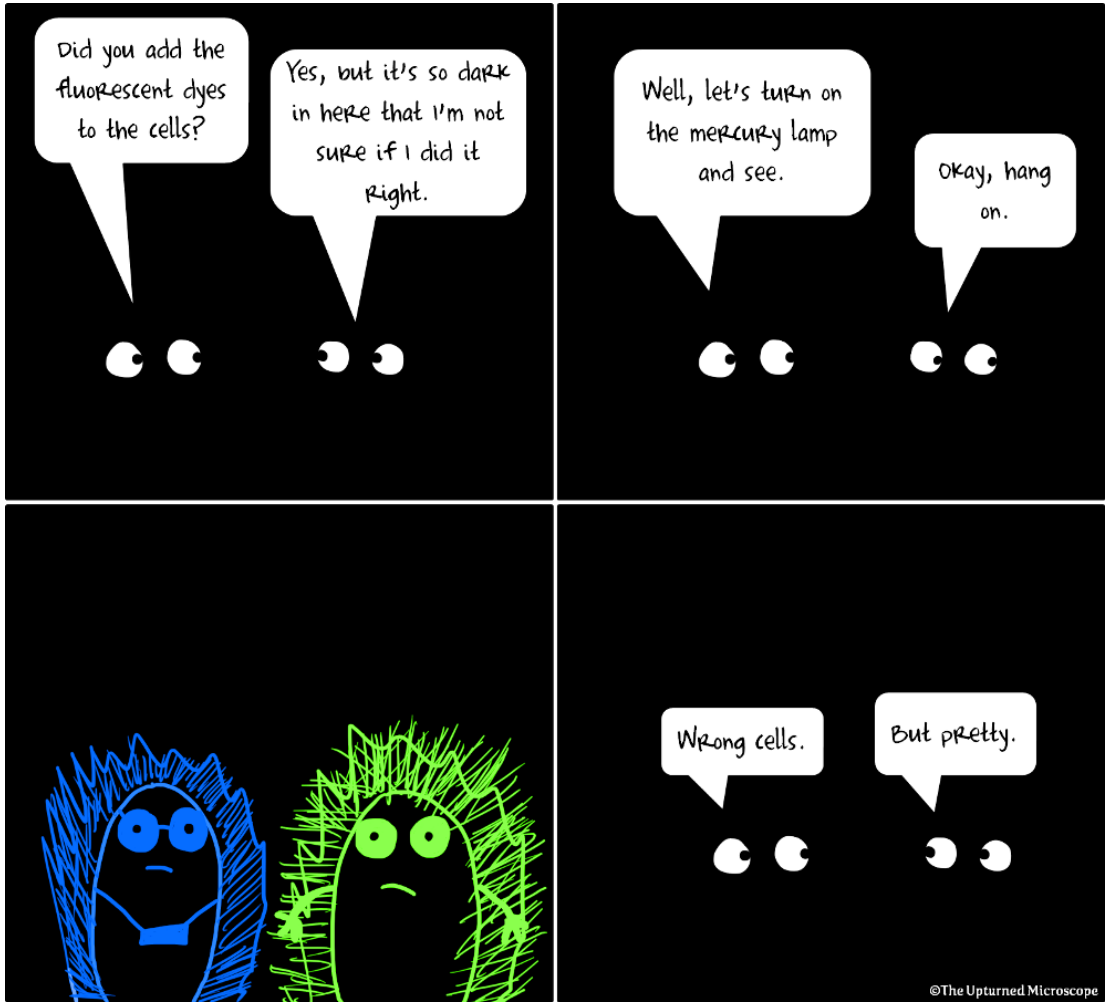
M1D3: Use immunofluorescence staining to assess repair foci experiment

1. Prelab
2. Antibody staining for γH2AX assay
3. Image coverslips



Mod1 Overview

Last lab:

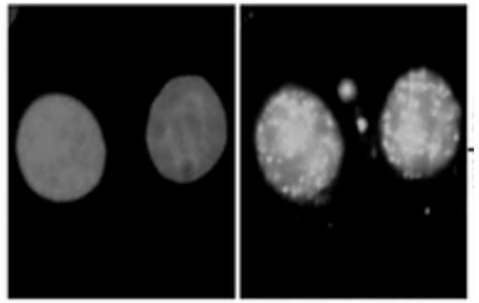
Treated MEF cells & fixed with PFA

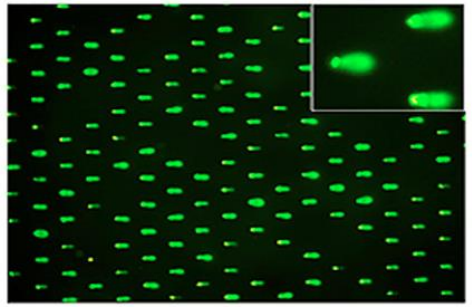
This lab:

Stain cells with fluorescent antibodies & mount coverslips

Next lab:

Image As&H202-treated MEF cells & begin CometChip





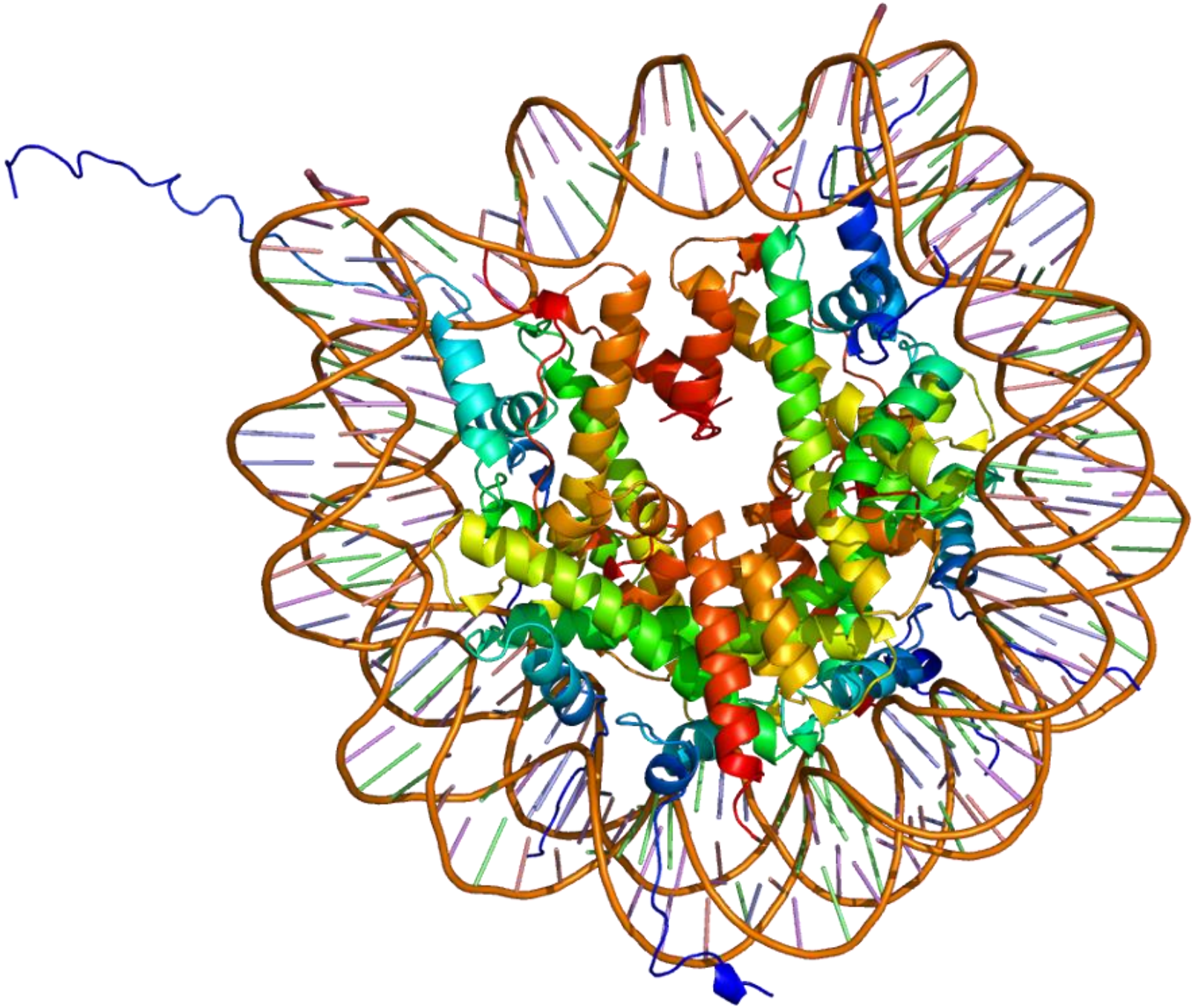
1. Use repair foci experiment to measure DNA breaks

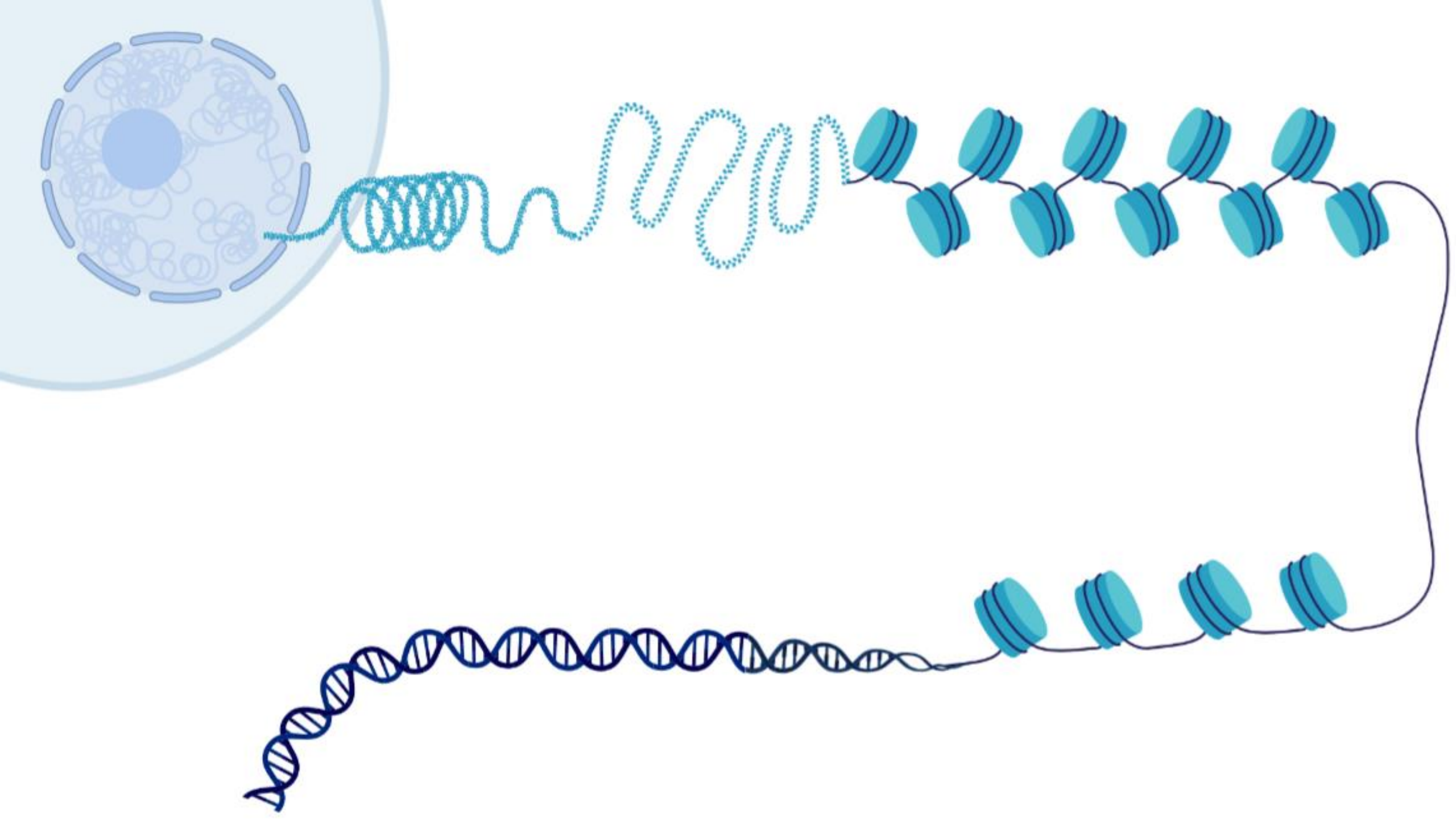
- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γH2AX foci formation

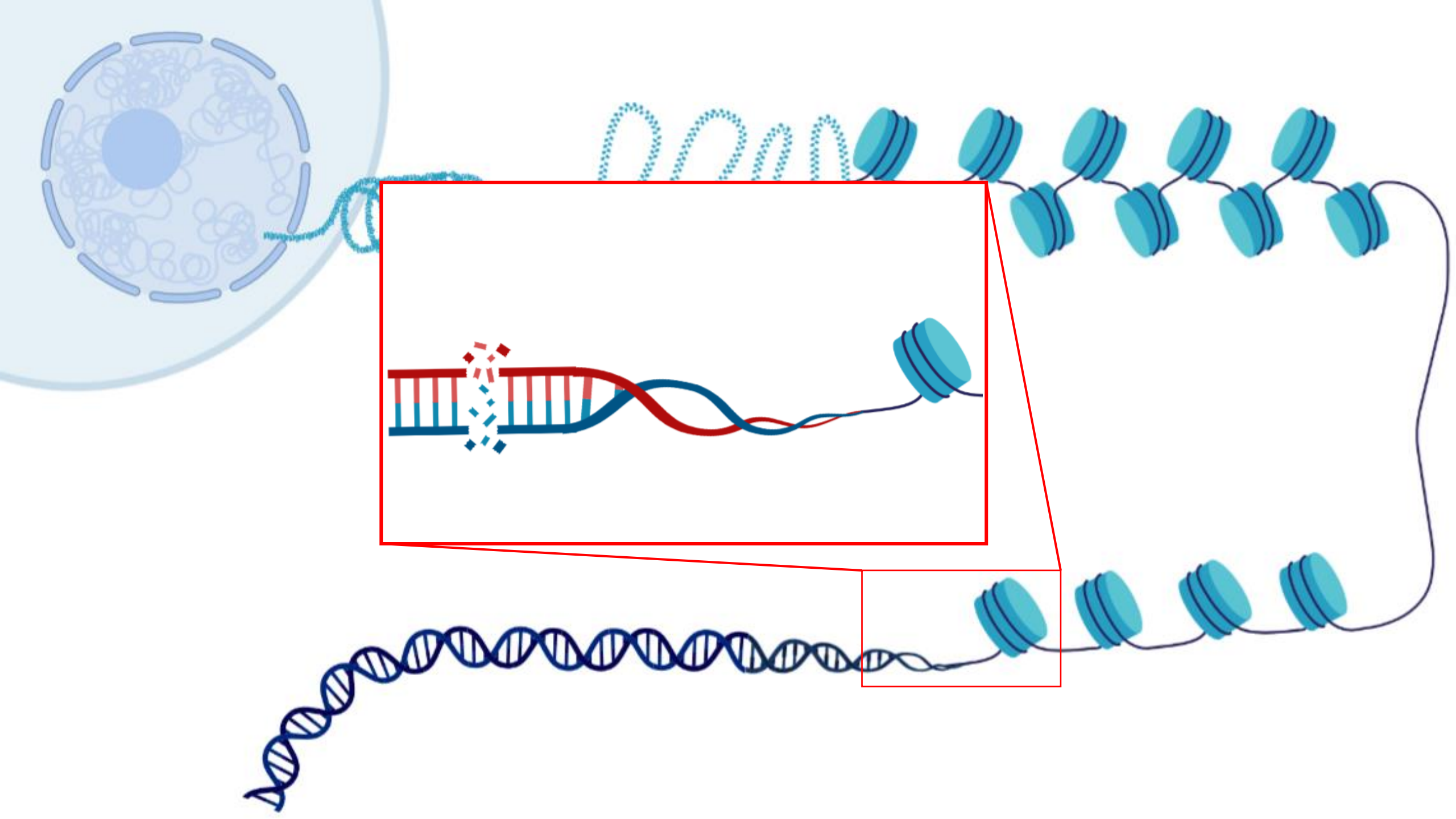
2. Use high-throughput genome damage assay to measure DNA damage

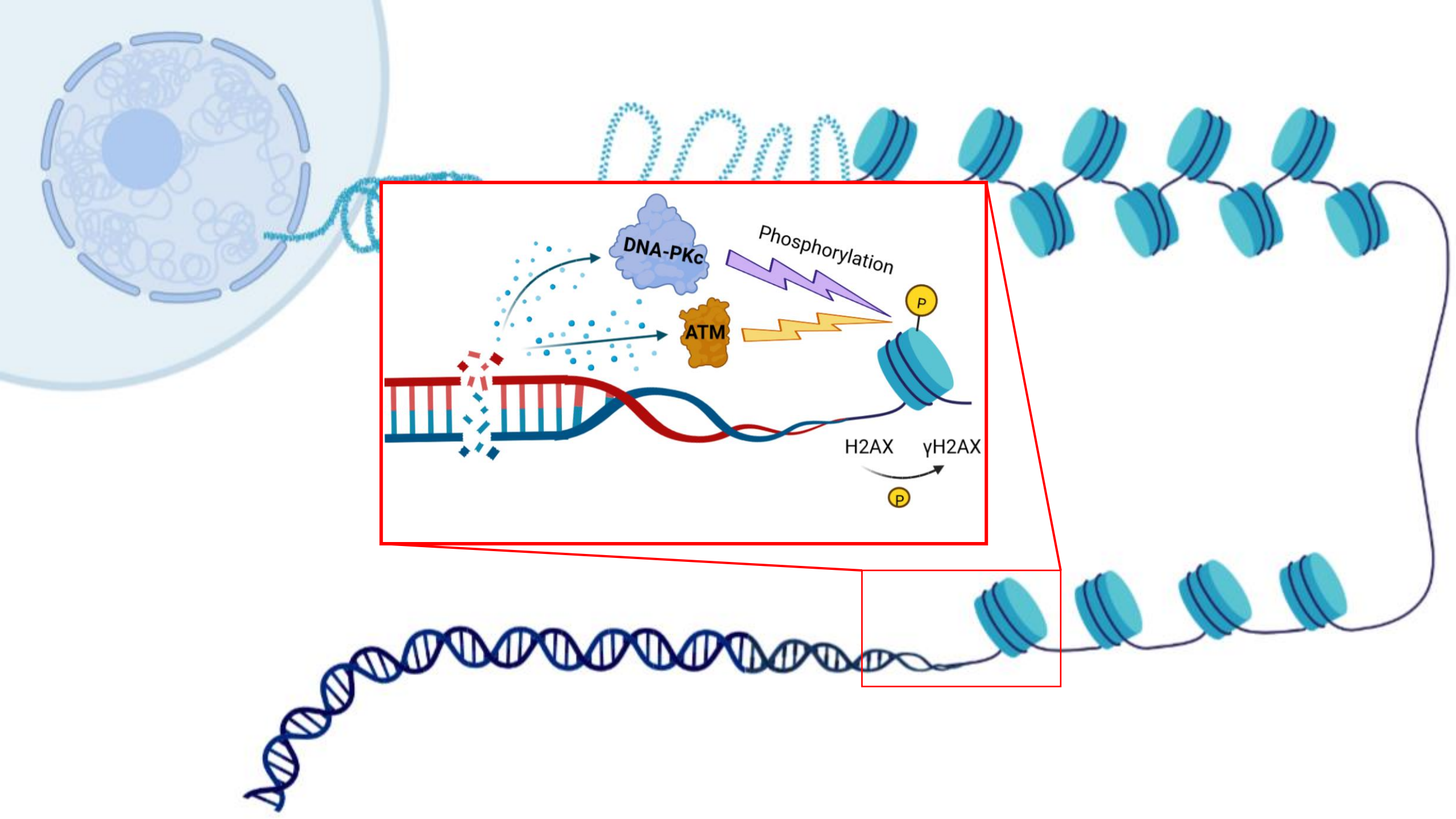
- Measure effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

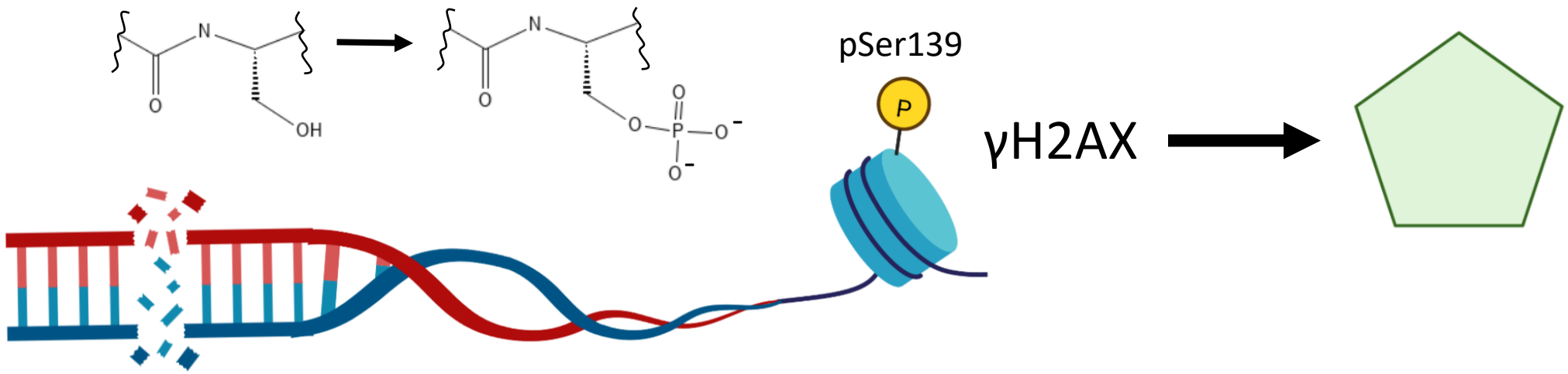
Structure of H2AX
PDB ID: 1AOI

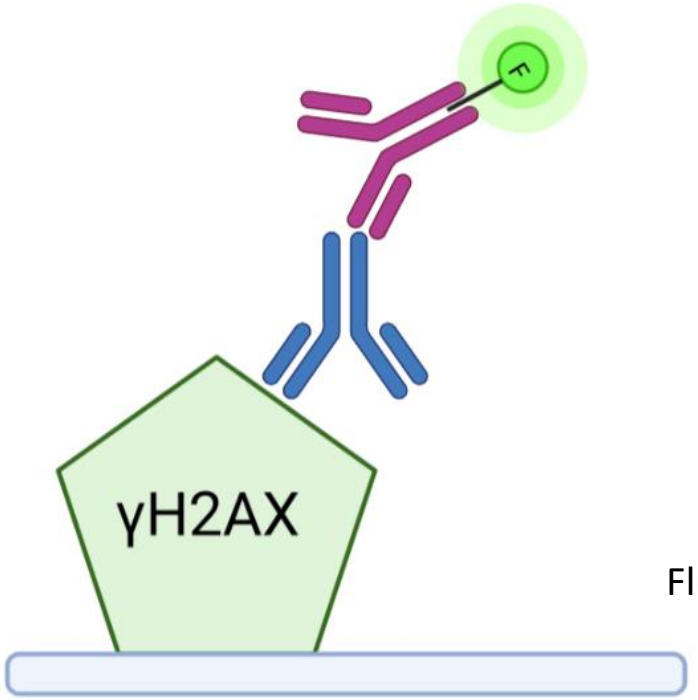







DNA-PKc
Phosphorylation
ATM
P
H2AX
γH2AX
P

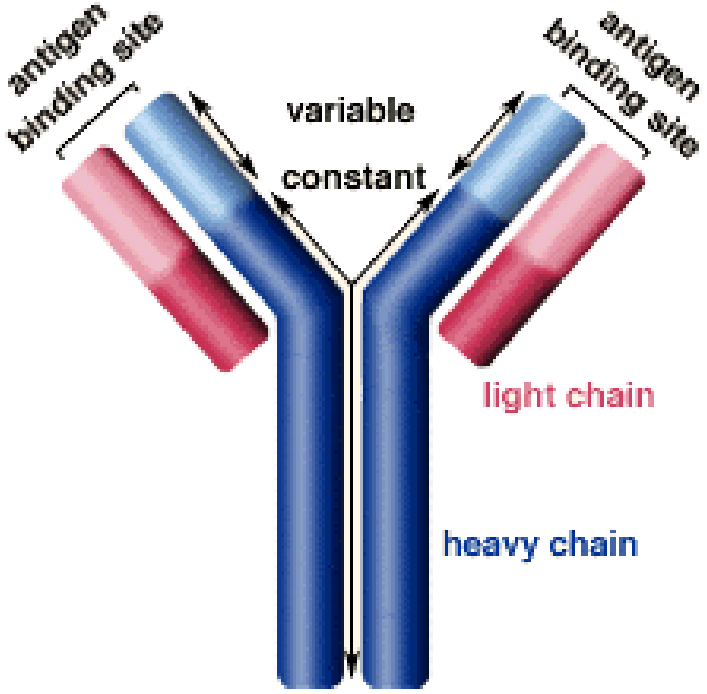




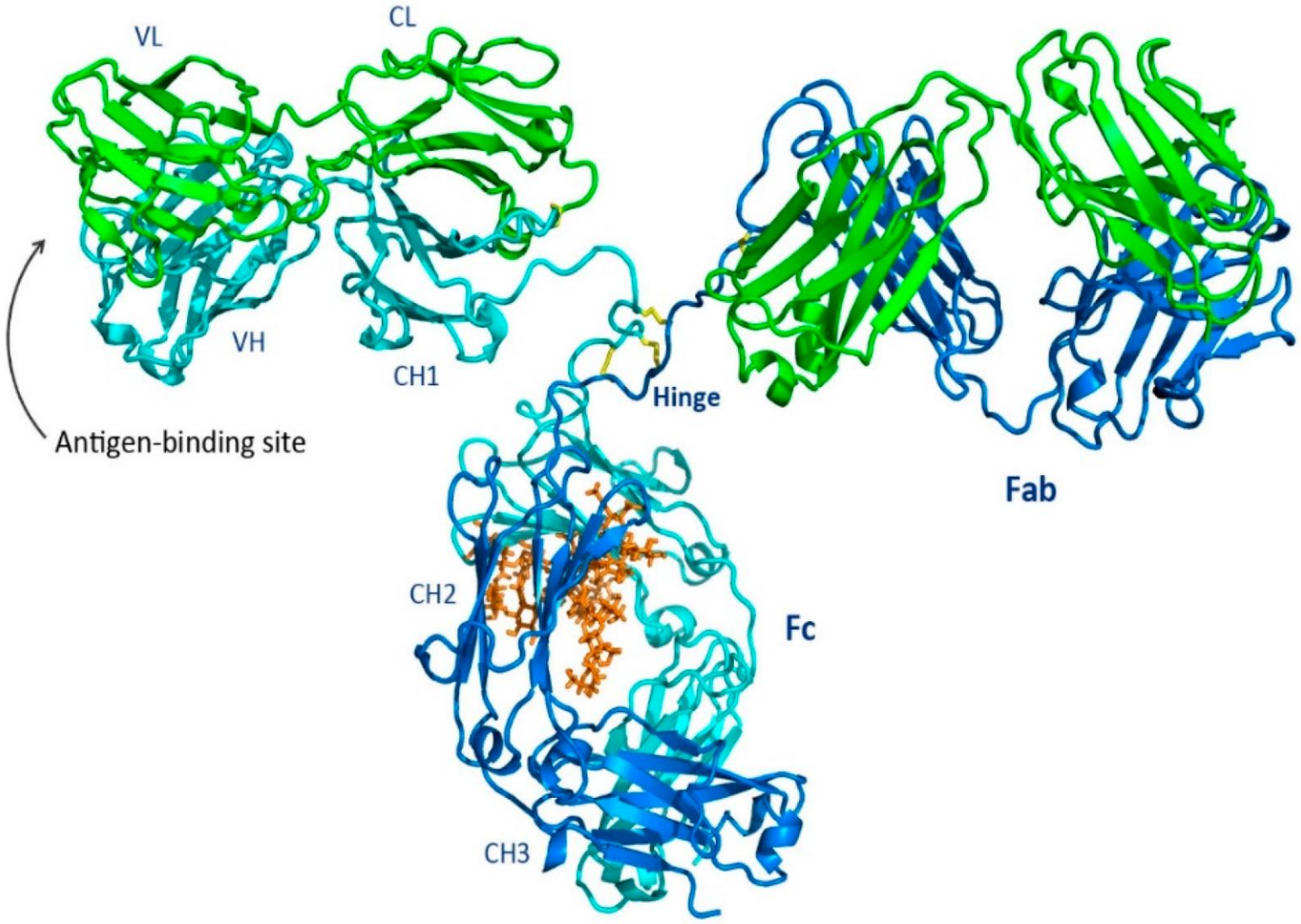
1° = Mouse anti-human γH2AX

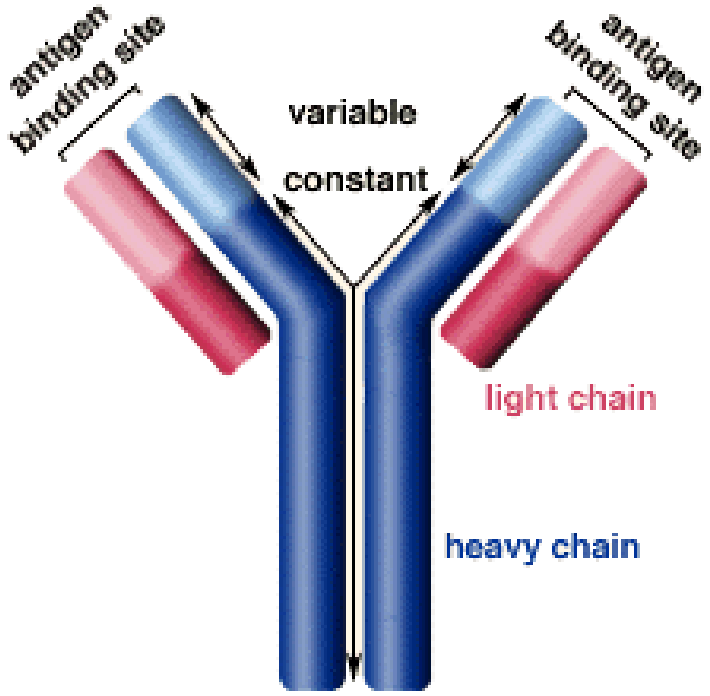
2° = Goat anti-mouse

Fluorophore F = 488nm/525nm (GFP/FITC/AF488)



The Biology Project, Univ. of Arizona (2000)





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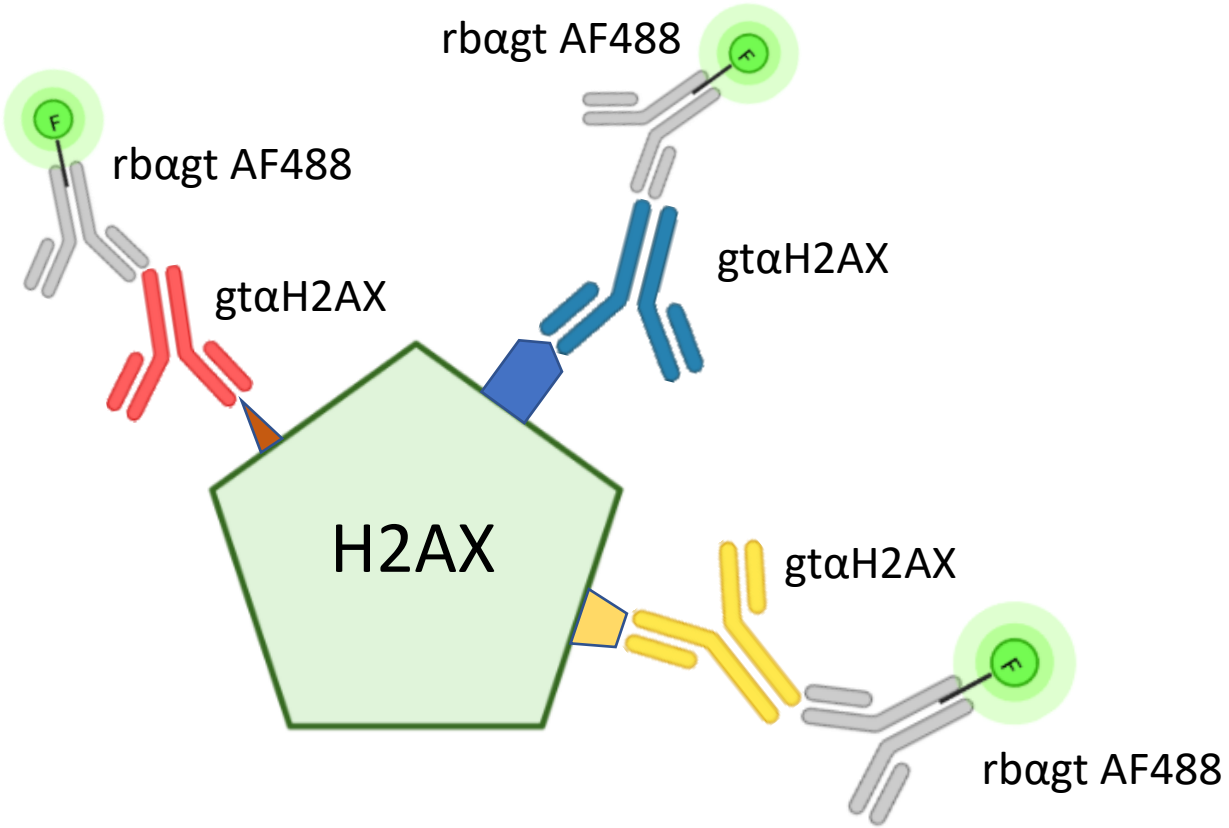
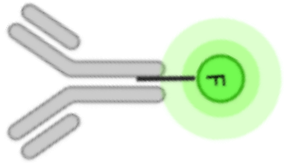


Primary antibody (1° Ab) **recognizes** and **characterizes** antigen

- Specific amino acid sequence/epitope
- Specific conformation of protein
- Specific state of protein (i.e. phosphorylation)



2° Ab recognizes the **species** of the 1° Ab (constant region)

- Often conjugated with tag for visualization
  Enzyme (luciferase) or fluorophore (AF488)
- Amplifies signal through multiple bindings
- Inexpensive to manufacture

Polyclonal Antibodies

Manufacturing:

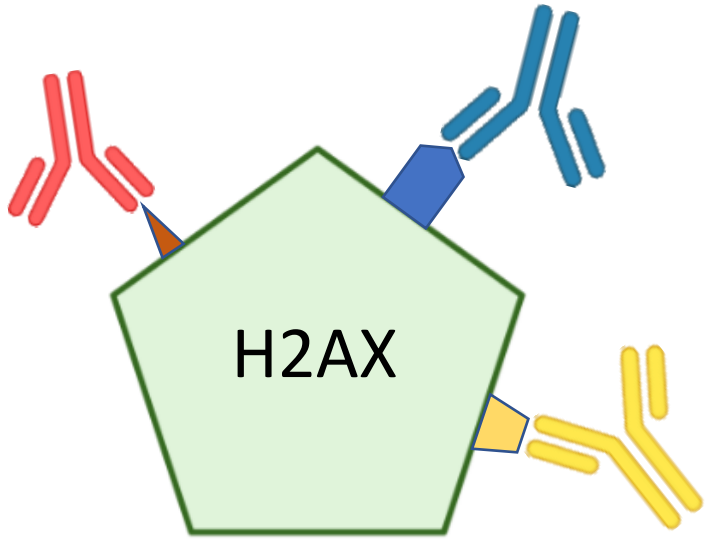
- Animal (often rabbit) immunized with antigen of interest then antibodies collected from blood serum and affinity purified

Advantages:

- Inexpensive and faster to produce than monoclonal
- Multiple antibodies in one polyclonal mixture **can increase antigen recognition by binding multiple epitopes**
    - Especially useful for proteins with low expression

Disadvantages:

- Variability from lot to lot



Polyclonal rbαH2AX

Monoclonal

Manufacturing:

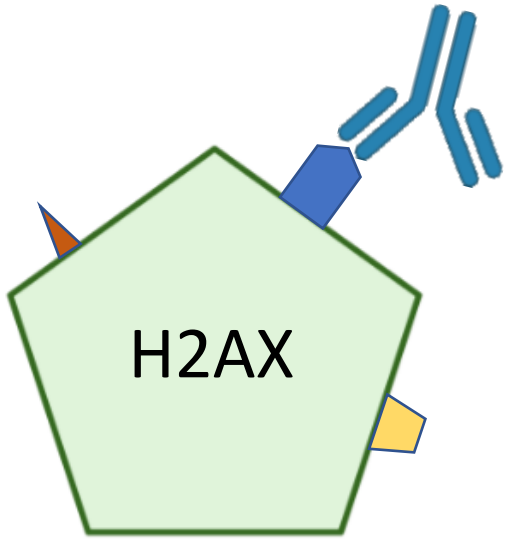
- Animal (often mouse) immunized with antigen of interest then B cells from spleen are harvested and fused with myeloma cells to create hybridoma cell line that will continually produce single antibody clone

Advantages:

- Very consistent
- Binds single epitope (**useful for assays** such as ELISA)

Disadvantages:

- More expensive and terminal for animal



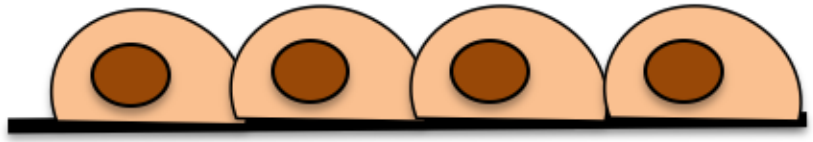
Monoclonal rbαH2AX [CL5039]

Slide Credit: Dr. Becky Meyer

Cellular Fluorescent Staining Protocol

1. Fixation

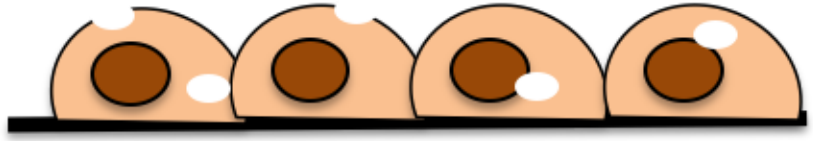
Paraformaldehyde (PFA), methanol



2. Permeabilization

Detergent:

Triton X-100, Tween20, saponin



3. Blocking

Serum:

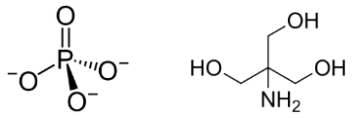
BSA, FBS, NRS, NGS, milk

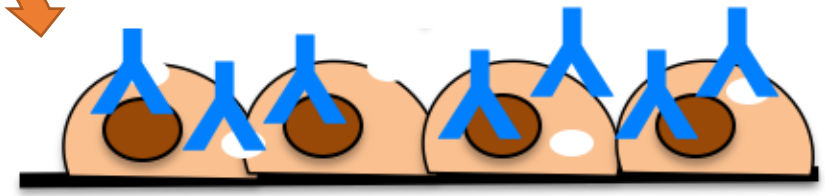
4. 1° Ab

α-γH2AX

5. Wash!

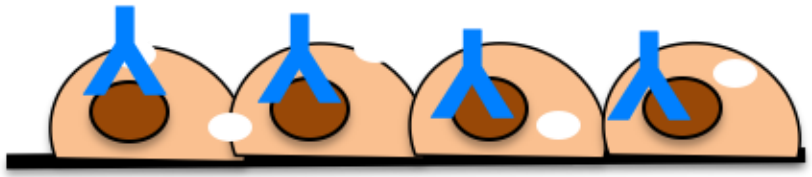
PBS, TBS





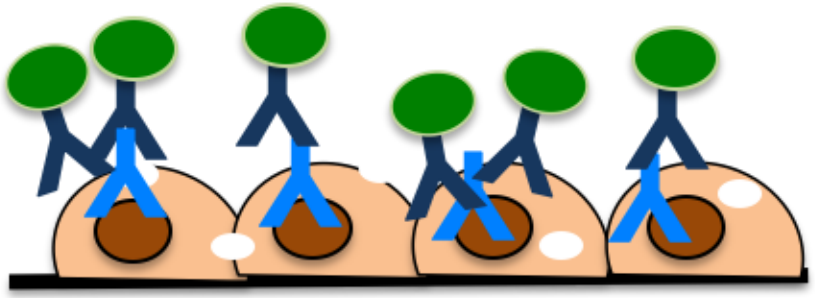
6. 2° Ab

Conjugated with Alexa Fluor 488



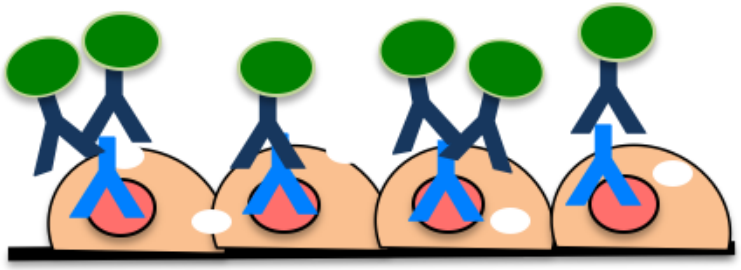
7. Nuclear staining

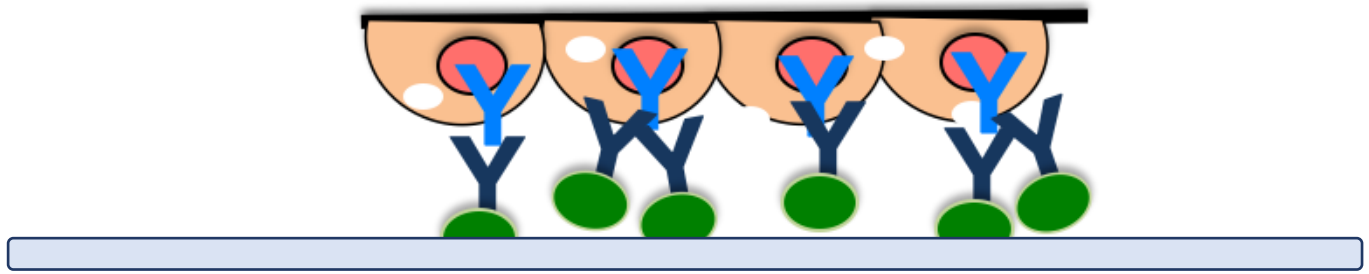
DAPI, Hoechst 33342, hematoxylin

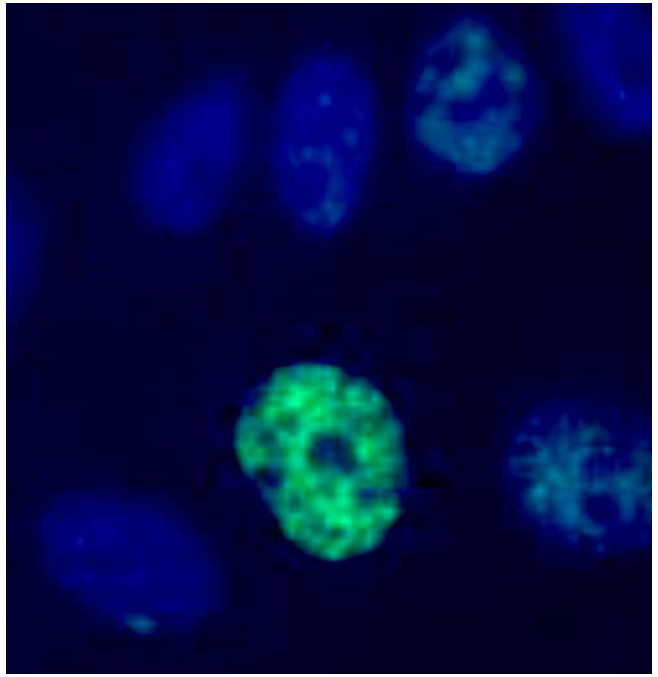


Slide Credit: Dr. Becky Meyer

Mounting media







In lab today:

1. Complete IF staining for H2AX
2. View H2AX images on 7th floor microscope

HW due M1D4 (Wednesday!)

(group)

- Create an experimental schematic for the H2AX staining process

(individual)

1. Write outline for Research Talk
2. Read paper for discussion (linked on M1D4)
3. Visit Comm Lab before M1D5

Notes on experimental schematics

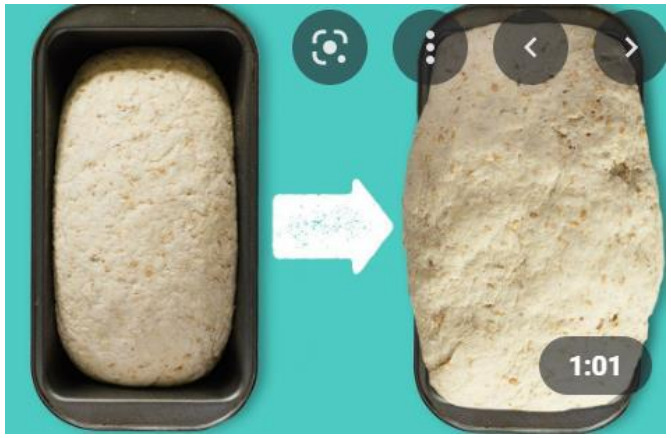
Research Question: What is the ideal flour hydration ratio for the best baguette?

Combine 1000 g All purpose flour, 1 tsp yeast, 1 tsp salt in a large mixing bowl

Add either 300g, 500g or 700g of warm water (37C)

Mix the dough in the mixing bowl, either by hand or using a rubber spatula

Turn the dough over onto a clean work surface and knead by pushing and folding the dough onto itself, creating gluten strands until smooth and elastic

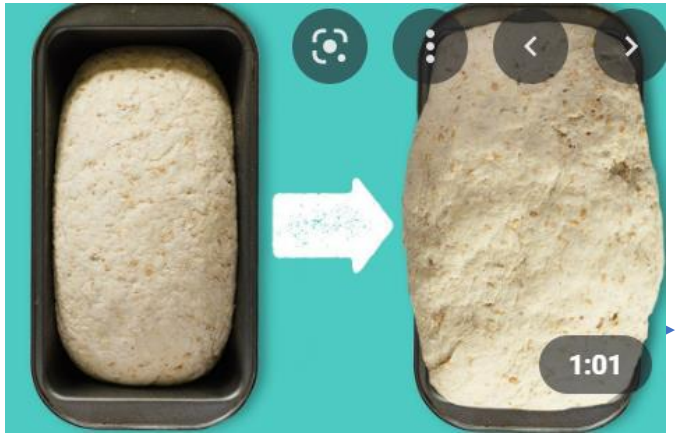


Allow your dough to prove by returning it to the mixing bowl, covering with a wet teacloth at room temperature until the dough has doubled in size

Notes on experimental schematics

Research Question: What is the ideal flour hydration ratio for the best baguette?

Combine Flour, yeast, salt & varying volumes of water into a dough and knead until smooth & elastic



Proof until doubled in size

Deflate the dough and shape into a long rectangle, let proof until doubled in size

Score the dough

Bake until a deep 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

Revised example:

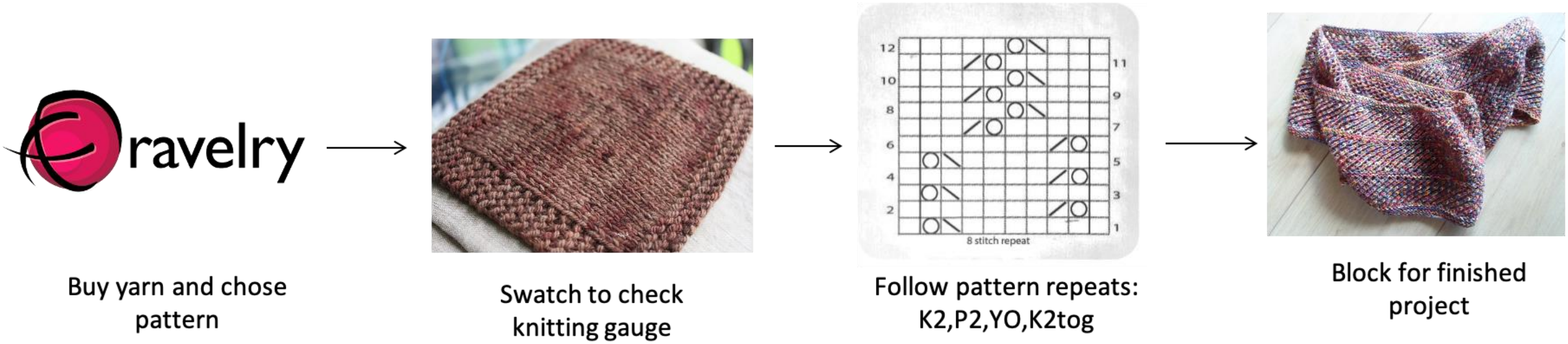


Figure 1: Becky's knitting process. Becky follows a specific protocol to knit a scarf. She choses her yarn and checks the pattern before following the written pattern and blocking to complete the project. K2= knit two, P2= purl 2, YO= yarn over, K2tog= knit two together

Research Talk due Saturday, Oct 1

- Prepare a video of you verbally discussing your research
  - Use any device or Zoom
  - No visuals / slides
  - Do not edit / splice the video
- Submit to Gmail account!
  - bioeng20.109@gmail.com
  - Remember to follow file name guidelines

Research Talk should be 3 min (+/- 15 sec)

- Introduce yourself
- Provide important background information
- Describe key results
  - Briefly describe critical methods used to generate important data
  - Use quantitative descriptions when discussing results
- Highlight the take-home message

What data / results should be included?

- How were the cells treated?
- How were the cells stained?
- How were your data analyzed?
- What are the results?

Review assignment description on wiki

Category	Elements of a strong presentation	Weight
Introduction	• Introduce yourself and the research • Summarize the background information necessary to understand the research • State the research question	25%
Methods & Data	• Provide ONLY the method information necessary to understand the results • Give complete and concise explanations of the results • Relate the results to the central question	25%
Summary & Conclusions	• Highlight the key finding(s) relevant to the central question / hypothesis	25%
Organization	• Give a logical, easy-to-follow narrative • Include transition statements	15%
Delivery	• Show confidence / enthusiasm and speak clearly • Use appropriate language (technical or informal, as appropriate) • Be mindful of the time limit (3 minutes +/- 15 seconds!)	10%

The Research talk will be graded by Dr. Noreen Lyell with input from Dr. Becky Meyer and Jamie Zhan.