# SUBE Annual Retreat

## Saturday 9/23/23

## Agenda

### Le Meridien in Central Square

- Engaging workshops
- Inspiring talks
- Interactive activities
- Breakfast & lunch provided

### Action Kitchen in Seaport

- Cook a chef-guided dinner as a group

## Recharge and grow community!

- Connect with peers in the Biological Engineering and Biology departments
- Expand your knowledge
- Make new friends

## RSVP

16 lucky participants chosen by lottery!

**Enter lottery here:**
https://forms.gle/kab4TEPRNLxxpAWJ6

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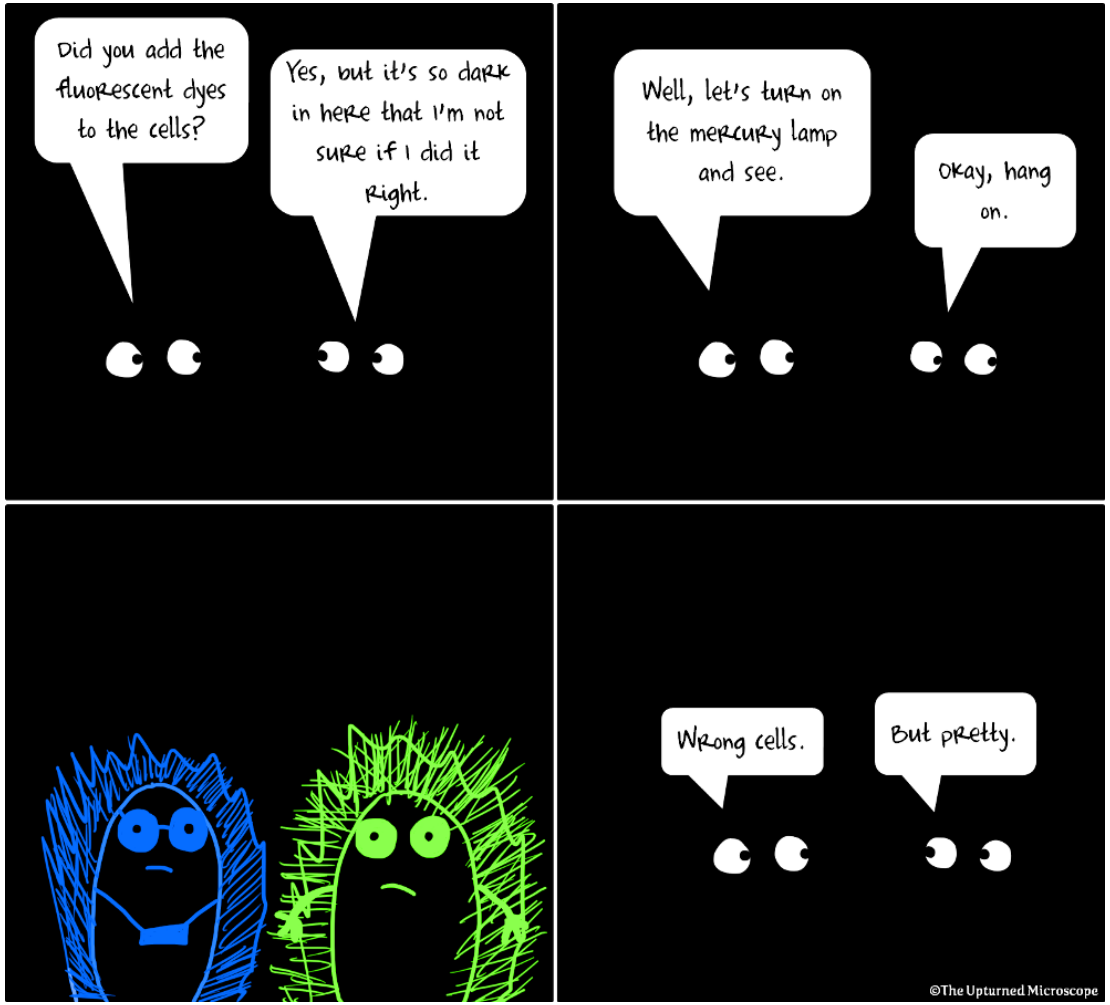
# M1D3: Use immunofluorescence staining to assess repair foci experiment

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




## Office Hours

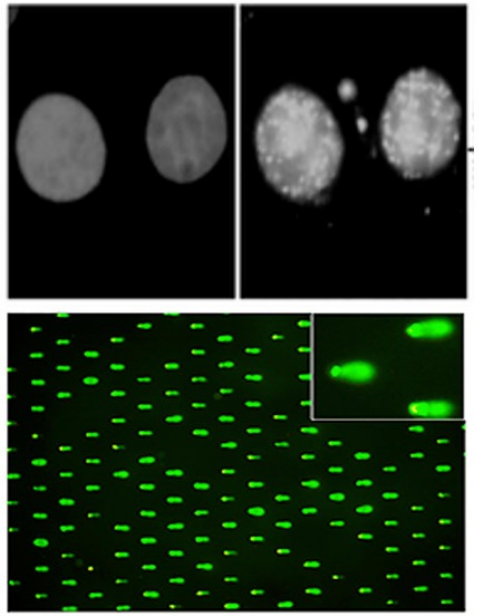
Jamie: 11a-12p Mondays
Noreen: 3-5pm Mondays
Becky: 10a-12p Wednesdays

# Mod1 Overview

Last lab:

This lab:

Next lab:



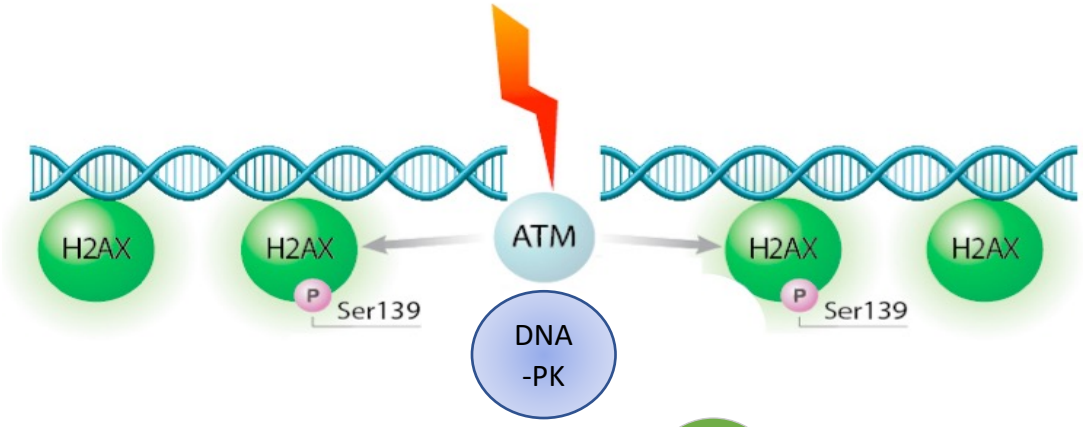
**1. Use repair foci experiment to measure DNA breaks**

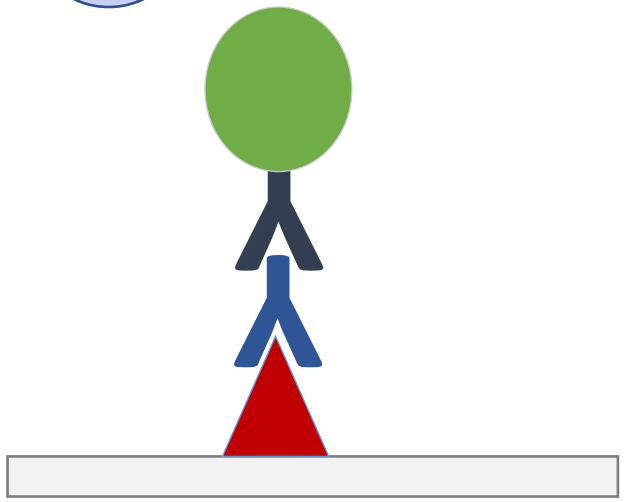
- Examine effect of $H_2O_2$ +/- As on double strand DNA breaks by measuring γH2AX foci formation

**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

# Using immunofluorescence: γH2AX assay to detect double-strand DNA breaks



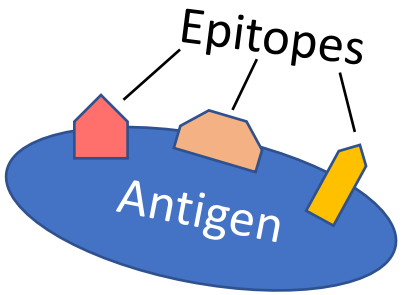



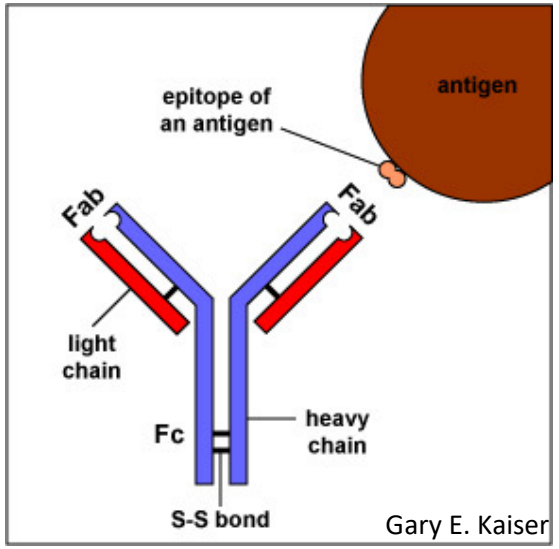
- Histone H2AX phosphorylated at Ser139 if DSB
- Antibodies against γH2AX (phosphorylated form)

| | |
|---|---|
| protein of interest | γH2AX |
| primary antibody | mouse anti-γH2AX |
| secondary antibody | goat anti-mouse |
| Fluorophore (conjugated to secondary antibody) exc./ em. wavelengths | 488/525 nm |

# Considerations for using antibodies in the lab

- Antibodies bind to specific epitopes on antigens
  - Antigens may have multiple epitopes

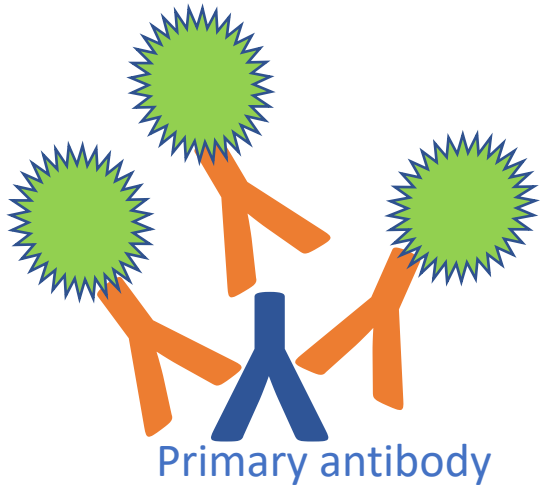



## Primary antibodies vs secondary antibodies

- Primary antibody recognizes the antigen
  - Specific protein sequence
  - Specific conformation of protein
  - Specific state of protein (i.e. phosphorylation)

- Secondary Ab recognizes the species of the primary Ab
  - Often conjugated to tag for visualization
    - Enzyme or fluorophore
  - Amplifies signal through multiple bindings
  - Consider sample species when choosing antibodies!

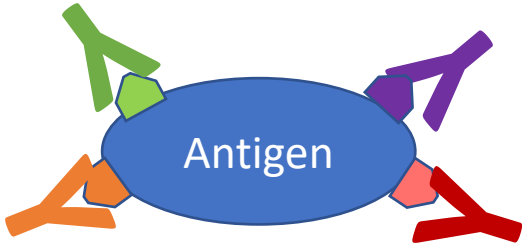



Secondary antibody conjugated to a fluorophore

# Polyclonal vs. monoclonal antibodies

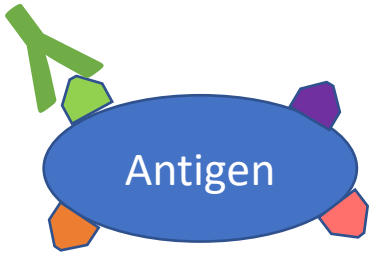
## Polyclonal

- How it's made: animal (often rabbit) immunized with antigen of interest then antibodies collected from blood sera and affinity purified
- Advantages:
  - Less expensive 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

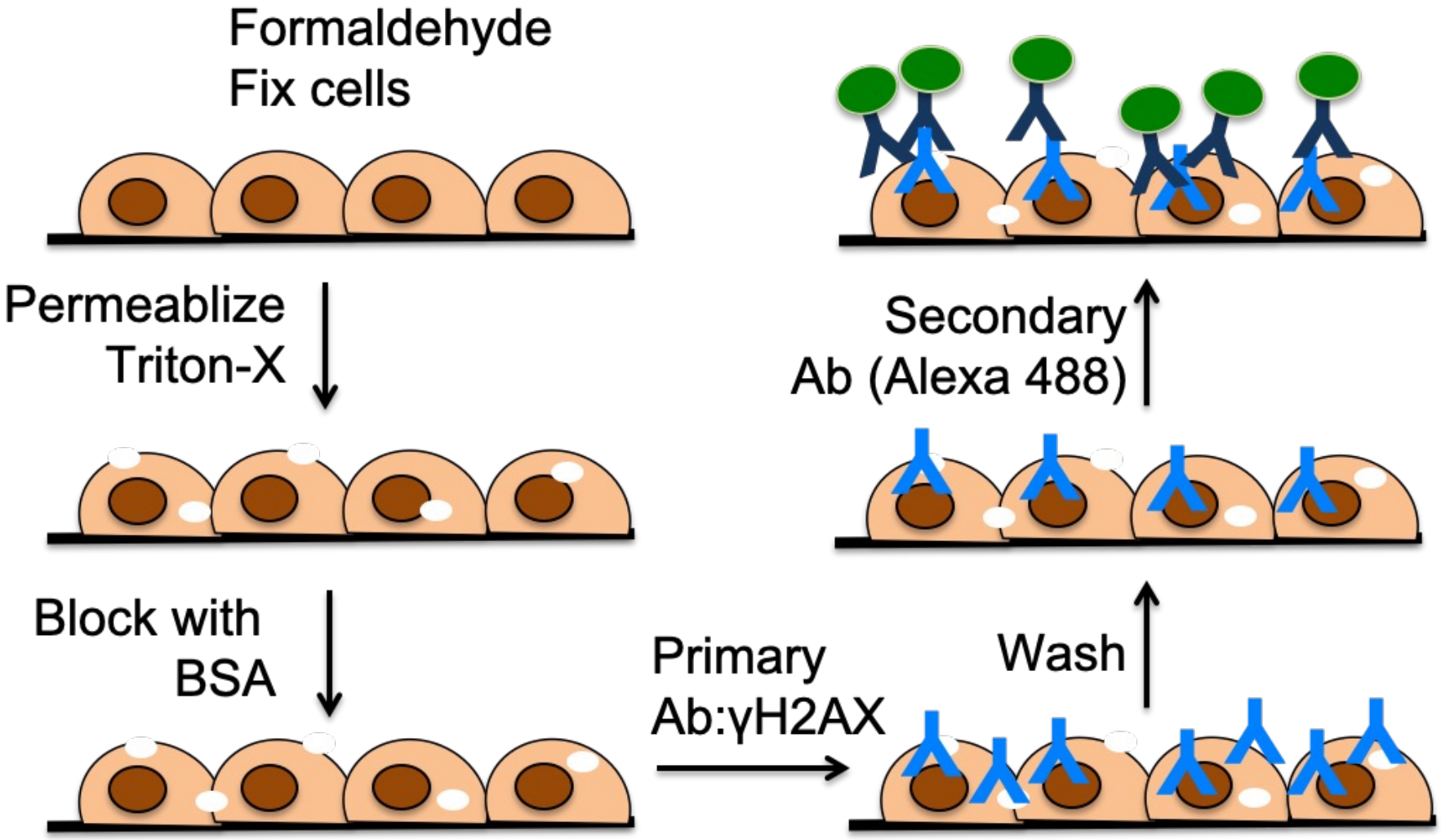



## Monoclonal

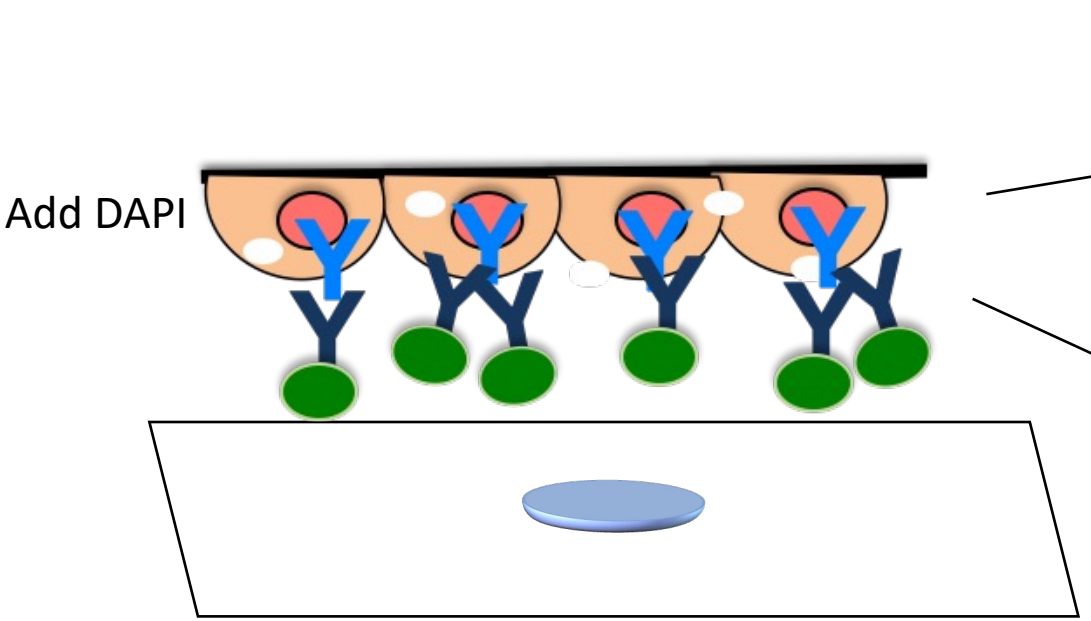
- How it's made: animal (usually mouse) immunized with antigen of interest then B cells from spleen are harvested and fused with myeloma cells to create hybridoma cell line that will continually produce single antibody clone
- Advantages:
  - Very consistent
  - Binds single epitope (can also be disadvantage)
- Disadvantages:
  - More expensive and requires animal sacrifice



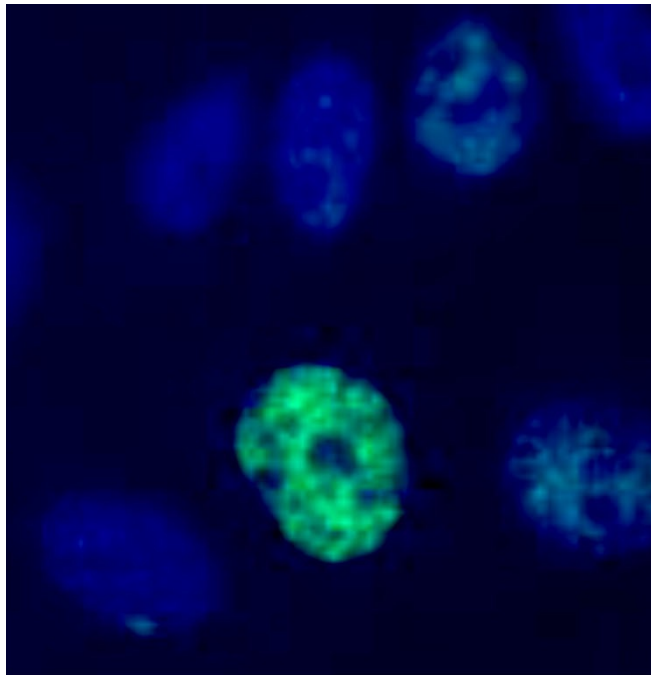
# Using immunofluorescence (IF): steps in protocol



# Finish IF by adding DAPI, then mount slides for imaging




Mount coverslip on glass slide
with mounting media



Blue= DAPI
Green= γH2AX

# Homework discussion

Experimental Schematics

# Notes on experimental schematics…

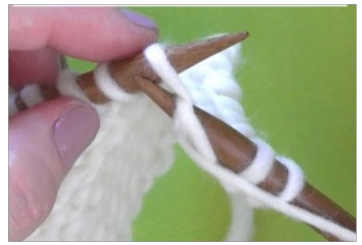
## How does Becky knit a scarf?

Buy beautiful yarn


Choose a pattern

Cast on 25 stitches

Knit



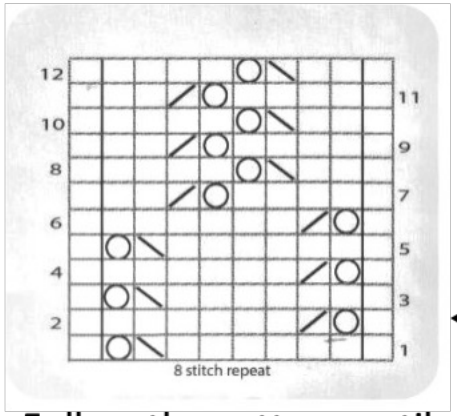
Purl

K & P & K & P

Measure size. It doesn't match the recommended gauge


Frog it!

Cast on 40 stitches




Follow the pattern until time to cast off

Block scarf to wear

# 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:

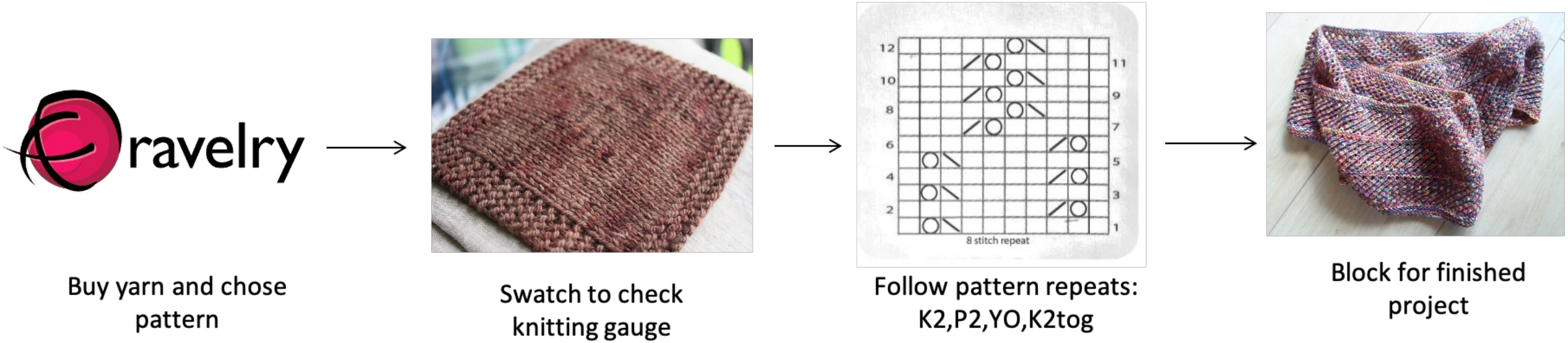



**Figure 1: Becky's knitting process.** Becky follows a specific protocol to knit a scarf. She choses her yarn and checks the pattern before following the written pattern and blocking to complete the project. K2= knit two, P2= purl 2, YO= yarn over, K2tog= knit two together

# In lab today:

1. Complete IF staining for H2AX
    1. Blocking: Noreen lecture on Research Talk
    2. Primary & Secondary Ab: View H2AX images on 7th floor microscope

## HW due M1D4

Tues 9/26!

Thursday =
Comm Lab lecture, no lab

(group)

- Create an experimental schematic for the H2AX staining process

(individual)

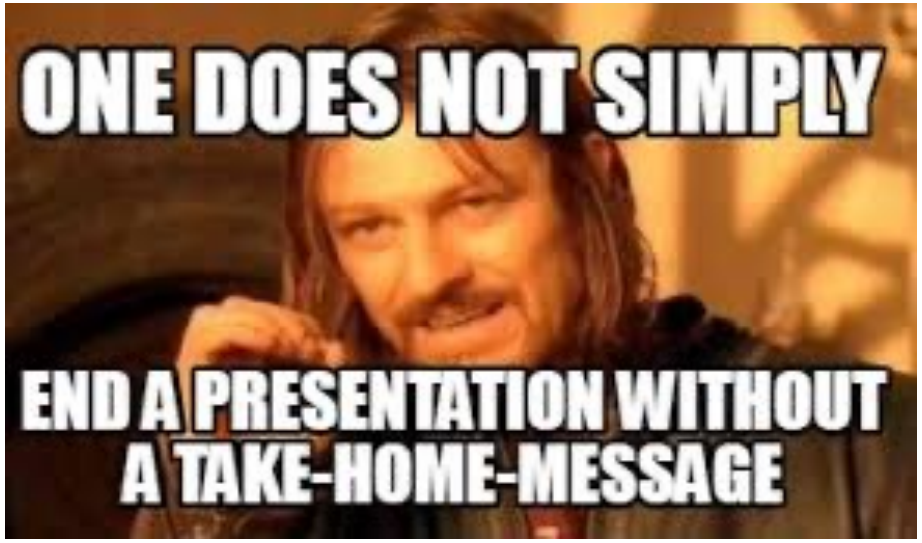
1. Write outline for Research Talk
2. Read paper for discussion (linked on M1D4)
3. Visit Comm Lab before M1D5

# Research Talk due Saturday, Sept 30

- 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

# Presentation 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?

Only include information on the gamma-H2AX experiment

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

# Review assignment description on wiki

| Category | Elements of a strong presentation | Weight |
|---|---|---|
| Introduction | • Introduce yourself and the research<br>• Summarize the background information necessary to understand the research<br>• State the research question | 25% |
| Methods & Data | • Provide ONLY the method information necessary to understand the results<br>• Give complete and concise explanations of the results<br>• 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<br>• Include transition statements | 15% |
| Delivery | • Show confidence / enthusiasm and speak clearly<br>• Use appropriate language (technical or informal, as appropriate)<br>• 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.