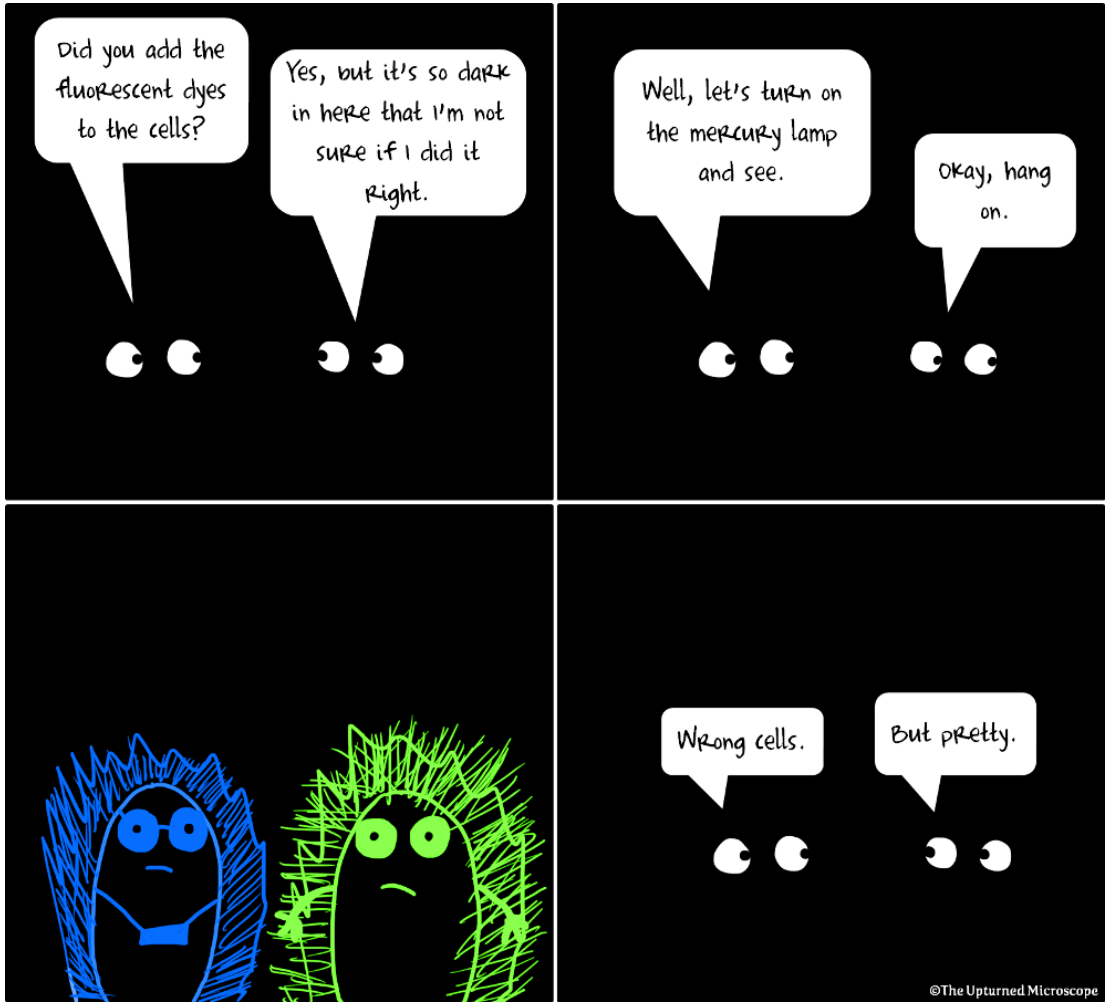
M1D3: Use immunofluorescence staining to assess repair foci experiment

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



Mod1 Overview

Last lab:

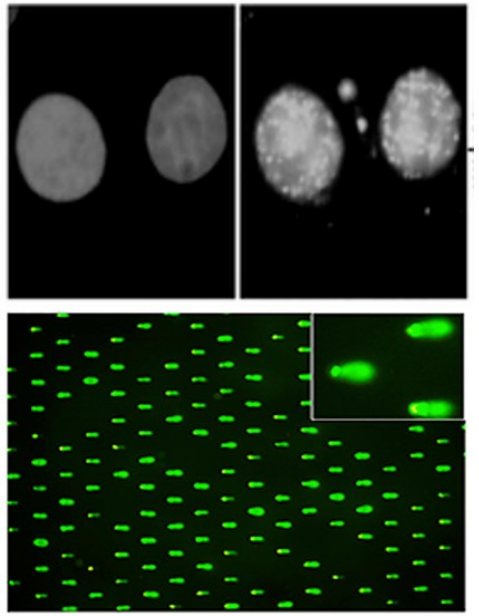
treated cells / fixed

This lab:

stain cells w/ IF

Next lab:

analysis / begin Comet chip



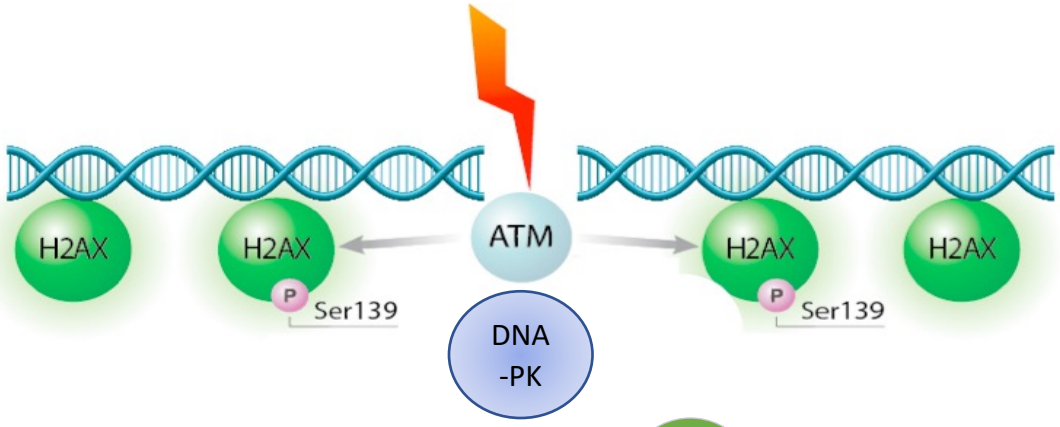
1. Use repair foci experiment to measure DNA breaks

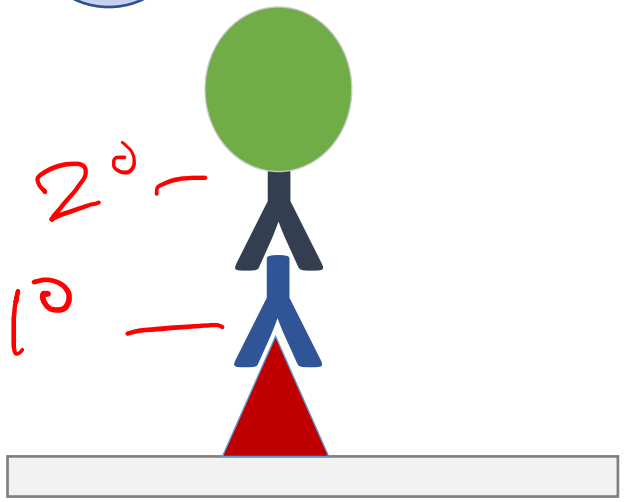
- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γH2AX foci formation

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



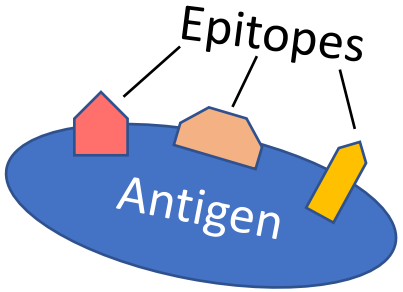


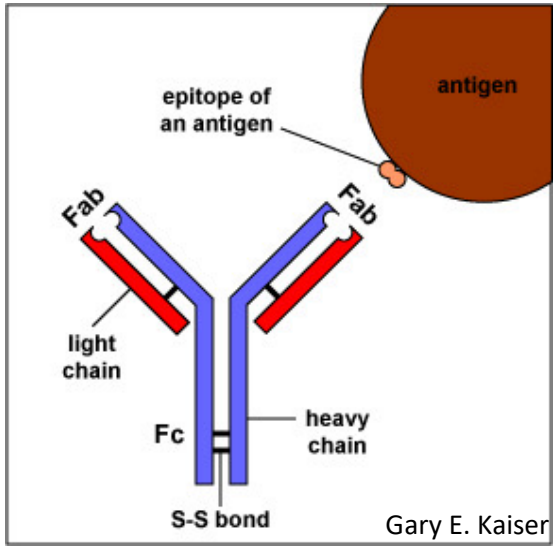
- Histone H2AX phosphorylated at Ser139 if DSB
- Antibodies against γH2AX (phosphorylated form)

protein of interest	γH2AX
primary antibody	mouse anti-human 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



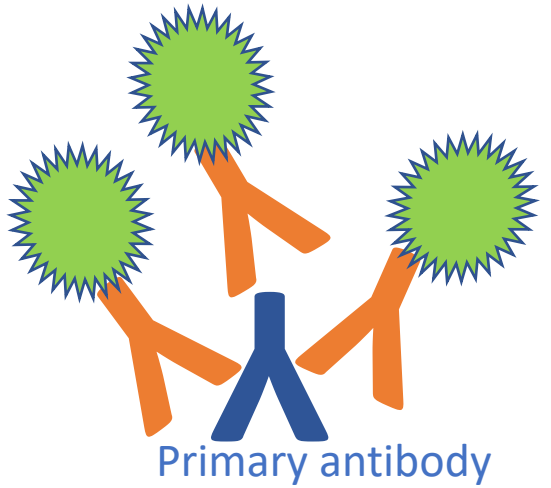


Primary antibodies vs secondary antibodies

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

highly specific

- 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!

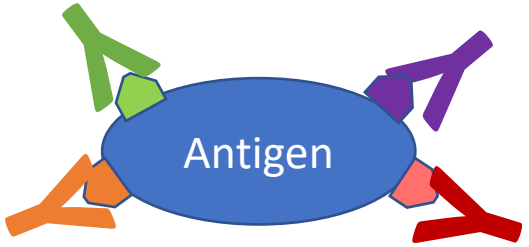


Secondary antibody conjugated to a fluorophore

Polyclonal vs. monoclonal antibodies

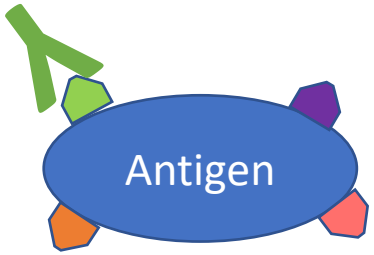
Polyclonal

- 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

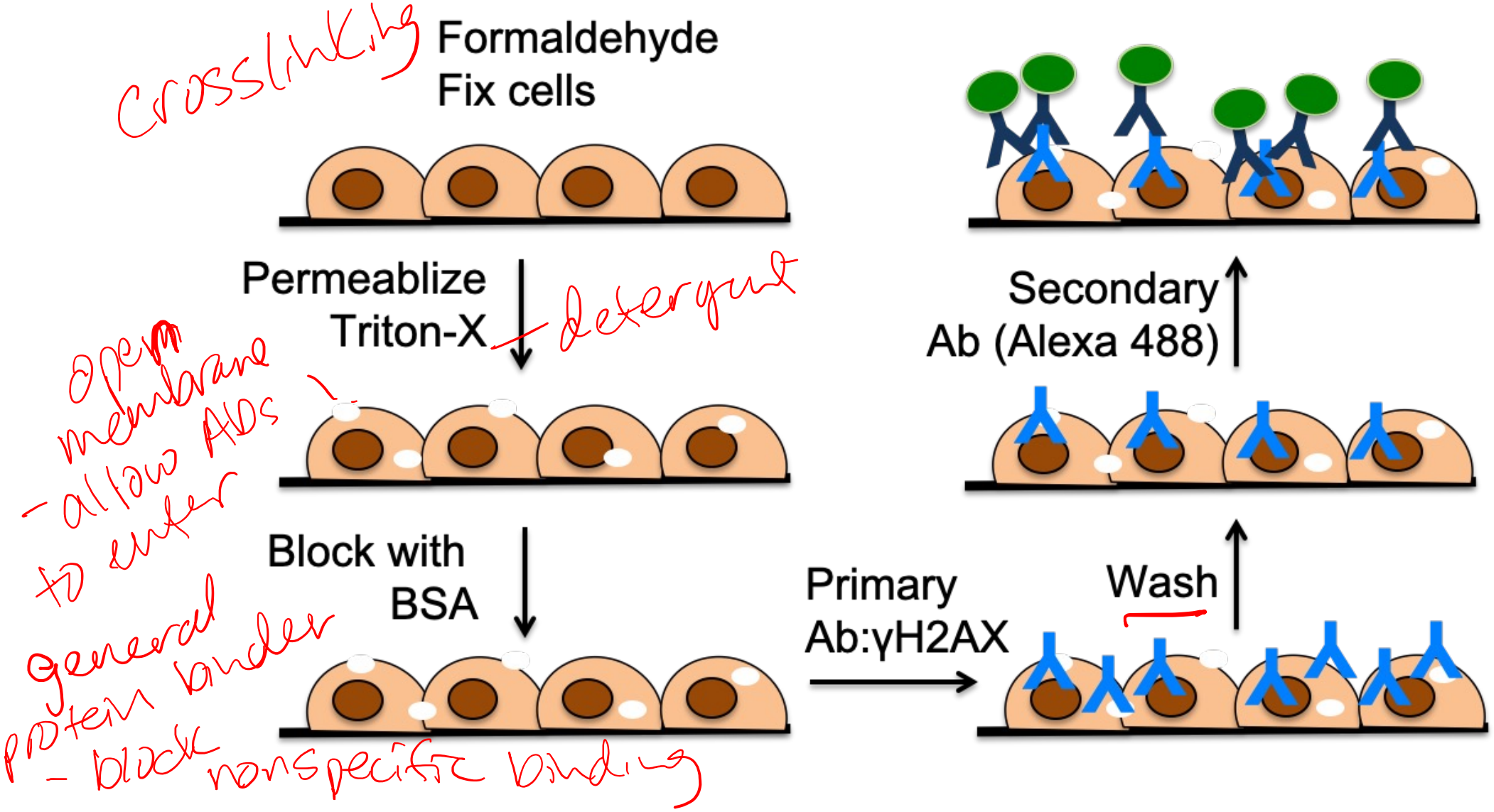


Monoclonal

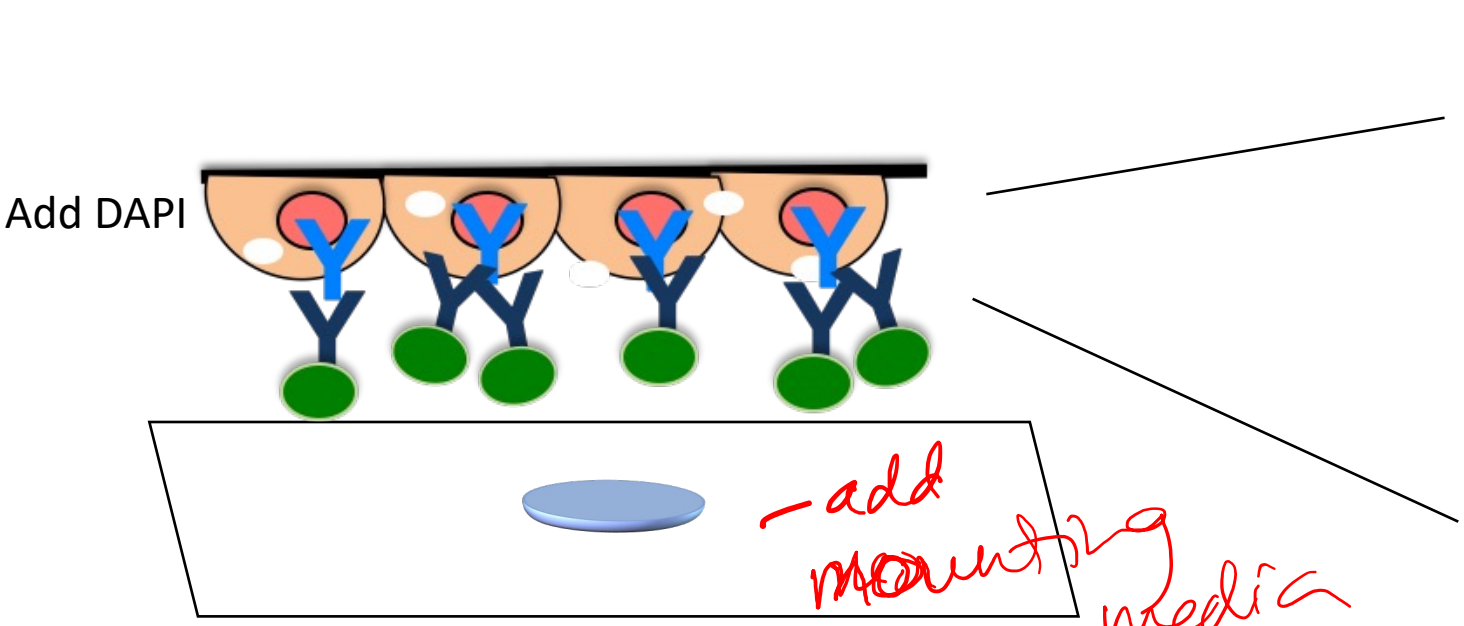
- How it's made: animal (usually mouse) immunized with antigen of interest then B cells from spleen are harvested and fused with myeloma cells to create hybridoma cell line that will continually produce single antibody clone
- Advantages:
  - Very consistent
  - Binds single epitope (can also be disadvantage)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



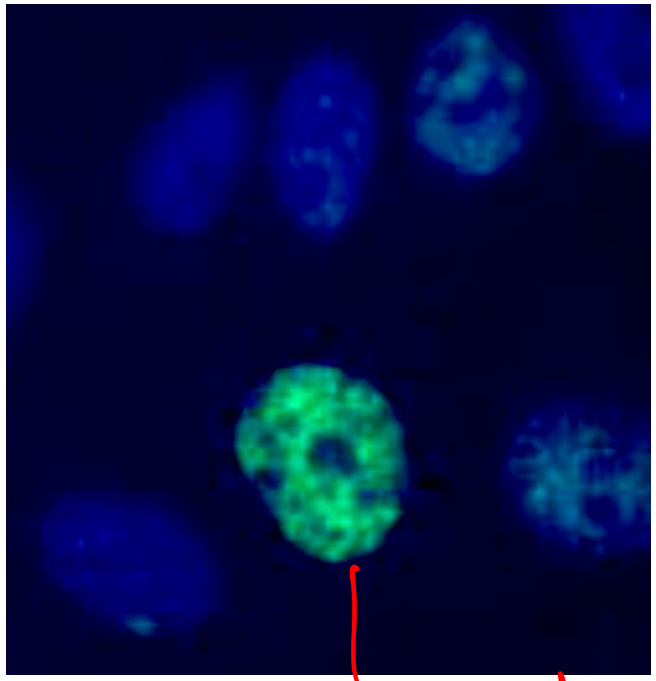
Using immunofluorescence (IF): steps in protocol



Finish IF by adding DAPI, then mount slides for imaging



Mount coverslip on glass slide with mounting media



Blue= DAPI
Green= γH2AX

pseudocolor green

coverslip

In lab today:

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

HW due M1D4 – Tuesday,

(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...

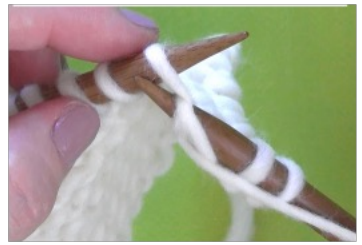
How does Becky knit a scarf?

Buy beautiful yarn

Choose a pattern

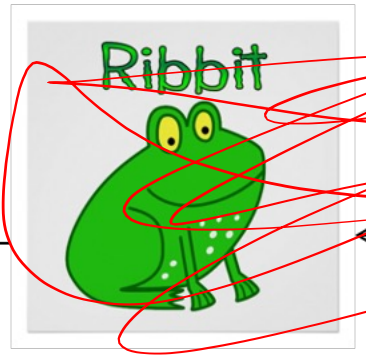
Cast on 25 stitches

Knit



Purl

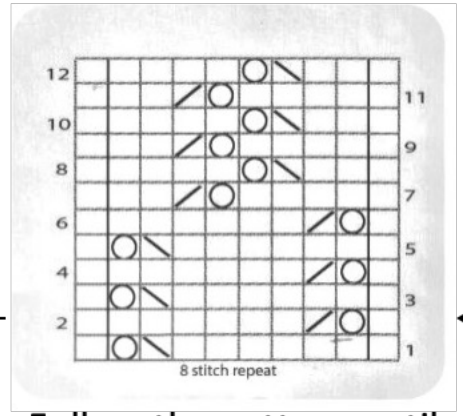
K & P & K & P



Frog it!

Measure size. It doesn't match the recommended gauge

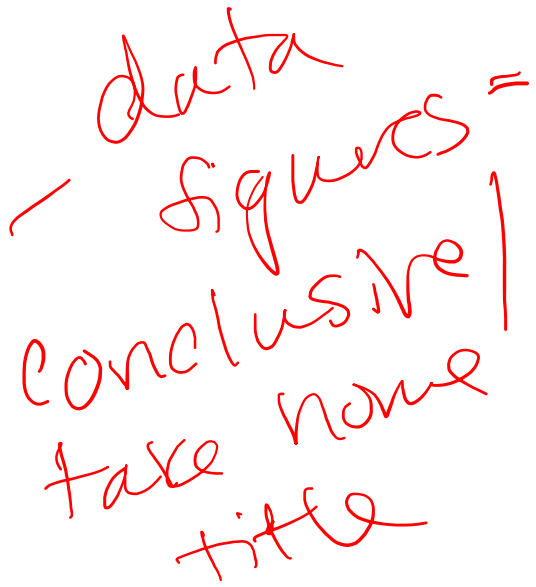
Cast on 40 stitches



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:

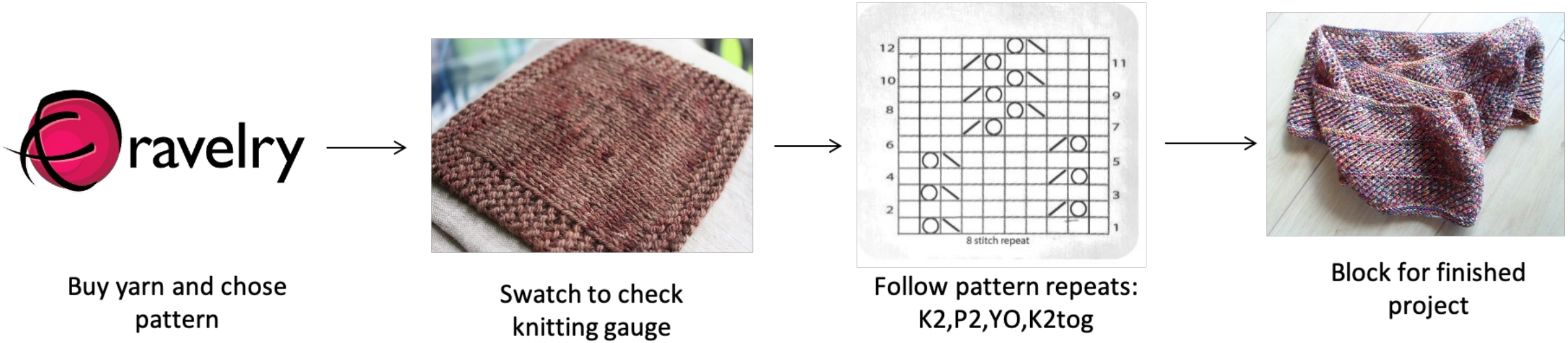


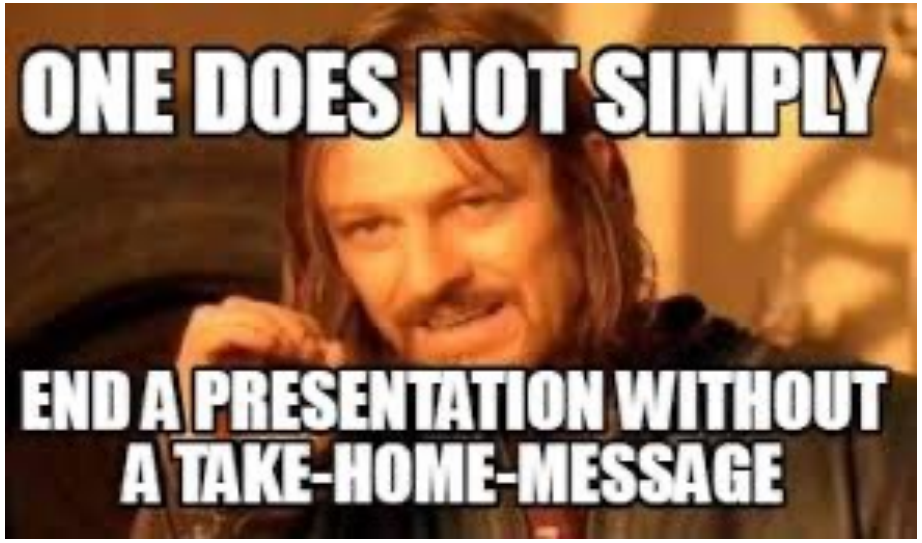
Figure 1: Becky's knitting process. Becky follows a specific protocol to knit a scarf. She choses her yarn and checks the pattern before following the written pattern and blocking to complete the project. K2= knit two, P2= purl 2, YO= yarn over, K2tog= knit two together

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

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 • 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.