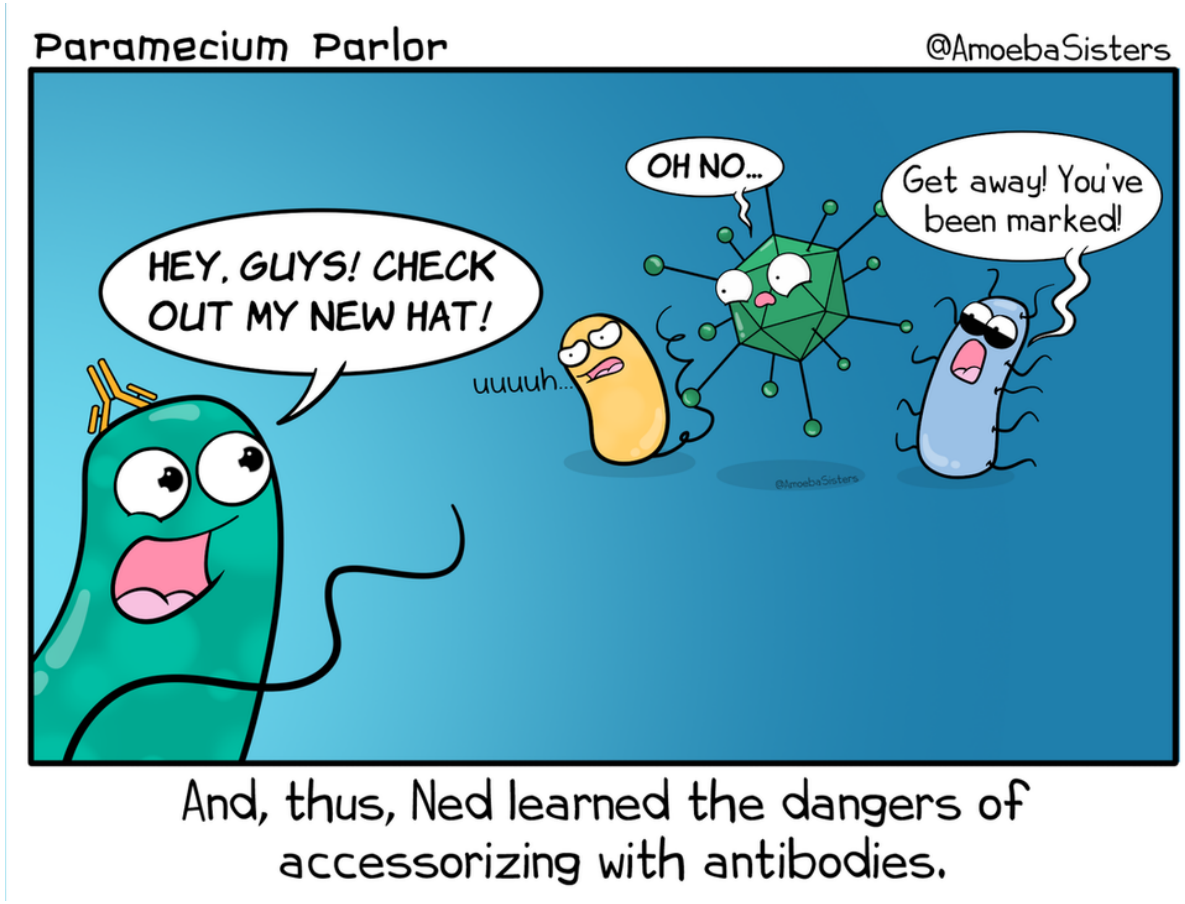
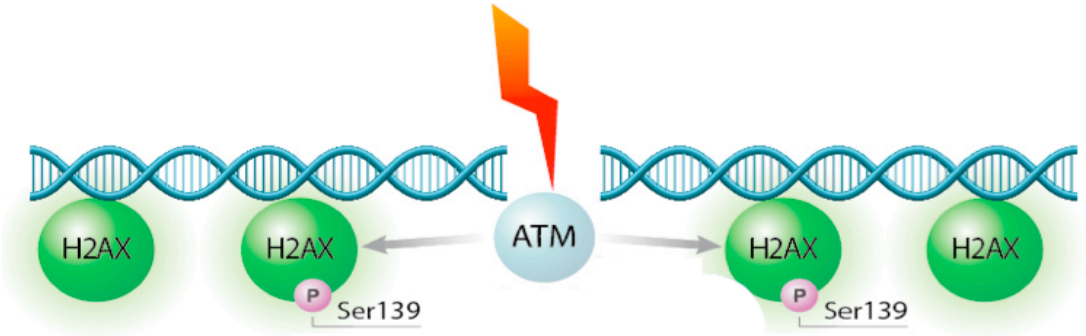
M1D3: Use immunofluorescence staining to assess repair foci experiment

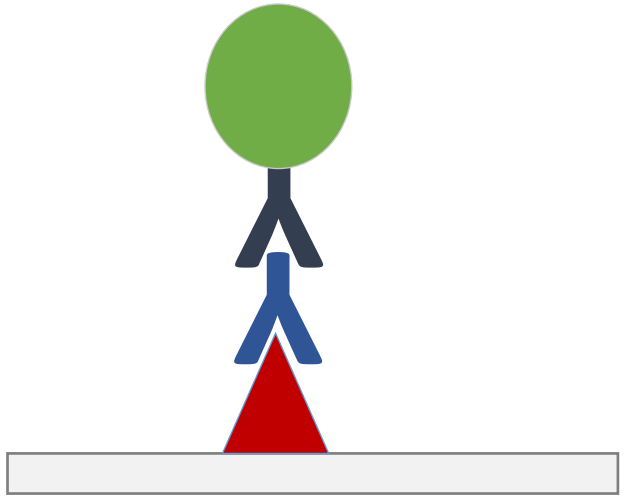
- Prelab
- Paper discussion
- Antibody staining for γH2AX assay



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



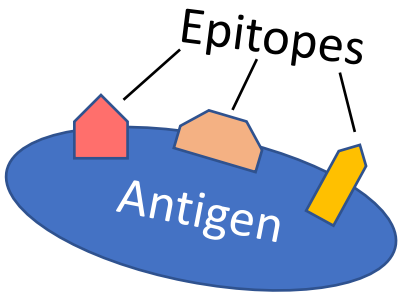
- Histone H2AX phosphorylated at Ser139 if DSB
- Antibodies against γH2AX (phosphorylated form)

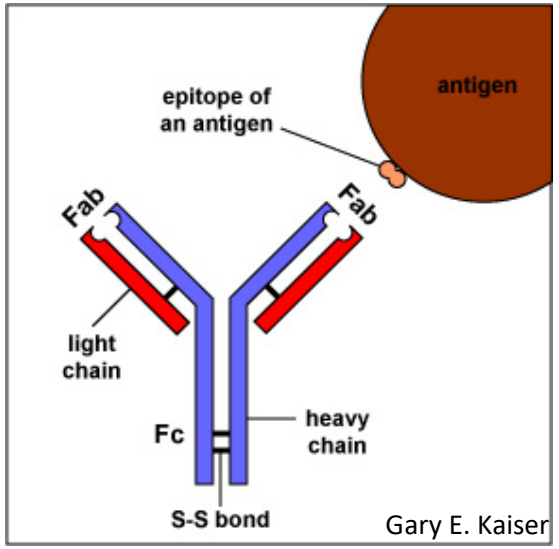


protein of interest	γH2AX
primary antibody	mouse anti-human anti-γH2AX
secondary antibody	goat anti-mouse
Fluorophore (conjugated to secondary antibody) exc./ em. wavelengths	488/525 nm

Considerations for using antibodies in the lab

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

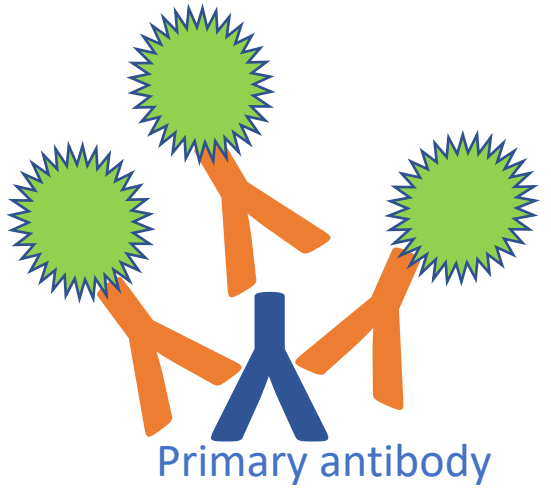




Primary antibodies vs secondary antibodies

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

- 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!

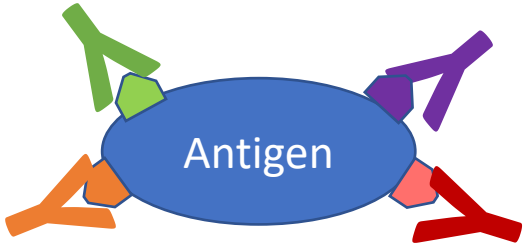


Secondary antibody conjugated to a fluorophore

Polyclonal vs. monoclonal antibodies

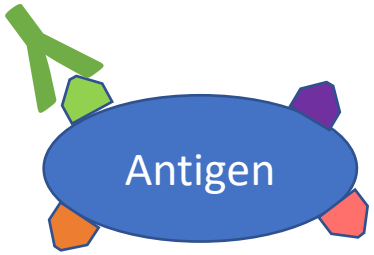
Polyclonal

- 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

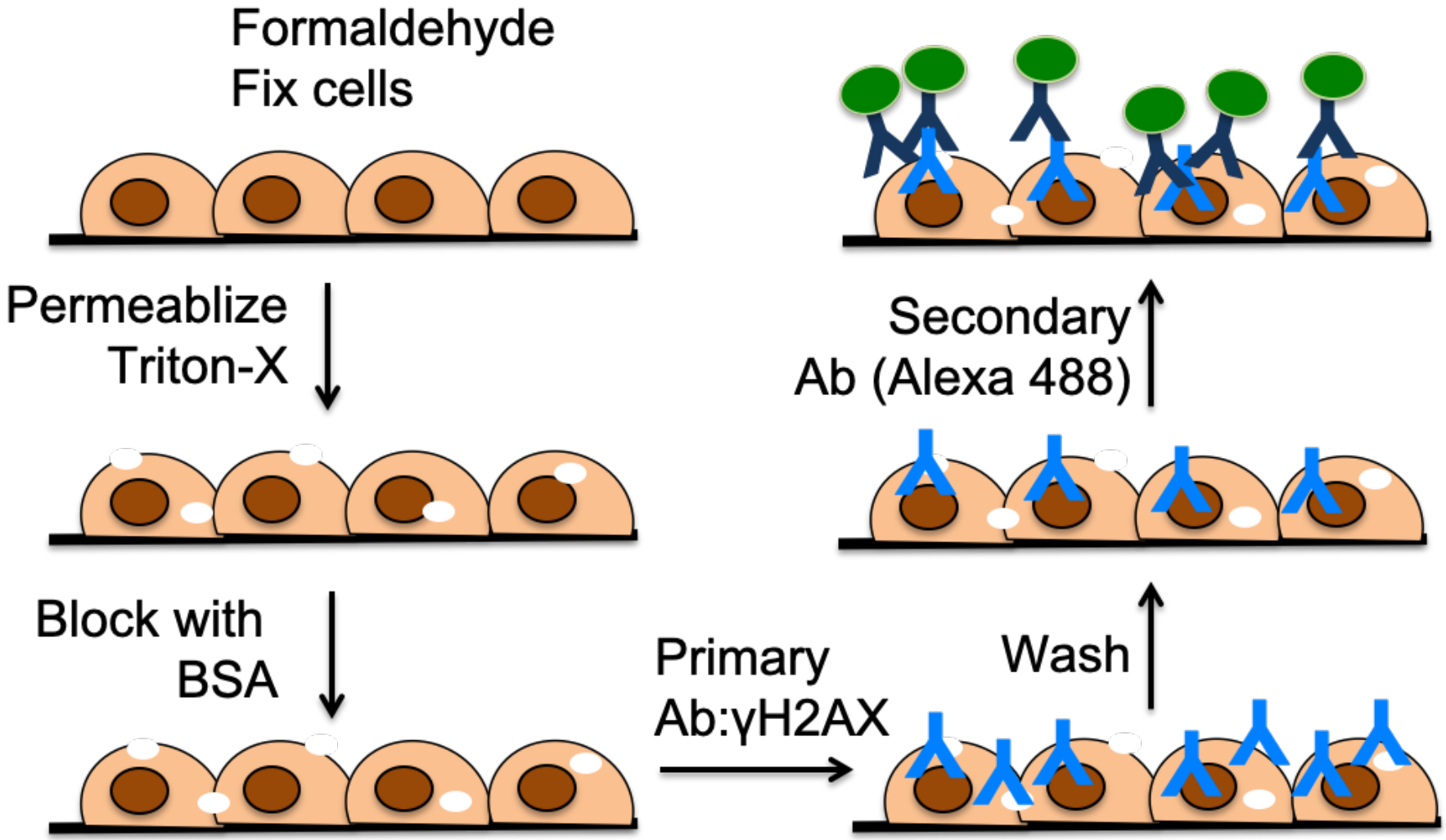


Monoclonal

- 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



Pro tips for writing a methods section

Include enough information to replicate the experiment

- List manufacturer's name (Company)
- Be **concise and clear** in your description

Use subsections with descriptive titles

- Put in logical order, rather than chronological order
- Begin with topic sentence to introduce purpose / goal of each experimental procedure

Use clear and concise full sentences

- NO tables or lists, all information should be provided in full sentences and paragraphs
- Write in passive voice and use past tense

Use the most flexible units

- Write concentrations (when known) rather than volumes

Eliminate 20.109 specific details

- Example "labeled Row A, Row B…"
- Do not include details about tubes and water!
- Assume reader has some biology experience
- Include parts of the protocol that the teaching faculty completed, but do not say "completed by teaching faculty."

How can you improve this example?

"Cells were grown in 12 mL of RMPI supplemented with FBS. We spun down the cells and counted them with a hemocytometer. Flasks were incubated in 37 C incubator."

How can you improve this example?

What cells? From where were the cells attained?

How much? What else was added to the media?

"Cells were grown in 12 mL of RPMI supplemented with FBS. We spun

Volume here does not have context as based on the flask used. When might flask / plate size be helpful??

Define all abbreviations and include supplier / manufacturer.

Use passive voice and avoid jargon!

down the cells and counted them with a hemocytometer. Flasks

Be specific about the purpose of each of the steps used…cells were harvested using centrifugation (be sure to include speed and time) then counted using a hemocytometer. And what else was used? At what final concentration / percent?

Be specific about the subject of each action / step.

were incubated in 37 C incubator."

Specific location / equipment used is not important, just the temperature conditions. What other growth conditions were maintained?

Revised example…

Maintaining MCL-5 cell line

Human lymphoblastoid cells (MCL-5) cells (gift of Engelward Laboratory, MIT) were grown in Roswell… (RPMI) (Manufacturer) supplemented with 10% fetal bovine serum (FBS) (Manufacturer) and 100 U / mL of penicillin and streptomycin (Manufacturer). To harvest, cells were centrifuged for 5 minutes at 300g and pelleted cells were resuspended in fresh media. Cells were counted using 10% (v/v) trypan blue and a hemocytometer. Cultures were maintained at 37 C, 5% CO_2, and 95% relative humidity.

In lab today:

1. Paper discussion
2. Work through IF staining steps on wiki
3. Work with your lab partner on methods homework
    1. Tell me your team name if haven't already!

HW due M1D4

(group)

1. Write methods section for protocols on M1D1 through M1D3
    - Consider how to divide the work amongst all of you
    - Follow guidelines discussed today

(individual)

1. Visit Comm Lab before M1D5.