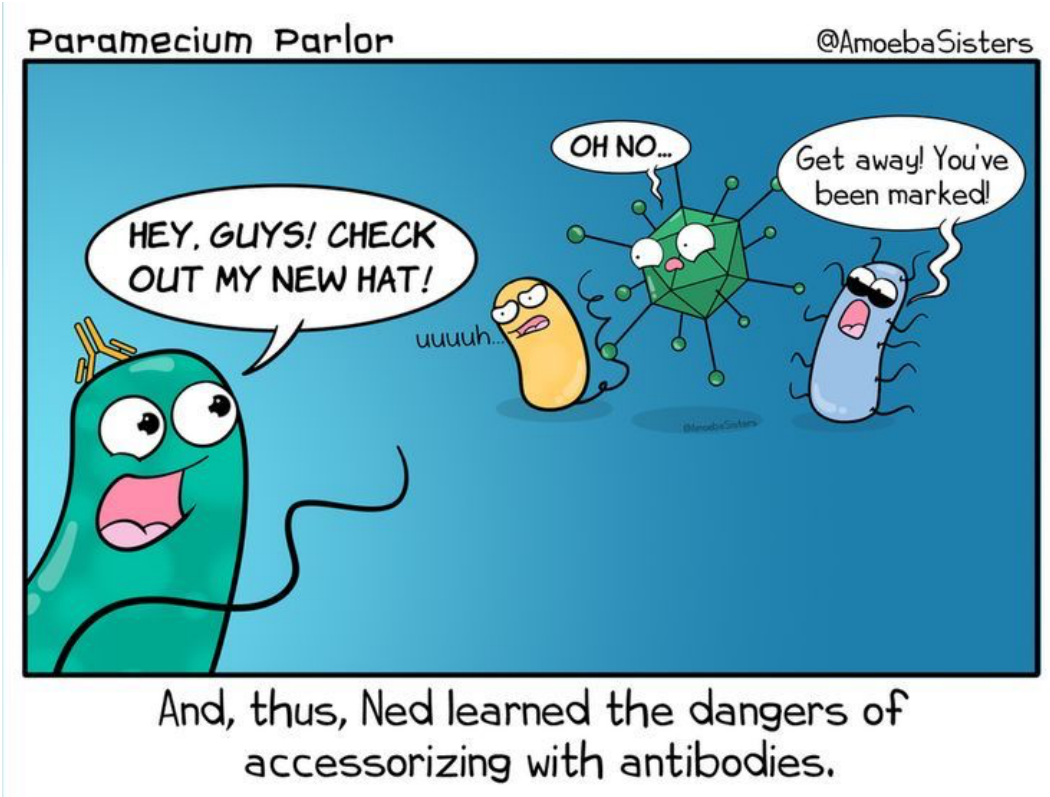
M1D3:
Use immunofluorescence staining to assess repair foci experiment

1. Prelab discussion
2. Paper discussion
3. Begin staining for gamma-H2AX experiment



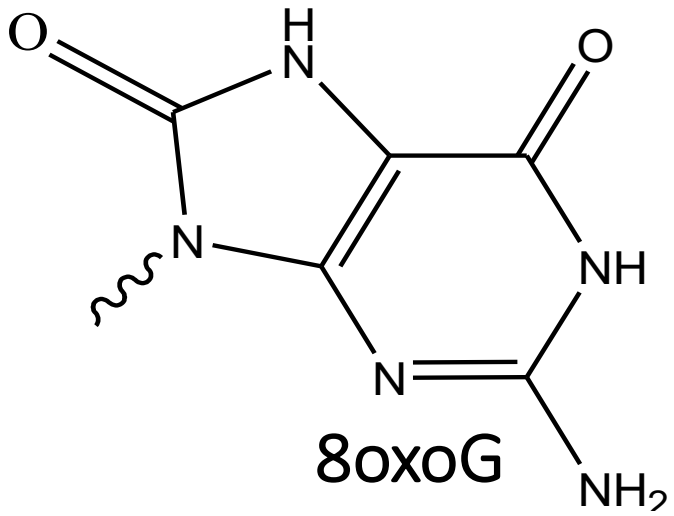
Let’s review…

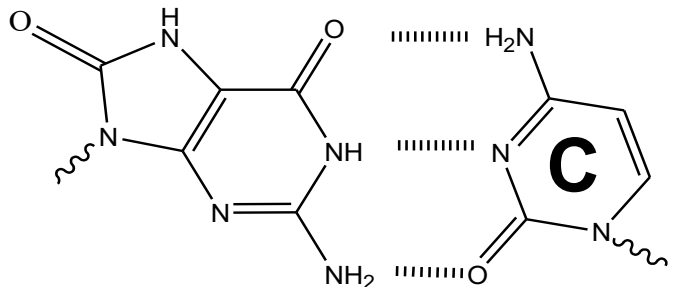
- What type of DNA damage is measured using the gamma-H2AX assay?

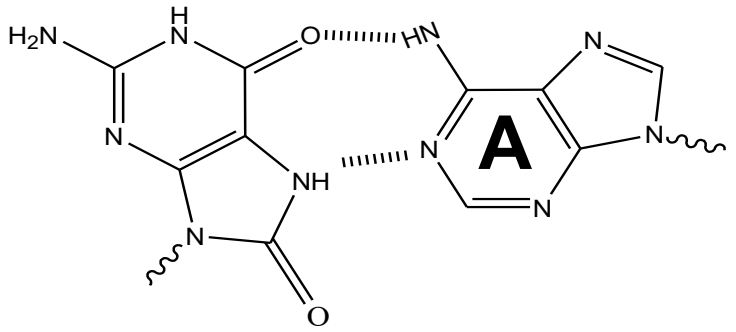
- How does H_2O_2 induce DNA damage? What type of damage?

- How does H_2O_2-induced DNA damage lead to mutations?

Follow-up for question re: base rotation

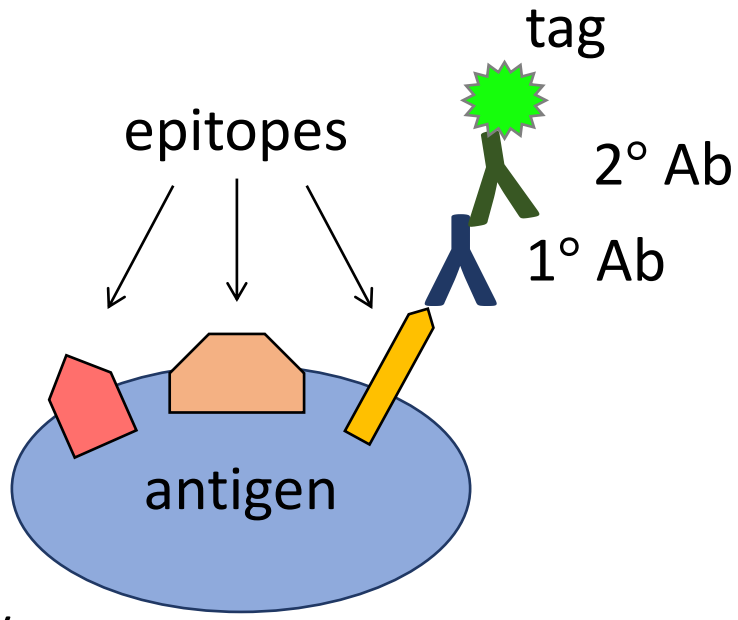






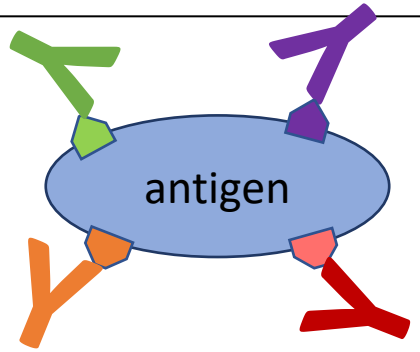
The language of antibodies

- Antibodies bind specific epitopes on antigens
- Primary antibodies recognize antigen
  - Specific protein sequence
  - Specific protein conformation
  - Specific protein state (i.e. phosphorylation)
- Secondary antibodies recognize primary antibody
  - Often conjugated to tag for visualization
  - Amplifies signal through multiple bindings



How are antibodies generated for research?

Polyclonal

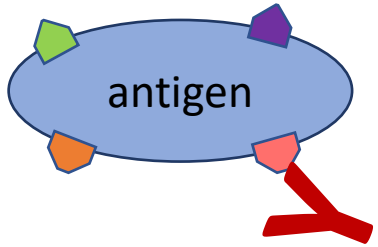


Production: animal injected with antigen and sera collected, affinity purified

Advantages: less expensive, more robust recognition

Disadvantages: variability between collections, mixed population

Monoclonal

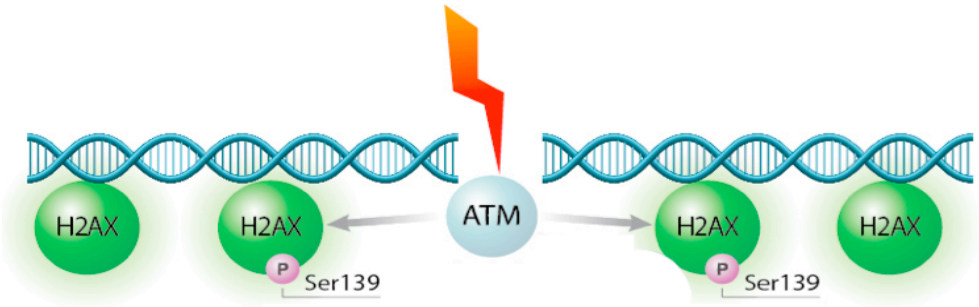


Production: B cells from injected animal harvested, fused to myeloma cell to create hybridoma

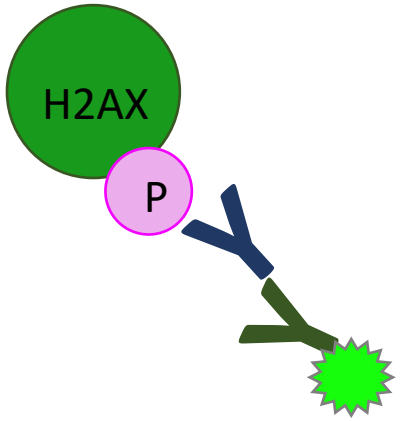
Advantages: consistent, specific to epitope

Disadvantages: expensive, specific to epitope

DSB visualized using immunofluorescence (IF)

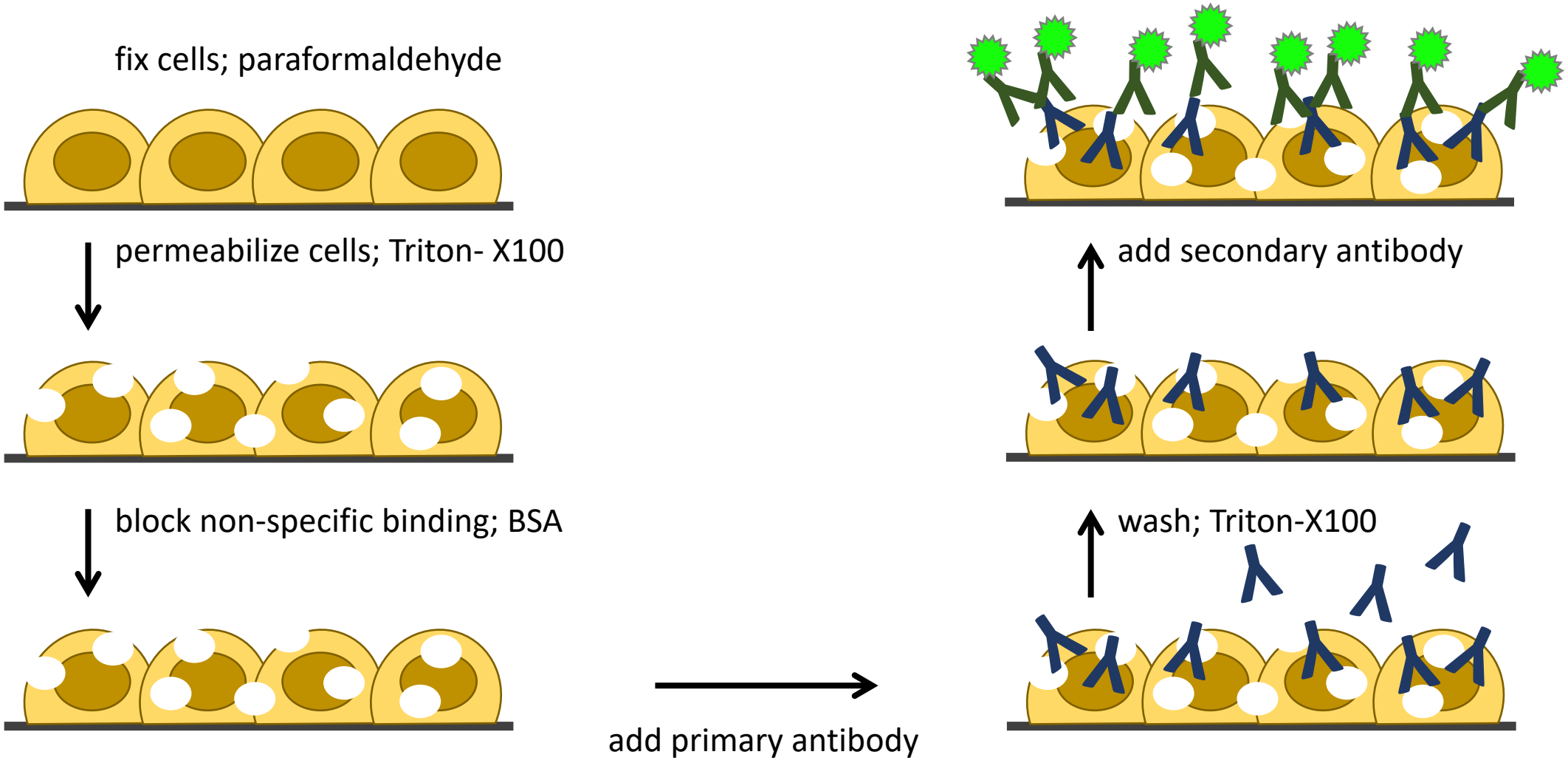


- Antibodies used to specifically tag phosphorylated H2AX histones (γH2AX)



- 1° Ab = mouse anti-human anti-γH2AX
- 2 ° Ab = goat anti-mouse IgG
- Fluorophore (ex. / em. wavelength) = FITC (488 /525 nm)

Procedure for using IF to quantify γH2AX foci?



For today…

- Group paper discussion
- Work through gamma-H2AX staining procedures / exercises
  - Be sure to record your notes in your laboratory notebook

For M1D4…

- Draft Methods section for protocols used M1D1 – M1D3 (in teams!)
- Visit the Communication Lab before M1D5

Notes on 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 RPMI supplemented with FBS. We spun down the cells and counted them with a hemocytometer. Flasks were incubated in 37 C incubator.”

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.