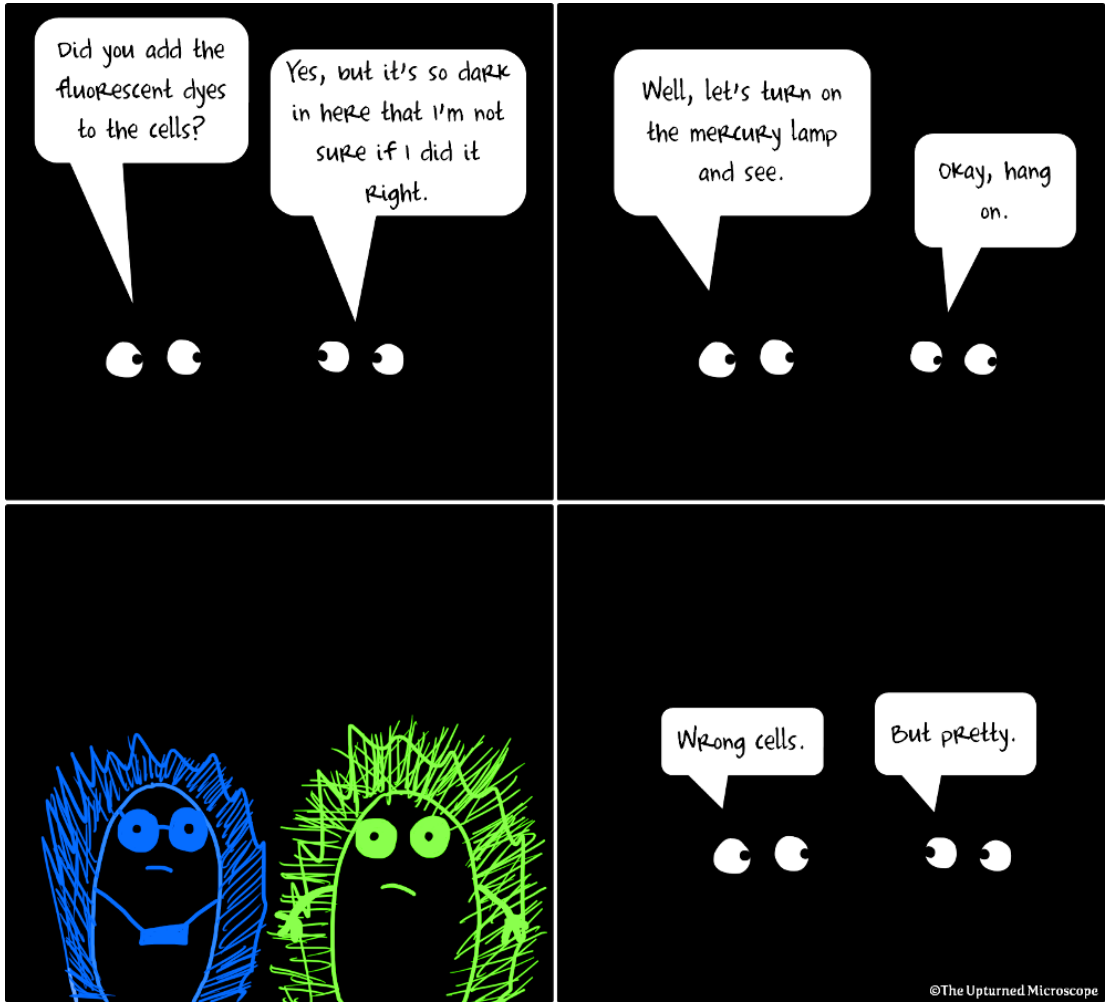
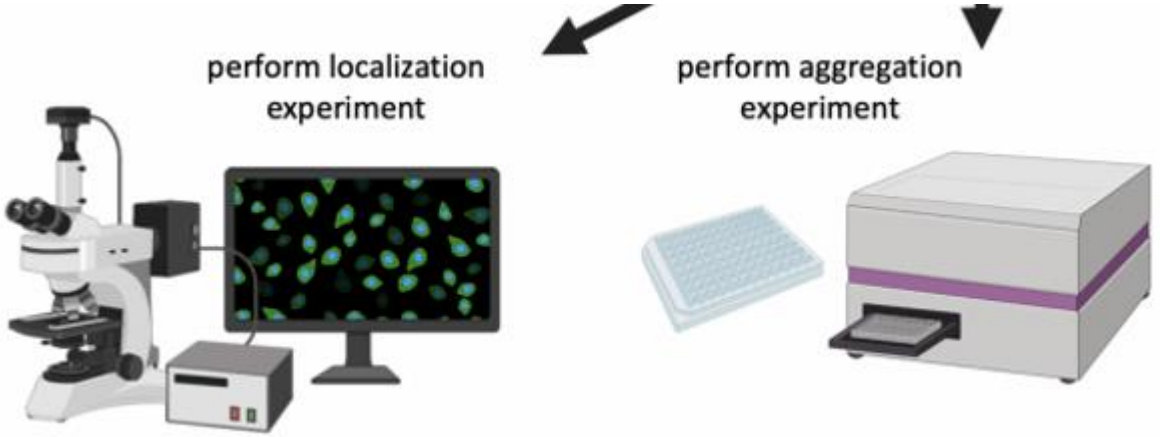
M1D7: Use immunofluorescence staining to assess repair foci experiment

1. Prelab
2. Antibody staining for TDP43 localization

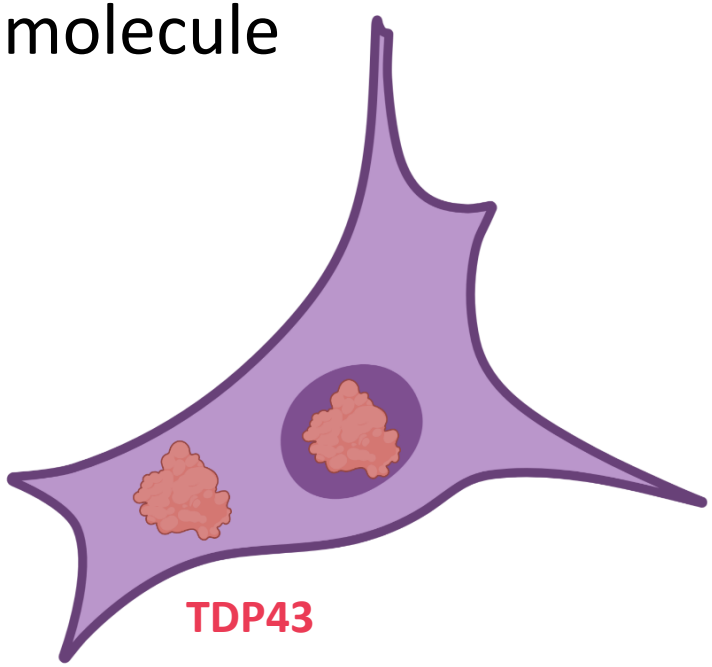


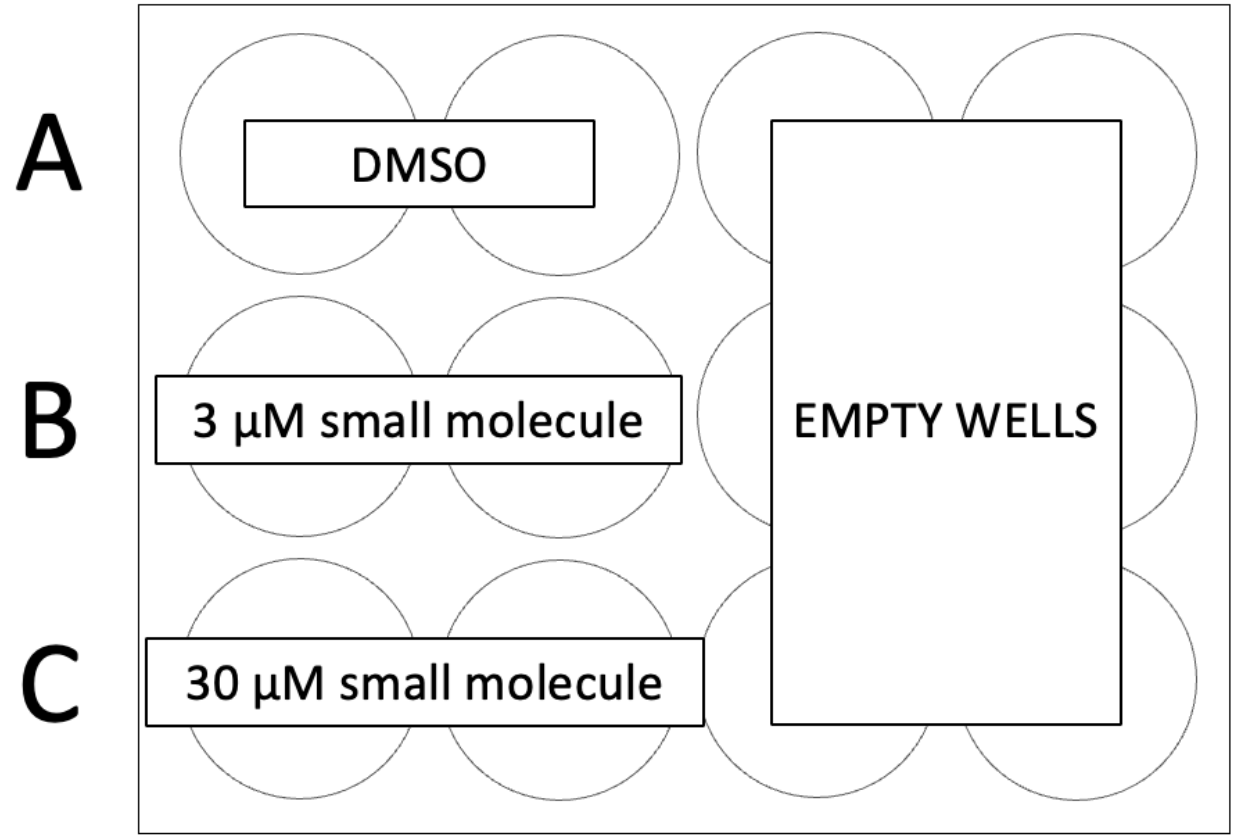
Independent Aims are vital for a smart research project



Using immunofluorescence: Localization of TDP43 in CAD cells

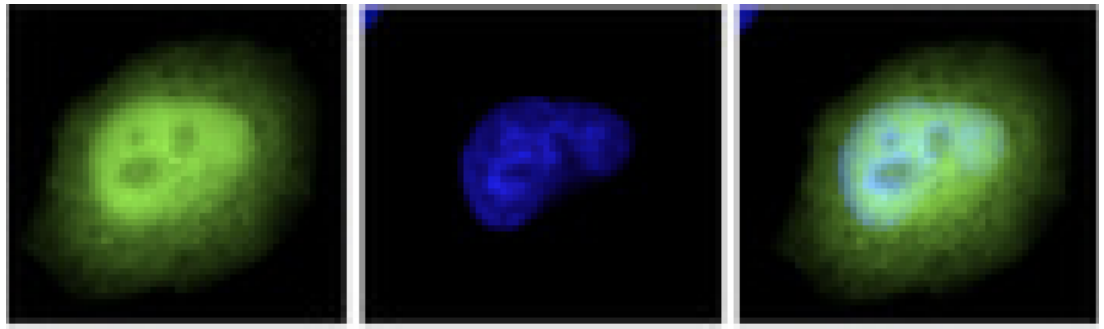
- CAD cells expressing endogenous TDP43 are treated for 1 hour with small molecule





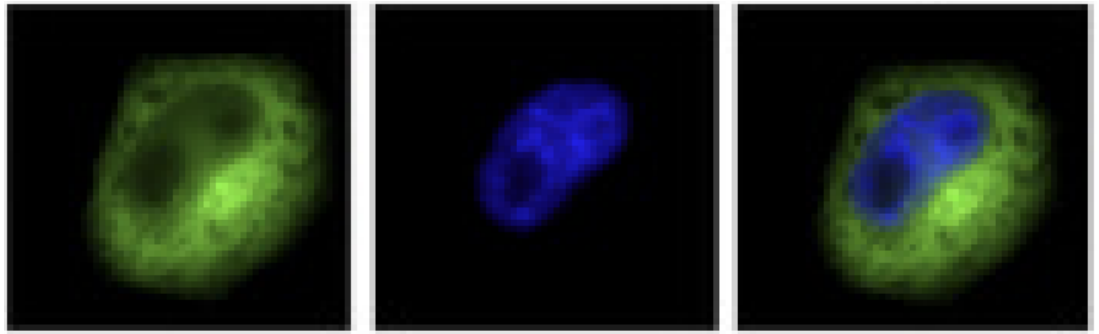
Using immunofluorescence: Localization of TDP43 in CAD cells

Condition 1:

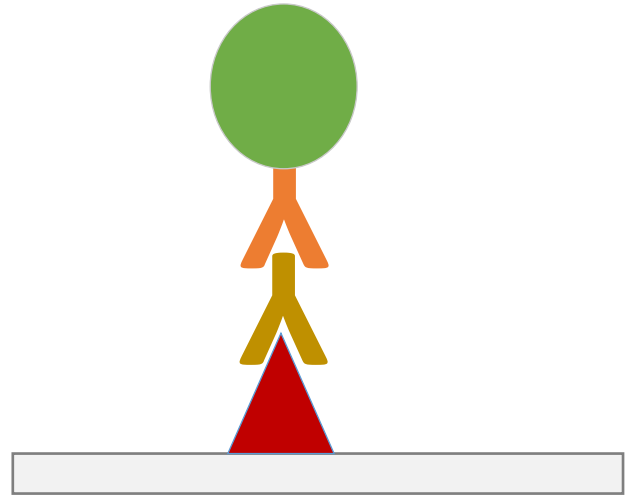


TDP43 DAPI Merge

Condition 2:

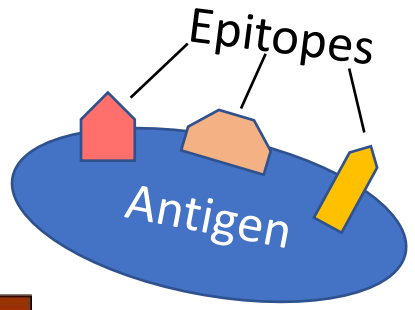


TDP43 DAPI Merge

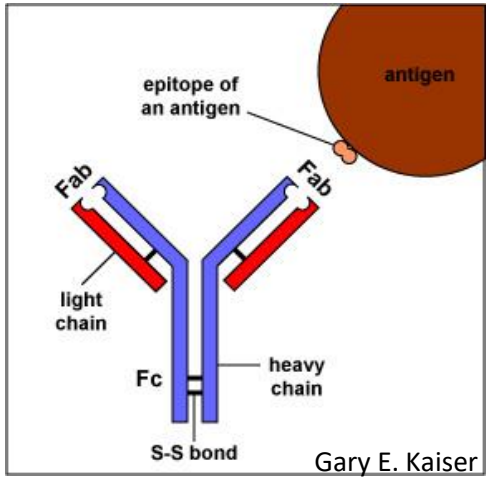


protein of interest	TDP43
primary antibody	rabbit anti-mouse anti-TDP43
secondary antibody	goat anti-rabbit
Fluorophore (conjugated to secondary antibody) exc./ em. wavelengths	488/525 nm

Considerations for using antibodies in the lab

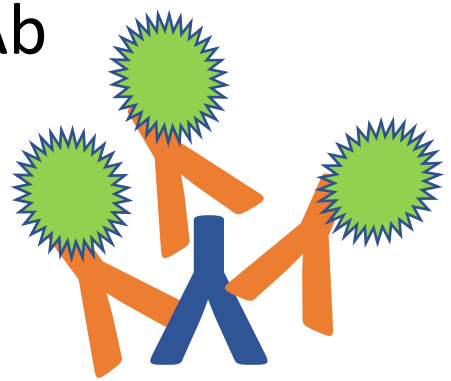


- Antibodies bind to specific epitopes on antigens
  - Antigens may have multiple epitopes



- Primary 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!



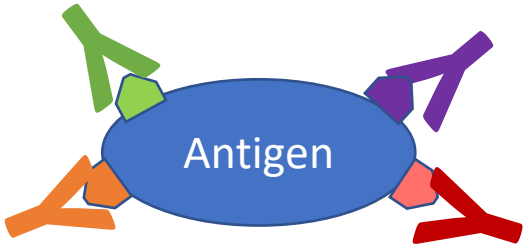
Secondary antibody conjugated to a fluorophore

Primary antibody

Polyclonal vs. monoclonal antibodies

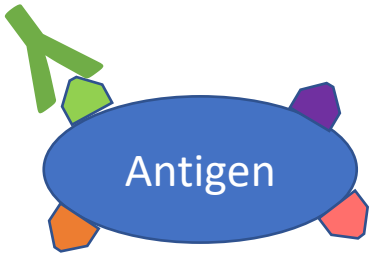
Polyclonal

- 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

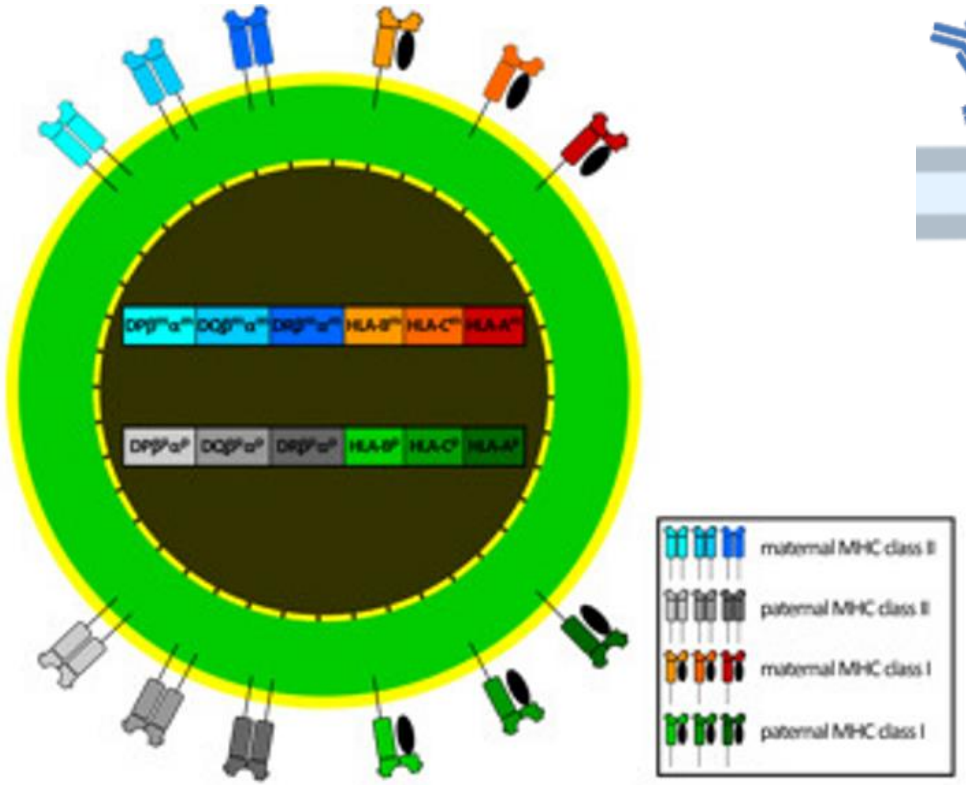


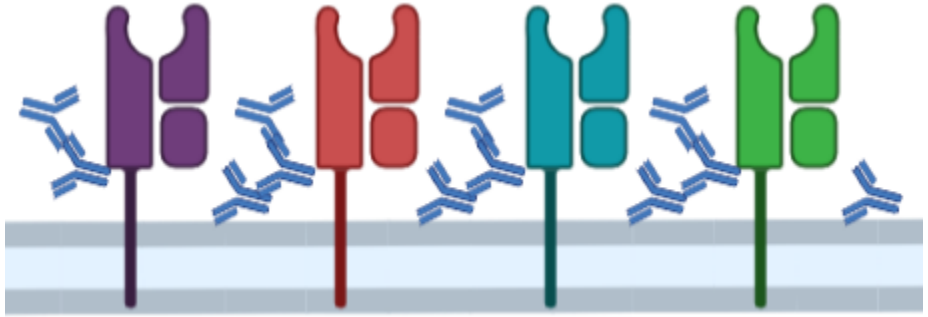
Monoclonal

- 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

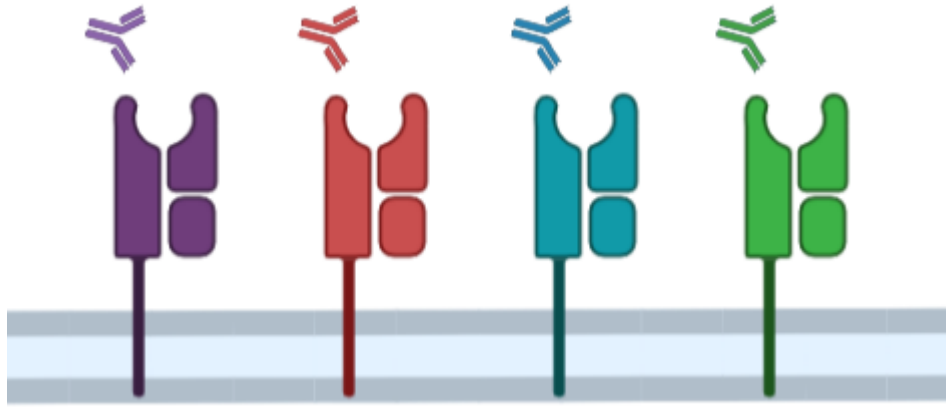


Case Study – MHCI (HLA in humans)





Polyclonal antibodies can target many MHCI proteins

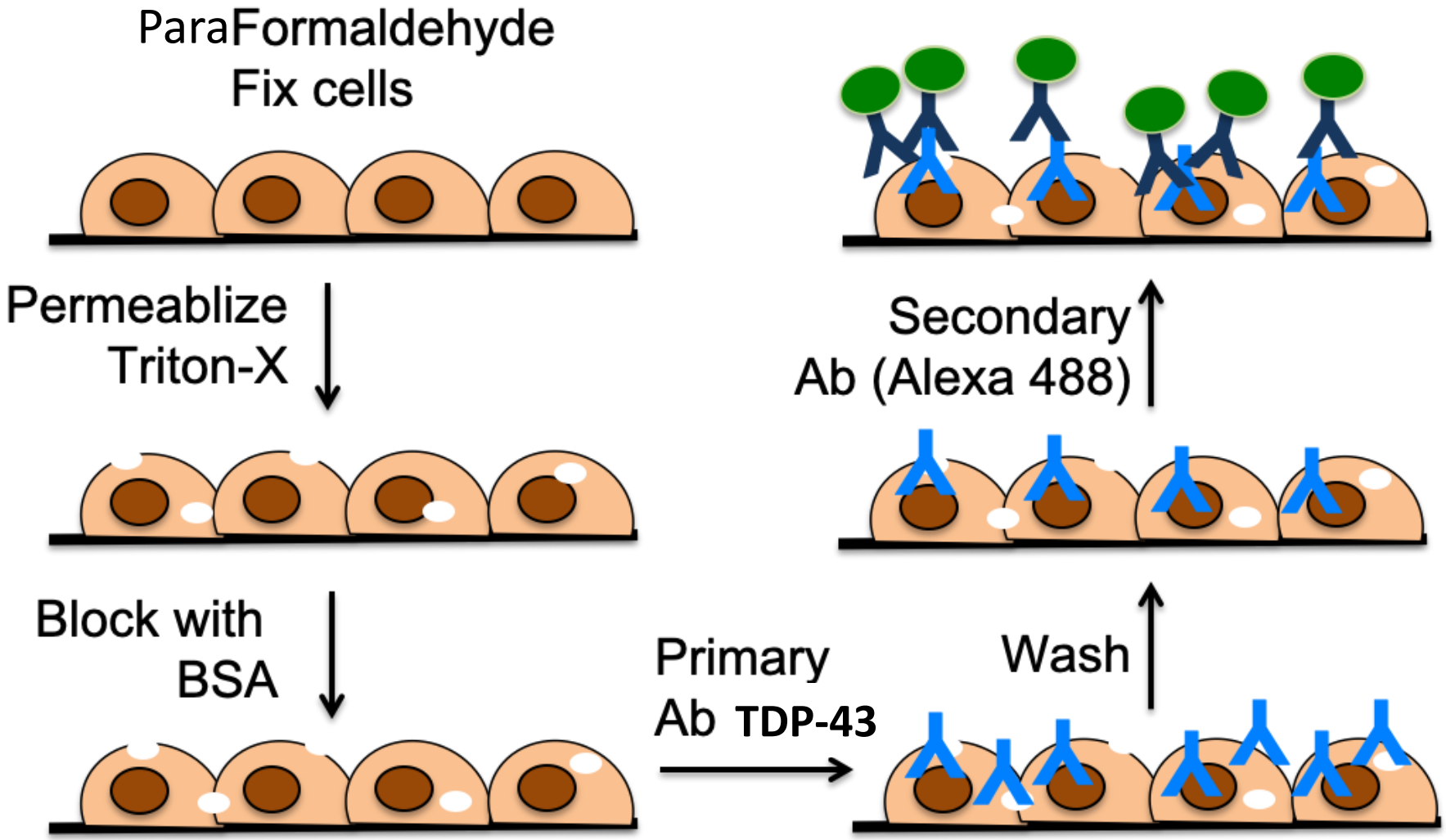


Monoclonal antibodies can target specific MHCI proteins

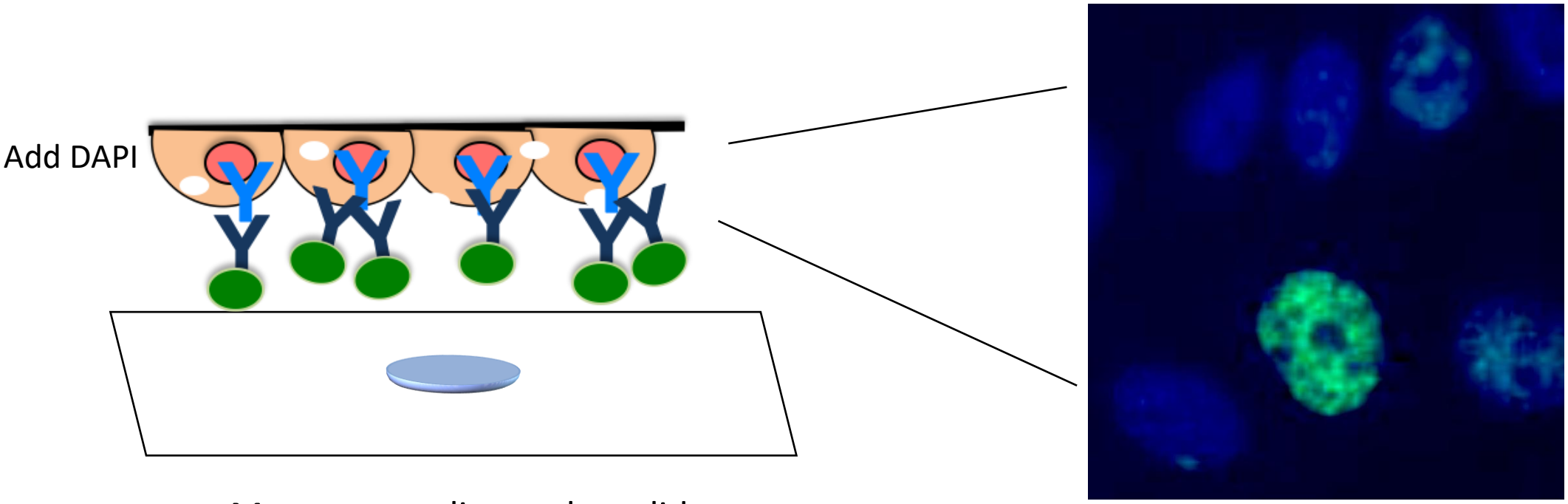
Optimizing Fixing & Staining is quite the decision tree

Item	Options
Fixation Buffer	**Paraformaldehyde**, Methanol, Ethanol….
Permeabilization Buffer	**Triton-X,** Tween…
Blocking Buffer	**BSA,** HI Goat Serum, Proprietary Formulas…
Primary	Mouse, Goat, **Rabbit**, Chicken, IgG, IgY, polyclonal, monoclonal… Invitrogen? Abcam? UCD? Avoid Matching Blocking Buffer Host…
Secondary	**Goat**, Mouse, Rabbit, Chicken-anti primary
Mountant	**Wet mount**, Dry Mount…

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= antibody staining

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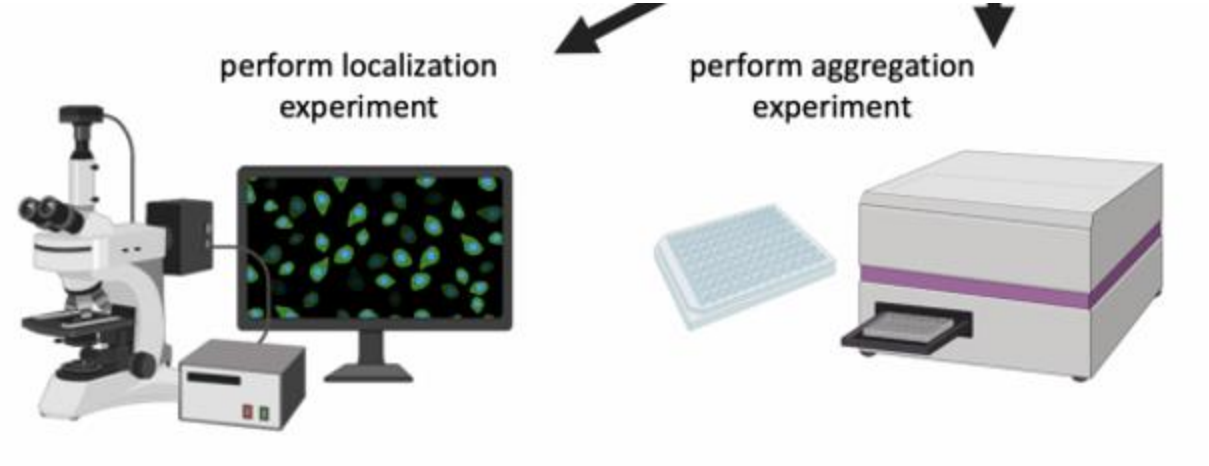

	SM prevents mislocalization	SM does NOT prevent mislocalization
SM stops aggregation		
SM does NOT stop aggregation		

For today:

1. Complete IF staining for TDP43 Localization
    1. Tyler will demo staining chamber assembly
2. Work on Methods revision with partner

For M1D8

1. Individually, answer the question prompts for the Implications and Future works section of your Data Summary
2. With your lab partner, revise your methods homework and add M1D4-M1D5