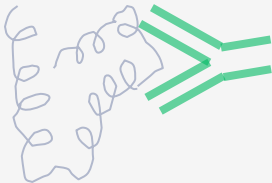


Immunolocalization (Antibody stain): visualize time and place of gene expression at the **protein** level



1. **Generate primary antibody** by injecting protein of interest into animal capable of making antibodies (often rabbit, mouse, rat, goat, donkey)

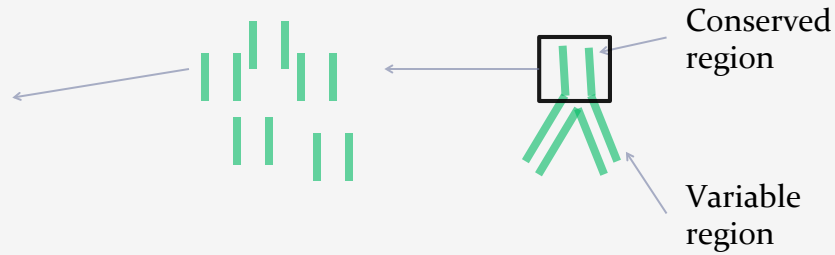


2. Mouse mounts immune response against protein, generates antibodies



3. Harvest primary antibodies from blood

Immunolocalization (Antibody stain): visualize time and place of gene expression at the **protein** level



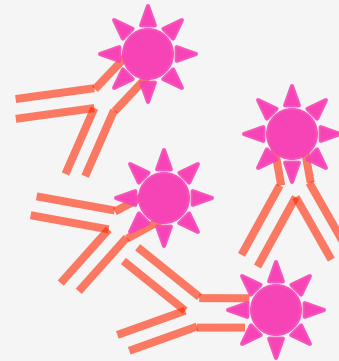
1. **Generate secondary antibody** by injecting conserved region of mouse antibody into a different animal capable of making antibodies



2. Rabbit mounts immune response, generates antibodies against conserved region of mouse antibody



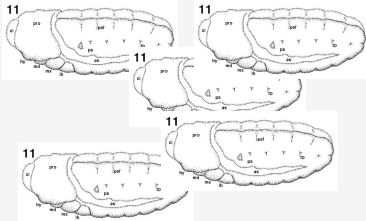
3. Harvest secondary antibodies from blood



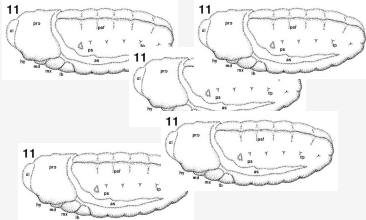
4. Covalently attach fluorescent protein

Antibody stain procedure

1. Live fly embryos

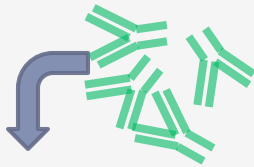


formaldehyde

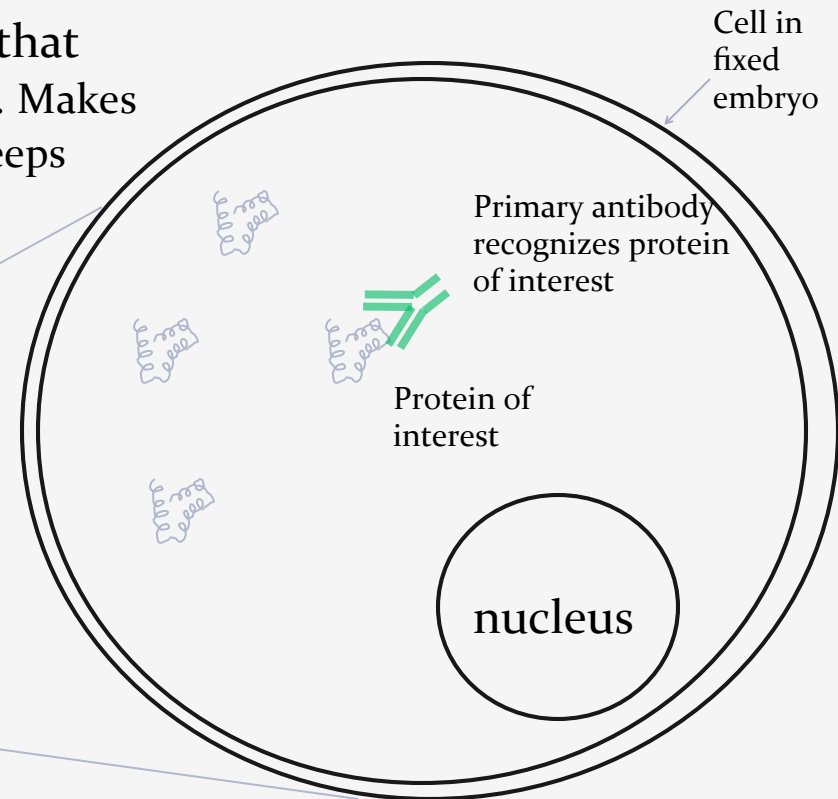


2. Fix tissue with chemicals that cross-link proteins together. Makes tissue “jelly-like”, less fragile, keeps mRNA in place

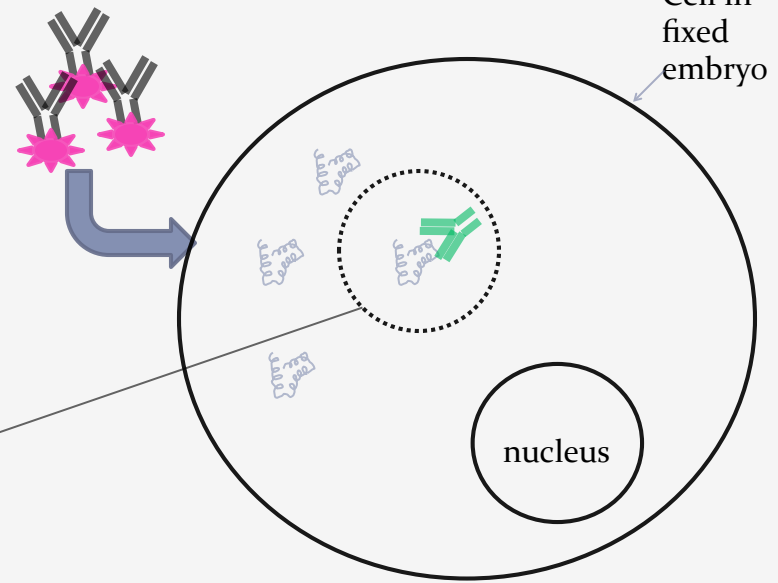
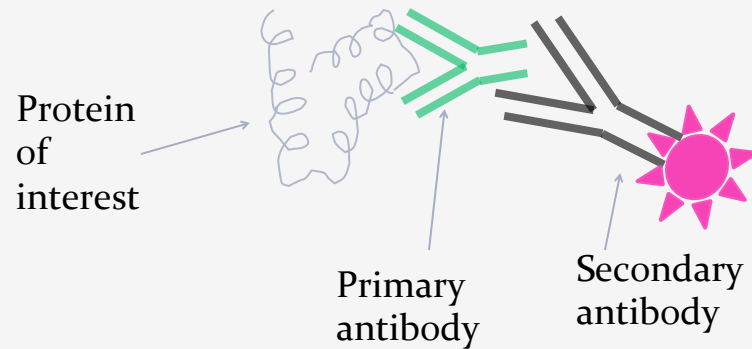
3. Add primary antibody to fixed tissue



4. If a cell is expressing the protein of interest, the primary antibody binds to it.



5. Add secondary antibody that recognizes the primary antibody



6. Secondary antibody has fluorescent label attached to it for visualization. Cells that are expressing the protein of interest will therefore fluoresce.

Note that this picture could be an in situ or an antibody stain: protein and mRNA are usually expressed in the same cells (sometimes mRNA is not made into protein, or only made into protein later in development)

