Blotting

Western Blot

Major Chemicals:

- Beta-Mercanthapal- Used to reduce disulfide bonds, smells very bad
- DTT(Dithiothreitol)- Used to reduce disulfide bonds
- EDTA- major chelator of ions, used to chelate all divalent ions, slows down protein and other chemical damage, also can be used to help dissolute tissue
- SDS(Sodium Dodcyl Sulfate)- A detergent(contains a sulfur end) used to linearize proteins and to dissolve lipid membranes
- Acrylamide Gel- Gel is made from acrylamide(potent neurotoxin) making small pores, as opposed to larger gel size made out of agarose which separates DNA fragments

General Procedure (2D-PAGE)

1. Proteins are first isolated from cells using a combination of EDTA, SDS, and other compounds. Proteins are **isoelectrically** focused in a PH tube(What happens when pH is below the isoelectric point? Is it positive or negative?)
2. After being isoelectrically separated, proteins are run through PAGE. Depending on distance traveled, protein weight can be determined.
3. Once proteins have run through PAGE, proteins are blotted to a nitrocellouse membrane.
4. Membrane is then blotted in milk. (Why?)
5. Membrane is then incubated in first primary and then secondary antibodies(Why are there two antibodies?)

General Problems

1. Why would your photographic film be completely black?
2. Why is DNA used in agarose gel while proteins are used in polyacrylamide gel?
3. Proteins often run different distances depending on the time run. How is that protein weight can be determined then?
4. How do think primary and secondary antibodies are made?
5. You run proteins A and B in your gel and find that A travels much farther then B. What is the weight of protein relative to B?
6. Assume you have an upright western blot apparatus. Which side would the cathode be on? The anode?

Southern/Northen Blotting

Major Chemicals

- Ethidium Bromide- DNA marker used to identify location/presence of DNA in gel electrophoresis.
- Agarose Gel- DNA is often run through agarose gel which often has many large holes.

General Procedure

1. DNA molecules are isolated through the miniprep. mRNA molecules are isolated using magnetic beads connected to dT nucleotides.
2. DNA are run through a gel. Presence is detected with ethidium bromide under UV light.
3. DNA/RNA is transferred into membrane.
4. DNA/RNA are incubated with radioactive primers.
5. The membrane is washed.
6. Photographic film is put next to membrane to measure locations of radioactivity

General Questions

1. Why is mRNA isolated using dT primers?
2. Ethidium is carcinogenic. Why do you think this is the case?
3. Why is the membrane washed?
4. What is the photographic film made out of?