PBC Recitation 5

I. Today in lab
   a. Set up and run gel
   b. Go to recitation
   c. Set up and run Western blot.
   d. Block Westen blot.

II. Electroblotting
   a. Transfers gel to membrane using an electrical current
      i. Nitrocellulose membrane used in PBC has high affinity for any and all proteins.
      ii. PVDF used in some applications.
   b. Pioneered by a guy named Southern for use with DNA
      i. Southern blot DNA blot. Detect specific DNA with radiolabeled oligonucleotide probe.
      ii. northern blot RNA blot. Also usually probed with radiolabeled oligo, although other methods are sometimes used. Will be done in DEV section.
      iii. western blot protein blot. May be probed in 2 different manners:
         1. Antibodies (immunoblot)
         2. Radiolabeled protein (Far western blot).
   c. Today s set up:
      i. Create gel sandwich. Refer to diagram in lab manual.
      ii. Place in blotting apparatus containing buffer.
      iii. Buffer components
         1. Glycine pushes protein out of gel. Where have we seen this before?
         2. Methanol helps smaller proteins transfer.
         3. Tris again, as a pH buffer.
   d. Monitoring transfer did the transfer work?
      i. Prestained MW standards if transfer successful, blue bands will show up on blot. Method preferred in 7.02.
      ii. Reversible dye usually Ponceau S. Red in color, binds non-specifically to proteins, washes off with water or any buffer that you ll use.
   e. Blot work up
      i. Blocking step use a cheap and abundant protein (non-fat dried milk (BLOTTO) or BSA) to fill in all sites on blot not taken up by other proteins. Prevents antibodies used in future steps from non-specifically binding to nitrocellulose membrane. (Antibodies are proteins too).
      ii. Primary antibody incubation add an antibody that binds specifically to the protein of interest. In this case, we re using a rabbit polyclonal anti-β-gal IgG.
      iii. Wash gets rid of excess primary antibody.
      iv. Secondary antibody incubation add an antibody that binds specifically to primary antibody. In this case, a goat anti-rabbit IgG antibody is used. This serves two purposes:
         1. The secondary antibody is linked to an enzyme (in this case Alkaline Phosphatase) that allows us to actually visualize the bands .
         2. Multiple secondary antibodies bind to each primary amplifies signal.
      v. Wash especially important. Gets rid of lingering secondary antibody.
      vi. Development Use a substrate for alkaline phosphatase. Enzyme bound to secondary antibody will convert the substrate into a blue precipitate allows visualization of β -gal bands.