Western Blotting

This protocol uses the Bio-Rad blotting system.

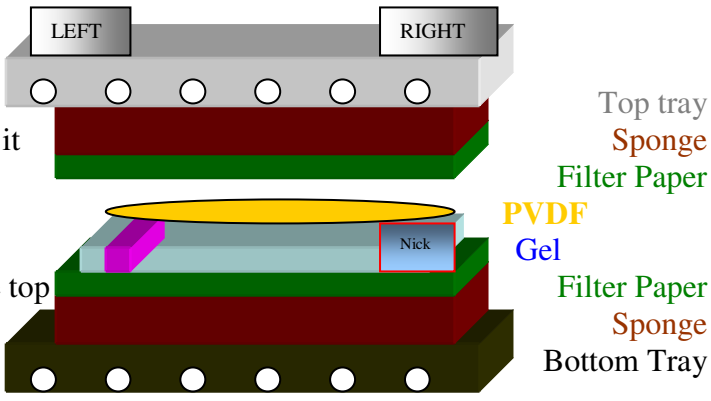
I. Running the Gel:

1. Put protein to be loaded into a 1.5 ml tube, and add Sample Buffer to reach 1x concentration. Boil tubes at 100°C for 5 minutes. Store at –20°C until ready for blotting.
2. In the first lane on each gel, add the protein ladder for molecular weight determinations.
   NOTE: the first lane is defined as the left-most lane looking from the outside of the box to the closest gel. Therefore, looking from the front, the gel in the back should be loaded from right to left.
3. Start the box at 100V. When all samples have run into the gel, increase voltage to 125V. Watch the gel to make sure the lanes are running evenly. Run the current until the dye front reaches the foot of the gel, which takes ~1-2 hrs depending on the percentage of acrylamide in the gel.

II. Transfer

1. Prepare fresh transfer buffer and locate an ice tray.
   1 L 1x transfer buffer:
   100 ml Transfer Buffer Stock (10x)
   200 ml MeOH
   700 ml dH20
   pH should be between 8.3 and 8.5.
2. Cut the top right corner of a PVDF membrane (7x10cm) and label the top left corner with the blot number.
3. Soak the PVDF in MeOH for ~1 minute. Wash with dH20 until the membrane loses its sheen.
4. Put the PVDF into 1x transfer buffer until ready for use.
5. Prepare the transfer apparatus: Add a small stir bar to the bottom and fill the box half full with transfer buffer.
6. Wet sponges and filter paper in transfer buffer. Prepare a sandwich as shown below:

To free the gel from the plastic binding, first remove the foot with a razor blade. Loosen the bindings with the spatula knife, and pull the two sides apart, letting the gel fall to whichever side it will.



Nick the top right corner of the gel to denote the top right side. Carefully transfer the gel to the filter paper (see figure), such that the top right corner is on the right and faces away from the hinge.

Transfer the PVDF membrane, now sitting in transfer buffer, on top of the gel to match the orientation of the gel (nicked corner of gel to nicked corner of PVDF). This will ensure transfer of protein from left to right on the PVDF, with marker on the left and samples numbering up.

Ensure the membrane and gel remain wet, and remove any bubbles in between them. Complete the transfer sandwich with filter paper and sponge, then clamp the tray closed. Close the transfer box, place it on a magnetic plate at 4ºC, and begin transfer.

7. Transfer 1 hr at 350 Amps.
8. After transferring is complete, remove membranes into 5% milk in TBST and block for 1 hr.

III. Antibody Detection

1. Rinse 2x briefly in TBST.
2. Add 1° Ab diluted in TBST for 1 hr (or overnight at 4°C).
3. Wash in TBST 3x10 min.
4. Add 2° Ab diluted in TBST for 1hr.
5. Wash in TBST 3x10 min.
6. Add Chemiluminescent compound onto the top of the PVDF and incubate with agitation.
7. Dab PVDF onto a paper towel to remove ECL, and place membrane protein-up onto saran wrap and cover.
8. Expose onto film: 5 sec, 15 sec, 30 sec, 1 min and adjust time as needed. If the signal is weak, leave one film in over night and develop the next day.

TBST is composed of 1x TBS and 0.1% to 0.05% Tween-20. PBST can be used interchangeably with TBST.

<u>10x Transfer Buffer:</u>
288.4g glycine (1.92 M)
60.5g Tris base (0.25 M)
2L H20