Western Blot Transfer

Prepare TBST and Blocking Buffer

TBST  500mL
50mM Tris pH 7.6  (25mL 1M Tris)
100mM NaCl  (10mL 5M NaCl)
.2% Tween  (1mL Tween)

Blocking Buffer
250mL TBST
12.5g milk powder

Let stir ‘till good and dissolved. Can prepare this early and just let stir and stir.

- Pull out the Criterion blotter, PVDF Immunoblot membrane (use flat-edged tweezers to handle), 2 pieces of Whatman paper, transfer tray.
- Cut membrane to match size of gel.
- Set transfer cartridge into tray w/ black on bottom, red propped up. Add transfer buffer to opposite side of tray. Add sponge to wet, then place as first layer on black side of cartridge.
- Pour some methanol into separate Tupperware. Place membrane in to soak. (If membrane has been mishandled, i.e. touched w/ bare hands, there will be areas that won’t wet. If this is the case, don’t use this membrane)
- Disassemble gel box, clean all components later, save plates for reuse
- Set 2 pieces of adequately sized pieces of filter paper into buffer bath
- Pull stack off gel with tweezers – scoop out – discard
- Pry plates apart w/ green tool – be very careful – gel should stick to one side or the other
- Place soaked filter paper against open side of gel
- Pull gel off other plate and onto filter paper with green tool
- Set onto sponge, gel side up, roll to expel air bubbles
- Remove membrane from methanol w/ tweezers and into transfer buffer bath, then onto gel (place onto gel while holding in a U shape to prevent air bubbles. Roll from middle out to expel any that did form
- Retrieve other piece of filter paper from transfer buffer, lay on top of membrane, roll out
- Wet 2nd sponge, layer it on top of everything else
- Close and latch cassette
- Insert cassette into chamber, red facing out, pour buffer in from tray
- Add ice pack, fill w/ more transfer buffer, put on lid
- Let run 45 minutes at 250mAmps.