Setting up Semi-Dry Transfer

Before removing the gel you ran from your gasket…..

Using the forceps, place membrane (white part, throw out the blue) and 6 filter sheets (in sets of 3 & 3) into Western Blot Buffer. Never touch the membrane with your fingers.
Let soak until wet throughout.

Place down onto the machine 3 filter sheets, then the membrane, then 3 more filter sheets. Roll with glass roller, fairly strongly, to remove bubbles. Use paper towel to absorb excess buffer that went off sides.

Remove your gel from the gasket and glass trays. Cut off the wells. Place into the buffer. Remove the top 3 filter papers and place into the buffer. Put the gel onto the membrane. Put the 3 filter papers back on top of that. *Gently* roll out bubbles. Use paper towel to soak up buffer that went off sides. Add ~1.5 mL transfer buffer dropwise to top of filter/membrane/gel stack.

Run at 0.15 Amp (150 mAmp) for 1 hr/gel (Typically only 2 gels per machine).

When it's done, remove the top 3 filter papers. Check to see that the ruler went onto the membrane. Cut off excess membrane with razor blade that the gel did not transfer to. Block the membrane, gently, in TBST + 5% Milk on the rocker for one hour. Throw away the gel.

*Note: After that hour, if you're busy you can put it in 4^{o} C fridge.

Wipe up remaining liquid, and place a paper towel down between the two main plates before closing up. This is important to prevent corrosion.

Take the baggy, cut two ends off and place your membrane inside. Seal those two edges, but so that the natural hole still exists. Place into it the solution with your antibody. For full mini-gel ~4-5 mL. If smaller portion of gel, 2-3 mL. Antibody diluted into TBST+5% Milk.
Concentration of antibody is variable, but good starting amount is 1 ug/mL.
Work out all the bubbles.
Heat seal the bag, and place in the cold room rocker at level ~20, rocking, overnight.

Next day….

Wash the membrane 3 times for 5 min. in TBS-T.

-Note: Save the milk + antibody that you get out of the bag for future use. Add to it the sodium azide preservative (1000 X). Only add sodium azide once. Each additional use do not re-add.

Put into the 2nd antibody (all of these antibodies (Rabbit or Mouse) are 5000X) in 5% Milk + TBST, and leave on shaker for 1 hr.
Do 3 washes, 10 min. each in TBS-T.

-Note, when you start these washes, take the ECL detection reagents out of the fridge so they can warm up.

Developing procedure:

-Rip 2 strips of cellophane. Place the membrane brighter-face up on the first sheet of cellophane.
-Combine equal parts white and black-topped bottles (1.2 ml + 1.2 ml for one membrane) into a conical tube. Very important to not contaminate the bottles between one another. Be very careful to use new pipettes when going into each bottle.
-Drip with a pipette onto the surface of your membrane. Let stand one minute (White that's sitting, put away the bottles in the fridge).
-Then use tweezers to blot off excess liquid onto a paper towel. Then place face DOWN onto the second sheet of cellophane. Fold edges over. Place membrane face UP onto tray. Tape down.
-Now take the tray, the film, scissors, and timer into dark room.

In dark room…

Cut off corner of film. Place onto the membrane, then shut contraption. Let it expose for various lengths of time. Then after you've exposed it, place film into the machine (NOTE: Lights must be off ANY TIME the film is exposed to light). Both red lights should be on on the machine. Once it beeps, you can turn on the lights.