Western Blots with ECL

I. Run appropriate % gel until dye at bottom with prestained markers in at least one lane.

II. Set up semi-dry transfer apparatus:

1. Cut 4 pieces of Whatmann paper and one piece of PVDF membrane approximately the same size as the gel

2. Float membrane in 100% methanol for 1 minute

3. Shake membrane in 50-100 mls Transfer Buffer (see over) for 5' or until membrane no longer repels Transfer Buffer. Also Soak Whatmann paper in Transfer Buffer until wet.

4. Wet bottom plate of transfer apparatus with Transfer Buffer.

5. Carefully stack two pieces of Whatmann so as to avoid bubbles. Gently roll out any bubbles with a pipet.

6. Next lay the membrane on top of Whatmann with gel on top of membrane. Again gently roll out bubbles (a little Transfer Buffer on top of gel will help avoid ripping)

7. Cover gel with last two pieces of Whatmann and gently roll. (don't press down)

8. Wet top of transfer apparatus with Transfer Buffer and place carefully on top.

9. Run at 150 mA for 1-1.5 hours (shorter time for minigels). Voltage should be ~5 V.

10. When complete, take apart apparatus and place membrane "gel side up" in Block (see below). Markers should be visible on membrane if transfer worked.

III. Blot with antibody

1. Incubate in Block for 2 hrs to overnight with gentle shaking (10-50 mls is sufficient for this and all following incubations depending on size of gel and container. Tupperware or tip box work well for small gels, glass dishes for large gel)

2. Pour off Block and add primary antibody appropriately diluted in LS-TBST. Shake 1-2 hrs.

3. Pour primary antibody solution into conical tube to keep (can be reused ~10x).

4. Wash blot 2 x 1 minute and 2 x 10 minutes with HS-TBST. (shake and then pour off)

5. Add HRP-conjugated secondary antibody appropriately diluted in LS-TBST. Shake 0.5-1 hr.

6. Wash blot 2 x 1 minute and 3 x 10 minutes with HS-TBST as in step 4.

7. Mix 2-5 mls each component of ECL kit (equal volume) together (use separate pipet to draw from each bottle) and immediately add to washed blot.

8. Shake by hand about 1 minute then hold membrane by one corner to let solution drip off briefly. Place membrane "gel side" down onto Saran wrap (NOT Glad wrap) and wrap Saran around membrane being careful not to introduce bubbles or folds (can roll with pipet)

9. Immediately take gel and ECL film to dark room to expose. Typical exposures are 1-5 minutes. Fluorescence decreases over time and will be gone by about 1 hour, so exposures longer than 1 hour don't add to signal.

Transfer Buffer
"Stock" =
2.9 g Glycine
5.8 g Tris base
3.7 mls 10% SDS
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bring up to 800 mls and store at 4 ^{o}C

To make 100 mls of Transfer Buffer use
80 mls of "Stock" plus 20 mls "good" Methanol

Block
5% BSA or Non-fat dry milk
in LS-TBST

LS-TBST
50 mM Tris pH 7.5
150 mM NaCl
0.1% Tween-20

HS-TBST
50 mM Tris pH 7.5
400 mM NaCl
0.1% Tween-20