# WESTERN BLOTTING

1. Equilibrate gel in **blotting buffer** for 20' at room temperature.

2. Make sandwich as follows:

   grey panel
   sponge
   whatman paper
   gel
   0.2 μm nitrocellulose (or nylon)
   whatman paper
   sponge
   clear panel

   Make sure no bubbles are present in any of the layers.

3. Place blotting sandwich in the tank with the grey panel next to the cathode (black).

4. Fill tank with **blotting buffer**, add ice pack and stirbar.

5. Run at 30V; 40 mA overnight or 100V; 250 mA for 1h. Stir buffer throughout run.

6. Disassemble sandwich and either store blot between filter paper at room temperature or use immediately.

**Blotting buffer** (1L)

| | |
|---|---|
| 25 mM Tris pH 8.3 | 3.03 g Tris |
| 192 mM glycine | 14.4 g glycine |
| 20% methanol | 200 ml methanol |