Western blot

1. Run gel at 200 V, 50 min.
2. Prepare for transfer of gel:
   a. cut Hybond ECL nitrocellulose membrane to size of gel
   b. cut four pieces of Whatman 3 MM filter paper to size of gel
   c. make transfer buffer (10% methanol in Laemmli buffer, without SDS)
   d. pre-soak Hybond ECL nitrocellulose membrane in transfer buffer (15 min, RT)
3. Set up transfer:
   a. equilibrate gel in transfer buffer (5 min, RT)
   b. wet 2 Whatman 3 MM filter papers with transfer buffer
   c. pour off the majority of transfer buffer from the glass tray which contains the gel
   d. overlay gel with the 2 wet Whatman 3 MM filter papers
   e. invert the tray and peel away the paper and adherent gel
   f. assemble the transfer on the black side of the cassette:
   g. close and secure the cassette, place within the transfer apparatus and orient correctly – black side of cassette against black side of transfer apparatus.
   h. to the bucket, add:
      transfer apparatus
      stir bar
      ice pack
      transfer buffer
   i. place on stir plate to help dissipate heat during the transfer.
4. Transfer gel at 100V, 30 min.
5. Make Ponceau S: 0.1% Ponceau S in 1% acetic acid
   (0.033 g Ponceau S + 10 ml 3% acetic acid + 20 ml water)
6. Make blocking solution: 3% BSA in TTBS (0.6 g in 20 ml TTBS)
   TTBS = 50 mM Tris, pH 7.4, 500 mM NaCl, 0.05% Tween 20
7. Incubate the membrane in Ponceau S (5 min, RT)
8. Rinse the membrane with water, mark membrane if necessary, and scan the stained blot.
9. Incubate the membrane in blocking solution (30 min, RT, with shaking)
10. Dilute the primary antibodies accordingly in TTBS + 1% BSA, typically 1:2000 and 10 ml is sufficient
11. Incubate the membrane in antibody solution (45 min, RT, with shaking)
12. Remove and save the antibody solution by adding 40 μl of 10% sodium azide and store at 4°C.
13. Wash membrane in 10 ml TTBS (2 times, 2 min/wash)
14. Dilute the secondary antibody in TTBS + 1% BSA, 1:5000 typically (Peroxidase AffiniPure F(ab')2 Fragment Goat anti-Mouse IgG + IgM (H+L), #115-036-068, 4 μl + 20 ml TTBS + 0.2 g BSA)
15. Incubate the membrane in secondary antibody (45 min, RT, with shaking)
16. Wash membrane in 10 ml TTBS (2 times, 2 min/wash)
17. Wash once with 50 mM Tris, pH 7.4 (5 min, with shaking)
18. Develop in freshly made substrate (20 ml 50 mM Tris, pH 7.4, 15 μl 30% H_2O_2, 9 mg 3, 3'-diaminobenzidine)
19 Rinse membrane with PBS to stop development.