IMMUNOLOCALIZATION OF PROTEINS (AP)

This is the Schneeberger/Freeling protocol modified by Miltos, then me, then Jill.

1. Dewax slides 2 x 10' in histoclear.

2. Rehydrate by passing through the following ethanol series (2' each):
   99% ethanol
   99% ethanol
   95% ethanol
   85% ethanol
   50% ethanol
   30% ethanol
   dH_2O

3. Drain slides, place in a large petri dish containing wet tissues around the side. Add 500 µl 100µgml^{-1} proteinase K in **PBS** to each slide and leave at room temperature for 10'.

4. Rinse briefly 3 x in **PBS**

5. Incubate in **PBS/BSA** for 30' at room temperature.

6. Incubate in **PBS/BSA plus 2.5-10% horse serum** for 2 h at room temperature.

7. Rinse in **PBS/BSA** for 15'

8. Drain slides, place back in petri dish and put 50-100 µl 1° antibody on
   each (1/10 – 1/100 dilution in **PBS/BSA**). Cover with coverslip, making sure there are no bubbles over the sections. Incubate overnight at 4°C.

8. Wash 2 x 15' in **PBS/BSA**.

9. Drain slides, place back in petri dish and put 100 µl 2° antibody

conjugated to alkaline phosphatase on each (1/50 dilution in **PBS/BSA**). Incubate for 2 h at room temperature.

10. Wash 2 x 15′ in **PBS/BSA**.

11. Wash 1 x 15′ in **PBS**.

12. Wash in **substrate buffer** for 5′.

13. Drain slides and transfer to a small square petri dish. Flood slides with 100 ml **substrate buffer** plus 200μl 75 $mgml^{-1}$ NBT and 150μl 50 $mgml^{-1}$ BCIP. Cover petri dish with foil and monitor reaction after 15 minutes and then every 1-2 hours as necessary.

14. When signal is clear, rinse slides with 10mM Tris, 1mM EDTA (TE) and then dehydrate back through the ethanol series (2′ each).

15. Air dry, add a drop of Entellan mountant and place a coverslip over sections.

PBS/BSA (1L)

0.15M NaCl	30 ml 5 M
10mM phosphate buffer pH 7.0	10 ml 1 M
0.1% BSA	1 g

Substrate buffer (1L)

100 mM Tris pH 9.5	50 ml 2 M
100 mM NaCl	20 ml 5 M
50 mM MgCl2	50 ml 1 M

NBT

Make up from powder in 50% dimethylformamide

BCIP

Buy solution in dimethylformamide