Qiu lab Immunohistochemistry protocol

(For labeling on mouse brain cryosections)

05/31/2013 (Following successful labeling of 5-HT, TH and GAD67)

1. Perfusion of mouse brain with 4% PFA in 0.1M PBS. Post fix in PFA over night.
2. Switch to 15%, 30% PFA sequentially, each for 24 hrs.
3. Section into 45 micron thickness slices using sliding microtome.
4. Wash sections with 0.01M PBS;
5. Blocking for 30 min in blocking solution (0.2% Triton X 100 +5% normal goat/donkey (depending on which species produce the secondary antibody) serum; make sure filter this solution with a syringe filter).
6. Make primary antibody dilution solution in the blocking solution. Use the 2ml color tube to do so, 1.5ml solution for each tube.
7. Primary antibody concentration, 5 ul->1.5 ml, that is 300X dilution for 5HT, TH and GAD67. Incubate brain sections in primary antibody for 24-48hrs.
8. Wash sections with PBS for 3X10 min inside a 6-well plate, keep everything clean.
9. Use another set of 2ml color tube, add secondary antibody (5ul->1.5ml antibody dilution solution), then transfer sections into the tubes.
10. Incubate at 4 C in the secondary antibody for overnight.
11. Wash sections in PBS in the 6 well plate.
12. Mount sections using the Vectashield mounting medium.