DEVELOPMENTAL STUDIES HYBRIDOMA BANK
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Protocol for Otx1 staining

1. Postnatal rats are perfused with 0.1M phosphate buffer and then 2% paraformaldehyde (the Fix).
2. Rat brains are dissected out and soaked in the Fix for more than one hour a 4C, before being transferred to 20% sucrose + the Fix. Leave in fridge overnight.
3. Fixed rat brains are either frozen in OCT (-80C) or sectioned at 25um using the stage-sliding microtome. Brain sections are collected in 4% paraformaldehyde and keet at 4C.
4. OCT frozen brains may be sectioned after 24 hours using the Cryostat (15-20um) and sections are mounted directly to "super sticky" slides (these probably can be replaced by commercially available treated slides such as Superfrost).
5. In either case, sections are washed with excessive PBS (three times, 5 minutes each) before incubating in the Blocker (0.1% BSA, 0.1% Saponin, 10% Goat Serum in PBS) for at least one hour (up to 4 or 5 hours) at room temperature (RT).
6. Incubate sections in primary antibody (5F5) diluted in the Blocker overnight at 4C. (Dilutions: 1:5-1:50 for floating sections and 1:1-1:10 for mounted sections.)
7. Wash brain sections with PBS (four times, one rinse and three 5-minute incubations) at RT (mild shaking).
8. Incubate sections in secondary antibodies diluted in the Blocker at RT for at least one hour (up to three hours). (Dilutions: 1:200-1:500, Cy2-Goat anti-mouse IgG or 1:150-1:300 Texas Red Goat anti-mouse IgG.)
9. Wash with PBS (repeat step 7)
10. Mount with Fluoromount.

DAB Staining

1. Continue after Step 7 in the above protocol. Incubate sections in biotinylated secondary (anti-mouse IgG, Vector Elite kit) diluted 1:1000 in blocker. RT for at least one hour.
2. Wash brain sections with PBS (repeat step 7 above)
3. Incubate in tertiary antibody (A+B solutions diluted in blocker minus azide, 1:500 each). RT for one hour. The tertiary antibody has to be prepared 30 minutes before use.
4. Wash with PBS (repeat step 7 above)
5. Prepare DAB solution: dissolve 10 mg DAB tablet in 10ml of water (make sure to break large chunks), filter through 0.2 um filter into 10 ml of 0.02M TBS (pH7.4). Add 3ul of 30% H202 to the 20 ml DAB-TBS solution just before use.
6. Incubate washed brain sections in freshly made DAB solution for 3-5 minutes, and stop the reaction by washing with excessive PBS.
7. Wash sections in water, mount to glass slides and let air-dry overnight.