Xu lab protocol and instructions

Indirect immunofluorescent staining

(e.g., Immunochemical Survey of GFP-positive Cells in Transgenic Mice)

General Procedures:

Part I. Section preparation (2 GFP mice)

1. Transcardical perfusion: Flushed with 1x PBS, fixed with 4% para
2. Take brains out and put them into 4% PFA for overnight
3. then transferred to 30% sucrose PBS overnight
4. Frozen cutting brains into 25 um thick sections at a coronal or parasagital plane

Part II. Immunochemical staining

Divide all the sections into different series

Stain cells for both GFP (1:500 chicken α-GFP, Aves Labs, Inc. GFP-1020) and one of the following biochemical contents: GAD65 (1:500 rabbit polyclonal antibody, Chemicon), GABA (1:1000 rabbit α-GABA polyclonal antibody, Sigma A2052);
calretinin (1:500 rabbit α-CalR, Swant #7698), VIP (1:1000 rabbit α-VIP, ImmunoStar), neuropeptide Y (1:500 rabbit α-NPY, Chemicon, AB1915), CCK (UCLA, mouse monoclonal antibody), NOS (1:250 rabbit α-NOS I polyclonal antibody, Chemicon, AB1552), parvalbumin (1:1000 rabbit α-Parv, Swant PV-28), cannabinoid receptor 1 (CBR1, 1:2000 from Dr. Ken Mackie, U of Washington), corticotropin releasing factor (1:2000 CRF, from Wiley Vale, Salk Institute).

For the control series, don't add either GFP antibody or the other antibody.

Steps:

- arrange series of sections in 6-well trays with tube nets.
- rinse sections 3 times in PBS
- blocking [blocker solution for 50 ml: Normal donkey serum 5 ml, BSA, 1 g, Triton X 100, 125 ul, 1xPBS, 45 ml]: incubate sections for 1-2 hours in room temperature (0.4 ml per well)
- incubate sections with the primary antibodies [1:500 Chicken α-GFP (Aves Lab) and an antibody for one content] for 4 hours at a shaker and then moved into the 4 deg fridge for 24-36 hours at 4 ºC. (0.4 ml per well)
- after a couple of quick washes, rinse sections 3 x 10 min in PBS and 2x10 min in work buffer [PBS with 2-5% blocker, e.g.: 5 ml blocker + 45 ml PBS]
- incubate sections in secondary antibodies [1:100 α-chicken conjugated to Cy2, and 1:200 α-rabbit or α-mouse conjugated to Cy3] in blocker solution for 4 hours in darkness (cover trays with foil). (0.4 ml per well)
- rinse sections 3-4 x5 min in PBS-0.1% Tx
- counterstain with 10 uM DAPI for 10 min (to distinguish cortical layers in sections) (to make DAPI solution, add 100 ul DAPI stock solution in 10 ml PBS).
- rinse 2x5min PBS
- wet mount sections on subbed slides, and cover-slip with Vectashield
- or dry overnight, then proceed with dehydration and defatting.
- keep slices in Xylenes until being cover-slipped with Krystalon in a chemical hood.
- examine sections with fluorescent scopes or confocal scope.