MEI Protocol

BrdU Labeling:

1. Check the plugs, and the date when the plug is found is E0.5.
2. Dissolve 5-bromo-2'-deoxyuridine (Sigma) in PBS 5mg/ml. Always make up **fresh** and incubate at 37^0C (approx. 20') for solubilization.
3. Female pregnant mice derived from timed matings are given a single intraperitoneal injection of BrdU using a 26 gauge needle and a 1cc syringe. We normally injected 0.6-0.8ml each mouse.
4. After 2 hours, one, two or three days according to your study, embryos are removed, fixed in 4% PFA from 2 hrs to overnight and sunk in 15% sucrose overnight.
5. The sections are processed and frozen sectioned.
6. Block endogenous peroxidases by incubation with 10% methanol, 3% H2O2 in PBS, 30', r.t. Rinse well in PBS: at lease 4X, 5' each.
6. Treat the slides with freshly prepared 0.02% pepsin (Sigma), 0.01 N HCL in PBS in a humidified chamber, 20'. (You can prepare 200 ml of solution and put all of the slides in it, since pepsin is not expensive).

Note-Ding YQ: BrdU is easily detected in young embryos (younger than E13.5) and a little bit hard in older ones (older than E14.5). I usually try two concentrations of pepsin: one is 0.03% and the other is 0.06-0.1%. Sometimes, both are working, sometimes one is working. I do not know why it happens.

7. Asperate off pepsin and denature DNA in 2N HCL, 45'. r.t.
8. Neutralize in 0.1 M sodium borate, pH8.5, 10'.
9. Wash with PBS, 10'.

Note: all subsequent steps utilize VectorStain kits from vector laboratories.

Blocking solutions (normal serum), biotinylated secondary Ab (horse anti-mouse IgG) (DingYQ, it does not matter, rabbit anti-mouse or horse anti-mouse or whichever anti-mouse), and ABC Elite Kit. You can purchase one kit in which all the stuffs are included.

10. Incublate with mouse anti-BrdU IgG (Becton-Dickinson #7580; Dako; Sigma; Ding: Molecular Probes is better) at 1:20 (Ding, 1:100-200, Molecular Probes) in 0.01 M PBS. The PBS contains 0.3% Triton X-100, 0.003% NaN3 and 1% normal horse or rabbit serum according to species of the secondary antibody you used in a humidified chamber, 4 C, o/n. As a control for Ab specificity, leave the PBS alone on one side, without Ab.

11. Aspirate off primary Ab (Ding: This can be reused several times), and rinse in PBS 3'X5.

12. Incubate all slides with biotinylated secondary Ab (1:200; Vector or Jackson Immunoreasearch) in 0.01M PBS containing 0.3% Triton X-100, 0.003% NaN3 and 1% normal serum for 2 hrs, r.t. in a humidified chamber.

13. Preparing avidin/biotin/peroxidase complex (ABC) and it takes approximately 20'. Take the same volume of A and B from ABC kit, mixed together, and dilute with 0.01M PBS into 1:200 (e.g. 5 ul of A and 5 ul of B, and then add 1000 ul PBS), and let sit for at least 15'. During period, A and B solution react and make ABC complex.

14. Aspirate off secondary antibody and rinse in PBS, 3' X5.

15. Incubate sections in ABC, 1 h at r.t.

16. Asperate off ABC, and rinse in PBS, 3'X3.

17. Incubate sections in 0.01 M PBS containing 0.01-0.02% DAB, 0.0003% H2O2 (0.3% stock solution at 4 C). It takes 5-30'. Check the reaction under microscopy.

18. Rinse in PBS for 5', and then dehydrate, clear (xylene), and coverslip. You can do conterstaining with neutral red (red color) or hematoxylin (dark blue).

By ChengZF