Immunofluorescent Analysis of the Mouse Neural Tube
(Protocol from Ben Allen)

Purpose: The purpose of this procedure is to visualize the specification of various neural cell progenitors in the neural tube using Abs directed against specific transcription factors that are expressed in distinct progenitor cell populations.

Protocol:

Day 1:

1. **Cut 12µm frozen sections using Microm HM550 cryostat (-15°C sample temperature, -15°C chamber temperature).**
2. **Let sections air dry completely (at least 20min). N.B.: can store slides indefinitely at -80°C at this point.**
3. **Wash slides 3 x 5 min with 1X PBS in a coplin jar.**
4. **Outline sections with ImmEdge hydrophobic pen (Vector Labs cat. #H-4000).**
5. **Block sections for 1hr at RT with 300µl/slide of IF block solution (1X PBS + 3% BSA + 1% HISS + 0.1% TX-100, pH 7.4).**
6. **Add 200µl/slide of the appropriate 1° Abs diluted in IF block solution.**
7. **Incubate in humidified tray overnight at 4°C. Alternatively, slides can be incubated at RT for 2hrs, although staining intensity will be significantly reduced for some Abs.**

Day 2:

8. **Remove 1° Ab solution, saving limited reagents for re-use.**
9. **Add 300µl/slide of DAPI stain diluted 1:30,000 in IF block solution to each slide.**
10. **Incubate slides 10min at RT.**
11. **Wash slides 2 x 5 min with 300µl/slide of 1X PBS.**
12. **Add 200µl/slide of the appropriate 2° Abs diluted 1:500 (for Alexa Dyes) in IF block solution.**
13. **Incubate slides in humidified tray for 1hr at RT.**
14. **Wash slides 3 x 5min with 300µl/slide of 1X PBS.**
15. **Add 3-4 drops of Immu-mount aqueous mounting medium (Fisher cat. # NC9496381) to each slide and apply coverslip (VWR Micro Cover Glasses, No. 1 ½).**
16. **Let dry for 1hr at RT.**
17. **Store at 4°C.**