Mulligan Lab

Deadpan/Prospero Staining of Larval Neuroblasts

***It is important to use age-matched third instar larvae (with spiracles) for this protocol.**

DAY 1

1. Dissect late Drosophila 3rd instar larval CNS

NOTE: There are several protocols for this. Links for a couple methods are below in the footnote.[1]

2. Fix in 4% formaldehyde in PBS for 20 mins at room temp.

3. Quick rinse in PBST followed by 3 washes in PBST (1X PBS + 0.1% Triton X-100) for 20 minutes at room temp.

4. Block non-specific binding sites and permeabilize cell membranes by incubating tissue in PBST + 5% BSA for 1 hour, rocking at room temperature.

5. Incubate in primary antibodies (mouse anti-Prospero; 1:100 and rat anti-Deadpan; 1:50) rocking at 4˚C overnight.

Prospero	6uL (for 2 tubes)
Deadpan	12uL (for 2 tubes)
PBST	582uL (for 2 tubes)
Total in tube	600uL

[1] https://www.youtube.com/watch?v=Y8py_dPMtao

https://www.youtube.com/watch?v=9WlloxxFuy0

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2504453/

DAY 2

1. Rinse 3x for 5 minutes in PBST at room temperature, followed by two 30 minute washes at 4˚C (possibly to equilibrate the tissue to 4˚C as the secondary antibody incubation is at this temperature)

2. Incubate the samples in secondary antibodies (Alexa488 goat anti-rat; 1:250 and Cy3 donkey anti-mouse; 1:200) rocking at room temperature for 2 hours or overnight at 4˚C.

Alexa488	2.4uL (for 2 tubes)
Cy3 donkey-anti mouse	3uL (for 2 tubes)
PBST	594.6uL (for 2 tubes)
Total in tube	600ul

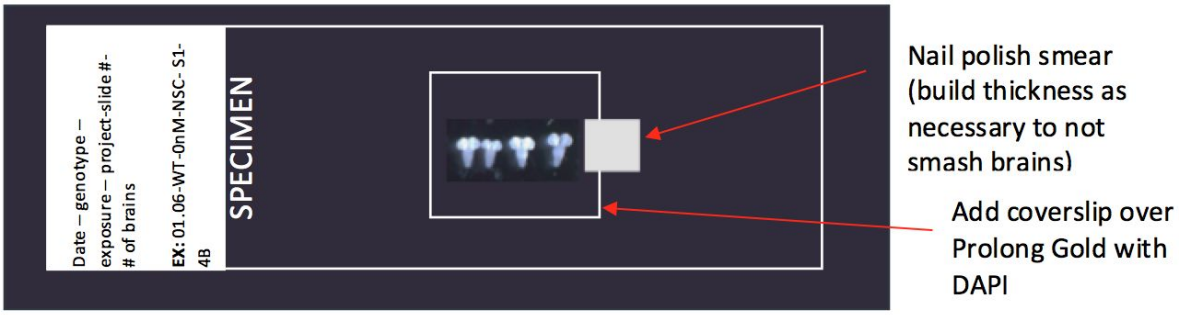
NOTE: Samples need to be protected from light after this point, fluorophores that are constantly

DAY 3

1. Wash brains 2x in PBST for 5 minutes at room temperature. Wash again in PBST 3x for 30 minutes at room temperature.

2. Transfer brains from Eppendorf tubes to dissection dishes and fill one well with 1% PBS-NDS and a second well with 1X PBS. Carefully remove any excess tissue or debris on brains with forceps in the well containing PBS-NDS. Transfer brains to the well containing 1X PBS, and then transfer them to a coverslip that has been treated with poly-l-lysine (from Day 2). Arrange the brains on the coverslip so the back of the ventral nerve cord is facing upward, and remove a small amount of the PBS liquid to ensure the brains adhere to the coverslip. Apply Prolong Gold antifade mounting media containing DAPI to the prepared slide, ensuring the space between the coverslip bridges is filled. Carefully

remove any more excess PBS liquid from the coverslip, and using a forcep, gently lift the coverslip and invert it against the slide so the brains become mounted in the Prolong Gold. Backfill with Prolong Gold underneath the coverslip if necessary. Allow slides to sit in the dark at room temperature for about 15 minutes, then transfer them to a dark box and store at 4 degrees Celsius. Wait at least 24 hours before imaging, as this allows the mounting media to cure properly. Label slides. Link in footnote.[1]



3. Capture immunofluorescent images using confocal microscope. Files should be saved onto the lab external hard drive.

4. Quantify type I and type II neuroblasts using Imaris Bitplane cell counting software.

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[2] https://www.youtube.com/watch?v=SdHapQHJo3Y