Mulligan Lab

FasII Staining of Adult Mushroom Bodies

DAY 1

***Before beginning, check vials to make sure there are flies of appropriate age ready for dissection**

Dissection

1. Prepare 4% paraformaldehyde (PFA) / phosphate buffered saline (PBS) solution. Keep on ice.
2. Prepare microcentrifuge tubes with PBS (as many as needed). Keep on ice.
3. Dissect 0-2 day old adult brains in 1% NDS/ PBS under microscope in glass well plates with sharp forceps.
1. Extract brains with cut micropipette tip and put them into the tube of PBS on ice.
2. Start timer for 25 minutes, dissect brains for only 25 minutes.
    - *If needed continue dissecting brains into new PBS tube and reset time to prevent tissue degradation
    - **Only 6 brains per tube to prevent the brains from clumping

Fixation

1. After 25 minutes is up remove PBS from tubes and add 1 mL of 4% PFA.
2. Let brains fix in 4% PFA for 25 minutes in tube laid on side at room temp.
    - *Make sure all brains are immersed in the PFA and separated from each other to avoid clumping

1st Round of Washes

1. After 25min fixation remove PFA under hood without disturbing brains.
2. Under the hood add 1 mL of PBST (PBS + 0.1% Triton X-100) into each tube for a quick wash.
3. Invert and put tube on rack for a minute or so to allow the brains to settle back to the bottom.
4. Remove the PBST and add 1mL 0.1% PBST.
    - *Now you may leave the hood, to continue the other washes
2. Put the tubes on the nutator for 15 min
3. Perform 2x15min additional washes (3x15min washes total)
4. Add 1 mL of blocking buffer (PBS + 0.3% Triton X-100 + 1% BSA) into the tubes.
5. Put tubes on nutator for 1 hour.

1° Antibody Incubation

1. Prepare antibody solution at 1:20 in PBST
    - *primary antibodies: mouse monoclonal antibody ID4 anti-FasII
2. Remove blocking buffer and add .4mL primary antibody solution to each tube
3. Incubate the tissues overnight at 4˚C (Fridge)

DAY 2

2nd Round of Washes

1. Wash tissues 3X for 20 minutes at room temperature in 0.1% PBST on nutator.

2° Antibody Incubation

1. Prepare secondary antibody solution at 1:1000 in PBST.
    - secondary antibodies: goat anti-mouse Alexa 488
2. Extract PBST from wash, then add .4mL of secondary antibody solution to each tube.
    - **The remaining steps should be done while limiting light exposure.**
3. Incubate tissues with secondary antibody solution overnight at 4˚C **in the dark.**

DAY 3

3nd Round of Washes

1. Wash 6X for 20 minutes in 0.1% PBST at room temperature **in dark** on nutator.

Mounting

1. Prepare slides with nail polish strokes to make barriers to prevent brains from being squished.

2. Once brains are ready for mounting, extract the brains from the microcentrifuge tube using a micropipette with a cut micropipette tip and place them in a well of a 9 well glass plate containing 1% PBS-NDS.

3. Using the micropipette, draw up a few brains from the well under a microscope and place them onto a slide.

4. Orient the brains with antennal lobes facing up under the microscope within the center of the slide slide with forceps.

5. Once the brains are oriented correctly, remove excess PBST carefully using either a micropipette or Kimwipe.

6. Add Prolong-gold over the brains and place a standard coverslip over the mounting area. There should be no gap between the prolong gold and the coverslip.

· **NOTE: Prolong-gold is very viscous and will create bubbles if forced out of bottle, so gently pipette up.

· Backfill with more Prolong-gold if necessary to have coverslip area completely filled.

7. Label the slide with the date of dissection, genotype, exposure, slide number, and number of brains on slide. (ex. 07.14-WT-20nM-S1-3B)