Immunofluorescence of *C. elegans* Gonads

Originally from the Dernburg lab, modified by Aya Sato 2013 Feb

Stock Solutions:

<u>10X Egg Buffer</u>
250 mM HEPES pH 7.4
1.18 M NaCl
480 mM KCl
20 mM EDTA
5 mM EGTA
Filter sterilize and store at room temperature.

<u>NPG-Glycerol</u>
2 g N-propyl gallate (Sigma)
50 ml Glycerol
Place on nutator and dissolve by agitating overnight, and store at room temperature.

Methanol (-20°)

5 M TRIS-base (not pH-ed)

Solutions made fresh the day of use:

<u>EBT (1ml)</u>
110 ml 10X Egg Buffer
10 ml 10% Tween
15 ml Tricane/Levamisole stock
865 ml milliQ water

<u>Fix (1ml)</u> – for 1% formaldehyde final
100 ml 10X Egg Buffer
200 ml 10% Formaldehyde stock
(The stock tube is mistakenly labeled as "8%" but it is actually 10%)
700 ml milliQ water

<u>1X PBST (1L)</u>
100ml 10X PBS
900ml milliQ water
1ml Tween 20 (final 0.1% Tween)

Homemade Mounting Medium (1ml)

50 ml 5M TRIS (not pH-adjusted)
450 ml NPG-glycerol
Mix this solution using a P-1000 tip that has been cut with a razor blade, vortex to mix further, then spin for 1-2 minutes at top speed to remove bubbles.

Before you start: make all your "day of" solutions and place a metal block on dry ice so it starts cooling.

1. Dissect age-matched adults. Pick worms into a 20ml drop of EBT on a No. 1 (18mm^2) coverslip on top of a glass slide. Use a scalpel blade (we use Feather brand #11) to cut the heads and/or the tails off of the worms to extrude the gonad.
2. Pipet 20ml of Fix solution into the drop of dissected worms. Pipetting up and down a few times may help release more gonads. Let worms fix for 2 minutes.
   * The fixation time depends on the antibody you use. Most of the antibodies we use work fine with 2 min fixation but a few (e.g. DPY-30 antibodies) antibodies require harder fixation (final 3.7% formaldehyde) and stronger permealization.
3. Pipet off excess liquid leaving around 15ml remaining. Pick up the drop by lightly touching a HistoBond (75x25x1mm from Lamb) microscope slide to the top of it. Wick away excess liquid from the edges of the coverslip using a torn piece of Whatman paper. The more liquid you remove, the better to worms will stick to the slide.
4. Freeze crack samples. Freeze the sample by placing it on the metal block chilled on dry ice for >5min. Normally I dissect all the worms and accumulate frozen slides on the metal block until I finish dissecting all. Carefully flick off the cover slip by catching the edge with a fresh razor blade.
5. Place the slide immediately in -20°C Methanol for 1 min, then move to a Coplin jar of PBST at room temperature. Repeat the process of flicking off the cover slip and methanol fixation.
6. Wash slides 3x (10 min/wash) by moving slides to fresh Coplin jars of PBST.
7. Block slides in BSA (0.5% in PBST, stock in 4°C) in a Coplin jar for 30 min at room temp. This blocking will prevent non-specific binding of antibodies. Remove excess liquid using Whatman paper (Do not touch the worms!).

8. Primary Antibody. Apply 50-100ml of 1° antibody in PBST (or PBST plus block), cover with a Parafilm coverslip, and incubate in a humid chamber for (2 hours at room temperature or) overnight at 4°C.
9. Remove the Parafilm coverslip by placing each slide in a jar of PBST and letting it float off. Wash slides 3x (10 min/wash) by moving slides to fresh Coplin jars of PBST. During the wash, prepare secondary antibodies. Dilute appropriate secondary antibodies (1/500-1/1000 dilution) in PBST (or PBST plus block) and centrifuge at the max speed

for 10min. Any aggregations of secondary antibodies will precipitate in this step. Use upper phase of the secondary antibody solution for the next step.

10. Secondary Antibody. Remove excess liquid from the slide using Whatman paper. Apply 50-100ml of 2° antibody in PBST (or PBST plus block), cover with a Parafilm coverslip, and incubate in a humid chamber for 2 hours at room temperature (or overnight at 4°C).
11. Wash and DAPI stain samples. Remove the Parafilm coverslip by placing each slide in a jar of PBST and letting it float off. Wash slides 10 min in a fresh Coplin jar of PBST, then move to Coplin jar of PBST plus 0.5mg/ml DAPI (add 5 ml of 0.5mg/ml stock to 50ml PBST in a Coplin jar) and incubate for >10min. Finally, wash slides >10 min in a fresh Coplin jar of PBST. During the wash, prepare mounting medium.
12. a Mount slides with Prolong Gold mounting medium. Remove excess liquid from the slide using the aspirator. Apply 30ul of Prolong Gold at the center of your sample, rotate/tilt the slide so that Prolong (heavy and sticky medium) spread out to cover the entire sample, remove the medium using the aspirator as much as possible. DO NOT SUCK UP WORMS! Pipet 10ul of Prolong Gold onto a clean Matsunami No. 1 ½ (22mm^2) coverslip (0.165-0175mm thickness, tested by Jessie, and plasma-cleaned if needed). Invert the slide and touch it to the Prolong Gold. Invert the slide again, and carefully aspirate off any excess mounting medium. Place a lint-free paper on the bench, invert the slide and place on the paper (cover slip is touching the paper), GENTLY touch (push) the back of slide to squeeze out any excess Prolong Gold. For OMX analysis, it's very important to have your samples as close to the coverslip (and the objective lens) as possible. Dry the slide mounted with Prolong at RT overnight (protected from light). Next morning, seal with clear nail polish (try to draw a THIN line of nail polish and minimize the amount of nail polish on the cover slip).

14.b Mount slides with cheaper (homemade) mounting medium
Pipet 10-15ml of Mounting Medium onto a clean Matsunami No. 1 ½ (22mm^2) coverslip. Remove the slide from buffer, remove as much buffer as possible without dessicating the tissue sample by wiping with a kim wipe and/or aspirating. BE CAREFUL NOT TO WIPE OFF YOUR WORMS!!! Invert the slide and touch it to the mounting medium. Invert the slide again, and carefully aspirate off any excess mounting medium (this step is very important! Suck up extra mouting medium as much as possible.). Seal with clear nail polish.