Dissecting and staining larval tissues

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Materials

Reagents

1) Paraformaldehyde 16% solution, EM grade (VWR, Cat: 15710)
2) DPBS modified (1X), without calcium, without magnesium (VWR, Cat: 82020-066)
3) Sucrose (Fisher Scientific, Cat: S5500)
4) Triton X-100 (Sigma, Cat: T8787)
5) Goat serum (Sigma, Cat: G9023)
6) Hoechst 33342, trihydrochloride trihydrate (Molecular Probes, Cat: H-1399)
7) VECTASHIELD mounting medium (Vector Laboratories, Cat: H-1000)
8) Clear nail polish (Electron Microscopy Sciences, Cat: 72180)

Equipments

1) Dissecting microscope
2) Nutator mixer
3) Forceps (Fine Science Tools, Cat: 11251-30)
4) Fisherbrand superfrost disposable microscope slides (Fisher Scientific, Cat: 12550143)
5) Fisherbrand cover glasses 22X22 (Fisher Scientific, Cat: 12-542-B)
6) Dissecting well
7) Petri dish
8) Thermo Scientific Samco fine tip transfer pipets (Fisher Scientific, Cat: 1371125)

Reagents setup

1) Fixative
   Dilute 1ml 16% paraformaldehyde (PFA) solution with 3ml 1X PBS to a working concentration of 4%. Store @4C. The 16% PFA stock solution should be aliquoted and stored @-20C.
2) 0.2% PBT
   Add 0.2ml Triton X-100 to 100ml PBS. Stir until fully dissolved. Store @ room temperature.
3) Blocking solution
   Add 10ul goat serum to 190ul 0.2% PBT to make 200ul blocking solution. Make right before use.
4) Hoechst solution
   Dissolve 2mg Hoechst 33342 in 1ml ddH_2O to make 2mg/ml stock solution. Protect from light. Store @4C. Before use, add 1ul stock solution to 400ul 0.2% PBT to make 5ug/ml working solution.
5) 25% sugar water
   Dissolve 100g sucrose in 300ml ddH_2O. Stir until fully dissolved. Bring the final volume to 400ml by adding ddH_2O.

Procedures

Collecting, sorting and inverting larvae

1. Pour some 25% sugar water in a small or large petri dish. Scoop larvae-containing food from the vial and place in the sugar water. Larvae will float on sugar water.
2. Fill the dissecting well with PBS. Transfer the larvae from sugar water to PBS using forceps. Sort the larvae if needed (eg. female vs. male, fluorescent markers).
3. Prepare a tube filled with 400ul fixative. Put it on ice.
4. Dissect larvae.
    1) For imaginal discs, brain, and salivary gland, tear the larvae at about one third back from the anterior head end. Discard the posterior end. Remove the protruding guts and fat body from the anterior end. Invert head, being careful not to disturb any discs or trachea (Fig 1). Remove fat body residue from the inverted anterior end. After each one is inverted, place it in the fixative tube on ice.
        Note: When transferring inverted larvae, gently grab cuticle with forceps.
    2) For gut, tear cuticle of the larvae at the very posterior end. Use two forceps to hold both ends of the larvae, pull them toward the opposite direction to extend the gut gently without breaking the gut. Carefully remove the fat body and the anterior cuticle. Put the gut and attached posterior end into the tube with fixative.
5. Fix as many larvae as you can do in 10min, with a maximum of about 10 larvae per tube. If additional larvae are needed, start step 4 again.

Staining inverted larvae

6. Incubate samples in fixative for 10min @RT.
    Note: Wash/incubation = An incubation of samples in liquid for some period of time, during which they should be rocked on nutator.
    Stop point: At this point, the fixed larvae can be stored at -20C until needed. For long-term storage, rinse the samples with 50% methanol in PBS once, then with 100% methanol twice. Store the fixed larvae in 100% methanol @-20C. Note: Some staining may not work in long-term methanol-stored samples (eg. Phalloidin staining)
7. Discard the fixative to PFA waste. Rinse the fixed samples with 400ul PBS twice.
    Note: Rinse = Add liquid, invert tube, let the samples settle, then remove liquid.
8. Wash samples with 400ul 0.2% PBT 2X20min.
9. Incubate samples with blocking solution for 30min @RT.
10. Incubate samples with primary antibody diluted in blocking solution @ 4C overnight.
11. Discard primary antibody solution. Wash with 400ul 0.2% PBT 3X10min.
12. Incubate samples with blocking solution for 30min @RT.
13. Incubate samples with secondary antibody diluted in blocking solution @RT for 1h. Keep the tubes in dark (eg. wrapped in aluminum foil).
14. Rinse samples with 0.2% PBT twice.

15. Incubate samples with 5ug/ml Hoechst solution for 10min @RT. Keep the tubes in dark.
16. Wash samples with 0.2% PBT 3X10min. Keep the tubes in dark.
17. Store @ 4C in 0.2% PBT or mount the interested tissues on slides immediately.

Mounting the interested tissues on slides

18. Fill the dissecting well with 0.2% PBT. Transfer the stained inverted larvae into the well. Dissect the tissues you are interested out (Fig 2).
19. Take a slide, wipe and label it.
20. Use a P20 pipet or a transfer pipet to transfer the dissected tissues from the dissecting well onto the slide. Carefully remove the extra liquid using the pipet or a small piece of Kimwipe.
21. Immediately add 20ul or 30ul mounting medium onto the tissues on slide. Use the forceps to gently position the tissues.
22. Put the coverslip on. Seal with nail polish.

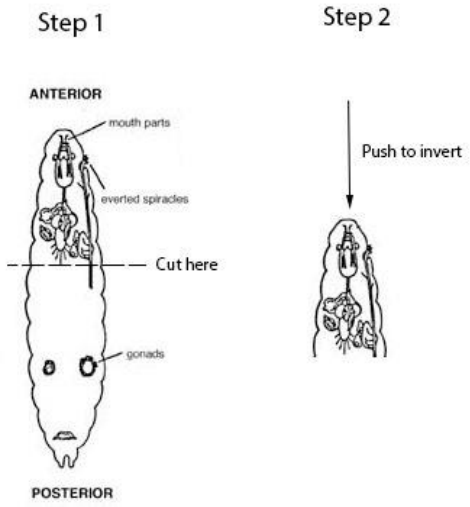

Fig 1. Inverting larvae anterior end for imaginal discs and brain.

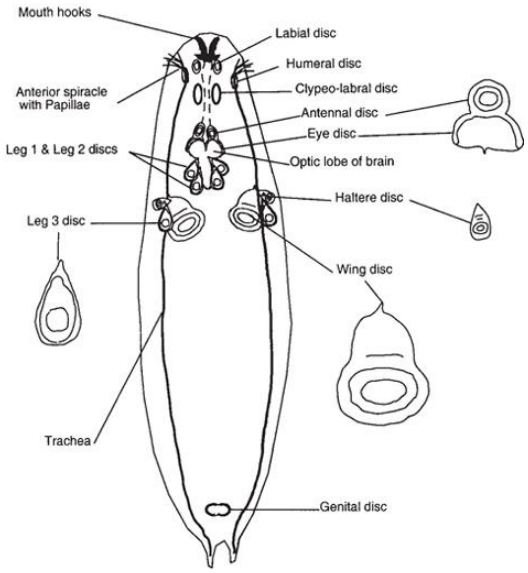

Fig 2. Schematic of third instar larvae showing the location of imaginal discs and brain. (Brigitte de Saint Phalle, 2004)

Reference

This protocol is modified from the one from Ken Irvine lab.