Collecting and Fixing Nuclear GFP/RFP in L1 Larva for Imaging

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[Abstract] In this protocol, L1 stage larvae are collected that carry nuclear-localized GFP/wCherry reporters. These can be fixed so that the GFP/wCherry maintains nuclear localization and stain nuclei by DAPI. This protocol therefore achieves the collection and fixation of nuclei in worm L1 larvae.

Materials and Reagents

1. Acetone
2. Formaldehyde
3. DAPI
4. Poly-lysine
5. Glycerol
6. Cytoseal 280 (Richard-Allan Scientific, catalog number: 8311-4)
7. Used Qiaquick Spin Column (QIAGEN)
8. 11.58 µm glassbeads (Whitehouse Scientific, catalog number: MS0012)
9. KCl
10. NaCl
11. Na_2EGTA
12. Triton X-100
13. EDTA
14. PIPES
15. 2x modified MRWB (see Recipes)
16. DAPI staining solution (see Recipes)
17. M9 buffer (see Recipes)
18. Tris triton buffer (TTB) (see Recipes)

Equipment

1. Standard bench top microcentrifuge
2. 16-slide glass staining jar (Thermo Fisher Scientific, catalog number: 08-810)

3. Spatula
4. Microscope
5. Glass container
6. Glass coverslip
7. Glass slide
8. 18 x 18 mm glass cover slip
9. 25 x 75 mm glass slide

Procedure

A. Preparing larva

1. Begin with a plate that contains many eggs (100+)
2. Use the spatula to remove any chunks which many retain worms. Be sure to flame between uses so no worms are transferred between plates.
3. Using a spatula, carefully displace and remove the agar from the plastic plate. Place the agar in a 16-slide glass-staining jar keeping the surface with worms facing upwards.
4. Rinse the agar in the glass container three times with deionized water, taking care that water does not directly hit the agar.
5. Using the spatula, place the agar back into the plastic container. Remember which side faces upward!
6. Check under the microscope to make sure no worms are left on the plate. If worms are left, repeat rinses until no worms are left. There should be plenty of eggs (100+).
7. Leave the plate at room temperature (RT) (25 °C) for 2 h.
8. Check that L1 larva has emerged.

B. Freezing and Fixing the Worms

9. Wash the plate with 500 µl M9 buffer and transfer to 1.5 ml centrifuge tube. Repeat twice.
10. Spin 3,000 rpm, 2 min. Remove supernatant taking care not to disturb worms at the bottom. Resuspend with 1 ml M9.
11. Spin 3,000 rpm, 2 min. Remove supernatant taking care not to disturb worms at the bottom. Resuspend with 500 µl M9.
12. Transfer to a used Qiaquick Spin Column. Spin 3,000 rpm, 2 min with lid open.
13. Close Qiaquick column cap and place column and collection tube separated into a bucket. Add liquid nitrogen. The next few steps should be performed as quickly as possible after liquid nitrogen is added.
14. Add 200 µl acetone (-20 °C) to the column and immediately spin 2,000 rpm, 30 sec.

15. Add 200 µl acetone (-20 °C) to the column and place in -20 °C freezer for 1 min. Then spin 2,000 rpm 30 sec.
16. Add 200 µl fresh MWRB/formaldehyde solution (50% 2x modified MRWB, 5% formaldehyde = 100 µl 2x modified MRWB, 100 µl 10% formaldehyde) and let sit at RT for 1 h. Then spin at 2,000 rpm, 30 sec.
17. Add 200 µl TTB and spin 2,000 rpm, 30 sec. Repeat to remove all formaldehyde.
18. Add 200 µl DAPI staining solution. Let sit at RT for 1 h. Spin 2,000 rpm, 30 sec.
19. Add 200 µl TTB and pipette up and down to resuspend the worms in the solution. Transfer to 1.5 ml centrifuge tube to collect worms.

C. Preparing the slides

20. Add 75 µl of .5% poly-lysine (in H_2O) to a 18 x 18 mm glass cover slip. Cover using plastic dish lid and let sit at RT for at least 30 min.
21. Recollect excess poly-lysine.
22. Wash cover slip using distilled H_2O and air dry.
23. Add a drop of well suspended 11.58 µm glass beads (in acetone) onto the treated surface of a 25 x 75 mm glass slide. Air dry.

D. Mounting the Worms

24. Add 20 µl of worms in TTB to the poly-lysine treated side of the coverslip. Leave at RT for 30 min to allow worms to stick to the coverslip.
25. Remove as much TTB as possible but observe this removal step under the microscope to make sure most worms are stuck to the poly-lysine.
26. Add 75 µl 50% glycerol to the coverslip. Carefully remove glycerol from the sides. Add 50 µl 50% glycerol to the middle of the coverslip. This helps to disperse an excess TTB, making the drop closer to 50% glycerol. Remove excess liquid from the sides.
27. Place slide over the cover slip and seal with Cytoseal 280.
28. Place in a slide holder and refrigerate until use.

Recipes

1. 2x modified MRWB
   160 mM KCl
   40 mM NaCl
   20 mM Na_2EGTA
   10 mM Spermidine HCl
   30 mM PIPES (pH 7.4)

2. TTB (Tris triton buffer)
   100 mM Tris-HCl (pH 7.4)
   1% Triton X-100
   1 mM EDTA
3. M9 buffer
   Refer to: common worm media and buffers
4. DAPI staining solution
   100 µl M9
   100 µl deionized H_2O
   0.1 µl 1 mg/ml DAPI

Acknowledgments

This protocol has been adapted from Rigaut *et al.* (1999) and Puig *et al.* (2001).

References

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2. Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M. and Seraphin, B. (2001). The tandem affinity purification (TAP) method: a general procedure of protein complex purification. *Methods* 24(3): 218-229.