General Whole mount ICC Protocol for Worms L1-Adult

Remember to have a negative control which is not exposed to primary antibody but is fixed and permeated so can test whether secondary's signal is real.

1. ~20-100 worms in M9 buffer
2. Wash bacteria away, may need 2 or more washes
3. Spin 2000 RPM, 1 min. Aspirate and add 1 ml Witches Brew 1-4% Formaldehyde (add fresh).
4. Freeze thaw in liquid N2 twice.
5. 30 min @ 4 C rocking or shaking to fix.
6. 2X wash in Triton buffer.
7. Shake worms at 37 C for 1h in 1 ml Triton Buffer + 1% β-ME
8. Wash in 1X Borate Buffer.
    a. Wash in 1X PBS to get pH right for RNase treatment
    b. Dilute RNase A/T1 Mix (thermo scientific) 1:100 in PBS incubate at 37 degrees for 2 hours
    c. Wash in 1X Borate Buffer
9. Shake worms at RT for 15 min in 1 ml Borate Buffer + 10mM DTT.
10. Wash in 1 X borate buffer.
11. Shake worms at RT for 15 min in 1 ml Borate Buffer +0.3% H202 (Sometimes caps pop at this step so you can lock them or put parafilm around the top).
12. Wash in Borate Buffer
13. Incubate while shaking at RT in PBST for 15 min-2 hours (depending on Antibody, usually start with 1h).
14. Add Ab+PBST (dilution has to be determined empirically, usually start with 10X more concentrated than western blot concentration). I try to incubate in as little volume as possible (20 - 50 ul). Incubate overnight @ RT. I've found that gentle agitation helps get a more even staining. I put them in a tube rack on a horizontal shaker and turn it up to maximum. Worms won't be moving too much in 20 ul of liquid.
15. Wash 4X, 25 min incubations in PBST while shaking (put tubes sideways on horizontal shaker).
16. Incubate fluorescent secondary 1-2h @ RT. The concentration for this step is the hardest to approximate. Sometimes needs to go as high as 1:20, but can also be 1:500. It is really important to add a – control that does not see primary antibody to determine whether secondary's signal is real.
17. Wash 2X 25 min incubations in PBST while shaking (put tubes sideways on horizontal shaker).
18. Wash 2X 25 min incubations in PBST + DAPI while shaking (put tubes sideways on horizontal shaker).
19. Mount worms on slides.

Solutions:

40X Borate Buffer:
1M H_3BO_3
0.5 M NaOH
make sure pH is 9.5, not lower

I put about a 1 ul drop of Tween 20 when I make up 50 ml of the 1X solution. Stops worms from sticking to tubes.

PBST
1X PBS
1% BSA (Pentax Fraction V)
0.5% Triton X
5 mM sodium azide
1 mM EDTA

2X Witches Brew
160 mM KCl
40 mM NaCl
20 mM Na_2EGTA
10 mM spermidine HCl
30 mM Na Pipes, pH 7.4
50% methanol

Add βME to 2% (fresh)

Tris Triton Buffer
100mM Tris Cl pH 7.4
1% Triton X-100
1 mM EDTA