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DNA FISH and DNA FISH followed by immunofluorescence on worms

Preparation of FISH probe.

1. Label probe by nick translation or random priming using fluorescent nucleotides, such as cy3-dCTP, cy5-dCTP (Amersham), or fluorescein-dUTP (Roche).
2. Remove unincorporated nucleotides using a spin column.
3. Ethanol precipitate probe together with 40 µL salmon sperm DNA (10mg/mL).
4. Wash pellet in 70% ethanol, and dry well (air dry or speed vac).
5. Resuspend pellet in 160 µL formamide.
6. Denature probe at 70-74°C, 10 minutes.
7. Chill on ice.
8. Add 160 µL 2X hyb (1part 20X SSC, 2 parts 10 mg/mL BSA, 2 parts 50% dextran sulfate)
9. The probe is ready to use as is and can be stored at –20°C for months.

Fixation of worms for staining the gut or the germline.

1. Pick about 40 animals onto a drop (5 µL) of sperm salts (50 mM PIPES pH7, 25 mM KCl, 1 mM $MgSO_4$, 45 mM NaCl, 2 mM $CaCl_2$) on a microscope slide. Cut to release gonads and gut. Add 5 μL of fixative (2-4% PFA in sperm salts) and fix for 5 minutes in a humid chamber.
2. Place an 18x18 mm coverslip on worms and place the slide on a dry ice block for 10 minutes.
3. Remove coverslip with a single-edged razor blade and immediately place the slide in PBST (1x PBS, I mM EDTA, 0.5% Triton X-100) at RT in a coplin jar.
4. Wash slides in PBST 3 x 10 minutes.
5. Place slides in 70% ethanol for 2 minutes.
6. 80% ethanol for 2 minutes, 95% ethanol for 2 minutes, 100% ethanol for 2 minutes.
7. Air dry. Proceed with FISH.

Fixation of embryos.

1. Bleach gravid hermaphrodites, wash and pellet embryos using standard methods.
2. Add and equal volume of fixative (4% PFA, 1x sperm salts) to the embryo pellet.
3. Pipette 10 μL of embryos to each slide and cover with an 18 mm x 18 mm coverslip. Incubate in humid chamber for a total of 5 minutes from the time fixative was added in step 2.
4. Place slides on a block of dry ice for 10 minutes.
5. Using a razor blade, remove coverslip and immerse slides in RT PBST.
6. Wash slides in PBST 3 times, 10 minutes. Handle slides carefully, otherwise embryos will fall off.
8. Place slides in 70% ethanol for 2 minutes.
9. 80% ethanol for 2 minutes, 95% ethanol for 2 minutes, 100% ethanol for 2 minutes.
7. Air dry. Proceed with FISH.

FISH.

1. Add 10-20 μL of FISH probe and cover with coverslip.
2. Place slide on a 95°C heat block for 3 minutes to denature the sample.
3. Incubate in humid chamber at 37°C overnight.
4. Wash slide in 2X SSC/50% formamide, 5 minutes at 39°C, 3 times.
5. Wash in 2X SSC, 5 minutes at 39°C, 3 times.
6. Wash in 1X SSC, 10 minutes at 39°C, once.
7. For immunofluorescence, proceed to the next section, otherwise wash slides once more in 4X SSC/0.01 μg/mL DAPI. Mount the slides using Vectashield (Vector Labs) and store them at –20°C.

Immunofluorescence.

(This part follows the Meyer lab staining protocol developed by Annette Chan.)

1. Wash slides in PBST.
2. Without letting the worms dry, wick off most of the PBST, add 30 μL primary antibody diluted in PBST, cover sample with a small piece of parafilm. Incubate in humid chamber at RT overnight, or 37°C for 4 hours. (Temperature and duration of incubation is antibody dependent.)
3. Wash slides 3 times in PBST, 10 minutes each.
4. Add 30 μL secondary antibody diluted in PBST, cover worms with parafilm, incubate at RT for 4 hours or 37°C for 1 hour in a humid chamber.
5. Wash slides 3 times in PBST, 10 minutes each. In the last wash, to 50 mL of PBST add 0.5 μL of 1 mg/mL DAPI to counterstain DNA.
6. Mount the slides using Vectashield, and store them at –20°C.

Immunofluorescence followed by DNA FISH on worms

Preparation of FISH probe.

9. Label probe by nick translation or random priming.
10. Remove unincorporated nucleotides using a spin column.
11. Ethanol precipitate probe together with 40 uL salmon sperm DNA (10mg/mL).
12. Wash pellet in 70% ethanol, and dry well (air dry or speed vac).
13. Resuspend pellet in 160 uL formamide.
14. Denature probe at 70-74°C, 10 minutes.
15. Chill on ice.
16. Add 160 uL 2X hyb (1part 20X SSC, 2 parts 10 mg/mL BSA, 2 parts 50% dextran sulfate)
9. The probe is ready to use as is and can be stored at –20°C for months.

Fixation of worms and FISH probe hybridization.

10. Pick animals onto a 5 uL of sperm salts on a microscope slide. Cut to release gonads and gut. Add 5 μL of fixative (2-4% PFA in sperm salts) and fix for 5 minutes.
11. Place a 18x18 mm coverslip on worms and place the slide on a dry ice block for 10 minutes.
12. Remove coverslip with using a single-edged razor blade and immediately place the slide in ethanol for 1 minute (optional).
13. Wash slides in PBST 3 x 10 minutes.
14. Perform immunostaining.

15. After your last wash, crosslink your antibodies to the specimen by immersing samples in 1-4 % PFA in PBS, RT, 10 minutes.
16. Place slides in 70% ethanol for 2 minutes.
17. 80% ethanol for 2 min, 95% ethanol for 2 min, 100% ethanol for 2 minutes.
18. Air dry.
19. Add 10-20 μL of FISH probe and cover with coverslip.
20. Place slide on a 95°C heat block for 3 minutes.
21. Incubate in humid chamber at 37°C overnight.
22. Wash slide in 2X SSC/50% formamide, 5 minutes at 39°C, 3 times.
23. Wash in 2X SSC, 5 minutes at 39°C, 3 times.
24. Wash in 1X SSC, 10 minutes at 39°C, once.
25. Counterstain and mount slides.

Note: Eliminating air drying (and essentially following Tammy's protocol) will preserve structure better but the signal is going to be weaker.