CBG
Max Planck Institute
of Molecular Cell Biology
and Genetics

Steps

1. Prepare slides by covering GoldSeal slides with poly-L-lysine; dry the layer on a hotplate and cook the slides for at least 1h at 110°C. Use a diamond-tipped pen to label the slides;
2. Pick up around 20-30 worms in a 2.5µl MQ water droplet on a poly-Lys glass slide;
3. Cover them with a 10x10mm coverslip, press them gently with a plastic tip till all the embrios come out of their mothers (but don't crush the embryos!) and snap-freeze in liquid N_2;
4. Pour cold (-20°C) absolute MeOH in a prechilled staining jar. After minimum 10min. of liquid N_2 treatment, get the slides out of the liquid N_2, pop the coverslip off using a scalpel and quickly dip the slides in MeOH. Fix for 10min in the -20°C freezer (no more than 5min if it's GFP);
5. Wash 2x in PBS for 5-10min;
6. Wash once in PBS-Tw for 5-10min;
7. Remove the slides, wipe off excess liquid **around** the worm parts and place them in a humid chamber; add 25µl of PBS-Tw + 2%BSA on the worm parts and cover with a square of Parafilm. Alternatively, draw a circle with the Pap-Pen and put the droplet in it. Incubate for 10min;
8. Remove the parafilm, wick off excess solution, add 25µl antibody in PBS-T 2%BSA; incubate for 1h to O/N at RT; don't forget to keep slides in the **dark** if you have fluorescently-labeled antibodies!
9. Wash twice in 1x PBS for 5-10min;
10. Remove the slides, wipe off excess liquid **around** the worm parts and place them in the humid chamber; add 25µl secondary antibodies in 1x PBS (**NO** Tween or BSA) on the worm parts and cover with a square of Parafilm. Incubate for >10min **in the dark**;
11. Wash twice in 1x PBS for 5-10min;
12. Dry off the non-worm parts of the slide; place 1.5µl of mounting medium on the worms; cover with an 18x18 coverslip. Let it sit for 1+ hour at RT, in the dark, to allow the mounting medium to spread under the coverslip; (if you want DAPI as counterstain, add it to the mounting medium);
13. Seal with nailpolish;
14. Store at -20°C for keeping.

Solutions

1. Sigma poly-L-Lys coated slides or GoldSeal slides and Sigma poly-L-Lys 0.1% solution;
2. Worms, forceps;
3. Liq. N_2;
4. COLD MeOH from the -20°C freezer (about 100ml) and a staining jar at -20°C;
5. PBS: 10x prepared as follows: 80gNaCl, 2gKCl, 14.4gNa_2HPO_4, 2.4gKH_2PO_4/1l H_2O; pH adjusted to 7.4 with HCl;
6. PBS-Tw: PBS with 0.05% Tween-20;
7. PBS-T + 2%BSA
8. Antibodies (primary and secondary), Hoechst etc

Bookings

Fluorescence microscope