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Vibratome Sections Protocol:

1. Fix embryos in 4% paraformaldehyde (either overnight at 4C or for 2 hours at room termperature).
2. Mount embryos in 4% low melting agarose/PBS (it is helpful to pre-heat the PBS in the microwave, add the agarose, swirl, and then keep warm at 70-72°C. It can take up to 2-4 hours with occasional mixing to fully dissolve the agarose)
3. Section the blocks on the vibratome (contact Marianne in the EM facility in Gonda)
   Section up to 300 um (antibodies staining does not work as well with sections greater than 300 um thick).
   Found that a higher frequency and lower speed worked best to keep the specimens in the sections.
4. Block sections for at least 2 hours in PBS/0.1% TritonX-100/10% goat serum (at 4C)
5. Wash sections twice with PBST/1% goat serum for 30 minutes each
6. Apply primary antibodies in PBST/1% goat serum and incubate for 1-2 days.
7. Wash sections 5x 30 minutes in PBST/1% goat serum
8. Apply secondary antibodies (in AB blocker?) for 1-2 days.
9. Wash sections 4x 30 minutes in PBST/1% goat serum
10. Do one last wash in PBS.
11. Post-fix sections in 4% paraformaldehyde in 0.1 M PBS for 20 minutes
12. Wash sections 2x 20 minutes in PBS
13. Begin glycerol washes:
    a. 30 minutes in 20% glycerol in PBS
    b. 30 minutes in 30% glycerol in PBS
    c. 1 hour in 50% glycerol in water
    d. 1 hour (or until sections sink) in 70% glycerol in water
14. Mount sections onto depression-well slides and image under the confocal.