Inclusion Gelatin-Sucrose.

1. Take the tissue and fix it in Fixer.

2. Rinse in PBS.

3. Transfer to Sucrose 15% in phosphate buffer. Leave during the day at 4 ° C.

4. Incubate 1/2 to 1 hour at 37 ° C in 15% Sucrose -7.5% Gelatin, previously made liquid in 40ºC water bath (or microwave-melted). .

5. In a suitably sized container (12-well plates), pour a 0.12M-Sucrose 15% phosphate pad -Gelatin-7.5% liquid base: cure at room temperature or set at 4 ° C to go faster.

6. Pour tissue in well and orient as desired with fire-blunted glass pipettes. Make sure that the temperature difference between the two layers of gelatin is not too large so as not to melt the base. Keep at 4ºC for 1h. The hardened block will have the consistency of jelly. Cut around the pieces with a scalpel and unmold the set to make parallelepipeds

7. Stick this block on a piece of carboard with OCT.

8. Cool isopentane (stored at -20 ° C) to -80 ° C in dry ice.

9. Immerse the block for 1 min in isopentane.

10. Keep the block at -80 ° C.

11. Before cutting balance the block between -20 ° C to -25 ° C in the cryostat.

12. Make sections of 7μm to 15μm at a temperature of -20 ° C to -25 ° C. Harvest the cuts on Superfrost + blades.

13. Allow the cuts to dry a good half hour before freezing at -80 ° C