Immunofluorescence
(Frozen Sections)

Tissue Processing Sectioning Procedure

1. Fix tissues in formalin immediately after collection 1hr/mm of tissue (perfused tissue is best)
2. Wash in 1x PBS for 3x10min, RT
3. Put into 5% sucrose (in PBS) o/n, agitating at 4C
4. Transfer into 3:1 mixture of 5%:20% sucrose, wash 1hr, agitating, 4C
5. Transfer into 1:1 mixture of 5%:20% sucrose, wash 1hr, agitating, 4C
6. Transfer into 1:3 mixture of 5%:20% sucrose, wash 1hr, agitating, 4C
7. Transfer into 20% sucrose, wash 1hr (or until sinks; sometimes o/n best), agitating, 4C
8. When ready to embed, pour off half the volume of 20% sucrose, and add O.C.T. medium such that it is a 1:1 ration, allow to mix/ agitate 1hr 4C
9. Make molds out of foil squares (double layered, using 15mL or 50mL tube cap impression); pour 20%sucrose/OCT with tissue in the foil mold, orient tissue as desired, then snap freeze using dry ice and 70%EtOH slurry ***Store blocks at -80°C***
5. Section with Cryostat (~6-10 µm), and proceed to stain OR store at -80°C for < 2 weeks

Day 1 (~2.5 hours)

6. PBS wash for 5 minutes in Coplin jar, RT

7. Block for 2 hours at room temperature with fresh (no longer than 4 weeks old) 1% BSA/0.3% Triton X-100 **OR** 5% heat-inactivated Goat Serum with 0.3% TritonX

8. Primary antibodies overnight (>16 hrs) at 4°C in 1% HIGS/0.3% Triton X-100. Use 50-200 ul of primary antibody solution per slide (sample size dependent). Best if in a level, moist container (blue slide containers, holds 20 slides each, or use tip boxes with 1 inch MQ water inside)

****Do NOT add Ab to your Secondary alone control here!**
Instead, incubate in 1% BSA/0.3% Triton X-100 **or** HIGS/Triton o/n

Day 2 (~5.5 hrs-6.5 hours)

9. Save primary Abs label with concentration and date – use for 3-4wks, keep at 4C

10. PBS wash 3 x 5 min, RT

11. Secondary antibodies for 2 hrs at room temperature in 1% BSA/0.3% Triton X-100 **OR** HIGS/0.3% Triton X-100. [50-200 ul per slide] Use moist container and **KEEP DARK with foil.**

12. PBS wash 3 x 5 min

13. DAPI stain (5000x dilution in PBS) for 5 min (still protect from light)

14. Coverslip with Prolong Gold Antifade or Fluoromount G (Aqueous, anti-fade mounting medium); Wipe off excess media, Seal with nail polish; Let dry at least 1 hour before imaging; store/ dry away from light