Immunofluorescence
Paraffin Sections
(w/ Trilogy)

Tissue Processing Procedure

1. Fix tissues in formalin (or 4%PFA) immediately after collection 1hr/mm of tissue (perfused tissue is best)
2. Wash in 1X PBS for 3x10 min
3. Put in 70% EtOH, process through EtOH steps, Histoclear & Wax (see Manual Processing Protocol)
4. Section embedded tissues to make 6um thick sections

Day 1 (~2.5 hours)

5. Deparaffinize slides:
   2x 10min in Histoclear (save Histoclear and re-use)
   Done using 250 ml of solution in staining buckets with slide rack
   Note – never let your slides dry out!!!!!! Throughout this whole procedure

6. Hydration and Antigen retrieval (Heat Induced Epitope Retrieval):
   a. In **Plastic** Coplin jar(s), place slides in **Trilogy Buffer**, fill additional Coplin jar(s) with Trilogy Buffer (you will need 1 additional jar per jar with your slides); antigen retrieve by placing Coplin jars with slides (and the additional jars with buffer) into pressure cooker for **15 min at HIGH pressure** (Cooker basin filled between the 6 and 8 mark with MQ water).
      i. When pressure cooker is done *(takes roughly 25 minutes)* – quick release the steam, allowing pressure to normalize
      ii. Take off lid, remove Coplin jar(s) – careful! They'll be hot, use oven mitt and set them on bench, remove lids and gently transfer slides from the Coplin jars to the fresh Coplin jars that were also pressure-cooked; place the Coplin jars with the fresh hot Trilogy & slides back into the cooker, close lid, and turn off the power – let incubate additional 5 mins
      iii. Remove Coplin jars from cooker, place on the bench top, remove lids, and allow them to cool to ~tepid RT; 20min maybe
      iv. Wait for water to cool before discarding from the cooker, put away

7. Wash 1x PBS 1x 5 min in Coplin jar, on bench, RT

8. Block for 1-2 hours at room temperature with fresh (no longer than 4 weeks old) 5% BSA/0.3% Triton X-100 (**OR** 10% heat-inactivated Goat Serum with 0.3% TritonX)
   Best to block by placing slide in a hydration chamber, using hydrophobic pen outline sample wish to stain, and add block solution to it. This saves BSA/ GS. But do this quickly and 1 sample at a time, so that the samples don't dry out!

9. Incubate samples with primary antibodies overnight (>16 hrs) at 4°C in 1% BSA/0.3% Triton X-100 **OR** 5% HIGS/0.1% 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). If used hydration chamber to block – simply aspirate blocking solution, and add desired antibody – no need to re-draw with hydrophobic pen.

Primary Antibody	Concentration
Pax 5	1:200
F4/80	1:400
E-cadherin	1:500

****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

<u>Day 2</u> (~3.5 hrs-4 hours)

7. Save primary Abs label with concentration and date – use for 3-4wks, keep at 4C
8. PBS wash 5 x 5 min, RT
9. Secondary antibodies for 2 hours at room temperature in 1% BSA/0.3% Triton X-100 **OR** 5% HIGS/0.3% Triton X-100. [50-200 ul per slide] Use moist container and **KEEP DARK with foil. (Most secondary antibodies are diluted 1:500)**
10. PBS wash 5 x 5 min with agitation, RT
11. DAPI stain (from 5000x stock → dilute to 1x in PBS) for 5 min (still protect from light)
12. 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