**IMMUNOFLUORESCENCE PROTOCOL**

*INTENDED FOR DOWNSTREAM USAGE IN BiliQML*

DAY 1:

**DEPARAFFINATION:**

1. Incubate the mice tissue slides at 55-60 C in oven for 60 mins.
2. Transfer to Xylene (1st container) for 10min, followed by 10min in 2nd container of Xylene.
3. Transfer to 100% ethanol (1st container) for 5min, followed by 5min in 2nd container of 100% ethanol.
   1. During this step, warm up the steamer/pressure cooker.
4. Transfer to a container containing 75% Ethanol for 5min.
5. Transfer to container with water or 1X PBS (does not matter) – briefly.

**ANTIGEN RETRIEVAL:**

1. Place slides in container with antigen retrieval solution (1x Citrate buffer; Vector Labs #H-300-250) and place container in the pressure cooker (High heat, sealed, 10min)
   1. Ensure that there is water up to 20% in the steamer/pressure cooker pot
2. Place container with slides (hot) on ice, or in container with cool water for 20 minutes
3. Gently add water to container, and rinse slides for ~2-3 minutes.
4. Mark circle around the tissue without touching the tissue with Liquid Blocker Super PAP pen.

**BLOCKING + 1o ANTIBODY INCUBATION:**

1. Rinse with 1xPBST (0.1% TWEEN-20 in 1xPBS) for 3 mins \* 3 times.
2. Rinse with 1xPBST (0.5% TWEEN-20 in 1xPBS) for 1 min.
   1. This is the only time in the protocol to use 0.5% TWEEN-20 in 1xPBS. For the remainder of PBST washes, use 1xPBST (0.1% TWEEN-20 in 1xPBS).
3. Block with 5% Goat Serum + 1% BSA in PBST for 1h at RT.
   1. It helps to block on some type of rotary, or shaking device (on low).
4. Incubate tissue with primary antibody (1-100)(Rat anti-Keratin-19; DSHB #TROMA-III) in blocking solution at 4C overnight.

DAY 2:

**WASHING + 2o ANTIBODY INCUBATION:**

1. Wash tissue with PBST 3X for 5 min each.
   1. It helps to wash on some type of rotary, or shaking device (on low).
2. Incubate with secondary antibody (1-100)(Goat anti-Rat; Alexa FluorTM 647; Thermo #A-21247) for 1h at RT.
3. Wash tissue with PBST 3X for 5 min each.

**DETECTION OF NUCLEI + COVERSLIPPING:**

1. Add DAPI (1:1000)(Invitrogen #D1306) in PBST for 30s-60s.
2. Wash tissue with PBST 1X for 2-3 min.
3. Add 17.5µl ProLongTM Gold Antifade Mountant medium (Invitrogen #P36930) to the tissue.
4. Place rectangular cover glass (22mm \* 50mm) gently on tissue and remove the excess vectashield media.
5. Seal the Coverslip with Nail polish on top and bottom edges of cover glass.
   1. Ensure that the nail polish is clear.
6. Leave it in a place without light until dry.