In vivo Immunofluorescent Analysis

A. Materials

- Paraffin embedded tissue sections on glass slides
- Xylene
- 100, 90, and 70 % Ethanol (EtOH)
- Deionized water (dH_2O)
- Microwave
- Dako Antigen retrieval buffer (S1700 Dako)
- Wax Pen
- 3% Bovine Serum Albumin (BSA) in Tris Buffered Saline (TBS)
- 0.3% BSA in TBS
- 0.05% Tween in PBS (PBS-T)
- ProLong Antifade Mounting Medium with DAPI (ThermoFisher Scientific)
- Coverslips

B. Deparaffinization

- Incubate slides in Xylene 3x 15 min
- Incubate in 100% EtOH 2X 10 min
- Incubate in 90% EtOH 1X 10 min
- Incubate in 70% EtOH 1X 10min
- Wash in dH_2O 2x 5 min

C. Antigen retrieval

- During 90% EtOH wash, heat Dako buffer in slide rack and a 250 mL bottle of dH_2O at a low setting (power setting of 4) for 30 min
- If buffer is at a rolling boil, place slides in Dako buffer and microwave on medium high setting (power setting of 7) for 30 min
    - Make sure antigen retrieval buffer is actively boiling when adding in slides
    - If not actively boiling, increase microwave setting to high until buffer is boiling
- Every 5 Min check to make sure tissue sections are covered by buffer; if the liquid is getting low, fill up container with boiling bottle of water also inside the microwave
- Remove slide rack and container from microwave and let cool to room temperature for 20 min
- Wash slide in PBS 1x 5 Min

D. Block and Primary

- Circle sections with wax pen
- Incubate sections with 3% BSA in TBS for 5 Hrs at RT in humidified chamber
- Wash 2x 5 min in PBS
- Incubated slides with 1:100 primary antibody in 0.3%BSA in TBS overnight at 4°C in humidified chamber

E. Washing and Secondary

- Incubate slides in PBS-T 3x 2 min with shaking
- Recircle sections and incubate with 1:200 secondary antibodies for 1 Hr at RT
- Incubate slides in PBS-T 5x3 min with shaking
- Wipe off wax pen from around the section
- Place a droplet of ProLong Antifade with DAPI mounting medium on each section and place coverslip on top of section
    - Ensure that bubbles are not enclosed on top of the mounted section.
    - If bubbles are observed gently force out the bubbles through pressure, directive bubbles to the edge of the coverslip
- Let cure in dark overnight and clean with 70% EtOH before imaging
- Store at room temperature for short periods of time, and at -20°C for long term