Immunofluorescence, 5-10um paraffin sections (Gillis)

Before you begin: heat one water bath to 60C, and a second water bath to 95C.

Rehydrate sections

Histosol – 5 minutes
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100% EtOH – 5 minutes
100% EtOH – 5 minutes
75% EtOH/PBS – 5 minutes
50% EtOH/PBS – 5 minutes
25% EtOH/PBS – 5 minutes
PBS + 0.1% triton – 3 x 5 minutes

Antigen retrieval

Warm slides to 60 degrees in preheated distilled water for 5 minutes. Transfer warmed slides to pre-warmed antigen retrieval solution (10mM sodium citrate, pH6.0) and incubate for 25min at 95C (important: this step must be done in a plastic slide container!). Upon completion, transfer the slide container to cool in -20 freezer for 30min (or less, depending on size of the container – make sure it doesn't freeze). Transfer slides back into a glass coplin jar and rinse 2 x 5 minutes in PBS + 0.1% triton.

Block

Place slides in a humidified chamber (or pink-top tube). Block with 10% sheep serum (in PBS + 0.1% triton) for 1 hour at room temperature.

1°AB

Dilute primary antibody in block solution. Apply ~150-200uL of antibody per slide. Coverslip with parafilm, and incubate in a humidified chamber overnight at 4C.

2°AB

Rinse 3 x 10 minutes in PBS + 0.1% triton. Dilute secondary antibody in block solution. Apply ~150-200uL of antibody per slide. Coverslip with parafilm, and incubate in a humidified at room temperature for four hours (in the dark).

Rinse and mount

Rinse 3 x 10 minutes in PBS, then do 3 x 30-60 minutes washes to thoroughly rinse out secondary antibody. Mount slides in Fluoromount G (with or without DAPI), coverslip, and leave to dry for ~24 hours before imaging.