Immunofluorescences staining protocol for oligomer imaging

Use only clean bottles, flasks, magnetic stirrers, tweezers, weighing spatulas, measuring cylinders – everything should be cleaned, dried and covered if left on the side before next use.

Everything should be handled with clean tweezers – gloves should not touch the samples, solutions and ideally anything placed into the solutions where the slides are.

1. Cut 8um tissue sections on a microtome and load onto glass slides

2. Dry slides overnight at 37 degrees **– cover over the top**

3. Before staining commences keep slides for a few hours but ideally overnight at 60 degrees

4. De-wax sections through three pots of xylene solution for 2 minutes each **fresh pots of xylene each time**

5. Take sections through two pots of 100% alcohol for 2 minutes each **fresh pots each time – methylated spirits**

6. Put slides into methanol + hydrogen peroxide (H2O2) solution (100 ml: 1 ml) for 10 minutes at RT **fresh pot each time**. This process will block any staining of endogenous peroxidase in the tissue sections.

7. Perform necessary antigen retrieval pretreatments by pressure cooking in citrate buffer

   Pressure cook sections in citrate buffer pH6 for 10 minutes at pressure (wait for it to release high pressure air) in **cleaned pressure cooker**.

   Cool under running **Milli Q** water – <u>never</u> tap water.

8. Block non-specific antigen/antibody binding by placing sections in **PBS and Goat Serum 10%, (taken from the DNA PAINT protocol)** for 30 minutes

9. Apply primary antibody for 1 hour

10. Wash 3 x 5 minutes in PBS clean squirty bottle with fresh buffer **(at least filtered if not cell culture grade)**

11. Apply secondary AlexaFluor antibody for 1 hour in the dark

12. Wash 3 x 5 minutes in PBS in the dark

13. Add **filtered (0.22 um)** 0.1% Sudan black solution (0.1% sudan black/70% ethanol) for 10 minutes at room temperature in the dark

14. Wash 2-3 times in 30% ethanol

15. Mount section with Vectashield and coverslip **(Plasma cleaned slides)**

16. Take for imaging