**Sequential smFISH – Allen Institute for Brain Science (Jennie Close, Zoe Maltzer)**

**Tissue and Sectioning**

10-14 um cryosections are taken from fresh-frozen tissue, which are collected on poly-lysine-treated #1 coverslips at room temperature (RT). After 5-10 min at RT, sections are placed at 4°C until sectioning is complete. At that point, proceed immediately to fixation and permeabilization.

**Fixation/Permeabilization:**

* Post-fix sections for 15 min with 4% PFA @ 4 C
* Wash with PBS 3X
* Permeabilize with cold methanol at -20 C for 10 min
* Air dry for 30 min in fume hood (Stopping point: store coverslips at -80C)
* Optional: Treat sections with 8% SDS/PBS for 10 minutes, followed by 3 – 5 rinses with PBSor 2XSSC
* Add 2ml 2X SSC

**Hybridization – pre-heat hyb oven to 37 C**

* Place sections in hyb buffer without probes
* Add 4 ul probe 400ul hyb buffer (specific to 6-well plate format – if using perfusion chamber, this volume can be reduced)
* Incubate at 37 C for 2H

**Wash-**

* Add 2 ml wash buffer to each well, incubate at 37 C for 15 min
* Remove wash buffer
* Add 2 ml fresh wash buffer and incubate at 37 C for 15 min
* Replace wash buffer with fresh wash buffer + DAPI (final 5ug/mL) and incubate at 37 C for 15 min
* (GLOX buffer step if performing antibody stain)
* Mount and image or store at 4 C in 2XSSC until imaging session

**Stripping**

* 65% formamide/2X SSC, 10 min X 3, 30 C
* 3 washes in 2XSSC
* Following stripping, proceed to hybridization step

Solutions

**Imaging buffer stock (can be stored at 4°C)**

0.4g glucose

48.5mL nuclease free water

1mL 1M Tris-HCl

500μL 5M NaCl

**Enzymes/Trolox (per 5mL of imaging buffer, added right before use)**

5μL glucose oxidase (3.7mg/mL stock)

8.78μL catalase (7mg/mL stock)

25μL Trolox (200mM in ethanol)

**Wash buffer**

5 ml 20X SSC

10 ml Formamide

35 ml deionized, nuclease free water

**Hybridization buffer**

7.3 ml deionized, nuclease free water

1 ml 20X SSC

1 g Dextran Sulfate

 (rotate on tube rotator until Dextran dissolves)

then add:

1 ml Formamide (can substitute 10% ethylene carbonate for formamide)

500 ul tRNA stock (20 mg/ml)

100 ul RVC stock (200 mM)

40ul BSA stock (50 mg/ml)