ISH

Day1

1. Bring frozen sections to RT and then dry then in a 50C oven for 15 min.
2. Place labeled slides in mailers and fix sections in ice cold 4%PFA for 10' (reuse fix for up to 1 week)
3. Wash x2 with PBST for 5min each at RT, shaking gently
4. Treat sections with 1µg/ml Proteinase K (Sigma P-6556) in PBS for 10' at RT, shaking gently (allow 15' for P0+)
5. Wash x2 for 5' with PBST at RT, shaking gently
6. Fix in ice cold 4% PFA for 10' as in step 2
7. Wash x2 for 5' with PBST at RT, shaking gently
8. Acetylate section in 0.1M triethanolamine/0.25% acetic anhydride for 15' at RT, shaking gently
9. Wash x2 for 5' with PBST at RT, shaking gently
10. Pre-hybe slides for 1-4hr at 60C (the longer the better)*
11. Add probe to hybe solution to a final concentration of 1-2µg/ml, heat at 85C for 3', and then keep at 60C until needed
12. Add ~150µl hybe/probe to each slide, coverslip, and incubate O/N at 60C

Day2

1. Wash slides in 300ml 65C 5x SSC in a wheaton glass for 10', shaking gently*
2. Wash slides in 50% formamide/1X SSC at 65C for 30', shaking gently
3. Wash slides in TNE for 10' at 37C, shaking gently
4. Incubate slides while gently shaking in TNE with 20µg/ml RNAse A (Sigma R-5503) at 37C for 30'
5. Wash in TNE for 10' at 37C, shaking gently
6. Wash slides in 2X SSC at 65C for 20', shaking gently
7. Wash slides x2 in 0.2X SSC at 65C for 20' each, shaking gently
8. Return slides to mailers and wash x2 in MABT at RT for 5' each, shaking gently
9. Block in MABT /20% heat-inactivated sheep serum (HISS) for 1hr or more at RT, shaking gently
10. Incubate slides at 4C in 1:2000 anti-DIG in MABT/10% HISS O/N, shaking gently

Day3

1. Rinse slides in MABT, transfer slides to clean mailers and then wash x2 in MABT for 5' each while shaking gently
2. Wash one more time for 10' in MABT
3. Wash slides in alkaline phosphatase buffer (AP) for 10', shaking gently
4. Replace AP with BM purple (warm up to 37C first)
5. When color develops completely rinse slides with AP buffer
6. Wash slides with PBS x2 for 5' each, shaking gently
7. Fix slides in 4% PFA as in day1
8. Rinse slides with PBS and then wash in PBS for 5', shaking gently
9. Mount slides

<u>Notes</u>

The Pre-hybe can be stored at 4C and used over again. Replace half of the volume with fresh Pre-hybe every fourth time or so that it is used

Cover slip as quick as possible to keep the probe warm (cooling causes nonspecific annealing).

During SSC washes do not let the slides cool (cooling will cause remaining probe to anneal nonspecifically). This is difficult when initially transferring the slides to Wheaton jars, so heat the first wash to 70C or so to allow room for cooling.

If BM purple turns purple during developing then it needs to be replaced with fresh BM purple (that's not purple).

Solutions:

Stop Buffer
1% SDS
20mM EDTA
20mM Tris pH 7.5
100mM NaCl

PFA (80ml):
4% PFA (10ml 32% Electron Microscopy Sciences paraformaldehyde)
77.4mM Na_2HPO_4 (6.2ml of 1M)
22.6mM NaH_2PO_4 (2ml of 1M)
61.8ml DEPC H_2O

PFA (40ml):
4% PFA(10ml 16% Electron Microscopy Sciences paraformaldehyde)
77.4mM Na_2HPO_4 (3.1ml of 1M)
22.6mM NaH_2PO_4 (1ml of 1M)
25.9ml DEPC H_2O

10X PBS (1L):
80g NaCl
2g KCl
6.1g Na_2HPO_4 (dibasic,anhydrous)
2g KH_2PO_4
800ml dH_2O
pH 7.4 via NaOH or HCl
DEPC treat and autoclave

PBST (1L):
100ml 10X PBS
10ml 10% Triton X-100
bring to volume via DEPC H_2O

1M Triethanolamine pH 8.0 (500ml):
300ml DEPC dH_2O
66.5ml triethanolamine (sigma T1377 à stock is 7.5M)
pH 8.0 via HCl
final volume 500ml via DEPC treated dH2O

0.1M Triethanolamine pH 8.0 / 0.25 % acetic anhydride(50ml):

45ml DEPC dH2O
5ml 1M triethanolamine pH 8.0
125µl acetic anhydride

Pre-hybe Solution (50ml):
10mM Tris (0.5ml of 1M Tris pH 7.5)
600mM NaCl (6ml of 5M)
1mM EDTA (100µl of 0.5M)
0.25% SDS (625µl of 20%)
1X Denhardt's (Sigma D-2532)(0.5ml of 100X)
50% formamide (Roche 1814320)(25ml)
17ml DEPC H_2O
300µg/ml Yeast tRNA (Sigma #R6750) (300µl 50mg/ml)

Hybridization Buffer (1ml)
0.5ml formamide
339.5µl 25% dextran sulfate
120µl 5M NaCl
10µl 100X Denhardts
10µl 1M Tris pH 7.5
0.25% SDS (12.5µl of 20%)
2µl 0.5M EDTA
0µl DEPC H_2O
300µg/ml Yeast tRNA (6µl 50mg/ml)

20X SSC (1L):
3M NaCl (175.3g)
0.3M $C_6H_5Na_3O_7 \cdot 2H_2O$ (dihydrous sodium citrate) (88.23g)
pH 7.0 via HCl
Final volume 1L autoclaved dH_2O
DO NOT AUTOCLAVE

10X TNE (1L):
100mM Tris pH 7.5 (12.11g)
5M NaCl (292.2g)
10mM EDTA (3.72g)

5X MABT (1L):
58g Maleic Acid
43.5g NaCl
pH 7.5 with Tris Base (need ~100g)
final volume 1L via autoclaved dH2O

Alkaline Phosphatase Buffer (100ml):
100mM Tris pH 9.5 (10ml of 1M)
50mM $MgCl_2$ (5ml of 1M)
100mM NaCl (2ml of 5M)
0.1% Tween 20 (1ml of 10%)