**ISH on (paraffin) sections. Modified from the Mallo lab**

DAY1

1. Deparaffinyze and rehydrate (PBS 20’ at 37ºC for CRYOSECTIONS)

2. 2xPBS

3. Postfix with 4% PFA 20’ RT in cytomailer.

4. 2xPBS

5. Permeabilization with PK 15 ug/ml 15’ at 37ºC in cytomailer(4 ug/ml 10’ for CRYOSECTIONS)

6. 2xPBS

7. Refix with 4% PFA 5’ RT (PFA from step 3 may be reused here)

8. 2xPBS

9. HCl 0.1N 15’ RT in cytomailer (SKIP for CRYOSECTIONS).

10. 2xPBS

11. Triethanolamine 0.1N pH8 + 0.25% acetic anhydride. 10’ RT in cytomailer.

12. 1xPBS

13. 1xH2O

14. Prehybridization buffer at 65ºC in humid chamber (50% formamide, SSC 5x), during 90’. 600 ul/slide.

15. Preheat probe in prehybridization buffer (1-2 ug/ml) at 85ºC 5’. Cool down in ice for 5’ and then place at 65ºC until use.

16. Remove prehybridization buffer and incubate o/n at 60–65ºC with the probe-buffer mix (100 ul/slide + coverslip OR 300 ul/slide without coverslip).

DAY2

17. Let the coverslips come off the slide in pre-warmed posthybridization buffer I (50% formamide, SSC 5x pH 5.5, 1% SDS).

18. Wash with posthybridization buffer I 2x30’ at 65ºC.

19. Wash with posthybridization buffer II (50% formamide, SSC 2x pH 5.5, 0.2% SDS) 2x30’ at 65ºC.

20. Let the slides cool down for 5’ at RT.

21. Wash with MABT 3x5’ RT.

22. Block with MABT + 10% goat serum + 1% blocking reagent (Roche 11096176001, prepare 10% stock in MAB 1X). 90’ at RT in humid chamber (water).

23. Incubate with anti-DIG antibody (1:2500 in MABT + 2% goat serum + 1% Blocking reagent) 2h at RT OR o/n 4ºC.

(DAY3)

24. Wash 2x5’ RT with MABT.

25. Wash 6x20’ RT with MABT.

26. Wash 2x10’ with NTMT (AP buffer) RT.

27. Develop with BM purple at 37ºC.

28. Wash with PBS.

29. Fix 10’ with PFA RT. Rinse in water

30. Counterstain 10’ with Nuclear Fast Red 0.005%.

31. Dehydrate, clear in xylene and mount with DPX.

REAGENTS

- Prehybridization buffer: (50% formamide, SSC 5x pH 5.5, 0.1% CHAPS, 0.05 mg/ml yeast tRNA, 0.05 mg/ml heparin, 1xDenhardt’s). Prepare 1 l, store as 50 ml aliquots at -80ºC.

- MAB 5x

4 l H2O

290.25 g maleic acid, 219.15 g NaCl

~70 g NaOH. Adjust pH to 7.5

Top to 5 l with H2O

- MABT1x

Dilute MAB5x in H2O. Add 0.1% Tween 20

- 100x Denhardt’s. For 50 ml: 1 gr ficoll (Sigma, F2637), 1 gr polyvinylpyrrolidone **(Sigma, P5288),** 1 **gr** BSA (Sigma, **A7906)**