Please cite the J.B. Lawrence Lab or publications for use of this protocol. Thanks!

RNA Hybridization

1. Dehydrate coverslips: cold 100% ETOH for 10min.

2. Air dry coverslips

3. **Probe**: Set up one Eppendorf tube for each coverslip:
   -10-15µl Human Cot-1 DNA (stock 1 ug/ul)
   -2 µl Salmon sperm DNA + tRNA (stock 10µg/µl of each)
   -5 µl of the Biotin or Digoxigenin labled probe (1µg/80µl concentration) (~50ng nick translated probe)

4. Dry Probe in the speed vac until completely dry

5. Resuspend probe with 10µl of 100% Formamide

6. Denature probe on 80°C heat block for 10 min

7. In separate tube make-up RNA Hybridization Buffer (Hybridization buffer with 2 units/µl of RNAsin)

   Stock Hybridization Buffer (4°c storage)
   1ml Albumin BSA (RNAse free)
   1ml 20xSSC
   1ml H20
   2ml Autoclaved 50% Dextran Sulfate

8. Add 10µls of the RNA Hyb buffer to each tube of denatured probe

9. Place 20µl of probe mix (total volume of probe + hyb buffer) onto a parafilmed lined glass plate

10. Place each coverslip, cells side down, on top of the probe mix

11. Cover with another sheet of parafilm, seal the sides like an envelope, and incubate overnight at 37°c in a humid incubator.

Washes

12. Rinse the coverslips in 50% Formamide, 2xSSC for 20min at 37°C

13. Rinse in 2xSSC for 20min at 37°C

14. Rinse in 1xSSC for 20min at room temp on a shaker

15. Rinse in 4xSSC for about 1 min at room temp

Detection

16. Thaw a 500µl aliquot of (stored at -20°C)

17. Add 1µl appropriate secondary antibody to 500µl 4xSSC/ 1% BSA. (can add 1unit/µl of RNAsin if worried about RNAse but usually it's not necessary)

18. Place 50-80µl of this secondary mix on parafilm lined glass plate

19. Place slips, cells down, on top of the secondary mix

20. Cover with and seal with another piece of parafilm, wrap entire plate with tin foil (keep dark) and incubate for 1 hour at 37°C.

Rinse

21. Rinse coverslips in 10ml of:
    4x SSC - 10min on shaker in the dark
    4x SSC / 0.1% Triton – 10min on shaker in the dark
    4x SSC – 10min on shaker in the dark

DAPI

22. Incubate in DAPI stain, 30sec-1 min, in dark

23. Rinse twice with 1xPBS

24. Mount coverslips onto slides using Vectashield (Vector Labs) mounting media and seal edges with fingernail polish.

25. Slides are stored in a slide folder at -20°C