Leslie Slota

Cohn Laboratory

6/13/11 to 8/19/11

In Situ Hybridization on Tissue Sections

- To visualize gene expression in sections of horseshoe crab embryos

Day 1
Lay slides flat

1. Let cryostat sections air dry
2. Draw barrier around slide with Pap pen
3. Wash 2 times DEPC-PBT 5 min each
4. Incubate in 5µg/mL Proteinase K 10 min
5. Wash DEPC-PBT 5 min
6. Fix in 4% Paraformaldehyde 5 min
7. Wash two times DEPC-PBT 5 min
8. Remove Pap pen with Kim wipe
9. Incubate in Prehybridization solution with 5% Dextran at 55˚C 15 min
10. Pre-warm probes in prehybe with dextran at 65˚C 5 min
11. Add 40 µL of probe/prehybe to slide and place parafilm coverslip on each slide.
12. Incubate at 65˚C overnight in humidity chamber
    a. Closed slide box with wet paper towels on inside work fine

Day 2

1. Remove parafilm coverslip and place slide in glass slidejars
2. Wash 3 times in 2X SSC + .1% chaps at 65˚C 30 min
3. Wash 3 times .2X SSC + .1% chaps at 65˚C 30 min
4. Wash KTBT at room temperature 5 min
5. Wash KTBT at room temp 30 min
6. Take slides out of glass jars and lay flat. Draw around slides with pap pen
7. Block slides in 10% goat serum/KTBT
    a. 4 ml goat serum: 16 ml KTBT
8. Incubate overnight in 1:2000 Anti-DIG A&P in 10% goat serum/KTBT overnight at 4˚C
    a. 7 µL anti-DIG: 20 ml goat serum

Day 3

1. Wash in KTBT 7 times one hour each
2. Incubate overnight at 4˚C in KTBT

Day 4

1. Wash NTMT at room temperature 10 min
2. Incubate NTMT at room temperature 30 min
3. Incubate in BM purple in dark at room temperature until color develops

Solutions:

DEPC-PBT
1 Liter DEPC PBS
1 mL Triton-X

2X SSC + .1% chaps- 50 mL X4
5 mL 20X SSC
50µL chaps
Dd water

.2X SSC + .1% chaps- 50 mL X 4
500 µL 20X SSC
50 µL chaps
dd water

KTBT- 250 mL
12.5 mL 1M Tris pH 7.5
7.5 mL 5M NaCl
1.25 mL 1M KCl
500 µL Trion- X
Dd water

NTMT- 50 mL
5 mL 1M Tris pH 9.5
1.25 mL 1M $MgCl_2$
500 µL 5M NaCl
500 µL Tween-20
Dd water

BM Purple- 40 mL
36 mL NTMT
4 mL Dimethylformamide
140 µL BCIP
136 µL NBT

NBT*: 75mg/ml in Dimethylformamide (store at -20^0C)

BCIP*: 50 mg/ml in dimethylformamide, 4-Tolouidine Salt (store at -20^0C)

Block Buffer: 10% Sheep serum, 2% BSA and 0.2% Sodium Azide, in PBT

Prehybridization solution: 50% Formamide, 5x SSC, 2% Boehringer blocking powder (cat. No. 1096176, dissolve directly into the mix. DO THIS BEFORE YOU ADD FORMAMIDE), 0.1% Trition X-100, 0.5% CHAPS (Sigma C-3023), 1mg/ml yeast RNA (sigma R-6625), 5mM EDTA, 50µg/ml heparin.