

## Embryo whole mount in situ w/ RNA probe (from Tautz/Boeh. Mann and E. Bier).

1. Take fixed 'bros from freezer.
  - Use <100  $\mu$ l 'bros in eppendorfs.
  - Use 1 ml soln volume.
2. Wash 3x in PBT for 5 minutes.
3. Incubate 3-5 min in 50  $\mu$ g/ml prot K in PBT - 100  $\mu$ l vol.  
Stop by:
  - a. 2x w/ 2 mg/ml glycine in PBT for 2 min.
  - b. 2x w/ PBT for 5 min.
4. Post fix with PBT + 5% formaldehyde.
  - (140  $\mu$ l 37% formaldehyde + 860  $\mu$ l PBT)
  - 20 min/ do not rock.
5. Rinse 5x in PBT for 1-2 min.
6. Rinse in hybe soln for 2 min.
7. Pre-hybe for 1 hr at 55-60°C.
  - 150  $\mu$ l hybe soln.
8. Heat denature probe. 85°C / 5 min then ice.  
(Final conc. 10 to 50 ng/100  $\mu$ l)
9. Try to remove all of pre-hybe s/n.
  - Add ~50-100  $\mu$ l probe in hybe.
  - Hybridize 18 hrs at 55-60°C.
10. Preabsorb anti-dig ab 1:2000 then use directly.
11. Remove probe (all washes done at 55-60°C):
  - a. 500/500 hybe/PBT 20 min.
  - b. 5x PBT 10-20 min.
12. Incubate in 200  $\mu$ l PBT + ab. > 1 hr RT or O/N at 4°C.
13.
  - a. Rinse 2x PBT.
  - b. Wash - 4x PBT for 20 min.
  - c. Wash - 2x In situ wash for 5 min.
14. To each sample ~ 400  $\mu$ l of following:
  - 1 ml in situ wash w/ 4.5  $\mu$ l NBT + 3.5  $\mu$ l X-phos.

*Watch color reaction under scope.*
15. Stop by rinsing sev'l times w/ PBT.
16. Clear in 80% glycerol. Let settle O/N.

## Solutions:

PBT: PBS plus 0.1 % Tween 20.

Hybe: 50% deionized formamide  
5x SSC

100 µg/ml sonicated, boiled salmon sperm DNA 100 µg/ml  
tRNA

50 µg/ml heparin  
0.1 % Tween 20

In situ wash:

2 ml 5 M NaCl

5 ml 1 M MgCl<sub>2</sub>

10 ml 1 M Tris pH 9.5

1 ml 0.1 M Levamisol

100 µl Tween 20

Bring to 100 ml      Keep at -20°C.

## Embryo whole mount in situ w/ RNA probe (combination of Tautz, Bier and Crews).

1. Take fixed 'bros from freezer.  
Use <100  $\mu$ l 'bros in eppendorfs.  
Use 1 ml soln volume.
2. Wash 3x in PBT for 5 minutes.
3. Incubate 3-5 min in 50  $\mu$ g/ml prot K in PBT - 100  $\mu$ l vol.  
Stop by washing: 2x w/ 2 mg/ml glycine in PBT for 2 min.  
2x w/ PBT for 5 min.
4. Post fix with PBT + 5% formaldehyde.  
20 min/ do not rock.
5. Rinse 5x in PBT for 1-2 min.
6. Rinse in hybridization (hybe) soln for 2 min.
7. Pre-hybe for 1 hr at 55-60°C.
  - 150  $\mu$ l volume
8. Heat denature probe in hybe soln. 85°C / 5 min then ice.
  - *Final conc. 10 to 50 ng/100  $\mu$ l*
9. Try to remove all of pre-hybe s/n.
  - a. Add ~50-100  $\mu$ l probe in hybe.
  - b. Hybridize 18 hrs at 55-60°C.
10. Preabsorb anti-dig ab 1:2000 then use directly.
11. Remove probe (all washes done at 55-60°C while rocking):
  - a. 500/500 hybe/PBT for 20 min
  - b. 5x PBT for 10-20 min.
12. Incubate in 200  $\mu$ l PBT + ab. > 1 hr RT or O/N at 4°C.
14. To each sample ~ 300  $\mu$ l of following:  
1 ml in situ wash w/ 4.5  $\mu$ l NBT  
3.5  $\mu$ l X-phos.

*Watch color reaction under scope. I see the signal within a few minutes.*

15. Stop by rinsing sev'l times w/ PBT.
16. Clear in 80% glycerol. Let settle O/N.

## Solutions:

PBT: PBS plus 0.1 % Tween 20.

Hybe: 50% deionized formamide  
5x SSC  
100 µg/ml sonicated, boiled salmon sperm DNA  
100 µg/ml tRNA  
50 µg/ml heparin  
0.1 % Tween 20

In situ wash: 2 ml 5 M NaCl  
5 ml 1 M MgCl<sub>2</sub>  
10 ml 1 M Tris pH 9.5 1 ml  
0.1 M Levamisol  
100 µl Tween 20  
Bring to 100 ml      Keep at -20°C.