In situ Hybridization on Imaginal Discs Using Riboprobes

Jen Scholz 2/00 Modifications from Ken Cadigan (7/97) and Grace Panganiban (6/93)

Hybridization buffer
50% deionized formamide
5x SSC (DEPC treated)
100 ug/ml sheared salmon sperm DNA
0.1% Tween 20

PBT
10 mM KPO4
140 mM NaCl
0.1% Tween 20
pH 7.0

Staining Buffer
100 mM NaCl
50 mM MgCl2
100 mM Tris 9.5
0.1% Tween 20

Preabsorbing Antibody

Fixed embryos are stored in 1 ml of Methanol or Ethanol. Remove 500µl of the Ethanol, and add an equal volume of PBT. Wash 5x with PBT so that there is no EtOH remaining. Preabsorb antibody in 10 times the concentration you will be using (e.g. with 4 tubes of discs, preabsorb 400µl of PBT with 4µl of antibody). Cover in foil and shake on an environmental shaker at 4°C for 24-48 hours.

Dissect larvae in PBS, removing all fat, within 20 minutes.

Fix in 4% formaldehyde (in PBS) for 15 minutes on ice (make fix immediately before use). Remove fix.

Fix in PBS + 4% formaldehyde + 0.1% DOC and 0.1% Triton X-100 for 15 minutes at room temperature on Nutator. Remove fix.

Add 500µl 0.3M NH4OAc and then 500µl EtOH. Mix well by inverting. Incubate for 2 minutes, and remove solution.

Wash in EtOH for 10 minutes.

Wash in Xylene/EtOH (1:1) for 10 minutes.

Wash 3 X 2 minutes in EtOH.

Wash 2 minutes in MeOH.

Wash 5 X 5 minutes in PBT.

Wash 2 X 10 minutes in PBT/hybridization buffer.

Was 10 minutes in hybridization buffer.

Prehybridize for at least one hour at 55°C.

Add heat denatured (5 minutes at 95°C) probe. Hybridize 48 hours, flicking tubes often (about once an hour during the day).

Wash 5 X 5 minutes each in 1 ml of hybridization buffer at 55°C. Let the last wash go over night.

Rinse discs once with 50% hybridization buffer: 50% PBT, and 3x with PBT at room temperature.

Dilute preabsorbed alkaline phosphatase-conjugated anti-digoxigenin F'ab fragment in PBT to a final concentration of 1:2000. Incubate discs with the antibody for 2 hours at room temperature on the Nutator.

Wash away excess antibody with at least 10 changes of PBT over 1 hour, on the Nutator.

Equilibrate discs with 2 changes of staining buffer.

Develop (ideally within 2 hours) in 1ml of staining buffer to which 4.5µl of NBT and 3.5µl of X-phosphate have been added. Change developing solution after 30 minutes.

Stop the developing by washing 5x with PBT + 50 mM EDTA.

Store in 10% glycerol, 50 mM EDTA at 4°C.