In Situ Hybridization On Mouse Cryostat sections

Modified by Lise Zakin and Eddy De Robertis 2008
From an original protocol by Henrique and Ish-Horowicz

Dissections and embedding

-Dissect embryos in 1X PBS
-Fix embryos in 50 ml fish fix o/n at 4°C in 50 ml falcon tubes
-Wash embryos 3x 5 minutes in buffer for fish fix
-Wash embryos in 15% sucrose in 1X PBS at 4°C until embryos sink to the bottom of the tube
-Wash embryos in 15% sucrose/7% gelatine in 1X PBS pre-warmed at 37°C (to dissolve the gelatine) for 30 minutes. Do not shake the solution too much to avoid the formation of bubbles
-Embed embryos in pre-warmed 15% sucrose/7% gelatine in 1X PBS o/n at 4°C
-Trim blocks, freeze progressively by immersing briefly several times in liquid nitrogen until the center of the block becomes opaque and finish the freezing process on dry ice (freezing too quickly might cause the block to crack)
-Store at -80°C (up to several months)
-Cut 15µm thick cryostat sections and transfer to superfrost/plus slides (Fisherbrand cat# 12-550-15)
-Allow sections to air dry for at least 2 hours
-Store sections at -80°C in a box containing desiccant (up to 1 year)

DAY ONE

Hybridization

-Defrost sections at room temperature for at least 1 hour
-Dilute DIG labeled RNA probe in hybridization buffer (0.1-1µg/ml). Denature the probe mix 5-10 minutes at 70°C. Calculate 100µl per section
-Add probe mix to each slide and cover slide with a coverslip (use 24x60mm number 1 coverslips)
-Hybridize o/n at 65°C in a sealed plastic slide box with 2 sheets of whatman paper wetted with 0.2X SSC + 50% formamide to prevent slides from drying

DAY TWO

Post-hybridization washes

-Transfer slides to a slide rack (one without a central support to allow the coverslips to fall off the slides) immersed in 200-300ml washing solution (enough to cover the slides) pre-warmed at 65°C
-Wash 1x 15 minutes at 65°C in washing solution to allow coverslips to fall off
-Wash 2x 30 minutes at 65°C in washing solution

-Wash 2x 30 minutes with 1X MABT at room temperature

Blocking and antibody staining

-Remove slides from the slide rack and place in a humidified chamber
-Block at least 1 hour in 1X MABT + 2% Blocking reagent + 20% heat inactivated sheep serum (goat serum works as well) at room temperature (no coverslips)
-Incubate o/n at 4°C with fresh 1X MABT + 2% Blocking reagent + 20% heat inactivated sheep serum containing a 1/2000 dilution of anti-DIG alkaline phosphatase antibody (Roche cat# 11093274910) in a humidified chamber. Use 100 µl antibody solution per section and coverslip

DAY THREE

Post-antibody washes

-Transfer slides to a slide rack (one without a central support to allow the coverslips to fall off the slides) immersed in 200-300ml 1X MABT (enough to cover the slides)
-Wash 4-5x 30 minutes each in 1X MABT

Staining reaction

-Reveal in humidified chamber protected from light, with 1 ml per slide BM purple AP substrate (Roche cat# 11 442 074 001) for 1-24 hours at 4°C or room temperature depending on the quality of the probe
-Wash 3x 5 minutes with PBS
-Mount slides for photography

SOLUTIONS

Fish fix: pre-warm buffer and then add paraformaldehyde. The fix is stable for one week at 4°C

8g sucrose
24 µl 1M CaCl2
77 ml 0.2M Na_2HPO_4
23 ml 0.2M NaH_2PO_4
8g paraformaldehyde
H_2O QSP 200 ml

Buffer for fish fix: as for fish fix minus paraformaldehyde

10X Salt: 2M NACl, 100 mM Tris-HCl pH 7.5**,** 100mM phosphate buffer pH 7.4**,** 50 mM EDTA pH8

100 ml 5M NaCl
25 ml 1M Tris-HCl pH7.5
25 ml 0.5M EDTA
19.35 ml 1M Na_2HPO_4
5.65 ml 1M NaH_2PO_4
H_2O QSP 250 ml

100X Denhardt's: stored aliquoted at -20°C

2g bovine serum albumin
2g Ficoll™
2g polyvinylpyrrolidone
H2O QSP 100 ml

Hybridization buffer: store 1-2 ml aliquots at -20°C

1X salt
50% formamide
10% dextran sulphate
Torula RNA 1 mg/ml (Roche cat# 1010950900)
1X Denhardt's

Washing solution: 1X SSC, 50% formamide, 0.1% Tween20

1X MABT: 100 mM maleic acid, 150mM NaCl, 0.1% Tween20, pH 7.5

5X MAB:

21.91g NaCl
29.02g Maleic Acid
pH to 7.5 with NaOH: first add 18g NaOH pellets, then adjust pH with concentrated NaOH solution
H_2O QSP 500ml

Blocking reagent: Roche cat# 11 096 176 001. Make 10% stocks in 1X MAB (no tween20) and store at -20°C