Whole Mount In Situ Hybridization On Mouse Embryos

Modified by Lise Zakin and Eddy De Robertis 2008
Henrique et al., 1995. Nature 375, 787-790.

Note: This protocol works well for embryos up to 10.5 days post-coïtum (d.p.c.). For older embryos, use in situ on sections.

Dissections

-Dissect embryos in 1X PBS; remove as much of the extra-embryonic membranes as possible

-Fix in 5-10 ml 4% PFA in PBS for 2 hours at room temperature or o/n at 4°C in vials with rubber lined closure (Fisherbrand cat# 03-339-25B, 15x45mm) for early stage embryos or 15 ml falcons for older embryos

-Wash 2x 5 minutes with cold PBT

-Wash successively with cold, 25% methanol (MetOH) in PBT, 50% MetOH in PBT, 75% MetOH in PBT 1x 5 minutes each and 2x 5 minutes in 100% MetOH

-Embryos of all stages can be stored at -20°C for several months in vials with rubber lined closure

DAY ONE

Pre-treatment and Hybridization

-Re-hydrate embryos successively in cold 75%, 50%, 25% MetOH in PBT, 1x 5 minutes each and 2x 5 minutes in cold PBT

-Treat embryos with 10 µg/ml proteinase K RNA grade (Invitrogen cat# 25530-049) in PBT at room temperature. Time of incubation depends on the stage of the embryos

15 minutes for 6.5 d.p.c. embryos
20 minutes for 7.5 d.p.c. embryos
25 minutes for 8.5 d.p.c. embryos
30 minutes for 9.5 d.p.c. embryos
35 minutes for 10.5 d.p.c. embryos

-Remove proteinase K solution, rinse briefly and carefully with PBT and post-fix embryos for 20 minutes in 4% PFA + 0.1% glutaraldehyde in PBT

-Rinse and wash 1x 5 minutes with PBT

-Rinse once with 1:1 PBT/hybridization mix. Let embryos settle

-Rinse with 1 ml hybridization mix. Let embryos settle

-Replace with 1 ml hybridization mix and incubate horizontally at least one hour at 70°C in a rotating hybridization oven. Vials can be immobilized horizontally inside falcon tubes fixed to the rotating axle. Watch for embryos stuck to the lid of the vials

-Replace with 1.5 ml pre-warmed hybridization mix containing 1 µg/ml DIG-labeled RNA probe

-Incubate horizontally o/n at 70°C in a rotating hybridization oven

DAY TWO

Post-hybridization washes

-Rinse 1x briefly and wash 4x 30 minutes with pre-warmed hybridization mix at 70°C in the rotating oven. Try to keep embryos at 70°C at all times during these washes
-Wash 1x 20 minutes with pre-warmed 1:1 hybridization mix/1X TBST
-Rinse 2x with 1X TBST at room temperature
-Wash 2x 30 minutes with 1X TBST
-Rinse 2x with 1X MABT

Blocking and antibody staining

-Pre-incubate at least 1 hour in 1X MABT + 2% Blocking reagent + 20% heat inactivated sheep serum (goat serum works as well) at room temperature
-Incubate o/n at 4°C in 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)

DAY THREE

Post-antibody washes

-Rinse 1x 5minutes in 1X MABT
-Wash 4x over a period of 48 to 72 hours in a rocking 15 ml falcon

Staining reaction

-Reveal in vials with rubber lined closure protected from light with 1-2 ml 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 PBT
-To clear embryos for photography transfer specimens into 25% to 50% glycerol in PBT progressively

SOLUTIONS

PBT: 1X PBS + 0.1% Tween20

4% PFA in PBS: dissolve 4g paraformaldehyde (PFA) in 100 ml 1X PBS on heat. PFA will go into solution when temperature reaches ~60°C. Remove immediately from heat when almost completely dissolved (a few grains should still be visible), filter and aliquot on ice. Freeze immediately or use the same day. Aliquots may only be thawed once

Hybridization mix: store at -20°C

50% Formamide
1.3X SSC pH 5 (pH 20X SSC with citric acid)
5mM EDTA pH 8
Torula RNA 50 µg/ml (Roche cat# 1010950900)
0.2% Tween20
0.5% CHAPS
100µg/ml Heparin

10X TBST:

8g NACl
0.2g KCl
25ml of 1M Tris-HCl pH 7.5
10ml Tween20
H_2O QSP 100ml

1X MABT: 100 mM maleic acid, 150mM Nacl, 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