Wholemount *In Situ* Hybridization Solutions

(Rivera lab)

DEPC treatment:

Add 1 ml DEPC/500 ml of solution.
Shake well, allow to sit overnight at room temperature and autoclave.

10X PBS (100 ml)

1.37 M	8.00 g	NaCl
27 mM	0.20 g	KCl
100 mM	1.44 g	Na_2HPO_4
20 mM	0.27 g	KH_2PO_4
	100.00 ml	ddH_2O

1X PBS for *in situ* should be adjusted to pH 7.4. Use 1N HCl.

PBT (50 ml):

1X	5.0 ml	10X PBS
0.1%	0.5 ml	10% Tween 20
	44.5 ml	depc ddH_2O

4% PFA/0.2% Glutaraldehyde in PBT (5 ml)

4.0%	5.0 ml	4% PFA in PBS
0.2%	40.0 µl	25% Glutaraldehyde
0.1%	50.0 µl	10% Tween 20

20X SSC pH 5.0 (100 ml):

3M	17.50 g	NaCl
0.3M	8.80 g	$Na_2Citrate2H_2O$

Dissolve in ~ 60 ml of ddH_2O.
Adjust to pH 5.0 with 0.2 M Citric Acid.
Add ddH_2O to 100 ml

HYBRIDIZATION SOLUTION

50%	5.00 ml	10.00ml	20.00 ml	25.00 ml	Formamide (Sigma F7503)
5 mM	0.10 ml	0.20 ml	0.40 ml	0.50 ml	0.5M EDTA pH 8.0
1.3X	0.65 ml	1.30 ml	2.60 ml	3.25 ml	20X SSC
0.2%	0.20 ml	0.40 ml	0.80 ml	1.00 ml	10% Tween 20
50 µg/ml	0.05 ml	0.10 ml	0.20 ml	0.25 ml	10 mg/ml Yeast tRNA (Sigma R5636)
0.5%	0.50 ml	1.00 ml	2.00 ml	2.50 ml	10% CHAPS
100 µg/ml	0.02 ml	0.04 ml	0.08 ml	0.10 ml	50 mg/ml Heparin (Sigma H9399)
	3.48 ml	6.96 ml	13.92 ml	17.40 ml	depc ddH_2O
	10.00 ml	20.00 ml	40.00 ml	50.00 ml	Final Volume

5X MAB (200 ml):

0.5 M	11.60g	Maleic acid
0.75 M	8.76g	NaCl

Add Maleic acid to ~150 ml of ddH_2O.
Adjust to approximately pH 7.0 with ~19 ml of 10 N NaOH.
Dissolve NaCl and allow solution to reach room temperature.
Adjust pH to 7.5 with 1 N NaOH. Steep slope close to 7.5, do not overshoot!
Add ddH_2O to 200 ml, autoclave and store at room temperature.

MABT (50 ml):

1X	10.0 ml	5X MAB
0.1%	0.5 ml	10% Tween 20
	39.5 ml	depc ddH_2O

Blocking Solution (10 ml)

2%	0.20 g	Roche Blocking Reagent (Cat # 1096176)
1X	2.00 ml	5X MAB
	8.0 ml	ddH_2O

Blocking Solution + NGS (10 ml)

2%	0.20 g	Roche Blocking Reagent (Cat # 1096176)
1X	2.00 ml	5X MAB
10%	1.00 ml	NGS
	7.0 ml	ddH_2O

NGS = Normal Goat Serum heated at 65 ºC for 1 hour.
OK if gels (denature) after heating, mix well.

NTMT (10 ml)

0.1 M	0.2 ml	5 M NaCl
0.1 M	1.0 ml	1 M Tris-HCl pH9.5
0.05 M	0.5 ml	1 M $MgCl_2$
1%	1.0 ml	10% Tween 20
	7.3 ml	ddH_2O

To make staining solution, add 20 µl/ml NBT/BCIP stock solution (Roche Cat. No. 1681451)