

**Whole-mount in situ hybridization for cricket embryos (24/3/10)**

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Day 1

100% / 75% / 50% / 25% MeOH / PT	r.t.	transfer embryos from a stock vial.	
2x PT buffer	r.t.	5 min	0 0
4% FA/PT	r.t.	20min	0
2x PT buffer	r.t.	5 min	0 0
Hybridization mix	70°C	5 min	0
Hybridization mix	70°C	2-4h	0
RNA probe (final 0.1ng/μl) / Hybridization mix		70°C	0 / N 0

Day 2

Solution1	70°C	20 min	0
3x Wash1	70°C	20 min	0 0 0
3x Wash2	70°C	20 min	0 0 0
3x KTBT	r.t.	10 min	0 0 0
1% Blocking Solution / KTBT	r.t.	60 min	0
Anti-DIG-AP, Anti-FITC-HRP / 1% Blocking Solution (1:2000)	r.t.	2h	0
2x KTBT	r.t.	1 min	0 0
2x KTBT	r.t.	30 min	0 0
2x KTBT	r.t.	60 min	0 0
1x KTBT+Hoechst	4C	0/N	0

Day 3

1x KTBT	r.t.	1 min	0
4x KTBT	r.t.	60 min	0 0 0 0
- Prepare NTMT and AP buffer for the following step during washing.			
1x KTBT	r.t.	15 min	0
1x NTMT (pH 9.5)	r.t.	15 min	0
1x NTMT – AP buffer (pH 9.5)	r.t.	15 min	0
2x AP buffer (pH 9.5)	r.t.	15 min	0 0
NBT / BCIP	r.t. or 4°C in the dark		
4x KTBT	r.t.	1min	0 0 0 0
4% FA/PT	r.t.	25min	0
4x PT	r.t.	1min	0 0 0 0

## Solutions

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### PT(pH7.4)

- a. PBS 1L
  - b. 0.1% Triton X-100 DEPC H<sub>2</sub>O 1mL
- Keep stirring the prepared solution O/N, because Triton X-100 is viscous.

### Hybridization Mix

	for 10ml	for 20ml	for 50ml
Blocking Reagent	0.2g	0.4g	1.0g
Formamide	5.0mL	10mL	25mL
20xSSC (pH 7.0)	2.5mL	5.0mL	12.5mL
10% TritonX-100	0.1mL	0.2mL	0.5mL
10% CHAPS	0.1mL	0.2mL	0.5mL
0.5M EDTA	0.1mL	0.2mL	0.5mL
Heparin (50 mg / mL)	10µL	20µL	50µL
yeast tRNA (10 mg / mL)	1.0µL	2.0µL	5.0µL
mQ	up to 10mL	up to 20mL	up to 50mL

- Do not vortex yeast tRNA solution.
- Measure Blocking Reagent first, and then add remaining reagents. Dissolve and keep them at 70°C until use.
- aliquots for hybridization buffer can be stored at -20C for up to 6 months (and probably longer)
- handle with care, toxic

### Solution1

	for 10mL	for 20mL
a. Formamide	5.0mL	10mL
b. 20xSSC (pH 4.5)	2.5mL	5.0mL
c. 10% SDS	1.0mL	2.0mL
d. mQ	1.5mL	3.0mL

- store aliquots at -20C for up to 6 months (and probably longer), handle with care, toxic

### Wash1

	for 500mL
a. 20xSSC (pH 7.0)	50mL
b. 10%CHAPS	5.0mL
c. DEPC H <sub>2</sub> O	up to 500mL

- store solution at room temperature

### Wash2

	for 500mL
a. 20xSSC (pH 7.0)	5.0mL
b. 10%CHAPS	5.0mL
c. mQ	up to 500mL

- store solution at room temperature

### KTBT

	for 1000mL
a. 10xKTBS	100mL
b. TritonX-100	1.0mL
c. mQ	up to 1000mL

- Keep stirring the prepared solution O/N, because Triton X-100 is viscous.

### 10X KTBS

Tris-HCL pH 7.5	500mM
NaCl	1500 mM

KCl 100 Mm

- autoclave and store at room temperature

10x blocking solution (Roche)

Dissolve 10 % (w/v) of the Blocking reagent (Roche, cat.no 11 096 176 001) in KTBT with stirring and heating to 65 °C, adjust the pH to 7.5 with drops of 1M NaOH, store in aliquots at -20°C

1% Blocking Solution / KTBT

	for 50mL	for 200mL
a. Blocking Reagent	0.50g	2.0g
b. KTBT	50mL	200mL

- Dissolve at 70°C. Store aliquots at -20°C.

- Do not freeze-thaw multiple times.

NTMT

	10 ml	15 ml	20 ml	30 ml	40 ml
a. 1M Tris-HCl (pH 9.5)	1 ml	1.5 ml	2 ml	3 ml	4 ml
b. 5M NaCl	0.2 ml	0.3 ml	0.4 ml	0.6 ml	0.8 ml
c. 10% TritonX-100	0.1 ml	0.15 ml	0.2 ml	0.3 ml	0.4 ml
d. 1M MgCl <sub>2</sub>	0.5 ml	0.75 ml	1 ml	1.5 ml	2 ml
e. mQ	8.2 ml	12.3 ml	16.4 ml	24.6 ml	32.8 ml

- Add 1M MgCl<sub>2</sub> just before use to avoid pH change. Always prepare NTMT fresh just before staining

NTMT-AP

Mix NTMT and AP buffer in 1:1 ratio.

AP buffer

	10 ml	15 ml	20 ml	30 ml	40 ml
a. 2x PVA in DEPC H <sub>2</sub> O (5%)	5.00 ml	7.50 ml	10.00 ml	15.00 ml	20.00 ml
b. 1M Tris-HCl (pH 9.5)	1.00 ml	1.50 ml	2.00 ml	3.00 ml	4.00 ml
c. 5M NaCl	0.20 ml	0.30 ml	0.40 ml	0.60 ml	0.80 ml
d. 10% TritonX-100	0.10 ml	0.15 ml	0.20 ml	0.30 ml	0.40 ml
e. 1M MgCl <sub>2</sub>	0.50 ml	0.75 ml	1.00 ml	1.50 ml	2.00 ml
f. mQ	3.20 ml	4.80 ml	6.40 ml	9.60 ml	12.80 ml

- Dissolve 10% Poly Vinyl Alcohol (PVA, Sigma 341584) in DEPC H<sub>2</sub>O at 90°C.

- Do not put on ice to avoid deposition of PVA.

- Add 1M MgCl<sub>2</sub> just before use to avoid pH change.

- Always prepare AP buffer fresh just before staining

Staining solution (NBT / BCIP)

	for 10mL	for 20mL	for 30mL
AP buffer	10mL	20mL	30mL
NBT	67.5µL	135µL	202.5µL
BCIP	30µL	60µL	90µL

- Protect from light with foil. Always prepare fresh

(NBT and BCIP: 50mg/ml)

- NBT/BCIP: toxic, careful!!