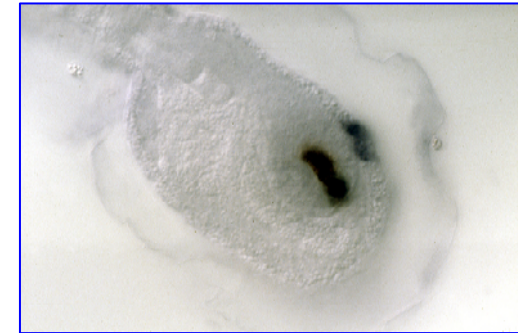


## InsituPro UP protocol ISH Ciona intestinalis

**Date:** May 2006

**System Configuration:** 30 small baskets

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Ciona intestinalis,

### Description

This protocol can be used for Ciona intestinalis embryos and larvae. Within the protocol, the embryos/larvae will be rehydrated through several steps (50% and 25% of alcohol), treated with Proteinase K, prehybridized and hybridized with sense and antisense probes.

Incubation with alkaline Phosphatase buffer and staining is performed outside the instrument. Buffers on position B, E, and F have to be replaced during the method! See buffer loading form for details.

## Method Listing

Step	Task	Time	Aliquoting	Thats what it means	Elapsed Time
1	SetTempReg		T0 (OFF)	Heating off, temperature to RT	2.5 h
2	PrimeNeedle		6000	Rinsing double needle	
3	IncubateVT	10 min	250µl EtOH 50%->Specimen <b>2x</b>	Hydrate with 50% ethanol	
4	IncubateVT	10 min	250µl EtOH 30%->Specimen <b>2x</b>	Hydrate with 30% ethanol	
5	IncubateVT	10 min	250µl PBT->Specimen <b>3x</b>	Wash with PBS-T	
6	IncubateVT	1 h	250µl para 4%->Specimen	Fixation with paraformaldehyde 4% in PBS	
7	IncubateVT	10 min	250µl PBT->Specimen <b>2x</b>	Wash with PBS-T	
8	SetTempReg		T1 (LOW)	Heating on, temperature to 37°C	
9	Wait	10 min		Time to equilibrate the heating block	25.5 h
10	IncubateVT	25 min	250µl PK->Specimen	Proteinase K digestion	
11	SetTempReg		T0 (OFF)	Heating off, temperature to RT	
12	Wait	5 min		Time to equilibrate the heating block	
13	IncubateVT	1 h	250µl para 4%->Specimen	Postfixation with paraformaldehyde 4% in PBS	
14	IncubateVT	10 min	250µl PBT->Specimen <b>3x</b>	Wash with PBS-T	
15	IncubateVT	10 min	250µl acetic an.->Specimen <b>3x</b>	Acetylation	
16	IncubateVT	10 min	250µl PBT->Specimen <b>3x</b>	Wash with PBS-T	
17	IncubateVT	10 min	150µl hyb. mix->Specimen	Wash with PBS-T / hyb. mix	
18	IncubateVT	20 min	250µl hyb. mix->Specimen	Wash with hyb. mix	
19	SetTempReg		T2 (HIGH)	Heating on, temperature to 55°C	
20	IncubateVT	2 h	250µl hyb. mix->Specimen	prehybridization	
21	IncubateVT	16 h	250µl probe->Specimen	hybridization	
22	IncubateVT	20 min	250µl 4X SSC->Specimen	wash with 4 X SSC (J)	
23	IncubateVT	20 min	250µl 4X SSC->Specimen	wash with 4 X SSC (K)	
24	IncubateVT	20 min	250µl 2X SSC->Specimen <b>2x</b>	wash with 2 X SSC	
25	SetTempReg		T1 (LOW)	Heating on, temperature to 37°C	
26	Wait	15 min		Time to equilibrate the heating block	
27	IncubateVT	10 min	250µl Sol. A->Specimen <b>3x</b>	wash with RNase buffer	
28	IncubateVT	30 min	250µl Sol. A->Specimen	wash with RNase solution	
29	IncubateVT	10 min	250µl Sol. A->Specimen	wash with RNase buffer	

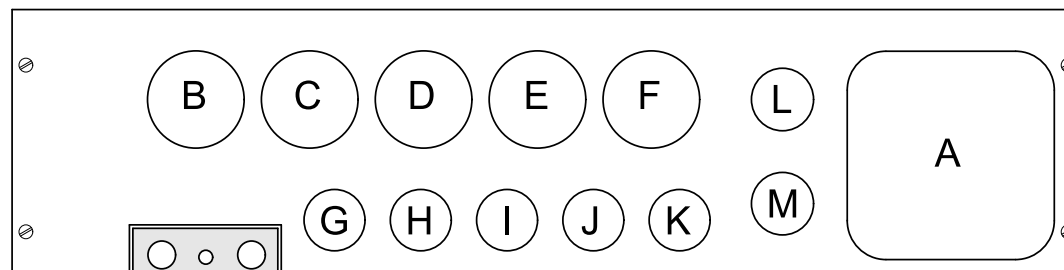
Step	Task	Time	Aliquoting	Thats what it means	Elapsed Time
30	SetTempReg		T2 (HIGH)	Heating on, temperature to 55°C	27 h ↓ 38
31	IncubateVT	15 min	250µl 2X SSC->Specimen	wash with 2 X SSC	
32	IncubateVT	10 min	250µl 0.5X SSC->Specimen <b>2x</b>	wash with 0.5 X SSC	
	SetTempReg		T0 (OFF)	Heating off, temperature to RT	
	Wait	15 min		Time to equilibrate the heating block	
	IncubateVT	10 min	150µl PBT->Specimen	wash with 0.5 X SSC / PBS-T	
	IncubateVT	10 min	250µl PBT->Specimen <b>3x</b>	wash with PBS-T	
	IncubateVT	1 h	250µl bloc.->Specimen	Blocking	
	IncubateVT	6 h	250µl antib.->Specimen	DIG antibody	
	IncubateVT	20 min	250µl PBT->Specimen <b>7x</b>	wash with PBS-T	
	PrimeNeedle		6000	Rinsing double needle	
	SetTempReg		T0 (OFF)	Heating off, temperature to RT	

## Buffer Loading

Method: Ciona intestinalis

User: Rita Marino

Date: May 2006



Position	Buffer	Amount
A	PBST	
B / B1	Paraformaldehyde 4% / <b>0.5 x SSC</b>	
C	Hyb. mix	
D	Acetic Anhydride	
E / E1	Ethanol 30% / <b>2 x SSC</b>	
F / F1	Ethanol 50% / <b>SoI. A</b>	
G	Proteinase K	

Position	Buffer	Amount
H	PBST	
I	RNAse A	
J	4 x SSC	
K	4 X SSC	
L	Blocking solution	
M	DIG antibody	
Probe		

Buffer printed in bold letters have to been put in while the method is running !

## Buffers

Buffer :	PBST	pH :	7.5
Substance	Concentration		
Na <sub>2</sub> HPO <sub>4</sub> x 2 H <sub>2</sub> O	10 mM		
NaCl	150 mM		
Triton X-100 or Tween 20	0.1 %		

Buffer :	50% EtOH	pH :	7.5
Substance	Concentration		
Ethanol p.A.	50%		
... in PBST			

Buffer :	30% EtOH	pH :	7.5
Substance	Concentration		
Ethanol p.A.	30%		
... in PBST			

Buffer :	4% PFA	pH :	
Substance	Concentration		
Paraformaldehyde	4%		
... in PBS			

Buffer :	Acetic anhydride	pH :	
Substance	Concentration		
Acetic anhydride	0.25%		
... in triethanolamin 0.1M			

Buffer :	Proteinase K	pH :	7.5
Substance	Concentration		
Proteinase K (Roche no. 03115887001)	0.043 units / ml about 4µg/ml		
... in PBST			

Buffer :	Hyb.-mix	pH :	7.5
Substance	Concentration		
Deionized formamide	50%		
20 X SSC stock solution	5 X		
tRNA	50 µg / ml		
Heparin	50 µg / ml		
50 X Denhardt's stock solution	5 X		
Tween 20	0.1 %		

Buffer :	4 X SSC	pH :	7.5
Substance	Concentration		
Deionized formamide	50%		
20 X SSC stock solution	4 X		
Tween 20	0.1 %		

Buffer :	2 X SSC	pH :	7.5
Substance	Concentration		
Deionized formamide	50%		
20 X SSC stock solution	2 X		
Tween 20	0.1 %		

<b>Buffer :</b> 0.5 X SSC	<b>pH :</b> 7.5
<b>Substance</b>	<b>Concentration</b>
Deionized formamide	50%
20 X SSC stock solution	0.5 X
Tween 20	0.1 %

<b>Buffer :</b> Sol. A	<b>pH :</b> 8.0
<b>Substance</b>	<b>Concentration</b>
Tris / HCl	10 mM
NaCl	0.5 M
tween 20	0.1%
EDTA	5 mM

<b>Buffer :</b> RNase	<b>pH :</b> 7.5
<b>Substance</b>	<b>Concentration</b>
RNase A	
. . . in Sol. A	