InsituPro VS protocol ISH Arabidopsis slides

Date: 11.11.2004
System Configuration: slide module for 60 slides

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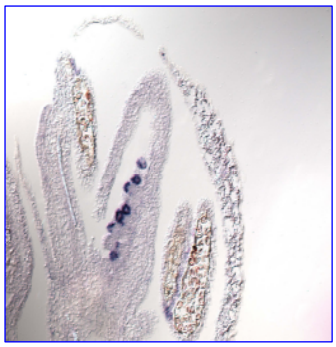


ISH on *A. thaliana*, WUS specific probe

Description:

This protocol can be used for in situ hybridization on *A. thaliana* thin sections.

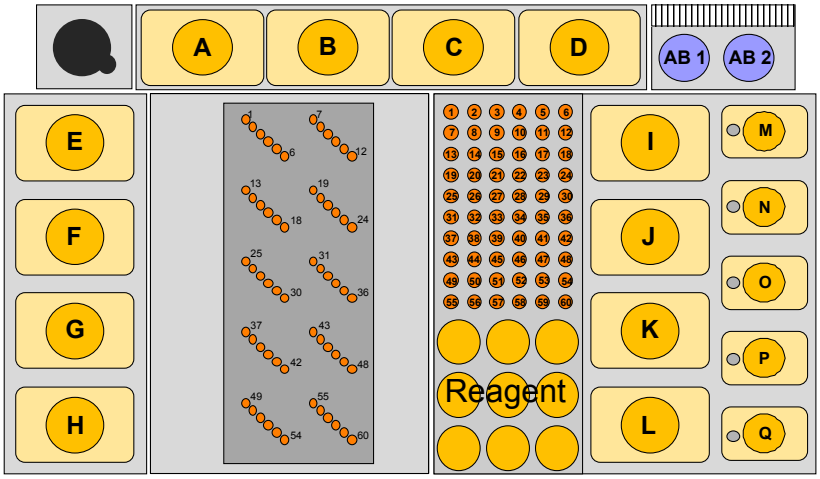
After embedding and sectioning of the plant tissue following standard protocols, slides were dewaxed and rehydrated before loading into the InsituPro VS. The slide chambers (slide with sections and spacer slide) are assembled in a water-filled beaker to avoid air bubbles on the slides. For colour detection, slides were removed from the InsituPro VS and incubated in buffer 4 with NBT/BCIP. The staining reaction was stopped by washing the slides with water. Slides were than dehydrated in an EtOH series and mounted with Entellan or similar mounting medium.

Step No.	Task	Time	Action	Proceeding
1	PrimeNeedle		12000	Wash needle
2	SetTempReg		T0 (OFF)	Set Temperature to RT
3	PrimeTub		60000	Fills tub with water
4	SetTempReg		T2 (HIGH)	Set temperature to 50 C
5	IncubateTS	5 min	400µl PBS -> Slides 2x	PBS wash
6	IncubateTS	20 min	250µl Prehyb Buffer -> Slides 2x	Prehybridization
7	PrimeTub		20000	
8	IncubateTS	10 h	250µl Probe -> Slides	Hybridization
9	PrimeTub		20000	
10	IncubateTS	15 min	250µl Formamide Wash Buffer -> Slides 6x	Formamide Wash
11	SetTempReg		T1 (LOW)	
12	Wait	15 min		Cooldown
13	IncubateTS	5 min	250µl NTE -> Slides 4x	NTE wash
14	IncubateTS	30 min	250µl RNAse -> Slides	RNAse treatment
15	IncubateTS	5 min	250µl NTE -> Slides 2x	PostRNAsewash
16	SetTempReg		T2 (HIGH)	
17	IncubateTS	15 min	250µl Formamide Wash Buffer->Slides 6x	Formamide Wash
18	SetTempReg		T0 (OFF)	
19	PrimeTub		60000	
20	IncubateTS	10 min	250µl NTE -> Slides 2x	NTE Wash

Step No.	Task	Time	Action	Proceeding
21	IncubateTS	10 min	250µl PBS -> Slides 2x	PBS wash
22	IncubateTS	5 min	250µl Buffer 1 -> Slides 2x	Buffer 1 wash
23	IncubateTS	30 min	250µl Buffer 2 -> Slides 2x	Buffer 2 blocking
24	IncubateTS	30 min	250µl Buffer 3 -> Slides 2x	Buffer 3 blocking
25	PrimeTub		20000	
26	IncubateTS	1 h	250µl Dig-Antibody -> Slides	Antibody incubation
27	PrimeTub		20000	
28	IncubateTS	10 min	250µl Buffer 1 –T ->Slides 10x	Buffer 1-T wash
29	IncubateTS	5 min	250µl Buffer 1 -> Slides2x	Buffer 1 wash
30	IncubateTS	5 min	250µl Buffer 4 -> Slides2x	Buffer 4 incubation
31	PrimeTub		60000	
32	RinseNeedle		12000	
33	SetTempReg		T0(OFF)	

Specimen and Buffer loading Form

Method: Plant slide 1 **User:** Rüdiger Simon **Date:** 09.2004



Buffer Loading:

Vial	Buffer	Volume
A	Formamide wash	
B		
C		
D		
E*	Hybridization solution	
F*	RNAse A	
G*	Buffer 2	
H*	Buffer 3	
I*		
J*		

Vial	Buffer	Volume
K*		
L*		
M	NTE	
N	Buffer 1	
O	Buffer 4	
P	PBS	
Q	Buffer 1 - T	
AB 1	AB	
AB 2		

Reagent

1		**4**		**7**	
2		**5**		**8**	
3		**6**		**9**	

Buffer amount can be reduced to 50 ml for positions E-H and I-L (labelled with *) by using the falcon adapters.

Buffers:

Buffer : 1	**pH : 7.5**
Substance	**Concentration**
Tris-HCl	100 mM
NaCl	150 mM

Buffer : PBS	**pH : 7.5**
Substance	**Concentration**
$Na_2HPO4 \times 2H_2O$	10 mM
NaCl	150 mM

Buffer : 1-T	**pH : 7.5**
Substance	**Concentration**
Tris-HCl	100 mM
NaCl	150 mM
Triton X-100	0,3%

Buffer : 2	**pH : 7.5**
Substance	**Concentration**
Tris-HCl	100 mM
NaCl	150 mM
Roche Digoxigenin Blocking reagent	0,5%

Buffer : 3	**pH : 7.5**
Substance	**Concentration**
Tris-HCl	100 mM
NaCl	150 mM
BSA	1%
Triton X-100	0,3%

Buffer : 4	**pH : 9.5**
Substance	**Concentration**
Tris-HCl	100 mM
NaCl	100 mM
$MgCl_2$	50 mM

Buffer : AB	**pH :**
Substance	**Concentration**
Tris-HCl	100 mM
NaCl	150 mM
BSA	1%
Triton X-100	0,3%
anti-DIG antibody	1:3000

Buffer: Formamide wash	**pH :**
Substance	**Concentration**
SSC	2 x
Formamide	50%

Buffer : NTE	**pH : 7.5**
Substance	**Concentration**
NaCl	500 mM
TrisHCl	10 mM
EDTA	1 mM

Buffer : RNAseA	**pH : 7.5**
Substance	**Concentration**
RNAseA	1 µg/ml
. . . in NTE	

Buffer : Hybridization sol.	**pH : 6,8**
Substance	**Concentration**
Tris-HCl	10 mM
NaCl	300 mM
$NaPO_4$-buffer	10 mM
Triton X-100	0,3%
EDTA	5 mM
tRNA	1 mg/ml
Denhards solution	1 x
Dextransulphate	10%
deionised formamide	50%