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# A protocol for mRNA in situ hybridization in rice tissue sections

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**Method Article**

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# Abstract

Nowadays, many methods had been developed to detect the expression levels of genes and proteins, such as q-PCR, northern blot, western blot and Immunofluorescence \(IF) et al. RNA _in situ_ hybridization as a classical method for detecting the localization of mRNAs is fond of the scientists. In our studies, we developed an efficient and systematic method to examine the mRNA expression level of genes using RNA _in situ_ in rice. Here we will describe this useful protocol in detail.


# Introduction

This _in situ_ hybridization was performed as described previously in Xu, YY. _et al_. \(2005)

# Reagents

1. Ethanol 2. Xylene 3. Chloroform 4. Paraffin wax 5. RNA polymerase \(T7 or SP6 polymerase) 6. RNase-free Dnase 7. 10mg/ml tRNA 8. 4M LiCl 9. 200mM $NaHCO_3$ 10. 200mM $Na_2CO_3$ 11. 1M pH4.7 NaAc buffer 12. 20 mg/ml Glycogen 13. Maleic acid buffer: 0.1M maleic acid in 0.15M pH7.5 NaCl buffer 14. RNA dilution buffer: 0.5ml DEPC water in 0.3ml 20×SSC and 0.2ml formaldehyd 15. Blocking solution: diluting 10% block reagent to 1% by malei acid buffer 16. Washing buffer: maleic acid buffer in 0.3% Tween20 17. TNM50: 0.58gNaCl; 1.0165g $MgCl_2.6H_2O$; 1.211g Tris adjust pH to 9.5 and add water to 100ml 18. Detection buffer: 2% NBT/BCIP stock solution in TNM50 19. 100ml 1M pH7.5 Tris-HCl 20. 100ml 500mM EDTA pH7.5 21. K buffer 200ml: 20ml 1M pH7.5 Tris-HCl; 20ml 500mM EDTA pH7.5; 160ml DEPC-$H_2O$ 22. 10×SSC 1000ml: 800ml DEPC-$H_2O$; 87.65g NaCl; 44.1g NaCit; 10N NaOH adjust pH to 7.0 add DEPC-$H_2O$ to 1000ml 23. 20×SSC 1000ml: 175.3g NaCl; 88.2g NaCit; 800ml d$H_2O$ and adjust pH to 7.0 24. 200ml 10×PBT: 2ml Tween 20; 5.8g $Na_2HPO_4$ .12$H_2O$; 0.6g $NaH_2PO_4$ .2$H_2O$; 15.2g NaCl; 0.04g KCl 25. 2.5U/ml antibody solution: 135ul 1×PBT; 15ul 10mg/ml BSA; 0.5ul Anti-DIG-AP 26. Detection buffer：2% NBT/BCIP stock solution in TNM50 \(150ul/2 slides)

# Equipment

Incubator \(60/42/37℃)

# Procedure

**Fixation and embedding** 1, Fixation: put the materials into the FAA fixative and drive away the air in the materials. 2, Dehydration: 50%, 70%, 80%, 90%, 95%, 100%, 100%, 100% ethanol, each for 30 min RT \(room temperation). 3,Transparent: remove the ethanol and replace with the mixture: 25% xylene - 75% ethanol, 50% xylene - 50% ethanol, 75% xylene - 25% ethanol, 90% xylene - 10% chloroform, 90% xylene-10% chloroform, 90% xylene-10% chloroform, 30min for each step. 4, Immerse the wax: 50% paraffin wax - 50% xylene, 42℃ for 4 - 16 hours and repeat once; then transfer to the 60℃ incubator and replace with pure paraffin wax, 60℃ 2 - 6 days, change the wax with the new one for at least four times. 5, Embedding

the materials. **Sectioning** 5 - 10 um sections, 42°C for at least 24 hours on the glasses with poly-lys and then stored at 4 °C. **Synthesis of probe** 1, Transcription \(20ul system): xul linearized template DNA \(1ug) 4ul 5 x transcription buffer \(containing rNTP mixture & DIG-UTP etc) 20-\(6+x) ul DEPC-water 2ul RNA polymerase \(T7 or SP6 polymerase), 42°C 2hours. 2, Digestion of template: 2 ul RNase-free Dnase at 37°Cfor 15min, Add 0.8ul 500mM EDTA to stop the reaction. 3, Precipitating the probe \(for 20ul reaction system): 1ul 10mg/ml tRNA \(It can be omitted) 2.5ul 4M LiCl 75ul 100% ethanol –20°C overnight□ 4°C 13000rpm spin down, washing 2 times with 70% ethanol and resuspended in 100ul DEPC-water. If necessary, incubate in 60°C 10min to get the probe into solution. 4, Hydrolysis of probe \(for 50ul probe solution): 20ul 200mM $NaHCO_3$ + 30ul 200mM $Na_2CO_3$ , hydrolyzed at 60°C. Stop the reaction by adding 10ul of 1M pH4.7 NaAc buffer \(or 3ul 3M pH6.0 sodium acetate and 5ul 10% glacial acetic acid) Precipitatethe probe: 1 µl 20 mg/ml Glycogen \(optional) + 10 µl 4 M LiCl + 300 µl Ethanol. Incubate at -20°C overnight. 4°C 13000rpm spin down, washing 2 times with 70% ethanol and resuspended in 100ul DEPC-water. 5, Semi-quantitative determination of probe according the protocol of RNA labeling Kit: apply a 1ul probe solution of the dilution series to the nylon membrane, fix the nucleic acid for 30min at 120°C, incubate in maleic acid buffer 2min, blocking for 30min at RT with agitation, combinding with antibody in Blocking solution for 30min at RT, pour off the antibody solution and wash with washing buffer for 2×15min, replaced the washing buffer with TNM50 and incubate for 3min, coloring in detection buffer \(NBT/BCIP) in dark for several minutes. **Prehybridization treatment** 1, Dewaxing and hydration: 100%xylene 20min at R.T., 100%xylene 20min at R.T., 66%xylene 2min, 33%xylene 2 min, 100%, 100%, 90%, 70%, 50%, 30%, 10% ethanol and $H_2O$, $H_2O$ 2min at RT for each step. 2, Digestion with Proteinase K: add the proteinase K to 37°C prewarmed K buffer to the final concentration of 1 5 ug/ml, and then washed three times with the sterilized ddH2~O. 3.Acetylation: 100mM pH8.0 triethanolamine\(2.68ml Triethanolamine per 200ml EDPC water, about 0.8ml concentrated HCl to adjust pH) 5min. Stiring the chloroform treated stir bar hard and add anhydride to final concentration of 0.25% \(500ul/200ml), after mixing for 5 seconds, incubate the slides for 5min at RT. 4.Dehydration: 2×SSC, 5min and then repeat once, 10% ethanol 2min at R.T. 30% ethanol 2min at R.T, 50% ethanol 2min at R.T, 70% ethanol 2min at R.T, 90%, 2min at R.T, 100%ethanol 2min at R.T, 100%ethanol 2min at R.T. Dry sections under vacuum at least 1h until hybridization. If essential pretreated sections can be stored at –20°C. **Hybridization** hybridization: 42°C overnight in humidified box with wet paper \(contains 0.3M NaCl□50□formamide). **Washing** 1, washing: 40ml 4xSSC RT, 5-10min 40ml 4xSSC RT, 5-10min 40ml 4xSSC RT, 5-10min 40ml 4xSSC RT, 5-10min. 2, RNase treatment: put slides in the prewarmed 37°C RNase buffer \(500Mm NaCl-1mM EDTA-10mM Tris.HCl pH 7.5), add RNase A to the final concentration of 25ug/ml for 30 min. 3, RNase buffer resine for 15min at 37°C and repeat twice. 4, low-stringency wash: 2XSSC resin for 30min and repeat once at R.T. with gentle stirring. 5, high-stringency wash: 0.1xSSC resin for 1hour at 60°C. **Detection** 1, 1×PBT pH7.5 RT 5min. 2, 0.5% Blocking Reagent in PBT 30~60min. 3, 1×PBT 1min. 4, combinding with anti-DIG-AP RT 30~120min in humidified box with wet paper with 1×PBT. 5. 1×PBT 250ml, 20minX2min. 6. 1×TNM50 5min. 7. Show the singal: RT in dark for 30min to 24 hours without shaking. 8.Cover the glasses.

## Timing

About 14 days: day 1, fixation the materials. day 2, dehydration, transparent and embedding. day 3-7, embedding \(synthesis the probes during this period). day 8, sectioning. day 9-10, prehybridization and hybridization. day 11, washing and detection. day 12-14, slides were photographed under a microscope.

# References

Wei-dong Yong, Yun-yuan Xu, Wen-Zhong Xu, Xin Wang, Ning Li, Jin-song Wu, Tie-bing Liang, Kang Chong, Zhi-hong Xu, Ke-hui Tan and Zhi-qing Zhu. Vernalization-induced flowering in wheat is mediated by a lectin-like gene VER2. Planta. 217, 261-270 \(2003). Xu, Y. Y. et al. Activation of the WUS gene induces ectopic initiation of floral meristems on mature stem surface in Arabidopsis thaliana. Plant Mol. Biol. 57, 773–784 \(2005).

# Figures

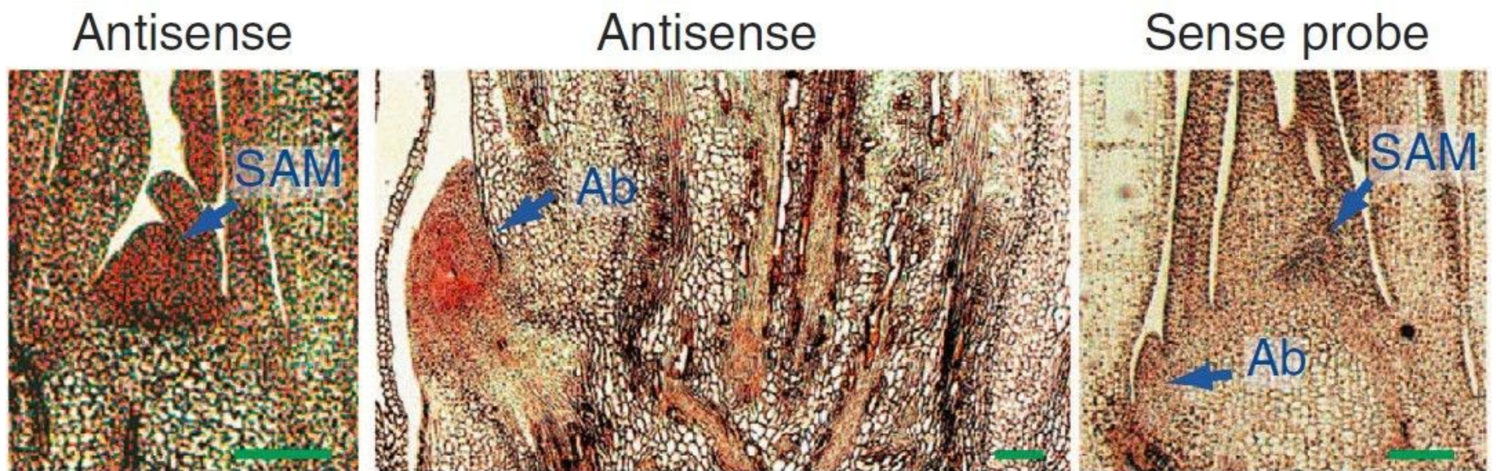


**Figure 1**

In situ