



Cold Spring Harbor Protocols

Whole-Mount In Situ Hybridization of RNA Probes to Plant Tissues

Jan Traas

Cold Spring Harb Protoc 2008; doi: 10.1101/pdb.prot4944

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>

Protocol

Whole-Mount In Situ Hybridization of RNA Probes to Plant Tissues

Jan Traas



This protocol was adapted from "How to Study Gene Expression," Chapter 7, in *Arabidopsis* (eds. Weigel and Glazebrook). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2002.

INTRODUCTION



The labeling of entire plantlets (whole-mount labeling) is very easy, but it can cause substantial artifacts. Because whole plants are labeled, certain tissues are not readily accessible to the probes, whereas others can often stain nonspecifically. In the hands of the author, for instance, the elongation zone of the root is labeled even in negative controls, whereas mature parts of the roots and hypocotyl tend to be more resistant to probe penetration. Whole-mount labeling should therefore only be used to monitor gene expression in a limited number of organs and tissues—in particular, root meristems, embryos, and very young primordia—and the results should be interpreted with care. A protocol for



whole-mount labeling is described here. Positive (e.g., constitutive probe) and negative controls should be included systematically.

RELATED INFORMATION



This protocol was adapted from Ludevid et al. (1992). Successful in situ localization of RNA and protein has been described for whole mounts of snapdragon floral meristems (Zachgo et al. 2000).

See *CSH Protocols* article **Preparation and Hydrolysis of Digoxigenin-Labeled Probes for In Situ Hybridization of Plant Tissues** (this issue) for probe preparation.

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <I>, and recipes for reagents marked with <R>.

Reagents

Acetone powder prepared from plant tissue (optional; see Step 18)
Antibody (anti-DIG, alkaline phosphatase-conjugated)
Blocking reagent (e.g. in DIG Nucleic Acid Detection kit [Roche Applied Science]) (optional; see Step 18)
5-bromo-4-chloro-3-indolyl-phosphate 4-toluidine (BCIP) (50 mg/mL)
<R>Detection buffer
<I>Dimethyl sulfoxide (DMSO) (optional; see Step 1)
Ethanol (series of 30%, 60%, 70%, 85%, 95%, 100%) (Steps 2 and 23)
<R><I>Formaldehyde (4%, v/v in PBS)
<I>Prepare fresh from paraformaldehyde.
<I>Histoclear

<R><!-- Hybridization buffer (whole-mount) (HBWM)
 <!-- Methanol
 <!-- 4-nitro blue tetrazolium chloride (NBT) (100 mg/mL)
 <R>PBT
 <R><!-- PBTF (PBT + 4% formaldehyde)
 <R><!-- PBTk (PBT + proteinase K, 40 µg/mL; may be reduced if necessary)
 Permout
 <R>Phosphate-buffered saline (PBS) (1X)
 Plant tissue of interest
Use only small samples such as young seedlings, young roots, meristems.
 Probe (DIG-labeled) (from **Preparation and Hydrolysis of Digoxigenin-Labeled Probes for In Situ Hybridization of Plant Tissues**)
 <R>TE buffer
 <!-- Xylene

Equipment

Heating block preset to 80°C
 Ice
 Microscope (dissecting)
 Pipettes (Pasteur)
 Tubes (microcentrifuge)
 Vials (scintillation)
 Water bath preset to 55°C

METHOD

Fixation and Dehydration

1. Fix plant tissue in a scintillation vial using 4% (v/v) formaldehyde in PBS as follows:
 - i. Fix the tissue for 30 min at room temperature; if necessary, place under vacuum for 15 min.
 - ii. Leave the tissue in fixative overnight at 4°C.
Adding 10% DMSO to the fixative can sometimes improve the results.
2. Dehydrate the samples through an ethanol series: 30%, 60%, 70%, 85%, 95%, 100%, 100%, for 30 min each.
3. Leave the samples in 100% ethanol for 2-4 d at -20°C.
This helps to reduce background staining, but it does make the samples brittle.
4. Transfer a small number of samples of tissue to a microcentrifuge tube (e.g., up to four 5-d-old plantlets per tube).

Pretreatment

5. Wash the samples twice with 100% ethanol.
If necessary, use a drawn-out Pasteur pipette to remove the ethanol. Be careful; the samples are fragile.
6. Wash the samples in xylene for 30 min.
7. Wash the samples four times in 100% ethanol, followed by twice in methanol.
8. Prepare 50% (v/v) methanol/50% (v/v) PBTF and transfer the tissue to this solution. Incubate for 5 min.
9. Post-fix the tissue in PBTF for 25 min and wash five times in PBT for 10 min per wash.

10. Incubate the samples in PBTK for 10 min and wash four times in PBT for 5 min per wash.
11. Post-fix the tissue in PBTF for 25 min and wash four times in PBT for 5 min per wash. Leave the tissue in PBT for 60 min. Add an equal volume of HBWM to the last wash.
12. Rinse the tissue twice in HBWM.

Hybridization and Wash

13. Prehybridize in HBWM for 3 h at 55°C in a water bath.
14. Denature the DIG-labeled probe for 2-5 min at 80°C, and place on ice.
15. Replace the buffer used for prehybridization with fresh prewarmed HBWM. Add the denatured DIG-labeled probe in 1-5 µL.
16. Hybridize for 18 h (i.e., overnight) at 55°C.
17. Wash the samples as follows:
 - i. Wash six times in HBWM for 30 min per wash.
 - ii. Prepare 75% (v/v) HBWM/25% (v/v) PBT. Wash for 20 min in this solution.
 - iii. Prepare 50% (v/v) HBWM/50% (v/v) PBT. Wash for 20 min in this solution.
 - iv. Prepare 25% (v/v) HBWM/75% (v/v) PBT. Wash for 20 min in this solution.
 - v. Wash four times in PBT for 5 min per wash.
18. Replace the PBT with 500 µL of PBT + antibody (diluted 1:2000).

If necessary, blocking reagent can be added according to the manufacturer's instruction. The antibody may also be preadsorbed with an acetone powder made from plant tissue. Incubate overnight in the cold.
19. Wash the tissue in PBT for at least 3 h, changing the PBT several times.
20. Equilibrate the tissue for 5 min in detection buffer.
21. Add 150 µL of NBT (100 mg/mL) and 150 µL of BCIP (50 mg/mL) per 100 mL of detection buffer. Incubate the tissue in the NBT/BCIP solution in the dark. Check under a dissecting microscope after 12 h. Incubate for a total of up to 36 h (or longer).
22. Stop the enzyme reaction in TE buffer.
23. Dehydrate the tissue through an ethanol series (90 sec each), followed by HistoClear, twice for 90 sec.
24. Mount with Permount.

REFERENCES

- | | |
|--|---|
| Ludevid, D., Höfte, H., Himmelblau, E., and Chrispeels, M.J. 1992. The expression pattern of the tonoplast intrinsic protein γ -TIP in <i>Arabidopsis thaliana</i> is correlated with cell enlargement. <i>Plant Physiol.</i> 100 : 1633–1639. | Zachgo, S., Perbal, M.C., Saedler, H., and Schwarz-Sommer, Z. 2000. In situ analysis of RNA and protein expression in whole mounts facilitates detection of floral gene expression dynamics. <i>Plant J.</i> 23 : 697–702. |
|--|---|