Appendix A

Reagents used

- Suitable restriction endonuclease enzyme, producing 5'-overhang or blunt end
- pEASY-Blunt Zero Cloning Kit (Transgene, cat. no. CB501-02)
- Universal DNA Purification Kit (Tiangen, cat. no. DP214-03)
- Phenol: Chloroform: Isoamyl alcohol (25:24:1) (Coolaber, cat. no. SL2040)
- NaAc (Sodium acetate) (Hushi, cat. no. 10018818)
- DEPC (Diethyl pyrocarbonate) (Sigma, cat. no. D5758)
- Promega Transcription System:

  4 µL 5×Buffer (Promega, cat. no. P1181)

  2 µL DIG-RNA-Labeling Mixture (Roche, cat. no.11277073910)

  2 µL DTT (0.1 mol/L) (Promega, cat. no. P1171)

  1 µL RNasin Plus RNase Inhibitor (Promega, cat. no. N2615)

  2 µL T7 RNA Polymerase (Promega, cat. no. P207B)
- RNase-free DNase I (Takara, cat. no. 2270A)
- $NaHCO_3$ (Sigma, cat. no. S6297)
- Na_2CO_3 (Sigma, cat. no. S7795)
- Acetic acid (Sigma, cat. no. 338826)
- Formamide, Deionized (Amresco, cat. no.0606)
- Paraformaldehyde (PFA) (Sigma, cat. no. P6148)
- Poly (ethylene glycol) distearate (Sigma, cat. no. 305413)
- 1-Hexadecanol (Sigma, cat. no. 258741)
- NaCl (Amresco, cat. no. 0241)
- Glycine (Amresco, cat. no. 0167)
- Na_2HPO_4 (Amresco, cat. no. 0404)
- NaH_2PO_4 (Amresco, cat. no. 0571)
- proteinase K (Roche, cat. no. 03115879001)
- Tris (Amresco, cat. no. 0497)
- EDTA (Amresco, cat. no. 0105)
- Dextran sulfate (Amresco, cat. no. 0198)
- 50×denhardt's (Sigma, cat. no. D9905)
- tRNA (Roche, cat. no. 10109541001)
- Na Citrate (Sigma, cat. no. S1804)

- RNase A (Takara, cat. no. 2158)
- Blocking reagent (Roche, cat. no. 11096176001)
- BSA (Amresco, cat. no. A7906)
- Triton X-100 (Amresco, cat. no. T8787)
- Anti-Digoxigenin AP-conjugate (Roche, cat. no. 11093274910)
- NBT/BCIP (Roche, cat. no. 11681451001)
- Glycerol (Sinopharm, cat. no. 10010618)
- 1×PBS (diluted with 10×PBS, see Reagent setup)
- 4% PFA (see Reagent setup)
- Proteinase K solution (see Reagent setup)
- 0.2%Glycine (see Reagent setup)
- Hybridization solution (see Reagent setup)
- 0.2×SSC (diluted with 20×SSC, see Reagent setup)
- 1×NTE (diluted with 5×NTE, see Reagent setup)
- RNase A solution (see Reagent setup)
- 1×TBS (diluted with 10×TBS, see Reagent setup)
- blocking solution (see Reagent setup)
- BSA solution (see Reagent setup)
- Detection buffer (see Reagent setup)
- TE buffer (see Reagent setup)

Special equipment

- Cell strainer (100 μm) (Biologix, cat. no. 15-1100)
- Petri-dish (60×15 mm) (Corning, cat. no. 430196)
- Petri-dish (35×10 mm) (Corning, cat. no. 430165)

Reagent setup

4%PFA Per 50 mL: 2 g PFA diluted in 50 mL 1×PBS, heating for 4 h to dissolve.

0.2%Glycine Per 50 mL: 2 g Glycine diluted in 50 mL 1×PBS.

2×Carbonate buffer (80 mmol/L $NaHCO_3$, 120 mmol/L Na_2CO_3) Per 200 μL: 16 μL 1 mol/L $NaHCO_3$, 24 μL 1 mol/L Na_2CO_3, 160 μL DEPC-H_2O.

10×PBS (1.3 mol/L NaCl, 70 mmol/L Na_2HPO_4, 30mmol/L NaH_2PO_4) Per 1 L: 75.97 g NaCl, 9.94 g Na_2HPO_4, 3.6 g NaH_2PO_4. Adjust pH to 7.0 and add DEPC-H_2O to 1 L.

Proteinase K solution (10 μg/mL proteinase K in 100 mmol/L Tris pH 8.0, 50 mmol/L EDTA) Per 50 mL: 5 mL 1 mol/L Tris pH 8.0, 5 mL 0.5 mol/L EDTA, 40 mL DEPC-H_2O, add 5 μL 100 mg/ml proteinase

K.

10×salts (3 mol/L NaCl, 100 mmol/L Tris-HCl pH 8.0, 100 mmol/L sodium phosphate, pH 6.8, 50 mmol/L EDTA) Per 5 mL: 3 mL 5 mol/L NaCl, 0.5 mL 1 mol/L Tris-HCl pH 8.0, 0.0306 g NaH_2PO_4, 0.0348 g Na_2HPO_4, 0.5 mL 0.5 mol/L EDTA, 1 mL DEPC-H_2O.

Hybridization solution (0.3 mol/L NaCl, 10 mmol/L Tris-HCl pH 8.0, 10 mmol/L sodium phosphate, pH 6.8, 5 mmol/L EDTA, 50% formamide, 10% dextran sulfate, 1×denhardt's, 1.25 mg/mL tRNA) Per 1.9 mL: 200 μL 10×salts, 950 μL formamide, 400 μL 50% dextran sulfate, 40 μL 50×denhardt's, 25 μL 100 mg/mL tRNA, 285 μL DEPC-H_2O.

20×SSC (3 mol/L NaCl, 300 mmol/L Na Citrate) Per 1 L: 175.3 g NaCl, 88.2 g Na Citrate, add DEPC-H_2O to 1 L.

5×NTE (2.5 mol/L NaCl, 50 mmol/L Tris-HCl pH 8.0, 5 mmol/L EDTA) Per 1 L: 146 g NaCl, 50 mL 1 mol/L Tris-HCl pH 8.0, 10 mL 0.5 mol/L EDTA, add DEPC-H_2O to 1 L.

RNase A solution (10 μg/mL RNase A in 1×NTE) Per 50 mL: 50 μL RNase A diluted in 50 mL 1×NTE.

10×TBS (1 mol/L Tris-HCl pH 8.0, 1.5 mol/L NaCl) Per 1 L: 121.16 g Tris, 87.66 g NaCl. Adjust pH to 7.0 and add DEPC-H_2O to 1 L.

Blocking solution (1% Blocking reagent in 1×TBS) Per 50 mL: 0.5 g Blocking reagent dissolved in 50 mL TBS, incubating for 1 h to dissolve.

BSA solution (1% BSA, 0.3% Triton X-100 in 1×TBS) Per 500 mL: 5 g BSA, 1.5 mL Triton X-100, add 1×TBS to 500 mL.

Antibody solution (1:1000) Per 20 mL: 20 μL anti-DIG-AP-antibody diluted in 20 mL BSA solution.

Detection buffer (100 mmol/L Tris-HCl pH 9.5, 100 mmol/L NaCl) Per 100 mL: 10 mL 1 mol/L Tris-HCl pH 9.5, 2 mL 5 mol/L NaCl, add DEPC-H_2O to 100 mL.

NBT/BCIP solution (1:1000) Per 10 mL: 200 μL NBT/BCIP diluted in 10 mL Detection buffer.

TE (10 mmol/L Tris-HCl pH 8.0, 1 mmol/L EDTA pH 8.0) Per 100 mL: 1 mL 1 mol/L Tris-HCl pH 8.0, 200 μL 0.5 mol/L EDTA, add DEPC-H_2O to 100 mL.

The whole step-by-step procedures of in situ hybridization are as following:

Probe preparation

Probe DNA templates prepare (1 day)

1. The DNA templates of probes are amplified from cDNA using primers designed based on the specific sequences of the genes of interest. The DNA fragments are inserted into a pEASY-Blunt vector. The constructed vector is linearized by restriction endonuclease digestion. The primer sequences and restriction endonucleases used in our study are given in Supplementary Table 1.

2. The linearized sequences of DNA templates are recovered using a Universal DNA Purification Kit; 10 µg of DNA is required to prepare probes.
3. To remove the RNase, the DNA solution is extracted by adding an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1), centrifuging for 10 min, and collecting the supernatant. Then, the DNA is precipitated by adding 2.5 times the volume of absolute ethanol and 1/10 the volume of 3 mol/L NaAc to the supernatant and refrigerating at -20°C overnight or at -80° C for 1 h.
4. After precipitation, samples are centrifuged at 4°C for 20 min to obtain a DNA pellet, which is then washed in 70% ethanol and centrifuged for 5 min. A pipette is used to remove as much 70% ethanol as possible, and the pellet is dried and then dissolved in DEPC water. The resulting RNase-free DNA templates for *in vitro* transcription can be stored at -80°C.

In vitro probe transcription (1 day)

5. DNA templates of 1 µg is filled with water to the volume of 9 µL, denatured at 65°C for 5 minutes and cooled down on ice immediately. RNA probe is synthesized in the Promega Transcription System of total 20 µL for incubating for 2 h at 37°C.
6. 1 µL of RNase-free DNase I (1 µL) is added for incubating at 37°C for 30 min to digest DNA templates and incubated at 65°C for 15 min to deactivate the DNase I. The RNA probe solution (1 µL) can be checked on an agarose gel.
7. The RNA probe solution is diluted to 200 µL with DEPC water and precipitated by adding 2.5 times the volume of absolute ethanol and 1/10 the volume of 3 mol/L NaAc at −20°C overnight or at −80°C for 1 h.
8. The RNA pellet is collected by centrifuging at 4°C for 20 min, washing with 70% ethanol, and centrifuging at 4°C for 5 min. A pipette is used to remove as much 70% ethanol as possible. The dry pellet is dried and then dissolved in 100 µL DEPC water. The resulting RNA probe can be stored at −80°C.

Probe hydrolysis (optional, 1 day)

9. The RNA probe is hydrolyzed in 2× carbonate buffer at 60 ℃ to the optimum final length of 150 bp. Hydrolyzation time = (initial length − 150) / (k × initial length × 150), k = rate constant = 110 bp/min.
10. After hydrolysis, the RNA probe is neutralized with 10 µL 10% acetic acid. Then, it is precipitated by adding 2.5 times the volume of absolute ethanol and 1/10 the volume of 3 mol/L NaAc at −20°C overnight or at −80°C for 1 h.
11. The RNA pellet is collected by centrifuging at 4°C for 20 min, washing with 70% ethanol, and centrifuging at 4°C for 5 min. A pipette is used to remove as much 70% ethanol as possible. The dry

RNA pellet is dried and then resuspended in 40 µL 50% formamide. The hydrolyzed RNA probe can be stored at −80 ℃ until use.

Tissues fixation (1 day)

1. Tissue samples (in our study, shoot apical meristem and floral meristem tissues of cucumber and Arabidopsis) are excised and placed into 50 mL centrifuge tubes containing 20 mL pre-cooled 4% PFA fixative.
2. Tissues are infiltrated by applying a vacuum for 1 h 2–10 times on ice until the tissue is at the bottom of the tube. At this stage, samples can be stored at 4°C in fresh 4% PFA.

Dehydration and Steedman's wax infiltration (1 day)

3. Fixation tissues are dehydrated through a 30, 50, 70, 85, 95 and 100% ethanol series on ice for 1 h each on a rotary shaker.
4. Firstly, polyethylene glycol (PEG) distearate is melted at 60°C in oven. Prepare 100 g of pure wax mixture by weighing 90 g of polyethylene glycol (PEG) distearate and 10 g of 1-hexadecanol and stirring the mixture at 65°C for at least 4 h.
5. Dehydrated tissue samples are placed in 10-mL pre-warmed pure ethanol at 42°C. The pure wax mixture (10 mL) is added carefully using a pipette without a tip along the tube wall. Do not mix the pure wax with ethanol and keep two phases: the upper phase is ethanol and the lower phase is pure wax.
6. The tissue samples are incubated in the ethanol and pure wax at 42°C overnight. At first, the tissue samples float at the interface between the two phases. Then, they gradually sink into the lower phase of pure wax.

Steedman's wax infiltration and embedding (1 day)

7. After the overnight incubation, the tissue samples should have sunk to the bottom of tubes. The ethanol and pure wax are removed separately using a pipette without tip.
8. Pre-warmed pure wax is then added to the tubes, and the tissue samples are incubated at 42°C for at least 8 h.
9. Tissue embedding is performed on a heating block pre-warmed to 42°C. Pre-warmed pure wax is poured into Petri dishes on the heating block. The tissues are placed in the Petri dishes in the required orientation.
10. The tissues in the Petri dishes are fully polymerized at room temperature (RT) overnight. The resulting blocks can be used directly for sectioning or stored at −20°C for a few weeks.

Tissue sectioning and hybridization (1 day)

11. The embedded tissues are cut into sections (10–20 μm) with a Leica RM 2015 Microtome.
12. Sections are collected into Petri dishes (60 × 15 mm) for the next pre-treatment step. An optional step is to examine and select appropriate sections with a microscope after dewaxing them in ethanol. Sections can be stored at 4°C.
13. Sections are dewaxed in ethanol directly and put into cell strainers in Petri dishes.
14. Sections are rehydrated in absolute ethanol twice for 5 min, then in a 95, 85, 70, 50, and 30% ethanol series for 1 min each, 0.85% NaCl for 2 min, and 1× PBS for 2 min.
15. Sections are placed in pre-warmed 10 μg/mL proteinase K solution at 37°C for 10 min. The treatment is stopped in 0.2% glycine for 2 min, and sections are washed in 1× PBS twice for 2 min.
16. The sections are post-fixed in 4% PFA for 10 min and washed again in 1× PBS twice for 2 min.
17. Sections are incubated in acetic anhydride solution for 10 min and washed again in 1× PBS twice for 2 min.
18. The hybridization solution is prepared by heating it to 80°C for 10 min and cooling it on ice.
19. The probes are denatured by heating them to 80°C for 2 min and quick-cooling them on ice.
20. Probes and hybridization solution are mixed to make a probe hybridization solution at a working concentration of 1 μg/mL.
21. Sections are hybridized in 2 mL probe hybridization solution at 50°C overnight in cell strainers in Petri dishes (35 × 10 mm), which are sealed using parafilm.

Washing, blocking, antibody incubation, and washing (1 day)

22. After hybridization, sections are washed in pre-warmed 0.2× SSC twice for 20 min at 55°C to remove any non-specifically bound probes.
23. Sections are rinsed in pre-warmed 1× NTE twice for 5 min at 37°C and then treated in RNase A for 30 min at 37°C, and washed again in 1× NTE twice for 5 min at 37°C and in 0.2× SSC for 20 min at 55°C.
24. Sections are incubated in 1× TBS for 5 min at RT. Then, they are blocked sequentially in blocking solution and BSA solution for 45 min each at RT.
25. Sections are incubated in anti-DIG-AP-antibody solution for 2 h at RT in cell strainers in Petri dishes (35 × 10 mm).
26. Sections are washed in BSA solution at least four times for 15 min to remove any non-specifically bound antibodies.

Signal detection and imaging (1 day)

27. Sections are incubated in detection buffer twice for 5 min and stained with NBT/BCIP in detection buffer for several hours in cell strainers in Petri dishes (35 × 10 mm).

28. Once probe signals are sufficiently developed, staining is stopped in TE buffer and sections are mounted onto microscope slides using forceps.
29. The slides are dried and covered in 50% (v/v) glycerol and a coverslip.
30. Images are captured using a Leica DM5500B microscope equipped with a digital camera.