

Appendix SI.

Pre-processing process:

1. Grill: 80°C Grill for 30 min;
2. Dewaxing: Enter the preheated 68°C dewaxing agent for 15 min;
3. Film washing: After removal, immerse in 100% ethanol at room temperature for 5 min;
4. Penetration: Take out the glass slide and immerse it in a preheated 90°C penetration agent for 20 min;
5. Film development: Take out the glass slide and immerse it in preheated 37°C deionized water for 3 min;
6. Enzyme digestion: Take out the slide and immerse it in preheated 37°C protease working solution for 10 to 40 min (protease working solution preparation: Take out 10X protease solution, shake well, dilute with protease working buffer to 1X protease solution, thoroughly mixed. Before preparation, the protease working buffer should be preheated to 37°C. The protease working solution is prepared and used immediately, and the solution is discarded after one use;
7. Film washing: Take out the glass slide and soak it in washing solution (2x rinse twice in SSC, 5 min/time);
8. Dehydration: After removing the glass slide, immerse it in 70, 80 and 100% gradient ethanol for 2 min each;
9. Dry slides: Take out the glass slides and let them dry at room temperature for hybridization experiments.

Transgenic hybridization:

1. Take out the probe, let it stand at room temperature for 5 min, mix the probe upside down and centrifuge briefly. Take 10 μ l and drop it onto the hybridization area, and immediately cover it with a 22x22-mm cover glass, the probe should be evenly spread under the cover glass without bubbles, and the edge should be sealed with rubber tape (the sealing must be thorough to prevent dry slides from affecting the detection results during the hybridization process);
2. Place the glass slide on the hybridizer and allow for a total of 5 min at 85°C (the hybridizer should be preheated to 85°C in advance). Hybridize at 42°C for 2 to 16 h.

Washing and re-dyeing:

The following operations need to be carried out in a dark room.

1. Take out the hybridized glass slide, remove the rubber glue from the cover slide and immerse the slice in 2x SSC for ~5 sec, gently remove the cover glass with tweezers;
2. Place the slices at room temperature 2x wash in SSC for 1 min;
3. Take out the 0.3% NP-40/0.4 preheated at 68°C in advance and wash in SSC solution for 2 min;
4. Take out the slide and immerse it in deionized water at 37°C for 1 min, then dry it naturally in the dark;
5. Drop 10 μ l of DAPI re-staining agent onto the hybridization area and immediately cover it with a cover glass.