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In situ hybridization

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1 Works for me dx.doi.org/10.17504/protocols.io.9qhh5t6

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ABSTRACT

This protocol provided the detail of in situ hybridization in the mantle tissue of Pinctada fucata martensii.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0226367

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Xiong X, Xie B, Zheng Z, Deng Y, Jiao Y, Du X (2019) *PfmPif97-like* regulated by Pfm-miR-9b-5p participates in shell formation in *Pinctada fucata martensii*. PLoS ONE 14(12): e0226367. doi: 10.1371/journal.pone.0226367

GUIDELINES

All tissue harvest and in situ hybridization should be performed using RNase-free reagents and tools.
Fix in time and add 0.1% DEPC into the stationary liquid to avoid that RNase decomposes mRNA.
The strict control of the temperature of the solution is necessary for each step. The washing process in each step needs to be gentle to prevent the sample from falling out of the slide.

BEFORE STARTING

Need to prepare the tissue sample, RNA probe, and some buffer.

Prepare stock solutions

1 **3% citric acid:** Add 3g citric acid into 100mL distilled water at a pH of around 2.0.

2X SSC: Add 17.6g NaCl and 8.8g Na3C6H5O7•2H2O into 1000mL distilled water.

0.5X SSC: Add 100mL 2X SSC into 300mL distilled water.

0.2X SSC: Add 30mL 2X SSC into 270mL distilled water.

20% glycerin: Add 20mL glycerin into 80mL distilled water.

0.5M PBS: Add 30g NaCl, 6g of Na2HPO4 • 12H2O, and 0.4g NaH2PO4 • 2H2O into 1000mL distilled water. Adjust pH to 7.2-7.6.

2 Prepare samples by paraffin sectioning.

3 Mix 30% H2O2 with CH3OH in the ratio of 1: 50. Deal the slide with the mixture at room temperature for 30 minutes to inactivate Endogenousperoxidase and wash with distilled water.

4 Exposure the fragments of mRNA nucleic acid. Pipette Pepsin that is diluted by 3% citric acid (Mix completely 1mL 30% citric acid with two drops of concentrated Pepsin) and digest at 37°C or room temperature for 3-30 minutes. Wash with 0.5M PBS 3 times for 5 minutes. (Adjustable according to the thickness of the visual specimen, old and new)

5 Prehybridization: Preparation for wet box—Add 40mL of 20% glycerin to the bottom of the dry hybridization chamber to keep moist. Apply 20 µl of prehybridization solution per sample in each slide. Incubate at 37°C incubator for 3 hours. Absorb excess liquid and do not wash.

6 Hybridization: dilute digoxin-labeled oligonucleotide probe with hybridization diluent solution at a concentration of typically 0.5-2 µg/ml. Apply 60 ng of the mixing solution per sample in each slide. Remove the protective film of the coverslip specialized for in situ hybridization and place it on the slide. Hybridize overnight at 37°C incubator.

7 Stand the slide from step 6 on 37°C 2X SSC, let the coverslip fall off naturally. A gentle vibration is allowed if necessary.

8 Wash: remove the coverslip and wash twice with 37°C 2X SSC for 5 minutes; wash once with 0.5X SSC for 15 minutes; wash once with 0.2X SSC for 15 minutes (Repeat as necessary).

9 Blocking: add blocking solution and blocking at 37°C for 30 minutes. Discard excess liquid and do not wash.

10 Add Biotinylated Mouse Anti-Digoxin: at 37°C for 1 hour or room temperature for 120 minutes. Wash 4 times with 0.5M PBS for 5 minutes. Do not wash with other buffers and distilled water.

11 Add SABC: at 37°C for 20 minutes. Wash 3 times with 0.5M PBS for 5 minutes. Do not wash with other buffers and distilled water.

12 Add Biotinylated Peroxidase: at 37°C for 20 minutes. Wash 4 times with 0.5M PBS for 5 minutes.

13 Color development: use DAB Chromogenic Substrate Kit (Catalog Number: AR1025) —add one drop(50µL) Reagent A (DAB Chromogen Concentrate), one drop (50µL) Reagent B (H2O2 Concentrate) and one drop(50µL) Reagent C (TBS buffer Concentrate) to 1mL distilled water and mix well. Then add the mixture to the specimen. Control color development under a microscope within 30 minutes. Continue to develop if there is no staining background. Wash the slide well with water.

14 Add 50 ul water-soluble mounting media (AR1018) to the slide for per coverslip. Remove the protective film of the coverslip specialized for in situ hybridization and place it on the slide.

15 Observed by using a microscope.