FISH Protocol

Paraffin-Fluorescence Probe

(For scientific research purposes only, not for clinical diagnosis!)

The Experiment Steps

1. Organization fixation: take out the organization, wash clean, then Immediately put in the fixed fluid (DEPC) above 12h.
2. Dehydration: The tissue is dehydrated by gradient alcohol, paraffin, embedding.
3. Section: The paraffin is sliced through the slicer, the piece of the slice machine and the 62 - degree oven roast for 2 hours.
4. Dewaxing and dehydration: Soak sections in 2 changes of Dewaxing Transparent Liquid, 15 minutes each. Dehydrate in 2 changes of pure ethanol for 5 minutes each.Then, followed respectively by dehydrating in gradient ethanol of 85%and 75% ethanol 5 minutes each. Wash in DEPC dilution.
5. Digestion: according to the tissue fixation time, the slices are boiled in the retrieval solution for 10-15 minutes and naturally cooled. Mark the objective tissue with liquid blocker pen, according to the characteristics of tissues, Add proteinase K(20 ug/ml) working solution to cover objectives and incubate at 37℃ formin. Wash in pure water, then wash three times in PBS (pH 7.4) on a Rocker device, 5 min each.
6. Pre-hybridization: add Pre-hybridization solution to each section and incubate for 1 h at 37℃.
7. Hybridization: remove the pre-hybridization solution, add the probe hybridization solution with concentration of , and incubate the section in a humidity chamber and hybridize overnight at℃.
8. Washing: remove the hybridization solution. Wash sections in 2×SSC for 10 min at 37℃ , Wash sections in 1×SSC two times for 5 min each at 37℃, and wash in 0.5×SSC for 10 min at room temperature. Formamide washing can be added if there are more non-specific hybrids.
9. Stain cell nuclei (counter stain): incubate with DAPI for 8min in the dark, and then mounting.
10. Microscopic examination and photography: to take photos with positive fluorescence microscope. DAPI glows blue by UV excitation wavelength 330-380 nm and emission wavelength 420 nm; FAM glows green by excitation wavelength 465-495 nm and emission wavelength 515-555 nm; CY3 glows red by excitation wavelength 510-560 nm and emission wavelength 590 nm.

Interpretation of Results

The nuclear stained by DAPI were blue under ultraviolet excitation, and the positive expression was a kind of fluorescence labeled by corresponding luciferin. FAM (488) is green light, cy3 is red light. The results of mRNA in situ hybridization were cytoplasmic positive and a few nuclear positive were normal. MicRNA and lncRNA were expressed differently. According to the expression, Different fluorescence brightness is strong or weak.