PNA FISH (Fluorescence In Situ Hybridization)

FISH (fluorescence *in situ* hybridization) is a cytogenetic technique to detect and localize the presence or absence of specific DNA sequences on chromosomes.

Because of its high affinity and specificity to target DNA, PNA (peptide nucleic acid) probes are ideal tools for FISH. The benefits are specific target binding, short hybridization time, low background, good reproducibility, and superior stability of the reagent. PNA FISH probes are also efficient at penetrating the tissues thanks to the small size, and also no need of denaturation of the probe itself since it is a short single stranded oligonucleotide.

PNA Bio offers telomere (TelC: C-rich or leading strand and TelG: G-rich or lagging strand) and centromere PNA probes with various fluorescence labels as catalog items. These probes can be also used to quantify the amount of specific target sequence (Q-FISH), to detect signal in flow (Flow FISH), to co-stain with antibodies (IF-FISH), or to detect strand specific hybridization (CO-FISH). Also custom designed PNA probes can be utilized for other types of detection. For more details, please refer to http://www.pnabio.com.

Preparation of reagents

1. PNA telomere and centromere probes
   a) Lyophilized PNA powder can be stored at – 20 °C.
   b) When ready to use, spin down the tube. Resuspend 5 nmole stock in 100 ul formamide to make 50uM stock or about 250 ug/ml (250x).
   c) Store aliquots in -70 °C, protecting from light.
   d) After thawing, heat at 55°C for 5 min to ensure complete dissolution.
   e) PNA probes should be stable >1 year if properly stored.
2. Hybridization buffer: 20 mM Tris, pH 7.4, 60% formamide, 0.1 µg/ml salmon sperm DNA or 0.5% of blocking reagent such as Roche 11096176001
3. RNase solution: 50 ul of 100 µg/ml RNase A in PBS
4. Wash solution: 2X SSC, 0.1% Tween-20
5. Pepsin solution: 0.005% pepsin in 10 mM HCl (make fresh and warm to 45 °C before use)
6. DAPI solution: 1/750 dilution of 0.5 mg/ml DAPI in 2x SSC
7. Ethanol (70%, 85%, 100%)

Hybridization of PNA probes

I. Pretreatment

1. Prepare the slide according to the recommended procedure for fixation. For FFPE section, deparaffinization using Xelene is required.
2. Wash in PBS twice for 2 min each.
3. (option) Add RNase solution and incubate for 20min at 37 °C. Make sure the slide does not dry out.
4. Wash in PBS twice and once with water, 2 min each.
5. (option) Immerse the slide in Pepsin solution for 5min at 37 °C.
6. Wash in PBS twice for 2min each.
7. Dehydrate the slide by incubating 2min each in 70%, 85%, and 100% cold Ethanol.
8. Air Dry the slide.

II. Hybridization

1. Preheat the incubator to 85 °C.
2. Prewarm the slide in incubator for 5 min.
3. Heat hybridization buffer to 85 °C for 5 min.
4. Prepare PNA probe in 20 ul hybridization buffer to final concentration of 200 nM.
5. Add the PNA probe in the buffer to the slide. Cover with cover slip.
6. Heat the slide for 10 min at 85 °C.
7. Place the slide at room temperature for 2hr in dark for hybridization. Use wet towels to prevent dry.

✓ Preheating the slide and PNA probe is critical to reduce background.

III. Washing

1. Immerse the slide in Wash solution to remove coverslip.
2. Wash the slide in Wash solution twice at 55~60 °C for 10 min.
3. Wash the slide with Wash solution at room temperature.

Counter-staining with DAPI

1. Add the DAPI solution for 10 min.
2. Wash the slide with 2X SSC, 1x SSC, and finally with water for 2 min each.
3. Dry the slide by quick centrifugation.
4. Add a drop of mounting media and cover with coverslip. Avoid air bubbles.
5. Observe in fluorescence microscope with appropriate filters.

References

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