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 for 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 cell sorter (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 in 100 ul formamide to make 50 uM stock or about 250 ug/ml (100x). Heat at 55 °C for 5 min.
   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 >2 year if properly stored.
2. Hybridization buffer: 20 mM Tris, pH 7.4, 60% formamide, 0.5% of blocking reagent (Roche 11096176001) or 0.1 µg/ml salmon sperm DNA plus 0.1% Tween-20
3. Wash solution: 2X SSC, 0.1% Tween-20
4. Ethanol (70%, 85%, 100%)
5. (option) RNase solution: 50 ul of 100 µg/ml RNase A in PBS
6. (option) Pepsin solution: 0.005% pepsin in 10 mM HCl (Make fresh and warm to 37 °C before use.)
7. (optional) DAPI solution: 1/750 dilution of 0.5 mg/ml DAPI in 2x SSC

Hybridization of PNA probes

I. Pretreatment

1. Prepare the slide according to the recommended procedure for fixation. For the FFPE section, deparaffinization using Xylene 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. For each slide, mix 0.2 ul of PNA probe in 20 ul hybridization buffer to a final concentration of 500 nM.
3. Prewarm the slide in an incubator at 85 °C for 5 min.
4. Heat the hybridization buffer containing the PNA probe at 85 °C for 5 min.
5. Add the hybridization buffer to the slide. Cover with a coverslip.
6. Keep the slide at 85 °C for 10 min.
7. Move the slide to room temperature and leave it for 1 hour in dark. Use wet towels to prevent drying during the hybridization.

✓ Preheating the slide and PNA probe is critical in order to minimize the background.

III. Washing

1. Immerse the slide in Wash solution to remove the 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 a coverslip. Avoid air bubbles.
5. Observe in fluorescence microscope with appropriate filters.

References

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4. **(Q-FISH)** Canela A, Vera E, Klatt P, Blasco MA (2007) High-throughput telomere length quantification by FISH and its application to human population studies. *Proc Natl Acad Sci USA* 104(13):5300-5.

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7. Lee WW, Nam KH, Terao K, and Yoshikawa Y (2002) Age-related telomere length dynamics in peripheral blood mononuclear cells of healthy cynomolgus monkeys measured by Flow FISH. *Immunology* 105: 458-465.

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