PNA FISH (Fluorescence In Situ Hybridization)

Fluorescence in situ hybridization (FISH) is a cytogenetic method used to detect and localize specific DNA sequences on chromosomes.

Due to their high affinity and specificity for target DNA, PNA probes are ideal for FISH applications. The advantages include precise target binding, short hybridization times, low background, excellent reproducibility, and exceptional reagent stability. Their small size allows for efficient tissue penetration.

PNA Bio provides telomere (TelC: C-rich/leading strand and TelG: G-rich/lagging strand) and centromere PNA probes labeled with various fluorophores as catalog items. These probes are suitable for multiple applications, including Q-FISH (quantitative analysis), Flow FISH (cell sorting), IF-FISH (antibody co-staining), and CO-FISH (strand-specific detection). Custom-designed PNA probes are also available for other detection needs. For more details, please visit http://www.pnabio.com.

Preparation of reagents

1. PNA FISH Probes
   - Lyophilized PNA powder can be stored at -20 °C.
   - When ready to use, spin down the tube. Resuspend 5 nmole in 100 µL formamide to make 50 µM stock or about 250 ug/ml (100x). Heat at 55 °C for 5 min.
   - Store aliquots in -20 °C, protecting from light. PNA can be stored for 3 years.
   - After thawing, heat at 65 °C for 5 minutes to ensure complete dissolution.
2. PNA FISH Hybridization Buffer (Cat No. PFB01)
   - Final hybridization buffer composition is 20 mM Tris, pH 7.4, 60% formamide.
3. Blocking buffer (Cat No. PFB05)
   - Alternatively, 0.5% of blocking reagent (Roche 11096176001) or 0.1 µg/ml salmon sperm DNA plus 0.1% Tween-20 can be used.
4. Wash solution: 2X SSC, 0.1% Tween-20
5. Ethanol series for dehydration (70%, 85%, 100%)
6. (Optional) RNase solution: 50 µL of 100 µg/ml RNase A in PBS
7. (Optional) Pepsin solution: 0.005% pepsin in 10 mM HCl, warm to 37 °C before use.
8. (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. To prepare cells for FISH, fix them with cold methanol-acetic acid (3:1), drop onto a clean slide, air dry. For metaphase spreads, treat cells with colcemid before harvesting.
2. (Optional) Add RNase solution and incubate for 20 minutes at 37°C. Make sure the slide does not dry out.
3. Wash in PBS twice and once with water, 2 minutes each.
4. (Optional) Immerse the slide in Pepsin solution for 5 minutes at 37°C.
5. Wash in PBS twice for 2 minutes each.
6. Dehydrate the slide by incubating 2minutes each in 70%, 85%, and 100% cold Ethanol.
7. Air Dry the slide.

II. Hybridization

1. Preheat the incubator to 60°C.
2. For each slide, aliquot 16.8 µL of PNA FISH Hybridization Buffer (PFB01) into a microcentrifuge tube.
3. Heat the PNA Blocking Buffer (PFB05) at 60°C for 5 minutes. Mix well and add 3 µL to the Hybridization Buffer.
4. Heat PNA probes (50 µM stock) at 60°C for 5 minutes to ensure complete dissolution.
5. Add 0.2 µL of the PNA probe to the hybridization buffer. For multiplexing, add 0.2 µL of each probe.
6. Prewarm the slide and hybridization buffer mix at 70~85°C for 5 minutes.
   - ✓ Preheating the slide and PNA probe is critical in order to minimize the background.
7. Add the hybridization buffer to the slide. Cover with a coverslip.
8. Incubate the slide at 70~85°C for 10 minutes.
9. Move the slide to room temperature and leave it for 30~60 minutes in dark. Use wet towels to prevent drying during the hybridization.

III. Washing

1. Immerse the slide in Wash solution (2X SSC, 0.1% Tween-20) 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 minutes 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

1. Tanemura K, Ogura A, Cheong C, Gotoh H, Matsumoto K, Sato E, Hayasi Y, Lee HW, and Kondo T (2005) Dynamic rearrangement of telomeres during spermatogenesis in mice. Dev Bio 281,196-207.

2. Goodwin EH and Meyne J (1993) Strand-specific FISH reveals the orientation of chromosomal breaks, duplications, deletions, and numerical abnormalities in the sperm of healthy men. *Am J Hum Genet* 67:862-872.

3. **(CO-FISH)** Bailey SM, Meyne J, Cornforth MN, McConnell TS, and Goodwin EH (1996) A new method for detecting pericentric inversions using COD-FISH. *Cytogenet Cell Genet* 75:248-253.

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.

5. **(Flow/Q-FISH)** Baerlocher GM, Vulto I, de Jong G, and Lansdorp PM (2006) Flow Cytometry and FISH to measure the average length of telomeres. *Nat Protoc.* 1(5):2365-76.

6. Brind'Amour J and Lansdorp PM (2011) Analysis of repetitive DNA in chromosomes by flow cytometry. *Nat Methods* 8(6):484-6

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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