TECHNICAL NOTE

Applications of PNA for FISH

PNA probes offer distinctive advantages over DNA probes in fluorescent *in situ* hybridization (FISH). The PNA probes due to their relative hydrophobic character and neutral backbone hybridize to their DNA or RNA targets with a higher affinity and specificity than their nucleic acids counterparts (1). The neutral character of the PNA backbone facilitates fast hybridization to target molecules and the high binding constant of the PNA/DNA (RNA) complex translates into more sensitive tests (2). PNA probes show better stability, higher reproducibility and lower background. PNA-FISH can discriminate between two DNA sequences that differ by a single base-pair (3). Due to the higher Tm of PNA/DNA duplexes short (12 to 18 mer) PNAs are widely used. A variety of fluorescent dyes, for example 5(6)-FAM, 5(6)-TAMRA, and biotin, can be used to label PNA probes for direct detection. Since PNAs labeled with rhodamine are more highly soluble compared to those labeled with fluorescein, the former dye is the preferred choice.

Quick procedure outline

1) Prepare the metaphase chromosomes.
2) Post-fix in methanol:acetic acid (3:1) for 1-2 hrs.
3) Dehydrate in cold ethanol series and air dry.
4) Wash in 1x PBS at 37°C for 5 min.
5) Denature chromosomes in 4% formaldehyde at 37°C for 1.5 to 2 min.
6) Dehydrate in the ethanol series and air dry.
7) Apply 15 to 25 μl of the PNA hybridization mixture to each slide.
8) Post-denature chromosomal preps at 80°C for 3 min.
9) Hybridize at room temperature or 37°C for 2 hrs.
10) Wash at room temperature (2x 15 min. with 70% formamide then 3x 5 min. with TNT).
11) Counter-stain slides with DAPI or PI for microscopic examination.

Materials:

PNA is dissolved in 100% deionized formamide at a concentration of either 10 μM (working solution) or 100 μM (stock). To convert from PNA OD_{260} to μmole follow the steps outlined in the PNA Conversion Technical Note (5). Store PNA solution in polypro tubes in the dark at 4°C or at –20°C. PNA is stable in formamide for at least two years.

PBS (Phosphate Buffered Saline)
Ethanol (70%, 80%, 90% and 100%)
37% formaldehyde
100% formamide
Blocking reagent, Cat# 1096176 (Roche Diagnostics)
DAPI I solution in antifade, Cat#32-804830 (Vysis)
PI solution in antifade, Cat#32-804829 (Vysis)

PNA-FISH Detailed protocol

1) Prepare the metaphase chromosomes by following standard cytogenic procedures (4). Use of freshly prepared slides is highly recommended.

2) Fix slides in methanol:acetic acid (3:1) for 1 to 2 hrs at room temperature, air dry, immerse in PBS for 5 min. and fix in 4% formaldehyde in PBS , pH 7.2 for 1.5-2 min.

3) After fixation, the slides are dehydrated using a series of cold ethanol washes (70%, 80%,90% and 100%) and air dried for 15 min..

4) To each slide add 15 to 20 μl of hybridization mixture containing 500 nM of labeled PNA probe in 70% formamide, 1% (wt/vol.) blocking reagent in 10 mM Tris, pH 7.2. Cover the area with a coverslip and seal with rubber cement. Denature the DNA on the slide again at 80°C for 3 min. followed by hybridization in the dark at 37°C in wet chamber for 2 hrs.

5) After hybridization, excessive exposure to light should be avoided to prevent florescent bleaching. The slides are washed three times with 70% formamide/10 mM Tris pH 7.2 for 10 min. and with TNT (0.05 M Tris/0.15 M NaCl/ 0.05% Tween-20, pH 7.5) for 5 min. Slides are dehydrated in the same ethanol series as previously used and dried in the dark.

6) Chromosomes are counter-stained with either 0.1 μg/ml of DAPI in antifade for rhodamine and fluorescein labeled probes or 0.6 μg/ml of PI in antifade for fluorescein labeled probes.

7) Slides can be stored at –20°C for two weeks.

Refrences:

1) Egholm et al. (1993) Nature, 365:566-8
2) Landsorp et al (1996) Human Mol. Genetics, 5:685-691
3) Chen et al. (1999) Mammalian Genome 10:13-18
4) Lawrence et al. (1990) Science 249:928-932
5) Technical Note “PNA Conversion from Optical Density$_{260}$ to μmole or μg”:

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