TECHNICAL NOTE

Applications of PNA for FISH (Alternative protocol)

The PNA probes offer distinctive advantage over DNA or RNA oligo probes for the detection and quantitation of telomere repeat sequences and for the detection and enumeration of specific chromosomes in human interphase and metaphase cells (1). The PNA probes, due to their relative hydrophobic character and neutral backbone, hybridize to their DNA targets with a higher affinity and specificity than their nucleic acids counterparts (2). A major advantage of using PNA for in situ hybridization procedures stems from the use of the low ionic strength hybridization conditions that can prevent reanealing of complementary genomic DNA strands. Under these conditions, PNA probes can hybridize in a quantitative manner to denatured chromosomal DNA before competition from renaturation occurs. This advantage is particularly important for hybridization with short probes that target repetitive sequences, because both, the probe length and nature of target sequence, will favor renaturation over hybridization of labeled probes.

Protocol outlined below was used with mixtures of PNA probes designed to detect chromosome specific repeat sequences in interphase and methaphase cells (1).

In Situ Hybridization

Pretreatment

1) Prepare slides by procedure recommended for a particular cell line and air dry overnight.
2) Rehydarte slides in PBS for 15 min, fix in 4% formaldehyde in PBS and rinse in PBS (3X5 min).
3) Treat slides with pepsin, pH 2.0 (1 mg/ml) for 10 min, wash in PBS (2X2 min) and fix again in 4% formaldehyde.
4) Rinse slides in PBS (3X5 min) and dehydrate in cold ethanol series (70%, 90%, 100%).
5) Air dry slides before an addition of hybridization mix.

Hybridization and washes

1) To each slide add 20 μl of hybridization mix containing 0.05 pM of each PNA probe in 10 mM $NaHPO_4$ pH 7.4, 10 mM NaCl, 20 mM Tris, pH 7.5, 70% formamide, 1X Denhardt's solution, 0.1 μg/ml each of tRNA and salmon sperm DNA.
2) Denature slides at 80° C for 3 min followed by hybridization for 30 min at room temperature in a humidified chamber.
3) Remove coverslips by rinsing slides in PBS, 0.1% Tween (PT) for 1-2 min at room temp.
4) Wash slides in preheated PT at 55-60° C for 30 min, rinsed with PBS and dehydrate.
5) Counterstain cells with DAPI [4,6-diamino-2-phenylindole] (Sigma Chemicals) and mount in 90% glycerol containing 2 mg/ml of DABCO (Sigma Chemicals) in PBS.

Buffers:

PBS: 2.7 mM KCl, 137 mM NaCl, 1.5 mM KH_2PO_4 , 8 mM Na_2HPO_4

Denhart solution, 100X : 10 g Ficoll 400, 10 g PVP, 10 g BSA (Pentax Fraction V), water to 500 ml.

PT: 0.1% Tween in PBS

Refrences:

1) Taneja et al. (2000) Genes, Chromosomes and Cancer (in press)
2) Egholm et al. (1993) Nature, 365:566-8