Protocol of PNA Telomere probe for FISH (Fluorescence in situ hybridization)

In principle, fluorescence *in situ* hybridization (FISH) should be able to provide information on the telomere length of individual chromosomes. Directly labeled oligonucleotide probes are attractive probes for such analysis because of their small size (good penetration properties), single strand nature (no denaturation of probe) and controlled synthesis. However the efficiency of oligonucleotide hybridizations for telomeric repeats has not been sufficient to extend this approach beyond qualitative studies of TTAGGG repeat sequences in chromosomes of various species. Recently, it was shown that peptide nucleic acid (PNA) oligonucleotide probes will hybridize with complementary oligonucleotide sequences and that the resulting duplexes are more stable than DNA/DNA or DNA/RNA duplexes. In PNA, the charged phosphate-(deoxy) ribose backbone of conventional DNA and RNA oligonucleotides is replaced by an uncharged backbone of repeating N-(2-amino ethyl)-glycine units linked by peptide bonds. In comparison with DNA oligonucleotides, PNA oligomers demonstrate the higher sequence specificity, improved stability, reproducibility, and lower background. Due to the higher Tm of PNA/DNA duplexes, short (18-mer) Telomere PNA $(CCCTAA)_3$ are widely used.

Things to do before starting

I. Preparation of the Telomere PNA probe

1. Centrifuge the tubes before opening them in order to collect lyophilized PNA probe at the bottom of the tubes.
2. Add formamide to the tube to obtain stock solution of PNA probe.
3. Dilute the stock solution in PNA hybridization buffer to a final concentration of 200 nM.
   ✓**Note**: Store the PNA probe solution in the dark at 4°C.

II. Preparation of solution

1. Hybridization buffer

   20 mM Na_2HPO_4, pH 7.4
   20 mM Tris, pH 7.4
   60% formamide
   2X SSC
   0.1 μg/ml salmon sperm DNA

2. RNase A solution

   100 μg/ml RNase A in 2X SSC

3. Pepsin 0.005% solution

   50 μl of 5% Pepsin stock in 50 ml 0.01 M HCl.
   ✓ **Note**: Make fresh! Warm to 45 ºC before use.

4. Washing solution

   2X SSC/0.1% Tween-20

Hybridization and Washes

I. Pretreatment

1. Prepare slide by procedure recommended for a particular cell line and air dry.
2. Immerse the slide in PBS for 15 min.
3. Fix the slide in 4% formaldehyde in PBS for 4 min at 37°C.
4. Wash in PBS for 5 min at 37°C (X2).
5. Add 500 μl of RNase A solution to a clean plate and put each slide face down on the RNase A solution for 1 hr at 37°C.
   ✓ **Note**: Be careful not to be dry.
6. Wash in 2X SSC (X3 repeat) and wash in DW.
7. Immerse the slide in 0.005% Pepsin for 4 min at 37°C.
8. Wash in PBS for 3 min at 37°C (X2).
9. Repeat step 3-4.
10. Wash in PBS for 5 min at room temperature.
11. Dehydrate slides in cold ethanol series (for 1 min in 70%, 85%, 100%).
12. Dry slide on air.

II. Hybridization

1. Place the slide in a pre-heated incubator at 80°C for 5 min.
2. Add 20 μl of PNA probe in Hybridization buffer to the marked area on each slide.
   ✓**Note**: Heat Hybridization buffer for 5 min at 90°C before use.
3. Cover the marked area on each slide with 18x18 mm cover slip.
   ✓ **Note**: Be careful not to be dry.
4. Denature slide for 10 min at 85°C.
   ✓**Note:** Denaturation should be performed between at minimum 80°C and maximum 90°C.
   Check the temperature of the incubator carefully.
   Denaturing temperature below 75°C impairs result seriously.
5. Place the slides in the dark at room temperature for 1hr.

III. Washing

1. Immerse the slide in Washing solution at room temperature to remove the coverslips.
2. Wash the slide in Washing solution for 10 min at 55-60°C **(X2)**.
   ✓**Note:** Wash should be performed at 55-60°C.
3. Wash the slide in Washing solution for 1 min at room temperature.

IV. Counterstaining

1. Stain the slide for 10 minutes in the DAPI/2X SSC.
2. Wash the slide in 2X SSC for 2 min.
3. Wash the slide in 1X SSC for 2 min.
4. Wash the slide in DW for 2 min.
5. Dry the slide by centrifuge.
6. A drop of mounting media to the target area of the slide.
7. Cover with a coverslip and allow the solution to spread evenly under the coverslip. Avoid air bubbles.
8. Observe the stained slide using an fluorescence microscope with the appropriate filters.

V. References

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Please contact us for any inquiry.
info@panagene.com
www.panagene.com
+82-42-861-9296