

## **PNA FISH probes are Powerful Tools for Chromosomal Analysis**

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Fluorescence *in situ* hybridization (FISH) provide information on the telomere length and centromere location in individual chromosomes. In this technique, a labelled DNA probe is hybridized to cytological targets such as metaphase chromosomes. Although FISH has proved to be a useful technique, it is a fairly time-consuming procedure with limitations in sensitivity.

Probes with higher DNA affinity may potentially improve the sensitivity of the FISH. Peptide nucleic acids (PNAs) are synthetic DNA mimics in which a peptide-like repeat of N-(2-aminoethyl) glycine unit replaces the sugar-phosphate backbone, thus conferring more stability and affinity to the PNA probes than to the DNA probes.

Cy3-labeled telomere and centromere PNA probes showed very strong signal, lower background, short hybridization time (30 min), and make it possible to hybridization reaction at the room temperature. The hybridization time of PNA probes was considerably shortened in comparison with FISH reaction using DNA probes, which requires an overnight hybridization in order to be efficiently completed on preparations.

PNA probes were shown to be excellent probes for FISH combining their high binding affinity and short hybridization time at room temperature.

Key words: PNA probe, FISH, telomere, centromere

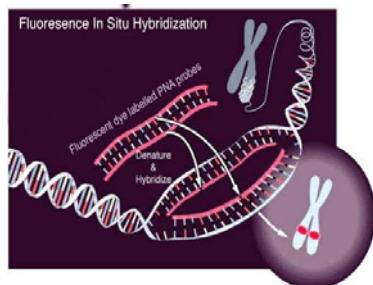
# PNA FISH probes are Powerful Tools for Chromosomal Analysis

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## INTRODUCTION

### ► FISH (Fluorescence in situ Hybridization)



<http://www.genome.gov/Pages/Hyperion/DIR/VIP/Glossary/Illustration/fish>

- Cell culture from human blood.
- Preparation of metaphase from cultured cells.
- Denaturation & Hybridization.
- Washing after hybridization.
- Counterstaining & Microscopy.

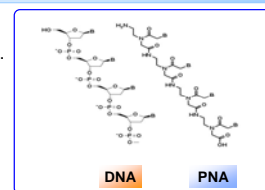
### ► Telomere & centromere

- Telomeres have important functions in the stability and replication of chromosomes.
- Telomere in all vertebrates are composed of a sequence of six nucleotides (TTAGGG) repeated from a few hundred to several thousand times.
- The centromere is a uniquely specialized region in the chromosome where the chromatids are joined.

### ► PNA (Peptide nucleic acid)

- High binding affinity to complementary DNA or RNA.
- More destabilizing effect by single-base mismatch.
- Resistant to nuclease and protease.
- Fast hybridization.

✓ Comparison of the structures of PNA and DNA



## MATERIALS & METHODS

### ► Metaphase chromosome preparation

#### → Human Blood cell Culture

- Human blood cell cultures at 37°C, 5% CO<sub>2</sub> incubator for 72 hours.

#### → Preparation of Metaphase from Cultured Cells

- Add colcemide to cultured cells and metaphase cell fix on glass slides.

### ► PNA FISH probes Fabrication & Hybridization

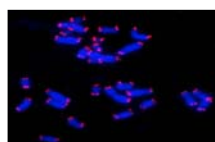
- PNA FISH probes were synthesized by PANAGENE Inc. for Chromosomal Analysis.

### ► FISH (Fluorescence in situ hybridization)

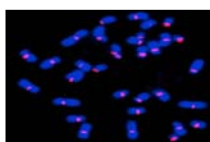
- FISH provide information on the telomere length and centromere location in individual chromosomes.
- Cy3-labeled PNA probes is hybridized to cytological targets such as metaphase chromosomes.

## RESULTS

### ► Telomere and Centromere PNA FISH



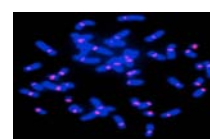
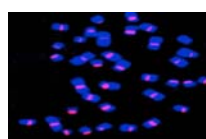
Specific telomere detection using PNA probes



Specific centromere detection using PNA probes

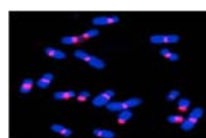
### ► Advantages of PNA FISH

- ✓ Excellent Hybridization Signal
- ✓ Hybridization at room temperature

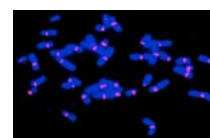


The use of Cy3-labelled PNA probe in FISH result in strong hybridization signal on chromosomes after hybridization for 30 min.

- ✓ Short Hybridization Time (<30min)

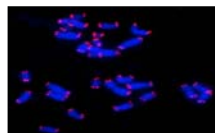


30 min

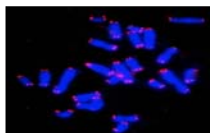


2 hour

- ✓ A variety of fluorescent dyes



Cy3-labelled PNA Probe



Biotin-labelled PNA Probe

A variety of fluorescent dyes, Cy3 and biotin can be used to label PNA probes for direct detection.

## CONCLUSION

- PNA probes showed excellent sensitive and specific detection and lower background.
- PNA probes showed short hybridization time (30min) at room temperature.

**PNA FISH technology enables sensitive, fast and specific detection at room temperature of chromosomal sequences directly on chromosomes.**

