PANArray™ for detection of lamivudine-resistant HBV

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Lamivudine resistant Hepatitis B virus (HBV) has caused major problem during the treatment of chronic patients. Accurate and early-stage detection of Lamivudine resistant HBV has been main issue in diagnostic field.

We have developed PNA array to detect point mutations of lamivudine-resistant HBV. PNA (Peptide nucleic acid) is DNA analogue which has the neutral backbone instead of ionic phosphate backbone. PNA has a high binding affinity complementary DNA. Due to its superior properties, PANArray™ gives rise to higher specificity, higher sensitivity and higher stability than DNA array.

We also developed ideal linker, spacer system, immobilization, and hybridization conditions for a PANArray™. The PANArray™ was tested with clones and clinical samples. Our result showed high specific signal and excellent discrimination of single base mismatch. In comparison with DNA array, the PANArray™ demonstrated 2.2 to 15 times more specific and about 10 times more sensitive than optimized DNA array. The rate of concordance between the PANArray™ and sequencing assay was 100%.

Additionally, PANArray™ can be stored more than one year at room temperature with high specificity and sensitivity.

In conclusion, PANArray™ is a highly reliable and efficient tool for lamivudine-resistant HBV detection in clinical diagnosis. Also PANArray™ will be promising tool for detection of point mutation.

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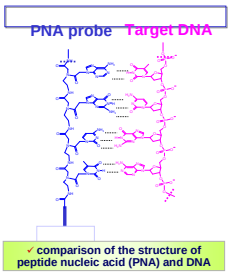
INTRODUCTION

What is PNA ?

- Discovered in 1991 by Egholm, Nielsen, Berg, and Buchardt .(1)
- Polyamide backbone N-(2-aminoethyl) glycine units
- Higher affinity to complementary nucleic acid (DNA, RNA)
- Strong hybridization independent of salt concentration
- Greater specificity and sensitivity of interaction
- Thermal and chemical stability
- Resistance to nucleases and proteases



✓ comparison of the structure of peptide nucleic acid (PNA) and DNA

Lamivudine-resistant HBV

HBV (Hepatitis B virus) is one of the major causes of liver disease. Lamivudine has been used to be effective antiviral agent for the treatment of HBV infection.(3) Lamivudine resistant HBV is main trouble during the treatment of HBV-infected patients. And It isoriginated from several viral point mutations. We developed a PNA Array for detection of point mutation

Table 1. Codon names related to lamivudine-resistant of HBV

	Region 1	Region 2	Region 3	Region 4
Wild types	180 W 180 T	204 VW	204 IW	207 W
Mutant types	180 M	204 V	204 I1 204 I2 204 I3	207 I1 207 I2

MATERIALS & METHODS

Preparation of PNA oligomer

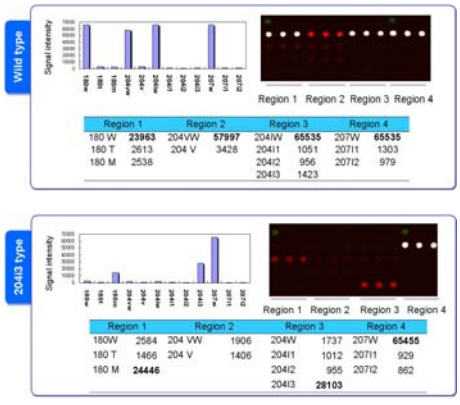
PNA probes were synthesized by Panagene Inc. 16 PNA probes were designed specifically to wild and mutant types of HBV.

Hybridization and analysis

Mixture of PCR product and PANArray™ hyb buffer (Panagene Inc.) was applied to PANArray™. And then hybridized for 2 hr at 45℃. We washed slide with PANArray™ wash buffer (Panagene Inc). Finally, the slide was analyzed to image and converted to signal intensity using fluorescence scanner (Genepix 4000B).

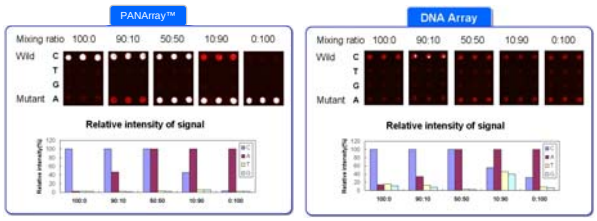
RESULTS

Specificities of PNA probes in the PANArray™ HBV



These results demonstrate that the PANArray™ HBV has high specificity.

Detection of mixed type of rtM204 & rtM204i3



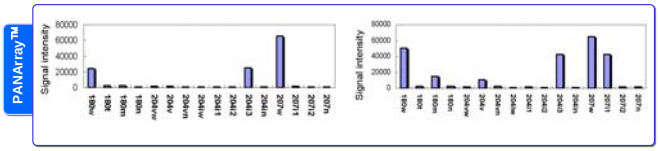
PANArray™ and DNA array assay with clinical samples

Sample #1

Region	1(180)	2(204v)	3(204i)	4(207)
DNA array	180m		204i3	207w
PNA array	180w		204i3	207w
Sequencing	180w		204i3	207w

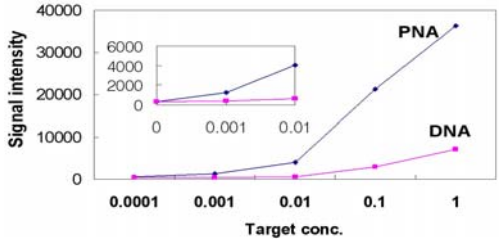
Sample #2

Region	1(180)	2(204v)	3(204i)	4(207)
DNA array	180>180m	204v	204i3	207w
PNA array	180>180m	204v	204i3	207w>207i1
Sequencing	180>180m	204v	204i3	207w>207i1



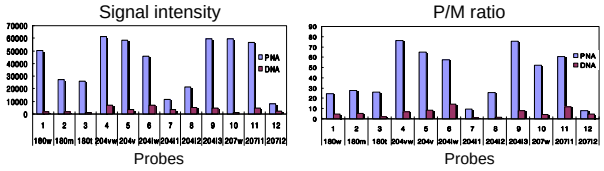
The rate of concordance between the assays with PANArray™ and sequencing was 100%.

Sensitivity of PANArray™ and DNA array



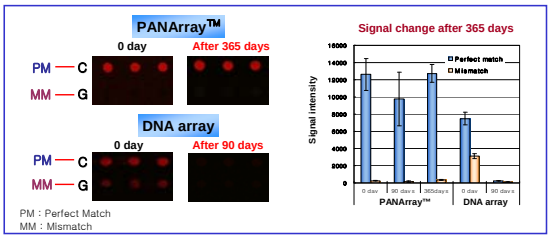
This result demonstrated that the PANArray™ was 10 times **more sensitive** and has 5-7 times **higher signal intensity** than DNA array.

Comparison of PANArray™ and DNA array



PNA probes have higher specific signal and signal-to-noise ratio than DNA probes for detection of point mutation

Long lasting Specificity & Sensitivity



PANArray™ is very stable even at room temperature.

CONCLUSION

PANArray™ HBV for Detection of Point Mutation of lamivudine-resistant HBV

1. Discriminated specifically between wild type and 7 mutant types.
2. The PANArray™ was about 10 times more sensitive and 2 to 45 times more specific than DNA array.
3. The high specificity and sensitivity lasts much longer than DNA array at RT.
4. The PANArray™ is greatly useful for detection of point mutation.

REFERENCES

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3. Park H., *et. al.*, J Clin Microbiol, (2005), v 43:1782-8.

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