# PNA Array 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, PNA array 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 PNA array. The PNA array 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 PNA array demonstrated 2.2 to 15 times more specific and about 10 times more sensitive than optimized DNA array. The rate of concordance between the PNA array and sequencing assay was 100%. Additionally, PNAarray can be stored more than one year at room temperature with high specificity and sensitivity.

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

# PANArray™ HBV for detection of lamivudine-resistant HBV

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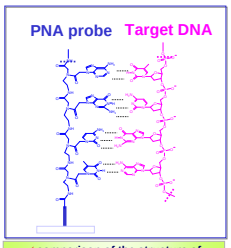
## INTRODUCTION

### 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<br>180 T | 204 VW | 204 IW | 207 W |
| Mutant types | 180 M | 204 V | 204 I1<br>204 I2<br>204 I3 | 207 I1<br>207 I2 |

## MATERIALS & METHODS

### Preparation of PNA oligomer

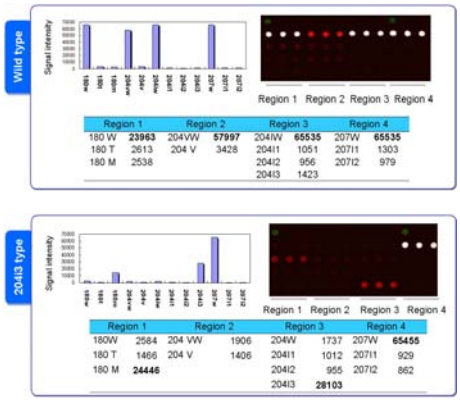
PNA probes were synthesized by Panagene.inc. 16 PNA probes were designed specifically to wild and mutant types of HBV.

### Hybridization and analysis

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

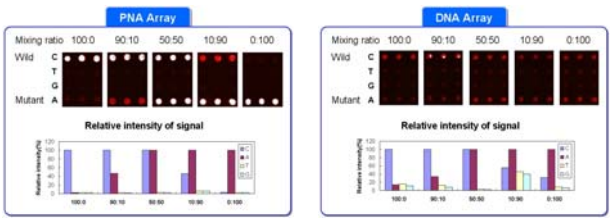
## RESULTS

### Specificities of PNA probes in the PNAArray ™ HBV PNA Array




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

### Detection of mixed type of rtM204 & rtM204i3



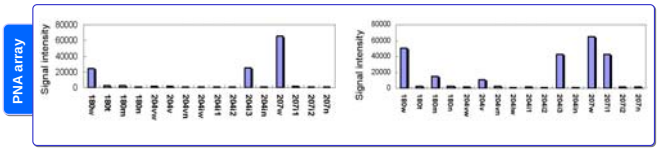

### PNA and DNA array assay with clinical samples

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 |

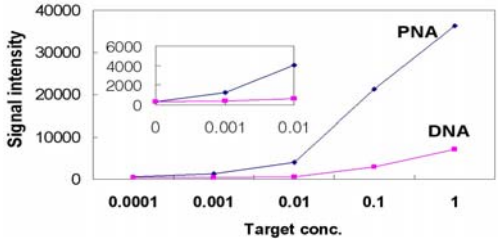
Sample #2

| 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 |



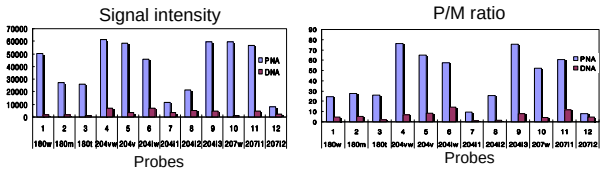

The rate of concordance between the assays with PNA array and sequencing was 100%.

### Sensitivity of PNA array and DNA array



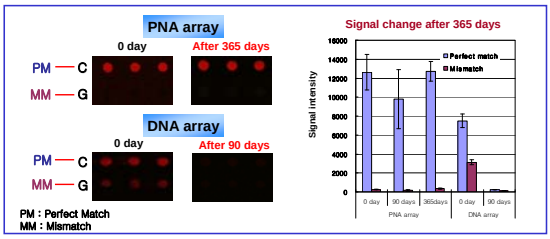

This result demonstrated that the PNA array was 10 times **more sensitive** and has 5-7 times **higher signal intensity** than DNA array.

### Comparison of PNA array 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




PNA array is very stable even at room temperature.

## CONCLUSION

The PANArray™ HBV PNA Array
for Detection of Point Mutation of lamivudine-resistant HBV

1. Discriminated specifically between wild type and 7 mutant types.
2. PNA array 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. PNA array is greatly useful for detection of point mutation.

## REFERENCES

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2. Brabdt O., *et. al.*,, Trends Biotechnol. 2004 Dec;22(12):617-22.
3. Park H., *et. al.*, J Clin Microbiol, (2005), v 43:1782-8.

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