
PNA Array for HPV Genotyping

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Infection with Human Papillomavirus (HPV) is one of the most common causes cervical cancer which was constitutes up to 25% of all female cancers. It is important that HPV genotypes be determined by a reliable method, because of strong association of certain HPV types with cervical cancers.

We have developed PNA based array to genotype HPVs using type-specific PNA probes. A PNA (peptide nucleic acid) is DNA mimics with neutral polypeptide backbone. The PNA hybridize to complementary DNA or RNA with higher affinity and specificity than traditional DNA.

The PANArrayTM HPV PNA array was able to discriminate specifically 32 HPV types including 19 high risk types and 13 low risk types. Moreover, S/N (Specific signal/Non-specific signal) ratios were ranged 8 to 85. The PNA array was about 10 times more sensitive and 2 to 30 times more specific than DNA array. For the more, the PNA array was shown longer shelf life than DNA array. Also, we compared the results of the PNA array and sequencing with clinical samples. The rate of concordance between the assays with PNA array and sequencing was 100%.

These results suggest that the PNA array was highly reliable for detection and genotyping of HPVs and accessible to all diagnostic laboratories.



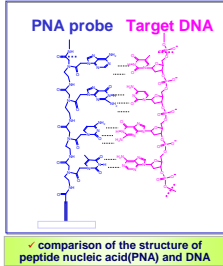
PANArray™ HPV for Genotyping of Human papillomavirus

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INTRODUCTION

What is PNA ?

- Discovered in 1991 by Egholm, Nielsen, Berg, and Buchardt .
- 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.



Human Papillomavirus

HPV has become one of the most common causes of sexually transmitted disease in both men and women.
HPV has been implicated in 99.7% of all cervical squamous cell cancer cases. Cervical cancer constitutes up to 25% of all female cancers.

Risk groups of HPV infection.

Risk groups	Types of HPVs
High risk	16, 18, 26, 31, 33, 39, 45, 51, 52, 53, 56, 56, 58, 59, 66, 68, 69, 70, 73,
Low risk	6, 11, 32, 34, 40, 42, 43, 44, 54, 55, 62, 81, 83

MATERIALS & METHODS

Preparation of PNA oligomer

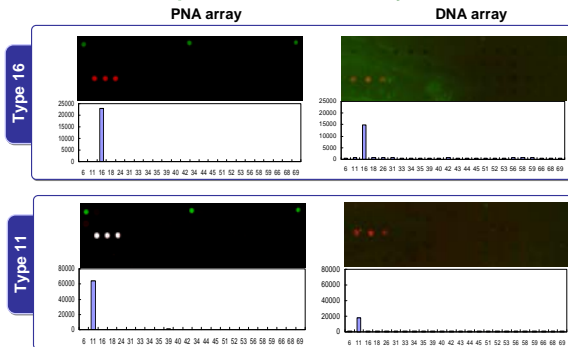
PNA probes were synthesized by Panagene.inc. 32 PNA probes were designed specifically HPV types.

Hybridization and analysis

Mixture of PCR product and PANArray™hyb buffer (Panagene) was applied to PNA array. And then hybridized for 2 hr at 40°C. 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).

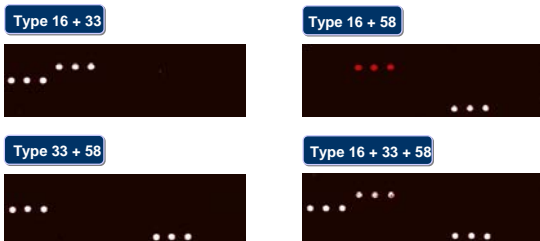
RESULTS

Specificities of PNA probes in the PANArray™ HPV PNA Array



The PANArray™HPV PNA Array has high specificity.

Detection of multi- infection of HPV types



PNA and DNA array assay with clinical samples

Diagnosis of clinical samples.

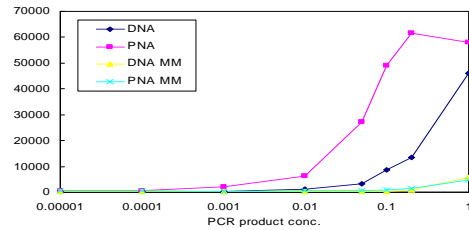
	C1	C2	C3	C4	C5	C6	C7	C8
DNA array	52	18,58	35	33	16	34	56	51
PNA array	52	18,58	35	33	16	56	56	51
Seq*	-	-	-	-	-	56	-	-

	C9	C10	C11	C12	C13	C14	C15	C16
DNA array	70	59	68	6	40,59,70	58	18	39,45
PNA array	70	59	53,68	6	40, 59,70	58	18	45
Seq*	-	-	-	-	-	-	-	-

* Sequencing results. Clinical sample were amplified by PCR and cloned. Cloned samples were done sequencing. Cloned clinical sample's type is only one of several types in a clinical sample.

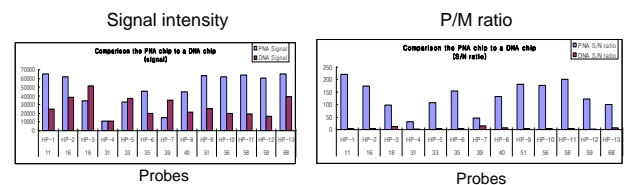
The rate of concordance between the assays with PNA array and sequencing was 100%.

Sensitivity of PNA array and DNA array



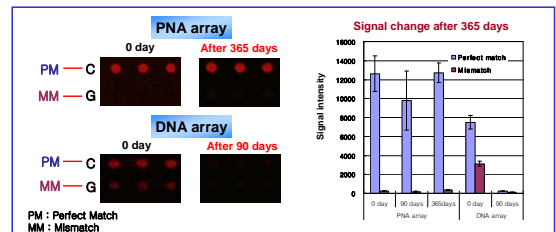
The PNA array was able to detect at 10 times lower target concentration than longer DNA probe.

Comparison of PNA array and DNA array



PNA probes have higher specific signal and signal-to-noise ratio than DNA probes

Long lasting Specificity & Sensitivity



PNA array is very stable even at room temperature.

CONCLUSION

The PANArray™ HPV for genotyping HPV

1. Discriminated specifically **32 types of HPV** including 19 high risk types and 13 low risk types.
2. PNA array was **more sensitive and 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 genotyping of HPVs**.

