

# **PANArray™ HPV Genotyping Chip**

## **for research use only**

Instruction manual for product # PHP-1001

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# I. PRODUCT SUMMARY

## Contents and storage

### PANArray™ HPV Kit #1 (Storage: Room temperature)

**PANArray™ HPV chip** [Catalog # PANArray-HPV-C-001]

: PANArray™ HPV chip consists of 12 slides attached each with an eight-hole cover slip.

**PANArray™ HPV hybridization buffer #1** [Catalog # PANArray-HPV-C-002]

: 1 bottle (10 ml)

**20X PANArray™ HPV washing buffer** [Catalog # PANArray-HPV-C-003]

: 1 bottle (200 ml)

### PANArray™ HPV Kit #2 (Storage: -20°C)

**PANArray™ HPV PCR mixture #1** [Catalog # PANArray-HPV-F-001]

: For amplification of HPV gene  
17 µl X 96 tubes

**PANArray™ HPV PCR mixture #2** [Catalog # PANArray-HPV-F-002]

: For amplification of human beta-globin gene  
17 µl X 96 tubes

**PANArray™ HPV PCR primer #1** [Catalog # PANArray-HPV-F-003]

: For amplification of HPV gene  
1 tube (300 µl)

**PANArray™ HPV PCR primer #2** [Catalog # PANArray-HPV-F-004]

: For amplification of human beta-globin gene  
1 tube (300 µl)

**PANArray™ HPV hybridization buffer #2** [Catalog # PANArray-HPV-F-005]

: 1 tube (400 µl)

**PANArray™ HPV positive control** [Catalog # PANArray-HPV-F-006]

: 1 tube (60 µl)

**PANArray™ HPV negative control** [Catalog # PANArray-HPV-F-007]

: 1 tube (60 µl)

## Additional equipment and reagents

- Slide warmer
- Hybridization incubator (50 ±1°C)
- PCR machine
- Shaker and magnetic stirrer
- Slide rack and glass jar
- Centrifuge (for slide desiccation)
- Fluorescence scanner GenePix 4000B (Axon Instrument Inc.)
- Third-distilled water and ice

## II. INTRODUCTION

### Human Papilloma Virus (HPV) and cervical cancer

Human Papilloma Virus (HPV) is a common virus and known to be over 100 genotypes. HPV is categorized into two groups; high risk group and low risk group. Even with HPV infection, most HPVs are known to be naturally cured with any indication or symptoms. Around 40 HPV genotypes may cause warts on hand, foot or skin. When the HPV infection by the high risk group persists, however, HPV may cause cervical cancers. Almost all cervical cancers (99.7%) are known to be implicated by presence of the high risk group HPV. Of the high risk groups, genotypes 16 and 18 are most commonly detected from the tissues of cervical cancers. These genotypes also detected from other tissues like vagina, anus, etc.

### Composition of PANArray™ HPV Genotyping Chip

PANArray™ HPV Genotyping Chip is immobilized with PNA probes specifically designed to each genotype on a glass slide. With PCR amplification of the HPV LI gene that includes DNA bases specific for each genotype, this amplified PCR product is hybridized to each PNA probe and determined for the HPV infection and its genotype. On one slide, 34 probes are positioned; 19 HPV genotypes with high risk (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 70 and 73) and 13 HPV genotypes with low risk (6, 11, 32, 34, 40, 42, 43, 44, 54, 55, 62, 81 and 83). In addition, a PNA probe to the beta-globin gene for PCR confirmation and four position markers are also immobilized on the same slide.

### Characteristics of PANArray™ HPV Genotyping Chip

PANArray™ HPV Genotyping Chip uses peptide nucleic acid (PNA) oligomers as probes instead of DNA oligonucleotides as used in DNA-based microarrays. PNA, a DNA analogue, has a strong binding affinity to its complementary DNA sequences and results in fast hybridization. In addition, due to its unique chemical structure, PNA is very stable chemically and biologically even at room temperature. Taking advantages of the PNA physicochemical characteristics, PANArray™ HPV

Genotyping Chip detects the target DNA at low concentration with high accuracy in a short time. PANArray™ HPV Genotyping Chip also precisely determines HPV genotypes for multiple infections by different genotypes. PANArray™ HPV Genotyping Chip can be stored for a long period, longer than one year, at room temperature.

### III. ADVANTAGES

PANArray™ HPV Genotyping Chip has the following advantages.

#### 1. High specificity

PANArray™ HPV Genotyping Chip precisely discriminates 32 HPV genotypes consisted of 19 high risk types and 13 low risk types.

#### 2. High sensitivity

PANArray™ HPV Genotyping Chip is able to detect at 10 copies of the target HPV in clinical samples.

#### 3. High reproducibility

PANArray™ HPV Genotyping Chip shows consistent results of the HPV genotyping with low cross hybridization and low noise and background signals.

#### 4. High stability

PANArray™ HPV Genotyping Chip has long shelf life, longer than one year at room temperature.

## IV. PROCEDURES

PANArray™ HPV Genotyping Chip is simple, accurate, fast and durable for your uses. Figure 1 shows a standard workflow for the PCR amplification, hybridization, washing and scanning processes.

### Workflow

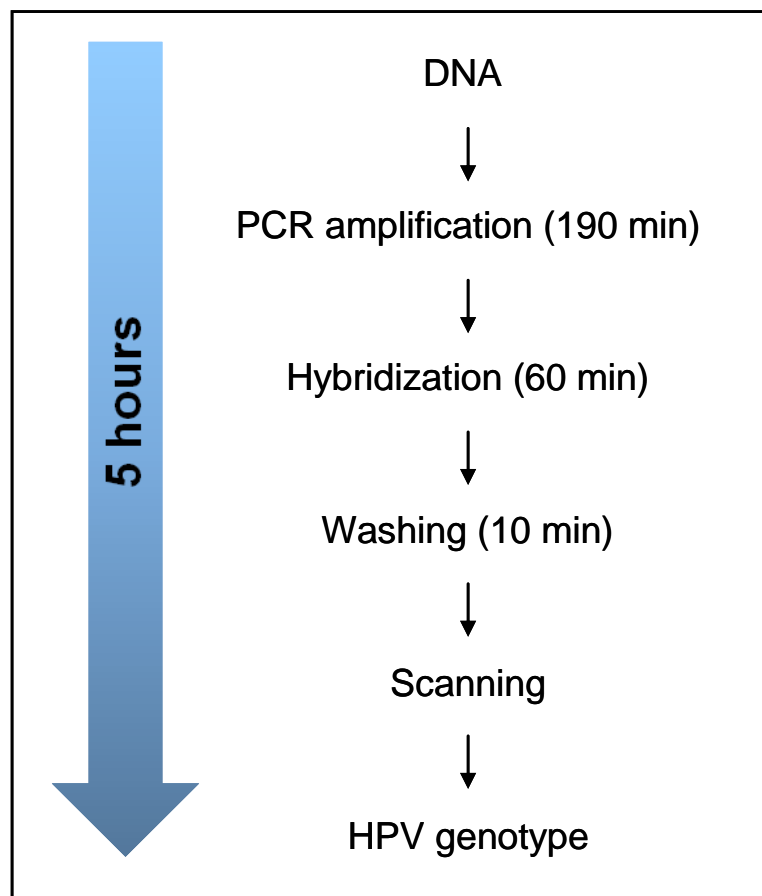


Figure 1. Workflow of PANArray™ HPV Genotyping Chip



## PCR amplification

**CAUTION!**

A careful PCR amplification is required to reduce contamination that may cause variation in results.

1. Prepare the same number of PCR mixture #1 [Catalog # PANArray-HPV-F-001] and #2 [Catalog # PANArray-HPV-F-002] tubes with specimen numbers at 4 °C.
2. Add 3 µl of PCR primer #1 [Catalog # PANArray-HPV-F-003] and #2 [Catalog # PANArray-HPV-F-003] for each specimen in each PCR mixture #1 and #2 tube, respectively.
3. Add 5 µl of each DNA extract in each PCR mixture.
4. Conduct PCR amplification with PCR mixture #1 and #2 under following conditions (Table 1).

**Table 1. PCR condition**

Step	Temp (°C)	Time (min)	Cycle No.
1	94	15	1
2	94	0.5	10
	60	0.5	
	72	0.5	
3	94	0.5	40
	47	1	
	72	0.5	
4	72	5	1
5	Keep at 12 °C		

5. Obtain the PCR product #1 from PCR mixture #1 and PCR product #2 from mixture #2 after PCR amplification.

## ✓ Preparation for next step

1. Preheat the hybridization incubator and slide warmer at 50 °C.
2. Preheat PANArray™ HPV chip [Catalog # PANArray-HPV-C-001] attached each with a cover slip in slide warmer at 50 °C for 10 min.
3. Preheat PANArray™ HPV hybridization buffer #1 [Catalog # PANArray-HPV-C-002] at 50 °C for 30 min.
4. Dilute the 20 X PANArray™ HPV washing buffer [Catalog # PANArray-HPV-C-003] into 1 X buffer.

## Hybridization

1. Add 5 µl of PCR product #1 and 5 µl of PCR product #2 in one tube [A].
2. Add 67 µl of PANArray™ HPV hybridization buffer #1 and 3 µl of PANArray™ HPV hybridization buffer #2 [Catalog # PANArray-HPV-F-005] in the tube [A] and mix thoroughly by vortexing.
3. Load 70 µl of the solution into each cover slip hole on the slide in slide warmer at 50 °C.
4. Hybridize at 50 °C for 1 hour. Prevent desiccation by maintaining humidity.

## Washing

1. Place the slide rack into a glass jar with 250 ml of 1x PANArray™ HPV washing buffer.
2. Remove the cover slips from each slides and immerse them in 1 X PANArray™ HPV washing buffer in a glass jar.
3. Wash the PANArray™ HPV chip at room temperature for 5 min by magnetic stirring on a shaker.
4. Change the buffer and wash for another 5 min.
5. Remove the buffer remaining on the slide by centrifugation.

## Scanning

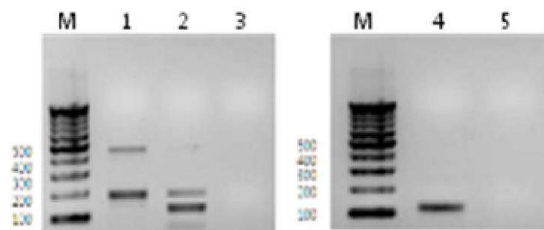
1. Set up GenePix 4000B scanner adjusted to 100% of laser outlet and 700 PMT Gain.
2. Place the slides and scan fluorescence for signal intensity.

## V. ANALYSIS OF RESULTS

### PCR result

1. Make 2% agarose gel as generally practiced.
2. Mix 2  $\mu$ l of loading buffer with 5  $\mu$ l of PCR product.
3. Conduct electrophoresis for 20 min at 130 volt.
4. Confirm bands for Gel documentation or UV trans-illuminator.

### ✓ Example



M: 100 bp DNA marker

Lane 1: HPV positive sample (200 bp and 450 bp)

Lane 2: HPV positive sample (200 bp, 130 bp)

Lane 3: HPV negative sample

Lane 4: beta-globin positive (130 bp)

Lane 5: beta-globin negative

## Probe arrangement of HPV genotypes

The signal fluorescence intensity is interpreted as to be positive when SMV (Signal Mean Variation; signal intensity – background signal) is greater than 1,500. HPV genotype is determined by the position map for each genotype in the following map.

16	16	51	51	PM	69	69	42	42
18	18	52	52		70	70	43	43
26	26	53	53		73	73	44	44
31	31	56	56	PM	6	6	54	54
33	33	58	58		11	11	55	55
35	35	59	59		32	32	62	62
39	39	66	66	PM	34	34	81	81
45	45	68	68		40	40	83	83
				PM	G	G		

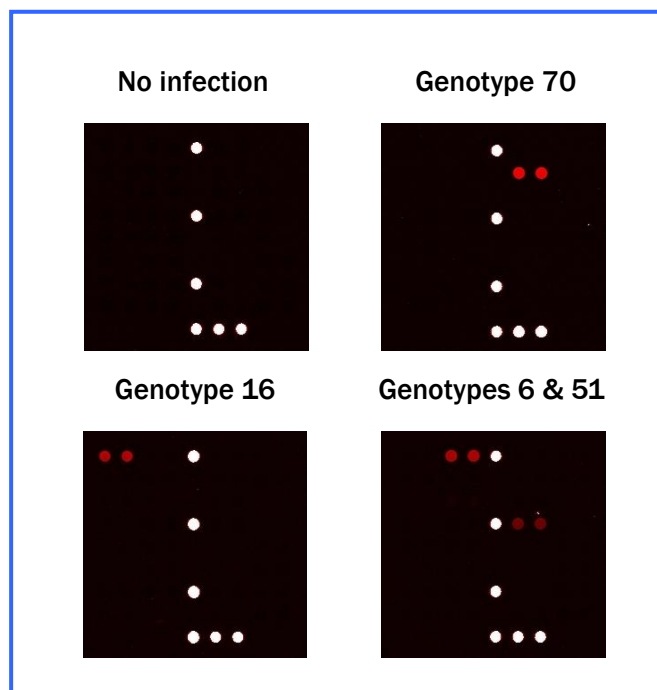
**G** beta-globin probe used for confirmation of PCR amplification

**PM** Position marker

 High risk HPV genotype

 Low risk HPV genotype

### ✓ Example



## VI. PRECAUTIONS

### Handling of PANArray™ HPV Genotyping Chip

- Wear powder-free gloves before handling the PANArray™ HPV Genotyping Chip.
- Use sterile pure water while preparing solutions.

### Weak signal intensity of fluorescence

- Check fluorescence of beta-globin probes. Low fluorescence of beta-globin probes indicates poor DNA extraction or PCR amplification.
- In this case, check your DNA extraction and PCR amplification and repeat the test.

### Detecting positive signals in the negative control

- Check any contamination during the DNA extraction or PCR amplification and repeat the test without any contamination.
- Be cautious for any contamination during PCR amplification. Wear powder-free gloves and keep the place clean. Using fresh new tips or filter tip may reduce contamination from pipetting.

For more information or any questions, please contact [info@panagene.com](mailto:info@panagene.com).

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