Design and Evaluation of High Definition Probe for HPV Genotyping Microarray

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Abstract

DNA microarray is one of the most useful tools in the Viral Genome Screening such as Human Papilomavirus(HPV). Using type specific probes of about 30 mer oligonucleotide DNA sequences based upon the L1 region of the HPV genome, we could genotyping the HPV which might be infected in the cervix. In this HPV DNA microarray technology, we used the amplicon of 150 bp of the HPV L1 region. We improved the performance of the type specific probe by triple probe sequence in the amplicon. New probes are composed of three types of sequences in the HPV L1 region. To make hybridization conditions amicable, we select the final probe set under melting temperature requirement. The new probes have shown more sensitive than the single sequence probe about 10 ~ 100 times.

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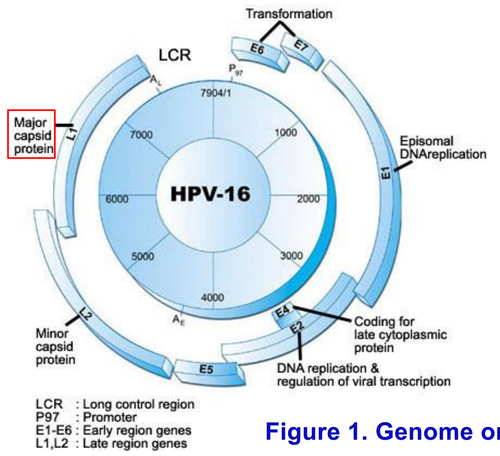
1. HPV(Human Papillomavirus)

HPVs, a group of circular double-stranded DNA virus with a size of about 8 Kb, are known to cause cervical cancers in women as well as various other malignant tumors in humans. Among over 70 genotypes of HPVs identified to date, the oncogenic high-risk HPVs (HPV-16, 18, 31, 33, 35, etc) are associated with invasive cervical carcinomas and their precursors, whereas the low-risk HPVs (HPV-6, 11, 34, 40, etc) cause genital warts and lesions rarely progress to malignancy. In addition, HPV 16 and 18 infections are a cause of a unique type of oropharyngeal (throat) cancer and are believed to cause 70% of cervical cancer.

2. Target L1 of HPV



HPV consists of early genes (E1, E2, E4, E5, E6, E7), late genes (L1, L2), and LCR (long control region), and most types of HPV L1 gene fragment (about 150 bp) can be amplified by consensus primers. The L1 is coding region of viral major capsid proteins

Figure 1. Genome organization of HPV 16type

3. Considerations for Probe Design

- **Number of oligos per target : 3**
  - **Three parts for triple oligonucleotide probes as shown in Fig.2**
  - **Probes were selected by using Cluster W ans MEGA 5.0 program.**

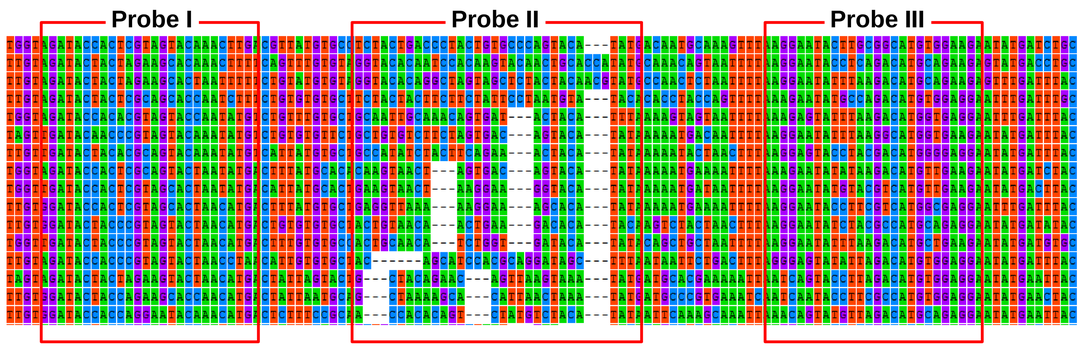


Figure 2. L1 sequence part of HPV types are aligned with each other.

- **Probe length**
  - **~20mer: many more matches than other lengths and may not be suitable for oligonucleotide design**
  - **30~80mer: specificity is reduced**
  - **20~30mer: increase specificity but could induce a lower sensitivity**
  - **20~25mer recommend**
- **Threshold to reject secondary structures**
- **GC content**
  - **<40% : signal low**
  - **>60% : signal too high**
  - **40~60% recommend**
- **Minimize repeated sequences**
- **Must not have more than 75% of similarity with a non-target**

Ref. GoArrays: highly dynamic and efficient microarray probe design 2005 Apr 1;21(7):1094-103. Epub 2004 Nov 5.

4. Test and Result

The HPV DNA Microarray was hybridized with the amplified target DNA (about 150 bp) by PCR in a humid incubator for 1 hr at 60C.

Standard Concentration	16type		18type		58type	
	Each	Mix	Each	Mix	Each	Mix
10^{-2} ng/ul						
10^{-3} ng/ul						
10^{-4} ng/ul						
10^{-5} ng/ul						
10^{-6} ng/ul						

Table1. Compare 3 single probes with Mix probe for HPV 16, 18 and 58 type

Standard Concentration	16type		18type		58type	
	A Chip*	B Chip**	A Chip	B Chip	A Chip	B Chip
10^{-2} ng/ul						
10^{-3} ng/ul						
10^{-4} ng/ul						
10^{-5} ng/ul						
10^{-6} ng/ul						

Table2. Compare A Chip with B Chip for HPV 16, 18 and 58 type
*** Above 30mer single sequence probe two dots Chip**
**** 20~25mer triple probe mix Chip**

5. Discussion

To improve the sensitivity and specificity of the HPV DNA Microarray, we adopted triple oligonucleotide probes for each targets and selected these probes not to have higher similarity of 75% with each others. These triple probes have shown 10 ~ 100 times higher sensitivities with comparable specificities than the conventional HPV DNA microarray of single oligonuclotide probe.