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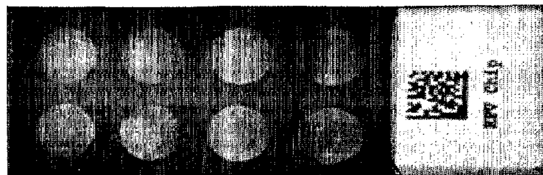
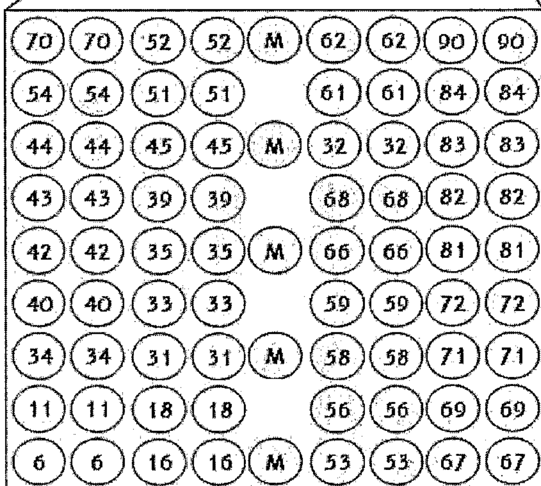
[Continued on next page]

(54) Title: PRIMER, PROBE, DNA CHIP CONTAINING THE SAME AND METHOD FOR DETECTING HUMAN PAPILLOMAVIRUS AND DETECTION KIT THEREOF

Fig. 1





(57) Abstract: The present invention discloses a primer, a probe, a DNA chip, and a test kit for diagnosis and genotyping of Human Papilloma Virus (HPV)as well as a method for testing HPV genotype. According to the present invention, it is possible to screen HPV genotypes with high sensitivity and specificity, and to diagnose infection by multiple HPV types which was not possible in other conventional HPV testing methods.

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【DESCRIPTION】

【Invention Title】

Primer, probe, DNA chip containing the same and method for detecting human papillomavirus and detection kit thereof

【Technical Field】

<1> The present invention relates to a primer, a probe and DNA chip containing them for genotyping of Human Papillomavirus (HPV). Specifically, the present invention relates to a PCR primer for amplification of HPV genes, a probe which specifically binds with the amplified genes, and a testing kit and method using them for diagnosis and/or test of HPV genes.

<2>

【Background Art】

<3> HPV has been found in papilloma of cottontail rabbit, and known as one of oncoviruses, a DNA virus on a double-helix with a size of about 45-55nm and 8kb. HPV belongs to Papovavirus family, and it is generally found in various kinds of animals other than rabbit, causing infections in the form of warts or condyloma in human.

<4> HPV has been known to cause cervical cancer in women, and be closely related to other malignant tumors such as skin cancer, laryngeal cancer or the like. HPV infection isvery commonly occurred in women, approximately 75% of women get infected as they become sexually active. Cervical cancer can be developed 10-20 years after a certain HPV infection. 20~25% of infections may progress to a precancerous stage.

<5> At present, approximately 120 types of different HPV genotypes have been known. Among HPV types, types prevalently infecting the genital area are following 45 types: HPV-16, -31, -33, -35, -52, -58, -67, -40, -43, -7, -32, -42, -6, -11, -74, -44, -55, -13, -61, -72, -62, -2, -27, -57, -3, -28, -29, -10, -54, -18, -39, -45, -59, -68, -70, -26, -69, -51, -30, -53, -56, -66, -34, -64 and -73. Among them, HPV types being closely associated with cervical cancer are: as a high-risk group of 22 types, HPV-16, -18, -26, -30,

-31, -33, -35, -45, -51, -52, -53, -56, -57, -58, -59, -61, -67, -68, -70, -73 and -74 and as a low-risk group, HPV-2a, -3, -6, -10, -11, -32, -34, -40, -43, -44, -54, -55, -66 and -69. 90% HPV types belonging to the high risk group are detected in epithelial tissues of cervical tumors.

<6> Knowing the HPV types infecting a patient, it is possible to expect how the infection would progress to diseases in the future. Therefore, it is important to test the presence of HPV and confirm the exact types thereof.

<7> As conventional methods for diagnosis of HPV infection, there are Southern blot hybridization, dot (slot) blot hybridization (ViraPap), in-situ hybridization, hybrid capture system, PCR (polymerase chain reaction) and the like.

<8> The conventional diagnosis methods are advantageous interms of being capable of partial quantification and convenience. However, for a diagnosis purpose, there are some disadvantages such that they require long time to get the result and complex experimental techniques, and diagnosis of HPV genotypes is not so convenient.

<9> A DNA chip method, which has been recently developed, is a method combined with DNA amplification, being capable of testing the HPV genotypes from a large amount of samples in convenient way, without problems of production economy or process time. Such advantages make this method very useful in early diagnosis, prevention and prognosis of cervical cancer.

<10> Since 2003, US FDA has recommended both PAP smear testing and HPV infection test at the same time for testing cervix cancer. At present, vaccines for HPV-16 and HPV-18 have been developed and now available. For monitoringeffects thereof, a device (kit) or a method for diagnosing HPV genotype is needed. Further, since tests on infection by various HPV genotypes are essential, a HPV diagnosis device (kit) and a test method for determining HPV genotypes are further required, at present.

<11>

【Disclosure】

【Technical Solution】

<12> The present invention is to provide a primer used in PCR for HPV amplification.

<13> Further, the present invention is to provide a probe for testing HPV genotypes with enhanced specificity.

<14> Still further, the present invention is to provide a DNA chip and kit for testing several tens of HPV genotypes in rapid way.

<15> Further, the present invention is to provide a method for testing several tens of HPV genotypes at once through a single test.

【Advantageous Effects】

<17> According to the presentinvention, it is possible to identify 36 HPV types present in cervical cells with enhanced specificity and further to detect multiple infections by different HPV types. The present invention can test HPV genotypes in a reduced time, taking about 6 hours or less from DNA extractionfrom cervical cell specimen to identification of the HPV genotype by using a DNA chip.

<18>

【Description of Drawings】

<19> Fig. 1is a view showing a DNA chip for a HPV genotype test according to one embodiment of the present invention.

<20> Fig. 2 is a view showing the gene amplification result by using a primer according to the present invention, JK1 set. HPV genotypes are as follows: from left to right, 6,11,16,18,31,32,33,34,35,39,40,42,43,44,45,51,52,53,54,56,58,59,61,62,66,67, 68,69,70,71,72,81,82,83,84,90, positive control, and negative control.

<21> Fig. 3 is a view showing the gene amplification result by using a primer according to the present invention, JK2 set.HPV genotypes are as follows: from left to right, 6,11,16,18,31,32,33,34,35,39,40,42,43,44,45,51,52,53,54,56,58,59,61,62,66,67, 68,69,70,71,72,81,82,83,84,90, positive control, and negative control.

<22> Fig. 4 is a view showing the gene amplification result by using a control primer set.HPV genotypes are as follows: from left to right,6,11,16,18,31,32,33,34,35,39,40,42,43,44,45,51,52,53,54,56,58,59,61,62, 66,67,68,69,70,71,72,81,82,83,84,90, positive control, and negative control.

<23> Figs. 5 and 6 are the results of HPV genotype tests with a DNA chip according to the present invention, appeared by a chip scanner.

<24> Fig. 7 is a DNA extraction reagent kit according to one embodiment of the present invention.

<25> Fig. 8 is a reagent kit containing a PCR primer according to one embodiment of the present invention.

<26> Fig. 9 is a DNA chip kit for testing HPV genotype according to one embodiment of the present invention.

<27> Figs. 10 to 22 are views showing the base sequencing results of PCR products according to each HPV genotype, obtained from Example 1-1.

<28>

【Mode for Invention】

<29> In one aspect, the present invention provides a primer for HPV (Human Papilloma Virus) amplification, which contains at least one base sequence selected from the group consisting of SEQ.ID.NO:1 to SEQ.ID.NO:6.

<30> The base sequence may have a fluorescent dye attached to 5' end thereof. The fluorescent dye can be selected from the group consisting of Cy3, Cy5, a biotin binding material, Alexa 647, Alexa 555, 5-(2-aminoethyl)amino-1-naphthalene sulfonic acid (EDANS), tetramethyl rhodamine (TMR), tetramethyl rhodamine isocyanate (TMRITC), x-rhodamine, fluorescein isocyanate(FITC)and Texas red.

<31> In another aspect, the present invention provides aprobe which forms complementary binding with HPV, containing at least one oligonucleotide selected from the group consisting of oligonucleotide having base sequences represented by SEQ.ID.NO:9 to SEQ.ID.NO:44, and oligonucleotidehaving base sequences complementary to said sequences.

<32> The oligonucleotide may correspond to the following HPV types: HPV-6,11,16,18,31,32,33,34,35,39,40,42,43,44,45,51,52,53,54,56,58,59,61,62,66,67,68,69,70,71,72,81,82,83,84 and 90.

<33> To the 5' end of the base sequences represented in SEQ.ID.NO:9 to SEQ.ID.NO:44, an amine group can be attached.

<34> In still other aspect, the present invention provides a DNA chip for testing HPV genotypes, containing the above-described probe.

<35> In another aspect, the present invention provides a kit for detecting or testing HPV genotype, containing:

<36> a reagent for DNA extraction;

<37> a PCR primer for amplification of the extracted DNA; and

<38> a DNA chip for testing HPV genotype containing a probe according to any one of claims 4 to 6.

<39> The primer for PCR can contain at least one base sequence selected from the group consisting of SEQ.ID.NO:1 to SEQ.ID.NO:6.

<40> The primer for PCR can be a set selected from: a set consisting of SEQ.ID.NO:1 and SEQ.ID.NO:2 (referred to 'JK1 set'); a set consisting of SEQ.ID.NO:3 and SEQ.ID.NO:4 (referred to 'JK2 set); and a set consisting of SEQ.ID.NO:5 and SEQ.ID.NO:6 (referred to 'JK3').

<41> In still other aspect, the present invention provides a method for testing HPV genotype, comprising the steps of:

<42> extracting DNA from cells taken from the cervix;

<43> amplifying the DNA by PCR involving a primer for HPV amplification;

<44> hybridization of the amplified DNA to a DNA chip containing a HPV probe which containsat least one oligonucleotide selected from the group consisting of oligonucleotide having base sequences represented bySEQ.ID.NO:9 to SEQ.ID.NO:44 and oligonucleotide having base sequences complementary to said sequences; and

<45> determining the HPV genotype through the product specifically hybridized with the probe.

<46> The method can further include, in the PCR amplification, a beta-globin primer set represented by SEQ.ID.NO:7 and SEQ.ID.NO:8, as a control.

<47> In the hybridization of the amplified DNA, an amine group can be attached to the 5' end of the base sequences represented in SEQ.ID.NO:9 to SEQ.ID.NO:44.

<48> In the step of determining the HPV genotype, the HPV genotype can be selected from the group consisting of HPV-6,11,16,18,31,32,33,34,35,39,40,42,43,44,45,51,52,53,54,56,58,59,61,62,66,67,68,69,70,71,72,81,82,83,84, 90 and mixtures thereof.

<49> The present invention is comprised of the following steps: amplification of DNA by PCR involving a primer for HPV amplification; hybridization of the amplified DNA to a DNA chip containing a HPV probe; and

determining HPV genotype, by assaying the product specifically hybridized with the probe. For achieving the invention, the present invention provides a novel primer, a probe, a DNA chip and a test kit. Hereinafter, the present invention is further illustrated in detail.

<50>

<51> The primer contains at least one base sequenceselected from the group consisting of SEQ.ID.NO:1 to SEQ.ID.NO:6. The primer is 5' primer or 3' primer specific to HPV genes, as represented in Table 1 below.

<54>

<55> 【Table 1】

<56>

SEQ. ID. No.	Primers		Sequence (5' → 3')
1	JK1	5'	5'- AGT GGS TCT ATG GTW WCY TC -3'
2		3'	5'- CAT AGT ATG TAW RTA KGY CAT-3'
3	JK2	5'	5'- GCC ACA AYA ATG GYA TWT GYT -3'
4		3'	5'- TGT AAA TCA TAT TCC TCM MCA TG -3'
5	JK3	5'	5'- CCA CCT ATA GGG GAA CAC TGG -3'
6		3'	5'- GAG GTA ACC ATA GAA CCA CT -3'

<57> <58> <59>

<60>

<61> The base sequence can be easily detected, owing to a fluorescent dye being attached to 5' end thereof. The fluorescent dye may be selected from the group consisting of Cy3, Cy5, a biotin binding material, Alexa 647, Alexa 555, 5-(2-aminoethyl)amino-1-naphthalene sulfonic acid (EDANS), tetramethyl rhodamine (TMR), tetramethyl rhodamine isocyanate (TMRITC), x-rhodamine, fluorescein isocyanate(FITC) and Texas red, without being limited to these.

<62> In the present invention, the primer can be one or moreselected from the group consisting of SEQ.ID.NO:1 to SEQ.ID.NO:6. Preferably, the primer is used as a primer set selected from: JK1 set consisting of SEQ.ID.NO:1 and

SEQ.ID.NO:2; JK2 set consistingof SEQ.ID.NO:3 and SEQ.ID.NO:4; and JK3 set consisting of SEQ.ID.NO:5 and SEQ.ID.NO:6.

<63> In addition to the primer, the present invention may further include a beta-globin primer set represented by SEQ.ID.NO:7 and SEQ.ID.NO:8, as a control gene. To the end of the 5' primer of beta-globin, a fluorescent dye can be attached.

<64> The probe of the present invention contains at least one oligonucleotide selected from the group consisting of oligonucleotide having base sequences represented as SEQ.ID.NO:9 to SEQ.ID.NO:44, and oligonucleotide having base sequences complementary to said sequences, as shown in Table 2. As seen from the following Table 2, the probes of the present invention can specifically bind with 36 types of HPV genotypes in total.

<65> To the 5' end of the base sequences represented in SEQ.ID.NO:9 to SEQ.ID.NO:44, an amine group can be attached so as to be usedin DNA chip fabrication. Such technique is well known in this field of art to which the present invention pertains, thereby eliminating detaileddescription thereof in this specification.

<66> The expression 'specifically hybridized' used herein, is generally used in this field of art, referring to a reaction of forming complementary binding between bases. Such specific binding reaction is called 'hybridization'.

<67> The primer, as described in the above, amplifies HPV gene (DNA), and then thus amplified gene isspecifically hybridized with the probe, resulting a product by which HPV genotype can be determined.

<68>

<69> 【Table 2】

<70>

SEQ. ID. No.	HPV types with which the probe is specifically hybridized	Sequence (5'- 3')
9	HPV6	ACC AAT TCT GAT TAT AAA GAG TAC ATG CGT
10	HPV11	TTC AGA TTA TAA GGA ATA CAT GCG CCA TGT
11	HPV16	CTA CAT ATA AAA ATA CTA ACT TTA AGG AGT
12	HPV18	CTC CTG TAC CTG GGC AAT ATG ATG CTA CCA
13	HPV31	CTA CAT TTA AAA GTA GTA ATT TTA AAG AGT
14	HPV32	GTG CTA CTG TAA CAA CTG AAG ACA CAT ACA
15	HPV33	CAC ACA AGT AAC TAG TGA CAG TAC ATA TAA
16	HPV34	TGC AAA CAG TAA TTT TAA GGA ATA CCT CAG
17	HPV35	CTG CTG TGT CTT CTA GTG ACA GTA CAT ATA
18	HPV39	TGA TCC TTC TAA GTT TAA GGA ATA TAC CAG
19	HPV40	TAA TAA CAG TAA TTT CAA GGA ATA TTT GCG
20	HPV42	CAG CTG CTA ATT TTA AGG AAT ATT TAA GAC
21	HPV43	TAT GAC AAT GCA AAG TTT AAG GAA TAC TTG
22	HPV44	TAC TAG TGA ACA ATA TAA GCA ATA CAT GCG
23	HPV45	CCT ACT AAG TTT AAG CAG TAT AGT AGA CAT
24	HPV51	TCC AAG TAA CTT TAA GCA ATA TAT TAG GCA
25	HPV52	TGA AAA TTT TAA GGA ATA CCT TCG TCA TGG
26	HPV53	AGC AAA TTA AAC AGT ATG TTA GAC ATG CAG
27	HPV54	TTC TGA CTT TAG GGA GTA TAT TAG ACA TGT
28	HPV56	TGC ACG AAA AAT TAA TCA GTA CCT TAG ACA
29	HPV58	AAA AAT GAT AAT TTT AAG GAA TAT GTA CGT
30	HPV59	CAC ACC TAC CAG TTT TAA AGA ATA TGC CAG
31	HPV61	TGT ATC TGA ATA TAA AGC CAC AAG CTT TAG
32	HPV62	TCC ACT GCT GCA GCA GAA TAC AAG GCT ACC
33	HPV66	ATG ATG CCC GTG AAA TCA ATC AAT ACC TTC
34	HPV67	TGT TCT GAG GAA AAA TCA GAG GCT ACA TAC
35	HPV68	TTA TGA TCC TAA TAA ATT TAA GGA ATA TAT
36	HPV69	TAA ACC ATC AGA TTA TAA GCA GTT TAT AAG
37	HPV70	TAG CCC TAC AAA GTT TAA GGA ATA TAC TAG
38	HPV71	GCC TCT AGT TTC ATG GAA TAT TTG AGA CAT
39	HPV72	GCG TCC TCT GTA TCA GAA TAT ACA GCT TCT
40	HPV81	GGC CTC TAA CTT TAA GGA ATT TCT GCG CCA
41	HPV82	CCA TCT GTT GCA CAA ACA TTT ACT CCA GC
42	HPV83	CAC AGG CTA ATG AAT ACA CAG CCT CTA ACT
43	HPV84	CAC CGA ATC AGA ATA TAA ACC TAC CAA TTT
44	HPV90	GAC ACA TAC AAG GCT TCC AAT TTT AAA GAG

<71> According to the present invention, a DNA chip for a genotype test may be fabricated by using said probe (Fig. 1). It will be further illustrated through the following examples.

<72> In another aspect, the present invention provides a kit for detecting or testing HPV genotype, containing: a reagent for DNA extraction; a PCR primer for amplification of the extracted DNA; and a DNA chip for testing HPV genotype, containing the above-described probe.

<73> Further, the presentinvention provides a method for testing HPV genotype, including the steps of: extracting DNA from cells taken from the cervix; amplifying the DNA by PCR involving a primer for HPV amplification; hybridization of the amplified DNA toa DNA chip containing the above-described HPV probe; and determining the HPV genotype through the product specifically hybridized with the probe.

<74> Specifically, a method for detecting HPV genes and/or testing HPV genotypes according to one embodiment of the present invention may include the following procedure, however it is not just limited to this example.

<75> 1) centrifuging the cervix cells taken from a vaginal swab to collect cells and lysing cells with a solutionfor cell lysis so as to obtain DNA,

<76> 2) amplifying a portion of the resulted DNA by using the 5' primer and 3' primer of the present invention, which are the primers specific to HPV genes, for HPV gene amplification, and

<77> 3) applying the amplified HPV genes to a 2.5% agarose gel, and determining the PCR products by the presence of fluorescence visualized by Et Br.

<78>

<79> A method for testing HPV genotypes by using a DNA chip according to one embodiment of the present invention may include the following procedure, however it is not limited to this example.

<80> 1) centrifuging cervix cells taken from a vaginal swab to collect cells and lysing cells with a solution for cell lysis so as to obtain DNA,

<81> 2) amplifying a portion of the resulted DNA by using the 5' primer and

3' primer of the present invention, which are primers specific to HPV genes, for HPV gene amplification,

<82> 3) fabricating a DNA chip for testing genotypes of the amplified HPV genes, by synthesizing oligonucleotides of 36 types of HPV probes which specifically bind to the 36 types of HPV genes, to the size as much as about 30 nucleotides,

<83> 4) placing each probe on a glass panel for chip fabrication in array at a certain interval by using a Microarrayer, and washing and fixing them to fabricate a chip,

<84> 5) hybridizing the PCR products of HPV genes from 2) to the resulted chip at a certain temperature and washing it so as to remove those unspecifically bound therefrom,

<85> 6) determining the HPV genotypes specifically bound to the HPV probes by using a chip scanner.

<86>

<87> Further, a kit for testing HPV genotypes according to one embodiment of the present invention may include a reagent, a device and equipment as follows, however it is not just limited to this example.

<88> 1) DNA extraction from cells:

<89> ① buffer for cell lysis(7% chelex, 20mM Tris HCl, 100ug/ml proteinase K, 10mM $CaCl_2$, pH 8.0)

<90> 2)PCR of HPV genes:

<91> ① 20pmol/$\mu\ell$ of each primer set

<92> ② 10X PCR buffer solution

<93> ③ Taq. DNA polymerase 250 unit (Solgent, South Korea)

<94> ④ 2.5mM dNTP(dATP, dGTP, dCTP, dUTP)

<95> ⑤ Uracil-N-Glycosylase 1U/$\mu\ell$

<96> 3) fabrication of a DNA chip for testing HPV genotypes:

<97> ① 36 types of oligonucleotide probes of HPV genes, wherein an amine group is attached to 5' end thereof (concentration: 50pmol/$\mu\ell$)

<98> ② a glass slide (silanated, amine-coated)

<99> ③ a 8-well hybridization chamber (Grace, US)

<100> ④ a blocking buffer (5XSSC, 1%BSA, 0.1%SDS)

<101> ⑤ isopropyl alcohol

<102> ⑥ distilled water

<103> 4) hybridization of PCR products of HPV genes with the HPV DNA chip:

<104> ① a 8-sectioned DNA chip for testing HPV genotype, fabricated in the above 3)

<105> ② hybridization buffer (0.4M Mes, 20mM $MgCl_2$, 0.15% SDS, 0.15% BSA, pH 6.5)

<106> 5) Washing DNA chip completed with the hybridization:

<107> ① washing solution I (2X SSC, 0.2% SDS)

<108> ② washing solution II (0.1X SSC)

<109> 6) HPVDNA chip reading:

<110> ① microarrayer scanner (GSI Lumonics, USA)

<111> By fabricating a DNA chip for a HPV genotype test from the above-described reagents, devices and equipment, it is possible to determine HPV genotype of samples taken from the cervix. Further, based on such DNA chip, a kit for HPV gene detection or a HPV genotype test can be formed by the following construction. However, the following description is only one embodimentof the present invention, and the present invention is by no means limited by such examples.

<112>

<113> 1) A kit for extracting DNA from cells:

<114> ① a 30ml volume tube containing a gene extract solution (7% (w/v) chelex, 20mM Tris HCl, 100ug/ml proteinase K, 10mM $CaCl_2$, pH 8.0)

<115> ② a 30ml volume tube containing a 10X washing solution (200mM Tris-HCl, 50mM KCl, 50mM NaH_2PO_4, 50mM Na_2HPO_4, pH 7.4)

<116> 2) A kit for PCR of HPV genes:

<117> ① a 1.5mℓ volume tube containing a primer set selected among JK 1, 2 and 3, at the concentration of 20pmol/μℓ

<118> ② a 1.5mℓ volume tube containing 10X PCR buffer solution

<119> ③ a 1.5mℓ volume tube containing Taq DNA polymerase 250 units (Tag., Solgent, South Korea)

<120> 3) A kit for a HPV genotype test of PCR products of HPV genes:

<121> ① a DNA chip for a HPV genotype test

<122> ② a multiwell hybridization chamber (8-well) (Sigma, US)

<123> For diagnosis of HPV infection, kits 1) and 2) may be used, and for a HPV genotype test, kits 1), 2) and 3) may be used. According to the present invention, it takes about 6 hours from extraction of DNA from the cervix cells and HPV gene amplification by PCR to HPV genotype assay by using the HPV DNA chip. With one HPV DNA chip, 8 samples can be tested simultaneously.

<124> Hereinafter, the present invention is further illustrated by the examples provided below. However, it should be understood that these examples only have illustrative purpose, by no means limiting the scope of the present invention.

<125>

<126> Examples

<127> Example 1: Detection of HPV genes by using HPV gene amplification

<128>

<129> 1-1: DNA extraction from cervix cell

<130> A cytobrush sample preserved in 5 mℓ of a washing solution (PBS) was severely vortexed to separate the cervix cells therefrom. The cells were centrifugedat 1,300g for 10 minutes, and the supernatant was removed therefrom. The cells were re-suspended in a washing buffer (200mM NaCl, 50mM KCl, 50mM NaH2PO4, 50mM Na2HPO4, pH 7.4), transferred to a 1.5mℓ-volume microtube, and centrifuged at 1,300g for 5 minutes. The supernatant was removed, and 200$\mu\ell$ of a chelex buffer (7% chelex, 20mM Tris HCl, 100 μg/ml proteinase K, 10mM $CaCl_2$, pH 8.0)were added thereto for re-suspension. The suspension was heated in a water bath at 55℃ for 3 hours, and then further heated at 95℃ for 20 minutes so as to eliminate the activity of proteinase K, finally obtaining DNA from the cervix cell.

<131>

<132> 1-2: HPV gene amplification by PCR

<133> For amplifyingthe DNA obtained from the above 1-1, JK1 and JK2 primer sets as shown in Table 1 were used independently. As a control, a β-globin primer represented in SEQ.ID.NO: 7 and SEQ.ID.NO: 8 was used, wherein Cy5 fluorescent dye was attached to the 5' end of the 3' primer (5'-Cy5-CAA GAC AGG TTT AAG GAG ACC A-3'/ 5'- GGT TGG CCA ATC TAC TCC CAG G-3').

<134> For gene amplification (PCR), into a 0.2mℓ PCR tube, 2.5μℓ 10×buffer (750mM Tris-HCl (pH9.0), 150mM $(NH_4)_2SO_4$, 25mM $MgCl_2$, 1mg/ml BSA) 5μℓ, 2.5mM dNTP 2.0μℓ, Taq polymerase (5 units) 0.3μℓ, 20pmolor JK2 primer set 1.0μℓ, respectively, template DNA 5μℓ were added, and by further adding distilled water thereto, 25μℓ of total volume was made. For pre-denaturation, the amplified gene mixture was heat-treated at 50℃ for 3 minutes and then 95℃ for 15 minutes. PCR thereof was conducted at 95℃ for 60 seconds, 53℃ for 60 seconds, and 72℃ for 60 seconds, and this cycle was repeated 40 times. Finally, it was treated at 72℃ for 30 minutes (9700, Applied Biosystems).

<135> The amplification of β-globin genes that was used as a control, was conducted under the conditions as same as above, except the different primer. After completing the PCR amplification, 3.0μℓ of the amplified gene products was applied to a 2.5% agarose gel for electrophoresis, and the image analysis was carried out (imageanalyzer, alphascan). The time elapsed was one and half hour or so. The gene amplification results using JK1, JK2 and control as a primer, were shown in Figs. 2 to 4. As seen from the results illustrated in Figs.2 to 4, the primer according to the present invention exhibited superior gene amplification effect.

<136>

<137> Example 2: Fabrication of a DNA chip for diagnosis and genotyping of HPV genes

<138>

<139> 2-1: Fabrication of a DNA chip for testing HPV genotype

<140> By using Microarrayer (Bio-Robotics, UK), a silanated, amine-coated

glass slide was divided into 8 sections, and 36 types of HPV genotype probes at the concentration of 50pmol/μℓ, respectively, (base sequences were shown in Table 2) attached to each section in identical way.

<141> The glass slide attached with the probes was kept in a dark place for 2 days; then baked at 80℃ for 4 hours or more; and subjected to shaking incubation in a blocking buffer (5XSSC, 1%BSA, 0.1%SDS) at 42℃ for 45 minutes. Isopropanol was added thereto and the mixture was subjected to shaking incubation for 1 minute. After removing the supernatant (reacted solution), shaking incubation in distilled water was carried out for 1 minute and it was repeated 5 times. The resulted slide was dried and kept in a closed container before use.

<142>

<143> <u>2-2: Testing genotypes of PCT products of HPV genes by using a DNA chip for a HPV genotype test</u>

<144> 1) Preparation of a DNA chip for a HPV genotype test

<145> To the HPV DNA chip fabricated in the above 2-1, a 8-well hybridization chamber (Grace, US) was rigidly fixed to obtain a 8-sectioned DNA chip (see, Fig. 1)

<146> 2) Hybridization of PCR products of HPV genes with the DNA chip

<147> For hybridization with the oligonucleotides spotted on the HPV DNA chip, PCR product 8μℓ and 2X hybridization buffer 32μℓ were added into a 1.5ml tube, wherein the PCR product was denatured at 95℃ for 5 minutes, and the hybridization buffer 32μℓ was allowed for reaction at 48℃ for 10 minutes just right before use. The hybridization buffer and the PCR product were well mixed together, and carefully added to each opening of a well on the DNA chip, (1 sample per well). The opening of the well was covered with Cellotape for preventing the hybridizationmixture from being dried out, allowing hybridization to occur at 48℃ for 1 hour.

<148> 3) Washing the DNA chip after completion of hybridization

<149> The DNA chip completed with hybridization was washed once with a washing solution I (2X SSC, 0.2% SDS) for 10 minutes, and once again with a

washing solution II (0.1X SSC) for 10 minutes. After washing, it was air-dried at room temperature or forcibly dried by using a positive displacement air pump for rapid dry, and then kept under shade.

<150> 4) Determining HPV genotypes on the DNA chip after washing

<151> After setting a chip scanner (GSI Lumonics, USA) to operate at 550nm excitation wavelength and 570nm emission wavelength, the DNA chip washed in the above 3) was read to identify the location of probes which developed color owing to the binding between the probes for HPV genotype and HPV gene products, determining the HPV genotypes (See, Figs. 5 and 6).

<152> By providing DNA chips and reagents for a HPV genotype test as a form of a kit, it is possible to easily conduct a HPV genotype test on a multiple amount of samples, while reducing effort and time. A method and a kit for a HPV test are also included in the scope of the present invention to be protected, and a method for providing them in the form of a kit is further illustrated in the following example 3.

<153>

<154> **Example 3: A kit for testing HPV genotype**

<155> A kit for a HPV genotype test according to the present was composed of 3 parts: a first part including a reagent for DNA extraction from cells taken from the cervix (Fig. 7) a second part including a reagent containing a primer for PCR of HPV genes (Fig. 8); and a third part including a DNA chip for a HPV genotypetest which could determine genotype of the PCR products of HPV genes.

<156>

<157> 3-1:Preparation of a DNA extraction kit

<158> With the reagents prepared as it has been described in examples 1 and 2, a kit was formed, wherein the kit was comprised of ① a 30ml volume tube of 10X washing solution, which contained 20ml PBS solution, and ② a 30ml volume tube containing a proteinase K (Sigma, US) at the concentration of 100 ㎍ of the DNA extraction solution/ml, 7% chelex, both of them being kept at room temperature. The kit can process 80 cervical cell samples, and the

method for operation and use the same was as described in the examples 1 and 2.

<159>

<160> 3-2: Preparation of a kit with reagents for PCR of HPV DNA amplification

<161> As described in example 2, reagents for PCR of HPV genes were provided as a kit.

<162> The kit was comprised of: ① 20pmol/$\mu\ell$ of primer selected from SEQ.ID.NO:1 to SEQ.ID.NO:6, these are PCR primers for HPV genes, ②a 10X PCR buffer solution, ③ a mixed solution of 2.5mM dNTP (dATP, dGTP, dCTP, dUTP), ④ an 1.5ml volume tube containing 100ul of a beta-globin primer at the concentration of 3pmol/$\mu\ell$, ⑤ a premix of primers for a HPV test, wherein the premix contained uracil-N-glycosylase (1U/$\mu\ell$) 20ul and template DNA 5ul, and as a control, ⑥ a 1.5ml tube containing Taq. DNA polymerase 250 units (Solgent, South Korea),⑦ a 1.5ml tube containing a 2X hybridization solution, ⑧ a 1.5ml tube containing distilled water 1ml. The kit containing all the above-described reagents was kept in a freezer at -20℃, and the method of use was the same as described in example 2.

<163>

<164> 3-3: Preparation of a kit with a DNA chip of HPV genes

<165> With one HPV DNA chip fabricated as in example 2, it was possible to test 8 samples. A kit was composed of 10 pieces of such DNA chips. A hybridization chamber was attached to the kit, for ready use, and the method of use was the same as described in example 2. The DNA chip kit for a HPV genotype test was composed of 10 DNA chips, being capable of processing 80 cervical cell samples (Fig. 9).

<166>

<167> **Example 4: Comparison between the DNA chip method and a base sequencing method**

<168>

<169> Based on the results obtained from the DNA chip according to the

present invention, each HPV genotype was determined. For confirming the genotype results determined by the DNA chip according to the present invention, a base sequence analysis was carried out.

<170> To 5μℓ of the PCR product of each sample corresponding to the HPV genotype of the HPV DNA chip (PCR products of Example 1-1), 8μℓ of a reagent (Bigdye), 1μℓ of 5 primer of each genes and the like were added, making a mixture to have the total volume of 20μℓ. The mixture was denatured at 95℃ for 2 minutes. Sequence analysis was conducted at 95℃, 20 seconds, 50℃, 5 seconds and 60℃, 2 minutes, 25 times, and then determined the base sequence (ABI3100, Applied Biosystems). The results were shown in Figs. 10 to 22. The results obtained from sequence analysis all agreed with the HPV genotype results from the HPV DNA chip method of the present invention.

【CLAIMS】

【Claim 1】

<172> A primer for HPV (Human Papilloma Virus) amplification, comprising at least one base sequence selected from the group consisting of SEQ.ID.NO:1 to SEQ.ID.NO:6

<173>

【Claim 2】

<174> The primer for HPV amplification according to claim 1, wherein the base sequence has a fluorescent dye attached to 5' end thereof.

<175>

【Claim 3】

<176> The primer for HPV amplification according to claim 2, wherein the fluorescent dye is selected from the group consisting of Cy3, Cy5, a biotin binding material, Alexa 647, Alexa 555, 5-(2-aminoethyl)amino-1-naphthalene sulfonic acid (EDANS), tetramethyl rhodamine (TMR), tetramethyl rhodamine isocyanate (TMRITC), fluorescein isocyanate(FITC), x-rhodamine and Texas red.

<177>

【Claim 4】

<178> A probe which forms complementary binding with HPV, comprising at least one oligonucleotide selected from the group consisting of oligonucleotide having base sequences represented as SEQ.ID.NO:9 to SEQ.ID.NO:44, and oligonucleotide having base sequences complementary to said sequences.

<179>

【Claim 5】

<180> The probe which forms complementary binding with HPV according to 4, wherein the oligonucleotide corresponds to the following HPV types: HPV-6,11,16,18,31,32,33,34,35,39,40,42,43,44,45,51,52,53,54,56,58,59,61,62,66,67,68,69,70,71,72,81,82,83,84 and 90.

<181>

【Claim 6】

<182> The probe which forms complementary binding with HPV according to

4,wherein an amine group is attached to the 5' end of the base sequences represented by SEQ.ID.NO:9 to SEQ.ID.NO:44.

<183>

【Claim 7】

<184> A DNA chip for testing HPV genotypes, comprising a probe according to any one of claims 4 to 6.

<185>

【Claim 8】

<186> A kit for detecting or testing HPV genotype, comprising:

<187> a reagent for DNA extraction;

<188> a PCR primer for amplification of the extracted DNA; and

<189> a DNA chip for testing HPV genotype comprising a probe according to any one of claims 4 to 6.

<190>

【Claim 9】

<191> The kit for detecting or testing HPV genotype according to claim 8,wherein the reagent for DNA extraction comprises proteinase and chelex 7% (w/v).

<192>

【Claim 10】

<193> The kit for detecting or testing HPV genotype according to claim 8,wherein the primer for PCR comprises at least one base sequence selected from the group consisting of SEQ.ID.NO:1 to SEQ.ID.NO:6.

<194>

【Claim 11】

<195> The kit for detecting or testing HPV genotype according to claim 8,wherein the primer for PCR is one selected from: JK1 set consisting of SEQ.ID.NO:1 and SEQ.ID.NO:2 JK2 set consisting of SEQ.ID.NO:3 and SEQ.ID.NO:4; and JK3 set consistingof SEQ.ID.NO:5 and SEQ.ID.NO:6.

<196>

【Claim 12】

<197> A method for testing HPV genotype, comprising the steps of:

<198> extracting DNA from cells taken from the cervix;

<199> amplifying the DNA by PCR involving a primer for HPV amplification;

<200> hybridization of the amplified DNA to a DNA chip comprising a HPV probe which comprises at least one oligonucleotide selected from the group consisting of oligonucleotide having base sequences represented as SEQ.ID.NO:9 to SEQ.ID.NO:44 and oligonucleotide having base sequences complementary to said sequences; and

<201> determining the HPV genotype through the product specifically hybridized with the probe.

<202>

【Claim 13】

<203> The method for testing HPV genotype according to 12, further comprising, in the PCR amplification, a beta-globin primer set represented by SEQ.ID.NO:7 and SEQ.ID.NO:8, as a control.

<204>

【Claim 14】

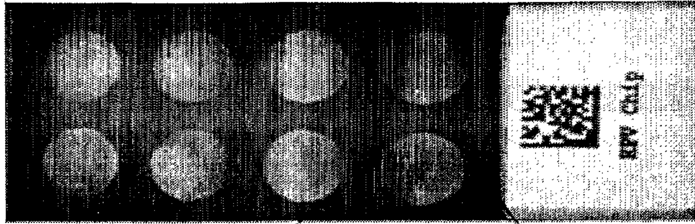
<205> The method for testing HPV genotype according to 12, wherein, in the hybridizationof the amplified DNA, an amine group is attached to the 5' end of the base sequences represented bySEQ.ID.NO:9 to SEQ.ID.NO:44.

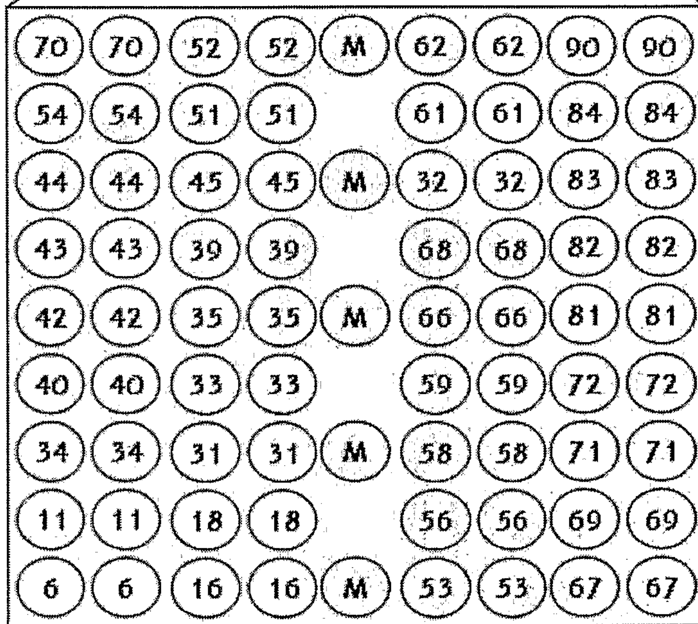
<206>

【Claim 15】

<207> The method for testing HPV genotype according to 12, wherein, in the step of determining the HPV genotype, the HPV genotype is selected from the group consisting of HPV-6,11,16,18,31,32,33,34,35,39,40,42,43,44,45,51,52,53,54,56,58,59,61,62,66,67,68,69,70,71,72,81,82,83,84,90 and mixtures thereof.

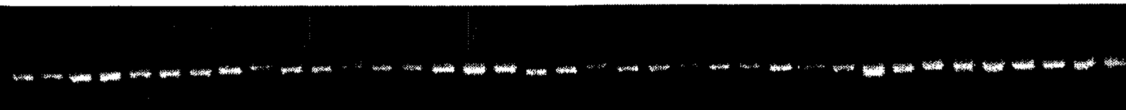
[Fig. 1]





[Fig. 2]

[Fig. 3]



[Fig. 4]

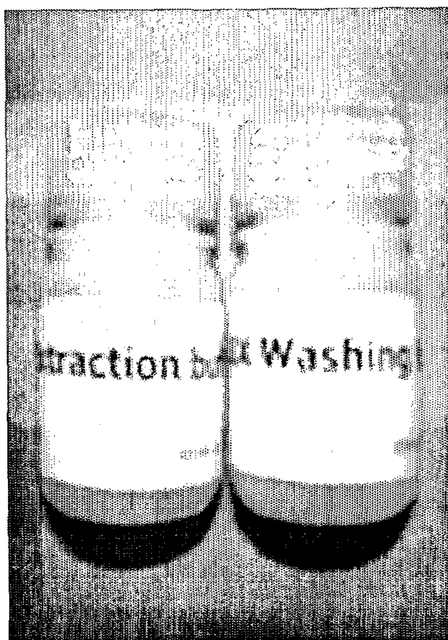
[Fig. 5]

HPV type	chip image	HPV type	chip image	HPV type	chip image	HPV type	chip image
6		34		53		67	
11		40		56		69	
54		42		68		71	
70		35		32		72	
16		39		58		81	

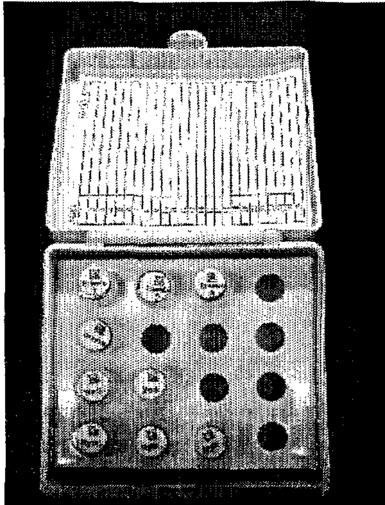
[Fig. 6]

HPV type	chip image	HPV type	chip image	HPV type	chip image	HPV type	chip image
66		45		61		82	
18		51		59		83	
31		43		62		84	
33		52		44		90	
co. 16,71		co. 56,62		co. 35,90		neg.	

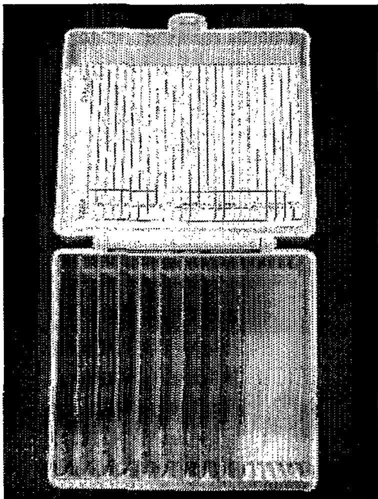
[Fig. 7]



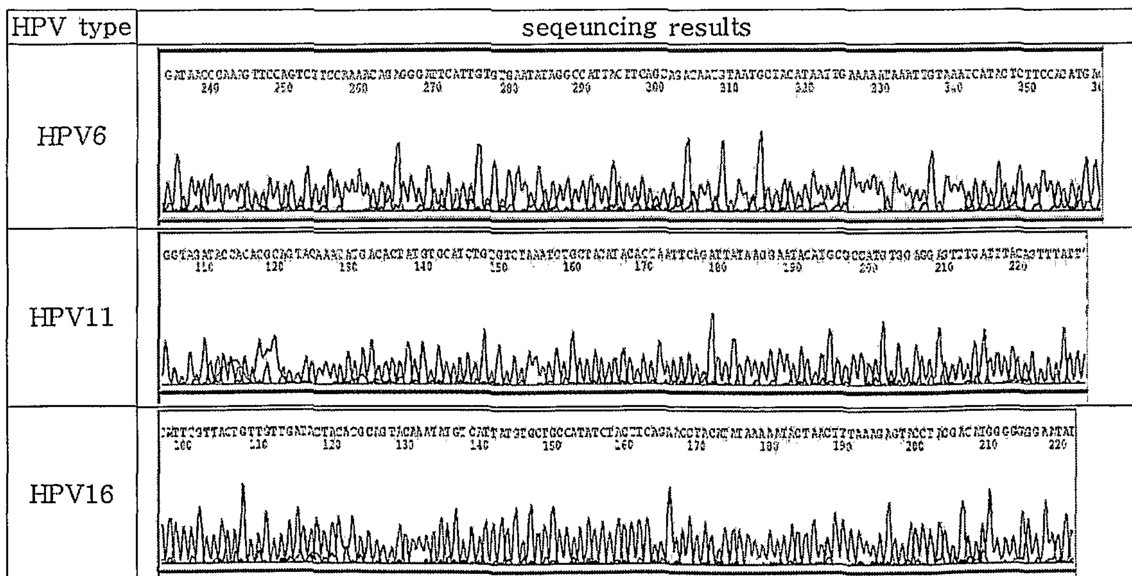
[Fig. 8]



[Fig. 9]



[Fig. 10]



HPV type	seqeuncing results
HPV6	
HPV11	
HPV16	

[Fig. 11]

HPV type	seqeuncing results
HPV18	
HPV31	
HPV32	

[Fig. 12]

HPV type	seqeuncing results
HPV33	
HPV34	
HPV35	

[Fig. 13]

HPV type	seqeuncing results
HPV39	
HPV40	
HPV42	

[Fig. 14]

HPV type	seqeuncing results
HPV43	
HPV44	
HPV45	

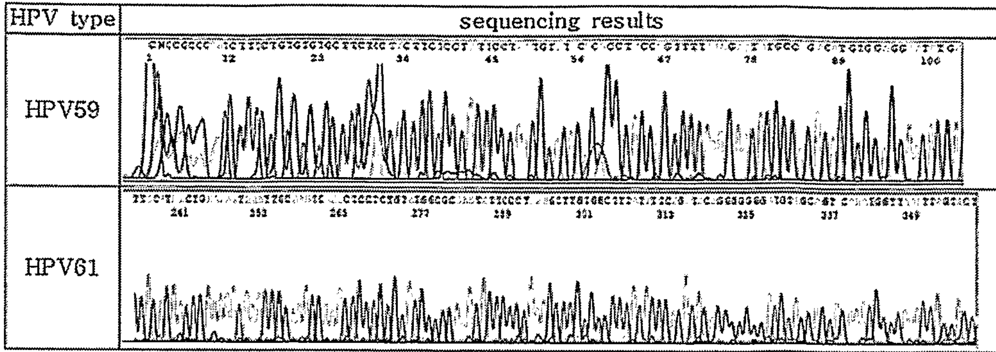
[Fig. 15]

HPV type	sequencing results
HPV51	
HPV52	
HPV53	

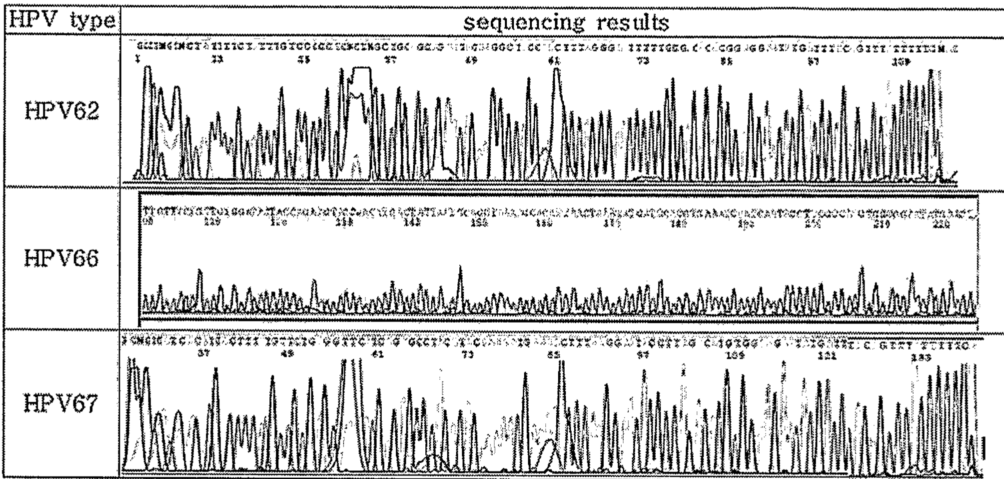
[Fig. 16]

HPV type	sequencing results
HPV54	
HPV56	
HPV58	

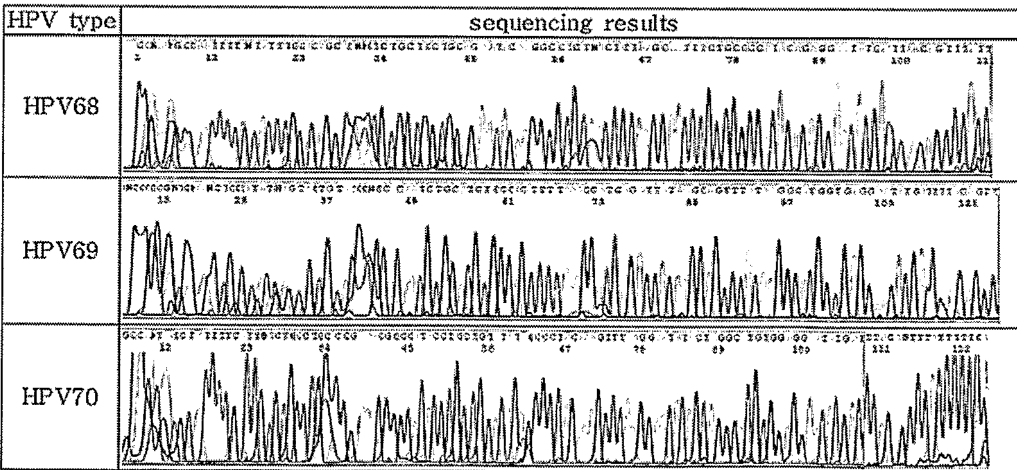
[Fig.17]



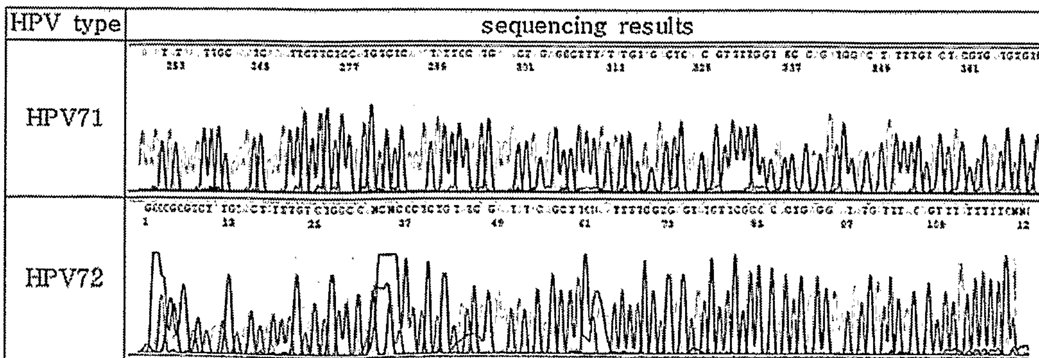
[Fig. 18]



[Fig. 19]



[Fig. 20]



[Fig. 21]

HPV type	sequencing results
HPV81	
HPV82	
HPV83	

[Fig. 22]

HPV type	sequencing results
HPV84	
HPV90	