

POLYVALENT IMMUNOGENICITY COMPOSITION FOR HUMAN PAPILLOMAVIRUS

TECHNICAL FIELD

5 [0001] The present invention relates to a multivalent immunogenic composition for preventing human papillomavirus (HPV) related diseases or infections and uses thereof.

BACKGROUND

10 [0002] Papilloma virus (PV) belongs to the *Papillomaviridae* Family and causes papillomas in humans, cattle, dogs, and rabbits. One of its member, human papilloma virus (HPV), is a non-enveloped DNA virus. The genome of this virus is a double-stranded closed circular DNA, about 7.2-8kb in size, with 8 open reading frames, which can be divided into three regions according to their
15 functions: (1) early region (E), about 4.5kb, encoding E1, E2, E4-E7, a total of 6 non-structural proteins related to viral replication, transcription and transformation; (2) late region (L), about 2.5kb, encoding the major capsid protein L1 and the minor capsid protein L2; (3) long regulatory region (LCR), which is located between the end of the L region and the beginning of the E region, is about
20 800-900 bp long and does not encode any protein, serving as DNA replication and expression regulatory elements.

[0003] The L1 proteins and the L2 proteins are synthesized late in the HPV infection cycle. The L1 protein is the major capsid protein and has a molecular weight of 55-60 kDa. The L2 protein is the minor capsid protein. 72 L1 protein
25 pentamers form the outer shell of the icosahedral HPV particle (45-55 nm in diameter), which encloses closed circular double-stranded DNA. The L2 protein

is located on the inner side of the L1 protein (Structure of Small Virus-like Particles Assembled from the L1 Protein of Human Papillomavirus 16 Chen, X.S., R. L.Garcea, Mol.Cell.5(3):557-567, 2000).

[0004] The ORF of the L1 protein, the most conserved gene in the PV genome, can be used to identify new PV types. A new PV type is identified if its complete genome is cloned and its L1 ORF DNA sequence differs from the closest known PV type by more than 10%. Homologies with differences between 2% and 10% are defined as different subtypes, and differences of less than 2% are defined as different variants of the same subtype (E.-M. de Villiers et al. / Virology 324 (2004) 17-27).

[0005] At late stages of HPV infection, newly synthesized L1 proteins in the cytoplasm are transported to the nucleus of terminally differentiated keratin where, together with L2 proteins, package the replicated HPV genomic DNA to form infectious viruses (Nelson, L.M, et al. 2002. Nuclear import strategies of high risk HPV16 L1 major capsid protein. J. Biol. Chem. 277: 23958-23964). This suggests that nuclear import of the L1 protein plays a very important role in HPV infection and production. The ability of the virus to enter the nucleus is determined by the nuclear localization signal (NLS) at the C-terminus of the HPV L1 protein, the NLS is characterized by its abundance of basic amino acids (Garcia-Bustos, J., et al. 1991. Nuclear protein localization. Biochimica et Biophysica Acta 1071: 83-101).

[0006] 15 high-risk (HR) HPV types can lead to cancers of cervix, anus, penis, vagina, vulva and oropharynx, among which HPV-16 and HPV-18 are by far the most common causes of cancers, accounting for approximately 70% of cervical cancers, and the other HR-HPV types (Types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) cause the rest. HPV-16 accounts for approximately 95% of HPV-positive oropharyngeal cancers (OPCs). The persistent low-risk genotypes

HPV-6 and HPV-11 cause most anogenital warts and respiratory papillomas, but are rarely associated with cancers (Human Papillomavirus in Cervical Cancer and Oropharyngeal Cancer: One Cause, Two Diseases Tara A. Berman and John T. Schiller, *PhD2 Cancer* 2017;123:2219-29).

5 **[0007]** The L1 protein can be recombinantly expressed by poxvirus, baculovirus, or yeast systems and then self-assembles to form virus-like particles (VLP) containing approximately 72 L1 proteins, similar to the virus capsid. VLP has no indication. VLP induces neutralizing antibodies in inoculated animals and protects experimental animals from subsequent attack by infectious viruses. Thus, 10 VLP appears to be an excellent candidate for papilloma virus vaccines (Structure of Small Virus-like Particles Assembled from the L1 Protein of Human Papillomavirus 16 Chen, X.S. , R. L. Garcea, *Mol. Cell.* 5(3):557-567, 2000).

[0008] Glaxo's CERVARIX®, a bivalent recombinant HPV vaccine, contains HPV Type 16 recombinant L1 protein and HPV Type 18 recombinant L1 protein. 15 The L1 protein is obtained by expression of a recombinant baculovirus expression vector system in insect cells of the nocturnal moth (*Trichoplusia ni*). The L1 protein self-assembles into virus-like particles for the prevention of cervical cancer, Grade 2 or 3 cervical intraepithelial neoplasia and adenocarcinoma in situ caused by HPV Types 16 and 18, and Grade 1 cervical intraepithelial neoplasia (oncogenic) in women aged 9-25 years 20 (<https://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM186981.pdf>).

[0009] GARDASIL® is a human papilloma virus quadrivalent (Types 6, 11, 16 and 18) recombinant vaccine from Merck for the prevention of cervical cancer, 25 genital warts (condyloma acuminata) and precancerous or proliferative abnormal lesions caused by HPV Types 6, 11, 16 and 18 in girls and women aged 9-26 years; and for the prevention of anal cancer, genital warts (condyloma

acuminatum) and pre-cancerous or abnormal developmental lesions caused by HPV Types 6, 11, 16, and 18 in boys and men aged 9-26 (<https://www.fda.gov/vaccines-blood-biologics/vaccines/gardasil>).

[0010] GARDASIL®9 is a nine-valent recombinant human papilloma virus vaccine from Merck that contains virus-like particles of L1 proteins of HPV Types 6, 11, 16, 18, 31, 33, 45, 52 and 58, the L1 protein is produced by fermentation of *Saccharomyces cerevisiae* and self-assembles into VLP. It is used in girls and women aged 9-45 years for the prevention of cervical cancer, vulvar cancer, vaginal cancer and anal cancer caused by HPV Types 16, 18, 31, 33, 45, 52 and 58, genital warts (condyloma acuminata) caused by HPV Types 6 and 11, and precancerous or proliferative abnormalities caused by HPV Types 6, 11, 16, 18, 31, 33, 45, 52 and 58; and in boys and men aged 9-45 years for the prevention of anal cancer caused by HPV Types 16, 18, 31, 33, 45, 52 and 58, genital warts (condyloma acuminatum) caused by HPV Types 6 and 11 and pre-cancerous or developmentally abnormal lesions caused by HPV Types 6, 11, 16, 18, 31, 33, 45, 52 and 58 (<https://www.fda.gov/vaccines-blood-biologics/vaccines/gardasil-9>).

[0011] The instruction for GARDASIL®9 announced that HPV Types 16 and 18 are the cause of about 70% of cervical cancers, with the remaining 20% of cases attributed to Types 31, 33, 45, 52 and 58, therefor GARDASIL®9 prevents 90% of cervical cancers (<https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm426445.htm>).

[0012] Industrial production of virus-like particles is critical to HPV vaccine development. The common systems for producing virus-like particles are mainly classified into the eukaryotic expression system and the prokaryotic expression system.

[0013] Commonly used eukaryotic expression systems include poxvirus expression systems, insect baculovirus expression systems, and yeast expression systems. The HPV L1 protein expressed in eukaryotic expression systems can be spontaneously assembled to virus-like particles as its natural conformation is less disrupted, but the yield thereof is low. The HPV L1 protein expressed in prokaryotic expression systems, mainly E. coli expression systems, is of high yields but mostly in the form of inclusion bodies, this form of protein cannot be easily purified thus makes the production process complicated.

[0014] Therefore, there is still a need to obtain high yields of HPV virus-like particles, so as to obtain HPV multivalent vaccines for broad-spectrum prevention of HPV-related diseases or infections, including those caused by the HPV types that are not currently covered by commercial available vaccines.

SUMMARY

[0015] In one aspect, the present invention provides a multivalent HPV immunogenic composition for the prevention of HPV-related disease or infection comprising: HPV virus-like particles assembled from L1 proteins of HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58; and one or more HPV virus-like particles assembled from L1 proteins of other pathogenic HPV types virus-like particles.

[0016] In another aspect, the present invention provides a method for preventing HPV-related disease or infection comprising: administering to a subject a multivalent HPV immunogenic composition.

[0017] In another aspect, the present invention provides the use of multivalent HPV immunogenic compositions in the preparation of vaccines or drugs for the prevention of HPV-related diseases or infections.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] **Figure 1A:** Expression of L1 protein of HPV 6 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0019] **Figure 1B:** Expression of L1 protein of HPV 11 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0020] **Figure 1C:** Expression of L1 protein of HPV 16 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0021] **Figure 1D:** Expression of L1 protein of HPV 18 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0022] **Figure 1E:** Expression of L1 protein of HPV 31 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0023] **Figure 1F:** Expression of L1 protein of HPV 33 L1. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0024] **Figure 1G:** Expression of L1 protein of HPV 35 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0025] **Figure 1H:** Expression of L1 protein of HPV 39 L1: 59C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0026] **Figure 1I:** Expression of L1 protein of HPV 45 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0027] **Figure 1J:** Expression of L1 protein of HPV 51 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0028] **Figure 1K:** Expression of L1 protein of HPV 52 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0029] **Figure 1L:** Expression of L1 protein of HPV 56 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0030] Figure 1M: Expression of L1 protein of HPV 58 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0031] Figure 1N: Expression of L1 protein of HPV 59 L1. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0032] Figure 2A: Transmission electron microscopy of HPV 6 L1: 33C virus-like particles.

[0033] Figure 2B: Transmission electron microscopy of HPV 11 L1: 33C virus-like particles.

[0034] Figure 2C: Transmission electron microscopy of HPV 16 L1: 33C virus-like particles.

[0035] Figure 2D: Transmission electron microscopy of HPV 18 L1: 33C virus-like particles.

[0036] Figure 2E: Transmission electron microscopy of HPV 31 L1: 33C virus-like particles.

[0037] Figure 2F: Transmission electron microscopy of HPV 33 L1 virus-like particles.

[0038] Figure 2G: Transmission electron microscopy of HPV 35 L1: 33C virus-like particles.

[0039] Figure 2H: Transmission electron microscopy of HPV 39 L1: 59C virus-like particles.

[0040] Figure 2I: Transmission electron microscopy of HPV 45 L1: 33C virus-like particles.

[0041] Figure 2J: Transmission electron microscopy of HPV 51 L1: 33C virus-like particles.

[0042] Figure 2K: Transmission electron microscopy of HPV 52 L1: 33C virus-like particles.

[0043] Figure 2L: Transmission electron microscopy of HPV 56 L1: 33C virus-like particles.

[0044] Figure 2M: Transmission electron microscopy of HPV 58 L1: 33C virus-like particles.

[0045] Figure 2N: Transmission electron microscopy of HPV 59 L1 virus-like particles.

[0046] Figure 3: The pseudovirus neutralizing titers in mice immunized with compositions of 14 HPV types of virus-like particles. Number of mice, N=10. GMT: Geometric Mean Titer.

[0047] Figure 4: Expression of C-terminal truncated HPV 16L1 (1-474). M: Marker; L: cell lysates; E-S: supernatant collected after centrifugation of the lysates.

DETAILED DESCRIPTION

[0048] In one aspect, the present invention provides a multivalent immunogenic composition for preventing papillomavirus-related diseases or infections. In one embodiment, the papillomavirus may be human papillomavirus. In another embodiment, the papillomavirus may be canine papillomavirus or rabbit papillomavirus.

[0049] In one aspect, the present invention provides a multivalent HPV immunogenic composition for preventing HPV-related diseases or infections, comprising: HPV virus-like particles assembled from L1 proteins of HPV Types 6, 11, 16, 18, 31, 33, 45, 52, and 58; and one or more HPV virus-like particles assembled from L1 proteins of other pathogenic HPV types.

[0050] In one embodiment, said L1 protein of each HPV type can be a naturally occurring L1 protein, or a non-naturally occurring L1 protein, or a chimeric HPV

L1 protein. In one embodiment, said HPV virus-like particles can be assembled from a single type of HPV L1 protein to form monovalent HPV virus-like particles, or can be assembled from multiple types of HPV L1 proteins to form multivalent HPV virus-like particles.

5 **[0051]** In one embodiment, said one or more other pathogenic HPV types are selected from HPV Types 35, 39, 51, 56, and 59.

[0052] In one embodiment, at least one of said HPV virus-like particle is a chimeric HPV virus-like particle, said chimeric HPV virus-like particle comprises one or more chimeric HPV L1 proteins; said chimeric HPV L1 protein comprises, from its N-terminus to C-terminus orientation: a. an N-terminal fragment derived from L1 protein of the first papilloma virus type, wherein said L1 protein of the first papilloma virus type is selected from HPV Types 6, 11, 16, 18, 31, 35, 39, 45, 51, 52, 56, or 58, said N-terminal fragment maintains the immunogenicity of the L1 protein of the corresponding HPV type; and b. a C-terminal fragment derived from the L1 protein of the second papilloma virus type, said L1 protein of the second papilloma virus type has a better expression level and solubility compared to L1 proteins of other types; wherein the chimeric L1 protein of HPV Types 6, 11, 16, 18, 31, 35, 39, 45, 51, 52, 56, or 58 has the immunogenicity of the L1 protein of the corresponding HPV type.

20 **[0053]** In one embodiment, said chimeric HPV virus-like particle may be assembled from a single type of chimeric HPV L1 proteins to form monovalent HPV virus-like particles, or may be assembled from multiple types of chimeric HPV L1 proteins to form multivalent HPV virus-like particles.

[0054] In one embodiment, said C-terminal fragment and N-terminal fragment may be freely combined as needed. In one embodiment, the chimeric HPV L1 protein may comprise one or plurality of C-terminal fragments. Said plurality of C-terminal fragments may be the same or different.

[0055] In one embodiment, said N-terminal fragment is a fragment obtained by truncating the C-terminus of the natural sequence of said L1 protein of the first papilloma virus type at any amino acid position within its $\alpha 5$ region, and a fragment having at least 98% identity therewith; said C-terminal fragment is a fragment obtained by truncating the N-terminus of the natural sequence of the L1 protein of the second papilloma virus type at any amino acid position within its $\alpha 5$ region and functional variants resulting from further mutations, deletions and/or additions to the fragment.

[0056] In another embodiment, said N-terminal fragment has at least 98.5%, 99%, 99.5% or 100% identity to a fragment obtained by truncating the C-terminus of the natural sequence of said L1 protein of the first papilloma virus type at any amino acid position within its $\alpha 5$ region.

[0057] In one embodiment, said C-terminal fragment comprises one or plurality of nuclear localization sequences.

[0058] In one embodiment, said L1 protein of the second papilloma virus type is selected from L1 proteins of HPV Types 1, 2, 3, 4, 6, 7, 10, 11, 13, 16, 18, 22, 26, 28, 31, 32, 33, 35, 39, 42, 44, 45, 51, 52, 53, 56, 58, 59, 60, 63, 66, 68, 73 or 82.

[0059] Preferably, said L1 protein of the second papilloma virus type is selected from L1 proteins of HPV Types 16, 28, 33, 59, or 68.

[0060] More preferably, said L1 protein of the second papilloma virus type is selected from L1 protein of HPV Type 33 or HPV Type 59.

[0061] In one embodiment, said L1 protein of the second papilloma virus type is an HPV Type 33 L1 protein, said C-terminal fragment is SEQ ID No: 2; or a fragment having a length of m1 amino acids, preferably a fragment covering amino acids 1-m1 position of SEQ ID No: 2; wherein m1 is an integer of 8-26; or said C-terminal fragment is SEQ ID No: 135; or a fragment having a length of

m2 amino acids, preferably a fragment covering amino acids 1-m2 position of SEQ ID No: 135; wherein m2 is an integer of 13-31.

[0062] In one embodiment, the C-terminal fragment of the HPV Type 33 L1 protein has a nuclear localization sequence. In another embodiment, the C-terminal fragment of the HPV Type 33 L1 protein has two nuclear localization sequences. In one embodiment, the amino acid sequences with amino acid No: 7-8 (KR) and the amino acid sequences amino with amino acid No: 20-23 (KRKK) of SEQ ID No: 2 are nuclear localization sequences of the C-terminal fragments of the HPV Type 33 L1 protein.

[0063] In one embodiment, said L1 protein of the second papilloma virus type is the HPV Type 59 L1 protein, said C-terminal fragment is SEQ ID No: 13; or a fragment having a length of n amino acids, preferably covering amino acids 1-n position of SEQ ID No: 13; wherein n is an integer of 16-38.

[0064] In one embodiment, the C-terminal fragment of the HPV Type 59 L1 protein has a nuclear localization sequence. In another embodiment, the C-terminal fragment of the HPV Type 59 L1 protein has two nuclear localization sequences. In some embodiments, the chimeric HPV L1 protein comprises one or plurality of C-terminal fragments of the HPV Type 59 L1 protein. Said plurality of C-terminal fragments of the HPV Type 59 L1 protein may be the same or different. In one embodiment, the amino acid sequence (RKR) of the 14th to 16th position of SEQ ID No: 13 and the amino acid sequence (KRVKRRK) of the 28th to 34th position of SEQ ID No: 13 are the nuclear localization sequences of the C-terminal fragment of the HPV Type 59 L1 protein.

[0065] In one embodiment, said chimeric HPV L1 protein comprises both a C-terminal fragment of the HPV Type 33 L1 protein and a C-terminal fragment of the HPV Type 59 L1 protein.

[0066] In one embodiment, said N-terminal fragment of said HPV Type 6 L1 protein has 98%, 98.5%, 99%, 99.5%, 99%, or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 1 at any amino acid site within its alpha 5 region.

5 **[0067]** Said N-terminal fragment of said HPV Type 11 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 14 at any amino acid site within its alpha 5 region.

10 **[0068]** Said N-terminal fragment of said HPV Type 16 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 27 at any amino acid site within its alpha 5 region.

15 **[0069]** Said N-terminal fragment of said HPV Type 18 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 40 at any amino acid site within its alpha 5 region.

20 **[0070]** Said N-terminal fragment of said HPV Type 31 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 53 at any amino acid site within its alpha 5 region.

[0071] Said N-terminal fragment of said HPV Type 35 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 69 at any amino acid site within its alpha 5 region.

25 **[0072]** Said N-terminal fragment of said HPV Type 39 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating

the C-terminus of the sequence shown in SEQ ID No: 82 at any amino acid site within its alpha 5 region.

[0073] Said N-terminal fragment of said HPV Type 45 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 95 at any amino acid site within its alpha 5 region.

[0074] Said N-terminal fragment of said HPV Type 51 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 108 at any amino acid site within its alpha 5 region.

[0075] Said N-terminal fragment of said HPV Type 52 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 121 at any amino acid site within its alpha 5 region.

[0076] Said N-terminal fragment of said HPV Type 56 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 134 at any amino acid site within its alpha 5 region; and

[0077] Said N-terminal fragment of said HPV Type 58 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 147 at any amino acid site within its alpha 5 region.

[0078] In one embodiment, the C-terminus of said N-terminal fragment is connected to the N-terminus of said C-terminal fragment directly or via a linker.

[0079] The linker does not affect the immunogenicity of said N-terminal fragment and does not affect the expression level or solubility of the protein. In one embodiment, said N-terminal fragment and said C-terminal fragment are

connected via a linker comprising 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids. In one embodiment, the linker is an artificial sequence. In another embodiment, the linker is a naturally occurring sequence in the HPV L1 protein. In another embodiment, the linker may be a partial sequence of the HPV Type 33 L1 protein. In another embodiment, the linker may be a partial sequence of the HPV Type 59 L1 protein.

[0080] In one embodiment, within the range of plus or minus 4 amino acid positions of the connection point when the C-terminus of said N-terminal fragment is connected to the N-terminus of said C-terminal fragment, there presents the following continuous amino acid sequence: RKFL; preferably, within the range of plus or minus 6 amino acid positions of the connection point, there presents the following continuous amino acid sequence: LGRKFL.

[0081] In some embodiments, said chimeric HPV L1 proteins of chimeric HPV Types 6, 11, 16, 18, 31, 35, 39, 45, 51, 52, 56, and 58 have 98%, 98.5%, 99%, 99.5% or 100% identity to SEQ ID No: 3, SEQ ID No: 16, SEQ ID No: 29, SEQ ID No: 42, SEQ ID No: 55, SEQ ID No: 71, SEQ ID No: 84, SEQ ID No: 97, SEQ ID No: 110, SEQ ID No: 123, SEQ ID No: 136 and SEQ ID No: 149, respectively; and the HPV Type 33 L1 protein and the HPV Type 59 L1 protein have 98%, 98.5%, 99%, 99.5%, or 100% identity to SEQ ID No: 66 and SEQ ID No: 160, respectively.

[0082] In one embodiment, at least one of said HPV virus-like particles is composed of a single type of chimeric HPV L1 protein, preferably is composed of said single type of chimeric HPV L1 protein having the same amino acid sequence.

[0083] In one embodiment, the chimeric HPV virus-like particle is the icosahedron comprising 72 pentamers of said chimeric HPV L1 protein. In one embodiment, the HPV virus-like particle has correctly formed disulfide bonds

and thus has a good natural conformation. In one embodiment, the chimeric HPV virus-like particles self-assemble in an *in vivo* expression system.

[0084] In one embodiment, said multivalent HPV immunogenic composition further comprises a physiologically acceptable carrier and, optionally, an adjuvant. In one embodiment, the adjuvant comprises one or more of aluminum salt, lipid A derivative, and ISCOM.

[0085] In one embodiment, said adjuvant is an aluminum phosphate adjuvant.

[0086] In one aspect, the present invention provides a method of preventing HPV-related diseases or infections, comprising: administering to a subject a multivalent HPV immunogenic composition. Said prevention may be considered as a treatment and they can be used interchangeably. In one embodiment, the subject is a human.

[0087] In one aspect, the present invention provides the use of the aforementioned multivalent HPV immunogenic compositions in the preparation of vaccines or drugs for preventing HPV-related diseases or infections.

[0088] Papillomavirus L1 proteins expressed by eukaryotic expression systems can spontaneously assemble to virus-like particles, but the low expression level make them not suitable for mass production.

[0089] The sequences of the L1 proteins of each HPV type can be easily obtained from <https://www.uniprot.org>. For a given HPV type, the L1 protein can be derived from different strains, thus the amino acid sequence thereof may have multiple versions, any version of the natural sequence can be used in the present invention. It is possible that the sequence of the HPV L1 protein of a given type used during the conception and design of the present invention may differ from the sequence used in the following examples, but such differences do not affect the decisions and conclusions of the inventors.

[0090] It is generally accepted by those of skill in the art that the C-terminus of the L1 protein does not contain major neutralizing antigenic epitope and therefore attempts have been made to increase expression by truncating the C-terminus of the HPV L1 protein, for example Glaxo's US patent US6361778B1, in which the C-terminus of the HPV16 L1 protein is truncated by 1-34 amino acids, preferably 26 amino acids, states that the yield of VLP is increased by many-fold, preferably at least by 10 folds, and in particular by about 10 to 100 folds. Inspired by this, the inventors attempted to truncate the C-terminus of HPV 16 L1 protein by 31 amino acids and named the truncated protein as HPV 16 L1 (1-474). The protein is in high expression level but poor solubility, and is difficult to be extract and purified (see Comparative Example).

[0091] The poor solubility of the protein due to this truncation may be caused by the deletion of the nuclear localization sequence located at the C-terminus, but the present invention is not binding to this speculation. During research and production, the inventor discovered that HPV Type 16 L1 protein, HPV Type 28 L1 protein, HPV Type 33 L1 protein, HPV Type 59 L1 protein and HPV Type 68 L1 protein have better expression levels and solubility than other HPV types L1 proteins. Inspired by the discovery, the inventors replaced the C-terminus of specific HPV types L1 proteins that are less extractable or less soluble with the C-terminus of specific HPV types L1 protein having better expression levels and solubility. That is, the inventors have constructed a chimeric protein: comprising, in the N-terminus to C-terminus orientation, an N-terminal fragment derived from the L1 protein of the first papilloma virus type (e.g. HPV L1 protein), which provides the immunogenicity of the first papilloma virus type (e.g. HPV), and a C-terminal fragment derived from the L1 protein of the second papilloma virus type (e.g. HPV L1 protein), which provides the features of better expression levels and solubility. These two fragments can be connected directly or via a linker.

[0092] The length of the N-terminal fragment of the HPV L1 protein appropriate for maintaining the immunogenicity of the The L1 protein of the first type and ensuring the formation of VLP is determined. The following reports relate to epitope studies of common HPV types.

5 **[0093]** Sunanda Baidya et al. reported that the epitopes of the L1 protein 48EEYDLQFIFQLCKITLTA65, 45RHGEEYDLQFIFQLCKITLTA65, 63LPDPNKF69, 79PETQRLVWAC88, 36PVPGQYDA43, 77YNPETQRLVWAC88, 188DTGYGAMD195, 36PVPGQYDATK45, 45KQDIPKVSAYQYRVFRV61, 130RDNVSVDYKQTQLCI144 and 49YSRHHVEEYDLQFIF62 can be used as tools for designing HPV 16 and 18 vaccines (see Epitope design of L1 protein for vaccine production against Human Papilloma Virus types 16 and 18, Bioinformation 13(3): 86-93 March 2017, incorporated herein by reference in its entirety).

15 **[0094]** Katharina Slupetzky et al. reported that the regions near aa 282-286 and 351-355 of HPV-16 contribute to the neutralization epitopes and that the latter is the immunodominant site (see Chimeric papilloma virus-like particles expressing a foreign epitope on capsid surface loops, Journal of General Virology (2001), 82, 2799-2804, incorporated herein by reference in its entirety).

20 **[0095]** Brooke Bishop et al. prepared the following three variants of the HPV 11, 16, 18 and 35 L1 proteins: deletion of 9 amino acids at its N terminus, deletion of $\alpha 4$ (corresponding to amino acid residues 404-436 of HPV 16), and deletion of 31 amino acids at its C terminus respectively, and reported that the former two could not be assembled into VLP, but this phenomenon has not been reported in the latter one (Crystal Structures of Four Types of Human Papillomavirus L1 Capsid Proteins UNDERSTANDING THE SPECIFICITY OF NEUTRALIZING MONOCLONAL ANTIBODIES, The Journal of Biological Chemistry, 282, 31803-31811. incorporated herein by reference in its entirety).

Each the α -helix, β -fold sheets and Loop Region of each type of HPV L1 protein can be conveniently determined by sequence analysis software commonly used in the field. Wherein the α -helix regions contains the $\alpha 1$ Region, $\alpha 2$ Region, $\alpha 3$ Region, $\alpha 4$ Region and $\alpha 5$ Region.

		β -B1	β -B2	BC-loop	β -C		
HPV16-L1	mslWlpseaTVYlPP-vpVskVVsTDeYVarThIyYHAgTSRLLaVGhPYfpikpnnhKilvPKVsglQYRVFfrihLPDPNKFgfPDTS					89	
HPV35-L1	mslWrsneaTVYlPP-vsVskVVsTDeYVtrTnIyYHAgSSRLLaVGhPYaikkqdsnKiavPKVsglQYRVFrvkLPDPNKFgfPDTS					89	
HPV11-L1	--mWrpsdsTVYvPPpnpVskVVaTdaYVkrTnIfYHAssSRLLaVGhPYysikkvn--KtvyPKVsgyQYRVFkvvLPDPNKFalPDSS					86	
HPV18-L1	malWrpsdnTVYlPP-psVarVvNTDdYVtpTsIfYHAgSSRLLTVGnPYfrvpaggggKqdiPKVSayQYRVFrvqLPDPNKFglPDTS					89	
		β -D	DE-loop	β -E	EF-		
HPV16-L1	fynPdtQRLVWAcVgEvGRGQPLGVGisGHPllNKlDDtEnasayaaNagvDnRecismDYKQTQLCligCkPpiGEHWgKGspCtnva					179	
HPV35-L1	fydPasQRLVWAcTgEvGRGQPLGVGisGHPllNKlDDtEnsnkyvgNsgtDnRecismDYKQTQLCligCkPpiGEHWgKGtpCnanq					179	
HPV11-L1	lfdPttQRLVWAcTGlEvGRGQPLGVGisGHPllNKyDDvEnsggyggNpggDnRvnvgmDYKQTQLCmvGCaPpiGEHWgKGtqCsnts					176	
HPV18-L1	iynPetQRLVWAcGgEiGRGQPLGVGisGHPfyNKlDDtEsshaatsNvseDvRdnvsvDYKQTQLCligCaPaiGEHWaKGtaCksrp					179	
		loop	$\alpha 1$	β -F	FG-		
HPV16-L1	vnpGdCPPLELIntViQDGMVDTFGAMdFttLQaNKseVPLDICTsiCKYPDYIkMvsePYGDSlFFyLRrEqmFvRHlENRAGavGe					269	
HPV35-L1	vkaGeCPPLELIntViQDGMVDTFGAMdFttLQaNKsdVPLDICssiCKYPDYIkMvsePYGDmlFFyLRrEqmFvRHlENRAGtvGe					269	
HPV11-L1	vqnGdCPPLELIntViQDGMVDTFGAMnFadLQTNKsdVPLDICgtvCKYPDYLqMaadPYGDr1FFyLRrEqmFaRHfENRAGtvGe					266	
HPV18-L1	lsqGdCPPLELkntVleDGMVDTFYGAMdFstLQdTKeVPLDICssiCKYPDYLqMsadPYGDSmFFcLRrEqLfaRHfwnNRAGtmGd					269	
		loop	G1	G2	β -H1	β -H2	HI-loop
HPV16-L1	nVPddLyikGsgstanlaSsnYfptPSGSmVtSdaQiFNKPYWLqraQGHNNgiCWgNgLFVTVVDDTrSTNmslCaaiatse-ttYknt						358
HPV35-L1	tVPadLyikG--ttgtlpStsYfptPSGSmVtSdaQiFNKPYWLqraQGHNNgiCWSNgLFVTVVDDTrSTNmsvCavsssd-stYknd						356
HPV11-L1	pVPddLlvKGgnnrssvaSsiYvhtPSGSlVsSeaQlFNKPYWLqkaQGHNNgiCWgNhLFVTVVDDTrSTNmtlCasvsksg--atYtns						354
HPV18-L1	tVPqsLyikGtgmpaspgScvYspsPSGsiVtSdsQlFNKPYWLhkaQGHNNgvCWhNgLFVTVVDDTtpSTNltiCastqspvPgqYdat						359
		β -I	$\alpha 2$	$\alpha 3$	$\alpha 4$		
HPV16-L1	nfKeYlRHgEEYDLQFIFQLCKITLAdVMtYIHsMNstiLEDWNFGlqPPPsgtLeDTYRfVtSqAiaCQKhptpapakDPlkkytFwe					448	
HPV35-L1	nfKeYlRHgEEYDLQFIFQLCKITLAdVMtYIHsMNpsiLEDWNFGltPPPsgtLeDTYRyVtSqAvtCQKpsapakDPlknytfwe					446	
HPV11-L1	dyKeYmRHVEEFDLQFIFQLCSITLSAeVMaYIHTMNpsvLEDWNFGlsPPPngtLeDTYRyVqSqAitCQKptpekeqDpykdmsFwe					444	
HPV18-L1	kfKqYsRHVEEFDLQFIFQLCTITLAdVMsYIHsMNssiLEDWNFGvpPPpttsLvDTYRfVqSvAitCQKdaapaenkDPydklkFwn					449	
		β -J	$\alpha 5$				
HPV16-L1	VnLKEKFSadLDQfPLGRKFLlQaGlkakpkftlgkr-katpttsststtakRkkrkl					505	
HPV35-L1	VdLKEKFSadLDQfPLGRKFLlQaGlkarpnfrlgkr-aapastksksstkrRkvks					502	
HPV11-L1	VnLKEKFSsleLDQfPLGRKFLlQsGyrgtrtsartgik-rpavskpstapkrkRtktkk					501	
HPV18-L1	VdLKEKFSldLDQyPLGRKFLvQaGlrrkptigprkrsapsattsskprkrvrvrark					507	

[0096] The inventors performed sequence alignments of L1 proteins of 14 HPV types (Types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and then performed secondary structure prediction according to the literature cited above (Crystal Structures of Four Types of Human Papillomavirus L1 Capsid Proteins UNDERSTANDING THE SPECIFICITY OF NEUTRALIZING MONOCLONAL ANTIBODIES, The Journal of Biological Chemistry, 282,31803-31811), the results of which are shown below, where the part between

the downward-pointing arrows corresponds to the regions which had been deleted for preparing the variants in that literature.

	β-B1 β-B2 BC-	
HPV6-L1	--MWRPSDSTVYVPPPNPVSKVVATDAYVTRTNIFYHASSRLLAVGHYPYFSIKRA----	54
HPV11-L1	--MWRPSDSTVYVPPPNPVSKVVATDAYVTRTNIFYHASSRLLAVGHYPYYSIKKV----	54
HPV52-L1	MSVWRPSEATVYLP-PVPVSKVVSTDEYVSRTSIYYAGSSRLLTVGHYPYFSIKNTSSGN	59
HPV58-L1	MSVWRPSEATVYLP-PVPVSKVVSTDEYVSRTSIYYAGSSRLLAVGNPYFSIKSP--NN	57
HPV33-L1	MSVWRPSEATVYLP-PVPVSKVVSTDEYVSRTSIYYAGSSRLLAVGHYPYFSIKNP--TN	57
HPV31-L1	MSLWRPSEATVYLP-PVPVSKVVSTDEYVTRTNIYYHAGSARLLTVGHYPYYSIPKS--DN	57
HPV16-L1	MSLWLPSEATVYLP-PVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGHYPYFPIKKP--NN	57
HPV35-L1	MSLWRSNEATVYLP-PVSVSKVVSTDEYVTRTNIYYHAGSSRLLAVGHYPYFAIKKQ--DS	57
HPV51-L1	MALWRTNDSKVYLP-PAPVSRIVNTEEYITRTGIYYAGSSRLITLGHYPYFPIPK----T	55
HPV56-L1	MATWRPSENKVYLP-PTPVSKVVATDSYVKRTSIFYHAGSSRLLAVGHYPYYSVTK----D	55
HPV39-L1	MAMWRSSDSMVYLP-PPSVAKVVNTDDYVTRTGIYYAGSSRLLTVGHYPYFKV--GM--NG	56
HPV59-L1	MALWRSSDNKVYLP-PPSVAKVVSTDEYVTRTSIFYHAGSSRLLTVGHYPYFKV--PK--GG	56
HPV18-L1	MALWRPSDNTVYLP-PPSVARVVNTDDYVTRTSIFYHAGSSRLLTVGNPYFRV--PA--GG	56
HPV45-L1	MALWRPSDSTVYLP-PPSVARVVNTDDYVSRTSIFYHAGSSRLLTVGNPYFRVPS--GA	57
	loop β-C β-D	
HPV6-L1	-NKTVPKVSQYQYRVFKVLPDPNKFALPDSSLFDPTTQRLVWACTGLEVGRGQPLGVG	113
HPV11-L1	-NKTVPKVSQYQYRVFKVLPDPNKFALPDSSLFDPTTQRLVWACTGLEVGRGQPLGVG	113
HPV52-L1	GKKVLVPKVSGLQYRVFRIKLDPNKFGLPDDTSFYNPDTQRLVWACTGLEIGRGQPLGVG	119
HPV58-L1	NKKVLVPKVSGLQYRVFVRVRLPDPNKFGLPDDTSFYNPDTQRLVWACVGLEIGRGQPLGVG	117
HPV33-L1	AKKLLVPKVSGLQYRVFVRVRLPDPNKFGLPDDTSFYNPDTQRLVWACVGLEIGRGQPLGVG	117
HPV31-L1	PKKIVVPKVSGLQYRVFVRVRLPDPNKFGLPDDTSFYNPDTQRLVWACVGLEVGRGQPLGVG	117
HPV16-L1	-NKILVPKVSGLQYRVFRIHLPDPNKFGLPDDTSFYNPDTQRLVWACVGVEVGRGQPLGVG	116
HPV35-L1	-NKIAYPKVSGLQYRVFVKLPDPNKFGLPDDTSFYDPAQRLVWACTGVEVGRGQPLGVG	116
HPV51-L1	STRAAIPKVSAYQYRVFRVQLPDPNKFGLPDPNLYNPDTQRLVWGCVGVEVGRGQPLGVG	115
HPV56-L1	NTKTNIPKVSAYQYRVFRVRLPDPNKFGLPDTNIYNPDQERLVWACVGLEVGRGQPLGAG	115
HPV39-L1	GRKQDIPKVSAYQYRVFRVTLDPDNKFSIPDASLYNPETQRLVWACVGVEVGRGQPLGVG	116
HPV59-L1	NGRQDVPKVSAYQYRVFRVRLPDPNKFGLPDPNTVYDPSQRLVWACVGVEIGRGQPLGVG	116
HPV18-L1	GNKQDIPKVSAYQYRVFRVQLPDPNKFGLPDTSIYNPETQRLVWACAGVEIGRGQPLGVG	116
HPV45-L1	GNKQAVPKVSAYQYRVFRVALPDPNKFGLPDTSIYNPETQRLVWACVMEIGRGQPLGIG	117
	DE-loop β-E EF-	
HPV6-L1	VSGHPFLNKYDDVENS-GSGNPGQDNRNVNMGMDYKQTLQCMVGCAPPLGEHWGKGKQCT	172
HPV11-L1	VSGHPLLNKYDDVENS-GGNGNPGQDNRNVNMGMDYKQTLQCMVGCAPPLGEHWGKGKQCS	173
HPV52-L1	ISGHPLLNFDDTETSNTKYAGKPGIDNRECLSMYKQTLQCLIGCKPPIGEHWGKGTPCN	179
HPV58-L1	VSGHPYLNKFDDETSTNRYPAQPGSDNRECLSMYKQTLQCLIGCKPPTGEHWGKGVAACN	177
HPV33-L1	ISGHPLLNFDDTETGNKYPGQPGADNRECLSMYKQTLQCLIGCKPPTGEHWGKGVACT	177
HPV31-L1	ISGHPLLNFDDTENSNTKYAGGPGTDNRECLSMYKQTLQCLIGCKPPIGEHWGKGSPCS	177
HPV16-L1	ISGHPLLNLDDTENASAYANAGVDNRECLSMYKQTLQCLIGCKPPIGEHWGKGSPCT	176
HPV35-L1	ISGHPLLNLDDTENSNTKYVGNSTDNRECLSMYKQTLQCLIGCRPPIGEHWGKGTPCN	176
HPV51-L1	LSGHPLFNKYDDTENSRIANGNAQDVRDNTSVDNKQTLQCLIGCAPPIGEHWGIGTTCK	175
HPV56-L1	LSGHPLFNRLDDTESSNLANNVIEDSRDNISVDGKQTLQCLIVGCTPAMGEHWTKGAVCK	175
HPV39-L1	ISGHPLYNRQDDTENSPPS-STTNKDSRDNVSDYKQTLQCLIGCVPAIGEHWGKGKACK	175
HPV59-L1	LSGHPLYNLDDTENSHVASAVDTKDRDNVSVDYKQTLQCLIGCVPAIGEHWTKGTACK	176
HPV18-L1	LSGHPPFYNLDDTESSHAATSNVSEDRDNVSDYKQTLQCLIGCAPAIGEHWAKGTACK	176
HPV45-L1	LSGHPPFYNLDDTESAHAATAVITQDVRDNVSDYKQTLQCLIGCVPAIGEHWAKGTACK	177
	loop	
HPV6-L1	NTPVQAGDCPPELITSVIQDGMVDTGFGAMNFADLTQNSDVPIDICGTTCKYPDYLQ	232
HPV11-L1	NTSVQNGDCPPELITSVIQDGMVDTGFGAMNFADLTQNSDVPIDICGTVCKYPDYLQ	233
HPV52-L1	NNSGNGDCPPELQINSVIQDGMVDTGFGCMDFTLQASKSDVPIDICSSVCKYPDYLQ	239
HPV58-L1	NNA-AATDCPPELNFNSIIEDGMVDTGFGCMDFTLQANKSDVPIDICNSTCKYPDYLK	236
HPV33-L1	NAA-PANDCPPELINTIIEDGMVDTGFGCMDFTLQANKSDVPIDICGSTCKYPDYLK	236
HPV31-L1	NNAITPGDCPPELKNNSVIQDGMVDTGFGAMDFTALQDTKSNVPLDICNSICKYPDYLK	237
HPV16-L1	NVAVNPGDCPPELINTVIQDGMVDTGFGAMDFTLQANKSEVPLDICTSICKYPDYLK	236
HPV35-L1	ANQVKAGECPPELINTVLQDGMVDTGFGAMDFTLQANKSDVPLDICSSICKYPDYLK	236
HPV51-L1	NTPVPPGDCPPELVSSVIQDGMIDTGFAMDFALQATKSDVPLDISQSVCKYPDYLK	235
HPV56-L1	STQVTGDCPPELALINTPIEDGMIDTGFAMDFKVLQESKAEPVPLDIVQSTCKYPDYLK	235
HPV39-L1	PNNVSTGDCPPELVNTPIEDGMIDTGFAMDFALQETKSEVPLDICQSICKYPDYLQ	235
HPV59-L1	PTTVVQDCPPELINTPIEDGMVDTGYGAMDFKLLQDNKSEVPLDICQSICKYPDYLQ	236
HPV18-L1	SRPLSQGDCPPELKNVTLEDGMVDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQ	236
HPV45-L1	PAQLQPGDCPPELKNITIEDGMVDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQ	237
	α1 β-F FG-loop	
HPV6-L1	MAADPYGDRLLFFFLRKEQMFARHFFNRAGEVGEPVPTLIIGKS--GNRTSVGSSIVVNT	290
HPV11-L1	MAADPYGDRLLFFFLRKEQMFARHFFNRAGTVGEPVPTDLIVKGG--NNRSSVASSIVVHT	291
HPV52-L1	MASEPYGDSLFFFLRREQMFVRHFFNRAGTLGDPVPGDLYIQGSNSGNTATVQSSAFFPT	299
HPV58-L1	MASEPYGDSLFFFLRREQMFVRHFFNRAGLGEAVPDDLYIGKS--GNTAVIQSSAFFPT	294
HPV33-L1	MTSEPYGDSLFFFLRREQMFVRHFFNRAGTLGEAVPDDLYIGKS--GTTASIQSSAFFPT	294
HPV31-L1	MVAEPYGDTLFFFLRREQMFVRHFFNRSGTVGESVPTDLYIGKS--GSTATLANSTVFPT	295

HPV16-L1	MVSEPYGDSLFFYLRRQMFVRHLFNRRAGAVGENVPDDLYIKGS--GSTANLASSNYFPT	294
HPV35-L1	MVSEPYGDMLEFFYLRRQMFVRHLFNRRAGTVGETVPADLYIKGT--T--GTLPTSTSYFPT	292
HPV51-L1	MSADTYGSMFFHLRRQMFARHYYNKLGVGEDIPNDYYIKGSG--NGRDPPIESYIYSAT	294
HPV56-L1	MSADAYGDSMWFYLRREQLFARHYFNRRAGKVGETIPAELYIKGS--NGREPPSSSVYVAT	293
HPV39-L1	MSADVYGDSMFFCLRRQMFARHFWNRGGMVGDIPAQLYIKGT--DIRANPGSSVYCPS	293
HPV59-L1	MSADAYGDSMFFCLRRQMFARHFWNRSGTMDQLPESLYIKGT--DIRANPGSYLYSPS	294
HPV18-L1	MSADPYGDSMFFCLRRQMFARHFWNRAGTMDGTVPQSLYIKGT--GMRASPGSCVYSPS	294
HPV45-L1	MSADPYGDSMFFCLRRQMFARHFWNRAGVMGDTVPTDLYIKGTSANMRETPGSCVYSPS	297

	G1 → G2 → β-H1 → β-H2 → HI- →	
HPV6-L1	PSGSLVSSEAQLFNKPYWLQKAQGHNNNGICWGNQLFVTVVDTTRSTNMTLCASVT--TSS	348
HPV11-L1	PSGSLVSSEAQLFNKPYWLQKAQGHNNNGICWGNHFLVTVVDTTRSTNMTLCASVS--KSA	349
HPV52-L1	PSGSMVTSESQLFNKPYWLQRAQGHNNNGICWGNQLFVTVVDTTRSTNMTLCAEVK--KES	357
HPV58-L1	PSGSIVTSESQLFNKPYWLQRAQGHNNNGICWGNQLFVTVVDTTRSTNMTLCETVT--KEG	352
HPV33-L1	PSGSMVTSESQLFNKPYWLQRAQGHNNNGICWGNQVFVTVVDTTRSTNMTLCQTVT--SDS	352
HPV31-L1	PSGSMVTSDAQIFNKPYWQRAQGHNNNGICWGNQLFVTVVDTTRSTNMSVCAAIAN--SDT	354
HPV16-L1	PSGSMVTSDAQIFNKPYWLQRAQGHNNNGICWGNQLFVTVVDTTRSTNMTLCAAI--SET	353
HPV35-L1	PSGSMVTSDAQIFNKPYWLQRAQGHNNNGICWSNQLFVTVVDTTRSTNMSVCSAVSS--SDS	351
HPV51-L1	PSGSMITSDSQIFNKPYWLHRAQGHNNNGICWNNQLFITCVDTTRSTNLTISTATAA--VSP	353
HPV56-L1	PSGSMITSEAQLFNKPYWLQRAQGHNNNGICWGNQLFVTVVDTTRSTNMTISTATE--QLS	351
HPV39-L1	PSGSMVTSDSQLFNKPYWLHKAQGHNNNGICWNNQLFVTVVDTTRSTNFTLSTLIESIPS	353
HPV59-L1	PSGSVVTSDSQLFNKPYWLHKAQGLNNGICWNNQLFVTVVDTTRSTNLSVCASTSSIPN	354
HPV18-L1	PSGSIVTSDSQLFNKPYWLHKAQGHNNNGICWNNQLFVTVVDTTRSTNLTICASTQSPVPG	354
HPV45-L1	PSGSITTSDSQLFNKPYWLHKAQGHNNNGICWNNQLFVTVVDTTRSTNLTICASTQNPNVPN	357

	loop → β-I → α2 → α3 →	
HPV6-L1	TYTNSDYKEYMRHVEEYDLQFIFQLCSITLSAEVMAYIHTMNPVLEDWNFGLSPPPNGT	408
HPV11-L1	TYTNSDYKEYMRHVEEYDLQFIFQLCSITLSAEVMAYIHTMNPVLEDWNFGLSPPPNGT	409
HPV52-L1	TYKNEFKEYLRHGEEDLQFIFQLCKITLTADVMTYIHKMDATILEDWQFGLTPPPSAS	417
HPV58-L1	TYKNEFKEYLRHVEEYDLQFIFQLCKITLTAEIMTYIHTMDSNILEDWQFGLTPPPSAS	412
HPV33-L1	TYKNEFKEYLRHVEEYDLQFIFQLCKITLTAEIMTYIHAMNPDILEDWQFGLTPPPSAS	412
HPV31-L1	TFKSSNFKEYLRHGEEDLQFIFQLCKITLSADIMTYIHSNMPAILEDWNFGLTPPPSGS	414
HPV16-L1	TYKNTNFKEYLRHGEEDLQFIFQLCKITLTADVMTYIHSMNSTILEDWNFGLQPPPGGT	413
HPV35-L1	TYKNEFKEYLRHGEEDLQFIFQLCKITLTADVMTYIHSNMPSTILEDWNFGLTPPPSGT	411
HPV51-L1	TFTPSNFQYLRHGEEDLQFIFQLCKITLTAEVMAYLHTMDPTILEQWNFGLTPPPSAS	413
HPV56-L1	KYDARKINQYLRHVEEYDLQFIFQLCKITLSAEVMAYLHNMNANLLEDWNIGLSPPVATS	411
HPV39-L1	TYDPSKFKEYLRHVEEYDLQFIFQLCTVTLTDDVMSYIHTMNSSILDNWNFAVAPPPSAS	413
HPV59-L1	VYTPTSFKEYLRHVEEYDLQFIFQLCKITLTAEVMSYIHNMTTILEDWNFGVTPPPPTS	414
HPV18-L1	QYDATKFQKYSRHEEYDLQFIFQLCTITLTADVMSYIHSNNSILEDWNFGVPPPPPTS	414
HPV45-L1	TYDPTKFHYSRHEEYDLQFIFQLCTITLTAEVMSYIHSNNSILENWNFGVPPPPPTS	417

	α4 → β-J → α5 →	
HPV6-L1	LEDTRYVQSQAITCQKPTPEKEKDPYKNLSFWEVNLKEKFSSELDQYPLGRKFLQSG	468
HPV11-L1	LEDTRYVQSQAITCQKPTPEKEKQDPYKDMFWEVNLKEKFSSELDQFPLGRKFLQSG	469
HPV52-L1	LEDTRYFVTSTAITCQKNTPPKGEDPLKDYMFWEVDLKEKFSADLDQFPLGRKFLQAG	477
HPV58-L1	LQDTRYFVTSTAITCQKTAPPEKEEDPLNKYTFWEVNLKEKFSADLDQFPLGRKFLQSG	472
HPV33-L1	LQDTRYFVTSTAITCQKTVPPEKEEDPLGKYTFWEVDLKEKFSADLDQFPLGRKFLQAG	472
HPV31-L1	LEDTRYFVTSTAITCQSAPQPKEDDPKDYVFWVNLKEKFSADLDQFPLGRKFLQAG	474
HPV16-L1	LEDTRYFVTSTAIACQKHTPPAPKEDPLKKYTFWEVNLKEKFSADLDQFPLGRKFLQAG	473
HPV35-L1	LEDTRYVTSQAIVTCQKPSAPKPKDDPLNKYTFWEVDLKEKFSADLDQFPLGRKFLQAG	471
HPV51-L1	LEDAYRFVRNAATSCQKDTTPQAKPDPLAKYKFDVNLKERFSLDLQFALGRKFLQVG	473
HPV56-L1	LEDKYRYVRSSTAITCQREPPTEKQDPLAKYKFDVNLQDSFSTDLDQFPLGRKFLMQLG	471
HPV39-L1	LVDTRYLQSAAITCQKDAPAEKKDPYDGLKFWNVDLREKFSLELDQFPLGRKFLQAR	473
HPV59-L1	LVDTRYFVQSAIVTCQKDTAPPVKQDPYDKLKFVVDLKERFSADLDQFPLGRKFLQLG	474
HPV18-L1	LVDTRYFVQSAITCQKDAAPAKNDPYDKLKFVVDLKEKFSLELDQYPLGRKFLVQAG	474
HPV45-L1	LVDTRYFVQSAIVTCQKDTTPPEKQDPYDKLKFVVDLKEKFSSELDQYPLGRKFLVQAG	477

HPV6-L1	YRGRSSIRTGVKRPVSKASAPKRKRKTKR--	500
HPV11-L1	YRGRSARTGIRPAVSKPSTAPKRKRKTKK--	501
HPV52-L1	LQARPKLKRPAASAPRTST---KKK-KVKR---	503
HPV58-L1	LKAKPRLKRSAPTTRAP-ST---KRK-KVKK---	498
HPV33-L1	LKAKPKLKRAAPTSTRSSA---KRK-KVKK---	499
HPV31-L1	YRARPKFKAGKRSAPSASTTTPAKRKRKTKK---	504
HPV16-L1	LKAKPKFTLGKRKATPTTSSSTTAKRKRKRKL---	505
HPV35-L1	LKARNPRLGKRAAPASTSKKSSTKRKRKVS---	502
HPV51-L1	VQRKPRPGLKRPAASASSSSSSAKRKRKVK---	504
HPV56-L1	TRSKPAVATSKKRSAPTSTS-TPAKRKR------	499
HPV39-L1	VRRRPTIGPRKRPAASTSSSSATKHKRKRVS---	505
HPV59-L1	ARPKPTIGPRKRAAPASTTPSPKRVRKRKSSRK---	508
HPV18-L1	LRRKPTIGPRKRAPSATSSSKPAKRVRVRARK---	507
HPV45-L1	LRRRPTIGPRKRPAASTSTASRPKRVRIRSKK---	510

[0097] In addition to the methods used by the inventors for sequence alignments, protein secondary structure prediction software that can be used for prediction includes, but is not limited to:

[0098] 1. JPred: <http://www.compbio.dundee.ac.uk/jpred/index.html>

[0099] 2. ProtPredicct: <http://predictprotein.org>

[00100] 3. PsiPred: <http://bioinf.cs.ucl.ac.uk/psipred>

[00101] 4. SCRATCH-1D: <http://download.igb.uci.edu>

[00102] 5. Nnpredict: <http://www.cmpharm.ucsf.edu/~nomi/nnpredict>

[00103] 6. Predictprotein: <http://www.embl-heidelberg.de/predictprotein/SOPMA>
<http://www.ibcp.fr/predict.html>

[00104] 7. SSPRED: http://www.embl-heidelberg.de/sspred/ssprd_info.html.

[00105] In one embodiment of the present invention, the inventors determine the length of the N-terminal fragment derived from the L1 protein of the first HPV type in the following manner: the natural sequence of the L1 protein is truncated within its $\alpha 5$ region and nearby regions thereof, with the sequence from its N-terminus to the newly generated C-terminus within the $\alpha 5$ region retained. Such a truncated sequence ensures that it has the immunogenicity of the first type and is capable of forming VLP.

[00106] The N-terminal fragment of the HPV L1 protein derived from the first type can be further modified as long as it retains the immunogenicity of the first type and is capable of forming VLP.

[00107] The length of the C-terminal fragment of the HPV L1 protein derived from the second type is determined in the following manner: The natural sequence of the L1 protein is truncated within its $\alpha 5$ region and nearby regions thereof, then the sequence from the newly generated N-terminus within its $\alpha 5$ region to the C-terminus is retained. Such a truncated sequence does not have the major

neutralizing antigenic epitope so that does not interfere with the immunogenicity of the resulting chimeric protein.

[00108] The C-terminal fragment of the HPV L1 protein derived from the second type can be further mutated, deleted and/or added, preferably retaining at least one of its nuclear localization sequences. Yang et al. predicted the nuclear localization sequences of 107 HPV subtypes (Yang et al. Predicting the nuclear localization signals of 107 types of HPV L1 proteins by bioinformatic analysis. *Geno. Prot. Bioinfo.* Vol. 4 No. 1 2006, incorporated herein by reference in its entirety). The nuclear localization sequences of each type of HPV L1 protein can be conveniently determined by sequence analysis software commonly used in the field.

[00109] The above-described connection of the N-terminal fragment to the C-terminal fragment occurs at the newly generated C-terminus of the former and at the newly generated N-terminus of the latter. This can be a direct connection or a connection via a linker. The site where the connection occurs is defined as the origin coordinate, the N-terminal side of the origin is regarded as minus while the C-terminal side as plus.

[00110] The following shows the sequence of amino acids 453-469 of the HPV6 L1 protein and the corresponding sequences of L1 proteins of other HPV types. These sequence overlap with their $\alpha 5$ region respectively. It can be seen these sequences are highly similar. The numbers in brackets indicate the positions of the last amino acid residue of the listed sequences, where for HPV Type 45, an additional 26 amino acids are present at the N-terminus of the L1 protein of some HPV Type 45 strains, while at the N-terminus of the L1 protein of other HPV Type 45 strains, the said additional 26 amino acids are not present, therefore the number is indicated as $(478) + 26$.

[00111] HPV6 ELDQYPLGRKFLLQSGY(469)
[00112] HPV11 ELDQFPLGRKFLLQSGY(470)
[00113] HPV16 DLDQFPLGRKFLLQAGL(474)
[00114] HPV18 DLDQYPLGRKFLVQAGL(475)
[00115] HPV31 DLDQFPLGRKFLLQAGY(475)
[00116] HPV35 DLDQFPLGRKFLLQAGL(472)
[00117] HPV39 ELDQFPLGRKFLLQARV (474)
[00118] HPV45 DLDQYPLGRKFLVQAGL(478)+26
[00119] HPV51 DLDQFALGRKFLLQVGV(474)
[00120] HPV52 DLDQFPLGRKFLLQAGL(478)
[00121] HPV56 DLDQFPLGRKFLLMQLGTRS(474)
[00122] HPV58 DLDQFPLGRKFLLQSGL(473)
[00123] HPV33

DLDQFPLGRKFLLQAGL(473)KAKPKLKRAAPTSTRTSSAKRKKVKK

wherein KR at positions 480-481 and KRKK at positions 493-496 are nuclear localization sequences.

[00124] HPV59

DLDQFPLGRKFLLQLGA(475)RPKPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK, wherein RKR at positions 484-486 and KRVKRRK at positions 498-504 are nuclear localization sequences.

[00125] In one embodiment of the present invention, the inventors have completed C-terminus substitutions of L1 proteins between different HPV types by taking advantage of sequence similarity of the $\alpha 5$ region and nearby regions between the HPV types.

[00126] In the most preferred embodiment of the present invention, the inventors have noted that L1 protein of each HPV type has a tetrapeptide RKFL or, more advantageously, a hexapeptide LGRKFL, at a similar position. The inventors

have skilfully exploited this highly conserved sequence to locate the chimeric protein connection point at any amino acid position within this oligopeptide. On one hand, the sequence starting from the N-terminus of the chimeric protein to RKFL or LGRKFL is identical to the sequence of the N-terminal fragment derived from the L1 protein of the first type, while on the other hand, the sequence starting from RKFL or LGRKFL to the C-terminus of the chimeric protein is identical to the sequence of the C-terminal fragment derived from the L1 protein of the second type.

[00127] The chimeric protein thus produced maintains a high degree of similarity to the natural HPV L1 protein, and it can be expected to perform well in production and even in medical or prophylactic procedures thereafter.

[00128] It will be understandable for those skilled in the art that there are different strains with different natural sequences of a given HPV type, chimeric proteins constructed using different strains also fall within the scope of the present invention.

[00129] It will be understandable for those skilled in the art that because of the high degree of similarity between L1 protein of different HPV types, if, during the construction of the chimeric proteins, the N-terminal fragment derived from the L1 protein of the first type is extended by more amino acid residues towards the C-terminus, or the C-terminal fragment derived from L1 protein of the second HPV virus type is extended by more amino acid residues towards the N-terminus, it is also possible to form chimeric proteins that are structurally identical to the present invention due to identical or similar amino acids at the corresponding sites. The chimeric proteins thus formed also fall within the scope of the present invention.

[00130] It will be understandable for those skilled in the art that on the basis of the chimeric proteins of the above described embodiments, variants of the

chimeric proteins may be formed by mutations, deletions and/or additions of amino acid residues. These variants are likely to have the immunogenicity of the L1 protein of the first type, can form VLP, and have a good yield and solubility. The chimeric proteins such formed also fall within the scope of the present invention.

[00131] The beneficial effects of the invention

[00132] The expression systems commonly used for producing virus-like particles are classified into eukaryotic expression systems and prokaryotic expression systems. The papilloma virus L proteins expressed by the eukaryotic expression systems can spontaneously assemble into viral-like particles, but have the disadvantage of low expression level thus are not suitable for mass production. The papilloma virus L protein expressed by the prokaryotic expression system requires *in vitro* processing to obtain virus-like particles because of often destroyed natural conformation, and has low yield, being difficult to be used in industrialization.

[00133] The present invention modifies the C-terminus of the L protein of the papilloma virus (e.g. human papilloma virus), for example by replacing it with the C-terminal fragment of HPV Type 16 L1 protein, HPV Type 28 L1 protein, HPV Type 33 L1 protein, HPV Type 59 L1 protein or HPV Type 68 L1 protein, thus can be used in expression systems (e.g. host cells, e.g. insect cells) to improve the expression level and the solubility of the papilloma virus L protein in expression systems (for example, host cells, e.g. insect cells). This can be used for the mass production of vaccines such as HPV vaccines.

[00134] The inventors found that the HPV Type 16 L1 protein, the HPV Type 28 L1 protein, the HPV Type 33 L1 protein, the HPV Type 59 L1 protein and the

HPV Type 68 L1 protein have increased expression level and increased solubility compared to L1 proteins of other HPV types, and that said increased protein expression level and increased solubility was found to be depend on the C-terminal sequence of said HPV L1 protein. Among 107 HPV Type L1 proteins, most of them have nuclear localization sequences at the C-termini, and the C-terminal sequences have some similarities.

[00135] For papilloma virus L proteins that are currently inexpressible, very low in expression level or insoluble after expression, replacement of their C-terminal fragments with C-terminal fragments of the HPV Type 16 L1 protein, HPV Type 28 L1 protein, HPV Type 33 L1 protein, HPV Type 59 L1 protein or HPV Type 68 L1 protein makes it possible for soluble expression and subsequent purification. This strategy can be used for the mass production of polyvalent vaccines (e.g. HPV vaccines), making it possible to provide more comprehensive protection against a wide range of papilloma virus infections, especially HPV.

[00136] There needs to increase the expression level and the solubility of the HPV L1 protein in insect cells for mass production purpose. In addition, the virus-like particles assembled by the HPV L protein lack good conformation in yeast cells due to failure to form correct disulphide bonds .

[00137] For the HPV L1 proteins that are poorly expressed and insoluble in insect cells, a significant increase in expression level and solubility can be resulted after the modification of its C-terminal fragment into the C-terminal fragment of the HPV Type 33 or 59 L1 protein, thus they could be used for mass production of HPV vaccines.

[00138] For HPV L1 proteins that are better expressed and better soluble in insect cells compared to L1 proteins of other HPV types, such as HPV Type 16 protein, HPV Type 28 L1 protein, HPV Type 68 L1 protein, etc., there are needs to further improve the expression level and the solubility in order to achieve mass

production of vaccines. In the present invention, for example, after modifying the C-terminal fragment of the HPV Type 16 L1 protein to the C-terminal fragment of the HPV Type 33 L1 protein, the expression level and the solubility of the modified chimeric HPV Type 16 protein are improved, which is conducive to the mass production of HPV vaccines.

[00139] To sum up, the chimeric HPV L1 proteins showed much higher expression level and solubility in insect cells compared to the unmodified HPV L1 protein. It can be used for the mass production of HPV vaccines. In addition, the chimeric HPV L1 proteins can correctly form disulfide bonds thus be assembled into HPV virus-like particles with good conformations in insect cells. This can improve the immunogenicity of HPV virus-like particles and trigger better immune responses.

[00140] The multivalent vaccine of the present invention can be used to prevent diseases or infections related with a wide range of HPV, including those HPV types that are not currently preventable.

[00141] Definition

[00142] Unless otherwise stated, all technical and scientific terms used herein have the meanings normally understood by a person of ordinary skill in the art to which the invention belongs. For the convenience of understanding the present invention, the following terms are cited below for their ordinary meaning.

[00143] When used herein and in the attached claims, the singular forms “a/an”, “another” and “said/the” include the plural designations of the objects unless the context clearly indicates otherwise. Unless otherwise expressly stated, the terms

“include/comprise/have”, “for example”, etc. are intended to convey inclusion rather than limitation.

[00144] The term “immunogenicity” refers to the ability of a substance, for example a protein or a peptide, to trigger an immune response, i.e. the ability to trigger the production of antibodies, in particular the ability to trigger humoral- or cell-mediated response.

[00145] The term “antibody” refers to an immunoglobulin molecule that binds an antigen. Antibodies may be polyclonal mixtures or monoclonal. Antibodies may be intact immunoglobulins of natural origin or of recombinant origin or may be immunoreactive portions of intact immunoglobulins. Antibodies may be present in a variety of forms including, for example, Fv, Fab’, F(ab’)2 and as single chains.

[00146] The term “antigenicity” refers to the ability of a substance, for example a protein or a peptide, to trigger the production of antibodies that bind specifically to it.

[00147] The term “epitope” includes any protein determinant cluster that specifically binds to an antibody or T-cell receptor. Epitope determinants typically consist of chemically active surface groups (e.g. amino acids or sugar side chains, or combinations thereof) of the molecule and typically have specific three-dimensional structural characteristics as well as specific charge characteristics.

[00148] The terms “subtype” or “type” are used interchangeably herein to refer to genetic variant of virus that allows it can be recognized by the immune system

as distinct antigen from type to type. For example, HPV 16 is immunologically distinguishable from HPV 33.

[00149] The term “HPV L1 protein”, as used herein, the term “HPV” and “human papilloma virus” refer to envelope-free double-stranded DNA viruses of the Papillomavirus family. Their genomes are circular and approximately 8 kilobase pairs in size. Most HPVs encode eight major proteins, six in the “early” region (E1-E2) and two in the “late” region (L1 (major capsid protein) and L2 (minor capsid protein)). Over 120 HPV types have been identified and they are labelled by numbers (e.g. HPV-16, HPV-18, etc.).

[00150] The term “HPV” or “HPV virus” refers to papilloma viruses of the *Papillomaviridae* Family, which are envelope-free DNA viruses with a double-stranded closed circular DNA genome of approximately 8 kb in size that is usually classified into three regions: (i) the early region (E), which contains six open reading frames E1, E2, E4-E7, encoding non-structural proteins related to viral replication, transcription and transformation, as well as open reading frames E3 and E8; (ii) the late region (L), which contains reading frames encoding the major capsid protein L1 and the minor capsid protein L2; and (iii) the long regulatory region (LCR), which does not encode any proteins but has the origin of replication and multiple transcription factor binding sites.

[00151] The terms “HPV L1 protein” and “HPV L2 protein” refer to proteins encoded by the late region (L) of the HPV gene and synthesized late in the HPV infection cycle. The L2 protein is the minor capsid protein. 72 L1 pentamers form

the outer shell of the icosahedral HPV particles, which encloses the closed circular double-stranded DNA microchromosome.

[00152] The term “virus-like particle” refers to a hollow particle containing one or plurality of structural proteins of a virus, without viral nucleic acids.

5 **[00153]** “HPV pseudovirus” is an ideal model for *in vitro* neutralization of HPV, by taking advantages of the non-specific nucleic acid encapsulation property of HPV VLP, HPV pseudovirus is formed by wrapping free DNA or introducing an exogenous plasmid into a VLP composed of intracellularly-expressed HPV L1 and L2.

10 **[00154]** The “pseudovirus neutralization assay” is a method for evaluating the neutralizing activity of antibodies. After incubation of immunized animal serum with a certain amount of pseudovirus and then infection of the cells, the amount of the cells decreases when serum neutralizing antibodies increases, showing a linear negative correlation in a certain range. The neutralizing activity of
15 antibodies in serum can therefore be assessed by measuring changes in the amounts of cells.

[00155] The term “fragment thereof” or “variant thereof” refers to a deletion, insertion and/or substitution of nucleotides or amino acids sequence of the present invention. Preferably, the fragment or variant of the polypeptide provided by the
20 present invention is capable of triggering the humoral- and/or the cellular-immune response in animals or humans.

[00156] The term “chimeric” means that sequences of polypeptides or nucleotides derived from different parental molecules are connected together by

-CO-NH- or 3', 5'-phosphodiester bonds, respectively. Preferably, they are not spaced by additional linker sequences, but are directly adjacent to each other.

[00157] The term “truncation” refers to the removal of one or plurality of amino acids from the N- and/or C-terminus of a polypeptide or the deletion of one or plurality of amino acids from the interior of a polypeptide.

[00158] The term “nuclear localization sequence” refers to an amino acid sequence that directs the protein into the nucleus. In some HPV L1 proteins, two tight clusters of basic residues (i.e. nuclear localization sequences) (e.g. one is KRKR, KRKK, KRKRK, KRKKRK, KRVKRRK, etc. and the other is KR, RKR, KRK, etc.) have a spacer region of 10-14 amino acids between them. The above clusters of basic residues belong to nuclear localization sequences. In some other HPV L1 proteins, the nuclear localization sequence is a tight cluster of basic residues formed by arginines and/or lysines. Nuclear localization sequences include, but are not limited to, examples of clusters of basic residues as described above. See Jun Yang et al, Predicting the Nuclear Localization Signals of 107 Types of HPV L1 Proteins by Bioinformatic Analysis, Genomics, Proteomics & Bioinformatics Volume 4, Issue 1, 2006, Pages 34-41, the entire contents of which are incorporated herein by reference.

[00159] The term “functional variant” refers to a version of a polypeptide or a protein that retains the desired activities or characteristics after truncation, mutation, deletion and/or addition.

[00160] “Sequence identity” between two sequences of polypeptides or nucleic acids indicates the number of identical residues between said sequences as a

percentage of the total number of residues, and is calculated based on the size of the smaller one of the compared molecules. When calculating the percentage identity, the sequences being compared are matched in such a way as to produce the maximum match between the sequences, with the vacant positions (if present) in the match being resolved by a specific algorithm. Preferred computer program methods for determining identity between two sequences include, but are not limited to, GCG program packages including GAP, BLASTP, BLASTN and FASTA (Altschul et al., 1990, J. Mol. Biol. 215: 403-410). The above programs are publicly available from the National Center of Biotechnology Information (NCBI) and other sources. The well-known Smith Waterman Algorithm can also be used to determine the identity.

[00161] Non-critical amino acids can be conservatively substituted without affecting the normal function of the protein. Conservative substitution means replacing amino acids with chemically or functionally similar amino acids. Tables for conservative substitutions that provide similar amino acids are well known in the art. By way of example, in some embodiments, the groups of amino acids provided in **Tables 1-3** are considered to be conservative substitutions for each other.

Table 1 In some embodiments, the selected groups of amino acids that are considered to be conservative substitutions for each other

Acid residues	D and E
Basic residues	K, R and H

Hydrophilic uncharged residues	S, T, N and Q
Aliphatic uncharged residues	G, A, V, L and I
Non-polar uncharged residues	C, M and P
Aromatic residues	F, Y and W

Table 2 In some embodiments, other selected groups of amino acids considered to be conservative substitutions for each other

Group 1	A, S and T
Group 2	D and E
Group 3	N and Q
Group 4	R and K
Group 5	I, L and M
Group 6	F, Y and W

5

Table 3 In some embodiments, other selected groups of amino acids considered to be conservative substitutions for each other

Group A	A and G
Group B	D and E
Group C	N and Q
Group D	R, K and H
Group E	I, L, M and V
Group F	F, Y and W

Group G	S and T
Group H	C and M

[00162] The term “amino acids” refers to the twenty common naturally occurring amino acids. Naturally occurring amino acids include alanine (Ala; A), arginine (Arg; R), asparagine (Asn; N), aspartic acid (Asp; D), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y) and valine (Val; V).

[00163] As used herein, a “physiologically acceptable carrier” is non-toxic to cells or mammals at the dose and concentration used. Usually it is a pH buffered aqueous solution, non-limiting examples of which include buffers; antioxidants; oligopeptides; proteins; hydrophilic polymers; amino acids; monosaccharides, disaccharides and other carbohydrates; chelating agents; sugar alcohols; salt-forming counterions such as sodium; and/or nonionic surface active agents.

[00164] The term “adjuvant” refers to a compound or mixture that enhances immune responses. In particular, a vaccine may comprise an adjuvant. Adjuvants for use in the present invention may include, but are not limited to, one or plurality of the following: mineral-containing adjuvant compositions, oil-emulsion adjuvants, saponin adjuvant formulations, derivatives of bacteria or microbes.

[00165] The term “vector” refers to a nucleic acid molecule capable of proliferating another nucleic acid connected to it. The term includes vectors as self-replicating nucleic acid structures and as vectors integrated into the genome of host cells into which the vector has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which such vectors are operatively connected.

[00166] The term “host cell” refers to a cell into which an exogenous nucleic acid has been introduced, as well as to the progeny of such a cell. Host cells include “transformants” (or “transformed cells”), “transfectants” (or “transfected cells”) or “infectants” (or “infected cells”), each of which includes primary transformed, transfected or infected cells and the progeny derived from them. Such progeny may not be identical to the parental cells in terms of nucleic acid content and may contain mutations.

[00167] The administration amount is preferably a “prophylactically effective amount” (herein the prophylaxis may be considered as treatment and the two may be used interchangeably), which is sufficient to show benefit to the individual.

[00168] Examples

[00169] Example 1 Construction of chimeric genes

[00170] Example 1.1: Construction of a chimeric gene with the C-terminus of HPV6 L1 substituted with the C-terminus of HPV33 L1

[00171] 1.1.1 Construction of pFB-HPV6 L1 as the template

[00172] The HPV6 L1 gene with the KpnI and XbaI cleavage sites at both ends of the synthesized sequences was synthesized by Thermo Fisher [formerly Invitrogen (Shanghai) Trading Co.], its sequence is shown in SEQ ID NO: 5. The plasmid pcDNA3-HPV6-L1 comprising the nucleotide sequence encoding amino acid 1-500 of HPV6 L1 was obtained by ligating the synthesized gene fragment with pcDNA3 vector (distributor: Thermo Fisher) at KpnI and XbaI cleavage sites.

[00173] The obtained pcDNA3-HPV6-L1 plasmid was subjected to double enzyme digestion with KpnI and XbaI to obtain a gene fragment of HPV6 L1 (1-500). The fragment was then ligated with the KpnI / XbaI double digested pFastBacTM1 vector (distributor: Thermo Fisher) to obtain a rod vector containing the HPV6 L1 (1-500) gene fragment, named as pFB-HPV6 L1.

[00174] 1.1.2 Construction of pFB-HPV33 L1 as the template

[00175] The HPV33 L1 gene with the KpnI and XbaI cleavage sites at both ends of the synthesized sequences was synthesized by Thermo Fisher [formerly Invitrogen (Shanghai) Trading Co.], its sequence is shown as SEQ ID NO: 6. The plasmid pcDNA3-HPV33-L1 comprising the nucleotide sequence encoding amino acids 1-499 of HPV33 L1 was obtained by ligating the synthesized gene fragment with pcDNA3 vector (distributor: Thermo Fisher) at Kpn I and XbaI cleavage sites.

[00176] The pcDNA3-HPV33-L1 plasmid was subjected to double enzyme digestion with KpnI and XbaI to obtain a fragment of the HPV33 L1 (1-499) gene. The fragment was then ligated to the KpnI and XbaI double digested pFastBacTM1

vector (distributor: Thermo Fisher) to obtain a rod vector containing the HPV33 L1 (1-499) gene fragment, named as pFB-HPV33 L1.

[00177] 1.1.3 Construction of pFB-HPV6 L1: 33C

[00178] Chimeric gene with HPV6 L1 C-terminus substituted with HPV33 L1 C-terminus: the constructed recombinant plasmid pFB-HPV6 L1 was used as the gene template to amplify a 1426 bp gene fragment using primers F1 and R1, the primer sequence F1 is shown in SEQ ID No: 7 and R1 is shown in SEQ ID No: 8.

[00179] This gene fragment contains a fragment encoding amino acids 1-469 of HPV6 L1, 10 bases overlapping with the gene fragment encoding amino acids 474-499 of HPV33 L1, and a fragment of the KpnI digest site (GGTAC[^]C), the amplified sequence is shown in SEQ ID No: 9.

[00180] PCR amplification parameters: pre-denaturation at 94°C for 5min; denaturation at 98°C for 10 s, annealing at 69°C for 15 s, 1 kb/1 min at 72°C, for 30 cycles; extension at 72°C for 5 min; end at 16°C.

[00181] The recombinant plasmid pFB-HPV33 L1 was used as the gene template to amplify a gene fragment of 101 bp in length using primers F2 and R2, the primer sequence of F2 is shown in SEQ ID No: 10 and the primer sequence of R2 is shown in SEQ ID No: 11.

[00182] This gene fragment contains a gene fragment encoding 26 C-terminal amino acids (474-499) of HPV33 L1, a 10 bp base overlapping with the gene fragment encoding the C-terminus of amino acids 1-469 of HPV6 L1 and the

XbaI (T[^]CTAGA) digest site, the amplified sequence is shown in SEQ ID No: 12.

[00183] PCR amplification parameters: pre-denaturation at 94°C for 5min; denaturation at 98°C for 10 s, annealing at 69°C for 15 s, 1 kb/1 min at 72°C, for 30 cycles; extension at 72°C for 5min; end at 16°C.

[00184] PCR ligating sequences:

[00185] The ligating primers were F1 and R2, and the fragments amplified using the above primers (amplified fragments of F1 and R1, amplified fragments of F2 and R2) were used as templates.

[00186] PCR ligating parameters: pre-denaturation at 94°C for 5 min; denaturation at 98°C for 10 s, annealing at 52°C for 15 s, 72°C for 1 kb/1 min, for 5 cycles; denaturation at 98°C for 10 s, annealing at 68°C for 15 s, 72°C for 1 kb/1 min, for 25 cycles; extension at 72°C for 5 min; end at 16°C.

[00187] The final result was SEQ ID NO: 4, a nucleotide sequence encoding amino acids 1-469 of HPV6 L1 and 26 C-terminal amino acids of HPV33 L1(aa 474-499), with KpnI and XbaI cleavage sites at both ends (hereafter referred to as the ligating sequence).

[00188] The recombinant plasmid pFB-HPV6 L1: 33C was obtained by double digesting the pFastBacTM1 vector and the ligating sequence fragment with KpnI+XbaI enzymes and cloning the ligating sequence into the pFastBacTM1 vector to obtain pFB-HPV6 L1: 33C, which is a chimeric gene with the C-terminus of HPV6 L1 substituted by the C-terminus of HPV33 L1.

[00189] Example 1.2 Construction of chimeric gene with the C-terminus of HPV11 L1 substituted by the C-terminus of HPV33 L1

[00190] The experimental methods and procedures were the same as in Example 1.1, see Appendix 2 for relevant sequences.

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[00191] Example 1.3 Construction of a chimeric gene with the C-terminus of HPV16 L1 substituted by the C-terminus of HPV33 L1

[00192] The experimental method and procedure were the same as in Example 1.1, see Appendix 3 for relevant sequences.

10

[00193] Example 1.4 Construction of a chimeric gene with the C-terminus of HPV18 L1 substituted by the C-terminus of HPV33 L1

[00194] The experimental method and procedure were the same as in Example 1.1, see Appendix 4 for relevant sequences.

15

[00195] Example 1.5 Construction of a chimeric gene with the C-terminus of HPV31 L1 substituted by the C-terminus of HPV33 L1

[00196] The experimental method and procedure were the same as in Example 1.1, see Appendix 5 for relevant sequences.

20

[00197] Example 1.6 Construction of HPV33 L1 gene

[00198] 1.6.1 Preparation of PFB-HPV33L1 gene

[00199] The HPV33L1 gene with the KpnI and XbaI cleavage sites at both ends of the synthesized sequences was synthesized by Thermo Fisher [formerly Invitrogen (Shanghai) Trading Co.], its sequence is shown in SEQ ID No: 68. The plasmid pcDNA3-HPV33-L1 comprising the nucleotide sequence encoding amino acid 1-499 of HPV33 L1 was obtained by ligating the synthesized gene fragment with pcDNA3 vector (distributor: Thermo Fisher) at KpnI and XbaI cleavage sites.

[00200] The obtained pcDNA3-HPV33-L1 plasmid was subjected to double enzyme digestion with KpnI and XbaI to obtain a gene fragment of HPV33 L1 (1-499). The fragment was then ligated with the KpnI / XbaI double digested pFastBacTM1 vector (distributor: Thermo Fisher) to obtain a rod vector containing the HPV33 L1 (1-499) gene fragment, named as pFB-HPV33 L1.

[00201] Example 1.7 Construction of a chimeric gene with the C-terminus of HPV35 L1 substituted by the C-terminus of HPV33 L1

[00202] The experimental methods and procedures were the same as in Example 1.1, see Appendix 7 for relevant sequences.

[00203] Example 1.8 Construction of chimeric gene with the C-terminus of HPV39 L1 substituted by the C-terminus of HPV59 L1

[00204] 1.8.1 Construction of pFB-HPV39 L1 used as the template

[00205] The HPV39 L1 gene with the KpnI and XbaI cleavage sites at both ends of the synthesized sequences was synthesized by Thermo Fisher [formerly

Invitrogen (Shanghai) Trading Co.), its sequence is shown as SEQ ID NO: 86. The plasmid pcDNA3-HPV39-L1 containing a nucleotide sequence encoding amino acids 1-505 of HPV39 L1 was obtained by ligating the synthesized gene fragment with pcDNA3 vector (distributor: Thermo Fisher) at KpnI and XbaI cleavage sites.

[00206] The pcDNA3-HPV39-L1 plasmid was subjected to double digestion with KpnI and XbaI to obtain a fragment of the HPV39 L1 (1-505) gene. The fragment was then ligated to the KpnI and XbaI double digested pFastBacTM1 vector (distributor: Thermo Fisher) to obtain a rod vector containing the HPV39 L1 (1-505) gene fragment, named as pFB-HPV39 L1.

[00207] 1.8.2 Construction of pFB-HPV59 L1 as the template

[00208] The HPV59 L1 gene with the KpnI and XbaI cleavage sites at both ends of the synthesized sequences was synthesized by Thermo Fisher [formerly Invitrogen (Shanghai) Trading Co.), its sequence is shown as SEQ ID NO: 87. The plasmid pcDNA3-HPV59-L1 containing a nucleotide sequence encoding amino acids 1-508 of HPV59 L1 was obtained by ligating the synthesized gene fragment with pcDNA3 vector (distributor: Thermo Fisher) at KpnI and XbaI cleavage sites.

[00209] The pcDNA3-HPV59-L1 plasmid was subjected to double digestion with KpnI and XbaI to obtain a fragment of the HPV59 L1 (1-508) gene. The fragment was then ligated to the KpnI and XbaI double digested pFastBacTM1 vector (distributor: Thermo Fisher) to obtain a rod vector containing the HPV59 L1 (1-508) gene fragment, named as pFB-HPV59 L1.

[00210] 1.8.3 Construction of pFB-HPV39 L1: 59C

[00211] Chimeric gene with HPV39 L1 C-terminus substituted with HPV59 L1 C-terminus: The constructed recombinant plasmid pFB-HPV39 L1 was used as the gene template to amplify a 1428 bp gene fragment using primers F1 and R1, the primer sequence F1 is shown in SEQ ID No: 88 and the primer sequence R1 is shown in SEQ ID No: 89.

[00212] This fragment contains a fragment encoding amino acids 1-469 of HPV39 L1, a 12-base overlapping with a fragment encoding amino acids 471-508 of HPV59 L1 and a segment of the KpnI digest site (GGTAC[^]C), the amplified sequence is shown in SEQ ID No: 90.

[00213] PCR amplification parameters: pre-denaturation at 94°C for 5min; denaturation at 98°C for 10 s, annealing at 69°C for 15 s, 1 kb/1 min at 72°C, for 30 cycles; extension at 72°C for 5min; end at 16°C.

[00214] The recombinant plasmid pFB-HPV59 L1 was used as the gene template to amplify a gene fragment of 139 bp in length using primers F2 and R2. The primer sequence F2 is shown in SEQ ID No: 91 and R2 is shown in SEQ ID No: 92.

[00215] This gene fragment contains a gene fragment encoding 38 C-terminal amino acids of the HPV59 L1(aa471-508), a 12 bp base overlapping with the gene fragment encoding amino acids 1-469 of HPV39 L1 and the XbaI (T[^]CTAGA) digest site, and the amplified sequence is shown in SEQ ID No: 93.

[00216] PCR amplification parameters: pre-denaturation at 94°C for 5min; denaturation at 98°C for 10 s, annealing at 69°C for 15 s, 1 kb/1 min at 72°C for 30 cycles; extension at 72°C for 5min; end at 16°C.

[00217] PCR ligating sequence

5 **[00218]** The ligating primers were F1 and R2, and the fragments amplified by using the above primers (F1 and R1 amplified fragments, F2 and R2 amplified fragments) were used as templates.

10 **[00219]** PCR ligating parameters: pre-denaturation at 94°C for 5 min; denaturation at 98°C for 10 s, annealing at 52°C for 15 s, 72°C for 1 kb/1 min, for 5 cycles; denaturation at 98°C for 10 s, annealing at 68°C for 15 s, 72°C for 1 kb/1 min, for 25 cycles; extension at 72°C for 5 min; end at 16°C.

15 **[00220]** The final result was SEQ ID NO: 85, a nucleotide sequence encoding amino acids 1-469 of HPV39 L1 and 38 C-terminal amino acids of HPV59 L1(471-508) with KpnI and XbaI enzyme cleavage sites at both ends (hereafter referred to as the ligating sequence).

20 **[00221]** The recombinant plasmid pFB-HPV39 L1: 59C was obtained by double digesting the pFastBacTM1 vector and the ligating sequence fragment with KpnI+XbaI enzymes and the ligating sequence was cloned into the pFastBacTM1 vector to obtain pFB-HPV39 L1: 59C, which is a chimeric gene with the C-terminus of HPV39 L1 substituted by the C-terminus of HPV59 L1.

[00222] Example 1.9 Construction of chimeric gene with the C-terminus of HPV45 L1 substituted by the C-terminus of HPV33 L1

[00223] The experimental methods and procedures were the same as in Example 1.1, see Appendix 9 for relevant sequences.

[00224] Example 1.10 Construction of a chimeric gene with the C-terminus of HPV51 L1 substituted by the C-terminus of HPV33 L1

[00225] The experimental methods and procedures were the same as in Example 1.1, see Appendix 10 for relevant sequences.

[00226] Example 1.11 Construction of a chimeric gene with the C-terminus of HPV52 L1 substituted by the C-terminus of HPV33 L1

[00227] The experimental methods and procedures were the same as in Example 1.1, see Appendix 11 for relevant sequences.

[00228] Example 1.12 Construction of a chimeric gene with the C-terminus of HPV56 L1 substituted by the C-terminus of HPV33 L1

[00229] The experimental methods and procedures were the same as in Example 1.1, see Appendix 12 for relevant sequences.

[00230] Example 1.13 Construction of a chimeric gene with the C-terminus of HPV58 L1 substituted by the C-terminus of HPV33 L1

[00231] The experimental methods and procedures were the same as in Example 1.1, see Appendix 13 for relevant sequences.

[00232] Example 1.14 Construction of HPV59 L1 gene

[00233] Example 1.14.1 Preparation of pFB-HPV59 L1 gene

[00234] The HPV59 L1 gene with the KpnI and XbaI cleavage sites at both ends of the synthesized sequences was synthesized by Thermo Fisher [formerly
5 Invitrogen (Shanghai) Trading Co.], its sequence is shown in SEQ ID No: 162. The plasmid pcDNA3-HPV59-L1 comprising the nucleotide sequence encoding amino acid 1-508 of HPV59 L1 was obtained by ligating the synthesized gene fragment with pcDNA3 vector (distributor: Thermo Fisher) at KpnI and XbaI cleavage sites.

10 [00235] The obtained pcDNA3-HPV59-L1 plasmid was subjected to double enzyme digestion with KpnI and XbaI to obtain a gene fragment of HPV59 L1 (1-508). The fragment was then ligated with the KpnI / XbaI double digested pFastBacTM1 vector (distributor: Thermo Fisher) to obtain a rod vector containing the HPV59 L1 (1-508) gene fragment, named as pFB-HPV59 L1.

15 [00236] Example 2 Recombinant baculovirus packaging

[00237] Example 2.1: HPV6 L1: 33C recombinant baculovirus packaging

[00238] The recombinant plasmid of pFB-HPV6 L1: 33C constructed in Example
20 1 was identified and sequenced to be correct, and was transformed into DH10Bac bacteria competent cells (Bac-to-Bac[®] kit, purchased from Thermo Fisher), incubated at 37°C for proliferation, and incubated in a flat dish for streak culture. White colonies was selected, incubated overnight. The bacterial culture was

collected and the recombinant baculovirus DNA was extracted using alkaline lysis method.

[00239] The recombinant baculovirus DNA was transfected into insect cells SF9 using a cationic transfection reagent (purchased from Sino Biological) to package the recombinant baculovirus virulent strains. The procedure was as follows:

[00240] a. SF9 cells at log phase were inoculated in dishes at a density of 0.6×10^6 cell/dish. The dish inoculated with SF9 cells were left at room temperature for 2 hrs. for the cell's adhering to the dish wall.

[00241] b. 20 μ L of extracted plasmid Bacmid DNA was added to 200 μ L Grace's Medium (no serum, no additives, purchased from Gibico) and mixed and inverted 5 times.

[00242] c. 25 μ L of 0.2x TF1 (transfection reagent, purchased from Sino Biological) was added dropwise to 200 μ L of Grace's Medium and mixed gently.

[00243] d. Mixed b and c. Incubated at room temperature for 15-45 min.

[00244] e. During the incubation of DNA with cellfectin (purchased from Sino Biological), the cell supernatant was discarded and 0.8 mL of Grace Medium (serum additive free) was added into the dish.

[00245] f. The incubated mixture of DNA and transfection reagent of d was added to the dish dropwise.

[00246] g. Incubated at 27°C for 2 hr.

[00247] h. Cell culture medium was discarded and 2.5mL complete growth media /dish (SCD6 SF + 10% FBS) (SCD6 SF was purchased from Sino Biological, FBS was purchased from Gibico) was added.

[00248] i. Culture was performed at 27°C for 7 days and whether the viral infection occurred was observed.

[00249] Virus supernatant was collected after visible lesions were observed in the transfected cells, typically after 7-11 days of culturing. The viral supernatant, i.e., the P1 generation virus strain of HPV6 L1: 33C is collected aseptically with a pipette. SF9 cells, at a density of 2×10^6 cells/mL, were infected using P1 generation virus strain of HPV6 L1: 33C at a ratio of 1: 50 (V/V), cultured at 27°C for 3 days, and centrifuged at $1000g \pm 200g$ for 10 min at room temperature. The collected virus supernatant was the P2 generation virus and could be used for infecting the host cells and production.

[00250] Example 2.2: Packaging of HPV11 L1: 33C recombinant baculovirus

[00251] The experimental methods and procedures were the same as in Example 2.1.

[00252] Example 2.3: Packaging of HPV 16L1: 33C recombinant baculovirus

[00253] The experimental methods and procedures are the same as those of Example 2.1.

[00254] Example 2.4: Packaging of HPV18 L1: 33C recombinant baculovirus

[00255] The experimental methods and procedures are the same as those of Example 2.1.

[00256] Example 2.5: Packaging of HPV31 L1: 33C recombinant baculovirus

[00257] The experimental methods and procedures are the same as those of Example 2.1.

5 **[00258]** Example 2.6: Packaging of HPV33 L1 recombinant baculovirus

[00259] The experimental methods and procedures are the same as those of Example 2.1.

[00260] Example 2.7: Packaging of HPV35 L1: 33C recombinant baculovirus

10 **[00261]** The experimental methods and procedures are the same as those of Example 2.1.

[00262] Example 2.8: Packaging of HPV39 L1: 59C recombinant baculovirus

15 **[00263]** The experimental methods and procedures are the same as those of Example 2.1.

[00264] Example 2.9: Packaging of HPV45 L1: 33C recombinant baculovirus

[00265] The experimental methods and procedures are the same as those of Example 2.1.

20

[00266] Example 2.10: Packaging of HPV51 L1: 33C recombinant baculovirus

[00267] The experimental methods and procedures are the same as those of Example 2.1.

[00268] Example 2.11: Packaging of HPV52 L1: 33C recombinant baculovirus

[00269] The experimental methods and procedures are the same as those of Example 2.1.

5

[00270] Example 2.12: Packaging of HPV56 L1: 33C recombinant baculovirus

[00271] The experimental methods and procedures are the same as those of Example 2.1.

10

[00272] Example 2.13: Packaging of HPV58 L1: 33C recombinant baculovirus

[00273] The experimental methods and procedures were the same as those of Example 2.1.

[00274] Example 2.14: Packaging of HPV59 L1 recombinant baculovirus

15

[00275] The experimental methods and procedures were the same as those of Example 2.1.

[00276] **Example 3 Expression of chimeric proteins or proteins**

20

[00277] Example 3.1: Expression of HPV6 L1: 33C

[00278] High Five cells were infected with baculovirus containing the HPV6 L1: 33C recombinant gene obtained in Example 2 at a ratio of 1: 200 (V/V), and the cell precipitate was collected by centrifugation at $1000g \pm 100g$ at room

temperature. The cells were broken up by sonication at low temperature for 3 min, centrifuged at >10,000g for 10 min and the supernatant was collected for SDS-PAGE. Lane 1: Marker (The marker is a mixture of 7 purified proteins with molecular weights ranging from 14.4 kDa to 116 kDa, produced by Thermo Scientific); Lane 2: cell lysate; Lane 3: supernatant of the lysate collected by centrifugation.

[00279] The result is shown in **Figure 1A**. The HPV6 L1: 33C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

[00280] Example 3.2: Expression and production of HPV11 L1: 33C

[00281] The experimental methods and procedures were the same as in Example 3.1.

[00282] The result is shown in **Figure 1B**. The HPV11 L1: 33C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

[00283] Example 3.3: Expression and production of HPV 16L1: 33C

[00284] The experimental methods and procedures were the same as in Example 3.1.

[00285] The result is shown in **Figure 1C**. The HPV 16L1: 33C L1 protein prepared by this method has a yield of > 100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

[00286] Example 3.4: Expression and production of HPV18 L1: 33C

[00287] The experimental methods and procedures were the same as in Example 3.1.

5 [00288] The result is shown in **Figure 1D**. The HPV18 L1: 33C L1 protein prepared method has a yield of > 100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

[00289] Example 3.5: Expression and production of HPV31 L1: 33C

10 [00290] The experimental methods and procedures were the same as in Example 3.1.

[00291] The result is shown in **Figure 1E**. The HPV31 L1: 33C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

15

[00292] Example 3.6: Expression and production of HPV33 L1

[00293] The experimental methods and procedures were the same as in Example 3.1.

[00294] The result is shown in **Figure 1F**. The HPV33 L1 L1 protein prepared
20 by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

[00295] Example 3.7: Expression and production of HPV35 L1: 33C

[00296] The experimental methods and procedures were the same as in Example 3.1.

[00297] The result is shown in **Figure 1G**. The HPV35 L1: 33C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

[00298] Example 3.8: Expression and production of HPV39 L1: 59C

[00299] The experimental methods and procedures were the same as in Example 3.1.

[00300] The result is shown in **Figure 1H**. The HPV39 L1: 59C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

[00301] Example 3.9: Expression and production of HPV45 L1: 33C

[00302] The experimental methods and procedures were the same as in Example 3.1.

[00303] The result is shown in **Figure 1I**. The HPV45 L1: 33C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

[00304] Example 3.10: Expression and production of HPV51 L1: 33C

[00305] The experimental methods and procedures were the same as in Example 3.1.

[00306] The result is shown in **Figure 1J**. The HPV51 L1: 33C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

5 [00307] Example 3.11: Expression and production of HPV52 L1: 33C

[00308] The experimental methods and procedures were the same as in Example 3.1.

[00309] The result is shown in **Figure 1K**. The HPV52 L1: 33C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of
10 approximate 56 KD, which can be used for mass production.

[00310] Example 3.12: Expression and production of HPV56 L1: 33C

[00311] The experimental methods and procedures were the same as in Example 3.1.

15 [00312] The result is shown in **Figure 1L**. The HPV56 L1: 33C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

[00313] Example 3.13: Expression and production of HPV58 L1: 33C

20 [00314] The experimental methods and procedures were the same as in Example 3.1.

[00315] The result is shown in **Figure 1M**. The HPV58 L1: 33C L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56 KD, which can be used for mass production.

5 [00316] Example 3.14: Expression and production of HPV59 L1

[00317] The experimental methods and procedures were the same as in Example 3.1.

[00318] The result is shown in **Figure 1N**. The HPV59 L1 L1 protein prepared by this method has a yield of >100 mg/L and a protein size of approximate 56
10 KD, which can be used for mass production.

[00319] **Example 4 Preparation of purified virus-like particles**

[00320] Example 4.1: Preparation of purified HPV6 L1: 33C virus-like particles

15 [00321] The HPV6 L1: 33C virus-like particles were purified by a two-step chromatography method, i.e. HS-MMA method, the supernatant collected in Example 3 was purified, and finally, high purity virus-like particles were obtained.

[00322] First step chromatography:

20 [00323] Medium: POROS® 50 HS strong cation exchange media produced by Thermo Fisher was used.

[00324] Medium volume: 150 mL of media volume, 30 mL/min of linear flow rate.

[00325] Chromatography conditions: equilibration buffer (pH 6.2, the salt concentration is 50 mM phosphate, 0.5 M NaCl); wash buffer (the salt concentration is 50 mM phosphate, 0.75 M NaCl, pH 6.2).

[00326] The chromatography column was first equilibrated with 5 CV of equilibration buffer and then the sample was loaded. After loading, the column was then eluted with 5 CV of equilibration buffer and wash buffer, respectively, to remove the protein impurities.

[00327] Elution conditions: a 50 mM phosphate buffer containing 50 mM arginine hydrochloride, pH 6.2, with an elution salt concentration being of 1.25 M NaCl, was used.

[00328] Second step chromatography.

[00329] Medium: MMA ion exchange media produced by Bestchrom (Shanghai) Biosciences Co., Ltd was used.

[00330] Medium volume: media volume is 150 mL, while linear flow rate is 30 mL/min.

[00331] Chromatography conditions: equilibration buffer: 50 mM PB, 1.25 M NaCl, pH 6.2. The chromatography column was first equilibrated with 4 CV equilibration buffer and then the sample was loaded. After loading, protein impurities were rinsed off with 5 CV equilibration buffer and then the target protein was eluted with elution buffer and collected.

[00332] Elution conditions: 100 mM NaAC, 150 mM NaCl, 0.01% Tween 80, pH 4.5.

[00333] Example 4.2: Preparation of purified HPV11 L1: 33C virus-like particles

[00334] The experimental methods and procedures were the same as in Example 4.1.

5 **[00335]** Example 4.3: Purification and preparation of HPV 16L1: 33C virus-like particles

[00336] The experimental methods and procedures were the same as in Example 4.1.

10 **[00337]** Example 4.4: Purification and preparation of HPV18 L1: 33C virus-like particles

[00338] The experimental methods and procedures were the same as in Example 4.1.

15 **[00339]** Example 4.5: Purification and preparation of HPV31 L1: 33C virus-like particles

[00340] The experimental methods and procedures were the same as in Example 4.1.

20 **[00341]** Example 4.6: Preparation of purified HPV33 L1 virus-like particles

[00342] The experimental methods and procedures were the same as in Example 4.1.

[00343] Example 4.7: Preparation of purified HPV35 L1: 33C virus-like particles

[00344] The experimental methods and procedures were the same as in Example 4.1.

5 **[00345]** Example 4.8: Preparation of purified HPV39 L1: 59C virus-like particles

[00346] The experimental methods and procedures were the same as in Example 4.1.

[00347] Example 4.9: Preparation of purified HPV45 L1: 33C virus-like particles

10 **[00348]** The experimental methods and procedures were the same as in Example 4.1.

[00349] Example 4.10: Preparation of purified HPV51 L1: 33C virus-like particles

15 **[00350]** The experimental methods and procedures were the same as in Example 4.1.

[00351] Example 4.11: Preparation of purified HPV52 L1: 33C virus-like particles

20 **[00352]** The experimental methods and procedures were the same as in Example 4.1.

[00353] Example 4.12: Preparation of purified HPV56 L1: 33C virus-like particles

[00354] The experimental methods and procedures were the same as in Example 4.1.

5

[00355] Example 4.13: Preparation of purified HPV58 L1: 33C virus-like particles

[00356] The experimental methods and procedures were the same as in Example 4.1.

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[00357] Example 4.14: Preparation of purified HPV59 L1 virus-like particles

[00358] The experimental methods and procedures were the same as in Example 4.1.

15

[00359] Example 5 Morphological observation of virus-like particles

[00360] Example 5.1: Morphological observation of HPV6 L1: 33C virus-like particles

20

[00361] 10 μ L sample was taken for transmission electron microscopy. The sample was fixed onto a carbon coated copper grid for 2 min, the rest liquid was absorbed off with filter paper, and then stained twice with phosphotungstic acid (Beijing Electron Microscopy China Technology Co., Ltd., concentration 2%, pH 6.5) for 30 seconds each time, the rest staining solution was absorbed off with

filter paper, left the sample for drying, then performed transmission electron microscopy observation. The transmission electron microscope (Brand: Hitachi, Model No.: H-7650) was 80KV with a magnification of 80,000x.

[00362] The electron microscopy observation is shown in **Figure 2A**. As can be seen in **Figure 2A**, the C-terminal-modified HPV6 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00363] Example 5.2: Morphological observation of HPV11 L1: 33C virus-like particles

[00364] The experimental methods and procedures were the same as in Example 5.1.

[00365] The electron microscopy observation is shown in **Figure 2B**. As can be seen in **Figure 2B**, the C-terminal-modified HPV11 L1: 33C can form uniform-sized virus-like particles, with an average diameter of approximate 60 nm.

[00366] Example 5.3: Morphological observation of HPV 16L1: 33C virus-like particles

[00367] The experimental methods and procedures were the same as in Example 5.1.

[00368] The electron microscopy observation is shown in **Figure 2C**. As can be seen in **Figure 2C**, the C-terminal-modified HPV 16L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00369] Example 5.4: Morphological observation of HPV18 L1: 33C virus-like particles

[00370] The experimental methods and procedures were the same as in Example 5.1.

5 [00371] The electron microscopy observation is shown in **Figure 2D**. As can be seen in **Figure 2D**, the C-terminal-modified HPV18 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

10 [00372] Example 5.5: Morphological observation of HPV31 L1: 33C virus-like particles

[00373] The experimental methods and procedures were the same as in Example 5.1.

15 [00374] The electron microscopy observation is shown in **Figure 2E**. As can be seen in **Figure 2E**, the C-terminal-modified HPV31 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00375] Example 5.6: Morphological observation of HPV33 L1 virus-like particles

20 [00376] The experimental methods and procedures were the same as in Example 5.1.

[00377] The electron microscopy observation is shown in **Figure 2F**. As can be seen in **Figure 2F**, the C-terminally modified HPV33 L1 can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00378] Example 5.7: Morphological observation of HPV35 L1: 33C virus-like particles

[00379] The experimental methods and procedures were the same as in Example 5.1.

[00380] The electron microscopy observation is shown in **Figure 2G**. As can be seen in **Figure 2G**, the C-terminally modified HPV35 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00381] Example 5.8: Morphological observation of HPV39 L1: 59C virus-like particles

[00382] The experimental methods and procedures were the same as in Example 5.1.

[00383] The electron microscopy observation is shown in **Figure 2H**. As can be seen in **Figure 2H**, the C-terminal-modified HPV39 L1: 59C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00384] Example 5.9: Morphological observation of HPV45 L1: 33C virus-like particles

[00385] The experimental methods and procedures were the same as in Example 5.1.

[00386] The electron microscopy observation is shown in **Figure 2I**. As can be seen in **Figure 2I**, the C-terminal-modified HPV45 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

5 [00387] Example 5.10: Morphological observation of HPV51 L1: 33C virus-like particles

[00388] The experimental methods and procedures were the same as in Example 5.1.

10 [00389] The electron microscopy observation is shown in **Figure 2J**. As can be seen in **Figure 2J**, the C-terminal-modified HPV51 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00390] Example 5.11: Morphological observation of HPV52 L1: 33C virus-like particles

15 [00391] The experimental methods and procedures were the same as in Example 5.1.

[00392] The electron microscopy observation is shown in **Figure 2K**. As can be seen in **Figure 2K**, the C-terminal-modified HPV52 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

20 [00393] Example 5.12: Morphological observation of HPV56 L1: 33C virus-like particles

[00394] The experimental methods and procedures were the same as in Example 5.1.

[00395] The electron microscopy observation is shown in **Figure 2L**. As can be seen in **Figure 2L**, the C-terminal-modified HPV56 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00396] Example 5.13: Morphological observation of HPV58 L1: 33C virus-like particles

[00397] The experimental methods and procedures were the same as in Example 5.1.

[00398] The electron microscopy observation is shown in **Figure 2M**. As can be seen in **Figure 2M**, the C-terminal-modified HPV58 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00399] Example 5.14: Morphological observation of HPV59 L1 virus-like particles

[00400] The experimental methods and procedures were the same as in Example 5.1.

[00401] The electron microscopy observation is shown in **Figure 2N**. As can be seen in **Figure 2N**, the C-terminal-modified HPV59 L1 : 33C

[00402] can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00403] Example 6 Immunogenicity evaluation of virus-like particles in animals

[00404] Example 6.1: Immunogenicity evaluation of HPV6 L1: 33C virus-like particles in animals

[00405] 6.1.1 Modeling of pseudovirus-neutralizing cells

[00406] As HPV is difficult to be cultured *in vitro* and has a strong host specificity, it is difficult to be reproduced in organisms other than humans, thus there is a lack of suitable animal models. Therefore, there is a need to establish suitable and effective *in vitro* neutralization experimental models for the evaluation of vaccine immunoprotectivity.

[00407] HPV pseudovirus is an ideal model for HPV *in vitro* neutralization: Thanks to the HPV VLP's characteristic of non-specifically encapsulating nucleic acids, HPV pseudovirus can be formed from the VLPs, composed of HPV L1 and L2 expressed in cells, by encapsulating free DNA or introducing exogenous plasmid.

[00408] The immunogenicity of immunized animal serum samples was analyzed by pseudovirus neutralization assay. The animal immunized with HPV6 virus-like particles can produce neutralizing antibodies against HPV6 which can neutralize HPV6 pseudovirus. When the immunized animal serum is incubated with a certain amount of pseudovirus and then infects cells, the number of cells capable of expressing GFP fluorescence decreases when neutralization antibodies in the serum increases, showing a linear negative correlation in a certain range,

so the neutralizing activity of antibodies in the serum can be evaluated by detecting the change in the number of cells expressing GFP.

[00409] Construction method of pseudovirus: The HPV6 pCMV3-3-HPV6 L1+L2 (L1 sequence was from Uniprot P69898, L2 sequence was from Uniprot Q84297) plasmid (purchased from Sino Biological) and the fluorescent plasmid (PSEU-GFP Spark, purchased from Sino Biological) were co-transfected into 293FT adherent cells (purchased from Thermo Fisher). The specific methods refer to the published literature (Pastrana D V, Buck C B, Pang Y S, Thompson C D, Castle P E, FitzGerald P C, Kjaer S K, Lowy D R, Schiller J T. Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papilloma virus neutralization assay for HPV16 and HPV18. [J] Virology 2004,321:205-216.). The pseudovirus supernatant was collected and aliquoted, and stored in a -80°C refrigerator for stock.

[00410] 6.1.2 Immunoprotective evaluation of HPV6 L1: 33C virus-like particles in animals

[00411] Immunization procedures in mice:

[00412] HPV6 L1: 33C virus-like particles were absorbed onto aluminum phosphate adjuvant, mixed, and used to immunize mice at a dose of 0.15 µg/200 µL per mouse, 10 mice in total. The mice were immunized with the diluted samples on Days 0, 7 and 21, with control mice immunized with blank serum. Blood was collected from the eyes of the mice on Day 28 and the sera were isolated for pseudovirus neutralization titers assay.

[00413] EC50 assay of mice:

[00414] The murine serum was inactivated at 56°C for 30 minutes, centrifuged at 6000g, 5 mins, and the supernatant was collected for assaying. 4-8 hours prior to the assay, 293FT cells were inoculated at a density of 15,000 cells/well into 96-well plates and incubated at 37°C in a CO₂ incubator with 5% CO₂. The post-immune murine serum and blank control serum were serial diluted with neutralizing media respectively, then mixed with the HPV6 pseudovirus prepared in 6.1 at a volume ratio of 1:1, incubated at 2-8°C for 1 hrs. , then 100 µL/well of the mixture were added to 293FT cells, which had been inoculated for 4-8 hrs in advance. Each sample was in replicate, and blank serum control group, pseudovirus positive control group and pseudovirus negative control group were used. The cells infected by pseudovirus were incubated at 37°C in a CO₂ incubator with 5% CO₂ for 62-96 hrs , fluorescence scanning photographed and counted in an ELISPOT analyser (Model No.: S6 Universal-V Analyzer, Manufacturer: CTL). On the basis of the neutralization inhibition of each murine serum sample, the maximum dilution of the serum at 50% neutralization inhibition was calculated for each murine serum sample according to the Reed-Muench method, i.e. the half efficacy dilution EC₅₀.

[00415] The results of the HPV6 serum pseudovirus neutralization titer assay are detailed in **Table 4**.

[00416] **Table 4 EC₅₀ of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

HPV Type	EC ₅₀ Value
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Type 6	4948±1831
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[00417] Notes:

[00418] 1. number of animals, N = 10.

[00419] 2. GMT (Geometric Mean Titer): geometric mean titer.

[00420] 3. SEM (Standard Error of Mean): standard error.

5 [00421] The above evaluation results show that the HPV6 L1: 33C virus-like particles prepared by the present invention have good immunogenicity and can produce neutralizing antibodies with high titers in animals, which can be used to prepare into a vaccine for preventing HPV infections.

10 [00422] Example 6.2: Evaluation of immunogenicity of HPV11 L1: 33C virus-like particles in animals

[00423] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P04012 and the L2 sequence was from Uniprot P04013.

15 [00424] The results of HPV11 serum pseudovirus neutralization titer assay are detailed in **Table 5**.

[00425] **Table 5 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00426]

HPV Type	EC ₅₀ Value
Type 11	15024±8400

[00427] The above evaluation results show that the HPV11 L1: 33C virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for the prevention of HPV infection.

5

[00428] Example 6.3: Evaluation of immunogenicity of HPV 16L1: 33C virus-like particles in animals

[00429] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P03101 and the L2 sequence was from Uniprot P03107.

10

[00430] The results of HPV16 serum pseudovirus neutralization titer assay are detailed in **Table 6**.

[00431] **Table 6 EC₅₀ of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00432]

HPV Type	EC ₅₀ Value
Type 16	32449±7224

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[00433] The above evaluation results show that the HPV 16L1: 33C virus-like particles prepared by the present invention have good immunogenicity and can produce high titers of neutralizing antibodies in animals, which can be used to prepare into a vaccine for the prevention of HPV infection.

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[00434] Example 6.4: Evaluation of immunogenicity of HPV18 L1: 33C virus-like particles in animals

[00435] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot Q80B70 and the L2 sequence was from Uniprot P06793.

[00436] The results of HPV18 serum pseudovirus neutralization titer assay are detailed in **Table 7**.

[00437] **Table 7 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00438]

HPV Type	EC ₅₀ Value
Type 18	18480±4051

[00439] The above evaluation results show that the HPV18 L1: 33C virus-like particles **prepared** by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for preventing HPV infection.

[00440] Example 6.5: Evaluation of immunogenicity of HPV31 L1: 33C virus-like particles in animals

[00441] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P17388 and the L2 sequence was from Uniprot P17389.

[00442] The results of HPV31 serum pseudovirus neutralization titer assay are detailed in **Table 8**.

[00443] **Table 8 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00444]

HPV Type	EC ₅₀ Value
Type 31	5210±1147

[00445] The above evaluation results show that the HPV31 L1: 33C virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for preventing HPV infection.

[00446] Example 6.6: Evaluation of immunogenicity of HPV33 L1 virus-like particles in animals

[00447] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P06416 and the L2 sequence was from Uniprot P06418.

[00448] The results of HPV33 serum pseudovirus neutralization titer assay are detailed in **Table 9**.

[00449] **Table 9 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00450]

HPV Type	EC ₅₀ Value
Type 33	4666±1555

[00451] The above evaluation results show that the HPV33 L1 virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for preventing HPV infection.

[00452] Example 6.7: Evaluation of immunogenicity of HPV35 L1: 33C virus-like particles in animals

[00453] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P27232 and the L2 sequence was from Uniprot P27234.

[00454] The results of HPV35 serum pseudovirus neutralization titer assay are detailed in **Table 10**.

[00455] **Table 10 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00456]

HPV Type	EC ₅₀ Value
Type 35	2293±1448

[00457] The above evaluation results show that the HPV35 L1: 33C virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for preventing HPV infection.

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[00458] Example 6.8: Immunogenicity evaluation of HPV39 L1: 59C virus-like particles in animals

[00459] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P24838 and the L2 sequence was from Uniprot P24839.

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[00460] The results of HPV39 serum pseudovirus neutralization titer assay are detailed in **Table 11**.

[00461] **Table 11 EC₅₀ of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00462]

HPV Type	EC ₅₀ Value
Type 39	25526±5857

[00463] The above evaluation results show that the HPV39 L1: 59C virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for preventing HPV infection.

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[00464] Example 6.9: Immunogenicity evaluation of HPV45 L1: 33C virus-like particles in animals

[00465] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P36741 and the L2 sequence was from Uniprot P36761.

[00466] The results of HPV45 serum pseudovirus neutralization titer assay are detailed in Table 12.

[00467] Table 12 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)

[00468]

HPV Type	EC ₅₀ Value
Type 45	755±935

[00469] The above evaluation results show that the HPV45 L1: 33C virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for preventing HPV infection.

[00470] Example 6.10: Evaluation of immunogenicity of HPV51 L1: 33C virus-like particles in animals

[00471] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P26536 and the L2 sequence was from Uniprot P26539.

[00472] The results of HPV51 serum pseudovirus neutralization titer assay are detailed in **Table 13**.

[00473] **Table 13 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00474]

HPV Type	EC ₅₀ Value
Type 51	5528±1572

[00475] The above evaluation results show that the HPV51 L1: 33C virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for preventing HPV infection.

[00476] Example 6.11: Evaluation of immunogenicity of HPV52 L1: 33C virus-like particles in animals

[00477] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot Q05138 and the L2 sequence was from Uniprot F8S4U2.

[00478] The results of HPV52 serum pseudovirus neutralization titer assay are detailed in **Table 14**.

[00479] **Table 14 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00480]

HPV Type	EC ₅₀ Value
Type 52	19019±8604

[00481] The above evaluation results show that the HPV52 L1: 33C virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for preventing HPV infection.

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[00482] Example 6.12: Evaluation of immunogenicity of HPV56 L1: 33C virus-like particles in animals

[00483] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P36743 and the L2 sequence was from Uniprot P36765.

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[00484] The results of HPV56 serum pseudovirus neutralization titer assay are detailed in **Table 15**.

[00485] **Table 15 EC₅₀ of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00486]

HPV Type	EC ₅₀ Value
Type 56	2497±612

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[00487] The above evaluation results show that the HPV56 L1: 33C virus-like particles prepared by the present invention have good immunogenicity, can

produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for preventing HPV infection.

[00488] Example 6.13: Evaluation of immunogenicity of HPV58 L1: 33C virus-like particles in animals

[00489] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot P26535 and the L2 sequence was from Uniprot B6ZB12.

[00490] The results of HPV58 serum pseudovirus neutralization titer assay are detailed in Table 16.

[00491] Table 16 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)

[00492]

HPV Type	EC ₅₀ Value
Type 58	19939±8459

[00493] The above evaluation results show that the HPV58 L1: 33C virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for the prevention of HPV infection.

[00494] Example 6.14: Evaluation of immunogenicity of HPV59 L1 virus-like particles in animals

[00495] The experimental methods and procedures were the same as in Example 6.1. The L1 sequence was from Uniprot Q81971 and the L2 sequence was from Uniprot Q81970.

[00496] The results of HPV59 serum pseudovirus neutralization titer assay are detailed in **Table 17**.

[00497] **Table 17 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00498]

HPV Type	EC ₅₀ Value
Type 59	1344±335

[00499] The above evaluation results show that the HPV59 L1 virus-like particles prepared by the present invention have good immunogenicity, can produce high titers of neutralizing antibodies in animals, and can be used to prepare into a vaccine for the prevention of HPV infection.

[00500] Example 6.15: Immunogenicity evaluation of 14-valent immunogenic composition of virus-like particles in mice

[00501] Immunization procedures in mice:

[00502] A sample consisted of the aforementioned virus-like particles of 14 types (HPV Types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) were diluted with aluminum phosphate adjuvant sterilely (for example, the HPV types which were of 20 µg in the sample, were diluted 80-fold to 0.5 µg/mL, the concentration of other HPV types in the samples being of different ration varied accordingly),

200 μ L of the diluted virus-like particle sample was used to immunize each mouse, and the doses are shown in **Table 18**. SPF-grade female Balb/c mice aged 6-8 weeks were divided into groups of 10 mice in each group (experimental group and control group), and were immunized with the diluted samples on Day 0, 7 and 21 respectively, with control mice immunized with blank serum. Blood was collected from the eyes of mice on Day 28, and the sera were isolated for pseudovirus neutralization titers assay.

[00503] Table 18 Doses of Each HPV Types in the Immunogenic Composition for Immunization in Each Mouse (μ g)

[00504]

HPV Type	6	11	16	18	31	33	45	52	58	35	39	51	56	59
Dose	0.15	0.2	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[00505] Serum neutralization titer assay in immunized mice by pseudovirus neutralization assay:

[00506] The murine serum was inactivated at 56°C for 30 minutes, centrifuged at 6000g, 5 mins, and the supernatant was collected for assaying. 4-8 hours prior to the assay, 293FT cells were inoculated at a density of 15,000 cells/well into 96-well plates and incubated at 37°C in a CO₂ incubator with 5% CO₂. The immunized murine serum and blank control serum were serial diluted with neutralizing media respectively, then mixed with the diluted 14 types of pseudovirus samples (the 14 types of HPV pseudovirus are those of HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59, prepared in **Examples 6.1-**

6.14) at a volume ratio of 1:1, incubated at 2-8°C for 1 h, then 100 µL/well of the mixture were added to 293FT cells, which had been inoculated for 4-8 h in advance. Each sample was in replicate; and blank serum control group, pseudovirus positive control group and pseudovirus negative control group were used. The cells infected by pseudovirus were incubated at 37°C in a CO₂ incubator with 5% CO₂ for 62-96 h, fluorescence scanning photographed and counted in an ELISPOT analyser (Model No.: S6 Universal-V Analyzer, Manufacturer: CTL). On the basis of the neutralization inhibition of each murine serum sample, the maximum dilution of the serum at 50% neutralization inhibition was calculated for each murine serum sample according to the Reed-Muench method, i.e. the half efficacy dilution EC₅₀. The results are shown in **Figure 3**.

[00507] As demonstrated **Figure 3**, the 14-valent immunogenic composition of virus-like particles can produce high titers of neutralizing antibodies in animals, and can be used to prepare a vaccine for the prevention of HPV infection.

[00508] Comparative Example 1: Expression of C-terminal truncated HPV16 L1 (aa 1-474)

[00509] The inventors attempted to truncate the C-terminus of HPV16 L1 by 31 amino acids and named it HPV16 L1 (1-474) (SEQ ID NO: 27). However, it was found in the study that the truncated HPV16 L1 (1-474) protein was highly expressed but has very poor solubility, and is difficult to extract and purify, the detailed results of expression and extraction are shown in **Figure 4**.

[00510] Although the present invention has been described in detail by way of illustration and embodiments in the foregoing, it is intended to facilitate understanding. It is obvious to those skilled in the art that various modifications and improvements can be made to the technical solutions of the present invention are apparently possible for those of ordinary skill in the art without deviating from the spirit or scope of the attached claims.

Appendix 1 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 6 L1 PROTEIN

SEQ ID No: 1	0601The amino acid sequence of HPV type 6 L1 protein aa 1-469 MWRPSDSTVYVPPNPVSKVVATDAYVTRTNIFYHASSSRL LAVGHPYFSIKRA NKTVPKVS GYQYRVFKVVLDPDNKFALPDSSLFDPTTQRLVWACTGLEVGRG QPLGVGVSGHPFLNKYDDVENS GSGGNPGQDNRVNVGMDYKQTQLCMVGCA PPLGEHWGKGKQCTNTPVQAGDCPPELITSVIQDGDMDVTGFGAMNFADLQT NKSDVPIDICGTTCKYPDYLQMAADPYGDRLFFFLRKEQMFARHFFNRAGEVG EPVPDTLIIKSGNRTSVGSSIIYVNTPSGSLVSSEAQLFNKPYWLQKAQGHNNGI CWGNQLFVTVDTTTRSTNMTLCASVTTSSSTYTNSDYKEYMRHVEEYDLQFIFQ LCSITLSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTLED TYRYVQSQAITCQKP TPEKEKPD PYKNLSFWEVNLKEKFSSELDQYPLGRKFLLQSGY
SEQ ID No: 2	0602 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 3	0603 Amino acid sequence of chimeric HPV type 6 L1 protein MWRPSDSTVYVPPNPVSKVVATDAYVTRTNIFYHASSSRL LAVGHPYFSIKRA NKTVPKVS GYQYRVFKVVLDPDNKFALPDSSLFDPTTQRLVWACTGLEVGRG QPLGVGVSGHPFLNKYDDVENS GSGGNPGQDNRVNVGMDYKQTQLCMVGCA PPLGEHWGKGKQCTNTPVQAGDCPPELITSVIQDGDMDVTGFGAMNFADLQT NKSDVPIDICGTTCKYPDYLQMAADPYGDRLFFFLRKEQMFARHFFNRAGEVG EPVPDTLIIKSGNRTSVGSSIIYVNTPSGSLVSSEAQLFNKPYWLQKAQGHNNGI CWGNQLFVTVDTTTRSTNMTLCASVTTSSSTYTNSDYKEYMRHVEEYDLQFIFQ LCSITLSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTLED TYRYVQSQAITCQKP TPEKEKPD PYKNLSFWEVNLKEKFSSELDQYPLGRKFLLQSGYKAKPKLKRAA

	PTSTRTSSAKRKKVKK
SEQ ID No: 4	0604 Nucleotide sequence of chimeric HPV type 6 L1 protein ATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGCTACAGA TGCCTATGTGACCAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGACACCCATA CTTCAGCATCAAGAGGGCTAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGTGTTC AAGG TGGTGCTGCCTGACCCAAACAAGTTTGCCCTGCCTGACTCCTCCCTGTTTGACCCAAACCACCCAGAGACTGG TGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGACACCCATTC CTGAACAAATATGATGATGTGGAGAACTCTGGCTCTGGAGGCAACCCTGGACAAGACAACAGGGTGAATG TGGGGATGGACTACAAGCAGACCCAACTTTGTATGGTGGGCTGTGCCCCCTCCACTGGGAGAACACTGGGGC AAGGGCAAGCAGTGTACCAACACACCTGTCCAGGCTGGAGACTGTCCTCCATTGGAAGTATTACCTCTGT GATTTCAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACTTTGCTGACCTCCAAACCAACAAGT CTGATGTGCCAATTGACATCTGTGGCACCATTGTAAATACCCTGACTACCTCCAAATGGCTGCTGACCCAT ATGGAGACAGACTGTTCTTCTCCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAGGGCTGGA GAGGTGGGAGAACCTGTGCCTGACACCCTGATTATCAAGGGCTCTGGCAACAGGACCTCTGTGGGCTCCAG CATCTATGTGAACACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAAGCCATACTGGCT CCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTTGTGACAGTGGTGGACACCA CCAGGAGCACCAATATGACCCTGTGTGCCTCTGTGACCACCTCCAGCACCTACACCAACTCTGACTACAAG GAATATATGAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGTAGCATCACCTGTCTGCT GAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGAACCTTTGGACTGAGCCCTCC TCCAAATGGCACCTTGGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTGAGAAGCCAACAC CTGAGAAGGAGAAGCCTGACCCATACAAGAACCTGTCCTTCTGGGAGGTGAACCTGAAAGAGAAGTTCTCC TCTGAACTGGACCAATACCCACTGGGCAGGAAGTTCCTGCTCCAATCTGGCTACAAAGCCAAGCCAAAAC GAAAAGGGCTGCCCCAACCAAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAGTAA
SEQ ID No: 5	0605 Synthetic HPV6 L1 gene

	ctgggtaccATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGGC TACAGATGCCTATGTGACCAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGAC ACCCATACTTCAGCATCAAGAGGGCTAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGTG TTCAAGGTGGTGTGCTGCCTGACCCAAACAAGTTTGCCCTGCCTGACTCCTCCCTGTTTGACCCAACCACCCAG AGACTGGTGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGAC ACCCATTCTGAACAAATATGATGATGTGGAGAACTCTGGCTCTGGAGGCAACCCTGGACAAGACAACAGG GTGAATGTGGGGATGGACTACAAGCAGACCCAACTTTGTATGGTGGGCTGTGCCCTCCACTGGGAGAACA CTGGGGCAAGGGCAAGCAGTGTACCAACACACCTGTCCAGGCTGGAGACTGTCCTCCATTGGAAGTGATTA CCTCTGTGATTACAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACTTTGCTGACCTCCAAACC AACAAGTCTGATGTGCCAATTGACATCTGTGGCACCCTTGTAATACCCTGACTACCTCCAAATGGCTGCT GACCCATATGGAGACAGACTGTTCTTCTCCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAG GGCTGGAGAGGTGGGAGAACCTGTGCCTGACACCCTGATTATCAAGGGCTCTGGCAACAGGACCTCTGTGG GCTCCAGCATCTATGTGAACACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAAGCCAT ACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTGTGACAGTGGTG GACACCACCAGGAGACCAATATGACCCTGTGTGCCTCTGTGACCACCTCCAGCACCTACACCAACTCTGA CTACAAGGAATATATGAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGTAGCATCACCT GTCTGCTGAGGTGATGGCTTACATCCACACAATGAACCATCTGTGTTGGAGGACTGGAACCTTTGGACTGA GCCCTCCTCCAAATGGCACCTTGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTCAGAAG CCAACACCTGAGAAGGAGAAGCCTGACCCATAACAAGAACCTGTCCTTCTGGGAGGTGAACCTGAAAGAGA AGTTCTCCTCTGAACTGGACCAATACCACTGGGCAGGAAGTTCCTGCTCCAATCTGGCTACAGGGGCAGG TCCAGCATCAGGACAGGAGTGAAGAGACCTGCTGTGAGCAAGGCATCTGCTGCCCCAAGAGGAAGAGGG CTAAGACCAAGAGGTAAActcgagctc
SEQ ID No: 6	0606 Synthetic HPV33 L1 gene ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG

	ACACCCATACTTCAGCATCAAGAACCCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTC AACAAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAAGTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 7	0607 HPV6 L1 F1 CTTGGTACCATGTGGAGACCATCTGACAGCACAGT
SEQ ID No: 8	0608 HPV6 L1 R1 GCTTGGCTTTGTAGCCAGATTGGAGCAGGAACTTCC
SEQ ID No: 9	0609 HPV6 L1 amplified sequence 1

	CTTGGTACC^[1] <u>ATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTG</u> <u>GTGGCTACAGATGCCTATGTGACCAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGA</u> <u>CTGCTGGCTGTGGGACACCCATACTTCAGCATCAAGAGGGCTAACAAGACAGTGGTGCCA</u> <u>AAGGTGTCTGGCTACCAATACAGGGTGTTC AAGGTGGTGTGCCTGACCCAAACAAGTTT</u> <u>GCCCTGCCTGACTCCTCCCTGTTTGACCCAACCACCCAGAGACTGGTGTGGGCTTGTACT</u> <u>GGATTGGAGGTGGGCAGGGGACAACCCTGGGAGTGGGAGTGTCTGGACACCCATTCTCTG</u> <u>AACAAATATGATGATGTGGAGA AACTCTGGCTCTGGAGGCAACCCTGGACAAGACAACAGG</u> <u>GTGAATGTGGGGATGGACTACAAGCAGACCCAACTTTGTATGGTGGGCTGTGCCCTCCA</u> <u>CTGGGAGA AACTGGGGCAAGGGCAAGCAGTGTACCAACACACCTGTCCAGGCTGGAGAC</u> <u>TGTCCTCCATTGGA AACTGATTACCTCTGTGATT CAGGATGGAGATATGGTGGACACAGGC</u> <u>TTTGGAGCTATGAACTTTGCTGACCTCCAAACCAACAAGTCTGATGTGCCAATTGACATC</u> <u>TGTGGCACCCTTGTA AATACCCTGACTACCTCCAAATGGCTGCTGACCCATATGGAGAC</u> <u>AGACTGTTCTTCTTCTCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAGGGCT</u> <u>GGAGAGGTGGGAGA AACTGTGCCTGACACCCTGATTATCAAGGGCTCTGGCAACAGGACC</u> <u>TCTGTGGGCTCCAGCATCTATGTGAACACACCATCTGGCTCCCTGGTGTCTCTGAGGCT</u> <u>CAACTTTTCAACAAGCCATACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGT</u> <u>TGGGGCAACCAACTTTTTGTGACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTG</u> <u>TGTGCCTCTGTGACCACCTCCAGCACCTACACCAACTCTGACTACAAGGAATATATGAGG</u> <u>CATGTGGAGGAATATGACCTCCAATTCATCTTCCA AACTTTGTAGCATCACCTGTCTGCT</u> <u>GAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGA AACTTTGGA</u> <u>CTGAGCCCTCCTCCAAATGGCACCTTGGAGGACCTACAGATATGTCCAGAGCCAGGCT</u> <u>ATCACTTGT CAGAAGCCAACACCTGAGAAGGAGAAGCCTGACCCATACAAGA AACTGTCC</u> <u>TTCTGGGAGGTGA AACTGAAAGAGAAGTTCTCCTCTGAACTGGACCAATACCCACTGGGC</u> AGGAAGTTCCTGCTCCAATCTGGCTAC^[2]AAAGCCAAGC^[3]
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	[1]: KpnI enzymatic cleavage site; [2]: the gene fragment encoding HPV6 L1 aa 1-469; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 10	0610 HPV6 L1 F2 ATCTGGCTACAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 11	0611 HPV6 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGGC
SEQ ID No: 12	0612 HPV6 L1 amplified sequence 2 <u>ATCTGGCTAC</u>^[1] <u>AAAGCCAAGCCAAACTGAAAAGGGCTGCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG</u>^[2] TAA <u>ATCTAGA</u>^[3]CAG. [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV6 L1 aa 1-469; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 13	0613 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKR VKRRKSSRK

Appendix 2 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 11 L1 PROTEIN

SEQ ID No: 14	1101 The amino acid sequence of HPV type 11 L1 protein aa 1-470 MWRPSDSTVYVPPNPVSKVVATDAYVKRTNIFYHASSRLLAVGHPYYSIKK VNKTVVPKVSGYQYRVFKVVLDPDNKFALPDSSLFDPTTQRLVWACTGLEVGR GQPLGVGVSGHPLLNKYDDVENS GGYGGNPGQDNRVNVGMDYKQTQLCMV GCAPPLGEHWGKGTQCSNTSVQNGDCPPELITSVIQDGDMDVDTGFGAMNFAD LQTNKSDVPLDICGTVCKYPDYLQMAADPYGDRLFFYLKEQM FARHFFNRA GTVGEPVPDDL LVKGGNNRSSVASSIYVHTPSGSLVSSEAQLFNKPYWLQKAQ GHNNGICWGNHLFVTVD TTRSTNMTLCASVSKSATYTNSDYKEYMRHVEEF DLQFIFQLCSITLSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTLED TYRYVQSQ AITCQKPTPEKEKQDPYKDMSFWEVNLKEKFSSELDQFPLGRKFLLQSGY
SEQ ID No: 15	1102 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTR TSSAKRKKVKK
SEQ ID No: 16	1103 Amino acid sequence of chimeric HPV type 11 L1 protein MWRPSDSTVYVPPNPVSKVVATDAYVKRTNIFYHASSRLLAVGHPYYSIKK VNKTVVPKVSGYQYRVFKVVLDPDNKFALPDSSLFDPTTQRLVWACTGLEVGR GQPLGVGVSGHPLLNKYDDVENS GGYGGNPGQDNRVNVGMDYKQTQLCMV GCAPPLGEHWGKGTQCSNTSVQNGDCPPELITSVIQDGDMDVDTGFGAMNFAD LQTNKSDVPLDICGTVCKYPDYLQMAADPYGDRLFFYLKEQM FARHFFNRA GTVGEPVPDDL LVKGGNNRSSVASSIYVHTPSGSLVSSEAQLFNKPYWLQKAQ GHNNGICWGNHLFVTVD TTRSTNMTLCASVSKSATYTNSDYKEYMRHVEEF DLQFIFQLCSITLSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTLED TYRYVQSQ AITCQKPTPEKEKQDPYKDMSFWEVNLKEKFSSELDQFPLGRKFLLQSGYKAKP

	KLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 17	1104 Nucleotide sequence of chimeric HPV type 11 L1 protein ATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGGCTACAGA TGCCTATGTGAAGAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGACACCCAT ACTACAGCATCAAGAAGGTGAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGTGTTCAA GGTGGTGCTGCCTGACCCAAACAAGTTTGCCCTGCCTGACTCCTCCCTGTTTGACCCAACCACCCAGAGACT GGTGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGACACCCA CTGCTGAACAAATATGATGATGTGGAGAACTCTGGAGGCTATGGAGGCAACCCTGGACAAGACAACAGGG TGAATGTGGGGATGGACTACAAGCAGACCCAACCTTGTATGGTGGGCTGTGCCCTCCACTGGGAGAACAC TGGGGCAAGGGCACCCAGTGTAGCAACACCTCTGTCCAGAATGGAGACTGTCTCCATTGGAAGTATTAC CTCTGTGATTCAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACTTTGCTGACCTCCAAACCA ACAAGTCTGATGTGCCACTGGACATCTGTGGCACAGTGTGTAAATACCCTGACTACCTCCAAATGGCTGCT GACCCATATGGAGACAGACTGTTCTTCTACCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAG GGCTGGCACAGTGGGAGAACCTGTGCCTGATGACCTGTGGTGAAGGGAGGCAACAACAGGTCTCTGTGG CATCCAGCATCTATGTGCATACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAAGCCAT ACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCACCTGTTTGTGACAGTGGTG GACACCACCAGGAGACCAATATGACCCTGTGTGCCTCTGTGAGCAAGTCTGCCACCTACACCAACTCTGA CTACAAGGAATATATGAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTCCAACCTTTGTAGCATCACCT GTCTGCTGAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGAACCTTGGACTGA GCCCTCCTCCAAATGGCACCTTGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTCAGAAG CCAACACCTGAGAAGGAGAAGCAGGACCCATACAAGGATATGAGTTTCTGGGAGGTGAACCTGAAAGAGA AGTTCTCCTCTGAACTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAATCTGGCTACAAAGCCAAGC CAAAACTGAAAAGGGGTGCCCAACCAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAGTA AA
SEQ ID No:	1105 Synthetic HPV11 L1 gene

18	ctgggtaccATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGGC TACAGATGCCTATGTGAAGAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGAC ACCCATACTACAGCATCAAGAAGGTGAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGT GTTCAAGGTGGTGTGCTGCCTGACCCAAACAAGTTTGCCCTGCCTGACTCCTCCCTGTTTGACCCAACCAACCA GAGACTGGTGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGA CACCCACTGCTGAACAAATATGATGATGTGGAGAACTCTGGAGGCTATGGAGGCAACCCTGGACAAGACA ACAGGGTGAATGTGGGGATGGACTACAAGCAGACCCAACCTTTGTATGGTGGGCTGTGCCCCCTCCACTGGGA GAACACTGGGGCAAGGGCACCCAGTGTAGCAACACCTCTGTCCAGAATGGAGACTGTCTCCATTGGAACCT GATTACCTCTGTGATTCAAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACCTTGTGACCTCCA AACCAACAAGTCTGATGTGCCACTGGACATCTGTGGCACAGTGTGTAATACCCTGACTACCTCCAAATGG CTGCTGACCCATATGGAGACAGACTGTTCTTCTACCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCA ACAGGGCTGGCACAGTGGGAGAACCTGTGCCTGATGACCTGCTGGTGAAGGGAGGCAACAACAGGTCTCTC TGTGGCATCCAGCATCTATGTGCATACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAA GCCATACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCACCTGTTTGTGACAG TGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTGCCTCTGTGAGCAAGTCTGCCACCTACACCAAC TCTGACTACAAGGAATATATGAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTCCAACCTTTGTAGCATC ACCCTGTCTGCTGAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGAACCTTGG ACTGAGCCCTCCTCCAAATGGCACCTTGGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTCTC AGAAGCCAACACCTGAGAAGGAGAAGCAGGACCCATACAAGGATATGAGTTTCTGGGAGGTGAACCTGAA AGAGAAGTTCTCCTCTGAACTGGACCAGTTTCCACTGGGCAGGAAGTTCTGCTCCAATCTGGCTACAGGG GCAGGACCTCTGCCAGGACAGGCATCAAGAGACCTGCTGTGAGCAAGCCAAGCACAGCCCCAAAGAGGAA GAGGACCAAGACCAAGAAGTAAActcgagctc
SEQ ID No: 19	1106 Synthetic HPV33 L1 gene ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG

	ACACCCATACTTCAGCATCAAGAACCCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTC AACAAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAAGTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 20	1107HPV11 L1 F1 CTTGGTACCATGTGGAGACCATCTGACAGCACAGT
SEQ ID No: 21	1108HPV11 L1 R1 GCTTGGCTTTGTAGCCAGATTGGAGCAGGAACTTCC
SEQ ID No:	1109 HPV11 L1 amplified sequence 1

CTTGGTACC^[1]

ATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGCTACAGA
TGCCTATGTGAAGAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGACACCCAT
ACTACAGCATCAAGAAGGTGAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGTGTTCAA
GGTGGTGCTGCCTGACCCAAACAAGTTTGCCCTGCCTGACTCCTCCCTGTTTGACCCAAACCACCCAGAGACT
GGTGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCCTGGGAGTGGGAGTGTCTGGACACCCA
CTGCTGAACAAATATGATGATGTGGAGAAGTCTGGAGGCTATGGAGGCAACCCTGGACAAGACAACAGGG
TGAATGTGGGGATGGACTACAAGCAGACCCAACTTTGTATGGTGGGCTGTGCCCTCCACTGGGAGAAACAC
TGGGGCAAGGGCACCCAGTGTAGCAACACCTCTGTCCAGAATGGAGACTGTCTCCATTGGAAGTATTAC
CTCTGTGATTCAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACTTTGCTGACCTCCAAACCA
ACAAGTCTGATGTGCCACTGGACATCTGTGGCACAGTGTGTAAATACCCTGACTACCTCCAAATGGCTGCT
GACCCATATGGAGACAGACTGTTCTTCTACCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAG
GGCTGGCACAGTGGGAGAACCTGTGCCTGATGACCTGCTGGTGAAGGGAGGCAACAACAGGTCTCTGTGG
CATCCAGCATCTATGTGCATACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAAGCCAT
ACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCACCTGTTTGTGACAGTGGTG
GACACCACCAGGAGACCAATATGACCCTGTGTGCCTCTGTGAGCAAGTCTGCCACCTACACCAACTCTGA
CTACAAGGAATATATGAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTCCAACCTTTGTAGCATCACCT
GTCTGCTGAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGAACCTTTGGACTGA
GCCCTCCTCCAAATGGCACCTTGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTCAGAAG
CCAACACCTGAGAAGGAGAAGCAGGACCCATACAAGGATATGAGTTTCTGGGAGGTGAACCTGAAAGAGA
AGTTCTCCTCTGAACTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAATCTGGCTAC^[2]AAAGCCAA

GC^[3]

[1]: Kpn I enzymatic cleavage site;

[2]: the gene fragment encoding of HPV11 L1 aa 1-470;

	[3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 23	1110HPV11 L1 F2 ATCTGGCTACAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 24	1111HPV11 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGGC
SEQ ID No: 25	1112 HPV11 L1 amplified sequence 2 <u>ATCTGGCTAC</u> ^[1] <u>AAAGCCAAGCCAAACTGAAAAGGGCTGCCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG</u> ^[2] TAA <u>ATCTAGA</u> ^[3] CAG. [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV11 L1 aa 1-470; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 26	1113 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

Appendix 3 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 16 L1 PROTEIN

SEQ ID No: 27	1601 The amino acid sequence of HPV type 16 L1 protein aa 1-474 MSLWLPSEATVYLPVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGHPYFPIKK PNNNKILVPKVSGLQYRVFRIHLPDPNKFGLPDTSFYNPDTQRLVWACVGVVEVG RGQPLGVGISGHPLLNLDDTENASAYAANAGVDNRECISMDYKQTQLCLIGC KPPIGEHWGKGSPCTNVAVNPGDCPPELINTVIQDGDMDVDTGFGAMDFTTLQ ANKSEVPLDICTSICKYPDYIKMVSEPYGDSLFFYLRRREQMFVRHLFNRAAGVG ENVPDDLYIKGSGSTANLASSNYFPTPSGSMVTSDAQIFNKPYWLQRAQGHNN GICWGNQLFVTVVDTTTRSTNMSLCAAISTSETTYKNTNFKEYLRHGEEYDLQFI FQLCKITLTADVMTYIHSMNSTILEDWNFGLQPPPGGTLEDYRFVTSQAIACQ KHTPPAPKEDPLKKYTFWEVNLKEKFSADLDQFPLGRKFLLQAGL
SEQ ID No: 28	1602 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 29	1603 Amino acid sequence of chimeric HPV type 16 L1 protein MSLWLPSEATVYLPVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGHPYFPIKK PNNNKILVPKVSGLQYRVFRIHLPDPNKFGLPDTSFYNPDTQRLVWACVGVVEVG RGQPLGVGISGHPLLNLDDTENASAYAANAGVDNRECISMDYKQTQLCLIGC KPPIGEHWGKGSPCTNVAVNPGDCPPELINTVIQDGDMDVDTGFGAMDFTTLQ ANKSEVPLDICTSICKYPDYIKMVSEPYGDSLFFYLRRREQMFVRHLFNRAAGVG ENVPDDLYIKGSGSTANLASSNYFPTPSGSMVTSDAQIFNKPYWLQRAQGHNN GICWGNQLFVTVVDTTTRSTNMSLCAAISTSETTYKNTNFKEYLRHGEEYDLQFI FQLCKITLTADVMTYIHSMNSTILEDWNFGLQPPPGGTLEDYRFVTSQAIACQ KHTPPAPKEDPLKKYTFWEVNLKEKFSADLDQFPLGRKFLLQAGLKAKPKLKR

	AAPTSTRTSSAKRKKVKK
SEQ ID No: 30	1604 Nucleotide sequence of chimeric HPV type 16 L1 protein ATGAGTCTGTGGCTGCCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTGAGCAC AGATGAATATGTGGCAAGGACCAACATCTACTACCATGCTGGCACCAGCAGACTGCTGGCTGTGGGACACC CATACTTTCCAATCAAGAAGCCAAACAACAACAAGATTCTGGTGCCAAAGGTGTCTGGACTCCAATACAGG GTGTTTCAGGATTCACCTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACCCTGACACC CAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG GACACCCACTGCTGAACAAACTGGATGACACAGAGAATGCCTCTGCCTATGCTGCCAATGCTGGAGTGGAC AACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACTTTGTCTGATTGGCTGTAAGCCTCCAATTGG AGAACTGTTGGGCAAGGGCAGCCCATGTACCAATGTGGCTGTGAACCCTGGAGACTGTCTCCATTGGAAC TGATAAACACAGTGATTTCAGGATGGAGATATGGTGGACACAGGCTTGGAGCTATGGACTTCACCACCTC CAAGCCAACAAGTCTGAGGTGCCACTGGACATCTGTACCAGCATCTGTAAATACCTGACTACATCAAGAT GGTGTCTGAACCATATGGAGACTCCCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACACCTGTT CAACAGGGCTGGAGCAGTGGGAGAGAATGTGCCTGATGACCTCTACATCAAGGGCTCTGGCAGCACAGCC AACCTGGCATCCAGCAACTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAAC AAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTTGTGAC AGTGGTGGACACCACCAGGAGCACCAATATGAGTCTGTGTGCTGCCATCAGCACCTCTGAGACCACCTACA AGAACACCAACTTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAACCTTGT AAGATTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACAGCACCATCTTGGAGGACTGGAA CTTTGGACTCCAACCTCCTCTGGAGGCACCTTGGAGGACACCTACAGGTTTGTGACCAGCCAGGCTATTGC CTGTCAGAAACACACACCTCCTGCCCCAAAGGAGGACCCACTGAAAAAATACACCTTCTGGGAGGTGAACC TGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCTGCTCCAAGCAGGACTG AAAGCCAAGCCAAAACGTGAAAAGGGCTGCCCCAACCAGCACCAGGACCTCCTTGCCAAGAGGAAGAAGG TGAAGAAGTAAA
SEQ ID No:	1605 Synthetic HPV16 L1 gene

31	ctgggtaccATGAGTCTGTGGCTGCCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGGCAAGGACCAACATCTACTACCATGCTGGCACCAGCAGACTGCTGGCTGTGGG ACACCCATACTTTCCAATCAAGAAGCCAAACAACAACAAGATTCTGGTGCCAAAGGTGTCTGGACTCCAAT ACAGGGTGTTCAGGATTACCTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACCCTG ACACCCAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCAT CTCTGGACACCCACTGCTGAACAAACTGGATGACACAGAGAATGCCTCTGCCTATGCTGCCAATGCTGGAG TGGACAACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACTTTGTCTGATTGGCTGTAAGCCTCCA ATTGGAGAACACTGGGGCAAGGGCAGCCCATGTACCAATGTGGCTGTGAACCTGGAGACTGTCCTCCATT GGAAGTATAACACAGTGATTGAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGGACTTCACCA CCCTCCAAGCCAACAAGTCTGAGGTGCCACTGGACATCTGTACCAGCATCTGTAAATACCCTGACTACATC AAGATGGTGTCTGAACCATATGGAGACTCCCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACA CCTGTTCAACAGGGCTGGAGCAGTGGGAGAGAATGTGCCTGATGACCTCTACATCAAGGGCTCTGGCAGCA CAGCCAACCTGGCATCCAGCAACTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTT TCAACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTT GTGACAGTGGTGGACACCACCAGGAGCACCAATATGAGTCTGTGTGCTGCCATCAGCACCTCTGAGACCAC CTACAAGAACACCAACTTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAAC TTTGTAAGATTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACAGCACCATCTTGAGGAGC TGGAACCTTTGGACTCCAACCTCCTCCTGGAGGCACCTTGGAGGACACCTACAGGTTTGTGACCAGCCAGGC TATTGCCTGTCAGAAACACACACCTCCTGCCCCAAAGGAGGACCCACTGAAAAAATACACCTTCTGGGAGG TGAACCTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCTGCTCCAAGCA GGACTGAAAGCCAAGCCAAAGTTACCCCTGGGCAAGAGGAAGGCTACACCAACCACCTCCAGCACCAGCA CCACAGCCAAGAGGAAGAAGAGGAAACTGTAAActcgagctc
SEQ ID No:	1606 Synthetic HPV33 L1 gene
32	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG

	ACACCCATACTTCAGCATCAAGAACCCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTC AACAAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAAGTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 33	1607HPV16 L1 F1 CTTGGTACCATGAGTCTGTGGCTGCCATCTGAGG
SEQ ID No: 34	1608HPV16 L1 R1 GCTTGGCTTTCAGTCCTGCTTGGAGCAGGAAGTTCC
SEQ ID No:	1609 HPV16 L1 amplified sequence 1

CTTGGTACC^[1]

ATGAGTCTGTGGCTGCCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTGAGCAC
AGATGAATATGTGGCAAGGACCAACATCTACTACCATGCTGGCACCAGCAGACTGCTGGCTGTGGGACACC
CATACTTTCCAATCAAGAAGCCAAACAACAACAAGATTCTGGTGCCAAAGGTGTCTGGACTCCAATACAGG
GTGTTTCAGGATTCACCTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACCCTGACACC
CAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG
GACACCCACTGCTGAACAAACTGGATGACACAGAGAATGCCTCTGCCTATGCTGCCAATGCTGGAGTGGAC
AACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACTTTGTCTGATTGGCTGTAAGCCTCCAATTGG
AGAACACTGGGGCAAGGGCAGCCCATGTACCAATGTGGCTGTGAACCCTGGAGACTGTCCTCCATTGGAAC
TGATAAACACAGTGATTTCAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGGACTTCACCACCTC
CAAGCCAACAAGTCTGAGGTGCCACTGGACATCTGTACCAGCATCTGTAAATACCCTGACTACATCAAGAT
GGTGTCTGAACCATATGGAGACTCCCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACACCTGTT
CAACAGGGCTGGAGCAGTGGGAGAGAATGTGCCTGATGACCTCTACATCAAGGGCTCTGGCAGCACAGCC
AACCTGGCATCCAGCAACTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAAC
AAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTTGTGAC
AGTGGTGGACACCACCAGGAGCACCAATATGAGTCTGTGTGCTGCCATCAGCACCTCTGAGACCACCTACA
AGAACACCAACTTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGT
AAGATTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACAGCACCATCTTGGAGGACTGGAA
CTTTGGACTCCAACCTCCTCCTGGAGGCACCTTGGAGGACACCTACAGGTTTGTGACCAGCCAGGCTATTGC
CTGTCAGAAACACACACCTCCTGCCCCAAAGGAGGACCCACTGAAAAAATACACCTTCTGGGAGGTGAACC
TGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCTGCTCCAAGCAGGACTG

²AAAGCCAAGC^[3]

[1]: Kpn I enzymatic cleavage site;

[2]: the gene fragment encoding of HPV16 L1 aa 1-474;

	[3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 36	1610HPV16 L1 F2 AGCAGGACTGAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 37	1611HPV16 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 38	1612 HPV16 L1 amplified sequence 2 <u>AGCAGGACTG^[1]</u> <u>AAAGCCAAGCCAAACTGAAAAGGGCTGCCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG^[2] TAA ATCTAGA^[3]CAG.</u> [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV16 L1 aa 1-474; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 39	1613 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKR VKRRKSSRK

Appendix 4 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 18 L1 PROTEIN

SEQ ID No: 40	1801 The amino acid sequence of HPV type 18 L1 protein aa 1-470 MALWRPSDNTVYLPPPSVARVVNTDDYVTRTSIFYHAGSSRLLTVGNPYFRVP AGGGNKQDIPKVSAYQYRVFRVQLPDPNKFGLPDTSIYNPETQRLVWACAGVE IGRGQPLGVGLSGHPFYNKLDDTESSHAATSNVSEDVRDENVSDYKQTQLCILG CAPAIGEHWAKGTACKSRPLSQGDCPPLELKNTVLEDGDMVDTGYGAMDFST LQDTKCEVPLDICQSICKYPDYQLQMSADPYGDSMFFCLRREQLFARHFWNRAG TMGDTVPPQSLYIKGTGMRASPGSCVYSPSPSGSIVTSDSQLFNKPYWLHKAQGH NNGVCWHNQLFVTVDTRSTNLTICASTQSPVPGQYDATKFKQYSRHVEEYD LQFIFQLCTITLTADVMSYIHSMNSSILEDWNFGVPPPPPTSLVDTYRFVQSVAIT CQKDAAPAENKDPYDKLKFWNVLDLKEKFSLDLDQYPLGRKFL
SEQ ID No: 41	1802 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 42	1803 Amino acid sequence of chimeric HPV type 18 L1 protein MALWRPSDNTVYLPPPSVARVVNTDDYVTRTSIFYHAGSSRLLTVGNPYFRVP AGGGNKQDIPKVSAYQYRVFRVQLPDPNKFGLPDTSIYNPETQRLVWACAGVE IGRGQPLGVGLSGHPFYNKLDDTESSHAATSNVSEDVRDENVSDYKQTQLCILG CAPAIGEHWAKGTACKSRPLSQGDCPPLELKNTVLEDGDMVDTGYGAMDFST LQDTKCEVPLDICQSICKYPDYQLQMSADPYGDSMFFCLRREQLFARHFWNRAG TMGDTVPPQSLYIKGTGMRASPGSCVYSPSPSGSIVTSDSQLFNKPYWLHKAQGH NNGVCWHNQLFVTVDTRSTNLTICASTQSPVPGQYDATKFKQYSRHVEEYD LQFIFQLCTITLTADVMSYIHSMNSSILEDWNFGVPPPPPTSLVDTYRFVQSVAIT CQKDAAPAENKDPYDKLKFWNVLDLKEKFSLDLDQYPLGRKFLKAKPKLKRAA

	PTSTRTSSAKRKKVKK
SEQ ID No: 43	1804 Nucleotide sequence of chimeric HPV type 18 L1 protein ATGGCCCTCTGGAGACCATCCGATAACACAGTGTACTTGCCCCACCCAGCGTCGCCCAGGTGGTGAACAC AGACGACTACGTACCCAGAACCTCAATCTTCTACCACGCCGGGTCCAGCCGGCTGCTGACCGTGGGCAACC CCTACTTCCGCGTGCCCGCCGGCGGCGGAAACAAACAAGACATCCCCAAAGTCAGCGCCTATCAGTACCGG GTGTTCCGCGTCCAACCTGCCCAGTCCCAACAAGTTTCGGCCTGCCCGACACCTCCATCTACAACCCGAGACC CAGAGGCTGGTCTGGGCTTGCGCCGGCGTCGAGATCGGGAGGGGGCAACCCCTGGGCGTGGGGTTGTCCGG CCACCCCTTCTACAACAAGCTGGACGATACCGAGTCCAGCCACGCAGCAACCAGCAACGTCTCCGAAGATG TGCGCGATAACGTACAGCTGGACTACAAACAAACCAACTGTGCATCCTGGGATGCGCACCCGCCATCGGC GAGCATTGGGCCAAGGGGACCGCCTGCAAGAGCAGGCCCTGAGCCAAGGGGACTGTCCACCCCTGGAGT TGAAGAATACCGTGCTCGAGGACGGCGACATGGTGGACACCGGTACGGCGCTATGGATTCTCCACCCCTC CAGGACACCAAGTGCGAAGTGCCCCTCGACATCTGCCAAAGCATCTGCAAGTACCCCGACTACCTCCAGAT GAGCGCCGACCCCTACGGCGACAGCATGTTCTTGTCTCAGAAGGGAACAATTGTTGCCCCGCCACTTCTG GAACCGGGCCGGCACAATGGGAGATACAGTCCCCCAGAGCCTGTACATCAAGGGGACCGGAATGAGGGCC AGCCCCGGGTCTGCGTCTACAGCCCAAGCCCCCTCCGGGAGCATCGTCACAAGCGATAGCCAACCTTTCAA CAAGCCCTACTGGCTCCACAAAGCCCAAGGCCACAATAACGGGGTGTGTTGGCACAACCAGCTGTTTCGTGA CCGTGCTGGACACAACCAGGTCCACAAACCTGACCATCTGCGCCAGCACCCAAAGCCCCGTGCCCGGCCAG TACGACGCCACAAAGTTCAAACAATACTCTCGGCACGTGGAAGAGTACGACCTCCAATTCATCTTCCAAC CTGCACCATCACCTCACCGCCGACGTGATGAGCTACATCCACTCCATGAACTCCTCCATCCTGGAAGACTG GAATTTGGCGTGCCACCACCCCTACCACCTCCCTCGTCGACACCTACAGATTCGTGCAGAGCGTGGCCAT CACATGCCAGAAAGACGCCGCCCCCGCCGAGAACAAGACCCATACGACAACTGAAATTCTGGAACGTC GACCTGAAAGAGAAATTCAGCCTGGATCTGGACCAGTACCCATTGGGCAGGAAGTTCTCAAAGCCAAGCC AAAAGTGAAGAGGGCTGCCCAACCAGCACCAAGGACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAGTAA A

SEQ ID No:	1805 Synthetic HPV18 L1 gene
44	ctgggtaccATGGCCCTCTGGAGACCATCCGATAACACAGTGTACTTGCCCCACCCAGCGTCGCCCCGGGTGGT GAACACAGACGACTACGTCACCAGAACCTCAATCTTCTACCACGCCGGGTCCAGCCGGCTGCTGACCGTGG GCAACCCCTACTTCCGCGTGCCCCGCCGGCGGCGGAAACAAACAAGACATCCCCAAAGTCAGCGCCTATCAG TACCGGGTGTTCGCGTCCAACCTGCCGATCCCAACAAGTTCGGCCTGCCGACACCTCCATCTACAACCCC GAGACCCAGAGGCTGGTCTGGGCTTGCGCCGGCGTTCGAGATCGGGAGGGGCCAACCCCTGGGCGTGGGGT TGTCCGGCCACCCCTTCTACAACAAGCTGGACGATACCGAGTCCAGCCACGCAGCAACCAGCAACGTCTCC GAAGATGTGCGCGATAACGTCAGCGTGGACTACAAACAAACCCAACTGTGCATCCTGGGATGCGCACCCGC CATCGGCGAGCATTGGGCCAAGGGGACCGCCTGCAAGAGCAGGCCCTGAGCCAAGGGGACTGTCCACCC CTGGAGTTGAAGAATACCGTGCTCGAGGACGGCGACATGGTGGACACCGGCTACGGCGCTATGGATTCTC CACCTCCAGGACACCAAGTGCGAAGTGCCCCCTGCACATCTGCCAAAGCATCTGCAAGTACCCGACTACC TCCAGATGAGCGCCGACCCCTACGGCGACAGCATGTTCTTGTCTCAGAAGGGAAACAATTGTTCCGCCGC CACTTCTGGAACCGGGCCGGCACAATGGGAGATACAGTCCCCCAGAGCCTGTACATCAAGGGGACCGGAA TGAGGGCCAGCCCCGGGTCTGCGTCTACAGCCCAAGCCCCTCCGGGAGCATCGTCACAAGCGATAGCCAA CTCTTCAACAAGCCCTACTGGCTCCACAAAGCCCAAGGCCACAATAACGGGGTGTGTTGGCACAACCAGCT GTTCTGTGACCGTCTGTGGACACAACCAGGTCCACAAACCTGACCATCTGCGCCAGCACCCAAAGCCCCGTGC CCGGCCAGTACGACGCCACAAAGTTCAACAATACTCTCGGCACGTGGAAGAGTACGACCTCCAATTTCATC TTCCAACCTCTGCACCATCACCTCACCGCCGACGTGATGAGCTACATCCACTCCATGAACTCCTCCATCCTG GAAGACTGGAATTCGGCGTGCCACCACCCCTACCACCTCCCTCGTCGACACCTACAGATTCTGTGCAGAG CGTGGCCATCACATGCCAGAAAGACGCCGCCCGCCGAGAACAAGACCCATACGACAAACTGAAATTC TGGAACGTGACCTGAAAGAGAAATTCAGCCTGGATCTGGACCAGTACCCATTGGGCAGGAAGTTCCTCGT GCAAGCCGGCCTCAGGAGAAAACCAACAATCGGGCCCAGGAAGAGGAGCGCCCCCAGCGCAACCACCAGC AGCAAGCCCGCAAAAAGGGTTCAGAGTGAGGGCACGCAATAAAActcgagetc
SEQ ID No:	1806 Synthetic HPV33 L1 gene
45	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG

	AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACC AATATGACCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGAGGACTGGCA GTTTGGACTGACACCTCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActgagctc
SEQ ID No: 46	1807HPV18 L1 F1 CTTGGTACCATGGCCCTCTGGAGACCATCCGATA
SEQ ID No: 47	1808HPV18 L1 R1 GCTTGGCTTTGAGGAACTTCCTGCCCAATGGGTAC

SEQ ID No:	1809 HPV18 L1 amplified sequence 1
48	CTTGGTACC^[1] <u>ATGGCCCTCTGGAGACCATCCGATAACACAGTGTACTTGCCCCACCCAGCGTCGCCCGGGTGGTGAACAC</u> <u>AGACGACTACGTCAACGAACTCAATCTTCTACCACGCCGGGTCCAGCCGGCTGCTGACCGTGGGCAACC</u> <u>CCTACTTCCGCGTGCCCGCCGGCGGCGGAAACAAACAAGACATCCCCAAAGTCAGCGCCTATCAGTACCGG</u> <u>GTGTTCCGCGTCCAAGTCCCCGATCCCAACAAGTTTCGGCCTGCCCGACACCTCCATCTACAACCCCGAGACC</u> <u>CAGAGGCTGGTCTGGGCTTGCGCCGGCGTCGAGATCGGGAGGGGCCAACCCCTGGGCGTGGGGTTGTCCGG</u> <u>CCACCCCTTCTACAACAAGCTGGACGATACCGAGTCCAGCCACGCAGCAACCAGCAACGTCTCCGAAGATG</u> <u>TGCGCGATAACGTCAGCGTGGACTACAAACAAACCCAACTGTGCATCCTGGGATGCGCACCCGCCATCGGC</u> <u>GAGCATTGGGCCAAGGGGACCGCCTGCAAGAGCAGGCCCTGAGCCAAGGGGACTGTCCACCCCTGGAGT</u> <u>TGAAGAATACCGTGCTCGAGGACGGCGACATGGTGGACACCGGTACGGCGCTATGGATTCTCCACCCCTC</u> <u>CAGGACACCAAGTGCGAAGTGCCCTCGACATCTGCCAAAGCATCTGCAAGTACCCCGACTACCTCCAGAT</u> <u>GAGCGCCGACCCCTACGGCGACAGCATGTTCTTCTGTCTCAGAAGGGAACAATTGTTGCCCGCCACTTCTG</u> <u>GAACCGGGCCGGCACAATGGGAGATACAGTCCCCCAGAGCCTGTACATCAAGGGGACCGGAATGAGGGCC</u> <u>AGCCCCGGGTCCTGCGTCTACAGCCCAAGCCCTCCGGGAGCATCGTCACAAGCGATAGCCAACCTTTCAA</u> <u>CAAGCCCTACTGGCTCCACAAAGCCCAAGGCCACAATAACGGGGTGTGTTGGCACAACCAGCTGTTTCGTGA</u> <u>CCGTCGTGGACACAACCAGGTCCACAAACCTGACCATCTGCGCCAGCACCCAAAGCCCGTGCCCGGCCAG</u> <u>TACGACGCCACAAAGTTCAAACAATACTCTCGGCACGTGGAAGAGTACGACCTCCAATTCATCTTCCAAC</u> <u>CTGCACCATCACCTCACCGCGACGTGATGAGCTACATCCACTCCATGAACTCCTCCATCCTGGAAGACTG</u> <u>GAATTTCCGCGTGCCACCACCCCTACCACCTCCCTCGTCGACACCTACAGATTCTGTCAGAGCGTGGCCAT</u> <u>CACATGCCAGAAAGACGCCGCCCGCCGAGAACAAAGACCCATACGACAAACTGAAATTCTGGAACGTC</u> <u>GACCTGAAAGAGAAATTCAGCCTGGATCTGGACCAGTACCCATTGGGCAGGAAGTTCCTC^[2]AAAGCCAAG</u> C^[3] [1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding of HPV18 L1 aa 1-470;

	[3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 49	1810HPV18 L1 F2 GAAGTTCCTCAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 50	1811HPV18 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 51	1812 HPV18 L1 amplified sequence 2 <u>GAAGTTCCTC</u> ^[1] <u>AAAGCCAAGCCAAACTGAAAAGGGCTGCCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG</u> ^[2] TAA <u>ATCTAGA</u> ^[3] CAG ₂ [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV18 L1 aa 1-470; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 52	1813 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKR VKRRKSSRK

Appendix 5 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 31 L1 PROTEIN

SEQ ID No: 53	3101 The amino acid sequence of HPV type 31 L1 protein aa 1-475 MSLWRPSEATVYLPVPVSKVVSTDEYVTRTNIIYHAGSARLLTVGHPYYSIPK SDNPKKIVVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPETQRLVWACVGLE VGRGQPLGVGISGHPLLNKFDDTENSNNRYAGGPGTDNRECISMDYKQTLCLL GCKPPIGEHWGKGSPCSNNAITPGDCPPLELKNSVIQDGMVDTGFGAMDFTA LQDTKSNVPLDICNSICKYPDYLKMVAEPTYGDTLFFYLRREQMFVRHFFNRS GT VGESVPTDLYIKGSGSTATLANSTYFPTPSGSMVTSDAQIFNKPYWMQRAQGH NNGICWGNQLFVTVVDTRSTNMSVCAAIANSDDTFKSSNFKEYLRHGEEFDL QFIFQLCKITLSADIMTYIHSMNPAILEDWNFGLTTPPSGSLEDYRFVTSQA ITC QKSAPQKPKEDPFKDYVFEVNLKEKFSADLDQFPLGRKFLLQAGY
SEQ ID No: 54	3102 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 55	3103 Amino acid sequence of chimeric HPV type 31 L1 protein MSLWRPSEATVYLPVPVSKVVSTDEYVTRTNIIYHAGSARLLTVGHPYYSIPK SDNPKKIVVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPETQRLVWACVGLE VGRGQPLGVGISGHPLLNKFDDTENSNNRYAGGPGTDNRECISMDYKQTLCLL GCKPPIGEHWGKGSPCSNNAITPGDCPPLELKNSVIQDGMVDTGFGAMDFTA LQDTKSNVPLDICNSICKYPDYLKMVAEPTYGDTLFFYLRREQMFVRHFFNRS GT VGESVPTDLYIKGSGSTATLANSTYFPTPSGSMVTSDAQIFNKPYWMQRAQGH NNGICWGNQLFVTVVDTRSTNMSVCAAIANSDDTFKSSNFKEYLRHGEEFDL QFIFQLCKITLSADIMTYIHSMNPAILEDWNFGLTTPPSGSLEDYRFVTSQA ITC QKSAPQKPKEDPFKDYVFEVNLKEKFSADLDQFPLGRKFLLQAGYKAKPKLK

	RAAPTSTRTSSAKRKKVKK
SEQ ID No:	3104 Nucleotide sequence of chimeric HPV type 31 L1 protein
56	ATGAGCCTGTGGAGGCCAGCGAGGCCACCGTGACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC CGACGAGTACGTGACCAGGACCAACATCTACTACCACGCCGGCAGCGCCAGGCTGCTGACCGTGGGCCACC CCTACTACAGCATCCCCAAGAGCGACAACCCCAAGAAGATCGTGGTGCCCAAGGTGAGCGGCCTGCAGTAC AGGGTGTTTACGGGTGAGGCTGCCCGACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACAACCCCGA GACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGGTGGGCAGGGGCCAGCCCCTGGGCGTGGGCATC AGCGGCCACCCCCTGCTGAACAAGTTCGACGACACCGAGAACAGCAACAGGTACGCCGGCGGCCCGGCA CCGACAACAGGGAGTGTCATCAGCATGGACTACAAGCAGACCCAGCTGTGCCTGCTGGGCTGCAAGCCCCC ATCGGCGAGCACTGGGGCAAGGGCAGCCCCTGCAGCAACAACGCCATCACCCCGGCGACTGCCCCCCCCT GGAGCTGAAGAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCGCCATGGACTTCACC GCCCTGCAGGACACCAAGAGCAACGTGCCCTGGACATCTGCAACAGCATCTGCAAGTACCCCGACTACCT GAAGATGGTGGCCGAGCCCTACGGCGACACCCTGTTCTTCTACCTGAGGAGGGAGCAGATGTTCTGTAGGC ACTTCTTCAACAGGAGCGGCACCGTGGGCGAGAGCGTGCCACCGACCTGTACATCAAGGGCAGCGGCAG CACCGCCACCCTGGCCAACAGCACCTACTTCCCCACCCCGAGCGGCAGCATGGTGACCAGCGACGCCAGA TCTTCAACAAGCCCTACTGGATGCAGAGGGGCCAGGGCCACAACAACGGCATCTGCTGGGGCAACCAGCTG TTCGTGACCGTGGTGGACACCACCAGGAGCACCAACATGAGCGTGTGCGCCGCCATCGCCAACAGCGACAC CACCTTCAAGAGCAGCAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCAGTTCATCTTCC AGCTGTGCAAGATCACCTGAGCGCCGACATCATGACCTACATCCACAGCATGAACCCCGCCATCCTGGAG GACTGGAACCTTCGGCCTGACCACCCCCCCCAGCGGCAGCCTGGAGGACACCTACAGGTTCTGTGACCAGCCA GGCCATCACCTGCCAGAAGTCCGCCCCCAGAAGCCCAAGGAGGACCCCTTCAAGGACTACGTGTTCTGGG AGGTGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCCTGCTGCAG GCCGGCTACAAAGCCAAGCCAAACTGAAAAGGGCTGCCCCAACCAGCACCAGGACCTCCTTGCCAAGA GGAAGAAGGTGAAGAAGTAAA

SEQ ID No:	3105 Synthetic HPV31 L1 gene
57	ctgggtaccATGAGCCTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGT GAGCACCGACGAGTACGTGACCAGGACCAACATCTACTACCACGCCGGCAGCGCCAGGCTGCTGACCGTG GGCCACCCCTACTACAGCATCCCCAAGAGCGACAACCCCAAGAAGATCGTGGTGCCCAAGGTGAGCGGCCT GCAGTACAGGGTGTTCAGGGTGAGGCTGCCCCACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACA ACCCCGAGACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGGTGGGCAGGGGCCAGCCCCTGGGCGT GGGCATCAGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGAACAGCAACAGGTACGCCGGCGGC CCCGGCACCGACAACAGGGAGTGCATCAGCATGGACTACAAGCAGACCCAGCTGTGCCTGCTGGGCTGCA AGCCCCCATCGGCGAGCACTGGGGCAAGGGCAGCCCCTGCAGCAACAACGCCATCACCCCCGGCGACTG CCCCCCCCTGGAGCTGAAGAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCGCCATGG ACTTACCGCCCTGCAGGACACCAAGAGCAACGTGCCCTGGACATCTGCAACAGCATCTGCAAGTACCCC GACTACCTGAAGATGGTGGCCGAGCCCTACGGCGACACCCTGTTCTTCTACCTGAGGAGGGAGCAGATGTT CGTGAGGGCACTTCTTCAACAGGAGCGGCACCGTGGGCGAGAGCGTGCCACCGACCTGTACATCAAGGGCA GCGGCAGCACCGCCACCCTGGCCAACAGCACCTACTTCCCCACCCCAGCGGCAGCATGGTGACCAGCGAC GCCAGATCTTCAACAAGCCCTACTGGATGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTGGGGCA ACCAGCTGTTCTGTGACCGTGGTGGACACCACCAGGAGCACCAACATGAGCGTGTGCGCCGCCATCGCCAAC AGCGACACCACCTTCAAGAGCAGCAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCAGTT CATCTTCCAGCTGTGCAAGATCACCTGAGCGCCGACATCATGACCTACATCCACAGCATGAACCCCGCCA TCCTGGAGGACTGGAACCTTCGGCCTGACCACCCCCCAGCGGCAGCCTGGAGGACACCTACAGGTTCTGTG ACCAGCCAGGCCATCACCTGCCAGAAGTCCGCCCCCAGAAGCCCAAGGAGGACCCCTTCAAGGACTACGT GTTCTGGGAGGTGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCC TGCTGCAGGCCGGCTACAGGGCCAGGCCCAAGTTCAAGGCCGGAAGAGGAGCGCCCCCAGCGCCAGCAC CACCACCCCGCCAAGAGGAAGAAGACCAAGAAGTAAActcgagctc
SEQ ID No:	3106 Synthetic HPV33 L1 gene

58	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCCCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No:	3107HPV31 L1 F1
59	CTTGGTACCATGAGCCTGTGGAGGCCAGCGAG
SEQ ID No:	3108HPV31 L1 R1

60	GCTTGGCTTTGTAGCCGGCCTGCAGCAGGAACTTCCTG
SEQ ID No:	3109 HPV31 L1 amplified sequence 1
61	CTTGGTACC^[1] <u>ATGAGCCTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC</u> <u>CGACGAGTACGTGACCAGGACCAACATCTACTACCACGCCGGCAGCGCCAGGCTGCTGACCGTGGGCCACC</u> <u>CCTACTACAGCATCCCCAAGAGCGACAACCCCAAGAAGATCGTGGTGCCCAAGGTGAGCGGCCTGCAGTAC</u> <u>AGGGTGTTTCAAGGTGAGGCTGCCCGACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACAACCCCGA</u> <u>GACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGGTGGGCAGGGGCCAGCCCCTGGGCGTGGGCATC</u> <u>AGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGAACAGCAACAGGTACGCCGGCGGCCCGGCA</u> <u>CCGACAACAGGGAGTGATCAGCATGGACTACAAGCAGACCCAGCTGTGCCTGCTGGGCTGAAGCCCCC</u> <u>ATCGGCGAGCACTGGGGCAAGGGCAGCCCCTGCAGCAACAACGCCATCACCCCGGCGACTGCCCCCCCCT</u> <u>GGAGCTGAAGAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCGCCATGGACTTCACC</u> <u>GCCCTGCAGGACACCAAGAGCAACGTGCCCTGGACATCTGCAACAGCATCTGCAAGTACCCCGACTACCT</u> <u>GAAGATGGTGGCCGAGCCCTACGGCGACACCCTGTTCTTCTACCTGAGGAGGGAGCAGATGTTCTGTAGGC</u> <u>ACTTCTTCAACAGGAGCGGCACCGTGGGCGAGAGCGTGCCACCGACCTGTACATCAAGGGCAGCGGCAG</u> <u>CACCGCCACCCTGGCCAACAGCACCTACTTCCCCACCCCGAGCGGCAGCATGGTGACCAGCGACGCCAGA</u> <u>TCTTCAACAAGCCCTACTGGATGCAGAGGGGCCAGGGCCACAACAACGGCATCTGTGGGGCAACCAGCTG</u> <u>TTCGTGACCGTGGTGGACACCACAGGAGCACCAACATGAGCGTGTGCGCCGCCATCGCCAACAGCGACAC</u> <u>CACCTTCAAGAGCAGCAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCAGTTCATCTTCC</u> <u>AGCTGTGCAAGATCACCTGAGCGCCGACATCATGACCTACATCCACAGCATGAACCCCGCCATCCTGGAG</u> <u>GACTGGAACCTTCGGCCTGACCACCCCCCCCAGCGGCAGCCTGGAGGACACCTACAGGTTCTGTGACCAGCCA</u> <u>GGCCATCACCTGCCAGAAGTCCGCCCCCAGAAGCCCAAGGAGGACCCCTTCAAGGACTACGTGTTCTGGG</u> <u>AGGTGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCCTGCTGCAG</u> <u>GCCGGCTAC^[2]AAAGCCAAGC^[3]</u> [1]: Kpn I enzymatic cleavage site;

	[2]: the gene fragment encoding of HPV31 L1 aa 1-475; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 62	3110HPV31 L1 F2 GGCCGGCTACAAAGCCAAGCCAAAAGTGAAGGG
SEQ ID No: 63	3111HPV31 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGGCAGAG
SEQ ID No: 64	3112 HPV31 L1 amplified sequence 2 <u>GGCCGGCTAC^[1]</u> <u>AAAGCCAAGCCAAAAGTGAAGGGCTGCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG^[2] TAA ATCTAGA^[3]CAG.</u> [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV31 L1 aa 1-475; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 65	3113 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

Appendix 6 : SEQUENCE LISTING - HUMAN PAPILLOMAVIRUS TYPE 33 L1 PROTEIN

SEQ ID No: 66	3301 The amino acid sequence of HPV type 33 L1 protein MSVWRPSEATVYLPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGHPYFSIKN PTNAKLLVPKVSGLQYRVFRVRLPDPNKFGLPDTSFYNPDTQRLVWACVGLEI GRGQPLGVGISGHPLLNFDDTETGNKYPGQPGADNRECLSM DYKQTQLC LLG CKPPTGEHWGKG VACTNAAPANDCPPLELINTIIEDGDMVDTGFGCMDFKTLQ ANKSDVPIDICGSTCKYPDYLKMTSEPYGDSLFFFLRREQMFVRHFFN RAGTLG EAVPDDLYIKSGTTASIQSSAFFPTPSGSMVTSESQLFNKPYWLQRAQGHNNGI CWGNQVFVTVD TTRSTNMTLCTQVTS DSTYKNENFKEYIRHVEEYDLQFVFQ LCKVTLTAEVMTYIHAMNPDILEDWQFGLTPPPSASLQD TYRFVTSQAITCQKT VPPKEKEDPLGKYTFWEVDLKEKFSADLDQFPLGRKFLLQAGLKAKPKLKRAA PTSTRTSSAKRKKVKK
SEQ ID No: 67	3302 Nucleotide sequence of HPV type 33 L1 protein ATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTGAGCAC AGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGGACACC CATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCCAATAC AGGGTGTT CAGGGTGAGACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACCCTGAC ACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGGCATCTC TGGACACCCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTGGAGCA GACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACTTTGTCTGCTGGGCTGTAAGCCTCCAAC AGGAGAACACTGGGGCAAGGGAGTGGCTGTACCAATGCTGCCCTGCCAATGACTGCCTCCATTGGAAC TGATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTGGCTGTATGGACTTCAAGACCCTC

	CAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGAAAAAT GACCTCTGAACCATATGGAGACTCCCTGTTCTTCTCCTGAGGAGGGAACAGATGTTTGTGAGACACTTCTT CAACAGGGCTGGCACCCCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACACAGCCA GCATCCAGTCCTCTGCCTTCTTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTCAACA AGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGTGACA TGTTGGTGGACACCACCAGGAGCACC AATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACAAGAA TGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGTAAGG TGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGAGGAGTGGCAGTTT GGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCACTTGT CAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCCACTGGGCAAATACACCTTCTGGGAGGTGGACCTGA AAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCTGCTCCAAGCAGGACTGAAA GCCAAGCCAAAACCTGAAAAGGGCTGCCCAACCAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAGGTGA AGAAGTAAA
SEQ ID No: 68	3303 Synthetic HPV33 L1 gene ctgggtaccATGAGTGTGTGGAGACCATCTGAGGTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA

	AAATGACCTCTGAACCATATGGGAGACTCCCTGTTCTTCTTCCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCCCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCA GCCAGCATCCAGTCCTCTGCCTTCTTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAAGTGAAGGGCTGCCCCAACCCAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
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Appendix 7 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 35 L1 PROTEIN

SEQ ID No: 69	3501 The amino acid sequence of HPV type 35 L1 protein aa 1-472 MSLWRSNEATVYLPPVSVSKVVSTDEYVTRTNIIYHAGSSRLLAVGHPYYAIK KQDSNKIAVPKVSGLQYRVFRVKLPDPNKFPGPDTSFYDPASQRLVWACTGVE VGRGQPLGVGISGHPLLNLDDTENS NKYVGNSGTDNRECISMDYKQTQLCLI GCRPPIGEHWGKGTPCNANQVKAGECPPELLNTVLQDGMVDTGFGAMDFT TLQANKSDVPLDICSSICKYPDYLKMOVSEPYGDMLFFYLRRREQMFVRHLFNRA GTVGETVPADLYIKGTTGTLPTSYFPTPSGSMVTSDAQIFNKPYWLQRAQGHN NGICWSNQLFVTVVDTTTRSTNMSVCSAVSSSDSTYKNDNFKEYLRHGEEYDLQ FIFQLCKITLTADVMTYIHSMNPSILEDWNFGLTPPPSGTLEDTYRYVTSQAVTC QKPSAPKPKDDPLKNYTFWEVDLKEKFSADLDQFPLGRKFLLQAGL
SEQ ID No: 70	3502 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 71	3503 Amino acid sequence of chimeric HPV type 35 L1 protein MSLWRSNEATVYLPPVSVSKVVSTDEYVTRTNIIYHAGSSRLLAVGHPYYAIK KQDSNKIAVPKVSGLQYRVFRVKLPDPNKFPGPDTSFYDPASQRLVWACTGVE VGRGQPLGVGISGHPLLNLDDTENS NKYVGNSGTDNRECISMDYKQTQLCLI GCRPPIGEHWGKGTPCNANQVKAGECPPELLNTVLQDGMVDTGFGAMDFT TLQANKSDVPLDICSSICKYPDYLKMOVSEPYGDMLFFYLRRREQMFVRHLFNRA GTVGETVPADLYIKGTTGTLPTSYFPTPSGSMVTSDAQIFNKPYWLQRAQGHN NGICWSNQLFVTVVDTTTRSTNMSVCSAVSSSDSTYKNDNFKEYLRHGEEYDLQ FIFQLCKITLTADVMTYIHSMNPSILEDWNFGLTPPPSGTLEDTYRYVTSQAVTC QKPSAPKPKDDPLKNYTFWEVDLKEKFSADLDQFPLGRKFLLQAGLKAKPKLK

	RAAPTSTRTSSAKRKKVKK
SEQ ID No: 72	3504 Nucleotide sequence of chimeric HPV type 35 L1 protein ATGAGTCTGTGGAGGAGCAATGAGGCTACAGTCTACCTGCCTCCTGTGTCTGTGAGCAAGGTGGTGAGCAC AGATGAATATGTGACCAGGACCAACATCTACTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGGGACACC CATACTATGCCATCAAGAAGCAGGACAGCAACAAGATTGCTGTGCCAAAGGTGTCTGGACTCCAATACAGG GTGTTTCAGGGTGAAACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTATGACCCTGCCAGC CAGAGACTGGTGTGGGCTTGTACTGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG GACACCCACTGCTGAACAAACTGGATGACACAGAGAACAGCAACAAATATGTGGGCAACTCTGGCACAGA CAACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACTTTGTCTGATTGGCTGTAGACCTCCAATTG GAGAACACTGGGGCAAGGGCACACCATGTAATGCCAACCAGGTGAAGGCTGGAGAGTGTCTCCATTGGA ACTGCTGAACACAGTGCTCCAAGATGGAGATATGGTGGACACAGGCTTGGAGCTATGGACTTCACCACCC TCCAAGCCAACAAGTCTGATGTGCCACTGGACATCTGTTCCAGCATCTGTAAATACCCTGACTACCTGAAA ATGGTGTCTGAACCATATGGAGATATGCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACACCT GTTCAACAGGGCTGGCACAGTGGGAGAGACAGTGCCTGCTGACCTCTACATCAAGGGCACCACAGGCACCC TGCCAAGCACCTCCTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAACAAGC CATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGAGCAACCAACTTTTTGTGACAGTG GTGGACACCACCAGGAGCACCAATATGAGTGTGTGTTCTGCTGTGTCCTCCTCTGACAGCACCTACAAGAA TGACAACCTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGTAAGA TTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACCAAGCATCTTGGAGGACTGGAACCTT GGACTGACACCTCCTCCATCTGGCACCTTGGAGGACACCTACAGATATGTGACCAGCCAGGCTGTGACTTG TCAGAAGCCATCTGCCCCAAAGCCAAAGGATGACCCACTGAAAACTACACCTTCTGGGAGGTGGACCTGA AAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACTGAAA GCCAAGCCAAAACCTGAAAAGGGCTGCCCAACCAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAGGTGA AGAAGTAAA

SEQ ID No:	3505 Synthetic HPV35 L1 gene
73	ctgggtaccATGAGTCTGTGGAGGAGCAATGAGGCTACAGTCTACCTGCCTCCTGTGTCTGTGAGCAAGGTGGT GAGCACAGATGAATATGTGACCAGGACCAACATCTACTACCATGTGGCTCCAGCAGACTGCTGGCTGTGG GACACCCATACTATGCCATCAAGAAGCAGGACAGCAACAAGATTGCTGTGCCAAAGGTGTCTGGACTCCAA TACAGGGTGTTCAGGGTGAAACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTATGACCCT GCCAGCCAGAGACTGGTGTGGGCTTGTA CTGGAGTGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCA TCTCTGGACACCCACTGCTGAACAAACTGGATGACACAGAGAACAGCAACAATATGTGGGCAACTCTGGC ACAGACAACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACCTTGTCTGATTGGCTGTAGACCTCC AATTGGAGAACACTGGGGCAAGGGCACACCATGTAATGCCAACCAGGTGAAGGCTGGAGAGTGTCTCCA TTGGAAC TGCTGAACACAGTGTCTCAAGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGGACTTCAC CACCTCCAAGCCAACAAGTCTGATGTGCCACTGGACATCTGTTCCAGCATCTGTAAATACCTGACTACCT GAAAAATGGTGTCTGAACCATATGGAGATATGCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGAC ACCTGTTCAACAGGGCTGGCACAGTGGGAGAGACAGTGCCTGCTGACCTCTACATCAAGGGCACCACAGGC ACCCTGCCAAGCACCTCCTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAAC AAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGAGCAACCAACTTTTTGTGAC AGTGGTGGACACCACCAGGAGCACCAATATGAGTGTGTGTTCTGCTGTGTCTCCTCTGACAGCACCTACA AGAATGACAACCTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGT AAGATTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACCCAAGCATCTTGGAGGACTGGAA CTTTGGACTGACACCTCCTCCATCTGGCACCTTGGAGGACACCTACAGATATGTGACCAGCCAGGCTGTGA CTTGTCAGAAGCCATCTGCCCCAAAGCCAAAGGATGACCCACTGAAAACTACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAGACCAAACTTCAGACTGGGCAAGAGGGCTGCCCTGCCAGCACCAGCAAGAAGTCCAGCACC AAGAGGAGGAAGGTGAAGAGCTAAActcgagetc

SEQ ID No:	3506 Synthetic HPV33 L1 gene
74	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACC AATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACCAAGCAGCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No:	3507HPV35 L1 F1
75	CTTGGTACCATGAGTCTGTGGAGGAGCAATGAGG

SEQ ID No: 76	3508HPV35 L1 R1 GCTTGGCTTTTCAGTCCTGCTTGGAGCAGGAACTTCC
SEQ ID No: 77	3509 HPV35 L1 amplified sequence 1 CTTGGTACC ^[1] ATGAGTCTGTGGAGGAGCAATGAGGCTACAGTCTACCTGCCTCCTGTGTCTGTGAGCAAGGTGGTGAGCAC AGATGAATATGTGACCAGGACCAACATCTACTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGGGACACC CATACTATGCCATCAAGAAGCAGGACAGCAACAAGATTGCTGTGCCAAAGGTGTCTGGACTCCAATACAGG GTGTTTCAGGGTGAAACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTATGACCCTGCCAGC CAGAGACTGGTGTGGGCTTGTACTGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG GACACCCACTGCTGAACAACTGGATGACACAGAGAACAGCAACAAATATGTGGGCAACTCTGGCACAGA CAACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACCTTTGTCTGATTGGCTGTAGACCTCCAATTG GAGAACTGGGGCAAGGGCACACCATGTAATGCCAACCAGGTGAAGGCTGGAGAGTGTCTCCATTGGA ACTGCTGAACACAGTGCTCCAAGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGGACTTCACCACCC TCCAAGCCAACAAGTCTGATGTGCCACTGGACATCTGTTCCAGCATCTGTAAATACCCTGACTACCTGAAA ATGGTGTCTGAACCATATGGAGATATGCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACACCT GTCAACAGGGCTGGCACAGTGGGAGAGACAGTGCCTGCTGACCTCTACATCAAGGGCACACAGGCACCC TGCCAAGCACCTCCTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAACAAGC CATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGAGCAACCAACTTTTTGTGACAGTG GTGGACACCACCAGGAGCACCAATATGAGTGTGTGTTCTGCTGTGTCCTCCTCTGACAGCACCTACAAGAA TGACAACTTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGTAAGA TTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACCCAAGCATCTTGGAGGACTGGAACCTT GGACTGACACCTCCTCCATCTGGCACCTTGGAGGACACCTACAGATATGTGACCAGCCAGGCTGTGACTTG TCAGAAGCCATCTGCCCCAAAGCCAAAGGATGACCCACTGAAAACTACACCTTCTGGGAGGTGGACCTGA AAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACTG ^[2] AA AGCCAAGC ^[3]

	[1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding of HPV35 L1 aa 1-472; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 78	3510HPV35 L1 F2 AGCAGGACTGAAAGCCAAGCCAAAAGTGAAGGG
SEQ ID No: 79	3511HPV35 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 80	3512 HPV35 L1 amplified sequence 2 <u>AGCAGGACTG</u>^[1] <u>AAAGCCAAGCCAAAAGTGAAGGGCTGCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG</u>^[2] TAA <u>ATCTAGA</u>^[3]CAG. [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV35 L1 aa 1-472; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 81	3513 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKRVRKRRKSSRK

Appendix 8 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 39 L1 PROTEIN

SEQ ID No: 82	3901 The amino acid sequence of HPV type 39 L1 protein aa 1-469 MAMWRSSDSMVYLPSPSVAKVVNTDDYVTRTGIYYYAGSSRLLTVGHPYFKV GMNGGRKQDIPKVSAYQYRVFRVTLDPNKFSPDASLYNPETQRLVWACVGV EVGRGQPLGVGISGHPLYNRQDDTENSFPSSSTTNKDSRDNVSDYKQTQLCHIG CVPAIGEHWGKGKACKPNNVSTGDCPPLELVNTPIEDGDMIDTGYGAMDFGAL QETKSEVPLDICQSICKYPDYLQMSADVYGDSMFFCLRREQLFARHFWNRGGM VGDAIPAQLYIKGTDIRANPGSSVYCPSPSGSMVTSDSQLFNKPYWLHKAQGHN NGICWHNQLFLTVDTTTRSTNFTLSTSISSIPSTYDPSKFKEYTRHVEEYDLQFI FQLCTVTLTTDVMSYIHTMNSSILDNWNFAVAPPPSASLVDYRYLQSAAITCQ KDAPAPEKKDPYDGLKFWNVDLREKFSLELDQFPLGRKFL
SEQ ID No: 83	3902 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARPKEPTIGPRKRAAPAPTSTPSPKRVRKRKSSRK
SEQ ID No: 84	3903 Amino acid sequence of chimeric HPV type 39 L1 protein MAMWRSSDSMVYLPSPSVAKVVNTDDYVTRTGIYYYAGSSRLLTVGHPYFKV GMNGGRKQDIPKVSAYQYRVFRVTLDPNKFSPDASLYNPETQRLVWACVGV EVGRGQPLGVGISGHPLYNRQDDTENSFPSSSTTNKDSRDNVSDYKQTQLCHIG CVPAIGEHWGKGKACKPNNVSTGDCPPLELVNTPIEDGDMIDTGYGAMDFGAL QETKSEVPLDICQSICKYPDYLQMSADVYGDSMFFCLRREQLFARHFWNRGGM VGDAIPAQLYIKGTDIRANPGSSVYCPSPSGSMVTSDSQLFNKPYWLHKAQGHN NGICWHNQLFLTVDTTTRSTNFTLSTSISSIPSTYDPSKFKEYTRHVEEYDLQFI FQLCTVTLTTDVMSYIHTMNSSILDNWNFAVAPPPSASLVDYRYLQSAAITCQ KDAPAPEKKDPYDGLKFWNVDLREKFSLELDQFPLGRKFLQLGARPKEPTIGPR

	KRAAPAPTSTPSPKRVKRRKSSRK
SEQ ID No: 85	3904 Nucleotide sequence of chimeric HPV type 39 L1 protein ATGGCTATGTGGAGGTCCTCTGACAGTATGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTGAACACA GATGACTATGTGACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGACAGTGGGACACCC ATACTTCAAGGTGGGGATGAATGGAGGCAGGAAGCAGGACATCCCAAAGGTGTCTGCCTACCAATACAGG GTGTTTCAGGGTGACCCTGCCTGACCCAAACAAGTTCAGCATCCCTGATGCCTCCCTCTACAACCTGAGACC CAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG GACACCCACTCTACAACAGACAGGATGACACAGAGAACAGCCCATTCTCCAGCACCACCAACAAGGACAG CAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCATTGGGTGTGTGCCTGCCATTGGAG AACACTGGGGCAAGGGCAAGGCTTGTAAGCCAAACAATGTGAGCACAGGAGACTGTCCTCCATTGGAAC GGTGAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTATGGAGCTATGGACTTTGGAGCCCTCC AAGAGACCAAGTCTGAGGTGCCACTGGACATCTGTCTGAGCATCTGTAAATACCCTGACTACCTCCAAATG AGTGCTGATGTCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACATTTTTCAGACACTTCTGG AACAGGGGAGGGATGGTGGGAGATGCCATCCCTGCCCAACTCTACATCAAGGGCACAGACATCAGGGCTA ACCCTGGCTCCTCTGTCTACTGTCCAAGCCCATCTGGCAGTATGGTGACCTCTGACAGCCAACCTTTTCAACA AGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACCTTTTCCTGACA GTGGTGGACACCACCAGGAGCACCAACTTCACCCTGAGCACCAGCATTGAGTCCAGCATCCCAAGCACCTA TGACCCAAGCAAGTTCAAGGAATACACCAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCAACCTT GTACTGTGACCTGACCACAGATGTGATGAGTTACATCCACACAATGAACTCCAGCATCCTGGACAACCTGG AACTTTGCTGTGGCTCCTCCTCCATCTGCCTCCCTGGTGGACACCTACAGATACCTCCAATCTGCTGCCATC ACTTGTGAGAAGGATGCCCCTGCCCCTGAGAAGAAGGACCCATATGATGGACTGAAGTTCTGGAATGTGGA CCTGAGGGAGAAGTTCTCCTTGGAACTGGACCAGTTTCCACTGGGCAGGAAGTTCTGCTCCAACCTTGGAG CCAGACCAAAGCCAACCATTTGGACCAAGGAAGAGGGCTGCCCTGCCCAACCAGCACACCAAGCCCAAA GAGGGTGAAGAGGAGGAAGTCCAGCAGGAAGTAAA

SEQ ID No:	3905 Synthetic HPV39 L1 gene
86	ctgggtaccATGGCTATGTGGAGGTCCTCTGACAGTATGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTG AACACAGATGACTATGTGACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGACAGTGGG ACACCCATACTTCAAGGTGGGGATGAATGGAGGCAGGAAGCAGGACATCCCAAAGGTGTCTGCCTACCAA TACAGGGTGTTCAGGGTGACCCTGCCTGACCCAAACAAGTTCAGCATCCCTGATGCCTCCCTCTACAACCCT GAGACCCAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCA TCTCTGGACACCCACTCTACAACAGACAGGATGACACAGAGAACAGCCCATTCTCCAGCACCACCAACAAG GACAGCAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCATTGGCTGTGTGCCTGCCAT TGGAGAACACTGGGGCAAGGGCAAGGCTTGTAAGCCAAACAATGTGAGCACAGGAGACTGTCCTCCATTG GAACTGGTGAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTATGGAGCTATGGACTTTGGAGC CCTCCAAGAGACCAAGTCTGAGGTGCCACTGGACATCTGTCTAGAGCATCTGTAAATACCCTGACTACCTCC AAATGAGTGCTGATGTCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGCCAGACACT TCTGGAACAGGGGAGGGATGGTGGGAGATGCCATCCCTGCCCAACTCTACATCAAGGGCACAGACATCAG GGCTAACCTGGCTCCTCTGTCTACTGTCCAAGCCCATCTGGCAGTATGGTGACCTCTGACAGCCAACTTTT CAACAAGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACTTTTCC TGACAGTGGTGGACACCACCAGGAGCACCAACTTCACCCTGAGCACCAGCATTGAGTCCAGCATCCCAAGC ACCTATGACCCAAGCAAGTTCAAGGAATACACCAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCA ACTTTGTA CTGTGACCCTGACCACAGATGTGATGAGTTACATCCACACAATGAACTCCAGCATCCTGGACA ACTGGAACCTTGCTGTGGCTCCTCCTCCATCTGCCTCCCTGGTGGACACCTACAGATACCTCCAATCTGCTG CCATCACTTGTGAGAAGGATGCCCTGCCCTGAGAAGAAGGACCCATATGATGGACTGAAGTTCTGGAAT GTGGACCTGAGGGAGAAGTTCTCCTTGGAAGTGGACCAAGTTCCACTGGGCAGGAAGTTCTGCTCCAAGC CAGGGTGAGGAGGAGACCAACCATTGGACCAAGGAAGAGACCTGCTGCCAGCACCTCCTCCTCTGCCA CCAAACACAAGAGGAAGAGGGTGAGCAAGTAAActcgagctc

SEQ ID No:	3906 Synthetic HPV59 L1 gene
87	ctgggtaccATGGCTCTGTGGAGGTCCTCTGACAACAAGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTG AGCACAGATGAATATGTGACCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGG ACACCCATACTTCAAGGTGCCAAAGGGAGGCAATGGCAGACAGGATGTGCCAAAGGTGTCTGCCTACCAAT ACAGGGTGTTCAAGGTGAAACTGCCTGACCCAAACAAGTTTGGACTGCCTGACAACACAGTCTATGACCCA AACAGCCAGAGACTGGTGTGGGCTTGTGTGGGAGTGAGATTGGCAGGGGACAACCACTGGGAGTGGGAC TGTCTGGACACCCACTCTACAACAACTGGATGACACAGAGAAGTCTCATGTGGCATCTGCTGTGGACACC AAGGACACCAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCATTGGCTGTGTGCCTGC CATTGGAGAAGACTGGACCAAGGGCACAGCCTGTAAGCCAACCACAGTGGTCCAGGGAGACTGTCCTCCAT TGGAAGTATAAACACACCAATTGAGGATGGAGATATGGTGGACACAGGCTATGGAGCTATGGACTTCAA ACTGCTCCAAGACAACAAGTCTGAGGTGCCACTGGACATCTGTCAGAGCATCTGTAAATACCCTGACTACC TCCAAATGAGTGCTGATGCCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAGGTGTTTGCCAGA CACTTCTGGAACAGGTCTGGCACAATGGGAGACCAACTTCCTGAGTCCCTCTACATCAAGGGCACAGACAT CAGGGCTAACCTGGCTCCTACCTCTACAGCCCAAGCCCATCTGGCTCTGTGGTGACCTCTGACAGCCAACT TTTCAACAAGCCATACTGGCTGCACAAGGCTCAAGGACTGAACAATGGCATCTGTTGGCACAACCAACTTT TCCTGACAGTGGTGACACCACCAGGAGCACCAACCTGTCTGTGTGTGCCAGCACCACTCCAGCATCCCA AATGTCTACACACCAACCTCCTTCAAGGAATATGCCAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTC CAACTTTGTAAGATTACCCTGACCACAGAGGTGATGAGTTACATCCACAATATGAACACCACCATCTTGGA GGACTGGAACCTTTGGAGTGACACCTCCTCCAACAGCCTCCCTGGTGGACACCTACAGGTTTGTCCAGTCTGC TGCTGTGACTTGTCAGAAGGACACAGCCCCTCCTGTGAAGCAGGACCCATATGACAAACTGAAGTTCTGGC CTGTGGACCTGAAAGAGAGGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAA CTTGAGCCAGACCAAGCCAACCATTTGGACCAAGGAAGAGGGCTGCCCCTGCCCCAACCCAGCACACCAA GCCCAAAGAGGGTGAAGAGGAGGAAGTCCAGCAGGAAGTAAActcgagctc
SEQ ID No:	3907HPV39 L1 F1

88	CTTGGTACCATGGCTATGTGGAGGTCCTCTGACAGTAT
SEQ ID No:	3908HPV39 L1 R1
89	TCCAAGTTGGAGCAGGAACCTCCTGCCCAGTGGAACCT
SEQ ID No:	3909 HPV39 L1 amplified sequence 1
90	CTTGGTACC^[1] <u>ATGGCTATGTGGAGGTCCTCTGACAGTATGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTGAACACA</u> <u>GATGACTATGTGACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGACAGTGGGACACCC</u> <u>ATACTTCAAGGTGGGGATGAATGGAGGCAGGAAGCAGGACATCCCAAAGGTGTCTGCCTACCAATACAGG</u> <u>GTGTTTCAGGGTGACCCTGCCTGACCCAAACAAGTTCAGCATCCCTGATGCCTCCCTCTACAACCTGAGACC</u> <u>CAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG</u> <u>GACACCCACTCTACAACAGACAGGATGACACAGAGAACAGCCCATTCTCCAGCACCACCAACAAGGACAG</u> <u>CAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCATTGGCTGTGTGCCTGCCATTGGAG</u> <u>AACACTGGGGCAAGGGCAAGGCTTGTAAAGCCAAACAATGTGAGCACAGGAGACTGTCCTCCATTGGAACCT</u> <u>GGTGAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTATGGAGCTATGGACTTTGGAGCCCTCC</u> <u>AAGAGACCAAGTCTGAGGTGCCACTGGACATCTGTCTAGAGCATCTGTAAATACCCTGACTACCTCCAAATG</u> <u>AGTGCTGATGTCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGGCCAGACACTTCTGG</u> <u>AACAGGGGAGGGATGGTGGGAGATGCCATCCCTGCCCCAACTCTACATCAAGGGCACAGACATCAGGGCTA</u> <u>ACCCTGGCTCCTCTGTCTACTGTCCAAGCCCATCTGGCAGTATGGTGACCTCTGACAGCCAACTTTTCAACA</u> <u>AGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACTTTTCCTGACA</u> <u>GTGGTGGACACCACCAGGAGCACCAACTTCACCCTGAGCACCAGCATTGAGTCCAGCATCCCAAGCACCTA</u> <u>TGACCCAAGCAAGTTCAAGGAATACACCAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCAACCTT</u> <u>GTACTGTGACCCTGACCACAGATGTGATGAGTTACATCCACACAATGAACTCCAGCATCCTGGACAACCTGG</u> <u>AACTTTGCTGTGGCTCCTCCTCCATCTGCCTCCCTGGTGGACACCTACAGATACCTCCAATCTGCTGCCATC</u> <u>ACTTGTCAGAAGGATGCCCCCTGCCCCCTGAGAAGAAGGACCCATATGATGGACTGAAGTTCTGGAATGTGGA</u> <u>CCTGAGGGAGAAGTTCTCCTTGGAACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTG^[2]</u>

	CTCCAACCTTGGGA^[3] [1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding of HPV39 L1 aa 1-469; [3]: 12 bases overlapping with the gene fragment encoding HPV59L1 aa 471-508.
SEQ ID No: 91	3910HPV39 L1 F2 AGGAAGTTCCTGCTCCAACCTTGGAGCCAGACCAAAGC
SEQ ID No: 92	3911HPV39 L1 R2 CTGTCTAGATTTACTTCCTGCTGGACTTCCTCCTCTTCAC
SEQ ID No: 93	3912 HPV39 L1 amplified sequence 2 <u>AGGAAGTTCCTG^[1]</u> <u>CTCCAACCTTGGAGCCAGACCAAAGCCAACCATTTGGACCAAGGAAGAGGGCTGCCCTGCCCCAACCAGCA</u> <u>CACCAAGCCCAAAGAGGGTGAAGAGGAGGAAGTCCAGCAGGAAG^[2] TAA ATCTAGA^[3]CAG.</u> [1]: 12bp overlapping with the gene fragment encoding the C-terminus of HPV39 L1 aa 1-469; [2]: the gene fragment encoding the C-terminal 38 amino acids of HPV59L1 aa 471-508; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 94	3913 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK

Appendix 9 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 45 L1 PROTEIN

SEQ ID No: 95	4501 The amino acid sequence of HPV type 45 L1 protein aa 1-478 MALWRPSDSTVYLPPPSVARVVNTDDYVSRTSIFYHAGSSRLLTVGNPYFRVVP SGAGNKQAVPKVSAYQYRVFRVALPDPNKFGLPDSTIYNPETQRLVWACVGM EIGRGQPLGIGLSGHPFYNKLDDTESAHAATAVITQDVRDNVSVDYKQTQLCIL GCVPAIGEHWAKGTLCKPAQLQPGDCPPELEKNTIIEDGDMVDTGYGAMDFST LQDTKCEVPLDICQSICKYPDYQLQMSADPYGDSMFFCLRREQLFARHFWNRAG VMGDTVPTDLYIKGTSANMRETPGSCVYSPSPSGSITTSDSQLFNKPYWLHKAQ GHNNGICWHNQLFVTVDTTTRSTNLTLCASTQNPVPNTYDPTKFKHYSRHVEE YDLQFIFQLCTITLTAEVMSYIHSMNSSILENWNFGVPPPPTTSLVDTYRFVQSV AVTCQKDTPPEKQDPYDKLKFWTVDLKEKFSSDLDQYPLGRKFLVQAGL
SEQ ID No: 96	4502 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 97	4503 Amino acid sequence of chimeric HPV type 45 L1 protein MALWRPSDSTVYLPPPSVARVVNTDDYVSRTSIFYHAGSSRLLTVGNPYFRVVP SGAGNKQAVPKVSAYQYRVFRVALPDPNKFGLPDSTIYNPETQRLVWACVGM EIGRGQPLGIGLSGHPFYNKLDDTESAHAATAVITQDVRDNVSVDYKQTQLCIL GCVPAIGEHWAKGTLCKPAQLQPGDCPPELEKNTIIEDGDMVDTGYGAMDFST LQDTKCEVPLDICQSICKYPDYQLQMSADPYGDSMFFCLRREQLFARHFWNRAG VMGDTVPTDLYIKGTSANMRETPGSCVYSPSPSGSITTSDSQLFNKPYWLHKAQ GHNNGICWHNQLFVTVDTTTRSTNLTLCASTQNPVPNTYDPTKFKHYSRHVEE YDLQFIFQLCTITLTAEVMSYIHSMNSSILENWNFGVPPPPTTSLVDTYRFVQSV AVTCQKDTPPEKQDPYDKLKFWTVDLKEKFSSDLDQYPLGRKFLVQAGLKA

	KPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 98	4504 Nucleotide sequence of chimeric HPV type 45 L1 protein ATGGCTCTGTGGAGACCATCTGACAGCACAGTCTACCTGCCTCCTCCATCTGTGGCAAGGGTGGTGAACAC AGATGACTATGTGAGCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGGCAACC CATACTTCAGGGTGGTGCCAAGTGAGCAGGCAACAAGCAGGCTGTGCCAAAGGTGTCTGCCTACCAATAC AGGGTGTTTCAAGGGTGGCTCTGCCTGACCCAAACAAGTTTGGACTGCCTGACAGCACCATCTACAACCCTGA GACCCAGAGACTGGTGTGGGCTTGTGTGGGGATGGAGATTGGCAGGGGACAACCACTGGGCATTGGACTGT CTGGACACCCATTCTACAACAACTGGATGACACAGAGTCTGCCCATGCTGCCACAGCAGTGATTACCCAG GATGTGAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTGTATCCTGGGCTGTGTGCCTGCCATT GGAGAACTGGGCTAAGGGCACCTGTGTAAGCCTGCCCAACTCCAACCTGGAGACTGTCCTCCATTGGA ACTGAAAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTATGGAGCTATGGACTTCAGCACCC TCCAAGACACCAAGTGTGAGGTGCCACTGGACATCTGTCTAGAGCATCTGTAAATACCCTGACTACCTCCAA ATGAGTGCTGACCCATATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGCCAGACACTTC TGGAACAGGGCTGGAGTGATGGGAGACACAGTGCCAACAGACCTCTACATCAAGGGCACCTCTGCCAATAT GAGGGAGACACCTGGCTCCTGTGTCTACAGCCCAAGCCCATCTGGCAGCATCACCACTCTGACAGCCAAC TTTTCAACAAGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACTT TTTGTGACAGTGGTGGACACCACCAGGAGCACCAACCTGACCCTGTGTGCCAGCACCCAGAACCCTGTGCC AAACACCTATGACCCAACCAAGTTCAAGCACTACAGCAGGCATGTGGAGGAATATGACCTCCAATTCATCT TCCAACCTTGTACCATCACCTGACAGCAGAGGTGATGAGTTACATCCACAGTATGAACTCCAGCATCTTGG AGAACTGGAACCTTGGAGTGCCTCCTCCTCCAACCACCTCCCTGGTGGACACCTACAGGTTGTCCAGTCTG TGGCTGTGACTTGTCTAGAAGGACACCACACCTCCTGAGAAGCAGGACCCATATGACAACTGAAGTTCTGG ACAGTGGACCTGAAAGAGAAGTTCTCCTCTGACCTGGACCAATACCCACTGGGCAGGAAGTTCTGGTCCA GGCTGGACTGAAAGCCAAGGCCAAAAGTGAAGGGCTGCCCAACCAGCACCAGGACCTCCTCTGCCAAG AGGAAGAAGGTGAAGAAGTAAA

SEQ ID No:	4505 Synthetic HPV45 L1 gene
99	ctgggtaccATGGCTCTGTGGAGACCATCTGACAGCACAGTCTACCTGCCTCCTCCATCTGTGGCAAGGGTGGTG AACACAGATGACTATGTGAGCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGG CAACCCATACTTCAGGGTGGTGCCAAGTGGAGCAGGCAACAAGCAGGCTGTGCCAAAGGTGTCTGCCTACC AATACAGGGTGTTCAGGGTGGCTCTGCCTGACCCAAACAAGTTTGGACTGCCTGACAGCACCATCTACAAC CCTGAGACCCAGAGACTGGTGTGGGCTTGTGTGGGGATGGAGATTGGCAGGGGACAACCACTGGGCATTG GACTGTCTGGACACCCATTCTACAACAACTGGATGACACAGAGTCTGCCCATGCTGCCACAGCAGTGATT ACCCAGGATGTGAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCCTGGGCTGTGTGCC TGCCATTGGAGAACACTGGGCTAAGGGCACCTGTGTAAGCCTGCCCAACTCCAACCTGGAGACTGTCCTC CATTGGAAGTGAACCAACCATCATTGAGGATGGAGATATGGTGGACACAGGCTATGGAGCTATGGACTTC AGCACCTCCAAGACACCAAGTGTGAGGTGCCACTGGACATCTGTCAGAGCATCTGTAAATACCCTGACTA CCTCCAAATGAGTGCTGACCCATATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGGCAG ACACTTCTGGAACAGGGCTGGAGTGATGGGAGACACAGTGCCAACAGACCTCTACATCAAGGGCACCTCTG CCAATATGAGGGAGACACCTGGCTCCTGTGTCTACAGCCCAAGCCATCTGGCAGCATCACCACCTCTGAC AGCCAACCTTTTCAACAAGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACA CCAACCTTTTGTGACAGTGGTGGACACCACAGGAGCACCAACCTGACCCTGTGTGCCAGACCCAGAACC CTGTGCCAAACACCTATGACCCAACCAAGTTCAAGCACTACAGCAGGCATGTGGAGGAATATGACCTCCAA TTCATCTTCCAACCTTGTACCATCACCTGACAGCAGAGGTGATGAGTTACATCCACAGTATGAACTCCAGC ATCTTGGAGAACTGGAACCTTGGAGTGCCTCCTCCTCCAACCACTCCCTGGTGGACACCTACAGGTTTGTG CAGTCTGTGGCTGTGACTTGTGAGAAGGACACCACCTCCTGAGAAGCAGGACCCATATGACAACTGAA GTTCTGGACAGTGGACCTGAAAGAGAAGTTCTCCTCTGACCTGGACCAATACCCACTGGGCAGGAAGTTCC TGGTCCAGGCTGGACTGAGGAGGAGACCAACCATTGGACCAAGGAAGAGACCTGCTGCCAGCACCAAGCAC AGCCAGCAGACCTGCCAAGAGGGTGAAGATTAGGAGCAAGAAGTAAA ctcgagctc
SEQ ID No:	4506 Synthetic HPV33 L1 gene

100	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCCCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 101	4507HPV45 L1 F1 CTTGGTACCATGGCTCTGTGGAGACCATCTGAC
SEQ ID No:	4508HPV45 L1 R1

102	GCTTGGCTTTCAGTCCAGCCTGGACCAGGAAC
SEQ ID No: 103	4509 HPV45 L1 amplified sequence 1 CTTGGTACC^[1] <u>ATGGCTCTGTGGAGACCATCTGACAGCACAGTCTACCTGCCTCCTCCATCTGTGGCAAGGGTGGTGAACAC</u> <u>AGATGACTATGTGAGCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGGCAACC</u> <u>CATACTTCAGGGTGGTGCCAAGTGAGCAGGCAACAAGCAGGCTGTGCCAAAGGTGTCTGCCTACCAATAC</u> <u>AGGGTGTTCAGGGTGGCTCTGCCTGACCCAAACAAGTTTGGACTGCCTGACAGCACCATCTACAACCCTGA</u> <u>GACCCAGAGACTGGTGTGGGCTTGTGTGGGGATGGAGATTGGCAGGGGACAACCACTGGGCATTGGACTGT</u> <u>CTGGACACCCATTCTACAACAACTGGATGACACAGAGTCTGCCCATGCTGCCACAGCAGTGATTACCCAG</u> <u>GATGTGAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTGTATCCTGGGCTGTGTGCCTGCCATT</u> <u>GGAGAACTGGGCTAAGGGCACCTGTGTAAGCCTGCCCAACTCCAACCTGGAGACTGTCTCCATTGGA</u> <u>ACTGAAAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTATGGAGCTATGGACTTCAGCACCC</u> <u>TCCAAGACACCAAGTGTGAGGTGCCACTGGACATCTGTGAGAGCATCTGTAAATACCTGACTACCTCCAA</u> <u>ATGAGTGCTGACCCATATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGCCAGACACTTC</u> <u>TGGAACAGGGCTGGAGTGATGGGAGACACAGTGCCAACAGACCTCTACATCAAGGGCACCTCTGCCAATAT</u> <u>GAGGGAGACACCTGGCTCCTGTGTCTACAGCCCAAGCCCATCTGGCAGCATCACCACTCTGACAGCCAAC</u> <u>TTTCAACAAGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACTT</u> <u>TTTGTGACAGTGGTGGACACCACCAGGAGCACCAACCTGACCCTGTGTGCCAGCACCCAGAACCCTGTGCC</u> <u>AAACACCTATGACCCAACCAAGTTCAAGCACTACAGCAGGCATGTGGAGGAATATGACCTCCAATTCATCT</u> <u>TCCAACCTTTGTACCATCACCTGACAGCAGAGGTGATGAGTTACATCCACAGTATGAACTCCAGCATCTTGG</u> <u>AGAACTGGAACCTTGGAGTGCCTCCTCCTCCAACCACCTCCCTGGTGGACACCTACAGGTTTGTCCAGTCTG</u> <u>TGGCTGTGACTTGTGAGAAGGACACCACACCTCCTGAGAAGCAGGACCCATATGACAACTGAAGTTCTGG</u> <u>ACAGTGGACCTGAAAGAGAAGTTCTCCTCTGACCTGGACCAATACCCACTGGGCAGGAAGTTCTGGTCCA</u> <u>GGCTGGACTG^[2]AAAGCCAAGC^[3]</u> [1]: Kpn I enzymatic cleavage site;

	[2]: the gene fragment encoding of HPV45 L1 aa 1-478; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 104	4510HPV45 L1 F2 GGCTGGACTGAAAGCCAAGCCAAAAGCTGAAAAGGG
SEQ ID No: 105	4511HPV45 L1 R2 CTGTCTAGATTACTTCTTCACCTTCTTCCTCTTGGC
SEQ ID No: 106	4512 HPV45 L1 amplified sequence 2 <u>GGCTGGACTG</u>^[1] <u>AAAGCCAAGCCAAAAGCTGAAAAGGGCTGCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG</u>^[2] TAA <u>ATCTAGA</u>^[3]CAG. [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV45 L1 aa 1-478; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 107	4513 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKR VKRRKSSRK

Appendix 10 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 51 L1 PROTEIN

SEQ ID No: 108	5101 The amino acid sequence of HPV type 51 L1 protein aa 1-474 MALWRTNSKVVYLPPAPVSRIVNTEEYITRTGIYYYAGSSRLITLGHYPFPIPKTS TRAAIPKVSFAFYRVFRVQLPDPNKFGLPDPNLYNPDTDRLVWGCVGVEVGRG QPLGVGLSGHPLFNKYDDTENSRIANGNAQQDVRDNTSVDNKQTQLCIIGCAPP IGEHWGIGTTCKNTPVPPGDCPPELVSSVIQDGDMDTGFGAMDFAALQATKS DVPLDISQSVCKYPDYLKMSADTYGNSMFFHLRREQIFARHYYNKLVGVGEDI PNDYYIKGSGNGRDPIESYIYSATPSGSMITSDSQIFNKPYWLHRAQGHNNGICW NNQLFITCVDTTTRSTNLTISTATAAVSPTFTPSNFKQYIRHGEEYELQFIFQLCKIT LTTEVMAYLHTMDPTILEQWNFGLTLPPSASLEDAYRFVRNAATSCQKDTPPQ AKPDPLAKYKFWVDVLKERFSLDLQFALGRKFLLQVGV
SEQ ID No: 109	5102 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 110	5103 Amino acid sequence of chimeric HPV type 51 L1 protein MALWRTNSKVVYLPPAPVSRIVNTEEYITRTGIYYYAGSSRLITLGHYPFPIPKTS TRAAIPKVSFAFYRVFRVQLPDPNKFGLPDPNLYNPDTDRLVWGCVGVEVGRG QPLGVGLSGHPLFNKYDDTENSRIANGNAQQDVRDNTSVDNKQTQLCIIGCAPP IGEHWGIGTTCKNTPVPPGDCPPELVSSVIQDGDMDTGFGAMDFAALQATKS DVPLDISQSVCKYPDYLKMSADTYGNSMFFHLRREQIFARHYYNKLVGVGEDI PNDYYIKGSGNGRDPIESYIYSATPSGSMITSDSQIFNKPYWLHRAQGHNNGICW NNQLFITCVDTTTRSTNLTISTATAAVSPTFTPSNFKQYIRHGEEYELQFIFQLCKIT LTTEVMAYLHTMDPTILEQWNFGLTLPPSASLEDAYRFVRNAATSCQKDTPPQ AKPDPLAKYKFWVDVLKERFSLDLQFALGRKFLLQVGVKAKPKLKRAAPTST

	RTSSAKRKKVKK
SEQ ID No: 111	5104 Nucleotide sequence of chimeric HPV type 51 L1 protein ATGGCTCTGTGGAGGACCAATGACAGCAAGGTCTACCTGCCTCCTGCCCCTGTGAGCAGGATTGTGAACAC AGAGGAATACATCACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGATTACCCTGGGACACC CATACTTTCCAATCCCAAAGACCAGCACCAGGGCTGCCATCCCAAAGGTGTCTGCCTTCCAATACAGGGTG TTCAGGGTCCAACCTTCTGACCCAAACAAGTTTGGACTGCCTGACCCAAACCTCTACAACCCTGACACAGA CAGACTGGTGTGGGGCTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGACTGTCTGGA CACCCACTGTTCAACAAATATGATGACACAGAGAACAGCAGGATTGCCAATGGCAATGCCCAACAGGATGT GAGGGACAACACCTCTGTGGACAACAAGCAGACCCAACTTTGTATCATTGGCTGTGCCCTCCAATTGGAG AACACTGGGGCATTGGCACCACTTGTAAGAACACACCTGTGCCTCCTGGAGACTGTCTCCATTGGAAGT GTGTCCTCTGTGATTCAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTTGCTGCCCTCCAA GCCACCAAGTCTGATGTGCCACTGGACATCAGCCAGTCTGTGTGTAAATACCCTGACTACCTGAAAATGAG TGCTGACACCTATGGCAACAGTATGTTCTTCCACCTGAGGAGGGAACAGATTTTGGCAGACACTACTACA ACAAACTGGTGGGAGTGGGAGAGGACATCCCAAATGACTACTACATCAAGGGCTCTGGCAATGGCAGGGA CCCAATTGAGTCCTACATCTACTCTGCCACACCATCTGGCAGTATGATTACCTCTGACAGCCAGATTTTCAA CAAGCCATACTGGCTGCACAGGGCTCAAGGACACAACAATGGCATCTGTTGGAACAACCAACTTTTCATCA CTTGTGTGGACACCACCAGGAGACCAACCTGACCATCAGCACAGCCACAGCAGCAGTGAGCCCAACCTTC ACACCAAGCAACTTCAAGCAATACATCAGACATGGAGAGGAATATGAACTCCAATTCTTCCAACCTTTG TAAGATTACCCTGACCACAGAGGTGATGGCTTACCTGCACACAATGGACCCAACCATCTTGGAACAGTGGA ACTTTGGACTGACCCTGCCTCCATCTGCCTCCTTGGAGGATGCCTACAGGTTTGTGAGGAATGCTGCCACCT CCTGTGAGAAGGACACACCTCCACAGGCTAAGCCTGACCCACTGGCTAAATACAAGTTCTGGGATGTGGAC CTGAAAGAGAGGTTCTCCCTGGACCTGGACCAGTTTGCCCTGGGCAGGAAGTTCCTGCTCCAAGTGGGAGT CAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACCAAGCAGCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAA

SEQ ID No:	5105 Synthetic HPV51 L1 gene
112	ctgggtaccATGGCTCTGTGGAGGACCAATGACAGCAAGGTCTACCTGCCTCCTGCCCCTGTGAGCAGGATTGT GAACACAGAGGAATACATCACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGATTACCCTGG GACACCCATACTTTCCAATCCCAAAGACCAGCACCAGGGCTGCCATCCCAAAGGTGTCTGCCTTCCAATAC AGGGTGTTCAAGGTCCAACCTCTGACCCAAACAAGTTTGGACTGCCTGACCCAAACCTCTACAACCTGA CACAGACAGACTGGTGTGGGGCTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGACTG TCTGGACACCCACTGTTCAACAAATATGATGACACAGAGAACAGCAGGATTGCCAATGGCAATGCCCAACA GGATGTGAGGGACAACACCTCTGTGGACAACAAGCAGACCCAACTTTGTATCATTGGCTGTGCCCTCCAA TTGGAGAACACTGGGGCATTGGCACCCTTGTAAGAACACACCTGTGCCTCCTGGAGACTGTCTCCATTG GAACTGGTGTCTCTGTGATTCAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTTGCTGCC CTCCAAGCCACCAAGTCTGATGTGCCACTGGACATCAGCCAGTCTGTGTGTAAATACCCTGACTACCTGAA AATGAGTGCTGACACCTATGGCAACAGTATGTTCTTCCACCTGAGGAGGGAACAGATTTTTGCCAGACACT ACTACAACAACTGGTGGGAGTGGGAGAGGACATCCCAAATGACTACTACATCAAGGGCTCTGGCAATGG CAGGGACCCAATTGAGTCCTACATCTACTTGCCACACCATCTGGCAGTATGATTACCTCTGACAGCCAGAT TTTCAACAAGCCATACTGGCTGCACAGGGCTCAAGGACACAACAATGGCATCTGTTGGAACAACCAACTTT TCATCACTTGTGTGGACACCACCAGGAGCACCAACCTGACCATCAGCACAGCCACAGCAGCAGTGAGCCCA ACCTTCACACCAAGCAACTTCAAGCAATACATCAGACATGGAGAGGAATATGAACTCCAATTCATCTTCCA ACTTTGTAAGATTACCCTGACCACAGAGGTGATGGCTTACCTGCACACAATGGACCCAACCATCTTGGAAC AGTGGAACCTTTGGACTGACCCTGCCTCCATCTGCCTCCTTGGAGGATGCCTACAGGTTTGTGAGGAATGCTG CCACCTCCTGTCAGAAGGACACACCTCCACAGGCTAAGCCTGACCCACTGGCTAAATACAAGTTCTGGGAT GTGGACCTGAAAGAGAGGTTCTCCCTGGACCTGGACCAGTTTGCCTGGGCAGGAAGTTCCTGCTCCAAGT GGGAGTCCAGAGGAAGCCAAGACCTGGACTGAAAAGACCTGCCTCCTCTGCCTCCTCCTCCTCCTCCTC TGCCAAGAGGAAGAGGGTGAAGAAGTAAActcgagctc

SEQ ID No:	5106 Synthetic HPV33 L1 gene
113	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACCAAGCAGCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No:	5107HPV51 L1 F1
114	CTTGGTACCATGGCTCTGTGGAGGACCAATGACA

SEQ ID No: 115	5108HPV51 L1 R1 GCTTGGCTTTGACTCCCCTTGGAGCAGGAAC
SEQ ID No: 116	5109 HPV51 L1 amplified sequence 1 <u>CTTGGTACC^[1]</u> <u>ATGGCTCTGTGGAGGACCAATGACAGCAAGGTCTACCTGCCTCCTGCCCCTGTGAGCAGGATTGTGAACAC</u> <u>AGAGGAATACATCACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGATTACCCTGGGACACC</u> <u>CATACTTTCCAATCCCAAAGACCAGCACCAGGGCTGCCATCCCAAAGGTGTCTGCCTTCCAATACAGGGTG</u> <u>TTCAGGGTCCAACCTCCTGACCCAAACAAGTTTGGACTGCCTGACCCAAACCTCTACAACCCTGACACAGA</u> <u>CAGACTGGTGTGGGGCTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGACTGTCTGGA</u> <u>CACCCACTGTTCAACAAATATGATGACACAGAGAACAGCAGGATTGCCAATGGCAATGCCAACAGGATGT</u> <u>GAGGGACAACACCTCTGTGGACAACAAGCAGACCCAACTTTGTATCATTGGCTGTGCCCCTCCAATTGGAG</u> <u>AACACTGGGGCATTGGCACCCTTGTAAGAACACACCTGTGCCTCCTGGAGACTGTCTCCATTGGAACCTG</u> <u>GTGTCCTCTGTGATTGAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTTGCTGCCCTCCA</u> <u>GCCACCAAGTCTGATGTGCCACTGGACATCAGCCAGTCTGTGTGTAAATACCCTGACTACCTGAAAATGAG</u> <u>TGCTGACACCTATGGCAACAGTATGTTCTTCCACCTGAGGAGGGAACAGATTTTGGCAGACACTACTACA</u> <u>ACAAACTGGTGGGAGTGGGAGAGGACATCCCAAATGACTACTACATCAAGGGCTCTGGCAATGGCAGGGA</u> <u>CCCAATTGAGTCCTACATCTACTCTGCCACACCATCTGGCAGTATGATTACCTCTGACAGCCAGATTTTCAA</u> <u>CAAGCCATACTGGCTGCACAGGGCTCAAGGACACAACAATGGCATCTGTTGGAACAACCAACTTTTCATCA</u> <u>CTTGTGTGGACACCACCAGGAGACCAACCTGACCATCAGCACAGCCACAGCAGCAGTGAGCCCAACCTTC</u> <u>ACACCAAGCAACTTCAAGCAATACATCAGACATGGAGAGGAATATGAACTCCAATTCATCTTCCAACCTTG</u> <u>TAAGATTACCCTGACCACAGAGGTGATGGCTTACCTGCACACAATGGACCCAACCATCTTGGAACAGTGGA</u> <u>ACTTTGGACTGACCCTGCCTCCATCTGCCTCCTTGGAGGATGCCTACAGGTTTGTGAGGAATGCTGCCACCT</u> <u>CCTGTCAGAAGGACACACCTCCACAGGCTAAGCCTGACCCACTGGCTAAATACAAGTTCTGGGATGTGGAC</u> <u>CTGAAAGAGAGGTTCTCCCTGGACCTGGACCAGTTTGCCCTGGGCAGGAAGTTCCTGCTCCAAGTGGGAGT</u> <u>C^[2]AAAGCCAAGC^[3]</u>

	[1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding of HPV51 L1 aa 1-474; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499
SEQ ID No: 117	5110HPV51 L1 F2 AGTGGGAGTCAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 118	5111HPV51 L1 R2 CTGTCTAGATTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 119	5112 HPV51 L1 amplified sequence 2 <u>AGTGGGAGTC^[1]</u> <u>AAAGCCAAGCCAAACTGAAAAGGGCTGCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG^[2] TAA ATCTAGA^[3]CAG.</u> [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV51 L1 aa 1-474; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 120	5113 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARPKPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

Appendix 11 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 52 L1 PROTEIN

SEQ ID No: 121	5201 The amino acid sequence of HPV type 52 L1 protein aa 1-478 MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLTVGHPYFSIKN TSSGNGKKVLVPKVSGLQYRVFRIKLDPNKFPGPDTSFYNPETQRLVWACTGL EIGRGQPLGVGISGHPLLNFDDTETS NKYAGKPGIDNRECLSMDYKQTQLCIL GCKPPIGEHWGKGTPC NNSGNPGDCPPLQLINSVIQDGDMDVDTGFGC MDFNT LQASKSDVPIDICSSVCKYPDYLQMASEPYGDSLFFFLRREQMFVRHFFNRAGT LGDPVPGDLYIQGSNSGNTATVQSSAFFPTPSGSMVTSESQLFNKPYWLQRAQG HNNGICWGNQLFVTVD TTRSTNMTLCAEVKKESTYKNENFKEYLRHGEEFDL QFIFQLCKITLTADVMTYIHKMDATILEDWQFGLTPPPSASLED TYRFVTSTAIT CQKNTPPKGKEDPLKDYMFEVDLKEKFSADLDQFPLGRKFLLQAGL
SEQ ID No: 122	5202 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTR TSSAKRKKVKK
SEQ ID No: 123	5203 Amino acid sequence of chimeric HPV type 52 L1 protein MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLTVGHPYFSIKN TSSGNGKKVLVPKVSGLQYRVFRIKLDPNKFPGPDTSFYNPETQRLVWACTGL EIGRGQPLGVGISGHPLLNFDDTETS NKYAGKPGIDNRECLSMDYKQTQLCIL GCKPPIGEHWGKGTPC NNSGNPGDCPPLQLINSVIQDGDMDVDTGFGC MDFNT LQASKSDVPIDICSSVCKYPDYLQMASEPYGDSLFFFLRREQMFVRHFFNRAGT LGDPVPGDLYIQGSNSGNTATVQSSAFFPTPSGSMVTSESQLFNKPYWLQRAQG HNNGICWGNQLFVTVD TTRSTNMTLCAEVKKESTYKNENFKEYLRHGEEFDL QFIFQLCKITLTADVMTYIHKMDATILEDWQFGLTPPPSASLED TYRFVTSTAIT CQKNTPPKGKEDPLKDYMFEVDLKEKFSADLDQFPLGRKFLLQAGLKAKPK

	LKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 124	5204 Nucleotide sequence of chimeric HPV type 52 L1 protein ATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC CGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGACCGTGGGCCACC CCTACTTCAGCATCAAGAACCAGCAGCGGCAACGGCAAGAAGGTGCTGGTGCCCAAGGTGAGCGGCCT GCAGTACAGGGTGTTCAGGATCAAGCTGCCCCACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACA ACCCCGAGACCCAGAGGCTGGTGTGGGCCTGCACCGGCCTGGAGATCGGCAGGGGCCAGCCCCTGGGCGT GGGCATCAGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGACCAGCAACAAGTACGCCGGCAAG CCCGGCATCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCATCCTGGGCTGCAA GCCCCCATCGGCGAGCACTGGGGCAAGGGCACCCCTGCAACAACAACAGCGGCAACCCCGGCGACTGC CCCCCCTGCAGCTGATCAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCTGCATGGA CTTCAACACCCTGCAGGCCAGCAAGAGCGACGTGCCATCGACATCTGCAGCAGCGTGTGCAAGTACCCCG ACTACCTGCAGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTTCTGAGGAGGGAGCAGATGTTT GTGAGGCACTTCTTCAACAGGGCCGGCACCCCTGGGCGACCCCGTGCCCGGCGACCTGTACATCCAGGGCAG CAACAGCGGCAACACCGCCACCGTGCAGAGCAGCGCCTTCTTCCCCACCCCAGCGGCAGCATGGTGACCA GCGAGAGCCAGCTGTTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTG GGGCAACCAGCTGTTCGTGACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCGCCGAGGTGA AGAAGGAGAGCACCTACAAGAACGAGAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCA GTTTATCTTCCAGCTGTGCAAGATCACCTGACCGCCGACGTGATGACCTACATCCACAAGATGGACGCCA CCATCCTGGAGGACTGGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGGAGGACACCTACAGGTTC GTGACCAGCACCGCCATCACCTGCCAGAAGAACACCCCCCAAGGGCAAGGAGGACCCCTGAAGGACT ACATGTTCTGGGAGGTGGACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCTCCCTGGGCAGGAAG TTCCTGCTGCAGGCCGGCCTGAAAGCCAAGCCAAAAGTGAAGGGGTGCCCAACCAGCACCAAGGACCTC CTCTGCCAAGAGGAAGAAGGTGAAGAAGTAAA

SEQ ID No:	5205 Synthetic HPV52 L1 gene
125	ctgggtaccATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGACCTGCCCCCGTGCCCGTGAGCAAGGTGGT GAGCACCGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGACCGTG GGCCACCCCTACTTCAGCATCAAGAACCAGCAGCGGCAACGGCAAGAAGGTGCTGGTGCCCAAGGTGA GCGGCCTGCAGTACAGGGTGTTCAGGATCAAGCTGCCCCGACCCCAACAAGTTCGGCTTCCCCGACACCAGC TTTACAACCCCGAGACCCAGAGGCTGGTGTGGGCCTGCACCGGCCTGGAGATCGGCAGGGGCCAGCCCT GGGCGTGGGCATCAGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGACCAGCAACAAGTACGCC GGCAAGCCCGGCATCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCATCCTGG GCTGCAAGCCCCCATCGGCGAGCACTGGGGCAAGGGCACCCCTGCAACAACAACAGCGGCAACCCCGG CGACTGCCCCCCCCTGCAGCTGATCAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCT GCATGGACTTCAACACCCTGCAGGCCAGCAAGAGCGACGTGCCCATCGACATCTGCAGCAGCGTGTGCAAG TACCCCGACTACCTGCAGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTTCTGAGGAGGGAGCA GATGTTCTGTGAGGCACTTCTTCAACAGGGCCGGCACCCCTGGGCGACCCCGTGCCCGGCGACCTGTACATCC AGGGCAGCAACAGCGGCAACACCGCCACCGTGCGAGAGCAGCGCCTTCTTCCCCACCCCAAGCGGCAGCATG GTGACCAGCGAGAGCCAGCTGTTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCA TCTGCTGGGGCAACCAGCTGTTCTGTGACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCGCC GAGGTGAAGAAGGAGAGCACCTACAAGAACGAGAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCG ACCTGCAGTTCATCTTCCAGCTGTGCAAGATCACCTGACCGCCGACGTGATGACCTACATCCACAAGATG GACGCCACCATCCTGGAGGACTGGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGGAGGACACCTA CAGGTTCGTGACCAGCACCGCCATCACCTGCCAGAAGAACACCCCCCAAGGGCAAGGAGGACCCCTG AAGGACTACATGTTCTGGGAGGTGGACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCTCCCTGGG CAGGAAGTTCCTGCTGCAGGCCGGCCTGCAGGCCAGGCCAAGCTGAAGAGGCCCGCCAGCAGCGCCCCC AGGACCAGCACCAAGAAGAAGAAGGTGAAGAGGTAAActcgagetc
SEQ ID No:	5206 Synthetic HPV33 L1 gene

126	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No:	5207HPV52 L1 F1
127	CTTGGTACCATGAGCGTGTGGAGGCCAGCGAGG
SEQ ID No:	5208HPV52 L1 R1

128	GCTTGGCTTTCAGGCCGGCCTGCAGCAGGAACTTC
SEQ ID No: 129	5209 HPV52 L1 amplified sequence 1 CTTGGTACC^[1] <u>ATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC</u> <u>CGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGACCGTGGGCCACC</u> <u>CCTACTTCAGCATCAAGAACACCAGCAGCGGCAACGGCAAGAAGGTGCTGGTGCCCAAGGTGAGCGGCCT</u> <u>GCAGTACAGGGTGTTCAGGATCAAGCTGCCCCGACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACA</u> <u>ACCCCGAGACCCAGAGGCTGGTGTGGGCCTGCACCGGCCTGGAGATCGGCAGGGGCCAGCCCTGGGCGT</u> <u>GGGCATCAGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGACCAGCAACAAGTACGCCGGAAG</u> <u>CCCGGCATCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCATCCTGGGCTGCAA</u> <u>GCCCCCATCGGCGAGCACTGGGGCAAGGGCACCCCTGCAACAACAACAGCGGCAACCCCGGCGACTGC</u> <u>CCCCCCTGCAGCTGATCAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCTGCATGGA</u> <u>CTTCAACACCCTGCAGGCCAGCAAGAGCGACGTGCCATCGACATCTGCAGCAGCGTGTGCAAGTACCCCG</u> <u>ACTACCTGCAGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTTCTGAGGAGGGAGCAGATGTTT</u> <u>GTGAGGCACTTCTTCAACAGGGCCGGCACCCCTGGGCGACCCCGTGCCCGGCGACCTGTACATCCAGGGCAG</u> <u>CAACAGCGGCAACACCGCCACCGTGCAGAGCAGCGCCTTCTTCCCCACCCCAAGCGGCGAGCATGGTGACCA</u> <u>GCGAGAGCCAGCTGTTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTG</u> <u>GGGCAACCAGCTGTTCGTGACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCGCCGAGGTGA</u> <u>AGAAGGAGAGCACCTACAAGAACGAGAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCA</u> <u>GTTTATCTTCCAGCTGTGCAAGATCACCCCTGACCGCCGACGTGATGACCTACATCCACAAGATGGACGCCA</u> <u>CCATCCTGGAGGACTGGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGGAGGACACCTACAGGTTC</u> <u>GTGACCAGCACCGCCATCACCTGCCAGAAGAACACCCCCCAAGGGCAAGGAGGACCCCTGAAGGACT</u> <u>ACATGTTCTGGGAGGTGGACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCTTGGGAGGAAAG</u> <u>TTCTGCTGCAGGCCGGCCTG</u>^[2]AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site;

	[2]: the gene fragment encoding of HPV52 L1 aa 1-478; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 130	5210HPV52 L1 F2 GGCCGGCCTGAAAGCCAAGCCAAAACTGAAAAGGG
SEQ ID No: 131	5211HPV52 L1 R2 CTGTCTAGATTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 132	5212 HPV52 L1 amplified sequence 2 <u>GGCCGGCCTG</u>^[1] <u>AAAGCCAAGCCAAAACTGAAAAGGGCTGCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG</u>^[2] TAA <u>ATCTAGA</u>^[3]CAG. [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV52 L1 aa 1-478; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site
SEQ ID No: 133	5213 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKR VKRRKSSRK

Appendix 12 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 56 L1 PROTEIN

SEQ ID No: 134	5601 The amino acid sequence of HPV type 56 L1 protein aa 1-467 MATWRPSENKVYLPPTPVSKVVATDSYVKRTSIFYHAGSSRLLAUGHPPYYSVT KDNTKTNIPKVSAYQYRVFRVRLPDPNKFGLPDTNIYNPDQERLVWACVGLEV GRGQPLGAGLSGHPLFNRLDDTESSNLANNVIEDSRDNISVDGKQTQLCIVGC TPAMGEHWTKGAVCKSTQVTTGDCPPLALINTPIEDGDMIDTGFGAMDFKVLQ ESKAEVPLDIVQSTCKYPDYLKMSADAYGDSMWFYLRREQLFARHYFNRAKG VGETIPAELYLKGSNGREPPSSVYVATPSGSMITSEAQLFNKPYWLQRAQGHN NGICWGNQLFVTVDTRSTNMTISTATEQLSKYDARKINQYLRHVEEYELQF VFQLCKITLSAEVMAYLHNMNANLLEDWNIGLSPPVATSLEDKYRYVRSTAITC QREQPTEKQDPLAKYKFWVDVNLQDSFSTDLDQFPLGRKFL
SEQ ID No: 135	5602 The amino acid sequence of HPV type 33 L1 protein aa 469-499 LQAGLKAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 136	5603 Amino acid sequence of chimeric HPV type 56 L1 protein MATWRPSENKVYLPPTPVSKVVATDSYVKRTSIFYHAGSSRLLAUGHPPYYSVT KDNTKTNIPKVSAYQYRVFRVRLPDPNKFGLPDTNIYNPDQERLVWACVGLEV GRGQPLGAGLSGHPLFNRLDDTESSNLANNVIEDSRDNISVDGKQTQLCIVGC TPAMGEHWTKGAVCKSTQVTTGDCPPLALINTPIEDGDMIDTGFGAMDFKVLQ ESKAEVPLDIVQSTCKYPDYLKMSADAYGDSMWFYLRREQLFARHYFNRAKG VGETIPAELYLKGSNGREPPSSVYVATPSGSMITSEAQLFNKPYWLQRAQGHN NGICWGNQLFVTVDTRSTNMTISTATEQLSKYDARKINQYLRHVEEYELQF VFQLCKITLSAEVMAYLHNMNANLLEDWNIGLSPPVATSLEDKYRYVRSTAITC QREQPTEKQDPLAKYKFWVDVNLQDSFSTDLDQFPLGRKFL

	RAAPTSTRTSSAKRKKVKK
SEQ ID No: 137	5604 Nucleotide sequence of chimeric HPV type 56 L1 protein ATGGGTACCTGGAGACCATCTGAGAACAAGGTCTACCTGCCTCCAACACCTGTGAGCAAGGTGGTGGCTAC AGACTCCTATGTGAAGAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGGGACACC CATACTACTCTGTGACCAAGGACAACACCAAGACCAACATCCCAAAGGTGTCTGCCTACCAATACAGGGTG TTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGACTGCCTGACACCAACATCTACAACCCTGACCAGGA GAGACTGGTGTGGGCTTGTGTGGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGCAGGACTGTCTGGA CACCCACTGTTCAACAGACTGGATGACACAGAGTCCAGCAACCTGGCTAACAACAATGTGATTGAGGACAG CAGGGACAACATCTCTGTGGATGGCAAGCAGACCCAACTTTGTATTGTGGGCTGTACTCCTGCTATGGGAG AACACTGGACCAAGGGAGCAGTGTGTAAGAGCACCCAGGTGACCACAGGAGACTGTCCTCCACTGGCTCTG ATAAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTCAAGGTGCTCCA AGAGAGCAAGGCTGAGGTGCCACTGGACATTGTCCAGAGCACTTGTAATAACCTGACTACCTGAAAATGA GTGCTGATGCCTATGGAGACAGTATGTGGTTCTACCTGAGGAGGGAACAACCTTTTGCCAGACACTACTTC AACAGGGCTGGCAAGGTGGGAGAGACCATCCCTGCTGAACTCTACCTGAAAGGCAGCAATGGCAGGGAAC CTCCTCCATCCTCTGTCTATGTGGCTACACCATCTGGCAGTATGATTACCTCTGAGGCTCAACTTTTCAACAA GCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTGTGACAG TGGTGGACACCACCAGGAGCACCAATATGACCATCAGCACAGCCACAGAACAACCTTAGCAAATATGATGC CAGGAAGATAAACCAATACCTGAGGCATGTGGAGGAATATGAACTCCAATTTGTGTCCAACCTTTGTAAGA TTACCCTGTCTGCTGAGGTGATGGCTTACCTGCACAATATGAATGCCAACCTGTTGGAGGACTGGAACATTG GACTGAGCCCTCCTGTGGCTACCTCCTTGGAGGACAAATACAGATATGTGAGGAGCACAGCCATCACTTGT CAGAGGGAACAACCTCCAACAGAGAAGCAGGACCCACTGGCTAAATACAAGTTCTGGGATGTGAACCTCC AAGACTCCTTCAGCACAGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACTGAAA GCCAAGCCAAAACCTGAAAAGGGCTGCCCAACCAGCACAGGACCTCCTCTGCCAAGAGGAAGAAGGTGA AGAAGTAAA

SEQ ID No:	5605 Synthetic HPV56 L1 gene
138	ctgggtaccATGGCTACCTGGAGACCATCTGAGAACAAAGGTCTACCTGCCTCCAACACCTGTGAGCAAGGTGGT GGCTACAGACTCCTATGTGAAGAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGG GACACCCATACTACTCTGTGACCAAGGACAACACCAAGACCAACATCCCAAAGGTGTCTGCCTACCAATAC AGGGTGTTTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGACTGCCTGACACCAACATCTACAACCCTGA CCAGGAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGCAGGACTG TCTGGACACCCACTGTTCAACAGACTGGATGACACAGAGTCCAGCAACCTGGCTAACAAACAATGTGATTGA GGACAGCAGGGACAACATCTCTGTGGATGGCAAGCAGACCCAACTTTGTATTGTGGGCTGTACTCCTGCTA TGGGAGAACTGGACCAAGGGAGCAGTGTGTAAGAGCACCCAGGTGACCACAGGAGACTGTCTCCACT GGCTCTGATAAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTCAAGG TGCTCCAAGAGAGCAAGGCTGAGGTGCCACTGGACATTGTCCAGAGCACTTGTAATAACCTGACTACCTG AAAATGAGTGCTGATGCCTATGGAGACAGTATGTGGTTCTACCTGAGGAGGGAACAACCTTTTTGCCAGACA CTACTTCAACAGGGCTGGCAAGGTGGGAGAGACCATCCCTGCTGAACTCTACCTGAAAGGCAGCAATGGCA GGGAACCTCCTCCATCCTCTGTCTATGTGGCTACACCATCTGGCAGTATGATTACCTCTGAGGCTCAACTTT TCAACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTT GTGACAGTGGTGGACACCACCAGGAGCACCAATATGACCATCAGCACAGCCACAGAACAACCTTAGCAAAT ATGATGCCAGGAAGATAAACCAATACCTGAGGCATGTGGAGGAATATGAACTCCAATTTGTGTTCCAACCT TGTAAGATTACCCTGTCTGCTGAGGTGATGGCTTACCTGCACAATATGAATGCCAACCTGTTGGAGGACTG GAACATTGGACTGAGCCCTCCTGTGGCTACCTCCTTGGAGGACAAATACAGATATGTGAGGAGCACAGCCA TCACTTGTCAGAGGGAACAACCTCCAACAGAGAAGCAGGACCCACTGGCTAAATACAAGTTCTGGGATGTG AACCTCCAAGACTCCTTCAGCACAGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGATGCAACTTGG CACCAGGAGCAAGCCTGCTGTGGCTACCAGCAAGAAGAGGTCTGCCCCAACAGCACCAGCACACCTGCC AAGAGGAAGAGGAGGTAAActegagctc
SEQ ID No:	5606 Synthetic HPV33 L1 gene
139	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG

	AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACC AATATGACCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 140	5607HPV56 L1 F1 CTTGGTACCATGGCTACCTGGAGACCATCTGAG
SEQ ID No: 141	5608HPV56 L1 R1 CTGCTTGGAGCAGGAACCTCCTGCCCAGTGG

SEQ ID No: 142	5609 HPV56 L1 amplified sequence 1 CTTGGTACC^[1] <u>ATGGGTACCTGGAGACCATCTGAGAACAAAGGTCTACCTGCCTCCAACACCTGTGAGCAAGGTGGTGGCTAC</u> <u>AGACTCCTATGTGAAGAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGGGACACC</u> <u>CATACTACTCTGTGACCAAGGACAACACCAAGACCAACATCCCAAAGGTGTCTGCCTACCAATACAGGGTG</u> <u>TTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGACTGCCTGACACCAACATCTACAACCTGACCAGGA</u> <u>GAGACTGGTGTGGGCTTGTGTGGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGCAGGACTGTCTGGA</u> <u>CACCCACTGTTCACAGACTGGATGACACAGAGTCCAGCAACCTGGCTAACACAATGTGATTGAGGACAG</u> <u>CAGGGACAACATCTCTGTGGATGGCAAGCAGACCCAACTTTGTATTGTGGGCTGTACTCCTGCTATGGGAG</u> <u>AACACTGGACCAAGGGAGCAGTGTGTAAGAGCACCCAGGTGACCACAGGAGACTGTCCTCCACTGGCTCTG</u> <u>ATAAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTCAAGGTGCTCCA</u> <u>AGAGAGCAAGGCTGAGGTGCCACTGGACATTGTCCAGAGCACTTGTAAATACCCTGACTACCTGAAAATGA</u> <u>GTGCTGATGCCTATGGAGACAGTATGTGGTTCTACCTGAGGAGGGAACAACCTTTTGCCAGACACTACTTC</u> <u>AACAGGGCTGGCAAGGTGGGAGAGACCATCCCTGCTGAACTCTACCTGAAAGGCAGCAATGGCAGGGAAC</u> <u>CTCCTCCATCCTCTGTCTATGTGGCTACACCATCTGGCAGTATGATTACCTCTGAGGCTCAACTTTTCAACAA</u> <u>GCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCACTTTTGTGACAG</u> <u>TGGTGGACACCACCAGGAGCACCAATATGACCATCAGCACAGCCACAGAACAACCTTAGCAAAATATGATGC</u> <u>CAGGAAGATAAACCAATACCTGAGGCATGTGGAGGAATATGAACTCCAATTTGTGTCCAACCTTTGTAAGA</u> <u>TTACCCTGTCTGCTGAGGTGATGGCTTACCTGCACAATATGAATGCCAACCTGTTGGAGGACTGGAACATTG</u> <u>GACTGAGCCCTCCTGTGGCTACCTCCTTGGAGGACAAATACAGATATGTGAGGAGCACAGCCATCACTTGT</u> <u>CAGAGGGAACAACCTCCAACAGAGAAGCAGGACCCACTGGCTAAATACAAGTTCTGGGATGTGAACCTCC</u> <u>AAGACTCCTTCAGCACAGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTG</u>^[2]CTCCAAGCAG^[3] [1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding of HPV56 L1 aa 1-467;

	[3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 469-499.
SEQ ID No: 143	5610HPV56 L1 F2 GAAGTTCCTGCTCCAAGCAGGACTGAAAGCCAAGCC
SEQ ID No: 144	5611HPV56 L1 R2 CTGTCTAGATTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 145	5612 HPV56 L1 amplified sequence 2 <u>GAAGTTCCTG</u> ^[1] <u>CTCCAAGCAGGACTGAAAGCCAAGCCAAAAGTGAAGGGCTGCCCCAACCAGCACCAGGACCTCCTCTG</u> <u>CCAAGAGGAAGAAGGTGAAGAAG</u> ^[2] TAA <u>ATCTAGA</u> ^[3] CAG. [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV56 L1 aa 1-467; [2]: the gene fragment encoding the C-terminal 31 amino acids of HPV33L1 aa 469-499; [3]: XbaI enzymatic cleavage site.
SEQ ID No: 146	5613 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKR VKRRKSSRK

Appendix 13 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 58 L1 PROTEIN

SEQ ID No: 147	5801 The amino acid sequence of HPV type 58 L1 protein aa 1-473 MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGNPYFSIKS PNNNKKVLVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPDTQRLVWACVGLE IGRGQPLGVGVSGHPYLNKFDDTETSNRYPAQPGSDNRECLSMDYKQTQLCLI GCKPPTGEHWGKGVACNNNAAATDCPPELFNSIIEDGDMVDTGFGCMDFGTL QANKSDVPIDICNSTCKYPDYLKMASEPYGDSLFFFLRREQMFVRHFFNRAGKL GEAVPDDLYIKGSGNTAVIQSSAFFPTPSGSIVTSESQFLNKPYPWLQRAQGHNN GICWGNQLFVTVVDTTTRSTNMTLCTEVTKEGTYKNDNFKEYVRHVEEYDLQF VFQLCKITLTAEIMTYIHTMDSNILEDWQFGLTPPPSASLQDTYRFVTSQAITCQ KTAPPKEKEDPLNKYTFWEVNLKEKFSADLDQFPLGRKFLLQSGL
SEQ ID No: 148	5802 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 149	5803 Amino acid sequence of chimeric HPV type 58 L1 protein MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGNPYFSIKS PNNNKKVLVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPDTQRLVWACVGLE IGRGQPLGVGVSGHPYLNKFDDTETSNRYPAQPGSDNRECLSMDYKQTQLCLI GCKPPTGEHWGKGVACNNNAAATDCPPELFNSIIEDGDMVDTGFGCMDFGTL QANKSDVPIDICNSTCKYPDYLKMASEPYGDSLFFFLRREQMFVRHFFNRAGKL GEAVPDDLYIKGSGNTAVIQSSAFFPTPSGSIVTSESQFLNKPYPWLQRAQGHNN GICWGNQLFVTVVDTTTRSTNMTLCTEVTKEGTYKNDNFKEYVRHVEEYDLQF VFQLCKITLTAEIMTYIHTMDSNILEDWQFGLTPPPSASLQDTYRFVTSQAITCQ KTAPPKEKEDPLNKYTFWEVNLKEKFSADLDQFPLGRKFLLQSGLKAKPKLKR

	AAPTSTRTSSAKRKKVKK
SEQ ID No: 150	5804 Nucleotide sequence of chimeric HPV type 58 L1 protein ATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC CGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGGCCGTGGGCAAC CCCTACTTCAGCATCAAGAGCCCCAACAACAACAAGAAGGTGCTGGTGCCCAAGGTGAGCGGCCTGCAGTA CAGGGTGTTCAAGGTGAGGCTGCCCCACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACAACCCCG ACACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGATCGGCAGGGGCCAGCCCCTGGGCGTGGGCGT GAGCGGCCACCCCTACCTGAACAAGTTCGACGACACCGAGACCAGCAACAGGTACCCCGCCAGCCCGGC AGCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCCTGATCGGCTGCAAGCCCC CCACCGGCGAGCACTGGGGCAAGGGCGTGGCCTGCAACAACAACGCCGCCGCCACCGACTGCCCCCCCCTG GAGCTGTTCAACAGCATCATCGAGGACGGCGACATGGTGGACACCGGCTTCGGCTGCATGGACTTCGGCAC CCTGCAGGCCAACAAGAGCGACGTGCCCATCGACATCTGCAACAGCACCTGCAAGTACCCCGACTACCTGA AGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTTCTGAGGAGGGAGCAGATGTTCTGTGAGGCAC TTCTTCAACAGGGCCGGCAAGCTGGGCGAGGCCGTGCCCGACGACCTGTACATCAAGGGCAGCGGCAACA CCGCCGTGATCCAGAGCAGCGCCTTCTTCCCCACCCCAAGCGGCAGCATCGTGACCAGCGAGAGCCAGCTG TTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTGGGGCAACCAGCTGTT CGTGACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCACCGAGGTGACCAAGGAGGGCACC TACAAGAACGACAACCTCAAGGAGTACGTGAGGCACGTGGAGGAGTACGACCTGCAGTTCGTGTTCCAGCT GTGCAAGATCACCTGACCGCCGAGATCATGACCTACATCCACACCATGGACAGCAACATCCTGGAGGACT GGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGCAGGACACCTACAGGTTCGTGACCAGCCAGGCC ATCACCTGCCAGAAGACCGCCCCCCCCAAGGAGAAGGAGGACCCCTGAACAAGTACACCTTCTGGGAGG TGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCCTGCTGCAGAGC GGCCTGAAAGCCAAGCCAAAAGTAAAAAGGGCTGCCCCAACCAAGCAGCAGGACCTCCTCTGCCAAGAGGA AGAAGGTGAAGAAGTAAA

SEQ ID No:	5805 Synthetic HPV58 L1 gene
151	ctgggtaccATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGT GAGCACCGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGGCCGTG GGCAACCCCTACTTCAGCATCAAGAGCCCCAACAACAACAAGAAGGTGCTGGTGCCCAAGGTGAGCGGCC TGCAGTACAGGGTGTTCAGGGTGAGGCTGCCCCGACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTAC AACCCCGACACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGATCGGCAGGGGCCAGCCCTGGGCG TGGGCGTGAGCGGCCACCCCTACCTGAACAAGTTCGACGACACCGAGACCAGCAACAGGTACCCGCCCCA GCCCCGGCAGCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCCTGATCGGCTGC AAGCCCCCACC GGCGAGCACTGGGGCAAGGGCGTGGCCTGCAACAACAACGCCGCCGCCACCGACTGCC CCCCCTGGAGCTGTTCAACAGCATCATCGAGGACGGCGACATGGTGGACACCGGCTTCGGCTGCATGGAC TTCGGCACCTGCAGGCCAACAAGAGCGACGTGCCATCGACATCTGCAACAGCACCTGCAAGTACCCCGA CTACCTGAAGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTTCTGAGGAGGGAGCAGATGTTCTG TGAGGCACTTCTTCAACAGGGCCGGCAAGCTGGGCGAGGCCGTGCCCGACGACCTGTACATCAAGGGCAGC GGCAACACCGCCGTGATCCAGAGCAGCGCCTTCTTCCCCACCCCAAGCGGCAGCATCGTGACCAGCGAGAG CCAGCTGTTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTGGGGCAACC AGCTGTTCTGTGACCGTGGTGGACACCACAGGAGCACCAACATGACCCTGTGCACCGAGGTGACCAAGGA GGGCACCTACAAGAACGACAACCTCAAGGAGTACGTGAGGCACGTGGAGGAGTACGACCTGCAGTTCGTG TTCCAGCTGTGCAAGATCACCTGACCGCCGAGATCATGACCTACATCCACACCATGGACAGCAACATCCT GGAGGACTGGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGCAGGACACCTACAGGTTCTGTACCA GCCAGGCCATCACCTGCCAGAAGACCGCCCCCCCCAAGGAGAAGGAGGACCCCTGAACAAGTACACCTT CTGGGAGGTGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCCTGC TGCAGAGCGGCCTGAAGGCCAAGCCCAGGCTGAAGAGGAGCGCCCCACCACAGGGCCCCCAGCACCAA GAGGAAGAAGGTGAAGAAGTAAA ctgagctc
SEQ ID No:	5806 Synthetic HPV33 L1 gene

152	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No:	5807HPV58 L1 F1
153	CTTGGTACCATGAGCGTGTGGAGGCCAGCGAGG
SEQ ID No:	5808HPV58 L1 R1

154	GCTTGGCTTTCAGGCCGCTCTGCAGCAGGAACTTCC
SEQ ID No: 155	5809 HPV58 L1 amplified sequence 1 CTTGGTACC^[1] <u>ATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC</u> <u>CGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGGCCGTGGGCAAC</u> <u>CCCTACTTCAGCATCAAGAGCCCCAACAACAACAAGAAGGTGCTGGTGCCCAAGGTGAGCGGCCTGCAGTA</u> <u>CAGGGTGTTCAAGGTGAGGCTGCCCCACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACAACCCCG</u> <u>ACACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGATCGGCAGGGGCCAGCCCTGGGCGTGGGCGT</u> <u>GAGCGGCCACCCCTACCTGAACAAGTTCGACGACACCGAGACCAGCAACAGGTACCCCGCCAGCCCGGC</u> <u>AGCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCCTGATCGGCTGCAAGCCCC</u> <u>CCACCGGCGAGCACTGGGGCAAGGGCGTGGCCTGCAACAACAACGCCGCCGCCACCGACTGCCCCCCCCCTG</u> <u>GAGCTGTTCAACAGCATCATCGAGGACGGCGACATGGTGACACCGGCTTCGGCTGCATGGACTTCGGCAC</u> <u>CCTGCAGGCCAACAAGAGCGACGTGCCCATCGACATCTGCAACAGCACCTGCAAGTACCCCGACTACCTGA</u> <u>AGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTTCTGAGGAGGGAGCAGATGTTTCGTGAGGCAC</u> <u>TTCTTCAACAGGGCCGGCAAGCTGGGGCAGGGCCGTGCCCGACGACCTGTACATCAAGGGCAGCGGCAACA</u> <u>CCGCCGTGATCCAGAGCAGCGCCTTCTTCCCCACCCCAAGCGGCAGCATCGTGACCAGCGAGAGCCAGCTG</u> <u>TTCAACAAGCCCTACTGGCTGCAGAGGGGCCAGGGCCACAACAACGGCATCTGCTGGGGCAACCAGCTGTT</u> <u>CGTGACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCACCGAGGTGACCAAGGAGGGCACC</u> <u>TACAAGAACGACAACCTTCAAGGAGTACGTGAGGCACGTGGAGGAGTACGACCTGCAGTTCGTGTTCCAGCT</u> <u>GTGCAAGATCACCTGACCGCCGAGATCATGACCTACATCCACACCATGGACAGCAACATCCTGGAGGACT</u> <u>GGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGCAGGACACCTACAGGTTCGTGACCAGCCAGGCC</u> <u>ATCACCTGCCAGAAGACCGCCCCCCCCAAGGAGAAGGAGGACCCCTGAACAAGTACACCTTCTGGGAGG</u> <u>TGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCCTGCTGCAGAGC</u> <u>GGCCTG</u>^[2]AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site;

	[2]: the gene fragment encoding of HPV58 L1 aa 1-473; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 156	5810HPV58 L1 F2 GAGCGGCCTGAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 157	5811HPV58 L1 R2 CTGTCTAGATTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 158	5812 HPV58 L1 amplified sequence 2 <u>GAGCGGCCTG</u>^[1] <u>AAAGCCAAGCCAAACTGAAAAGGGCTGCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG</u>^[2] TAA <u>ATCTAGA</u>^[3]CAG. [1]: 10bp overlapping with the gene fragment encoding the C-terminus of HPV58 L1 aa 1-473; [2]: the gene fragment encoding the C-terminal 26 amino acids of HPV33L1 aa 474-499; [3]: XbaI enzymatic cleavage site
SEQ ID No: 159	5813 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARPKPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

Appendix 14: SEQUENCE LISTING - HUMAN PAPILLOMAVIRUS TYPE 59 L1 PROTEIN

SEQ ID No: 160	5901 The amino acid sequence of HPV type 59 L1 protein MALWRSSDNKVYLPPPSVAKVVSTDEYVTRTSIFYHAGSSRLLTVGHPYFKVP KGGNGRQDVPKVSAYQYRVFRVKLPDPNKFGLPDNTVYDPNSQRLVWACVG VEIGRGQPLGVGLSGHPLYNKLDDTENS HVASAVDTKDTRDNVSVDYKQTQL CHIGCVPAIGEHWTKGTACKPTTVVQGDCPPLELINTPIEDGDMVDTGYGAMDF KLLQDNKSEVPLDICQSICKYPDYLQMSADAYGDSMFFCLRREQVFARHFWNR SGTMGDQLPESLYIKGTDIRANPGSYLYSPSPSGSVVTSDSQLFNKPYWLHKAQ GLNNGICWHNQLFLT VVDTTTRSTNLSVCASTTSSIPNVYTPTSFKEYARHVEEF DLQFIFQLCKITLTTEVMSYIHNMNNTTILEDWNFGVTPPTASLVD TYRFVQSAA VTCQKDTAPPVKQDPYDKLKFWPVDLKERFSADLDQFPLGRKFLLQLGARPKP TIGPRKRAAPAPTSTPSPKRVKRRKSSRK
SEQ ID No: 161	5902 Nucleotide sequence of HPV type 59 L1 protein ATGGCTCTGTGGAGGTCTCTGACAACAAGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTGAGCACA GATGAATATGTGACCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGGACACCC ATACTTCAAGGTGCCAAAGGGAGGCAATGGCAGACAGGATGTGCCAAAGGTGTCTGCCTACCAATACAGG GTGTTTCAGGGTGAAACTGCCTGACCCAAACAAGTTTGGACTGCCTGACAACACAGTCTATGACCCAAACAG CCAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGATTGGCAGGGGACAACCACTGGGAGTGGGACTGTCT GGACACCCACTCTACAACAACTGGATGACACAGAGAACTCTCATGTGGCATCTGCTGTGGACACCAAGGA CACCAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCATTGGCTGTGTGCCTGCCATTGG AGAACACTGGACCAAGGGCACAGCCTGTAAGCCAACCACAGTGGTCCAGGGAGACTGTCCTCCATTGGAA CTGATAAACACACCAATTGAGGATGGAGATATGGTGGACACAGGCTATGGAGCTATGGACTTCAAAC TGCT

	CCAAGACAACAAGTCTGAGGTGCCACTGGACATCTGTGAGAGCATCTGTAAATACCCTGACTACCTCCAAA TGAGTGCTGATGCCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAGGTGTTTGCCAGACACTTCT GGAACAGGTCTGGCACAATGGGAGACCAACTTCCTGAGTCCCTCTACATCAAGGGCACAGACATCAGGGCT AACCCTGGCTCCTACCTCTACAGCCCAAGCCCATCTGGCTCTGTGGTGACCTCTGACAGCCAACTTTTCAAC AAGCCATACTGGCTGCACAAGGCTCAAGGACTGAACAATGGCATCTGTTGGCACAACCAACTTTTCTGAC AGTGGTGGACACCACCAGGAGCACCAACCTGTCTGTGTGTGCCAGCACCACTCCAGCATCCCAAATGTCT ACACACCAACCTCCTTCAAGGAATATGCCAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTCCAACCTT GTAAGATTACCCTGACCACAGAGGTGATGAGTTACATCCACAATATGAACACCACCATCTTGGAGGACTGG AACTTTGGAGTGACACCTCCTCCAACAGCCTCCCTGGTGGACACCTACAGGTTTGTCCAGTCTGCTGCTGTG ACTTGTGAGAAGGACACAGCCCCCTCTGTGAAGCAGGACCCATATGACAACTGAAGTTCTGGCCTGTGGA CCTGAAAGAGAGGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAACCTGGAG CCAGACCAAAGCCAACCATTTGGACCAAGGAAGAGGGCTGCCCCTGCCCCAACCCAGCACACCAAGCCCCAA GAGGGTGAAGAGGAGGAAGTCCAGCAGGAAGTAAA
SEQ ID No: 162	5903 Synthetic HPV59 L1 gene ctgggtaccATGGCTCTGTGGAGGTCCTCTGACAACAAGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTG AGCACAGATGAATATGTGACCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGG ACACCCATACTTCAAGGTGCCAAAGGGAGGCAATGGCAGACAGGATGTGCCAAAGGTGTCTGCCTACCAAT ACAGGGTGTTCAAGGTGAAACTGCCTGACCCAAACAAGTTTGGACTGCCTGACAACACAGTCTATGACCCA AACAGCCAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGATTGGCAGGGGACAACCACTGGGAGTGGGAC TGTCTGGACACCCACTCTACAACAACTGGATGACACAGAGAACTCTCATGTGGCATCTGCTGTGGACACC AAGGACACCAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACCTTGTATCATTGGCTGTGTGCCTGC CATTGGAGAACACTGGACCAAGGGCACAGCCTGTAAGCCAACCACAGTGGTCCAGGGAGACTGTCCTCCAT TGGAAC TGATAAACACACCAATTGAGGATGGAGATATGGTGGACACAGGCTATGGAGCTATGGACTTCAA ACTGCTCCAAGACAACAAGTCTGAGGTGCCACTGGACATCTGTGAGAGCATCTGTAAATACCCTGACTACC

	TCCAAATGAGTGCTGATGCCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAGGTGTTTGCCAGA CACTTCTGGAACAGGTCTGGCACAATGGGAGACCAACTTCCTGAGTCCCTCTACATCAAGGGCACAGACAT CAGGGCTAACCCTGGCTCCTACCTCTACAGCCCAAGCCCATCTGGCTCTGTGGTGACCTCTGACAGCCAACT TTTCAACAAGCCATACTGGCTGCACAAGGCTCAAGGACTGAACAATGGCATCTGTTGGCACAACCAACTTT TCCTGACAGTGGTGACACCACCAGGAGCACCAACCTGTCTGTGTGTGCCAGCACCACTCCAGCATCCCA AATGTCTACACCAACCTCCTTCAAGGAATATGCCAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTC CAACTTTGTAAGATTACCCTGACCACAGAGGTGATGAGTTACATCCACAATATGAACACCACCATCTTGGA GGACTGGAACCTTTGGAGTGACACCTCCTCCAACAGCCTCCCTGGTGACACCTACAGGTTTGTCCAGTCTGC TGCTGTGACTTGTCAGAAGGACACAGCCCCTCCTGTGAAGCAGGACCCATATGACAAACTGAAGTTCTGGC CTGTGGACCTGAAAGAGAGGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAA CTTGAGCCAGACCAAAGCCAACCATTGACCAAGGAAGAGGGCTGCCCCTGCCCCAACCAGCACACCAA GCCCAAAGAGGGTGAAGAGGAGGAAGTCCAGCAGGAAGTAAActcgagctc
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CLAIMS

What is claimed is:

1. A multivalent HPV immunogenic composition for preventing HPV-related diseases or infections, comprising:

HPV virus-like particles assembled from the L1 protein of HPV Types 6, 11, 16, 18, 31, 33, 45, 52 and 58; and

One or more HPV virus-like particles assembled from L1 proteins of other pathogenic HPV types.

2. The multivalent HPV immunogenic composition according to claim 1, wherein said one or more other pathogenic HPV types are selected from HPV Types 35, 39, 51, 56 and 59.

3. The multivalent HPV immunogenic composition according to claims 1 or 2, wherein at least one of said HPV virus-like particles is a chimeric HPV virus-like particle, said chimeric HPV L1 protein comprises, from its N-terminus to C-terminus orientation:

a. an N-terminal fragment derived from L1 protein of the first papilloma virus type, said N-terminal fragment maintains the immunogenicity of the L1 protein of the first papilloma virus type, wherein said L1 protein of the first

papilloma virus type is selected from HPV Types 6, 11, 16, 18, 31, 35, 39, 45, 51, 52, 56, 58, and one or more other pathogenic HPV types; and

b. a C-terminal fragment derived from L1 protein of the second papilloma virus type, said L1 protein of the second papilloma virus type has a better expression level and solubility compared to L1 proteins of other types;

Wherein the chimeric HPV L1 protein has the immunogenicity of the L1 protein of the first papilloma virus type.

4. The multivalent HPV immunogenic composition according to claim 3, wherein

Said N-terminal fragment is a fragment obtained by truncating the C-terminus of the natural sequence of said L1 protein of the first papilloma virus type at any amino acid position within its $\alpha 5$ region, and a fragment having at least 98% identity therewith; and

Said C-terminal fragment is a fragment obtained by truncating the N-terminus of the natural sequence of said L1 protein of the second papilloma virus type at any amino acid position within its $\alpha 5$ region and functional variants resulting from further mutations, deletions and/or additions to the fragment.

5. The multivalent HPV immunogenic composition according to claim 4, wherein said C-terminal fragment contains one or more nuclear localization sequences.

6. The multivalent HPV immunogenic composition according to claim 3,

Wherein said L1 protein of the first papilloma virus type is selected from L1 proteins of HPV Types 6, 11, 16, 18, 31, 35, 39, 45, 51, 52, 56 or 58;

Wherein said L1 protein of the second papilloma virus type is selected from L1 proteins of HPV Types 1, 2, 3, 4, 6, 7, 10, 11, 13, 16, 18, 22, 26, 28, 31, 32, 33, 35, 39, 42, 44, 45, 51, 52, 53, 56, 58, 59, 60, 63, 66, 68, 73 or 82;

Preferably, said L1 protein of the second papilloma virus type is selected from L1 proteins of HPV Types 16, 28, 33, 59, or 68;

More preferably, said L1 protein of the second papilloma virus type is selected from L1 proteins of HPV Type 33 or HPV Type 59.

7. The multivalent HPV immunogenic composition according to claim 6, wherein said C-terminal fragment is SEQ ID No: 2; or a fragment having a length of m1 amino acids, preferably a fragment covering amino acids 1-m1 position of SEQ ID No: 2; wherein m1 is an integer of 8-26; or said C-terminal fragment is SEQ ID No: 135; or a fragment having a length of m2 amino acids, preferably a fragment covering amino acids 1-m2 position of SEQ ID No: 135; wherein m2 is an integer of 13-31.

8. The multivalent HPV immunogenic composition according to claim 6,

wherein said C-terminal fragment is SEQ ID No: 13; or a fragment having a length of n amino acids, preferably covering amino acids 1-n position of SEQ ID No: 13; wherein n is an integer of 16-38.

5 9. The multivalent HPV immunogenic composition according to claim 3, wherein

 Said N-terminal fragment of said HPV Type 6 L1 protein has 98%, 98.5%, 99%, 99.5%, 99%, or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 1 at any amino acid site within
10 its alpha 5 region;

 Said N-terminal fragment of said HPV Type 11 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 14 at any amino acid site within its alpha 5 region;

15 Said N-terminal fragment of said HPV Type 16 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 27 at any amino acid site within its alpha 5 region;

 Said N-terminal fragment of said HPV Type 18 L1 protein has 98%, 98.5%,
20 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 40 at any amino acid site within its alpha 5 region;

Said N-terminal fragment of said HPV Type 31 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 53 at any amino acid site within its alpha 5 region;

5 Said N-terminal fragment of said HPV Type 35 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 69 at any amino acid site within its alpha 5 region;

10 Said N-terminal fragment of said HPV Type 39 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 82 at any amino acid site within its alpha 5 region;

15 Said N-terminal fragment of said HPV Type 45 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 95 at any amino acid site within its alpha 5 region;

20 Said N-terminal fragment of said HPV Type 51 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 108 at any amino acid site within its alpha 5 region;

Said N-terminal fragment of said HPV Type 52 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 121 at any amino acid site within

its alpha 5 region;

Said N-terminal fragment of said HPV Type 56 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 134 at any amino acid site within its alpha 5 region; and

Said N-terminal fragment of said HPV Type 58 L1 protein has 98%, 98.5%, 99%, 99.5%, 99% or 100% identity to the fragment obtained by truncating the C-terminus of the sequence shown in SEQ ID No: 147 at any amino acid site within its alpha 5 region.

10. The multivalent HPV immunogenic composition according to claim 3, wherein the C-terminus of said N-terminal fragment is connected to the N-terminus of said C-terminal fragment directly or via a linker.

11. The multivalent HPV immunogenic composition according to claim 3, wherein when the C-terminus of said N-terminal fragment is connected to the N-terminus of said C-terminal fragment, the continuous amino acid sequence RKFL is present within a range of plus or minus 4 amino acid positions of the connection point,

Preferably, the continuous amino acid sequence LGRKFL is present within a range of plus or minus 6 amino acid positions of the connection point.

12. The multivalent HPV immunogenic composition according to claim 3, wherein

Said chimeric HPV L1 proteins of chimeric HPV Types 6, 11, 16, 18, 31, 35, 39, 45, 51, 52, 56, and 58 have 98%, 98.5%, 99%, 99.5% or 100% identity to SEQ ID No: 3, SEQ ID No: 16, SEQ ID No: 29, SEQ ID No: 42, SEQ ID No: 55, SEQ ID No: 71, SEQ ID No: 84, SEQ ID No: 97, SEQ ID No: 110, SEQ ID No: 123, SEQ ID No: 136 and SEQ ID No: 149, respectively; and

The HPV Type 33 L1 protein and the HPV Type 59 L1 protein have 98%, 98.5%, 99%, 99.5%, or 100% identity to SEQ ID No: 66 and SEQ ID No: 160, respectively.

13. The multivalent HPV immunogenic composition according to claim 3, wherein at least one of said HPV virus-like particles is composed of a single type of chimeric HPV L1 protein, preferably is composed of said single type of chimeric HPV L1 protein having the same amino acid sequence.

14. The multivalent HPV immunogenic composition according to any one of claims 1-13, wherein said multivalent HPV immunogenic composition further comprises a physiologically acceptable carrier and, optionally, an adjuvant.

15. The multivalent HPV immunogenic composition according to claim 14, wherein said adjuvant is an aluminum phosphate adjuvant.

16. A method of preventing HPV- related disease or infection comprising.

Administering to a subject the multivalent HPV immunogenic composition of any one of claims 1-15.

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17. The use of the multivalent HPV immunogenic composition of any one of claims 1-15 in the preparation of vaccines or drugs for preventing HPV-related diseases or infections.

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