

CHIMERIC PAPILLOMA VIRUS L1 PROTEIN

FIELD OF THE INVENTION

5 **[0001]** The present invention relates to the papilloma virus (HPV) L1 protein and the polynucleotide encoding the same, and also to HPV virus-like particles and the preparation methods thereof.

BACKGROUND OF THE INVENTION

10 **[0002]** Papilloma virus (PV) belongs to the *Papillomaviridae* Family and causes papillomas in humans, cattles, 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 functions: (1) early region (E), about 4.5kb, encoding E1, E2, E4-E7, a total of 6 non-
15 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 800-900 bp long and does not encode any protein, serving as DNA replication and expression regulatory elements.

20 **[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-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, PhD *Cancer* 2017;123:2219-29).

[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, 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. 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 (<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, 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 can not be easily purified thus makes the production process complicated.

5 [0014] Therefore, there is still a need to obtain high yields of HPV virus-like particles.

SUMMARY OF THE INVENTION

[0015] In one aspect, the present invention provides a chimeric papilloma virus L1 protein comprising, 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 corresponding human papilloma virus type; 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 the L1 proteins of other types; wherein said chimeric papilloma virus L1 protein has the immunogenicity of the L1 protein of the first papilloma virus type.

[0016] In another aspect, the present invention provides a papilloma virus-like particle comprising a chimeric papilloma virus L1 protein.

[0017] In another aspect, the present invention provides an immunogenic composition for the prevention of papilloma virus-associated diseases or infections, comprising papilloma virus-like particles and adjuvants as previously described.

[0018] In another aspect, the present invention provides an isolated polynucleotide encoding a chimeric papilloma virus L1 protein.

[0019] In a further aspect, the present invention provides a vector comprising a polynucleotide encoding a chimeric papilloma virus L1 protein.

[0020] In a further aspect, the present invention provides a baculovirus comprising a polynucleotide encoding a chimeric papilloma virus L1 protein.

5 [0021] In another aspect, the present invention provides a host cell comprising said polynucleotides, said vectors, or said baculovirus as previously described.

[0022] In another aspect, the present invention provides a method of preparing a papilloma virus-like particle, comprising culturing host cells as previously described to express said chimeric papilloma virus L1 proteins and to assemble into virus-like particles; and purifying said papilloma virus-like particles.

10

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] 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.

15 [0024] 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.

[0025] 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.

[0026] 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.

20

[0027] 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.

[0028] Figure 1F: Expression of L1 protein of HPV 35 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0029] Figure 1G: Expression of L1 protein of HPV 39 L1: 59C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

5 **[0030]** Figure 1H: Expression of L1 protein of HPV 45 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0031] Figure 1I: Expression of L1 protein of HPV 51 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

10 **[0032]** Figure 1J: Expression of L1 protein of HPV 52 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0033] Figure 1K: Expression of L1 protein of HPV 56 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

[0034] Figure 1L: Expression of L1 protein of HPV 58 L1: 33C. M: Marker; L: cell lysate; E-S: supernatant collected after centrifugation of the lysate.

15 **[0035]** Figure 2A: Transmission electron microscopy of HPV 6 L1: 33C virus-like particles.

[0036] Figure 2B: Transmission electron microscopy of HPV 11 L1: 33C virus-like particles.

[0037] Figure 2C: Transmission electron microscopy of HPV 16 L1: 33C virus-like particles.

[0038] Figure 2D: Transmission electron microscopy of HPV 18 L1: 33C virus-like particles.

[0039] Figure 2E: Transmission electron microscopy of HPV 31 L1: 33C virus-like particles.

20 **[0040]** Figure 2F: Transmission electron microscopy of HPV 35 L1: 33C virus-like particles.

[0041] Figure 2G: Transmission electron microscopy of HPV 39 L1: 59C virus-like particles.

[0042] Figure 2H: Transmission electron microscopy of HPV 45 L1: 33C virus-like particles.

[0043] Figure 2I: Transmission electron microscopy of HPV 51 L1: 33C virus-like particles.

[0044] Figure 2J: Transmission electron microscopy of HPV 52 L1: 33C virus-like particles.

[0045] Figure 2K: Transmission electron microscopy of HPV 56 L1: 33C virus-like particles.

[0046] Figure 2L: Transmission electron microscopy of HPV 58 L1: 33C virus-like particles.

[0047] Figure 3: Expression of C-terminal truncated HPV 16L1 (1-474). M: Marker; L: cell lysates; E-S: supernatant collected after centrifugation of the lysates.

SPECIFIC EMBODIMENTS

[0048] In one aspect, the present invention provides a chimeric papilloma virus L1 protein comprising, from its N-terminus to C-terminus orientation: a. an N-terminal fragment derived from L1 protein of the corresponding human papilloma virus type, said N-terminal fragment maintains the immunogenicity of the L1 protein of the corresponding type of HPV; 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 said chimeric papilloma virus L1 protein has the immunogenicity of L1 protein of the first papilloma virus type.

[0049] 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 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.

[0050] 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.

[0051] In one embodiment, said C-terminal fragment comprises one or plurality of nuclear localization sequences.

[0052] In one embodiment, said papilloma virus L1 protein is an HPV L1 protein.

[0053] 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.

[0054] Preferably, said L1 protein of the second papilloma virus type is selected from L1 proteins of HPV Types 16, 28, 33, 59, or 68.

[0055] More preferably, said L1 protein of the second papilloma virus type is selected from L1 protein of HPV Type 33 or HPV Type 59.

[0056] 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: 132; or a fragment having a length of m2 amino acids, preferably a fragment covering amino acids 1-m2 position of SEQ ID No: 132; wherein m2 is an integer of 13-31.

[0057] 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 some embodiments, the chimeric papilloma virus L1 protein comprises one or plurality of C-terminal fragments of the HPV Type 33 L1

protein. Said plurality of C-terminal fragments of the HPV Type 33 L1 protein may be the same or different. 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.

5 **[0058]** In another 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.

10 **[0059]** 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 papilloma virus 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.

15 **[0060]** In one embodiment, 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.

[0061] 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.

20 **[0062]** 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.

[0063] 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.

[0064] In one aspect, the present invention provides a papilloma virus-like particle, comprising a chimeric papilloma virus L1 protein as previously described. In one embodiment, the papilloma virus-like particle is the HPV virus-like particle, and in one embodiment, the 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 HPV virus-like particles self-assemble in an *in vivo* expression system.

[0065] In one aspect, the present invention provides an immunogenic composition for the prevention of papilloma virus-related disease or infections, comprising papilloma virus-like particles and adjuvants as previously described. Said prevention may be considered as a treatment and they can be used interchangeably.

[0066] In one aspect, the above-described immunogenic composition is administered to a subject. In one embodiment, the subject is a human. In one embodiment, the subject is a rabbit. In one embodiment, the subject is a dog.

[0067] In one aspect, the present invention provides an isolated polynucleotide encoding a chimeric papilloma virus L1 protein as previously described. In one embodiment, the

polynucleotide is a codon-optimized polynucleotide for varieties of expression systems. In one embodiment, the polynucleotide is a codon-optimized polynucleotide for an insect baculovirus expression system.

[0068] In one aspect, the present invention provides a vector, comprising a polynucleotide as previously described. In one embodiment, the vector is a baculovirus vector. In one embodiment, the vector may be a transfer vector for a baculovirus expression system. In another embodiment, the vector may be an expression vector for a baculovirus expression system. In another embodiment, the vector may be a recombinant vector for the baculovirus expression system.

[0069] In one aspect, the present invention provides a baculovirus, comprising a polynucleotide as previously described.

[0070] In one aspect, the present invention provides a host cell, comprising the polynucleotide, the vector, or the baculovirus as previously described. In one embodiment, the host cell is an insect cell, preferably, said insect cell is selected from Sf9 cells, Sf21 cells, Hi5 cells and S2 cells.

[0071] In one aspect, the present invention provides a method for preparing papilloma virus-like particles as previously described, comprising: culturing the host cells as previously described to express said chimeric papilloma virus L1 proteins which is assembled into virus-like particles; and purifying said papilloma virus-like particles.

[0072] In one embodiment, the host cells are insect cells. In one embodiment, the host cells are Hi5 cells. In one embodiment, the chimeric papilloma L1 proteins are chimeric HPV L1 proteins that self-assemble into HPV virus-like particles in host cells. In one embodiment, the chimeric HPV L1 proteins self-assemble into HPV virus-like particles in host cells having an icosahedron comprising 72 pentamers of said chimeric HPV L1 proteins. In one embodiment, the HPV virus-like particles have correctly formed disulfide bonds and thus have a good natural conformation.

[0073] In one embodiment, the purification is carried out using cation exchange chromatography.

In one embodiment, the purification is carried out using strong cation exchange chromatography.

In another embodiment, the purification is carried out using weak cation exchange chromatography. In one embodiment, the purification is carried out using a combination of

multiple cation exchange chromatography. In one embodiment, the purification is carried out using HS strong cation exchange chromatography. In another embodiment, the purification is carried out using MMA ion exchange chromatography. In another embodiment, purification is performed using HS-MMA two-step chromatography.

[0074] 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.

[0075] 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.

[0076] 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).

5 **[0077]** The poor solubility of the protein due to this truncation may be due to 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.

10 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 L1 protein of the first papilloma virus type (e.g. HPV L1 protein), which provides the

15 immunogenicity of the first papilloma virus type (e.g. HPV), and a C-terminal fragment derived from 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.

20 **[0078]** The length of the N-terminal fragment of the HPV L1 protein appropriate for maintaining the immunogenicity of the L1 protein of the first HPV type and ensuring the formation of VLP is determined. The following reports relate to epitope studies of common HPV types.

[0079] Sunanda Baidya et al. reported that the epitopes of the L1 protein 48EEYDLQFIFQLCKITLTA65, 45RHGEEYDLQFIFQLCKITLTA65, 63LPDPNKF69,

79PETQRLVWAC88, 36PVPQGQYDA43, 77YNPETQRLVWAC88, 188DTGYGAMD195, 36PVPQGQYDATK45, 45KQDIPKVSAYQYRVFRV61, 130RDNVSVDYKQTQLCI144 and 49YSRHVEEYDLQFIF62 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).

[0080] 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).

[0081] 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-vpVskVVsTDeYVarThIyYHAgtsRLLaVGhPYfpikxpnnhKilvPKVsglQYRVFvrihLPDPNKFgfPDTS	89							
HPV35-L1	mslWrsneaTVYlPP-vsVskVVsTDeYVtrThIyYHAgtsRLLaVGhPYaikkqdsnKiavPKVsglQYRVFvrvkLPDPNKFgfPDTS	89							
HPV11-L1	--mWrpsdsTVYvPPnpVskVvTaDaYVkrThIfYHAssSRLLaVGhPYsikkvn--KtvvPKVsgyQYRVFkvvLPDPNKFalPDSS	86							
HPV18-L1	malWrpsdnTVYlPP-psVarVvTDDYVtpTsIfYHAgtsRLLtVGnPYfrvpagggnKqdiPKVsayQYRVFvrvqLPDPNKFglPDTS	89							
			β -D	DE-loop	β -E	EF-			
HPV16-L1	fynPdtQRLVWACvGvEvGRGQPLGVGisGHPllNKlDDtEnasayaaNagvDnRecismDYKQTQLClIGCKPpiGEHWgKGspCtnva	179							
HPV35-L1	fydPasQRLVWACtGvEvGRGQPLGVGisGHPllNKlDDtEnsnkyvgNsgtDnRecismDYKQTQLClIGCrPpiGEHWgKGtpCnang	179							
HPV11-L1	lfdPttQRLVWACtGleEvGRGQPLGVGisGHPllNKyDDvEnsggyggNpggDnRvnvgmDYKQTQLCmvGCaPpiGEHWgKGtqCsnts	176							
HPV18-L1	iynPetQRLVWACaGvEiGRGQPLGVGisGHPfyNKlDDtEsshaatsNvseDvRdnvsvDYKQTQLClIGCaPalGEHWaKGtaCksrp	179							
	loop				α 1	β -F	FG-		
HPV16-L1	vnpGdCPPLELIntViqDGDMDVTGFGAMdFttLQaNKseVPLDICTsiCKYPDYIkMvsePYGdsiFFyLRrEQmFvRHlfNRAGavGe	269							
HPV35-L1	vkaGeCPPLELIntViqDGDMDVTGFGAMdFttLQaNKsdVPLDICssiCKYPDYIkMvsePYGdmIFFyLRrEQmFvRHlfNRAGavGe	269							
HPV11-L1	vqnGdCPPLELiTsViqDGDMDVTGFGAMnFadLQTNKsdVPLDICgtvCKYPDYlQMaadPYGDrIFFyLRrEQmFaRHfNRAGavGe	266							
HPV18-L1	lsqGdCPPLELkntVleDGDMDVTGYGAMdFstLQdTKceVPLDICqsiCKYPDYlQMsadPYGdsMFFcLRrEQlFaRHfNRAGtmGd	269							
	loop	G1	G2		β -H1	β -H2	HI-loop		
HPV16-L1	nVPddLyikGsgstanlaSsnYfptPSGSmVtSdaQiFNKPYWLqraQGHNNGiCWgNgLFVTVVDTrSTNmSlCaaistse-ttYknt	358							
HPV35-L1	tVPadLyikG--ttgtlpStsYfptPSGSmVtSdaQiFNKPYWLqraQGHNNGiCWsNgLFVTVVDTrSTNmSvCsavsssd-stYknd	356							
HPV11-L1	pVPddLlvKGgnnrssvaSsiYvhtPSGSlVsSeaQlFNKPYWLqraQGHNNGiCWgNhLFVTVVDTrSTNmTlCasvsk--atYtns	354							
HPV18-L1	tVPqsLyikGtgmpaspgScvYspPSGSiVtSdsQlFNKPYWLhkaQGHNNGvCWNgLFVTVVDTrSTNltiCastqspvPgqYdat	359							
	β -I	α 2	α 3		α 4				
HPV16-L1	nfKeYlRHgEEYDLQFIFQLCKITLAdVMtYIHsMNsileDWNFGlqPPPggtLeDTYRfVtSqAiaCQKhtppapkeDPlkkytFwe	448							
HPV35-L1	nfKeYlRHgEEYDLQFIFQLCKITLAdVMtYIHsMNsileDWNFGltPPPggtLeDTYRyVtSqAvtCQKpsapkpkdDPlknytfwe	446							
HPV11-L1	dyKeYmRHvEEfDLQFIFQLCsITLsAeVMaYIHtMnpsvLEDWNFGlsPPPggtLeDTYRyVqSqAitCQKptpekekqDPykdmFwe	444							
HPV18-L1	kfKqYsRHvEEYDLQFIFQLCTITLAdVMsYIHsMNsileDWNFGvpPPPttsLvDTYRfVqSvAitCQKdaapaenkDpydklkFwn	449							
	β -J	α 5							
HPV16-L1	VnLKEKFSadLDQfPLGRKFLlQaGlkakpkftlgkr-katpttsststtakRkkrkl	505							
HPV35-L1	VdLKEKFSadLDQfPLGRKFLlQaGlkarpnfrlgkr-aapastskksstkrRkvks	502							
HPV11-L1	VnLKEKFSsleLDQfPLGRKFLlQsGygrtsartgik-rpavskpstapkrRtktkk	501							
HPV18-L1	VdLKEKFSldLDQyPLGRKFLvQaGlrrkptigprkrsapsattsskpaKrvRvrark	507							

[0082] 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	--MWRPSDSTVYVPPNPVSKVVATDAYVTRTNIFYHASSRLLAVGHPYFSIKRA----	54
HPV11-L1	--MWRPSDSTVYVPPNPVSKVVATDAYVTRTNIFYHASSRLLAVGHPYYSIKV----	54
HPV52-L1	MSVWRPSEATVYLP-PVPVSKVVSTDEYVSRTSIYYAGSSRLLTVGHPYFSIKNTSSGN	59
HPV58-L1	MSVWRPSEATVYLP-PVPVSKVVSTDEYVSRTSIYYAGSSRLLAVGNPYFSIKSP--NN	57
HPV33-L1	MSVWRPSEATVYLP-PVPVSKVVSTDEYVSRTSIYYAGSSRLLAVGHPYFSIKNP--TN	57
HPV31-L1	MSLWRPSEATVYLP-PVPVSKVVSTDEYVTRTNIYYHAGSARLLTVGHPYYSIPKS--DN	57
HPV16-L1	MSLWLPSEATVYLP-PVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGHPYFPIKKP--NN	57
HPV35-L1	MSLWRSNEATVYLP-PVSVSKVVSTDEYVTRTNIYYHAGSSRLLAVGHPYFAIKKQ--DS	57
HPV51-L1	MALWRTNDSKVYLP-PAPVSRIVNTTEYITRTGIYYAGSSRLITLGHYPYFPIPK----T	55
HPV56-L1	MATWRPSENKVYLP-PTPVSKVVATDSYVKRTSIFYHAGSSRLLAVGHPYYSVTK----D	55
HPV39-L1	MAMWRSSDSMVYLP-PPSVAKVVNTDDYVTRTGIYYAGSSRLLTVGHPYFKV--GM--NG	56
HPV59-L1	MALWRSSDNKVYLP-PPSVAKVVSTDEYVTRTSIFYHAGSSRLLTVGHPYFKV--PK--GG	56
HPV18-L1	MALWRPSDNTVYLP-PPSVARVVNTDDYVTRTSIFYHAGSSRLLTVGNPYFRV--PA--GG	56
HPV45-L1	MALWRPSDSTVYLP-PPSVARVVNTDDYVSRTSIFYHAGSSRLLTVGNPYFRVPS--GA	57
	loop β-C β-D	
HPV6-L1	-NKTVVPKVSGYQYRVFKVLPDPNKFALPDSSLFDPPTQRLVWACTGLEVGRGQPLGVG	113
HPV11-L1	-NKTVVPKVSGYQYRVFKVLPDPNKFALPDSSLFDPPTQRLVWACTGLEVGRGQPLGVG	113
HPV52-L1	GKKVLVPKVSGLQYRVFRVRIKLPDPNKFPGPDTSFYNPETQRLVWACTGLEIGRGQPLGVG	119
HPV58-L1	NKKVLVPKVSGLQYRVFRVRLPDPNKFPGPDTSFYNPDTQRLVWACVGLEIGRGQPLGVG	117
HPV33-L1	AKKLLVPKVSGLQYRVFRVRLPDPNKFPGPDTSFYNPDTQRLVWACVGLEIGRGQPLGVG	117
HPV31-L1	PKKIVVPKVSGLQYRVFRVRLPDPNKFPGPDTSFYNPETQRLVWACVGLEVGGRGQPLGVG	117
HPV16-L1	-NKILVPKVSGLQYRVFRVRIHLPDPNKFPGPDTSFYNPDTQRLVWACVGVEVGRGQPLGVG	116
HPV35-L1	-NKIAYPKVSGLQYRVFRVRLPDPNKFPGPDTSFYDPAQRLVWACTGVEVGRGQPLGVG	116
HPV51-L1	STRAAIPKVSAYQYRVFRVQLPDPNKFGLPDPNLYNPDTDLRVWCGVGEVGRGQPLGVG	115
HPV56-L1	NTKTNIKVSAYQYRVFRVRLPDPNKFGLPDTNIYNPDQERLVWACVGLEVGRGQPLGAG	115
HPV39-L1	GRKQDIPKVSAYQYRVFRVTLDPDNKFSIPDASLYNPETQRLVWACVGVEVGRGQPLGVG	116
HPV59-L1	NGRQDVPKVSAYQYRVFRVRLPDPNKFGLPDTNVDYDPSQRLVWACVGEIGRGQPLGVG	116
HPV18-L1	GNKQDIPKVSAYQYRVFRVQLPDPNKFGLPDTSIYNPETQRLVWACAGVEIGRGQPLGVG	116
HPV45-L1	GNKQAVPKVSAYQYRVFRVALPDPNKFGLPDTSIYNPETQRLVWACVGEIGRGQPLGIG	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	LSGHPLFNKYDDTENSRIANGNAQQDVRDNTSVDNKQTLQCLIGCAPPIGEHWGIGTTCK	175
HPV56-L1	LSGHPLFNRLDDTESSNLANNVIEDSRDNTSVDGKQTLQCLIVGCTPAMGEHWTKGAVCK	175
HPV39-L1	ISGHPLYNRQDDTENSPPFS-STTNKDSRDNVSDYKQTLQCLIGCVPAIGEHWGKGKACK	175
HPV59-L1	LSGHPLYNLDDTENSHVASAVDTKDRDNTSVSDYKQTLQCLIGCVPAIGEHWTKGTACK	176
HPV18-L1	LSGHPPFYNLDDTESSHAATSNVSEDRDNTSVSDYKQTLQCLIGCAPAIGEHWAKGTACK	176
HPV45-L1	LSGHPPFYNLDDTESAHAATAVITQDVRDNTSVSDYKQTLQCLIGCVPAIGEHWAKGTACK	177
	loop	
HPV6-L1	NTPVQAGDCPPELITSVIQDGMVDTGFGAMNFADLTQNSDVPIDICGTTCKYPDYLQ	232
HPV11-L1	NTSVQNGDCPPELITSVIQDGMVDTGFGAMNFADLTQNSDVPIDICGTVCKYPDYLQ	233
HPV52-L1	NNSGNGDCPPELQINSVIQDGMVDTGFGCMDFTLQASKSDVPIDICSSVCKYPDYLQ	239
HPV58-L1	NNA-AATDCPPELFNSIIEDGMVDTGFGCMDFTLQANKSDVPIDICNSTCKYPDYLK	236
HPV33-L1	NAA-PANDCPPELINTIIEDGMVDTGFGCMDFTLQANKSDVPIDICGSTCKYPDYLK	236
HPV31-L1	NNAITPGDCPPELKNVSIQDGMVDTGFGAMDFTALQDTKSNVPLDICSICKYPDYLK	237
HPV16-L1	NVAVNPGDCPPELINTVIQDGMVDTGFGAMDFTLQANKSEVPLDICTSICKYPDYLK	236
HPV35-L1	ANQVKAGECPPELINTVLQDGMVDTGFGAMDFTLQANKSDVPLDICSICKYPDYLK	236
HPV51-L1	NTPVPPGDCPPELVSSVIQDGMIDTGFAMDFALQATKSDVPLDISQSVCKYPDYLK	235
HPV56-L1	STQVTGDCPPELALINTPIEDGMIDTGFAMDFKLVQESKAEPVPLDIVQSTCKYPDYLK	235
HPV39-L1	PNNVSTGDCPPELVNTPIEDGMIDTGFAMDFALQETKSEVPLDICSICKYPDYLK	235
HPV59-L1	PTTVVQDCPPELINTPIEDGMVDTGYGAMDFKLLQDNKSEVPLDICSICKYPDYLQ	236
HPV18-L1	SRPLSQGDCPPELKNVTLEDGMVDTGYGAMDFSTLQDTKCEVPLDICSICKYPDYLQ	236
HPV45-L1	PAQLQPGDCPPELKNVTLEDGMVDTGYGAMDFSTLQDTKCEVPLDICSICKYPDYLQ	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	MSADTYGNSMFFHLRRQMFARHYYNKLGVGEDIPNDYYIKGSG--NGRDPPIESYIYSAT	294
HPV56-L1	MSADAYGDSMWFYLRREQLFARHYFNRRAGKVGETIPAELYIKGS--NGREPPSSSVYVAT	293
HPV39-L1	MSADVYGDSMFFCLRRQMFARHFWNRGGMVGDIPAQLYIKGT--DIRANPGSSVYCPS	293
HPV59-L1	MSADAYGDSMFFCLRRQMFARHFWNRSGTMDQLPESLYIKGT--DIRANPGSYLYSPS	294
HPV18-L1	MSADPYGDSMFFCLRRQMFARHFWNRAGTMDGTVPQSLYIKGT--GMRASPGSCVYSPS	294
HPV45-L1	MSADPYGDSMFFCLRRQMFARHFWNRAGVMGDVTPDLYIKGTANMRETPGSCVYSPS	297

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

	loop → β-I → α2 → α3 →	
HPV6-L1	TYTNSDYKEYMRHVEEYDLQFIFQLCSITLSAEVMAYIHTMNPVLEDWNFGLSPPNGT	408
HPV11-L1	TYTNSDYKEYMRHVEEYDLQFIFQLCSITLSAEVMAYIHTMNPVLEDWNFGLSPPNGT	409
HPV52-L1	TYKNENFKEYLRHGEEDLQFIFQLCKITLTADVMTYIHKMDATILEDWQFGLTPPPSAS	417
HPV58-L1	TYKDNFKEYVRHVEEYDLQFVFQLCKITLTAEIMTYIHTMDSNILEDWQFGLTPPPSAS	412
HPV33-L1	TYKNENFKEYIRHVEEYDLQFVFQLCKVTLTAEVMTYIHAMNPDILEDWQFGLTPPPSAS	412
HPV31-L1	TFKSSNFKEYLRHGEEDLQFIFQLCKITLSADIMTYIHSNMPAILEDWNFGLTPPPSGS	414
HPV16-L1	TYKNTNFKEYLRHGEEDLQFIFQLCKITLTADVMTYIHSMNSTILEDWNFGLQPPPGGT	413
HPV35-L1	TYKDNFKEYLRHGEEDLQFIFQLCKITLTADVMTYIHSMNPSILEDWNFGLTPPPSGT	411
HPV51-L1	TFTPSNFQYIRHGEYELQFIFQLCKITLTTEVMAYLHTMDPTILEQWNFGLTLPPSAS	413
HPV56-L1	KYDARKINQYLRHVEEYELQFVFQLCKITLSAEVMAYLHNNMNLLEDWNIGLSPPVATS	411
HPV39-L1	TYDPSKFKEYTRHVEEYDLQFIFQLCTVTLTDDVMSYIHTMNSSILDNNWFAVAPPPSAS	413
HPV59-L1	VYTPTSFKEYARHVEEYDLQFIFQLCKITLTTEVMSYIHNMTTILEDWNFGVTPPPPTS	414
HPV18-L1	QYDATKFQKYSRHEEYDLQFIFQLCTITLTADVMSYIHSMNSSILEDWNFGVPPPPPTS	414
HPV45-L1	TYDPTKFHYSRHEEYDLQFIFQLCTITLTAEVMSYIHSMNSSILENNWFGVPPPPPTS	417

	α4 → β-J → α5 →	
HPV6-L1	LEDTRYVQSQAITCQKPTPEKEKDPYKNLSFWEVNLKEKFSSELDQYPLGRKFLQSG	468
HPV11-L1	LEDTRYVQSQAITCQKPTPEKEKQDPYKDMSFWEVNLKEKFSSELDQFPLGRKFLQSG	469
HPV52-L1	LEDTRYFVTSTAITCQKNTPPKGEDPLKDYMFWEVDLKEKFSADLDQFPLGRKFLQAG	477
HPV58-L1	LQDTRYFVTSAITCQKTAPPEKEEDPLNKYTFWEVNLKEKFSADLDQFPLGRKFLQSG	472
HPV33-L1	LQDTRYFVTSAITCQKTVPPEKEEDPLGKYTFWEVDLKEKFSADLDQFPLGRKFLQAG	472
HPV31-L1	LEDTRYFVTSAITCQSAPQPKEDDPKDYVFWVNLKEKFSADLDQFPLGRKFLQAG	474
HPV16-L1	LEDTRYFVTSAIACQKHTPPAPKEDPLKKYTFWEVNLKEKFSADLDQFPLGRKFLQAG	473
HPV35-L1	LEDTRYVTSQAIVTCQKPSAPKPKDDPLNKYTFWEVDLKEKFSADLDQFPLGRKFLQAG	471
HPV51-L1	LEDAYRFVRNAATSCQKDTTPQAKPDPLAKYKFDVNLKERFSLDLQFALGRKFLQVG	473
HPV56-L1	LEDKYRYVRSTAITCQREQPPEKQDPLAKYKFDVNLQDSFSTDLDQFPLGRKFLMQLG	471
HPV39-L1	LVDTRYLQSAAITCQKDAPAEKKDPYDGLKFWNVDLREKFSLELDQFPLGRKFLQAR	473
HPV59-L1	LVDTRYFVQSAAVTCQKDTAPPVKQDPYDKLKFVWVNLKERFSADLDQFPLGRKFLQLG	474
HPV18-L1	LVDTRYFVQSAITCQKDAAPAENKDPYDKLKFVWVNLKEKFSLELDQYPLGRKFLVQAG	474
HPV45-L1	LVDTRYFVQSAVTCQKDTTPPEKQDPYDKLKFVWVNLKEKFSSELDQYPLGRKFLVQAG	477

HPV6-L1	YRGRSSIRTGVKRPVSKASAPKRKRKTKR--	500
HPV11-L1	YRGRTSARTGIRPAVSKPSTAPKRKRKTKK--	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	LKARNPRLGKRAAPASTSKKSSTKRKRKVK---	502
HPV51-L1	VQRKPRPGLKRPAASASSSSSSAKRKRKVK---	504
HPV56-L1	TRSKPAVATSKKRSAPTSTS-TPAKRKR---	499
HPV39-L1	VRRRPITIGPRKRPAASTSSSATKHKRKRVS---	505
HPV59-L1	ARPKPTIGPRKRAAPASTTPSPKRVRKRKSSRK	508
HPV18-L1	LRRKPTIGPRKRSAPSATTSSKPAKRVRVRARK-	507
HPV45-L1	LRRRPITIGPRKRPAASTSTASRPKRVRIRSKK-	510

[0083] 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:

[0084] 1. JPred: <http://www.compbio.dundee.ac.uk/jpred/index.html>

5 **[0085]** 2. ProtPredicct: <http://predictprotein.org>

[0086] 3. PsiPred: <http://bioinf.cs.ucl.ac.uk/psipred>

[0087] 4. SCRATCH-1D: <http://download.igb.uci.edu>

[0088] 5. Nnpredict: <http://www.cmpharm.ucsf.edu/~nomi/nnpredict>

[0089] 6. Predictprotein: <http://www.embl-heidelberg.de/predictprotein/SOPMA>

10 <http://www.ibcp.fr/predict.html>

[0090] 7. SSPRED: http://www.embl-heidelberg.de/sspred/ssprd_info.html.

[0091] In one embodiment of the present invention, the inventors determine the length of the N-terminal fragment of the HPV L1 protein derived from the first 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.

15 **[0092]** 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.

20 **[0093]** 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.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] HPV6 ELDQYPLGRKFLLQSGY(469)

[0098] HPV11 ELDQFPLGRKFLLQSGY(470)

[0099] HPV16 DLDQFPLGRKFLLQAGL(474)

[00100] HPV18 DLDQYPLGRKFLVQAGL(475)

[00101] HPV31 DLDQFPLGRKFLLQAGY(475)

[00102] HPV35 DLDQFPLGRKFLLQAGL(472)

5 [00103] HPV39 ELDQFPLGRKFLLQARV (474)

[00104] HPV45 DLDQYPLGRKFLVQAGL(478)+26

[00105] HPV51 DLDQFALGRKFLLQVGV(474)

[00106] HPV52 DLDQFPLGRKFLLQAGL(478)

[00107] HPV56 DLDQFPLGRKFLMQLGTRS(474)

10 [00108] HPV58 DLDQFPLGRKFLLQSGL(473)

[00109] HPV33 DLDQFPLGRKFLLQAGL(473) KAKPKLKRAAPTSTRTSSAKRKKVKK

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

[00110] HPV59 DLDQFPLGRKFLLQLGA(475)

15 RPKPTIGPRKRAAPAPTSTPSPKRVRKRKSSRK wherein RKR at positions 484-486 and KRVKRRK at positions 498-504 are nuclear localization sequences.

[00111] 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.

20 [00112] 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 L1 protein of the first HPV 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 L1 protein of the second type.

[00113] 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.

[00114] 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.

[00115] 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 L1 protein of the first HPV 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 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.

[00116] 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 L1 protein of the first HPV 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.

[00117] The beneficial effects of the invention

5 **[00118]** 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
10 *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.

[00119] 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
15 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.

[00120] The inventors found that the HPV Type 16 L1 protein, the HPV Type 28 L1 protein, the
20 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.

[00121] 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.

[00122] 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.

[00123] 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.

[00124] 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.

[00125] 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.

[00126] Definition

[00127] 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.

[00128] 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.

[00129] 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.

[00130] 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.

[00131] 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.

5 **[00132]** 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.

10 **[00133]** 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.

[00134] 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
15 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.).

[00135] The term “HPV” or “HPV virus” refers to papilloma viruses of the *Papillomaviridae*
20 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.

5 **[00136]** 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.

10 **[00137]** The term “virus-like particle” refers to a hollow particle containing one or plurality of structural proteins of a virus, without viral nucleic acids.

[00138] “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.

15 **[00139]** 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 antibodies in serum can therefore be assessed by measuring changes in the amounts of
20 cells.

[00140] 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 present invention is capable of triggering the humoral- and/or the cellular-immune response in animals or humans.

[00141] 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.

[00142] 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.

[00143] 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.

[00144] 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.

[00145] “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.

[00146] 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.

[00147] 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

[00148] 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 **[00149] 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

[00150] 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).

[00151] 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.

[00152] 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.

[00153] 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.

[00154] 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.

5 **[00155] Examples**

[00156] Example 1 Construction of chimeric genes

[00157] Example 1.1: Construction of a chimeric gene with the C-terminus of HPV6 L1 substituted with the C-terminus of HPV33 L1

[00158] 1.1.1 Construction of pFB-HPV6 L1 as the template

10 **[00159]** 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.

15 **[00160]** 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.

[00161] 1.1.2 Construction of pFB-HPV33 L1 as the template

20 **[00162]** The HPV33 L1 gene with the Kpn I 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 KpnI and XbaI cleavage sites.

[00163] 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.

[00164] 1.1.3 Construction of pFB-HPV6 L1: 33C

[00165] 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.

[00166] 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.

[00167] 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.

[00168] 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.

[00169] 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.

[00170] 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.

[00171] PCR ligating sequences:

[00172] 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.

[00173] 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.

[00174] 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).

[00175] 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.

[00176] Example 1.2 Construction of chimeric gene with the C-terminus of HPV11 L1 substituted by the C-terminus of HPV33 L1

[00177] The experimental methods and procedures were the same as in Example 1.1, see Appendix 2 for relevant sequences.

[00178] Example 1.3 Construction of a chimeric gene with the C-terminus of HPV16 L1 substituted by the C-terminus of HPV33 L1

[00179] The experimental method and procedure were the same as in Example 1.1, see Appendix 3 for relevant sequences.

[00180] Example 1.4 Construction of a chimeric gene with the C-terminus of HPV18 L1 substituted by the C-terminus of HPV33 L1

[00181] The experimental method and procedure were the same as in Example 1.1, see Appendix 4 for relevant sequences.

[00182] Example 1.5 Construction of a chimeric gene with the C-terminus of HPV31 L1 substituted by the C-terminus of HPV33 L1

[00183] The experimental method and procedure were the same as in Example 1.1, see Appendix 5 for relevant sequences.

[00184] Example 1.6 Construction of a chimeric gene with the C-terminus of HPV35 L1 substituted by the C-terminus of HPV33 L1

[00185] The experimental methods and procedures were the same as in Example 1.1, see Appendix 6 for relevant sequences.

[00186] Example 1.7 Construction of chimeric gene with the C-terminus of HPV39 L1 substituted by the C-terminus of HPV59L1

[00187] 1.7.1 Construction of pFB-HPV39 L1 used as the template

[00188] 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: 83. 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 Kpn I and XbaI cleavage sites.

[00189] 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.

[00190] 1.7.2 Construction of pFB-HPV59L1 as the template

[00191] The HPV59L1 gene was synthesized by Thermo Fisher [formerly Invitrogen (Shanghai) Trading Co.] to obtain plasmid pcDNA3-HPV59-L1 containing the nucleotide sequence encoding amino acids 1-508 of HPV59L1.

[00192] The pcDNA3-HPV59-L1 plasmid was double digested with KpnI and XbaI to obtain a fragment of the HPV59L1 (1-508) gene. The fragment was then ligated to the KpnI / XbaI double digested pFastBacTM1 vector (distributor: Thermo Fisher) to obtain a rod vector containing the HPV59L1 (1-508) gene fragment, named as pFB-HPV59L1.

[00193] 1.7.3 Construction of pFB-HPV39 L1: 59C

[00194] Chimeric gene with HPV39 L1 C-terminus substituted with HPV59L1 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: 85 and the primer sequence R1 is shown in SEQ ID No: 86.

[00195] 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 HPV59L1 and a segment of the KpnI digest site (GGTAC[^]C), the amplified sequence is shown in SEQ ID No: 87.

[00196] 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.

[00197] The recombinant plasmid pFB-HPV59L1 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: 88 and R2 is shown in SEQ ID No: 89.

[00198] This gene fragment contains a gene fragment encoding 38 C-terminal amino acids (471-508) of the HPV59L1, 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: 90.

[00199] 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.

[00200] PCR ligating sequence

[00201] 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.

[00202] 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.

[00203] The final result was SEQ ID NO: 82, a nucleotide sequence encoding amino acids 1-469 of HPV39 L1 and 38 C-terminal amino acids of HPV59L1(471-508) with KpnI and XbaI enzyme cleavage sites at both ends (hereafter referred to as the ligating sequence).

[00204] 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 HPV59L1.

[00205] Example 1.8 Construction of chimeric gene with the C-terminus of HPV45L1 substituted by the C-terminus of HPV33 L1

[00206] The experimental methods and procedures were the same as in Example 1.1, see Appendix 8 for relevant sequences.

[00207] Example 1.9 Construction of a chimeric gene with the C-terminus of HPV51L1 substituted by the C-terminus of HPV33 L1

[00208] The experimental methods and procedures were the same as in Example 1.1, see Appendix 9 for relevant sequences.

[00209] Example 1.10 Construction of a chimeric gene with the C-terminus of HPV52L1 substituted by the C-terminus of HPV33 L1

[00210] The experimental methods and procedures were the same as in Example 1.1, see Appendix 10 for relevant sequences.

[00211] Example 1.11 Construction of a chimeric gene with the C-terminus of HPV56L1 substituted by the C-terminus of HPV33 L1

[00212] The experimental methods and procedures were the same as in Example 1.1, see Appendix 11 for relevant sequences.

[00213] Example 1.12 Construction of a chimeric gene with the C-terminus of HPV58L1 substituted by the C-terminus of HPV33 L1

[00214] The experimental methods and procedures were the same as in Example 1.1, see Appendix 12 for relevant sequences.

[00215] Example 2 Recombinant baculovirus packaging

[00216] Example 2.1: HPV6 L1: 33C recombinant baculovirus packaging of

[00217] The recombinant plasmid of pFB-HPV6 L1: 33C constructed in Example 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.

[00218] 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:

[00219] 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.

[00220] 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.

[00221] 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.

[00222] d. Mixed b and c. Incubated at room temperature for 15-45 min.

[00223] 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.

[00224] f. The incubated mixture of DNA and transfection reagent of d was added to the dish dropwise.

[00225] g. Incubated at 27°C for 2 hr.

[00226] 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.

[00227] i. Culture was performed at 27°C for 7 days and whether the viral infection occurred was observed.

[00228] 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),
5 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.

[00229] Example 2.2: Packaging of HPV11 L1: 33C recombinant baculovirus

10 **[00230]** The experimental methods and procedures were the same as in Example 2.1.

[00231] Example 2.3: Packaging of HPV 16L1: 33C recombinant baculovirus

[00232] The experimental methods and procedures were the same as those of Example 2.1.

15 **[00233]** Example 2.4: Packaging of HPV18 L1: 33C recombinant baculovirus

[00234] The experimental methods and procedures were the same as those of Example 2.1.

[00235] Example 2.5: Packaging of HPV31 L1: 33C recombinant baculovirus

[00236] The experimental methods and procedures were the same as those of Example 2.1.

20 **[00237]** Example 2.6: Packaging of HPV35 L1: 33C recombinant baculovirus

[00238] The experimental methods and procedures were the same as those of Example 2.1.

[00239] Example 2.7: Packaging of HPV39 L1: 59C recombinant baculovirus

[00240] The experimental methods and procedures were the same as those of Example 2.1.

[00241] Example 2.8: Packaging of HPV45 L1: 33C recombinant baculovirus

5 [00242] The experimental methods and procedures were the same as those of Example 2.1.

[00243] Example 2.9: Packaging of HPV51 L1: 33C recombinant baculovirus

[00244] The experimental methods and procedures were the same as those of Example 2.1.

10 [00245] Example 2.10: Packaging of HPV52 L1: 33C recombinant baculovirus

[00246] The experimental methods and procedures were the same as those of Example 2.1.

[00247] Example 2.11: Packaging of HPV56 L1: 33C recombinant baculovirus

[00248] The experimental methods and procedures were the same as those of Example 2.1.

15

[00249] Example 2.12: Packaging of HPV58 L1: 33C recombinant baculovirus

[00250] The experimental methods and procedures were the same as those of Example 2.1.

[00251] **Example 3 Expression of chimeric proteins**

20 [00252] Example 3.1: Expression of HPV6 L1: 33C

[00253] 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.

5 **[00254]** 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.

[00255] Example 3.2: Expression and production of HPV11 L1: 33C

10 **[00256]** The experimental methods and procedures were the same as in Example 3.1.

[00257] 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.

15 **[00258]** Example 3.3: Expression and production of HPV 16L1: 33C

[00259] The experimental methods and procedures were the same as in Example 3.1.

[00260] 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.

20

[00261] Example 3.4: Expression and production of HPV18 L1: 33C

[00262] The experimental methods and procedures were the same as in Example 3.1.

[00263] 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.

5 **[00264]** Example 3.5: Expression and production of HPV31 L1: 33C

[00265] The experimental methods and procedures were the same as in Example 3.1.

[00266] 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.

10 **[00267]** Example 3.6: Expression and production of HPV35 L1: 33C

[00268] The experimental methods and procedures were the same as in Example 3.1.

[00269] The result is shown in Figure 1F. 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.

15 **[00270]** Example 3.7: Expression and production of HPV39 L1: 59C

[00271] The experimental methods and procedures were the same as in Example 3.1.

[00272] The result is shown in Figure 1G. 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.

20 **[00273]** Example 3.8: Expression and production of HPV45 L1: 33C

[00274] The experimental methods and procedures were the same as in Example 3.1.

[00275] The result is shown in Figure 1H. 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.

5

[00276] Example 3.9: Expression and production of HPV51 L1: 33C

[00277] The experimental methods and procedures were the same as in Example 3.1.

[00278] The result is shown in Figure 1I. 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.

10

[00279] Example 3.10: Expression and production of HPV52 L1: 33C

[00280] The experimental methods and procedures were the same as in Example 3.1.

[00281] The result is shown in Figure 1J. The HPV52 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

[00282] Example 3.11: Expression and production of HPV56 L1: 33C

[00283] The experimental methods and procedures were the same as in Example 3.1.

[00284] The result is shown in Figure 1K. 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.

20

[00285] Example 3.12: Expression and production of HPV58 L1: 33C

[00286] The experimental methods and procedures were the same as in Example 3.1.

[00287] The result is shown in Figure 1L. 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.

[00288] Example 4 Preparation of purified virus-like particles

[00289] Example 4.1: Preparation of purified HPV6 L1: 33C virus-like particles

[00290] 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.

[00291] First step chromatography:

[00292] Medium: POROS® 50 HS strong cation exchange media produced by Thermo Fisher was used.

[00293] Medium volume: 150 mL of media volume, 30 mL/min of linear flow rate.

[00294] 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).

[00295] 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.

[00296] 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.

[00297] Second step chromatography.

[00298] Medium: MMA ion exchange media produced by Bestchrom (Shanghai) Biosciences Co., Ltd was used.

[00299] Medium volume: media volume is 150 mL, while linear flow rate is 30 mL/min.

5 **[00300]** 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.

[00301] Elution conditions: 100 mM NaAC, 150 mM NaCl, 0.01% Tween 80, pH 4.5.

10

[00302] Example 4.2: Preparation of purified HPV11 L1: 33C virus-like particles

[00303] The experimental methods and procedures were the same as in Example 4.1.

[00304] Example 4.3: Purification and preparation of HPV 16L1: 33C virus-like particles

15

[00305] The experimental methods and procedures were the same as in Example 4.1.

[00306] Example 4.4: Purification and preparation of HPV18 L1: 33C virus-like particles

[00307] The experimental methods and procedures were the same as in Example 4.1.

20

[00308] Example 4.5: Purification and preparation of HPV31 L1: 33C virus-like particles

[00309] The experimental methods and procedures were the same as in Example 4.1.

[00310] Example 4.6: Preparation of purified HPV35 L1: 33C virus-like particles

[00311] The experimental methods and procedures were the same as in Example 4.1.

[00312] Example 4.7: Preparation of purified HPV39 L1: 59C virus-like particles

[00313] The experimental methods and procedures were the same as in Example 4.1.

5

[00314] Example 4.8: Preparation of purified HPV45 L1: 33C virus-like particles

[00315] The experimental methods and procedures were the same as in Example 4.1.

[00316] Example 4.9: Preparation of purified HPV51 L1: 33C virus-like particles

10

[00317] The experimental methods and procedures were the same as in Example 4.1.

[00318] Example 4.10: Preparation of purified HPV52 L1: 33C virus-like particles

[00319] The experimental methods and procedures were the same as in Example 4.1.

15

[00320] Example 4.11: Preparation of purified HPV56 L1: 33C virus-like particles

[00321] The experimental methods and procedures were the same as in Example 4.1.

[00322] Example 4.12: Preparation of purified HPV58 L1: 33C virus-like particles

[00323] The experimental methods and procedures were the same as in Example 4.1.

20

[00324] **Example 5 Morphological observation of virus-like particles**

[00325] Example 5.1: Morphological observation of HPV6 L1: 33C virus-like particles

[00326] 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.

[00327] 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.

[00328] Example 5.2: Morphological observation of HPV11 L1: 33C virus-like particles

[00329] The experimental methods and procedures were the same as in Example 5.1.

[00330] 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.

[00331] Example 5.3: Morphological observation of HPV 16L1: 33C virus-like particles

[00332] The experimental methods and procedures were the same as in Example 5.1.

[00333] 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.

[00334] Example 5.4: Morphological observation of HPV18 L1: 33C virus-like particles

[00335] The experimental methods and procedures were the same as in Example 5.1.

[00336] 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.

[00337] Example 5.5: Morphological observation of HPV31 L1: 33C virus-like particles

[00338] The experimental methods and procedures were the same as in Example 5.1.

[00339] 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.

[00340] Example 5.6: Morphological observation of HPV35 L1: 33C virus-like particles

[00341] The experimental methods and procedures were the same as in Example 5.1.

[00342] The electron microscopy observation is shown in Figure 2F. As can be seen in Figure 2F, the C-terminally modified HPV35 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00343] Example 5.7: Morphological observation of HPV39 L1: 59C virus-like particles

[00344] The experimental methods and procedures were the same as in Example 5.1.

[00345] The electron microscopy observation is shown in Figure 2G. As can be seen in Figure 2G, the C-terminal-modified HPV39 L1: 59C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00346] Example 5.8: Morphological observation of HPV45 L1: 33C virus-like particles

[00347] The experimental methods and procedures were the same as in Example 5.1.

[00348] The electron microscopy observation is shown in Figure 2H. As can be seen in Figure 2H, the C-terminal-modified HPV45 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00349] Example 5.9: Morphological observation of HPV51 L1: 33C virus-like particles

[00350] The experimental methods and procedures were the same as in Example 5.1.

[00351] The electron microscopy observation is shown in Figure 2I. As can be seen in Figure 2I, the C-terminal-modified HPV51 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00352] Example 5.10: Morphological observation of HPV52 L1: 33C virus-like particles

[00353] The experimental methods and procedures were the same as in Example 5.1.

[00354] The electron microscopy observation is shown in Figure 2J. As can be seen in Figure 2J, the C-terminal-modified HPV52 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

[00355] Example 5.11: Morphological observation of HPV56 L1: 33C virus-like particles

[00356] The experimental methods and procedures were the same as in Example 5.1.

[00357] The electron microscopy observation is shown in Figure 2K. As can be seen in Figure 2K, the C-terminal-modified HPV56 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

5 [00358] Example 5.12: Morphological observation of HPV58 L1: 33C virus-like particles

[00359] The experimental methods and procedures were the same as in Example 5.1.

[00360] The electron microscopy observation is shown in Figure 2L. As can be seen in Figure 2L, the C-terminal-modified HPV58 L1: 33C can form uniform-sized virus-like particles with an average diameter of approximate 60 nm.

10 **[00361] Example 6 Immunogenicity evaluation of virus-like particles in animals**

[00362] Example 6.1: Immunogenicity evaluation of HPV6 L1: 33C virus-like particles in animals

[00363] 6.1.1 Modeling of pseudovirus-neutralizing cells

[00364] As HPV is difficult to be cultured *in vitro* and has a strong host specificity, it is difficult
15 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.

[00365] 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
20 formed from the VLPs, composed of HPV L1 and L2 expressed in cells, by encapsulating free DNA or introducing exogenous plasmid.

[00366] 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.

[00367] 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.

[00368] 6.1.2 Immunoprotective evaluation of HPV6 L1: 33C virus-like particles in animals

[00369] Immunization procedures in mice:

[00370] HPV6 L1: 33C virus-like particles were adsorpted onto aluminium 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.

[00371] EC50 assay of mice:

[00372] 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.

[00373] The results of the HPV6 serum pseudovirus neutralization titer assay are detailed in Table 4.

[00374] **Table 4 EC₅₀ of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

HPV Type	EC ₅₀ Value
Type 6	4948±1831

[00375] Notes:

[00376] 1. number of animals, N = 10.

[00377] 2. GMT (Geometric Mean Titer): geometric mean titer.

[00378] 3. SEM (Standard Error of Mean): standard error.

[00379] 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.

5 [00380] Example 6.2: Evaluation of immunogenicity of HPV11 L1: 33C virus-like particles in animals

[00381] 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.

10 [00382] The results of HPV11 serum pseudovirus neutralization titer assay are detailed in Table 5.

[00383] **Table 5 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00384]

HPV Type	EC ₅₀ Value
Type 11	15024±8400

15 [00385] 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.

[00386] Example 6.3: Evaluation of immunogenicity of HPV 16L1: 33C virus-like particles in animals

20 [00387] 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.

[00388] The results of HPV16 serum pseudovirus neutralization titer assay are detailed in Table 6.

[00389] **Table 6 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00390]

HPV Type	EC ₅₀ Value
Type 16	32449±7224

5 [00391] 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.

10 [00392] Example 6.4: Evaluation of immunogenicity of HPV18 L1: 33C virus-like particles in animals

[00393] 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.

15 [00394] The results of HPV18 serum pseudovirus neutralization titer assay are detailed in Table 7.

[00395] **Table 7 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00396]

HPV Type	EC ₅₀ Value
Type 18	18480±4051

[00397] 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.

5 [00398] Example 6.5: Evaluation of immunogenicity of HPV31 L1: 33C virus-like particles in animals

[00399] 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.

10 [00400] The results of HPV31 serum pseudovirus neutralization titer assay are detailed in Table 8.

[00401] **Table 8 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00402]

HPV Type	EC ₅₀ Value
Type 31	5210±1147

15 [00403] 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.

[00404] Example 6.6: Evaluation of immunogenicity of HPV35 L1: 33C virus-like particles in animals

20 [00405] 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.

[00406] The results of HPV35 serum pseudovirus neutralization titer assay are detailed in Table 9.

[00407] **Table 9 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00408]

HPV Type	EC ₅₀ Value
Type 35	2293±1448

5

[00409] 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.

10

[00410] Example 6.7: Immunogenicity evaluation of HPV39 L1: 59C virus-like particles in animals

[00411] 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.

[00412] The results of HPV39 serum pseudovirus neutralization titer assay are detailed in Table 10.

15

[00413] **Table 10 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00414]

HPV Type	EC ₅₀ Value
Type 39	25526±5857

[00415] 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.

5 [00416] Example 6.8: Immunogenicity evaluation of HPV45 L1: 33C virus-like particles in animals

[00417] 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.

10 [00418] The results of HPV45 serum pseudovirus neutralization titer assay are detailed in Table 11.

[00419] **Table 11 EC₅₀ of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00420]

HPV Type	EC ₅₀ Value
Type 45	755±935

15 [00421] 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.

[00422] Example 6.9: Evaluation of immunogenicity of HPV51 L1: 33C virus-like particles in animals

20 [00423] 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.

[00424] The results of HPV51 serum pseudovirus neutralization titer assay are detailed in Table 12.

[00425] **Table 12 EC₅₀ of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00426]

HPV Type	EC ₅₀ Value
Type 51	5528±1572

[00427] 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.

[00428] Example 6.10: Evaluation of immunogenicity of HPV52 L1: 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 Q05138 and the L2 sequence was from Uniprot F8S4U2.

[00430] The results of HPV52 serum pseudovirus neutralization titer assay are detailed in Table 13.

[00431] **Table 13 EC₅₀ of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00432]

HPV Type	EC ₅₀ Value
Type 52	19019±8604

[00433] 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.

[00434] Example 6.11: Evaluation of immunogenicity of HPV56 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 P36743 and the L2 sequence was from Uniprot P36765.

[00436] The results of HPV56 serum pseudovirus neutralization titer assay are detailed in Table 14.

[00437] **Table 14 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00438]

HPV Type	EC ₅₀ Value
Type 56	2497±612

[00439] 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.

[00440] Example 6.12: Evaluation of immunogenicity of HPV58 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 P26535 and the L2 sequence was from Uniprot B6ZB12.

[00442] The results of HPV58 serum pseudovirus neutralization titer assay are detailed in Table 15.

[00443] **Table 15 EC50 of Serum Neutralization Titer Assay in Mice (GMT±SEM)**

[00444]

HPV Type	EC ₅₀ Value
Type 58	19939±8459

[00445] 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.

[00446] Comparative Example 1: Expression of C-terminal truncated HPV16L1 (aa 1-474)

[00447] The inventors attempted to truncate the C-terminus of HPV16L1 by 31 amino acids and named it HPV16L1 (1-474) (SEQ ID NO: 27). However, it was found in the study that the truncated HPV16L1 (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 3.

[00448] 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	0601 The amino acid sequence of HPV type 6 L1 protein aa 1-469 MWRPSDSTVYVPPPNPVSKVVATDAYVTRTNIFYHASSSRLLAVGHPYFSIKRA NKTVPKVSQYQYRVFKVVLDPNKFALPDSSLFDPTTQRLVWACTGLEVGRG QPLGVGVSGHPFLNKYDDVENSQSGGNPGQDNRVNVGMDYKQTQLCMVGCA PPLGEHWGKGKQCTNTPVQAGDCPPELITSVIQDGDMDVDTGFGAMNFADLQT NKSDVPIDICGTTCKYPDYLQMAADPYGDRLFFFLRKEQMFARHFFNRAGEVG EPVPDTLIIKSGNRTSVGSSIIYVNTPSGSLVSSEAQLFNKPYWLQKAQGHNNGI CWGNQLFVTVDTTTRSTNMTLCASVTTSSSTYTNSDYKEYMRHVEEYDLQFIFQ LCSITLSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTLEDYRYVQSQAITCQKP TPEKEKPDYPKNLSFWEVNLKEKFSSELDQYPLGRKFLLQSGY
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 MWRPSDSTVYVPPPNPVSKVVATDAYVTRTNIFYHASSSRLLAVGHPYFSIKRA NKTVPKVSQYQYRVFKVVLDPNKFALPDSSLFDPTTQRLVWACTGLEVGRG QPLGVGVSGHPFLNKYDDVENSQSGGNPGQDNRVNVGMDYKQTQLCMVGCA PPLGEHWGKGKQCTNTPVQAGDCPPELITSVIQDGDMDVDTGFGAMNFADLQT NKSDVPIDICGTTCKYPDYLQMAADPYGDRLFFFLRKEQMFARHFFNRAGEVG EPVPDTLIIKSGNRTSVGSSIIYVNTPSGSLVSSEAQLFNKPYWLQKAQGHNNGI CWGNQLFVTVDTTTRSTNMTLCASVTTSSSTYTNSDYKEYMRHVEEYDLQFIFQ LCSITLSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTLEDYRYVQSQAITCQKP TPEKEKPDYPKNLSFWEVNLKEKFSSELDQYPLGRKFLLQSGYKAKPKLKRAA PTSTRTSSAKRKKVKK

SEQ ID No: 4	0604 Nucleotide sequence of chimeric HPV type 6 L1 protein ATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGCTACAGA TGCCTATGTGACCAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGACACCCATA CTTCAGCATCAAGAGGGCTAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGTGTTC AAGG TGGTGCTGCCTGACCCAAACAAGTTTGGCCCTGCCTGACTCCTCCCTGTTTGACCCAAACCACCCAGAGACTGG TGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGACACCCATTC CTGAACAAATATGATGATGTGGAGA AACTCTGGCTCTGGAGGCAACCCTGGACAAGACAACAGGGTGAATG TGGGGATGGACTACAAGCAGACCCAACTTTGTATGGTGGGCTGTGCCCCCTCCACTGGGAGA AACTTGGGGC AAGGGCAAGCAGTGTACCAACACACCTGTCCAGGCTGGAGACTGTCTCCATTGGA AACTGATTACCTCTGT GATTCAAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACTTTGCTGACCTCCAAACCAACAAGT CTGATGTGCCAATTGACATCTGTGGCACC AACTGTAAATACCCTGACTACCTCCAAATGGCTGCTGACCCAT ATGGAGACAGACTGTTCTTCTTCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAGGGCTGGA GAGGTGGGAGAACCTGTGCCTGACACCCTGATTATCAAGGGCTCTGGCAACAGGACCTCTGTGGGCTCCAG CATCTATGTGAACACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAAGCCATACTGGCT CCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTTGTGACAGTGGTGGACACCA CCAGGAGCACCAATATGACCCTGTGTGCCTCTGTGACCACCTCCAGCACCTACACCAACTCTGACTACAAG GAATATATGAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCA AACTTTGTAGCATCACCTGTCTGCT GAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGA AACTTTGGACTGAGCCCTCC TCCAAATGGCACCTTGGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTCAGAAGCCAACAC CTGAGAAGGAGAAGCCTGACCCATACAAGAACCTGTCCTTCTGGGAGGTGAACCTGAAAGAGAAGTTCTCC TCTGAACTGGACCAATACCCACTGGGCAGGAAGTTCCTGCTCCAATCTGGCTACAAAGCCAAGCCAAA AACT GAAAAGGGCTGCCCAACCAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAGTAA
SEQ ID No: 5	0605 Synthetic HPV6 L1 gene ctgggtaccATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGCC

	TACAGATGCCTATGTGACCAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGAC ACCCATACTTCAGCATCAAGAGGGCTAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGTG TTCAAGGTGGTGTGCTGCCTGACCCAAACAAGTTTGCCCTGCCTGACTCCTCCCTGTTTGACCCAACCAACCCAG AGACTGGTGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGAC ACCCATTCTGAACAAATATGATGATGTGGAGAACTCTGGCTCTGGAGGCAACCCTGGACAAGACAACAGG GTGAATGTGGGGATGGACTACAAGCAGACCCAACTTTGTATGGTGGGCTGTGCCCTCCACTGGGAGAACA CTGGGGCAAGGGCAAGCAGTGTACCAACACACCTGTCCAGGCTGGAGACTGTCCTCCATTGGAAGTATTA CCTCTGTGATTCAAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACCTTGTCTGACCTCCAAACC AACAAGTCTGATGTGCCAATTGACATCTGTGGCACCCTTGTAATACCCTGACTACCTCCAAATGGCTGCT GACCCATATGGAGACAGACTGTTCTTCTCCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAG GGCTGGAGAGGTGGGAGAACCTGTGCCTGACACCCTGATTATCAAGGGCTCTGGCAACAGGACCTCTGTGG GCTCCAGCATCTATGTGAACACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAAGCCAT ACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTGTGACAGTGGTG GACACCACCAGGAGACCAATATGACCCTGTGTGCCTCTGTGACCACCTCCAGCACCTACACCAACTCTGA CTACAAGGAATATATGAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGTAGCATCACCT GTCTGCTGAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGAACCTTTGGACTGA GCCCTCTCCAAATGGCACCTTGGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTGAGAAG CCAACACCTGAGAAGGAGAAGCCTGACCCATACAAGAACCTGTCCTTCTGGGAGGTGAACCTGAAAGAGA AGTTCTCCTCTGAACTGGACCAATACCACTGGGCAGGAAGTTCCTGCTCCAATCTGGCTACAGGGGCAGG TCCAGCATCAGGACAGGAGTGAAGAGACCTGCTGTGAGCAAGGCATCTGCTGCCCCAAGAGGAAGAGGG CTAAGACCAAGAGGTAAActcgagctc
SEQ ID No: 6	0606 Synthetic HPV33 L1 gene ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC

	AATACAGGGTGTTTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGAGGACTGGCA GTTTGGACTGACACCTCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC 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]

ATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTG
GTGGCTACAGATGCCTATGTGACCAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGA
CTGCTGGCTGTGGGACACCCATACTTCAGCATCAAGAGGGCTAACAAGACAGTGGTGCCA
AAGGTGTCTGGCTACCAATACAGGGTGTTCAGGTGGTGTGCCTGACCCAAACAAGTTT
GCCCTGCCTGACTCCTCCCTGTTTGACCCAACCACCCAGAGACTGGTGTGGGCTTGTACT
GGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGACACCCATTCTCTG
AACAAATATGATGATGTGGAGAAGTCTGGCTCTGGAGGCAACCCTGGACAAGACAACAGG
GTGAATGTGGGGATGGACTACAAGCAGACCCAACTTTGTATGGTGGGCTGTGCCCTCCA
CTGGGAGAAGCACTGGGGCAAGGGCAAGCAGTGTACCAACACACCTGTCCAGGCTGGAGAC
TGTCCTCCATTGGAAGTATTACCTCTGTGATTGAGGATGGAGATATGGTGGACACAGGC
TTTGGAGCTATGAACTTTGCTGACCTCCAAACCAACAAGTCTGATGTGCCAATTGACATC
TGTGGCACCCTTGTAATACCTGACTACCTCCAAATGGCTGCTGACCCATATGGAGAC
AGACTGTTCTTCTTCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAGGGCT
GGAGAGGTGGGAGAAGCTGTGCCTGACACCTGATTATCAAGGGCTCTGGCAACAGGACC
TCTGTGGGCTCCAGCATCTATGTGAACACACCATCTGGCTCCCTGGTGTCTCTGAGGCT
CAACTTTTCAACAAGCCATACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGT
TGGGGCAACCAACTTTTTGTGACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTG
TGTGCCTCTGTGACCACCTCCAGCACCTACACCAACTCTGACTACAAGGAATATATGAGG
CATGTGGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGTAGCATCACCTGTCTGCT
GAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGAACCTTTGGA
CTGAGCCCTCCTCCAAATGGCACCTTGGAGGACACCTACAGATATGTCCAGAGCCAGGCT
ATCACTTGTGAGAAGCCAACACCTGAGAAGGAGAAGCCTGACCCATACAAGAACCTGTCC
TTCTGGGAGGTGAACCTGAAAGAGAAGTTCTCCTCTGAACTGGACCAATACCCACTGGGC
AGGAAGTTCCTGCTCCAATCTGGCTAC^[2] AAAGCCAAGC^[3]

[1]: Kpn I 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 ATCTGGCTACAAAGCCAAGCCAAAAGCTGAAAAGGG
SEQ ID No: 11	0611 HPV6 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGGC
SEQ ID No: 12	0612 HPV6 L1 amplified sequence 2 <u>ATCTGGCTAC</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 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 KPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

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 MWRPSDSTVYVPPPNPVSKVVATDAYVKRTNIFYHASSSRLLAVGHPYYSIKK VNKTVVPKVSGYQYRVFKVVLDPDNKFALPDSSLFDPTTQRLVWACTGLEVGR GQPLGVGVSGHPLLNKYDDVENSNGGYGGNPGQDNRVNVGMDYKQTQLCMV GCAPPLGEHWGKGTQCSNTSVQNGDCPPELITSVIQDGDGMVDTGFGAMNFAD LQTNKSDVPLDICGTVCKYPDYLQMAADPYGDRLFFYLRLKEQMFARHFFNRA GTVGEPVPDDLVLKGGNNRSSVASSIYVHTPSGSLVSSEAQLFNKPYWLQKAQ GHNNGICWGNHLFVTVDTTTRSTNMTLCASVSKSATYTNSDYKEYMRHVEEF DLQFIFQLCSITLSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTLEDTYRYVQSQ AITCQKPTPEKEKQDPYKDMSFWEVNLKEKFSSELDQFPLGRKFLLQSGY
SEQ ID No: 15	1102 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 16	1103 Amino acid sequence of chimeric HPV type 11 L1 protein MWRPSDSTVYVPPPNPVSKVVATDAYVKRTNIFYHASSSRLLAVGHPYYSIKK VNKTVVPKVSGYQYRVFKVVLDPDNKFALPDSSLFDPTTQRLVWACTGLEVGR GQPLGVGVSGHPLLNKYDDVENSNGGYGGNPGQDNRVNVGMDYKQTQLCMV GCAPPLGEHWGKGTQCSNTSVQNGDCPPELITSVIQDGDGMVDTGFGAMNFAD LQTNKSDVPLDICGTVCKYPDYLQMAADPYGDRLFFYLRLKEQMFARHFFNRA GTVGEPVPDDLVLKGGNNRSSVASSIYVHTPSGSLVSSEAQLFNKPYWLQKAQ GHNNGICWGNHLFVTVDTTTRSTNMTLCASVSKSATYTNSDYKEYMRHVEEF DLQFIFQLCSITLSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTLEDTYRYVQSQ AITCQKPTPEKEKQDPYKDMSFWEVNLKEKFSSELDQFPLGRKFLLQSGYKAKP KLKRAAPTSTRTSSAKRKKVKK

SEQ ID No: 17	1104 Nucleotide sequence of chimeric HPV type 11 L1 protein ATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGCTACAGA TGCCTATGTGAAGAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGACACCCAT ACTACAGCATCAAGAAGGTGAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGTGTTCAA GGTGGTGCTGCCTGACCCAAACAAGTTTGCCCTGCCTGACTCCTCCCTGTTTGACCCAAACCACCCAGAGACT GGTGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGACACCCA CTGCTGAACAAATATGATGATGTGGAGAACTCTGGAGGCTATGGAGGCAACCCTGGACAAGACAACAGGG TGAATGTGGGGATGGACTACAAGCAGACCCAACCTTTGTATGGTGGGCTGTGCCCTCCACTGGGAGAACAC TGGGGCAAGGGCACCCAGTGTAGCAACACCTCTGTCCAGAATGGAGACTGTCTCCATTGGAAGTATTAC CTCTGTGATTCAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACTTTGCTGACCTCCAAACCA ACAAGTCTGATGTGCCACTGGACATCTGTGGCACAGTGTGTAAATACCCTGACTACCTCCAAATGGCTGCT GACCCATATGGAGACAGACTGTTCTTCTACCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAG GGCTGGCACAGTGGGAGAACCTGTGCCTGATGACCTGCTGGTGAAGGGAGGCAACAACAGGTCTCTGTGG CATCCAGCATCTATGTGCATACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAAGCCAT ACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCACCTGTTTGTGACAGTGGTG GACACCACCAGGAGACCAATATGACCCTGTGTGCCTCTGTGAGCAAGTCTGCCACCTACACCAACTCTGA CTACAAGGAATATATGAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTCCAACCTTTGTAGCATCACCT GTCTGCTGAGGTGATGGCTTACATCCACACAATGAACCATCTGTGTTGGAGGACTGGAACCTTTGGAAGTGA GCCCTCCTCCAAATGGCACCTTGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTCAGAAG CCAACACCTGAGAAGGAGAAGCAGGACCCATACAAGGATATGAGTTTCTGGGAGGTGAACCTGAAAGAGA AGTTCTCCTCTGAAGTGGACCAAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAATCTGGCTACAAAGCCAAGC CAAAACTGAAAAGGGCTGCCCCAACCAAGCAGGACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAGTA AA
SEQ ID No: 18	1105 Synthetic HPV11 L1 gene ctgggtaccATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGCC

	TACAGATGCCTATGTGAAGAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGAC ACCCATACTACAGCATCAAGAAGGTGAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGT GTTCAAGGTGGTGTCTGCCTGACCCAAACAAGTTTGCCCTGCCTGACTCCTCCCTGTTTGACCCAACCAACCA GAGACTGGTGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGA CACCCACTGCTGAACAAATATGATGATGTGGAGAACTCTGGAGGCTATGGAGGCAACCCTGGACAAGACA ACAGGGTGAATGTGGGGATGGACTACAAGCAGACCCAACCTTTGTATGGTGGGCTGTGCCCCCTCCACTGGGA GAACACTGGGGCAAGGGCACCCAGTGTAGCAACACCTCTGTCCAGAATGGAGACTGTCTCCATTGGAAC GATTACCTCTGTGATTGAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACTTTGCTGACCTCCA AACCAACAAGTCTGATGTGCCACTGGACATCTGTGGCACAGTGTGTAATACCCTGACTACCTCCAAATGG CTGCTGACCCATATGGAGACAGACTGTTCTTCTACCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCA ACAGGGCTGGCACAGTGGGAGAACCTGTGCCTGATGACCTGCTGGTGAAGGGAGGCAACAACAGGTCTCTC TGTGGCATCCAGCATCTATGTGCATACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAA GCCATACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCACCTGTTTGTGACAG TGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTGCCTCTGTGAGCAAGTCTGCCACCTACACCAAC TCTGACTACAAGGAATATATGAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTCCAACCTTTGTAGCATC ACCCTGTCTGCTGAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGAACCTTTGG ACTGAGCCCTCCTCCAAATGGCACCTTGGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTG AGAAGCCAACACCTGAGAAGGAGAAGCAGGACCCATACAAGGATATGAGTTTCTGGGAGGTGAACCTGAA AGAGAAGTTCTCCTCTGAACTGGACCAGTTTCCACTGGGCAGGAAGTTCTGCTCCAATCTGGCTACAGGG GCAGGACCTCTGCCAGGACAGGCATCAAGAGACCTGCTGTGAGCAAGCCAAGCACAGCCCCAAAGAGGAA GAGGACCAAGACCAAGAAGTAAActcgagctc
SEQ ID No: 19	1106 Synthetic HPV33 L1 gene ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC

	AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 20	1107 HPV11 L1 F1 CTTGGTACCATGTGGAGACCATCTGACAGCACAGT
SEQ ID No: 21	1108 HPV11 L1 R1 GCTTGGCTTTGTAGCCAGATTGGAGCAGGAACTTCC
SEQ ID No: 22	1109 HPV11 L1 amplified sequence 1 CTTGGTACC ^[1]

	<u>ATGTGGAGACCATCTGACAGCACAGTCTATGTGCCTCCTCCAAACCCTGTGAGCAAGGTGGTGCTACAGA</u> <u>TGCCTATGTGAAGAGGACCAACATCTTCTACCATGCCTCCTCCAGCAGACTGCTGGCTGTGGGACACCCAT</u> <u>ACTACAGCATCAAGAAGGTGAACAAGACAGTGGTGCCAAAGGTGTCTGGCTACCAATACAGGGTGTTCAA</u> <u>GGTGGTGCTGCCTGACCCAAACAAGTTTGCCCTGCCTGACTCCTCCCTGTTTGACCCAAACCACCCAGAGACT</u> <u>GGTGTGGGCTTGTACTGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGAGTGTCTGGACACCCA</u> <u>CTGCTGAACAAATATGATGATGTGGAGAACTCTGGAGGCTATGGAGGCAACCCTGGACAAGACAACAGGG</u> <u>TGAATGTGGGGATGGACTACAAGCAGACCCAACTTTGTATGGTGGGCTGTGCCCTCCACTGGGAGAACAC</u> <u>TGGGGCAAGGGCACCCAGTGTAGCAACACCTCTGTCCAGAATGGAGACTGTCTCCATTGGAAGTATTAC</u> <u>CTCTGTGATTCAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGAACTTTGCTGACCTCCAAACCA</u> <u>ACAAGTCTGATGTGCCACTGGACATCTGTGGCACAGTGTGTAATACCCTGACTACCTCCAAATGGCTGCT</u> <u>GACCCATATGGAGACAGACTGTTCTTCTACCTGAGGAAGGAACAGATGTTTGCCAGACACTTCTTCAACAG</u> <u>GGCTGGCACAGTGGGAGAACCTGTGCCTGATGACCTGCTGGTGAAGGGAGGCAACAACAGGTCTCTGTGG</u> <u>CATCCAGCATCTATGTGCATACACCATCTGGCTCCCTGGTGTCTCTGAGGCTCAACTTTTCAACAAGCCAT</u> <u>ACTGGCTCCAAAAGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCACCTGTTTGTGACAGTGGTG</u> <u>GACACCACCAGGAGACCAATATGACCCTGTGTGCCTCTGTGAGCAAGTCTGCCACCTACACCAACTCTGA</u> <u>CTACAAGGAATATATGAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTCCAACCTTTGTAGCATCACCCCT</u> <u>GTCTGCTGAGGTGATGGCTTACATCCACACAATGAACCCATCTGTGTTGGAGGACTGGAACCTTTGGACTGA</u> <u>GCCCTCCTCCAAATGGCACCTTGGAGGACACCTACAGATATGTCCAGAGCCAGGCTATCACTTGTCAGAAG</u> <u>CCAACACCTGAGAAGGAGAAGCAGGACCCATACAAGGATATGAGTTTCTGGGAGGTGAACCTGAAAGAGA</u> <u>AGTTCTCCTCTGAACTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAATCTGGCTAC</u>^[2] AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding HPV11 L1 aa 1-470; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No:	1110 HPV11 L1 F2

23	ATCTGGCTACAAAGCCAAGCCAAAAGCTGAAAAGGG
SEQ ID No:	1111 HPV11 L1 R2
24	CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGGC
SEQ ID No:	1112 HPV11 L1 amplified sequence 2
25	<u>ATCTGGCTAC</u>^[1] <u>AAAGCCAAGCCAAAAGCTGAAAAGGGCTGCCCCAACCAGCACCAGG</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:	1113 The amino acid sequence of HPV type 59 L1 protein aa 471-508
26	LQLGARP KPTIGPRKRAAPAPTSTPSPKR VKRRKSSRK

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 MSLWLPSEATVYLPPVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGHPYFPIKK PNNNKILVPKVSGLQYRVFRIHLPDPNKFGFPDTSFYNPDTQRLVWACVGVEVG RGQPLGVGISGHPLLNLDDTENASAYAANAGVDNRECISMDYKQTQLCLIGC KPPIGEHWGKGSPCTNVAVNPGDCPPELINTVIQDGDMDVDTGFGAMDFTTLQ ANKSEVPLDICTSICKYPDYIKMVSEPYGDSLFFYLRRQMFVRHLFNRAAGVG ENVPDDLYIKGSGSTANLASSNYFPTPSGSMVTSDAQIFNKPYWLQRAQGHNN GICWGNQLFVTVVDTTTRSTNMSLCAAISTSETTYKNTNFKEYLRHGEEYDLQFI FQLCKITLTADVMTYIHSMNSTILEDWNFGLQPPPGGTLEDTYRFVTSQAIACQ 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 MSLWLPSEATVYLPPVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGHPYFPIKK PNNNKILVPKVSGLQYRVFRIHLPDPNKFGFPDTSFYNPDTQRLVWACVGVEVG RGQPLGVGISGHPLLNLDDTENASAYAANAGVDNRECISMDYKQTQLCLIGC KPPIGEHWGKGSPCTNVAVNPGDCPPELINTVIQDGDMDVDTGFGAMDFTTLQ ANKSEVPLDICTSICKYPDYIKMVSEPYGDSLFFYLRRQMFVRHLFNRAAGVG ENVPDDLYIKGSGSTANLASSNYFPTPSGSMVTSDAQIFNKPYWLQRAQGHNN GICWGNQLFVTVVDTTTRSTNMSLCAAISTSETTYKNTNFKEYLRHGEEYDLQFI FQLCKITLTADVMTYIHSMNSTILEDWNFGLQPPPGGTLEDTYRFVTSQAIACQ KHTPPAPKEDPLKKYTFWEVNLKEKFSADLDQFPLGRKFLLQAGLKAKPKLKR AAPTSTRTSSAKRKKVKK

SEQ ID No:	1604 Nucleotide sequence of chimeric HPV type 16 L1 protein
30	ATGAGTCTGTGGCTGCCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTGAGCAC AGATGAATATGTGGCAAGGACCAACATCTACTACCATGCTGGCACCAGCAGACTGCTGGCTGTGGGACACC CATACTTTCCAATCAAGAAGCCAAACAACAACAAGATTCTGGTGCCAAAGGTGTCTGGACTCCAATACAGG GTGTTTCAGGATTACCTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACCCTGACACC CAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG GACACCCACTGCTGAACAACTGGATGACACAGAGAATGCCTCTGCCTATGCTGCCAATGCTGGAGTGGAC AACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACTTTGTCTGATTGGCTGTAAGCCTCCAATTGG AGAACACTGGGGCAAGGGCAGCCCATGTACCAATGTGGCTGTGAACCCTGGAGACTGTCCTCCATTGGAAC TGATAAACACAGTGATTTCAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGGACTTCACCACCCTC CAAGCCAACAAGTCTGAGGTGCCACTGGACATCTGTACCAGCATCTGTAAATACCTGACTACATCAAGAT GGTGTCTGAACCATATGGAGACTCCCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACACCTGTT CAACAGGGCTGGAGCAGTGGGAGAGAATGTGCCTGATGACCTCTACATCAAGGGCTCTGGCAGCACAGCC AACCTGGCATCCAGCAACTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAAC AAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTGTGAC AGTGGTGGACACCACCAGGAGCACCAATATGAGTCTGTGTGCTGCCATCAGCACCTCTGAGACCACCTACA AGAACACCAACTTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAACCTTGT AAGATTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACAGCACCATCTTGGAGGACTGGAA CTTTGGACTCCAACCTCCTCCTGGAGGCACCTTGGAGGACACCTACAGGTTTGTGACCAGCCAGGCTATTGC CTGTGAGAAACACACACCTCCTGCCCCAAAGGAGGACCCACTGAAAAAATACACCTTCTGGGAGGTGAACC TGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACTG AAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACCAGCACCAGGACCTCCTTGCCAAGAGGAAGAAGG TGAAGAAGTAAA
SEQ ID No:	1605 Synthetic HPV16 L1 gene
31	ctgggtaccATGAGTCTGTGGCTGCCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG

	AGCACAGATGAATATGTGGCAAGGACCAACATCTACTACCATGCTGGCACCAGCAGACTGCTGGCTGTGGG ACACCCATACTTTCCAATCAAGAAGCCAAACAACAACAAGATTCTGGTGCCAAAGGTGTCTGGACTCCAAT ACAGGGTGTTTCAGGATTCACCTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACCCTG ACACCCAGAGACTGGTGTGGGCTTGTGTGGGAGTGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCAT CTCTGGACACCCACTGCTGAACAACTGGATGACACAGAGAATGCCTCTGCCTATGCTGCCAATGCTGGAG TGGACAACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACTTTGTCTGATTGGCTGTAAGCCTCCA ATTGGAGAACACTGGGGCAAGGGCAGCCCATGTACCAATGTGGCTGTGAACCTGGAGACTGTCCTCCATT GGAAGTGAATAACACAGTGATTGAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGGACTTCACCA CCCTCCAAGCCAACAAGTCTGAGGTGCCACTGGACATCTGTACCAGCATCTGTAAATACCCTGACTACATC AAGATGGTGTCTGAACCATATGGAGACTCCCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACA CCTGTTCAACAGGGCTGGAGCAGTGGGAGAGAATGTGCCTGATGACCTCTACATCAAGGGCTCTGGCAGCA CAGCCAACCTGGCATCCAGCAACTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTT TCAACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTT GTGACAGTGGTGGACACCACCAGGAGCACC AATATGAGTCTGTGTGCTGCCATCAGCACCTCTGAGACCAC CTACAAGAACACCAACTTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAAC TTTGTAAGATTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACAGCACCATCTTGAGGAGC TGGAACCTTTGGACTCCAACCTCCTCCTGGAGGCACCTTGAGGACACCTACAGGTTTGTGACCAGCCAGGC TATTGCCTGTCAGAAACACACACCTCCTGCCCCAAAGGAGGACCCACTGAAAAAATACACCTTCTGGGAGG TGAACCTGAAAGAGAAGTTCTCTGCTGACCTGGACCAAGTTTCCACTGGGCAGGAAGTTCTGCTCCAAGCA GGACTGAAAGCCAAGCCAAAGTTACCCCTGGGCAAGAGGAAGGCTACACCAACCACCTCCAGCACCAGCA CCACAGCCAAGAGGAAGAAGAGGAAACTGTAAActcgagctc
SEQ ID No: 32	1606 Synthetic HPV33 L1 gene ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC

	AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 33	1607 HPV16 L1 F1 CTTGGTACCATGAGTCTGTGGCTGCCATCTGAGG
SEQ ID No: 34	1608 HPV16 L1 R1 GCTTGGCTTTCAGTCCTGCTTGGAGCAGGAACCTTCC
SEQ ID No: 35	1609 HPV16 L1 amplified sequence 1 CTTGGTACC ^[1]

	<u>ATGAGTCTGTGGCTGCCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTGAGCAC</u> <u>AGATGAATATGTGGCAAGGACCAACATCTACTACCATGCTGGCACCAGCAGACTGCTGGCTGTGGGACACC</u> <u>CATACTTTCCAATCAAGAAGCCAAACAACAACAAGATTCTGGTGCCAAAGGTGTCTGGACTCCAATACAGG</u> <u>GTGTTTCAGGATTACCTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACCCTGACACC</u> <u>CAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG</u> <u>GACACCCACTGCTGAACAACTGGATGACACAGAGAATGCCTCTGCCTATGCTGCCAATGCTGGAGTGGAC</u> <u>AACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACTTTGTCTGATTGGCTGTAAGCCTCCAATTGG</u> <u>AGAACACTGGGGCAAGGGCAGCCCATGTACCAATGTGGCTGTGAACCCTGGAGACTGTCCTCCATTGGAAC</u> <u>TGATAAACACAGTGATTTCAGGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGGACTTCACCACCTC</u> <u>CAAGCCAACAAGTCTGAGGTGCCACTGGACATCTGTACCAGCATCTGTAAATACCCTGACTACATCAAGAT</u> <u>GGTGTCTGAACCATATGGAGACTCCCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACACCTGTT</u> <u>CAACAGGGCTGGAGCAGTGGGAGAGAATGTGCCTGATGACCTCTACATCAAGGGCTCTGGCAGCACAGCC</u> <u>AACCTGGCATCCAGCAACTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAAC</u> <u>AAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTTGTGAC</u> <u>AGTGGTGGACACCACCAGGAGCACCAATATGAGTCTGTGTGCTGCCATCAGCACCTCTGAGACCACCTACA</u> <u>AGAACACCAACTTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGT</u> <u>AAGATTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACAGCACCATCTTGGAGGACTGGAA</u> <u>CTTTGGACTCCAACCTCCTCCTGGAGGCACCTTGGAGGACACCTACAGGTTTGTGACCAGCCAGGCTATTGC</u> <u>CTGTCAGAAACACACACCTCCTGCCCCAAAGGAGGACCCACTGAAAAAATACACCTTCTGGGAGGTGAACC</u> <u>TGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCTGCTCCAAGCAGGACTG</u> ^[2] AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding HPV16 L1 aa 1-474; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No:	1610 HPV16 L1 F2

36	AGCAGGACTGAAAGCCAAGCCAAAAGCTGAAAAGGG
SEQ ID No:	1611 HPV16 L1 R2
37	CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No:	1612 HPV16 L1 amplified sequence 2
38	<u>AGCAGGACTG^[1]</u> <u>AAAGCCAAGCCAAAAGCTGAAAAGGGCTGCCCAACCAGCACCAGG</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:	1613 The amino acid sequence of HPV type 59 L1 protein aa 471-508
39	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 LQDTKCEVPLDICQSICKYPDYLQMSADPYGDSMFFCLRREQLFARHFWNRAG TMGDTVPPQSLYIKGTGMRASPGSCVYSPSPSGSIVTSDSQLFNKPYWLHKAQGH NNGVCWHNQLFVTVDTTTRSTNLTICASTQSPVPGQYDATKFKQYSRHVEEYD LQFIFQLCTITLTADVMSYIHSMNSSILEDWNFGVPPPPPTSLVDTYRFVQSVAIT CQKDAAPAENKDPYDKLKFWNVDLKEKFSLDLDQYPLGRKFL
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 LQDTKCEVPLDICQSICKYPDYLQMSADPYGDSMFFCLRREQLFARHFWNRAG TMGDTVPPQSLYIKGTGMRASPGSCVYSPSPSGSIVTSDSQLFNKPYWLHKAQGH NNGVCWHNQLFVTVDTTTRSTNLTICASTQSPVPGQYDATKFKQYSRHVEEYD LQFIFQLCTITLTADVMSYIHSMNSSILEDWNFGVPPPPPTSLVDTYRFVQSVAIT CQKDAAPAENKDPYDKLKFWNVDLKEKFSLDLDQYPLGRKFLKAKPKLKRAA PTSTRTSSAKRKKVKK

SEQ ID No:	1804 Nucleotide sequence of chimeric HPV type 18 L1 protein
43	ATGGCCCTCTGGAGACCATCCGATAACACAGTGTACTTGCCCCACCCAGCGTCGCCCAGGTGGTGAACAC AGACGACTACGTACCCAGAACCTCAATCTTCTACCACGCCGGGTCCAGCCGGCTGCTGACCGTGGGCAACC CCTACTTCCGCGTGCCCGCCGGCGGCGGAAACAAACAAGACATCCCCAAAGTCAGCGCCTATCAGTACCGG GTGTTCCGCGTCCAACCTGCCCCGATCCCAACAAGTTGCGCCTGCCCGACACCTCCATCTACAACCCGAGACC CAGAGGCTGGTCTGGGCTTGCGCCGGCGTCGAGATCGGGAGGGGCCAACCCCTGGGCGTGGGGTTGTCCGG CCACCCCTTCTACAACAAGCTGGACGATACCGAGTCCAGCCACGCAGCAACCAGCAACGTCTCCGAAGATG TGCGCGATAACGTCAGCGTGGACTACAAACAAACCCAACTGTGCATCCTGGGATGCGCACCCGCCATCGGC GAGCATTGGGCCAAGGGGACCGCTGCAAGAGCAGGCCCTGAGCCAAGGGGACTGTCCACCCCTGGAGT TGAAGAATACCGTGCTCGAGGACGGCGACATGGTGGACACCGGTACGGCGCTATGGATTCTCCACCCCTC CAGGACACCAAGTGCGAAGTGCCCTCGACATCTGCCAAAGCATCTGCAAGTACCCCGACTACCTCCAGAT GAGCGCCGACCCCTACGGCGACAGCATGTTCTTCTGTCTCAGAAGGGAACAATTGTTGCCCGCCACTTCTG GAACCGGGCCGGCACAATGGGAGATACAGTCCCCCAGAGCCTGTACATCAAGGGGACCGGAATGAGGGCC AGCCCCGGGTCCTGCGTCTACAGCCCAAGCCCTCCGGGAGCATCGTCACAAGCGATAGCCAACCTTTCAA CAAGCCCTACTGGCTCCACAAAGCCCAAGGCCACAATAACGGGGTGTGTTGGCACAACCAGCTGTTTCGTGA CCGTGCTGGACACAACCAGGTCCACAAACCTGACCATCTGCGCCAGCACCCAAAGCCCCGTGCCCGGCCAG TACGACGCCACAAAGTTCAAACAATACTCTCGGCACGTGGAAGAGTACGACCTCCAATTCATCTTCCAAC CTGCACCATCACCTCACCGCCGACGTGATGAGCTACATCCACTCCATGAACTCCTCCATCCTGGAAGACTG GAATTTGCGCGTGCCACCACCCCTACCACCTCCCTCGTCGACACCTACAGATTCTGTCAGAGCGTGGCCAT CACATGCCAGAAAGACGCCGCCCCCGCGAGAACAAGACCCATACGACAACTGAAATTCTGGAACGTC GACCTGAAAGAGAAATTCAGCCTGGATCTGGACCAGTACCCATTGGGCAGGAAGTTCCTCAAAGCCAAGCC AAAAGTGAAGAGGGCTGCCCCAACCCAGCACCAAGACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAGTAA A
SEQ ID No:	1805 Synthetic HPV18 L1 gene

44	ctgggtaccATGGCCCTCTGGAGACCATCCGATAACACAGTGTACTTGCCCCACCCAGCGTCGCCCCGGGTGGT GAACACAGACGACTACGTCACCAGAACCTCAATCTTCTACCACGCCGGGTCCAGCCGGCTGCTGACCGTGG GCAACCCCTACTTCCGCGTGCCCCGCCGGCGGGAACAAACAAGACATCCCCAAAGTCAGCGCCTATCAG TACCGGGTGTTCGCGTCCAACCTGCCGATCCCAACAAGTTCGGCCTGCCGACACCTCCATCTACAACCCC GAGACCCAGAGGCTGGTCTGGGCTTGCGCCGGCGTCGAGATCGGGAGGGGCCAACCCCTGGGCGTGGGGT TGTCCGGCCACCCCTTCTACAACAAGCTGGACGATACCGAGTCCAGCCACGCAGCAACCAGCAACGTCTCC GAAGATGTGCGGATAACGTCAGCGTGGACTACAAACAAACCAACTGTGCATCCTGGGATGCGCACCCGC CATCGGCGAGCATTGGGCCAAGGGGACCGCCTGCAAGAGCAGGCCCTGAGCCAAGGGGACTGTCCACCC CTGGAGTTGAAGAATACCGTGCTCGAGGACGGCGACATGGTGGACACCGGCTACGGCGCTATGGATTCTC CACCTCCAGGACACCAAGTGCGAAGTGCCCCCTCGACATCTGCCAAAGCATCTGCAAGTACCCGACTACC TCCAGATGAGCGCCGACCCCTACGGCGACAGCATGTTCTTCTGTCTCAGAAGGGAAACAATTGTTGCCCCG CACTTCTGGAACCGGGCCGGCACAATGGGAGATACAGTCCCCCAGAGCCTGTACATCAAGGGGACCGGAA TGAGGGCCAGCCCCGGGTCTGCGTCTACAGCCCAAGCCCCCTCCGGGAGCATCGTCACAAGCGATAGCCAA CTCTTCAACAAGCCCTACTGGCTCCACAAAGCCCAAGGCCACAATAACGGGGTGTGTTGGCACAACCAAGT GTTCTGTACCGTCTGTGGACACAACCAAGGTCCACAAACCTGACCATCTGCGCCAGCACCCAAAGCCCCGTGC CCGGCCAGTACGACGCCACAAAGTTCAACAATACTCTCGGCACGTGGAAGAGTACGACCTCCAATTTCATC TTCCAACCTCTGCACCATCACCTCACCGCCGACGTGATGAGCTACATCCACTCCATGAATCCTCCATCCTG GAAGACTGGAATTCGGCGTGCCACCACCCCTACCACCTCCCTCGTCGACACCTACAGATTCGTGCAGAG CGTGGCCATCACATGCCAGAAAGACGCCGCCCCCGCCGAGAACAAGACCCATACGACAAACTGAAATTC TGGAACGTGACCTGAAAGAGAAATTCAGCCTGGATCTGGACCAGTACCCATTGGGCAGGAAGTTCCTCGT GCAAGCCGGCCTCAGGAGAAAACCAACAATCGGGCCCAGGAAGAGGAGCGCCCCAGCGCAACCACCAGC AGCAAGCCCGCAAAAAGGGTCAGAGTGAGGGCACGCAATAAActcgagctc
SEQ ID No: 45	1806 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 GTGAAGAAGTAAActgagctc
SEQ ID No: 46	1807 HPV18 L1 F1 CTTGGTACCATGGCCCTCTGGAGACCATCCGATA
SEQ ID No: 47	1808 HPV18 L1 R1 GCTTGGCTTTGAGGAACTTCCTGCCCAATGGGTAC
SEQ ID No:	1809 HPV18 L1 amplified sequence 1

48	CTTGGTACC^[1] <u>ATGGCCCTCTGGAGACCATCCGATAACACAGTGTACTTGCCCCACCCAGCGTCGCCCGGGTGGTGAACAC</u> <u>AGACGACTACGTCAACCAGAACCTCAATCTTCTACCACGCCGGGTCCAGCCGGCTGCTGACCGTGGGCAACC</u> <u>CCTACTTCCGCGTGCCCGCCGGCGGCGGAAACAAACAAGACATCCCCAAAGTCAGCGCCTATCAGTACCGG</u> <u>GTGTTCCGCGTCCAACCTGCCCCGATCCCAACAAGTTCGGCCTGCCCACACCTCCATCTACAACCCCGAGACC</u> <u>CAGAGGCTGGTCTGGGCTTGCGCCGGCGTCGAGATCGGGAGGGGGCAACCCCTGGGCGTGGGGTTGTCCGG</u> <u>CCACCCCTTCTACAACAAGCTGGACGATACCGAGTCCAGCCACGCAGCAACCAGCAACGTCTCCGAAGATG</u> <u>TGCGCGATAACGTCAGCGTGGACTACAAACAAACCCAACTGTGCATCCTGGGATGCGCACCCGCCATCGGC</u> <u>GAGCATTGGGCCAAGGGGACCGCCTGCAAGAGCAGGCCCTGAGCCAAGGGGACTGTCCACCCCTGGAGT</u> <u>TGAAGAATACCGTGCTCGAGGACGGCGACATGGTGGACACCGGTACGGCGCTATGGATTCTCCACCCCTC</u> <u>CAGGACACCAAGTGCGAAGTGCCCCCTCGACATCTGCCAAAGCATCTGCAAGTACCCCGACTACCTCCAGAT</u> <u>GAGCGCCGACCCCTACGGCGACAGCATGTTCTTCTGTCTCAGAAGGGAACAATTGTTGCCCCGCCACTTCTG</u> <u>GAACCGGGCCGGCACAATGGGAGATACAGTCCCCCAGAGCCTGTACATCAAGGGGACCGGAATGAGGGCC</u> <u>AGCCCCGGGTCTGCGTCTACAGCCCAAGCCCTCCGGGAGCATCGTCACAAGCGATAGCCAACCTTTCAA</u> <u>CAAGCCCTACTGGCTCCACAAAGCCCAAGGCCACAATAACGGGGTGTGTTGGCACAACCAGCTGTTTCGTGA</u> <u>CCGTCTGTGGACACAACCAGGTCCACAAACCTGACCATCTGCGCCAGCACCCAAAGCCCGTGCCCGGCCAG</u> <u>TACGACGCCACAAAGTTCAAACAATACTCTCGGCACGTGGAAGAGTACGACCTCCAATTCATCTTCCAAC</u> <u>CTGCACCATCACCCCTACCGCCGACGTGATGAGCTACATCCACTCCATGAACTCCTCCATCCTGGAAGACTG</u> <u>GAATTTGCGCGTGCCACCACCCCTACCACCTCCCTCGTCGACACCTACAGATTCTGTCAGAGCGTGGCCAT</u> <u>CACATGCCAGAAAGACGCCGCCCCCGCGAGAACAAGACCCATACGACAAACTGAAATTCTGGAACGTC</u> <u>GACCTGAAAGAGAAAATTCAGCCTGGATCTGGACCAGTACCCATTGGGCAGGAAGTTCCTC</u> ^[2] AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding HPV18 L1 aa 1-470; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
----	---

SEQ ID No: 49	1810 HPV18 L1 F2 GAAGTTCCTCAAAGCCAAGCCAAAAGCTGAAAAGGG
SEQ ID No: 50	1811 HPV18 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 51	1812 HPV18 L1 amplified sequence 2 <u>GAAGTTCCTC</u>^[1] <u>AAAGCCAAGCCAAAAGCTGAAAAGGGCTGCCCCAACCCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG</u>^[2] TAA ATCTAGA^[3]CAG₂ [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 LQLGARPKPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

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 MSLWRPSEATVYLPVPVSKVVSTDEYVTRTNIYYHAGSARLLTVGHPYYSIPK SDNPKKIVVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPETQRLVWACVGLE VGRGQPLGVGISGHPLLNKFDDTENSNNRYAGGPGTDNRECISMDYKQTQLCLL GCKPPIGEHWGKGSPCSNNAITPGDCPPELKNLSVIQDGDMDVDTGFGAMDFTA LQDTKSNVPLDICNSICKYPDYLKMVAEPTYGDTLFFYLRREQMFVRHFFNRSRG VGESVPTDLYIKGSGSTATLANSTYFPTPSGSMVTSDAQIFNKPYWMQRAQGH NNGICWGNQLFVTVVDTRSTNMSVCAAIANSDDTFKSSNFKEYLRHGEEFDL QFIFQLCKITLSADIMTYIHSMNPAILEDWNFGLTTPPSGSLEDYRFVTSQAITC 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 MSLWRPSEATVYLPVPVSKVVSTDEYVTRTNIYYHAGSARLLTVGHPYYSIPK SDNPKKIVVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPETQRLVWACVGLE VGRGQPLGVGISGHPLLNKFDDTENSNNRYAGGPGTDNRECISMDYKQTQLCLL GCKPPIGEHWGKGSPCSNNAITPGDCPPELKNLSVIQDGDMDVDTGFGAMDFTA LQDTKSNVPLDICNSICKYPDYLKMVAEPTYGDTLFFYLRREQMFVRHFFNRSRG VGESVPTDLYIKGSGSTATLANSTYFPTPSGSMVTSDAQIFNKPYWMQRAQGH NNGICWGNQLFVTVVDTRSTNMSVCAAIANSDDTFKSSNFKEYLRHGEEFDL QFIFQLCKITLSADIMTYIHSMNPAILEDWNFGLTTPPSGSLEDYRFVTSQAITC QKSAPQKPKEDPFKDYVFEVNLKEKFSADLDQFPLGRKFLLQAGYKAKPKLK RAAPTSTRTSSAKRKKVKK

SEQ ID No:	3104 Nucleotide sequence of chimeric HPV type 31 L1 protein
56	ATGAGCCTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC CGACGAGTACGTGACCAGGACCAACATCTACTACCACGCCGGCAGCGCCAGGCTGCTGACCGTGGGCCACC CCTACTACAGCATCCCCAAGAGCGACAACCCCAAGAAGATCGTGGTGCCCAAGGTGAGCGGCCTGCAGTAC AGGGTGTTTCAAGGTGAGGCTGCCCGACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACAACCCCGA GACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGGTGGGCAGGGGCCAGCCCCTGGGCGTGGGCATC AGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGAACAGCAACAGGTACGCCGGCGGCCCGGCA CCGACAACAGGGAGTGCATCAGCATGGACTACAAGCAGACCCAGCTGTGCCTGCTGGGCTGCAAGCCCCC ATCGGCGAGCACTGGGGCAAGGGCAGCCCCTGCAGCAACAACGCCATCACCCCGGCGACTGCCCCCCCCT GGAGCTGAAGAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCGCCATGGACTTCACC GCCCTGCAGGACACCAAGAGCAACGTGCCCTGGACATCTGCAACAGCATCTGCAAGTACCCCGACTACCT GAAGATGGTGGCCGAGCCCTACGGCGACACCTGTTCTTCTACCTGAGGAGGGAGCAGATGTTCTGTAGGC ACTTCTTCAACAGGAGCGGCACCGTGGGCGAGAGCGTGCCACCGACCTGTACATCAAGGGCAGCGGCAG CACCGCCACCCCTGGCCAACAGCACCTACTTCCCCACCCCGAGCGGCAGCATGGTGACCAGCGACGCCAGA TCTTCAACAAGCCCTACTGGATGCAGAGGGGCCAGGGCCACAACAACGGCATCTGCTGGGGCAACCAGCTG TTCGTGACCGTGGTGGACACCACAGGAGCACCAACATGAGCGTGTGCGCCGCCATCGCCAACAGCGACAC CACCTTCAAGAGCAGCAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCAGTTCATCTTCC AGCTGTGCAAGATCACCTGAGCGCCGACATCATGACCTACATCCACAGCATGAACCCCGCCATCCTGGAG GACTGGAACCTTCGGCCTGACCACCCCCCCCAGCGGCAGCCTGGAGGACACCTACAGGTTCTGTGACCAGCCA GGCCATCACCTGCCAGAAGTCCGCCCCCAGAAGCCCAAGGAGGACCCCTTCAAGGACTACGTGTTCTGGG AGGTGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCCTGCTGCAG GCCGGCTACAAAGCCAAGCCAAAAGTGAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGA GGAAGAAGGTGAAGAAGTAAA
SEQ ID No:	3105 Synthetic HPV31 L1 gene

57	ctgggtaccATGAGCCTGTGGAGGCCCAGCGAGGCCACCGTGACCTGCCCCCGTGCCCGTGAGCAAGGTGGT GAGCACCGACGAGTACGTGACCAGGACCAACATCTACTACCACGCCGGCAGCGCCAGGCTGCTGACCGTG GGCCACCCCTACTACAGCATCCCCAAGAGCGACAACCCCAAGAAGATCGTGGTGCCCAAGGTGAGCGGCCT GCAGTACAGGGTGTTCAGGGTGAGGCTGCCCCACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACA ACCCCGAGACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGGTGGGCAGGGGCCAGCCCCTGGGCGT GGGCATCAGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGAACAGCAACAGGTACGCCGGCGGC CCCGGCACCGACAACAGGGAGTGCATCAGCATGGACTACAAGCAGACCCAGCTGTGCCTGCTGGGCTGCA AGCCCCCATCGGCGAGCACTGGGGCAAGGGCAGCCCCTGCAGCAACAACGCCATCACCCCCGGCGACTG CCCCCCCCTGGAGCTGAAGAACAGCGTGATCCAGGACGGCGACATGGTGACACCGGCTTCGGCGCCATGG ACTTCACCGCCCTGCAGGACACCAAGAGCAACGTGCCCTGGACATCTGCAACAGCATCTGCAAGTACCCC GACTACCTGAAGATGGTGGCCGAGCCCTACGGCGACACCCTGTTCTTCTACCTGAGGAGGGAGCAGATGTT CGTGAGGGCACTTCTTCAACAGGAGCGGCACCGTGGGCGAGAGCGTGCCACCGACCTGTACATCAAGGGCA GCGGCAGCACCGCCACCCTGGCCAACAGCACCTACTTCCCCACCCCCAGCGGCAGCATGGTGACCAGCGAC GCCAGATCTTCAACAAGCCCTACTGGATGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTGGGGCA ACCAGCTGTTCTGTGACCGTGGTGACACCACCAGGAGCACCAACATGAGCGTGTGCGCCGCCATCGCCAAC AGCGACACCACCTTCAAGAGCAGCAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCAGTT CATCTTCCAGCTGTGCAAGATCACCTGAGCGCCGACATCATGACCTACATCCACAGCATGAACCCCGCCA TCCTGGAGGACTGGAACCTTCGGCCTGACCACCCCCCCCAGCGGCAGCCTGGAGGACACCTACAGGTTCTGTG ACCAGCCAGGCCATCACCTGCCAGAAGTCCGCCCCCAGAAGCCCAAGGAGGACCCCTTCAAGGACTACGT GTTCTGGGAGGTGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCC TGCTGCAGGCCGGCTACAGGGCCAGGCCAAAGTTCAAGGCCGGAAGAGGAGCGCCCCCAGCGCCAGCAC CACCACCCCCGCCAAGAGGAAGAAGACCAAGAAGTAAActcgagctc
SEQ ID No:	3106 Synthetic HPV33 L1 gene
58	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG

	AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACC AATATGACCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 59	3107 HPV31 L1 F1 CTTGGTACCATGAGCCTGTGGAGGCCAGCGAG
SEQ ID No: 60	3108 HPV31 L1 R1 GCTTGGCTTTGTAGCCGGCCTGCAGCAGGAACTTCCTG

SEQ ID No:	3109 HPV31 L1 amplified sequence 1
61	CTTGGTACC^[1] <u>ATGAGCCTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC</u> <u>CGACGAGTACGTGACCAGGACCAACATCTACTACCACGCCGGCAGCGCCAGGCTGCTGACCGTGGGCCACC</u> <u>CCTACTACAGCATCCCCAAGAGCGACAACCCCAAGAAGATCGTGGTGCCCAAGGTGAGCGGCCTGCAGTAC</u> <u>AGGGTGTTTCAGGGTGAGGCTGCCCGACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACAACCCCGA</u> <u>GACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGGTGGGCAGGGGCCAGCCCCTGGGCGTGGGCATC</u> <u>AGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGAACAGCAACAGGTACGCCGGCGGCCCGGCA</u> <u>CCGACAACAGGGAGTGCATCAGCATGGACTACAAGCAGACCCAGCTGTGCCTGCTGGGCTGCAAGCCCCC</u> <u>ATCGGCGAGCACTGGGGCAAGGGCAGCCCCTGCAGCAACAACGCCATCACCCCGGCGACTGCCCCCCCCT</u> <u>GGAGCTGAAGAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCGCCATGGACTTCACC</u> <u>GCCCTGCAGGACACCAAGAGCAACGTGCCCTGGACATCTGCAACAGCATCTGCAAGTACCCCGACTACCT</u> <u>GAAGATGGTGGCCGAGCCCTACGGCGACACCCTGTTCTTCTACCTGAGGAGGGAGCAGATGTTTCGTGAGGC</u> <u>ACTTCTTCAACAGGAGCGGCACCGTGGGCGAGAGCGTGCCACCGACCTGTACATCAAGGGCAGCGGCAG</u> <u>CACCGCCACCCTGGCCAACAGCACCTACTTCCCCACCCCGAGCGGCAGCATGGTGACCAGCGACGCCAGA</u> <u>TCTTCAACAAGCCCTACTGGATGCAGAGGGGCCAGGGCCACAACAACGGCATCTGCTGGGGCAACCAGCTG</u> <u>TTCGTGACCGTGGTGGACACCACAGGAGCACCAACATGAGCGTGTGCGCCGCCATCGCCAACAGCGACAC</u> <u>CACCTTCAAGAGCAGCAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCAGTTCATCTTCC</u> <u>AGCTGTGCAAGATCACCTGAGCGCCGACATCATGACCTACATCCACAGCATGAACCCCGCCATCCTGGAG</u> <u>GACTGGAACCTTCGGCCTGACCACCCCCCAGCGGCAGCCTGGAGGACACCTACAGGTTTCGTGACCAGCCA</u> <u>GGCCATCACCTGCCAGAAGTCCGCCCCCAGAAGCCCAAGGAGGACCCCTTCAAGGACTACGTGTTCTGGG</u> <u>AGGTGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCTGGGCAGGAAGTTCCTGCTGCAG</u> <u>GCCGGCTAC</u> ^[2] AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding HPV31 L1 aa 1-475;

	[3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 62	3110 HPV31 L1 F2 GGCCGGCTACAAAGCCAAGCCAAAAGTGAAGGG
SEQ ID No: 63	3111 HPV31 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTCCTCTTGGCAGAG
SEQ ID No: 64	3112 HPV31 L1 amplified sequence 2 <u>GGCCGGCTAC</u> ^[1] <u>AAAGCCAAGCCAAAAGTGAAGGGCTGCCCCAACCAGCACCAGG</u> <u>ACCTCCTCTGCCAAGAGGAAGAAGGTGAAGAAG</u> ^[2] TAA <u>ATCTAGA</u> ^[3] CAG. [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 -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 35 L1 PROTEIN

SEQ ID No: 66	3501 The amino acid sequence of HPV type 35 L1 protein aa 1-472 MSLWRSNEATVYLPPVSVSKVVSTDEYVTRTNIYYHAGSSRLLAVGHPYYAIK KQDSNKIAVPKVSGLQYRVFRVKLPDPNKFGFPDTSFYDPASQRLVWACTGVE VGRGQPLGVGISGHPLLNKLDDTENS NKYVGNSGTDNRECISMDYKQTQLCLI GCRPPIGEHWGKGTPCNANQVKAGECPPELLNTVLQDGMVDTGFGAMDFT TLQANKSDVPLDICSSICKYPDYLKMVSEPYGDMLFFYLRRREQMFVRHLFNRA GTVGETVPADLYIKGTTGTL PSTSYFPTPSGSMVTSDAQIFNKPYWLQRAQGHN NGICWSNQLFVTTVVDTTRSTNMSVCSAVSSSDSTYKNDNFKEYLRHGEEYDLQ FIFQLCKITLTADVMTYIHSMNPSILEDWNFGLTPPPSGTLED TYRYVTSQAVTC QKPSAPKPKDDPLKNYTFWEVDLKEKFSADLDQFPLGRKFLLQAGL
SEQ ID No: 67	3502 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTR TSSAKRKKVKK
SEQ ID No: 68	3503 Amino acid sequence of chimeric HPV type 35 L1 protein MSLWRSNEATVYLPPVSVSKVVSTDEYVTRTNIYYHAGSSRLLAVGHPYYAIK KQDSNKIAVPKVSGLQYRVFRVKLPDPNKFGFPDTSFYDPASQRLVWACTGVE VGRGQPLGVGISGHPLLNKLDDTENS NKYVGNSGTDNRECISMDYKQTQLCLI GCRPPIGEHWGKGTPCNANQVKAGECPPELLNTVLQDGMVDTGFGAMDFT TLQANKSDVPLDICSSICKYPDYLKMVSEPYGDMLFFYLRRREQMFVRHLFNRA GTVGETVPADLYIKGTTGTL PSTSYFPTPSGSMVTSDAQIFNKPYWLQRAQGHN NGICWSNQLFVTTVVDTTRSTNMSVCSAVSSSDSTYKNDNFKEYLRHGEEYDLQ FIFQLCKITLTADVMTYIHSMNPSILEDWNFGLTPPPSGTLED TYRYVTSQAVTC QKPSAPKPKDDPLKNYTFWEVDLKEKFSADLDQFPLGRKFLLQAGLKAKPKLK RAAPTSTR TSSAKRKKVKK

SEQ ID No:	3504 Nucleotide sequence of chimeric HPV type 35 L1 protein
69	ATGAGTCTGTGGAGGAGCAATGAGGCTACAGTCTACCTGCCTCCTGTGTCTGTGAGCAAGGTGGTGAGCAC AGATGAATATGTGACCAGGACCAACATCTACTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGGGACACC CATACTATGCCATCAAGAAGCAGGACAGCAACAAGATTGCTGTGCCAAAGGTGTCTGGACTCCAATACAGG GTGTTTCAGGGTGAAACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTATGACCCTGCCAGC CAGAGACTGGTGTGGGCTTGTACTGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG GACACCCACTGCTGAACAACTGGATGACACAGAGAACAGCAACAAATATGTGGGCAACTCTGGCACAGA CAACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACTTTGTCTGATTGGCTGTAGACCTCCAATTG GAGAACTGGGGCAAGGGCACACCATGTAATGCCAACCAGGTGAAGGCTGGAGAGTGTCTCCATTGGA ACTGCTGAACACAGTGCTCCAAGATGGAGATATGGTGGACACAGGCTTGGAGCTATGGACTTCACCACCC TCCAAGCCAACAAGTCTGATGTGCCACTGGACATCTGTTCCAGCATCTGTAAATACCCTGACTACCTGAAA ATGGTGTCTGAACCATATGGAGATATGCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACACCT GTTCAACAGGGCTGGCACAGTGGGAGAGACAGTGCCTGCTGACCTCTACATCAAGGGCACCACAGGCACCC TGCCAAGCACCTCCTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAACAAGC CATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGAGCAACCAACTTTTTGTGACAGTG GTGGACACCACCAGGAGCACCAATATGAGTGTGTGTTCTGCTGTGTCCTCCTGACAGCACCTACAAGAA TGACAACTTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGTAAGA TTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACCCAAGCATCTTGGAGGACTGGAACCTT GGACTGACACCTCCTCCATCTGGCACCTTGGAGGACACCTACAGATATGTGACCAGCCAGGCTGTGACTTG TCAGAAGCCATCTGCCCCAAAGCCAAAGGATGACCCACTGAAAACTACACCTTCTGGGAGGTGGACCTGA AAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACTGAAA GCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAGGTGA AGAAGTAAA

SEQ ID No:	3505 Synthetic HPV35 L1 gene
70	ctgggtaccATGAGTCTGTGGAGGAGCAATGAGGCTACAGTCTACCTGCCTCCTGTGTCTGTGAGCAAGGTGGT GAGCACAGATGAATATGTGACCAGGACCAACATCTACTACCATGTGGCTCCAGCAGACTGCTGGCTGTGG GACACCCATACTATGCCATCAAGAAGCAGGACAGCAACAAGATTGCTGTGCCAAAGGTGTCTGGACTCCAA TACAGGGTGTTCAGGGTGAAACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTATGACCCT GCCAGCCAGAGACTGGTGTGGGCTGTACTGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCA TCTCTGGACACCCACTGCTGAACAAACTGGATGACACAGAGAACAGCAACAAATATGTGGGCAACTCTGGC ACAGACAACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACTTTGTCTGATTGGCTGTAGACCTCC AATTGGAGAACACTGGGGCAAGGGCACACCATGTAATGCCAACCAGGTGAAGGCTGGAGAGTGTCTCCA TTGGAAGTGTGAACACAGTGTCTCAAGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGGACTTCAC CACCTCCAAGCCAACAAGTCTGATGTGCCACTGGACATCTGTTCCAGCATCTGTAAATACCCTGACTACCT GAAAAATGGTGTCTGAACCATATGGAGATATGCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGAC ACCTGTTCAACAGGGCTGGCACAGTGGGAGAGACAGTGCCTGCTGACCTCTACATCAAGGGCACCACAGGC ACCCTGCCAAGCACCTCCTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAAC AAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGAGCAACCAACTTTTTGTGAC AGTGGTGGACACCACCAGGAGCACCAATATGAGTGTGTGTTCTGCTGTGTCCTCTGACAGCACCTACA AGAATGACAACCTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCCAACCTTTGT AAGATTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACCCAAGCATCTTGGAGGACTGGAA CTTTGGACTGACACCTCCTCCATCTGGCACCTTGGAGGACACCTACAGATATGTGACCAGCCAGGCTGTGA CTTGTCAGAAGCCATCTGCCCCAAAGCCAAAGGATGACCCACTGAAAACTACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAGACCAAACTTCAGACTGGGCAAGAGGGCTGCCCTGCCAGCACCAGCAAGAAGTCCAGCACC AAGAGGAGGAAGGTGAAGAGCTAAActcgagctc
SEQ ID No:	3506 Synthetic HPV33 L1 gene

71	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 72	3507 HPV35 L1 F1 CTTGGTACCATGAGTCTGTGGAGGAGCAATGAGG
SEQ ID No:	3508 HPV35 L1 R1

73	GCTTGGCTTTCAGTCCTGCTTGGAGCAGGAACTTCC
SEQ ID No:	3509 HPV35 L1 amplified sequence 1
74	CTTGGTACC^[1] <u>ATGAGTCTGTGGAGGAGCAATGAGGCTACAGTCTACCTGCCTCCTGTGTCTGTGAGCAAGGTGGTGAGCAC</u> <u>AGATGAATATGTGACCAGGACCAACATCTACTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGGGACACC</u> <u>CATACTATGCCATCAAGAAGCAGGACAGCAACAAGATTGCTGTGCCAAAGGTGTCTGGACTCCAATACAGG</u> <u>GTGTTCAGGGTGAAACTGCCTGACCCAAACAAGTTTGGCTTTCCTGACACCTCCTTCTATGACCCTGCCAGC</u> <u>CAGAGACTGGTGTGGGCTTGACTGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG</u> <u>GACACCCACTGCTGAACAACTGGATGACACAGAGAACAGCAACAAATATGTGGGCAACTCTGGCACAGA</u> <u>CAACAGGGAGTGTATCAGTATGGACTACAAGCAGACCCAACCTTTGTCTGATTGGCTGTAGACCTCCAATTG</u> <u>GAGAACTGGGGCAAGGGCACACCATGTAATGCCAACCAGGTGAAGGCTGGAGAGTGTCTCCATTGGA</u> <u>ACTGCTGAACACAGTGCTCCAAGATGGAGATATGGTGGACACAGGCTTTGGAGCTATGGACTTCACCACCC</u> <u>TCCAAGCCAACAAGTCTGATGTGCCACTGGACATCTGTTCCAGCATCTGTAAATACCCTGACTACCTGAAA</u> <u>ATGGTGTCTGAACCATATGGAGATATGCTGTTCTTCTACCTGAGGAGGGAACAGATGTTTGTGAGACACCT</u> <u>GTCAACAGGGCTGGCACAGTGGGAGAGACAGTGCCTGCTGACCTCTACATCAAGGGCACACAGGCACCC</u> <u>TGCCAAGCACCTCCTACTTTCCAACACCATCTGGCAGTATGGTGACCTCTGATGCCCAGATTTTCAACAAGC</u> <u>CATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGAGCAACCAACTTTTTGTGACAGTG</u> <u>GTGGACACCACCAGGAGCACCAATATGAGTGTGTGTTCTGCTGTGTCCTCCTCTGACAGCACCTACAAGAA</u> <u>TGACAACTTCAAGGAATACCTGAGACATGGAGAGGAATATGACCTCCAATTCATCTTCAACTTTGTAAGA</u> <u>TTACCCTGACAGCAGATGTGATGACCTACATCCACAGTATGAACCCAAGCATCTTGGAGGACTGGAACCTT</u> <u>GGACTGACACCTCCTCCATCTGGCACCTTGGAGGACACCTACAGATATGTGACCAGCCAGGCTGTGACTTG</u> <u>TCAGAAGCCATCTGCCCCAAAGCCAAAGGATGACCCACTGAAAACTACACCTTCTGGGAGGTGGACCTGA</u> <u>AAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACTG</u> ^[2] AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site;

	[2]: the gene fragment encoding HPV35 L1 aa 1-472; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 75	3510 HPV35 L1 F2 AGCAGGACTGAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 76	3511 HPV35 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 77	3512 HPV35 L1 amplified sequence 2 <u>AGCAGGACTG</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 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: 78	3513 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKR VKRRKSSRK

Appendix 7 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 39 L1 PROTEIN

SEQ ID No: 79	3901 The amino acid sequence of HPV type 39 L1 protein aa 1-469 MAMWRSSDSMVYLPPPSVAKVVNTDDYVTRTGIYYYYAGSSRLLTVGHPYFKV GMNGGRKQDIPKVSAYQYRVFRVTLDPDNKFSIPDASLYNPETQRLVWACVGV EVGRGQPLGVGISGHPLYNRQDDTENSFPSSSTTNKDSRDNVSDYKQTQLCIIG CVPAIGEHWGKGKACKPNNVSTGDCPPELVNTPIEDGDMIDTGYGAMDFGAL QETKSEVPLDICQSICKYPDYLQMSADVYGDSMFFCLRREQLFARHFWNRGGM VGDAIPAQLYIKGTDIRANPGSSVYCPSPSGSMVTSDSQLFNKPYWLHKAQGHN NGICWHNQLFLTVDTTTRSTNFTLSTSISSIPSTYDPSKFKEYTRHVEEYDLQFI FQLCTVTLTDDVMSYIHTMNSSILDNWNFAVAPPPSASLVDYRYLQSAAITCQ KDAPAPEKKDPYDGLKFWNVDLREKFSLELDQFPLGRKFL
SEQ ID No: 80	3902 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARPKEPTIGPRKRAAPAPTSTPSPKRVRKRKSSRK
SEQ ID No: 81	3903 Amino acid sequence of chimeric HPV type 39 L1 protein MAMWRSSDSMVYLPPPSVAKVVNTDDYVTRTGIYYYYAGSSRLLTVGHPYFKV GMNGGRKQDIPKVSAYQYRVFRVTLDPDNKFSIPDASLYNPETQRLVWACVGV EVGRGQPLGVGISGHPLYNRQDDTENSFPSSSTTNKDSRDNVSDYKQTQLCIIG CVPAIGEHWGKGKACKPNNVSTGDCPPELVNTPIEDGDMIDTGYGAMDFGAL QETKSEVPLDICQSICKYPDYLQMSADVYGDSMFFCLRREQLFARHFWNRGGM VGDAIPAQLYIKGTDIRANPGSSVYCPSPSGSMVTSDSQLFNKPYWLHKAQGHN NGICWHNQLFLTVDTTTRSTNFTLSTSISSIPSTYDPSKFKEYTRHVEEYDLQFI FQLCTVTLTDDVMSYIHTMNSSILDNWNFAVAPPPSASLVDYRYLQSAAITCQ KDAPAPEKKDPYDGLKFWNVDLREKFSLELDQFPLGRKFL LQLGARPKEPTIGPRKRAAPAPTSTPSPKRVRKRKSSRK

SEQ ID No:	3904 Nucleotide sequence of chimeric HPV type 39 L1 protein
82	ATGGCTATGTGGAGGTCCTCTGACAGTATGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTGAACACA GATGACTATGTGACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGACAGTGGGACACCC ATACTTCAAGGTGGGGATGAATGGAGGCAGGAAGCAGGACATCCCAAAGGTGTCTGCCTACCAATACAGG GTGTTCAAGGTGACCCTGCCTGACCCAAACAAGTTCAGCATCCCTGATGCCTCCCTCTACAACCTGAGACC CAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG GACACCCACTCTACAACAGACAGGATGACACAGAGAACAGCCATTCTCCAGCACCACCAACAAGGACAG CAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCATTGGCTGTGTGCCTGCCATTGGAG AACACTGGGGCAAGGGCAAGGCTTGTAAGCCAAACAATGTGAGCACAGGAGACTGTCCTCCATTGGAAT GGTGAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTATGGAGCTATGGACTTTGGAGCCCTCC AAGAGACCAAGTCTGAGGTGCCACTGGACATCTGTCTGAGAGCATCTGTAAATACCCTGACTACCTCCAAATG AGTGCTGATGTCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACATTTTGGCCAGACACTTCTGG AACAGGGGAGGGATGGTGGGAGATGCCATCCCTGCCCAACTCTACATCAAGGGCACAGACATCAGGGCTA ACCCTGGCTCCTCTGTCTACTGTCCAAGCCCATCTGGCAGTATGGTGACCTCTGACAGCCAACCTTTTCAACA AGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACCTTTTCTGACA GTGGTGGACACCACCAGGAGCACCAACTTCACCCTGAGCACCAGCATTGAGTCCAGCATCCCAAGCACCTA TGACCCAAGCAAGTTCAAGGAATACACCAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCAACCTT GTACTGTGACCTGACCACAGATGTGATGAGTTACATCCACACAATGAACTCCAGCATCCTGGACAACCTGG AACTTTGCTGTGGCTCCTCCTCCATCTGCCTCCCTGGTGGACACCTACAGATACCTCCAATCTGCTGCCATC ACTTGTGAGAAGGATGCCCCTGCCCCTGAGAAGAAGGACCCATATGATGGACTGAAGTTCTGGAATGTGGA CCTGAGGGAGAAGTTCTCCTTGGAACTGGACCAGTTTCCACTGGGCAGGAAGTCTGCTCCAACCTTGGAG CCAGACCAAAGCCAACCATTTGGACCAAGGAAGAGGGCTGCCCCTGCCCCAACCAAGGACACCAAGCCCAA GAGGGTGAAGAGGAGGAAGTCCAGCAGGAAGTAAA

SEQ ID No:	3905 Synthetic HPV39 L1 gene
83	ctgggtaccATGGCTATGTGGAGGTCCTCTGACAGTATGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTG AACACAGATGACTATGTGACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGACAGTGGG ACACCCATACTTCAAGGTGGGGATGAATGGAGGCAGGAAGCAGGACATCCCAAAGGTGTCTGCCTACCAA TACAGGGTGTTCAGGGTGACCCTGCCTGACCCAAACAAGTTCAGCATCCCTGATGCCTCCCTCTACAACCCT GAGACCCAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCA TCTCTGGACACCCACTCTACAACAGACAGGATGACACAGAGAACAGCCCATTCTCCAGCACCACCAACAAG GACAGCAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCATTGGCTGTGTGCCTGCCAT TGGAGAACACTGGGGCAAGGGCAAGGCTTGTAAGCCAAACAATGTGAGCACAGGAGACTGTCCTCCATTG GAACTGGTGAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTATGGAGCTATGGACTTTGGAGC CCTCCAAGAGACCAAGTCTGAGGTGCCACTGGACATCTGTGAGCATCTGTAAATACCCTGACTACCTCC AAATGAGTGCTGATGTCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGGCAGACACT TCTGGAACAGGGGAGGGATGGTGGGAGATGCCATCCCTGCCCAACTCTACATCAAGGGCACAGACATCAG GGCTAACCTGGCTCCTCTGTCTACTGTCCAAGCCCATCTGGCAGTATGGTGACCTCTGACAGCCAACTTTT CAACAAGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACTTTTCC TGACAGTGGTGGACACCACCAGGAGCACCAACTTCACCCTGAGCACCAGCATTGAGTCCAGCATCCCAAGC ACCTATGACCCAAGCAAGTTCAAGGAATACACCAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCA ACTTTGTACTGTGACCCTGACCACAGATGTGATGAGTTACATCCACACAATGAACTCCAGCATCCTGGACA ACTGGAACCTTGCTGTGGCTCCTCCTCCATCTGCCTCCCTGGTGGACACCTACAGATACCTCCAATCTGCTG CCATCACTTGTGAGAAGGATGCCCTGCCCTGAGAAGAAGGACCCATATGATGGACTGAAGTTCTGGAAT GTGGACCTGAGGGAGAAGTTCTCCTTGGAACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGC CAGGGTGAGGAGGAGACCAACCATTGGACCAAGGAAGAGACCTGCTGCCAGCACCTCCTCCTCCTGCCA CCAAACACAAGAGGAAGAGGGTGAGCAAGTAAActcgagctc
SEQ ID No:	3906 Synthetic HPV59 L1 gene

84	ctgggtaccATGGCTCTGTGGAGGTCCTCTGACAACAAGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTG AGCACAGATGAATATGTGACCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGG ACACCCATACTTCAAGGTGCCAAAGGGAGGCAATGGCAGACAGGATGTGCCAAAGGTGTCTGCCTACCAAT ACAGGGTGTTTCAGGGTGAAACTGCCTGACCCAAACAAGTTTGGACTGCCTGACAACACAGTCTATGACCCA AACAGCCAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGATTGGCAGGGGACAACCACTGGGAGTGGGAC TGTCTGGACACCCACTCTACAACAACTGGATGACACAGAGAAGTCTCATGTGGCATCTGCTGTGGACACC AAGGACACCAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCATTGGCTGTGTGCCTGC CATTGGAGAACACTGGACCAAGGGCACAGCCTGTAAGCCAACCACAGTGGTCCAGGGAGACTGTCCTCCAT TGGAAGTATAAACACACCAATTGAGGATGGAGATATGGTGGACACAGGCTATGGAGCTATGGACTTCAA ACTGCTCCAAGACAACAAGTCTGAGGTGCCACTGGACATCTGTCAGAGCATCTGTAAATACCCTGACTACC TCCAAATGAGTGCTGATGCCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAGGTGTTTGCCAGA CACTTCTGGAACAGGTCTGGCACAATGGGAGACCAACTTCCTGAGTCCCTCTACATCAAGGGCACAGACAT CAGGGCTAACCTGGCTCCTACCTCTACAGCCCAAGCCCATCTGGCTCTGTGGTGACCTCTGACAGCCAACT TTTCAACAAGCCATACTGGCTGCACAAGGCTCAAGGACTGAACAATGGCATCTGTTGGCACAACCAACTTT TCCTGACAGTGGTGGACACCACCAGGAGCACCAACCTGTCTGTGTGTGCCAGCACCACTCCAGCATCCCA AATGTCTACACACCAACCTCCTTCAAGGAATATGCCAGGCATGTGGAGGAGTTTGACCTCCAATTCATCTTC CAACTTTGTAAGATTACCCTGACCACAGAGGTGATGAGTTACATCCACAATATGAACACCACCATCTTGGA GGACTGGAACCTTTGGAGTGACACCTCCTCCAACAGCCTCCCTGGTGGACACCTACAGGTTTGTCCAGTCTGC TGCTGTGACTTGTGAGAAGGACACAGCCCCTCCTGTGAAGCAGGACCCATATGACAAACTGAAGTTCTGGC CTGTGGACCTGAAAGAGAGGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAA CTTGAGCCAGACCAAAGCCAACCATTTGGACCAAGGAAGAGGGCTGCCCTGCCCAACCAGCACACCAA GCCCAAAGAGGGTGAAGAGGAGGAAGTCCAGCAGGAAGTAAActcgagctc
SEQ ID No: 85	3907 HPV39 L1 F1 CTTGGTACCATGGCTATGTGGAGGTCCTCTGACAGTAT

SEQ ID No: 86	3908 HPV39 L1 R1 TCCAAGTTGGAGCAGGAACTTCCTGCCCAGTGGAAGT
SEQ ID No: 87	3909 HPV39 L1 amplified sequence 1 CTTGGTACC ^[1] ATGGCTATGTGGAGGTCTCTGACAGTATGGTCTACCTGCCTCCTCCATCTGTGGCTAAGGTGGTGAACACA GATGACTATGTGACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGACAGTGGGACACCC ATACTTCAAGGTGGGGATGAATGGAGGCAGGAAGCAGGACATCCCAAAGGTGTCTGCCTACCAATACAGG GTGTTCAAGGTGACCTGCCTGACCCAAACAAGTTCAGCATCCCTGATGCCTCCCTCTACAACCTGAGACC CAGAGACTGGTGTGGGCTTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGCATCTCTG GACACCCACTCTACAACAGACAGGATGACACAGAGAACAGCCCATTCTCCAGCACCACCAACAAGGACAG CAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCATTGGCTGTGTGCCTGCCATTGGAG AACTACTGGGGCAAGGGCAAGGCTTGTAAAGCCAAACAATGTGAGCACAGGAGACTGTCCTCCATTGGAAT GGTGAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTATGGAGCTATGGACTTTGGAGCCCTCC AAGAGACCAAGTCTGAGGTGCCACTGGACATCTGTCAGAGCATCTGTAAATACCCTGACTACCTCCAAATG AGTGCTGATGTCTATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGGCAGACACTTCTGG AACAGGGGAGGGATGGTGGGAGATGCCATCCCTGCCCAACTCTACATCAAGGGCACAGACATCAGGGCTA ACCCTGGCTCCTCTGTCTACTGTCCAAGCCCATCTGGCAGTATGGTGACCTCTGACAGCCAACCTTTTCAACA AGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACCTTTTCTGACA GTGGTGGACACCACCAGGAGCACCAACTTCACCCTGAGCACCAGCATTGAGTCCAGCATCCCAAGCACCTA TGACCCAAGCAAGTTCAAGGAATACACCAGGCATGTGGAGGAATATGACCTCCAATTCATCTTCCAACCTT GTACTGTGACCCTGACCACAGATGTGATGAGTTACATCCACACAATGAACTCCAGCATCCTGGACAACCTGG AACTTTGCTGTGGCTCCTCCTCCATCTGCCTCCCTGGTGGACACCTACAGATACCTCCAATCTGCTGCCATC ACTTGTGAGAAGGATGCCCCTGCCCCTGAGAAGAAGGACCCATATGATGGACTGAAGTTCTGGAATGTGGA CCTGAGGGAGAAGTTCCTTGGAACTGGACCAGTTTCCACTGGGCAGGAAGTTCCTG ^[2] CTCCAACCTGGA ^[3]

	[1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding HPV39 L1 aa 1-469; [3]: 12 bases overlapping with the gene fragment encoding HPV59L1 aa 471-508
SEQ ID No: 88	3910 HPV39 L1 F2 AGGAAGTTCCTGCTCCAACCTGGAGCCAGACCAAAGC
SEQ ID No: 89	3911 HPV39 L1 R2 CTGTCTAGATTTACTTCCTGCTGGACTTCCTCCTCTTCAC
SEQ ID No: 90	3912 HPV39 L1 amplified sequence 2 <u>AGGAAGTTCCTG</u>^[1] <u>CTCCAACCTGGAGCCAGACCAAAGCCAACCATTGGACCAAGGAAGAGGGCTGCCCCTGCCCCAACCAGCA</u> <u>CACCAAGCCCAAAGAGGGTGAAGAGGAGGAAGTCCAGCAGGAAG</u>^[2] TAA <u>ATCTAGA</u>^[3]CAG. [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: 91	3913 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK

Appendix 8 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 45 L1 PROTEIN

SEQ ID No: 92	4501 The amino acid sequence of HPV type 45 L1 protein aa 1-478 MALWRPSDSTVYLPPPSVARVVNTDDYVSRTSIFYHAGSSRLLTVGNPYFRVVP SGAGNKQAVPKVSAYQYRVFRVALPDPNKFGLPDSTIYNPETQRLVWACVGM EIGRGQPLGIGLSGHPFYNKLDDTESAHAATAVITQDVRDNVSDYKQTQLCIL GCVPAIGEHWAKGTLCKPAQLQPGDCPPELEKNTIIEDGDMVDTGYGAMDFST LQDTKCEVPLDICQSICKYPDYLQMSADPYGDSMFFCLRREQLFARHFWNRAG VMGDTVPTDLYIKGTSANMRETPGSCVYSPSPSGSITTSDSQLFNKPYWLHKAQ GHNNGICWHNQLFVTVDTTTRSTNLTLCASTQNPVPNTYDPTKFKHYSRHVEE YDLQFIFQLCTITLTAEVMSYIHSMNSSILENWNFGVPPPPTTSLVDTYRFVQSV AVTCQKDTPPEKQDPYDKLKFWTVDLKEKFSSDLQYPLGRKFLVQAGL
SEQ ID No: 93	4502 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 94	4503 Amino acid sequence of chimeric HPV type 45 L1 protein MALWRPSDSTVYLPPPSVARVVNTDDYVSRTSIFYHAGSSRLLTVGNPYFRVVP SGAGNKQAVPKVSAYQYRVFRVALPDPNKFGLPDSTIYNPETQRLVWACVGM EIGRGQPLGIGLSGHPFYNKLDDTESAHAATAVITQDVRDNVSDYKQTQLCIL GCVPAIGEHWAKGTLCKPAQLQPGDCPPELEKNTIIEDGDMVDTGYGAMDFST LQDTKCEVPLDICQSICKYPDYLQMSADPYGDSMFFCLRREQLFARHFWNRAG VMGDTVPTDLYIKGTSANMRETPGSCVYSPSPSGSITTSDSQLFNKPYWLHKAQ GHNNGICWHNQLFVTVDTTTRSTNLTLCASTQNPVPNTYDPTKFKHYSRHVEE YDLQFIFQLCTITLTAEVMSYIHSMNSSILENWNFGVPPPPTTSLVDTYRFVQSV AVTCQKDTPPEKQDPYDKLKFWTVDLKEKFSSDLQYPLGRKFLVQAGLKA KPKLKRAAPTSTRTSSAKRKKVKK

SEQ ID No:	4504 Nucleotide sequence of chimeric HPV type 45 L1 protein
95	ATGGCTCTGTGGAGACCATCTGACAGCACAGTCTACCTGCCTCCTCCATCTGTGGCAAGGGTGGTGAACAC AGATGACTATGTGAGCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGGCAACC CATACTTCAGGGTGGTGCCAAGTGAGCAGGCAACAAGCAGGCTGTGCCAAAGGTGTCTGCCTACCAATAC AGGGTGTTTCAGGGTGGCTCTGCCTGACCCAAACAAGTTTGGACTGCCTGACAGCACCATCTACAACCTGA GACCCAGAGACTGGTGTGGGCTTGTGTGGGGATGGAGATTGGCAGGGGACAACCACTGGGCATTGGACTGT CTGGACACCCATTCTACAACAACTGGATGACACAGAGTCTGCCCATGCTGCCACAGCAGTGATTACCCAG GATGTGAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCCTGGGCTGTGTGCCTGCCATT GGAGAACTGGGCTAAGGGCACCTGTGTAAGCCTGCCCAACTCCAACCTGGAGACTGTCTCCATTGGA ACTGAAAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTATGGAGCTATGGACTTCAGCACCC TCCAAGACACCAAGTGTGAGGTGCCACTGGACATCTGTCTAGAGCATCTGTAAATACCTGACTACCTCAA ATGAGTGCTGACCCATATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGGCCAGACACTTC TGGAACAGGGCTGGAGTGATGGGAGACACAGTGCCAACAGACCTCTACATCAAGGGCACCTCTGCCAATAT GAGGGAGACACCTGGCTCCTGTGTCTACAGCCCAAGCCCATCTGGCAGCATCACCACTCTGACAGCCAAC TTTTCAACAAGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACTT TTTGTGACAGTGGTGGACACCACAGGAGCACCAACCTGACCCTGTGTGCCAGCACCCAGAACCCTGTGCC AAACACCTATGACCCAACCAAGTTCAAGCACTACAGCAGGCATGTGGAGGAATATGACCTCCAATTCATCT TCCAACCTTTGTACCATCACCTGACAGCAGAGGTGATGAGTTACATCCACAGTATGAACTCCAGCATCTTGG AGAACTGGAACCTTTGGAGTGCCTCCTCCTCCAACCACCTCCCTGGTGGACACCTACAGGTTTGTCCAGTCTG TGGCTGTGACTTGTGAGAAGGACACCACACCTCCTGAGAAGCAGGACCCATATGACAACTGAAGTTCTGG ACAGTGGACCTGAAAGAGAAGTTCTCCTCTGACCTGGACCAATACCCACTGGGCAGGAAGTTCTGGTCCA GGCTGGACTGAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAG AGGAAGAAGGTGAAGAAGTAAA
SEQ ID No:	4505 Synthetic HPV45 L1 gene

96	ctgggtaccATGGCTCTGTGGAGACCATCTGACAGCACAGTCTACCTGCCTCCTCCATCTGTGGCAAGGGTGGTG AACACAGATGACTATGTGAGCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGG CAACCCATACTTCAGGGTGGTGCCAAGTGGAGCAGGCAACAAGCAGGCTGTGCCAAAGGTGTCTGCCTACC AATACAGGGTGTTCAGGGTGGCTCTGCCTGACCCAAACAAGTTTGGACTGCCTGACAGCACCATCTACAAC CCTGAGACCCAGAGACTGGTGTGGGCTTGTGTGGGGATGGAGATTGGCAGGGGACAACCACTGGGCATTG GACTGTCTGGACACCCATTCTACAACAACTGGATGACACAGAGTCTGCCCATGCTGCCACAGCAGTGATT ACCCAGGATGTGAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCCTGGGCTGTGTGCC TGCCATTGGAGAACTGGGCTAAGGGCACCTGTGTAAGCCTGCCAACTCCAACCTGGAGACTGTCCTC CATTGGAAGTGAACCACTGGGCTAAGGGCACCTGTGTAAGCCTGCCAACTCCAACCTGGAGACTGTCCTC AGCACCTCCAAGACACCAAGTGTGAGGTGCCACTGGACATCTGTGAGAGCATCTGTAAATACCCTGACTA CCTCCAAATGAGTGCTGACCCATATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGCCAG ACACTTCTGGAACAGGGCTGGAGTGATGGGAGACACAGTGCCAACAGACCTCTACATCAAGGGCACCTCTG CCAATATGAGGGAGACACCTGGCTCCTGTGTCTACAGCCCAAGCCCATCTGGCAGCATCACCACCTCTGAC AGCCAACCTTTTCAACAAGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGGCATCTGTTGGCACA CCAACCTTTTGTGACAGTGGTGGACACCACAGGAGCACCAACCTGACCCTGTGTGCCAGACCCAGAACC CTGTGCCAAACACCTATGACCCAACCAAGTTCAAGCACTACAGCAGGCATGTGGAGGAATATGACCTCCAA TTCATCTTCCAACCTTGTACCATCACCTGACAGCAGAGGTGATGAGTTACATCCACAGTATGAACTCCAGC ATCTTGGAGAACTGGAACCTTGGAGTGCCTCCTCCTCCAACCACCTCCCTGGTGGACACCTACAGGTTTGTG CAGTCTGTGGCTGTGACTTGTGAGAAGGACACCACACCTCCTGAGAAGCAGGACCCATATGACAACTGAA GTTCTGGACAGTGGACCTGAAAGAGAAGTTCTCCTCTGACCTGGACCAATACCCACTGGGCAGGAAGTTCC TGGTCCAGGCTGGACTGAGGAGGAGACCAACCATTGGACCAAGGAAGAGACCTGTGCCAGCACCAGCAC AGCCAGCAGACCTGCCAAGAGGGTGAGGATTAGGAGCAAGAAGTAAA ctcgagctc
SEQ ID No:	4506 Synthetic HPV33 L1 gene
97	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG

	AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGACCAATATGACCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 98	4507 HPV45 L1 F1 CTTGGTACCATGGCTCTGTGGAGACCATCTGAC
SEQ ID No: 99	4508 HPV45 L1 R1 GCTTGGCTTTCAGTCCAGCCTGGACCAGGAAC

SEQ ID No:	4509 HPV45 L1 amplified sequence 1
100	CTTGGTACC^[1] <u>ATGGCTCTGTGGAGACCATCTGACAGCACAGTCTACCTGCCTCCTCCATCTGTGGCAAGGGTGGTGAACAC</u> <u>AGATGACTATGTGAGCAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGACAGTGGGCAACC</u> <u>CATACTTCAGGGTGGTGCCAAGTGGAGCAGGCAACAAGCAGGCTGTGCCAAAGGTGTCTGCCTACCAATAC</u> <u>AGGGTGTTCAGGGTGGCTCTGCCTGACCCAAACAAGTTTGGACTGCCTGACAGCACCATCTACAACCTGA</u> <u>GACCCAGAGACTGGTGTGGGCTTGTGTGGGGATGGAGATTGGCAGGGGACAACCACTGGGCATTGGACTGT</u> <u>CTGGACACCCATTCTACAACAACTGGATGACACAGAGTCTGCCCATGCTGCCACAGCAGTGATTACCCAG</u> <u>GATGTGAGGGACAATGTGTCTGTGGACTACAAGCAGACCCAACTTTGTATCCTGGGCTGTGTGCCTGCCATT</u> <u>GGAGAACTGGGCTAAGGGCACCTGTGTAAGCCTGCCCAACTCCAACCTGGAGACTGTCTCCATTGGA</u> <u>ACTGAAAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTATGGAGCTATGGACTTCAGACCCC</u> <u>TCCAAGACACCAAGTGTGAGGTGCCACTGGACATCTGTGAGGCATCTGTAAATACCTGACTACCTCCAA</u> <u>ATGAGTGCTGACCCATATGGAGACAGTATGTTCTTCTGTCTGAGGAGGGAACAACCTTTTGCCAGACACTTC</u> <u>TGGAACAGGGCTGGAGTGATGGGAGACACAGTGCCAACAGACCTCTACATCAAGGGCACCTCTGCCAATAT</u> <u>GAGGGAGACACCTGGCTCCTGTGTCTACAGCCCAAGCCCATCTGGCAGCATCACCACTCTGACAGCCAAC</u> <u>TTTTCAACAAGCCATACTGGCTGCACAAGGCTCAAGGACACAACAATGGCATCTGTTGGCACAACCAACTT</u> <u>TTTGTGACAGTGGTGGACACCACCAGGAGCACCAACCTGACCTGTGTGCCAGACCCAGAACCCTGTGCC</u> <u>AAACACCTATGACCCAACCAAGTTCAAGCACTACAGCAGGCATGTGGAGGAATATGACCTCCAATTCATCT</u> <u>TCCAACCTTTGTACCATCACCTGACAGCAGAGGTGATGAGTTACATCCACAGTATGAACTCCAGCATCTTGG</u> <u>AGAACTGGAACCTTTGGAGTGCCTCCTCCTCCAACCACCTCCCTGGTGGACACCTACAGGTTTGTCCAGTCTG</u> <u>TGGCTGTGACTTGTGAGAAGGACACCACACCTCCTGAGAAGCAGGACCCATATGACAAACTGAAGTTCTGG</u> <u>ACAGTGGACCTGAAAGAGAAGTTCTCCTCTGACCTGGACCAATACCCACTGGGCAGGAAGTTCTGGTCCA</u> <u>GGCTGGACTG</u> ^[2] AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding HPV45 L1 aa 1-478;

	[3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 101	4510 HPV45 L1 F2 GGCTGGACTGAAAGCCAAGCCAAAAGTGAAGGG
SEQ ID No: 102	4511 HPV45 L1 R2 CTGTCTAGATTACTTCTTCACCTTCTTCCTCTTGGC
SEQ ID No: 103	4512 HPV45 L1 amplified sequence 2 <u>GGCTGGACTG</u> ^[1] <u>AAAGCCAAGCCAAAAGTGAAGGGCTGCCCCAACCAGCACCAGG</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: 104	4513 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

Appendix 9 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 51 L1 PROTEIN

SEQ ID No: 105	5101 The amino acid sequence of HPV type 51 L1 protein aa 1-474 MALWRTNDSKVYLPPAPVSRIVNTEYYITRTGIYYYAGSSRLITLGHYPYFPIPKTS TRAAIPKVSAFQYRVFRVQLPDPNKFGLPDPNLYNPDTDRLVWGCVGVEVGRG QPLGVGLSGHPLFNKYDDTENSRIANGNAQQDVRDNTSVDNKQTQLCIIGCAPP IGEHWGIGTTCKNTPVPPGDCPPELVSSVIQDGDMDTGFGAMDFAALQATKS DVPLDISQSVCKYPDYLKMSADTYGNSMFFHLRREQIFARHYYNKLVGVGEDI PNDYYIKGSGNGRDPIESYIYSATPSGSMITSDSQIFNKPYWLHRAQGHNNGICW NNQLFITCVDTTTRSTNLTISTATAAVSPTFTPSNFKQYIRHGEEYELQFIFQLCKIT LTTEVMAYLHTMDPTILEQWNFGLTLPPSASLEDAYRFVRNAATSCQKDTPPQ AKPDPLAKYKFWVDVLDKERFSLDLQFALGRKFLLQVGV
SEQ ID No: 106	5102 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 107	5103 Amino acid sequence of chimeric HPV type 51 L1 protein MALWRTNDSKVYLPPAPVSRIVNTEYYITRTGIYYYAGSSRLITLGHYPYFPIPKTS TRAAIPKVSAFQYRVFRVQLPDPNKFGLPDPNLYNPDTDRLVWGCVGVEVGRG QPLGVGLSGHPLFNKYDDTENSRIANGNAQQDVRDNTSVDNKQTQLCIIGCAPP IGEHWGIGTTCKNTPVPPGDCPPELVSSVIQDGDMDTGFGAMDFAALQATKS DVPLDISQSVCKYPDYLKMSADTYGNSMFFHLRREQIFARHYYNKLVGVGEDI PNDYYIKGSGNGRDPIESYIYSATPSGSMITSDSQIFNKPYWLHRAQGHNNGICW NNQLFITCVDTTTRSTNLTISTATAAVSPTFTPSNFKQYIRHGEEYELQFIFQLCKIT LTTEVMAYLHTMDPTILEQWNFGLTLPPSASLEDAYRFVRNAATSCQKDTPPQ AKPDPLAKYKFWVDVLDKERFSLDLQFALGRKFLLQVGVKAKPKLKRAAPTST RTSSAKRKKVKK

SEQ ID No:	5104 Nucleotide sequence of chimeric HPV type 51 L1 protein
108	ATGGCTCTGTGGAGGACCAATGACAGCAAGGTCTACCTGCCTCCTGCCCCTGTGAGCAGGATTGTGAACAC AGAGGAATACATCACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGATTACCCTGGGACACC CATACTTTCCAATCCCAAAGACCAGCACCAGGGCTGCCATCCCAAAGGTGTCTGCCTTCCAATACAGGGTG TTCAGGGTCCAACTTCCTGACCCAAACAAGTTTGGACTGCCTGACCCAAACCTCTACAACCCTGACACAGA CAGACTGGTGTGGGGCTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGACTGTCTGGA CACCCACTGTTCAACAAATATGATGACACAGAGAACAGCAGGATTGCCAATGGCAATGCCCAACAGGATGT GAGGGACAACACCTCTGTGGACAACAAGCAGACCCAACTTTGTATCATTGGCTGTGCCCTCCAATTGGAG AACACTGGGGCATTGGCACCACTTGTAAGAACACACCTGTGCCTCCTGGAGACTGTCTCCATTGGAAGTG GTGTCCTCTGTGATTGAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTTGCTGCCCTCCAA GCCACCAAGTCTGATGTGCCACTGGACATCAGCCAGTCTGTGTGTAAATACCCTGACTACCTGAAAATGAG TGCTGACACCTATGGCAACAGTATGTTCTTCCACCTGAGGAGGGAACAGATTTTGGCAGACACTACTACA ACAAACTGGTGGGAGTGGGAGAGGACATCCCAAATGACTACTACATCAAGGGCTCTGGCAATGGCAGGGA CCCAATTGAGTCCTACATCTACTCTGCCACACCATCTGGCAGTATGATTACCTCTGACAGCCAGATTTTCAA CAAGCCATACTGGCTGCACAGGGCTCAAGGACACAACAATGGCATCTGTTGGAACAACCAACTTTTCATCA CTTGTGTGGACACCACCAGGAGCACCAACCTGACCATCAGCACAGCCACAGCAGCAGTGAGCCCCAACCTTC ACACCAAGCAACTTCAAGCAATACATCAGACATGGAGAGGAATATGAACTCCAATTCATCTTCCAACTTTG TAAGATTACCCTGACCACAGAGGTGATGGCTTACCTGCACACAATGGACCCAACCATCTTGGAACAGTGGA ACTTTGGACTGACCCTGCCTCCATCTGCCTCCTTGGAGGATGCCTACAGGTTTGTGAGGAATGCTGCCACCT CCTGTGAGAAGGACACACCTCCACAGGCTAAGCCTGACCCACTGGCTAAATACAAGTTCTGGGATGTGGAC CTGAAAGAGAGGTTCTCCCTGGACCTGGACCAGTTTGCCCTGGGCAGGAAGTTCCTGCTCCAAGTGGGAGT CAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAA

SEQ ID No:	5105 Synthetic HPV51 L1 gene
109	ctgggtaccATGGCTCTGTGGAGGACCAATGACAGCAAGGTCTACCTGCCTCCTGCCCCTGTGAGCAGGATTGT GAACACAGAGGAATACATCACCAGGACAGGCATCTACTACTATGTGGCTCCAGCAGACTGATTACCCTGG GACACCCATACTTTCCAATCCCAAAGACCAGCACAGGGCTGCCATCCCAAAGGTGTCTGCCTTCCAATAC AGGGTGTTTCAAGGTCCAACCTCTGACCCAAACAAGTTTGGACTGCCTGACCCAAACCTCTACAACCTGA CACAGACAGACTGGTGTGGGGCTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGACTG TCTGGACACCCACTGTTCAACAAATATGATGACACAGAGAACAGCAGGATTGCCAATGGCAATGCCCAACA GGATGTGAGGGACAACACCTCTGTGGACAACAAGCAGACCCAACTTTGTATCATTGGCTGTGCCCTCCAA TTGGAGAACACTGGGGCATTGGCACCACTTGTAAGAACACACCTGTGCCTCCTGGAGACTGTCTCCATTG GAACTGGTGTCTCTGTGATTCAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTTGTGCC CTCCAAGCCACCAAGTCTGATGTGCCACTGGACATCAGCCAGTCTGTGTGTAAATACCCTGACTACCTGAA AATGAGTGCTGACACCTATGGCAACAGTATGTTCTTCCACCTGAGGAGGGAACAGATTTTTGCCAGACACT ACTACAACAACTGGTGGGAGTGGGAGAGGACATCCCAAATGACTACTACATCAAGGGCTCTGGCAATGG CAGGGACCCAATTGAGTCCTACATCTACTCTGCCACACCATCTGGCAGTATGATTACCTCTGACAGCCAGAT TTTCAACAAGCCATACTGGCTGCACAGGGCTCAAGGACACAACAATGGCATCTGTTGGAACAACCAACTTT TCATCACTTGTGTGGACACCACCAGGAGCACCAACCTGACCATCAGCACAGCCACAGCAGCAGTGAAGCCCA ACCTTCACACCAAGCAACTTCAAGCAATACATCAGACATGGAGAGGAATATGAACTCCAATTCATCTTCCA ACTTTGTAAGATTACCCTGACCACAGAGGTGATGGCTTACCTGCACACAATGGACCCAACCATCTTGGAAC AGTGGAACCTTTGGACTGACCCTGCCTCCATCTGCCTCCTTGGAGGATGCCTACAGGTTTGTGAGGAATGCTG CCACCTCCTGTCAGAAGGACACACCTCCACAGGCTAAGCCTGACCCACTGGCTAAATACAAGTTCTGGGAT GTGGACCTGAAAGAGAGGTTCTCCCTGGACCTGGACCAGTTTGCCTGGGCAGGAAGTTCCTGCTCCAAGT GGGAGTCCAGAGGAAGCCAAGACCTGGACTGAAAAGACCTGCCTCCTCTGCCTCCTCCTCCTCCTCCTC TGCCAAGAGGAAGAGGGTGAAGAAGTAAActcgagctc
SEQ ID No:	5106 Synthetic HPV33 L1 gene

110	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 111	5107 HPV51 L1 F1 CTTGGTACCATGGCTCTGTGGAGGACCAATGACA
SEQ ID No:	5108 HPV51 L1 R1

112	GCTTGGCTTTGACTCCCCTTGGAGCAGGAAC
SEQ ID No:	5109 HPV51 L1 amplified sequence 1
113	CTTGGTACC^[1] <u>ATGGCTCTGTGGAGGACCAATGACAGCAAGGTCTACCTGCCTCCTGCCCCTGTGAGCAGGATTGTGAACAC</u> <u>AGAGGAATACATCACCAGGACAGGCATCTACTACTATGCTGGCTCCAGCAGACTGATTACCCTGGGACACC</u> <u>CATACTTTCCAATCCCAAAGACCAGCACCAGGGCTGCCATCCCAAAGGTGTCTGCCTTCCAATACAGGGTG</u> <u>TTCAGGGTCCAACCTCCTGACCCAAACAAGTTTGGACTGCCTGACCCAAACCTCTACAACCCTGACACAGA</u> <u>CAGACTGGTGTGGGGCTGTGTGGGAGTGGAGGTGGGCAGGGGACAACCACTGGGAGTGGGACTGTCTGGA</u> <u>CACCCACTGTTCAACAAATATGATGACACAGAGAACAGCAGGATTGCCAATGGCAATGCCCAACAGGATGT</u> <u>GAGGGACAACACCTCTGTGGACAACAAGCAGACCCAACTTTGTATCATTGGCTGTGCCCCTCCAATTGGAG</u> <u>AACACTGGGGCATTGGCACCCTTGTAAGAACACACCTGTGCCTCCTGGAGACTGTCTCCATTGGAACCTG</u> <u>GTGTCCTCTGTGATTGAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTTGCTGCCCTCCA</u> <u>GCCACCAAGTCTGATGTGCCACTGGACATCAGCCAGTCTGTGTGTAAATACCCTGACTACCTGAAAATGAG</u> <u>TGCTGACACCTATGGCAACAGTATGTTCTTCCACCTGAGGAGGGAACAGATTTTGCCAGACACTACTACA</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</u>^[2] AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site;

	[2]: the gene fragment encoding HPV51 L1 aa 1-474; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499
SEQ ID No: 114	5110 HPV51 L1 F2 AGTGGGAGTCAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 115	5111 HPV51 L1 R2 CTGTCTAGATTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 116	5112 HPV51 L1 amplified sequence 2 <u>AGTGGGAGTC</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 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: 117	5113 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

Appendix 10 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 52 L1 PROTEIN

SEQ ID No: 118	5201 The amino acid sequence of HPV type 52 L1 protein aa 1-478 MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLTVGHPYFSIKN TSSGNGKKVLVPKVSGLQYRVFRIKLDPNKFPGPDTSFYNPETQRLVWACTGL EIGRGQPLGVGISGHPLLNFDDTETSNKYAGKPGIDNRECLSMDYKQTQLCIL GCKPPIGEHWGKGTPCENNNSGNPGDCPPLQLINSVIQDGDGMVDTGFGCMDENT LQASKSDVPIDICSSVCKYPDYLQMASEPYGDSLFFFLRREQMFVRHFFNRAGT LGDPVPGDLYIQGSNSGNTATVQSSAFFPTPSGSMVTSESQLFNKPWYLQRAQG HNNGICWGNQLFVTVVDTTTRSTNMTLCAEVKKESTYKNENFKEYLRHGEEFDL QFIFQLCKITLTADVMTYIHKMDATILEDWQFGLTPPPSASLEDTYRFVTSTAIT CQKNTPPKGKEDPLKDYMFEVDLKEKFSADLDQFPLGRKFLLQAGL
SEQ ID No: 119	5202 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 120	5203 Amino acid sequence of chimeric HPV type 52 L1 protein MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLTVGHPYFSIKN TSSGNGKKVLVPKVSGLQYRVFRIKLDPNKFPGPDTSFYNPETQRLVWACTGL EIGRGQPLGVGISGHPLLNFDDTETSNKYAGKPGIDNRECLSMDYKQTQLCIL GCKPPIGEHWGKGTPCENNNSGNPGDCPPLQLINSVIQDGDGMVDTGFGCMDENT LQASKSDVPIDICSSVCKYPDYLQMASEPYGDSLFFFLRREQMFVRHFFNRAGT LGDPVPGDLYIQGSNSGNTATVQSSAFFPTPSGSMVTSESQLFNKPWYLQRAQG HNNGICWGNQLFVTVVDTTTRSTNMTLCAEVKKESTYKNENFKEYLRHGEEFDL QFIFQLCKITLTADVMTYIHKMDATILEDWQFGLTPPPSASLEDTYRFVTSTAIT CQKNTPPKGKEDPLKDYMFEVDLKEKFSADLDQFPLGRKFLLQAGLKAKPK LKRAAPTSTRTSSAKRKKVKK

SEQ ID No:	5204 Nucleotide sequence of chimeric HPV type 52 L1 protein
121	ATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC CGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGACCGTGGGCCACC CCTACTTCAGCATCAAGAACCAGCAGCGGCAACGGCAAGAAGGTGCTGGTGCCCAAGGTGAGCGGCCT GCAGTACAGGGTGTTCAGGATCAAGCTGCCCCGACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACA ACCCCGAGACCCAGAGGCTGGTGTGGGCCTGCACCGGCCTGGAGATCGGCAGGGGCCAGCCCCTGGGCGT GGGCATCAGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGACCAGCAACAAGTACGCCGGCAAG CCCGGCATCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCATCCTGGGCTGCAA GCCCCCATCGGCGAGCACTGGGGCAAGGGCACCCCTGCAACAACAACAGCGGCAACCCCGGCGACTGC CCCCCCTGCAGCTGATCAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCTGCATGGA CTTCAACACCCTGCAGGCCAGCAAGAGCGACGTGCCCATCGACATCTGCAGCAGCGTGTGCAAGTACCCCG ACTACCTGCAGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTCCTGAGGAGGGAGCAGATGTTT GTGAGGCACTTCTTCAACAGGGCCGGCACCCCTGGGCGACCCCGTGCCCGGCGACCTGTACATCCAGGGCAG CAACAGCGGCAACACCGCCACCGTGCAGAGCAGCGCCTTCTTCCCCACCCCAGCGGCAGCATGGTGACCA GCGAGAGCCAGCTGTTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTG GGGCAACCAGCTGTTCGTGACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCGCCGAGGTGA AGAAGGAGAGCACCTACAAGAACGAGAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCA GTTTATCTTCCAGCTGTGCAAGATCACCTGACCGCCGACGTGATGACCTACATCCACAAGATGGACGCCA CCATCCTGGAGGACTGGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGGAGGACACCTACAGGTTT GTGACCAGCACCGCCATCACCTGCCAGAAGAACACCCCCCAAGGGCAAGGAGGACCCCTGAAGGACT ACATGTTCTGGGAGGTGGACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCTCCCTGGGCAGGAAG TTCTGCTGCAGGCCGGCCTGAAAGCCAAGCCAAAAGTGAAGGGGTGCCCAACCAGCACCAGGACCTC CTCTGCCAAGAGGAAGAAGGTGAAGAAGTAAA

SEQ ID No:	5205 Synthetic HPV52 L1 gene
122	ctgggtaccATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGT GAGCACCGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGACCGTG GGCCACCCCTACTTCAGCATCAAGAACCAGCAGCGGCAACGGCAAGAAGGTGCTGGTGCCCAAGGTGA GCGGCCTGCAGTACAGGGTGTTCAGGATCAAGCTGCCCCAGCCCCAACAAAGTTTCGGCTTCCCCGACACCAGC TTCTACAACCCCGAGACCCAGAGGCTGGTGTGGGCCTGCACCGGCCTGGAGATCGGCAGGGGCCAGCCCT GGGCGTGCGCATCAGCGGCCACCCCTGCTGAACAAGTTTCGACGACACCGAGACCAGCAACAAGTACGCC GGCAAGCCCGGCATCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCATCCTGG GCTGCAAGCCCCCATCGGCGAGCACTGGGGCAAGGGCACCCCTGCAACAACAACAGCGGCAACCCCGG CGACTGCCCCCCCCTGCAGCTGATCAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCT GCATGGACTTCAACACCCTGCAGGCCAGCAAGAGCGACGTGCCCATCGACATCTGCAGCAGCGTGTGCAAG TACCCCGACTACCTGCAGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTTCTGAGGAGGGAGCA GATGTTCTGTGAGGCACTTCTTCAACAGGGCCGGCACCCCTGGGCGACCCCGTGCCCGGCACCTGTACATCC AGGGCAGCAACAGCGGCAACACCGCCACCGTGCAGAGCAGCGCCTTCTTCCCCACCCCGAGCGGCAGCATG GTGACCAGCGAGAGCCAGCTGTTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCA TCTGCTGGGGCAACCAGCTGTTCGTGACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCGCC GAGGTGAAGAAGGAGAGCACCTACAAGAACGAGAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCG ACCTGCAGTTCATCTTCCAGCTGTGCAAGATCACCTGACCGCCGACGTGATGACCTACATCCACAAGATG GACGCCACCATCCTGGAGGACTGGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGGAGGACACCTA CAGGTTCGTGACCAGCACCGCCATCACCTGCCAGAAGAACACCCCCCAAGGGCAAGGAGGACCCCTG AAGGACTACATGTTCTGGGAGGTGGACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCTCCCTGGG CAGGAAGTTCCTGCTGCAGGCCGGCCTGCAGGCCAGGCCAAAGCTGAAGAGGCCCGCCAGCAGCGCCCC AGGACCAGCACCAAGAAGAAGAAGGTGAAGAGGTAAActcgagetc
SEQ ID No:	5206 Synthetic HPV33 L1 gene
123	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG

	AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 124	5207 HPV52 L1 F1 CTTGGTACCATGAGCGTGTGGAGGCCAGCGAGG
SEQ ID No: 125	5208 HPV52 L1 R1 GCTTGGCTTTCAGGCCGGCCTGCAGCAGGAACTTC

SEQ ID No:	5209 HPV52 L1 amplified sequence 1
126	CTTGGTACC^[1] <u>ATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC</u> <u>CGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGCGAGCAGCAGGCTGCTGACCGTGGGCCACC</u> <u>CCTACTTCAGCATCAAGAACCAGCAGCGGCAACGGCAAGAAGGTGCTGGTGCCCAAGGTGAGCGGCCT</u> <u>GCAGTACAGGGTGTTCAGGATCAAGCTGCCCCACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACA</u> <u>ACCCCGAGACCCAGAGGCTGGTGTGGGCCTGCACCGGCCTGGAGATCGGCAGGGGCCAGCCCCTGGGCGT</u> <u>GGGCATCAGCGGCCACCCCTGCTGAACAAGTTCGACGACACCGAGACCAGCAACAAGTACGCCGGCAAG</u> <u>CCCCGCATCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCATCCTGGGCTGCAA</u> <u>GCCCCCATCGGCGAGCACTGGGGCAAGGGCACCCCTGCAACAACAACAGCGGCAACCCCGCGCACTGC</u> <u>CCCCCCTGCAGCTGATCAACAGCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCTGCATGGA</u> <u>CTTCAACACCCTGCAGGCCAGCAAGAGCGACGTGCCATCGACATCTGCAGCAGCGTGTGCAAGTACCCCG</u> <u>ACTACCTGCAGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTCCTGAGGAGGGAGCAGATGTTT</u> <u>GTGAGGCACTTCTTCAACAGGGCCGGCACCCCTGGGCGACCCCGTGCCCGCGACCTGTACATCCAGGGCAG</u> <u>CAACAGCGGCAACACCGCCACCGTGCAGAGCAGCGCCTTCTTCCCCACCCCAAGCGGCAGCATGGTGACCA</u> <u>GCGAGAGCCAGCTGTTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTG</u> <u>GGGCAACCAGCTGTTCGTGACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCGCCGAGGTGA</u> <u>AGAAGGAGAGCACCTACAAGAACGAGAACTTCAAGGAGTACCTGAGGCACGGCGAGGAGTTCGACCTGCA</u> <u>GTTTATCTTCCAGCTGTGCAAGATCACCTGACCGCCGACGTGATGACCTACATCCACAAGATGGACGCCA</u> <u>CCATCCTGGAGGACTGGCAGTTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGGAGGACACCTACAGGTTC</u> <u>GTGACCAGCACCGCCATCACCTGCCAGAAGAACACCCCCCAAGGGCAAGGAGGACCCCTGAAGGACT</u> <u>ACATGTTCTGGGAGGTGGACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCTCCCTGGGCAGGAAG</u> <u>TTCCTGCTGCAGGCCGGCCTG</u>^[2] AAAGCCAAGC^[3] [1]: Kpn I enzymatic cleavage site; [2]: the gene fragment encoding HPV52 L1 aa 1-478;

	[3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 127	5210 HPV52 L1 F2 GGCCGGCCTGAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 128	5211 HPV52 L1 R2 CTGTCTAGATTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 129	5212 HPV52 L1 amplified sequence 2 <u>GGCCGGCCTG</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 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: 130	5213 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKR VKRRKSSRK

Appendix 11 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 56 L1 PROTEIN

SEQ ID No: 131	5601 The amino acid sequence of HPV type 56 L1 protein aa 1-467 MATWRPSENKVYLPPTPVSKVVATDSYVKRTSIFYHAGSSRLLAVGHPYYSVT KDNTKTNIPKVSAYQYRVFRVRLPDPNKFGLPDTNIYNPDQERLVWACVGLEV GRGQPLGAGLSGHPLFNRLDDTESSNLANNVIEDSRDNISVDGKQTQLCIVGC TPAMGEHWTKGAVCKSTQVTTGDCPPLALINTPIEDGDMIDTGFGAMDFKVLQ ESKAEVPLDIVQSTCKYPDYLKMSADAYGDSMWFYLRREQLFARHYFNRAAGK VGETIPAELYLKGSNGREPPSSVYVATPSGSMITSEAQLFNKPYWLQRAQGHN NGICWGNQLFVTVDTRSTNMTISTATEQLSKYDARKINQYLRHVEEYELQF VFQLCKITLSAEVMAYLHNMNANLLEDWNIGLSPPVATSLEDKYRYVRSTAITC QREQPPTEKQDPLAKYKFWDVNLQDSFSTDLDQFPLGRKFL
SEQ ID No: 132	5602 The amino acid sequence of HPV type 33 L1 protein aa 469-499 LQAGLKAKPKLKRAAPTSTRTSSAKRKKVKK
SEQ ID No: 133	5603 Amino acid sequence of chimeric HPV type 56 L1 protein MATWRPSENKVYLPPTPVSKVVATDSYVKRTSIFYHAGSSRLLAVGHPYYSVT KDNTKTNIPKVSAYQYRVFRVRLPDPNKFGLPDTNIYNPDQERLVWACVGLEV GRGQPLGAGLSGHPLFNRLDDTESSNLANNVIEDSRDNISVDGKQTQLCIVGC TPAMGEHWTKGAVCKSTQVTTGDCPPLALINTPIEDGDMIDTGFGAMDFKVLQ ESKAEVPLDIVQSTCKYPDYLKMSADAYGDSMWFYLRREQLFARHYFNRAAGK VGETIPAELYLKGSNGREPPSSVYVATPSGSMITSEAQLFNKPYWLQRAQGHN NGICWGNQLFVTVDTRSTNMTISTATEQLSKYDARKINQYLRHVEEYELQF VFQLCKITLSAEVMAYLHNMNANLLEDWNIGLSPPVATSLEDKYRYVRSTAITC QREQPPTEKQDPLAKYKFWDVNLQDSFSTDLDQFPLGRKFL LQAGLKAKPKLKRAAPTSTRTSSAKRKKVKK

SEQ ID No:	5604 Nucleotide sequence of chimeric HPV type 56 L1 protein
134	ATGGCTACCTGGAGACCATCTGAGAAACAAGGTCTACCTGCCTCCAACACCTGTGAGCAAGGTGGTGGCTAC AGACTCCTATGTGAAGAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGGGACACC CATACTACTCTGTGACCAAGGACAACACCAAGACCAACATCCCAAAGGTGTCTGCCTACCAATACAGGGTG TTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGACTGCCTGACACCAACATCTACAACCCTGACCAGGA GAGACTGGTGTGGGCTTGTGTGGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGCAGGACTGTCTGGA CACCCACTGTTCAACAGACTGGATGACACAGAGTCCAGCAACCTGGCTAACAACAATGTGATTGAGGACAG CAGGGACAACATCTCTGTGGATGGCAAGCAGACCCAACTTTGTATTGTGGGCTGTACTCCTGCTATGGGAG AACACTGGACCAAGGGAGCAGTGTGTAAGAGCACCCAGGTGACCACAGGAGACTGTCCTCCACTGGCTCTG ATAAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTCAAGGTGCTCCA AGAGAGCAAGGCTGAGGTGCCACTGGACATTGTCCAGAGCACTTGTAATAACCTGACTACCTGAAAATGA GTGCTGATGCCTATGGAGACAGTATGTGGTTCTACCTGAGGAGGGAACAACCTTTTGCCAGACACTACTTC AACAGGGCTGGCAAGGTGGGAGAGACCATCCCTGCTGAACTCTACCTGAAAGGCAGCAATGGCAGGGAAC CTCCTCCATCCTCTGTCTATGTGGCTACACCATCTGGCAGTATGATTACCTCTGAGGCTCAACTTTTCAACAA GCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCACTTTTGTGACAG TGGTGGACACCACCAGGAGCACCAATATGACCATCAGCACAGCCACAGAACAACCTTAGCAAAATATGATGC CAGGAAGATAAACCAATACCTGAGGCATGTGGAGGAATATGAACTCCAATTTGTGTCCAACCTTTGTAAGA TTACCCTGTCTGCTGAGGTGATGGCTTACCTGCACAATATGAATGCCAACCTGTTGGAGGACTGGAACATTG GACTGAGCCCTCCTGTGGCTACCTCCTTGGAGGACAAATACAGATATGTGAGGAGCACAGCCATCACTTGT CAGAGGGAACAACCTCCAACAGAGAAGCAGGACCCACTGGCTAAATACAAGTTCTGGGATGTGAACCTCC AAGACTCCTTCAGCACAGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACTGAAA GCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAGGTGA AGAAGTAAA
SEQ ID No:	5605 Synthetic HPV56 L1 gene

135	ctgggtaccATGGCTACCTGGAGACCATCTGAGAACAAAGGTCTACCTGCCTCCAACACCTGTGAGCAAGGTGGT GGCTACAGACTCCTATGTGAAGAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGG GACACCCATACTACTCTGTGACCAAGGACAACACCAAGACCAACATCCCAAAGGTGTCTGCCTACCAATAC AGGGTGTTTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGACTGCCTGACACCAACATCTACAACCCTGA CCAGGAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGCAGGACTG TCTGGACACCCACTGTTCAACAGACTGGATGACACAGAGTCCAGCAACCTGGCTAACAAACAATGTGATTGA GGACAGCAGGGACAACATCTCTGTGGATGGCAAGCAGACCCAACTTTGTATTGTGGGCTGTACTCCTGCTA TGGGAGAACACTGGACCAAGGGAGCAGTGTGTAAGAGCACCCAGGTGACCACAGGAGACTGTCTCCTCACT GGCTCTGATAAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTCAAGG TGCTCCAAGAGAGCAAGGCTGAGGTGCCACTGGACATTGTCCAGAGCACTTGTAATACCTGACTACCTG AAAATGAGTGCTGATGCCTATGGAGACAGTATGTGGTTCTACCTGAGGAGGGAACAACCTTTTTGCCAGACA CTACTTCAACAGGGCTGGCAAGGTGGGAGAGACCATCCCTGCTGAACTCTACCTGAAAGGCAGCAATGGCA GGGAACCTCCTCCATCCTCTGTCTATGTGGCTACACCATCTGGCAGTATGATTACCTCTGAGGCTCAACTTT TCAACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTT GTGACAGTGGTGGACACCACCAGGAGCACCAATATGACCATCAGCACAGCCACAGAACAACCTTAGCAAAT ATGATGCCAGGAAGATAAACCAATACCTGAGGCATGTGGAGGAATATGAACTCCAATTTGTGTTCCAACCT TGTAAGATTACCCTGTCTGCTGAGGTGATGGCTTACCTGCACAATATGAATGCCAACCTGTTGGAGGACTG GAACATTGGACTGAGCCCTCCTGTGGCTACCTCCTTGGAGGACAAATACAGATATGTGAGGAGCACAGCCA TCACTTGTCAGAGGGAACAACCTCCAACAGAGAAGCAGGACCCACTGGCTAAATACAAGTTCTGGGATGTG AACCTCCAAGACTCCTTCAGCACAGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGATGCAACTTGG CACCAGGAGCAAGCCTGCTGTGGCTACCAGCAAGAAGAGGTCTGCCCCAACCAGCACCAGCACACCTGCC AAGAGGAAGAGGAGGTAAActegagctc
SEQ ID No: 136	5606 Synthetic HPV33 L1 gene ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG

	ACACCCATACTTCAGCATCAAGAACCCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTC AACAAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAAGTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 137	5607 HPV56 L1 F1 CTTGGTACCATGGCTACCTGGAGACCATCTGAG
SEQ ID No: 138	5608 HPV56 L1 R1 CTGCTTGGAGCAGGAACCTCCTGCCCAGTGG

SEQ ID No:	5609 HPV56 L1 amplified sequence 1
139	CTTGGTACC^[1] <u>ATGGCTACCTGGAGACCATCTGAGAAACAAGGTCTACCTGCCTCCAACACCTGTGAGCAAGGTGGTGGCTAC</u> <u>AGACTCCTATGTGAAGAGGACCAGCATCTTCTACCATGCTGGCTCCAGCAGACTGCTGGCTGTGGGACACC</u> <u>CATACTACTCTGTGACCAAGGACAACACCAAGACCAACATCCCAAAGGTGTCTGCCTACCAATACAGGGTG</u> <u>TTCAGGGTGAGACTGCCTGACCCAAACAAGTTTGGACTGCCTGACACCAACATCTACAACCTGACCAGGA</u> <u>GAGACTGGTGTGGGCTTGTGTGGGATTGGAGGTGGGCAGGGGACAACCACTGGGAGCAGGACTGTCTGGA</u> <u>CACCACTGTTCACAGACTGGATGACACAGAGTCCAGCAACCTGGCTAACAACAATGTGATTGAGGACAG</u> <u>CAGGGACAACATCTCTGTGGATGGCAAGCAGACCCAACTTTGTATTGTGGGCTGTACTCCTGCTATGGGAG</u> <u>AACACTGGACCAAGGGAGCAGTGTGTAAGAGCACCCAGGTGACCACAGGAGACTGTCCTCCACTGGCTCTG</u> <u>ATAAACACACCAATTGAGGATGGAGATATGATTGACACAGGCTTTGGAGCTATGGACTTCAAGGTGCTCCA</u> <u>AGAGAGCAAGGCTGAGGTGCCACTGGACATTGTCCAGAGCACTTGTAATAACCTGACTACCTGAAAATGA</u> <u>GTGCTGATGCCTATGGAGACAGTATGTGGTTCTACCTGAGGAGGGAACAACCTTTTGCCAGACACTACTTC</u> <u>AACAGGGCTGGCAAGGTGGGAGAGACCATCCCTGCTGAACTCTACCTGAAAGGCAGCAATGGCAGGGAAC</u> <u>CTCCTCCATCCTCTGTCTATGTGGCTACACCATCTGGCAGTATGATTACCTCTGAGGCTCAACTTTTCAACAA</u> <u>GCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAACTTTTGTGACAG</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 HPV56 L1 aa 1-467; [3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 469-499.

SEQ ID No: 140	5610 HPV56 L1 F2 GAAGTTCCTGCTCCAAGCAGGACTGAAAGCCAAGCC
SEQ ID No: 141	5611 HPV56 L1 R2 CTGTCTAGATTTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 142	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: 143	5613 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARPKPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

Appendix 12 : SEQUENCE LISTING -CHIMERIC HUMAN PAPILLOMAVIRUS TYPE 58 L1 PROTEIN

SEQ ID No: 144	5801 The amino acid sequence of HPV type 58 L1 protein aa 1-473 MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGNPYFSIKS PNNNKKVLVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPDTQRLVWACVGLE IGRGQPLGVGVSGHPYLNKFDDTETSNRYPAQPGSDNRECLSM DYKQTQLCLI GCKPPTGEHWGKG VACNNNAATDCPPELFNSIIEDGDMVDTGFGCMDFGTL QANKSDVPIDICNSTCKYPDYLKMASEPYGDSLFFFLRREQMFVRHFFNRAGKL GEAVPDDL YIKGSGNTAVIQSSAFFPTPSGSIVTSESQLFNKPYWLQRAQGHNN GICWGNQLFVTVVDTTTRSTNMTLCTEVTKEGTYKNDNFKEYVRHVEEYDLQF VFQLCKITLTAEIMTYIHTMDSNILEDWQFGLTPPPSASLQD TYRFVTSQAITCQ KTAPPKEKEDPLNKYTFWEVNLKEKFSADLDQFPLGRKFLLQSGL
SEQ ID No: 145	5802 The amino acid sequence of HPV type 33 L1 protein aa 474-499 KAKPKLKRAAPTSTR TSSAKRKKVKK
SEQ ID No: 146	5803 Amino acid sequence of chimeric HPV type 58 L1 protein MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGNPYFSIKS PNNNKKVLVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPDTQRLVWACVGLE IGRGQPLGVGVSGHPYLNKFDDTETSNRYPAQPGSDNRECLSM DYKQTQLCLI GCKPPTGEHWGKG VACNNNAATDCPPELFNSIIEDGDMVDTGFGCMDFGTL QANKSDVPIDICNSTCKYPDYLKMASEPYGDSLFFFLRREQMFVRHFFNRAGKL GEAVPDDL YIKGSGNTAVIQSSAFFPTPSGSIVTSESQLFNKPYWLQRAQGHNN GICWGNQLFVTVVDTTTRSTNMTLCTEVTKEGTYKNDNFKEYVRHVEEYDLQF VFQLCKITLTAEIMTYIHTMDSNILEDWQFGLTPPPSASLQD TYRFVTSQAITCQ KTAPPKEKEDPLNKYTFWEVNLKEKFSADLDQFPLGRKFLLQSGLKAKPKLKR AAPTSTR TSSAKRKKVKK

SEQ ID No: 147	5804 Nucleotide sequence of chimeric HPV type 58 L1 protein ATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGTGAGCAC CGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGGCCGTGGGCAAC CCCTACTTCAGCATCAAGAGCCCCAACAACAACAAGAAGGTGCTGGTGCCCAAGGTGAGCGGCCTGCAGTA CAGGGTGTTCAAGGTGAGGCTGCCCCACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTACAACCCCG ACACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGATCGGCAGGGGCCAGCCCCTGGGCGTGGGCGT GAGCGGCCACCCCTACCTGAACAAGTTCGACGACACCGAGACCAGCAACAGGTACCCCGCCAGCCCGGC AGCGACAACAGGGAGTGCCTGAGCATGGACTACAAGCAGACCCAGCTGTGCCTGATCGGCTGCAAGCCCC CCACCGGCGAGCACTGGGGCAAGGGCGTGGCCTGCAACAACAACGCCGCCGCCACCGACTGCCCCCCCCCTG GAGCTGTTCAACAGCATCATCGAGGACGGCGACATGGTGGACACCGGCTTCGGCTGCATGGACTTCGGCAC CCTGCAGGCCAACAAGAGCGACGTGCCCATCGACATCTGCAACAGCACCTGCAAGTACCCCGACTACCTGA AGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTTCTGAGGAGGGAGCAGATGTTTCGTGAGGCAC TTCTTCAACAGGGCCGGCAAGCTGGGCGAGGCCGTGCCCGACGACCTGTACATCAAGGGCAGCGGCAACA CCGCCGTGATCCAGAGCAGCGCCTTCTTCCCCACCCCAAGCGGCAGCATCGTGACCAGCGAGAGCCAGCTG TTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTGGGGCAACCAGCTGTT CGTGACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCACCGAGGTGACCAAGGAGGGCACC TACAAGAACGACAACCTTAAGGAGTACGTGAGGCACGTGGAGGAGTACGACCTGCAGTTCGTGTTCCAGCT GTGCAAGATCACCTGACCGCCGAGATCATGACCTACATCCACACCATGGACAGCAACATCCTGGAGGACT GGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGCAGGACACCTACAGGTTCGTGACCAGCCAGGCC ATCACCTGCCAGAAGACCGCCCCCCCCAAGGAGAAGGAGGACCCCTGAACAAGTACACCTTCTGGGAGG TGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCCTGCTGCAGAGC GGCCTGAAAGCCAAGCCAAAAGTAAAAAGGGCTGCCCCAACCAAGCAGCAGGACCTCCTCTGCCAAGAGGA AGAAGGTGAAGAAGTAAA
SEQ ID No:	5805 Synthetic HPV58 L1 gene

148	ctgggtaccATGAGCGTGTGGAGGCCAGCGAGGCCACCGTGTACCTGCCCCCGTGCCCGTGAGCAAGGTGGT GAGCACCGACGAGTACGTGAGCAGGACCAGCATCTACTACTACGCCGGCAGCAGCAGGCTGCTGGCCGTG GGCAACCCCTACTTCAGCATCAAGAGCCCCAACAACAACAAGAAGGTGCTGGTGCCCAAGGTGAGCGGCC TGCAGTACAGGGGTTCAGGGTGAGGCTGCCCCACCCCAACAAGTTCGGCTTCCCCGACACCAGCTTCTAC AACCCCGACACCCAGAGGCTGGTGTGGGCCTGCGTGGGCCTGGAGATCGGCAGGGGCCAGCCCTGGGCG TGGGCGTGAGCGGCCACCCCTACCTGAACAAGTTCGACGACACCGAGACCAGCAACAGGTACCCGCCCA GCCCCGGCAGCGACAACAGGGAGTGCTGAGCATGGACTACAAGCAGACCCAGCTGTGCCTGATCGGCTGC AAGCCCCCACC GGCGAGCACTGGGGCAAGGGCGTGCCCTGCAACAACAACGCCGCCGCCACCGACTGCC CCCCCTGGAGCTGTTCAACAGCATCATCGAGGACGGCGACATGGTGGACACCGGCTTCGGCTGCATGGAC TTCGGCACCTGCAGGCCAACAAGAGCGACGTGCCATCGACATCTGCAACAGCACCTGCAAGTACCCCGA CTACCTGAAGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTTCTGAGGAGGGAGCAGATGTTCTG TGAGGCACTTCTTCAACAGGGCCGGCAAGCTGGGCGAGGCCGTGCCCGACGACCTGTACATCAAGGGCAGC GGCAACACCGCCGTGATCCAGAGCAGCGCCTTCTTCCCCACCCCAAGCGGCAGCATCGTGACCAGCGAGAG CCAGCTGTTCAACAAGCCCTACTGGCTGCAGAGGGCCCAGGGCCACAACAACGGCATCTGCTGGGGCAACC AGCTGTTCTGTACCGTGGTGGACACCACCAGGAGCACCAACATGACCCTGTGCACCGAGGTGACCAAGGA GGGCACCTACAAGAACGACAACCTCAAGGAGTACGTGAGGCACGTGGAGGAGTACGACCTGCAGTTCGTG TTCCAGCTGTGCAAGATCACCTGACCGCCGAGATCATGACCTACATCCACCATGGACAGCAACATCCT GGAGGACTGGCAGTTCGGCCTGACCCCCCCCCCAGCGCCAGCCTGCAGGACACCTACAGGTTCTGTACCA GCCAGGCCATCACCTGCCAGAAGACCGCCCCCCCCAAGGAGAAGGAGGACCCCTGAACAAGTACACCTT CTGGGAGGTGAACCTGAAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCAGGAAGTTCCTGC TGCAGAGCGGCCTGAAGGCCAAGCCCAGGCTGAAGAGGAGCGCCCCACCACCAGGGCCCCCAGCACCAA GAGGAAGAAGGTGAAGAAGTAAA ctgagctc
SEQ ID No:	5806 Synthetic HPV33 L1 gene
149	ctgggtaccATGAGTGTGTGGAGACCATCTGAGGCTACAGTCTACCTGCCTCCTGTGCCTGTGAGCAAGGTGGTG

	AGCACAGATGAATATGTGAGCAGGACCAGCATCTACTACTATGCTGGCTCCAGCAGACTGCTGGCTGTGGG ACACCCATACTTCAGCATCAAGAACCAACCAATGCCAAGAACTGCTGGTGCCAAAGGTGTCTGGACTCC AATACAGGGTGTTCAGGGTGAGACTGCCTGACCCAAAACAAGTTTGGCTTTCCTGACACCTCCTTCTACAACC CTGACACCCAGAGACTGGTGTGGGCTTGTGTGGGATTGGAGATTGGCAGGGGACAACCACTGGGAGTGGG CATCTCTGGACACCACTGCTGAACAAGTTTGATGACACAGAGACAGGCAACAAATACCCTGGACAACCTG GAGCAGACAACAGGGAGTGTCTGAGTATGGACTACAAGCAGACCCAACCTTTGTCTGCTGGGCTGTAAGCCT CCAACAGGAGAACACTGGGGCAAGGGAGTGGCTTGTACCAATGCTGCCCTGCCAATGACTGTCCTCCATT GGAAGTATAAACACCATCATTGAGGATGGAGATATGGTGGACACAGGCTTTGGCTGTATGGACTTCAAGA CCCTCCAAGCCAACAAGTCTGATGTGCCAATTGACATCTGTGGCAGCACTTGTAATACCCTGACTACCTGA AAATGACCTCTGAACCATATGGAGACTCCCTGTTCTTCTTCTGAGGAGGGAACAGATGTTTGTGAGACT TCTTCAACAGGGCTGGCACCTGGGAGAGGCTGTGCCTGATGACCTCTACATCAAGGGCTCTGGCACCACA GCCAGCATCCAGTCCTCTGCCTTCTTCCAACACCATCTGGCAGTATGGTGACCTCTGAGAGCCAACCTTTC AACAAGCCATACTGGCTCCAAAGGGCTCAAGGACACAACAATGGCATCTGTTGGGGCAACCAGGTGTTTGT GACAGTGGTGGACACCACCAGGAGCACCAATATGACCTGTGTACCCAGGTGACCTCTGACAGCACCTACA AGAATGAGAACTTCAAGGAATACATCAGGCATGTGGAGGAATATGACCTCCAATTTGTGTTCCAACCTTGT AAGGTGACCCTGACAGCAGAGGTGATGACCTACATCCATGCTATGAACCCTGACATCTTGGAGGACTGGCA GTTTGGACTGACACCTCCTCCATCTGCCTCCCTCCAAGACACCTACAGGTTTGTGACCAGCCAGGCTATCAC TTGTCAGAAGACAGTGCCTCCAAAGGAGAAGGAGGACCACTGGGCAAATACACCTTCTGGGAGGTGGAC CTGAAAGAGAAGTTCTCTGCTGACCTGGACCAAGTTTCCACTGGGCAGGAAGTTCCTGCTCCAAGCAGGACT GAAAGCCAAGCCAAAACCTGAAAAGGGCTGCCCCAACAGCACCAGGACCTCCTCTGCCAAGAGGAAGAAG GTGAAGAAGTAAActcgagctc
SEQ ID No: 150	5807 HPV58 L1 F1 CTTGGTACCATGAGCGTGTGGAGGCCAGCGAGG
SEQ ID No: 151	5808 HPV58 L1 R1 GCTTGGCTTTCAGGCCGCTCTGCAGCAGGAACTTCC

SEQ ID No:	5809 HPV58 L1 amplified sequence 1
152	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>GAGCTGTTCAACAGCATCATCGAGGACGGCGACATGGTGGACACCGGCTTCGGCTGCATGGACTTCGGCAC</u> <u>CCTGCAGGCCAACAAGAGCGACGTGCCCATCGACATCTGCAACAGCACCTGCAAGTACCCCGACTACCTGA</u> <u>AGATGGCCAGCGAGCCCTACGGCGACAGCCTGTTCTTCTCCTGAGGAGGGAGCAGATGTTCTGTGAGGCAC</u> <u>TTCTTCAACAGGGCCGGCAAGCTGGGCGAGGCCGTGCCCGACGACCTGTACATCAAGGGCAGCGGCAACA</u> <u>CCGCCGTGATCCAGAGCAGCGCCTTCTTCCCCACCCCCAGCGGCAGCATCGTGACCAGCGAGAGCCAGCTG</u> <u>TTCAACAAGCCCTACTGGCTGCAGAGGGGCCAGGGCCACAACAACGGCATCTGCTGGGGCAACCAGCTGTT</u> <u>CGTGACCGTGGTGGACACCACGAGCACCAACATGACCCTGTGCACCGAGGTGACCAAGGAGGGCACC</u> <u>TACAAGAACGACAACCTTCAAGGAGTACGTGAGGCACGTGGAGGAGTACGACCTGCAGTTCTGTGTTCCAGCT</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 HPV58 L1 aa 1-473;

	[3]: 10 bases overlapping with the gene fragment encoding HPV33L1 aa 474-499.
SEQ ID No: 153	5810 HPV58 L1 F2 GAGCGGCCTGAAAGCCAAGCCAAACTGAAAAGGG
SEQ ID No: 154	5811 HPV58 L1 R2 CTGTCTAGATTACTTCTTCACCTTCTTCCTCTTGG
SEQ ID No: 155	5812 HPV58 L1 amplified sequence 2 <u>GAGCGGCCTG</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 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: 156	5813 The amino acid sequence of HPV type 59 L1 protein aa 471-508 LQLGARP KPTIGPRKRAAPAPTSTPSPKRVKRRKSSRK

What is claimed is:

1. A chimeric papilloma virus L1 protein comprising, 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; 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 better expression level and solubility compared to the L1 proteins of other types;

wherein said chimeric papilloma virus L1 protein has the immunogenicity of the L1 protein of the first papilloma virus type.

2. The chimeric papilloma virus L1 protein according to Claim 1, 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;

Preferably, 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.

3. The chimeric papilloma virus L1 protein according to Claim 2, wherein said C-terminal fragment contains one or plurality of nuclear localization sequences.

4. The chimeric papillomavirus L1 protein according to any one of Claims 1-3, wherein said papilloma virus L1 protein is an HPV L1 protein.

5. The chimeric papilloma virus L1 protein according to any one of Claims 1-4, 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 HPV 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.

6. The chimeric papilloma virus L1 protein according to Claim 5, 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: 132; or a fragment having a length of m2 amino acids, preferably covering amino acids 1-m2 position of SEQ ID No: 132; wherein m2 is an integer of 13-31.

7. The chimeric papilloma virus L1 protein according to Claim 5, wherein said C-terminal fragment is SEQ ID No: 13; or a fragment thereof having n amino acids in length, preferably a fragment covering amino acids 1-n of SEQ ID No: 13; wherein n is an integer of 16-38.

8. The chimeric papilloma virus L1 protein according to any one of Claims 1-7, 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,

preferably, selected from

HPV type 6 L1 protein encoded by the gene thereof shown in SEQ ID No: 5,
HPV type 11 L1 protein encoded by the gene thereof shown in SEQ ID No: 18,
HPV type 16 L1 protein encoded by the gene thereof shown in SEQ ID No: 31,
HPV type 18 L1 protein encoded by the gene thereof shown in SEQ ID No: 44,
HPV type 31 L1 protein encoded by the gene thereof shown in SEQ ID No: 57,
HPV type 35 L1 protein encoded by the gene thereof shown in SEQ ID No: 70,

HPV type 39 L1 protein encoded by the gene thereof shown in SEQ ID No: 83,
HPV type 45 L1 protein encoded by the gene thereof shown in SEQ ID No: 96,
HPV type 51 L1 protein encoded by the gene thereof shown in SEQ ID No: 109,
HPV type 52 L1 protein encoded by the gene thereof shown in SEQ ID No: 122,
HPV type 56 L1 protein encoded by the gene thereof shown in SEQ ID No: 135, or
HPV type 58 L1 protein encoded by the gene thereof shown in SEQ ID No: 148.

9. The chimeric papilloma virus L1 protein according to Claim 8, wherein the L1 protein of the first papilloma virus type is selected from

HPV type 6 L1 protein shown in SEQ ID No: 1,
HPV type 11 L1 protein shown in SEQ ID No: 14,
HPV type 16 L1 protein shown in SEQ ID No: 27,
HPV type 18 L1 protein shown in SEQ ID No: 40,
HPV type 31 L1 protein shown in SEQ ID No: 53,
HPV type 35 L1 protein shown in SEQ ID No: 66
HPV type 39 L1 protein shown in SEQ ID No: 79,
HPV type 45 L1 protein shown in SEQ ID No: 92,
HPV type 51 L1 protein shown in SEQ ID No: 105,
HPV type 52 L1 protein shown in SEQ ID No: 118,
HPV type 56 L1 protein shown in SEQ ID No: 131, or
HPV type 58 L1 protein shown in SEQ ID No: 144.

10. The chimeric papilloma virus L1 protein according to any one of Claims 1 to 9, said 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 chimeric papilloma virus L1 protein according to any one of Claims 1 to 10, 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 chimeric papillomavirus L1 protein according to claim 11, selected from chimeric HPV L1 protein of HPV Types 6, 11, 16, 18, 31, 35, 39, 45, 51, 52, 56, and 58 has 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: 68, SEQ ID No: 81, SEQ ID No: 94, SEQ ID No: 107, SEQ ID No: 120, SEQ ID No: 133 and SEQ ID No: 146, respectively.

13. A papilloma virus-like particle, comprising the chimeric papilloma virus L1 protein of any one of Claims 1 to 12.

14. An immunogenic composition for the prevention of papilloma virus-associated diseases or infections, comprising the papilloma virus-like particles according to Claim 13 and adjuvants.

15. An isolated polynucleotide encoding the chimeric papilloma virus L1 protein of any one of Claims 1 to 12.

16. The polynucleotide according to claim 15, selected from
the nucleotide sequence encoding the chimeric HPV type 6 L1 protein which is shown in SEQ ID No: 4,
the nucleotide sequence encoding the chimeric HPV type 11 L1 protein which is shown in SEQ ID No: 17,
the nucleotide sequence encoding the chimeric HPV type 16 L1 protein which is shown in SEQ ID No: 30,
the nucleotide sequence encoding the chimeric HPV type 18 L1 protein which is shown in SEQ ID No: 43,
the nucleotide sequence encoding the chimeric HPV type 31 L1 protein which is shown in SEQ ID No: 56,
the nucleotide sequence encoding the chimeric HPV type 35 L1 protein which is shown in SEQ ID No: 69,
the nucleotide sequence encoding the chimeric HPV type 39 L1 protein which is shown

in SEQ ID No: 82,
the nucleotide sequence encoding the chimeric HPV type 45 L1 protein which is shown
in SEQ ID No: 95,
the nucleotide sequence encoding the chimeric HPV type 51 L1 protein which is shown
in SEQ ID No: 108,
the nucleotide sequence encoding the chimeric HPV type 52 L1 protein which is shown
in SEQ ID No: 121,
the nucleotide sequence encoding the chimeric HPV type 56 L1 protein which is shown
in SEQ ID No: 134, or
the nucleotide sequence encoding the chimeric HPV type 58 L1 protein which is shown
in SEQ ID No: 147.

17. A vector comprising the polynucleotide according to Claim 15 or 16.

18. The vector according to Claim 17, wherein said vector is a baculovirus
vector.

19. A baculovirus comprising the polynucleotide according to Claim 15 or 16.

20. A host cell comprising a polynucleotide according to Claim 15 or 16, a
vector according to Claims 17 or 18, or a baculovirus according to Claim 19.

21. A host cell according to Claim 20, which is an insect cell, preferably said
insect cell is selected from Sf9 cells, Sf21 cells, Hi5 cells and S2 cells.

22. A method for preparing a papilloma virus-like particle according to Claim
11, comprising,

culturing host cells of any one of Claims 20-21 to express said chimeric
papilloma virus L1 protein and to assemble into virus-like particles; and
purifying said papillomavirus-like particles.

23. The method according to Claim 22, wherein said purification is carried out

using cation exchange chromatography, preferably, said cation exchange chromatography is HS-MMA two-step chromatography.