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(54) **TRUNCATED L1 PROTEIN OF HUMAN PAPILLOMAVIRUS TYPE 6**

(71) Applicants: **XIAMEN UNIVERSITY**, Xiamen, Fujian Province (CN); **BEIJING WANTAI BIOLOGICAL PHARMACY ENTERPRISE CO., LTD.**, Changping District, Beijing (CN)

(72) Inventors: **Shaowei Li**, Xiamen (CN); **Huirong Pan**, Xiamen (CN); **Bo Liu**, Xiamen (CN); **Jun Zhang**, Xiamen (CN); **Ji Miao**, Xiamen (CN); **Ningshao Xia**, Xiamen (CN)

(73) Assignees: **XIAMEN UNIVERSITY**, Fujian Province (CN); **BEIJING WANTAI BIOLOGICAL PHARMACY ENTERPRISE CO., LTD.**, Beijing (CN)

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(51) **Int. Cl.**

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CPC ***C07K 14/005*** (2013.01); ***A61K 39/12*** (2013.01); ***C12N 7/00*** (2013.01); ***C12P 21/00*** (2013.01); *A61K 2039/5258* (2013.01); *A61K 2039/55566* (2013.01); *A61K 2039/70* (2013.01); *C07K 14/025* (2013.01); *C12N 2700/00* (2013.01); *C12N 2710/00* (2013.01); *C12N 2710/20022* (2013.01); *C12N 2710/20034* (2013.01); *G01N 2333/025* (2013.01)

(58) **Field of Classification Search**

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See application file for complete search history.

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Primary Examiner — Rachel B Gill

(74) *Attorney, Agent, or Firm* — Pillsbury Winthrop Shaw Pittman LLP

(57) **ABSTRACT**

The invention relates to a truncated L1 protein of the Human Papillomavirus Type 6, a virus-like particle consisting of the truncated L1 protein, a vaccine comprising said virus-like particle, and the use of the vaccine in the prevention of condyloma acuminatum or HPV infections.

15 Claims, 7 Drawing Sheets

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FIG. 1

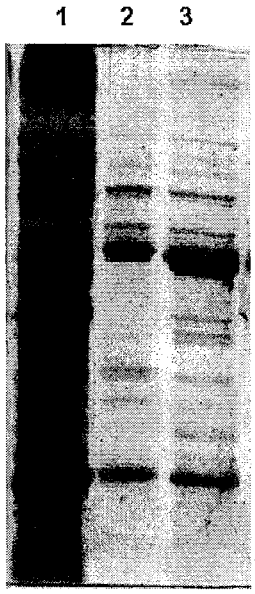


FIG. 2

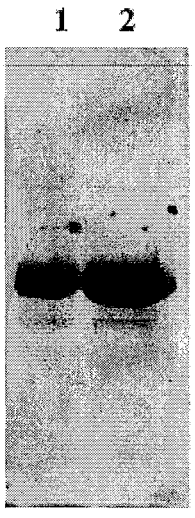


FIG. 3

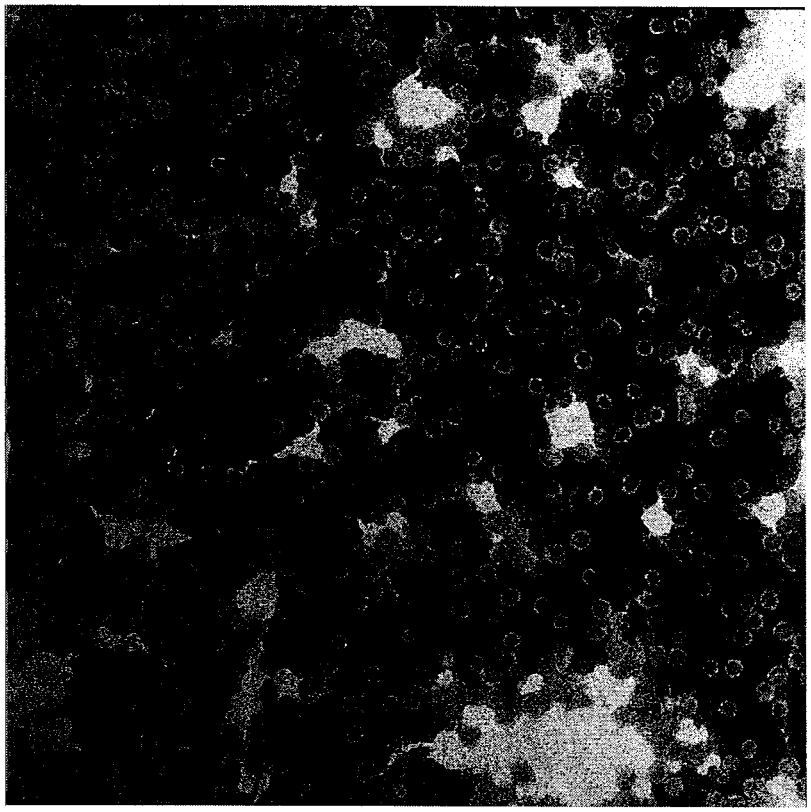
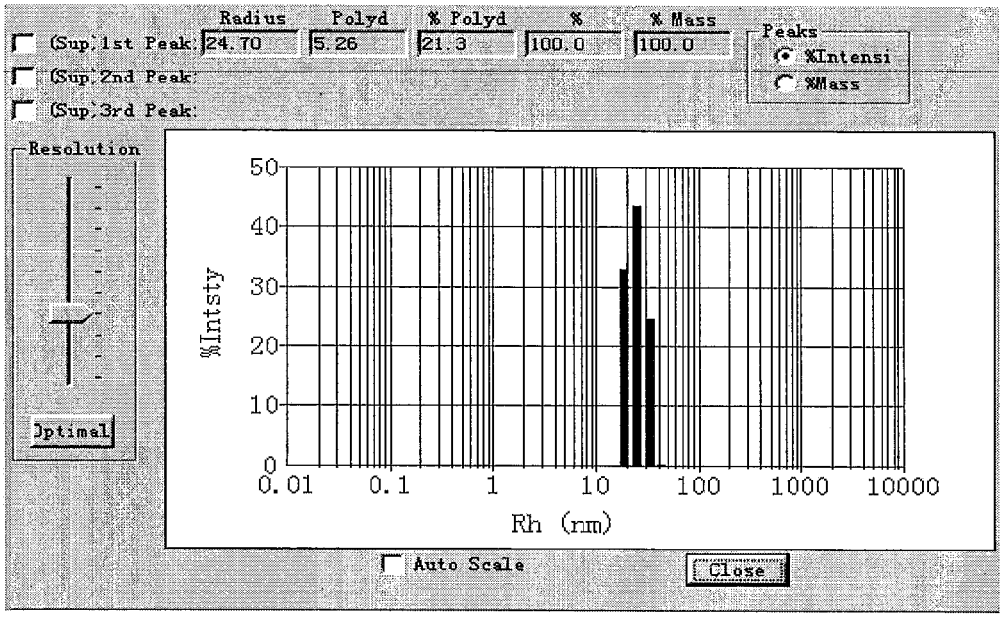


FIG. 4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Polyd
% Polyd
%
% Mass
(Sup)1st Peak:
24.70
5.26
21.3
100.0
100.0
(Sup)2nd Peak:
(Sup)3rd Peak:
Peaks
%Intensi
%Mass
Resolution
Optimal
%Intsty
50
40
30
20
10
0
0.01
0.1
1
10
100
1000
10000
Rh (nm)
Auto Scale
Close

FIG. 5

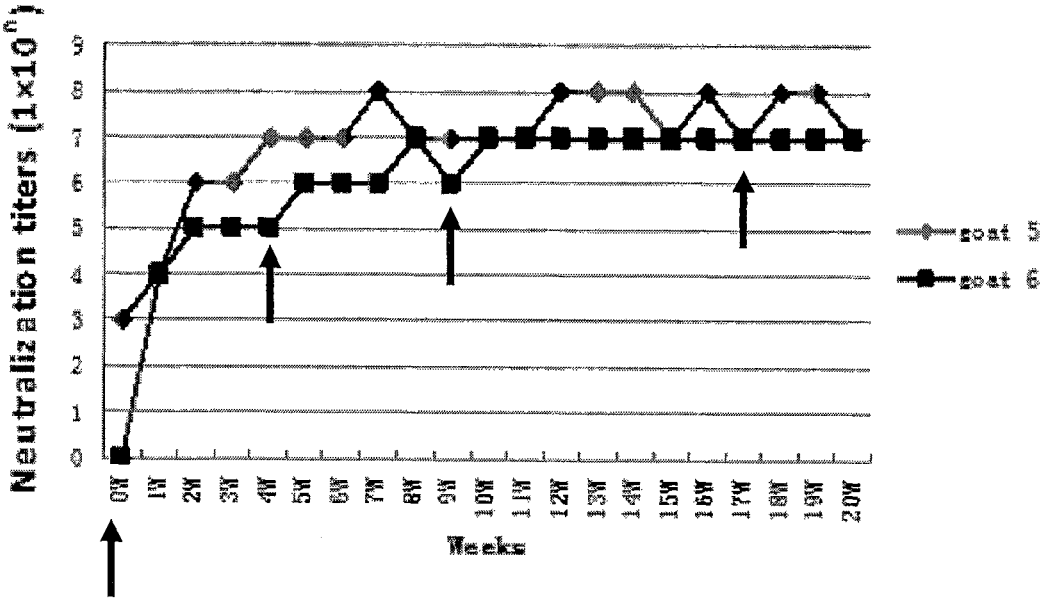

Neutralization titers (1×10^n)
0
1
2
3
4
5
6
7
8
9
0W
1W
2W
3W
4W
5W
6W
7W
8W
9W
10W
11W
12W
13W
14W
15W
16W
17W
18W
19W
20W
Weeks
goat 5
goat 6

FIG. 6

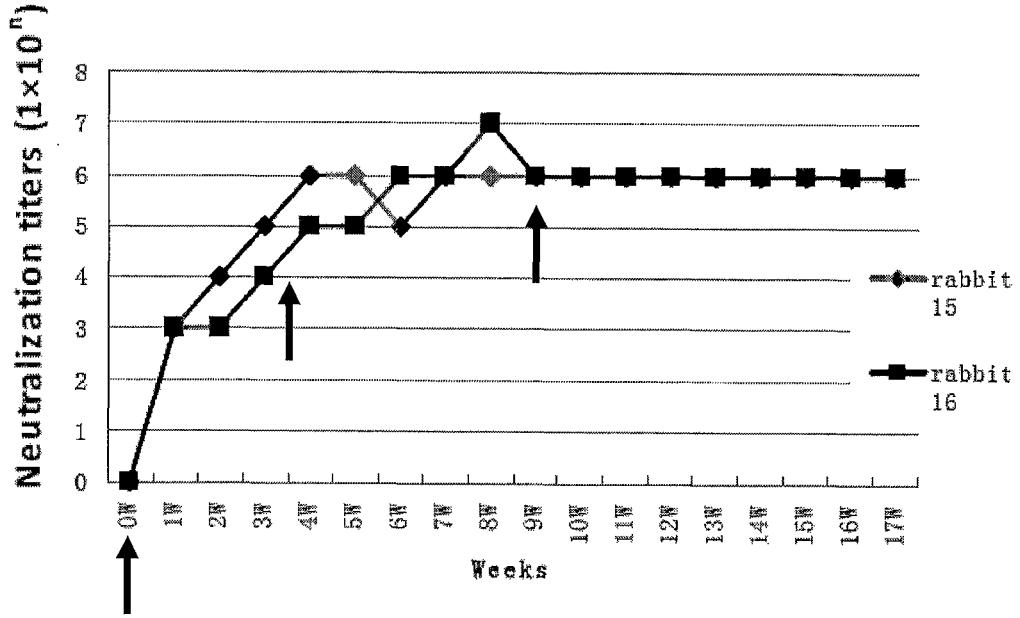

Neutralization titers (1×10^n)
0
1
2
3
4
5
6
7
8
0W
1W
2W
3W
4W
5W
6W
7W
8W
9W
10W
11W
12W
13W
14W
15W
16W
17W
Weeks
rabbit 15
rabbit 16

FIG. 7

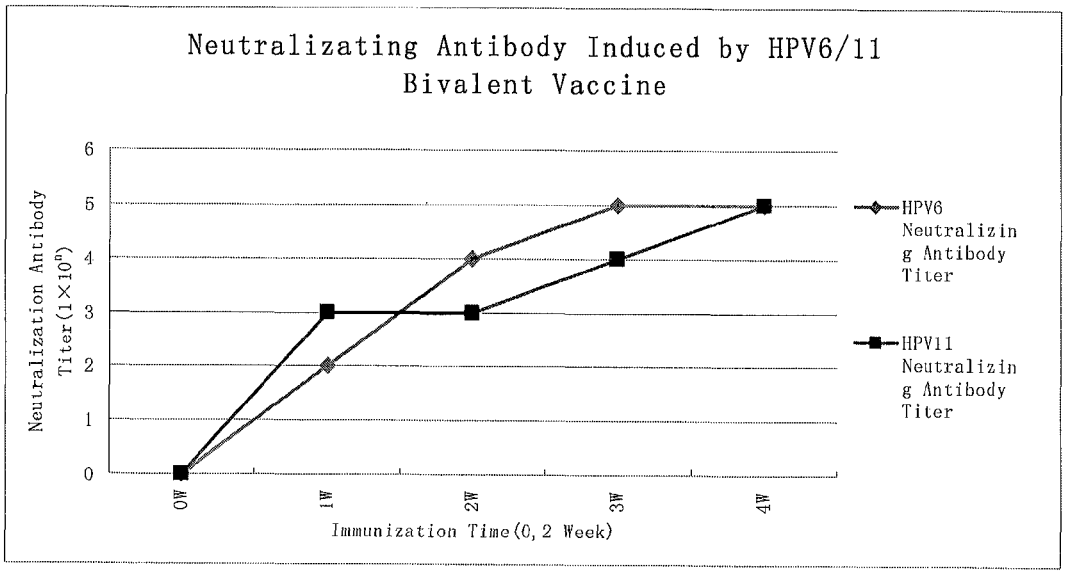

Neutralizating Antibody Induced by HPV6/11
Bivalent Vaccine
Neutralization Antibody
Titer(1×10n)
0
1
2
3
4
5
6
0W
1W
2W
3W
4W
Immunization Time(0, 2 Week)
HPV6
Neutralizin
g Antibody
Titer
HPV11
Neutralizin
g Antibody
Titer

FIG. 8

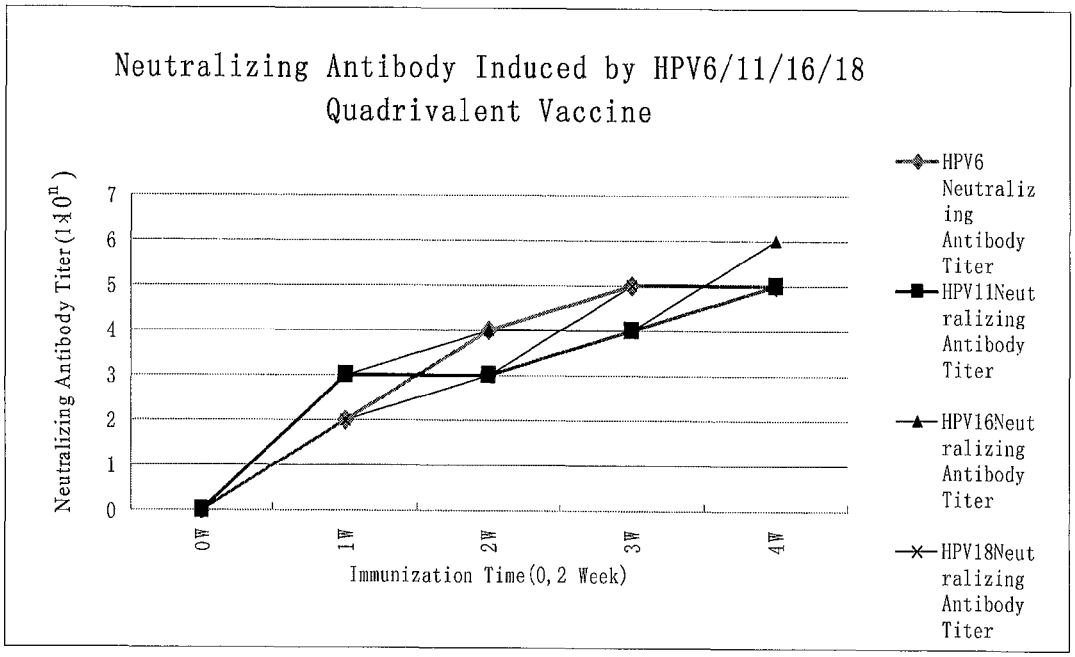

Neutralizing Antibody Induced by HPV6/11/16/18
Quadrivalent Vaccine
Neutralizing Antibody Titer(1×10n)
0
1
2
3
4
5
6
7
0W
1W
2W
3W
4W
Immunization Time(0, 2 Week)
HPV6
Neutraliz
ing
Antibody
Titer
HPV11Neut
ralizing
Antibody
Titer
HPV16Neut
ralizing
Antibody
Titer
HPV18Neut
ralizing
Antibody
Titer

FIG. 9

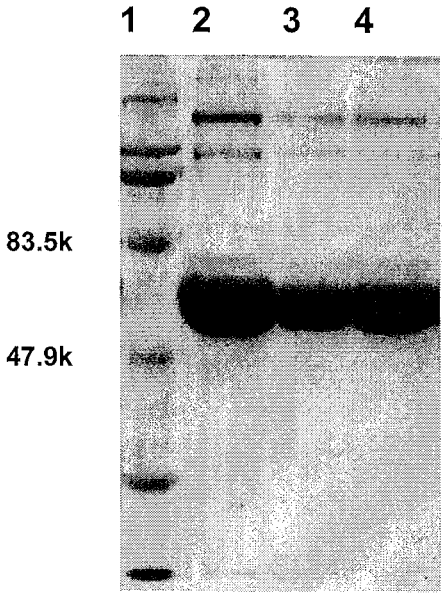


FIG. 10

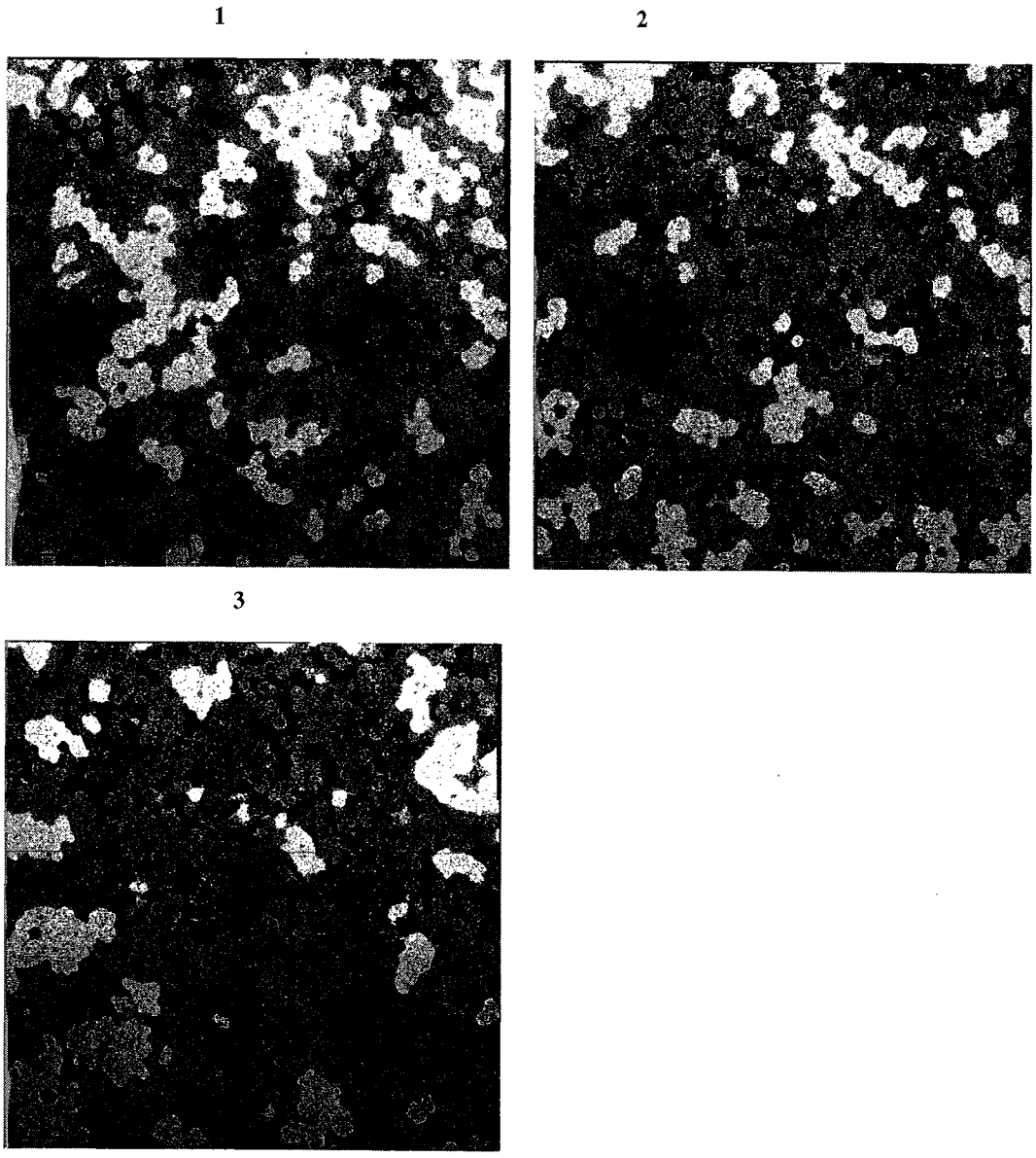
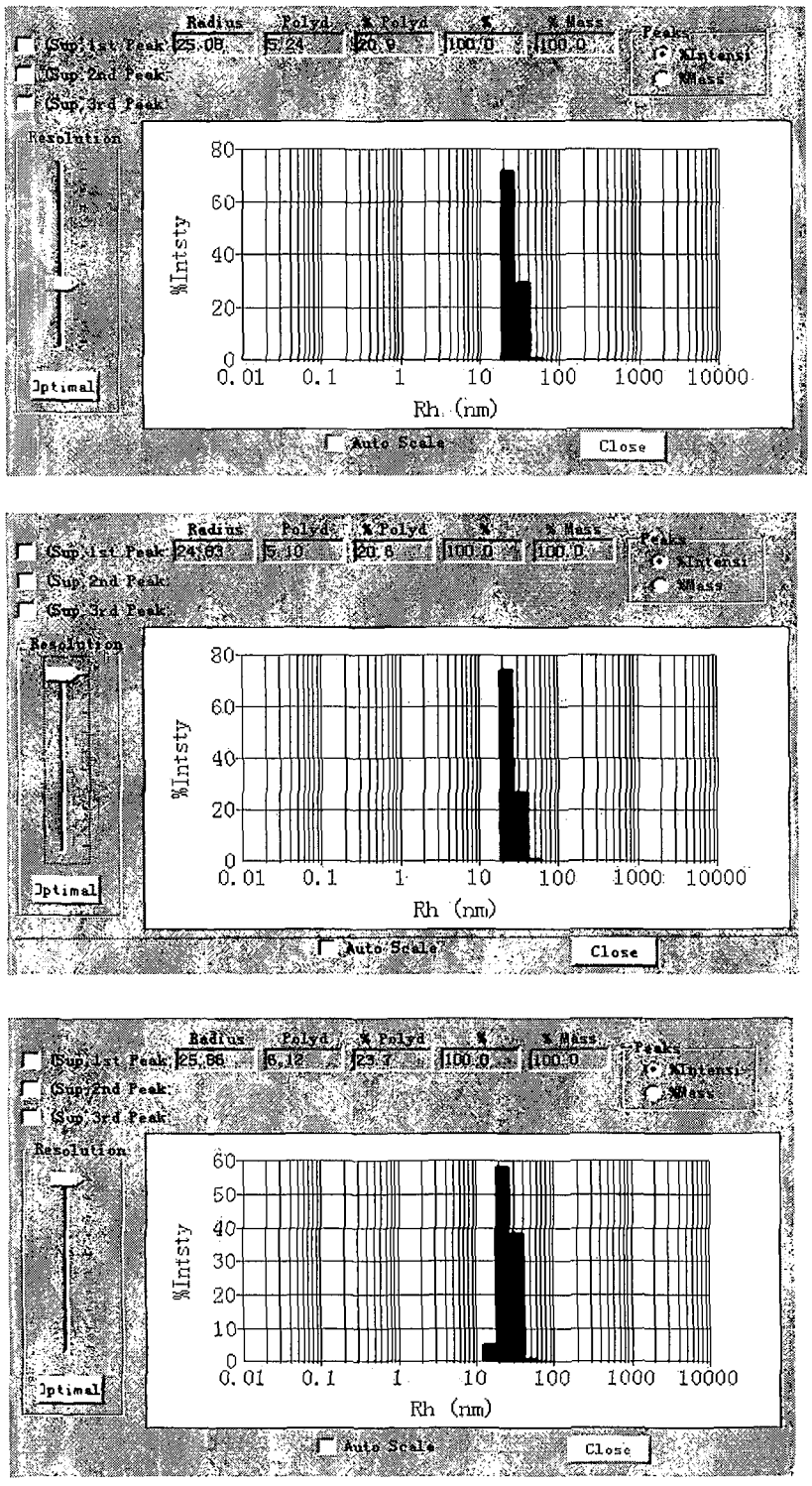


FIG. 11


1
Radius
Polyd.
% Polyd
%
% Mass
Peaks
(Sup)1st Peak
(Sup)2nd Peak
(Sup)3rd Peak
Resolution
Optimal
%Intsty
Rh (nm)
Auto Scale
Close
2
Radius
Polyd.
% Polyd
%
% Mass
Peaks
(Sup)1st Peak
(Sup)2nd Peak
(Sup)3rd Peak
Resolution
Optimal
%Intsty
Rh (nm)
Auto Scale
Close
3
Radius
Polyd.
% Polyd
%
% Mass
Peaks
(Sup)1st Peak
(Sup)2nd Peak
(Sup)3rd Peak
Resolution
Optimal
%Intsty
Rh (nm)
Auto Scale
Close

TRUNCATED L1 PROTEIN OF HUMAN PAPILLOMAVIRUS TYPE 6

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation of co-pending U.S. application Ser. No. 12/601,972, filed Dec. 30, 2009, which is a U.S. National Stage Application of PCT/CN2008/001050, filed May 29, 2008, which in turn claims priority to Chinese Patent Application No. 200710105764.7, filed May 29, 2007, the entire contents of all applications are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

The invention relates to a truncated L1 protein of the Human Papillomavirus Type 6, a virus-like particle consisting of the protein, a vaccine comprising said virus-like particle, and the use of the vaccine in the prevention of condyloma acuminatum and HPV (especially HPV6) infection.

BACKGROUND OF THE INVENTION

The human papillomavirus, a non-enveloped, deoxyribonucleic acid (DNA) virus, belongs to the genus of papovaviridae. The viral genome is a closed circle, double-stranded DNA, which is approximately 7.2-8 kb in length and contains 8 open reading frames (ORFs). The genome can be divided into three parts in terms of function: (1) the early region (E), approximately 4.5 Kb in length, coding for 6 non-structural proteins E1, E2, E4~E7 associated with virus replication, transcription and transformation; (2) the late region (L), approximately 2.5 Kb in length, coding for the major capsid protein L1 and the minor capsid protein L2; (3) the long control region (LCR), located between the end of the L region and the initiating terminal of the E region, approximately 800-900 bp in length, and comprising regulator elements for DNA replication and expression instead of coding for proteins. Viral particles are 45-55 nm in diameter, wherein the nucleocapsid, consisting of L1 and L2, exhibits icosahedral symmetry and comprise 72 capsomers.

Currently, there are over 90 different types of HPV, mainly causing papillary disease in the skin and mucosa of human. HPV types are divided into three groups depending on their relation with tumorigenesis: (1) group of low or no cancerogenic risk, containing types 6, 11, 39, 41, 42, and 43; (2) group of medium cancerogenic risk, containing types 31, 33, 35, 51, and 52; and (3) group of high cancerogenic risk, containing types 16, 18, 45, and 56.

Epidemiological investigation reveals that HPV (such as HPV6, 11) infection in the anal-genital mucosa is the third most common sexually transmitted disease following trichommoniasis and chlamydia. Pathological changes caused by HPV types 6 and 11 account for about 90% of these cases. In America, HPV infection of genital meatus among women occurs most frequently when they are 15-25 years old and is highly related to the infected person's sexual behavior. In China, HPV infection among women occurs most frequently when they are 20-29 years old, and the infection rate is 1606.1/100,000. Women are less infected with HPV as they grow older than 35. However, since the majority of HPV infections are sub-clinical, it is difficult to accurately estimate the infection rate. As estimated by the US CDC, the risk is approximately 10% during the whole life. In addition, there is little data regarding HPV infection among men, due to the difficulty of sample collection and the lesser severity of consequences. Currently, HPV infection rate among men is believed to be close to the one among women. In the United States, condyloma acuminatum can be found in 1% of sexually active adult men. Therefore, the development of a safe, efficient vaccine for HPV 6 and 11 would be an effective way to prevent sexually transmitted diseases.

HPV L1 protein, with a molecular weight of 55-60 kDa, is the major capsid protein of the human papillomavirus and the main target protein of the HPV vaccine. HPV L1 protein expressed in multiple different expression systems can form Virus-like particles (VLPs) which resemble native HPV particles morphologically, without the assistance of the L2 protein. The VLP, consisting of 72 pentamers of the L1 proteins, exhibits icosahedral symmetry. Since the VLPs retain the native epitopes of the viral particles, they are highly immunogenic and can induce the generation of neutralizing antibodies against homologous HPV (Kirnbauer, R., F. Booy, et al. 1992 Proc Natl Acad Sci USA 89(24): 12180-4). Furthermore, the VLPs are safe and have no potential cancergenic risk as they contain no viral DNA. Therefore, VLP vaccines become the primary candidate for an HPV vaccine.

The key for development of a vaccine is to efficiently produce VLP vaccines of HPV in large-scale. Currently, the most commonly used expression systems are eukaryotic expression systems and prokaryotic expression systems.

The commonly used eukaryotic systems comprise poxvirus, insect baculovirus and yeast vectors. HPV L1 protein expressed in eukaryotic systems shows little conformational difference from that of the native virus, and can self-assemble into VLPs. Thus, purified VLPs can be easily obtained after gradient density centrifugation. It brings a lot of convenience to the purification work. However, due to the high culture costs and low expression level, it is quite difficult to product industrially on a large-scale. The HPV vaccine Gardasil®, which came into the market recently, is more expensive than others due to low expression level and high production cost of the *Saccharomyces cerevisiae* expression system employed in its manufacture.

The expression of HPV L1 protein in a prokaryotic system such as *E. coli* has been previously reported. Banks, Matlashewski, et al. published a paper regarding the expression of HPV 16 L1 by employing *E. coli* (Banks, L., G. Matlashewski, et al. (1987). J Gen Virol 68 (Pt 12): 3081-9). However, most HPV L1 proteins expressed by *E. coli* lose their native conformation and cannot induce the generation of protective antibodies against HPV. Alternatively, although HPV VLPs can be obtained from the incorrectly folded proteins by steps such as purification from inclusion bodies and refolding, it is difficult to apply this method to large-scale production, as the protein is largely lost during the refolding process and the yield is low (Kelsall, S. R. and J. K. Kulski (1995). J Virol Methods 53(1): 75-90). Although HPV L1 protein may be expressed in a soluble form with a correct conformation in *E. coli* and dissolved in the supernatants of *E. coli* lysate, the expression level is low. Moreover, since there are large number and amount of impure proteins, it is difficult to isolate the proteins of interest from them. Although it is reported that the expression level of L1 protein can be increased in the supernatants by means of GST fusion expression and the purification of the protein of interest is facilitated (Li, M., T. P. Cripe, et al. (1997), J Virol 71(4): 2988-95), it still cannot be applied to large-scale production because expensive enzymes are required to cleave the fusion protein.

Therefore, a HPV L1 protein capable of inducing the generation of protective antibodies against HPV, and a virus-like particle consisting of the same are still needed in the art, so that it is possible to produce vaccines for condyloma acuminatum industrially on a large scale.

DESCRIPTION OF THE INVENTION

This invention aims to provide a novel HPV type 6 L1 protein, the virus-like particles (VLPs) consisting of it, and a vaccine comprising the VLPs.

During research, it was found by chance that the *E. coli* expression system can produce a truncated HPV 6 L1 protein that can induce the generation of neutralizing antibodies against HPV 6. After purification, the truncated HPV6 L1 protein can be produced in high yield, with at least 50% purity. Further treatment of the purified HPV L1 protein can produce VLPs, which can induce the production of neutralizing antibodies against HPV6. The invention has been completed based on the above.

Therefore, the first aspect of the invention relates to HPV 6 L1 proteins with 2, 3, 4, or 5 amino acids truncated at N-terminal as compared to a wild type HPV 6 L1 protein. Preferably, the truncated protein has the sequence set forth in SEQ ID Nos:1, 2, 3, or 4, especially the sequence set forth in SEQ ID NO:1.

A further aspect of the invention relates to a polynucleotide encoding the truncated protein according to the invention, and a vector containing the polynucleotide.

A further aspect of the invention relates to a cell comprising the vector.

The invention also relates to a composition comprising the truncated protein, the polynucleotide, the vector, or the cell.

A further aspect of the invention relates to a HPV 6 VLP, comprising or consisting of a HPV 6 L1 protein with 2, 3, 4, or 5 amino acids truncated at the N terminal such as a HPV 6 L1 protein having a sequence set forth in SEQ ID NOs: 1, 2, 3, or 4.

A further aspect of the invention relates to a method for obtaining the HPV 6 L1 protein, comprising the expression of a truncated HPV 6 L1 gene fragment in an *E. coli* system and the subsequent purification of the protein from the lysate supernatant.

In a preferred embodiment of the invention, a method for obtaining HPV 6 L1 protein comprises:

a) expressing the truncated HPV 6 L1 gene fragment in a *E. coli* expression system;

b) disrupting *E. coli*, which has expressed the truncated HPV 6 L1 protein, in a salt solution at a concentration of from 100 mM to 600 mM, and isolating the supernatant;

c) decreasing the salt concentration of the supernatant in b) to from 100 mM to 0, inclusive, by using water or a low salt solution, and collecting a precipitate;

d) redissolving the precipitation in c) in a salt solution at a concentration of from 150 mM to 2500 mM, with a reductant added, and then isolating the resultant solution, wherein the solution contains the truncated HPV 6 L1 protein with a purity of at least 50%.

More generally, the invention also relates to a method for obtaining a HPV L1 protein, such as the HPV 6 L1 protein according to the invention, comprising:

a) expressing a HPV L1 gene encoding the HPV L1 protein in an *E. coli* expression system;

b) disrupting *E. coli*, which has expressed the HPV L1 protein, in a salt solution at a concentration of from 100 mM to 600 mM, and isolating the supernatant;

c) decreasing the salt concentration of the supernatant in b) to from 100 mM to 0, inclusive, by using water or a low salt solution, and collecting a precipitate;

d) redissolving the precipitation of c) in a salt solution at a concentration of from 150 mM to 2500 mM, with a reductant added, and then isolating the resultant solution, wherein the solution contains the HPV L1 protein with a purity of at least 50%.

The invention also relates to a vaccine for the prevention of condyloma acuminatum or HPV infection, comprising VLPs of HPV 6 L1 proteins according to the invention. Preferably, the vaccine further comprises at least one VLPs selected from VLPs of HPV 18, 11, 16, 31, 33, 45, 52, and 58 L1 proteins. Generally, the vaccine further contains excipients or vectors for vaccine.

Preferably, the vaccine comprises HPV 6 VLPs and HPV 11 VLPs, especially the HPV 6 VLPs comprising or consisting of a protein having an amino acid sequence set forth in SEQ ID No: 4, and the HPV 11 VLPs comprising or consisting of a protein having an amino acid sequence set forth in SEQ ID No: 7. More preferably, the vaccine further comprises HPV 16 VLPs and HPV 18 VLPs, especially the HPV 16 VLPs comprising or consisting of a protein having an amino acid sequence set forth in SEQ ID No: 8, and the HPV 18 VLPs comprising or consisting of a protein having an amino acid sequence set forth in SEQ ID No: 9.

In a specially preferred embodiment, the vaccine comprises the HPV 6 VLPs comprising or consisting of a protein having an amino acid sequence set forth in SEQ ID No: 4, the HPV 11 VLPs comprising or consisting of a protein having an amino acid sequence set forth in SEQ ID No: 7, the HPV 16 VLPs comprising or consisting of a protein having an amino acid sequence set forth in SEQ ID No: 8, and the HPV 18 VLPs comprising or consisting of a protein having an amino acid sequence set forth in SEQ ID No: 9.

The invention further relates to the use of the HPV 6 L1 protein or the VLPs thereof in the manufacture of a vaccine for the prevention of condyloma acuminatum or HPV infections.

The invention further relates to a method for preventing condyloma acuminatum or HPV infections, comprising administrating a vaccine comprising an preventively effective amount of HPV 6 L1 protein to a human or animal in need of preventing condyloma acuminatum or HPV infections.

The invention involves a method for obtaining VLPs of the HPV 6 L1 protein, comprising:

e) further purifying the truncated HPV 6 L1 protein with a purity of at least 50% by subjecting it to a chromatography;

f) removing the reductant from the HPV 6 L1 protein obtained in e).

This invention involves a method for preparing a vaccine for preventing condyloma acuminatum or HPV infections, comprising blending the VLPs above, and optionally, one or more VLPs selected from the group consisting of VLPs of HPV 11, 16, 18, 31, 33, 45, 52 and 58, with carriers or excipients useful for vaccines.

Definitions of the Term in Present Invention

According to the invention, the term "*E. coli* expression system" refers to a expression system consisting of *E. coli* (strains) and vectors, wherein the *E. coli* (strains) include, but are not limited to: GI698, ER2566, BL21 (DE3), B834 (DE3), and BLR (DE3), which are available on the market.

According to the invention, the term "vectors" refers to the nucleic acid carrier tools which can have a polynucleotide encoding a protein inserted therein and allow for the expression of the protein. The "vector" can have the carried genetic material expressed in a host cell by transformation, transduction, and transfection into the host cell. For example, "vectors" include plasmids, phages, cosmids and the like.

According to the invention, the term "a gene fragment of a truncated HPV 6 L1 protein" refers to the nucleic acids with the nucleotide(s) encoding one or more amino acid sequences deleted at 5' or 3' terminal of the wild-type HPV 6 L1 gene (cDNA). The full-length gene sequence of the wild-type HPV 6 L1 gene can be found in, but not limited to, the following NCBI sequences: AF067042.1, AF092932.1, L41216.1 and X00203.1.

The term "truncated HPV 6 L1 protein" refers to the protein with one or more amino acids deleted at the N- and/or C-terminal of the wild-type HPV 6 L1 protein. The full-length gene sequence of the wild-type HPV 6 L1 protein can be found in, but not limited to, the full-length L1 proteins encoded by the following NCBI sequences: AF067042.1, AF092932.1, L41216.1 and X00203.1.

According to the invention, the term "carriers and excipients useful for vaccines" refers to one or more reagents, including but not limited to: pH regulators, surfactants, adjuvants, and ionic strength enhancers. For example, pH regulators include, but are not limited to, phosphate buffers; surfactants include, but are not limited to: anion surfactants, cation surfactants, non-ionic surfactants (for example, but not limited to Tween-80); adjuvants include, but are not limited to, aluminum hydroxide and Freund's complete adjuvant; and Ionic strength enhancers include, but are not limited to, NaCl.

According to the invention, the term "chromatography" includes, but is not limited to: ion exchange chromatography (e.g. cation-exchange chromatography), hydrophobic interaction chromatography, absorbent chromatography (e.g. hydroxyapatite chromatography), gel filtrate chromatography (gel exclusion chromatography), and affinity chromatography.

According to the invention, the truncated HPV 6 L1 proteins may be obtained preferably by the following steps:

a) disrupting *E. coli*, which expresses truncated HPV 6 L1 protein, in a buffer containing 100-600 mM salt, preferably 200-500 mM;

b) isolating the supernatant from the disrupted solution, then decreasing the salt concentration of the supernatant to 100 mM-0M with water or a low-salt buffer (generally, with a salt concentration lower than the one of the buffer for disrupting);

c) separating a precipitant from the supernatant with a salt concentration as low as 100 mM-0;

d) redissolving the precipitant in a solution containing a reductant and having a salt concentration of 150-2000 mM, preferably greater than 200 mM;

e) isolating a solution of the truncated HPV 6 L1 proteins with a purity of at least 50%, preferably at least 70%, more preferably at least 80%.

According to the invention, in the method for obtaining the truncated HPV 6 L1 proteins, the term "buffer" refers to a solution which can maintain pH value stable within a certain range, including but not limited to: Tris buffers, phosphate buffers, HEPES buffers, and MOPS buffers.

According to the invention, the disrupting of the prokaryotic host cell can be achieved by methods including, but not limited to one or more of homogenizer disrupting, ultrasonic treatment, grinding, high pressure extrusion, and lysozyme treatment.

According to the invention, in the method for obtaining the truncated HPV 6 L1 proteins, the salts used include, but are not limited to: one or more of neutral salts, especially alkali metal salt, ammonium salts, hydrochlorides, sulfates, bicarbonates, phosphate salts or hydrogenphosphates, especially NaCl, KCl, NH_4Cl, $(NH_4)_2SO_4$. NaCl are preferred. The reductant used includes, but is not limited to, DTT and 2-mercaptoethanol, in an amount of including, but not limited to, 10-100 mM.

According to the invention, the VLPs of the truncated HPV 6 L1 protein may be produced by the following steps: further purifying the truncated HPV 6 L1 protein with a purity of at least 50% by subjecting it to a chromatography, and thereby obtaining a purified truncated HPV 6 L1 protein solution; and removing the reductant from the purified HPV 6 L1 protein solution, and thereby obtaining VLPs of the truncated HPV 6 L1. Methods for removing the reductant include, but are not limited to, known techniques in the art, such as dialysis, ultrafiltration, and chromatography.

According to the invention, the truncated HPV L1 protein preferably has the sequence set forth in SEQ ID NO:1.

According to the invention, the vaccine may be administrated in a patient-acceptable form, including but not limited to oral and injection, preferably injection.

According to the invention, the vaccine is preferably used in a unit dose. Each unit dose contains 5-80 μg truncated HPV 6 L1 VLP, preferably 20-40 μg.

Beneficial Effect

Presently, the expression systems useful for preparing HPV VLPs include eukaryotic and prokaryotic expression systems.

HPV L1 proteins expressed in eukaryotic expression systems retain their native conformation, and can form VLPs on their own. In most cases, VLP with a correct conformation can be obtained by simple purification. Nevertheless, eukaryotic expression systems, such as the baculovirus and yeast expression systems, are difficult to be applied in large-scale industrial production due to low expression levels and high costs.

Prokaryotic expression systems, such as *E. coli* systems, have the advantages of high expression levels at a lower cost. However, when expressed in a prokaryotic system, the HPV L1 protein usually loses its native conformation and is expressed in a form of inclusion bodies in the precipitant. Renaturation of the protein from inclusion bodies is still a problem worldwide. Due to the difficulty and inefficiency of renaturation, this method is limited to small-scale lab research and cannot be applied on a large scale so as to obtain VLP with a correct conformation from the inclusive bodies. Although the HPV L1 protein can exist in its native conformation in the supernatant of *E. coli* lysate, its expression levels are low. Moreover, it is quite difficult to purify the HPV L1 protein from the numerous soluble proteins in the *E. coli* lysate supernatant. Generally, the purification is completed by means such as fusion expression and affinity chromatography which are not feasible for industrial-scale processes due to expensive enzymes employed therein.

In this invention, N-truncated HPV 6 L1 protein is expressed in an *E. coli* expression system and is selectively precipitated from the *E. coli* lysate supernatant under mild conditions. The HPV 6 L1 protein is then redissolved in a salt buffer to significantly improve its purity while still

retaining its native conformation. The redissolved protein of interest can be immediately subjected to ion-exchange or hydrophobic interaction chromatography so as to obtain the pure protein. The purified, truncated HPV 6 L1 protein obtained from these steps, can self-assemble into VLPs with good immunogenicity and the ability to induce neutralizing antibodies of a high titer against HPV 6, which is a good vaccine for preventing human from HPV 6 infection. In addition, the truncated HPV 6 L1 protein used in the present invention, with the antigenicity and particle-self assembly ability of the full-length HPV 6 L1 protein retained, is easily expressed in an *E. coli* expression system, and can be economically purified without using expensive enzymes. Furthermore, because the protein of interest is not subjected to the intensive procedures of denaturation and renaturation during purification, the method can be applied industrially on a large scale due to low loss.

The invention will be more apparent after referring to the detailed description and the drawings as follows. All public references are incorporated hereby by reference in their entirety.

DESCRIPTION OF DRAWINGS

FIG. **1** shows the SDS-PAGE result of HPV6N3C-L1 protein during steps a)-d) of the method according to the invention. Lane 1: Lysate supernatant; Lane 2: HPV6N3C-L1 protein precipitated by tangential flow; Lane 3: Redissolved HPV6N3C-L1 in a re-suspension solution. The result shows that the purity of HPV6N3C-L1 reached about 70% following the steps of precipitation and re-dissolution.

FIG. **2** shows the SDS-PAGE result of HPV6N3C-L1 that was obtained in step d) and was further purified according to step e). Lane 1: HPV6N3C-L1 purified according to step e), 10 μL; Lane 2: HPV6N3C-L1 purified according to step e), 20 μL. The result shows that HPV6N3C-L1 purified according to step e) reached a purity of about 98%.

FIG. **3** shows the transmission electron microscopy (TEM) photograph of HPV6N3C-L1 VLPs obtained in step f), taken at 50,000× magnification. A great deal of VLPs in a radius of about 25 nm were observed in visual field, wherein the particle size was consistant with the theoretic size and the particles were homogenous.

FIG. **4** shows the dynamic light-scattering measurement result of HPV6N3C-L1 VLPs obtained in step f). The result shows that HPV6N3C-L1 VLP had a hydrodynamic radius of 24.70 nm and a particle assembly rate of 100%.

FIG. **5** shows titers of neutralizing antibodies in serum at different stages after vaccination of goat with HPV6N3C-L1 VLPs. Vaccination times are indicated with arrows. The titer of neutralizing antibodies increased rapidly a week after the first vaccination, and reached a peak level of 10^7-10^8 after a booster.

FIG. **6** shows titers of neutralizing antibodies in serum at different stages a week after vaccination of rabbit with HPV6N3C-L1 VLPs. Vaccination times are indicated with arrows. The titer of neutralizing antibodies increased rapidly a week after the first vaccination, and reached a peak level of 10^6 after a booster.

FIG. **7** shows the titers of neutralizing antibodies against HPV 6 and HPV11 in serum at different times after vaccination of mice with HPV6/11 bivalent vaccine obtained in Example 5. Vaccine was administered at 0 and 2 weeks. The titers of neutralizing antibodies against HPV6 and HPV 11 increased rapidly after the first vaccination, reaching 10^4-10^5.

FIG. **8** shows the titers of neutralizing antibodies against HPV 6, HPV 11, HPV 16 and HPV 18 in serum at different times after vaccination of mice with HPV6/11/16/18 quadrivalent vaccine obtained in Example 5. Vaccine was administered at 0 and 2 weeks. The titers of neutralizing antibodies against HPV 6, HPV 11, HPV 16 and HPV 18 increased rapidly after the first vaccination, reaching 10^5-10^6.

FIG. **9** shows the SDS-PAGE results of HPV6N2C-L1, HPV6N4C-L1 and HPV6N5C-L1 protein separately having 2, 4 and 5 amino acids truncated at the N-terminal of HPV 6 L1 protein (the amino acid sequences thereof set forth in SEQ ID Nos: 2, 3 and 4, respectively) during steps a)-e) of the method according to the invention. Lane 1: Molecular Weight Marker; Lane 2: HPV6N2C-L1 purified according to step a)-e), 10 μL; Lane 3: HPV6N4C-L1 purified according to step a)-e), 10 μL; Lane 4: HPV6N5C-L1 purified according to step a)-e), 10 μL. The result shows that the purity of HPV6N2C-L1, HPV6N4C-L1 and HPV6N5C-L1 protein separately having 2, 4 and 5 amino acids truncated at the N-terminal of HPV 6 L1 protein, reached about 98% following the steps a)-e).

FIG. **10** the transmission electron microscopy (TEM) photographs of the VLPs of HPV6N2C-L1, HPV6N4C-L1 and HPV6N5C-L1 protein separately having 2, 4 and 5 amino acids truncated at the N-terminal of HPV 6 L1 protein obtained after steps a)-f), taken at 50,000× magnification. 1. The transmission electron microscopy (TEM) photographs of HPV6N2C-L1 VLPs obtained after steps a)-f), taken at 50,000× magnification. 2 The transmission electron microscopy (TEM) photographs of HPV6N4C-L1 VLPs obtained after steps a)-f), taken at 50,000× magnification. 3. The transmission electron microscopy (TEM) photographs of HPV6N5C-L1 VLPs obtained after steps a)-f), taken at 50,000× magnification. The results show that a great deal of VLPs in a radius of about 25 nm were observed in visual field, wherein the particle size was consistant with the theoretic size and the particles were homogenous.

FIG. **11** shows the dynamic light-scattering measurement result of the VLPs of HPV6N2C-L1, HPV6N4C-L1 and HPV6N5C-L1 protein separately having 2, 4 and 5 amino acids truncated at the N-terminal of HPV 6 L1 protein obtained after steps a)-f). 1. The dynamic light-scattering measurement result of HPV6N2C-L1 VLPs obtained after steps a)-f). 2. The dynamic light-scattering measurement result of HPV6N4C-L1 VLPs obtained after steps a)-f). 3. The dynamic light-scattering measurement result of HPV6N5C-L1 VLPs obtained after steps a)-f). The result shows that HPV6N2C-L1 VLPs, HPV6N4C-L1 VLPs and HPV6N5C-L1 VLPs had a hydrodynamic radius of about 25 nm and a particle assembly rate of 100%.

SEQUENCES

SEQ ID NO: 1:

```
  1  MPSDSTVYVP PPNPVSKVVA TDAYVTRTNI FYHASSSRLL
     AVGHPYFSIK RANKTVVPKV

 61  SGYQYRVFKV VLPDPNKFAL PDSSLFDPTT QRLVWACTGL
     EVGRGQPLGV GVSGHPFLNK

121  YDDVENSGSG GNPGQDNRVN VGMDYKQTQL CMVGCAPPLG
     EHWGKGKQCT NTPVQAGDCP

181  PLELITSVIQ DGDMVDTGFG AMNFADLQTN KSDVPIDICG
     TTCKYPDYLQ MAADPYGDRL
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SEQUENCES

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  241  FFFLRKEQMF ARHFFNRAGE VGEPVPDTLI IKGSGNRTSV
       GSSIYVNTPS GSLVSSEAQL

  301  FNKPYWLQKA QGHNNGICWG NQLFVTVVDT TRSTNMTLCA
       SVTTSSTYTN SDYKEYMRHV

  361  EEYDLQFIFQ LCSITLSAEV VAYIHTMNPS VLEDWNFGLS
       PPPNGTLEDT YRYVQSQAIT

  421  CQKPTPEKQK PDPYKNLSFW EVNLKEKFSS ELDQYPLGRK
       FLLQSGYRGR SSIRTGVKRP

  481  AVSKASAAPK RKRAKTKR

SEQ ID NO: 2
    1  MRPSDSTVYV PPPNPVSKVV ATDAYVTRTN IFYHASSSRL
       LAVGHPYFSI KRANKTVVPK

   61  VSGYQYRVFK VVLPDPNKFA LPDSSLFDPT TQRLVWACTG
       LEVGRGQPLG VGVSGHPFLN

  121  KYDDVENSGS GGNPGQDNRV NVGMDYKQTQ LCMVGCAPPL
       GEHWGKGKQC TNTPVQAGDC

  181  PPLELITSVI QDGDMVDTGF GAMNFADLQT NKSDVPIDIC
       GTTCKYPDYL QMAADPYGDR

  241  LFFFLRKEQM FARHFFNRAG EVGEPVPDTL IIKGSGNRTS
       VGSSIYVNTP SGSLVSSEAQ

  301  LFNKPYWLQK AQGHNNGICW GNQLFVTVVD TTRSTNMTLC
       ASVTTSSTYT NSDYKEYMRH

  361  VEEYDLQFIF QLCSITLSAE VVAYIHTMNP SVLEDWNFGL
       SPPPNGTLED TYRYVQSQAI

  421  TCQKPTPEKQ KPDPYKNLSF WEVNLKEKFS SELDQYPLGR
       KFLLQSGYRG RSSIRTGVKR

  481  PAVSKASAAP KRKRAKTKR

SEQ ID NO: 3
    1  MSDSTVYVPP PNPVSKVVAT DAYVTRTNIF YHASSSRLLA
       VGHPYFSIKR ANKTVVPKVS

   61  GYQYRVFKVV LPDPNKFALP DSSLFDPTTQ RLVWACTGLE
       VGRGQPLGVG VSGHPFLNKY

  121  DDVENSGSGG NPGQDNRVNV GMDYKQTQLC MVGCAPPLGE
       HWGKGKQCTN TPVQAGDCPP

  181  LELITSVIQD GDMVDTGFGA MNFADLQTNK SDVPIDICGT
       TCKYPDYLQM AADPYGDRLF

  241  FFLRKEQMFA RHFFNRAGEV GEPVPDTLII KGSGNRTSVG
       SSIYVNTPSG SLVSSEAQLF

  301  NKPYWLQKAQ GHNNGICWGN QLFVTVVDTT RSTNMTLCAS
       VTTSSTYTNS DYKEYMRHVE

  361  EYDLQFIFQL CSITLSAEVV AYIHTMNPSV LEDWNFGLSP
       PPNGTLEDTY RYVQSQAITC

  421  QKPTPEKQKP DPYKNLSFWE VNLKEKFSSE LDQYPLGRKF
       LLQSGYRGRS SIRTGVKRPA

  481  VSKASAAPKR KRAKTKR

SEQ ID NO: 4
    1  MDSTVYVPPP NPVSKVVATD AYVTRTNIFY HASSSRLLAV
       GHPYFSIKRA NKTVVPKVSG

   61  YQYRVFKVVL PDPNKFALPD SSLFDPTTQR LVWACTGLEV
       GRGQPLGVGV SGHPFLNKYD

  121  DVENSGSGGN PGQDNRVNVG MDYKQTQLCM VGCAPPLGEH
       WGKGKQCTNT PVQAGDCPPL

  181  ELITSVIQDG DMVDTGFGAM NFADLQTNKS DVPIDICGTT
       CKYPDYLQMA ADPYGDRLFF

  241  FLRKEQMFAR HFFNRAGEVG EPVPDTLIIK GSGNRTSVGS
       SIYVNTPSGS LVSSEAQLFN

  301  KPYWLQKAQG HNNGICWGNQ LFVTVVDTTR STNMTLCASV
       TTSSTYTNSD YKEYMRHVEE

  361  YDLQFIFQLC SITLSAEVVA YIHTMNPSVL EDWNFGLSPP
       PNGTLEDTYR YVQSQAITCQ

  421  KPTPEKQKPD PYKNLSFWEV NLKEKFSSEL DQYPLGRKFL
       LQSGYRGRSS IRTGVKRPAV

  481  SKASAAPKRK RAKTKR

SEQ ID NO: 5
    1  ATGTGGCGGC CTAGCGACAG CACAGTATAT GTGCCTCCTC
       CTAACCCTGT ATCCAAAGTT

   61  GTTGCCACGG ATGCTTATGT TACTCGCACC AACATATTTT
       ATCATGCCAG CAGTTCTAGA

  121  CTTCTTGCAG TGGGTCATCC TTATTTTTCC ATAAAACGGG
       CTAACAAAAC TGTTGTGCCA

  181  AAGGTGTCAG GATATCAATA CAGGGTATTT AAGGTGGTGT
       TACCAGATCC TAACAAATTT

  241  GCATTGCCTG ACTCGTCTCT TTTTGATCCC ACAACACAAC
       GTTTGGTATG GGCATGCACA

  301  GGCCTAGAGG TGGGCAGGGG ACAGCCATTA GGTGTGGGTG
       TAAGTGGACA TCCTTTCCTA

  361  AATAAATATG ATGATGTTGA AAATTCAGGG AGTGGTGGTA
       ACCCTGGACA GGATAACAGG

  421  GTTAATGTTG GTATGGATTA TAAACAAACA CAATTATGCA
       TGGTTGGATG TGCCCCCCCT

  481  TTGGGCGAGC ATTGGGGTAA AGGTAAACAG TGTACTAATA
       CACCTGTACA GGCTGGTGAC

  541  TGCCCGCCCT TAGAACTTAT TACCAGTGTT ATACAGGATG
       GCGATATGGT TGACACAGGC

  601  TTTGGTGCTA TGAATTTTGC TGATTTGCAG ACCAATAAAT
       CAGATGTTCC TATTGACATA

  661  TGTGGCACTA CATGTAAATA TCCAGATTAT TTACAAATGG
       CTGCAGACCC ATATGGTGAT

  721  AGATTATTTT TTTTTCTACG GAAGGAACAA ATGTTTGCCA
       GACATTTTTT TAACAGGGCT

  781  GGCGAGGTGG GGGAACCTGT GCCTGATACT CTTATAATTA
       AGGGTAGTGG AAATCGAACG

  841  TCTGTAGGGA GTAGTATATA TGTTAACACC CCAAGCGGCT
       CTTTGGTGTC CTCTGAGGCA

  901  CAATTGTTTA ATAAGCCATA TTGGCTACAA AAAGCCCAGG
       GACATAACAA TGGTATTTGT

  961  TGGGGTAATC AACTGTTTGT TACTGTGGTA GATACCACAC
       GCAGTACCAA CATGACATTA

 1021  TGTGCATCCG TAACTACATC TTCCACATAC ACCAATTCTG
       ATTATAAAGA GTACATGCGT
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-continued

SEQUENCES

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 1081 CATGTGGAAG AGTATGATTT ACAATTTATT TTTCAATTAT
      GTAGCATTAC ATTGTCTGCT

 1141 GAAGTAATGG CCTATATTCA CACAATGAAT CCCTCTGTTT
      TGGAAGACTG GAACTTTGGG

 1201 TTATCGCCTC CCCCAAATGG TACATTAGAA GATACCTATA
      GGTATGTGCA GTCACAGGCC

 1261 ATTACCTGTC AAAAGCCCAC TCCTGAAAAG CAAAAGCCAG
      ATCCCTATAA GAACCTTAGT

 1321 TTTTGGGAGG TTAATTTAAA AGAAAAGTTT TCTAGTGAAT
      TGGATCAGTA TCCTTTGGGA

 1381 CGCAAGTTTT TGTTACAAAG TGGATATAGG GGACGGTCCT
      CTATTCGTAC CGGTGTTAAG

 1441 CGCCCTGCTG TTTCCAAAGC CTCTGCTGCC CCTAAACGTA
      AGCGCGCCAA AACTAAAAGG

 1501 TAA

SEQ ID NO: 6
    1 ATGCCTAGCG ACAGCACAGT ATATGTGCCT CCTCCTAACC
      CTGTATCCAA

   51 AGTTGTTGCC ACGGATGCTT ATGTTACTCG CACCAACATA
      TTTTATCATG

  101 CCAGCAGTTC TAGACTTCTT GCAGTGGGTC ATCCTTATTT
      TTCCATAAAA

  151 CGGGCTAACA AAACTGTTGT GCCAAAGGTG TCAGGATATC
      AATACAGGGT

  201 ATTTAAGGTG GTGTTACCAG ATCCTAACAA ATTTGCATTG
      CCTGACTCGT

  251 CTCTTTTTGA TCCCACAACA CAACGTTTGG TATGGGCATG
      CACAGGCCTA

  301 GAGGTGGGCA GGGGACAGCC ATTAGGTGTG GGTGTAAGTG
      GACATCCTTT

  351 CCTAAATAAA TATGATGATG TTGAAAATTC AGGGAGTGGT
      GGTAACCCTG

  401 GACAGGATAA CAGGGTTAAT GTTGGTATGG ATTATAAACA
      AACACAATTA

  451 TGCATGGTTG GATGTGCCCC CCCTTTGGGC GAGCATTGGG
      GTAAAGGTAA

  501 ACAGTGTACT AATACACCTG TACAGGCTGG TGACTGCCCG
      CCCTTAGAAC

  551 TTATTACCAG TGTTATACAG GATGGCGATA TGGTTGACAC
      AGGCTTTGGT

  601 GCTATGAATT TTGCTGATTT GCAGACCAAT AAATCAGATG
      TTCCTATTGA

  651 TATATGTGGC ACTACATGTA AATATCCAGA TTATTTACAA
      ATGGCTGCAG

  701 ACCCTTATGG TGATAGATTA TTTTTTTTTC TACGGAAGGA
      ACAAATGTTT

  751 GCCAGACATT TTTTTAACAG GGCTGGCGAG GTGGGGGAAC
      CTGTGCCTGA

  801 TACTCTTATA ATTAAGGGTA GTGGAAATCG AACGTCTGTA
      GGGAGTAGTA

  851 TATATGTTAA CACCCCAAGC GGCTCTTTGG TGTCCTCTGA
      GGCACAATTG

  901 TTTAATAAGC CATATTGGCT ACAAAAAGCC CAGGGACATA
      ACAATGGTAT

  951 TTGTTGGGGT AATCAACTGT TTGTTACTGT GGTAGATACC
      ACACGCAGTA

 1001 CCAACATGAC ATTATGTGCA TCCGTAACTA CATCTTCCAC
      ATACACCAAT

 1051 TCTGATTATA AAGAGTACAT GCGTCATGTG GAAGAGTATG
      ATTTACAATT

 1101 TATTTTTCAA TTATGTAGCA TTACATTGTC TGCTGAAGTA
      GTGGCCTATA

 1151 TTCACACAAT GAATCCCTCT GTTTTGGAAG ACTGGAACTT
      TGGGTTATCG

 1201 CCTCCCCCAA ATGGTACATT AGAAGATACC TATAGGTATG
      TGCAGTCACA

 1251 GGCCATTACC TGTCAAAAGC CCACTCCTGA AAAGCAAAAG
      CCAGATCCCT

 1301 ATAAGAACCT TAGTTTTTGG GAGGTTAATT TAAAAGAAAA
      GTTTTCTAGT

 1351 GAATTGGATC AGTATCCTTT GGGACGCAAG TTTTTGTTAC
      AAAGTGGATA

 1401 TAGGGGACGG TCCTCTATTC GTACCGGTGT TAAGCGCCCT
      GCTGTTTCCA

 1451 AAGCCTCTGC TGCCCCTAAA CGTAAGCGCG CCAAAACTAA
      AAGGTAA
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The description is further illustrated in combination with the Examples, wherein it is not limited to the Examples.

EXAMPLE 1

Expression of the Truncated HPV6 L1 Protein (SEQ ID NO. 1)

Preparation of HPV6 L1 Gene Fragments as PCR Template

The full-length gene of HPV-6 L1 was synthesized by Shanghai Boya Bio Co. The synthesized gene fragment has a full length of 1503 by and has a sequence of SEQ ID NO:5. Based on the synthetic full-length gene fragment of HPV-6 L1, the truncated HPV 6 L1 protein according to the invention was prepared as a template.

Construction of Non-Fusion Expression Vector of Truncated HPV6 L1 Gene

The full-length gene fragment of HPV-6 L1 synthesized in the previous step were used as the template for the next PCR reaction. The forward primer was 6N3F: 5'-CAT ATG CCT AGC GAC AGC ACA GTA TA-3' (SEQ ID NO:10), at the 5' terminal of which the restriction endonuclease NdeI site was introduced. The sequence of NdeI site was CAT ATG, wherein ATG was the initiation codon in *E. coli* system. The reverse primer was 6CR: 5'-GTC GAC TTA CCT TTT AGT TTT GGC GC-3' (SEQ ID NO:11), at the 5' terminal of which the restriction endonuclease Sa1I site was introduced. Amplification was performed in a Biometra T3 PCR thermocycler using the following parameters:

94° C. denaturation 5 min	1 cycle
94° C. denaturation 50 sec	25 cycles
57° C. annealing 50 sec	
72° C. elongation 2 min	
72° C. elongation 10 min	1 cycle

The DNA fragments, about 1.5 kb in length, were obtained after amplification. The PCR products were linked to the pMD 18-T vector (Takara Biosciences). After digestion with NdeI/Sa1I, it was identified that positive colonies, wherein the truncated HPV6 L1 gene was inserted, were obtained, designated as pMD 18-T-HPV6N3C-L1.

The nucleotide sequence of interest, which was inserted into the plasmid pMD 18-T-HPV6N3C-L1, was determined as SEQ ID NO: 6 by Shanghai Boya Bio Co. through using M13 +/− primers. SEQ ID NO:6 encodes the amino acid sequence set forth in SEQ ID NO:1 which corresponds to a HPV 6 L1 protein having 3 amino acids truncated at its N-terminal and no amino acid truncated at its C-terminal and was designated as HPV6N3C-L1.

The truncated HPV6N3C-L1 gene fragments were obtained by NdeI/Sa1I digestion of plasmid pMD 18-T-HPV6N3C-L1 The fragments were linked to the prokaryotic expression vector pTrxFus digested with NdeI/Sa1I (Invitrogen). Since the fusion protein was cleaved, the protein of interest was expressed immediately after initiating the expression of the amino acid Met, without other fusion proteins included. Colonies were screened with NdeI/Sa1I digestion. Positive colonies containing the insert of the L1 gene fragment were labeled pTRX-HPV6N3C-L1. 1 μL plasmid pTRX-HPV6N3C-L1 (0.15 mg/ml) was used to transform 40 μL competent *E. coli* GI698 (Invitrogen) prepared by Calcium chloride method, and then were coated on solid CAA media (dissolving 6 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 20 g casein hydrolysate, 0.095 $MgCl_2$, 1.5 g agar powder, and 20 ml 50% glycerinin 900 ml deionized water, and was added) containing benzyl chloride (at a final concentration of 100 mg/ml, the same as below). Plates were incubated at 30° C. for about 10-12 h until single colonies could be observed clearly. Single colonies from the plates were transferred to a tube containing 4 ml liquid IMC medium containing benzyl chloride. Cultures were incubated in a shaking incubator at 220 rpm for 10 h at 25° C., and then 1 ml bacterial solution was freeze-dried and stored at −70° C.

Expression of HPV6N3C-L1 in Large Scale

E. coli transformed with pTRX-HPV6N3C-L1 was taken from freeze-dried stain at −70° C., and diluted with a little sterile water, and then incubated in 50 mL IMC medium containing benzyl amine at 200 rpm and 30° C. for 8 h. Then, the cultures were transferred to ten flasks (5 ml cultures per flask), each of which contains 500 mL LB medium, and were incubated in a shaking incubator overnight at 200 rpm and 30° C.

A 50 L fermenter made by Shanghai Baoxing Biological Ltd. was used in large-scale incubation. pH electrode was calibrated. 30 L LB medium was prepared and transferred into the fermenter, sterilized at 121° C. for 30 minutes. Dissolved oxygen electrode was calibrated, wherein the value was determined as 0 before introduction of air after sterilization and as 100% prior to vaccination after introduction of air while stirring at 100 rpm at the beginning.

Preparation of the feed: 30 g casein hydrolysates was dissolved in 100 mL deionized water to prepare a solution (30%), and 50 g glucose was dissolved in 100 ml deionized water to prepared a glucose solution (50%). The two mixtures were sterilized at 121° C. for 20 min.

On the second day, the starter cultures in the ten flasks (for a total of 5 L) were transferred to the fermenter. At 30° C. and pH 7.0, the dissolved O_2 was maintained at >40% by regulating agitation rate or air supply manually.

Flow Feed: 50% glucose and 30% casein hydrolysates were mixed at a 2:1 mass ratio.

Flow rates were as follows:

25 ml/min was defined as 100%.

1 h: 5%

2 h: 10%

3 h: 20%

4 h: 40%

5 h to the end: 60%

When OD_{600} reached about 10.0, the culture temperature was lowered to 25° C. and 4 g tryptophan was added to begin an induction culture of 4 h. Fermentation was halted when OD_{600} reached about 40. The culture was then centrifuged to obtain strains (about 2.5 kg).

IMC medium (1 liter):

Na_2HPO_4	6 g
KH_2PO_4	3 g
NaCl	0.5 g
NH_4Cl	1 g
Casein Hydrolysates	20 g
$MgCl_2$	0.095 g

EXAMPLE 2

Preparation of HPV6N3C-L1 with a Purity of about 70%

1 g strains were re-suspended in 10 ml lysis buffer (20 mM tris buffer pH 7.2, 300 mM NaCl). Strains were disrupted by passing through a APV homogenizer (Invensys Group) for five times at a pressure of 600 bar. The homogenate was centrifuged at 30,000 g (13,500 rpm in JA-14 rotor) for 15 min. The supernatant was subjected to SDS-PAGE on a 10% gel. At this stage, the HPV6N3C-L1 had a purity of about 10%. The supernatant was dialyzed by a Centrasette 5 Tangential Flow Filter (Pall Co.) running at a pressure of 0.5 psi, a flow rate of 500 ml/min, and a tangential flow rate of 200 mL/min, wherein the retention molecular weight was 30 kDa, the dialysate was 10 mM phosphate buffer pH 6.0, and the dialysis volume was three times as large as the volume of supernatant. After thorough dialysis, the mixture was centrifuged at 12,000 g (9500 rpm in JA-10 rotor (Beckman J25 high speed centrifuge)) for 20 min, and the precipitation was collected. The precipitation was re-suspended in 10 mM phosphate buffer pH 7.0 containing 10 mM DTT and 300 mM NaCl, wherein the volume of the buffer was 1/10 times as large as the volume of the supernatant. The mixture was stirred for 30 min and centrifuged at 30,000 g (13,500 rpm in JA-14 rotor (Beckman J25 high speed centrifuge)) for 20 min. The supernatant passes through a 0.22 μm filter membrane. The sample was used for cation exchange chromatography of the next step. 30 μL of 6× loading buffer was added to 150 μL of the filtered supernatant, and the result solution was mixed. After heating in a water bath at 80° C. for 10 min, 10 ul of the sample was subjected to SDS-PAGE on a 10% gel at 120V for 120 min. The electrophoretic bands were stained by Coomassie brilliant blue. The result was shown in FIG. **1**. According to the

analysis of SDS-PAGE, HPV6N3C-L1 protein was purified and enriched after the steps of precipitation and re-dissolution, with the purity of about 70%.

EXAMPLE 3

Chromatography Purification of HPV6N3C-L1

Purification of HPV6N3C-L1 by Cation exchange Chromatography

Equipment: AKTA Explorer 100 preparative liquid chromatography system (GE Healthcare, i.e. the original Amershan Pharmacia Co.)

Chromatographic media: SP Sepharose 4 Fast Flow

Column Volume: 5.5 cm×20 cm

Buffer: 20 mM phosphate buffer pH 7.0, 10 mM DTT

20 mM phosphate buffer pH 7.0, 10 mM DTT, 2M NaCl

Flow Rate: 25 mL/min

Detector Wavelength: 280 nm

Sample: 3 L 70% pure HPV6N3C-L1 solution

Elution protocol: eluting undesired proteins with 200 mM NaCl, eluting the protein of interest with 500 mM NaCl, collecting 500 mM NaCl elutate, and finally getting about 900 mL purified HPV6N3C-L1 sample.

Purification of HPV6N3C-L1 by CHT-II Chromatography

Equipment: AKTA Explorer 100 preparative liquid chromatography system (GE Healthcare, i.e. the original Amershan Pharmacia Co.)

Chromatographic media: CHT-II (Bio-Rad)

Column Volume: 5.5 cm×20 cm

Buffer: 10 mM phosphate buffer pH 7.0, 10 mM DTT, 0.5M NaCl

Flow Rate: 20 mL/min

Detector Wavelength: 280 nm

Sample: 500 mM NaCl elutate from SP Sepharose 4 Fast Flow

Elution protocol: directly collecting the pass-through containing the protein of interest.

The pass-through, which contains HPV6N3C-L1, was collected and about 1000 mL purified HPV6N3C-L1 was obtained. 30 μL 6× loading buffer was added to 150 μL HPV6N3C-L1 sample purified according to the method of the Example, and then the result solution was mixed thoroughly. After heating the solution in a water bath at 80° C. for 10 min, a 10 uL sample was subjected to SDS-PAGE on a 10% gel at 120V for 120 min. The electrophoretic bands were stained by Coomassie brilliant blue. The result was shown in FIG. **2**. The concentration of the protein of interest was about 0.7 mg/ml, and the purity was greater than 98% according to SDS-PAGE.

EXAMPLE 4

Assembly of HPV6N3C-L1 VLPs

Equipment: Centrasette 5 Tangential Flow Filter (Pall Co.), retention MW 30 kDa.

Sample: 1000 mL HPV6N3C-L1 obtained in Example 3

Sample Concentration Sample was concentrated to 800 mL with the system tangential flow rate was adjusted to 50 mL/min

Sample renaturation: Sample Renaturation: Sample buffer was exchanged with 10 L renaturation buffer (20 mM PB pH 6.0, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.5M NaCl, 0.003% Tween-80) thoroughly. When running the Tangential Flow Filter, the pressure was 0.5 psi and the tangential flow rate was 10 mL/min. When exchange was finished, the sample buffer was replaced with storage buffer (20 L PBS: 20 mM PB pH 6.5, 0.5M NaCl). The exchange volume was 20 L. The running pressure was 0.5 psi and the tangential flow rate was 25 mL/min. When the liquid exchange was finished, the sample was aseptically filtrated with a Pall filter (0.20 μm), and thereby obtaining HPV6N3C-L1 VLPs. The HPV6N3C-L1 VLPs were stored at 4° C. for further use.

EXAMPLE 5

Determination of the Morphology and Immunogenicity of HPV6N3C-L1 VLPs

Transmission Electron Microscopy (TEM) of HPV6N3C-L1 VLPs

The equipment was a JEOL 100 kV Transmission Electron Microscope (100,000× magnification). HPV6N3C-L1 VLPs were negatively stained with 2% phosphotungstic acid at pH 7.0, and fixed on a copper grid. Results were shown in FIG. **3**. It could be seen that the VLPs obtained in Example 4 had a radius of approximately 25 nm, and were homogenous and in a hollow form.

Dynamic Light-Scattering Measurement of HPV6N3C-L1 VLPs

DynaPro MS/X dynamic light-scattering instrument (including a temperature controller) (US Protein Solutions Co.) was used for light-scattering measurements. The regulation algorithm was used in the measurements. The sample was the one obtained in Example 4. The sample was passed through a 0.22 μm filter membrane prior to the measurement. Results were shown in FIG. **4**. The result shows that HPV6N3C-L1 VLPs had a Hydrodynamic radius of 25.46 nm.

Establishment a Model of Pseudovirion Neutralization Assay for HPV6:

HPV can hardly be cultured in vitro, and the HPV host had a strong specificity. Thus, HPV can hardly be propagated in hosts other than human. That is, there was not an appropriate animal model for HPV. Therefore, in order to evaluate the immune productivity of HPV vaccine quickly, there was a need to establish a efficient model for in vitro neutralization assays.

In Vitro Infection Model of Pseudovirion: According to the characteristic that HPV VLP can package nucleic acids non-specifically, HPV pseudivirion was formed by expressing HPV L1 and L2 protein in cells, and by packaging viral DNA of episome or introducing reporter plasmids heterologously. Methods include expression systems based on recombinant viruses and cotransfection of multi-plasmids (see Yeager, M. D, Aste-Amezaga, M. et al (2000) Virology (278) 570-7).

The invention utilizes cotransfection of a multi-plasmid system. Some improvements were made as follows. An optimized calcium phosphate transfection method was established for the 293 FT cell line, with a transfection efficiency of above 90%, which facilitate the production on a large scale. The resultant codon-optimized expression plasmid of HPV protein could express HPV L1 and L2 gene efficiently in mammalian cell lines, facilitating efficient assembly of pseudovirion.

1. Construction of HPV Pseudovirion:

P6L1h, p6L2h and pN31-EGFP (donated by Professor John T. Schiller of NIH) contain genes for HPV6L1, HPV6L2, and GFP, respectively. These plasmids were purified using CsCl density gradient centrifugation as described

in The Molecular Cloning Experiment Guide, (3rd edition). The purification procedure was as follows:

Plasmids were used to transform *E. coli* DH5α;

Single colonies were transferred into 500 mL LB culture medium and incubated in a shaking flask at 37° C. for 16 h;

Culture medium was centrifuged at 9,000 g for 5 min and the stains were collected;

The following substances were successively added to bacteria in each 1000 mL LB: 40 mL solution I (50 mM glucose, 25 mM Tris-Cl pH 8.0, 10 mM EDTA pH 8.0) and 2 ml 1 μg/μL RNase A), 40 mL solution II (0.2M NaOH, 1% SDS), and 48 mL solution III (60.0 mL 5M potassium acetate, 11.5 mL acetic acid, and 28.5 mL deionized water);

After placing on ice for 10 min, the mixture was centrifuged at 15,000 g for 20 min at 4° C.;

The supernatant was mixed with 0.6 volume of isopropyl alcohol, then was centrifuged again at 15,000 g for 30 min;

The supernatant was decanted into waste and the precipitation was washed with 70% ethanol;

The precipitation was dissolved in TE and the content of DNA was determined;

CsCl was dissolved in the solution of DNA (1 g DNA per 1.01 g CsCl), and then 100 μL 10 mg/mL EB solution was also dissolved in it;

The mixture was centrifuged using a Beckman NVT65 centrifuge at 62,000 rpm for 10 hr at 20° C.;

Closed circle DNA section was collected using an injector pinhead;

EB was extracted with equivalent volume of Isoamyl alcohol repeatedly for four times;

Three volumes of deionized water and eight volumes of dry ethanol were added to one volume of DNA solution, and then the mixture was centrifuged at 20000 g for 30 min at 4° C.;

The precipitation was collected and washed with 75% ethanol, and then dissolved in 1 mL TE;

The concentration of the DNA solution was determined, then the solution was stored in small packages at −20° C.

The purified p6L1h, p6L2h and pN31-EGFP co-transfected 293 FT cells (Invitrogen) cultured on a 10 cm cell culture plate by calcium phosphate method. The calcium phosphate method was described as follows. 40 μg p6L1h, 40 μg p6L2h and 40 μg pN31-EGFP were separately added to the mixture of 1 mL HEPES solution (125 μL 1M HEPES/50 mL deionized water, at pH7.3 and 4° C.) and 1 mL 0.5M $CaCl_2$ solution. After mixing, 2 mL 2× HeBS solution (0.28M NaCl (16.36 g), 0.05M HEPES (11.9 g), 1.5 mM Na_2HPO_4 (0.213 g), dissolved in 1000 mL deionized water, at pH 6.96 and −70° C.) was added dropwise. After standing at room temperature for 1 min, the mixture was added to the 10 cm cell culture plate where the 293 FT cells were cultured. The original culture medium was replaced with 10 ml complete medium (Invitrogen Co.) 6 hours later. 48 hours after transfection, the medium was decanted and the cells were washed twice with PBS. Then, the cells were collected and counted. Every 10^8 cells were suspended in 1 mL cytolytic solution (0.25% Brij58, 9.5 mM $MgCl_2$). After lysing, cell lysate was centrifugated at 5,000 g for 10 min and the supernatant was collected. The Pseudovirion solution was obtained after adding 5M NaCl to the supernatant to a final concentration of 850 mM, then was stored in small packages at −20° C.

293 FT cells (Invitrogen) were spread on a 96-well cell culture plate (1.5×10^4 cells/well), Neutralization assay was performed five hours later. Serum samples were serially diluted with 10% DMEM half-by-half. 50 μL diluted samples were separately mixed with 50 μL Pseudovirion solutions diluted with 10% DMEM (moi=0.1). After incubating at 4° C. for 1 h, the mixture was added to the 96-well cell culture plate spread with 293 FT cells. The mixture was then incubated for 72 h at 37° C. Neutralization titers of samples were estimated by observing fluorescence. Infection percentage of cells in each well was checked by flow cytometry (EPICS XL, American Beckman Coulter Co.). The exact titers of monoclonal antibodies or polyclonal antibodies were calculated. Infection percentage was the percentage of cells in the positive region minus the uninfected cells in the positive region.

Infection control percentage=(1−infection percentage of sample cell/infection percentage of negative cell)×100%

Neutralization titer was defined as the highest dilution multiple by which the infection control percentage was just above 50%. Monoclonal and polyclonal antibodies were considered as having neutralizing capacity if their infection control percentage was above 50% after 50 times dilutions.

Measurement of Immune Protection of Animals Vaccinated with HPV6 VLPs

Female rabbits (general level), 6-8 weeks old, were purchased from the Disease Prevention and Control Center of Guangxi province, where they were raised. HPV6N3C-L1 VLPs prepared in Example 4, were mixed with equal amount of complete Freund's Adjuvant for the first immunization. For the booster, HPV6N3C-L1 VLPs were mixed with incomplete Freund's Adjuvant. Rabbits were immunized via muscle injection, with 100 μg per rabbit for the first immunization, and with 50 μg per rabbit for the booster separately at week 4, 10. After immunization, external vein blood was collected every week, and serum was separated and stored for detection.

Female goats (general level), 6-8 weeks old, were purchased from the Disease Prevention and Control Center of Guangxi province, where they were raised. HPV6N3C-L1 VLPs as prepared in Example 4, were mixed with equal amount of complete Freund's adjuvant for the first immunization. For the booster, HPV6N3C-L1 VLPs were mixed with incomplete Freund's adjuvant. Goats were immunized via muscle injection, with 1 mg per goat for the first immunization, and with 0.5 mg per goat for the booster separately at weeks 4, and 18. After immunization, external vein blood was collected, and serum was separated and stored for detection.

Neutralization titers of the anti-serums were evaluated using a pseudovirion-based neutralization cell model assay. As shown in FIGS. **5** and **6**, the vaccine produced by mixing HPV6N3C-L1 VLPs prepared in Example 4 with Freund's adjuvants (aluminum hydroxide or aluminum phosphate adjuvants available commercially or self-prepared may be used besides Freund's adjuvants) had good immunogenicity, could induce neutralizing antibodies with a high titer in animals, and could be used as an effective vaccine for the prevention of HPV infection.

Measurement of Immune Protection of Mice Vaccinated with HPV6/11 Bivalent Vaccine.

Four SPF BALB/c mice, 4-5 weeks old, were used. HPV6N5C-L1 and HPV11N4C-L1 VLPs, prepared according to the method similar to that of Examples 1-4, were mixed at a ratio of 1:2 (by weight), wherein the final

concentrations of them were 40 μg/mL and 80 μg/mL, respectively. The vaccine was mixed with an equal amount of complete Freund's adjuvant for the first immunization, and was mixed with an equal amount of incomplete Freund's adjuvant for the booster.

Mice were immunized by muscle injection. The amount for the first immunization was 10 μg HPV6N5C-L1 and 20 μg HPV11N4C-L1 per mouse. The booster was administered every two weeks. The amount for the booster was 20 μg HPV6N5C-L1 and 40 μg HPV11N4C-L1 per mouse.

After immunization, external vein blood was collected every week and serum was separated. The titers of neutralizing antibodies against HPV6 and HPV 11 in immunized mice were separately determined according to the method of Example 5.

Results were shown in FIG. **7**, indicating that HPV6/11 bivalent vaccine, prepared by blending HPV6N5C-L1 and HPV11N4C-L1 VLPs as prepared in Examples 1-4, had good immunogenicity, could induce neutralizing antibodies with a high titer against HPV 6 and HPV 11 in animals, and could be used as an effective vaccine for the prevention of HPV6/HPV11 infection (besides the Freund's adjuvants used in the experiments, the vaccine may be prepared by blending the two HPV6N5C-L1 and HPV11N4C-L1, with aluminum hydroxide or aluminum phosphate adjuvants available commercially or self-prepared).

The Amino Acid Sequence of HPV11N4C-L1 is showed in SEQ ID NO: 7 as follows.

```
Met Ser Asp Ser Thr Val Tyr Val Pro Pro Pro Asn Pro Val Ser Lys
1               5                  10                  15

Val Val Ala Thr Asp Ala Tyr Val Lys Arg Thr Asn Ile Phe Tyr His
            20                  25                  30

Ala Ser Ser Ser Arg Len Leu Ala Val Gly His Pro Tyr Tyr Ser Ile
        35                  40                  45

Lys Lys Val Asn Lys Thr Val Val Pro Lys Val Ser Gly Tyr Gln Tyr
    50                  55                  60

Arg Val Phe Lys Val Val Leu Pro Asp Pro Asn Lys Phe Ala Leu Pro
65                  70                  75                  80

Asp Ser Ser Leu Phe Asp Pro Thr Thr Gln Arg Leu Val Trp Ala Cys
                85                  90                  95

Thr Gly Leu Glu Val Gly Arg Gly Gln Pro Leu Gly Val Gly Val Ser
            100                 105                 110

Gly His Pro Leu Leu Asn Lys Tyr Asp Asp Val Glu Asn Ser Gly Gly
        115                 120                 125

Tyr Gly Gly Asn Pro Gly Gln Asp Asn Arg Val Asn Val Gly Met Asp
130                 135                 140

Tyr Lys Gln Thr Gln Leu Cys Met Val Gly Cys Ala Pro Pro Leu Gly
145                 150                 155                 160

Glu His Trp Gly Lys Gly Thr Gln Cys Ser Asn Thr Ser Val Gln Asn
                165                 170                 175

Gly Asp Cys Pro Pro Leu Glu Leu Ile Thr Ser Val Ile Gln Asp Gly
            180                 185                 190

Asp Met Val Asp Thr Gly Phe Gly Ala Met Asn Phe Ala Asp Leu Gln
        195                 200                 205

Thr Asn Lys Ser Asp Val Pro Leu Asp Ile Cys Gly Thr Val Cys Lys
    210                 215                 220

Tyr Pro Asp Tyr Leu Gln Met Ala Ala Asp Pro Tyr Gly Asp Arg Leu
225                 230                 235                 240

Phe Phe Tyr Leu Arg Lys Glu Gln Met Phe Ala Arg His Phe Phe Asn
                245                 250                 255

Arg Ala Gly Thr Val Gly Glu Pro Val Pro Asp Asp Leu Leu Val Lys
            260                 265                 270

Gly Gly Asn Asn Arg Ser Ser Val Ala Ser Ser Ile Tyr Val His Thr
        275                 280                 285

Pro Ser Gly Ser Leu Val Ser Ser Glu Ala Gln Leu Phe Asn Lys Pro
    290                 295                 300

Tyr Trp Leu Gln Lys Ala Gln Gly His Asn Asn Gly Ile Cys Trp Gly
305                 310                 315                 320
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-continued

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Asn His Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser Thr Asn Met
                325                 330                 335

Thr Leu Cys Ala Ser Val Ser Lys Ser Ala Thr Tyr Thr Asn Ser Asp
            340                 345                 350

Tyr Lys Glu Tyr Met Arg His Val Glu Glu Phe Asp Leu Gln Phe Ile
        355                 360                 365

Phe Gln Len Cys Ser Ile Thr Leu Ser Ala Glu Val Met Ala Tyr Ile
    370                 375                 380

His Thr Met Asn Pro Ser Val Leu Glu Asp Trp Asn Phe Gly Leu Ser
385                 390                 395                 400

Pro Pro Pro Asn Gly Thr Leu Glu Asp Thr Tyr Arg Tyr Val Gln Ser
                405                 410                 415

Gln Ala Ile Thr Cys Gln Lys Pro Thr Pro Glu Lys Glu Lys Gln Asp
            420                 425                 430

Pro Tyr Lys Asp Met Ser Phe Trp Glu Val Asn Leu Lys Glu Lys Phe
        435                 440                 445

Ser Ser Glu Leu Asp Gln Phe Pro Leu Gly Arg Lys Phe Leu Leu Gln
    450                 455                 460

Ser Gly Tyr Arg Gly Arg Thr Ser Ala Arg Thr Gly Ile Lys Arg Pro
465                 470                 475                 480

Ala Val Ser Lys Pro Ser Thr Ala Pro Lys Arg Lys Arg Thr Lys Thr
                485                 490                 495

Lys Lys
```

Measurement of Immune Protection of Mice Vaccinated with HPV6/11/16/18Quadrivalent Vaccine

Four SPF BALB/c mice, 4-5 weeks old, were used. HPV6N5C-L1, HPV11N4C-L1, HPV16N30C-L1 and HPV18N65C-L1 VLPs, prepared according to the method similar to that of Examples 1-4, were mixed at a ratio of 1:2:2:1 (by weight), wherein the final concentrations of them were 40 μg/mL, 80 μg/mL, 80 μg/mL and 40 μg/mL, respectively. The vaccine was mixed with an equal amount of complete Freund's adjuvant for the first immunization, and was mixed with an equal amount of incomplete Freund's adjuvant for the booster.

Mice were immunized by muscle injection. The amount for the first immunization was 10 μg HPV6N5C-L1, 10 μg HPV18N65C-L1, 20 μg HPV11N4C-L1, and 20 μg HPV16N30C-L1 per mouse. The booster was administered every two weeks. The amount for the booster was 20 μg HPV6N5C-L1, 20 μg HPV18N65C-L1, 40 μg HPV11N4C-L1, and 40 μg HPV16N30C-L1 per mouse.

After immunization, external vein blood was collected every week and serum was separated. The titers of neutralizing antibodies against HPV6, HPV 11, HPV 16 and HPV 18 in immunized mice were separately determined according to the method of Example 5.

Results were shown in FIG. **8**, indicating that HPV6/11/16/18 quadrivalent vaccine, prepared by blending HPV6N5C-L1, HPV11N4C-L1, HPV16N30C-L1 and HPV18N65C-L1 VLPs as prepared in Examples 1-4, had good immunogenicity, could induce neutralizing antibodies with a high titer against HPV 6, HPV 11, HPV 16, and HPV 18 in animals, and could be used as a effective vaccine for the prevention of HPV6/HPV11/HPV16/HPV18 infection (in addition to the Freund's adjuvants used in the experiments, the vaccine could be prepared by blending the four HPV6N5C-L1, HPV11N4C-L1, HPV16N30C-L1 and HPV18N65C-L1 VLPs with aluminum hydroxide or aluminum phosphate adjuvants available commercially or self-prepared).

The Amino Acid Sequence of L1 of HPV6N5C-L1 is showed in SEQ ID NO 4 as follows.

The Amino Acid Sequence of L1 of HPV16N30C-L1 is showed in SEQ ID NO 8 as follows.

```
Met Leu Pro Ser Glu Ala Thr Val Tyr Leu Pro Pro Val Pro Val Ser
1               5                   10                  15

Lys Val Val Ser Thr Asp Glu Tyr Val Ala Arg Thr Asn Ile Tyr Tyr
            20                  25                  30

His Ala Gly Thr Ser Arg Leu Leu Ala Val Gly His Pro Tyr Phe Pro
        35                  40                  45

Ile Lys Lys Pro Asn Asn Asn Lys Ile Leu Val Pro Lys Val Ser Gly
    50                  55                  60

Leu Gln Tyr Arg Val Phe Arg Ile His Leu Pro Asp Pro Asn Lys Phe
65                  70                  75                  80
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-continued

```
Gly Phe Pro Asp Thr Ser Phe Tyr Asn Pro Asp Thr Gln Arg Leu Val
                85                  90                  95

Trp Ala Cys Val Gly Val Glu Val Gly Arg Gly Gln Pro Leu Gly Val
            100                 105                 110

Gly Ile Ser Gly His Pro Leu Leu Asn Lys Leu Asp Asp Thr Glu Asn
        115                 120                 125

Ala Ser Ala Tyr Ala Ala Asn Ala Gly Val Asp Asn Arg Glu Cys Ile
    130                 135                 140

Ser Met Asp Tyr Lys Gln Thr Gln Leu Cys Leu Ile Gly Cys Lys Pro
145                 150                 155                 160

Pro Ile Gly Glu His Trp Gly Lys Gly Ser Pro Cys Thr Asn Val Ala
                165                 170                 175

Val Asn Pro Gly Asp Cys Pro Pro Leu Glu Leu Ile Asn Thr Val Ile
            180                 185                 190

Gln Asp Gly Asp Met Val Asp Thr Gly Phe Gly Ala Met Asp Phe Thr
        195                 200                 205

Thr Leu Gln Ala Asn Lys Ser Glu Val Pro Leu Asp Ile Cys Thr Ser
    210                 215                 220

Ile Cys Lys Tyr Pro Asp Tyr Ile Lys Met Val Ser Glu Pro Tyr Gly
225                 230                 235                 240

Asp Ser Leu Phe Phe Tyr Leu Arg Arg Glu Gln Met Phe Val Arg His
                245                 250                 255

Leu Phe Asn Arg Ala Gly Ala Val Gly Asp Asn Val Pro Asp Asp Leu
            260                 265                 270

Tyr Ile Lys Gly Ser Gly Ser Thr Ala Asn Leu Ala Ser Ser Asn Tyr
        275                 280                 285

Phe Pro Thr Pro Ser Gly Ser Met Val Thr Ser Asp Ala Gln Ile Phe
    290                 295                 300

Asn Lys Pro Tyr Trp Leu Gln Arg Ala Gln Gly His Asn Asn Gly Ile
305                 310                 315                 320

Cys Trp Gly Asn Gln Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser
                325                 330                 335

Thr Asn Met Ser Leu Cys Ala Ala Ile Ser Thr Ser Glu Thr Thr Tyr
            340                 345                 350

Lys Asn Thr Asn Phe Lys Glu Tyr Leu Arg His Gly Glu Glu Tyr Asp
        355                 360                 365

Leu Gln Phe Ile Phe Gln Leu Cys Lys Ile Thr Leu Thr Ala Asp Ile
    370                 375                 380

Met Thr Tyr Ile His Ser Met Asn Ser Thr Ile Leu Glu Asp Trp Asn
385                 390                 395                 400

Phe Gly Leu Gln Pro Pro Pro Gly Gly Thr Leu Glu Asp Thr Tyr Arg
                405                 410                 415

Phe Val Thr Ser Gln Ala Ile Ala Cys Gln Lys His Thr Pro Pro Ala
            420                 425                 430

Pro Lys Glu Asp Pro Leu Lys Lys Tyr Thr Phe Trp Glu Val Asn Len
        435                 440                 445

Lys Glu Lys Phe Ser Ala Asp Leu Asp Gln Phe Pro Leu Gly Arg Lys
    450                 455                 460

Phe Leu Leu Gln Ala Gly Leu Glu Ala Lys Pro Lys Phe Thr Leu Gly
465                 470                 475                 480

Lys Arg Lys Ala Thr Pro Thr Thr Ser Ser Thr Ser Thr Thr Ala Lys
                485                 490                 495

Arg Lys Lys Arg Lys Leu
            500
```

The Amino Acid Sequence of L1 of HPV18N65C-L1 is showed in SEQ ID NO 9 as follows.

```
Met Arg Pro Ser Asp Asn Thr Val Tyr Leu Pro Pro Pro Ser Val Ala
1               5                  10                  15

Arg Val Val Asn Thr Asp Asp Tyr Val Thr Arg Thr Ser Ile Phe Tyr
            20                  25                  30

His Ala Gly Ser Ser Arg Leu Leu Thr Val Gly Asn Pro Tyr Phe Arg
        35                  40                  45

Val Pro Ala Gly Gly Gly Asn Lys Gln Asp Ile Pro Lys Val Ser Ala
    50                  55                  60

Tyr Gln Tyr Arg Val Phe Arg Val Gln Leu Pro Asp Pro Asn Lys Phe
65                  70                  75                  80

Gly Leu Pro Asp Thr Ser Ile Tyr Asn Pro Glu Thr Gln Arg Leu Val
                85                  90                  95

Trp Ala Cys Ala Gly Val Glu Ile Gly Arg Gly Gln Pro Leu Gly Val
            100                 105                 110

Gly Leu Ser Gly His Pro Phe Tyr Asn Lys Leu Asp Asp Thr Glu Ser
        115                 120                 125

Ser His Ala Ala Thr Ser Asn Val Ser Glu Asp Val Arg Asp Asn Val
    130                 135                 140

Ser Val Asp Tyr Lys Gln Thr Gln Leu Cys Ile Leu Gly Cys Ala Pro
145                 150                 155                 160

Ala Ile Gly Glu His Trp Ala Lys Gly Thr Ala Cys Lys Ser Arg Pro
                165                 170                 175

Leu Ser Gln Gly Asp Cys Pro Pro Leu Glu Leu Lys Asn Thr Val Leu
            180                 185                 190

Glu Asp Gly Asp Met Val Asp Thr Gly Tyr Gly Ala Met Asp Phe Ser
        195                 200                 205

Thr Leu Gln Asp Thr Lys Cys Glu Val Pro Leu Asp Ile Cys Gln Ser
    210                 215                 220

Ile Cys Lys Tyr Pro Asp Tyr Leu Gln Met Ser Ala Asp Pro Tyr Gly
225                 230                 235                 240

Asp Ser Met Phe Phe Cys Leu Arg Arg Glu Gln Leu Phe Ala Arg His
                245                 250                 255

Phe Trp Asn Arg Ala Gly Thr Met Gly Asp Thr Val Pro Gln Ser Leu
            260                 265                 270

Tyr Ile Lys Gly Thr Gly Met Arg Ala Ser Pro Gly Ser Cys Val Tyr
        275                 280                 285

Ser Pro Ser Pro Ser Gly Ser Ile Val Thr Ser Asp Ser Gln Leu Phe
    290                 295                 300

Asn Lys Pro Tyr Trp Leu His Lys Ala Gln Gly His Asn Asn Gly Val
305                 310                 315                 320

Cys Trp His Asn Gln Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser
                325                 330                 335

Thr Asn Leu Thr Ile Cys Ala Ser Thr Gln Ser Pro Val Pro Gly Gln
            340                 345                 350

Tyr Asp Ala Thr Lys Phe Lys Gln Tyr Ser Arg His Val Glu Glu Tyr
        355                 360                 365

Asp Leu Gln Phe Ile Phe Gln Leu Cys Thr Ile Thr Leu Thr Ala Asp
    370                 375                 380

Val Met Ser Tyr Ile His Ser Met Asn Ser Ser Ile Leu Glu Asp Trp
385                 390                 395                 400

Asn Phe Gly Val Pro Pro Pro Pro Thr Thr Ser Leu Val Asp Thr Tyr
                405                 410                 415
```

-continued

```
Arg Phe Val Gln Ser Val Ala Ile Ala Cys Gln Lys Asp Ala Ala Pro
            420                 425                 430

Ala Glu Asn Lys Asp Pro Tyr Asp Lys Leu Lys Phe Trp Asn Val Asp
        435                 440                 445

Leu Lys Glu Lys Phe Ser Leu Asp Leu Asp Gln Tyr Pro Leu Gly Arg
    450                 455                 460

Lys Phe Leu Val Gln Ala Gly Leu Arg Arg Lys Pro Thr Ile Gly Pro
465                 470                 475                 480

Arg Lys Arg Ser Ala Pro Ser Ala Thr Thr Ala Ser Lys Pro Ala Lys
                485                 490                 495

Arg Val Arg Val Arg Ala Arg Lys
            500
```

The Amino Acid Sequence of HPV11N4C-L1 VLP is shown in SEQ ID NO:7, as described above.

EXAMPLE 6

The truncated HPV6L1 proteins set forth in SEQ ID NOs: 2, 3 and 4 were prepared according to the techniques used in examples 1-5. All these truncated proteins could be purified to an extent of above 98% and could be assembled into VLPs with a radius of about 25 nm. The results are shown in FIGS. **9**, **10** and **11**.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 11

<210> SEQ ID NO 1
<211> LENGTH: 498
<212> TYPE: PRT
<213> ORGANISM: Artifical
<220> FEATURE:
<223> OTHER INFORMATION: HPV

<400> SEQUENCE: 1

```
Met Pro Ser Asp Ser Thr Val Tyr Val Pro Pro Pro Asn Pro Val Ser
1               5                   10                  15

Lys Val Val Ala Thr Asp Ala Tyr Val Thr Arg Thr Asn Ile Phe Tyr
            20                  25                  30

His Ala Ser Ser Ser Arg Leu Leu Ala Val Gly His Pro Tyr Phe Ser
        35                  40                  45

Ile Lys Arg Ala Asn Lys Thr Val Val Pro Lys Val Ser Gly Tyr Gln
    50                  55                  60

Tyr Arg Val Phe Lys Val Val Leu Pro Asp Pro Asn Lys Phe Ala Leu
65                  70                  75                  80

Pro Asp Ser Ser Leu Phe Asp Pro Thr Thr Gln Arg Leu Val Trp Ala
                85                  90                  95

Cys Thr Gly Leu Glu Val Gly Arg Gly Gln Pro Leu Gly Val Gly Val
            100                 105                 110

Ser Gly His Pro Phe Leu Asn Lys Tyr Asp Asp Val Glu Asn Ser Gly
        115                 120                 125

Ser Gly Gly Asn Pro Gly Gln Asp Asn Arg Val Asn Val Gly Met Asp
    130                 135                 140

Tyr Lys Gln Thr Gln Leu Cys Met Val Gly Cys Ala Pro Pro Leu Gly
145                 150                 155                 160

Glu His Trp Gly Lys Gly Lys Gln Cys Thr Asn Thr Pro Val Gln Ala
                165                 170                 175
```

-continued

```
Gly Asp Cys Pro Pro Leu Glu Leu Ile Thr Ser Val Ile Gln Asp Gly
            180                 185                 190

Asp Met Val Asp Thr Gly Phe Gly Ala Met Asn Phe Ala Asp Leu Gln
        195                 200                 205

Thr Asn Lys Ser Asp Val Pro Ile Asp Ile Cys Gly Thr Thr Cys Lys
    210                 215                 220

Tyr Pro Asp Tyr Leu Gln Met Ala Ala Asp Pro Tyr Gly Asp Arg Leu
225                 230                 235                 240

Phe Phe Phe Leu Arg Lys Glu Gln Met Phe Ala Arg His Phe Phe Asn
                245                 250                 255

Arg Ala Gly Glu Val Gly Glu Pro Val Pro Asp Thr Leu Ile Ile Lys
            260                 265                 270

Gly Ser Gly Asn Arg Thr Ser Val Gly Ser Ser Ile Tyr Val Asn Thr
        275                 280                 285

Pro Ser Gly Ser Leu Val Ser Ser Glu Ala Gln Leu Phe Asn Lys Pro
    290                 295                 300

Tyr Trp Leu Gln Lys Ala Gln Gly His Asn Asn Gly Ile Cys Trp Gly
305                 310                 315                 320

Asn Gln Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser Thr Asn Met
                325                 330                 335

Thr Leu Cys Ala Ser Val Thr Thr Ser Ser Thr Tyr Thr Asn Ser Asp
            340                 345                 350

Tyr Lys Glu Tyr Met Arg His Val Glu Glu Tyr Asp Leu Gln Phe Ile
        355                 360                 365

Phe Gln Leu Cys Ser Ile Thr Leu Ser Ala Glu Val Val Ala Tyr Ile
    370                 375                 380

His Thr Met Asn Pro Ser Val Leu Glu Asp Trp Asn Phe Gly Leu Ser
385                 390                 395                 400

Pro Pro Pro Asn Gly Thr Leu Glu Asp Thr Tyr Arg Tyr Val Gln Ser
                405                 410                 415

Gln Ala Ile Thr Cys Gln Lys Pro Thr Pro Glu Lys Gln Lys Pro Asp
            420                 425                 430

Pro Tyr Lys Asn Leu Ser Phe Trp Glu Val Asn Leu Lys Glu Lys Phe
        435                 440                 445

Ser Ser Glu Leu Asp Gln Tyr Pro Leu Gly Arg Lys Phe Leu Leu Gln
    450                 455                 460

Ser Gly Tyr Arg Gly Arg Ser Ser Ile Arg Thr Gly Val Lys Arg Pro
465                 470                 475                 480

Ala Val Ser Lys Ala Ser Ala Ala Pro Lys Arg Lys Arg Ala Lys Thr
                485                 490                 495

Lys Arg


<210> SEQ ID NO 2
<211> LENGTH: 499
<212> TYPE: PRT
<213> ORGANISM: Artifical
<220> FEATURE:
<223> OTHER INFORMATION: HPV

<400> SEQUENCE: 2

Met Arg Pro Ser Asp Ser Thr Val Tyr Val Pro Pro Pro Asn Pro Val
1               5                   10                  15

Ser Lys Val Val Ala Thr Asp Ala Tyr Val Thr Arg Thr Asn Ile Phe
            20                  25                  30

Tyr His Ala Ser Ser Ser Arg Leu Leu Ala Val Gly His Pro Tyr Phe
```

-continued

```
        35                  40                  45

Ser Ile Lys Arg Ala Asn Lys Thr Val Val Pro Lys Val Ser Gly Tyr
    50                  55                  60

Gln Tyr Arg Val Phe Lys Val Val Leu Pro Asp Pro Asn Lys Phe Ala
65                  70                  75                  80

Leu Pro Asp Ser Ser Leu Phe Asp Pro Thr Thr Gln Arg Leu Val Trp
                85                  90                  95

Ala Cys Thr Gly Leu Glu Val Gly Arg Gly Gln Pro Leu Gly Val Gly
            100                 105                 110

Val Ser Gly His Pro Phe Leu Asn Lys Tyr Asp Asp Val Glu Asn Ser
        115                 120                 125

Gly Ser Gly Gly Asn Pro Gly Gln Asp Asn Arg Val Asn Val Gly Met
    130                 135                 140

Asp Tyr Lys Gln Thr Gln Leu Cys Met Val Gly Cys Ala Pro Pro Leu
145                 150                 155                 160

Gly Glu His Trp Gly Lys Gly Lys Gln Cys Thr Asn Thr Pro Val Gln
                165                 170                 175

Ala Gly Asp Cys Pro Pro Leu Glu Leu Ile Thr Ser Val Ile Gln Asp
            180                 185                 190

Gly Asp Met Val Asp Thr Gly Phe Gly Ala Met Asn Phe Ala Asp Leu
        195                 200                 205

Gln Thr Asn Lys Ser Asp Val Pro Ile Asp Ile Cys Gly Thr Thr Cys
    210                 215                 220

Lys Tyr Pro Asp Tyr Leu Gln Met Ala Ala Asp Pro Tyr Gly Asp Arg
225                 230                 235                 240

Leu Phe Phe Phe Leu Arg Lys Glu Gln Met Phe Ala Arg His Phe Phe
                245                 250                 255

Asn Arg Ala Gly Glu Val Gly Glu Pro Val Pro Asp Thr Leu Ile Ile
            260                 265                 270

Lys Gly Ser Gly Asn Arg Thr Ser Val Gly Ser Ser Ile Tyr Val Asn
        275                 280                 285

Thr Pro Ser Gly Ser Leu Val Ser Ser Glu Ala Gln Leu Phe Asn Lys
    290                 295                 300

Pro Tyr Trp Leu Gln Lys Ala Gln Gly His Asn Asn Gly Ile Cys Trp
305                 310                 315                 320

Gly Asn Gln Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser Thr Asn
                325                 330                 335

Met Thr Leu Cys Ala Ser Val Thr Thr Ser Ser Thr Tyr Thr Asn Ser
            340                 345                 350

Asp Tyr Lys Glu Tyr Met Arg His Val Glu Glu Tyr Asp Leu Gln Phe
        355                 360                 365

Ile Phe Gln Leu Cys Ser Ile Thr Leu Ser Ala Glu Val Val Ala Tyr
    370                 375                 380

Ile His Thr Met Asn Pro Ser Val Leu Glu Asp Trp Asn Phe Gly Leu
385                 390                 395                 400

Ser Pro Pro Pro Asn Gly Thr Leu Glu Asp Thr Tyr Arg Tyr Val Gln
                405                 410                 415

Ser Gln Ala Ile Thr Cys Gln Lys Pro Thr Pro Glu Lys Gln Lys Pro
            420                 425                 430

Asp Pro Tyr Lys Asn Leu Ser Phe Trp Glu Val Asn Leu Lys Glu Lys
        435                 440                 445

Phe Ser Ser Glu Leu Asp Gln Tyr Pro Leu Gly Arg Lys Phe Leu Leu
    450                 455                 460
```

```
Gln Ser Gly Tyr Arg Gly Arg Ser Ser Ile Arg Thr Gly Val Lys Arg
465                 470                 475                 480

Pro Ala Val Ser Lys Ala Ser Ala Ala Pro Lys Arg Lys Arg Ala Lys
                485                 490                 495

Thr Lys Arg


<210> SEQ ID NO 3
<211> LENGTH: 497
<212> TYPE: PRT
<213> ORGANISM: Artifical
<220> FEATURE:
<223> OTHER INFORMATION: HPV

<400> SEQUENCE: 3

Met Ser Asp Ser Thr Val Tyr Val Pro Pro Pro Asn Pro Val Ser Lys
1               5                   10                  15

Val Val Ala Thr Asp Ala Tyr Val Thr Arg Thr Asn Ile Phe Tyr His
            20                  25                  30

Ala Ser Ser Ser Arg Leu Leu Ala Val Gly His Pro Tyr Phe Ser Ile
        35                  40                  45

Lys Arg Ala Asn Lys Thr Val Val Pro Lys Val Ser Gly Tyr Gln Tyr
    50                  55                  60

Arg Val Phe Lys Val Val Leu Pro Asp Pro Asn Lys Phe Ala Leu Pro
65                  70                  75                  80

Asp Ser Ser Leu Phe Asp Pro Thr Thr Gln Arg Leu Val Trp Ala Cys
                85                  90                  95

Thr Gly Leu Glu Val Gly Arg Gly Gln Pro Leu Gly Val Gly Val Ser
            100                 105                 110

Gly His Pro Phe Leu Asn Lys Tyr Asp Asp Val Glu Asn Ser Gly Ser
        115                 120                 125

Gly Gly Asn Pro Gly Gln Asp Asn Arg Val Asn Val Gly Met Asp Tyr
    130                 135                 140

Lys Gln Thr Gln Leu Cys Met Val Gly Cys Ala Pro Pro Leu Gly Glu
145                 150                 155                 160

His Trp Gly Lys Gly Lys Gln Cys Thr Asn Thr Pro Val Gln Ala Gly
                165                 170                 175

Asp Cys Pro Pro Leu Glu Leu Ile Thr Ser Val Ile Gln Asp Gly Asp
            180                 185                 190

Met Val Asp Thr Gly Phe Gly Ala Met Asn Phe Ala Asp Leu Gln Thr
        195                 200                 205

Asn Lys Ser Asp Val Pro Ile Asp Ile Cys Gly Thr Thr Cys Lys Tyr
    210                 215                 220

Pro Asp Tyr Leu Gln Met Ala Ala Asp Pro Tyr Gly Asp Arg Leu Phe
225                 230                 235                 240

Phe Phe Leu Arg Lys Glu Gln Met Phe Ala Arg His Phe Phe Asn Arg
                245                 250                 255

Ala Gly Glu Val Gly Glu Pro Val Pro Asp Thr Leu Ile Ile Lys Gly
            260                 265                 270

Ser Gly Asn Arg Thr Ser Val Gly Ser Ser Ile Tyr Val Asn Thr Pro
        275                 280                 285

Ser Gly Ser Leu Val Ser Ser Glu Ala Gln Leu Phe Asn Lys Pro Tyr
    290                 295                 300

Trp Leu Gln Lys Ala Gln Gly His Asn Asn Gly Ile Cys Trp Gly Asn
305                 310                 315                 320
```

-continued

```
Gln Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser Thr Asn Met Thr
                325                 330                 335

Leu Cys Ala Ser Val Thr Thr Ser Ser Thr Tyr Thr Asn Ser Asp Tyr
            340                 345                 350

Lys Glu Tyr Met Arg His Val Glu Glu Tyr Asp Leu Gln Phe Ile Phe
        355                 360                 365

Gln Leu Cys Ser Ile Thr Leu Ser Ala Glu Val Val Ala Tyr Ile His
    370                 375                 380

Thr Met Asn Pro Ser Val Leu Glu Asp Trp Asn Phe Gly Leu Ser Pro
385                 390                 395                 400

Pro Pro Asn Gly Thr Leu Glu Asp Thr Tyr Arg Tyr Val Gln Ser Gln
                405                 410                 415

Ala Ile Thr Cys Gln Lys Pro Thr Pro Glu Lys Gln Lys Pro Asp Pro
            420                 425                 430

Tyr Lys Asn Leu Ser Phe Trp Glu Val Asn Leu Lys Glu Lys Phe Ser
        435                 440                 445

Ser Glu Leu Asp Gln Tyr Pro Leu Gly Arg Lys Phe Leu Leu Gln Ser
    450                 455                 460

Gly Tyr Arg Gly Arg Ser Ser Ile Arg Thr Gly Val Lys Arg Pro Ala
465                 470                 475                 480

Val Ser Lys Ala Ser Ala Ala Pro Lys Arg Lys Arg Ala Lys Thr Lys
                485                 490                 495

Arg
```

<210> SEQ ID NO 4
<211> LENGTH: 496
<212> TYPE: PRT
<213> ORGANISM: Artifical
<220> FEATURE:
<223> OTHER INFORMATION: HPV

<400> SEQUENCE: 4

```
Met Asp Ser Thr Val Tyr Val Pro Pro Pro Asn Pro Val Ser Lys Val
1               5                   10                  15

Val Ala Thr Asp Ala Tyr Val Thr Arg Thr Asn Ile Phe Tyr His Ala
            20                  25                  30

Ser Ser Ser Arg Leu Leu Ala Val Gly His Pro Tyr Phe Ser Ile Lys
        35                  40                  45

Arg Ala Asn Lys Thr Val Val Pro Lys Val Ser Gly Tyr Gln Tyr Arg
    50                  55                  60

Val Phe Lys Val Val Leu Pro Asp Pro Asn Lys Phe Ala Leu Pro Asp
65                  70                  75                  80

Ser Ser Leu Phe Asp Pro Thr Thr Gln Arg Leu Val Trp Ala Cys Thr
                85                  90                  95

Gly Leu Glu Val Gly Arg Gly Gln Pro Leu Gly Val Gly Val Ser Gly
            100                 105                 110

His Pro Phe Leu Asn Lys Tyr Asp Asp Val Glu Asn Ser Gly Ser Gly
        115                 120                 125

Gly Asn Pro Gly Gln Asp Asn Arg Val Asn Val Gly Met Asp Tyr Lys
    130                 135                 140

Gln Thr Gln Leu Cys Met Val Gly Cys Ala Pro Pro Leu Gly Glu His
145                 150                 155                 160

Trp Gly Lys Gly Lys Gln Cys Thr Asn Thr Pro Val Gln Ala Gly Asp
                165                 170                 175

Cys Pro Pro Leu Glu Leu Ile Thr Ser Val Ile Gln Asp Gly Asp Met
```

-continued

```
            180                 185                 190

Val Asp Thr Gly Phe Gly Ala Met Asn Phe Ala Asp Leu Gln Thr Asn
        195                 200                 205

Lys Ser Asp Val Pro Ile Asp Ile Cys Gly Thr Thr Cys Lys Tyr Pro
    210                 215                 220

Asp Tyr Leu Gln Met Ala Ala Asp Pro Tyr Gly Asp Arg Leu Phe Phe
225                 230                 235                 240

Phe Leu Arg Lys Glu Gln Met Phe Ala Arg His Phe Phe Asn Arg Ala
                245                 250                 255

Gly Glu Val Gly Glu Pro Val Pro Asp Thr Leu Ile Ile Lys Gly Ser
            260                 265                 270

Gly Asn Arg Thr Ser Val Gly Ser Ser Ile Tyr Val Asn Thr Pro Ser
        275                 280                 285

Gly Ser Leu Val Ser Ser Glu Ala Gln Leu Phe Asn Lys Pro Tyr Trp
    290                 295                 300

Leu Gln Lys Ala Gln Gly His Asn Asn Gly Ile Cys Trp Gly Asn Gln
305                 310                 315                 320

Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser Thr Asn Met Thr Leu
                325                 330                 335

Cys Ala Ser Val Thr Thr Ser Ser Thr Tyr Thr Asn Ser Asp Tyr Lys
            340                 345                 350

Glu Tyr Met Arg His Val Glu Glu Tyr Asp Leu Gln Phe Ile Phe Gln
        355                 360                 365

Leu Cys Ser Ile Thr Leu Ser Ala Glu Val Val Ala Tyr Ile His Thr
    370                 375                 380

Met Asn Pro Ser Val Leu Glu Asp Trp Asn Phe Gly Leu Ser Pro Pro
385                 390                 395                 400

Pro Asn Gly Thr Leu Glu Asp Thr Tyr Arg Tyr Val Gln Ser Gln Ala
                405                 410                 415

Ile Thr Cys Gln Lys Pro Thr Pro Glu Lys Gln Lys Pro Asp Pro Tyr
            420                 425                 430

Lys Asn Leu Ser Phe Trp Glu Val Asn Leu Lys Glu Lys Phe Ser Ser
        435                 440                 445

Glu Leu Asp Gln Tyr Pro Leu Gly Arg Lys Phe Leu Leu Gln Ser Gly
    450                 455                 460

Tyr Arg Gly Arg Ser Ser Ile Arg Thr Gly Val Lys Arg Pro Ala Val
465                 470                 475                 480

Ser Lys Ala Ser Ala Ala Pro Lys Arg Lys Arg Ala Lys Thr Lys Arg
                485                 490                 495


<210> SEQ ID NO 5
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Artifical
<220> FEATURE:
<223> OTHER INFORMATION: HPV

<400> SEQUENCE: 5

atgtggcggc ctagcgacag cacagtatat gtgcctcctc ctaaccctgt atccaaagtt     60

gttgccacgg atgcttatgt tactcgcacc aacatatttt atcatgccag cagttctaga    120

cttcttgcag tgggtcatcc ttatttttcc ataaaacggg ctaacaaaac tgttgtgcca    180

aaggtgtcag gatatcaata cagggtattt aaggtggtgt taccagatcc taacaaattt    240

gcattgcctg actcgtctct ttttgatccc acaacacaac gtttggtatg ggcatgcaca    300
```

-continued

ggcctagagg tgggcagggg acagccatta ggtgtgggtg taagtggaca tcctttccta 360

aataaatatg atgatgttga aaattcaggg agtggtggta accctggaca ggataacagg 420

gttaatgttg gtatggatta taaacaaaca caattatgca tggttggatg tgcccccct 480

ttgggcgagc attggggtaa aggtaaacag tgtactaata cacctgtaca ggctggtgac 540

tgcccgccct tagaacttat taccagtgtt atacaggatg gcgatatggt tgacacaggc 600

tttggtgcta tgaattttgc tgatttgcag accaataaat cagatgttcc tattgacata 660

tgtggcacta catgtaaata tccagattat ttacaaatgg ctgcagaccc atatggtgat 720

agattatttt tttttctacg gaaggaacaa atgtttgcca gacatttttt taacagggct 780

ggcgaggtgg gggaacctgt gcctgatact cttataatta agggtagtgg aaatcgaacg 840

tctgtaggga gtagtatata tgttaacacc ccaagcggct ctttggtgtc ctctgaggca 900

caattgttta ataagccata ttggctacaa aaagcccagg gacataacaa tggtatttgt 960

tggggtaatc aactgtttgt tactgtggta gataccacac gcagtaccaa catgacatta 1020

tgtgcatccg taactacatc ttccacatac accaattctg attataaaga gtacatgcgt 1080

catgtggaag agtatgattt acaatttatt tttcaattat gtagcattac attgtctgct 1140

gaagtaatgg cctatattca cacaatgaat ccctctgttt tggaagactg gaactttggg 1200

ttatcgcctc ccccaaatgg tacattagaa gatacctata ggtatgtgca gtcacaggcc 1260

attacctgtc aaaagcccac tcctgaaaag caaaagccag atccctataa gaaccttagt 1320

ttttgggagg ttaatttaaa agaaaagttt tctagtgaat tggatcagta tcctttggga 1380

cgcaagtttt tgttacaaag tggatatagg ggacggtcct ctattcgtac cggtgttaag 1440

cgccctgctg tttccaaagc ctctgctgcc cctaaacgta agcgcgccaa aactaaaagg 1500

taa 1503

<210> SEQ ID NO 6
<211> LENGTH: 1497
<212> TYPE: DNA
<213> ORGANISM: Artifical
<220> FEATURE:
<223> OTHER INFORMATION: HPV

<400> SEQUENCE: 6

atgcctagcg acagcacagt atatgtgcct cctcctaacc ctgtatccaa agttgttgcc 60

acggatgctt atgttactcg caccaacata ttttatcatg ccagcagttc tagacttctt 120

gcagtgggtc atccttattt ttccataaaa cgggctaaca aaactgttgt gccaaaggtg 180

tcaggatatc aatacagggt atttaaggtg gtgttaccag atcctaacaa atttgcattg 240

cctgactcgt ctcttttga tcccacaaca caacgtttgg tatgggcatg cacaggccta 300

gaggtgggca ggggacagcc attaggtgtg ggtgtaagtg gacatccttt cctaaataaa 360

tatgatgatg ttgaaaattc agggagtggt ggtaaccctg gacaggataa cagggttaat 420

gttggtatgg attataaaca aacacaatta tgcatggttg gatgtgcccc ccctttgggc 480

gagcattggg gtaaaggtaa acagtgtact aatacacctg tacaggctgg tgactgcccg 540

cccttagaac ttattaccag tgttatacag gatggcgata tggttgacac aggctttggt 600

gctatgaatt ttgctgattt gcagaccaat aaatcagatg ttcctattga tatatgtggc 660

actacatgta aatatccaga ttatttacaa atggctgcag acccttatgg tgatagatta 720

ttttttttttc tacggaagga acaaatgttt gccagacatt tttttaacag ggctggcgag 780

gtgggggaac ctgtgcctga tactcttata attaagggta gtggaaatcg aacgtctgta 840

-continued

gggagtagta tatatgttaa caccccaagc ggctctttgg tgtcctctga ggcacaattg 900

tttaataagc catattggct acaaaaagcc cagggacata acaatggtat ttgttggggt 960

aatcaactgt ttgttactgt ggtagatacc acacgcagta ccaacatgac attatgtgca 1020

tccgtaacta catcttccac atacaccaat tctgattata aagagtacat gcgtcatgtg 1080

gaagagtatg atttacaatt tatttttcaa ttatgtagca ttacattgtc tgctgaagta 1140

gtggcctata ttcacacaat gaatccctct gttttggaag actggaactt tgggttatcg 1200

cctcccccaa atggtacatt agaagatacc tataggtatg tgcagtcaca ggccattacc 1260

tgtcaaaagc ccactcctga aaagcaaaag ccagatccct ataagaacct tagtttttgg 1320

gaggttaatt taaaagaaaa gttttctagt gaattggatc agtatccttt gggacgcaag 1380

tttttgttac aaagtggata taggggacgg tcctctattc gtaccggtgt taagcgccct 1440

gctgtttcca aagcctctgc tgcccctaaa cgtaagcgcg ccaaaactaa aaggtaa 1497

<210> SEQ ID NO 7
<211> LENGTH: 498
<212> TYPE: PRT
<213> ORGANISM: Artifical sequence
<220> FEATURE:
<223> OTHER INFORMATION: HPV

<400> SEQUENCE: 7

Met Ser Asp Ser Thr Val Tyr Val Pro Pro Pro Asn Pro Val Ser Lys
1 5 10 15

Val Val Ala Thr Asp Ala Tyr Val Lys Arg Thr Asn Ile Phe Tyr His
20 25 30

Ala Ser Ser Ser Arg Leu Leu Ala Val Gly His Pro Tyr Tyr Ser Ile
35 40 45

Lys Lys Val Asn Lys Thr Val Val Pro Lys Val Ser Gly Tyr Gln Tyr
50 55 60

Arg Val Phe Lys Val Val Leu Pro Asp Pro Asn Lys Phe Ala Leu Pro
65 70 75 80

Asp Ser Ser Leu Phe Asp Pro Thr Thr Gln Arg Leu Val Trp Ala Cys
85 90 95

Thr Gly Leu Glu Val Gly Arg Gly Gln Pro Leu Gly Val Gly Val Ser
100 105 110

Gly His Pro Leu Leu Asn Lys Tyr Asp Asp Val Glu Asn Ser Gly Gly
115 120 125

Tyr Gly Gly Asn Pro Gly Gln Asp Asn Arg Val Asn Val Gly Met Asp
130 135 140

Tyr Lys Gln Thr Gln Leu Cys Met Val Gly Cys Ala Pro Pro Leu Gly
145 150 155 160

Glu His Trp Gly Lys Gly Thr Gln Cys Ser Asn Thr Ser Val Gln Asn
165 170 175

Gly Asp Cys Pro Pro Leu Glu Leu Ile Thr Ser Val Ile Gln Asp Gly
180 185 190

Asp Met Val Asp Thr Gly Phe Gly Ala Met Asn Phe Ala Asp Leu Gln
195 200 205

Thr Asn Lys Ser Asp Val Pro Leu Asp Ile Cys Gly Thr Val Cys Lys
210 215 220

Tyr Pro Asp Tyr Leu Gln Met Ala Ala Asp Pro Tyr Gly Asp Arg Leu
225 230 235 240

Phe Phe Tyr Leu Arg Lys Glu Gln Met Phe Ala Arg His Phe Phe Asn

-continued

```
               245                 250                 255

Arg Ala Gly Thr Val Gly Glu Pro Val Pro Asp Asp Leu Leu Val Lys
            260                 265                 270

Gly Gly Asn Asn Arg Ser Ser Val Ala Ser Ser Ile Tyr Val His Thr
        275                 280                 285

Pro Ser Gly Ser Leu Val Ser Ser Glu Ala Gln Leu Phe Asn Lys Pro
    290                 295                 300

Tyr Trp Leu Gln Lys Ala Gln Gly His Asn Asn Gly Ile Cys Trp Gly
305                 310                 315                 320

Asn His Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser Thr Asn Met
                325                 330                 335

Thr Leu Cys Ala Ser Val Ser Lys Ser Ala Thr Tyr Thr Asn Ser Asp
            340                 345                 350

Tyr Lys Glu Tyr Met Arg His Val Glu Glu Phe Asp Leu Gln Phe Ile
        355                 360                 365

Phe Gln Leu Cys Ser Ile Thr Leu Ser Ala Glu Val Met Ala Tyr Ile
    370                 375                 380

His Thr Met Asn Pro Ser Val Leu Glu Asp Trp Asn Phe Gly Leu Ser
385                 390                 395                 400

Pro Pro Pro Asn Gly Thr Leu Glu Asp Thr Tyr Arg Tyr Val Gln Ser
                405                 410                 415

Gln Ala Ile Thr Cys Gln Lys Pro Thr Pro Glu Lys Glu Lys Gln Asp
            420                 425                 430

Pro Tyr Lys Asp Met Ser Phe Trp Glu Val Asn Leu Lys Glu Lys Phe
        435                 440                 445

Ser Ser Glu Leu Asp Gln Phe Pro Leu Gly Arg Lys Phe Leu Leu Gln
    450                 455                 460

Ser Gly Tyr Arg Gly Arg Thr Ser Ala Arg Thr Gly Ile Lys Arg Pro
465                 470                 475                 480

Ala Val Ser Lys Pro Ser Thr Ala Pro Lys Arg Lys Arg Thr Lys Thr
                485                 490                 495

Lys Lys


<210> SEQ ID NO 8
<211> LENGTH: 502
<212> TYPE: PRT
<213> ORGANISM: Artifical sequence
<220> FEATURE:
<223> OTHER INFORMATION: HPV

<400> SEQUENCE: 8

Met Leu Pro Ser Glu Ala Thr Val Tyr Leu Pro Pro Val Pro Val Ser
1               5                   10                  15

Lys Val Val Ser Thr Asp Glu Tyr Val Ala Arg Thr Asn Ile Tyr Tyr
            20                  25                  30

His Ala Gly Thr Ser Arg Leu Leu Ala Val Gly His Pro Tyr Phe Pro
        35                  40                  45

Ile Lys Lys Pro Asn Asn Asn Lys Ile Leu Val Pro Lys Val Ser Gly
    50                  55                  60

Leu Gln Tyr Arg Val Phe Arg Ile His Leu Pro Asp Pro Asn Lys Phe
65                  70                  75                  80

Gly Phe Pro Asp Thr Ser Phe Tyr Asn Pro Asp Thr Gln Arg Leu Val
                85                  90                  95

Trp Ala Cys Val Gly Val Glu Val Gly Arg Gly Gln Pro Leu Gly Val
            100                 105                 110
```

-continued

```
Gly Ile Ser Gly His Pro Leu Leu Asn Lys Leu Asp Asp Thr Glu Asn
        115                 120                 125

Ala Ser Ala Tyr Ala Ala Asn Ala Gly Val Asp Asn Arg Glu Cys Ile
    130                 135                 140

Ser Met Asp Tyr Lys Gln Thr Gln Leu Cys Leu Ile Gly Cys Lys Pro
145                 150                 155                 160

Pro Ile Gly Glu His Trp Gly Lys Gly Ser Pro Cys Thr Asn Val Ala
                165                 170                 175

Val Asn Pro Gly Asp Cys Pro Pro Leu Glu Leu Ile Asn Thr Val Ile
            180                 185                 190

Gln Asp Gly Asp Met Val Asp Thr Gly Phe Gly Ala Met Asp Phe Thr
        195                 200                 205

Thr Leu Gln Ala Asn Lys Ser Glu Val Pro Leu Asp Ile Cys Thr Ser
    210                 215                 220

Ile Cys Lys Tyr Pro Asp Tyr Ile Lys Met Val Ser Glu Pro Tyr Gly
225                 230                 235                 240

Asp Ser Leu Phe Phe Tyr Leu Arg Arg Glu Gln Met Phe Val Arg His
                245                 250                 255

Leu Phe Asn Arg Ala Gly Ala Val Gly Asp Asn Val Pro Asp Asp Leu
            260                 265                 270

Tyr Ile Lys Gly Ser Gly Ser Thr Ala Asn Leu Ala Ser Ser Asn Tyr
        275                 280                 285

Phe Pro Thr Pro Ser Gly Ser Met Val Thr Ser Asp Ala Gln Ile Phe
    290                 295                 300

Asn Lys Pro Tyr Trp Leu Gln Arg Ala Gln Gly His Asn Asn Gly Ile
305                 310                 315                 320

Cys Trp Gly Asn Gln Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser
                325                 330                 335

Thr Asn Met Ser Leu Cys Ala Ala Ile Ser Thr Ser Glu Thr Thr Tyr
            340                 345                 350

Lys Asn Thr Asn Phe Lys Glu Tyr Leu Arg His Gly Glu Glu Tyr Asp
        355                 360                 365

Leu Gln Phe Ile Phe Gln Leu Cys Lys Ile Thr Leu Thr Ala Asp Ile
    370                 375                 380

Met Thr Tyr Ile His Ser Met Asn Ser Thr Ile Leu Glu Asp Trp Asn
385                 390                 395                 400

Phe Gly Leu Gln Pro Pro Pro Gly Gly Thr Leu Glu Asp Thr Tyr Arg
                405                 410                 415

Phe Val Thr Ser Gln Ala Ile Ala Cys Gln Lys His Thr Pro Pro Ala
            420                 425                 430

Pro Lys Glu Asp Pro Leu Lys Lys Tyr Thr Phe Trp Glu Val Asn Leu
        435                 440                 445

Lys Glu Lys Phe Ser Ala Asp Leu Asp Gln Phe Pro Leu Gly Arg Lys
    450                 455                 460

Phe Leu Leu Gln Ala Gly Leu Glu Ala Lys Pro Lys Phe Thr Leu Gly
465                 470                 475                 480

Lys Arg Lys Ala Thr Pro Thr Thr Ser Ser Thr Ser Thr Thr Ala Lys
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Arg Lys Lys Arg Lys Leu
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<210> SEQ ID NO 9
<211> LENGTH: 504

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<212> TYPE: PRT
<213> ORGANISM: Artifical sequence
<220> FEATURE:
<223> OTHER INFORMATION: HPV

<400> SEQUENCE: 9

Met Arg Pro Ser Asp Asn Thr Val Tyr Leu Pro Pro Pro Ser Val Ala
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Arg Val Val Asn Thr Asp Asp Tyr Val Thr Arg Thr Ser Ile Phe Tyr
20 25 30

His Ala Gly Ser Ser Arg Leu Leu Thr Val Gly Asn Pro Tyr Phe Arg
35 40 45

Val Pro Ala Gly Gly Gly Asn Lys Gln Asp Ile Pro Lys Val Ser Ala
50 55 60

Tyr Gln Tyr Arg Val Phe Arg Val Gln Leu Pro Asp Pro Asn Lys Phe
65 70 75 80

Gly Leu Pro Asp Thr Ser Ile Tyr Asn Pro Glu Thr Gln Arg Leu Val
85 90 95

Trp Ala Cys Ala Gly Val Glu Ile Gly Arg Gly Gln Pro Leu Gly Val
100 105 110

Gly Leu Ser Gly His Pro Phe Tyr Asn Lys Leu Asp Asp Thr Glu Ser
115 120 125

Ser His Ala Ala Thr Ser Asn Val Ser Glu Asp Val Arg Asp Asn Val
130 135 140

Ser Val Asp Tyr Lys Gln Thr Gln Leu Cys Ile Leu Gly Cys Ala Pro
145 150 155 160

Ala Ile Gly Glu His Trp Ala Lys Gly Thr Ala Cys Lys Ser Arg Pro
165 170 175

Leu Ser Gln Gly Asp Cys Pro Pro Leu Glu Leu Lys Asn Thr Val Leu
180 185 190

Glu Asp Gly Asp Met Val Asp Thr Gly Tyr Gly Ala Met Asp Phe Ser
195 200 205

Thr Leu Gln Asp Thr Lys Cys Glu Val Pro Leu Asp Ile Cys Gln Ser
210 215 220

Ile Cys Lys Tyr Pro Asp Tyr Leu Gln Met Ser Ala Asp Pro Tyr Gly
225 230 235 240

Asp Ser Met Phe Phe Cys Leu Arg Arg Glu Gln Leu Phe Ala Arg His
245 250 255

Phe Trp Asn Arg Ala Gly Thr Met Gly Asp Thr Val Pro Gln Ser Leu
260 265 270

Tyr Ile Lys Gly Thr Gly Met Arg Ala Ser Pro Gly Ser Cys Val Tyr
275 280 285

Ser Pro Ser Pro Ser Gly Ser Ile Val Thr Ser Asp Ser Gln Leu Phe
290 295 300

Asn Lys Pro Tyr Trp Leu His Lys Ala Gln Gly His Asn Asn Gly Val
305 310 315 320

Cys Trp His Asn Gln Leu Phe Val Thr Val Val Asp Thr Thr Arg Ser
325 330 335

Thr Asn Leu Thr Ile Cys Ala Ser Thr Gln Ser Pro Val Pro Gly Gln
340 345 350

Tyr Asp Ala Thr Lys Phe Lys Gln Tyr Ser Arg His Val Glu Glu Tyr
355 360 365

Asp Leu Gln Phe Ile Phe Gln Leu Cys Thr Ile Thr Leu Thr Ala Asp
370 375 380

-continued

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Val Met Ser Tyr Ile His Ser Met Asn Ser Ser Ile Leu Glu Asp Trp
385                 390                 395                 400

Asn Phe Gly Val Pro Pro Pro Pro Thr Thr Ser Leu Val Asp Thr Tyr
                405                 410                 415

Arg Phe Val Gln Ser Val Ala Ile Ala Cys Gln Lys Asp Ala Ala Pro
            420                 425                 430

Ala Glu Asn Lys Asp Pro Tyr Asp Lys Leu Lys Phe Trp Asn Val Asp
        435                 440                 445

Leu Lys Glu Lys Phe Ser Leu Asp Leu Asp Gln Tyr Pro Leu Gly Arg
    450                 455                 460

Lys Phe Leu Val Gln Ala Gly Leu Arg Arg Lys Pro Thr Ile Gly Pro
465                 470                 475                 480

Arg Lys Arg Ser Ala Pro Ser Ala Thr Thr Ala Ser Lys Pro Ala Lys
                485                 490                 495

Arg Val Arg Val Arg Ala Arg Lys
            500


<210> SEQ ID NO 10
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artifical sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 10

catatgccta gcgacagcac agtata                                        26


<210> SEQ ID NO 11
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artifical sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic

<400> SEQUENCE: 11

gtcgacttac cttttagttt tggcgc                                        26
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The invention claimed is:

1. A method for producing a human papillomavirus (HPV) L1 protein, consisting of:

a) expressing a HPV L1 gene encoding the HPV L1 protein in an *E. coli* expression system;

b) disrupting the *E. coli*, which has expressed the HPV L1 protein, in a salt solution having a salt concentration of from 200 mM to 500 mM, centrifuging the solution comprising the disrupted *E. coli*, and isolating the supernatant without using chromatography;

c) precipitating the HPV L1 protein from the supernatant of b) by decreasing the salt concentration of the supernatant of b) to from 100 mM to 0, inclusive, by using water or a low salt solution, and collecting the resultant precipitate;

d) redissolving the precipitate of c) in a salt solution having a salt concentration of from 150 mM to 25006 mM, adding a reductant to it, and then centrifuging the resultant solution, and isolating the supernatant without using chromatography, wherein the supernatant contains the HPV L1 protein with a purity of at least 50%.

2. The method of claim **1**, wherein the HPV L1 protein is HPV6 L1 protein.

3. The method of claim **2**, wherein the HPV L1 protein is HPV6 L1 protein with 2, 3, 4, or 5 amino acids truncated at its N-terminal.

4. The method o claim **1**, wherein the HPV L1 protein is HPV6 L1 protein having a sequence set forth in SEQ ID NOs:1, 2, 3, or 4.

5. The method of claim **1**, wherein the salt concentration in step d) is 200 mM-2000 mM.

6. The method of claim **1**, wherein the purity is at least 70%.

7. The method of claim **1**, wherein the salt in steps b), c) or d) is one or more of neutral salts.

8. The method of claim **1**, wherein the salt in steps b), c) or d) is selected from alkali metal salt, ammonium salts, hydrochlorides, sulfates, bicarbonates, phosphate salts or hydrogenphosphates.

9. The method of claim **1**, wherein the salt in steps b), c) or d) is selected from NaCl, KCl, NH_4Cl, and $(NH_4)_2SO_4$.

10. The method of claim **2**, wherein the salt concentration in step d) is 200 mM-2000 mM.

11. The method of claim **2**, wherein the purity is at least 70%.

12. The method of claim **2**, wherein the salt in steps b), c) or d) is one or more of neutral salts.

13. The method of claim **2**, wherein the salt in steps b), c) or d) is selected from alkali metal salt, ammonium salts, hydrochlorides, sulfates, bicarbonates, phosphate salts or hydrogenphosphates.

14. The method of claim **2**, wherein the salt in steps b), c) or d) is selected from NaCl, KCl, NH_4Cl, and $(NH_4)SO_4$.

15. A method for obtaining a virus-like particles (VLP) of a human papillomavirus (HPV) 6 L1 protein, the method consisting of:

a) expressing a HPV L1 gene encoding the HPV6 L1 protein in an *E. coli* expression system;

b) disrupting the *E. coli*, which has expressed the HPV6 L1 protein, in a salt solution having a salt concentration of from 200 mM to 600 mM, centrifuging the solution comprising the disrupted *E. coli*, and isolating the supernatant without using chromatography;

c) precipitating the HPV L1 protein from the supernatant of b) by decreasing the salt concentration of the supernatant of b) to from 100 mM to 0, inclusive, by using water or a low salt solution, and collecting the resultant precipitate;

d) redissolving the precipitate of c) in a salt solution having a salt concentration of from 150 mM to 2500 mM, adding a reductant to it, and then centrifuging the resultant solution, and isolating the supernatant without using chromatography, wherein the supernatant contains the HPV6 L1protein with a purity of at least 50%;

e) further purifying the HPV 6 L1 protein with a purity of at least 50% obtained in step d) by chromatography; and

f) removing the reductant from the HPV 6L1 protein obtained in step e).

* * * * *