



REVIEW ARTICLE

Virus like Particles as Vaccines

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ABSTRACT

Virus Like Particles are formed by the structural elements of viruses representing a specific class of subunit vaccine that mimic the structure of authentic virus particle. They are recognized readily by the immune system and presents viral antigens in a more authentic conformation. Virus Like Particles have therefore shown dramatic effectiveness as a candidate vaccine. To date, a wide variety of VLP-based candidate vaccines targeting various viral, bacterial, parasitic and fungal pathogens, as well as non-infectious diseases, have been produced in different expression systems. Some VLPs have entered clinical development and a few have been licensed and commercialized. The aim of the present review is to give an account of achievements brought about by the availability of Virus Like Particles to serve as Vaccines. The main objective of the topic is to review the basic idea about the production of virus like particles used as vaccine against various diseases. Here we study various methods and processes involved in the production of virus like particles. Production of VLP-based vaccine for Human Pappilomavirus, Influenza and Hepatitis B using different expression systems is been reviewed in these manuscript.

KEYWORDS

Virus like particle, VLP, HPV VLP, HEP B VLP, Influenza Virus VLP

INTRODUCTION

Vaccination is the most effective way to control and prevent infectious diseases. The principle of vaccination is to generate sufficient immunity to protect from infectious disease. At present, the majority of vaccines are based on inactivated or live attenuated pathogens. Although these vaccines are highly effective, they often induce some side effects. With the advances in recombinant DNA technologies and genetic engineering, a new class for vaccines, i.e. subunit vaccines are developed which are based on specific components of pathogen and not the whole pathogen.

Therefore, subunit vaccines are safer than full pathogen based inactivated or live attenuated vaccines. Virus like Particles represents advancement in development of subunit vaccines with appreciable safety and enhanced immunogenicity.

A Virus like Particle (VLPs) is a biological construct, designed to look like a virus, but which is not infectious¹. These are a highly effective type of subunit vaccine that mimics the overall structure of virus particles. These complex macromolecular structures are formed by viral envelope proteins (pseudocapsids) or nucleoproteins (core particles).² When expressed in a suitable heterologous system, viral structural proteins involved in capsid and envelop formation self-assemble in Virus like particle in the absence of other viral

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components, such as viral genomes and multiple structural or non-structural proteins³. The chemically synthesized peptides are capable of eliciting the protective antibodies against the antigens from which they are derived.

Virus like particles elicits strong humoral response at lower doses of antigen relative to subunit vaccines. These particles are potent immunogens because they display a number of features that contribute to their efficacy as T cell and B cell-stimulating antigens. This includes:⁴

(1) A stable and compact structure containing hundreds of copies of the same protein antigen that are focused to produce an immune response.

(2) A highly ordered structure of antigenic epitopes present on the particles forming proteins that can trigger multi-specific immune responses.

(3) Interactions between CD4+ ‘helper’ T cells and CD8+ ‘effector’ T cells as well as B cells, presented by the Virus like particles produces immune response.

Division of VLPs by Structure^{5,6,7,8}

Virus like Particles is composed of proteins that coat a virus. The protein shell that coats a virus is called as “capsid”. Capsids are the most important component of a Virus like Particles Vaccines, because these surface proteins acts as antigen and are responsible to produce protective antibodies, thereby eliciting immune response. Virus like particle vaccines consists of multiple copies of a protein antigen, when assembled together; mimic the structure of a native virus.⁶ Based on the structure of their parental viruses, VLPs can be divided into two major categories:

(1) Non-enveloped VLPs

(2) Enveloped VLPs

Non-Enveloped VLPs⁹

Non-enveloped VLPs are composed of one or more components of a pathogen. They possess the ability to self-assemble into particles and do not include any host components. For number of non-enveloped viruses, capsids are formed by

only one or two major proteins. Examples of these are, VLPs formed by expression of major capsid proteins of papilloma virus, Parvovirus, Calcivirus, Circovirus, Polyomavirus, Rotavirus, Norwalk virus (NV), Hepatitis E virus, Hepatitis B virus.

Chimeric non-enveloped VLPs can also be developed, where antigens are expressed on the surface of particles, which consists of components of pathogens which can self-assemble on the surface of particles.

Non-enveloped VLPs can consist of single or multiple components of a target pathogen or a single or multiple vaccine target antigens displayed on the VLP surface as a fusion to a heterologous viral protein with the ability to self-assemble.

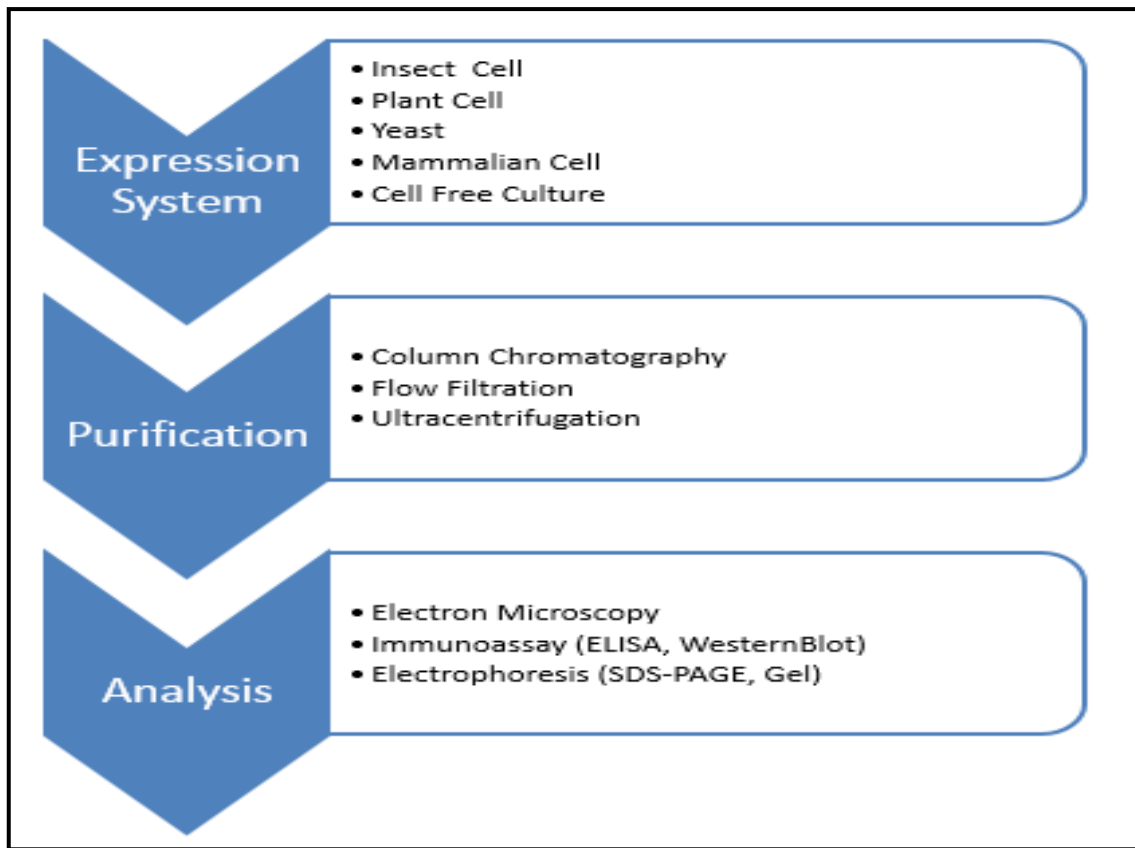
Enveloped VLPs^{5,9}

Enveloped VLPs are complex structures than non-enveloped VLPs. Enveloped VLPs consist of the host cell membrane (an envelope) with antigens on the outer surface. Enveloped VLPs provide surface for integration of more antigens from the same or different pathogens.

Examples of enveloped VLPs are VLPs produced to express antigens from influenza viruses, retroviruses and hepatitis C virus. Production of enveloped VLPs requires expression of several structural viral proteins, their assembly into particles, incorporation into host membranes and release of particles from the cell membrane. The enveloped VLPs produced, resembles native virus particles by size and structure of the surface.

In addition, the assembly and release of enveloped VLPs is also seen for retroviruses including HIV and Simian Immunodeficiency Virus (SIV). The Gag proteins of HIV and SIV have been shown form VLPs in insect cells. The enveloped VLPs containing antigens from heterologous viruses, such as SIV Gag protein and HIV Envelop protein, can also be produced.

Enveloped VLPs represent complex structures which consist of multiple components of pathogens and host membrane components and resemble the structure of pathogens.



Flow Chart 1: Major Processes Involved In VLP Production

General Steps Involved in Production of Virus like Particles

1. Genes producing capsid proteins in the virus are isolated from the viral DNA/RNA.
2. Isolated DNA is incorporated in vectors and cloned vector is selected.
3. Transformation of target gene by cloned vector into yeast, bacteria, baculo virus, plants, etc., depending upon the expression system selected.
4. The recombinant microbe is grown in large amount.
5. As these recombinant microbes grow, it produces the desired capsid proteins.
6. The chemistry of these capsid proteins is such that, they self-assemble into a particle, resembling to native virus.
7. These particles formed are a virus like particles, empty protein shells without

DNA/RNA.

8. VLPs thus produced are purified by downstream processing to obtain from the expression system. Various purification techniques are employed such as, chromatography, centrifugation, etc.
9. Analysis of the proteins produced is done with various techniques such as, ELISA, Western blot, electron microscopy, etc.
10. Thus Production of Virus like Particle can be said as “molecular cut and paste.” i.e., cut the gene from DNA/RNA of virus and paste in another microbe to synthesize VLPs.

Expression Systems¹⁰

A very broad range of biological systems, including microorganisms; insects, plants and mammalian cell lines; insect, plant and mammalian viruses and multi-cellular organisms such as plants are used as expressions systems by biotechnologists. In general, these

biological systems are extremely important for the biotransformation phases of various processes.

Of the many different replicable expression units which can be used for production of various proteins for cloned genes, the insect, plant, yeast and mammalian cells are commonly used as host cells in virus like particle production. Moreover, in many instances, these genetically modified host cells are the actual commercial products. Cell free culture is also used for expression of viral proteins to produce virus like particle against some viruses.

Expression Systems used in VLPs Production

1. Insect cell expression system
2. Plant cell expression system
3. Yeast expression system
4. Mammalian cell expression system
5. Cell free expression system

Insect Cell Expression System^{11, 12}

Insect cell expression system is a widely used expression system for VLP production. Insect cells can be used for manufacturing of both non enveloped and enveloped VLPs. Enveloped VLP vaccines produced in insect cells are among the most advanced in clinical development. Insect cells are equally effective in producing non-enveloped VLPs.

Insect Cell system is characterized by a two-phase process, where insect cells are first grown to a desired viable cell density and then infected by recombinant baculoviruses for protein expression. In these system, multiple recombinant baculoviruses containing the required genes of interest for the production of different viral proteins and vectors with different promoters/enhancers can be used to infect insect cells (e.g., *Spodopterafrugiperda* 9, Sf21 or Hi5) to express large amounts of the required viral proteins and assemble these protein units into the correspondent VLP. Multiple recombinant baculoviruses containing the required genes of interest for the production

of different viral proteins and multi-cistronic vectors with different

Promoters/enhancers can be used to infect insect cells (e.g., *Spodopterafrugiperda* 9, Sf21 or Hi5) to express large amounts of the required viral proteins and assemble this multimeric units into the correspondent VLP

Advantages

- 1) Extremely large amount of recombinant proteins can be produced in high-density cell-culture conditions in eukaryotic cell.
- 2) The insect cell system possesses eukaryotic-type Post Transitional Modifications including glycosylation, which can accommodate high-level accumulation of foreign proteins.

Disadvantages

- 1) Contamination of target with co-produced enveloped baculovirus particles which require the development of more complex schemes for VLP purification.
- 2) Handling of insects is more difficult and tedious than yeast and plant cells.

Plant Cell Expression System^{13, 14, 15}

Plants serve as a cost-effective alternative with several benefits for the commercial production of vaccines. Firstly, production in plants can be easily scaled up with increase in demand for the vaccines and secondly, produced proteins are free from contamination by human or animal pathogens, toxins and oncogenic sequences. In addition, plants provide a convenient environment for protein expression and storage including the possibility of direct administration as edible vaccine if expressed in the appropriate plant tissue.

Antigens having the ability to assemble VLPs are of particular interest, in plant expression system because:

- 1) The compact and highly ordered structures of VLPs may prevent degradation by enzymes in the gut.

- 2) The particulate nature of VLPs allows them to get engulfed by the cells of the gut epithelium that transport antigens across the mucosal barrier; and
- 3) The presence of a structure that mimics the authentic viral particle may produce a “danger signal” stimulate a potent immune responses.

Advantages

- 1) Fast and more economic than other expression systems.
- 2) Large scale production VLP is possible.
- 3) Contamination from pathogens or toxins is avoided.

Yeast Expression System^{16, 17}

Yeast is a eukaryotic organism which can be grown to very high cell mass densities in well-defined medium. Recombinant proteins in yeast can be expressed so the product is secreted from the cell and available for recovery in the fermentation solution. Proteins secreted by yeasts are heavily glycosylated at glycosylation sites. Thus, expression of recombinant proteins in yeast systems has been confined to proteins where post-translations glycosylation patterns do not affect the function of proteins.

The yeast species, *Saccharomyces cerevisiae* has proven to be extremely useful for expression and analysis of eukaryotic proteins. *S. cerevisiae*, mostly used yeast in VLP production, is listed as “generally recognized as safe” (GRAS) organism. Therefore, use of these organism, does not require experimentations as for unapproved host cells. These single-celled eukaryotic organisms grow quickly in defined medium, are easier and less expensive to work with than insect or mammalian cells, and are easily adapted to fermentation. Yeast expression systems are ideally suited for large-scale production of recombinant eukaryotic proteins.

Several yeast expression systems are used for recombinant protein expression, including

Saccharomyces, *Scizosacchomycespombe*, *Pichiapastoris* and *Hansanuelapolyomorpha*.

Advantages

- 1) Can be grown in both small culture vessels and large scale bioreactors.
- 2) Capable of carrying out many posttranslational modifications.
- 3) Easy and less expensive to work with than mammalian cells.

Disadvantages

- 1) Fermentation required for very high yields.
- 2) Growth conditions may require optimization.

Mammalian Expression System^{18, 19}

Many proteins require trimming, editing of amino acids, addition of sugars and many others. These alterations after the initial translation of the proteins are called posttranslational modifications. Sometimes they are crucial for the desired effect or enhance the effect of the substance. Mammalian cells have the ability to perform post-translational modifications and to secrete glycoproteins that are correctly folded and contain complex antennary oligosaccharide. Mammalian cells are widely used in the production of recombinant proteins, antibodies, virus, viral-subunit proteins, and gene-therapy vectors.

Advantages

- 1) Highest level of correct post-translational modifications.
- 2) Highest probability of obtaining fully functional human proteins.

Disadvantages

- 1) Very low yield for VLPs production.
- 2) Culture conditions required are more complex than other expression systems.
- 3) Cost of production is high.

Cell-Free Expression System²⁰

It is known that disrupted cells are still capable

of synthesizing proteins. Based on these observation, Cell-free expression system has been developed to obtain proteins. Cell-free expression system opens way to the synthesis of molecules such as peptides which contains non-natural amino acids. For example, modified amino acids artificially linked to suppressor tRNA can be incorporated at specific sites. For production of proteins from recombinant genes, the cell-free gene expression mixture can be totally reconstructed *in-vitro* to obtain purified proteins

Advantages

- 1) Rapid expression directly from plasmid.
- 2) Open system- easy addition of components to enhance solubility or functionality.

Morphological Characteristics of Virus Like Particles²¹

VLP vaccines combine many advantages of live attenuated vaccines and recombinant subunit vaccines, along with the features enhancing their immunogenicity, safety and protective potential. Various features of VLP vaccines that underlay their immunogenicity, safety and protective potential are:

- 1) Well-defined geometry and uniformity of surface structures.
- 2) Repetitive and ordered surface structures.
- 3) Particulate and multivalent nature.
- 4) Preservation of native antigenic conformation.
- 5) Safe, non-infectious and non-replicating as VLPs lack the DNA or RNA genome of the virus.
- 6) Higher stability than soluble antigens in extreme environmental conditions.
- 7) Serve as carriers for foreign epitopes.

VLP Vaccines on the Market and in the Clinical Development²²

VLPs have been shown to be highly immunogenic and have recently come into focus for their diverse applications in vaccination, targeted drug delivery, gene therapy and

immune therapy. All four recombinant vaccines that are on the market, GlaxoSmithKline (GSK)'s Engerix (hepatitis B virus [HBV]) and Cervarix (human papillomavirus [HPV]) and Merckand Co., Inc.'s Recombivax HB (HBV)and Gardasil (HPV), are based on highly purified VLPs. Additionally, a number of VLP-based vaccine candidates, including GSK's anti-malaria vaccine RTS,S, are in clinical development, while many others, targeting pathogens such as influenza virus, rotavirus (RV)and human immunodeficiency virus (HIV), are undergoing preclinical evaluation.

To date, VLPs, non-enveloped and enveloped, have been produced for a number of targets using mammalian, plant, insect, yeast or cell-free platforms. Marketed products of VLPs as Vaccines at licensed or advanced stages are presented in tables focusing on HPV, HBV, Influenza and Malaria Virus like particle as vaccine and other VLPs.

VLPs Discussed in this Review

Following VLPs against the respective diseases are discussed and studied under these review. Different methods, processes and techniques for production of VLPs against following viruses are mentioned.

- Human Papillomavirus VLP
- Influenza virus VLP
- Hepatitis B virus VLP

Human Papillomavirus VLP^{23,24,25,26,27,28,29}

Human papillomaviruses (HPVs) are a group of more than 150 related viruses, each designated as a type and numbered in order of discovery. They are called papillomaviruses because certain types may cause warts, or papillomas, which are benign (noncancerous) growths. Some types of HPV are associated with certain types of cancer. These are called high-risk, oncogenic, or carcinogenic HPVs.

Human papillomavirus infection is the primary cause of cervical cancer, the second most common cancer in women worldwide. It is also associated with vaginal, vulvar, anal and oral cancers. Two types (HPV-6 and HPV-11)

account for 90 percent of genital wart cases, where as 15 to 20 types may be associated with cervical cancer. Four types of HPV (16, 18, 31, and 45) accounted for 80 percent of all cervical cancer. There is a strong demand for the development of an HPV preventive vaccine.

Many viral envelop or capsid proteins have the ability to self-assemble into non-infectious empty VLP in different culture systems. Human papilloma virus virion is composed of capsid proteins L1 and L2. The L1 protein can self-assemble into virus-like particles, which are structurally similar to native HPV virions. Both prokaryotic and eukaryotic expressions systems can be employed to generate empty HPV VLP. HPV VLPs can be expressed in insect, yeast and bacterial cell. When expressed by recombinant DNA technology, capsid proteins of HPV forms recombinant VLP that exhibit ordered repetitive structures of epitopes on the surface.

Influenza Virus VLP^{30,31,32}

Influenza virus contains a RNA genome and belongs to the *Orthomyxoviridae* family. It is a lipid enveloped RNA virus, surrounded by lipid membrane containing two major glycoproteins the hemagglutinin (HA) and neuraminidase (NA), and a minor but essential ion-channel protein M2 (matrix proteins).

There are three types of influenza viruses: A, B, and C.

- 1) Influenza type A viruses:-There are 16 known HA subtypes and 9 known NA subtypes of influenza A viruses. Only some influenza A subtypes (i.e., H1N1, H1N, and H3N2) are currently in general circulation among the people.
- 2) Influenza Type B:-Influenza B viruses can cause morbidity and mortality among humans, less severe epidemics than influenza A viruses.
- 3) Influenza Type C:-Influenza type C viruses cause mild illness in humans and do not cause epidemics or pandemics.

Influenza virus-like particles are not infectious, but they are immunogenic: when injected into animals, they induce the production of anti-viral antibodies that can block infection. VLPs are assembled in producer cells and released into culture medium mimicking the viral budding process, which incorporates viral glycoproteins on their surfaces. Influenza VLPs resemble intact virions in structure and morphology, and contain functionally active and immunologically relevant structural proteins. It is likely that the self-assembled macrostructure of VLPs can present native conformational epitopes of surface proteins to the immune system comparable to those of live virions. The non-infectious nature of VLPs and their lack of viral genomic material represent a desirable safety feature as a vaccine candidate that can be repeatedly administered to all populations including high-risk groups. In addition, VLPs as particulate antigens can activate antigen-presenting cells such as dendritic cells capturing the antigens for presentation to both T and B lymphocytes.

Hepatitis B Virus VLP^{33, 34}

HBV is an enveloped virus with three envelope proteins. These proteins are the co-translational products of the HBV genome, termed the S gene. These proteins self-assemble into sub-viral particles commonly called HBsAg particles. HBsAg proteins include a major polypeptide and the small HBV envelope protein (S), in both glycosylated and non-glycosylated forms. A protective immune response against Hepatitis B infection can be obtained through the administration of a single viral polypeptide, the Hepatitis B surface antigen (HBsAg). Thus, the Hepatitis B vaccine is generated through the utilization of recombinant DNA technology, preferentially by using yeast-based expression systems. However, the polypeptide needs to assemble into spherical particles, so called virus-like particles (VLPs), to elicit the required protective immune response.

HPV VLP Production: Insect Cell Expression System³⁵⁻⁴¹

Steps Involved (Chart 2)

Expression System

- a) Insect Cells Growth
- b) Polymerase chain reaction
- c) Generation of Baculovirus Recombinants
- d) Transfection of selection of recombinant baculoviruses

Purification

- a. Virus-like particle production and purification

Analysis

- a. Sedimentation analysis
- b. SDS-PAGE
- c. Electron microscopy
- d. Immunofluorescence
- e. ELISA

Expression Systems

Insect Cells Growth

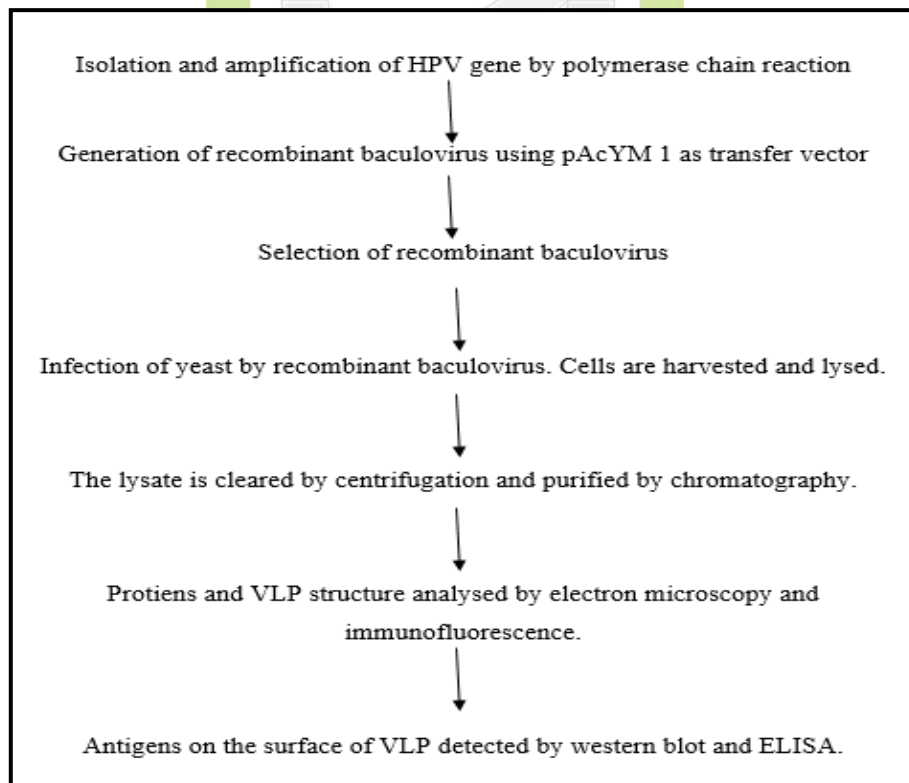
Insect cells are grown in suspension and maintained on insect medium. Some widely used insect cells are *Trichoplusia ni* High Five (TN Hi-5) and *Spodoptera frugiperda*.

Polymerase Chain Reaction

HPV genes are amplified by the polymerase chain reaction. The HPV16 E7 ORF, is amplified from pHPV16 using primers with EcoRI and Bg/II restriction sites at the 5' and the 3' ends. An amplified product containing E6 and E7 ORFs are generated using tailed oligonucleotide with restriction sites for BgfII added to the 5' and 3' ends.

Generation of Baculovirus Recombinants (Figure 1)

The transfer vector used is pVL 1393. The target gene pHPV16 is isolated. Plasmids pVL16E6 and pVL16E7 are constructed by insertion of amplified E6 and E7 ORFs into Pvl 1393 at the EcoRI/ PstI site.



Flow Chart 2: HPV VLP Production: Insect Cell Expression System

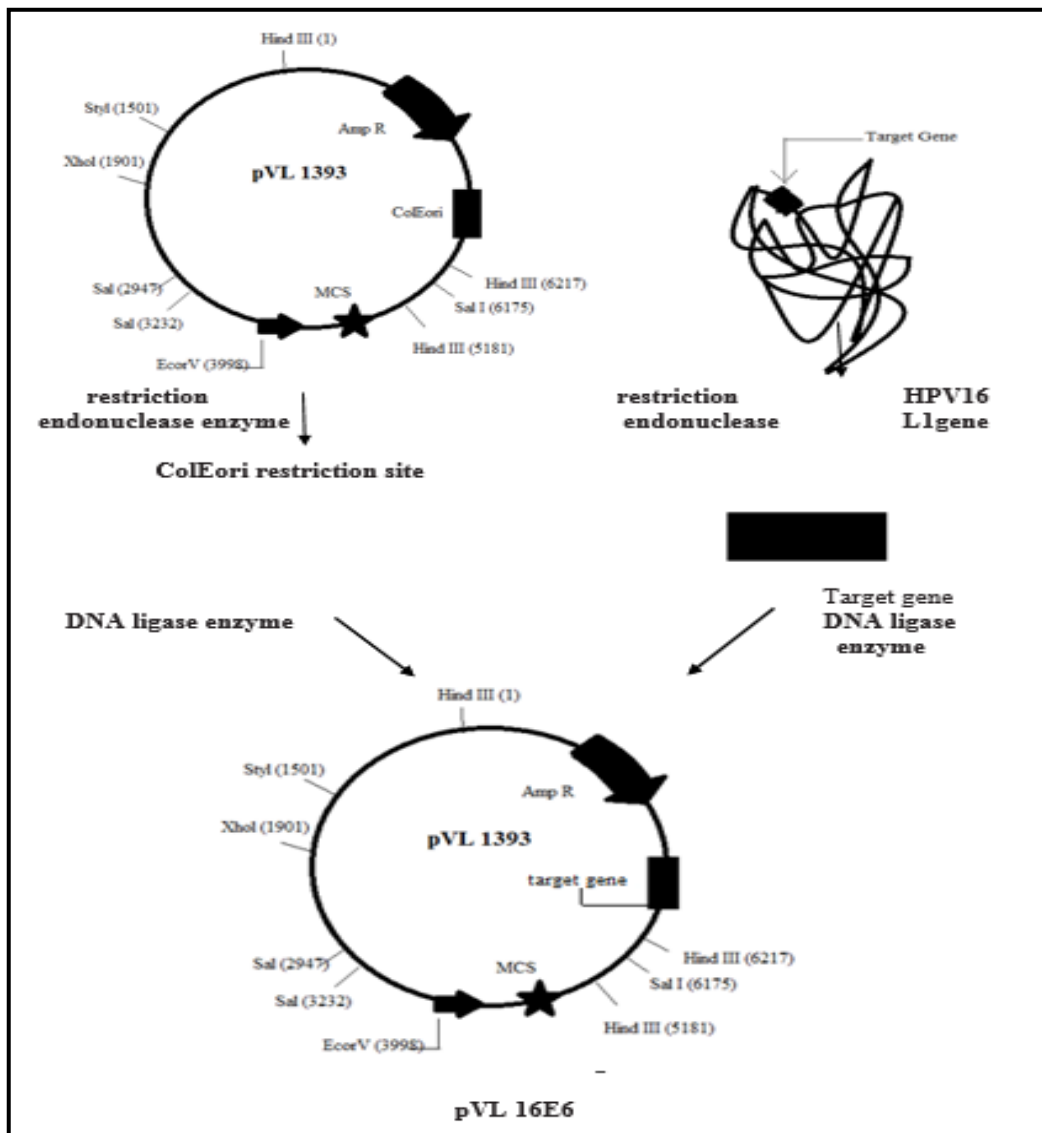


Figure 1: Generation of recombinant transfer vector pVL 16E

Transfection and Selection of Recombinant Baculoviruses

Sf-21 cells are transfected with a mixture of purified AcNPV DNA and a recombinant transfer vector. Virions lacking the polyhedron gene produce distinctive zone of cell lysis (occlusion negative plaques) from which recombinant baculovirus can be isolated.

Purification

Virus-Like Particle Production and Purification

After infection, cells are harvested and lysed. The lysate is cleared by centrifugation. The interphase is collected. To ascertain the production of VLPs, SDS-PAGE is carried out

before purification. The samples are further purified by chromatography by separation of proteins. Elution of VLPs is analyzed by SDS-PAGE and western-blot analysis. The capsid quality is analyzed by electron microscopy.

Analysis

Sedimentation Analysis

Samples are loaded onto a linear gradient of 5–50 % sucrose and centrifuged. Fractions are collected from the bottom of the gradient and analysed by SDS-PAGE and immunoblotting.

SDS-PAGE

This technique is employed to detect proteins and separate VLPs produced. Insect cells

infected with recombinant baculovirus are harvested in phosphate-buffered saline (PBS) and disrupted by repeated passage through a gauge needle. Sample added to SDS/PAGE sample buffer and electrophoresed in 12.5% SDS-polyacrylamide gels. Following electrophoresis, gels are either stained or transferred to nitrocellulose. Blots are blocked with 5% skim milk powder in PBS and reacted with HPV-specific antisera or monoclonal antibodies temperature. Bound antibodies are detected by ELISA.

Electron Microscopy

VLPs stained with uranyl acetate are analysed using a transmission electron microscope. Electron microscopy is employed to study protein produced, check the VLP formation and size of VLP particles produced.

Immunofluorescence

VLPs structures and surface proteins are analysed by immunofluorescence. Insect cells are infected with recombinant baculovirus, centrifuged and mounted on microscope slides by means of cytospin. Cells are fixed with 4% paraformaldehyde and washed with phosphate buffered saline. Immunostaining is performed with the primary and secondary antibodies. Cells are washed with phosphate buffered saline after incubation with each antibody. Slides are washed and mounted in PBS-glycerol and viewed under ultraviolet illumination.

ELISA

ELISA is employed to detect antigens on surface of VLPs by detection of antibodies produced. HPV-specific monoclonal antibodies diluted in PBS are sampled into ELISA plates and incubated. Plates are washed with PBS and lysates of baculovirus infected cells are added. Anti-HPV sera are added and plates incubated. Plates are washed with PBS and conjugated secondary antibodies are added. Plates are washed and bound antibodies are detected.

HPV VLP Production: Plant Cell Expression System⁴²⁻⁴⁵

Steps Involved (Chart 3)

Expression System

- a. Plant material
- b. Plasmid construction
- c. Binary vector system
- d. Plant transformation
- e. Selection of transformed plant cells

Purification of VLPs

Analysis

- a) Western blot analysis.
- b) Enzyme-linked immunosorbent assay (ELISA).
- c) Electron microscopy

Expression System

Plant Material

Most widely used plants for plant expression system are Tobacco plants (*Nicotiana tabacum*) and potato plants (*Solanum tuberosum* family *solanaceae*).

Plasmid Construction (Figure 2)

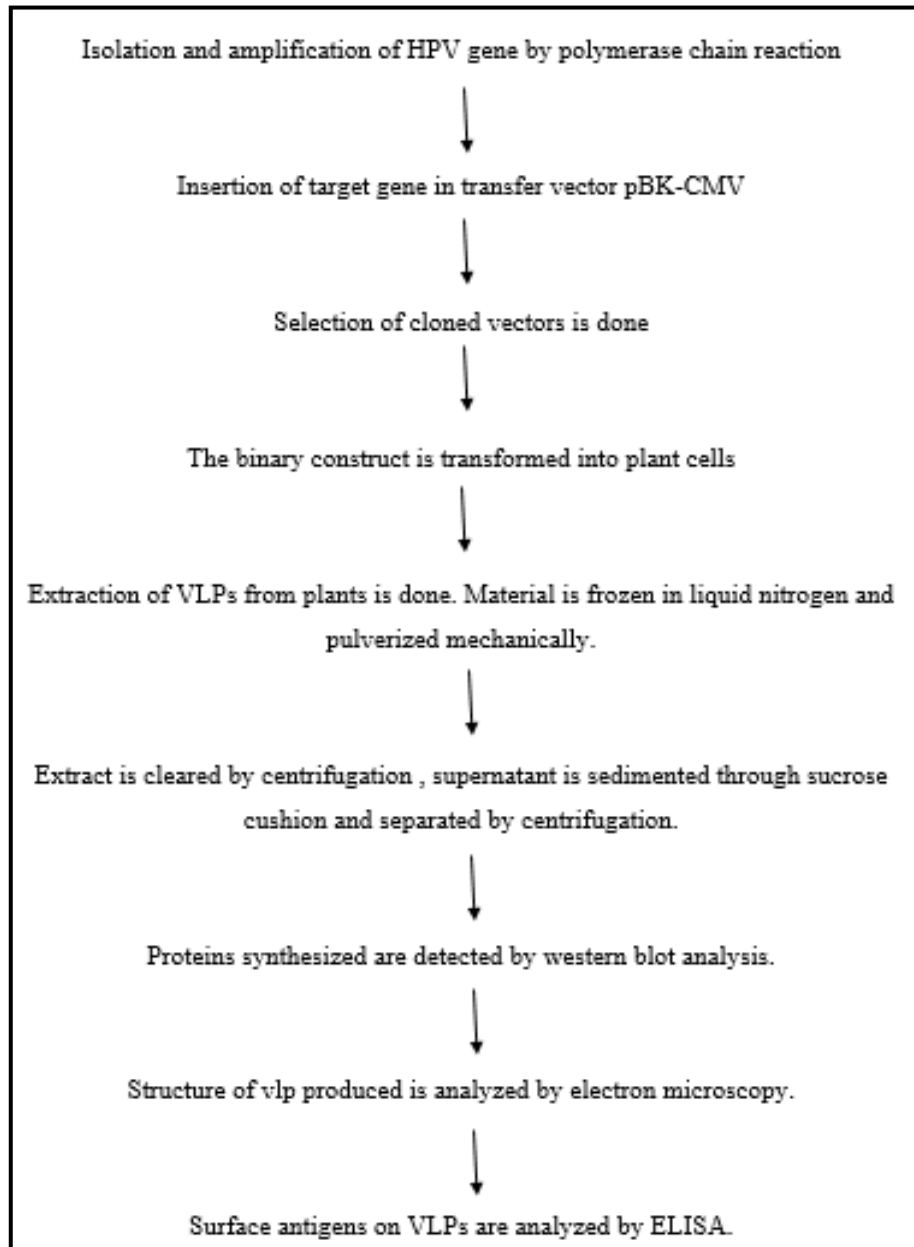
The unmodified and the codon-optimized HPV-16 L1 genes are excised and inserted into a transfer vector. Transfer vector used is pBK-CMV. Plasmid pBK-16L1 is produced by insertion of pHPV16L1 gene at *SalI/ KpnI* restriction sites.

Binary Vector System

The cloning vectors lack vir genes and they cannot affect transfer and integration of the T-DNA region into recipient plant cells by themselves. Hence, a binary cloning vector is formed, which contains both *E. coli* and *A. tumefaciens* origin of DNA replication.

Plant Transformation

The binary constructs are transformed into *Agrobacterium tumefaciens* strain CV58C1, which carries the virulence plasmid pGV2260. Transformation of tobacco and potato plants using *Agrobacterium*-mediated gene transfer is done.



Flow Chart 3: HPV VLP Production: Plant Cell Expression System

Selection of Transformed Plant Cells

Ti-plasmid present in most of the *A. tumefaciens* strains consists of selection marker gene, such as neomycin phosphotransferase, that confers kanamycin resistance to transformed plant cells.

Purification of VLPs

VLPs from plant cells are extracted from leaf or tubers depending upon the expression plant used. Leaf material is frozen in liquid nitrogen and pulverized using a mortar and pestle.

For potato plant, tubers are sliced and mechanically homogenized. Plant material is then subjected to further extraction. Extracts are cleared by centrifugation. L1-containing supernatant is sedimented through a sucrose cushion and further purified by centrifugation.

Analysis

Western Blot Analysis

This technique is employed to detect L1 proteins produced in VLPs. Leaf disks or tuber

slices are homogenized in extraction buffer and centrifuged. Sodium dodecyl sulfate (SDS)-containing sample buffer is added, and after heat-denaturation protein is separated on SDS-Polyacrylamide gels. Proteins are transferred onto nitrocellulose membranes and incubated with antiserum raised against insect cell-derived VLPs. Immunodetection is performed using the western blot.

Enzyme-Linked Immunosorbent Assay (ELISA)

To analyze whether the plant that produced L1 is able to induce a humoral immune response and to detect HPV-16 L1-specific antibodies ELISA is carried out. ELISA plates are coated

overnight with phosphate-buffered saline (PBS) containing VLPs derived from insect cells. Antisera is added and incubated. After washing, peroxidase-conjugated antibodies for HPV-VLP 16 are added. Plates are washed and stained with ABTS (2,2'-azino-bis-3-ethylbenzthiazolin-6-sulfonic acid) substrate solution and allowed to develop.

Electron Microscopy

VLPs are analyzed for structure and size produced by electron microscopy. Supernatant collected from the centrifuged sample are stained with 30 µl of a 2% uranyl acetate solution. After air-drying, specimens are inspected with an electron microscope.

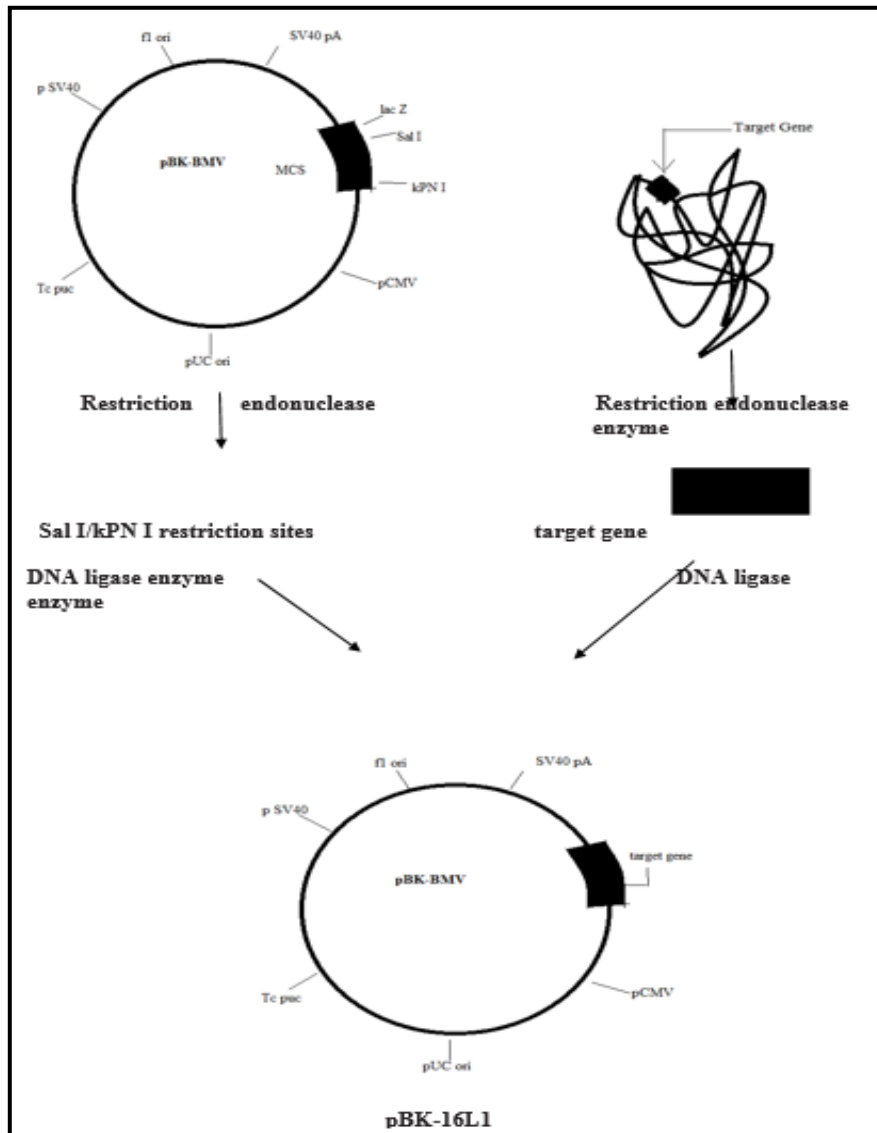


Figure 2: Generation of recombinant transfer vector pBK- 16L1

HPV VLP Production: Yeast Expression System⁴⁶⁻⁵²

Steps Involved (Chart 4)

Expression System

- Cloning of HPV-16 L1 gene in yeast expression vector pGal426
- Transformation of host and expression of protein

Purification and Characterization of VLP

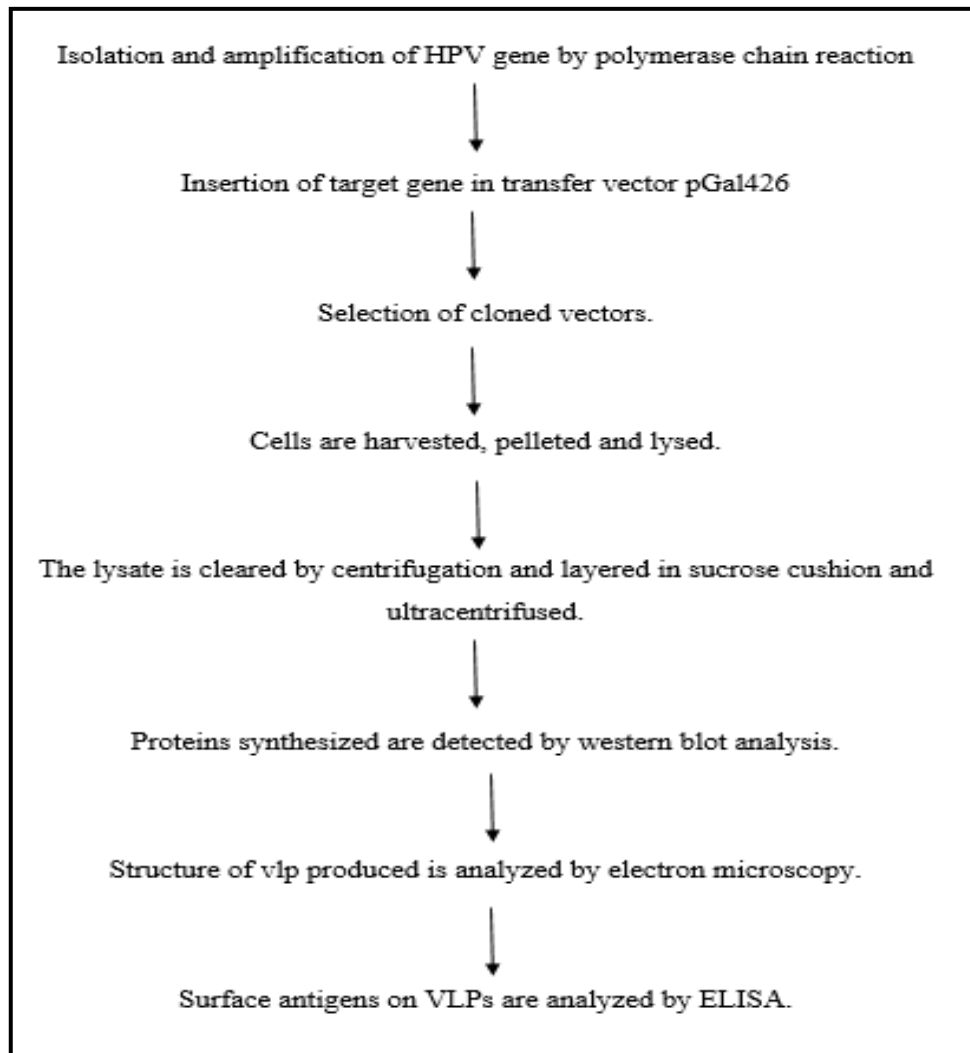
Analysis

- Western blotting
- Transmission electron microscopy
- ELISA

Expression System

Cloning of HPV-16 L1 Gene in Yeast Expression Vector pGal426 (Figure 3)

Amplification of isolated HPV gene is done using Polymerase chain Reaction. Full length L1 sequence are PCR amplified from genomic DNA of one HPV16 by cloning vector pTZ57R followed by subcloning in the yeast shuttle expression vector. Transfer vector used is pGal426 which contains the galactose inducible promoter Gal1 and uracil synthesizing gene (*ura3*) as selection marker. pTZ57R-L1 are digested with SacI and HindIII to release the genes. Cloned genes are inserted at SmaI and HindIII sites of pGal426. Recombinant ampicillin resistant pGal426-L1 clones are produced.



Flow Chart 4: HPV VLP Production: Yeast Expression System

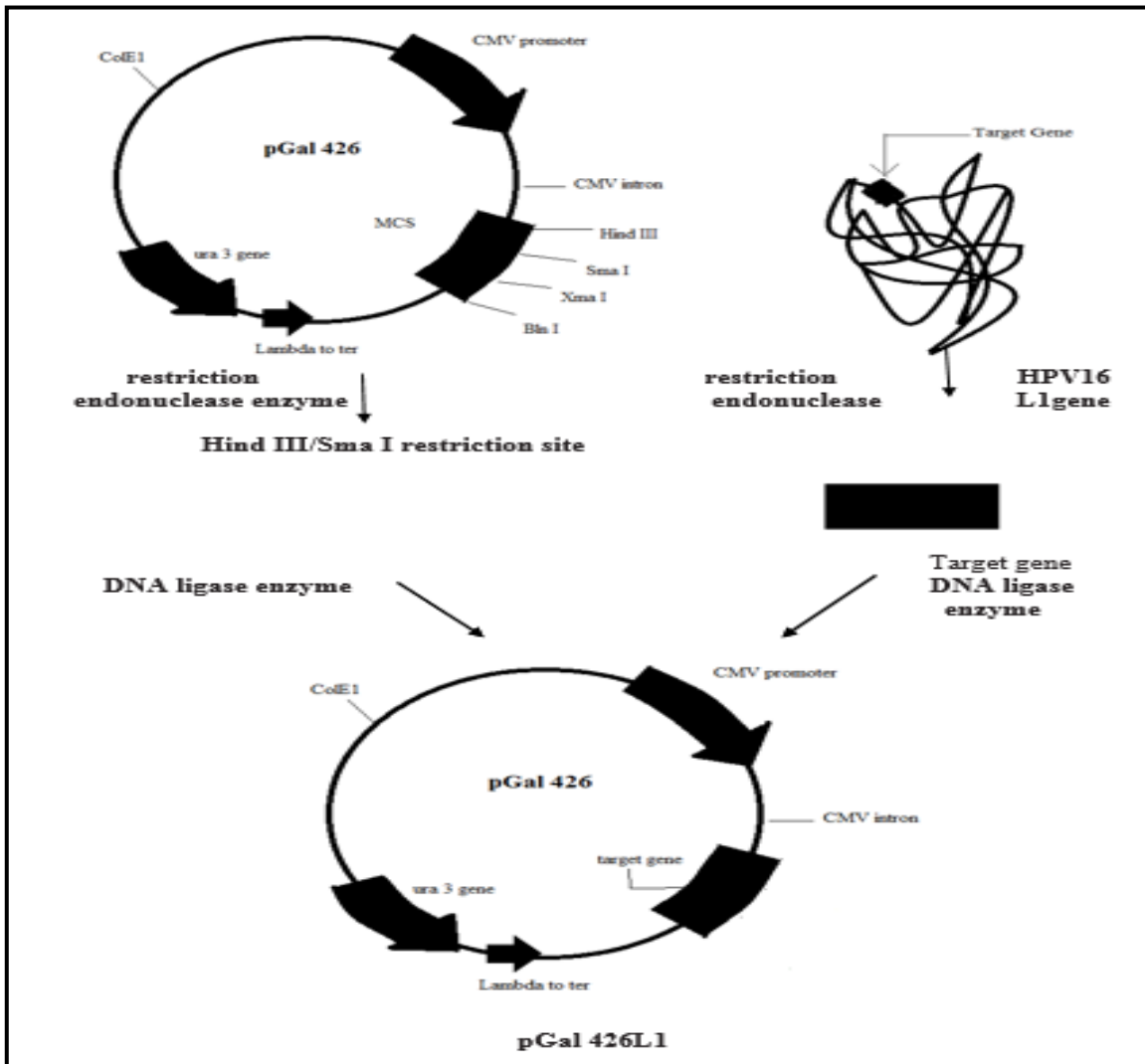


Figure 3: Generation of recombinant transfer vector pGal 16E

Transformation of Host and Expression of Protein

Saccharomyces cerevisiae is used as host system to express the L1 protein by transformation of yeast. Cells are pelleted, washed and incubated. Cells are inoculated in selective complete medium lacking uracil (SC-Ura). These medium is selected to select and differentiate recombinant pGal426-L1 clones, as pGal426 contains uracil synthesizing gene (*ura3*) which can be used as selection marker. The cells are harvested by centrifugation and washed in sterile distilled water. The yeast cells are lysed and the lysate is clarified by centrifugation.

Purification and Characterization of VLP

The transformed yeast cells are inoculated in SC-Ura medium. Yeast cells are harvested, lysed and clarified by centrifugation. The cell lysate are layered onto a cushion of 45 per cent (wt/vol) sucrose. The sucrose suspension is centrifuged in ultracentrifuge. The resulting pellet is resuspended in PBS and used for detection of L1 protein.

Western Blotting

This technique is employed to detect the L1 proteins in VLPs. Recombinant HPV-16 L1 expression proteins from the crude cell lysate of the yeast clones are obtained by Immuneblot. In

these, the cell lysate is sampled on a 10 per cent SDS-PAGE gel and transferred onto nitrocellulose membrane. The blot is washed in PBS and reacted with anti-HPV-16 L1 monoclonal antibody. The blot is washed with blotting buffer and PBS, the blot is reacted with substrate solution consisting 3, 3'-Diaminobenzidine tetrahydrochloride (DAB), NiCl₂ and H₂O₂ till (dark blue) color development.

Transmission Electron Microscopy

This technique is employed to analyze structure and size of the VLPs produced. Purified HPV 16 L1 are washed with PBS, absorbed to carbon-coated grid and stained with 2% phosphotungstic acid. Transmission electron microscopy (TEM) is performed using a transmission electron microscope.

ELISA

To analyze whether the plant that produced L1 is able to induce a humoral immune response and to detect HPV-16 L1-specific antibodies ELISA is carried out. ELISA immuno-stripes are coated with VLPs and washed with PBS buffer and nonspecific binding sites were blocked with PBS containing 0.5 per cent non-fat milk powder. Anti human IgG anti-sera is added and incubated. Plates are washed with ortho-phenylene diamine (OPD) in substrate buffer with H₂O₂ and incubated to develop.

Influenza VLP Production: Insect Cell Expression System⁵³⁻⁶¹

Steps Involved (Chart 5)

Expression System

- a. Cells and viruses
- b. Cloning of , NA and M1 genes
- c. selection of recombinant baculovirus

VLP Production and Purification

Analysis

- a. Chromatography
- b. Electron microscopy
- c. Enzyme-linked immunosorbent assay(ELISA)

Expression System

Cells and Viruses

Spodoptera frugiperda (Sf-9) and High-fiveTM (Hi-5) cells are maintained and grown as suspension cultures.

Cloning of HA, NA and M1 genes (Figure 4)

The HA gene is cloned as a *Bam*HI-*Kpn*I DNA fragment within pFastBac1 bacmid transfer vector digested with *Bam*HI and *Kpn*I. The NA and M1 genes are cloned as *Eco*RI-*Eco*RI DNA fragments into *Eco*RI-digested pFastBac1 plasmid DNA.

Selection of Recombinant Baculovirus

The pFastBac 1 vector possesses' ampicillin and gentamycin resistance genes which are used as selection markers to isolate recombinant baculoviruses for further transfection.

VLP Production and Purification

Cells are infected with recombinant baculovirus and harvested. Cells are pelleted and lysed. Supernatants are collected and clarified by centrifugation. The pellets are resuspended and purified by using 20% (w/v) sucrose gradient in phosphate-buffered saline (PBS). Viral particles are precipitated via ultracentrifugation. Proteins within VLP preparations are separated using 4–12% gradient SDS-PAGE, Proteins are analyzed by SDS-PAGE and Western blotting.

Analysis

Chromatography

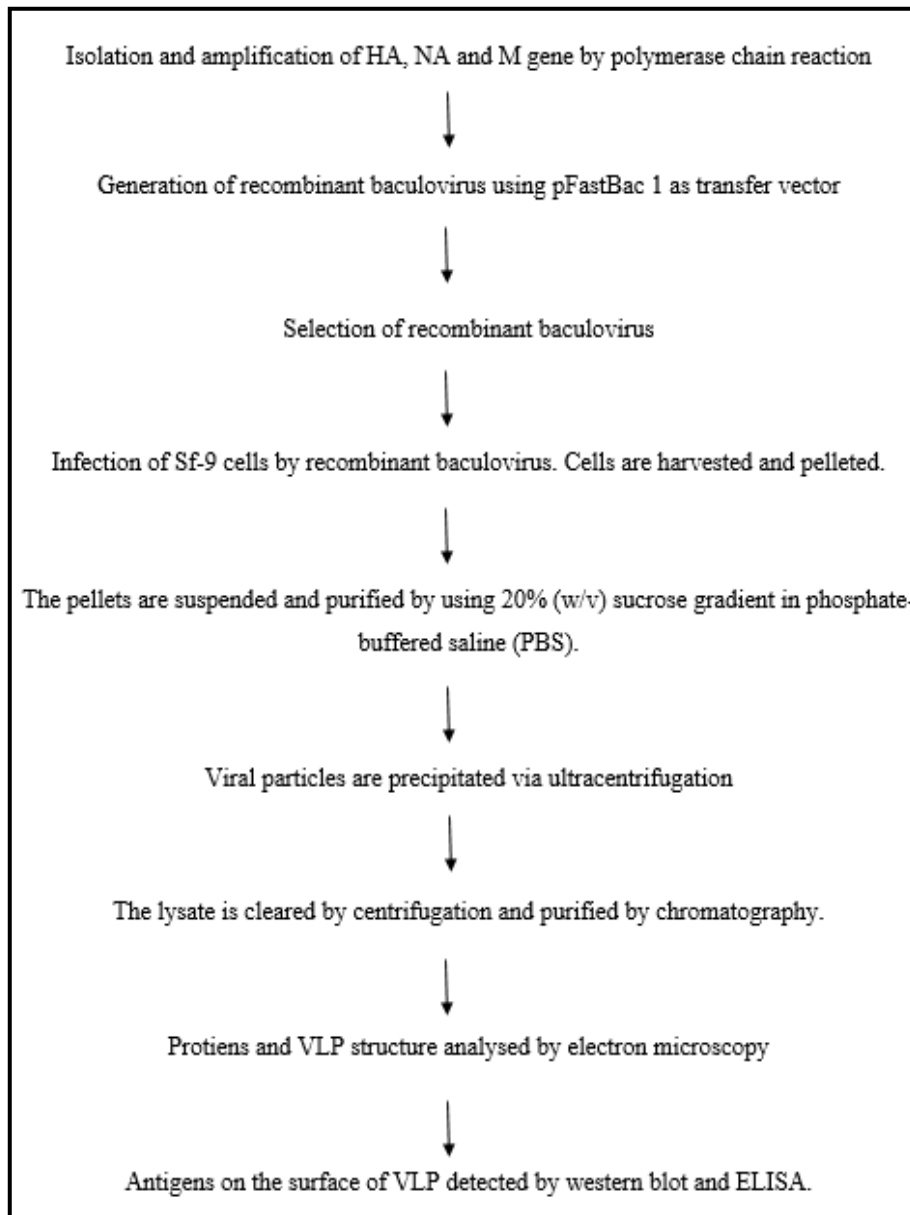
Gel filtration chromatography is performed to evaluate the sizes of influenza VLPs in the sucrose gradient fractions. The Sepharose CL-4B resins are packaged into an empty column and mounted onto a Fast flow Column System. Before sample loading, the column was pre-equilibrated with PBS buffer and calibrated with molecular weight markers. Void volume of the column was determined using dextran blue 2000. The sample is then loaded onto the column. Fractions were collected and analyzed by SDS-PAGE and Western blotting.

Electron Microscopy

Quantification of VLPs produced can be done by electron microscopy. Supernatant from sucrose gradient fractions is treated with 2% glutaraldehyde in PBS, adsorbed on carbon-coated grids, and washed with deionized water. Washed samples are stained with 2% sodium phosphotungstate. Stained VLPs are observed by transmission electron microscope.

Enzyme-Linked Immunosorbent Assay (ELISA)

HA, NA and M1-specific immunoglobulin G (IgG) antibodies are detected by ELISA. Plates are coated with the control antigens. The plates are blocked with 1% PBS/BSA, serial dilutions of the serum are performed. Plates are incubated. Antigen specific IgG is detected by incubating the plates with goat anti-human IgG antibody. The assay is developed by adding TMB substrate stopped by adding sulphuric acid.



Flow Chart 5: Influenza VLP Production: Insect Cell Expression System

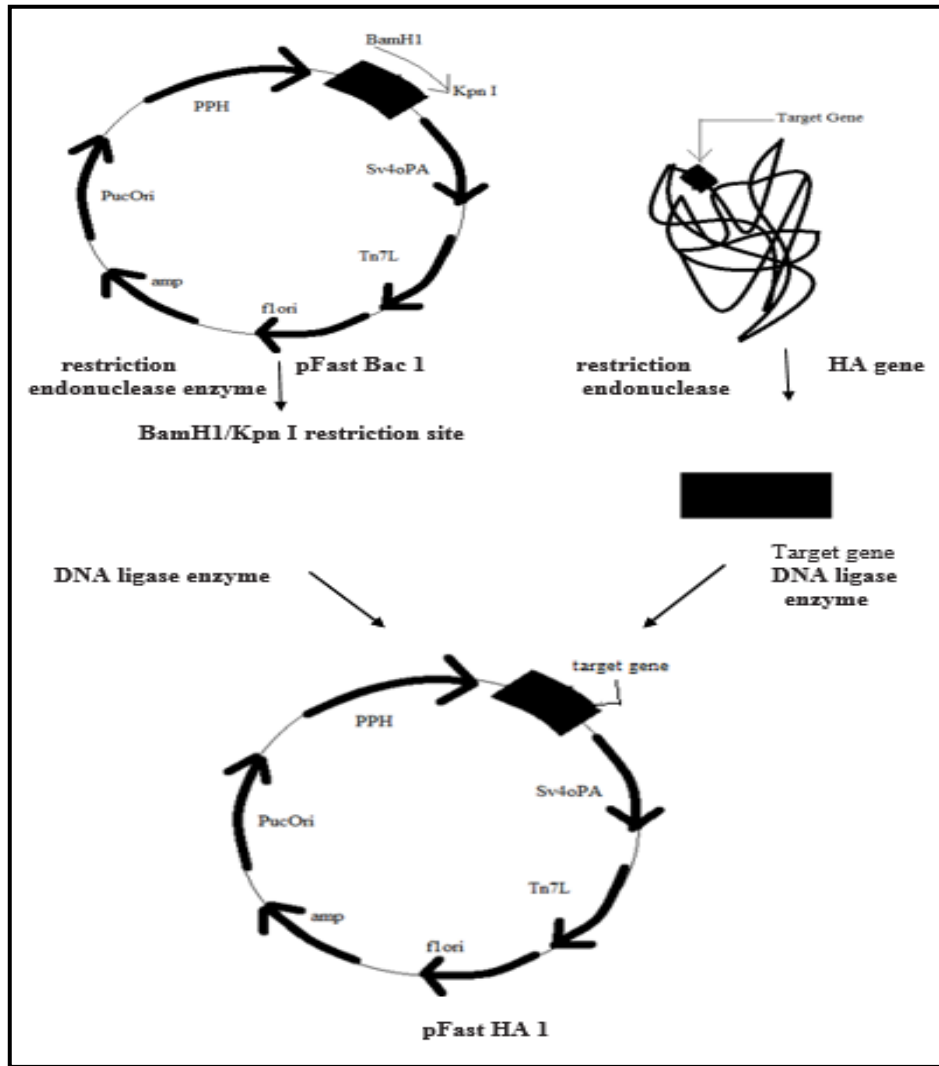


Figure 4: Generation of recombinant transfer vector pFast HA 1

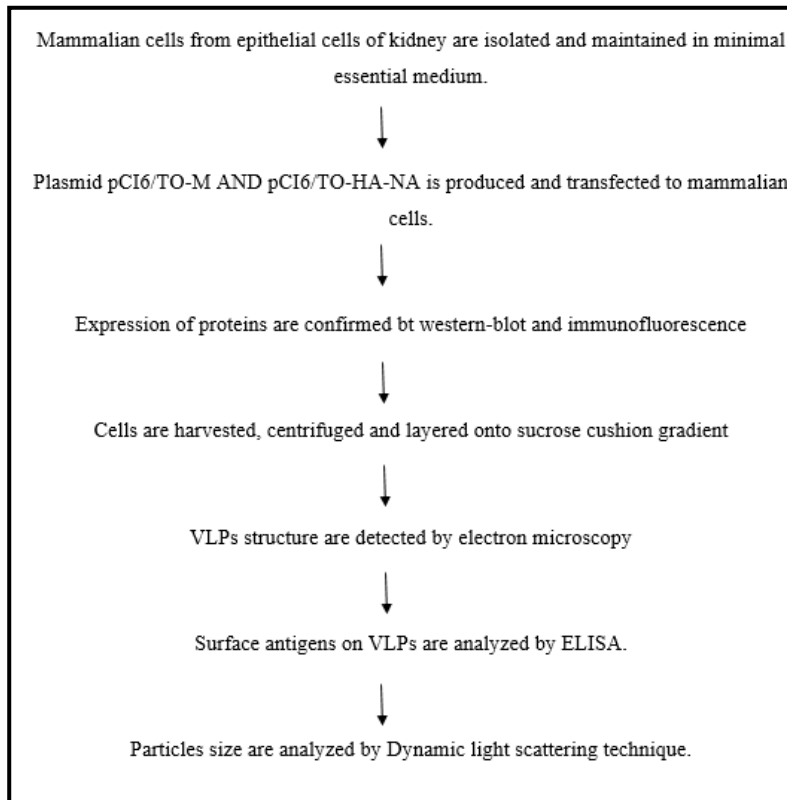
Influenza VLP Production: Mammalian Cell Expression System

Steps Involved (Chart 6)

- a. Cells and plasmids
- b. Purification of mammalian VLPs
- c. Analysis
 - i. Electron microscopy of purified mammalian VLPs
 - ii. ELISA
 - iii. Dynamic light scattering (DLS) determination of particle size

Cells and Plasmids (Figure 5)

For influenza VLP production, the mammalian cell used here is kidney epithelial cells. These cells are obtained from African green monkey, and named as vero cells. Cells are maintained in minimal essential medium. For the mammalian H3N2- and H5N1-VLPs producer cells, the plasmid pCI6/TO-M1-M2 is stably transfected into Vero cells to derive a founder cell line, which is further transfected with HA-NA expression vectors to obtain the co-expression cell line with HA, NA, M1, and M2 proteins. Expression of the proteins is confirmed by Western blot analyses and immunofluorescence staining as follows. The cells are fixed in 4% paraformaldehyde and immersed in 0.05%



Flow Chart 6: Influenza VLP Production: Mammalian Cell Expression System

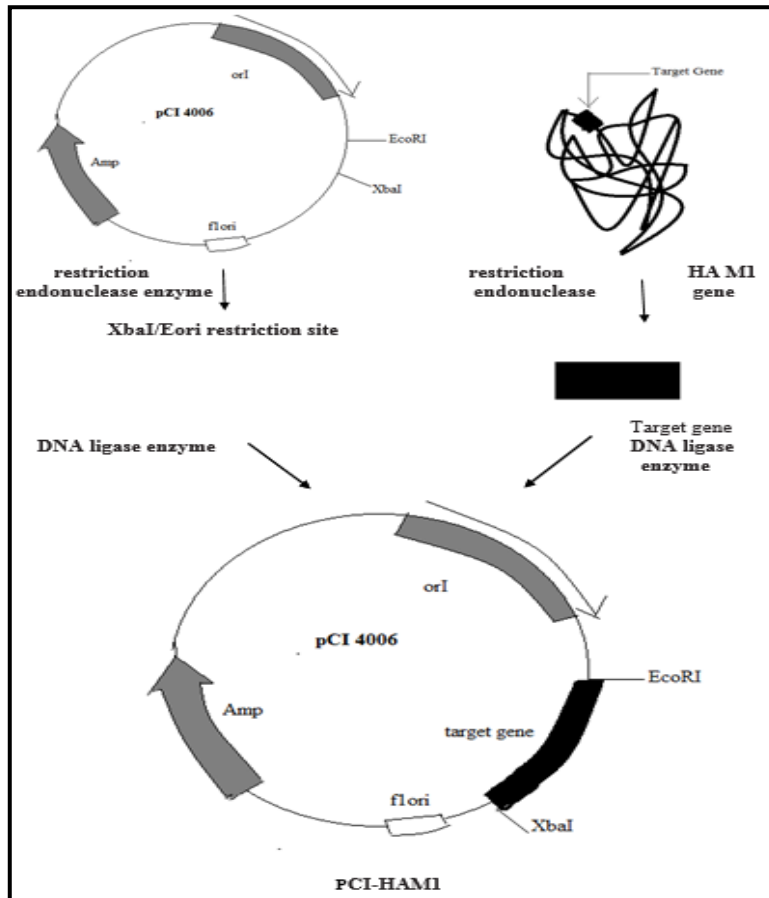


Figure 5: Generation of recombinant transfer vector pCI-HA-M1

Triton-X 100. After blocking with 1% gelatin, the cells are incubated with distinct primary specific antibodies, followed by incubation with goat anti-mouse or goat anti-rabbit IgG conjugated with Cy3 dye.

Purification of Mammalian VLPs

Cells are maintained in minimal essential medium supplemented with 10% fetal bovine serum. Cells was harvested, centrifuged and then layered onto a 30% sucrose cushion. Following centrifugation, the resulting pellet is resuspended in TNE buffer, and further purified over a 60% sucrose gradient. Finally, the banded VLPs are collected, dialyzed with TNE buffer.

Analysis

Electron Microscopy of Purified Mammalian VLPs

Sucrose gradient purified VLPs are adsorbed onto carbon-coated nickel grids and washed with TBS buffer. The sample was blocked with 1% BSA in TBS. Primary antibody is diluted in 1% BSA/TBS and adsorbed onto the grid. Washed with TBS, secondary gold-conjugated antibody is added. The grids are then washed twice with TBS, fixed with 1% glutaraldehyde, washed with water, and negatively stained with 2% uranyl acetate.

ELISA

HA, NA-specific immunoglobulin G (IgG) antibodies are detected by ELISA. ELISA plates are coated with indicated H5 glycoprotein, VLPs blocked with 1% casein in PBS. ELISA plates are then incubated with serum samples of indicated dilution, traced with HRP-conjugated secondary Ab, and developed with TMB substrate.

Dynamic Light Scattering (DLS) Determination of Particle Size

Mammalian VLPs are diluted to phosphate buffer at passed through filters and analyzed on a Nano ZS particle-size analyzer. For each sample analyzed by DLS, two consecutive measurements are taken on a single sample and measured with a light-scattering data collection

time. The average diameters of VLPs or virus were then calculated as the mean size of particle population \pm standard deviation (SD) of three independent experiments.

Hepatitis B VLP Production: Yeast Expression System⁶⁵⁻⁶⁷

Steps Involved (Chart 7)

Expression system

- a. Construction of an expression vector
- b. Selection of clones

VLP production and purification

- a. VLP Production
- b. Purification of HBsAg VLPs

Analysis

- a. Chromatography
- b. SDS-PAGE
- c. Electron microscopy

Expression Systems

Construction of an Expression Vector (Figure 6)

The HBsAg gene from plasmid pHB320 containing the full HBV Genome is PCR-amplified and ligated into the BamHI/Eco105I-treated vector pPIC3.5K, under control of the AOX1 promoter. After sequencing, the resulting pPIC-HBsAg plasmid is linearized with Ecl136II in AOX1 promoter region and used for transformation of the *P. pastoris*.

Selection of Clones

Transformants are replica-plated onto yeast extract peptone dextrose (YEPD) agarized medium containing increased concentrations of the G418 antibiotic. This is used as a selection marker for recombinant vectors.

VLP Production and Purification

VLP Production

Selected clones are incubated on buffered complex glycerol medium (BMGY). Cells are then harvested by low-speed centrifugation and resuspended in the same volume of buffered

complex methanol medium (BMMY) containing 0.5% methanol.

Debris is separated by low-speed centrifugation, and the supernatant is serially diluted for an immunodiffusion assay using a polyclonal rabbit anti-HBc antibody to detect surface antigens.

Purification of HBsAg VLPs

The yeast cells are resuspended in lysis buffer and disrupted. The soluble fraction is separated by centrifugation. The supernatant is incubated and subsequently centrifuged. Solid ammonium sulfate is then added to the supernatant to 40% saturation, which is then incubated and centrifuged.

Analysis

Chromatography

Gel filtration chromatography is performed to evaluate the sizes of influenza VLPs in the sucrose gradient fractions. The sediment is dissolved in a minimal amount of phosphate buffer and loaded onto a pre-packed anion-exchange DEAE resins. The column is equilibrated with phosphate-buffered saline.

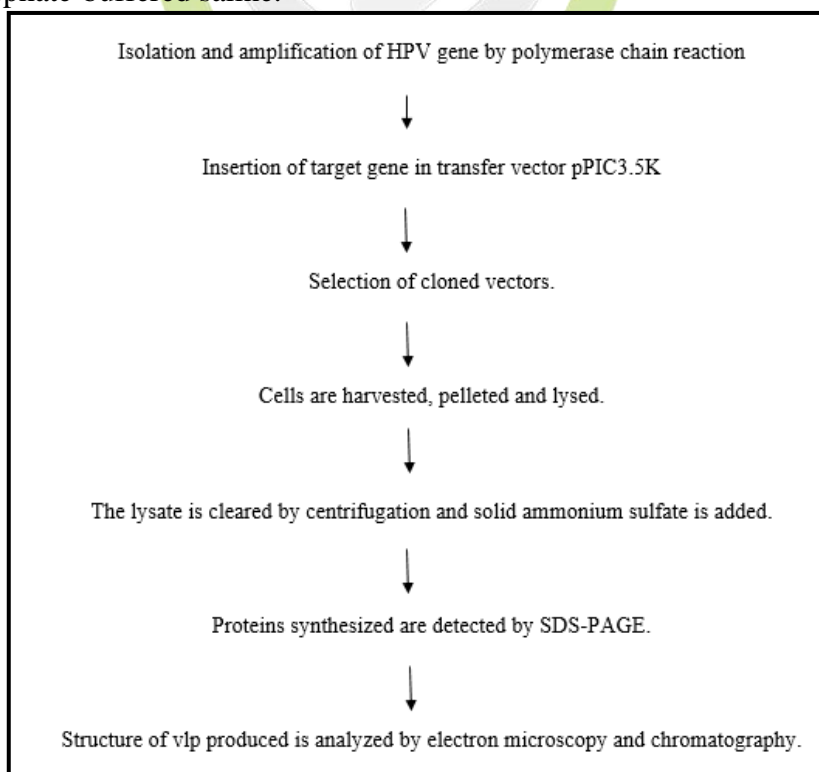
Column-bound proteins are eluted by a linear gradient with phosphate buffer.

SDS-Page

Detection and separation of VLPs produced is done by SDS-PAGE. Protein samples are monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, with a 4% stacking and 15% separating polyacrylamide gel (PAAG). To visualize protein bands, the gels are stained with Coomassie Brilliant Blue (CBB) G-250. Alternatively, separated proteins are transferred onto nitrocellulose membranes and detected by immunoblotting with the monoclonal anti-HBc antibody and the anti-mouse IgG peroxidase conjugate.

Electron Microscopy

Quantification of VLPs produced and structure analysis of VLP produced is done by electron microscopy. The protein samples are adsorbed on carbon–formvar-coated copper grids and negatively stained with 1% uranyl acetate aqueous solution. The grids are examined with electron microscope.



Flow Chart 7: Hepatitis B VLP Production: Yeast Expression System

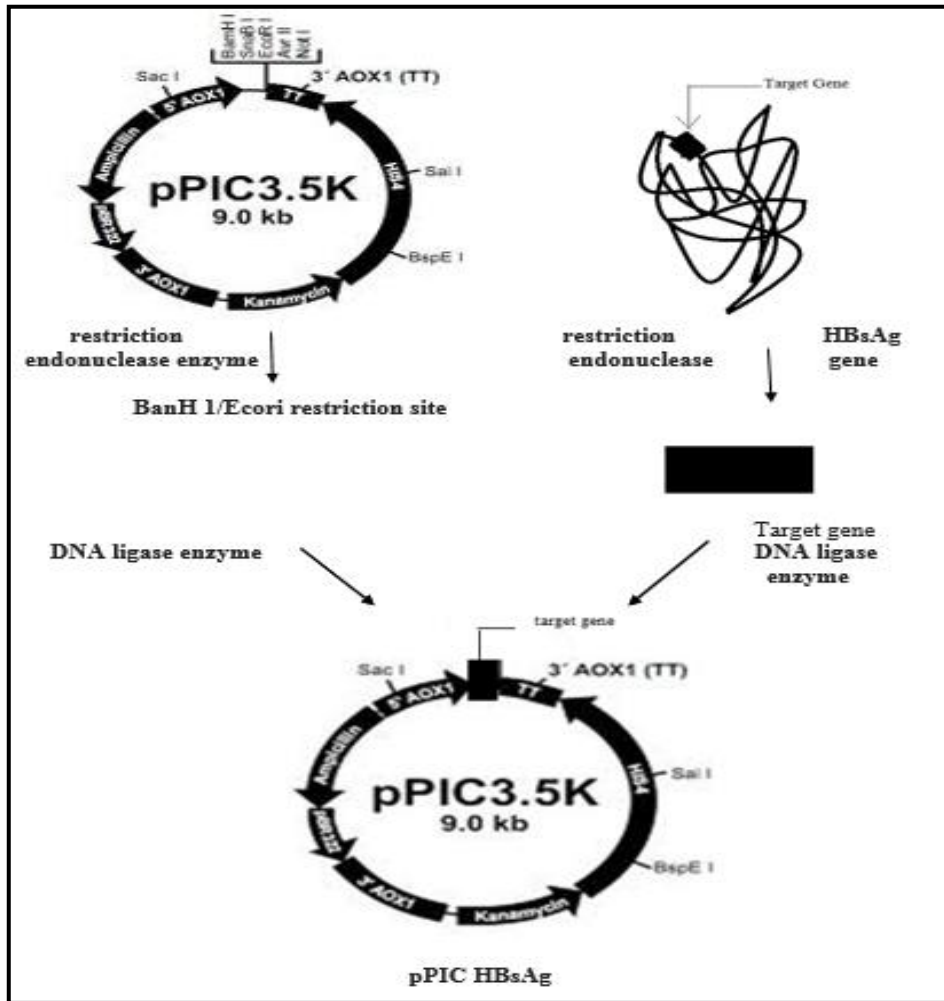


Figure 6: Generation of recombinant transfer vector pPIC HBsAg

Hepatitis B VLP Production: Plant Expression System⁶⁸⁻⁷⁰

Steps Involved (Chart 8)

Expression System

- Plasmid construction
- Selection of Cloned vectors
- Agrobacterium* -mediated transient expression assay and plant transformation

Production and purification of VLPs

- Selection of Explant
- Sucrose gradient and partial purification of Antigens

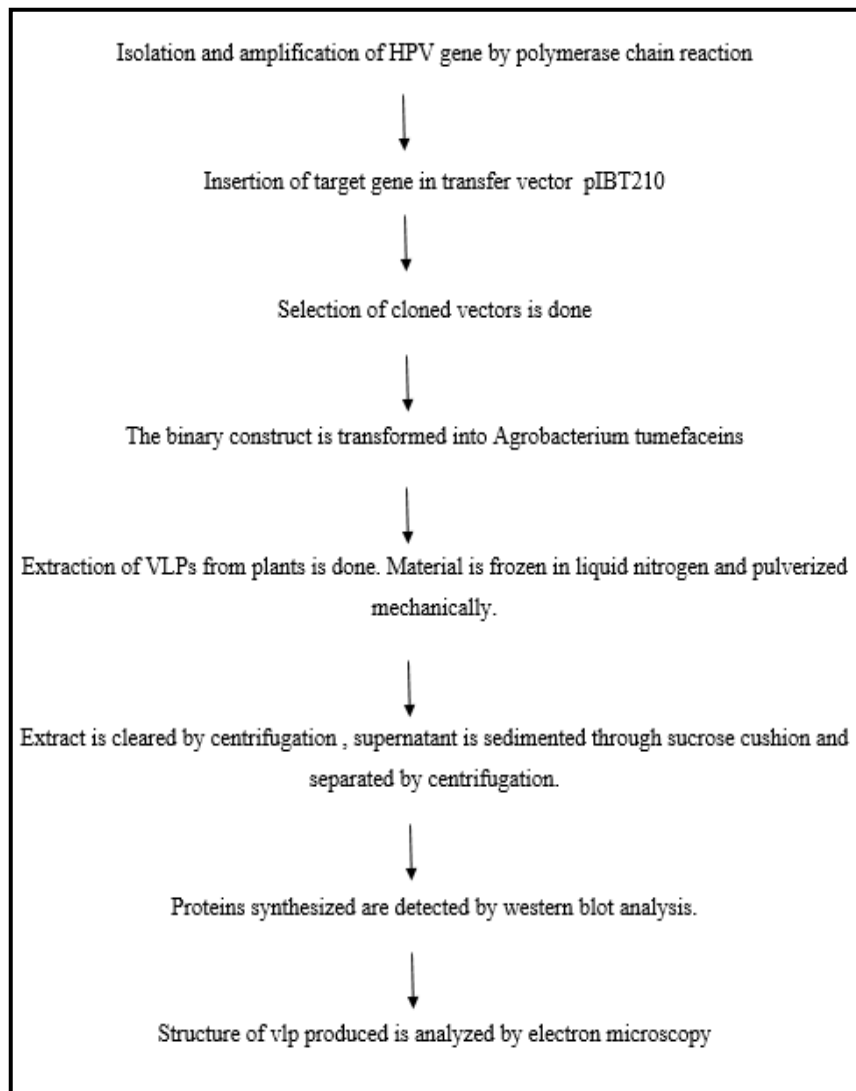
Analysis

- Western blot analysis

Expression System

Plasmid Construction (Figure 7)

pHB117 is a plant vector that provides expression of a HBsAg S-protein coding sequence. Plasmid pHBV48 encodes M-HBsAg gene. The M-HBsAg gene is amplified from pHBV48 using primer containing BamHI and KpnI sites. The resulting PCR product is digested with BamHI and KpnI, and inserted into pIBT210 between the 35S promoter and VSP terminator, giving rise to pIBT-HBsAg. The MHB gene is then released by XbaI/KpnI digestion and inserted into binary vector pPS1, resulting in pHBsAg.



Flow Chart 8: Hepatitis B VLP Production: Plant Cell Expression System

Selection of Cloned Vectors

Selection of cloned genes for HBsAg S-protein and M-protein is done using western blot technique. Yeast derived proteins are used as reference antigens. HBsAg S protein from the pHB117 leaf extract co-migrated with monomeric form of the yeast derived S protein; while the pMHB extract yielded a band with slightly higher molecular weight, representing the M protein.

Agrobacterium-Mediated Transient Expression Assay and Plant Transformation

Binary vectors are introduced into *Agrobacterium tumefaciens* LBA4404 by electroporation. For transient expression, the

resulting *Agrobacterium* strains are used to infiltrate *Nicotiana benthamiana* leaves. For *N. benthamiana* transformation, *A. tumefaciens* LBA4404 strains harboring plasmids pHB117 or pMHB are grown in Yeast extract peptone medium. Leaf discs of in vitro-grown *N. benthamiana* plants are submerged in *Agrobacterium* culture and then put on co-cultivation medium.

Production and Purification

Selection of Explant

The explants are transferred to shoot regeneration and selection medium. Kanamycin-resistant transgenic lines are produced for each construct and screened by Auszyme monoclonal ELISA. The regenerated shoots are transferred

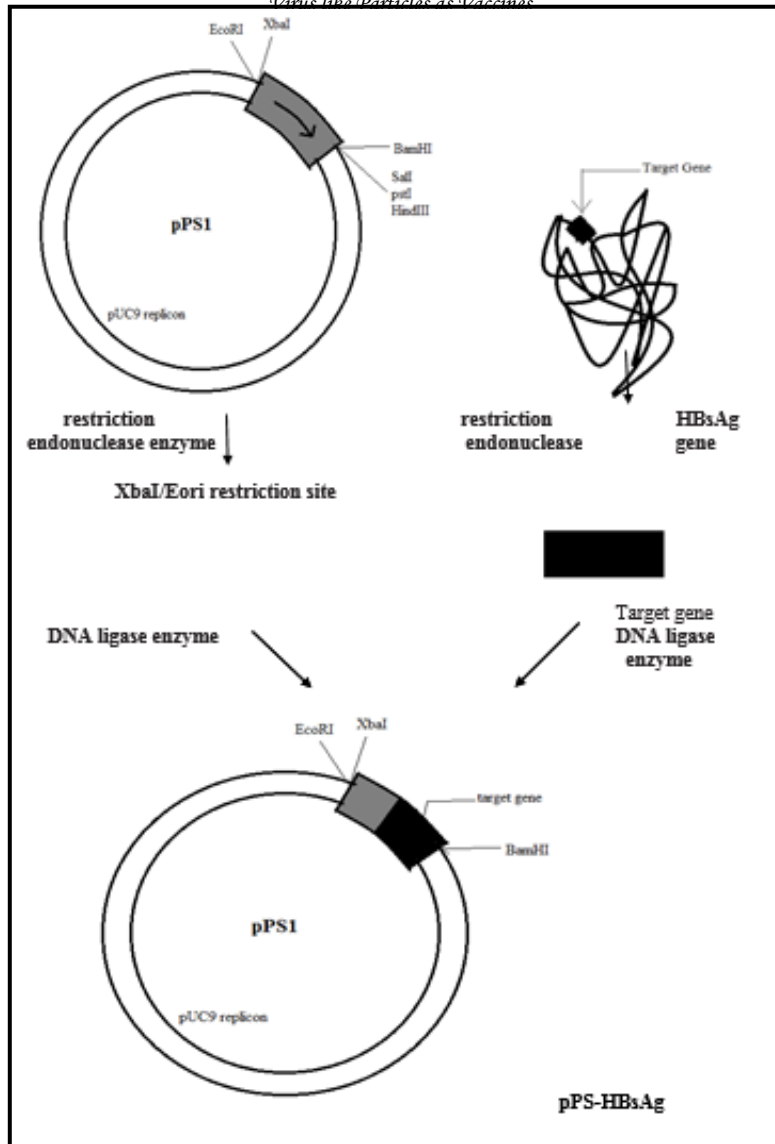


Figure 7: Generation of recombinant transfer vector PPS-HBsAg

to rooting medium. Plantlets rooting on selective medium are tested for HBsAg expression, and the high expressors are then planted in soil and grown in greenhouse.

Sucrose Gradient and Partial Purification of Antigens

To determine whether plant-expressed protein assembled as VLP, sucrose gradient sedimentation of extracts from the transgenic plants is performed. Leaves of HBsAg transgenic plants are homogenized with liquid N₂, and the resulting powder is extracted with extraction buffer. The extract is centrifuged and supernatant is layered onto linear 5 mL 10–50% sucrose gradients in phosphate-buffered saline.

The presence of the M protein in the sucrose gradient fractions is confirmed by Western blot probed with polyclonal goat anti-HBsAg serum.

Analysis

Western Blot Analysis

This technique is employed to detect the HBsAg proteins in VLPs. Crude transgenic plant extract or yeast-derived HBsAg reference standard is added to sample buffer subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidenedifluoride membranes. For detection of HBsAg, membranes were probed with goat anti-HBsAg followed by rabbit anti-goat IgG conjugated to horseradish peroxidase.

Comparison of Expression Systems⁷¹⁻⁷⁵

	Yeast	Insect	Mammalian	Plant	Cell-free
Cloning	E. coli is used	E. coli is used	E. coli is used	A. tumefaciens is used	E. coli is used
Yield	Average	High	Very low	High	Average
Transformation	Electroporation	Baculovirus	Electroporation	Agrobacterium	Electroporation
Selection	Antibiotics/Auxotrophic	Antibiotics/Auxotrophic	Antibiotics	Antibiotics/herbicides	Antibiotics
Purification	Recovery from supernatant	Cell lysis and recovery	Recovery from supernatant	Cell lysis recovery of media	Recovery from supernatant
Cell growth	Rapid (90 min)	Slow (24 hrs)	Slow (24 hrs)	Very Slow	Slow
Complexity of growth medium	Minimum	Complex	Very complex	Minimum	Complex
PTM					
Protein folding	Refolding required	Proper refolding	Proper refolding	Proper refolding	Refolding required
Glycosylation	Shows	Shows	Shows	Shows	Depending on growth medium
Phosphorylation	Shows	Shows	Shows	Shows	Depending on growth medium
Acetylation	Shows	Shows	Shows	Shows	Shows
Carboxylation	Does not show	Does not show	Shows	Shows	shows

VLP Vaccines on the Market and in the Clinical Development

HPV VLP Vaccines

Table 1: HPV VLP Vaccines

Vaccine Name	Company	Expression System	Vaccine Antigen	Current Status
Gardasil	Merck	Yeast (<i>S. cerevisiae</i>)	HPV 6/11/16/18 L1	Licensed
Cervarix	GSK	Insect (<i>Hi-5</i> cells)	HPV 16/18 L1	Licensed
V503	Merck	Yeast (<i>S. cerevisiae</i>)	HPV11/16/18/ 31/33/45/52/58 L1	Phase 3

Influenza VLP Vaccine

Table 2: Influenza VLP Vaccine

VACCINE NAME	COMPANY	EXPRESSION SYSTEM	VACCINE ANTIGEN	CURRENT STATUS
	Novavax	Insect (<i>Sf-9</i> cells)	A/California/04/09 (H1N1)	Phase 2
	Medicago	Plant(transient <i>N.benthamiana</i>)	A/California/04/09 (H1N1)	Phase 1
	Novavax	Insect (<i>Sf-9</i> cells)	A/Indonesia/05/05 (H5N1)	Phase 1/2a
	Medicago	Plant (transient <i>N.benthamiana</i>)	A/Indonesia/05/05 (H5N1)	Phase 2
	Novavax	Insect (<i>Sf-9</i> cells)	A/Brisbane/59/07 (H1N1)	Phase 2a
Inflexal V	Crucell	Cell-Free	A(H1N1), A(H3N2)	Licensed
ACAM-FLU-A	Sanofi Pasteur	Bacteria (<i>E. coli</i>)	Influenza A M2e	Phase 1

Hepatitis B VLP Vaccines

Table 3: Hepatitis B VLP Vaccines

Vaccine name	Company	Expression system	Vaccine Antigen	Current status
Genevac B	Pasteur-Merieux Aventis	Mammalian (CHO cells)	HBsAg	Licensed
Bio-Hep B	BTE (SciGen,FDS Pharma)	Mammalian (CHO cell)	HBsAg	Licensed
DTP-Hep B	P.T.Bio Farma	Yeast (<i>P.pastoris</i>)	HBsAg	Licensed
Engerix-B	GSK	Yeast (<i>S.cerevisiae</i>)	HBsAg	Licensed
Enivac HB	Panacea Biotec	Yeast (<i>P.pastoris</i>)	HBsAg	Licensed
Euvax B	LG Life Sciences	Yeast (<i>S.cerevisiae</i>)	HBsAg	Licensed
Gene Vac-B	Serum Inst of India	Yeast (<i>H.polymorpha</i>)	HBsAg	Licensed
Heberbiovac HB	CIGB-Heber Biotech	Yeast (<i>P.pastoris</i>)	HBsAg	Licensed

Vaccine name	Company	Expression system	Vaccine Antigen	Current status
Hepavax-Gene	Crucell	Yeast (<i>H.polymorpha</i>)	HBsAg	Licensed
Recombivax HB	Merck	Yeast (<i>S.cerevisiae</i>)	HBsAg	Licensed
Revac-B	Bharat Biotech International	Yeast (<i>P.pastoris</i>)	HBsAg	Licensed
Shanvac-B	Shantha Biotechnics	Yeast (<i>P.pastoris</i>)	HBsAg	Licensed

Malaria VLP Vaccine

Table 4: Malaria VLP Vaccine

Vaccine name	Company	Expression system	Vaccine Antigen	Current status
MalariVax	Apovia	Bacteria (<i>E. coli</i>)	<i>P.falciparum</i> CSP	Phase 1
RTS,S	GSK/PATH	Yeast (<i>S.cerevisiae</i>)	<i>P.falciparum</i> CSP	Phase 3
PEV	Pevion Biotech	Cell-Free	<i>P.falciparum</i> CSP	Phase ½

CSP-circumsporozoite protein

Norovirus VLP Vaccine

Table 5: Norovirus VLP Vaccine

Vaccine name	Company	Expression system	Vaccine Antigen	Current status
	Baylor College of Medicine	Insect (<i>Sf-9</i> cells)	Norwalk virus coat protein	Phase 1
	Ligocyte	Insect (<i>Sf-9</i> cells)	Norwalk virus coat protein	Phase 1
	Ligocyte	Insect (<i>Sf-9</i> cells)	Norwalk virus coat protein	Phase 1
	University of Maryland	Plant (Tg Potato)	Norwalk virus coat protein	Phase 1

HIV VLP vaccine

Table 6: HIV VLP vaccine

Vaccine name	Company	Expression system	Vaccine Antigen	Current status
	British Biotech/ NIAID	Yeast (<i>S. cerevisiae</i>)	HIV-1 Gag p17/p24	Phase 2
MYM-V101	PevionBiotech/ MymeticsCorpo ration	Cell-Free	HIV-1 Gag p41	Phase 1

VLP Vaccines against different Diseases

Table 7: VLP Vaccines against different Diseases

Vaccine name	Company	Expression system	Vaccine Antigen	Current status
Alzheimer's Disease				
CAD106	Cytos Biotech/Novarti s	Bacteria (<i>E. coli</i>)	Aβ1-6	Phase 2
Type II Diabetes mellitus				
CYTO13- IL1bQβ	Cytos Biotech	Bacteria (<i>E. coli</i>)	IL-1β	Phase 1/2a
Allergic rhinoconjunctivitis and asthma				
CYT003- QβG10	Cytos Biotech	Bacteri (<i>E. coli</i>)	G10 (CpG DNA)	Phase 2
Breast Cancer				
	Pevion Biotech	Cell-Free	Her2/neu	Phase 1
<i>C. albicans</i>				
PEV7	Pevion Biotech	Cell-Free	<i>C. albicans</i> SAP2	Phase 1
Hepatitis A				
Epaxal	Crucell	Cell-Free	Inactivated HAV RG-SB	Licensed

Hepatitis C				
	Pevion Biotech	Cell-Free	HCV Peptides	Phase 1
Human parvovirus B19				
VAI-VP705	NIH/Meridian Life Science	Insect (<i>Sf-9</i> cells)	B19 VP1, VP2	Phase 1/2
Hypertension				
CYT006-AngQ β	Cytos Biotech	Bacteria (<i>E. coli</i>)	Ang II	Phase 2
Malignant melanoma				
CYT004-MelQ β G10	Cytos Biotech	Bacteria (<i>E. coli</i>)	Melan-4, G10DNA	Phase 2
Nicotin addiction				
NIC002	Cytos Biotech/Novartis/Duke university	Bacteria (<i>E. coli</i>)	Nicotin	Phase 2
Rabies				
	Thomas Jefferson University	Plant (Tg spinach)	Rabies GP/NP	Phase 1
Respiratory syncytial virus				
	Novavax	Insect (<i>Sf-9</i> cells)	RSV F protein	Phase 1

GP-glycoprotein: NP-nucleocapsid protein

RECENT ADVANCES

VLPs have structural characteristics and antigenicity similar to the parental virus, and some have already proven successful as vaccines against the viral infection. The structural components of some VLPs have are amenable to the insertion or fusion of foreign antigenic sequences, allowing the production of chimeric VLPs exposing the foreign antigen on their surface. Chimeric VLPs provide a means for the incorporation of heterologous antigens into VLPs, including antigens that are unable to self-assemble in a particulate form and antigens from viruses where the intact virus particle may not have optimal immunogenicity. Other VLPs can be used as carriers for foreign antigens including non-protein antigens to enhance efficacy and utility of virus like particle to serve as vaccine.

CONCLUSION

VLPs are established tools to induce immune responses and being able to prevent infection by their naturally occurring counterparts for associated diseases. As there is increasing awareness of the structure, assembly and production of VLPs along with interaction with cells to induce immune response it is expected that the development of VLPs as vaccines, as vehicles for delivering small molecules and as an epitope carrier for foreign antigen will lead to development of VLPs with greater efficiency.

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