

Use of Virus-Like-Particles in Biotechnology

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Etimology and Definition of Virus

Latin *virus*: poison, nocive substance.

Acelular microscope infectious agent that only can multiple in the cells of others organism.

Can infect to all forms of life (plants, animals, prokaryotes, eukaryotes, fungi, protist, even other viruses).

History

- In the mid-nineteenth century, Louis Pasteur proposed the germ theory of disease:

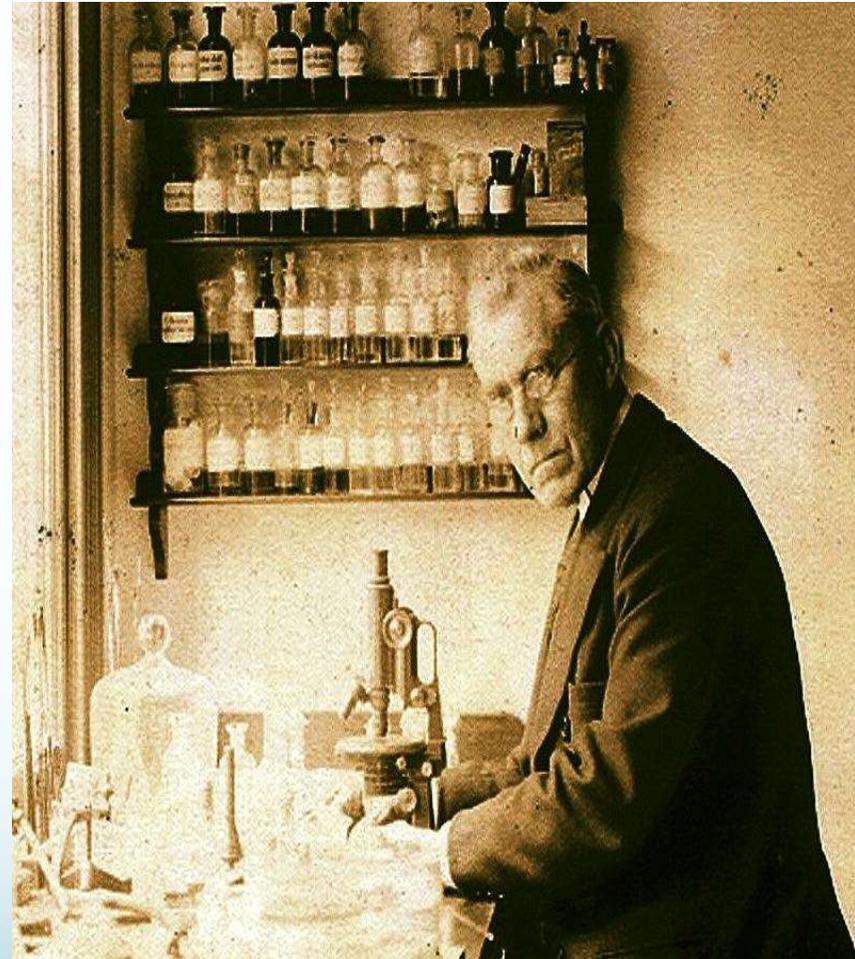
"Diminute type of life" that multiplies in the diseased organism, pass from one to another and made them sick.

- He work with the rabies disease and discovered that although the disease was contagious and contracted by the bite of a rabid animal, could not see the germ anywhere.
- Pasteur concluded that the germ was there, but it was too small to be observed.



History

- In 1899, Martinus Beijerinck repeated the experiments of Ivanovski and was convinced that it was a new form of infectious agent.
- He observed that the agent only multiplied within living cells, but its experiments did not show that it was composed of particles.
- He introduced the term "virus".



History

- With the invention of electron microscopy in 1931 by Ernst Ruska and Max Knoll, the first virus images were obtained.

Ruska-Knoll Microscope



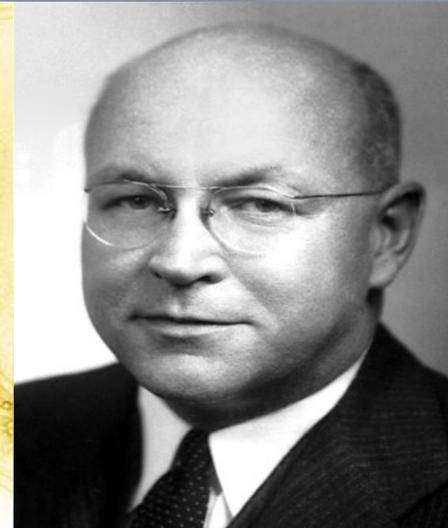
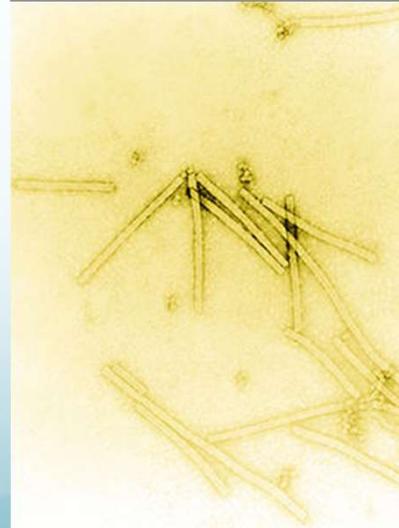
1906–1988



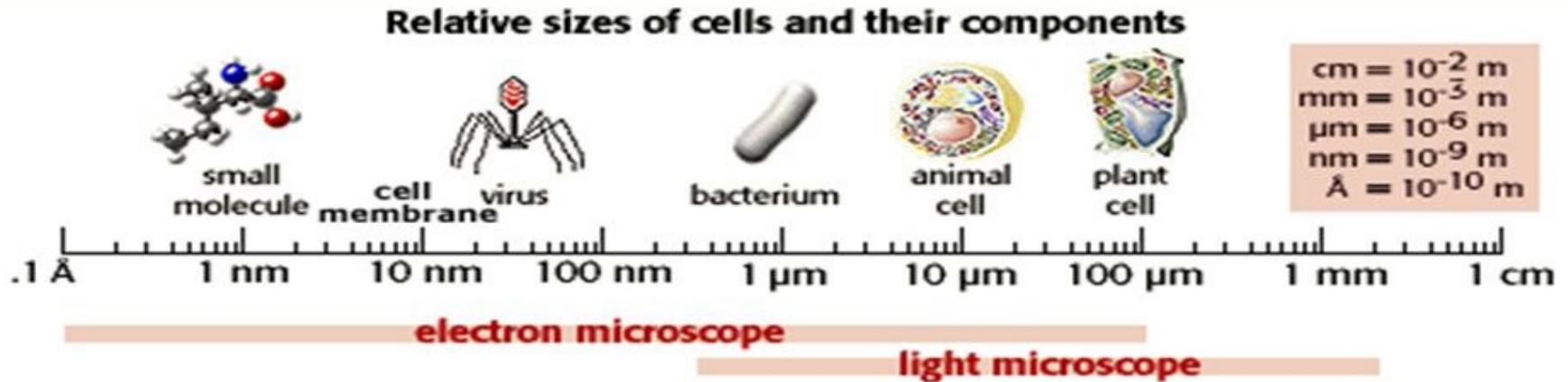
1897–1961



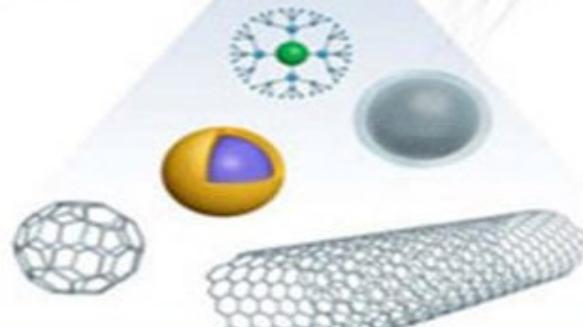
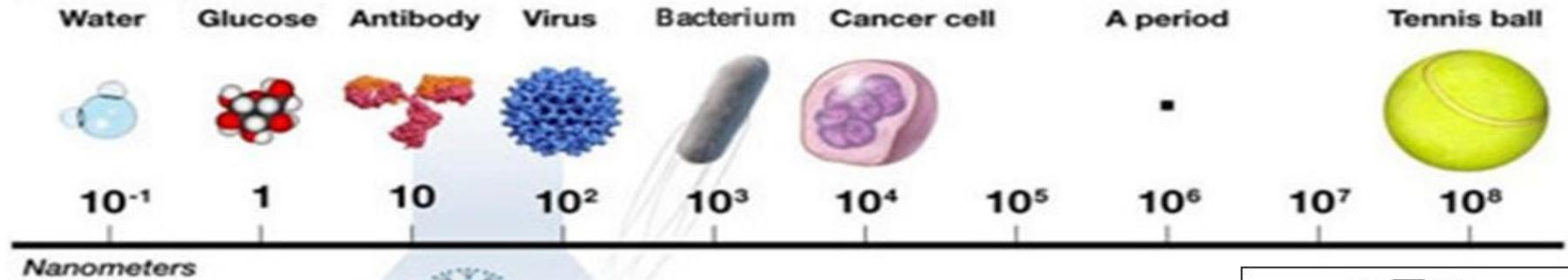
- In 1935, Wendell Stanley demonstrated that viruses are particles composed mostly of proteins.



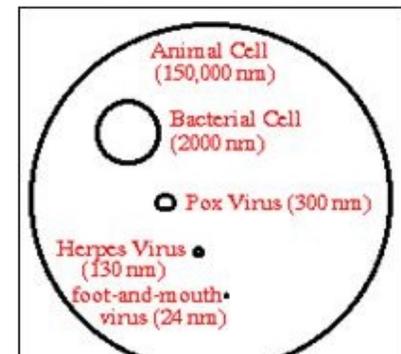
Size Comparison



How Small Is Small?

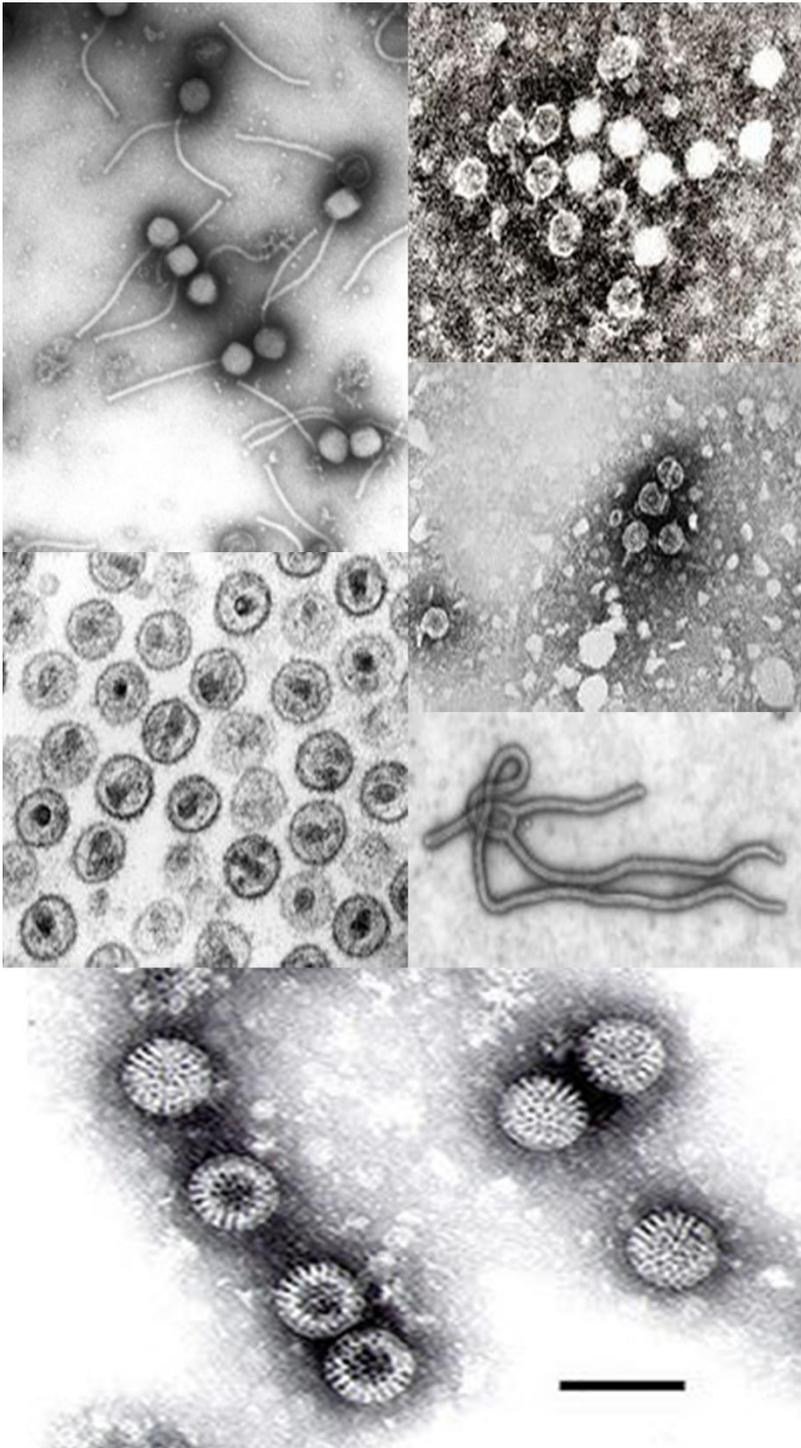


Nanodevices
 Nanopores
 Dendrimers
 Nanotubes
 Quantum dots
 Nanoshells



History

- The second half of the twentieth century was the golden age of virus discovery, and most of the recognized species of animal, plant and bacterial viruses were discovered during these years.
- Today more than 3700 types are known.
- The origin of virus is unknown and the theories about it are based in inferences since they do not fossilize.



Virus-Like-Particles

- VLPs are multisubunit self-assembly competent protein structures with identical or highly related overall structure to their corresponding native viruses.
- VLPs can also be defined as replication incompetent macromolecular protein assemblies that consist of one or several structural proteins of different viruses and can be obtained from recombinant organisms via the expression of the corresponding cloned genes.

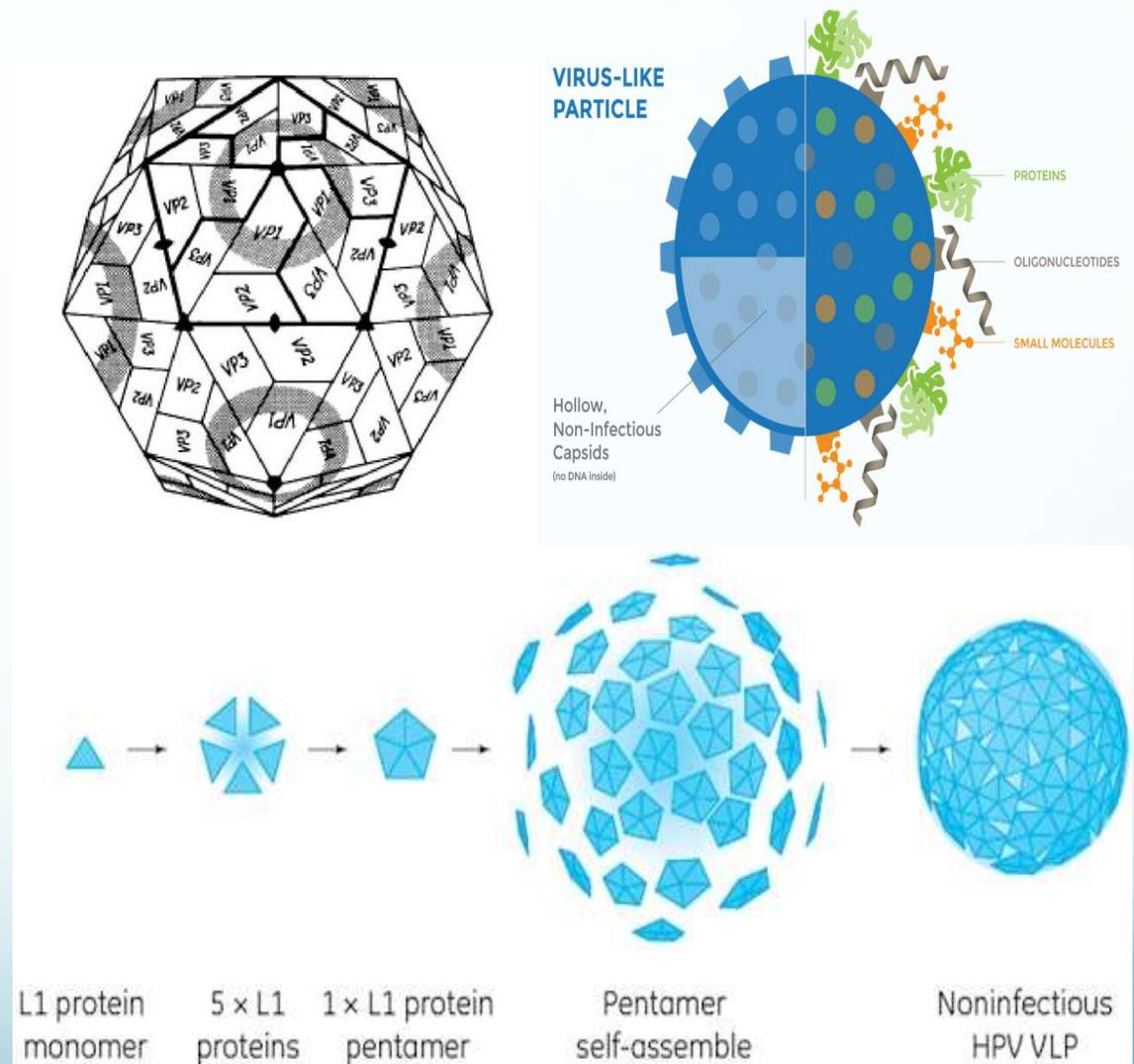
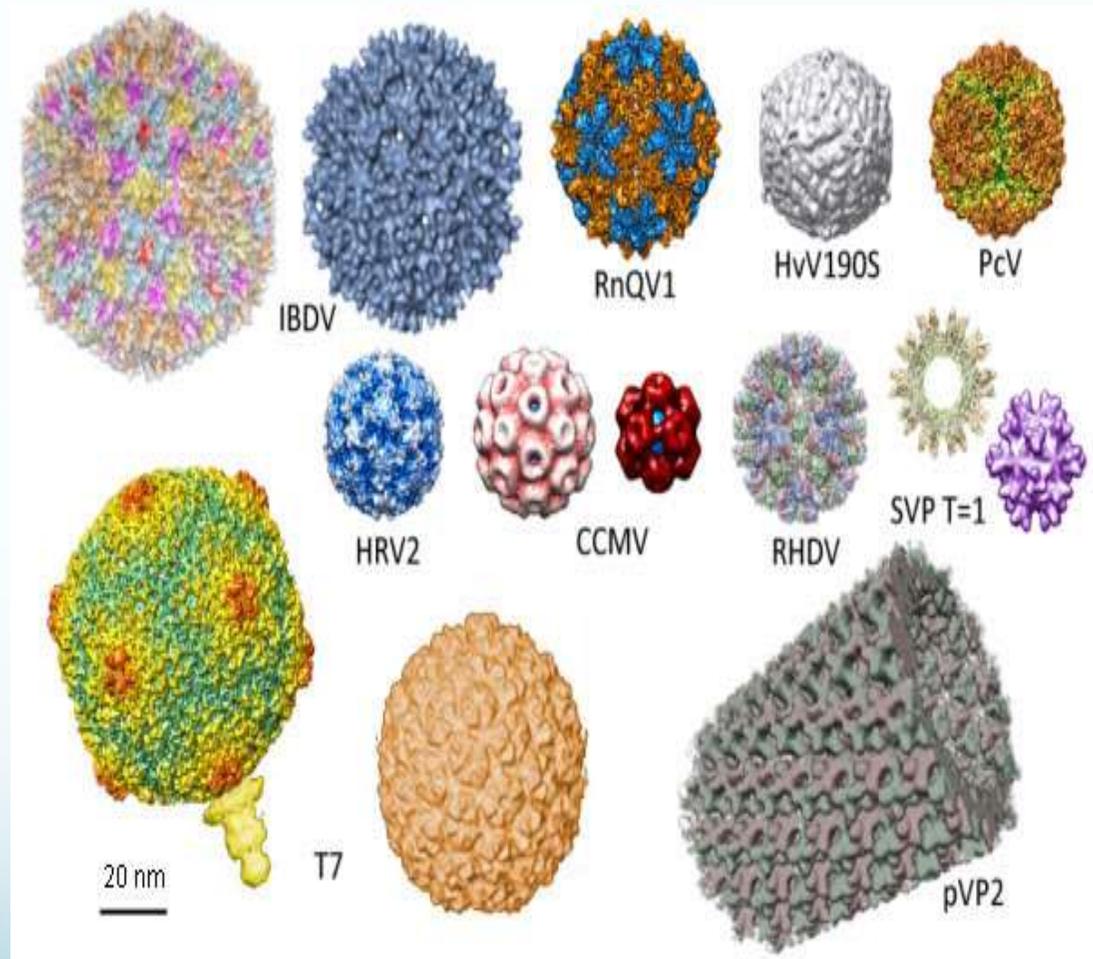


Fig 1. Schematic picture of formation of L1 proteins into HPV VLP.

Virus-Like-Particles

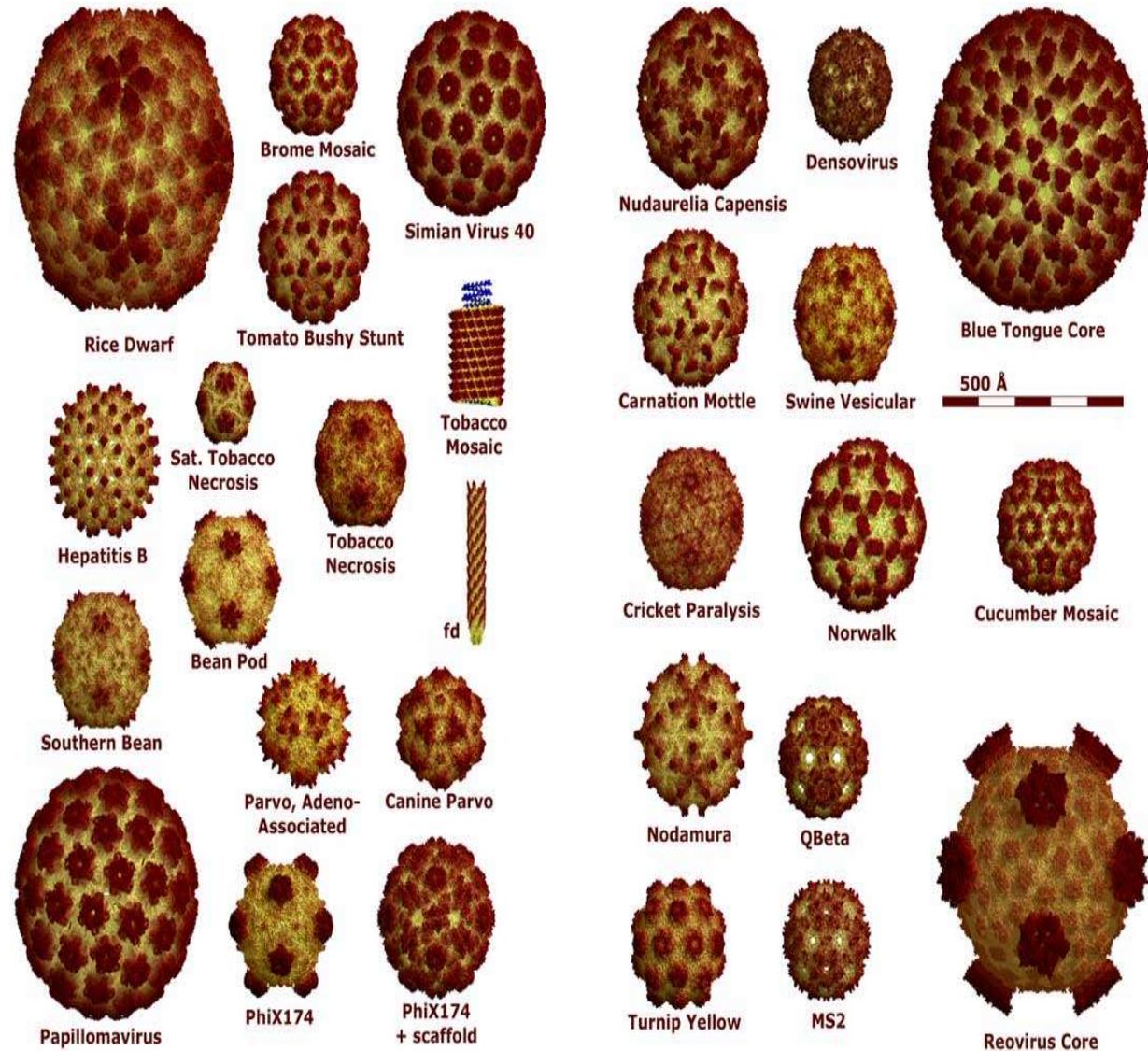
The term VLP has been used to describe a number of biological objects, such as:

- Uncharacterized structures with viral morphology that are found in biological samples.
- Empty structures of viral origin that are not composed of nucleic acids.
- Noninfectious, self-assembled gene products resulting from the cloning and expression of viral structural genes in heterologous host systems.



Recombinant VLPs

- Since the beginning of the 1980s, over 100 VLPs that originated from microbial, plant, insect, and mammalian viruses have been constructed and characterized.
- The aim of these studies was to use these particles for a variety of purposes, from basic virus assembly and structure studies to the production of commercial human and animal vaccines.



Virus-Like-Particles

A cumulative search for the term virus-like, which is the most accepted name for these particles, in Google Scholar yielded:

- 15,700 publication records through 1995.
- Approximately 42,000 through 2005.
- At least 102,000 publications through the middle of 2012.
- In 2017 approximately 2,500,000 results are obtained.



Commercial Use

VLP-based vaccines are currently commercialized worldwide.

- Glaxo Smith Kline's Engerix (HBV) and Cervarix (HPV).
- Merck and Co., Inc.'s Recombivax HB (HBV) and Gardasil (HPV).



GlaxoSmithKline



MERCK



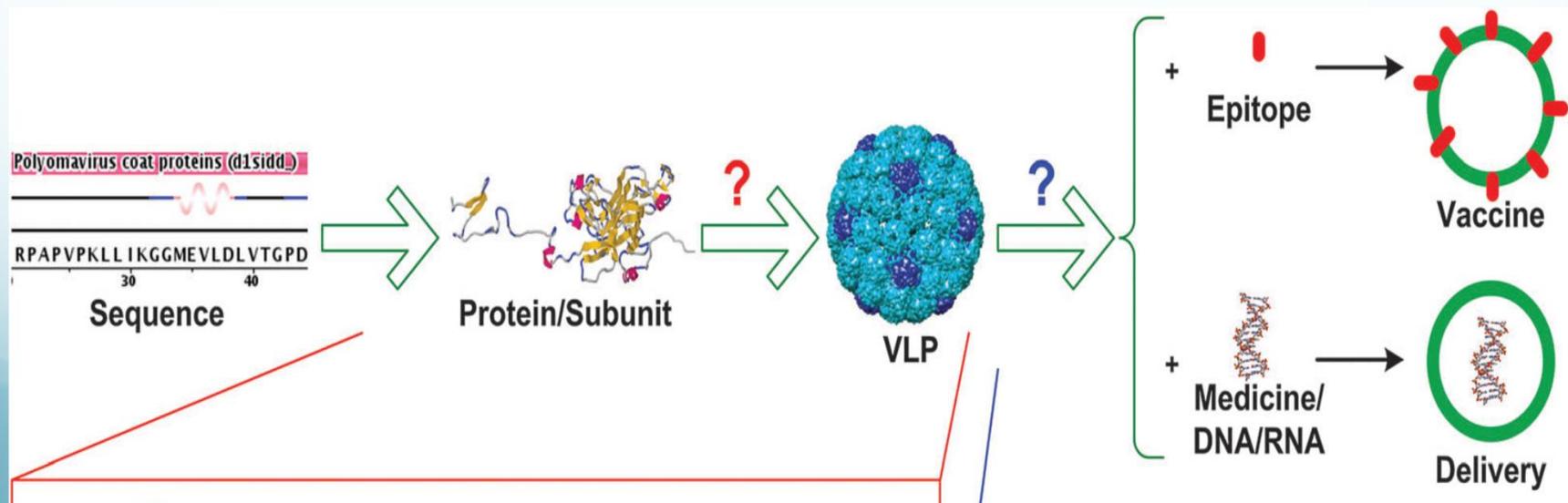
Commercial Use

- Recently, several VLP-based vaccine candidates have entered different stages of clinical investigations with the aim to develop VLP-based vaccines for medical and veterinary purposes in the near future.



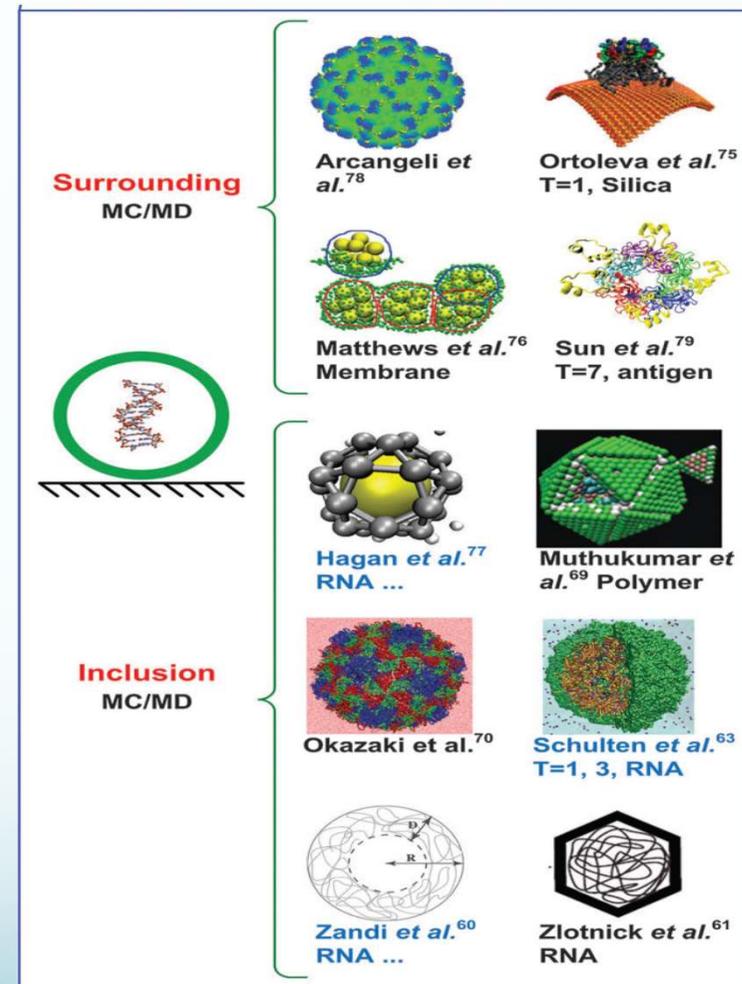
VLPs and New Vaccines

- The key to the success of virus-like particle (VLP) vaccines resides in their accurate mimicry of wild-type viral particles.
- VLPs are supra-molecular assemblages incorporating the key immunologic features of viruses which include repetitive surface patterns and particulate structures with potential for induction of innate and adaptive immunity through activation of pattern recognition receptors and antigen-specific B-cell receptors, respectively.



VLPs and New Vaccines

- In addition to being effective vaccines against analogous viruses from which they are derived, VLPs can also be used to present foreign epitopes to the immune system.
- This can be achieved by genetic fusion or chemical conjugation between target antigens and structure viral proteins that can self-assemble into VLPs, offering new perspectives in vaccine development.

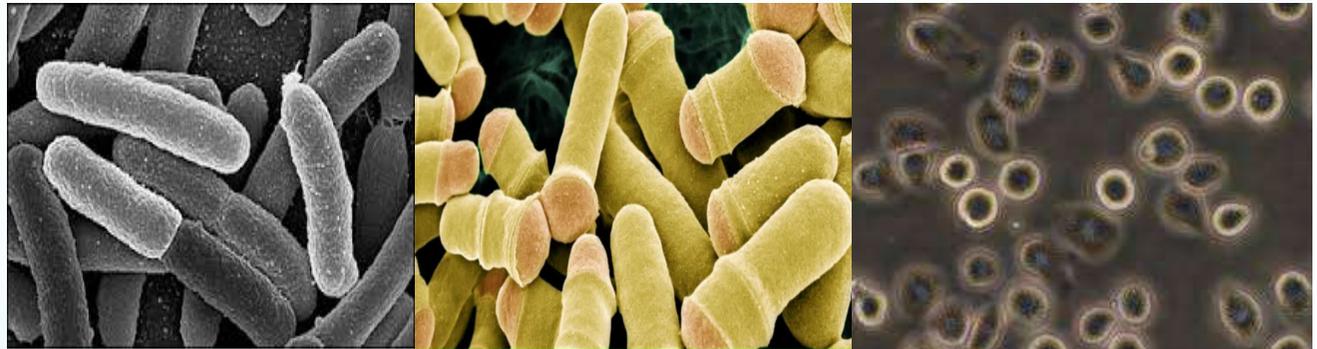


Expression Host Systems

One of the crucial factors in the construction of new VLPs is the choice of expression system:

- Bacterial (used particularly for the production of bacterial and plant VLPs.)

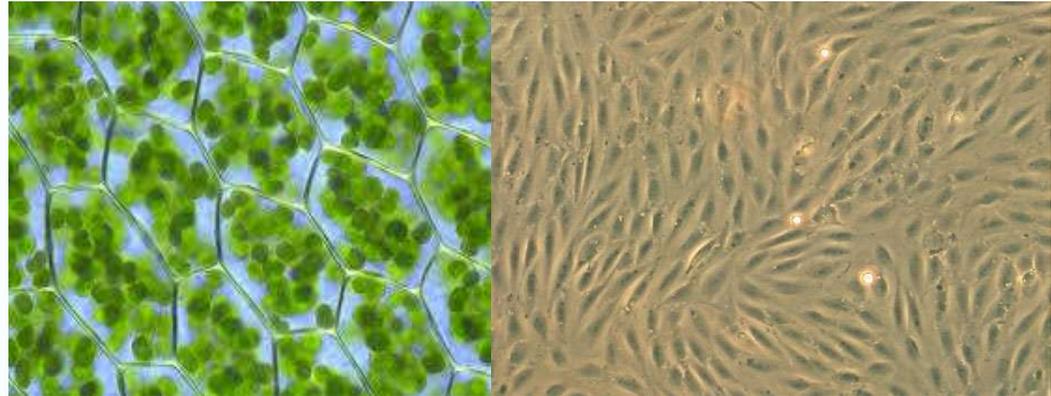
- Yeast *
- Insect *



These systems* are more universal, and these expression hosts have been successfully used for the construction of VLPs from different sources.

Expression Host Systems

- Plant
- Mammalian Cell

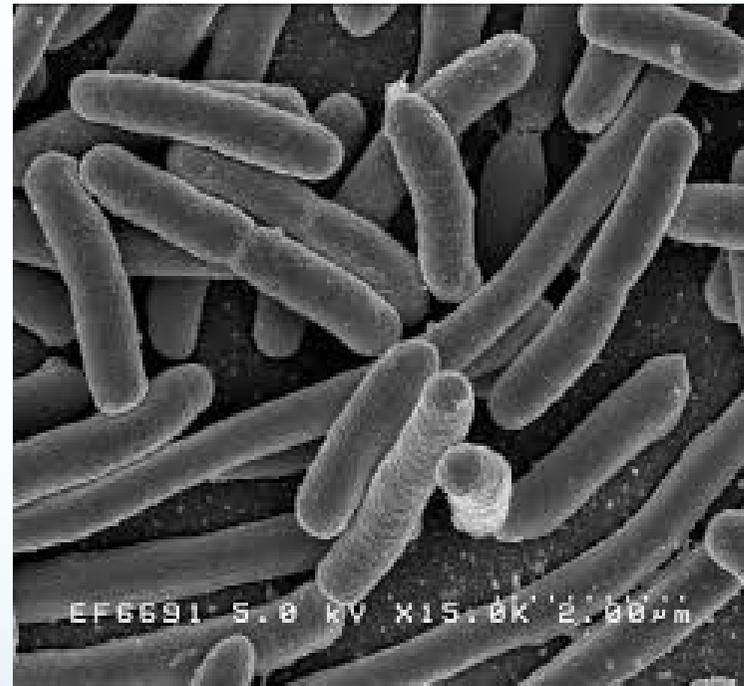


Are typically used to insure the production of VLPs with specific properties.



Bacterial Systems

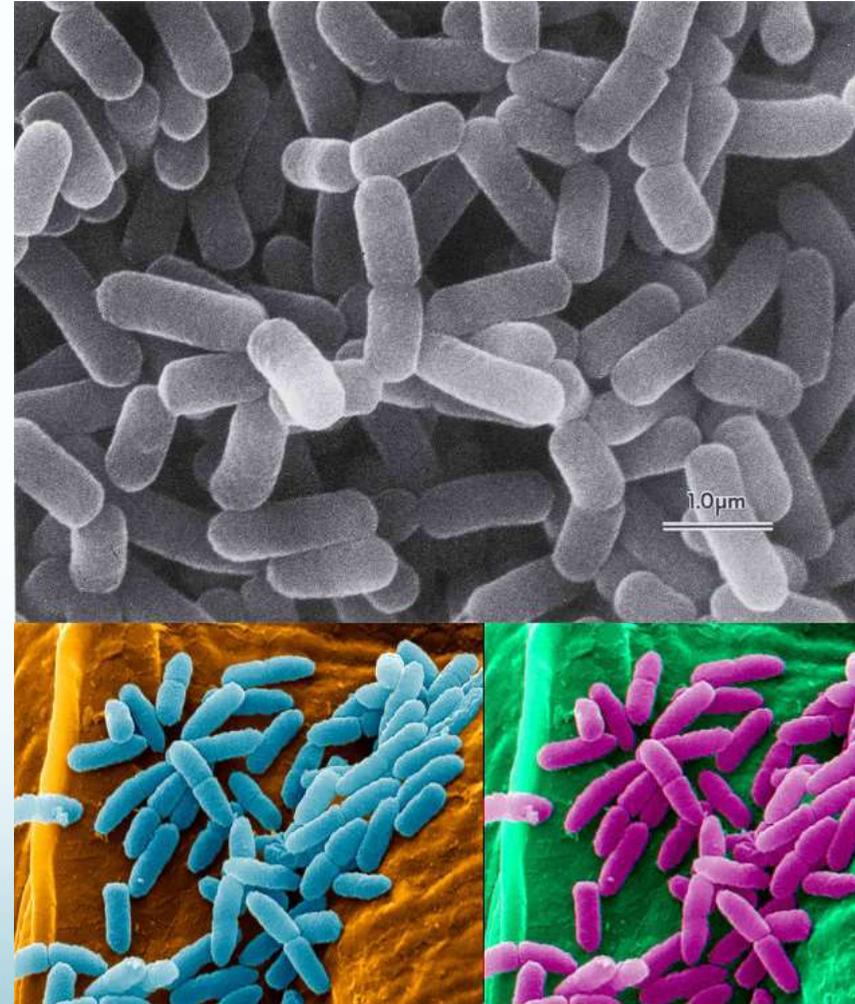
- Are mostly based on well-characterized commercial *Escherichia coli* strains (BL21 DE3, HT115 DE3, etc.) and expression vectors.
- A successful VLP production using *E. coli* is likely achievable in cases in which the target VLPs contain only one coat protein that is soluble in the cells after cultivation.



Bacterial Systems

Other prokaryotic organisms can also be used for VLP production.

- Lactobacillus has been used for the production of the HPV L1 protein to generate edible vaccine candidates.
- Pseudomonas was used to improve the low solubility of the CP of CCMV.



Synthesis of VLPs in *E. coli*

Cloning:

- Isolation of viral genetic material
- Amplification of the capsid gene (PCR)
- Ligation
- Transformation
- Induction of the recombinant protein expression
- Purification of viral capsid protein

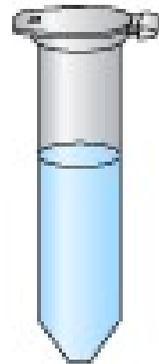
Isolation of Viral Genetic Material

Homogenization and Lysis



Add sample and lysis buffer to NucleoSpin Bead Tube, vortex or use homogenizer

Removal of Contaminants



Precipitate contaminants

Bind – Wash – Elute



Load supernatant onto NucleoSpin Inhibitor Removal Columns

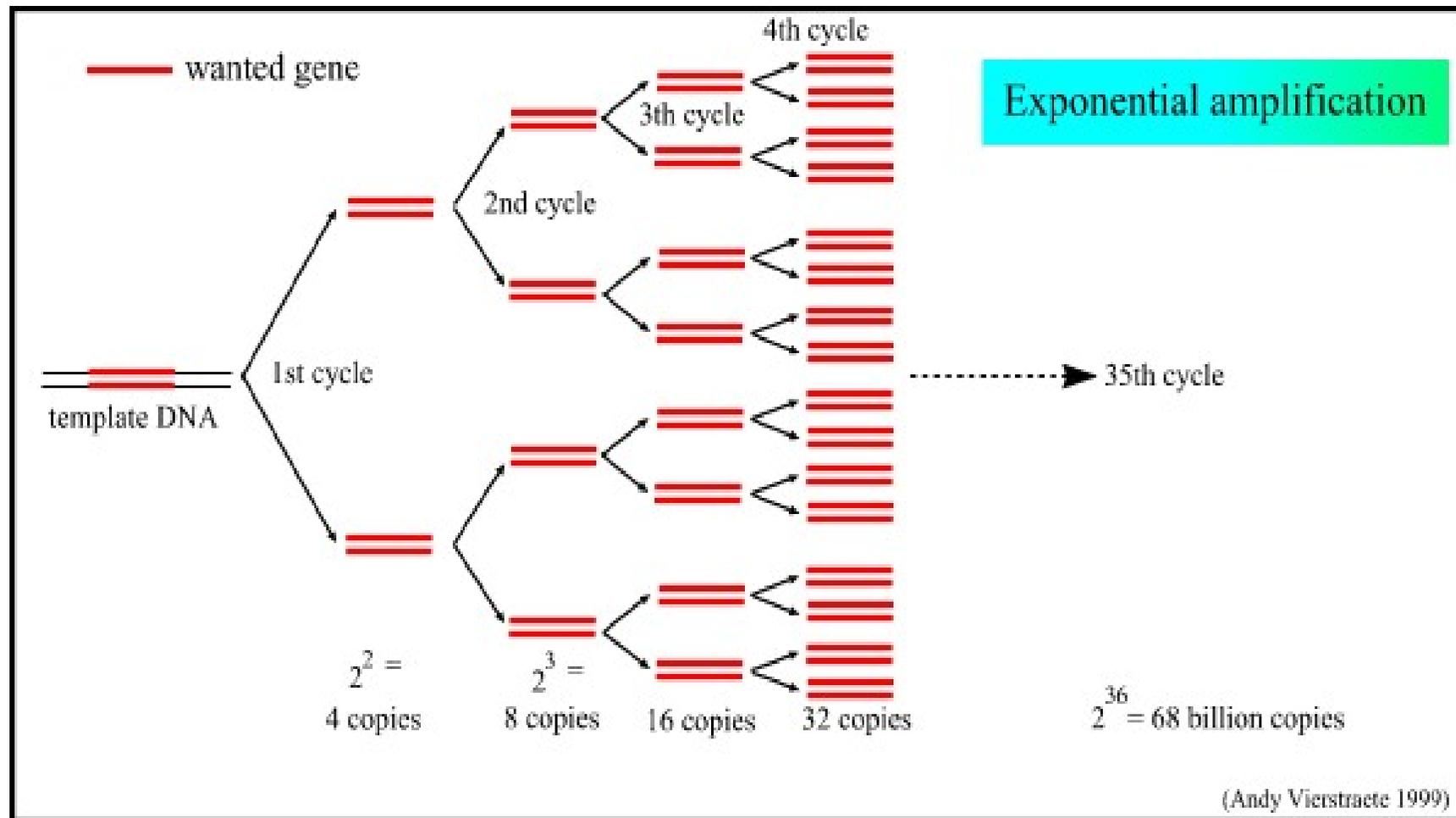


Bind DNA to NucleoSpin Soil Column, apply efficient wash steps, dry the membrane



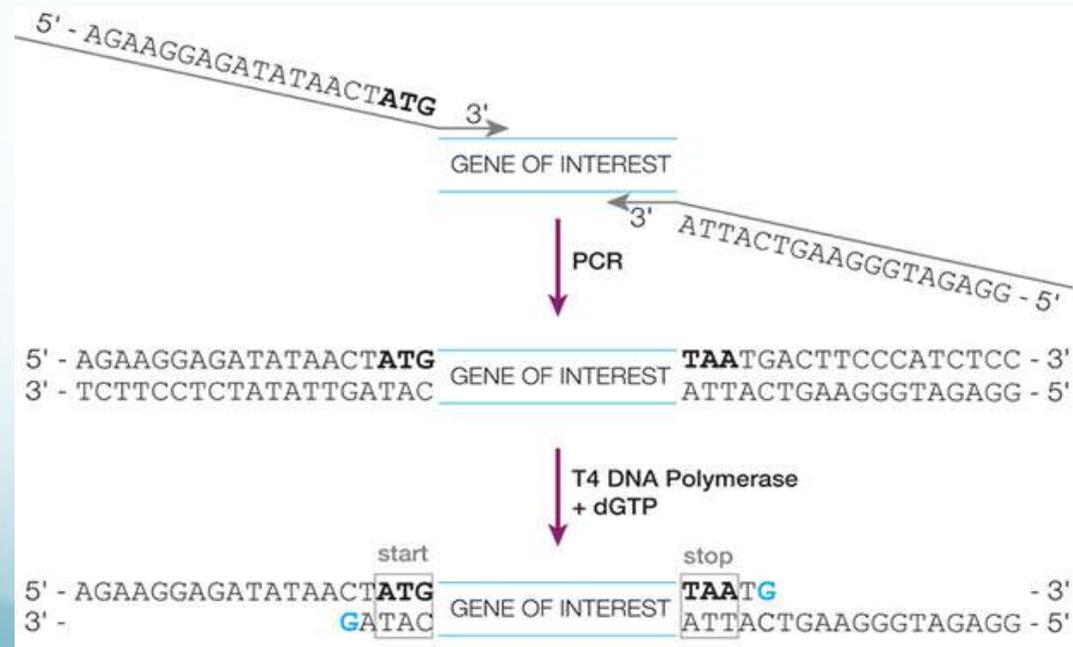
Elute pure DNA ready to use in downstream PCR

PCR

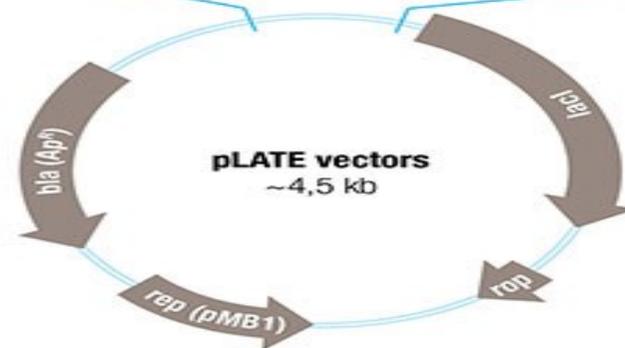
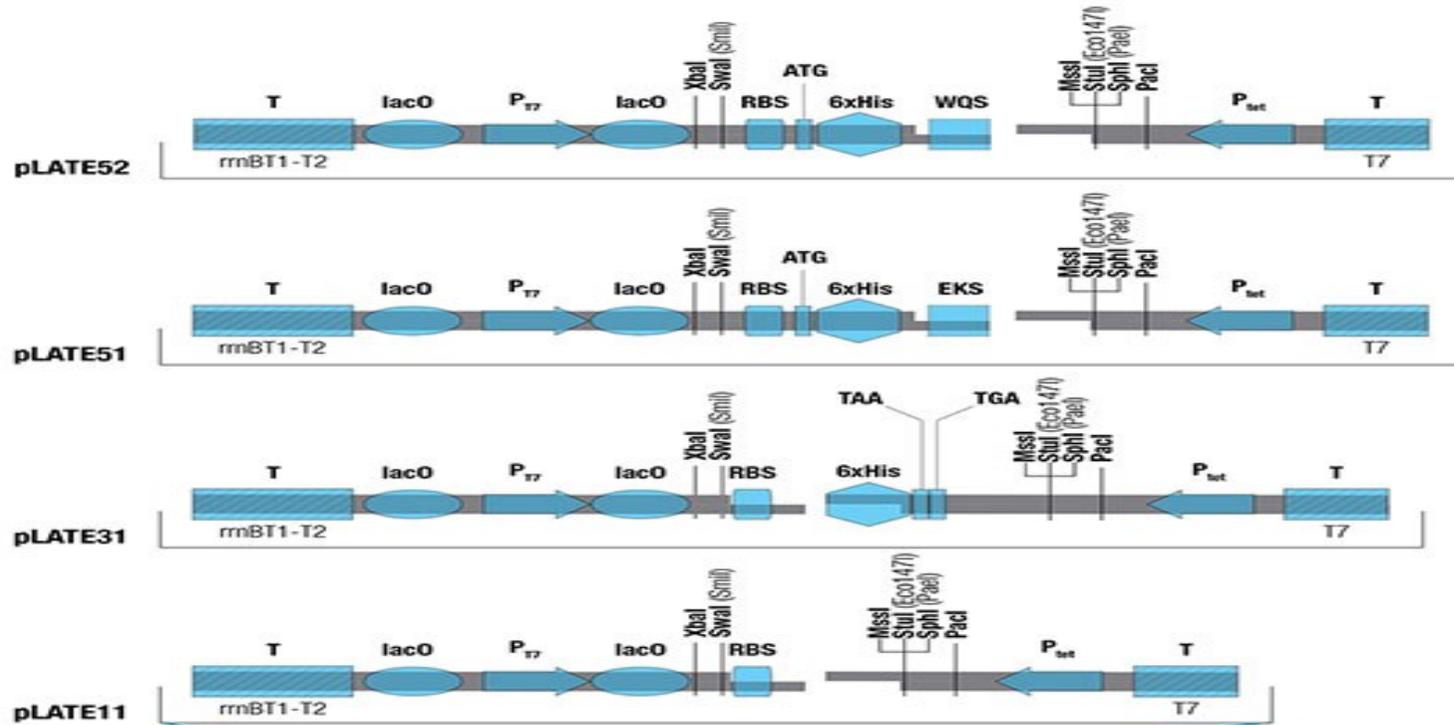


Synthesis of VLPs in *E. coli*

Expression vector pLATE51 (Ligation Independent Cloning and Expression System)

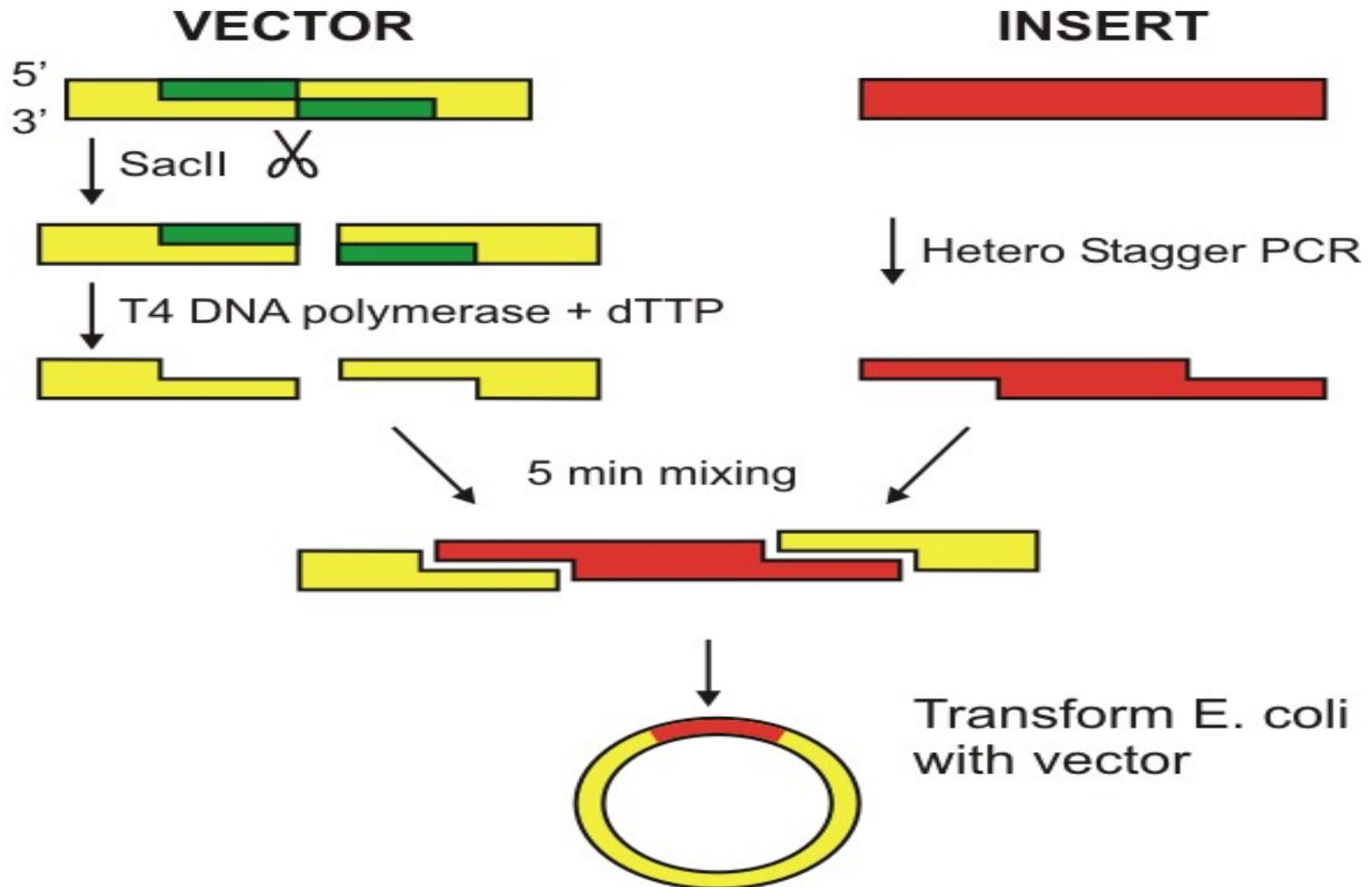


Expresion Vector pLATE51



P_{T7} - T7 RNA polymerase promoter
T rrnBT1-T2 - Transcription terminator
lacO - lac operator
RBS - Ribosome binding site
EKS - Enterokinase recognition sequence
WQS - WELOut protease recognition sequence
P_{tet} - P_{tet} promoter
T T7 - T7 terminator
lacI - lac repressor
rop - rop protein regulates plasmid copy number
rep (pMB1) - Origin of replication (*rep*) of the pMB1 plasmid
bla (Ap^r) - β -lactamase gene
6xHis - Polyhistidine tag

Ligation

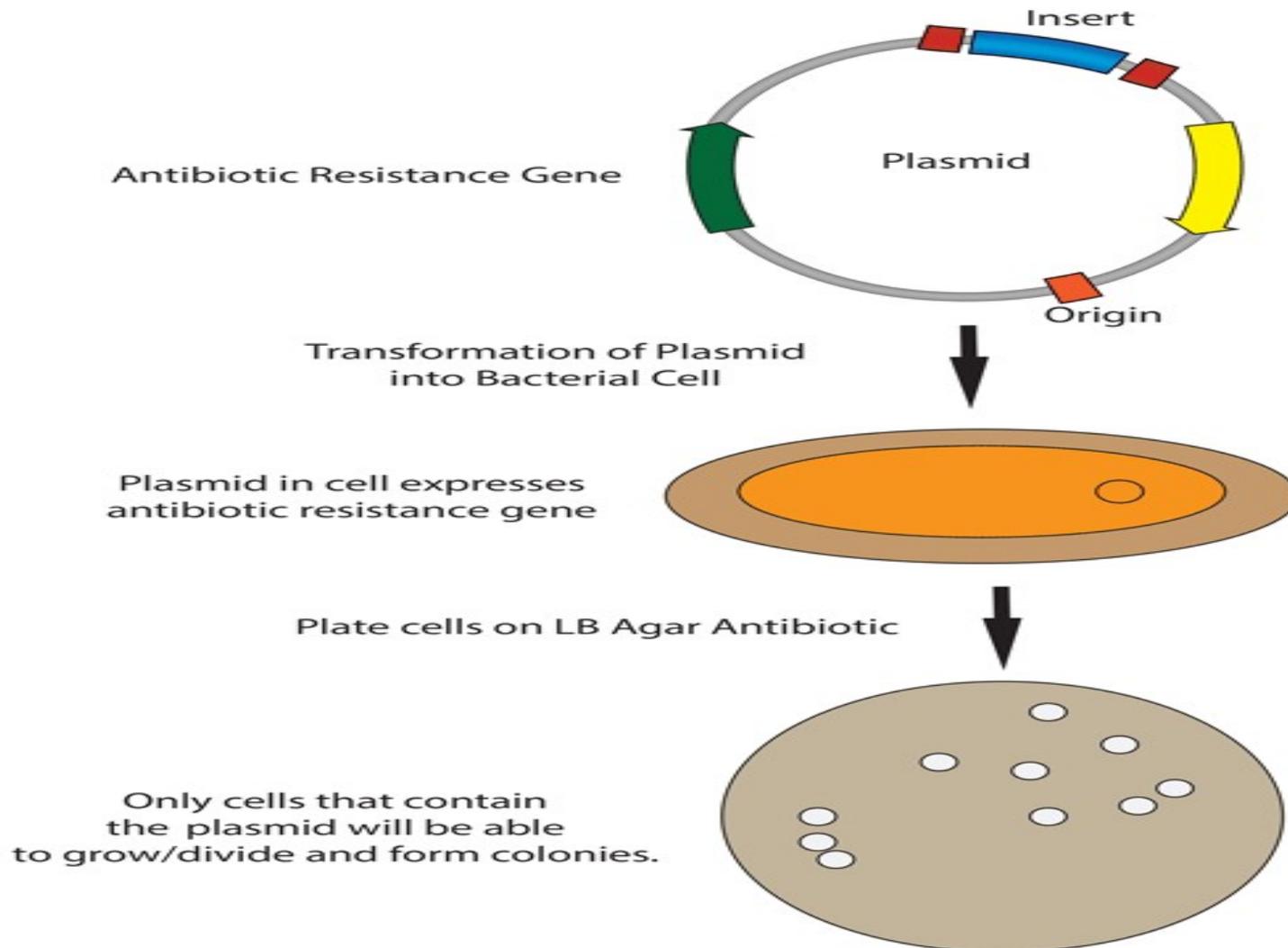


Transformation Protocol

- 5 μL of the ligation to 50 μL of competent cells (BL21 DE3) which have been defrost on ice or on the hand palms.
- Keep on ice for 20 min
- Heat shock at 42°C for 45 seg.
- Put on ice and plate on LB agar prewarmed plates at 37°C (with ampicillin $100\ \mu\text{g}/\mu\text{l}$).



Transformation



Induction of Recombinant Protein Expression

- An overnight culture of *E. coli* BL21 (DE3) is used to inoculated fresh LB-Amp medium (1:50 dilution).
- Incubate at 37° C with shaking (220 rpm) for 2 h (until OD₆₀₀ between 0.5-0.6).
- To induce protein expression, IPTG is added to a final concentration of between 1 and 0.1 mM and incubated at 37 ° C with shaking (220 rpm) for 3 h.

System of Expression T7

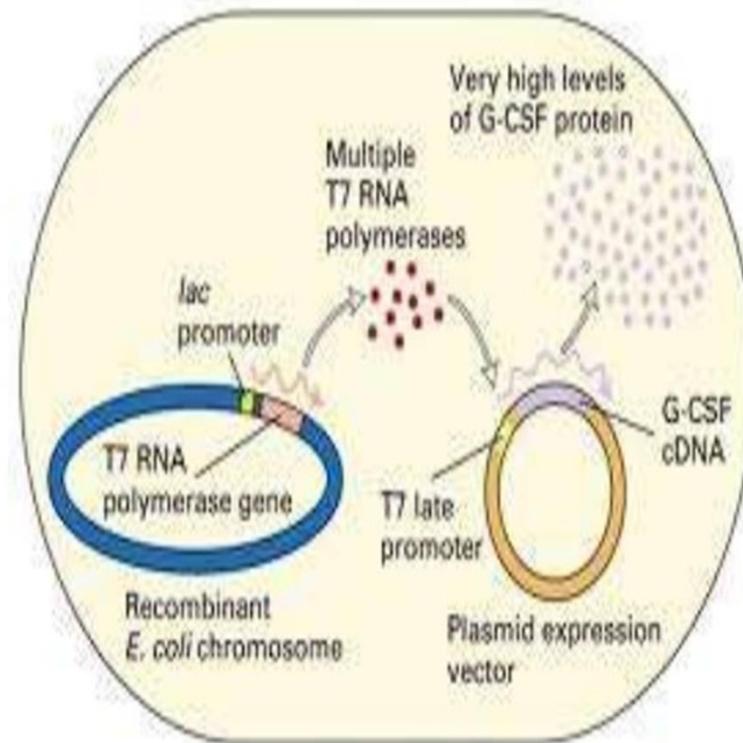
pLATE expression vectors use elements from bacteriophage T7 to control expression of heterologous genes in *E. coli*.

The expression of the gene of interest is driven by a strong bacteriophage T7 promoter that is specifically recognized by T7 RNA polymerase.

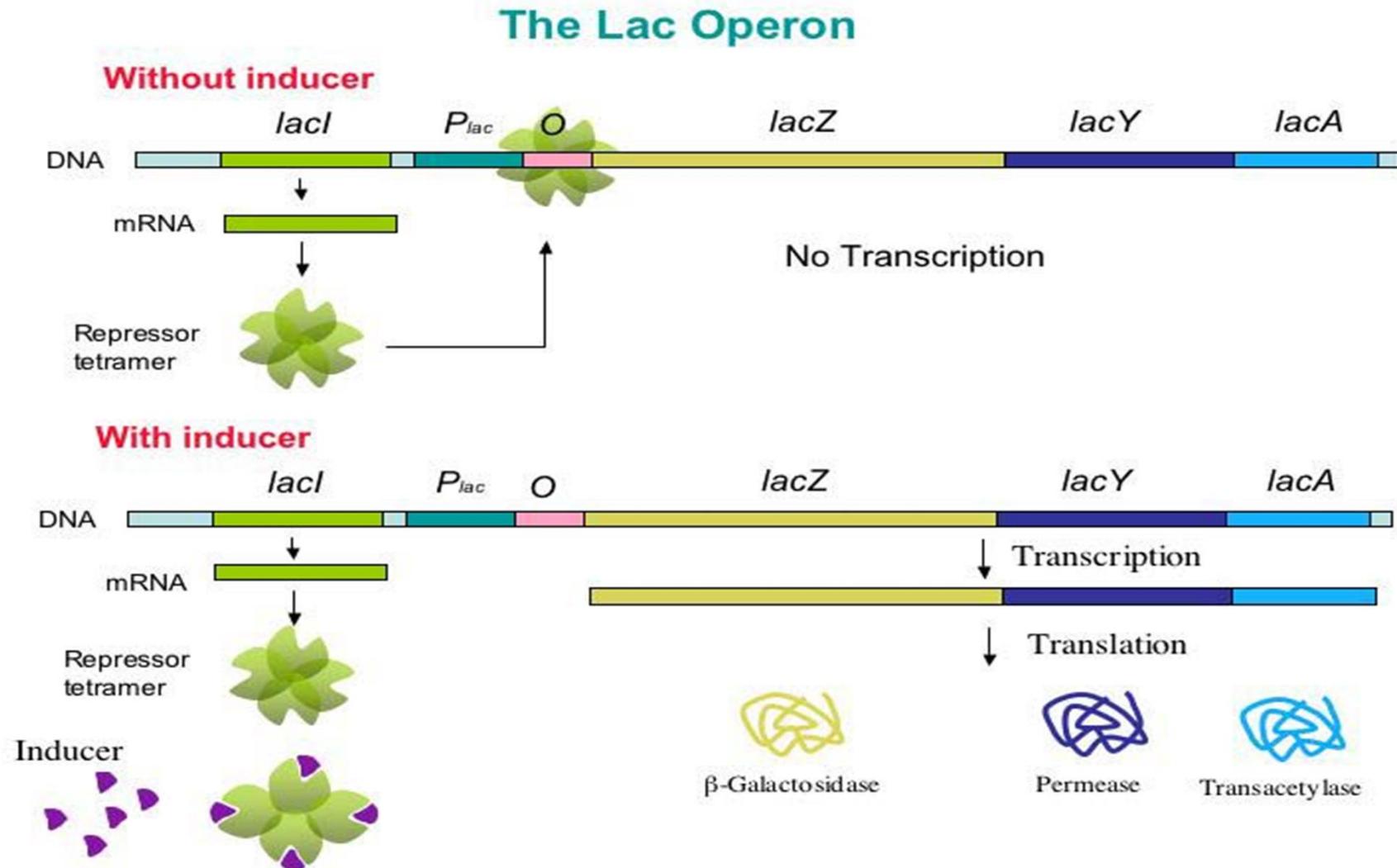
To express the gene of interest, *E. coli* strains such as BL21 (DE3), HMS 174 (DE3) must be used, in which expression of T7 RNA polymerase gene is under the control of an inducible promoter such as lacUV5.

After IPTG induction, the T7 RNA polymerase is expressed within the cell, and begins transcription of genes under the T7 promoter.

The basal expression level of pLATE vectors is very low; therefore it is not necessary to use lysozyme for T7 polymerase repression.



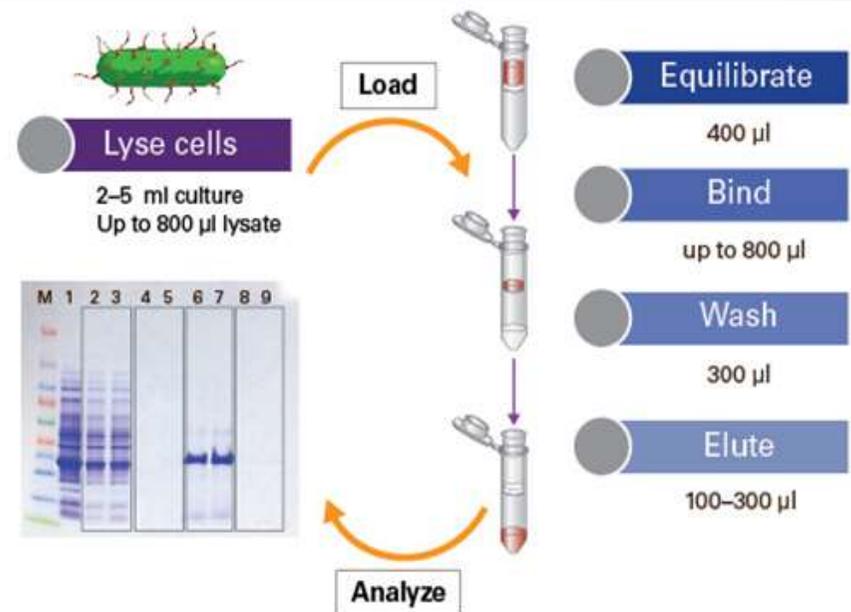
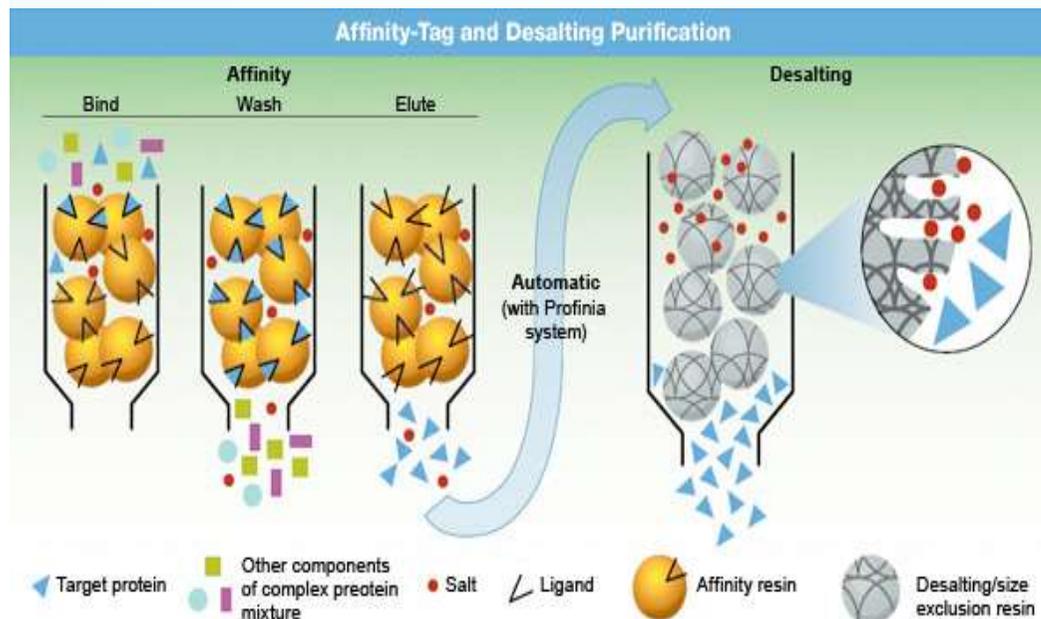
Induction Mechanism by IPTG



Purification of Viral Capsid Protein

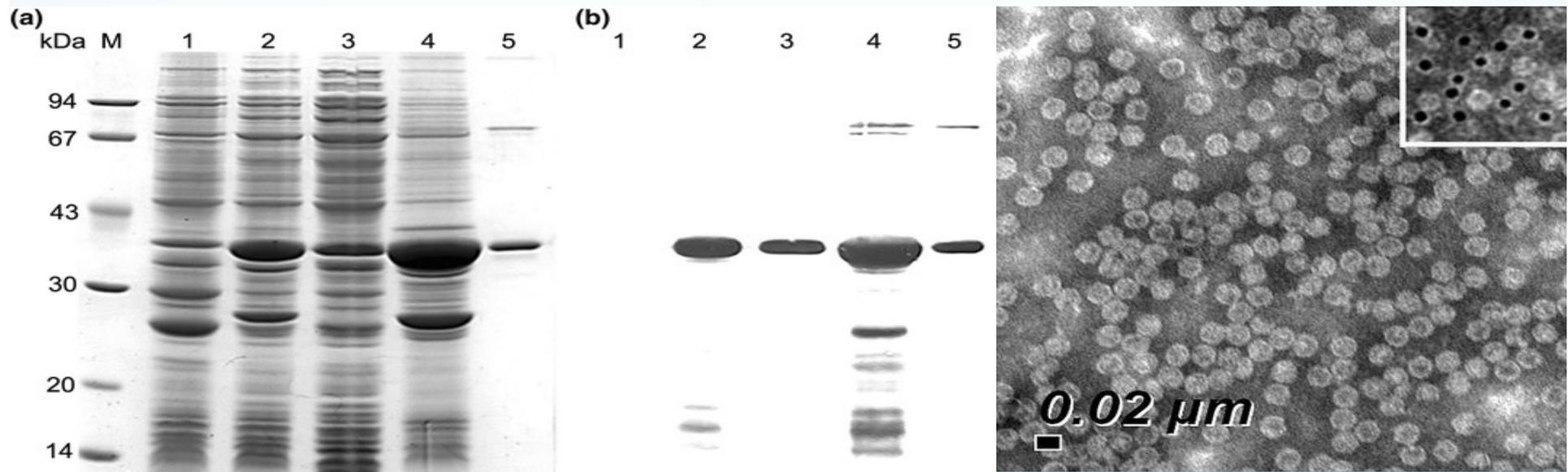
Affinity Chromatography

His-Spin Protein Miniprep (Zymo Research)

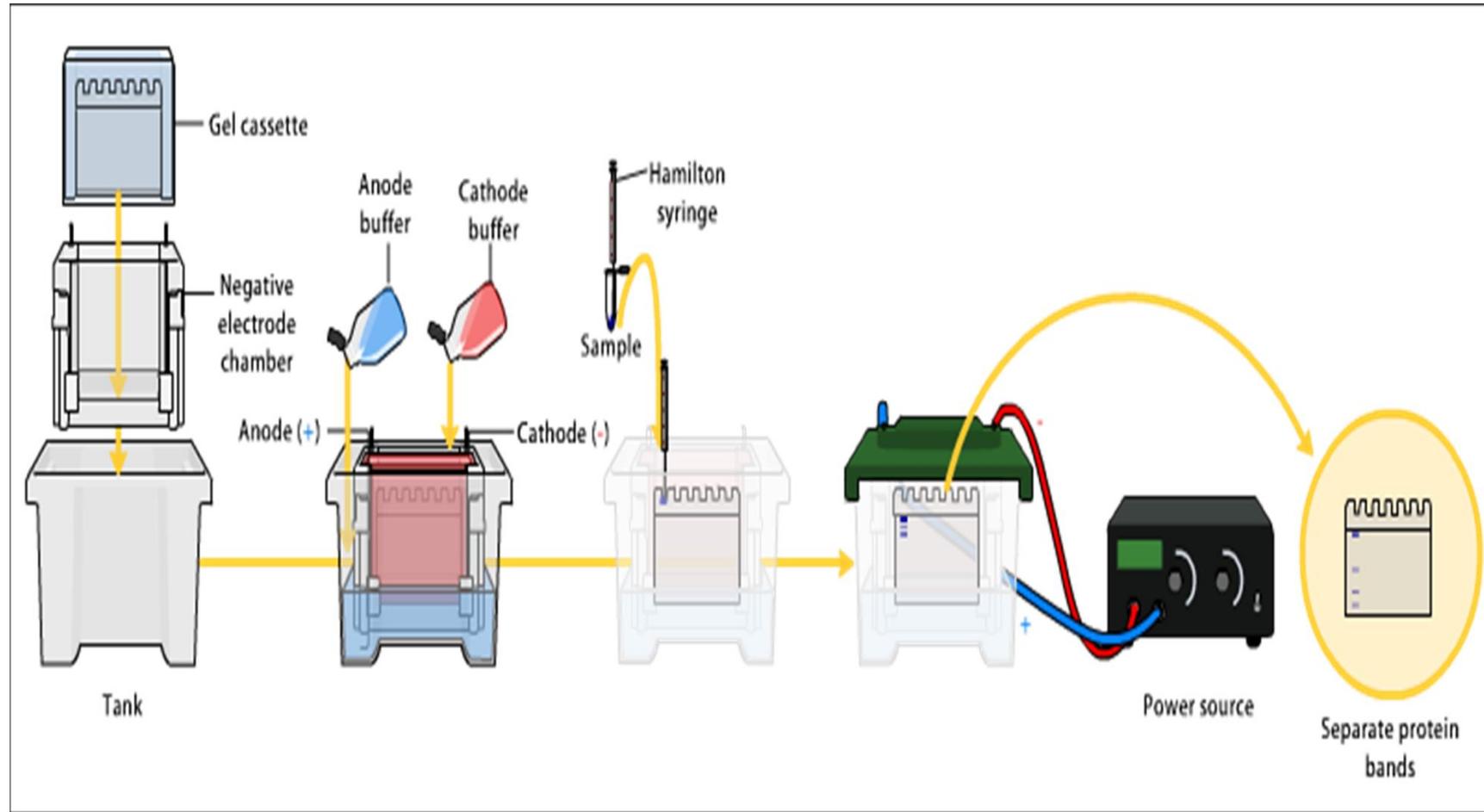


Verification of the Presence of the Recombinant Protein and the Correct Assembly of the VLPs

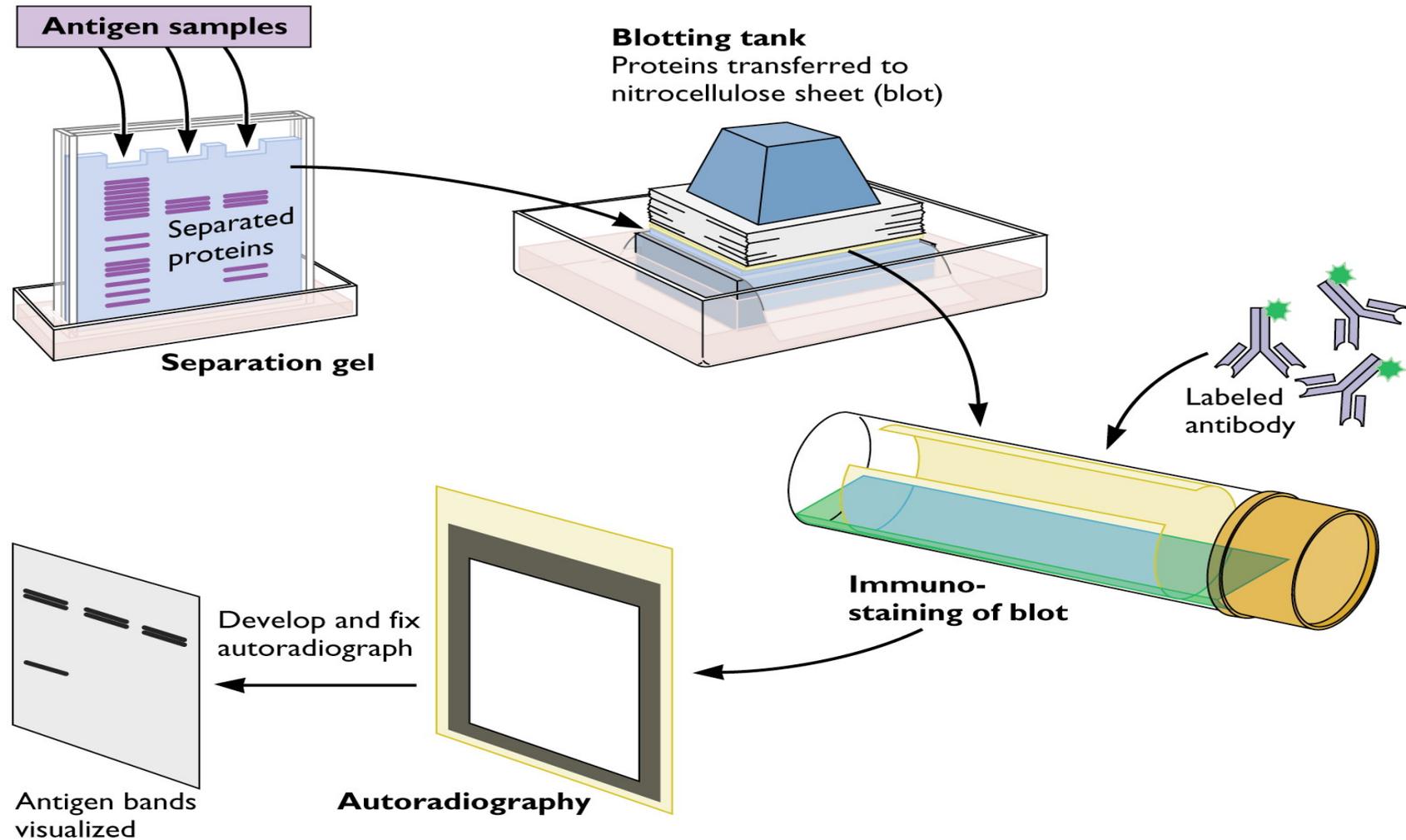
- SDS-PAGE, Western Blot
- Electronic microscopy (TEM)



SDS PAGE



Western Blot



Other Applications

VLPs have great potential for a number of applications in addition to vaccines.

The treatment of many hereditary and acquired genetic disorders by different **gene therapy tools** is one of the most important challenges that are being addressed through the progress of nanotechnology.

- **Nanocontainers** (icosahedral, filamentous, and lipid-enveloped virus-like vectors are the most promising gene carrier).

Other Applications

- The current success of gene therapy is largely based on viral vectors and synthetic liposomes, although both have a number of limitations, such as restricted packaging capacity, production difficulties, and undesirable immunological properties.
- However, due to their construction flexibility, VLPs can be engineered to overcome these disadvantages, which include the use of chemical modifications to reduce unwanted immunological responses after repeated use.

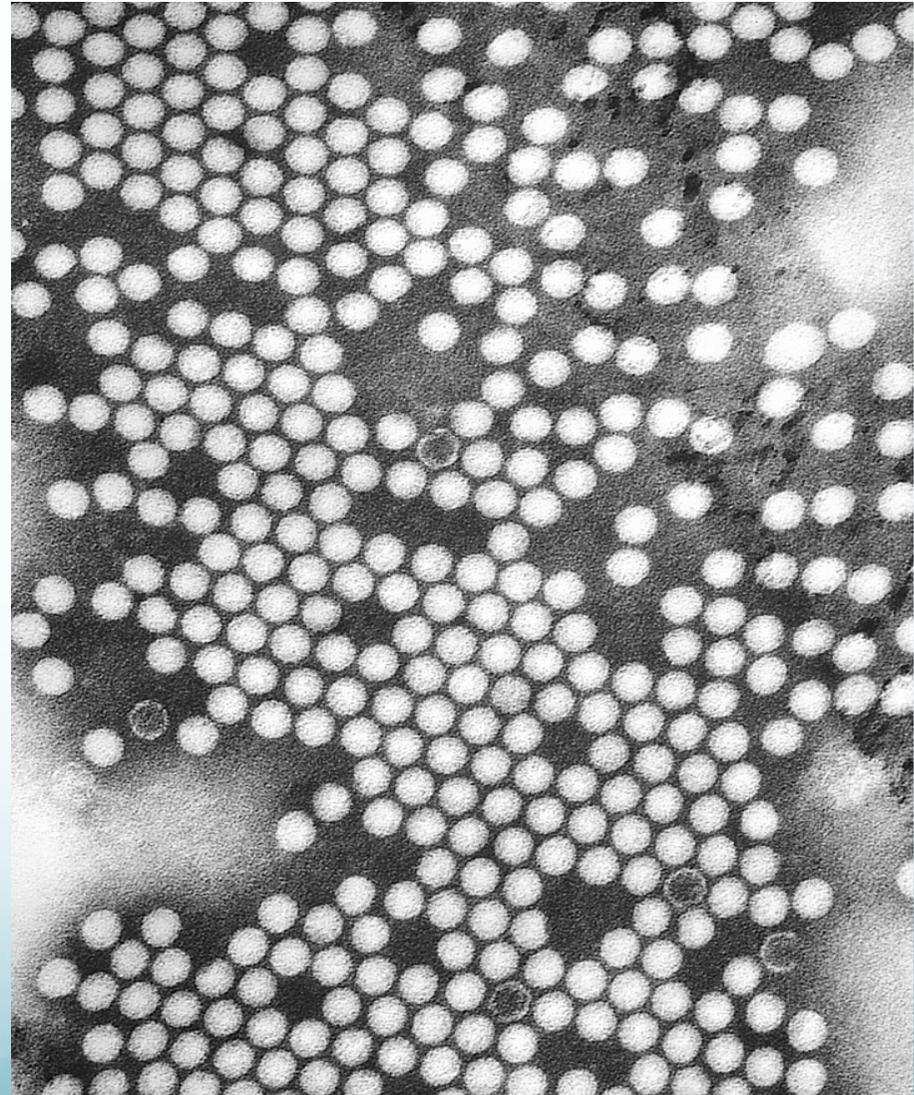
Other Applications

- Particular interest has recently been paid to the development of new **nanomaterials** that are composed of different viruses and VLPs.
- This new area of biotechnology, which is called **bionanotechnology**, represents a broad multidisciplinary approach that employs concepts and methodologies from biology, chemistry, physics, and engineering.

Other Applications

As new nanomaterials, viruses and VLPs possess several advantages over the products that are produced by chemical synthesis,

- Size, which ranges from 10 to 2000 nm.
- Availability of high-resolution 3D models of their structure.
- Construction flexibility through chemical and molecular biological techniques.
- High- production yields in natural and/or heterologous hosts.
- Structural uniformity



Bibliography

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Thanks



Erasmus+

L'UTILITÀ DEL NON-ESSERE

Trenta raggi si uniscono in un solo mozzo
e nel suo non-essere si ha l'utilità del carro,
s'impasta l'argilla per fare un vaso
e nel suo non-essere si ha l'utilità del vaso,
s'aprono porte e finestre per fare una casa
e nel suo non-essere si ha l'utilità della casa.
Perciò l'essere costituisce l'oggetto
e il non-essere costituisce l'utilità.

Lao Tzu
Tao Te Ching