# Capsid p24 protein - HIV-1

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## 1.0 INTRODUCTION

Human immunodeficiency virus (HIV) is a virus that targets immune system cells, making an individual more susceptible to various illnesses and infections. It is shared through sharing injection equipment or through direct contact with the bodily fluids of an infected individual, most frequently during unprotected sex (sex without the use of a condom or HIV medication to prevent or treat HIV).

Moreover, HIV cannot be eliminated by the human body, and there is no HIV treatment that works. Therefore, if you have HIV, you will always have it. Antiretroviral therapy, or ART, an effective HIV treatment option, is fortunately available. HIV medications have the ability to significantly lower the viral load, another name for the amount of HIV in the blood, if taken as directed. The term for this is viral suppression. A person is said to have an undetected viral load if their viral load is so low that a typical lab is unable to detect it. People with HIV can live long, healthy lives and will not spread the virus through intercourse to their HIV-negative partners if they take their HIV medications as directed and achieve and maintain an undetectable viral load.

Additionally, there are effective ways to avoid contracting HIV through sex or drug use, such as pre-exposure prophylaxis (PrEP), a drug that people who are at risk for contracting HIV take to avoid contracting HIV through sex or injecting drugs, and post-exposure prophylaxis

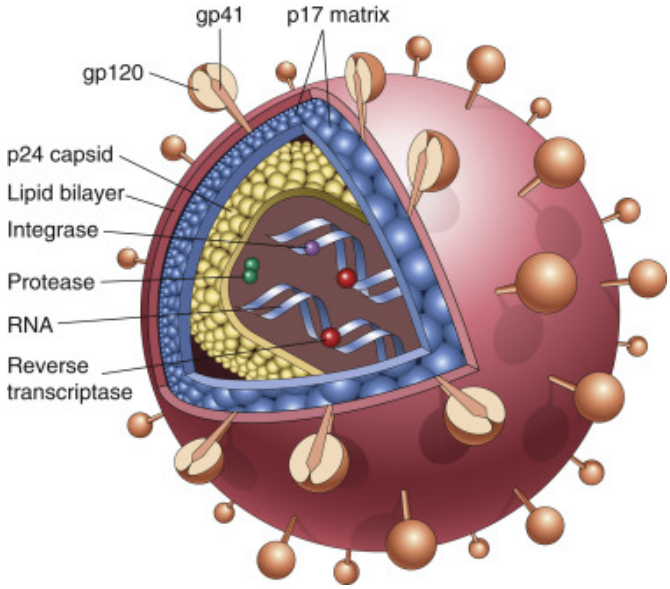
(PEP), a drug that HIV-positive people must take within 72 hours of a potential exposure to stop the virus from establishing a foothold.

## 2.0 SEQUENCE OF THE DNA

**Sequence :** HIV-1 is divided into three major groups: M, N, and O, which can be up to 40% divergent. The M group is subdivided into subtypes A-K5, and inter-subtype recombination has been shown to be very common in geographic regions where more than one subtype is prevalent.
**Gene symbol :** HTATSF1
**Gene description :** The HIV genome is made up of nine genes that code for fifteen viral proteins. These are synthesized as polyproteins that produce proteins for the virion interior known as Gag, group specific antigen; viral enzymes (Pol, polymerase); or virion env glycoproteins (envelope).

| | Sequence (5′→3′) |
|---|---|
| | AAATCTCTAGCAGTGGCGCCCGAACAG |
| | GCGGAGGCTAGAAGGAGAGAGATGG |
| | AGAAATTGCAGGGCCCCTAGGAA |
| | AGANCAGAGCCAACAGCCCCACCA |
| | CCTCAAATCACTCTTTGGCARCGAC |
| | AGGACCTACRCCTGTCAACATAATTGG |
| | TTTCCCCACTAACTTCTGTATGTCATTGACA |
| | GAATCTCTCTGTTTTCTGCCAGTTC |
| | AGARGAYAGATGGAACAAGCCCCAG |
| | TGGAAGCATCCRGGAAGTCAGCCT |
| | CTCTCATTGCCACTGTCTTCTGCTC |
| | GGTACCCCATAATAGACTGTRACCCACAA |
| | GTGTGTAGTTCTGCCAATCAGGGAA |
| | GCACTCAAGGCAAGCTTTATTGAGGCTT |

## 3.0 SIZE OF THE DNA OR PROTEIN OF INTEREST




Capsid protein (CA), or P24 antigen of human immunodeficiency virus type 1 (HIV-1) has the most abundant viral protein, each of the virus contain around 1500 to 3000 p24 molecules and weighing around 24 kDa which is about 10^4 virus per picogram

During the virus particular assembly, the capsid domain interacts with gag polyprotein and undergoes proteolytic cleavage, then transforms immature particles to mature virions. Upon mature virions, around 1500 p24 monomers assemble into a lattice p24 hexamers and pentamers package the RNA genome and other proteins.

The size of the capsid protein (p24) is approximately 24 kDa(kilodaltons) , weighing about 24,000 daltons. The capsid protein (p24) has a diameter of around 120 nm (nanometers). The capsid, often known as the virus's outer shell, is composed of several copies of the p24 protein. The p24 protein is composed of 228 amino acids. With around 25% of the total viral protein, the p24 protein is one of the most prevalent proteins in HIV-1 virions. Additionally, it is the first protein to be seen in the blood of an infected person, making it a crucial indicator of HIV-1 infection.

## 4.0 DISEASE RELATED TO THE GENE OR PROTEIN

Acquired immunodeficiency syndrome (AIDS) is a chronic, potentially life-threatening condition caused by the human immunodeficiency virus (HIV). By damaging your immune system, HIV interferes with your body's ability to fight infection and disease. Moreover, AIDS is a late stage of HIV infection that develops when the immune system of the body has been severely compromised by the virus. When a person with HIV has progressed to AIDS, their CD4 cell count falls below 200 cells per cubic millimeter of blood (200 cells/mm3). (CD4 counts in people with a healthy immune system range between 500 and 1,600 cells/mm3.) Alternatively, regardless of CD4 count, they develop one or more opportunistic infections. Without HIV medicine, people with AIDS typically survive about 3 years. Once someone has a dangerous opportunistic illness, life expectancy without treatment falls to about 1 year. HIV medicine can still help people at this stage of HIV infection, and it can even be lifesaving. But people who start HIV medicine soon after they get HIV experience more benefits.

Primary infection (Acute HIV) symptoms are within 2 to 4 weeks of the virus entering the body; some HIV-infected people develop a flu-like illness. This condition, known as primary (acute) HIV infection, can last for several weeks. Possible signs and symptoms include fever, headache, muscle aches and joint pain, rash, sore throat and painful mouth sores, swollen lymph glands, diarrhea, weight loss, cough and night sweats. These symptoms can be so mild that you might not even notice them. However, the amount of virus in your bloodstream (viral load) is quite high at this time. As a result, the infection spreads more easily during primary infection than during the next stage.

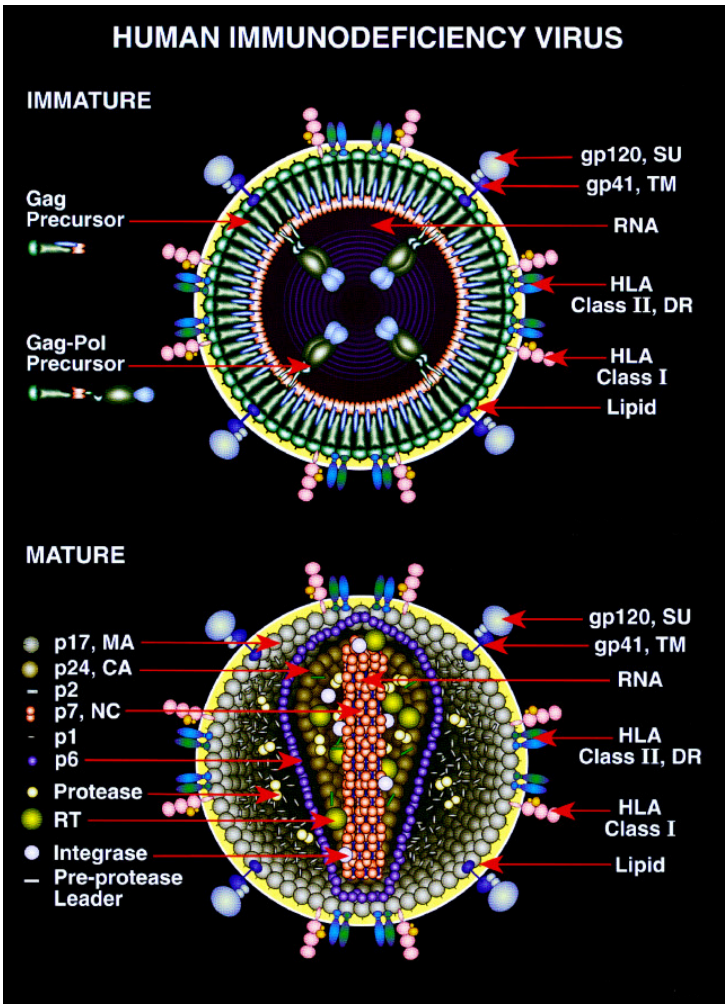
Furthermore, clinical latent infection (Chronic HIV) is when HIV remains in the body and in white blood cells. However many people may not experience any symptoms or infections during this time. If you are receiving antiretroviral therapy (ART), this stage can last for many years. Some people experience much more severe disease much sooner.

Moreover, symptomatic HIV infection when the virus continues to multiply and destroy your immune cells which are the cells in your body that help fight off germs. You may develop mild infections or chronic signs and symptoms such as fever, fatigue, swollen lymph nodes, diarrhea, weight loss, oral yeast infection (thrush), shingles (herpes zoster) and pneumonia.

Besides, progression to AIDS when your immune system is severely weakened. You'll be more susceptible to diseases that would not normally cause illness in someone with a healthy immune system. These are known as opportunistic infections or cancers. The signs and symptoms of some of these infections may include sweats, chills, recurring fever, chronic diarrhea, swollen lymph glands, persistent white spots or unusual lesions on your tongue or in your mouth, unexplained fatigue, weakness, weight loss and skin rashes or bumps.

## 5.0 ROLE OF THE GENE OR PROTEIN OF INTEREST IN THE PATHOGENESIS

Reverse transcriptase and other enzymes are used by the RNA virus HIV to change its genome from RNA to chromosomally integrated proviral DNA. Its viral core includes three viral enzymes (protease, reverse transcriptase, and integrase), two copies of the viral RNA, the main capsid protein p24, and nucleocapsid proteins. The viral capsid protein p24 is crucial to HIV pathogenesis.



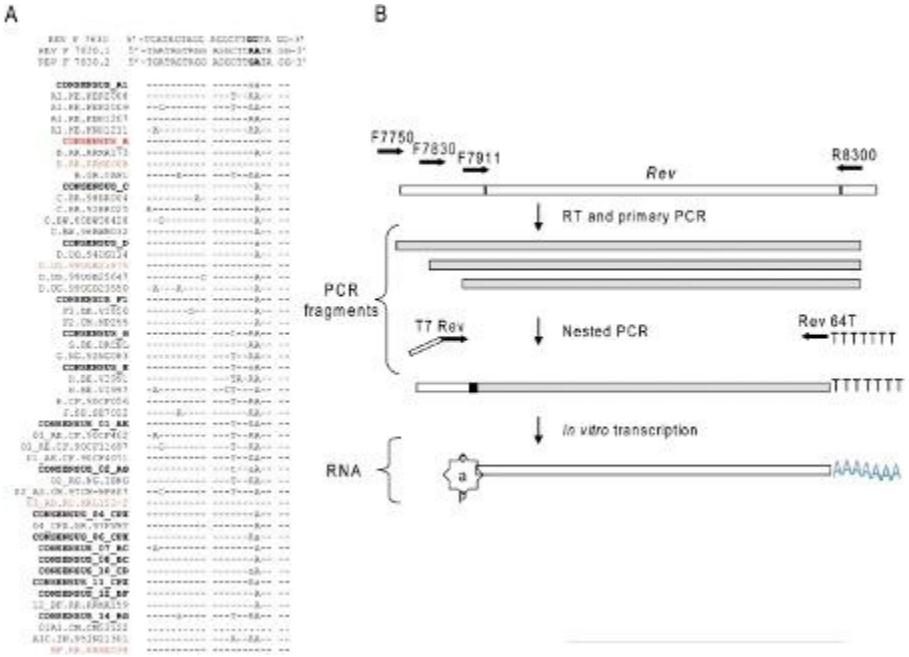

HIV destroys CD4 cells commonly known as T cells or helper cells which are essential to the immune system. The body is protected from common diseases and infections by CD4 cells. HIV affects the cells that usually defend against invaders. In the process of replicating, the virus destroys the infected CD4 cell and produces more viruses to infect other CD4 cells. The body's

immune system will eventually be overpowered by this, leaving it completely vulnerable to disease. Once the immune system has been severely weakened, then he or she may be diagnosed with AIDS.

## 6.0 CLONING METHODS EMPLOYED

### 6.1 PCR amplification of the gene of interest and plasmid vector

In molecular biology, PCR amplification, also known as polymerase chain reaction amplification, is a common method for amplifying and producing several copies of a target section of DNA. In the case of HIV-1 (Capsid p24 protein),Multiplex RT-PCR Amplification from blood tests are the most common way to diagnose the human immunodeficiency virus (HIV), the virus that causes acquired immunodeficiency syndrome (AIDS). The test should be carried out in a series of steps.

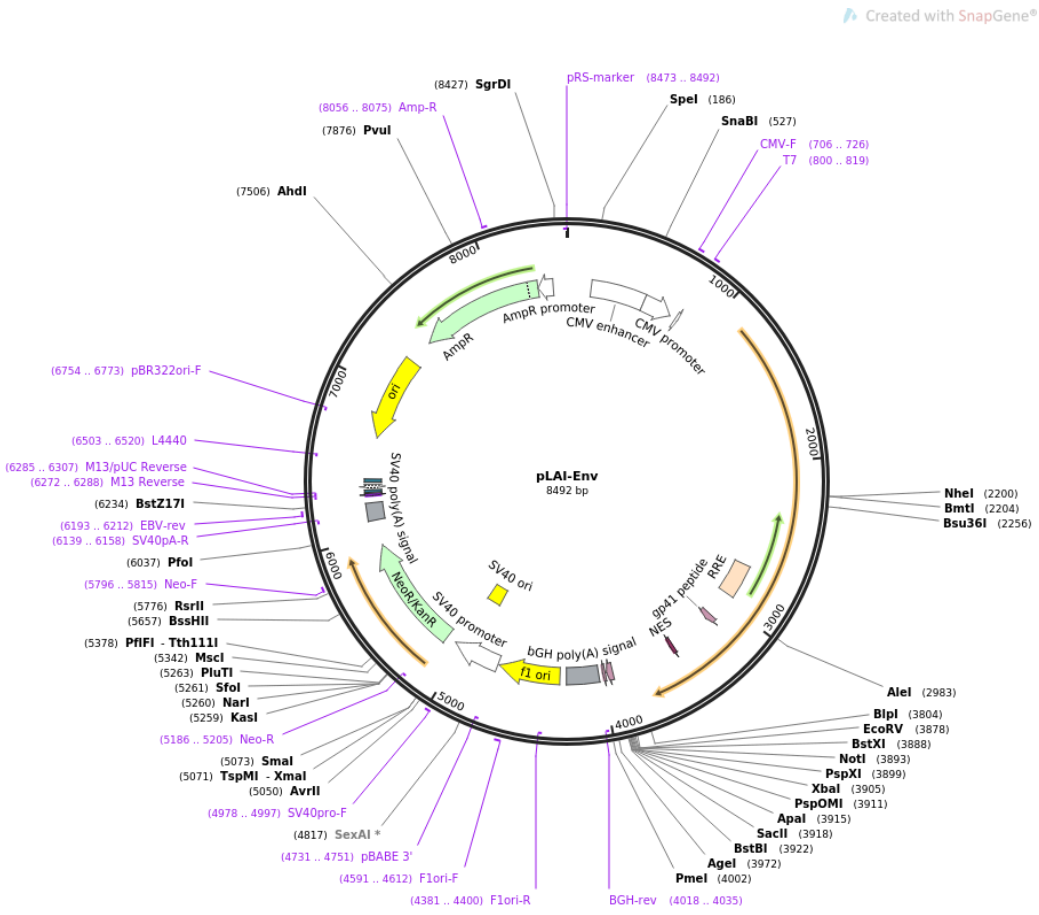



Three procedures are carried out during PCR amplification which is denaturation, annealing, and extension. Denaturing is done by heating the DNA strands. Denaturation of double-stranded DNA of the template into single strands using the heat at least 94°C (201.2°F)

will separate the double stranded DNA causing the original DNA sample's hydrogen bonds to dissolve.Next annealing is process when DNA strands cooled between 50 to 60°C (122 to 140°F) allowing the DNA primers and the DNA polymerase enzyme to bind to the individual strands of DNA that were separated by the heat. Last process is an extension. Extension forms a new complementary strand of DNA. New duplicate double-stranded DNA molecule has been formed from each of the single strands of the original sample molecule. The temperature cycles from 95°C to 50 to 60°C. The cycle is then repeated about 35 to 40 times using the thermal cycle which automatically repeats the denaturation and annealing cycles of the process.This three-step process is done 35 to 40 times to amplify the DNA or RNA into millions of duplicate segments.

**Plasmid vector**

The chosen plasmid vector is pLAI-Env. The target sequence is recognised by the restriction enzyme and cut into fragments. Following this, PCR is amplified and proceeds via denaturation, annealing, and extension.



**6.2 Purification of the DNA**

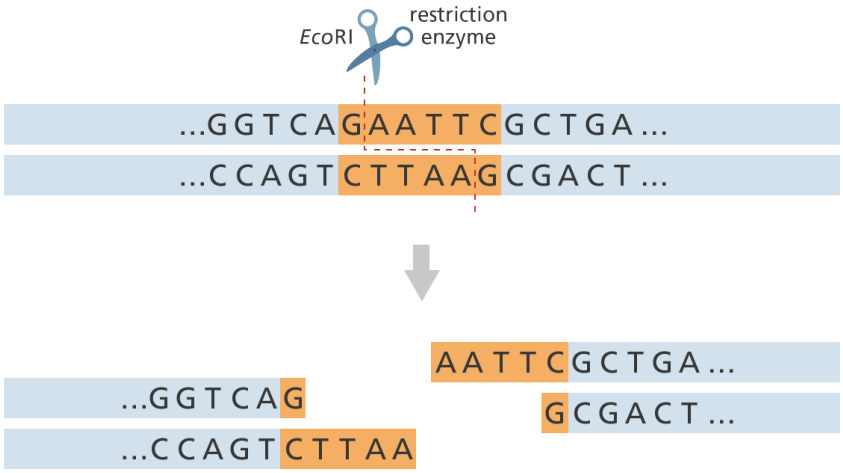
DNA purification is a process of isolating DNA samples from cells and impurities. There are two general procedures to purify the DNA which are centrifugation and chemical extraction. For HIV-1, we will be using the EasyPure HiPure Plasmid MiniPrep Kit to extract and purify the DNA. Before started we will be needed six buffer which are Resuspension Buffer (RB), Lysis Buffer (LB), Neutralization Buffer (NB), ToxinOut Buffer (TB), Wash Buffer (WB), Elution Buffer (EB) and 10 mg/ml of RNase A. Firstly, add 5 ml overnight bacterial culture to a microcentrifuge tube and centrifuge at 10 000×g for 1 minute and discard the supernatant. Then, add 250 µl of RB that has been mixed with RNase A to the cell pellet and resuspend it completely by pipetting. After that, add 250 µl of LB, immediately mix gently by inverting the tube 4-6 times. In this step, the color of the lysate should change from opaque to bright blue. Then, add 350 µl of NB, mix gently by inverting the tube 5-6 times. In this step, the color of the lysate will turn into yellow when the neutralization is completed and a yellowish precipitate will be formed. Incubate the lysate at room temperature for 2 minutes.

Then, centrifuge at 12 000xg for 5 minutes, gently transfer the supernatant to a spin column. Centrifuge at 12 000xg for 1 minute and discard the flow-through. After that, add 250 µl TB, incubate at room temperature for 10 minutes. Centrifuge at 12 000xg for 1 minute and discard the flow-through. Add 650 µl of WB to the column to check to make sure that ethanol has been added. Centrifuge at 12 000xg for 1 minute. Centrifuge the column at 12 000xg for 1-2 minutes to remove residual WB completely. Place the spin column in a clean microcentrifuge tube, add 30-50 µl of EB or sterile, distilled water directly to the center of the column matrix. Finally, centrifuge the column at 10 000xg for 1 minute to elute DNA. Isolated plasmid DNA is ready to use or can be stored at -20℃.

**6.3 Cutting up the DNA**

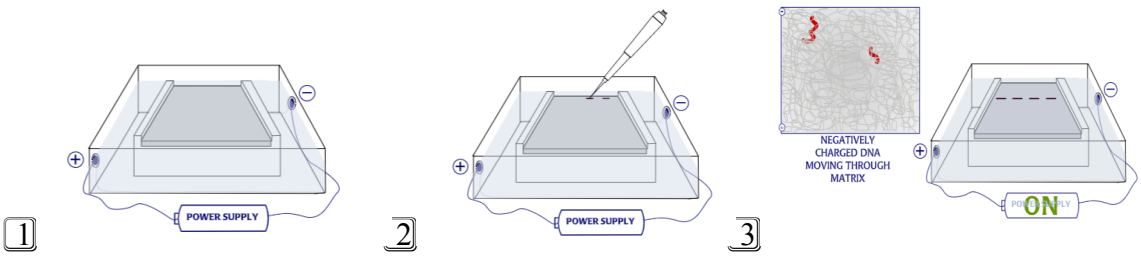
In genetic engineering, only a fragment of a gene is needed. Therefore, a restriction enzyme must be used to cut the DNA into manageable size. Restriction enzyme is an endonuclease found in bacteria that recognizes palindromic sequences and cuts both strands of DNA at the recognition site to produce blunt end or sticky end. In HIV-1, the restriction enzyme, EcoRI, is used as a restriction enzyme. EcoRI recognizes the palindromic code GAATTC and

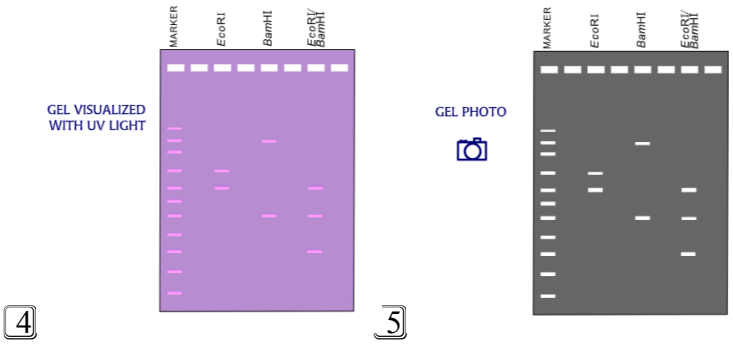
cuts the DNA strands between the base A and G. This results in two DNA fragments with their sticky ends with the base sequence "AATT" or 'TTAA'.




### 6.4 Separation of the DNA

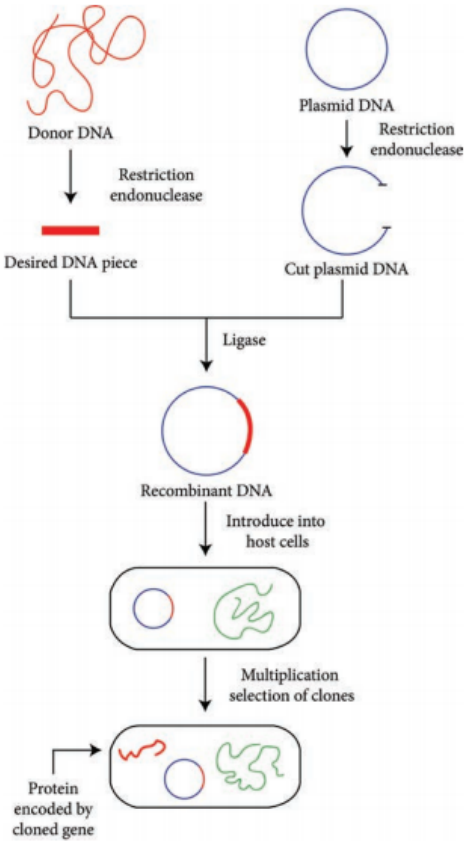
After cutting up a long piece of DNA, the pieces need to be separated from one another by using gel electrophoresis. Electrophoresis is a migration of charged molecules such as amino acids, proteins and fragments of DNA through a gel by using an electric field. Agarose gel is submersed in a tank filled with a buffer that conducts electricity. Using a pipette, DNA samples are loaded into slots made in the agarose gel. The DNA samples are colorless, therefore, a fluorescent dye, ethidium bromide can be added. This makes it easier to load the samples and visually track the DNA migration through the gel. The fluorescent dye binds tightly to the DNA double helix and glows when illuminated with ultraviolet light. The phosphate groups in the DNA backbone carry negative-charged oxygen. This gives a DNA molecule an overall negative charge. In an electric current, the negatively-charged DNA moves from the negative pole toward the positive pole of the electrophoresis chamber. The DNA molecules move through the pores of the agarose matrix. Smaller DNA fragments migrate faster and further over a given period of time than the larger fragments do. This is how DNA fragments can be separated by size in an agarose gel. The size of the DNA is then determined by comparing it to the marker DNA fragments of known sizes.






### 6.5 DNA Ligation (Cloning into plasmid vector)

DNA cloning is a process to produce multiple identical copies of a specific gene or DNA fragment that usually involves plasmid as vector and bacteria as host cell. In DNA cloning, a DNA fragment of interest is first inserted into a circular piece of DNA called a plasmid and then produces recombinant DNA.



Basic principles of recombinant DNA technology

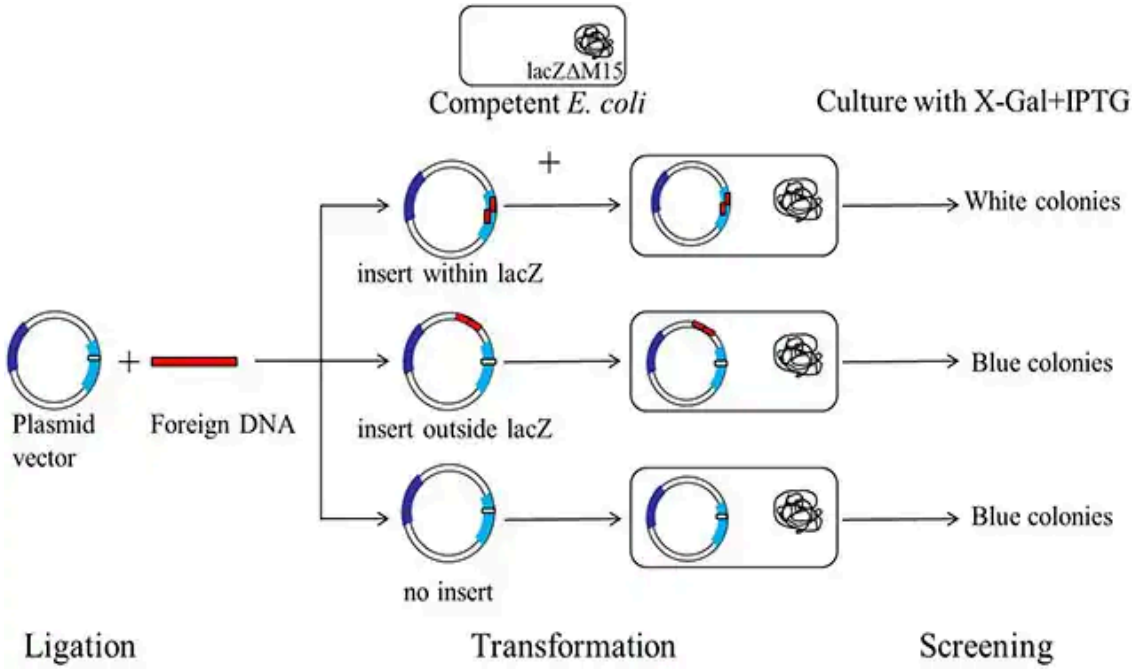
Firstly, isolation of target DNA and plasmid vectors. The restriction enzyme cuts the DNA of HIV-1 at the specific target sequences to develop a specific cohesive end which is the sticky end. The plasmid is also cut with the same restriction enzyme so that the cohesive end will be complemented with the cohesive end of the DNA. Insertion of DNA fragments into plasmid. Next, the isolated and amplified DNA will be placed into a vector in order to create recombinant DNA. In order to do this, ligation is needed. Ligation is the process of joining two different DNA strands together. DNA ligase makes the bond permanent by attaching restriction fragments and DNA vectors to each other with phosphodiester bonds. Thus, both plasmid vector and target gene, Capsid protein will join together and form a recombinant plasmid.

Next, in the transformation process, a recombinant plasmid is transformed into the competent host cell. Host cell that uptakes the plasmid DNA is known as a transformed cell. The host cell will be given heat shock to help the host cell uptake of HIV-1 DNA. After the heat shock, the host cell is incubated at 37℃ to let it recover and allow the production of antibiotic

resistance genes. The host cell that uptakes the recombinant DNA then grows colonies into the desired DNA.

### 6.6 Selection for the correct clones and verification

Using a color screening method, the plasmid pLAI-Env in E. coli can be identified and verified to present. The pLAI-Env plasmid, which initially contains the lacZ gene, will eventually produce *B-galactosidase*. Start with a plasmid containing the lacZ gene for *B-galactosidase* and add a polylinker very close to the start of the lacZ coding sequence. You can obtain an active enzyme as long as the polylinker is inserted without destroying the reading frame. Later, this enzyme would convert X-gal into a coloured product, turning the colony blue. The EcoR1 restriction enzyme is then used to cleave the plasmid and the DNA of the Capsid p24 protein-HIV-1 virus, and the DNA is then inserted into the plasmid. Due to a lost piece during the restriction, the sequence of the lacZ gene is not entirely present at this time. Since the plasmid has amp-resistance, the lacZ gene is inactivated, which prevents the production of *B-galactosidase,* and the inserted Capsid p24 protein-HIV-1 DNA, it is now referred to as recombinant DNA. Last but not least, recombinant DNA is disseminated on an agar plate containing X-gal. If *B-galactosidase* isn't functioning, the sole colony that appears on the X-gal agar is white. If the colony turns blue, the attempt at cloning fails; however, if the colony turns white, it is successful. And now we can selectively use the white colonies and place them on a different plate and work with them since we know that our DNA insert should contain the pLAI-Env plasmid.



## 7.0 CONCLUSION

The prevalence of HIV is widespread. Despite new developments in the fight against this virus, numerous new infections occur annually. Some of the symptoms include fatigue, fever, swollen lymph nodes — often one of the first signs of HIV infection, weight loss, diarrhea and pneumonia. You might not even be aware of these symptoms because they can be so minor. However, the amount of virus in your bloodstream (viral load) is fairly high at this point. Thus, everyone should be aware of how it spreads and how to prevent it. All are urged to get tested for HIV right away if engaging in any risky activities. Additionally, you should educate people on the value of prevention and testing, such as choosing less risky sexual behavior, use condoms every time you have sex, get tested and treated for STDs and talk to your health care provider about pre-exposure prophylaxis (PrEP). There is no treatment for this infection, thus ignorance of the issue is not a solution.

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