Lentivirus and Lentiviral Vectors

Family: *Retroviridae*
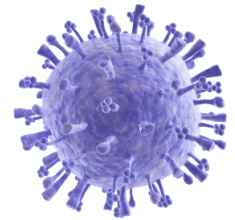*Genus: Lentivirus*
Enveloped
Size: ~ 80 - 120 nm in diameter
Genome: Two copies of positive-sense ssRNA inside a conical capsid
Risk Group: 2

Lentivirus Characteristics

Lentivirus (*lente*-, latin for "slow") is a group of retroviruses characterized for a long incubation period. They are classified into five serogroups according to the vertebrate hosts they infect: bovine, equine, feline, ovine/caprine and primate. Some examples of lentiviruses are Human (HIV), Simian (SIV) and Feline (FIV) Immunodeficiency Viruses.

Lentiviruses can deliver large amounts of genetic information into the DNA of host cells and can integrate in both dividing and non-dividing cells. The viral genome is passed onto daughter cells during division, making it one of the most efficient gene delivery vectors.
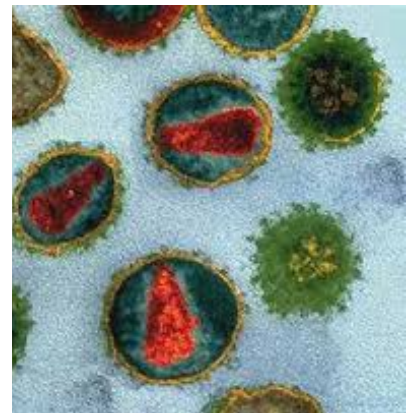
Most lentiviral vectors are based on the Human Immunodeficiency Virus (HIV), which will be used as a model of lentiviral vector in this fact sheet.

Structure of the HIV Virus
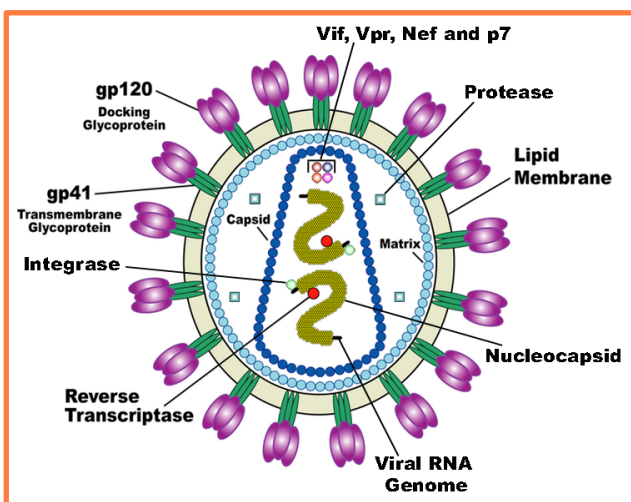
The structure of HIV is different from that of other retroviruses. HIV is roughly spherical with a diameter of ~120 nm. HIV is composed of two copies of positive ssRNA that code for nine genes enclosed by a conical capsid containing 2,000 copies of the p24 protein. The ssRNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion: reverse transcriptase (RT), proteases (PR), ribonuclease and integrase (IN). A matrix composed of p17 surrounds the capsid ensuring the integrity of the virion. This, in turn, is surrounded by an envelope composed of two layers of phospholipids taken from the membrane of a human cell when a newly formed virus particle buds from the cell. Embedded in the viral envelope are proteins from the host cell and about 70 copies of a complex HIV protein, known as Env, that protrudes through the surface of the virus particle. Env consists of a cap made of three gp120 molecules, and a stem consisting of three gp41 molecules that anchor the structure into the viral envelope. The

glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle.

Genome Organization of the HIV-1 Virus
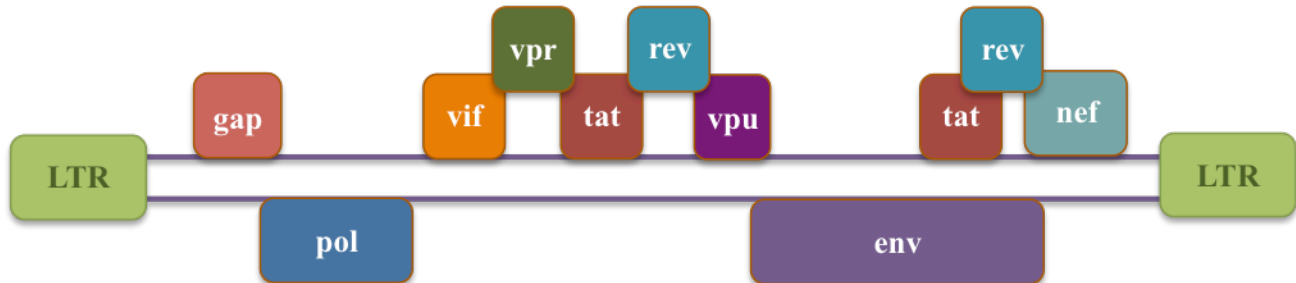
The HIV-1 genome contains 9,749 bp. In addition to the **gap**, **pol**, and **env** genes common to all retroviruses, HIV-1 contains:

❖ Two regulatory genes - **tat** and **rev** - indispensable for virus replication, and
❖ Four accessory genes - **vif**, **vpr**, **vpu** and **nef** - that, while dispensable for *in vitro* virus growth, are key for *in vivo* replication and pathogenesis.

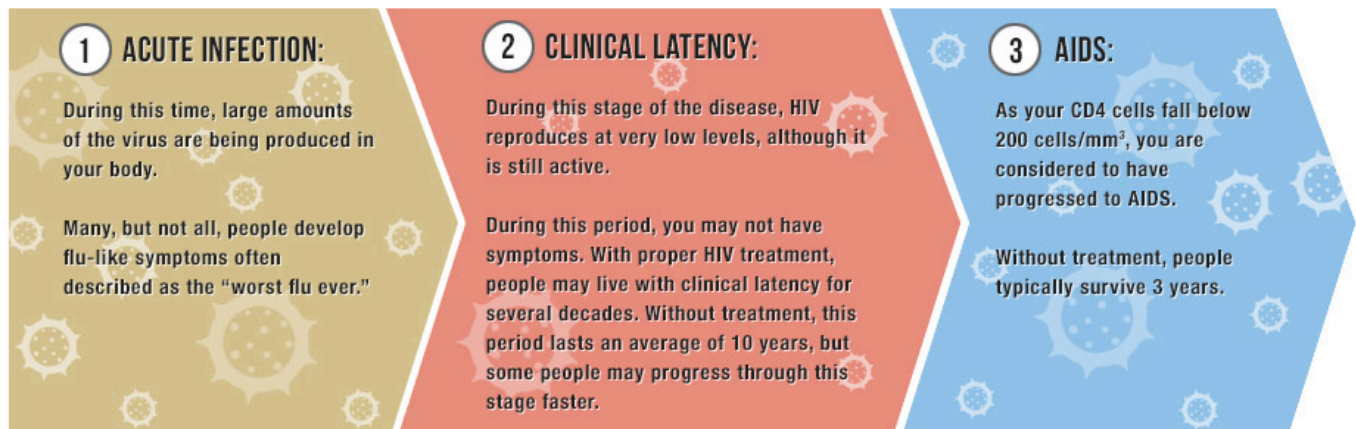|		GENE	PRECURSOR PROTEINS → PRODUCTS
Essential Genes & Regulatory Elements	gag	**Group-specific antigen**	**gag → MA, CA, SP1, NC, SP2, P6**
	pol	**Polymerase**	**pol → RT, RNase H, IN, PR**
	env	**Envelope**	**gp160 → gp120, gp41**
	tat	**HIV Transactivator**	**Positive regulator of transcription**
	rev	**Regulator of expression of virion proteins**	**Important for synthesis of major viral proteins and essential for viral replication**
Accessory Genes	vif	**Viral infectivity**	**Required for infectivity in some cell types**
	vpr	**Virus protein R**	**Nuclear import of pre-integration complex and host cell cycle arrest**
	vpu	**Virus protein U**	**Proteasomal degradation of CD44 and release of virions from infected cells**
	nef	**Negative factor**	**Role in apoptosis and key in increasing virus infectivity**

Life Cycle of the HIV Virus
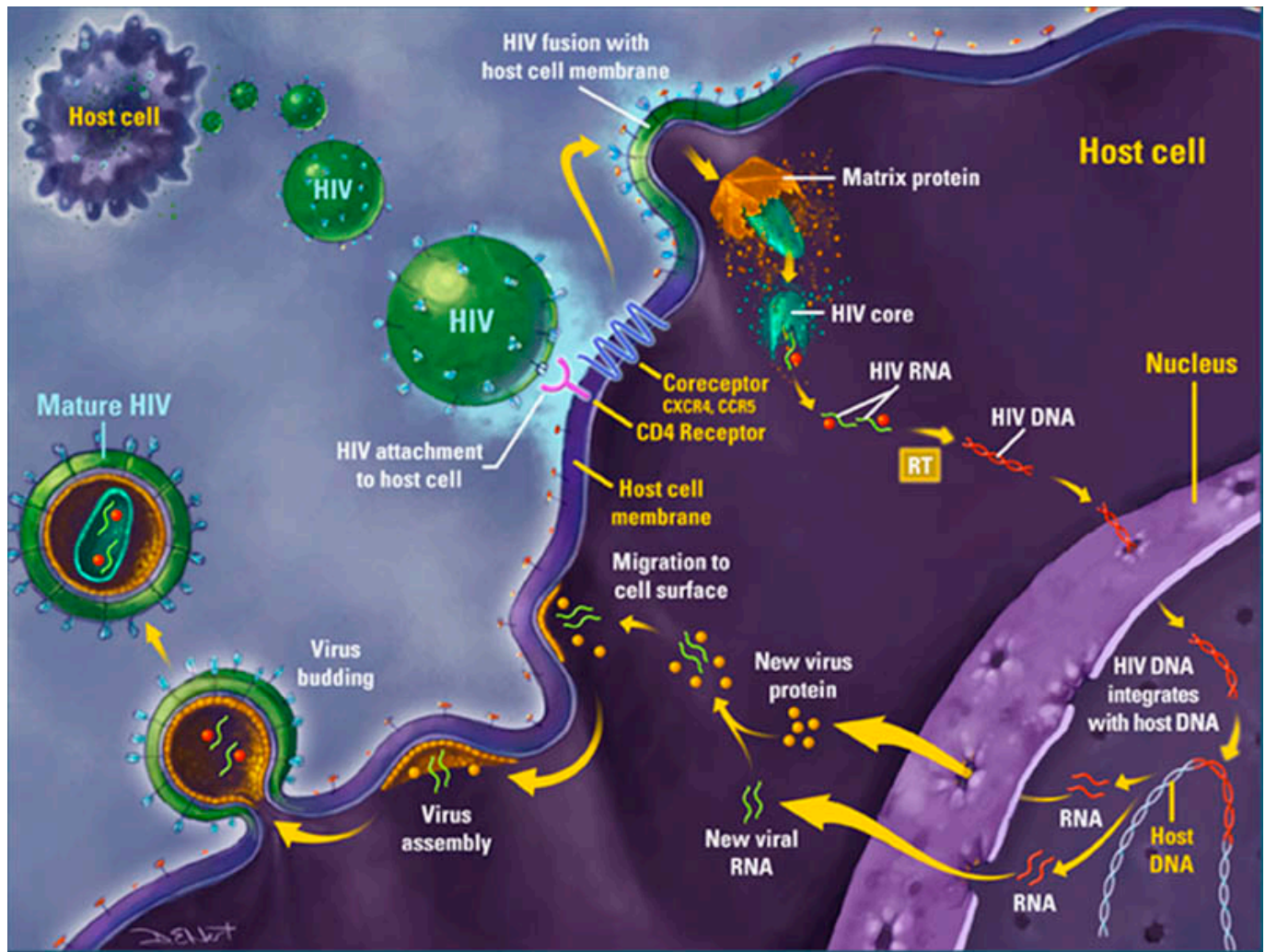
The life cycle of HIV begins with viral entry, a multi-step interaction between the HIV envelope and the host target cell surface receptors. In the initial step of entry, the HIV gp120 protein binds to the host target cell CD4 receptor, thereby anchoring HIV to the host cell. This interaction generates a conformational change in the HIV envelope that stimulates HIV binding with a host cell co-receptor; the main co-receptors used by HIV are CCR5 and CXCR4. Subsequently, the viral and host membranes fuse, the viral capsid enters the cell, and the HIV core dissolves releasing the two copies of single-stranded HIV RNA. The next step, reverse transcription, involves the conversion of the HIV ssRNA to double-stranded DNA (dsDNA) by the HIV enzyme reverse transcriptase (RT). The RT uses the cellular nucleotides as the building blocks for synthesizing HIV DNA. Next, the HIV DNA complexed with other HIV proteins migrates inside the host nucleus. The HIV integrase (IN) enzyme then catalyzes the integration of the HIV DNA into the host DNA. Once the HIV DNA has integrated into the host genome, it is referred to as proviral DNA. The HIV provirus remains part of the host DNA and is perceived by the cell as normal host cellular DNA. The cellular enzymes transcribe the proviral DNA into messenger RNA (mRNA) and genomic RNA. The control of the transcription of proviral DNA involves multiple factors, including the HIV Tat protein and cellular modulators. The viral mRNA then is exported out of the nucleus into the host cell cytoplasm where cellular enzymes translate the

viral mRNA into viral proteins. The larger viral proteins require cleaving into smaller, functional proteins, a step performed by the HIV enzyme protease (PR). The multiple components of the HIV are then assembled and, as the HIV buds off from the cell, further processing occurs to complete the viral life cycle, with the final product consisting of a mature HIV virion capable of infecting other cells.

Routes of Transmission of HIV

HIV is primarily transmitted through direct contact of mucous membranes and non-intact skin with the virus. Percutaneous exposure (e.g., needlesticks, sharps injuries) is an important route of exposure in clinical and research settings.

Stages of HIV Infection

1 ACUTE INFECTION:
During this time, large amounts of the virus are being produced in your body.

Many, but not all, people develop flu-like symptoms often described as the "worst flu ever."

2 CLINICAL LATENCY:
During this stage of the disease, HIV reproduces at very low levels, although it is still active.

During this period, you may not have symptoms. With proper HIV treatment, people may live with clinical latency for several decades. Without treatment, this period lasts an average of 10 years, but some people may progress through this stage faster.

3 AIDS:
As your CD4 cells fall below 200 cells/mm³, you are considered to have progressed to AIDS.

Without treatment, people typically survive 3 years.

The symptoms of **acute infection** are usually non-specific. Some individuals develop flu-like symptoms approximately two to four weeks after infection, while others are asymptomatic. During this period, HIV replicates rapidly destroying numerous CD4+ cells until reaching a *viral set point* - a relatively stable level of HIV virus in the body.

During the **clinical latency** stage, the virus continues to reproduce at very low levels - reason why this stage is also known as "asymptomatic or chronic HIV infection".

In a final stage, individuals progress from a chronic HIV infection to full-blown **AIDS** (Acquired Immunodeficiency Syndrome). AIDS patients are susceptible to a wide range of opportunistic infections and HIV-related cancers because their immune systems are seriously damaged. A CD4+ count lower than 200 cells/mm^3, or the development of one or more opportunistic infections, is indicative of AIDS in HIV+ individuals.

Cell Tropism of HIV and Pseudotyping

HIV can infect a wide variety of human immune cells like CD4+ T-cells, macrophages and microglial cells. Viral entry into target cells occurs through interaction between HIV's gp120 and the CD4 molecules and chemokine co-receptors (CCR5 or CXCR4) present in the surface of the host cells. The picture below shows HIV particles (pink) on a human cell (brown). Since CD4 is the major receptor for binding to the native HIV envelope glycoproteins, the tropism for lentiviral
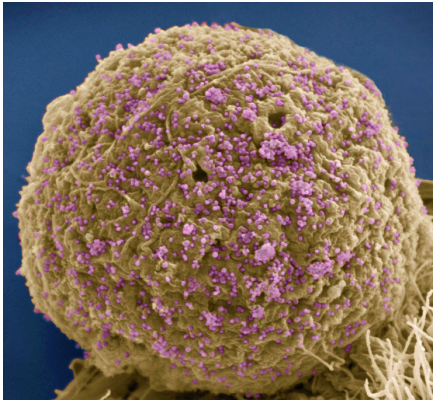
vectors is very restricted. In order to infect cells without CD4 expression, pseudotyping with other heterologous envelope proteins has been used.

The Vesicular Stomatitis Virus glycoprotein G (VSV-G), which allows gene transfer to a broad array of cell types and species, is frequently used for pseudotyping of lentiviral vectors. Though advantageous for research purposes, this poses an increased risk of infection in case of exposure to VSV-G-pseudotyped lentiviral vectors for lab workers, since these vectors will be able to target a larger range of cells.

For transfection of murine cells, lentiviral vectors can be pseudotyped with a murine ecotropic envelope, which would eliminate the exposure risk in humans.

Lentiviral Vector Construction

Lentiviruses have high mutation and recombination rates, so the likelihood that HIV could self-replicate and be produced during vector manufacturing by recombination is a serious safety concern. To reduce that probability:
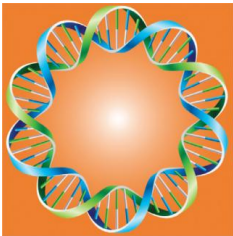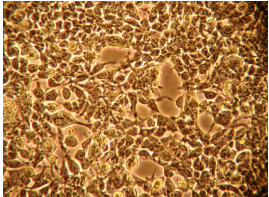
- ❖ Essential genes are separated into different plasmids, and
- ❖ The four viral accessory genes (vif, vpr, vpu and nef) are deleted.

That way, multiple recombination events would be necessary to reconstitute a replication-competent lentivirus (RCL).

Several components are essential to generate a lentiviral vector, including:

- ❖ A lentiviral backbone, a.k.a. transfer vector plasmid or lentiviral construct: with LTRs and the Packaging Signal Psi (Ψ)
- ❖ The transgene of interest: e.g., a cDNA, miRNA, or shRNA cloned into the backbone
- ❖ Helper plasmids: packaging and envelope plasmids, and
- ❖ A packaging cell line: "factory" in which the viral vector production takes place. The transfer vector with the transgene and helper plasmids are transiently transfected into a packaging cell line such as HEK-293 cells, where they get assembled.

The table below illustrates each of these components:

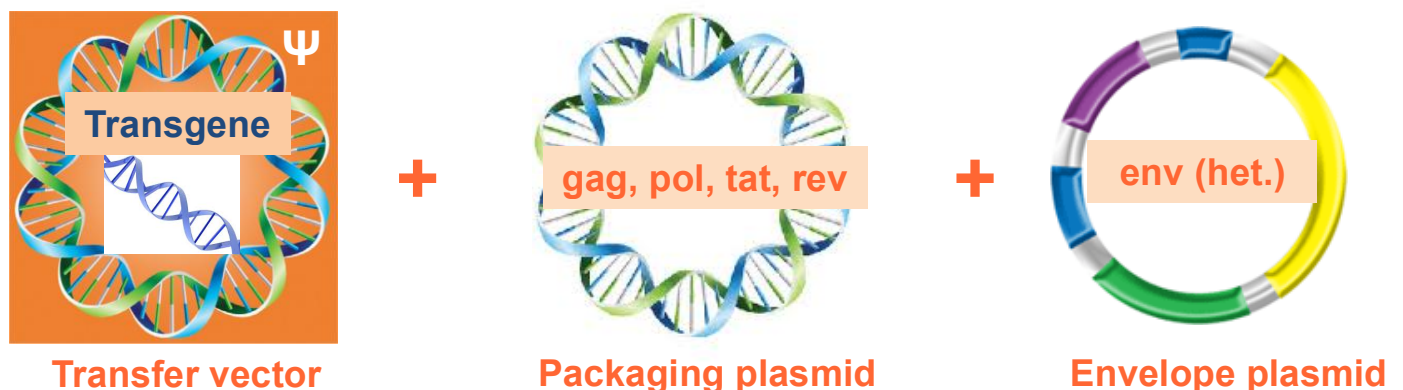| Lentiviral Backbone Transfer Vector Plasmid	Transgene	Helper Plasmids		Packaging Cell Line
	cDNA, shRNA	Packaging	Envelope	E.g., HEK-293 cells

Lentiviral Vector Generations

The use of lentiviral vectors for gene transfer is not exempt of risks, since the viral genomic material can integrate into the host DNA. This can disturb the function of host cell genes by leading to the repression or overexpression of such genes and insertional mutagenesis. As technology advanced and biosafety risks were discovered, newer generations of lentiviral vectors with enhanced safety features were designed. These generations are described in the next paragraphs, with the understanding that "the higher the generation, the safer the vector". In all three generations the envelope gene is usually heterologous, i.e., from a different virus, such as VSV-G (not an HIV gene).


Vesicular Stomatitis virus (VSV)
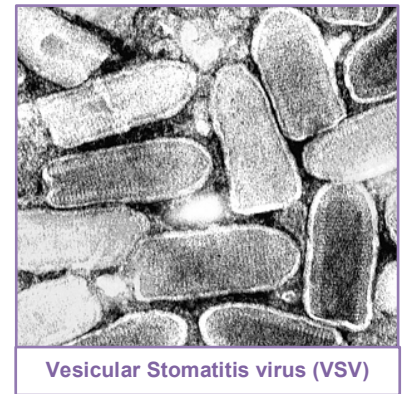
First-generation: includes a packaging system with all HIV genes except for the env gene (usually heterologous) that is included in another vector.

Transfer vector + **gag, pol, tat, rev & accessory genes** (Packaging plasmid) + **env (het.)** (Envelope plasmid)

Transfer vector   **Packaging plasmid**   **Envelope plasmid**

Second-generation: Researchers discovered that the four HIV accessory genes - vif, vpr, vpu and nef - were not required for HIV replication in immortalized cell lines. This led to the engineering of second-generation vectors. In this system, the four accessory genes were eliminated leaving the gag and pol reading frames and the tat and rev genes. In general, lentiviral vectors with a wild-type 5' LTR need the 2nd generation packaging system because they need tat for activation

Transfer vector + **gag, pol, tat, rev** (Packaging plasmid) + **env (het.)** (Envelope plasmid)

Transfer vector   **Packaging plasmid**   **Envelope plasmid**

Third-generation / Self-Inactivating (SIN): In a third-generation vector, the 3' LTR is modified, with tat being eliminated and rev provided in a separate plasmid. Since the HIV promoter in the 5' LTR depends on tat, a vector without tat needs to have its wild-type promoter replaced with a heterologous enhancer/promoter to ensure transcription. Such promoter could be either viral (like CMV) or cellular (like EF1-α).

Transfer vector + **Packaging plasmids** + **Envelope plasmid**

Transgene + gag, pol + rev + env (het.)
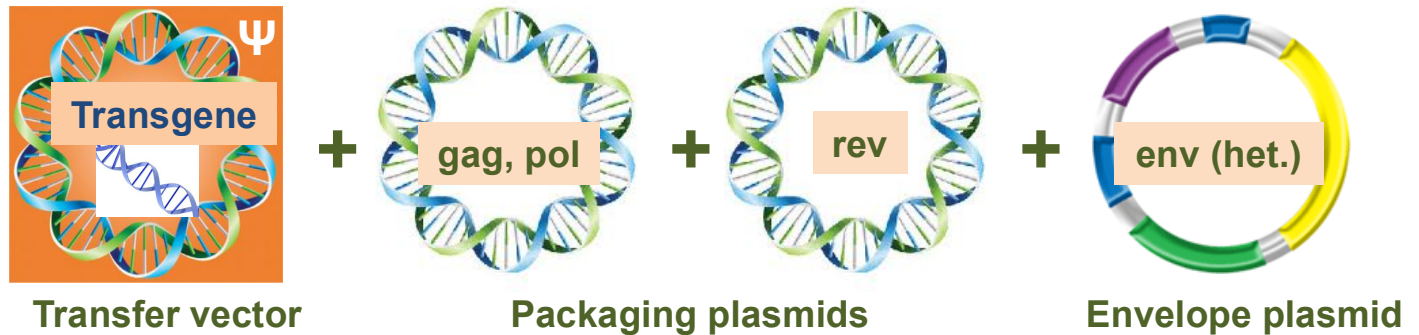
Lentiviral Vector Generations Summary Table

	First Generation	Second Generation	Third Generation
Plasmids	3	3	4
Deletion in 3' LTR - SIN	No	No	Yes
Packaging plasmids with HIV genes	1	1	2
Accessory genes: vif, vpr, vpu, nef	All absent	All absent	All absent
tat and rev genes	On a single packaging plasmid	On a single packaging plasmid	tat is absent; rev on a separate plasmid
gag and pol genes	Same plasmid	Same plasmid	Same plasmid
Recombination events needed to generate Replication Competent Lentiviruses (RCL)*	2 recombinations	3 recombinations	4 recombinations between plasmids without homology & must pick a promoter to complement SIN deletion

* The risk of formation of RCLs exists not only during lentiviral vector production, but also during experiments involving materials infected with wild-type HIV. Recombination between the lentiviral vector and HIV can lead to the generation of new viruses with unknown safety

consequences. For that reason, experiments involving human materials not screened for HIV pose an enhanced risk for laboratory workers.

Pros and Cons of Lentiviral Vectors

ADVANTAGES	DISADVANTAGES
Can carry large transgenes (up to 8 Kb)	Potential for generation of RCL
Efficient gene transfer	Potential for insertional mutagenesis: Even replication-incompetent lentiviruses with human tropism are able to infect human cells and integrate their genome into the host cells → risk in case of accidental exposure
Infects dividing and non-dividing cells	
No immunogenic proteins generated	
Stable integration into the host genome and stable expression of the transgene	

Use of Lentiviral Vectors in the Laboratory

Environmental Stability and Disinfection

Lentiviral vectors are highly susceptible to desiccating conditions due to the presence of a lipid envelope. Thus, in theory, lentiviral vectors can be inactivated with alcohol-based disinfectants like a 70% solution of isopropanol or ethanol. In practice, alcohols are not appropriate for disinfection of large quantities of viral vectors (e.g., a spill) because of the difficulties in achieving enough contact time due to alcohol evaporation. In addition, alcoholic disinfectants have limited efficacy against organic materials (e.g., cells, bovine serum) that may be present together with the lentiviral vectors.

2% Jodopax, sodium hypochlorite, iodine and phenolic disinfectants are the preferred methods of disinfection when working with lentiviral vectors.

Containment Level

Though the parent virus for most lentiviral vectors belongs to risk group 3 (e.g., HIV), BSL-2 or BSL-2+ practices are commonly used for such work. A final determination of the BSL level will be made based on a risk assessment.

Rodents treated with lentiviral vectors must be housed at ABSL2 conditions and cages marked with a goldenrod card stating the day, time, and dose of lentivirus administration. Animal care workers will not change the cages of treated animals for a period of 72 hours. Afterwards, all waste should be collected as biohazardous following appropriate procedures.

Routes of Exposure to Lentiviral Vectors in the Laboratory

Parenteral exposure poses the highest risk of infection in the laboratory when working with lentiviral vectors. This usually occurs through needlesticks, cuts with sharps, contamination of non-intact skin, scratches… Failure to properly restrain a rodent while performing inoculations has a very high probability of resulting in worker self-inoculation.

Mucous membrane exposure with viral vector preparations during certain steps of the vector preparation process can also result in infection of the worker. The adequate use of biosafety cabinets and PPE can prevent this risk.

Post-Exposure Prophylaxis

Lentiviral vectors can infect the cells of a lab worker in case of accidental exposure. Though the vector may be replication-incompetent, it is still able to cause a one-time infection of the worker's cells and deliver its genetic contents into them. Insertion of the viral genetic material into the host cell's genes can result in mutations (insertional mutagenesis), even if the transgene is apparently innocuous (e.g., cell markers such as GFP). In addition, overexpression of transgenic oncogenes or repression of cellular tumor-suppressor genes can lead to the development of oncogenesis.

All Principals Investigators (PI) who work with lentiviral vectors must have an approved IBC protocol including an SOP for lentiviral work and how to respond in case of exposure. All lab workers must be properly trained on safe work with lentivirus and emergency response. The current guidelines for managing occupational exposure to HIV-based viral vectors relies on the use of antivirals targeting the pre-integration steps, in order to prevent insertional mutagenesis. Post-exposure prophylaxis (PEP) should be administered as soon as possible - within the first 4 hours after exposure. Other antivirals that inhibit the reverse transcriptase (RT) or HIV Integrase (IN) can be used as PEP.

Resources

- http://researchcompliance.uc.edu/training/adenovirus/story.html
- http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/hiv-vih-eng.php
- http://www.genetherapynet.com/viral-vectors/lentiviruses.html
- http://en.wikipedia.org/wiki/HIV
- http://depts.washington.edu/hivaids/arvrx/case2/discussion.html
- http://www.aids.gov/hiv-aids-basics/just-diagnosed-with-hiv-aids/hiv-in-your-body/stages-of-hiv/
- http://en.wikipedia.org/wiki/Insertional_mutagenesis