

# Viral vectors and gene therapy III

## Retroviral vectors

Retroviral vectors derived from Oncoviruses

Design and construction

Important aspects

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Recombination

Role of cell division

Promoter methylation

Formation of envelope pseudotypes

Retroviral vectors derived from Lentiviruses

Retroviral vectors derived from Foamy viruses

### General features of retroviruses

A unifying feature of these viruses is that replication involves the process of conversion of the viral RNA genome into double-stranded DNA, hence, the designation retro- (backward) viruses. Prior to the discovery of reverse transcriptase, the viral-coded enzyme that mediates synthesis of DNA from RNA, the central dogma held that RNA was transcribed from DNA but not the reverse.

The retroviral genome (ssRNA) is reverse transcribed to produce a linear dsDNA molecule, which is then permanently integrated into the host chromosome. When infected/transduced cells divide, the integrated retrovirus (named "provirus") will be vertically transmitted like normal, cellular gene. Thus, retroviral vectors offer the unique advantage of being able to transduce target cells in a permanent fashion.

### Retroviral vectors derived from Oncoviruses

#### Design of retroviral vectors

The structural genes of retroviral vectors are typically split into 3 constructs (see figure below).

Typically, the gag/pol and env constructs are transfected stably into packaging cells of murine origin. The transfer vector, containing the therapeutic gene, is then transfected into packaging cells, which are then selected for antibiotic resistance.

The transfer vector must contain the following cis acting elements:

- Primer binding site, PBS
- Poly-purine tract, PPU
- Long terminal repeats, LTR (viral promoter, poly (A) site, R repeat for replication/RT, integration site).
- RNA packaging signal,  $\Psi$

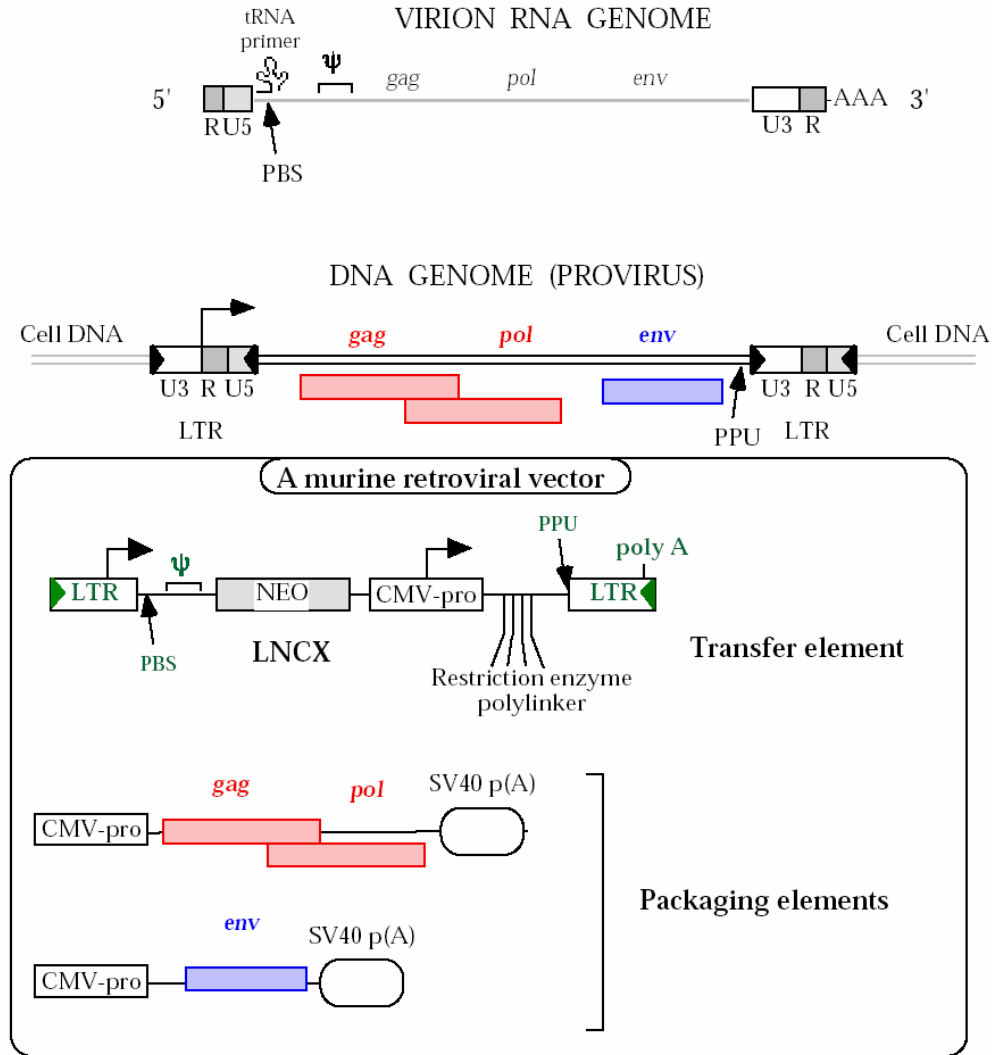
The transfer vector also needs to encode gene of interest, and a promoter (can use LTR or heterologous promoter, such as CMV or SV40).

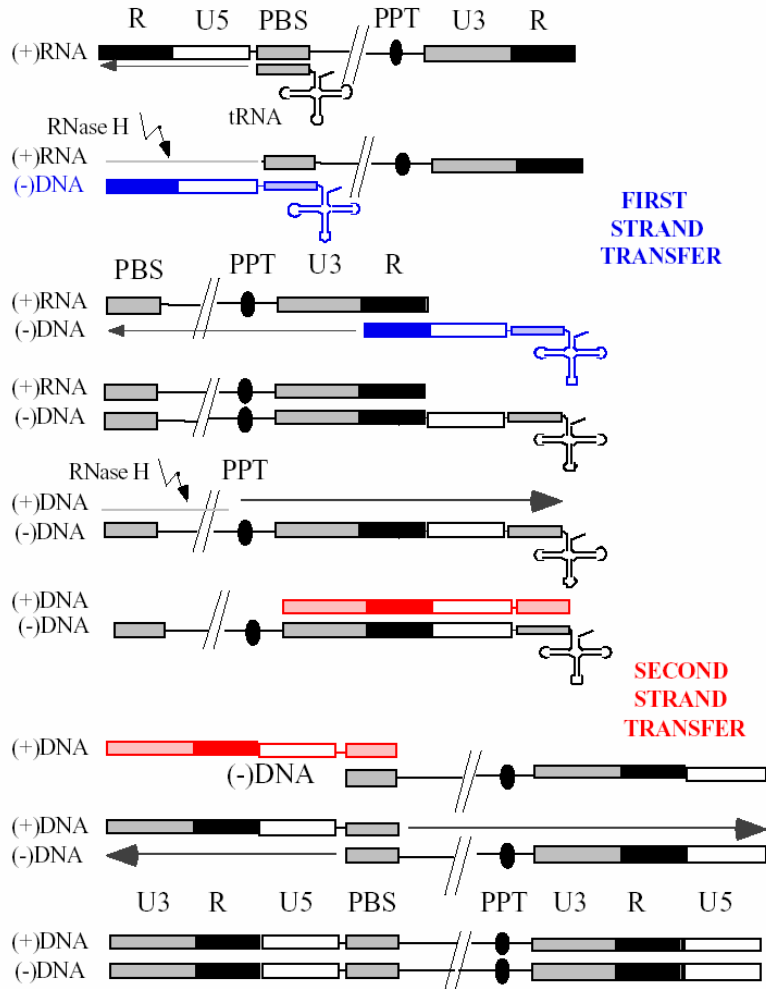
Packaging elements need to encode viral structural proteins: Gag, Pol, Env. For safety, Gag/Pol gene has been separated from Env (split-genome strategy). This way, in order for replication competent recombinant (RCR) to be produced, multiple cross-overs have to occur. This is unlikely but not impossible (see below).

**Packaging lines.** Typically, retroviral vectors are produced in packaging cell lines. PA317 is a commonly used packaging line originated from NIH-3T3 mouse fibroblasts. NIH-3T3 cells

were stably transfected with two constructs expressing Gag/Pol and Env, respectively, from the murine leukemia virus (MuLV). To produce vector, a transfer plasmid (LNCX) is stably transfected into PA317 cells and selected with Neo.

Supernatants from these cells contain titers of retroviral vectors in the order of  $10^5$  to  $10^6$  infectious units per ml.





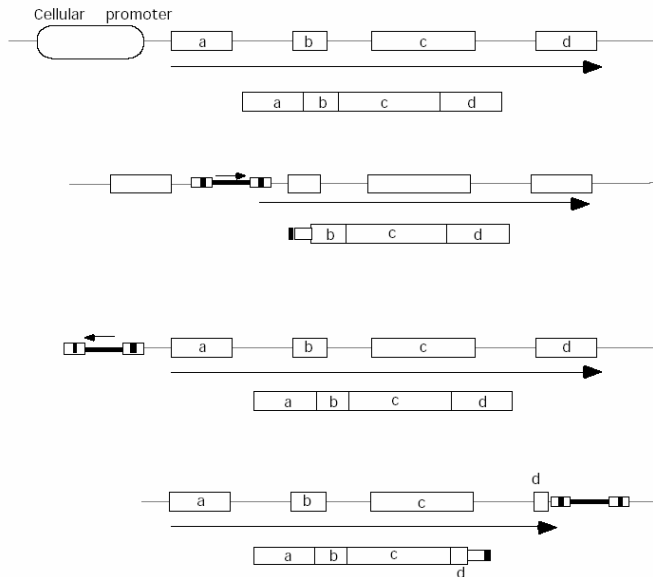
**Reverse transcription.** Retroviruses are highly prone to recombination “by definition”. Retroviruses use strand-transfers or “jumps” to complete reverse transcription of their genome. Strand transfers are recombinatorial in essence because they can occur intra-chain and inter-chain with similar frequency. In addition to the “obligate” strand transfers, the RT enzyme can switch templates at any point in this process.

## Safety issues regarding design of lentiviral vectors.

### 1. Effects derived from integration

- Promoter insertion
- Enhancer insertion
- Poly A site insertion
- Insertional inactivation of cellular genes

*The importance of promoter, enhancer, and poly (a) insertion lies in the potential for disregulation (constitutive activation) of genes involved in cell proliferation.*



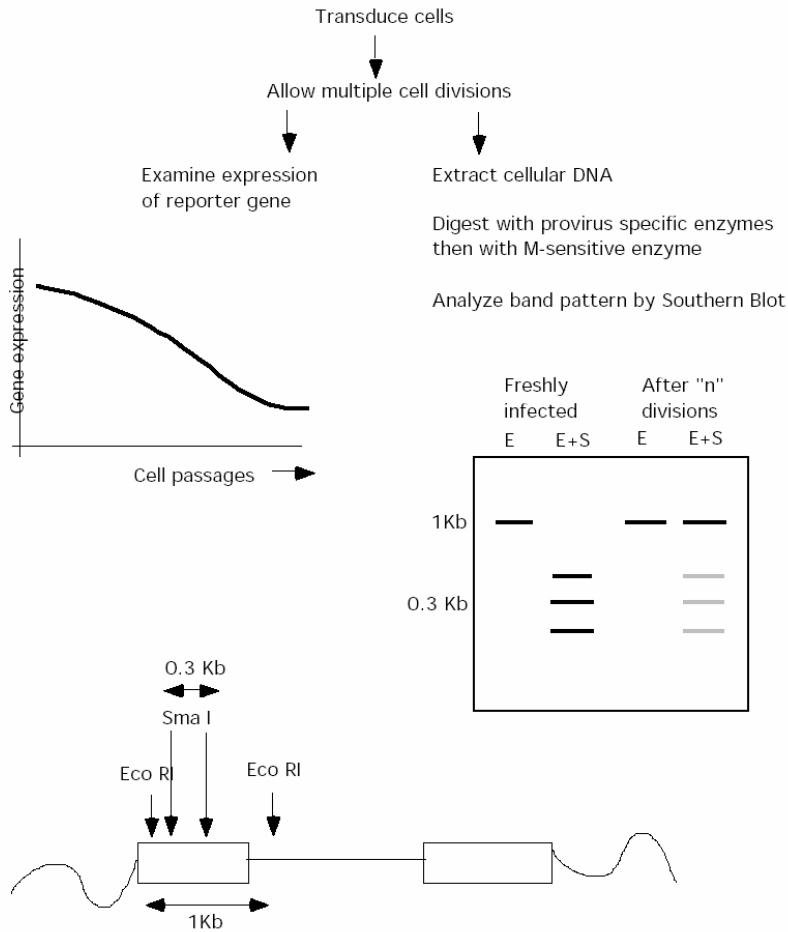
### Effects derived from insertion of retroviral LTRs

**2. RCR (replication-competent retrovirus).** The most urgent issue regarding safety of lentiviral vectors is the potential for recombination leading to replication-competent (sometimes also referred to as “helper”) virus. Generation of helper virus in preparations of replication-defective vectors has been documented in numerous instances involving oncoviruses. In later generations of vectors in which viral protein-coding regions were split in the packaging cells, the frequency of recombination leading to appearance of helper virus was decreased, but not eliminated (see Otto, Vanin and Donahue references). Helper virus has the potential for inducing pathogenesis as demonstrated by studies in which monkeys were infused with transduced bone marrow cells after ablation of endogenous marrow with gamma irradiation. In these studies, replication-competent virus gave rise to lymphoma in monkeys.

### Role of cell division (will be discussed in the context of lentivirus vectors)

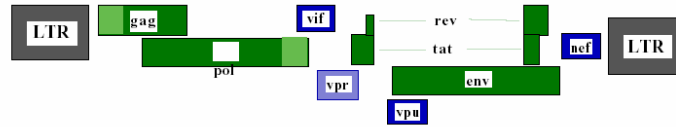
#### Promoter methylation

After transduction with retroviral (onco) vectors, the viral promoter becomes increasingly methylated at CpG sites. Methylation is associated with downregulation of the expression levels of the promoters overlapping methylation.

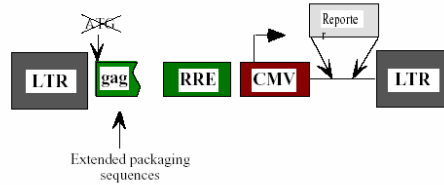


### Production of envelope pseudotypes

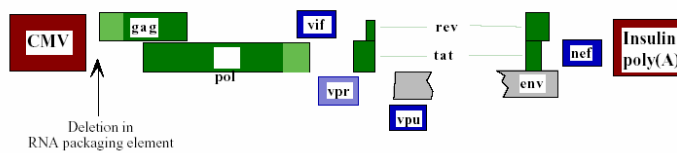
Genome-containing core particles from a particular retrovirus can incorporate the envelope glycoprotein of almost any other retrovirus. The envelope glycoproteins of viruses other than retroviruses can also be utilized to produce retroviral particles. Examples are VSV glycoprotein G and the Ebola virus glycoprotein.



#### Transfer construct



#### Packaging construct



#### Envelope construct



Three elements of a lentiviral vector (See Naldini et al reference)

## Retroviral vectors derived from Lentiviruses

*(Will stress unique aspects of lentiviruses)*

### Infection of non-dividing cells

Terminally differentiated stem cells and other non-dividing cell types (hepatocytes, neurons, primary macrophages) are not efficiently transduced by onco-retroviral vectors. Lentiviral vectors encode multiple nuclear localization/transport signals that determine active transport of pre-integration complexes to the nucleus.

### The structural genes of HIV are extremely toxic to mammalian cells

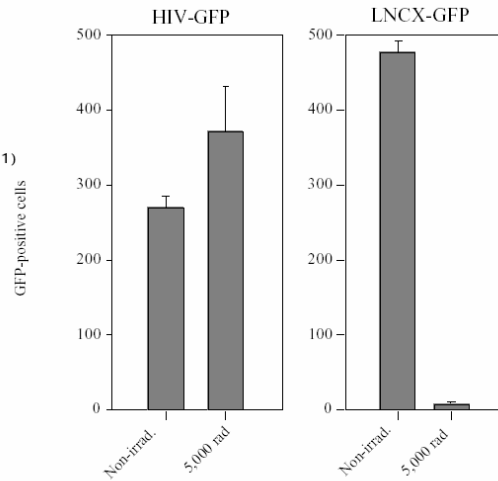
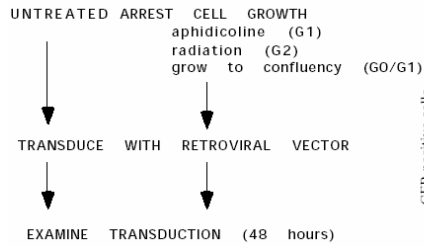
The immediate consequence of this is that the production of packaging cells will require the presence of regulated promoters. A popular way to circumvent the toxicity problem is to generate lentivirus vectors by transient transfection of cells.

### HIV is a human, lethal pathogen

1. Delete accessory genes (vpr, vpu, vif, nef) from packaging construct
2. Delete Tat (transactivator of the LTR) -> provide a strong internal promoter (CMV)
3. Delete Rev -> need to provide the transfer vector and packaging vector with cis acting sequences that replace Rev/RRE function
4. Provide an env from a non-retrovirus (VSV)
5. Use of self-inactivating (SIN) LTR
6. Develop vectors from lentiviruses that are not human pathogens

SIV  
FIV

BIV  
EIAV



Differences between lenti-and onco viral vectors in terms of infection of non-dividing cells

**ADVANTAGES AND DISADVANTAGES OF VARIOUS VECTOR SYSTEMS:**

System	Titers	Manipulate tropism	Integration	Immunogenicity	Inf. non-div. cells
Adenovirus	10 <sup>10</sup>	Terrific	No	Very high	Yes
Retrovirus	10 <sup>7</sup>	Good	Yes	Low	Only lentiviruses
Herpes	10 <sup>7</sup>	Not so good	No	Low	Yes
Adeno - assoc.	10 <sup>8</sup>	Not so good	Yes	Low	Yes

**References**

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- Vanin, E. F., M. Kaloss, C. Broscius, and A. W. Nienhuis. 1994. Characterization of replication-competent retroviruses from nonhuman primates with virus-induced T-cell lymphomas and observations regarding the mechanism of oncogenesis. *J Virol.* 68:4241-50.
- Naldini, L., U. Blomer, P. Gallay, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma, and D. Trono. 1996. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science.* 272:263-7.