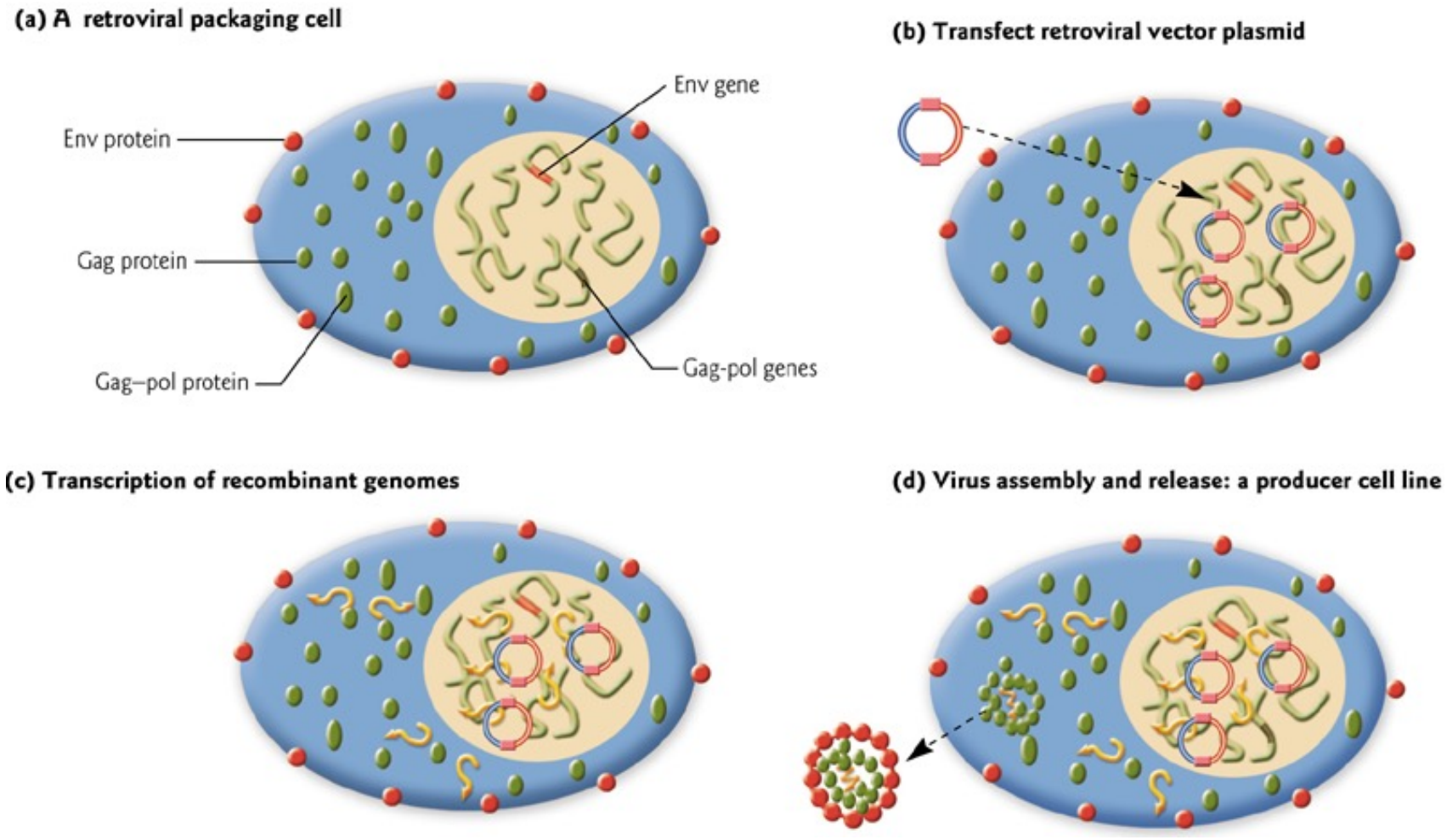
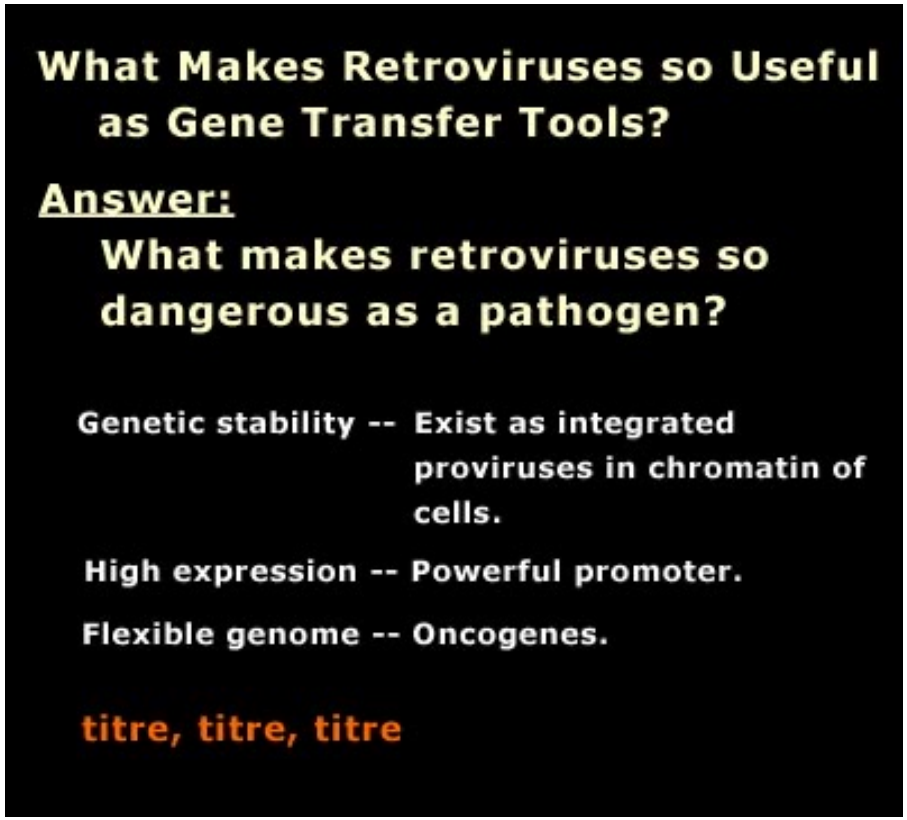
Most common viral vectors

- Retroviruses: can infect only dividing cells; stable, random integration into host genome.
- Lentiviruses: derived from HIV can infect quiescent cells; stable, random integration into host genome.
- Adenoviruses: high level expression, do not integrate, induce important immune reactions.
- Adeno-associated viruses (AAV): site-specific integration and stable expression over time; difficult to produce at high titer.
- Herpes simplex viruses: very selective infection of neurons, but significant cytotoxic effects.

Retroviral Vectors

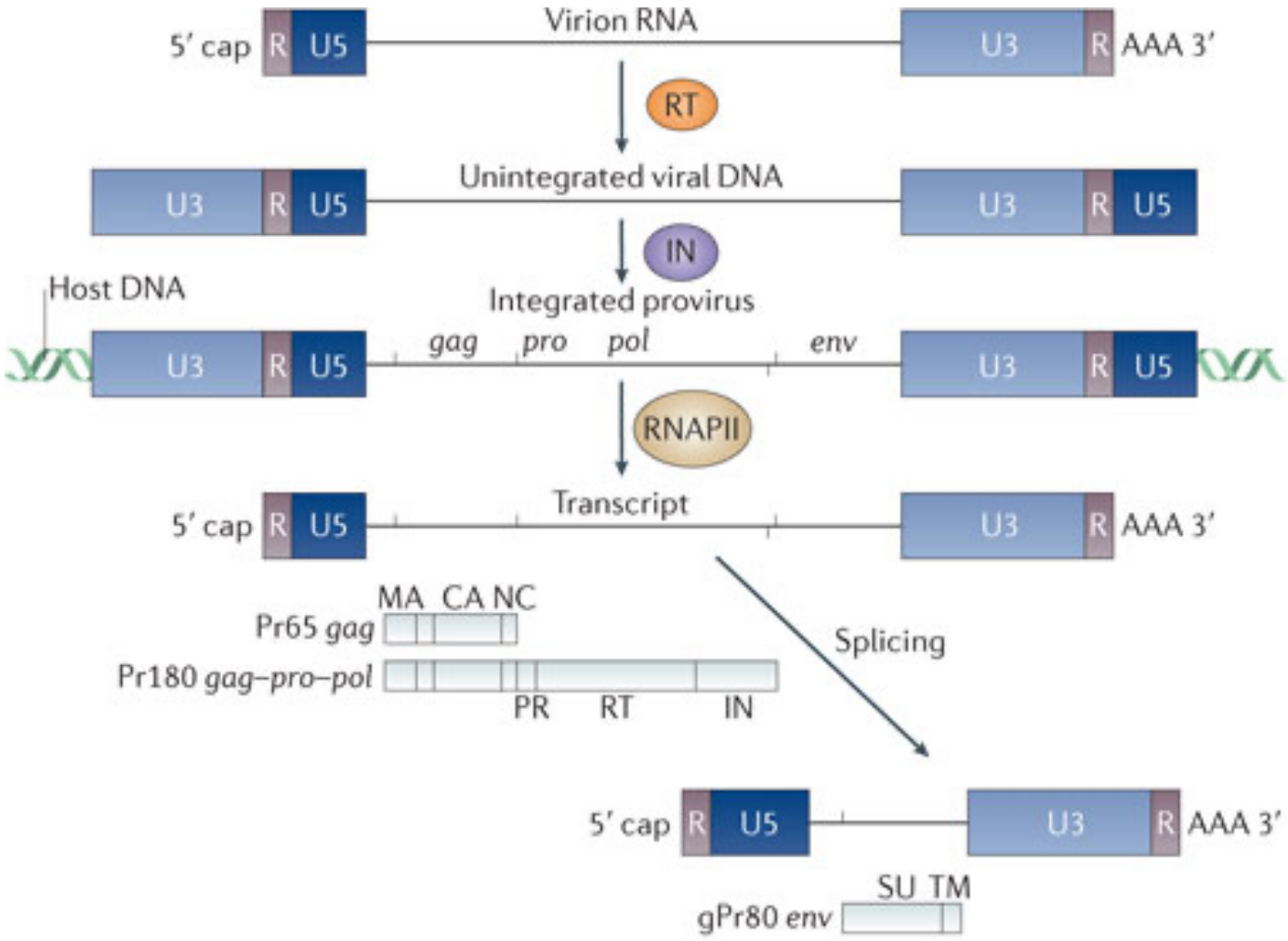


Retroviral Vectors

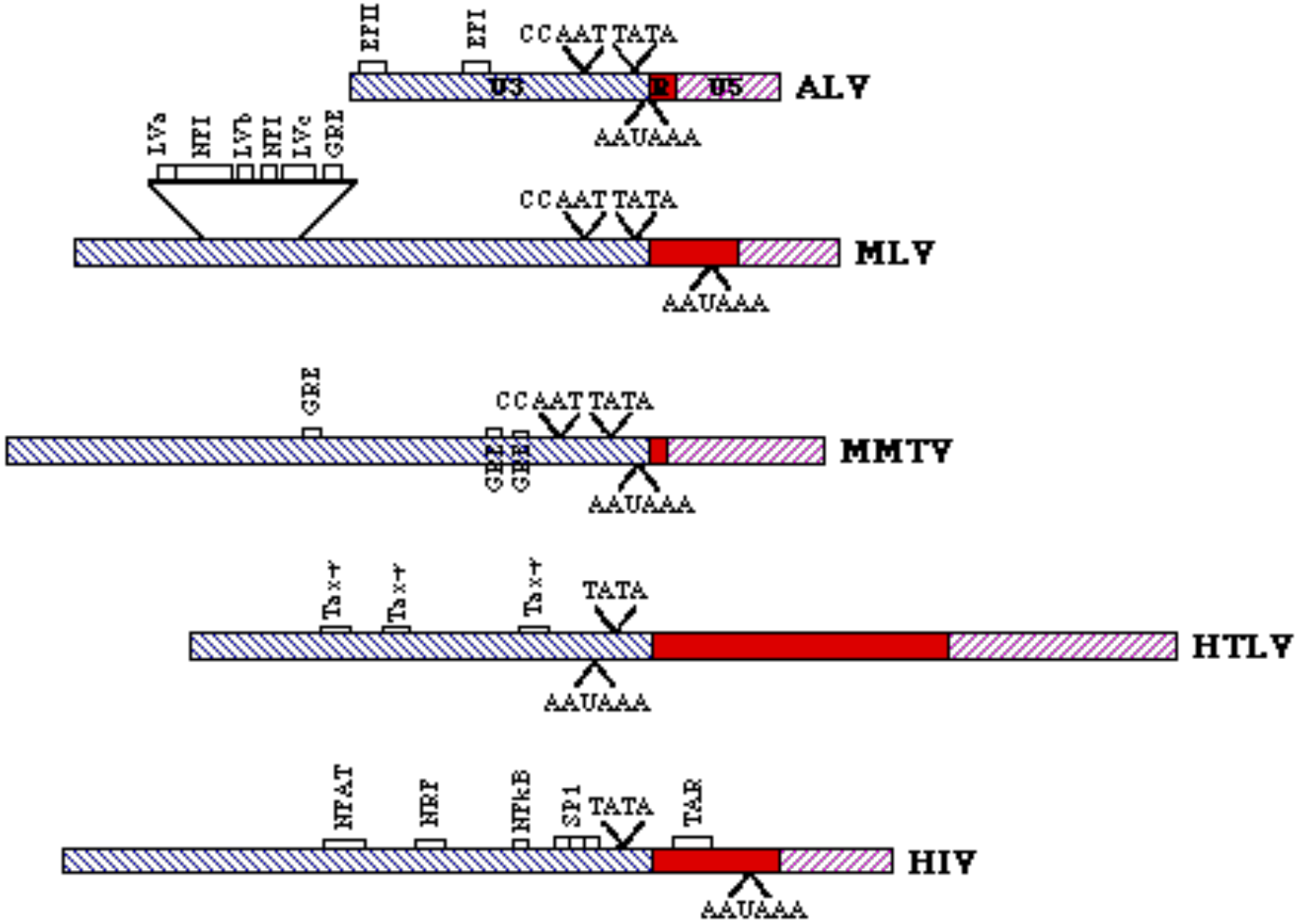


Several characteristics of retroviruses make them unique as vectors for gene transfer. Their replication cycle involves the integration of proviral DNA into the host genome. Depending on the context of chromatin, the viral promoters ensure the expression of genes with high efficiency. As revealed by the discovery of retroviral oncogenes, the retroviral genome can tolerate changes in its configuration and contain genes derived from host genome. For these and other reasons, retroviruses are potentially the most useful tools for transferring genes into target cells with high efficiency, allowing stable and permanent expression of the genetic elements introduced.

Genetic stability-exist as integrated proviruses in chromatin of cells



The retroviral regulatory regions (contained in U3) ensure the expression of genes with high efficiency.



Flexible genome

Some retroviruses contains extra genes

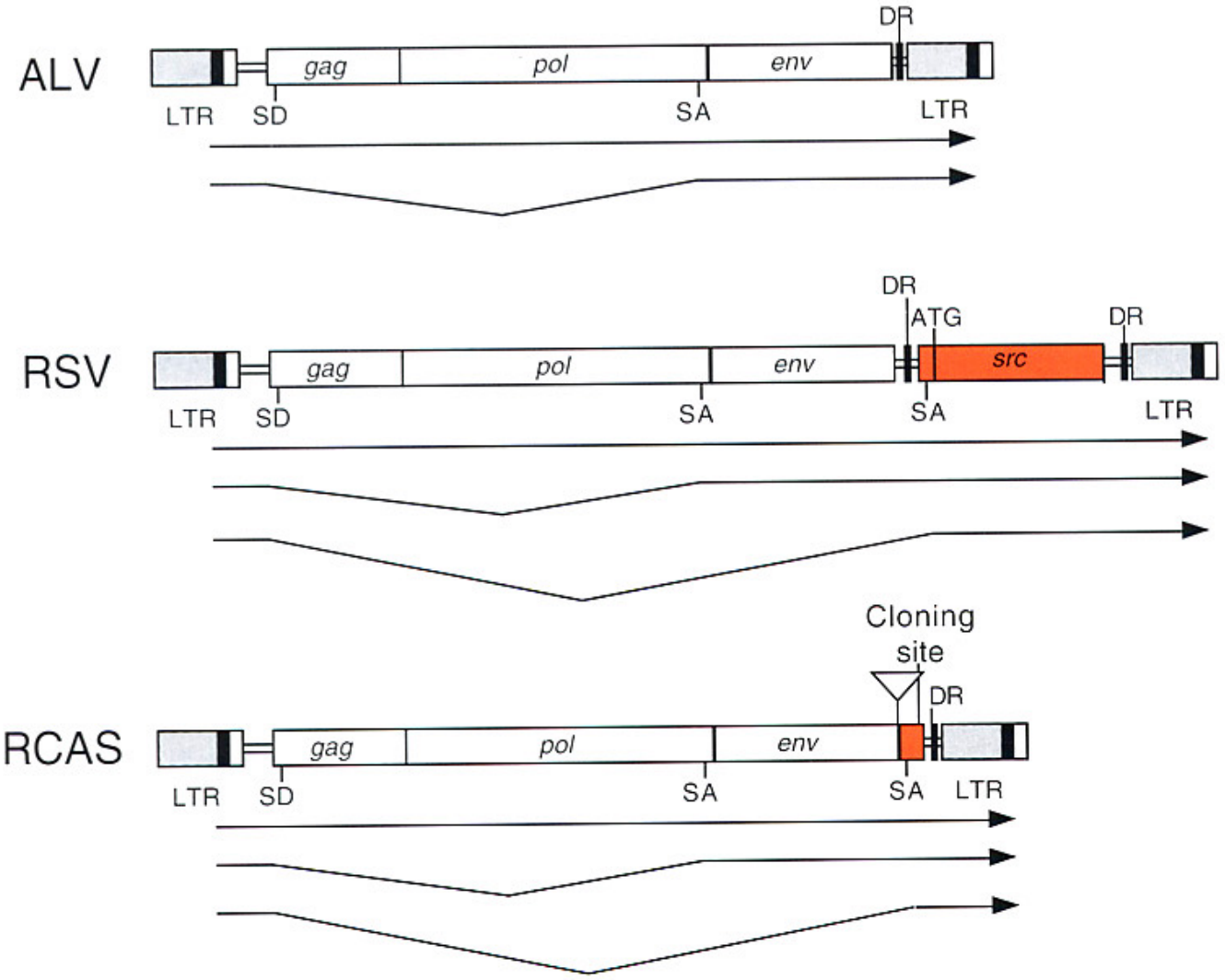
Retrovirus “normale”

R U5 GAG POL ENV U3 R

Rous Sarcoma Virus

R U5 GAG POL ENV SRC U3 R

Avian replication competent retroviral vector



From Coffin et al. *Retroviruses* (1997). CSHL Press

The host sequence replaced viral genes in most of acute transforming retroviruses, so….

Avian Myeloblastosis Virus

R U5 GAG POL **MYB** U3 R

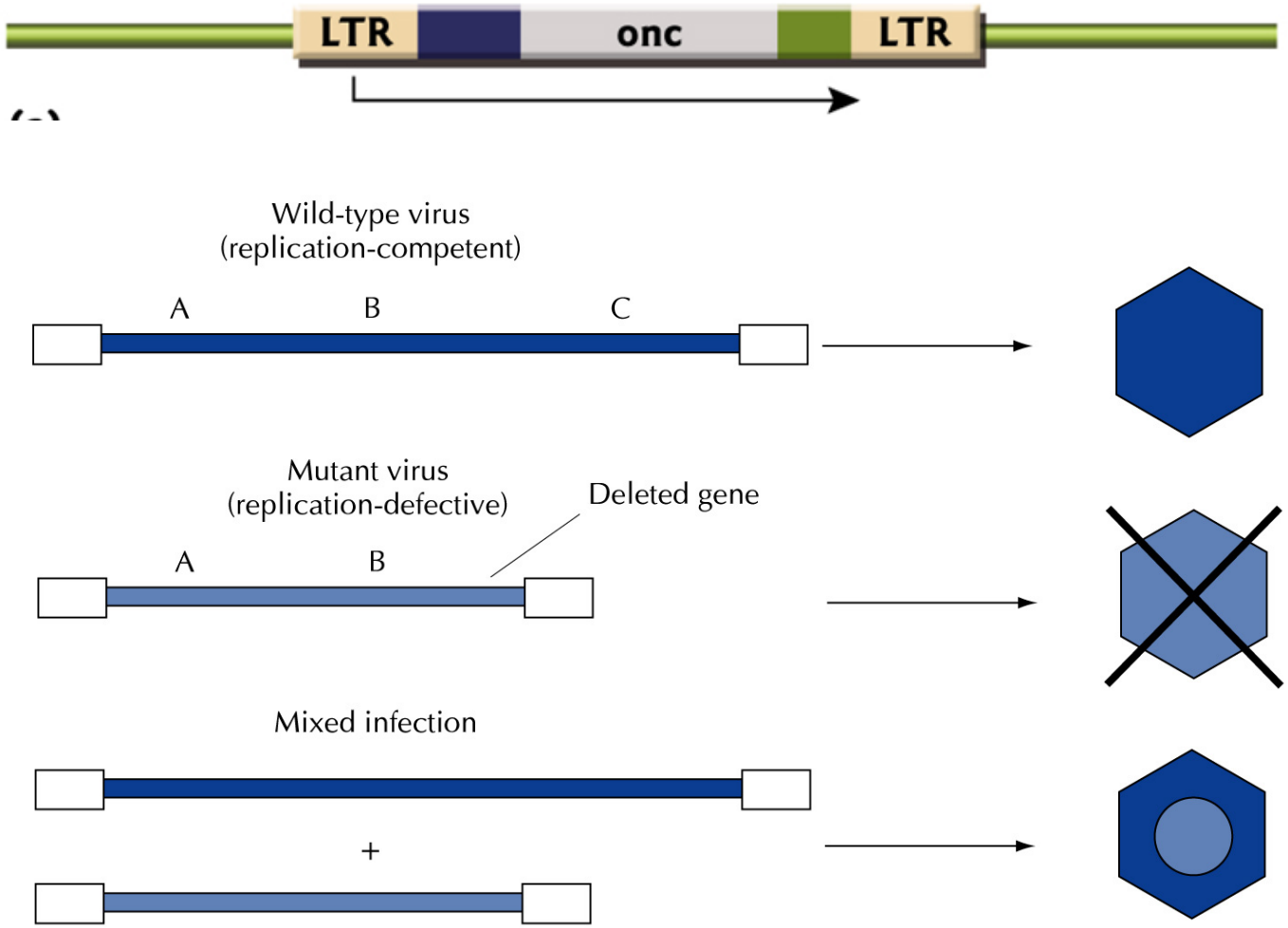
Feline Sarcoma Virus (FSV)

R U5 **dGAG** **FMS** **dENV** U3 R

Avian Myelocytoma Virus (MC29)

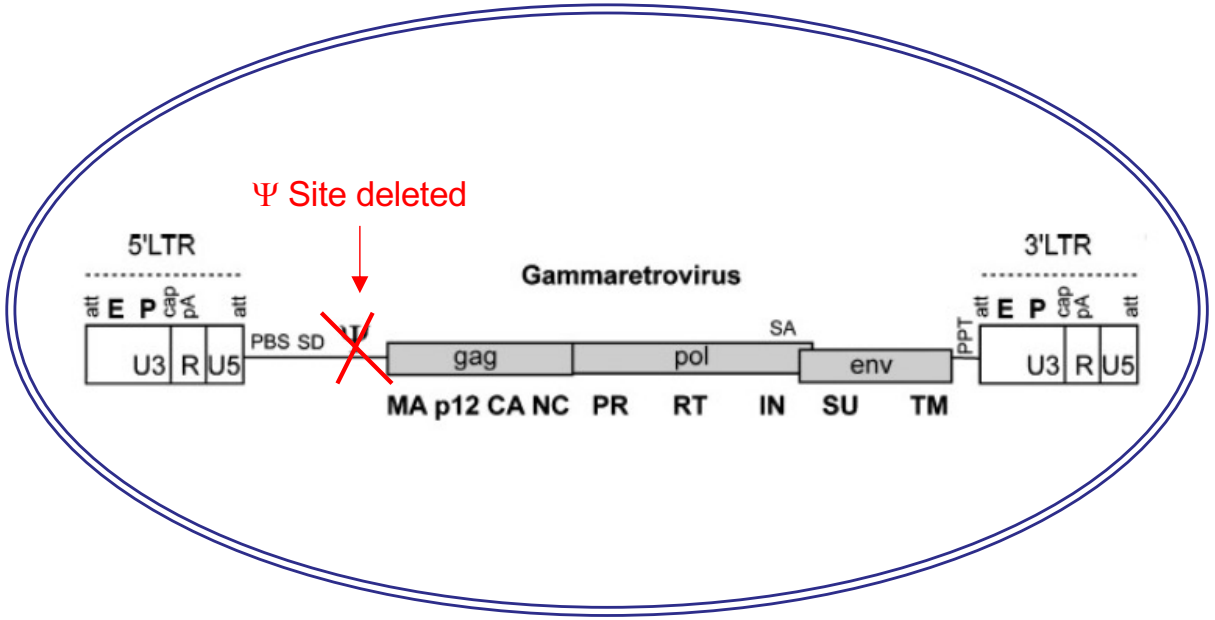
R U5 dGAG **MYC** dENV U3 R

……they need a helper virus in order to produce new viral particles



From Cann *Principles of molecular virology* (2001). Academic Press

Helper cells or packaging cell lines



The use of recombinant retroviruses began with David Baltimore and Richard Mulligan in 1983 when they reported the construction of the first packing cell line Psi-2 (Ψ-2) and other similar packaging systems, based on the stable modification of NIH 3T3 cells. These packaging lines, defective for the helper, are able to provide in trans all the necessary proteins (gag, pol, and env) required for packaging of the recombinant genome [which in turn possesses in cis the packaging signal (Ψ site)], the processing, reverse transcription and integration.

What do I need to generate a recombinant retrovirus?

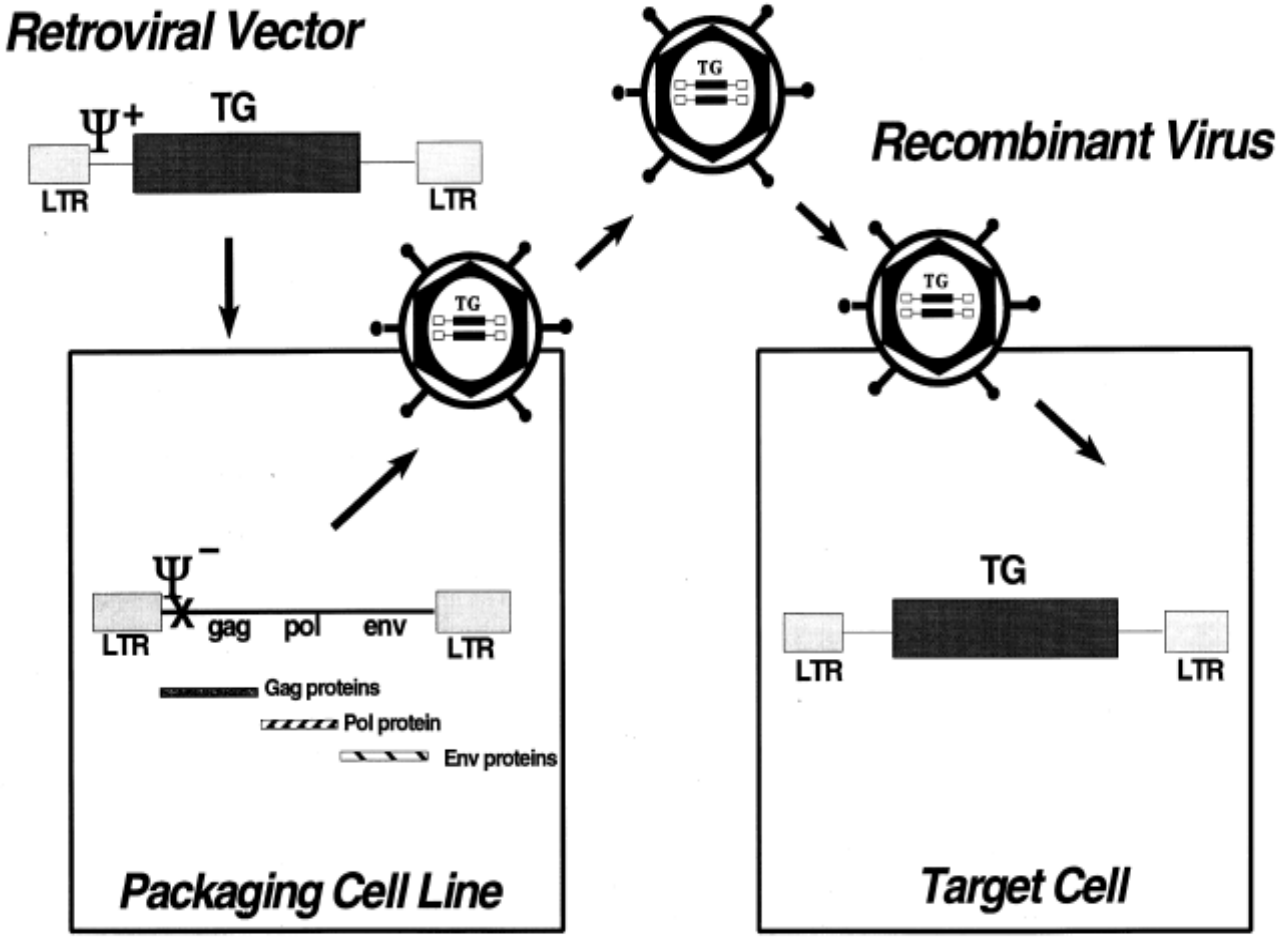
Packaging Line (PL):

Provides packaging proteins
(gag-pol-env)

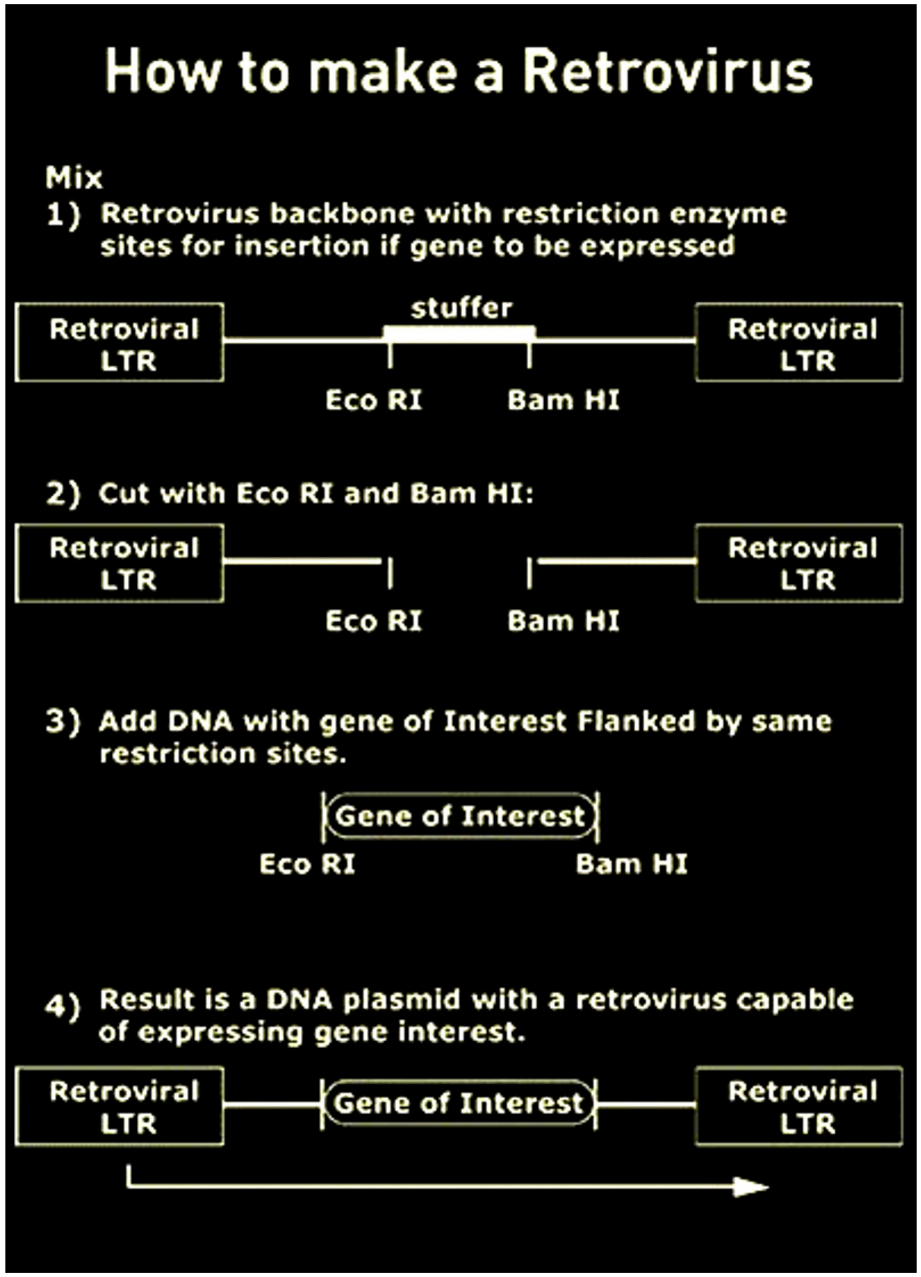
Recombinant Retroviral Vector:

Provides RNA encoding genetic elements of interest (genes) and requisite *cis* elements for packaging, reverse transcription, and integration.

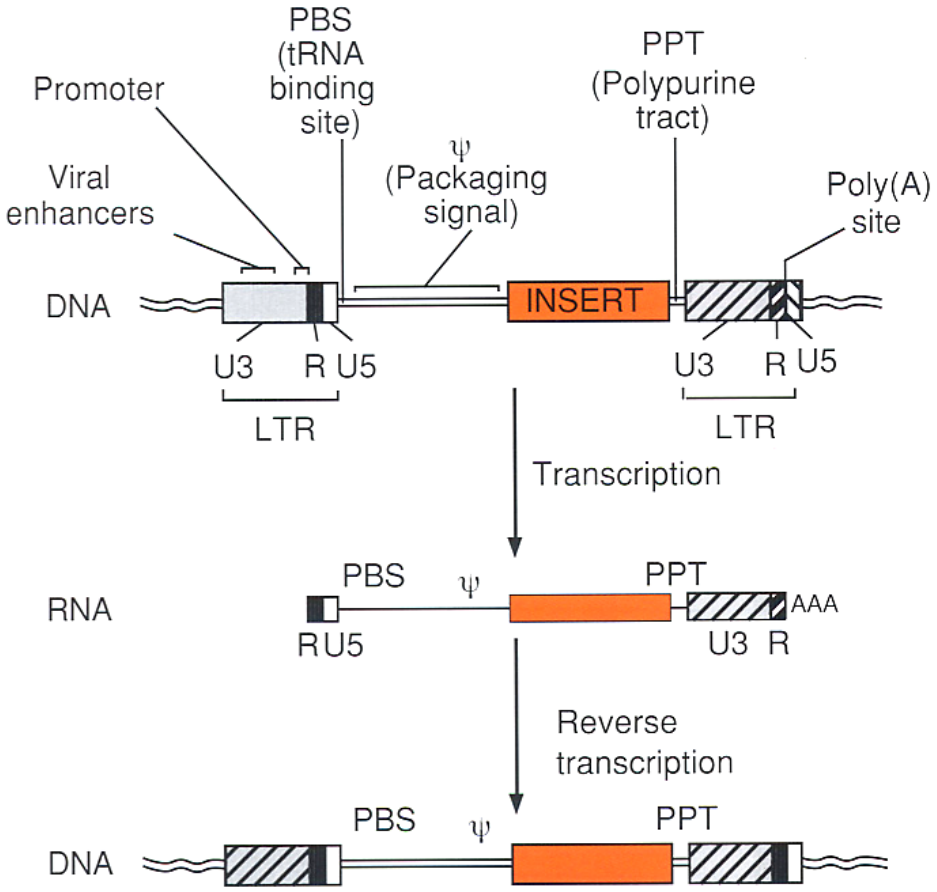
So that….


Retroviral Vector
Ψ+
TG
LTR
LTR
TG
Recombinant Virus
TG
Ψ−
LTR
gag
pol
env
LTR
Gag proteins
Pol protein
Env proteins
Packaging Cell Line
TG
LTR
LTR
Target Cell

To generate the recombinant retrovirus, it is necessary to provide the packaging cells with the RNA genome containing the sequences, derived from the proviral DNA LTR, necessary for retrotranscription, integration and expression of the transgene in the target cell.



The retroviral vector

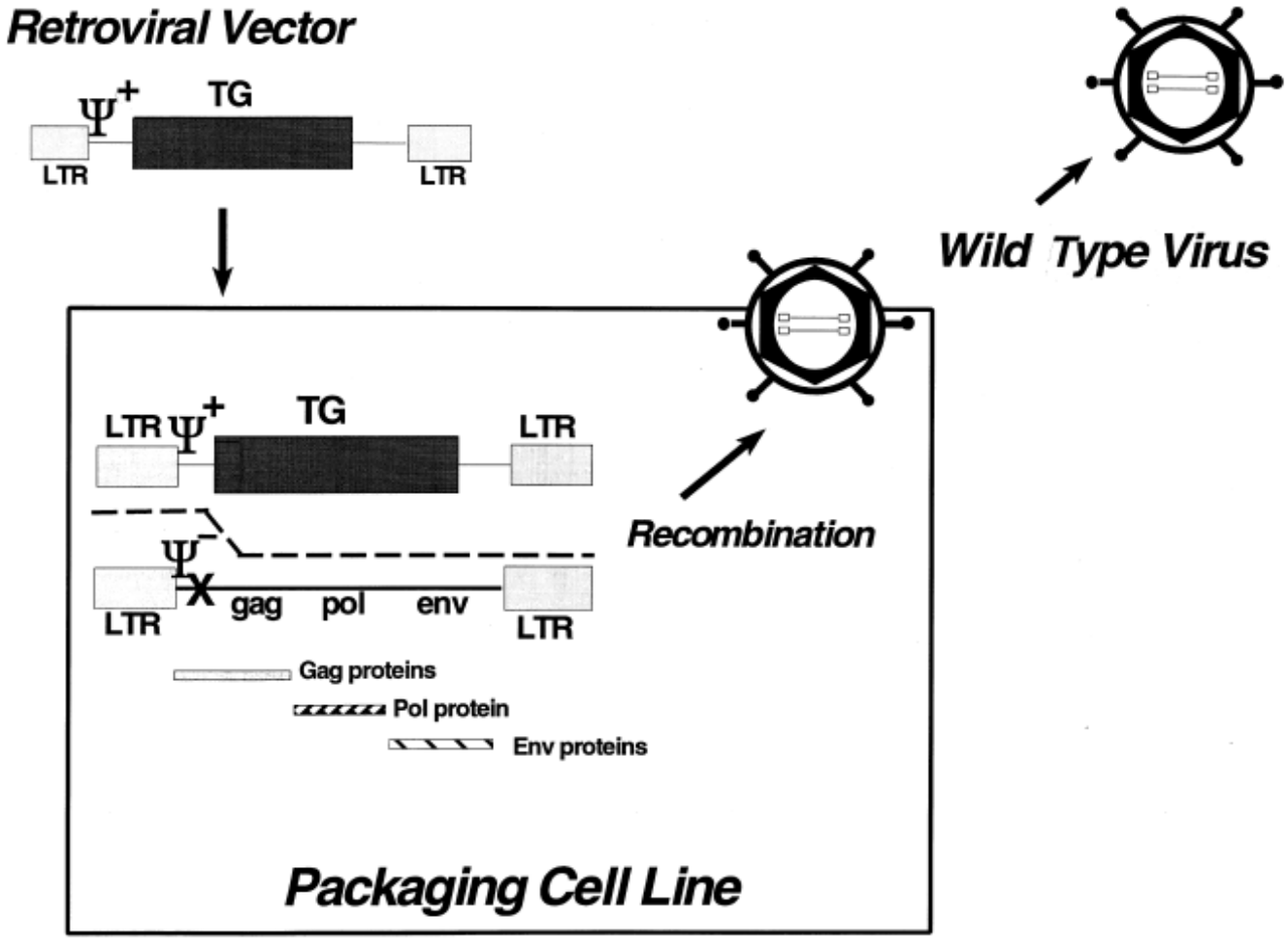


Transfected plasmid into the packaging cell line (provirus DNA)

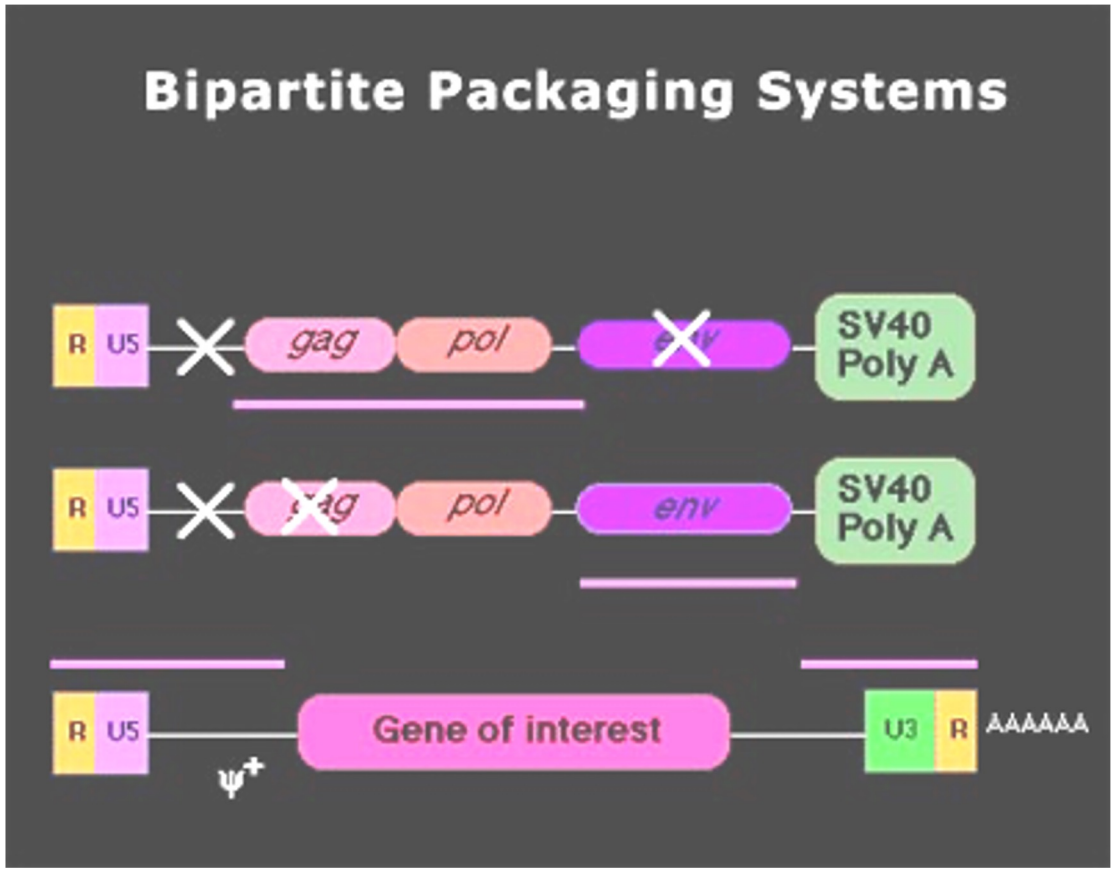
Ricombinant retroviral genome packaged into virus particles

Integrated provirus into target cell genome

From Coffin et al. Retroviruses (1997). CSHL Press

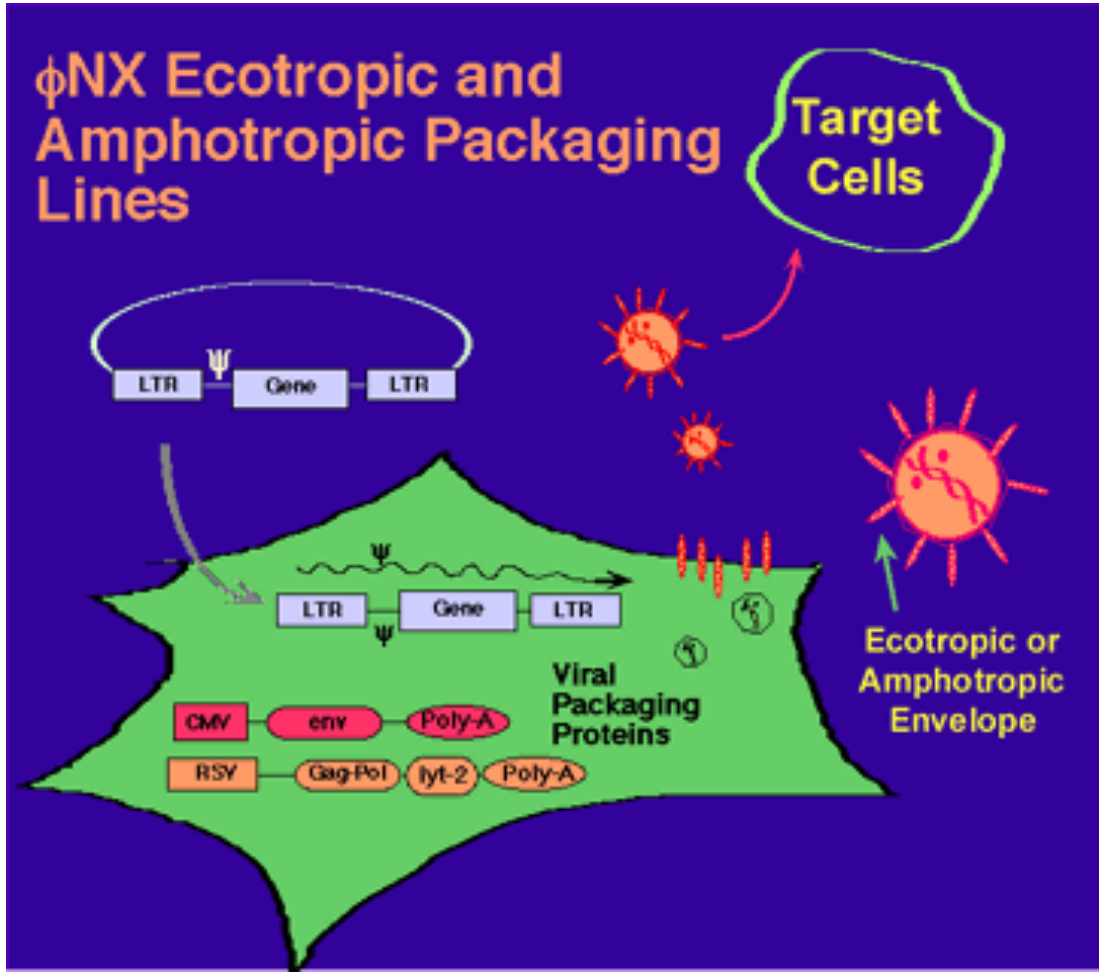


Initial retroviral vector systems often rapidly became contaminated with replication-competent virus due to at least one homologous recombination event (dotted line) occurring between the vector construct and the packaging construct in the packaging cells. The probability of this happening depends on the degree of homology shared between the two constructs.



Bipartite packaging systems were established to minimize the potential for recombination overlap and creation of a wild-type virus. Such systems consist of three components, for example two independent constructs, one of which expresses the Gag and Pol gene products and a second that is used for expression of the Env proteins.

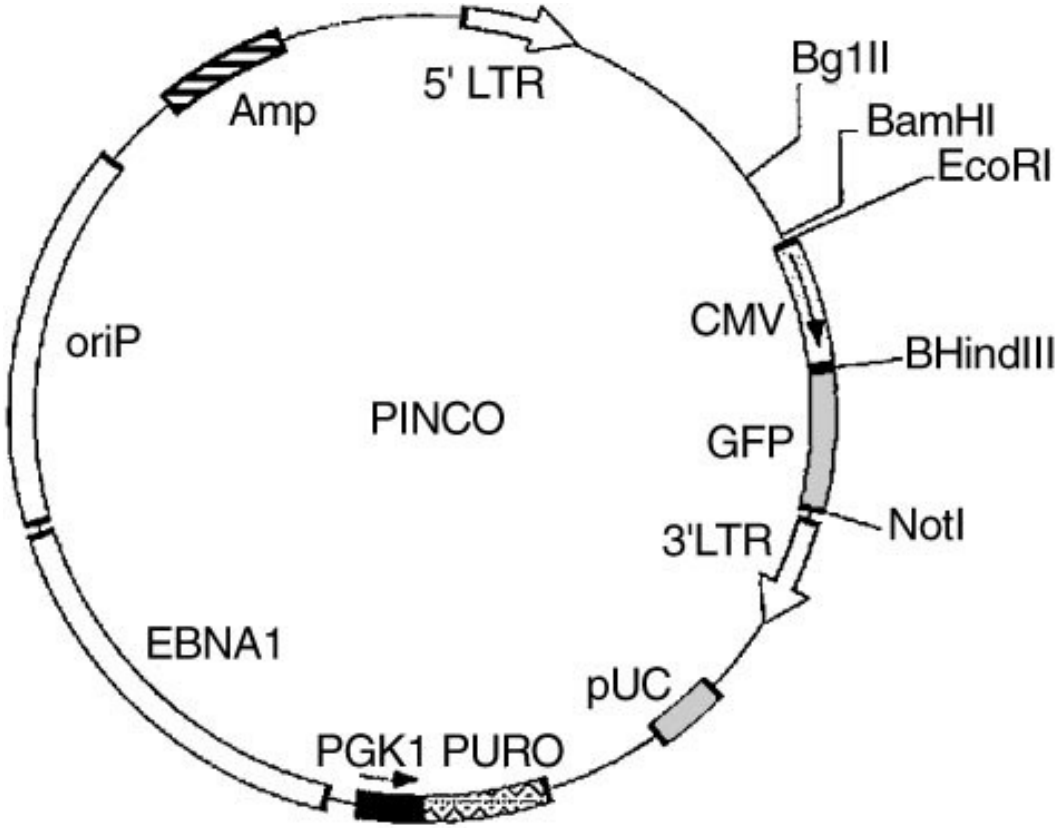
http://www.stanford.edu/group/nolan/



PhNX have been derived from the human embryo kidney cells HEK 293, harboring the adenovirus E1 region and containing a ts mutant di SV40 Large T. Both Phoenix-ECO and Phoenix-AMPHO were tested for helper virus production and established as being helper-virus free and are fully compatible with transient and episomal stable retroviral gene transfer experiments.

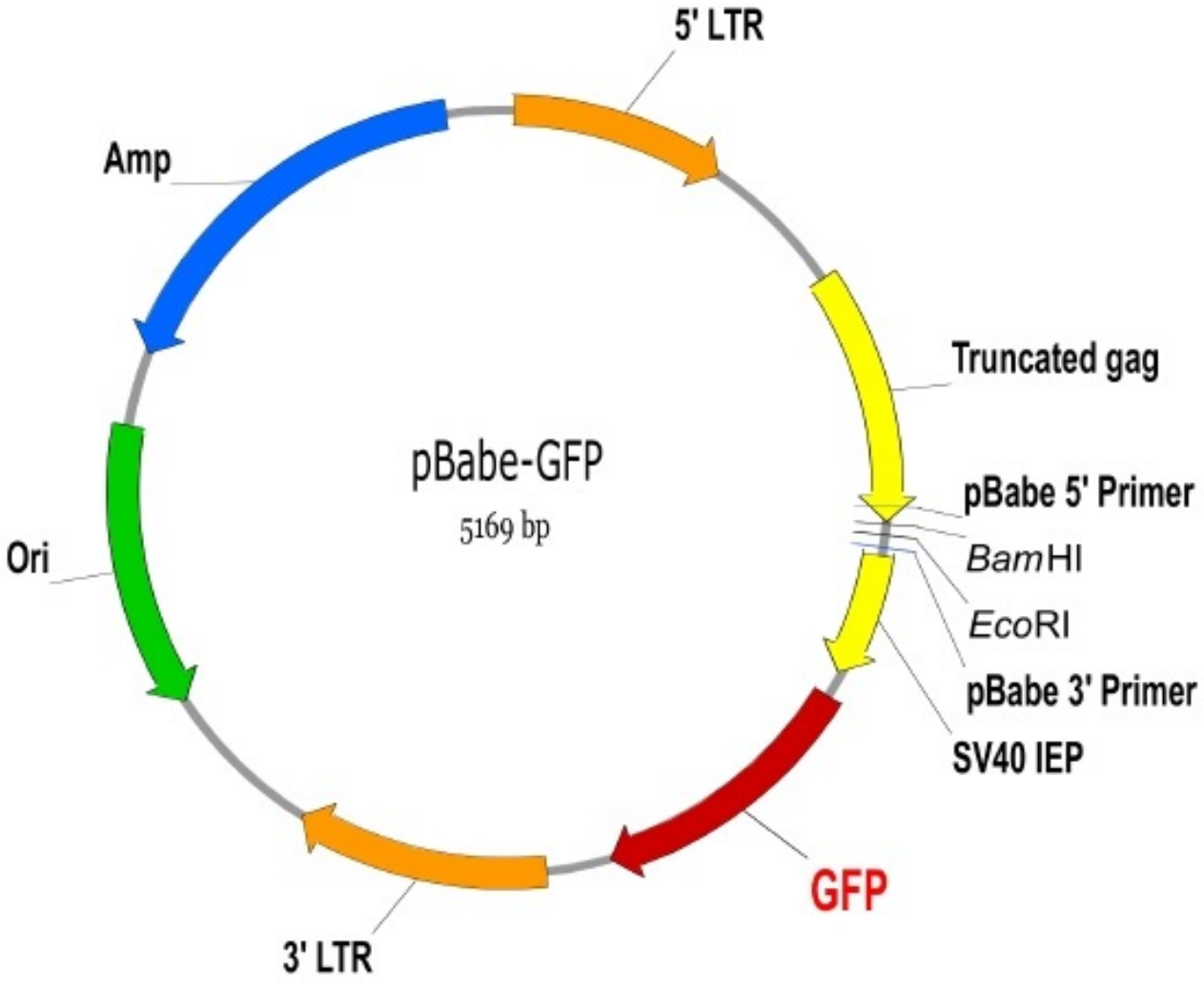
http://www.stanford.edu/group/nolan/

Episomal retroviral vector



http://www.stanford.edu/group/nolan/

pBABE retroviral vectors



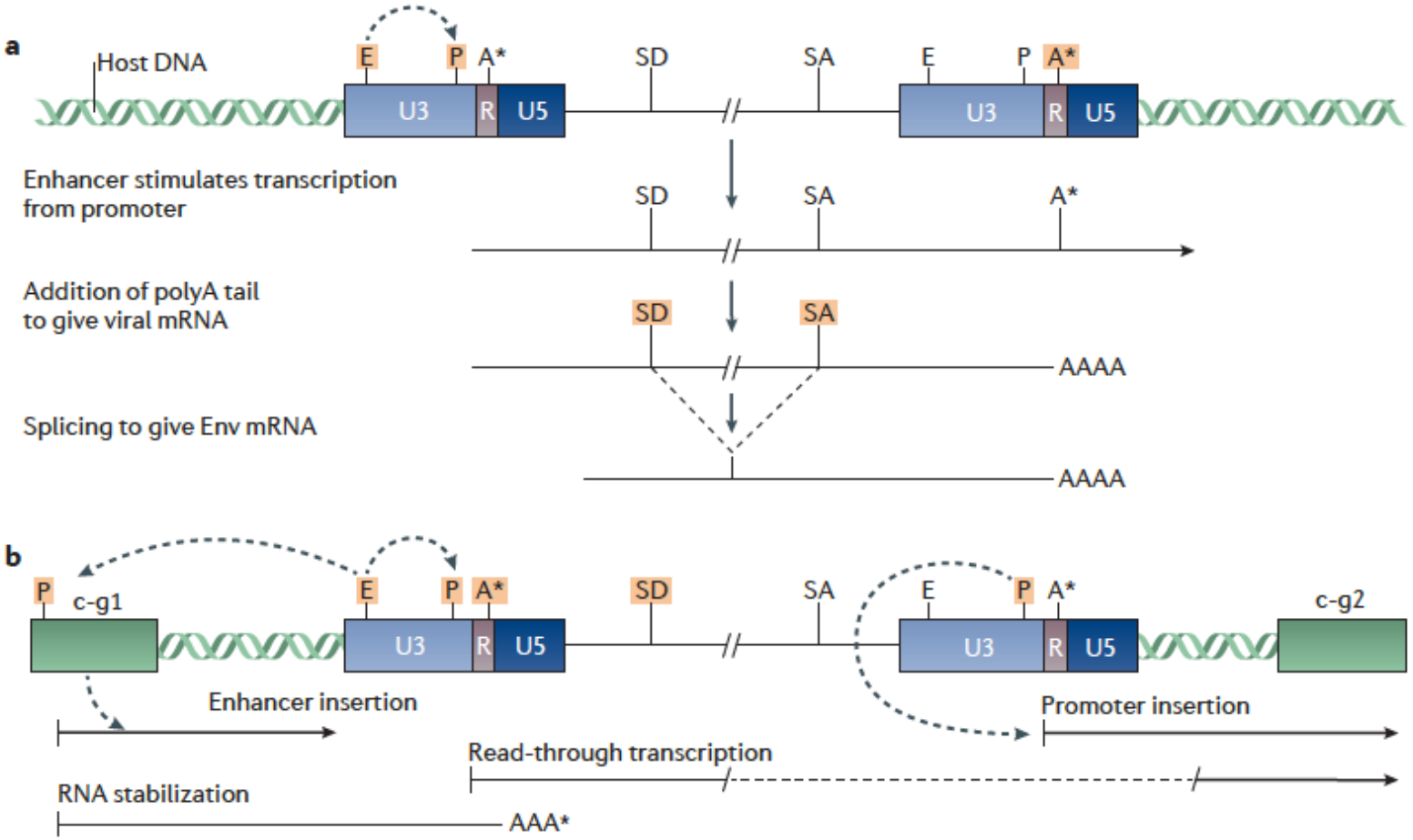
“Random Integration”
of
Retroviruses

NO!!!

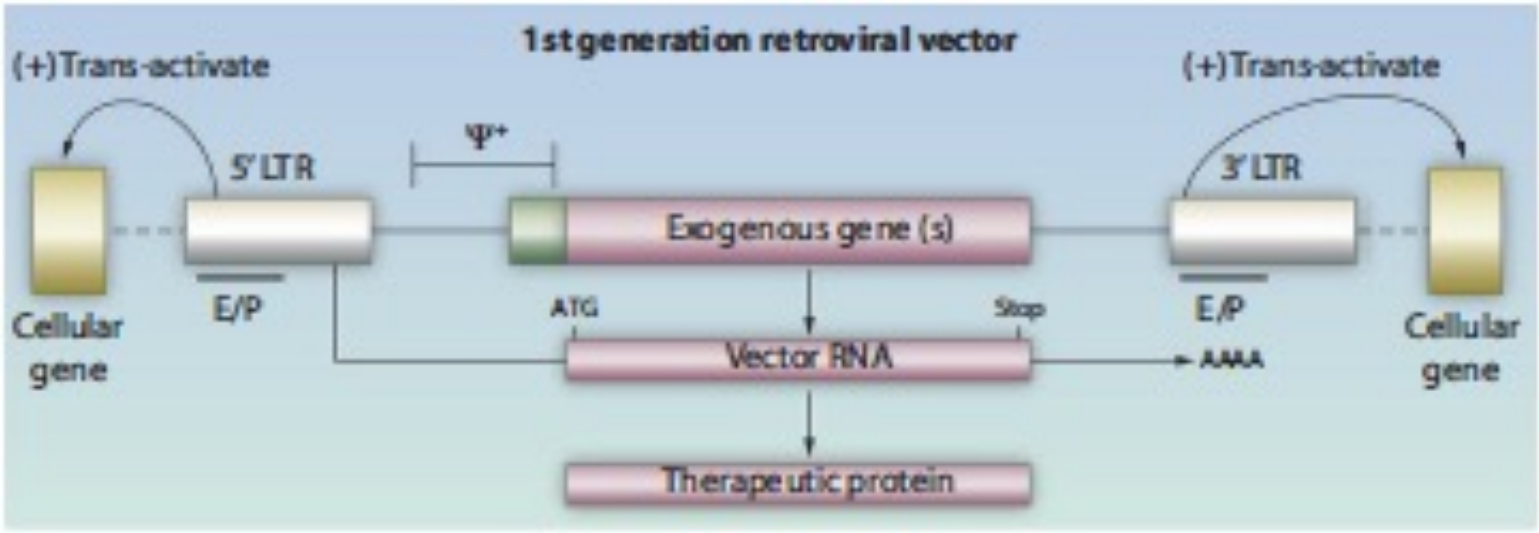
Insertion into

“Open”
Transcriptionally Active
Chromatin

Insertional mutagenesis



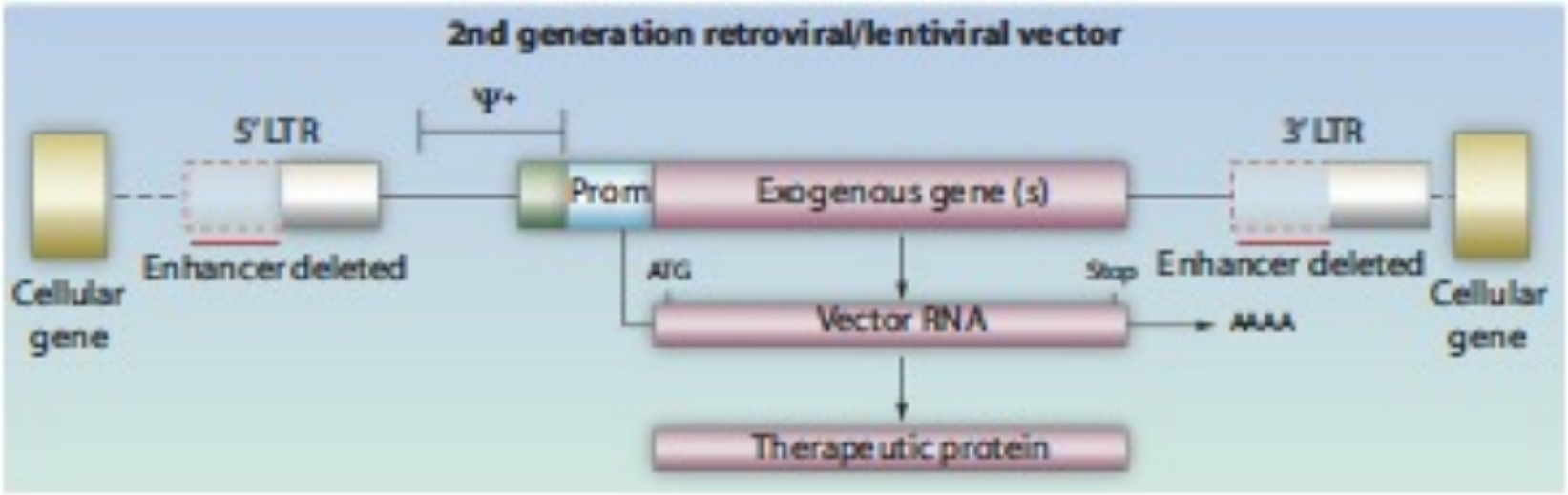
First generation γ-retroviral and lentiviral vectors



First-generation γ-retroviral vectors used the enhancer and promoters (E/P) of the LTRs from the retroviruses to drive transcription of the exogenous gene. The strong enhancers are capable of trans-activating cellular protooncogenes that are adjacent to the sites of vector integration into cellular chromosomes (dashed lines).

Sci. Transl. Med. 3, 97ps36 (2011)

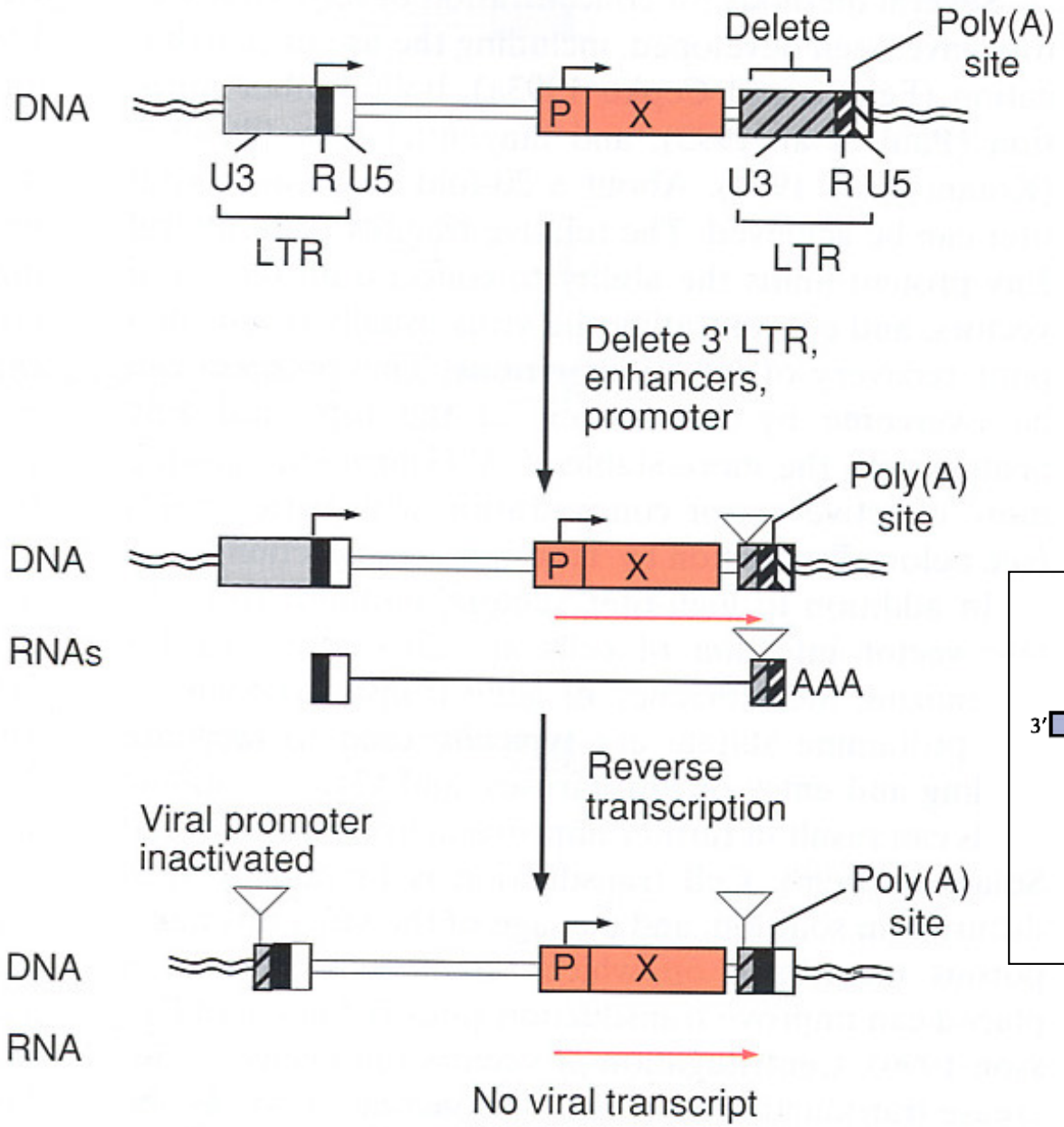
Second-generation γ-retroviral and lentiviral vectors

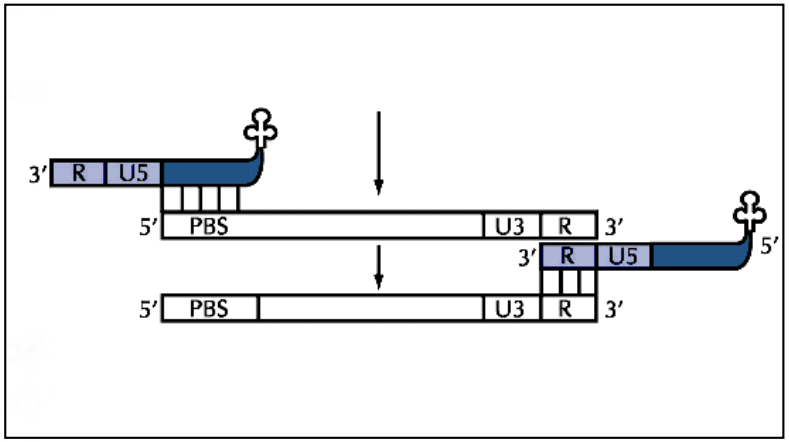


Second-generation γ-retroviral and lentiviral vectors have the enhancers of the LTR deleted and drive transcription of the exogenous gene using internal promoters (prom) that lack strong enhancer activity. These types of vectors have shown minimal potential to trans-activate cellular genes in preclinical models.

Sci. Transl. Med. 3, 97ps36 (2011)

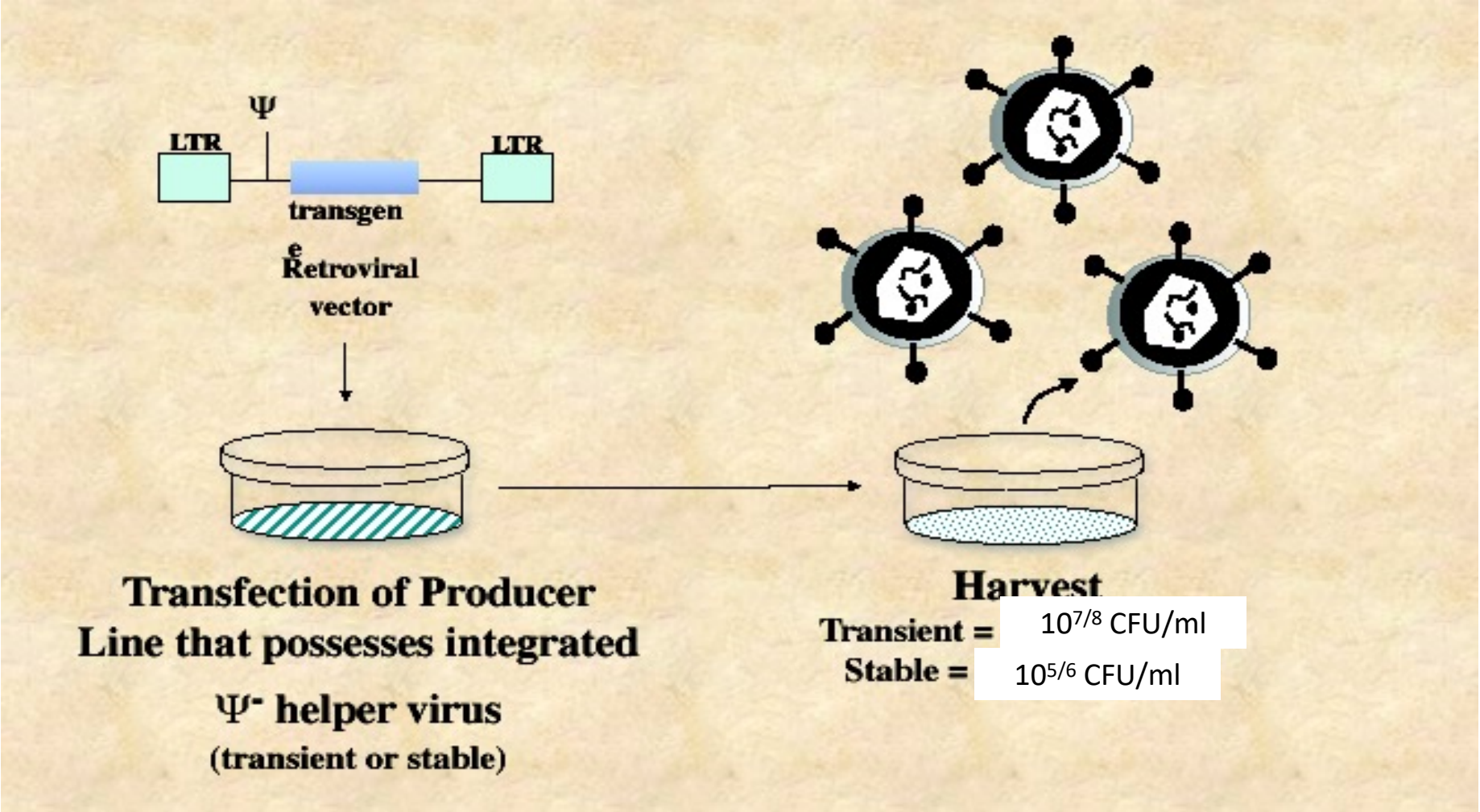
SIN (self inactivating) vectors



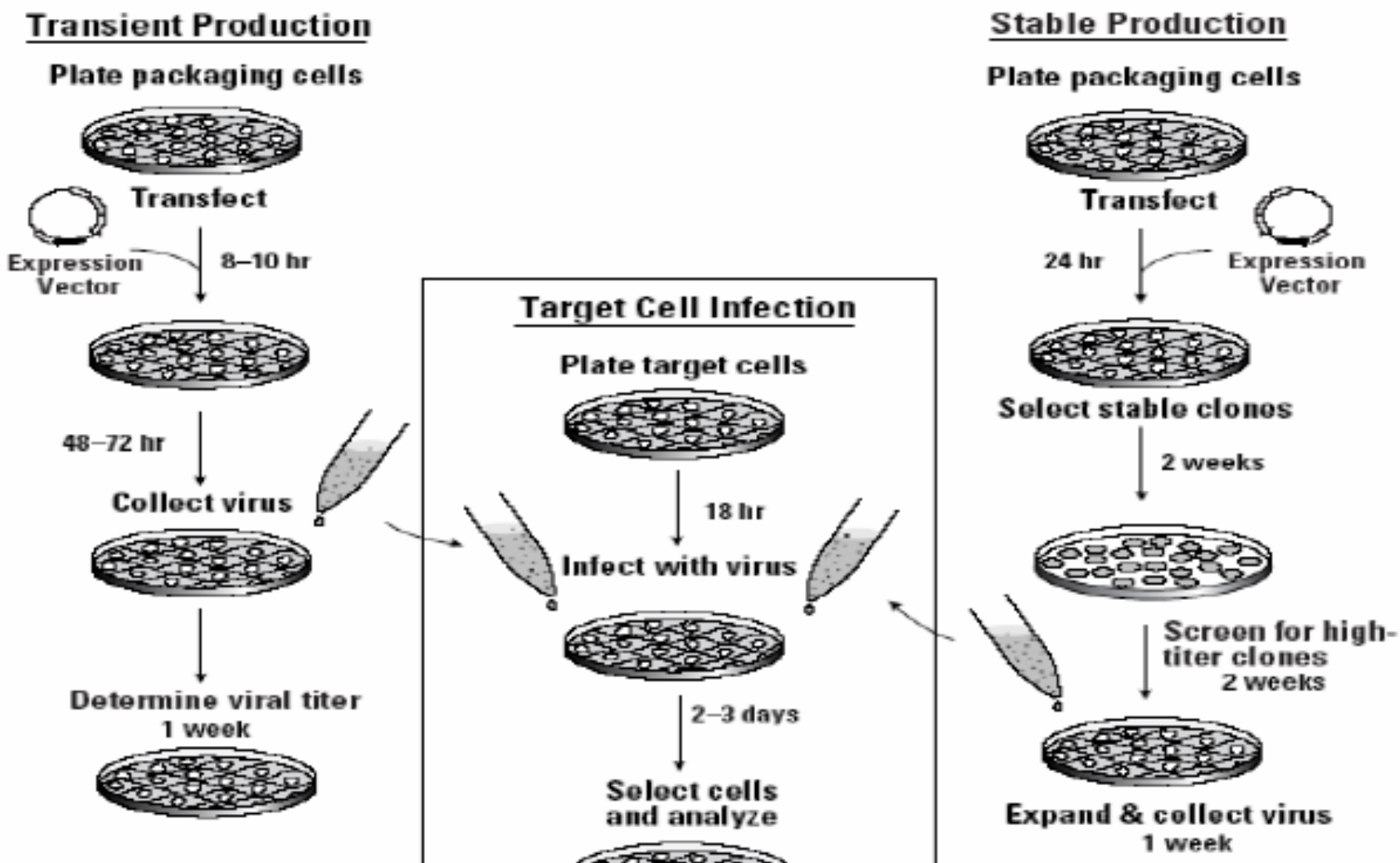


From Coffin et al. *Retroviruses* (1997). CSHL Press

How it works


Ψ
LTR
LTR
transgen
e
Retroviral
vector
Transfection of Producer
Line that possesses integrated
Ψ- helper virus
(transient or stable)
Harvest
Transient = 10^7/8 CFU/ml
Stable = 10^5/6 CFU/ml

Transient and stable virus production



Transfect an expression vector into a packaging cell line.

After 48–72 hours, collect virus and infect a target cell line.

Alternatively, use antibiotic selection to develop clones that stably produce retrovirus.

Tissue tropism

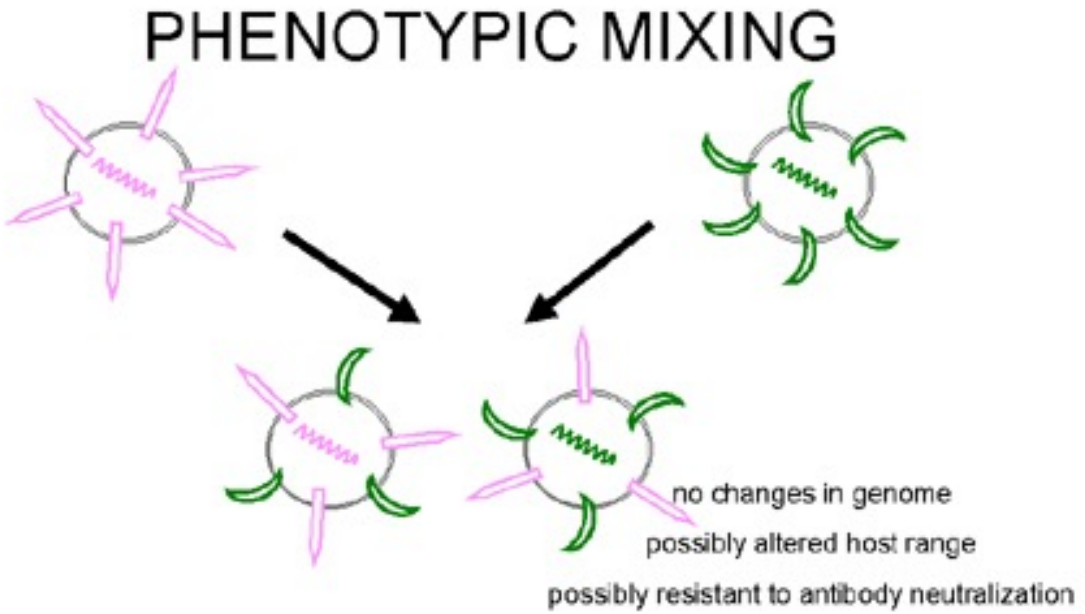
The viral *env* gene, expressed by the packaging cell line, encodes the envelope protein, which determines the range of infectivity (tropism) of the packaged recombinant virus. Viral envelopes are classified according to the receptors used to enter host cells and, thus, the host cell range:

Ecotropic viruses can recognize a receptor found on only mouse and rat cells.

Amphotropic viruses recognize a receptor found on a broad range of mammalian cell types.

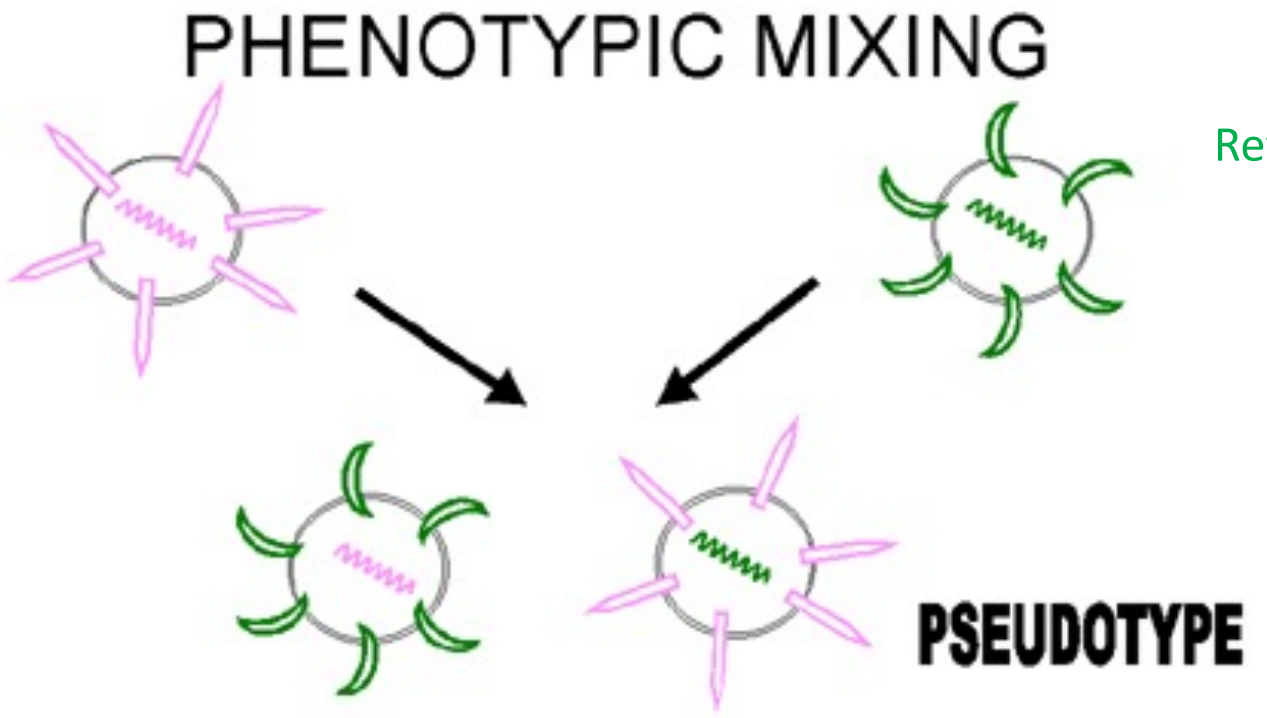
Pantropic viruses can infect both mammalian and non-mammalian cells.

Generating a pantropic recombinant retrovirus



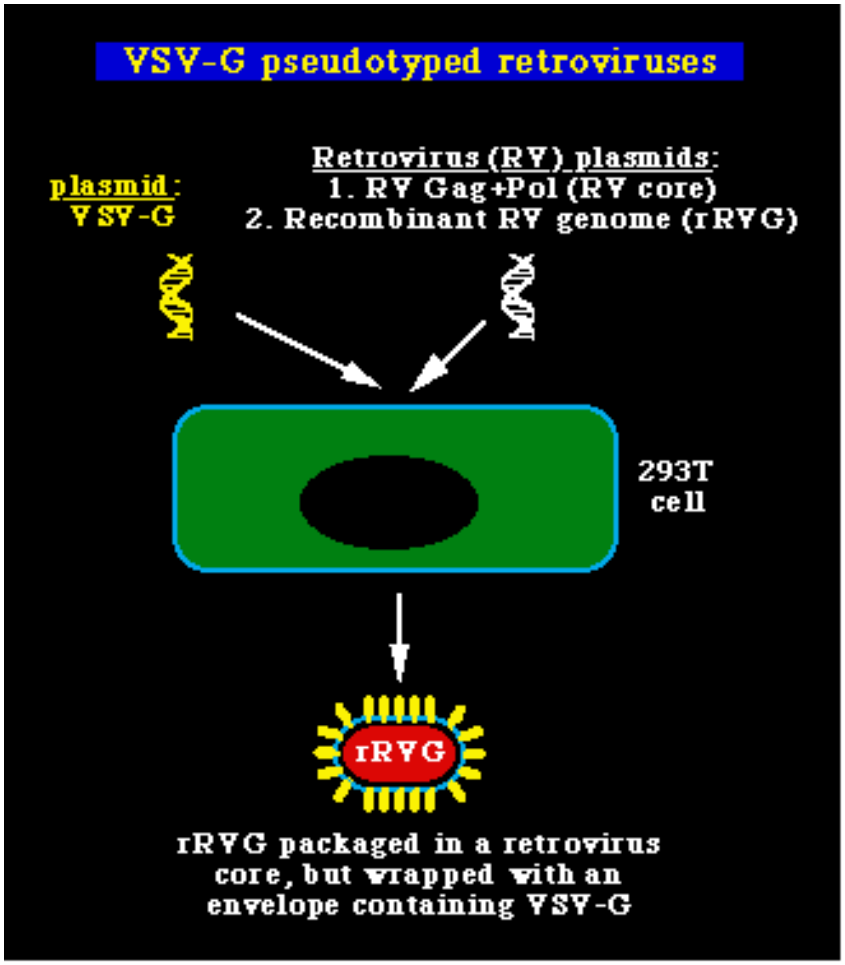
Phenotypic mixing may occur between related viruses, e.g. different members of the Picornavirus family, or between genetically unrelated viruses, e.g. Rhabdo- and Paramyxo- viruses. In the latter case the two viruses involved are usually enveloped since it seems there are fewer restraints on packaging nucleocapsids in other viruses' envelopes than on packaging nucleic acids in other viruses' icosahedral capsids.

Generating a pantropic recombinant retrovirus

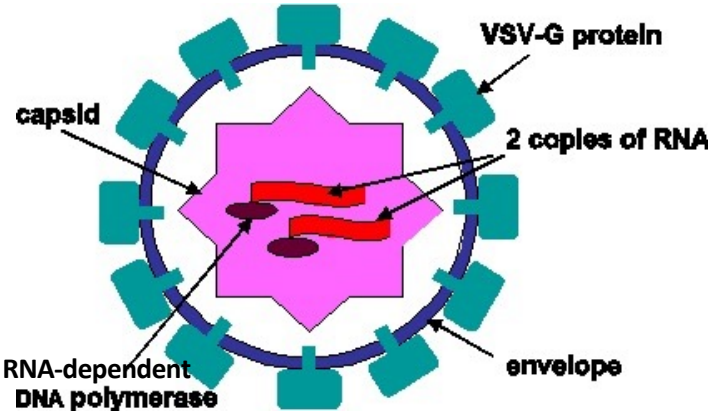


We can also get the situation where a coat is entirely that of another virus, e.g. a retrovirus nucleocapsid in a rhabdovirus envelope. This kind of phenotypic mixing is sometimes referred to as pseudotype (pseudovirion) formation. The pseudotype described above will show the adsorption-penetration-surface antigenicity characteristics of the rhabdovirus and will then, upon infection, behave as a retrovirus and produce progeny retroviruses. This results in pseudotypes having an altered host range/tissue tropism on a temporary basis

Modify env gene by
creation a pseudotyped vector



Vesicular
stomatitis virus (VSV):
phospholipid component of membrane as a receptor (phosphatidyl serine)

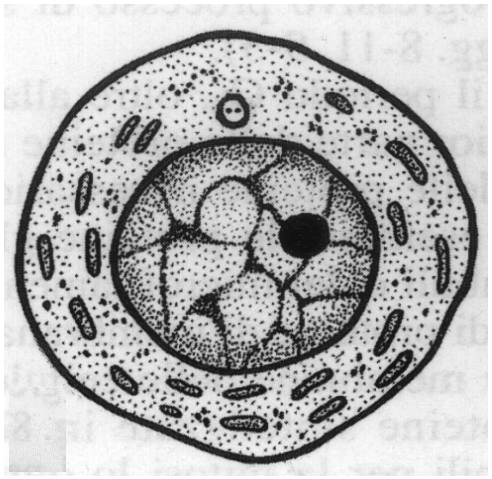


hybrid virion
with retrovirus core
and **VSV envelope protein**

How to infect post-mitotic cells?

Lentivirus
(HIV-1)

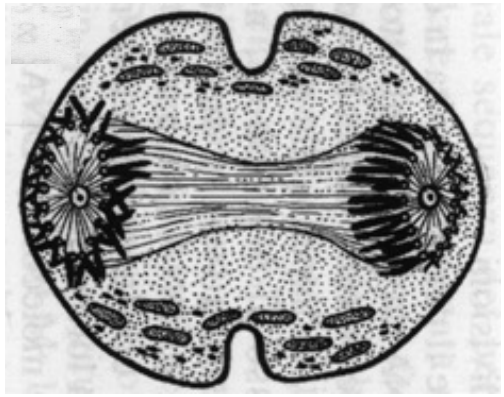
Oncoretrovirus



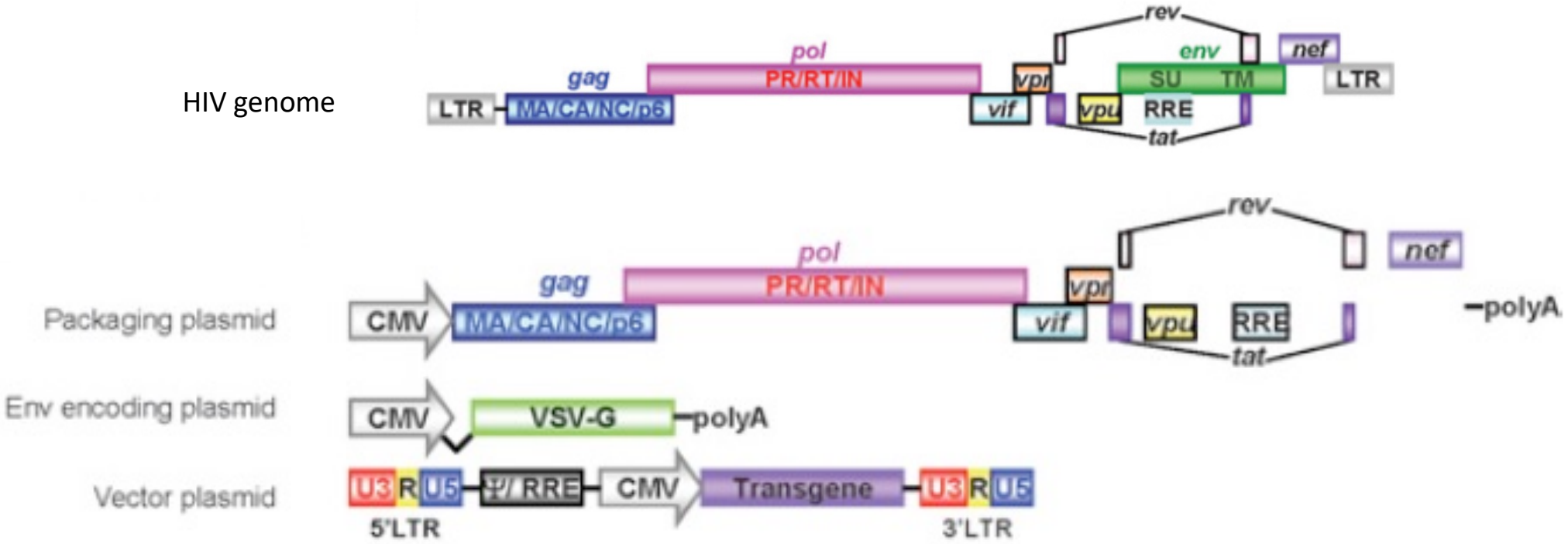
Nuclear transport
Dependent on

- Int (MA, CA, Vpr)
- cPPT-DNA flap

Mitosis-
dependent

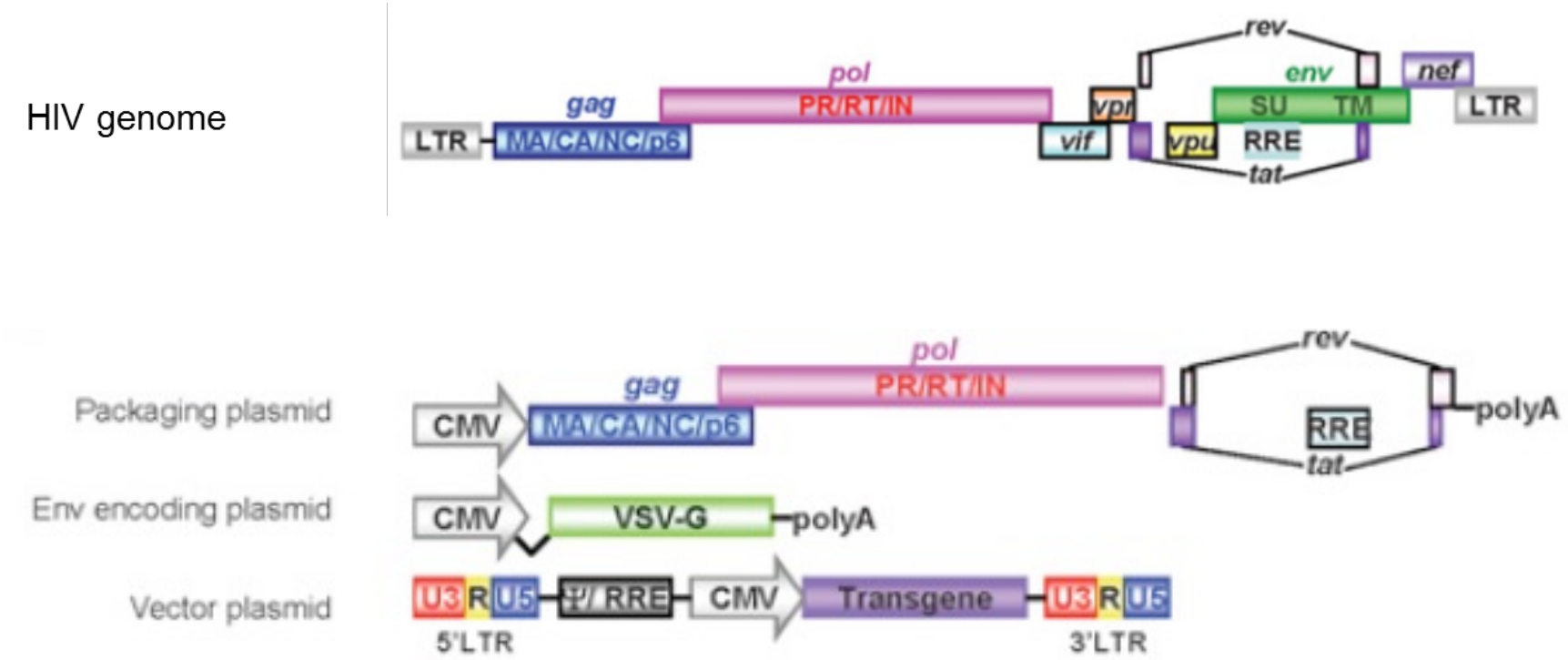


First generation lentiviral vectors



The packaging construct expresses HIV Gag, Pol and regulatory/accessory proteins from a strong mammalian promoter to generate viral particles. The Env plasmid expresses a viral glycoprotein, such as VSV-G, to provide the vector particles with a receptor-binding protein. These two plasmids have been specifically engineered without either a packaging signal or LTRs to avoid their transmission into vector particles and to reduce the production of RCL in vector preparations. The transfer vector plasmid contains the transgene(s) and all of the essential cis-acting elements (LTRs, ψ and RRE) for packaging/reverse transcription/integration), but expresses no HIV proteins. Since the transactivator Tat is not encoded by the transfer genome, the promoter activity by the 5'-LTR is minimal. Instead, transfer genomes use an internal promoter to express transgenes in transduced cells. Biochem. J. (2012)

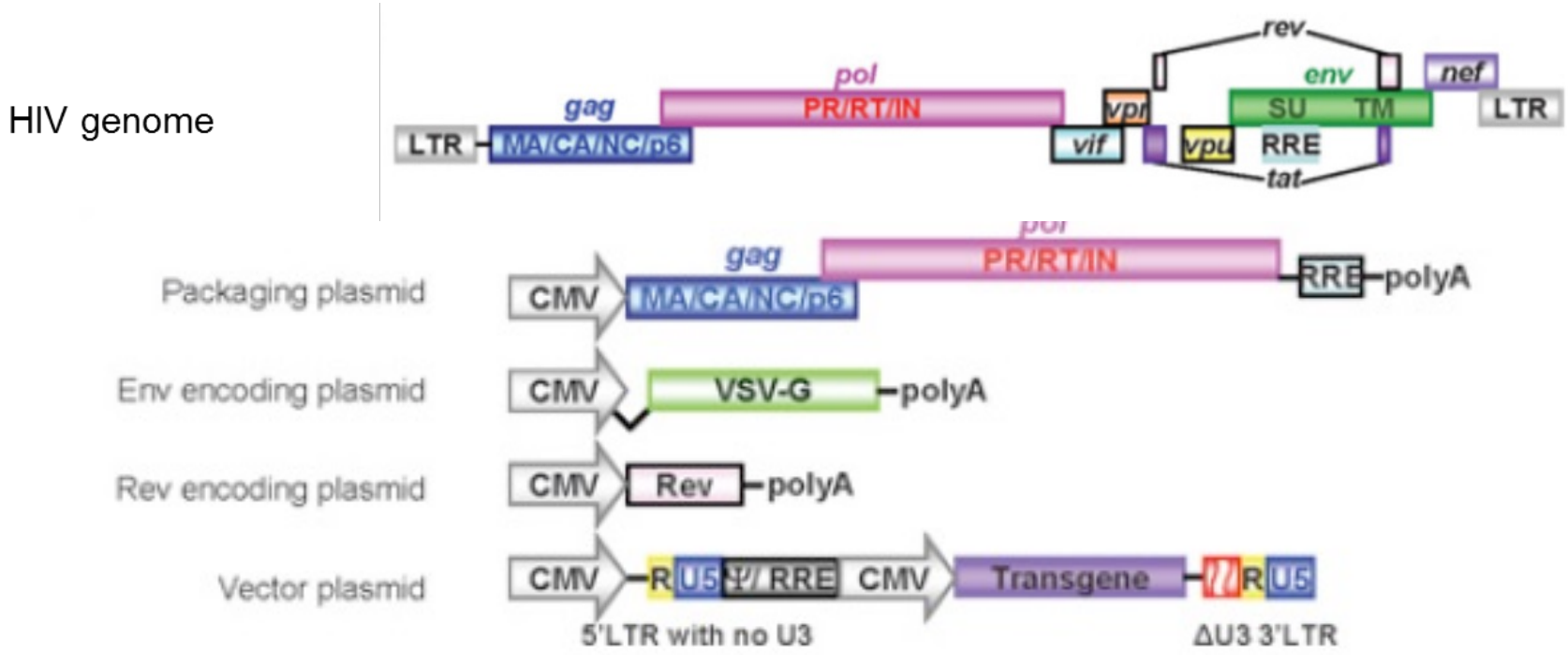
Second generation lentiviral vectors



To increase safety further, second-generation vectors have been developed by modifying accessory genes in the system. Although HIV-1 Vif, Vpu, Vpr and Nef are important for HIV as a pathogen, they can be deleted in second-generation lentivectors. By replacing HIV-1 Env with VSVG, these second-generation vectors include only four of the nine HIV genes: gag, pol, tat and rev

Biochem. J. (2012)

Third generation lentiviral vectors



To increase safety, third-generation vectors have been designed to be Tat-independent with Rev provided from a separate plasmid Tat-independence is achieved by replacing the U3 promoter region of the 5'-LTR in the transfer vector with strong viral promoters from CMV or RSV (rous sarcoma virus). The four plasmids used to generate third-generation vectors are: (i) a packaging construct containing only gag and pol genes; (ii) a plasmid expressing Rev; (iii) an Env plasmid; and (iv) a transgene plasmid driven by a heterologous strong promoter. The enhancer/promoter region (U3) of 3'-LTR is also removed to add the SIN property.

Biochem. J. (2012)

Transfer vector

RSV-R-U5: 5’ LTR chimera
Ψ-Δgag: packaging sequence, including DIS (dimerization-iniziation site)
RRE: Rev responsive element
cPPT/cTS: creation of a plus strand overlap: the central DNA flap
WPRE: Woodchuck Hepatitis Virus Post-transcriptional Regulatory Element
ΔU3: the enhancer/promoter region (U3) of 3’-LTR is removed to add the SIN property.

Transfer vector