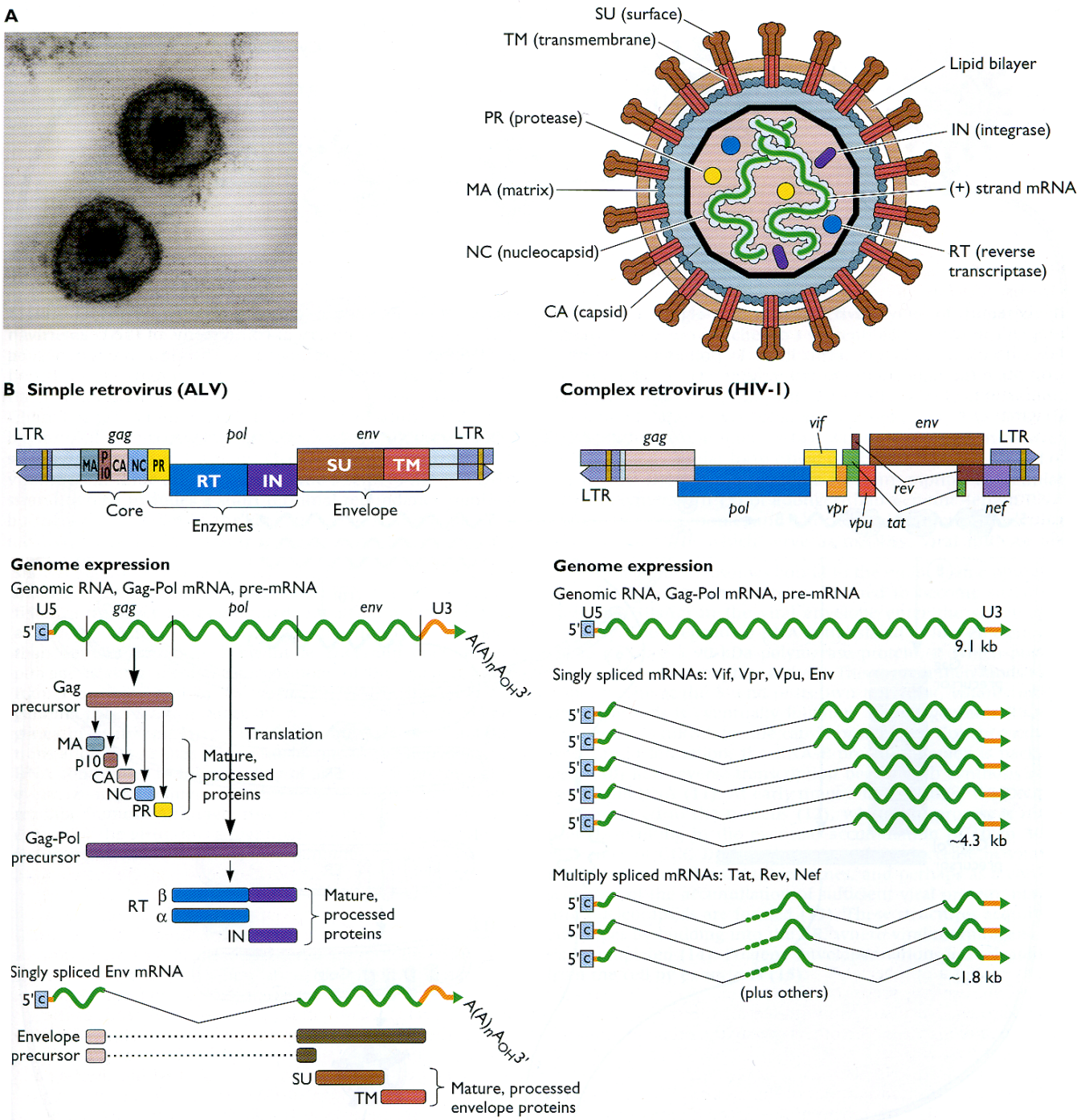
VIROLOGY

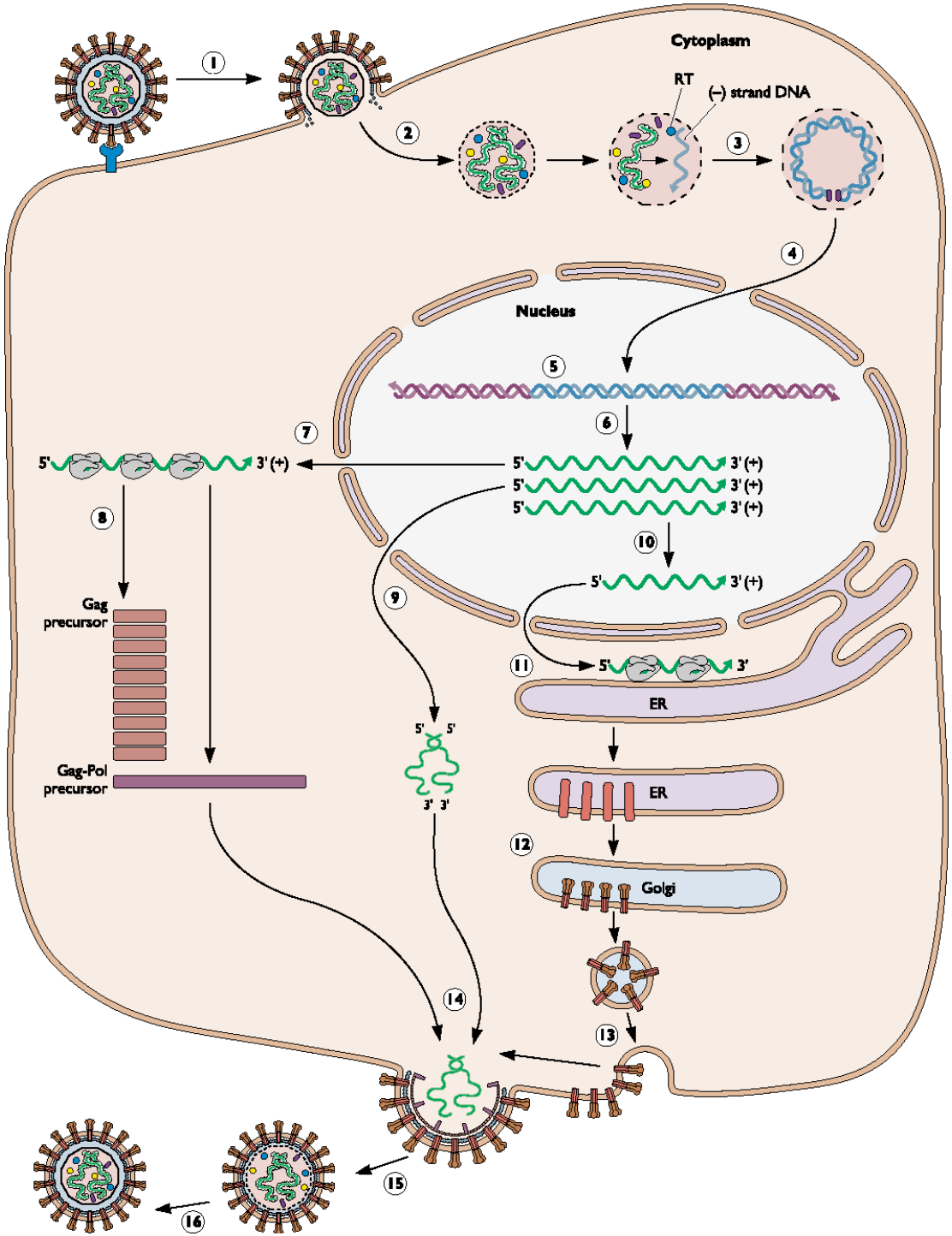
Engineering Viral Genomes: Retrovirus Vectors

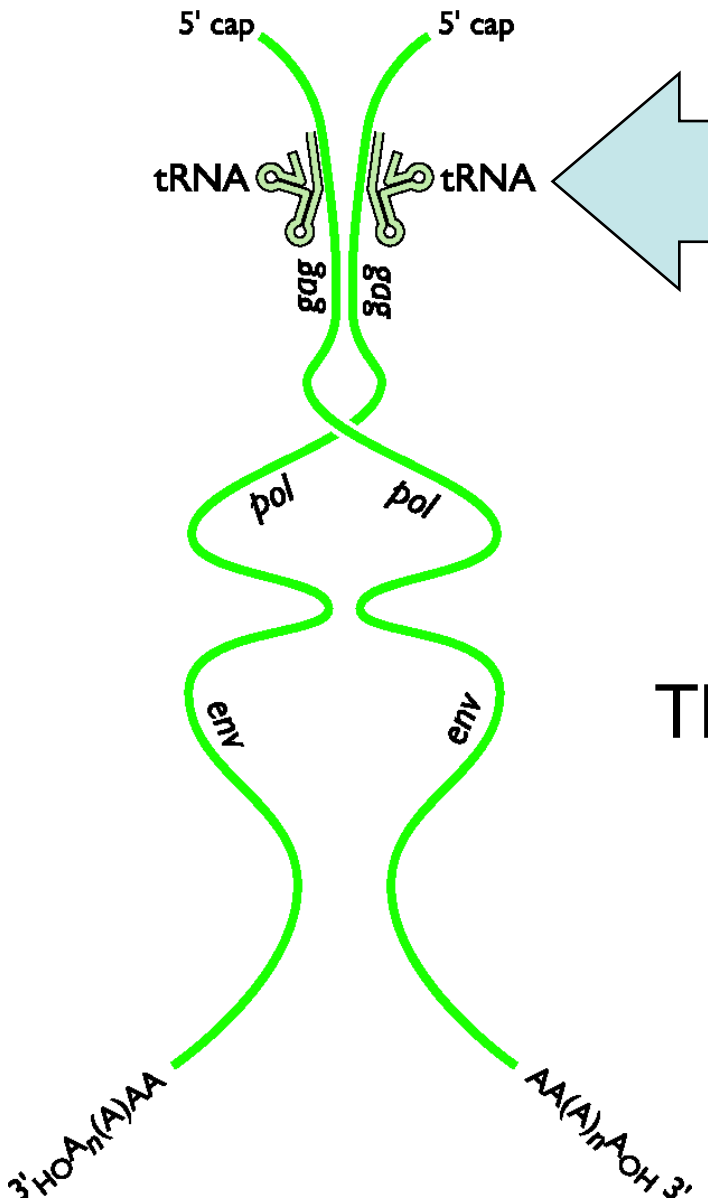
Viral vectors

Virus	Insert size	Integration	Duration of expression	Advantages	Disadvantages
Adeno-associated virus	~4.5–9 (?) kb	Low efficiency	Long	Nonpathogenic, episomal, infects nondividing cells	Immunogenic, toxicity
Adenovirus	2–38 kb	No	Short	Efficient gene delivery	Transient, immunogenic
Alphavirus	~5 kb	No	Short	Broad host range, high-level expression	Virulence
Herpes simplex virus	~30 kb	No	Long in central nervous system, short elsewhere	Neurotropic, large capacity	Virulence, persistence in neurons
Influenza virus	Unknown	No	Short	Strong immune response	Virulence
Lentivirus	7–18 kb	Yes	Long	Stable integration; infects nondividing and terminally differentiated mammalian cells	Insertional mutagenesis
Poliovirus	~300 bp for helper-free virus; ~3 kb for defective virus	No	Short	Excellent mucosal immunity	Limited capacity, reversion to neurovirulence
Retrovirus	1–7.5 kb	Yes	Shorter than formerly believed	Stable integration	May rearrange genome, insertional mutagenesis, require cell division
Rhabdovirus	Unknown	No	Short	High-level expression, rapid cell killing	Virulence, highly cytopathic
Vaccinia virus	At least ~25 kb, probably ~75–100 kb	No	Short	Wide host range, ease of isolation, large capacity, high-level expression	Transient, immunogenic


A
SU (surface)
TM (transmembrane)
Lipid bilayer
PR (protease)
IN (integrase)
MA (matrix)
(+) strand mRNA
NC (nucleocapsid)
RT (reverse transcriptase)
CA (capsid)
B Simple retrovirus (ALV)
LTR
gag
pol
env
LTR
MA
p10
CA
NC
PR
RT
IN
SU
TM
Core
Enzymes
Envelope
Complex retrovirus (HIV-1)
gag
vif
env
LTR
LTR
rev
pol
vpr
vpu
tat
nef
Genome expression
Genomic RNA, Gag-Pol mRNA, pre-mRNA
U5
gag
pol
env
U3
5'
c
A(A)mOH3'
Gag precursor
MA
p10
CA
NC
PR
Translation
Mature, processed proteins
Gag-Pol precursor
RT
β
α
IN
Mature, processed proteins
Singly spliced Env mRNA
Envelope precursor
SU
TM
Mature, processed envelope proteins
Genome expression
Genomic RNA, Gag-Pol mRNA, pre-mRNA
9.1 kb
Singly spliced mRNAs: Vif, Vpr, Vpu, Env
~4.3 kb
Multiply spliced mRNAs: Tat, Rev, Nef
~1.8 kb
(plus others)

Retrovirus replicative cycle



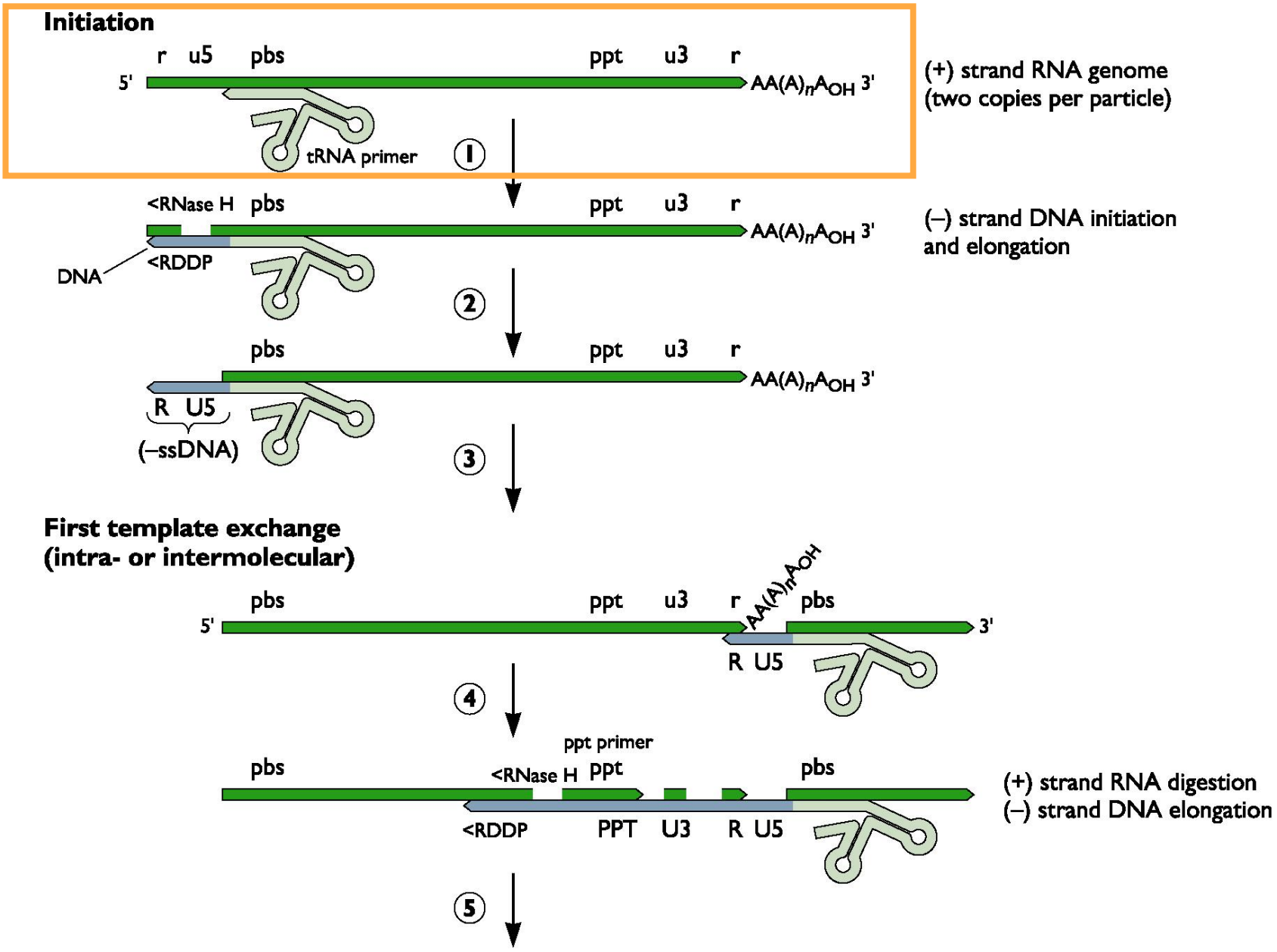


Most mammalian retroviruses use $tRNA^{PRO}$, $tRNA^{Lys3}$, $tRNA^{Lys1,2}$

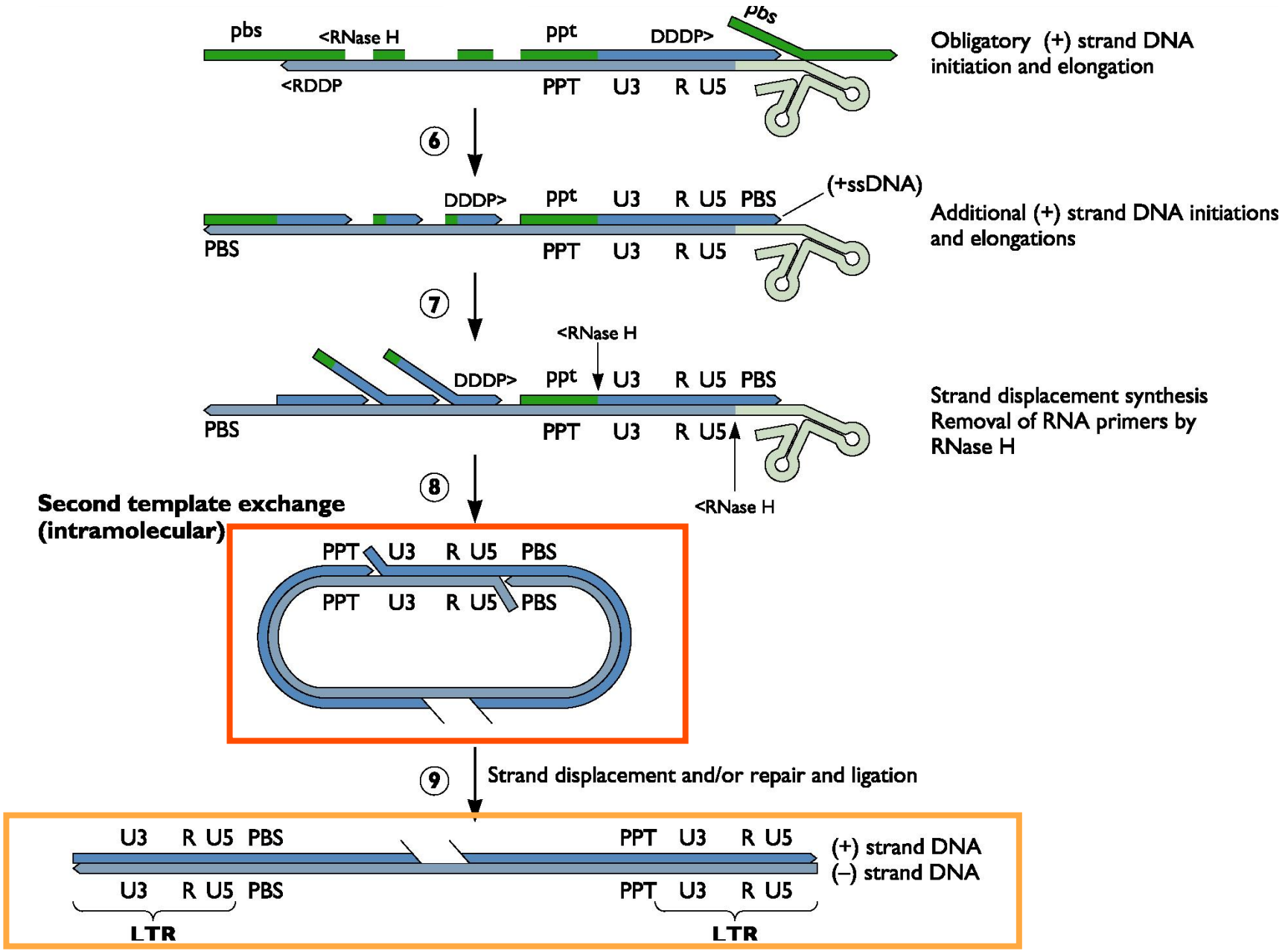
The partially unfolded tRNA is annealed via at least 18 nt at its 3' end to a site on RNA genome called the **primer-binding site (pbs)**

The diploid retroviral genome

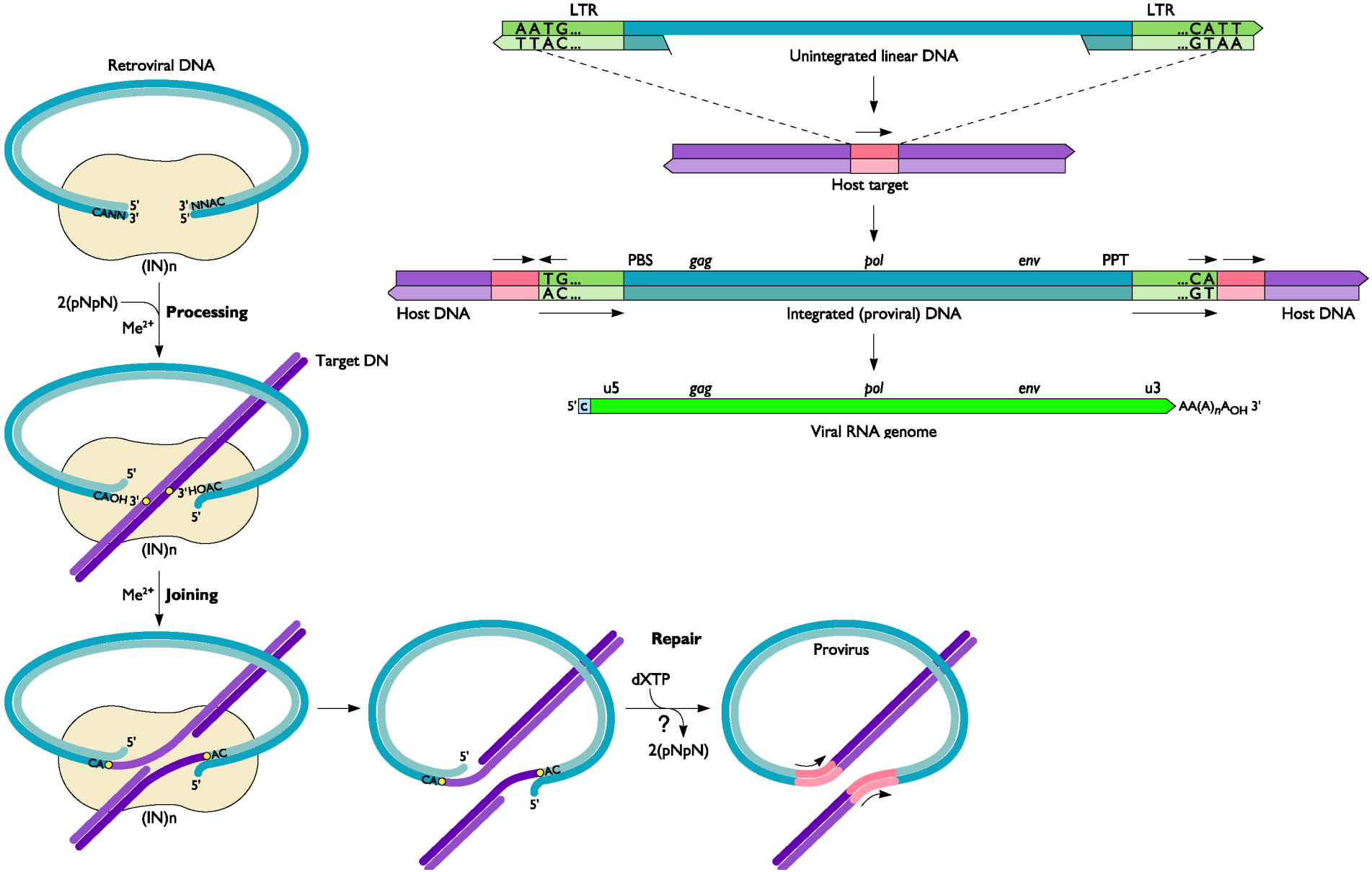
The reverse transcription process -1



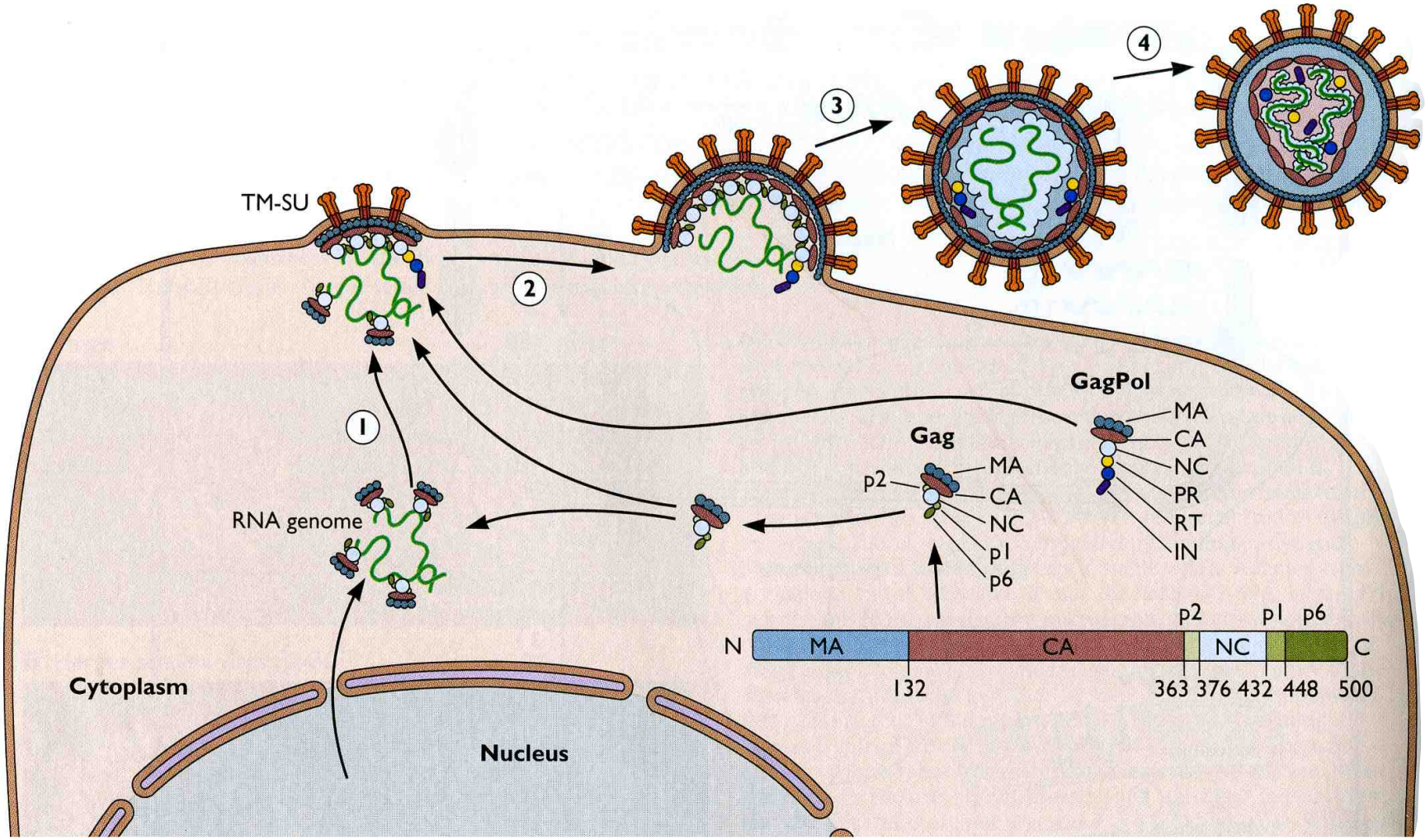
The reverse transcription process -2



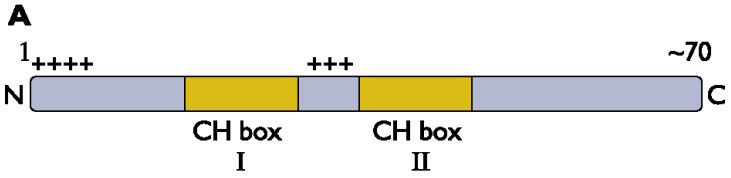
Characteristics of retroviral integration

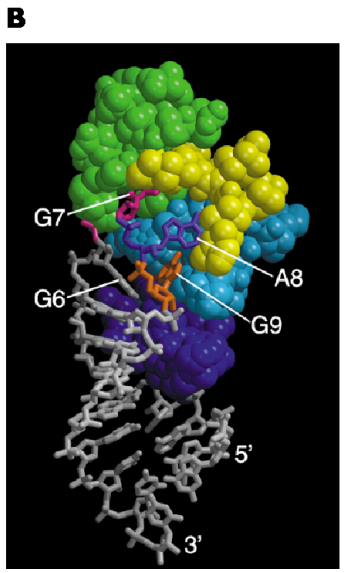


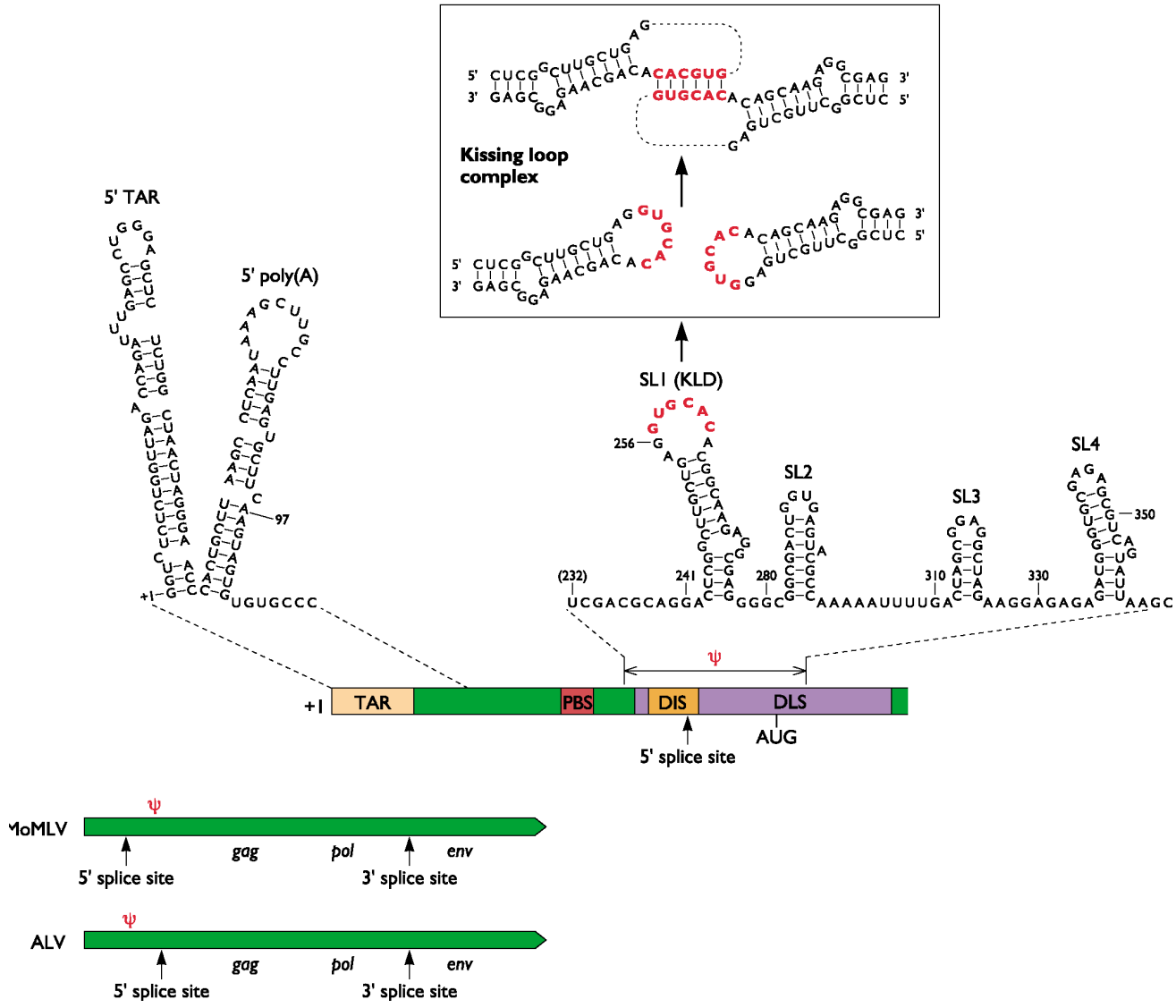
Assembly of a retrovirus from polyprotein precursors



Sequences important in packaging of retroviral genomes







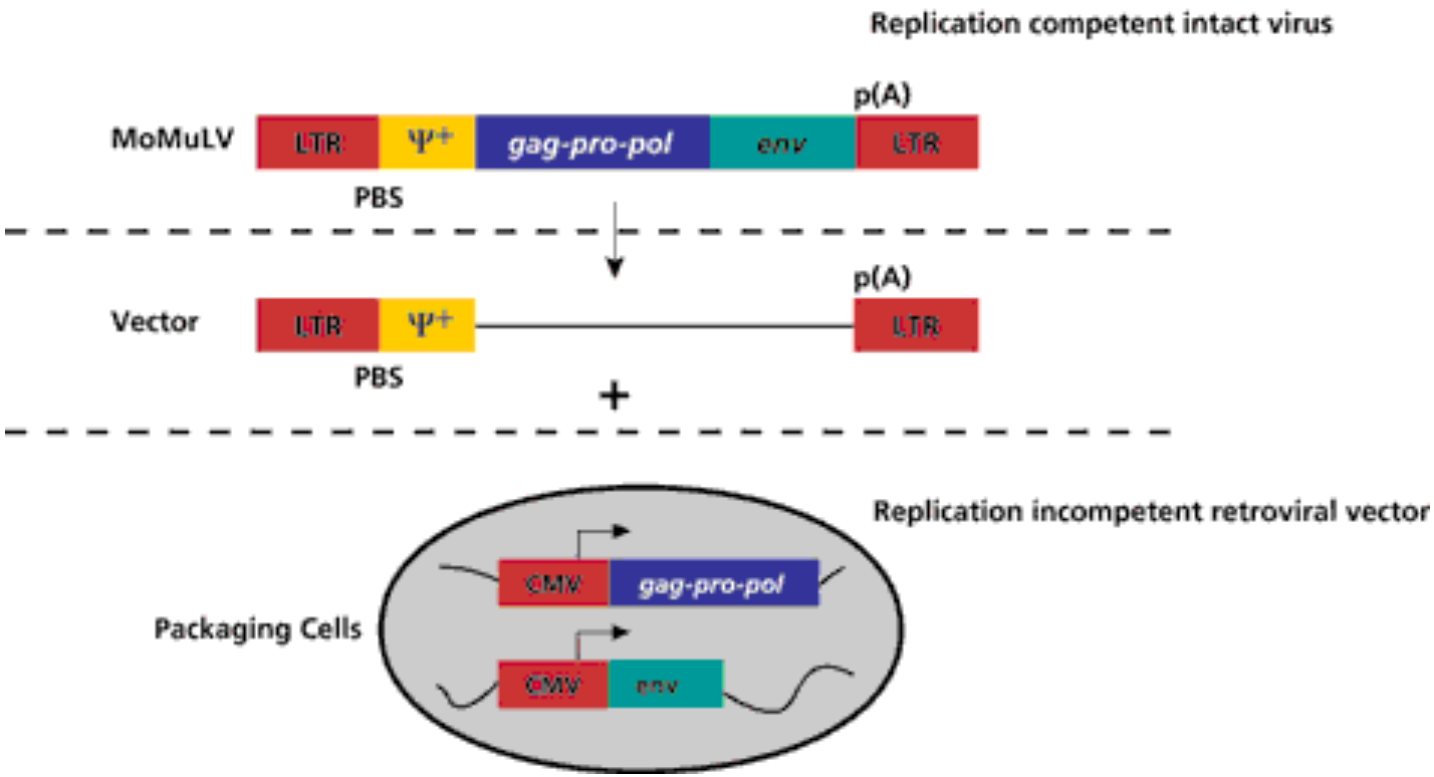
Favorable Features of Retroviruses as Vectors

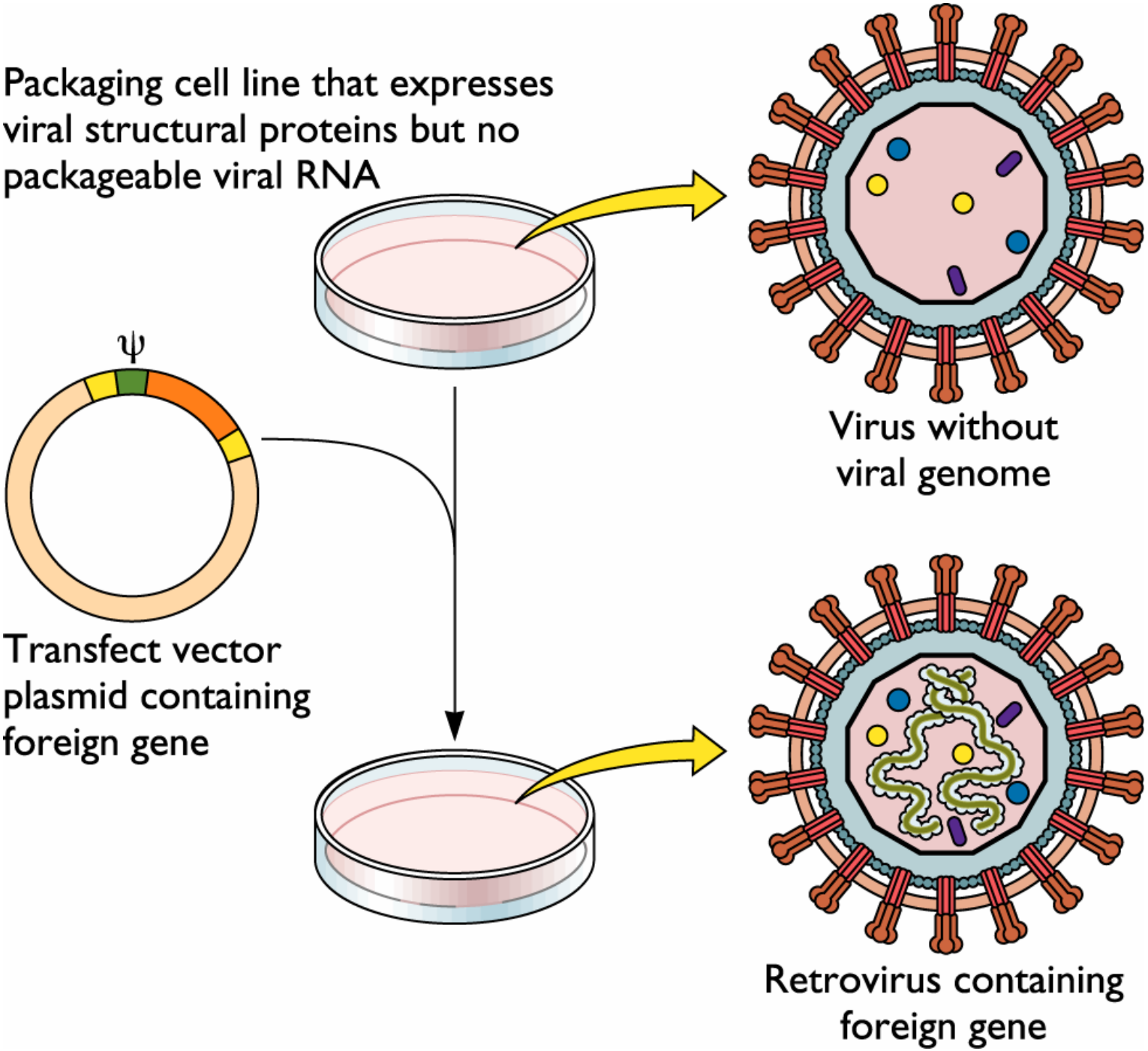
- Well characterized
- Easily to manipulate (genomes 7-9 kb)
- They require 3 trans (gag, pol, env) and 7 major cis-active control elements (U3, R, U5, PBS, SD, Ψ, SA) in order to replicate
- Stability of recombinants vectors (plasmids)
- High efficiency of gene transfer
- Most are replication-defective
- Stable and precise integration of the transgene
- Low immunogenicity
- Can be pseudotyped to infect a broad range of cells

Retrovirus as vectors

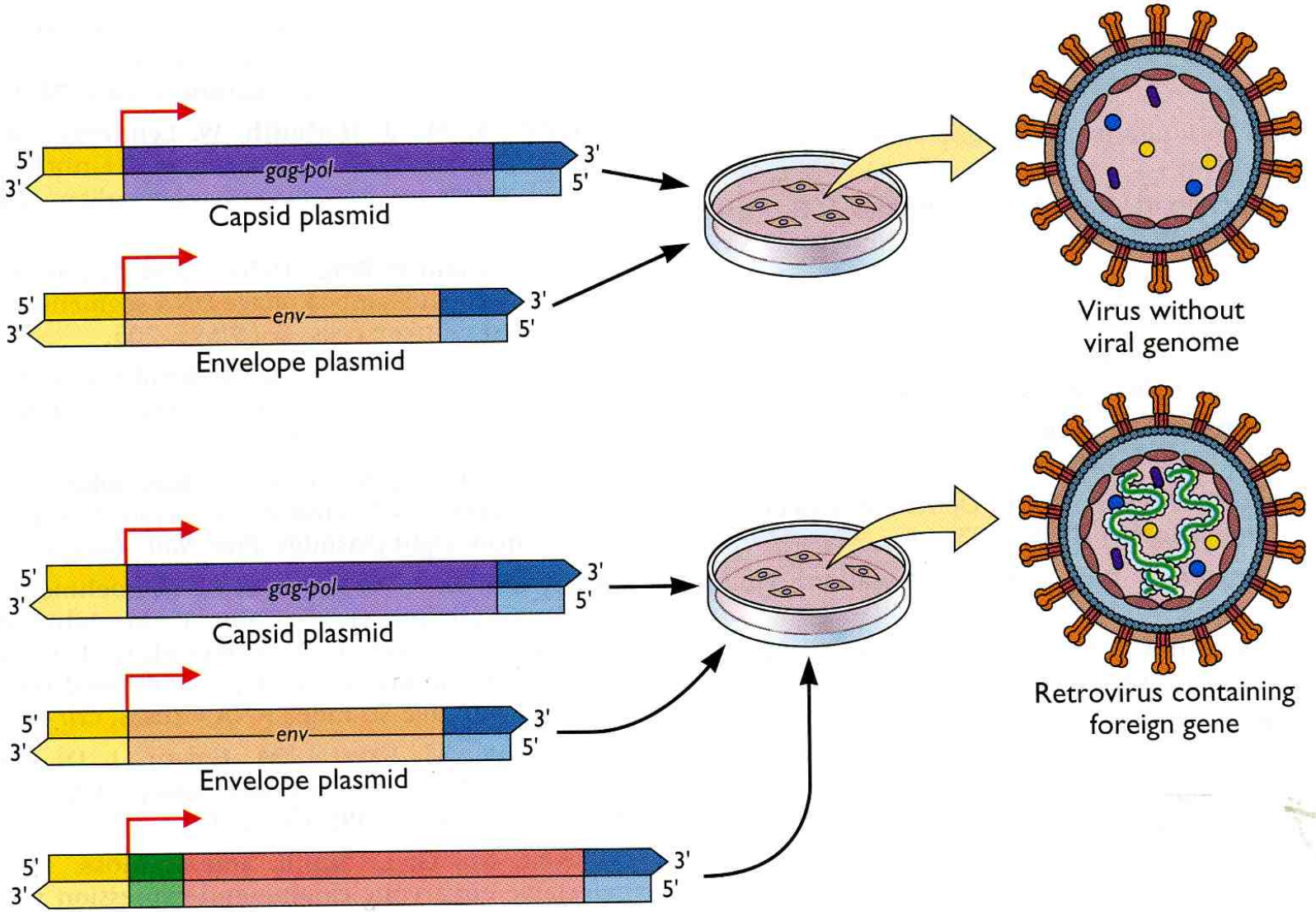
- Replication-incompetent vectors
  - -They bear deletions of some or all of the viral genes
  - -They retain *cis*-acting viral sequences necessary for transmission (U3, R, U5, PBS, SD, Ψ, SA)
  - -They need to be propagated in "packaging" cell lines that provide in *trans* gag, pol, env
- Replication-competent vectors (RCR)
  - -Avian vectors (up to 2 kb inserts)

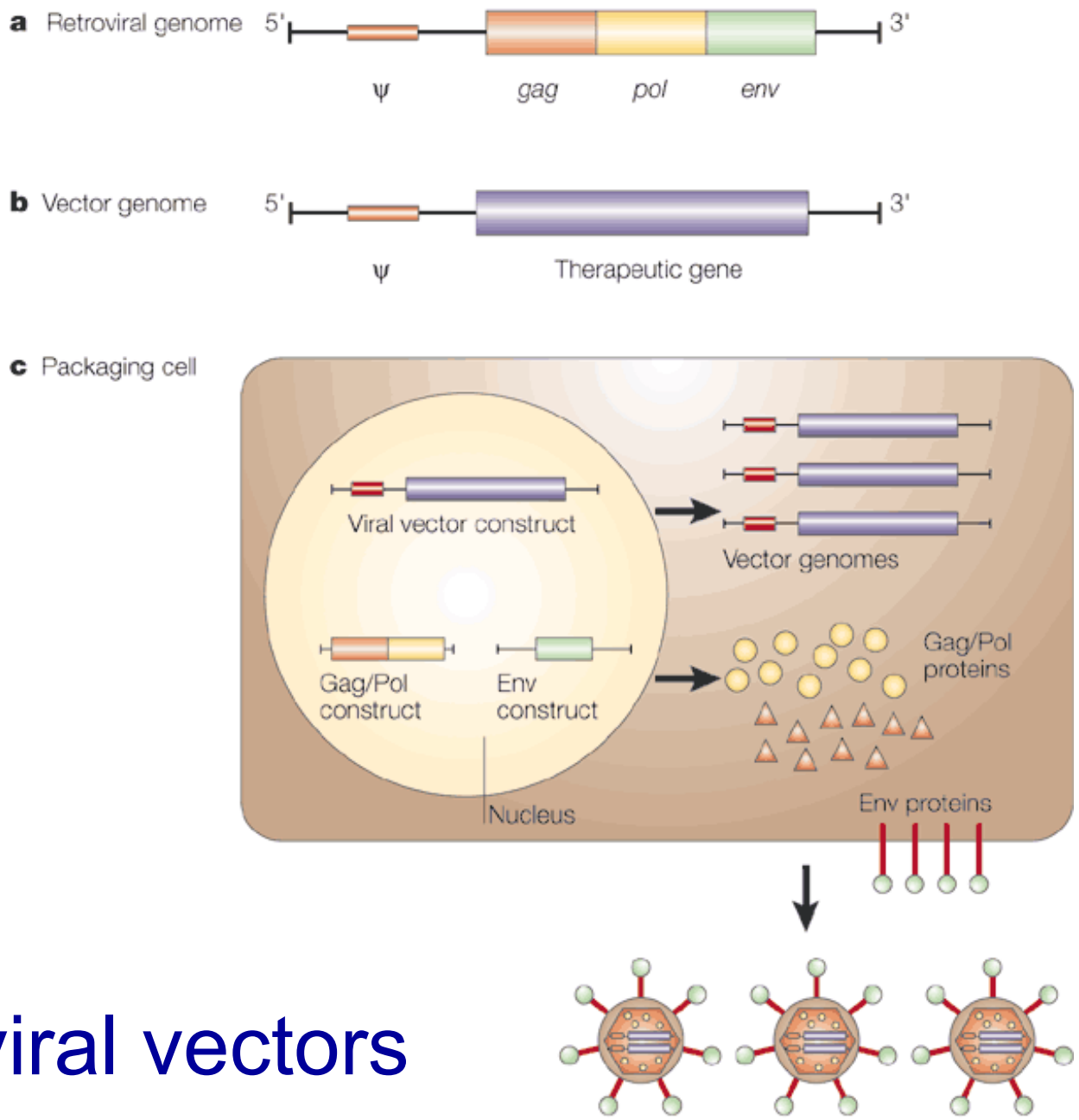
Replication-incompetent vectors




Packaging cell line that expresses viral structural proteins but no packageable viral RNA
ψ
Transfect vector plasmid containing foreign gene
Virus without viral genome
Retrovirus containing foreign gene

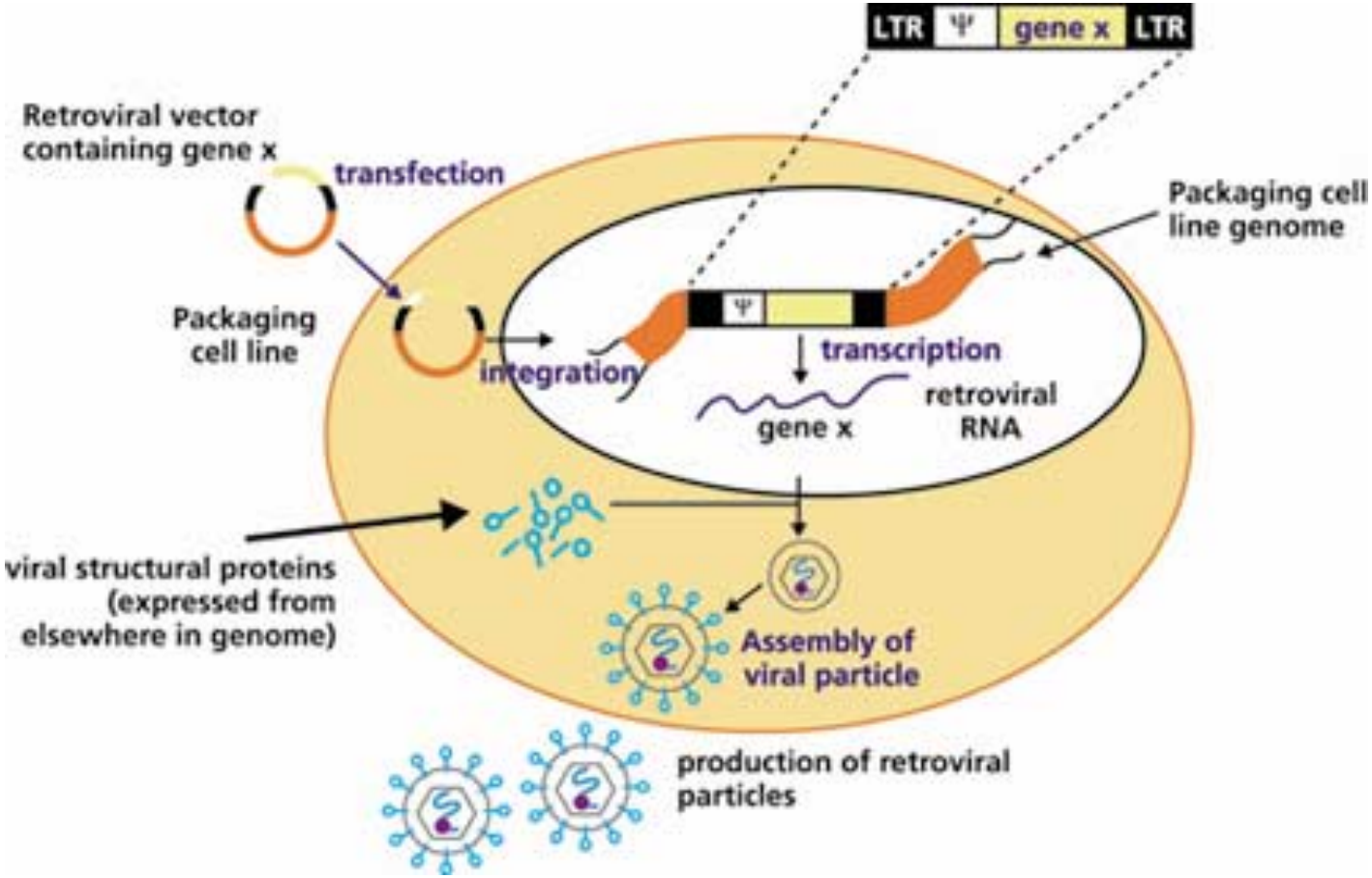
Retroviral vectors



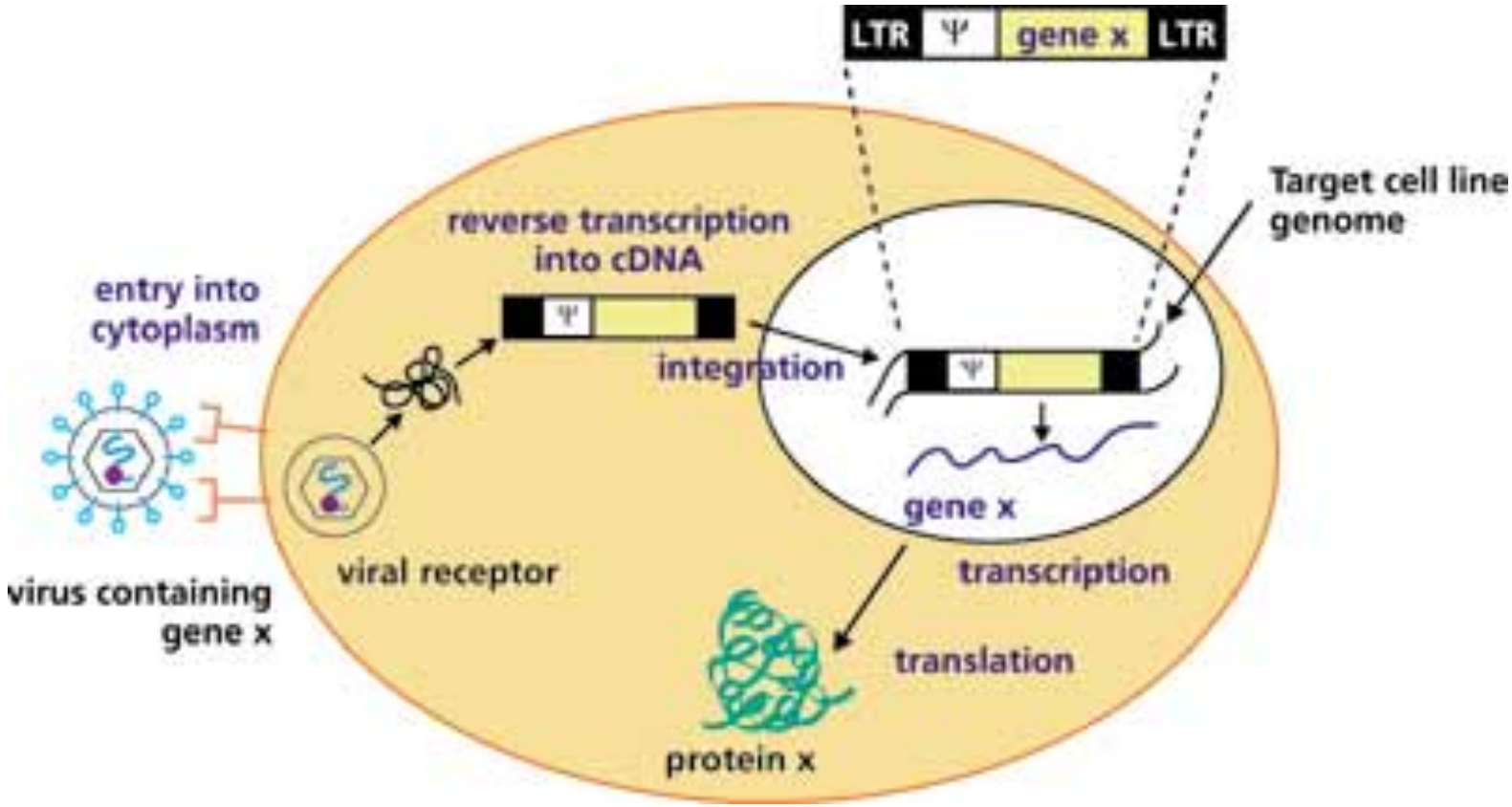


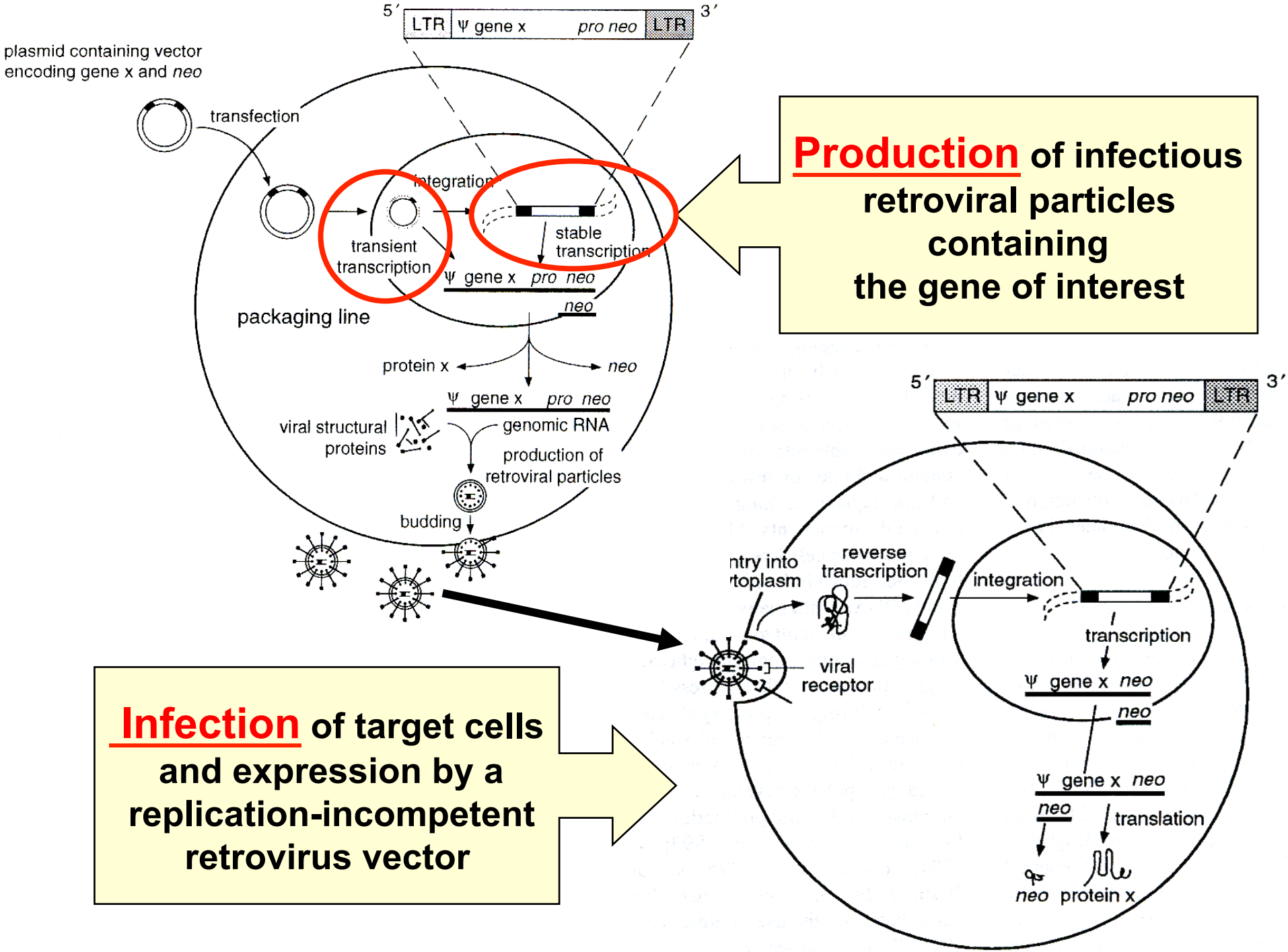
Retroviral vectors

Production of Recombinant Retrovirus in the Packaging Cell



Infection of a Target cell and Expression by a Replication-Incompetent Retrovirus Vector




plasmid containing vector
encoding gene x and *neo*
transfection
5′
LTR
ψ gene x
pro neo
LTR
3′
integration
transient
transcription
stable
transcription
ψ gene x *pro neo*
neo
packaging line
protein x
neo
ψ gene x *pro neo*
viral structural
proteins
genomic RNA
production of
retroviral particles
budding
Production of infectious retroviral particles containing the gene of interest
5′
LTR
ψ gene x
pro neo
LTR
3′
ntry into
toplasm
reverse
transcription
integration
transcription
viral
receptor
ψ gene x *neo*
neo
ψ gene x *neo*
neo
translation
neo
protein x
Infection of target cells and expression by a replication-incompetent retrovirus vector

Tropism of Retrovirus Vectors

Retrovirus tropism is determined at three levels:

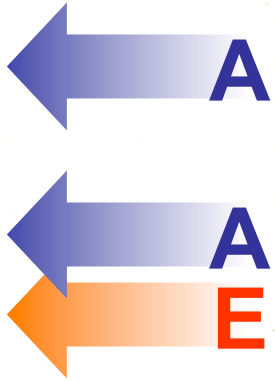
1) Viral envelope proteins (gpSU);

2) Nuclear translocation and integration
   -defined by structural features of $p30^{CA}$

3) Transcriptional activity of the LTR in the transfected cell
   - MLV LTR is active in most mammalian cell types

Retroviridae host-cell receptors and co-receptors

Human immunodeficiency virus type 1	CD4	Ig-like	Chemokine receptors (Ccr5, Cxcr4, Ccr3)
	Galactosylceramide	Glycolipid	
Human immunodeficiency virus type 2	CD4	Ig-like	Chemokine receptors
	Cxcr4	7-transmembrane superfamily	
Simian immunodeficiency virus	CD4	Ig-like	Chemokine receptors
Gibbon ape leukemia virus	Glvr1	Sodium-dependent phosphate transport protein	
Feline leukemia virus B	Glvr1	Sodium-dependent phosphate transport protein	
Amphotropic murine leukemia virus	Ram-1	Sodium-dependent phosphate transport protein	
Ecotropic murine leukemia virus	Cat	Cationic amino acid transport protein	
Subgroup A avian leukosis and sarcoma virus	Tva	Low-density lipoprotein receptor protein family	
Subgroup B and D avian leukosis and sarcoma viruses	Car1	Tnf receptor family protein superfamily	
Bovine leukemia virus	BLVRcp 1	Unknown	
Feline immunodeficiency virus	Cxcr4	7-transmembrane superfamily	
Visna virus	Major histocompatibility complex class II molecule	Ig-like	



The envelope determines which cells the retrovirus enter

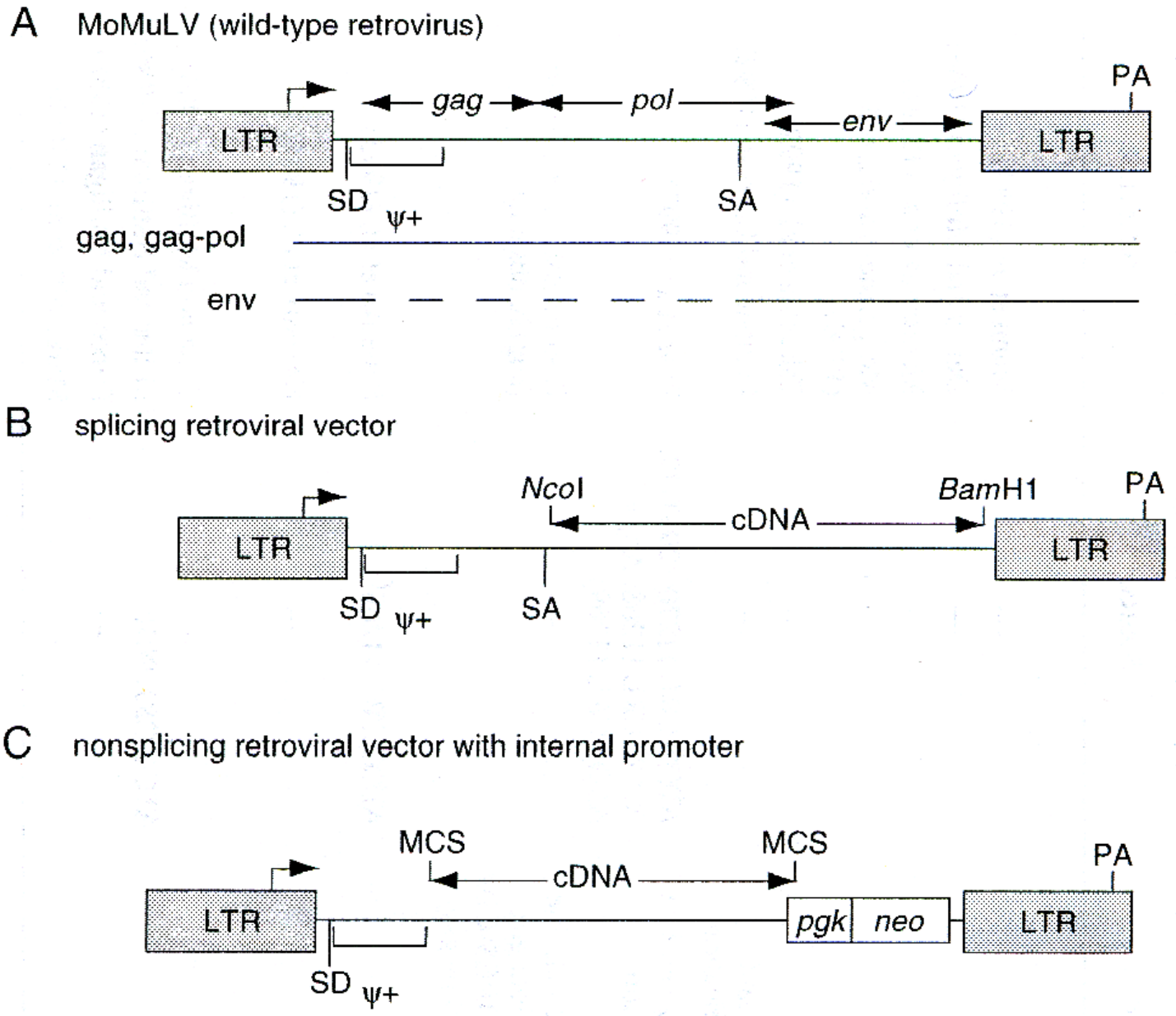
Host-range of MoMuLV-derived Vectors

- **Ecotropic** glycoprotein, gp70, allows infection of rat and mouse cells
- **Amphotropic** glycoprotein gp70 endows a murine virus with a very broad host range (mouse, human, chicken, dog, cat, mink cells)
- **Polytropic** receptors can be utilized for retrovirus entry. Pseudotyping the retroviral envelope with the VSV G protein confers a host range capable to infect mammalian, fish, frogs and insect cells

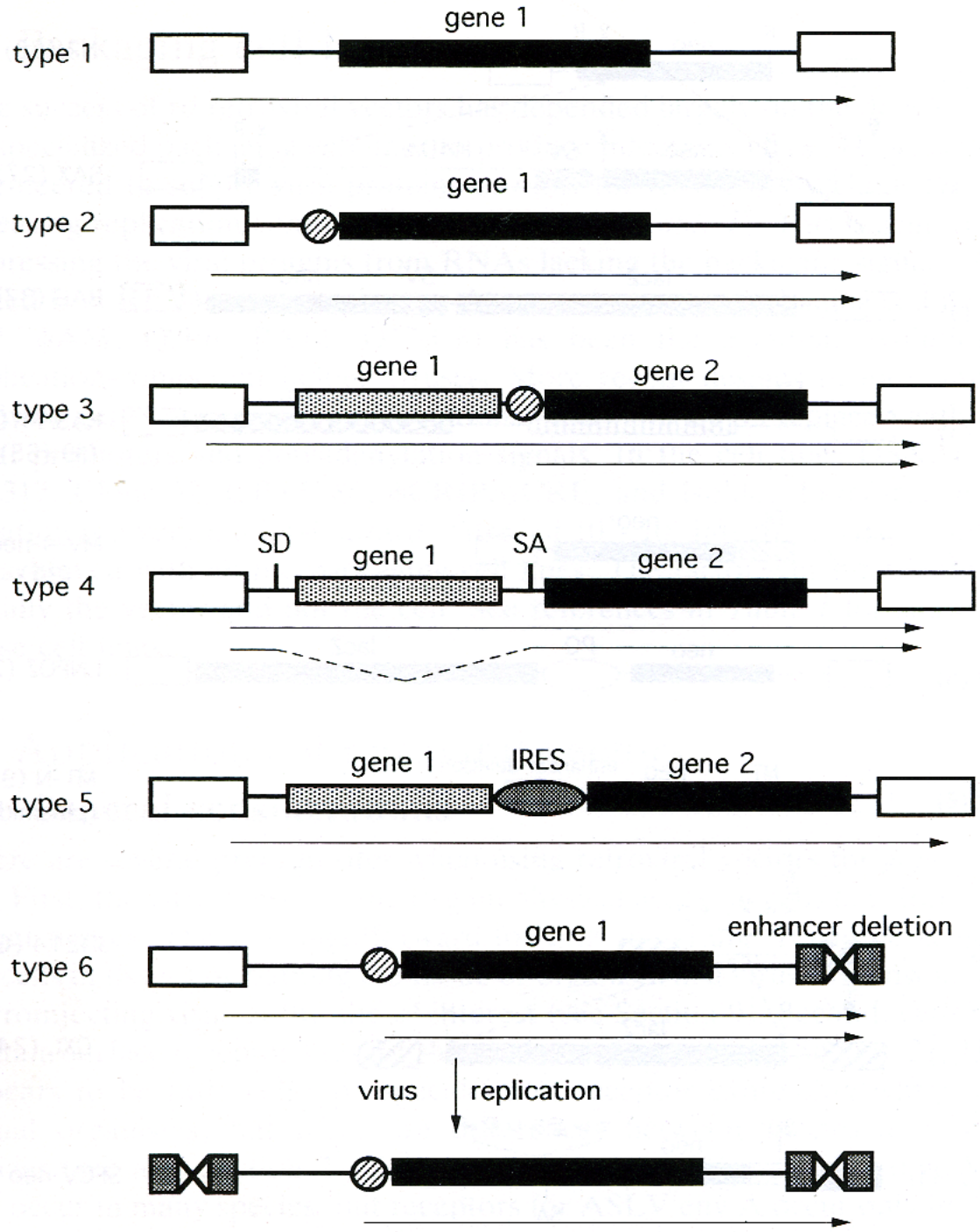
Host-range of Retroviral Vectors

Vector pseudotype	Cells that can be transduced	
	Mouse	Human
Ecotropic	Yes	No
Amphotropic	Yes	Yes
GALV	No	Yes
VSV G	Yes	Yes
RD114	No	Yes
10A1	Yes	Yes

Development of retroviral vector design



Replication-defective retroviral vectors

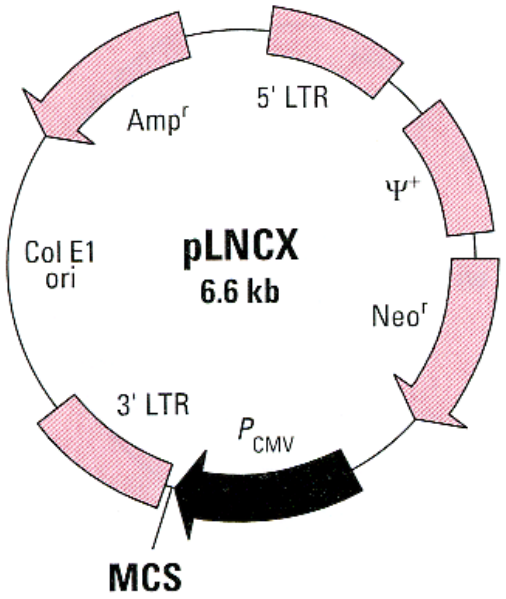


LTR

Internal promoter

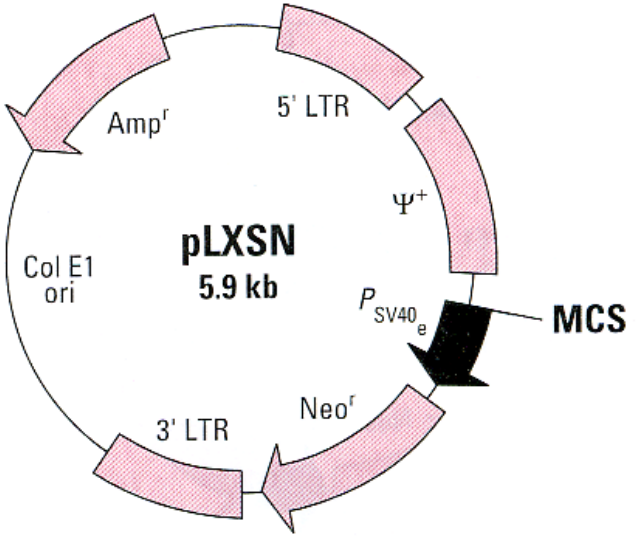
IRES

Retroviral expression vectors



GCGGCCCCAAGCTTGTTAACATCGATAAAATA

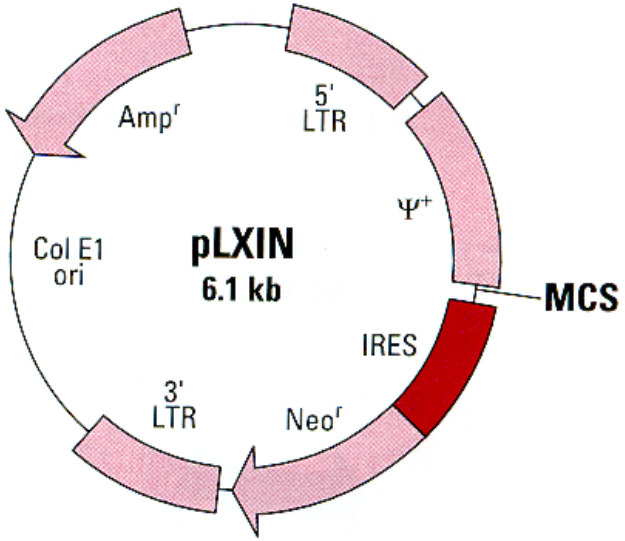
Hind III *Hpa* I *Cla* I

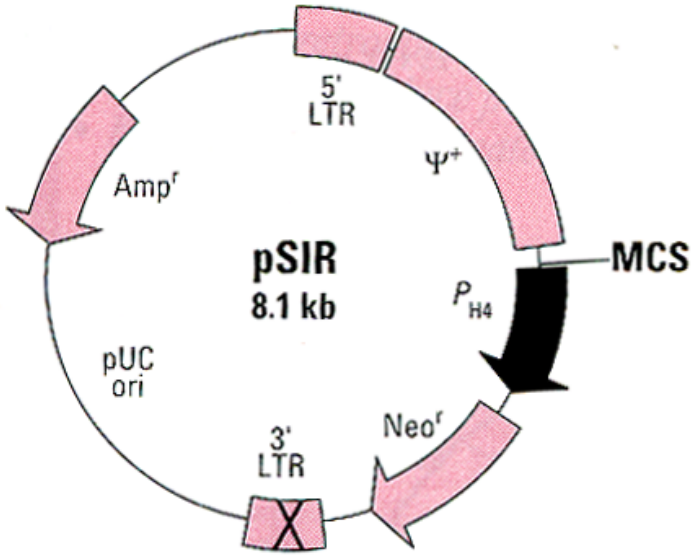


GCGCCGGAATTCGTTAACTCGAGGATCCGGCTGTG

*Eco*R I *Hpa* I *Xho* I *Bam*H I

Retroviral expression vectors



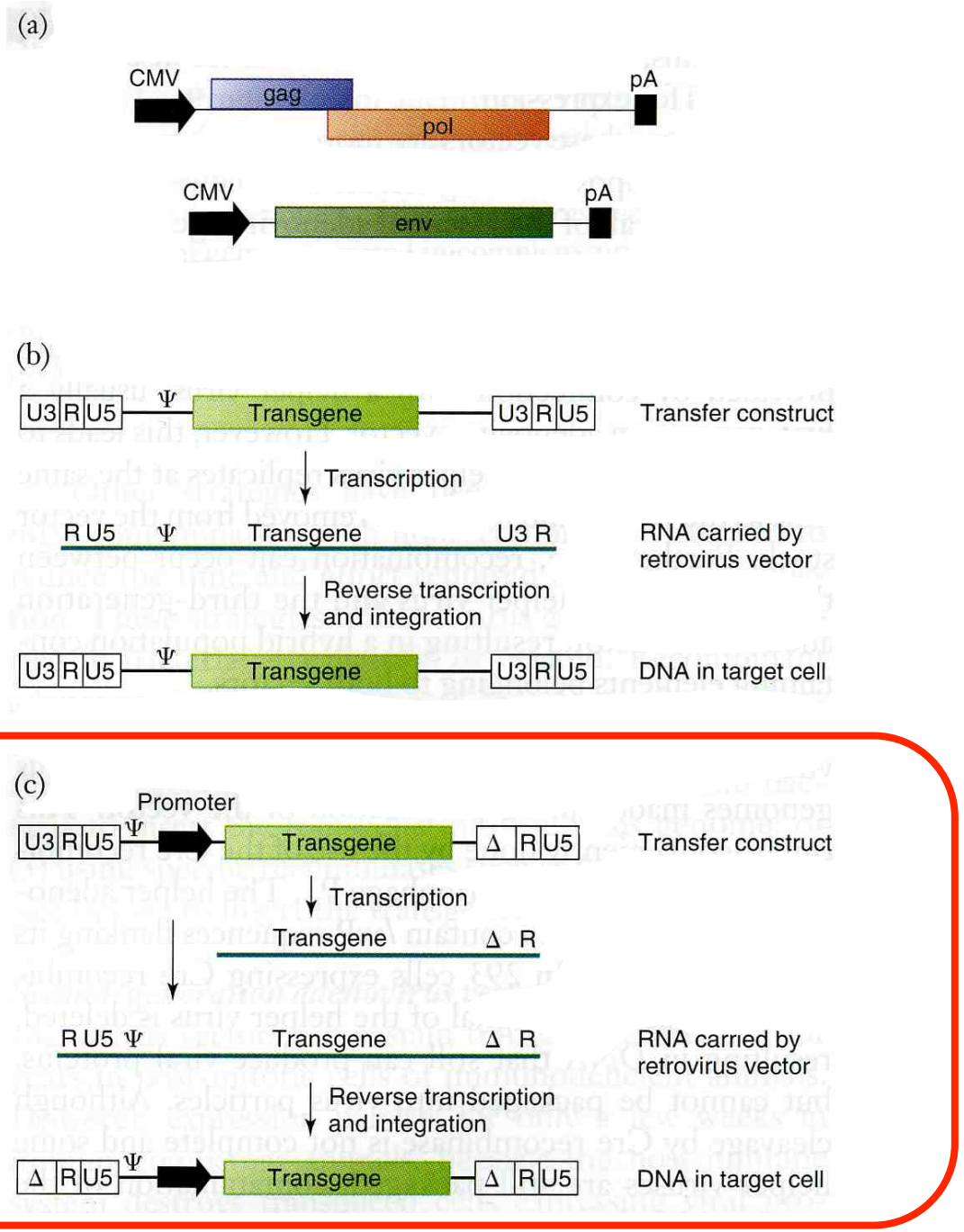


GGAATTCGTTAACTCGAGGATCCACTAGTAACGGCCGCCAGAATTCG

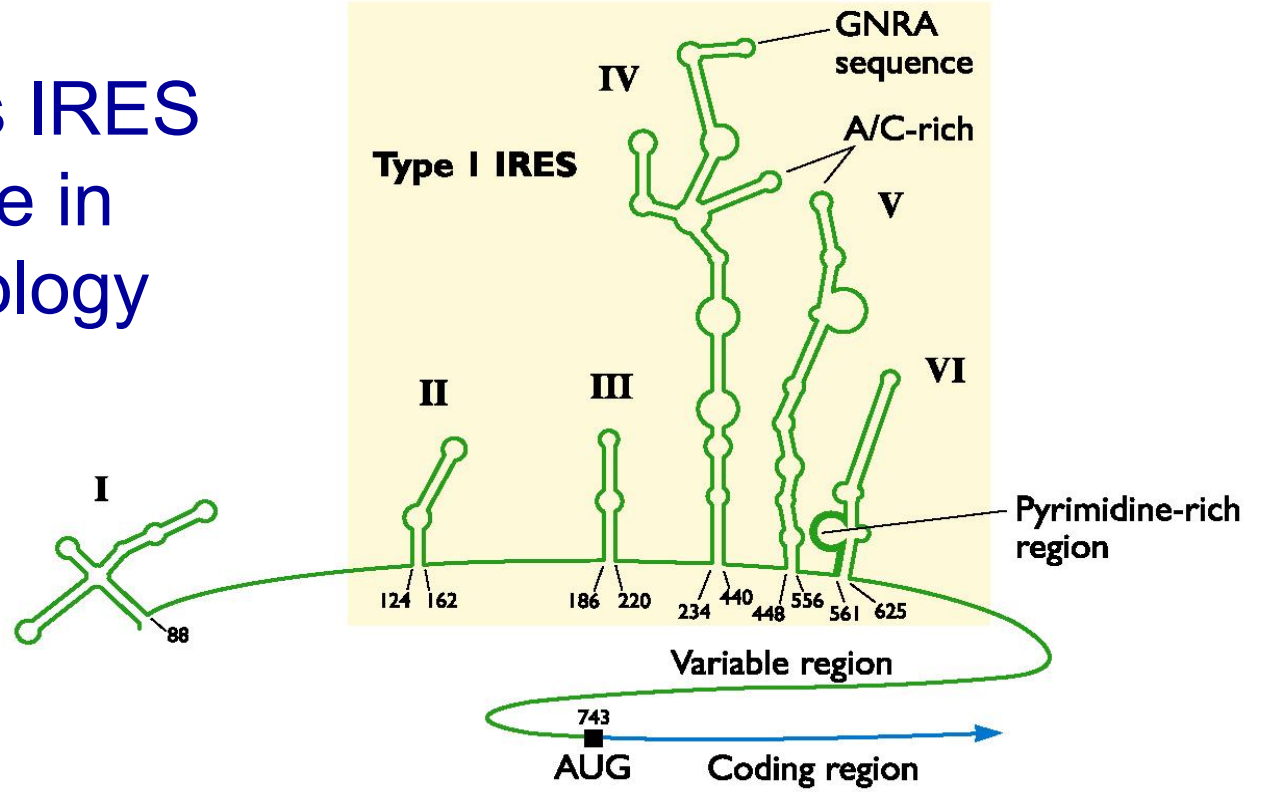
*Eco*R I *Hpa* I *Xho* I *Bam*H I *Eco*R I

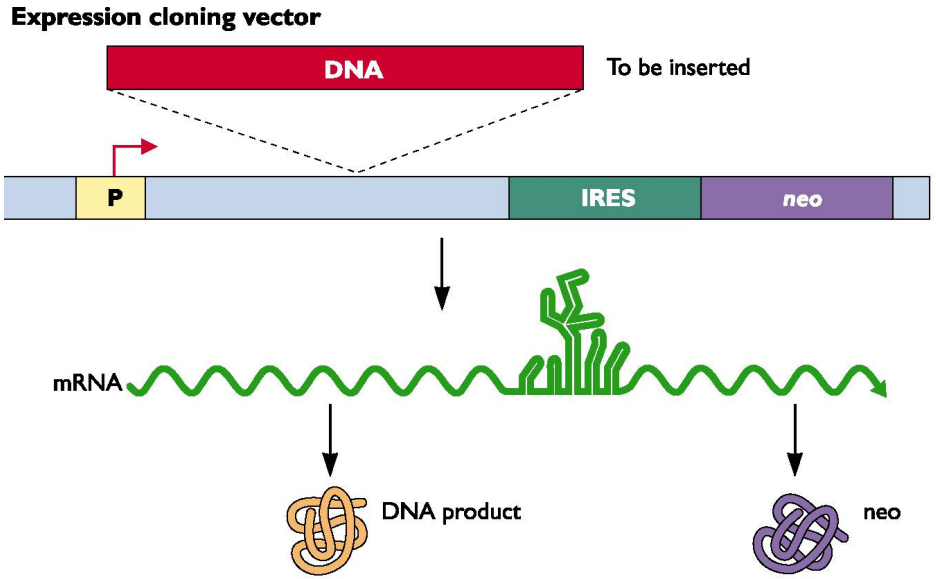
CCCCTCGAGAAGCTTGTCGACGGATCCGAATTC

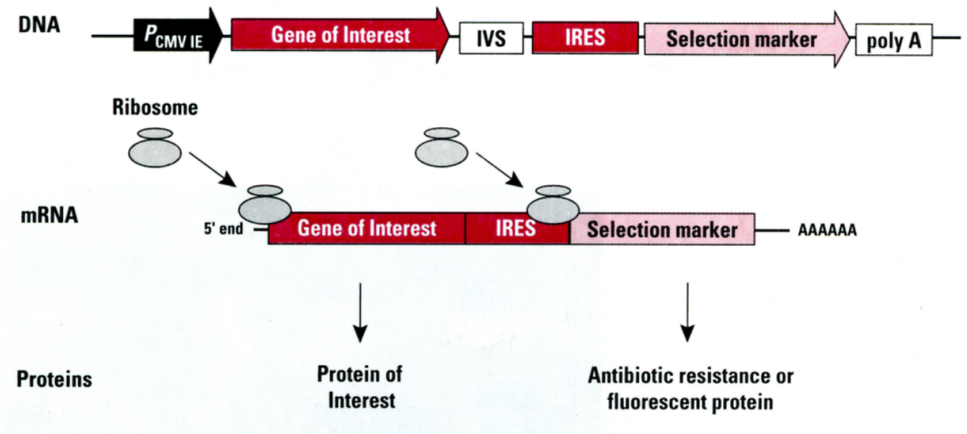
Xho I *Hind* III *Bam*H I *Eco*R I

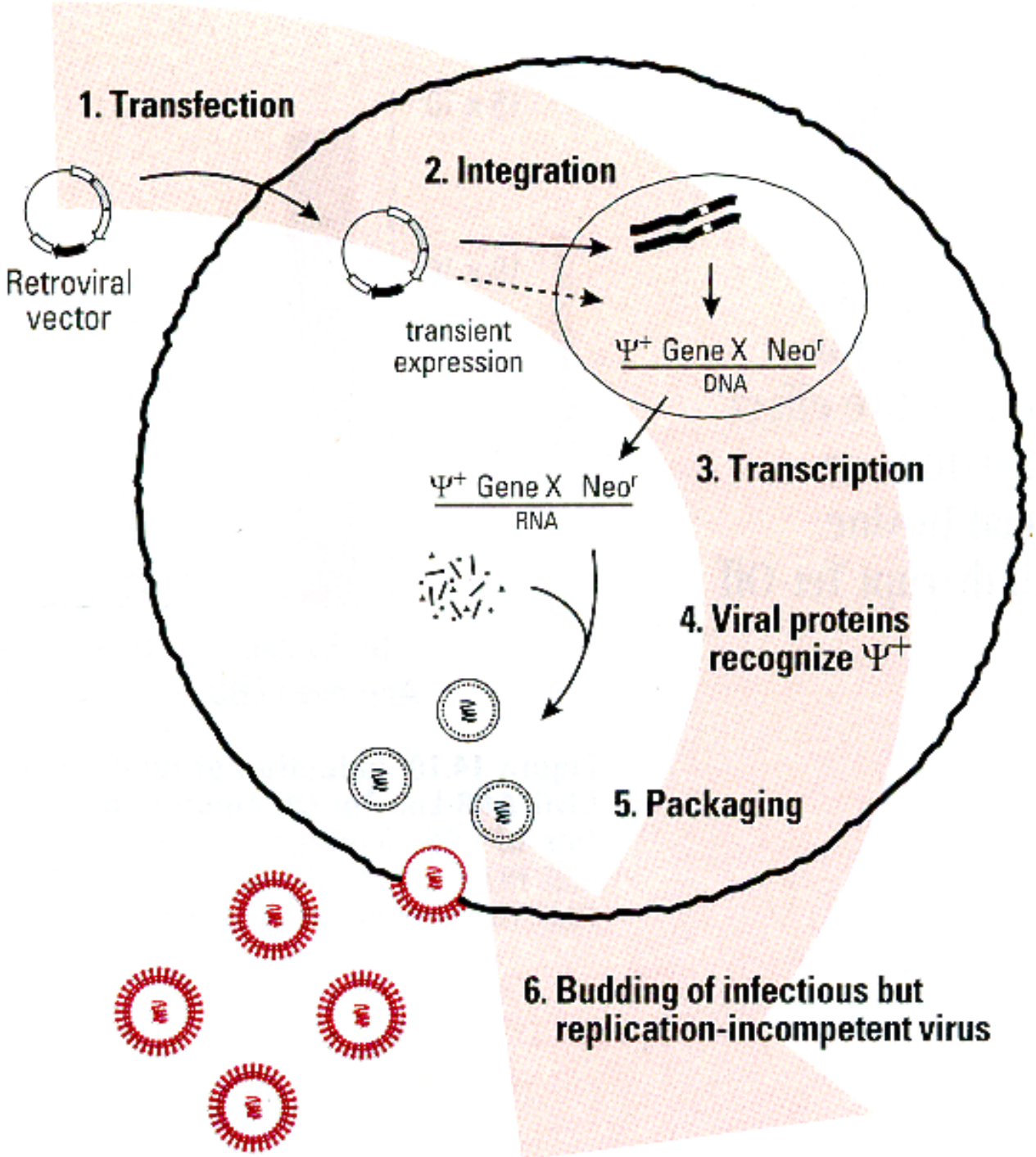

(a)
CMV
gag
pol
pA
CMV
env
pA
(b)
U3 R U5
Ψ
Transgene
U3 R U5
Transfer construct
Transcription
R U5
Ψ
Transgene
U3 R
RNA carried by retrovirus vector
Reverse transcription and integration
U3 R U5
Ψ
Transgene
U3 R U5
DNA in target cell
(c)
Promoter
U3 R U5
Ψ
Transgene
Δ R U5
Transfer construct
Transcription
Transgene
Δ R
R U5
Ψ
Transgene
Δ R
RNA carried by retrovirus vector
Reverse transcription and integration
Δ R U5
Ψ
Transgene
Δ R U5
DNA in target cell

Picornaviruses IRES and their use in molecular biology



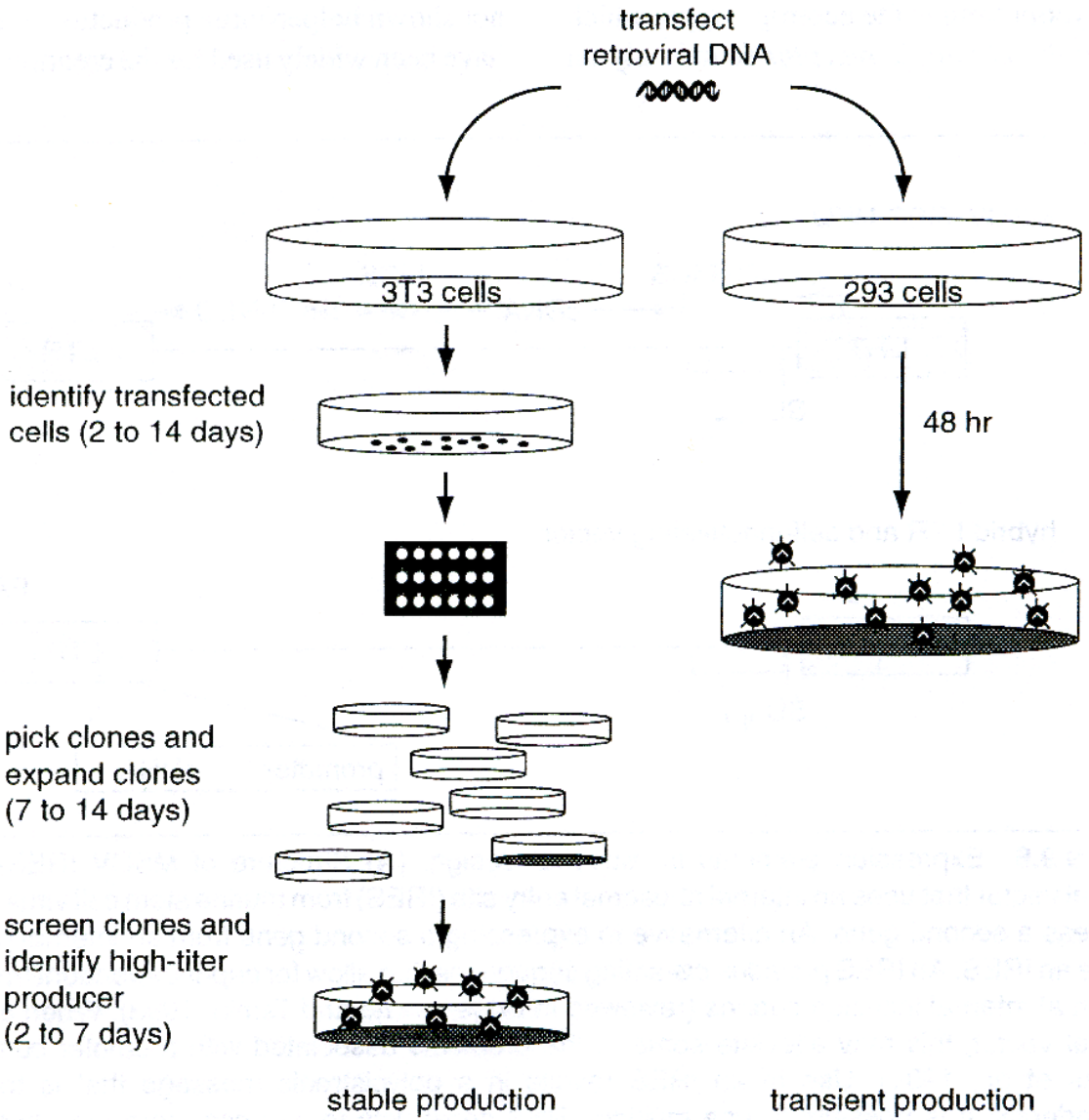


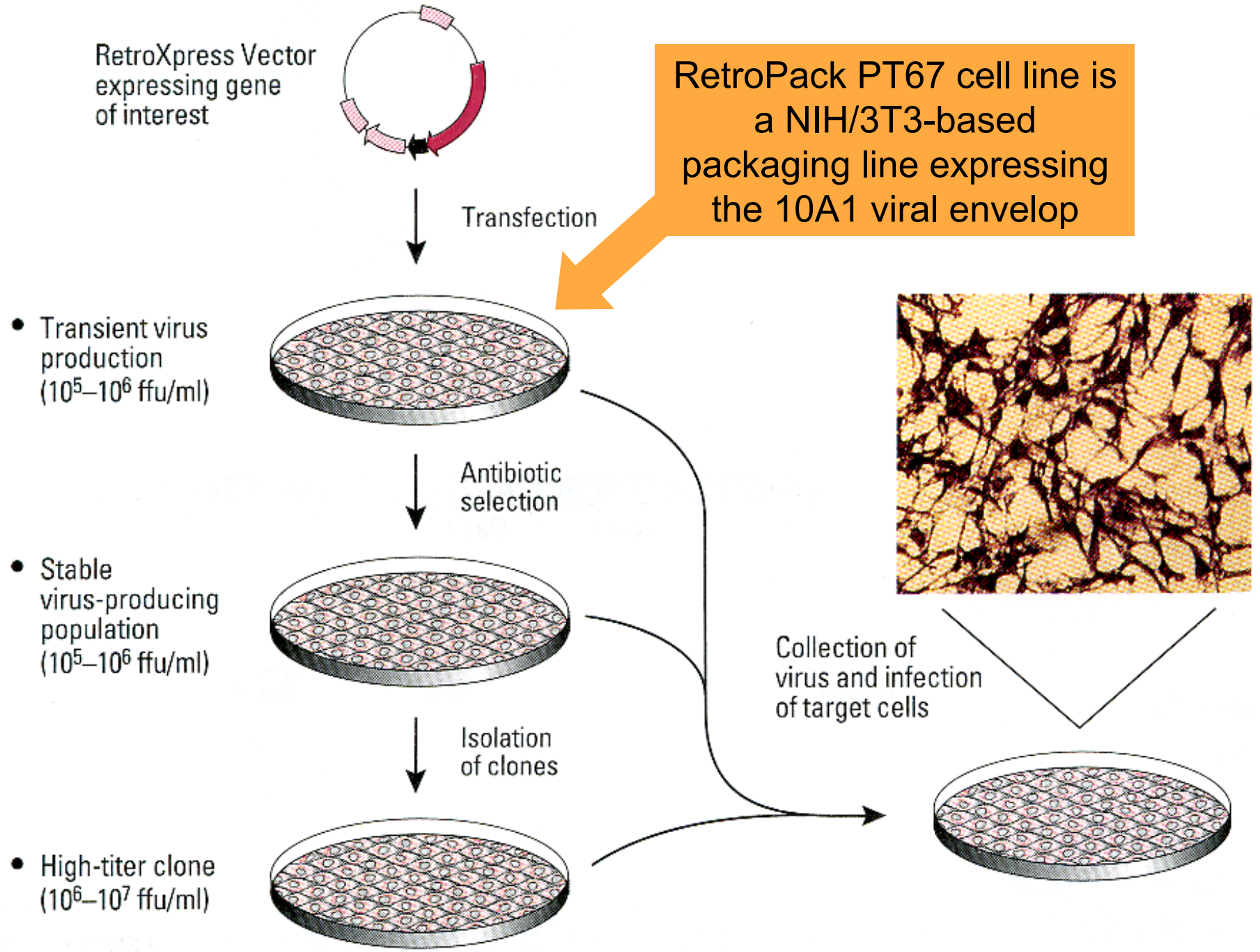




Packaging cell: produces viral proteins from stably integrated genes

Production of recombinant retroviral stocks by stable and transient producer cell lines




RetroXpress Vector
expressing gene
of interest
Transfection
RetroPack PT67 cell line is
a NIH/3T3-based
packaging line expressing
the 10A1 viral envelop
• Transient virus
production
(10⁵–10⁶ ffu/ml)
Antibiotic
selection
• Stable
virus-producing
population
(10⁵–10⁶ ffu/ml)
Isolation
of clones
• High-titer clone
(10⁶–10⁷ ffu/ml)
Collection of
virus and infection
of target cells

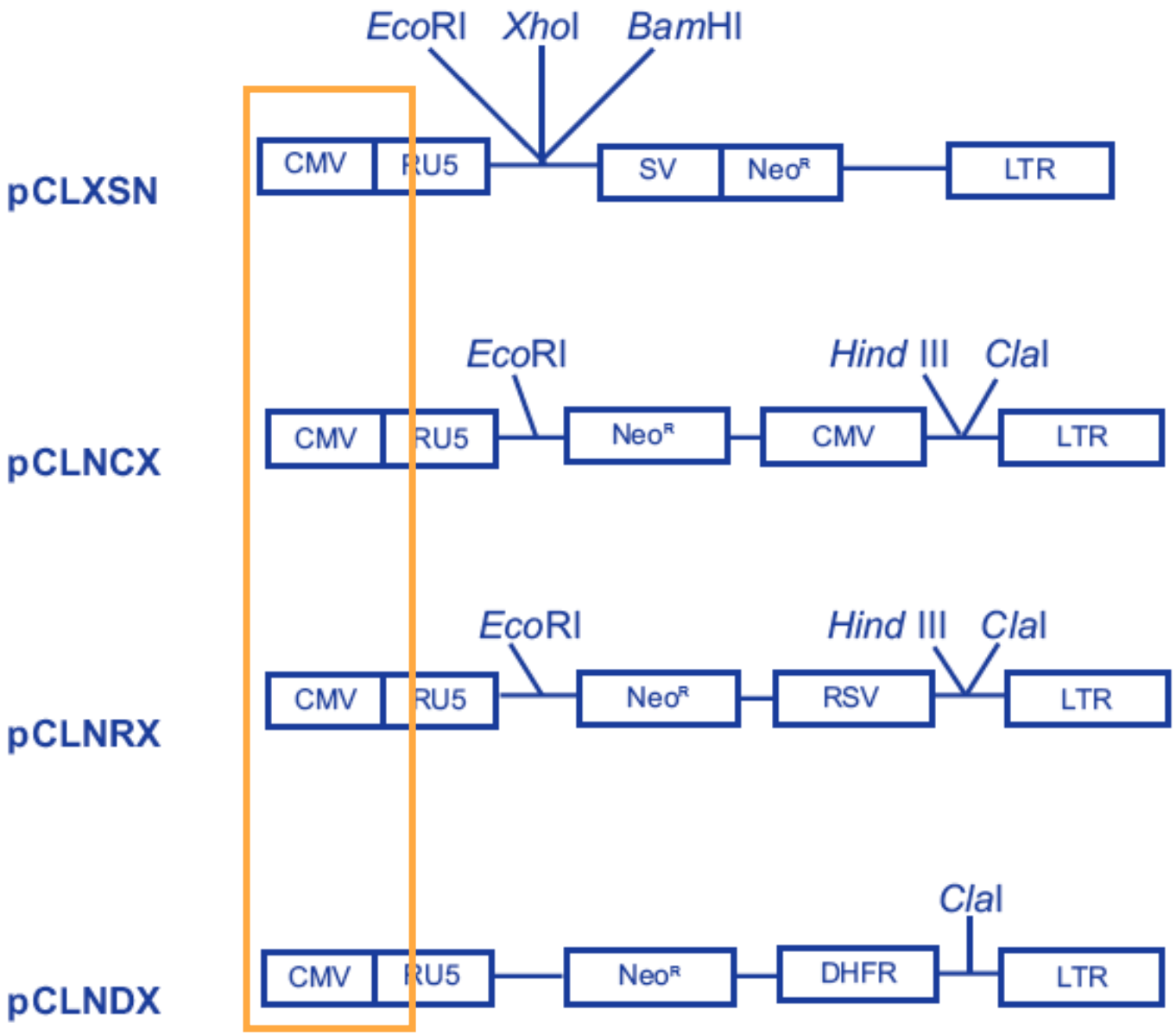
Disadvantages of Retrovirus Transduction System

- Post-mitotic cells cannot be transduced
- Unable to transduce large (>11kb) DNA fragments
- Random integration and genome rearrangement (risk of insertional activation of cellular genes)

Retrovirus vectors - a research lab application:

generation of a GFP-expressing retroviral vector by using the Retromax system

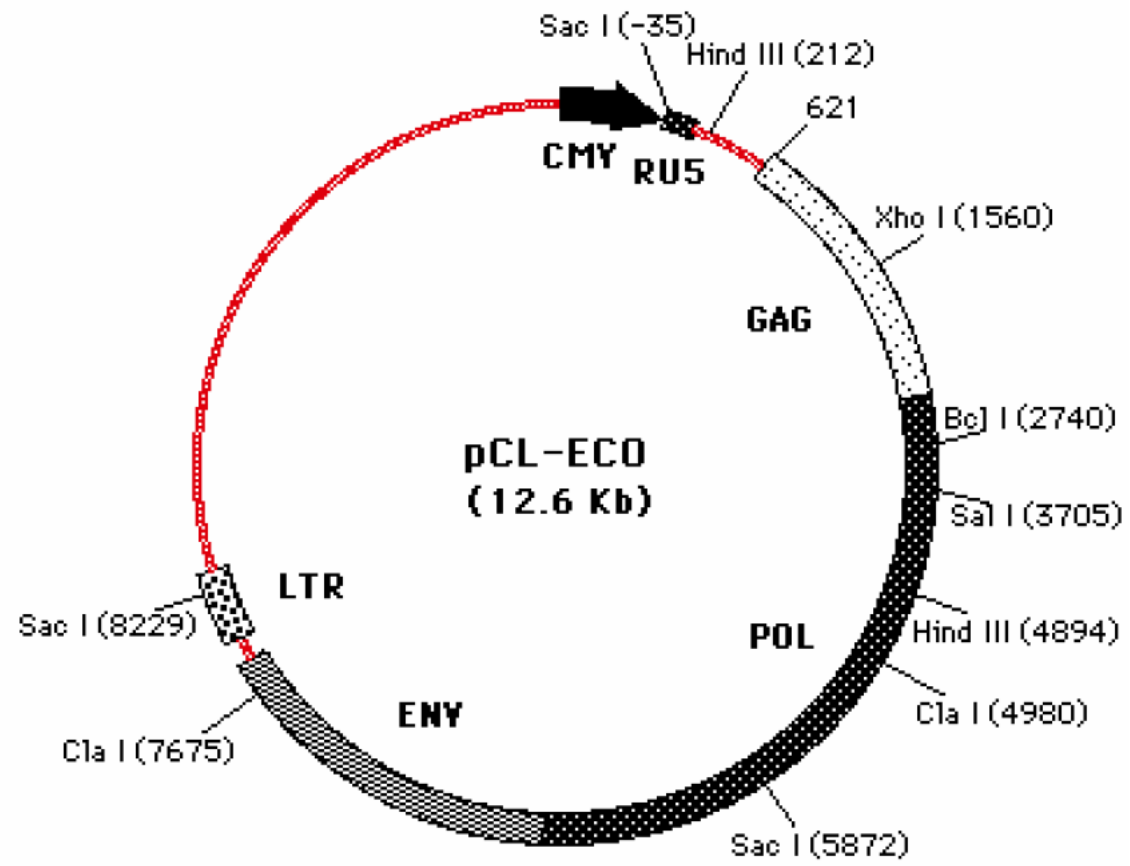
Retromax system: choice of vectors



Retromax system: choice of packaging vectors

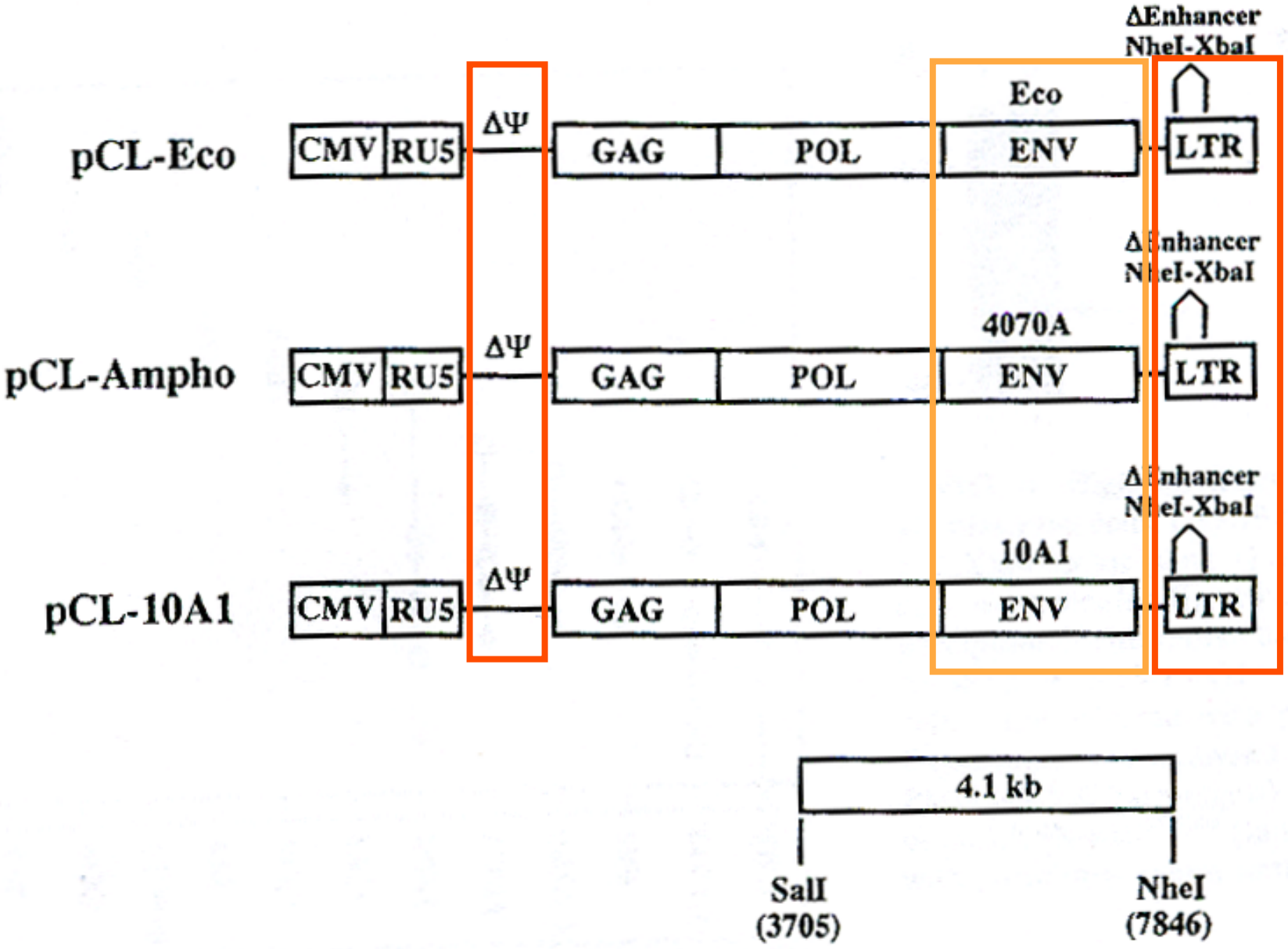
Ecotropic (usually (MoMuLV)	mouse and rat cells only (not human)
Amphotropic (from 4070 MulV)	most mammalian cells (no hamster)
Gibbon Ape leukemia virus (GALV)	many mammalian cells (including hamster)
10A1 (MuLV)	most mammalian cells (including hamster)

Retromax system: choice of packaging vectors

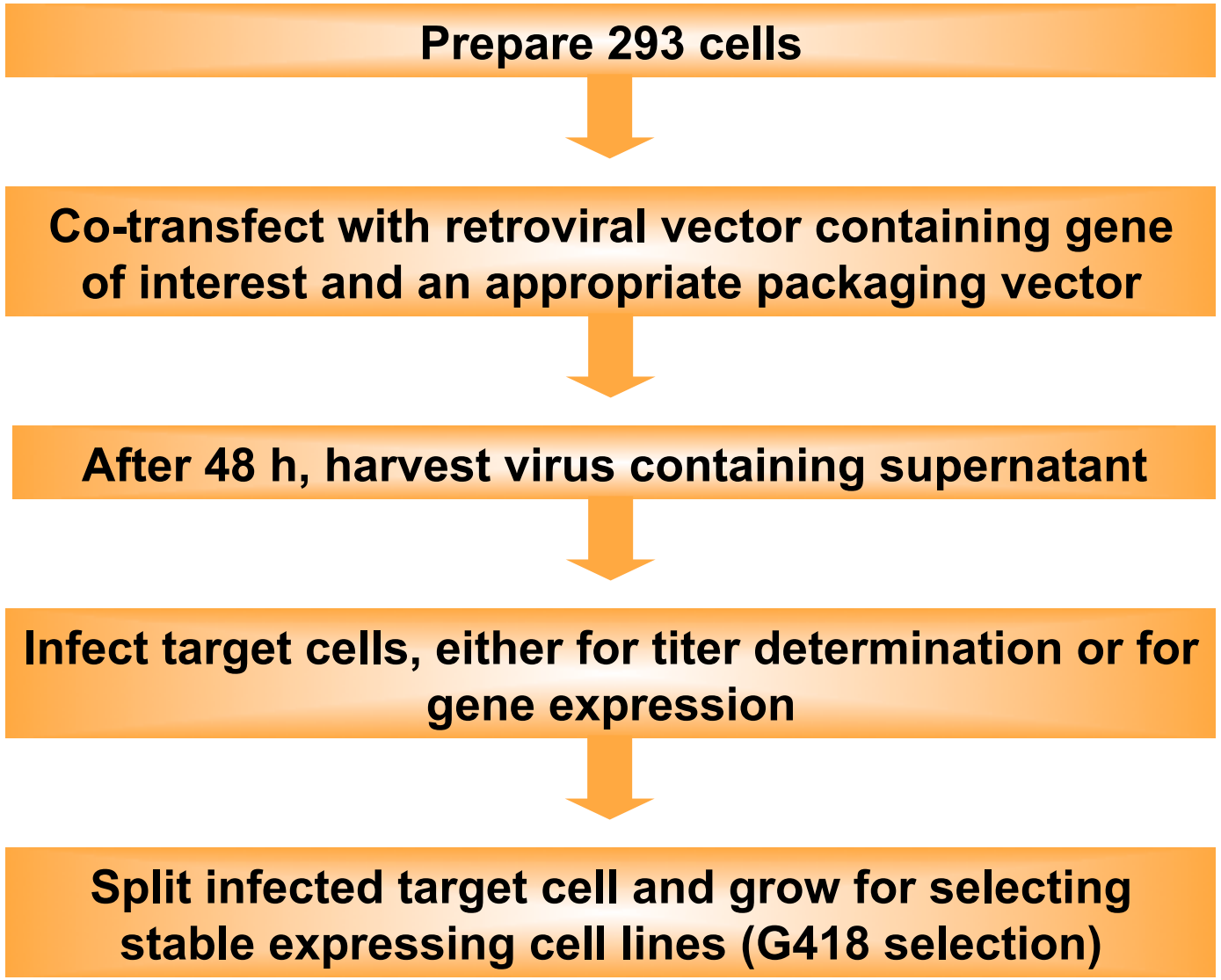


The **pCL-Eco** packaging vector. The gene coding for **env** was replaced with env gene from different MULV strains (4070A and 10A1) to create **pCL-Ampho** and **pCL-10A1** packaging vectors

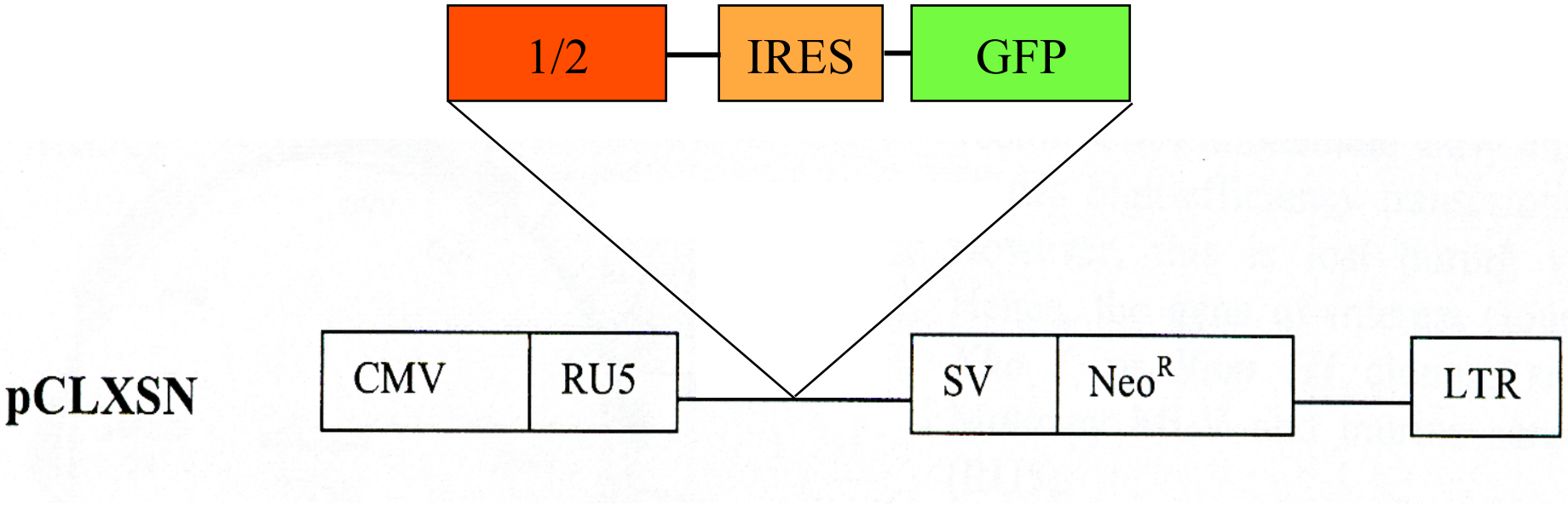
Retromax system: pCL packaging vectors



Retromax system: outline of the procedure

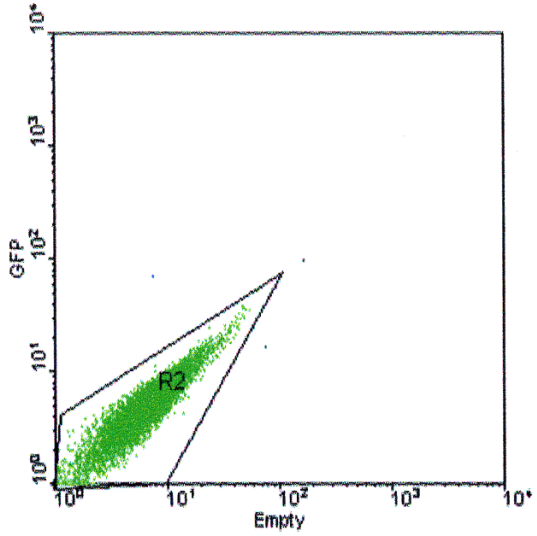


Retromax system: construction of pCLXSN-GFP vectors

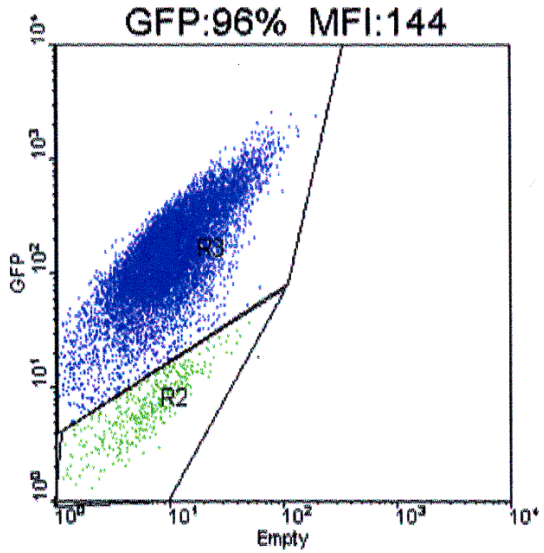


GFP exp in infected HUVEC after 1 wk of G418 selection

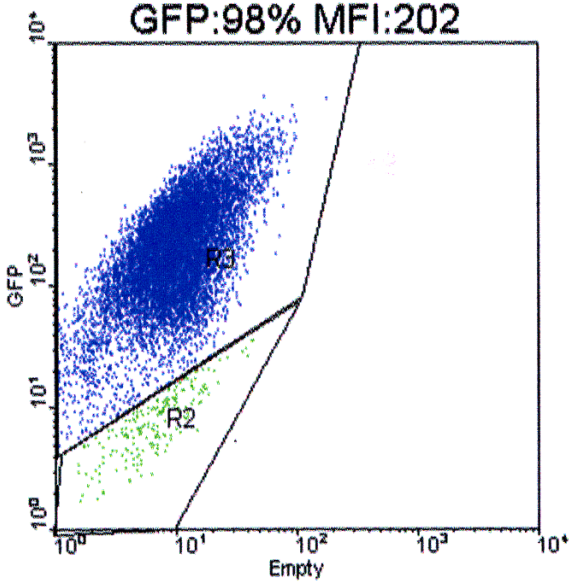
Mock



P1



P2



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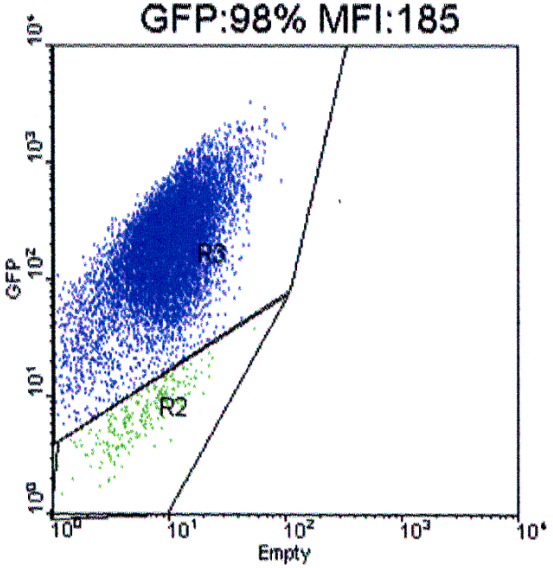


VIROLOGY

Engineering Viral Genomes: Lentivirus Vectors

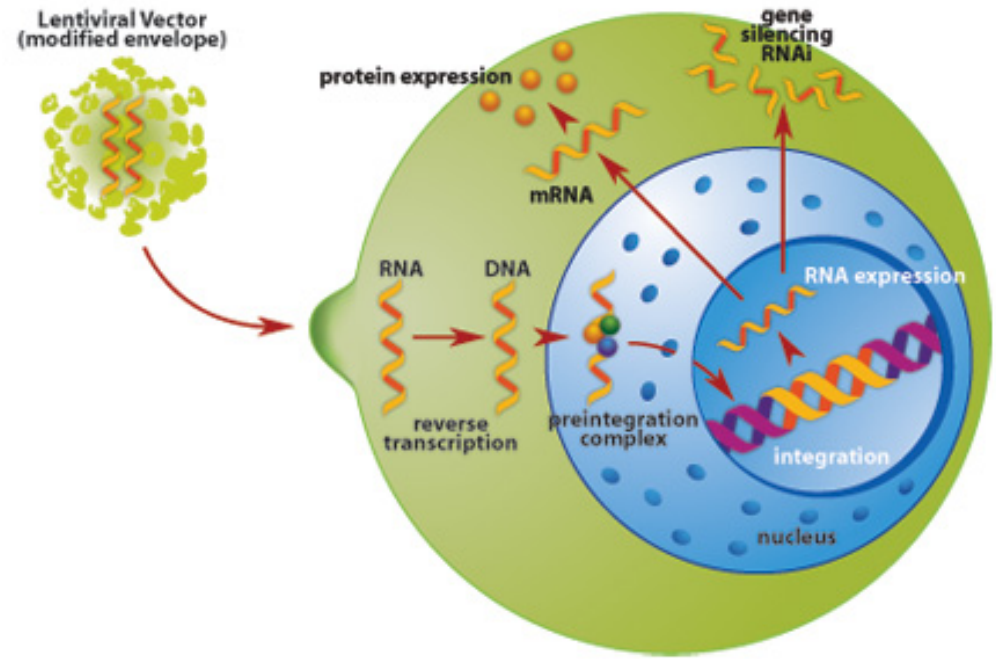
Viral vectors

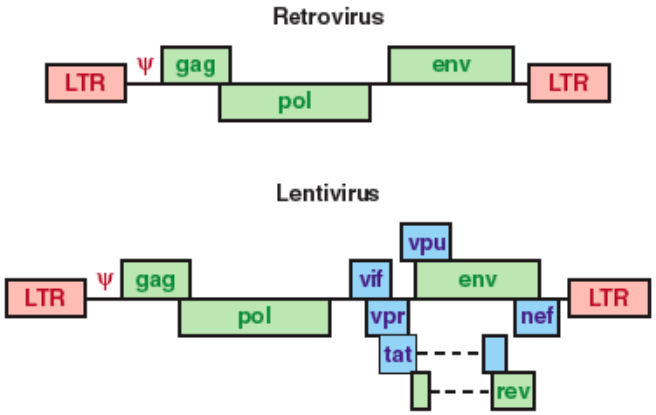
Virus	Insert size	Integration	Duration of expression	Advantages	Disadvantages
Adeno-associated virus	~4.5–9 (?) kb	Low efficiency	Long	Nonpathogenic, episomal, infects nondividing cells	Immunogenic, toxicity
Adenovirus	2–38 kb	No	Short	Efficient gene delivery	Transient, immunogenic
Alphavirus	~5 kb	No	Short	Broad host range, high-level expression	Virulence
Herpes simplex virus	~30 kb	No	Long in central nervous system, short elsewhere	Neurotropic, large capacity	Virulence, persistence in neurons
Influenza virus	Unknown	No	Short	Strong immune response	Virulence
Lentivirus	7–18 kb	Yes	Long	Stable integration; infects nondividing and terminally differentiated mammalian cells	Insertional mutagenesis
Poliovirus	~300 bp for helper-free virus; ~3 kb for defective virus	No	Short	Excellent mucosal immunity	Limited capacity, reversion to neurovirulence
Retrovirus	1–7.5 kb	Yes	Shorter than formerly believed	Stable integration	May rearrange genome, insertional mutagenesis, require cell division
Rhabdovirus	Unknown	No	Short	High-level expression, rapid cell killing	Virulence, highly cytopathic
Vaccinia virus	At least ~25 kb, probably ~75–100 kb	No	Short	Wide host range, ease of isolation, large capacity, high-level expression	Transient, immunogenic

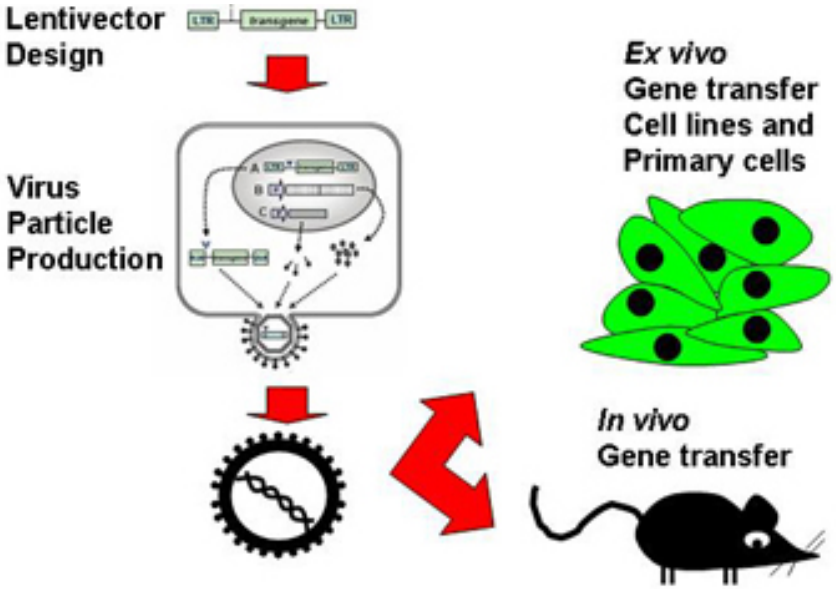
Retrovirus and Lentivirus vectors

Viral System	Transient expression		Stable expression			
	Dividing Cells	Non Dividing Cells	Dividing Cells	Neuronal Cells	Drug or Growth Arrested Cells	Contact Inhibited Cells
Adenovirus	•	•				
Retrovirus	•		•			
Lentivirus	•	•	•	•	•	•

Lentiviral vectors



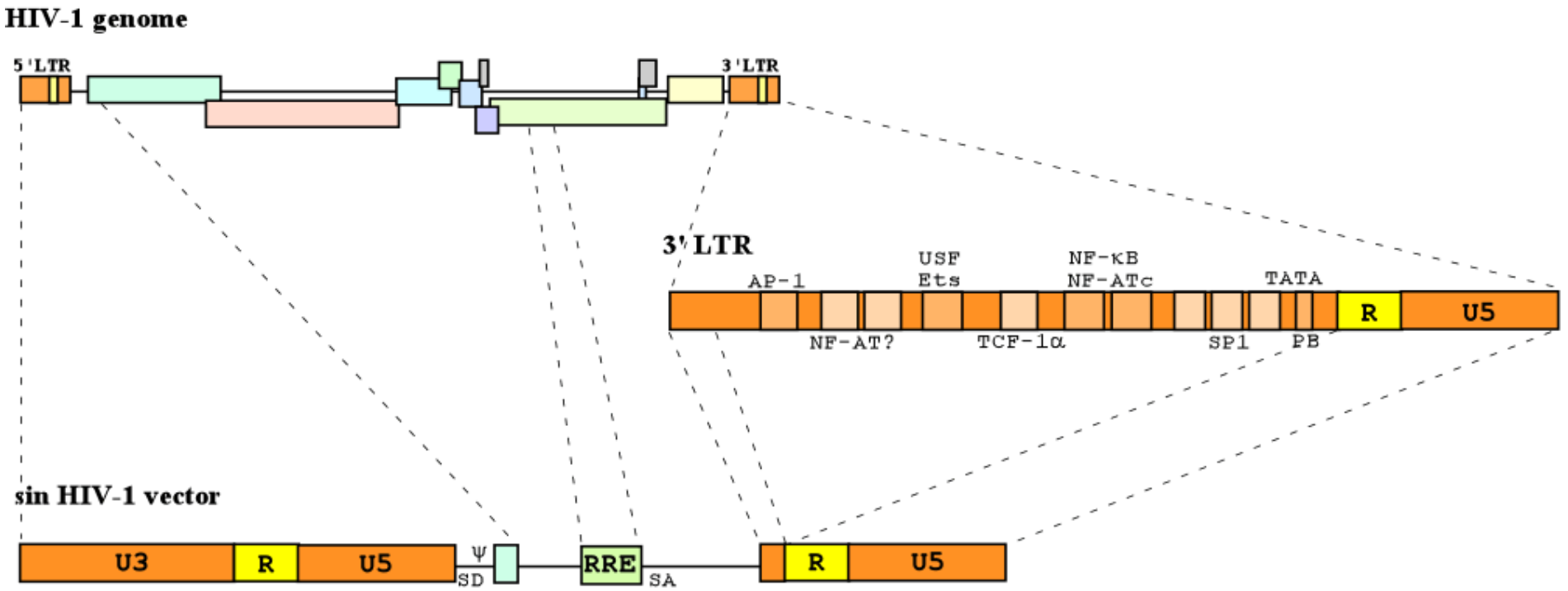




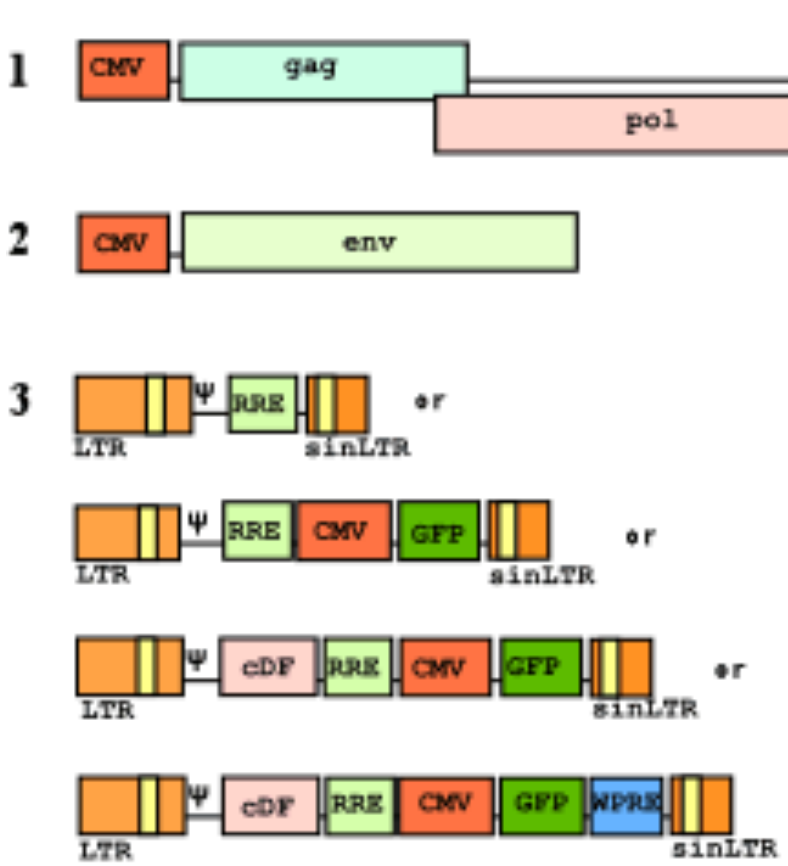
Favorable Features of Lentivirus Vectors

- HIV-1 integrates its DNA and completes a replication cycle in fully differentiated, non dividing cells (macrophages).
- So, this virus must have a mechanims for the active transport of preintegration complexes into the nucleus.
- Vpr and a minor, phosphorylated, form of the matrix (MA/p17) protein direct nuclear import of the HIV-1 preintegration complex.
- Nuclear localization signals have been found in the IN protein of HIV-1

Structure of a non-RCR (SIN) HIV-1 based vector



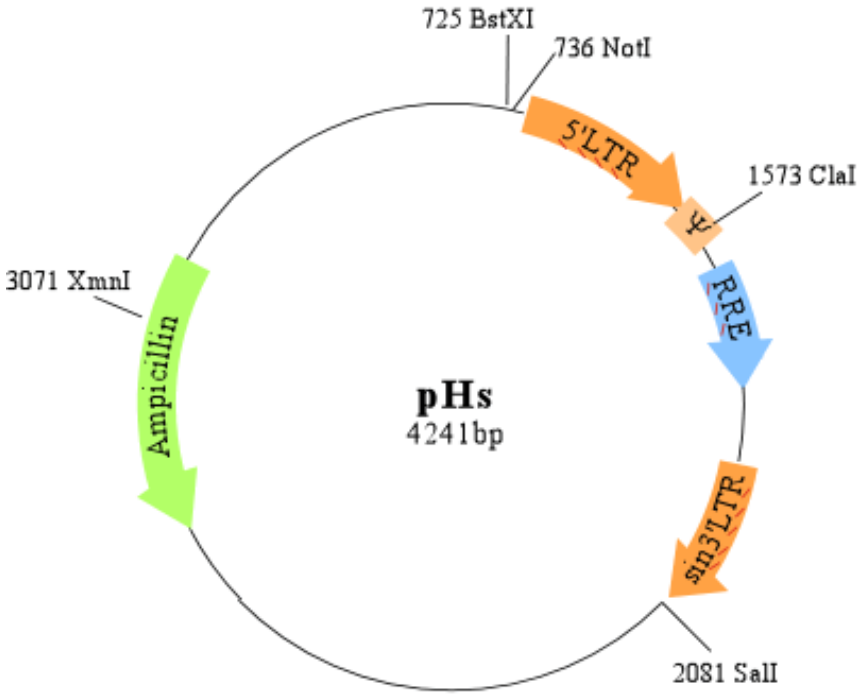
Development of self-inactivating vectors or SIN vectors



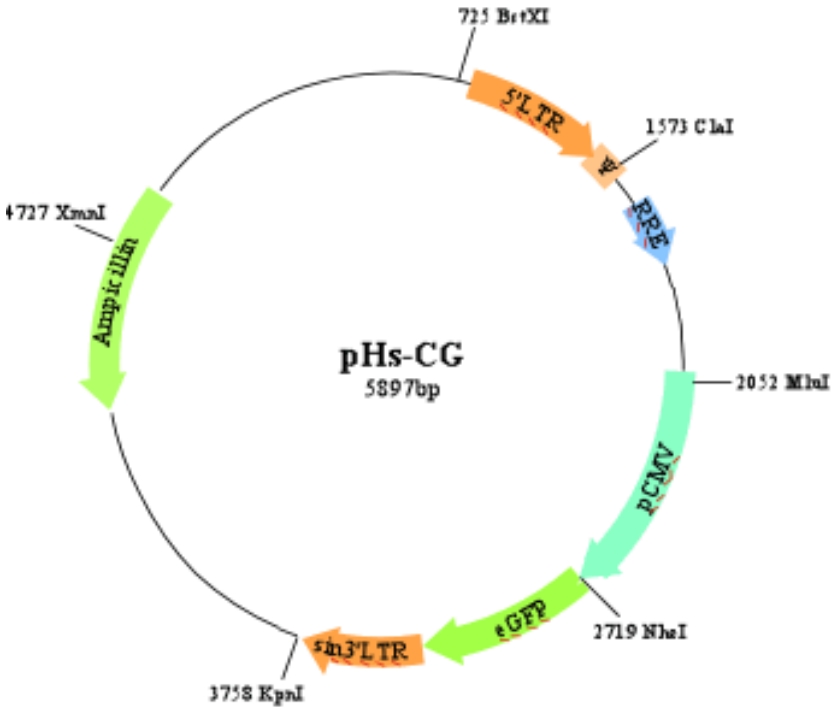
Packaging plasmids

Transfer vectors

Lentiviral SIN vectors

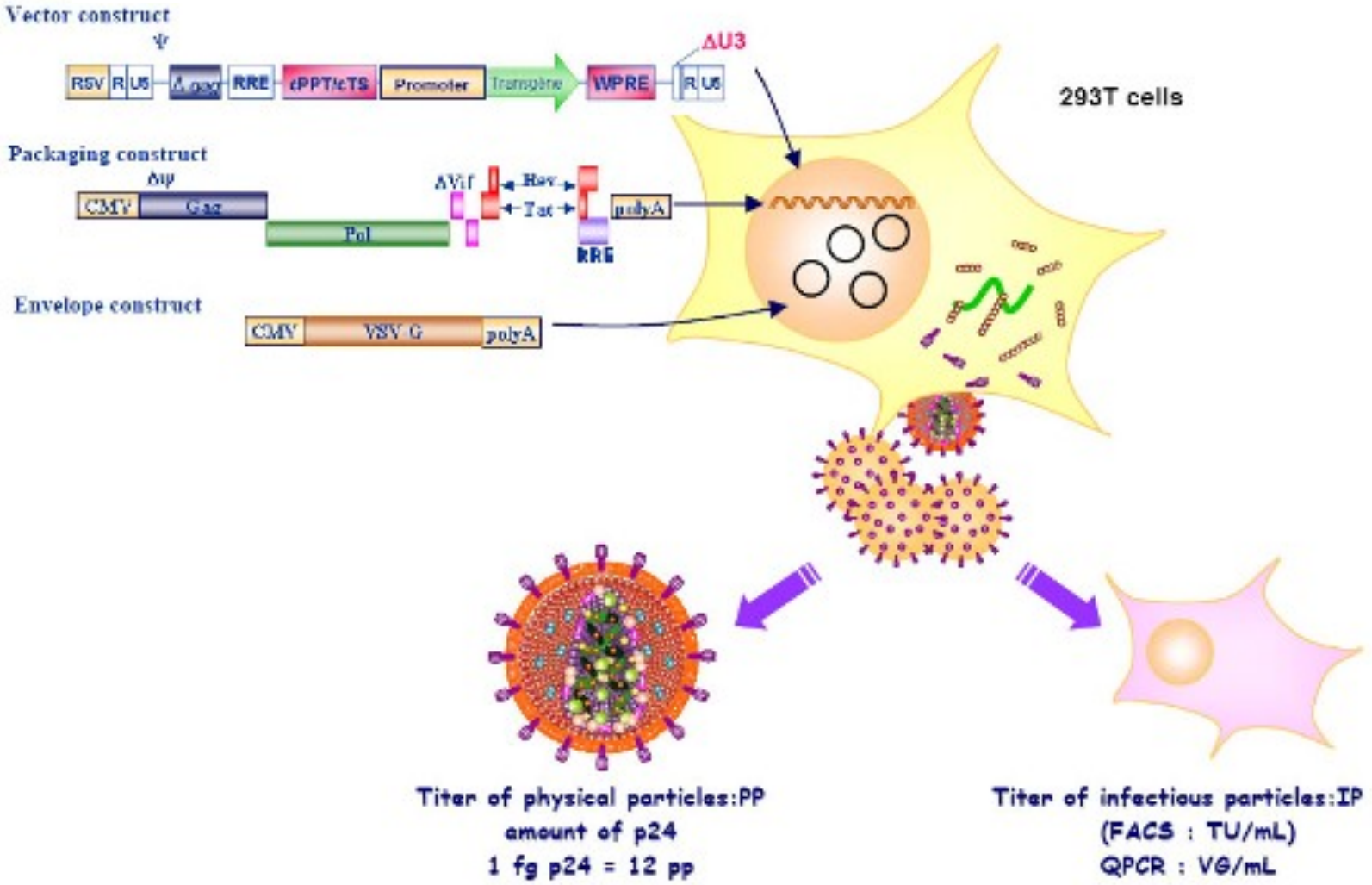


Basic HIV-1 based sin vector.

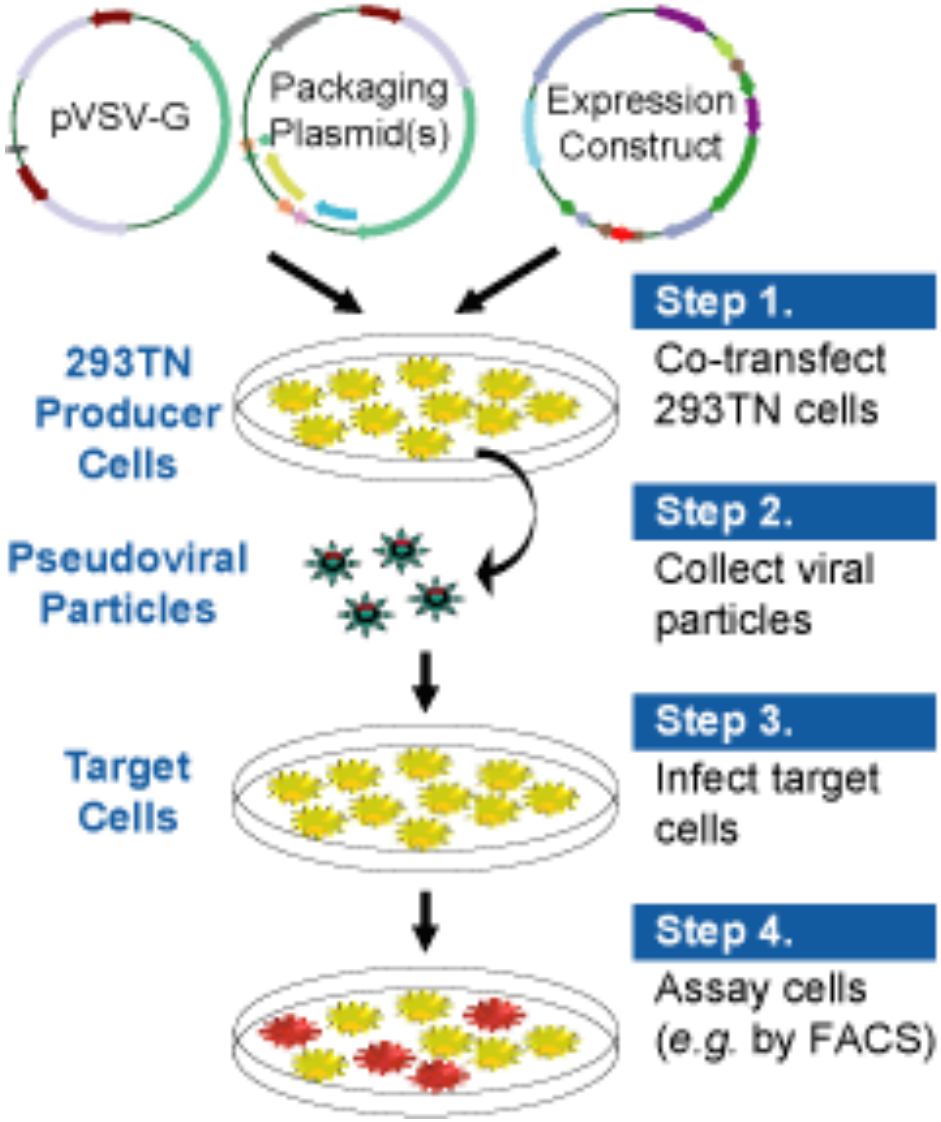


Basic HIV-1 based sin vector with the GFP marker driven by pCMV.

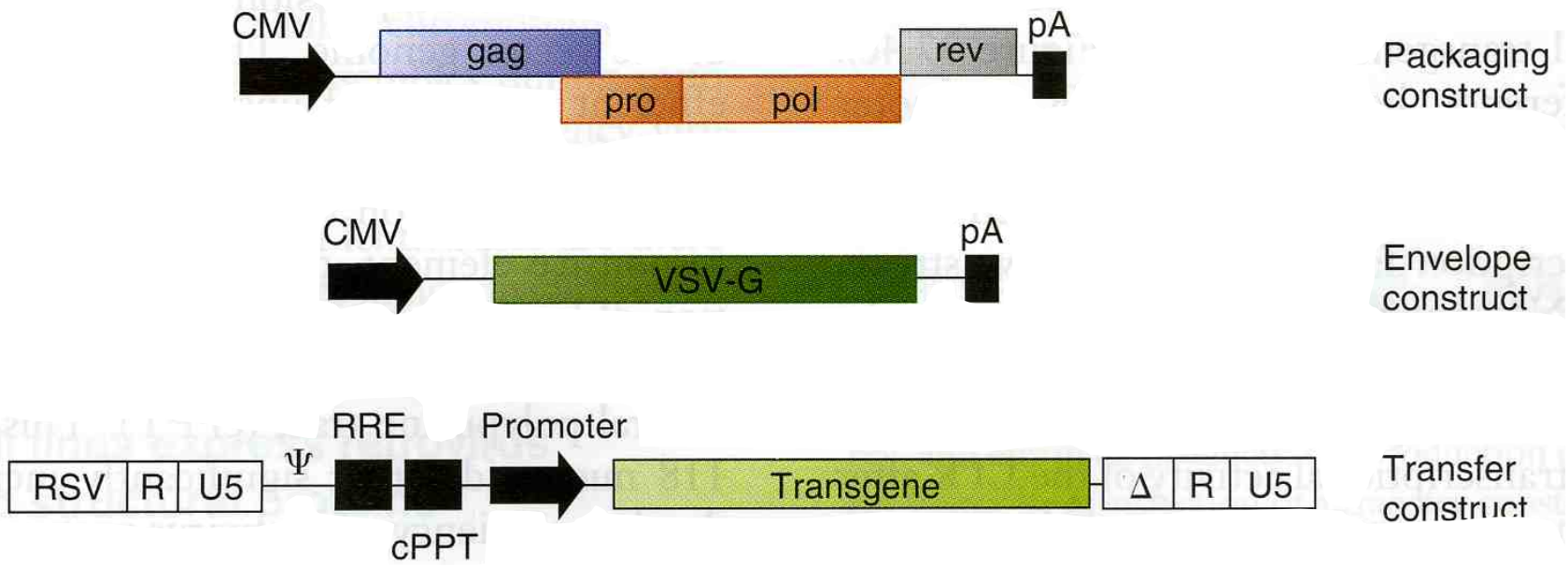
Production of a 2nd generation Lentiviral SIN vector



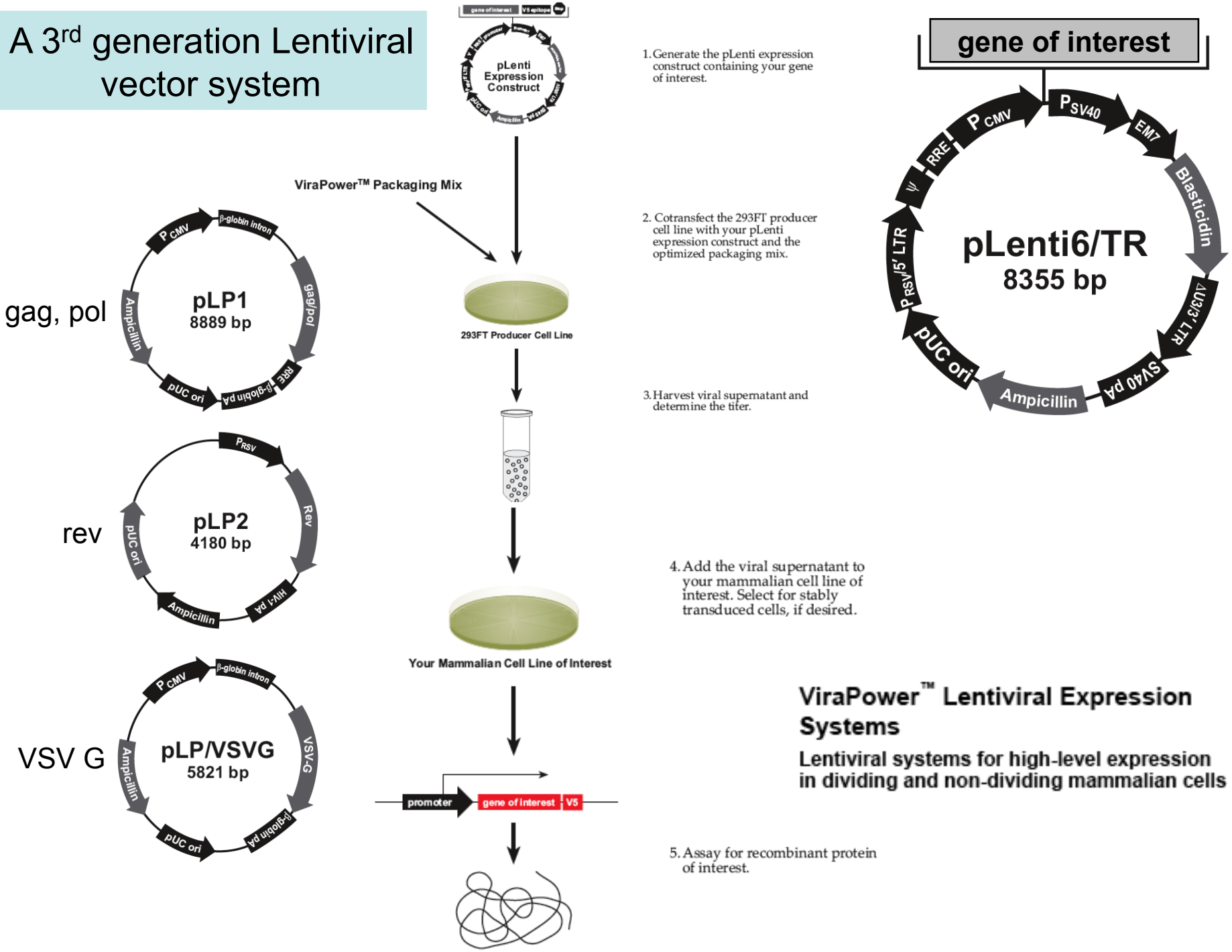
Flow-chart production of a recombinant lentiviral vector



A 3rd generation Lentiviral vector system



A 3rd generation Lentiviral vector system

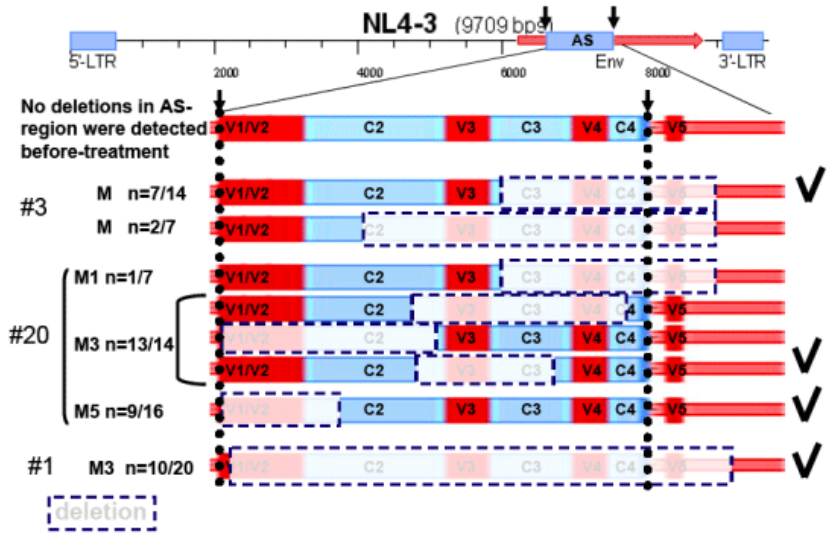


Biosafety Considerations for Research with Lentiviral Vectors

Biosafety Considerations and Risk Levels

Biosafety Considerations	Higher Risk	Lower Risk
Vector Design	• Vector packaging functions on two plasmids • Expression of viral genes	• Vector and packaging functions separated onto multiple plasmids • Deletion of viral genes
Transgene	• Oncogene	• Non-oncogene
Vector Generation	• Large scale	• Laboratory scale
Animal Hosts	• Permissive host • Animals engrafted with human cells	• Non-permissive host
Animal Manipulation	• Vector administration (e.g., use of sharps during injection	• Housing and husbandry (no use of sharps)

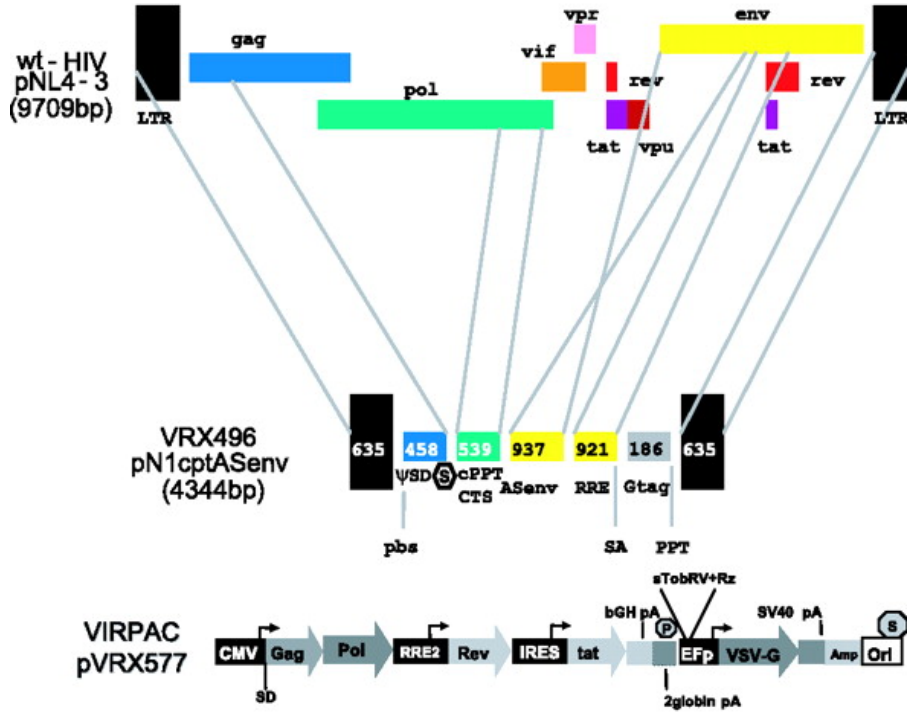
VRX496 Anti-sense Mechanism Deletes Env

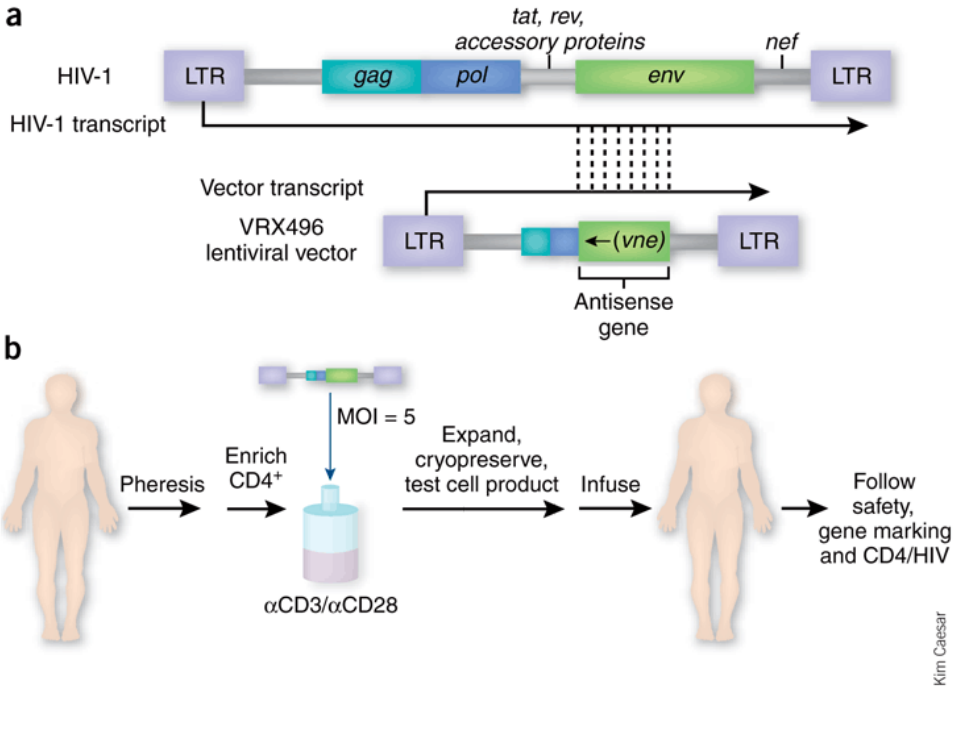


The first clinical trial of a lentiviral vector highlights the promise of this new class of gene-therapy vector

VIRxSYS

Delivering the promise of genetic medicine





Lexgenleucel-T (VRX496):

Autologous CD4+ Cells Transduced with a Lentiviral Vector Encoding a 937 Base Antisense Sequence Targeting HIV Envelope

VRX496 Anti-HIV T Cell Transplantation

Conclusions from Clinical Trials

- No safety issues
- Reduced viral in treatment failures
- Reduced viral infectivity
- Sustained increases in CD4 counts in 10B bolus
- Additional clinical trials being developed

VRX496 Clinical Studies – Summary

Clinical Trial	Infusion Schedule	Cell Dose	Status
Phase I Failed ≥2 HAART CD4 ≥150; VL ≥ 5000	Single dose	~10 billion	Completed**
Phase II Failed ≥1 HAART CD4 ≥150 VL ≥ 5000	Repeat 4 or 8 doses	10 billion per dose	Completed
	Single dose	10 billion 20 billion 30 billion	Ongoing
Phase I/II Virologically Controlled CD4 ≥350; VL ≤ 50	Repeated 6 doses	10 billion per dose	Ongoing

• U.S. multi-center study: University of Pennsylvania, Stanford University, University of Kentucky, Jacobi Medical Center, Mercy Medical Center, Circle Medical Center

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