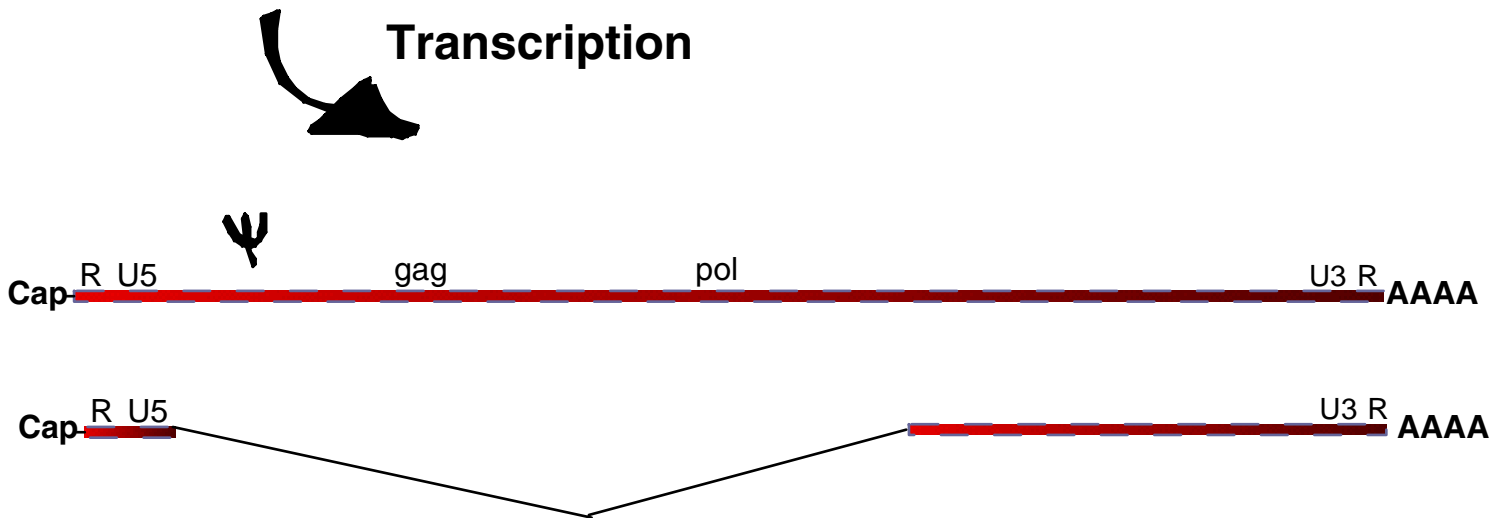
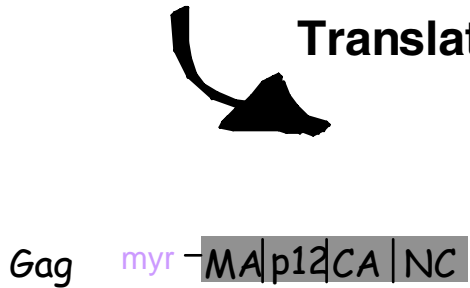


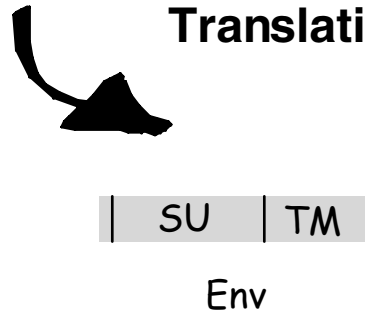
Transcription



Translation

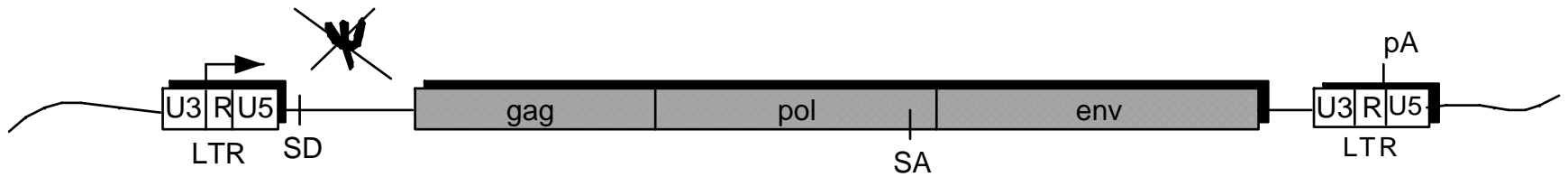


Translation

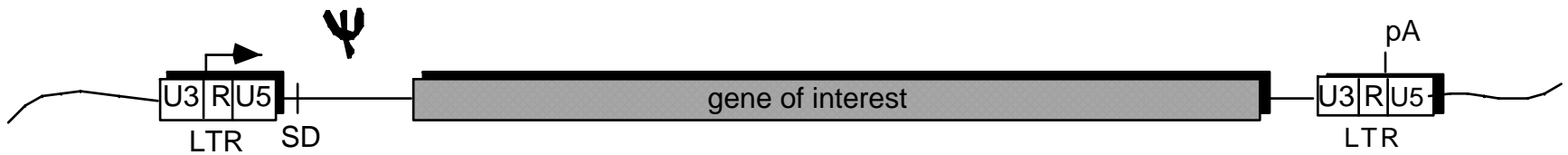


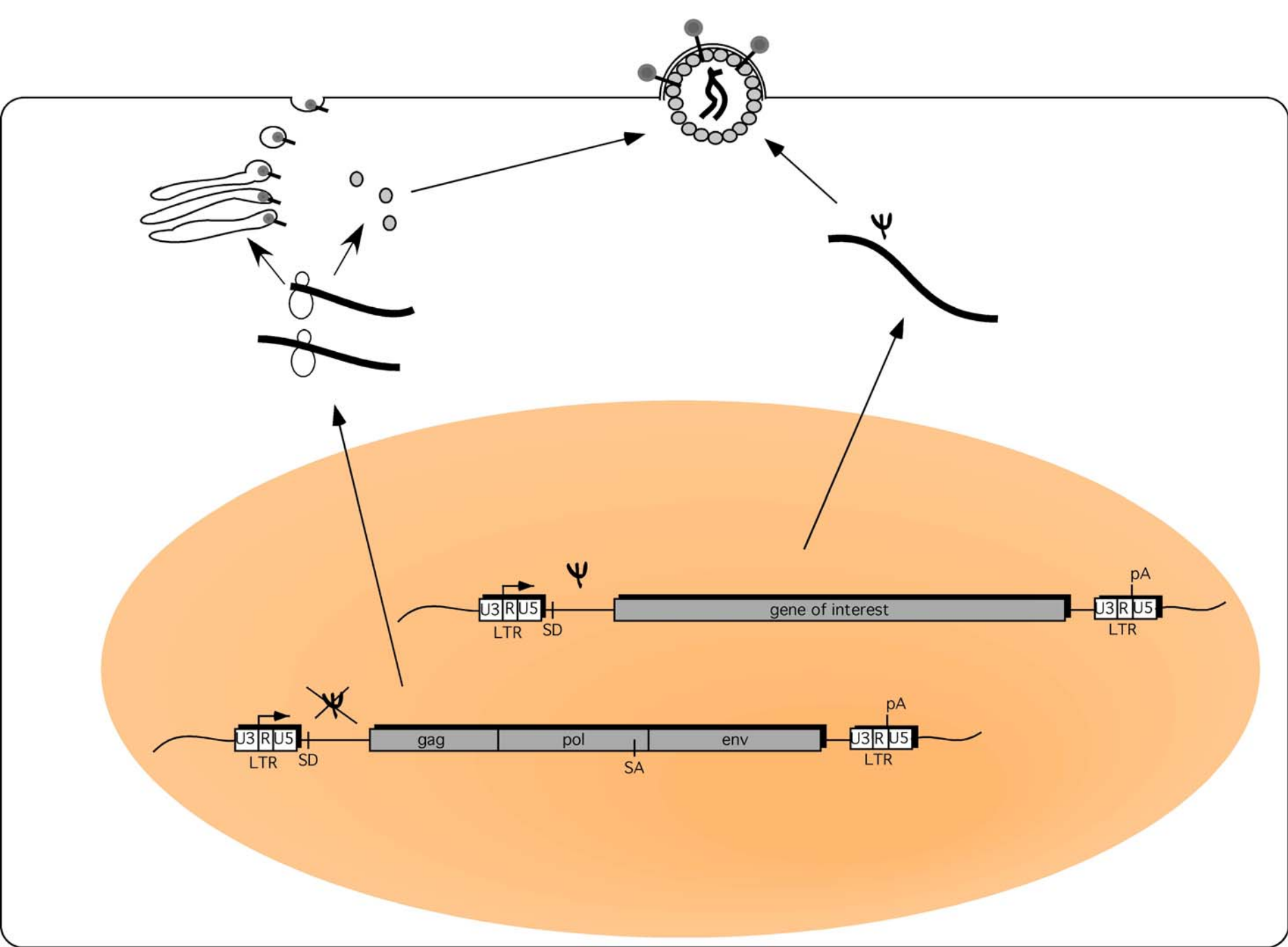
Retrovirus	Receptor	Type	Function
HIV, SIV	CD4 and CXCR4, CCR5, others	TM1, TM7	Immune recognition, G protein-coupled chemokine receptors
E-MLV	CAT-1 (SLC7A1)	TM14	Basic amino acid transport
GALV, 10A1, MLV, FeLV-B, woolly monkey virus	Pit1 (SLC20A1)	TM10-13	Phosphate transport
A-MLV, 10A1, MLV, FeLV-B	Pit2 (SLC20A2)	TM10--13	Phosphate transport
BLV	Blvr	TM1	?
ASLV-A	Tva	TM1	LDL receptor-like protein
ASLV-B, ASLV-D, ASLV-E	Tvb	TM1	Fas/NFR-like receptor
MMTV	Mtvr	TM1	?
RD-114, type D SRV, BaEV, HERV-W	RDR (SLC1A5) or RDR2 (SLC1A4)	TM9-10 TM9-10	Neutral amino acid transport Glutamate, neutral amino acid transport
Xenotropic and polytropic MLVs	XPR1	TM8	G protein-coupled signaling?, transport?
FeLV-C	Flvcr	TM12	Organic anion transporter?
FeLV-T FeLIX and Pit1	(SLC20A1)	Soluble TM10-13	Env-like protein Phosphate transport
JSRV	HYAL2	GPI anchored	Hyaluronidase (weak)

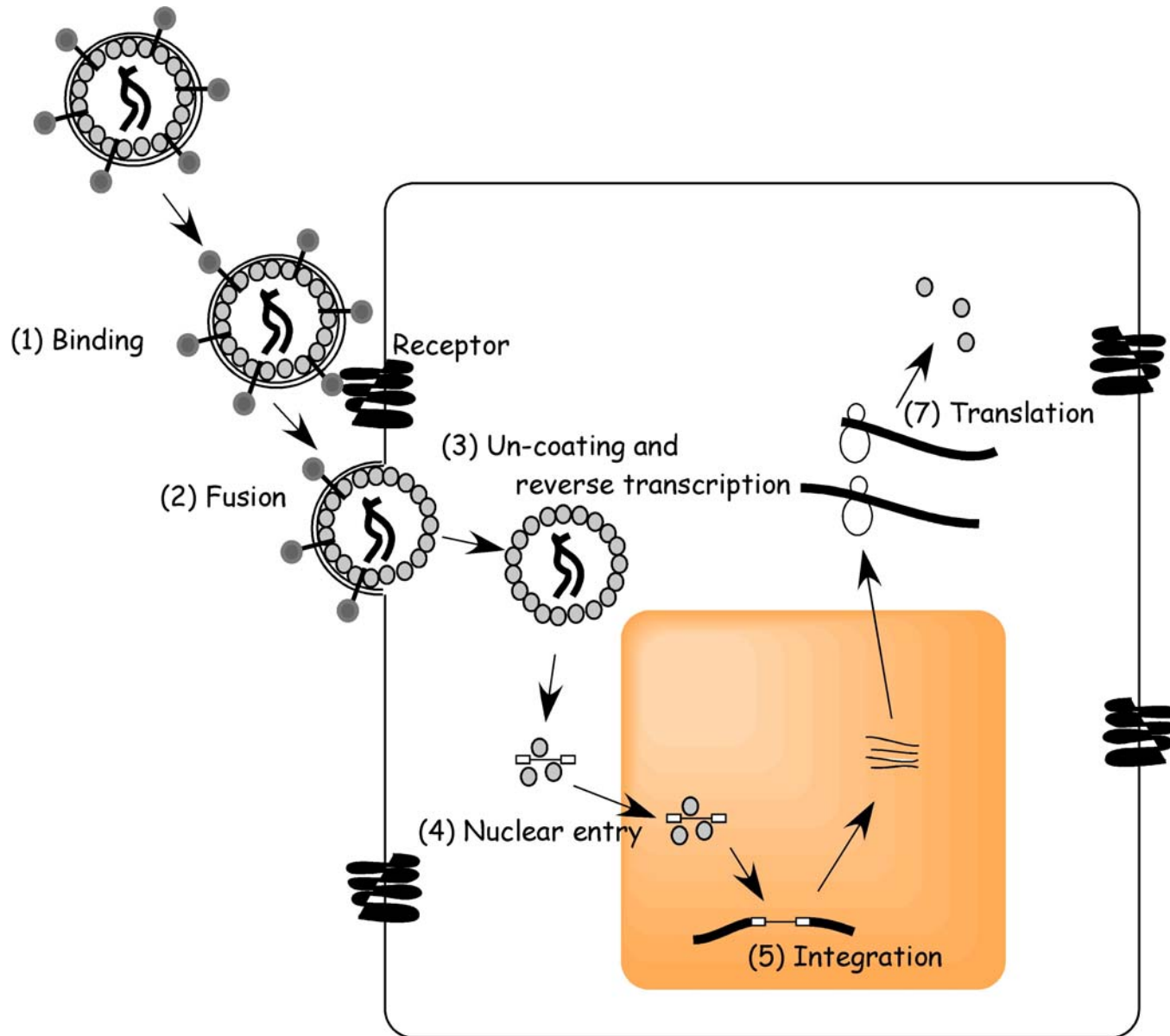
The Helper:



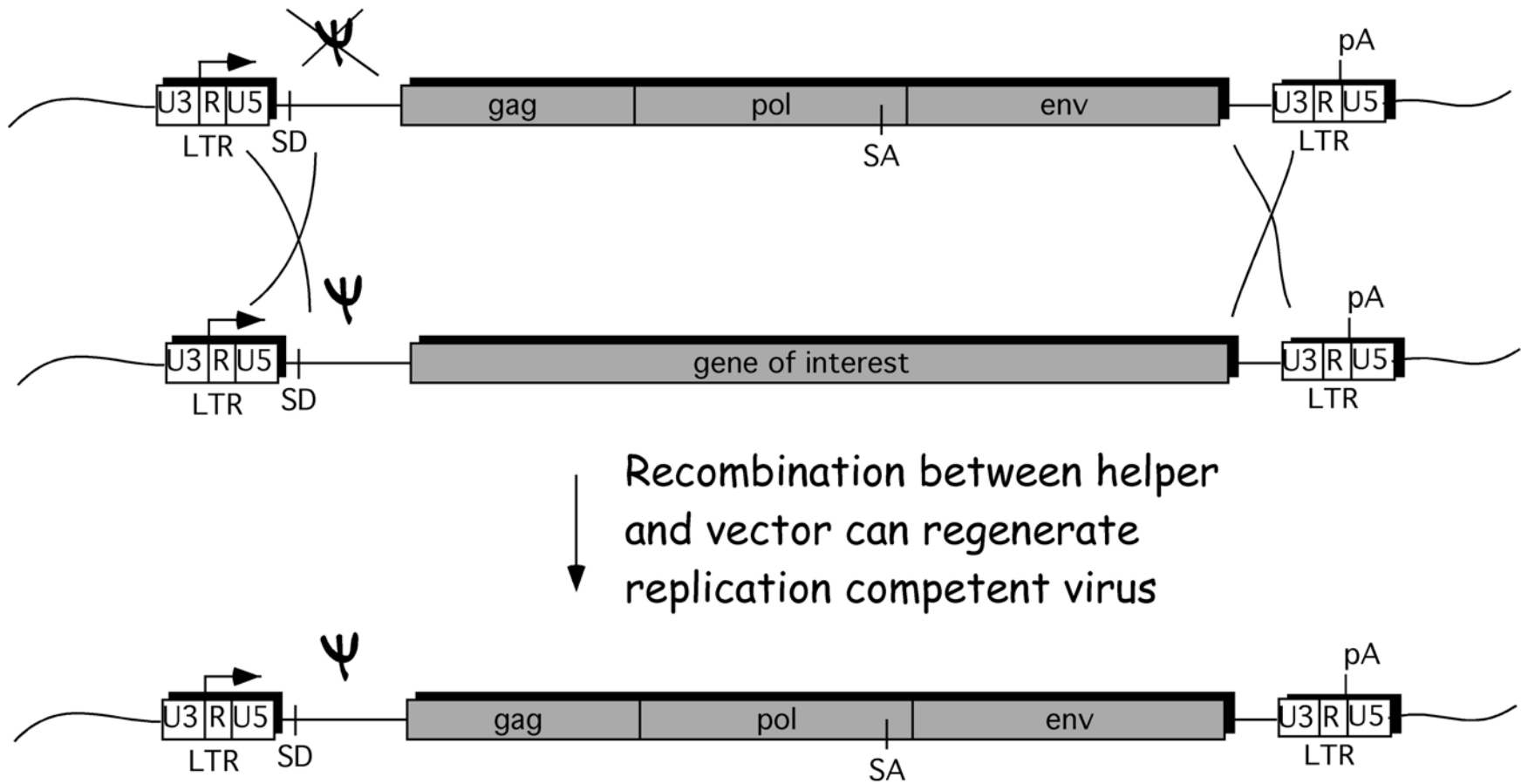
The Vector:



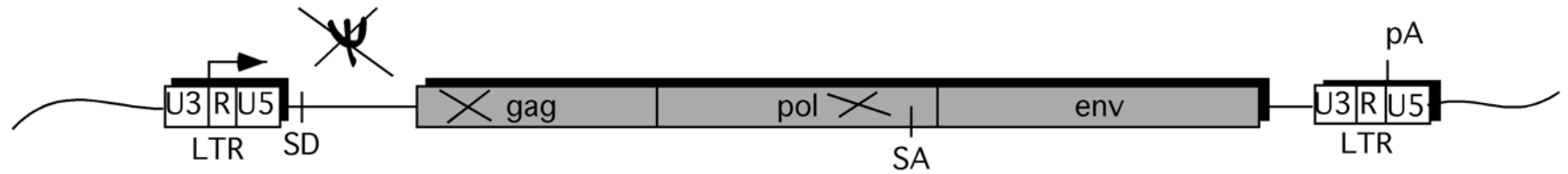




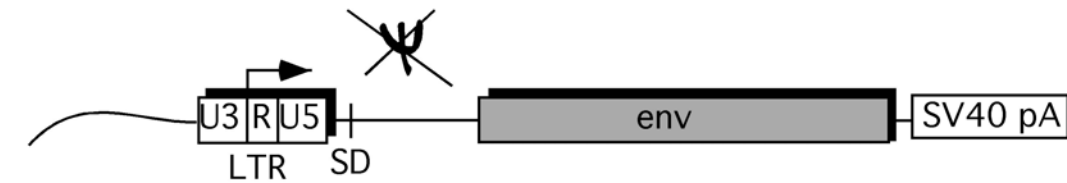
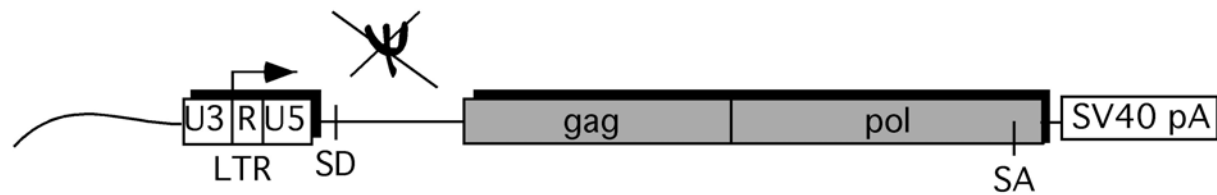
The problem:



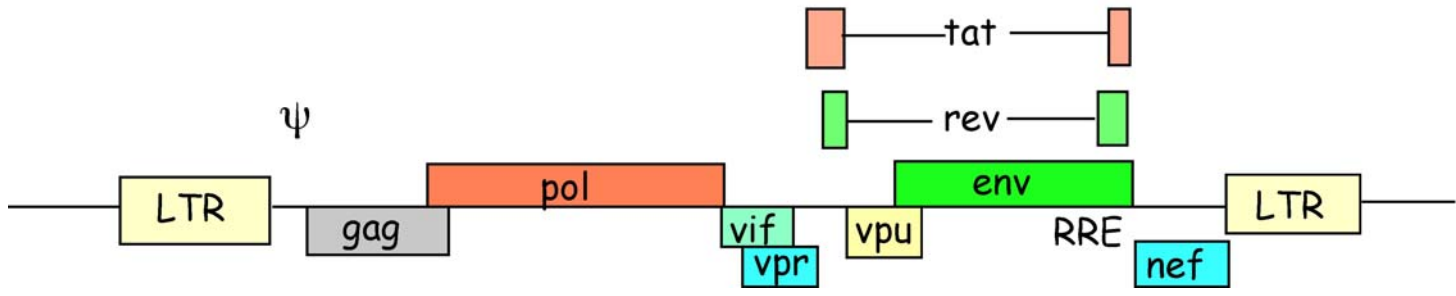
Solutions: Split genomes



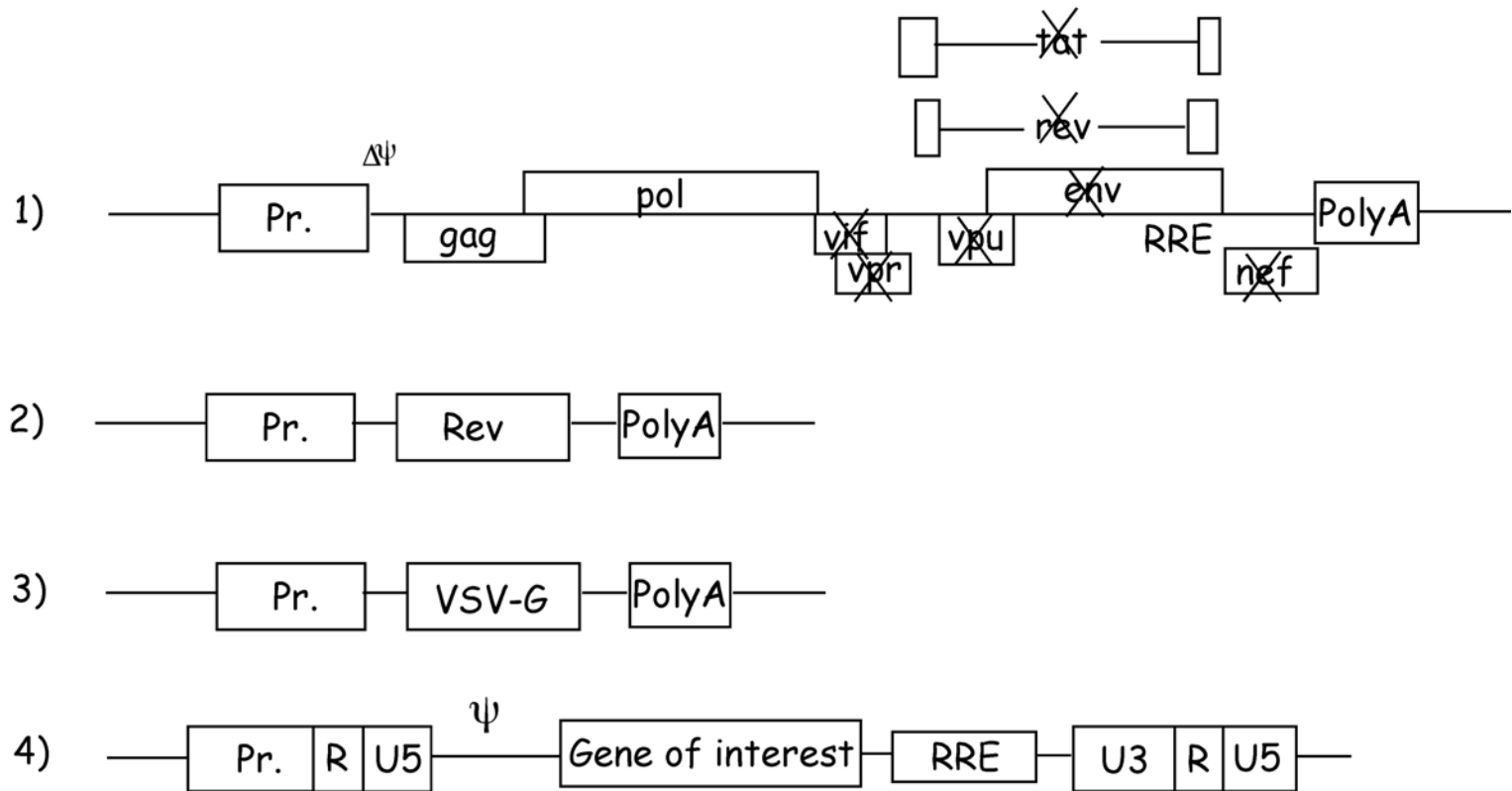
OR



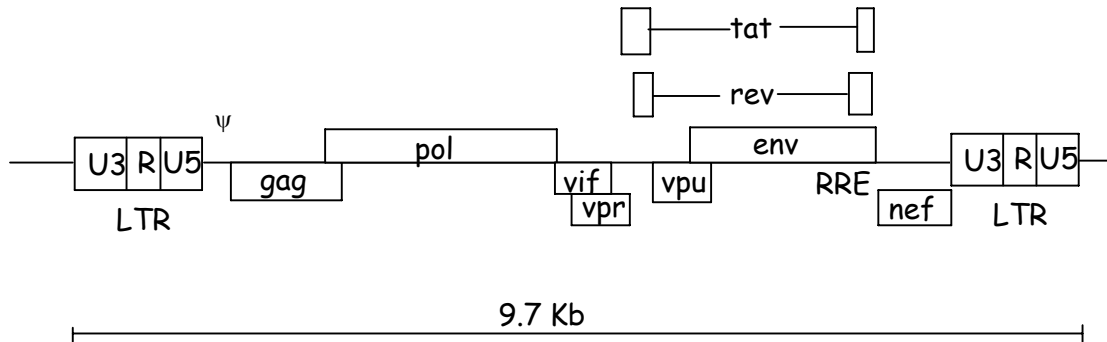
HIV - an upgrade in retroviral vectors



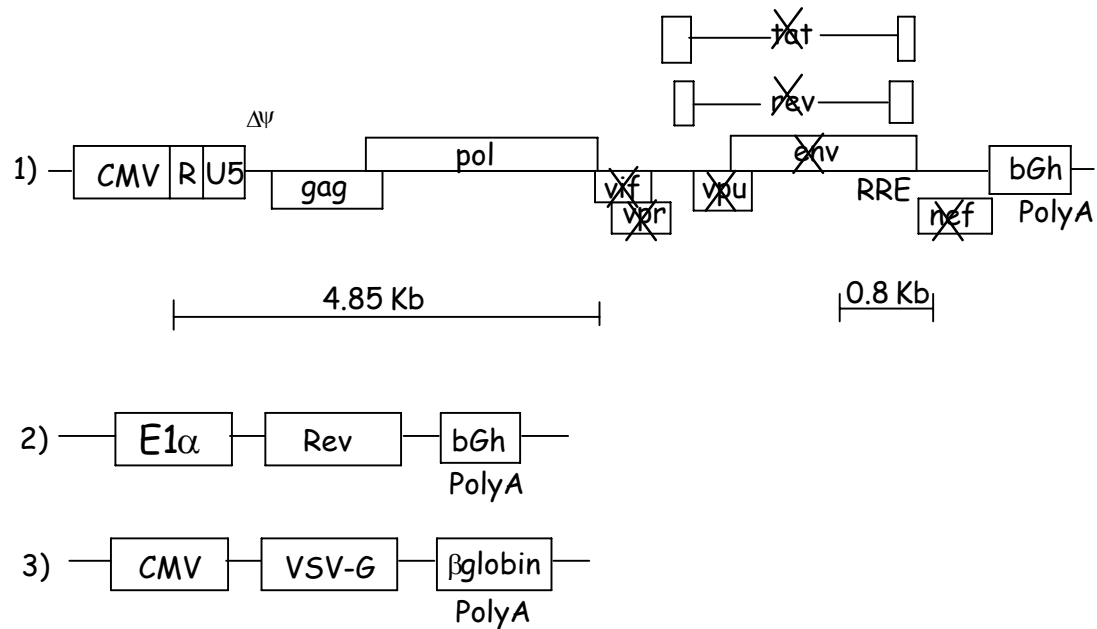
HIV - vectors production

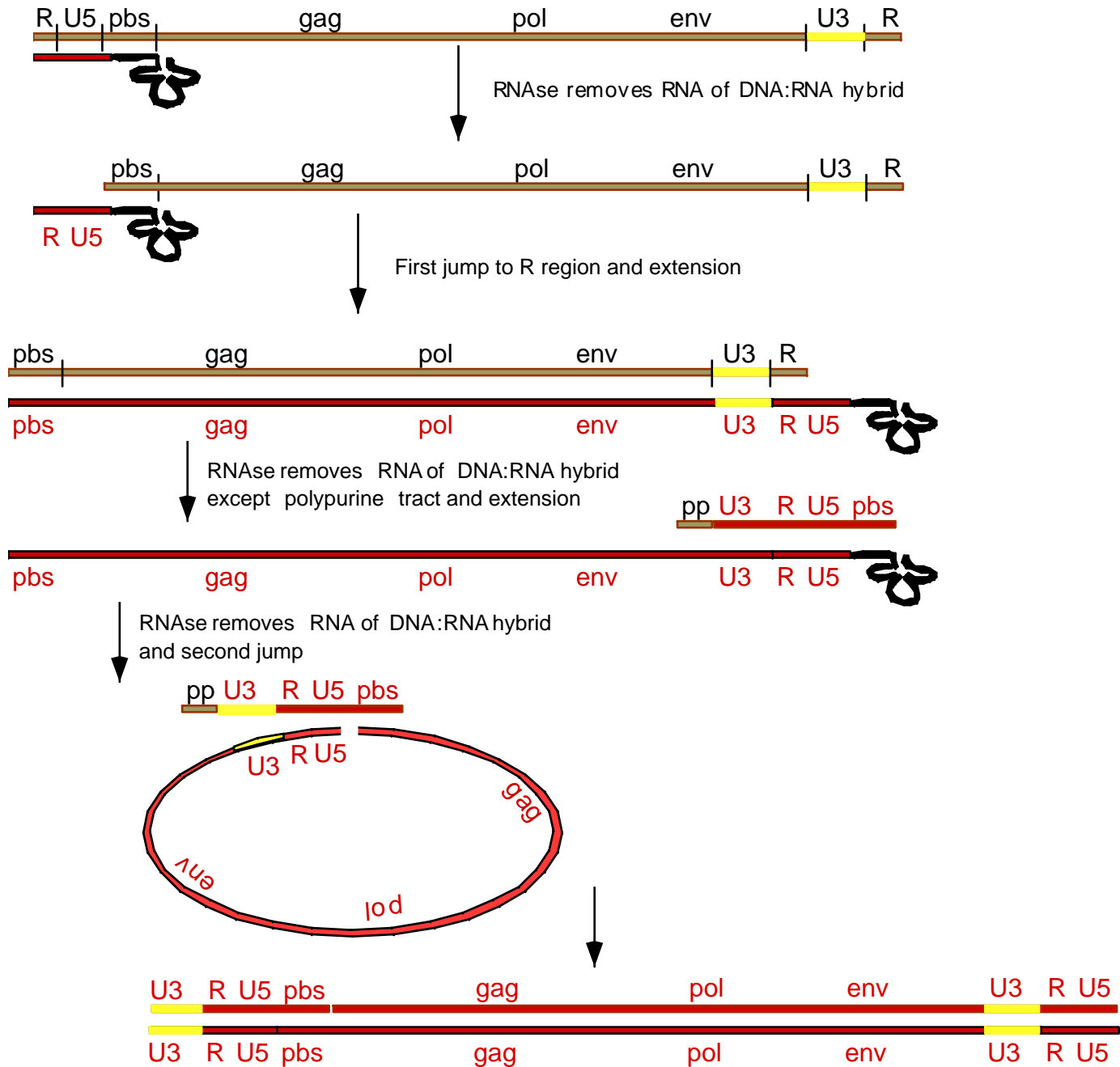


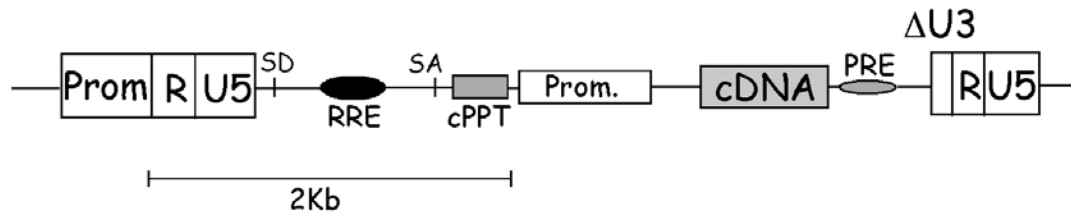
HIV genome



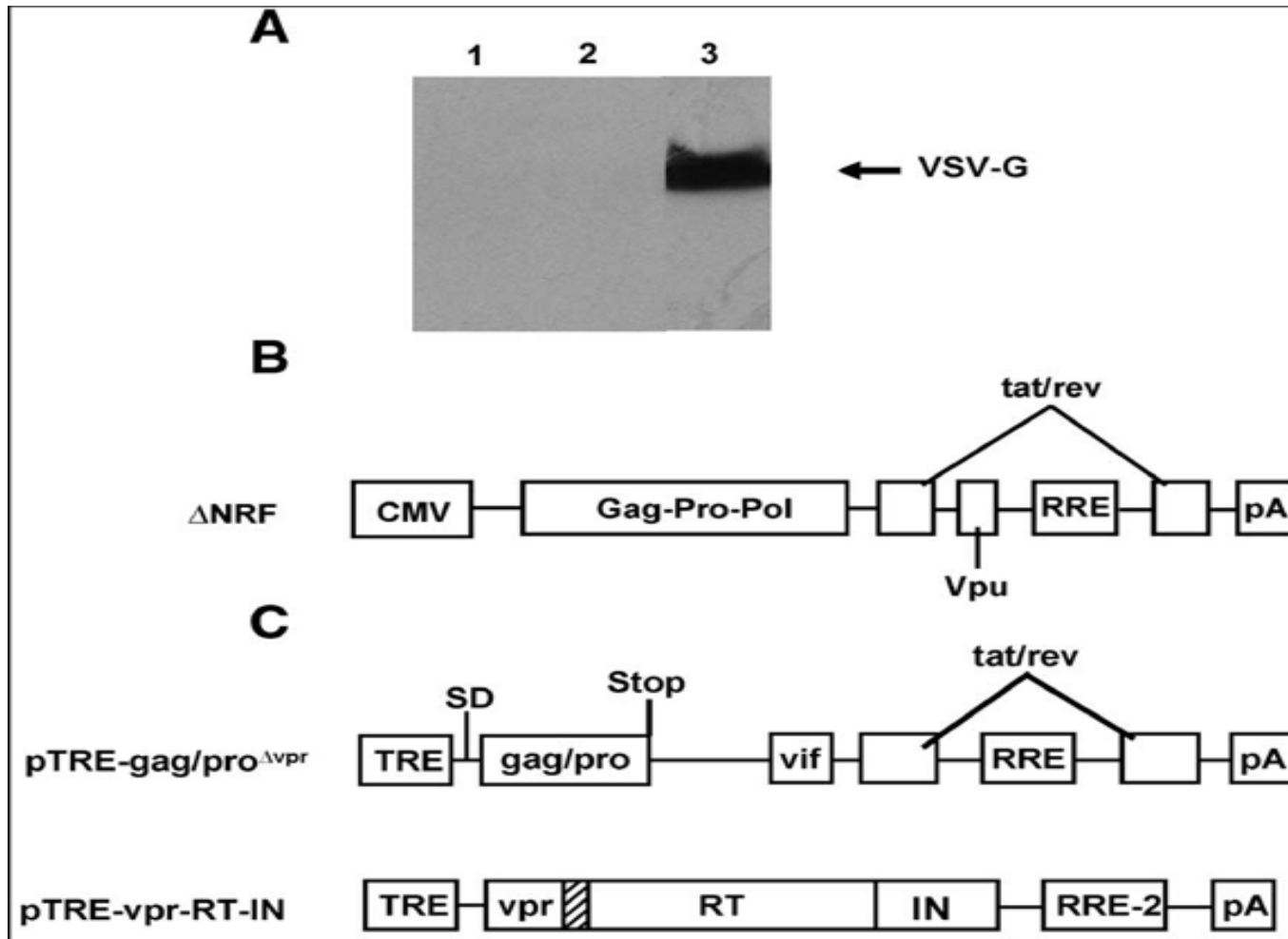
Helper constructs







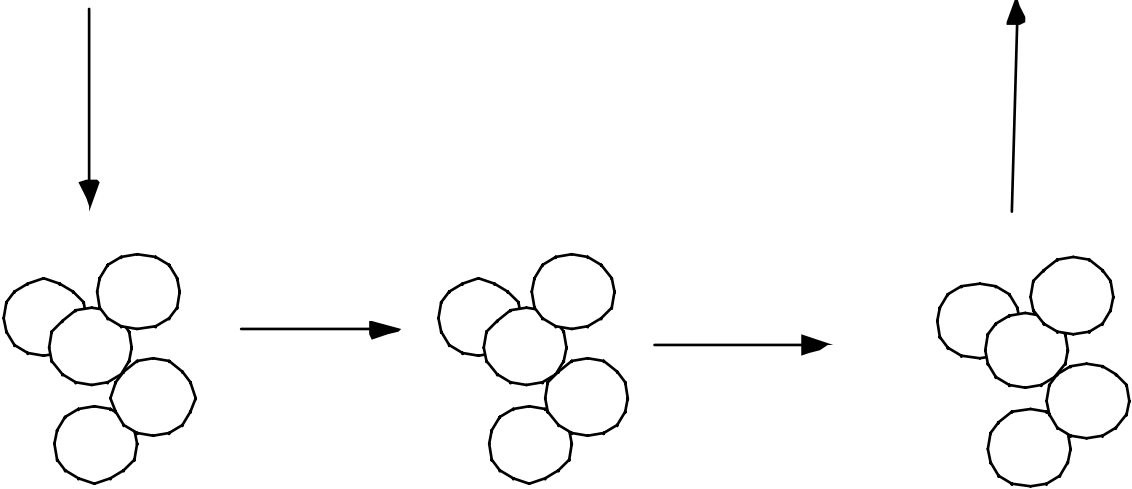
- RRE Rev Responsive element
- cPPT central polypurine tract
- EF-1 α elongation factor 1 α promoter
- PRE post-transcriptional regulatory element
- SD/SA Splice donor and acceptor sequences
- Prom Transcriptional promoter/enhancer element



From: Cockrell et al., *Molecular Therapy* (2006)

Infect with vector with
suspect RCL

Test for p24, RT, Vector



Growth and Amplification

JOURNAL OF VIROLOGY, Nov. 2000, p. 9868–9877

0022-538X/00/\$04.00+0

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Vol. 74, No. 21

Multiple Blocks to Human Immunodeficiency Virus Type 1 Replication in Rodent Cells

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Received 12 June 2000/Accepted 6 August 2000

Lentiviral vectors are derived from:

Primates HIV-1, HIV-2 and SIV

Non-primates FIV, EIAV and BIV

Recommendations:

BL2 for manipulation of generation 3 vectors

ABL1 for housing animals injected with vector

Exceptions:

Animals with human cells that may allow replication of HIV-1 RCL (though non has been detected)

At the discretion of the IBC.

Discussion Questions

The aim of the discussion is to develop some general guidance to assist Institutional Biosafety Committees in conducting risk assessments and determination of appropriate containment for lentiviral vector research.

- What factors should be considered in a biosafety assessment of lentiviral vector research with regard to
 - The parental virus the vector is derived from (e.g., HIV-1, FIV, EIAV, etc.)
 - Safety modifications to the vector system (e.g., deletion of viral genes, sequence overlap creating potential for recombination, SIN vector, etc.)
 - The system used to generate vector (e.g., 2, 3, or 4 plasmid transient transfections, stable packaging cell lines, etc.)
 - Pseudotyping (e.g., VSV-G, etc.)

- What factors should be considered in determining containment for different types of manipulations of lentiviral vectors such as
 - Vector generation
 - Tissue culture
 - Small and large animal work
 - Vector administration
 - Housing
 - Animals permissive for viral replication
 - Large scale production

Discussion Questions

- What consideration should be given to the type of transgene expressed from the vector?
- When should testing for replication competent lentivirus (RCL) be considered?
- What RCL assays may be useful?
- For research in which enhanced BL2 containment is considered appropriate, what types of enhancements to practices would be most useful?