Disciplina: BMM0586 - Virologia

Vírus como ferramentas: os vetores virais

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- ✓ **Os vetores virais são uma ferramenta promissora para a entrega eficaz de material genético em células.**
- ✓ **Consideram a capacidade natural de um vírus de transmitir a carga genética para as células**

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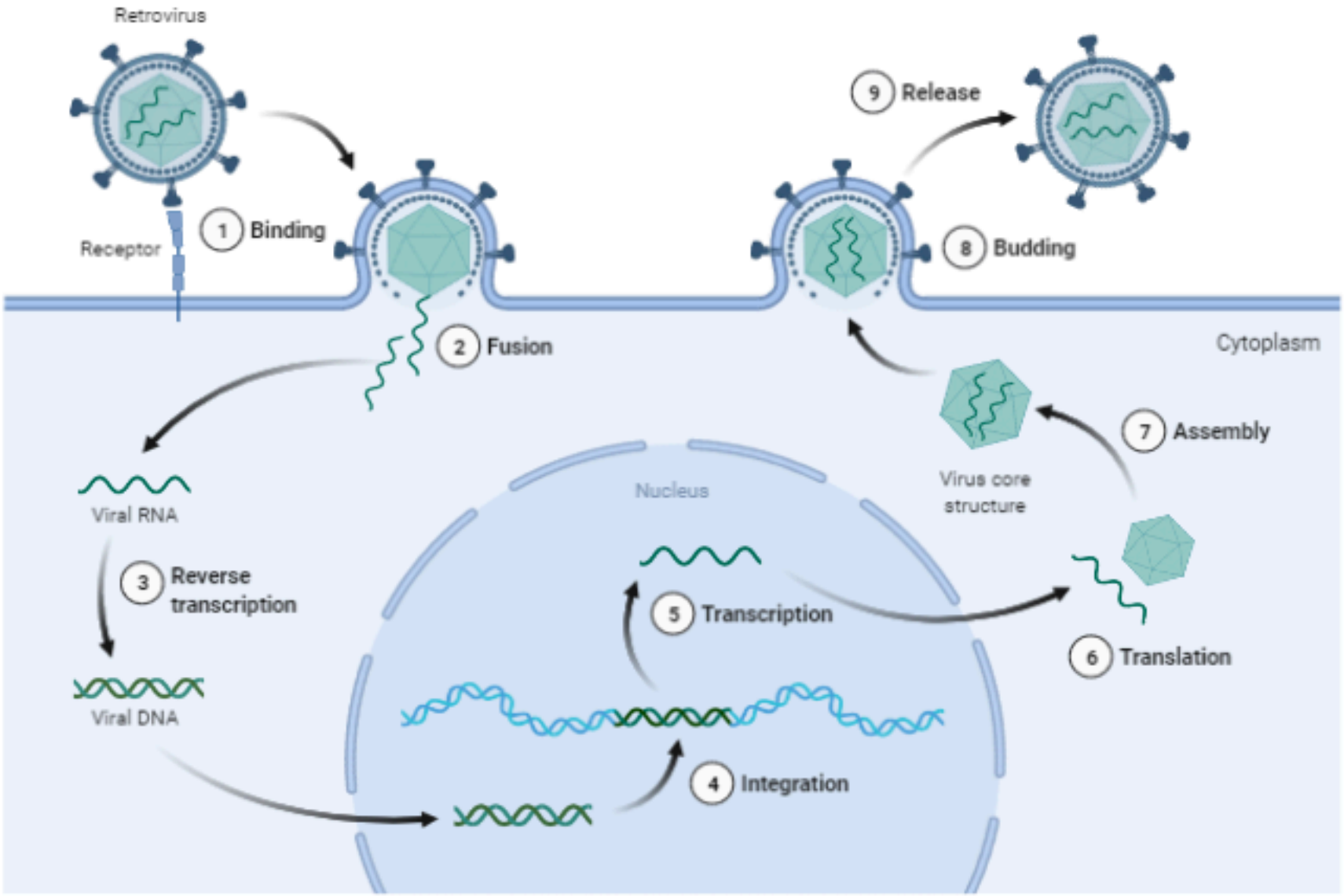
HIV



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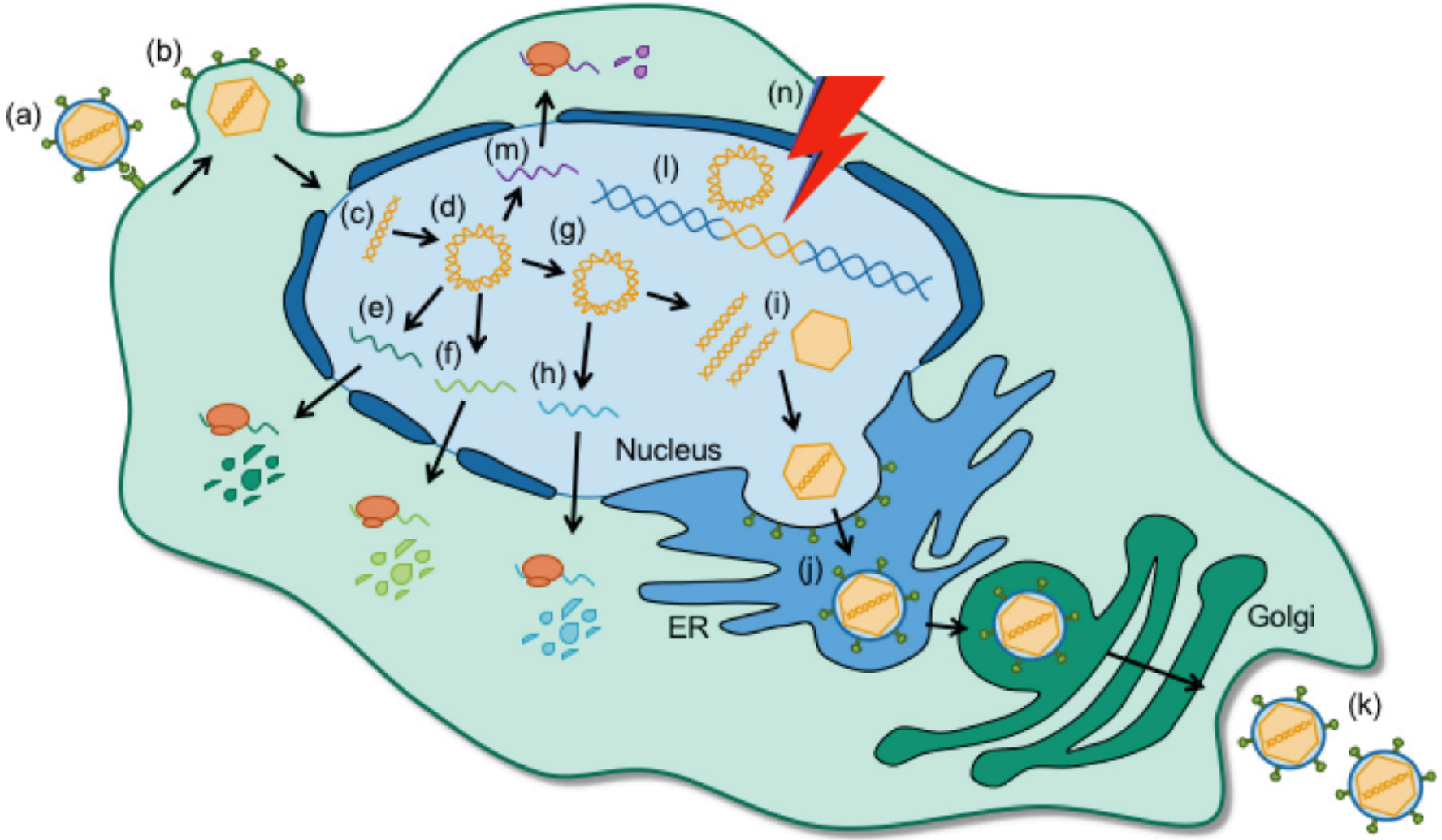
Herpes vírus (HSV)



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	Adenoviridae	*Parvoviridae*	*Retroviridae*	*Retroviridae*
				
	ADENOVIRUS	AAV	γ-RETROVIRUS	LENTIVIRUS
SIZE	~90-100 nm	~25 nm	~80-100 nm	~80-100 nm
GENOME	dsDNA	ssDNA	ssRNA	ssRNA
PACKAGING CAPACITY	~8 kb – 36 kb	~4.7 kb	10 kb	8 kb
TRANSDUCTION	Dividing and non-dividing cells	Dividing and non-dividing cells	Dividing cells	Dividing and non-dividing cells
TRANSDUCTION EFFICIENCY	High	Moderate	Moderate	Moderate
INTEGRATION	Non-integrating	Non-integrating	Integrating	Integrating
EXPRESSION	Transient	Transient or stable	Stable	Stable
BIOSAFETY LEVEL	BSL-2	BSL-1	BSL-2	BSL-2
IMMUNOGENICITY	High	Low	Moderate-High	Moderate-High
GENE THERAPY STRATEGY	*In vivo*	*In vivo*	*Ex vivo*	*Ex vivo*

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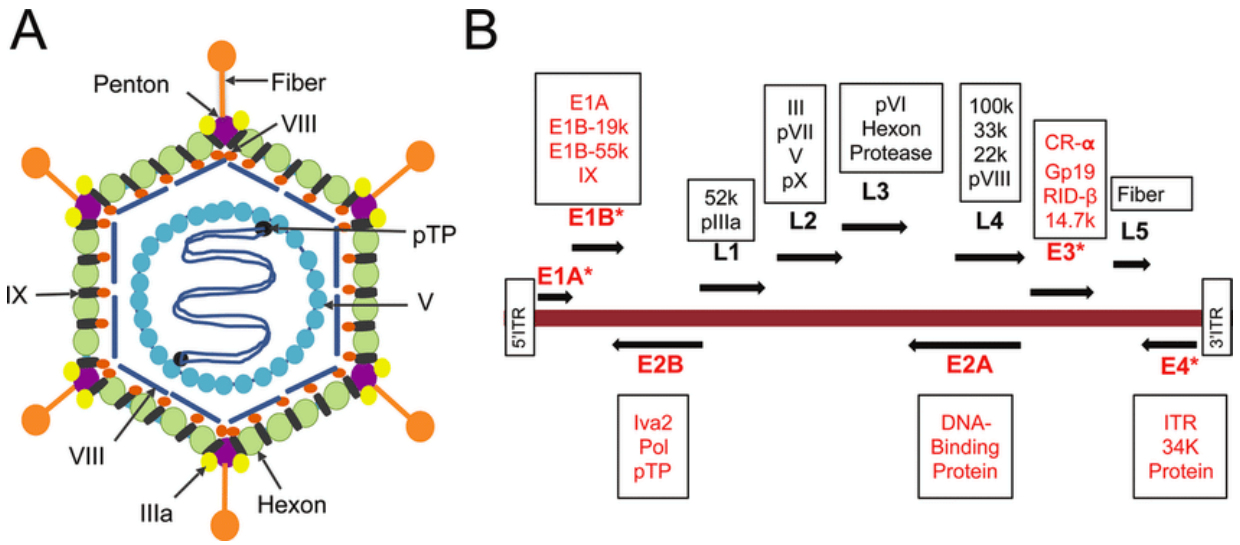
ADENOVIRUS

- ✓ DNA, 26 a 45 kb, flanqueado por ITR (inverted terminal repeats)
- ✓ DNA encapsulado em um icosaédrico não envelopado
- ✓ Capsídeo (tamanho de 90 nm de diâmetro).
- ✓ Mais usado é o Ad5
- ✓ Permanece episssomal



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ADENOVIRUS

Vantagens

- ✓ Empacota cerca de 30 kb
- ✓ Episssomal.
- ✓ *Dividing and non dividing cells*

Desvantagens

- ✓ Alta resposta imune do hospedeiro contra o capsídeo, resultando em toxicidade e morte das células transduzidas
- ✓ Estratégia para diminuir resposta imune: uso com PEG, polietileno glicol e imunossupressores

Uso

- ✓ Vacinas

AAV

- ✓ Parvovírus não patogênico/ *helper-Ad dependent virus*
- ✓ Composto de uma fita simples de 4,7 kb
- ✓ DNA encapsulado em um icosaédrico não envelopado
- ✓ Capsídeo (tamanho de 20 a 25 nm de diâmetro).
- ✓ Adsorção em ácido siálico, galactose, ou heparan sulfato e proteínas (AAV receptor- AAVR)
- ✓ AAV8 (fígado); AAV1 e AAV5 em SNC (Asokan, Schaffer, & Samulski, 2012

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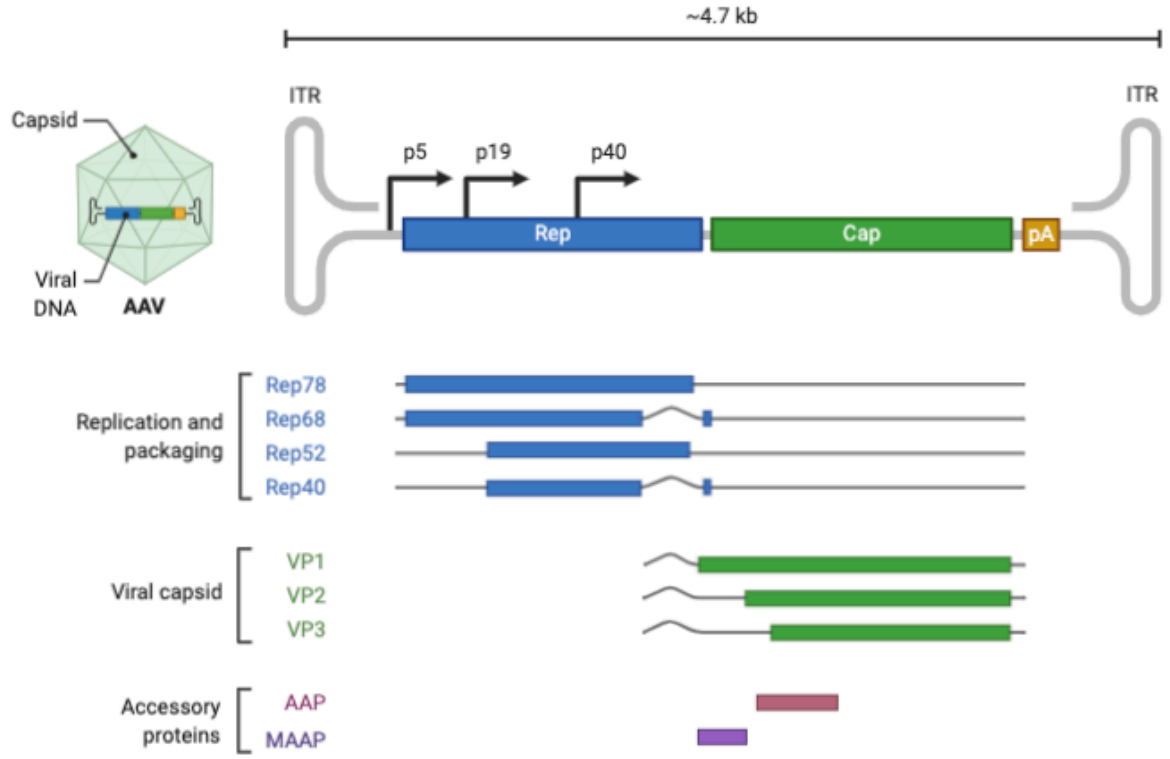
AAV

✓ O genoma viral é composto por três genes: Rep, Cap e AAP, flanqueado por terminal invertido repetições (ITRs) que funcionam como o vírus origem de replicação e o sinal de empacotamento.

- *REP: genes que codificam proteínas não estruturais, relacionados a replicação*
- *Cap: genes que codificam proteínas estruturais*
- *AAP: genes associados a montagem (assembly activating protein)*



AAV

Vantagens

- ✓ Não patogênico ou citotóxico
- ✓ In vivo é pouco imunogênico
- ✓ Epissomal persite por longos períodos em células que não se dividem
- ✓ *Low integration rate*: baixa tumorigenicidade (mutagênese)

Desvantagens

- ✓ Empacota < 5 kb

Uso

- ✓ Gene replacement in vivo (gene theraphy)
- ✓ Knock in ou knoch down
- ✓ Hemofilia, cegueira, neurobiologia e anatomia do SNC (Sizemore, Seeger-Armbruster, Hughes, & Parr-Brownlie, 2016)

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RETROVIRUS

- ✓ ssRNA, 2 cópias de RNA +
- ✓ Composto de uma fita simples de 4,7 kb
- ✓ DNA encapsulado em um icosaédrico envelopado
- ✓ Capsídeo e envolope (tamanho de 80 a 120 nm de diâmetro).
- ✓ Lentivirus (non-dividing cells) HIV, gamma retrovírus (dividing cells) Moloney Murine Leukenia Virus (MoMLV). feline immunodeficiency virus (FIV), and equine infectious anaemia virus (EIAV)

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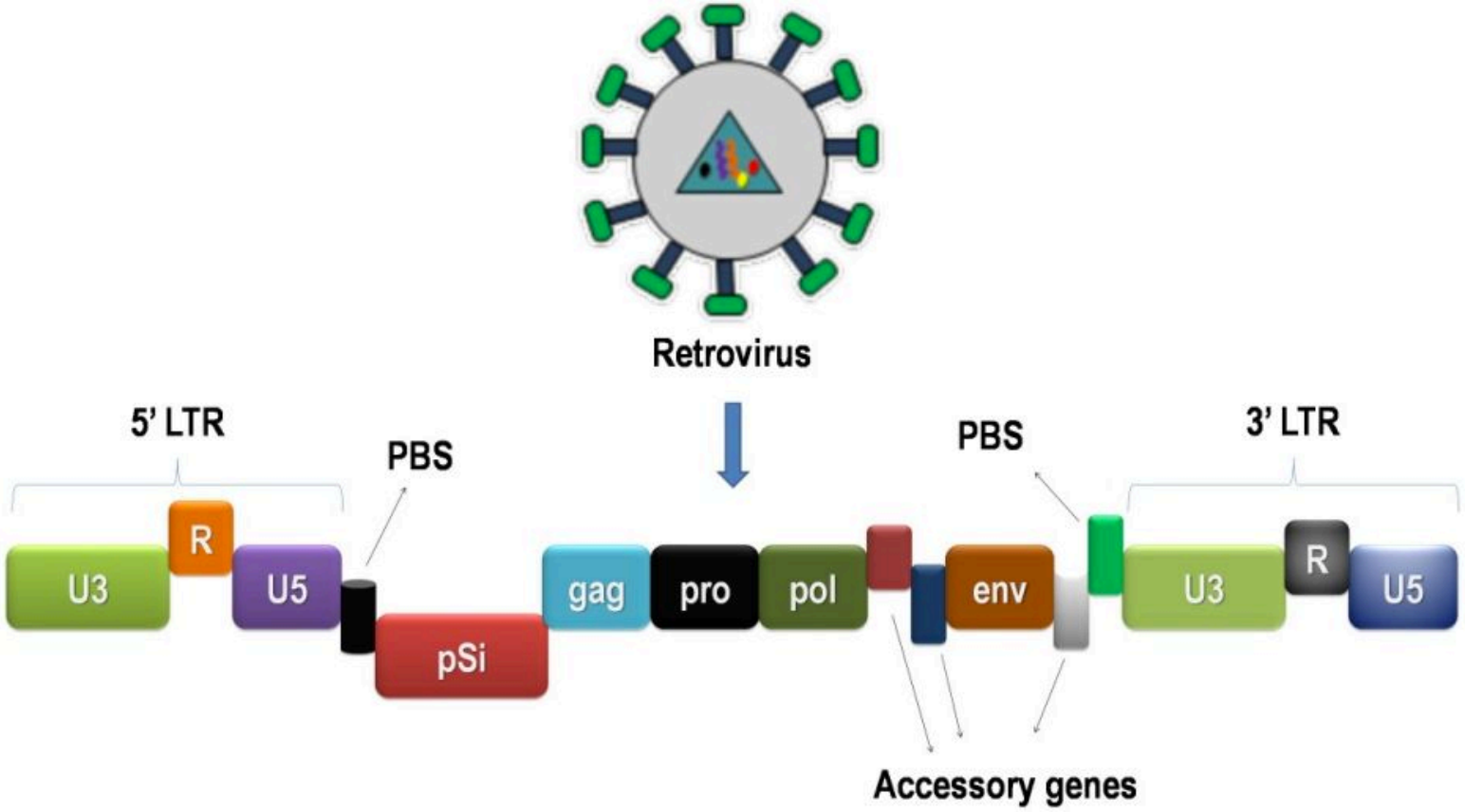
RETROVIRUS

- ✓ O genoma viral é composto por três genes: *gag, pol e env* e genes acessórios (*tat, rev, vpr, vpu, nef, and vif*) flanqueados por terminal longo invertido repetições (LTRs)
- ✓ Trasncriptase reversa faz cDNA



RETROVIRUS

Vantagens

- ✓ Empacota cerca de 9kb
- ✓ Integra no genoma, *long-term gene expression.*

Desvantagens

- ✓ Pode ser silenciado
- ✓ Integra no genoma, mutagênese

Uso

- ✓ Ex vivo delivery (gene theraphy)
- ✓ Doenças do sangue, SNC, metabólicas
- ✓ CARs terapia anti câncer (leucemia)

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Vector	MoMLV retroviral	Lentiviral	Adenoviral	Helper-dependent adenoviral	Recombinant HSV	HSV amplicon	AAV
Family	Retroviridae	Retroviridae	Adenoviridae	Adenoviridae	Herpesviridae	Herpesviridae	Parvoviridae
Particle size (nm)	100	100	70-120	70-120	120-300	120-300	20-25
Cargo	RNA	RNA	dsDNA	dsDNA	dsDNA	dsDNA	ssDNA
Packaging capacity (kB)	7-8	7-9	8-10	Up to 36	30-50	Up to 150	4.8
Vector yield (transducing units ml-1)	1.00E+09	1.00E+09	1.00E+12	1.00E+12	1.00E+11	1.00E+08	1.00E+13
Chromosomal integration?	Yes	Yes	No	No	No	No	No
Oncolytic?	No	No	Yes/no	No	Yes/no	No	No
Infects post-mitotic cells?	No	Yes	Yes	Yes	Yes	Yes	Yes
Risk of oncogene activation?	Yes	Yes	No	No	No	No	No

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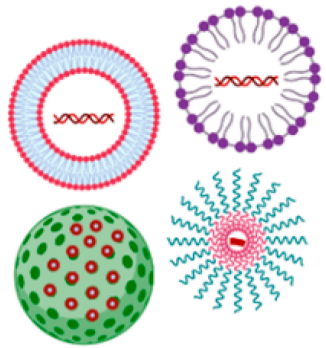
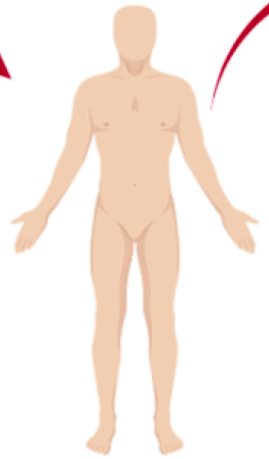
In vivo gene therapy

Ex vivo gene therapy

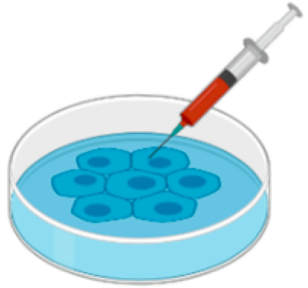
Non-viral delivery system



Nanoparticles that can potentially be used for HCC gene therapy



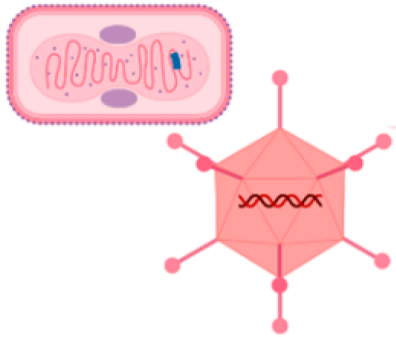
cells isolated from patient



cells modified *in vitro*

Modified cells injected back into the patient

Viral delivery systems



Viruses currently being used for HCC gene therapy

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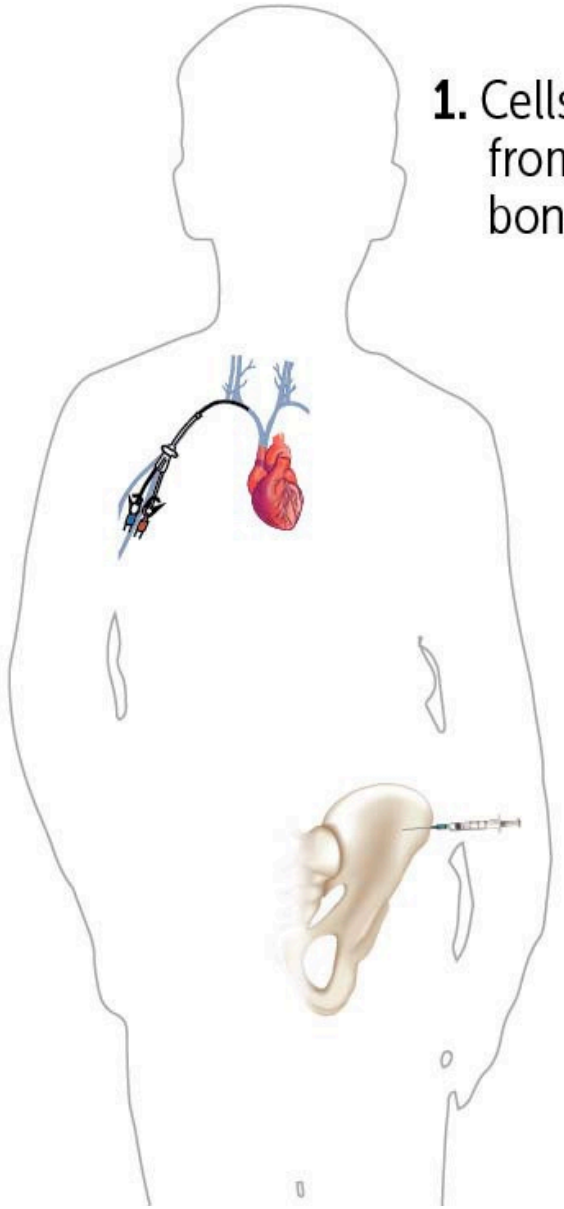
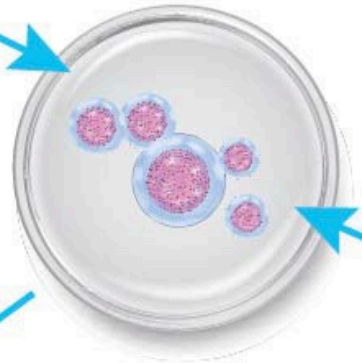
Ex-Vivo Gene Therapy - Cell-Based Delivery

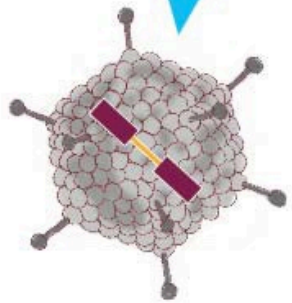


1. Cells are collected from blood or bone marrow

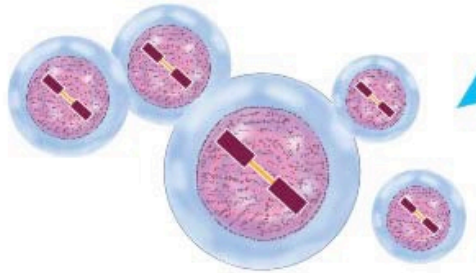
2. Patient's target cells (e.g. stem cells or T cells) are isolated



3. A theraputic gene is packaged into a delivery vehicle (vector), such as an engineered virus



4. Vector is introduced to the patient's stem cells



5. Genetically modified cells are returned to the patient who may require specific conditioning

Boston Children's Hospital

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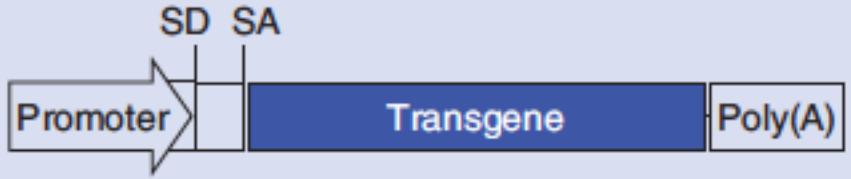
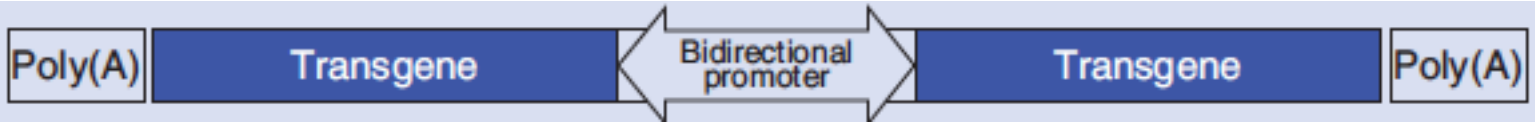
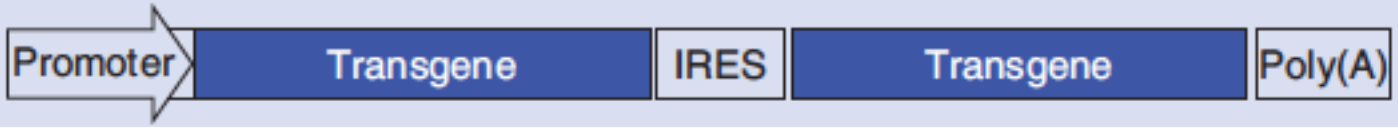
Como construir um vetor viral?

BOX 2. TRANSGENE EXPRESSION CASSETTES







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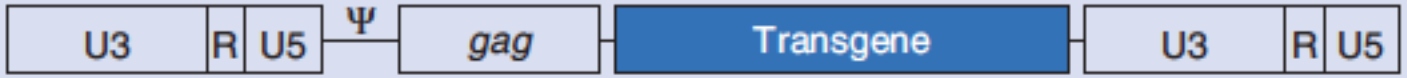
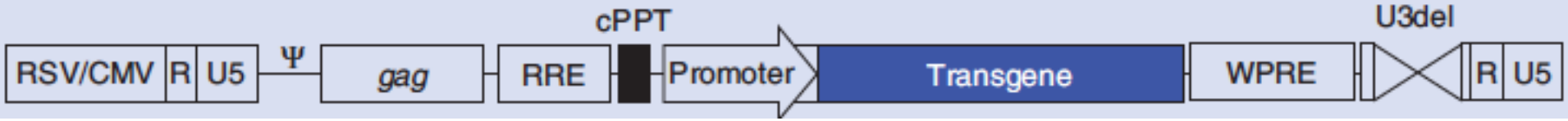
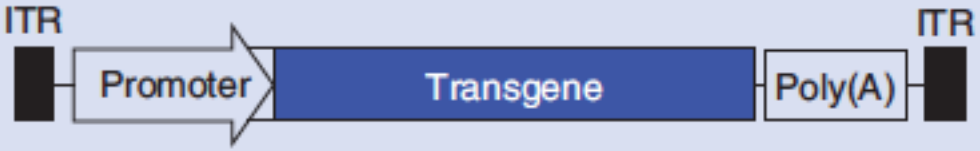
Retrovirus Vectors



Lentivirus Vectors

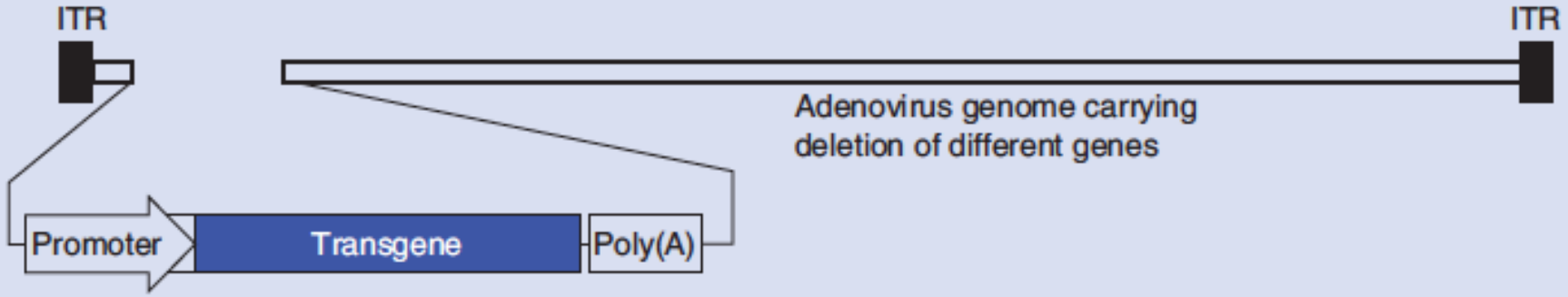


AAV Vectors



Adenovirus Vectors

Standard Ad vector



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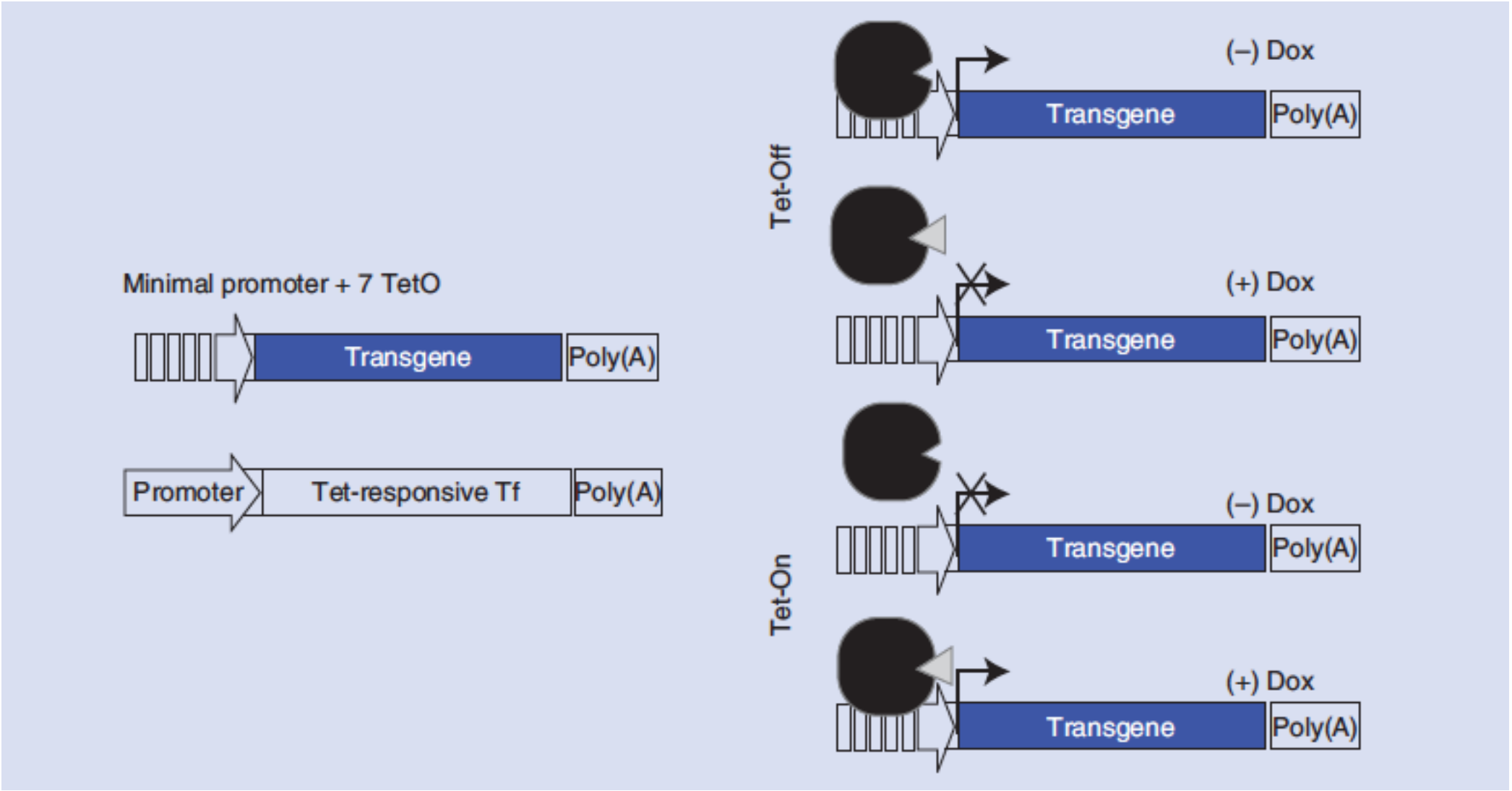
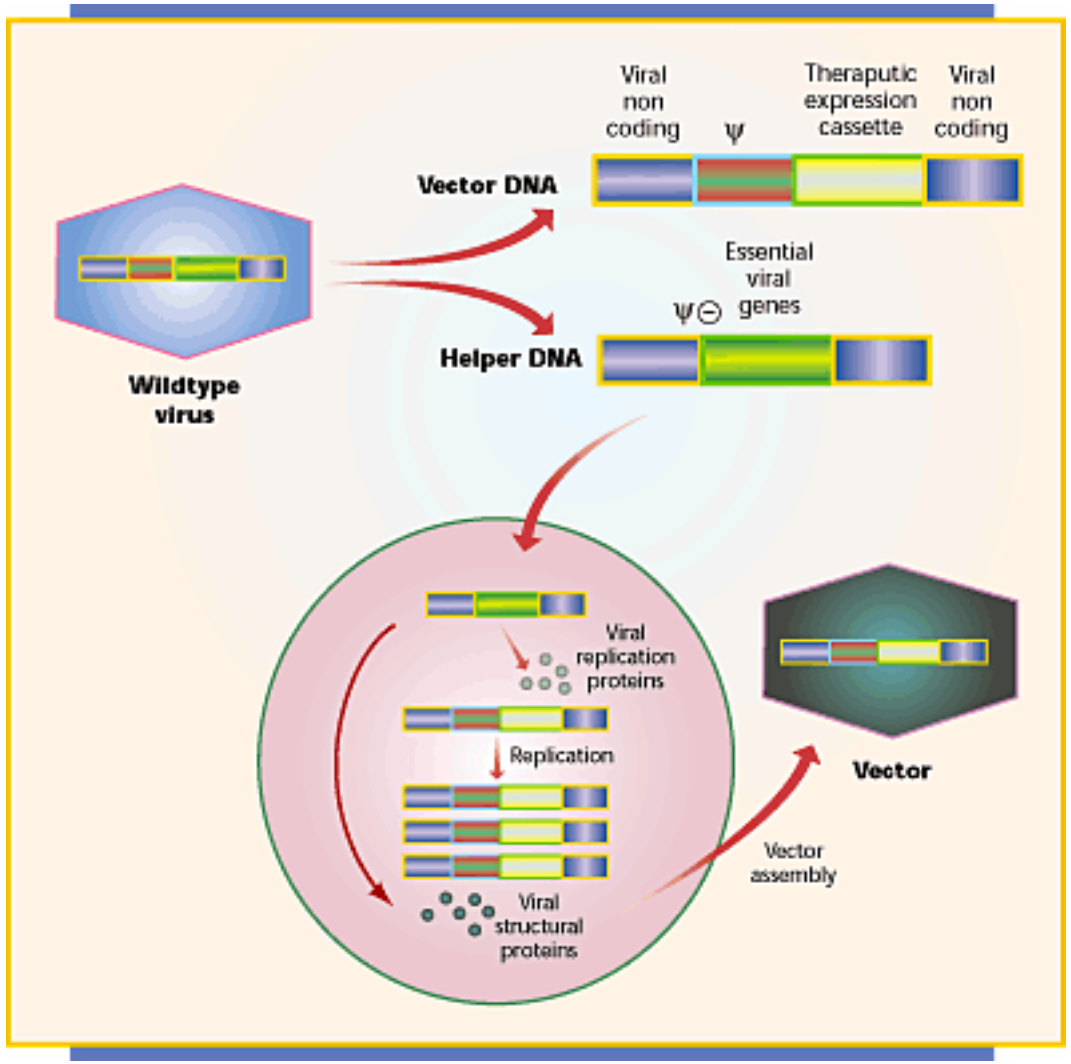
Vetores com a expressão controlada



- ✓ **Os vetores virais são geneticamente modificados de modo que seu capacidade de replicação é prejudicada ou removida**



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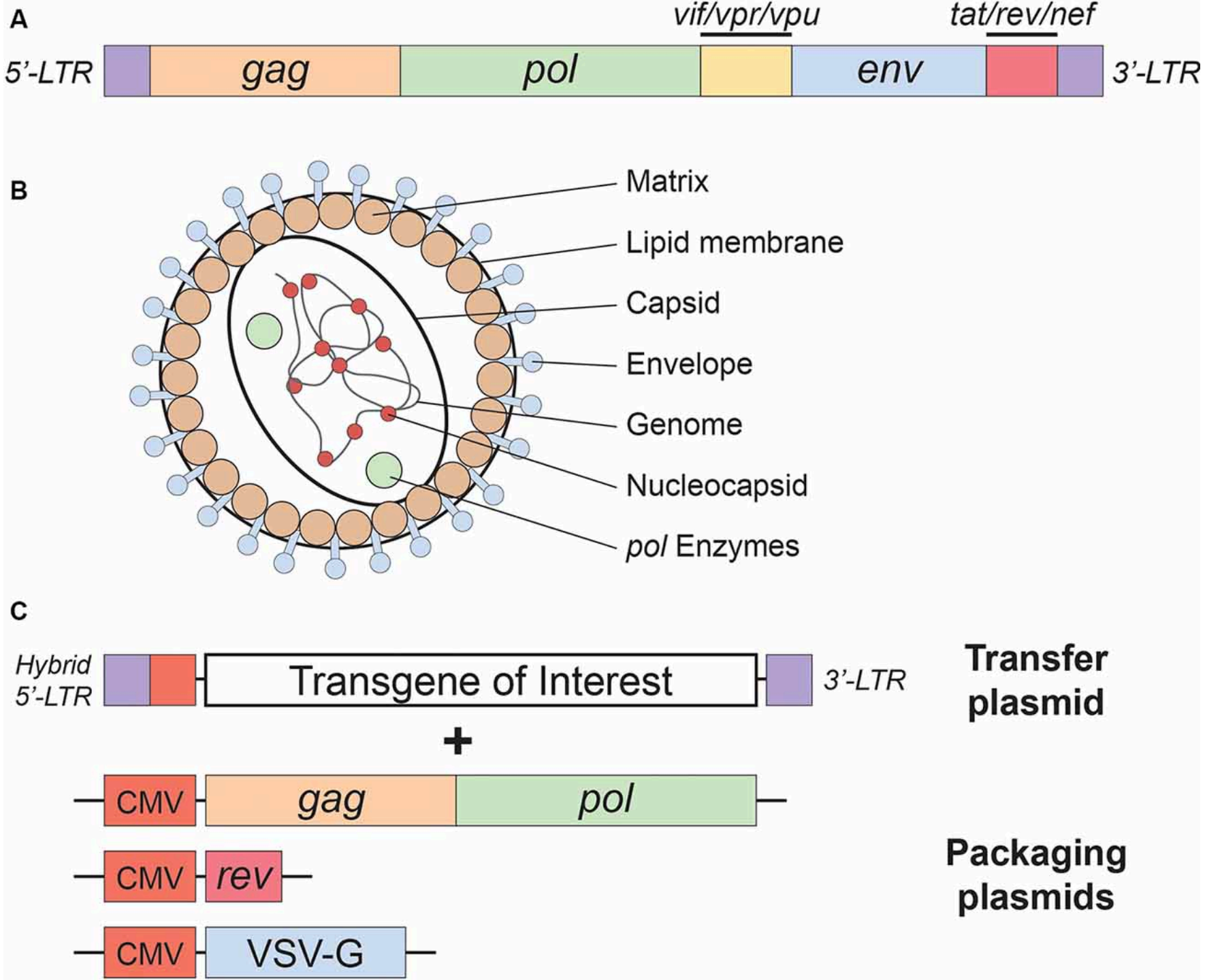
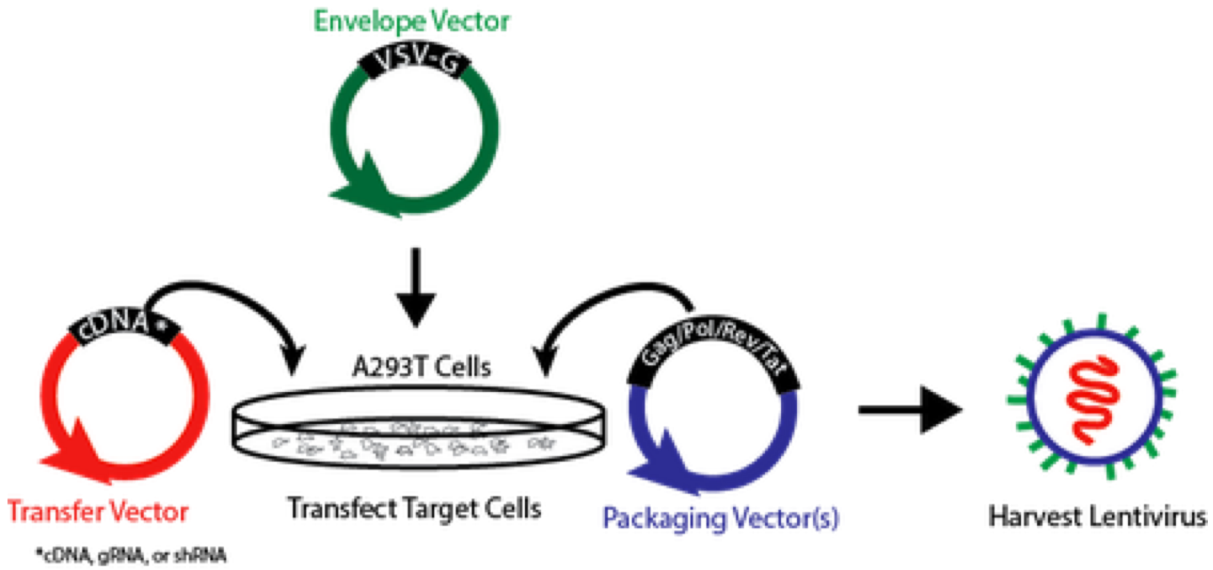
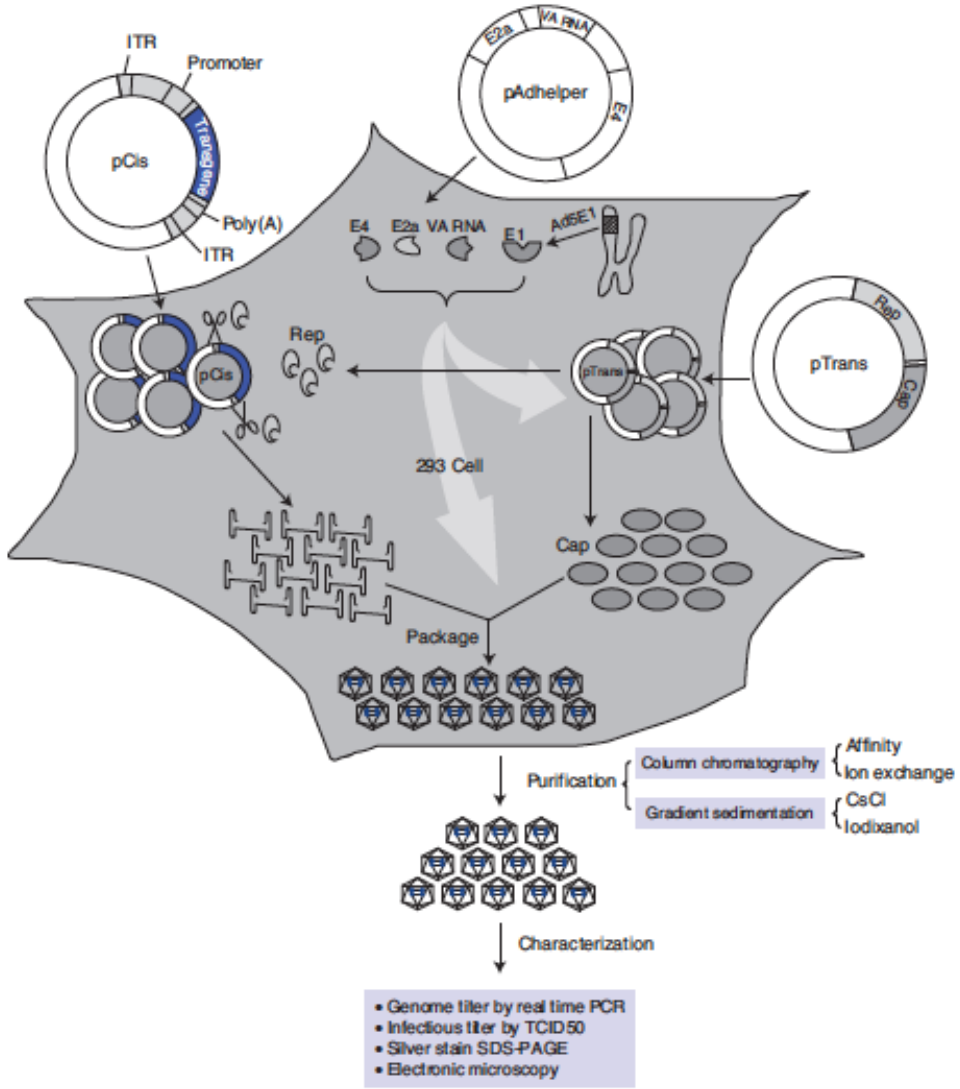
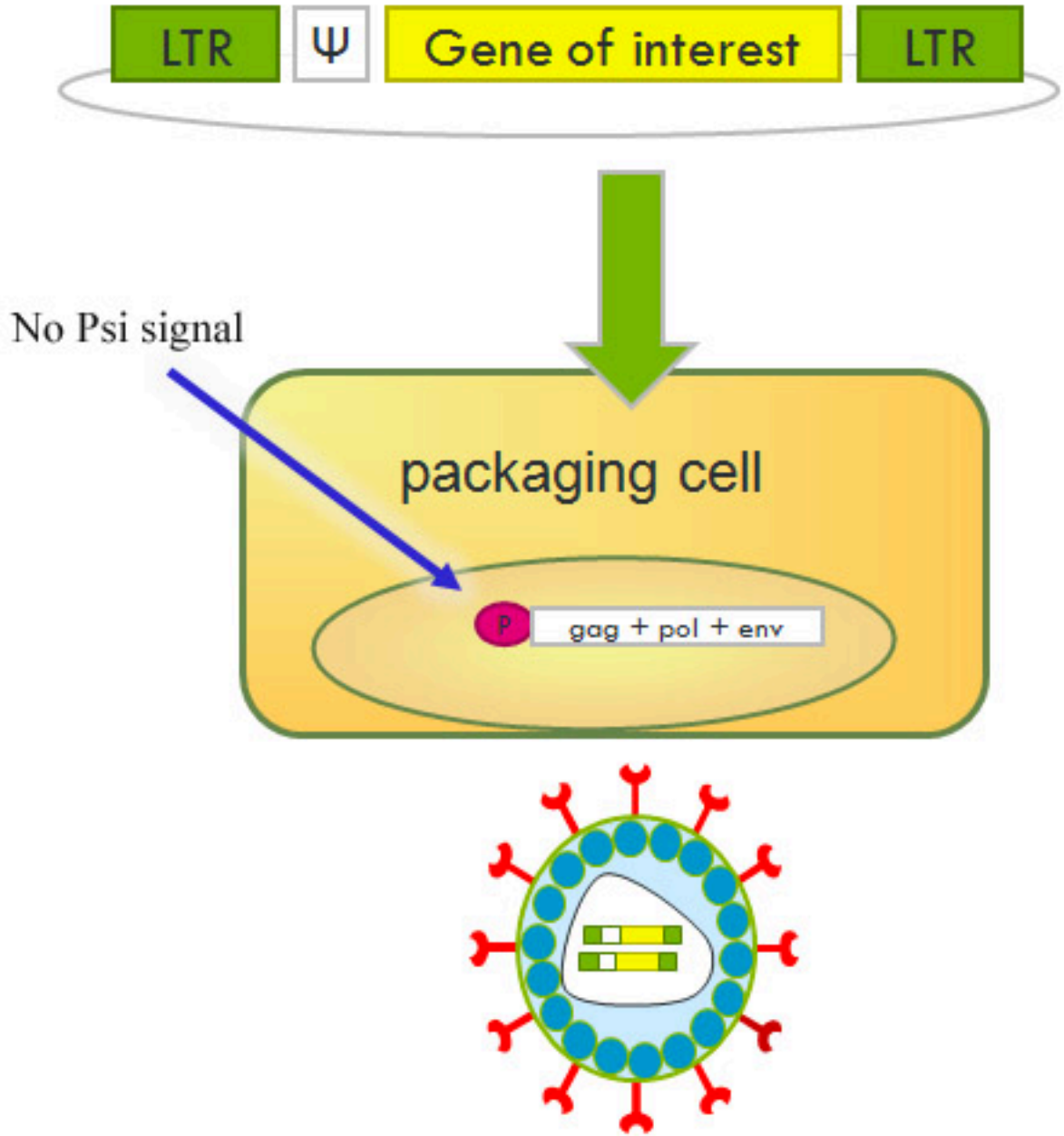


Tripla transfecção





Células empacotadoras



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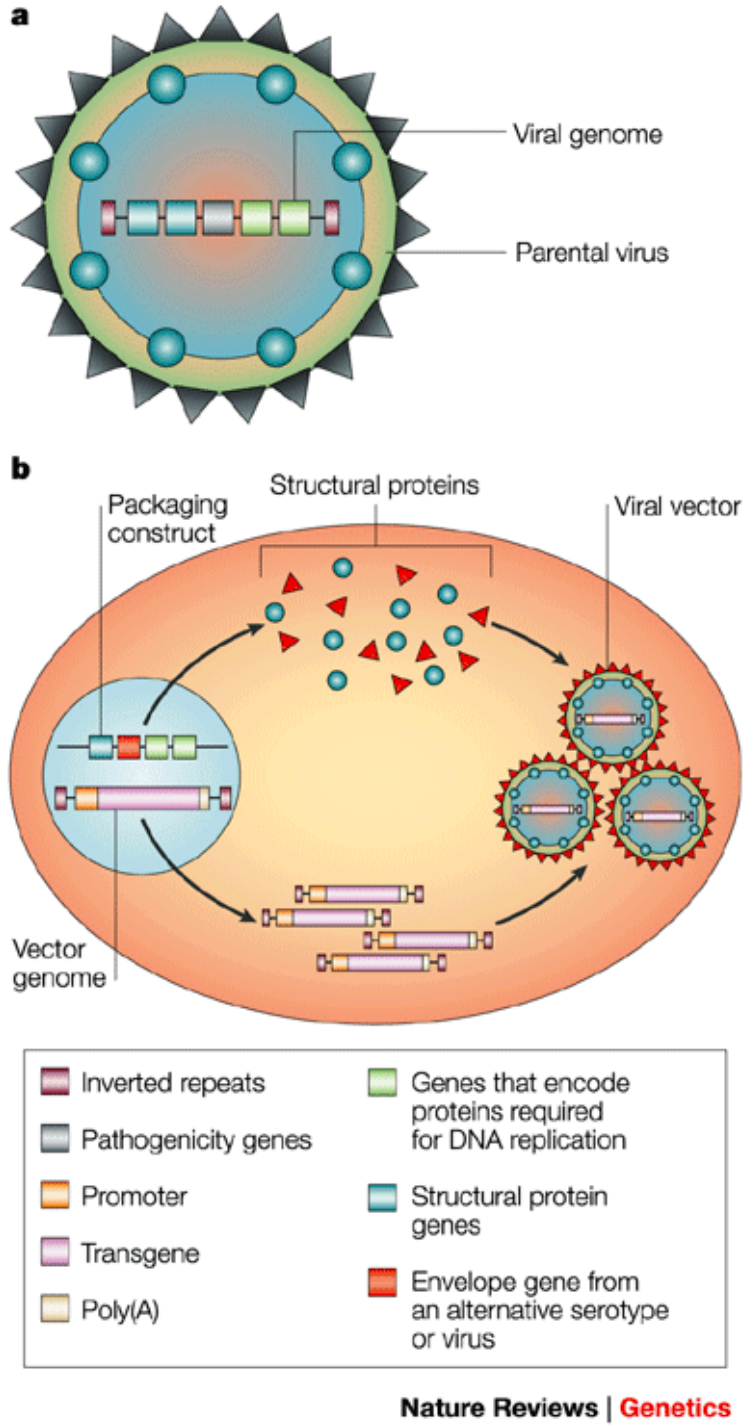
Células empacotadoras



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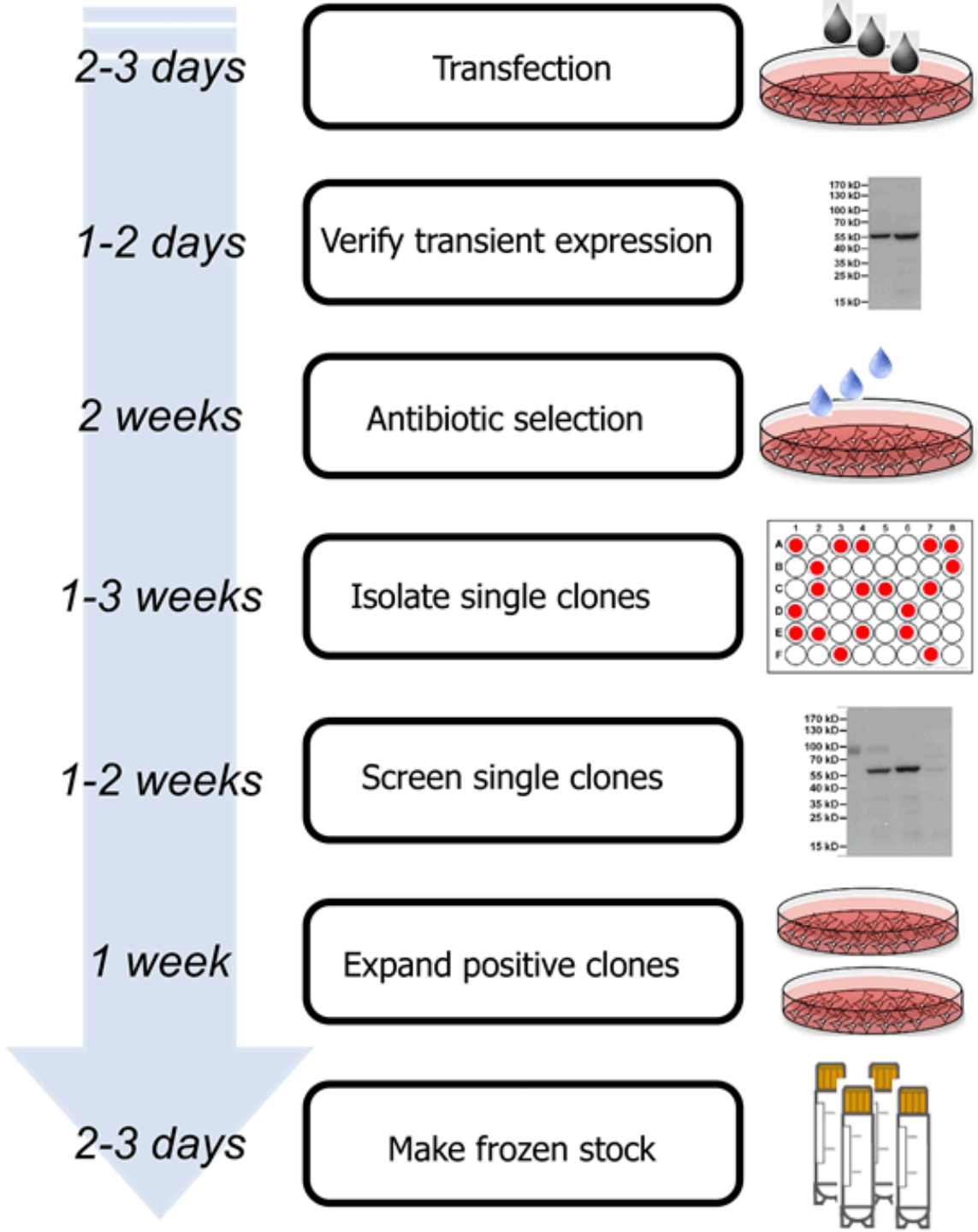
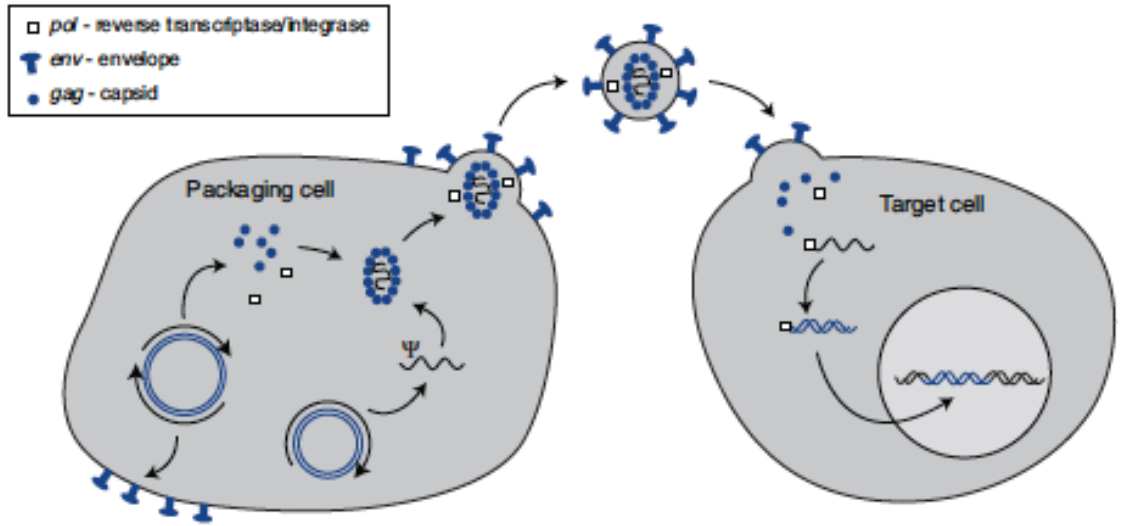


Produção de vírus recombinantes em células empacotadoras e posterior infecção na célula-alvo



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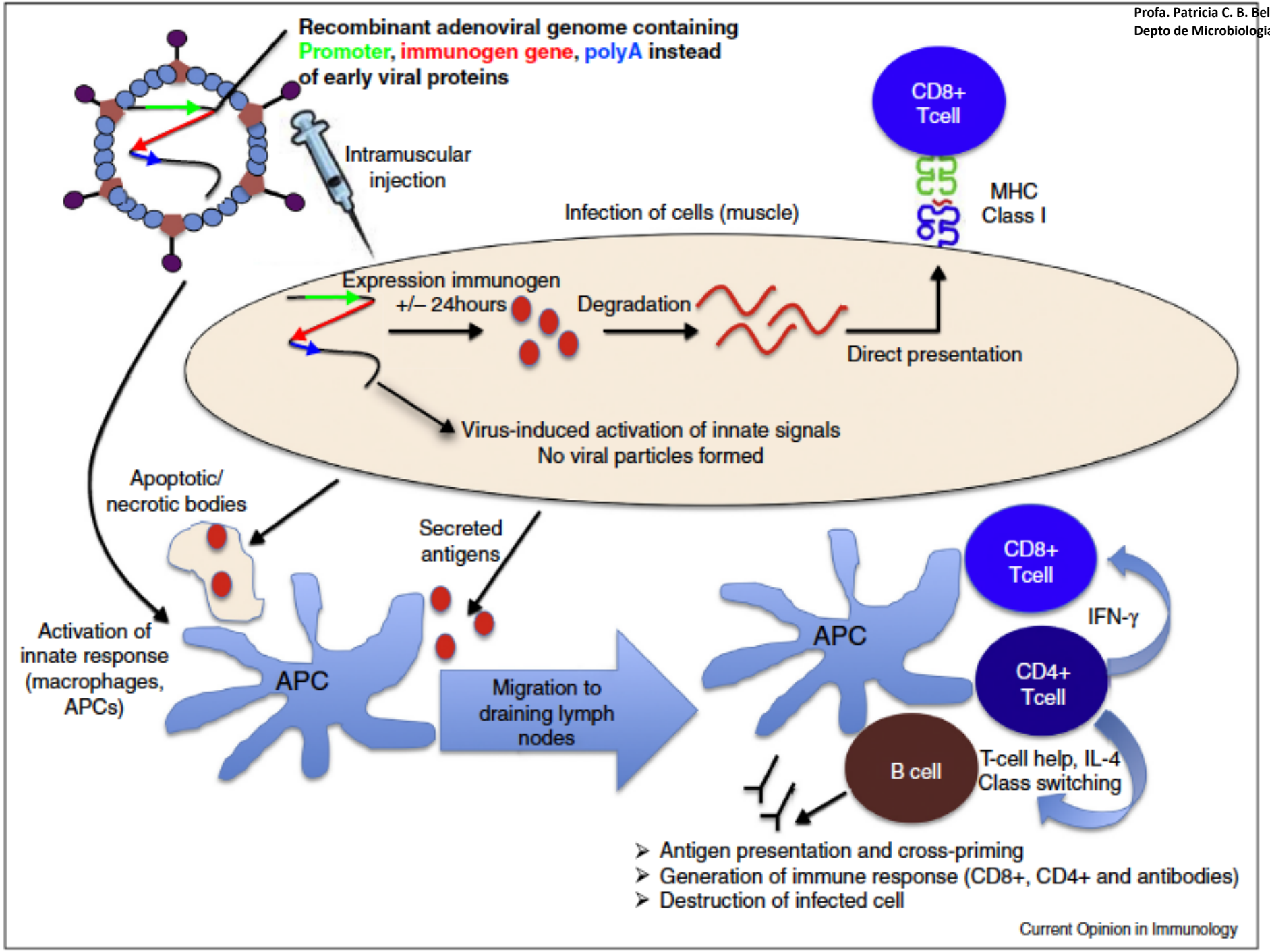
Vírus recombinantes e Vacinas



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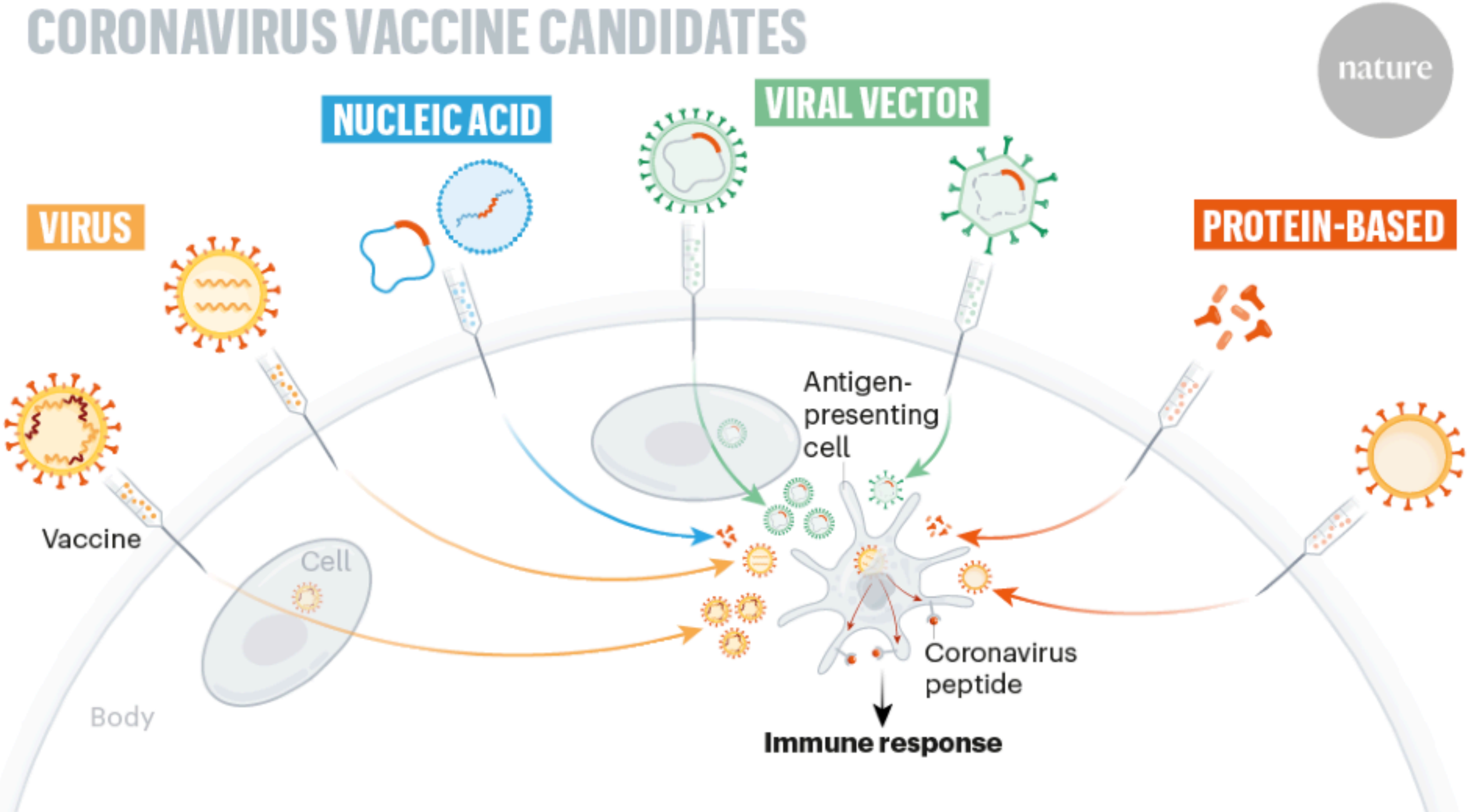
CORONAVIRUS VACCINE CANDIDATES



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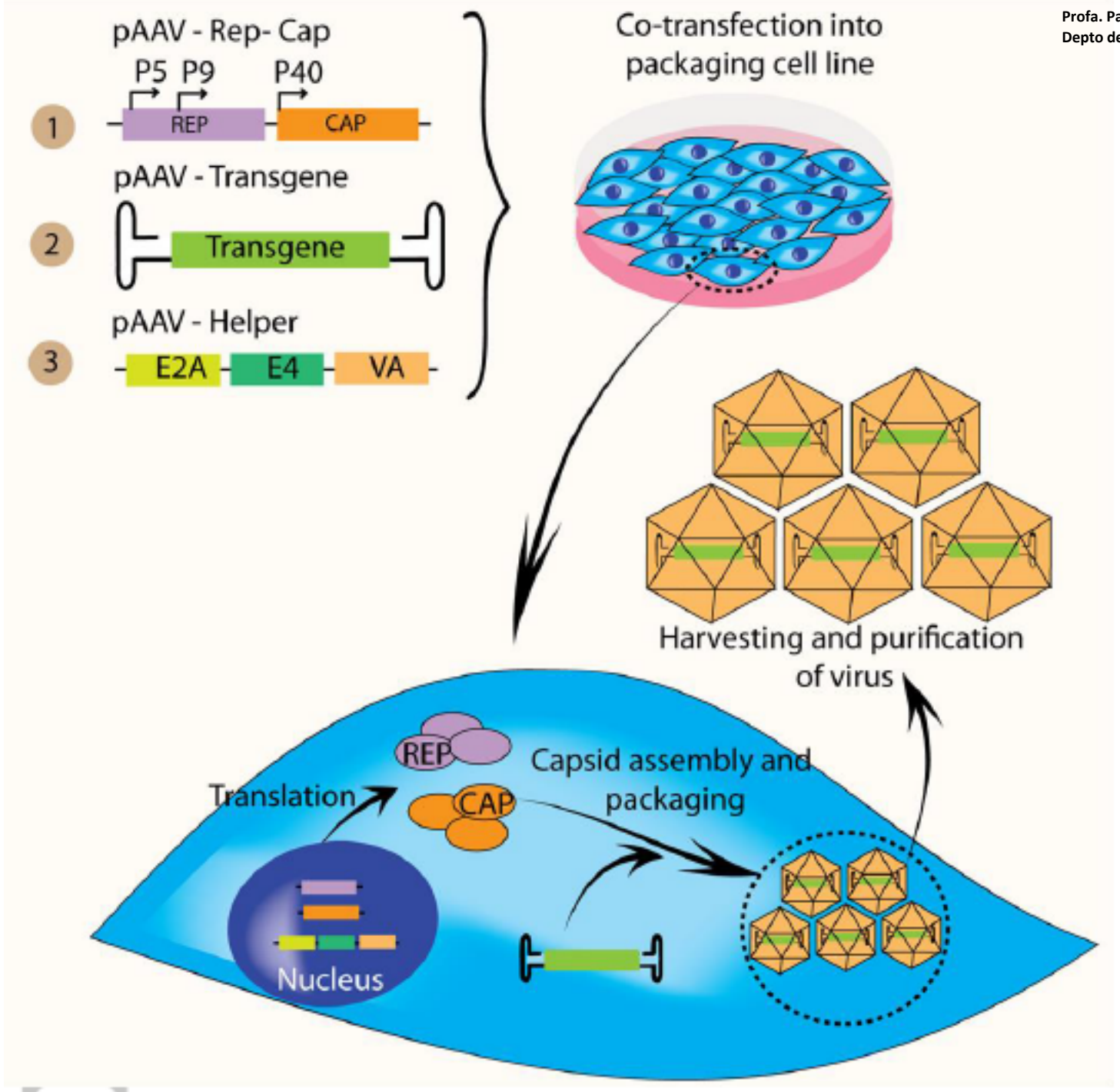
***Harvesting* de vírus recombinantes**



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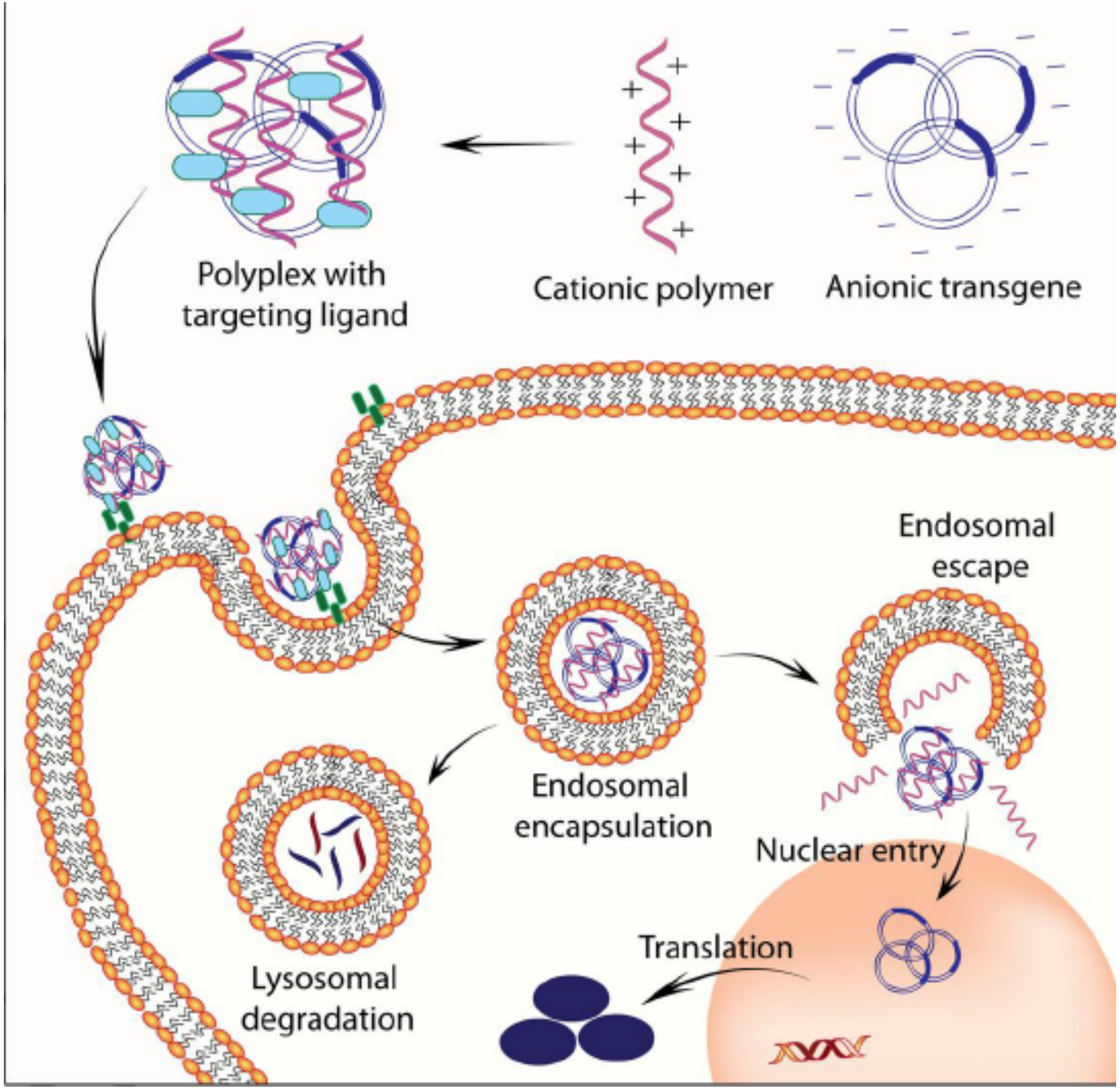
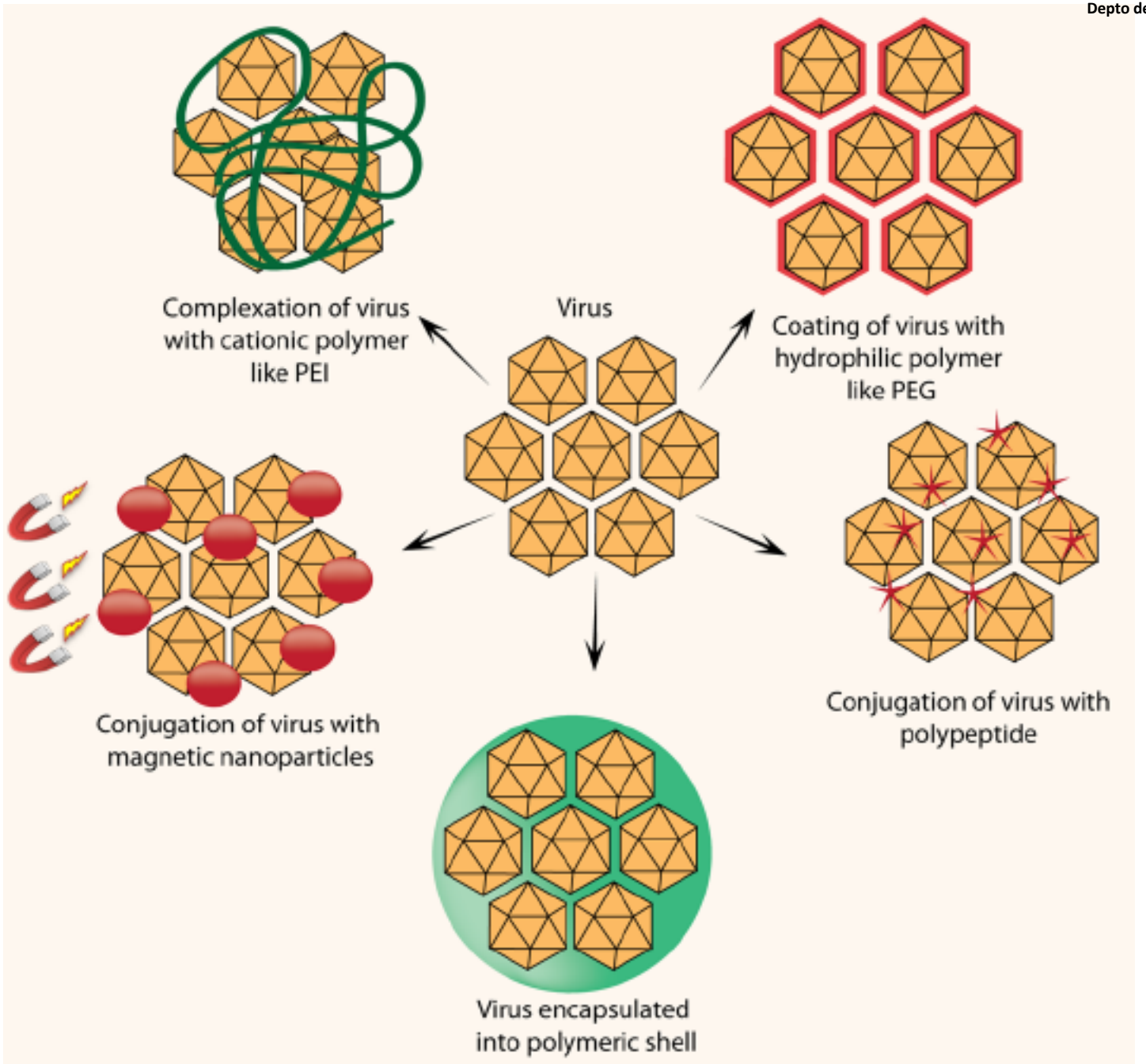
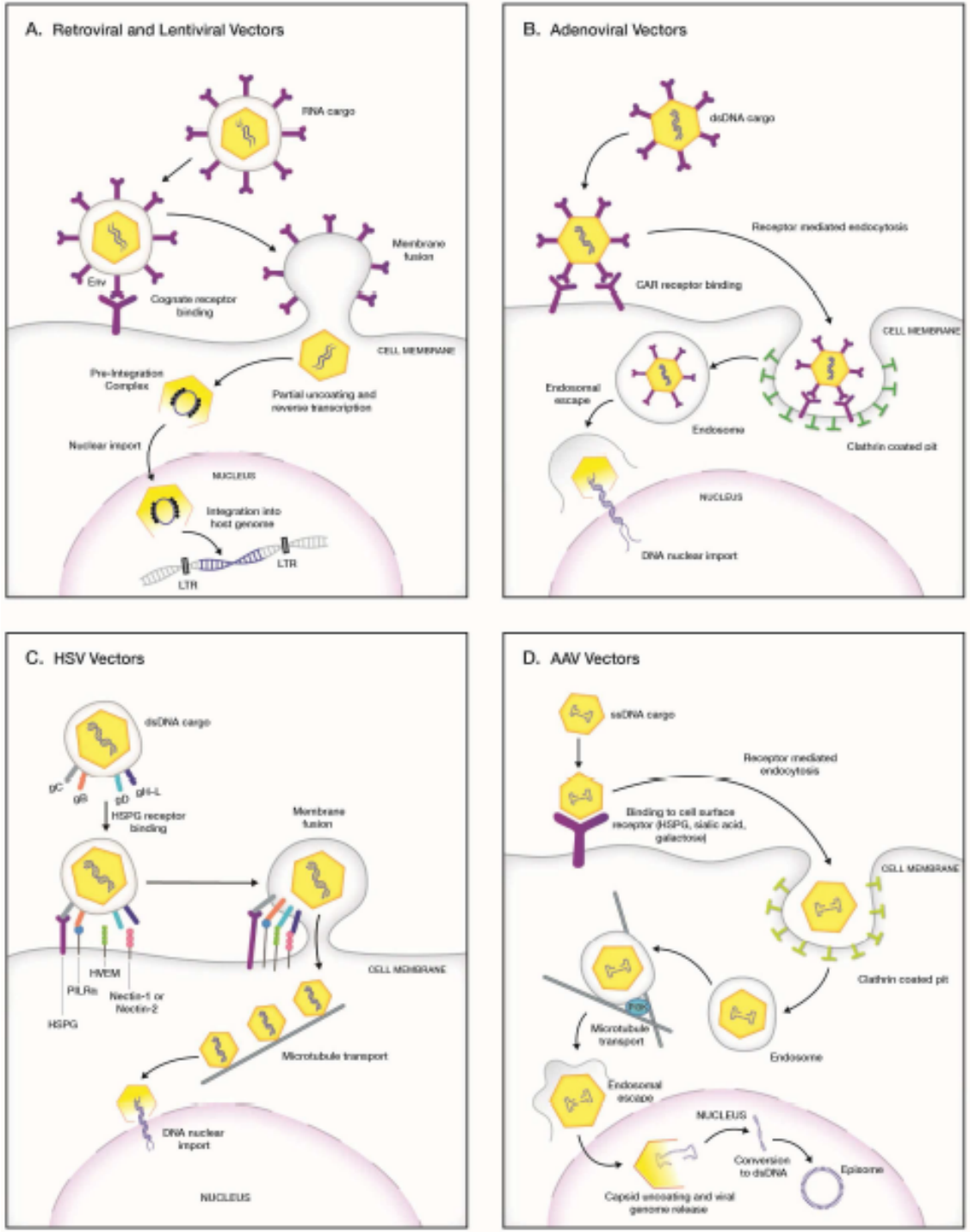
***Harvesting* de vírus recombinantes**



Harvesting de vírus recombinantes




A. Retroviral and Lentiviral Vectors
RNA cargo
Membrane fusion
Env
Cognate receptor binding
CELL MEMBRANE
Pre-Integration Complex
Partial uncoating and reverse transcription
Nuclear import
NUCLEUS
Integration into host genome
LTR
LTR
B. Adenoviral Vectors
dsDNA cargo
Receptor mediated endocytosis
CAR receptor binding
CELL MEMBRANE
Endosomal escape
Endosome
Clathrin coated pit
NUCLEUS
DNA nuclear import
C. HSV Vectors
dsDNA cargo
gC
gB
gD
gH-L
HSPG receptor binding
Membrane fusion
CELL MEMBRANE
HVEM
PILRa
Nectin-1 or Nectin-2
HSPG
Microtubule transport
DNA nuclear import
NUCLEUS
D. AAV Vectors
ssDNA cargo
Receptor mediated endocytosis
Binding to cell surface receptor (HSPG, sialic acid, galactose)
CELL MEMBRANE
Clathrin coated pit
Microtubule transport
Endosome
Endosomal escape
NUCLEUS
Conversion to dsDNA
Episome
Capsid uncoating and viral genome release

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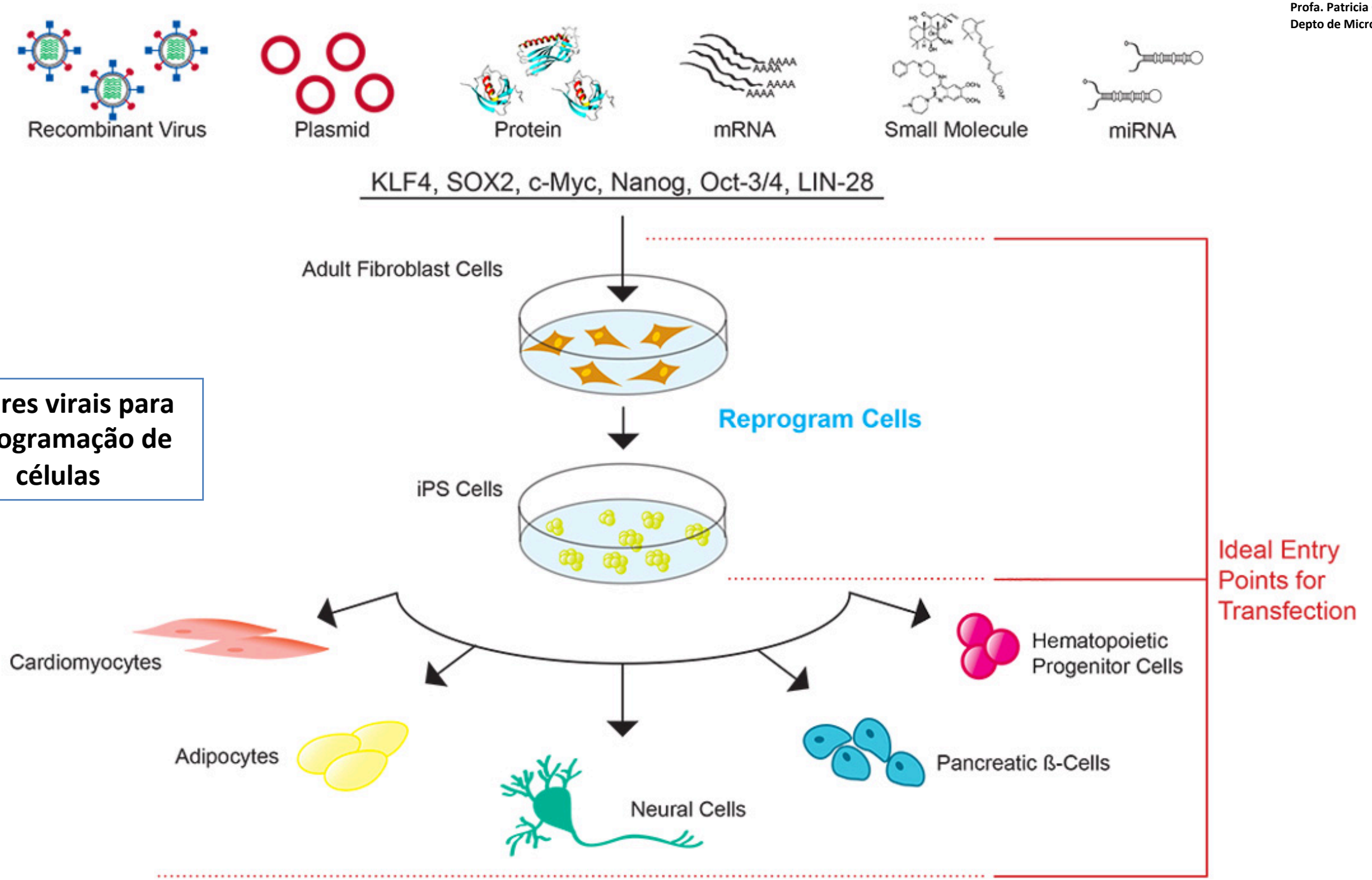
Vetores virais para reprogramação de células



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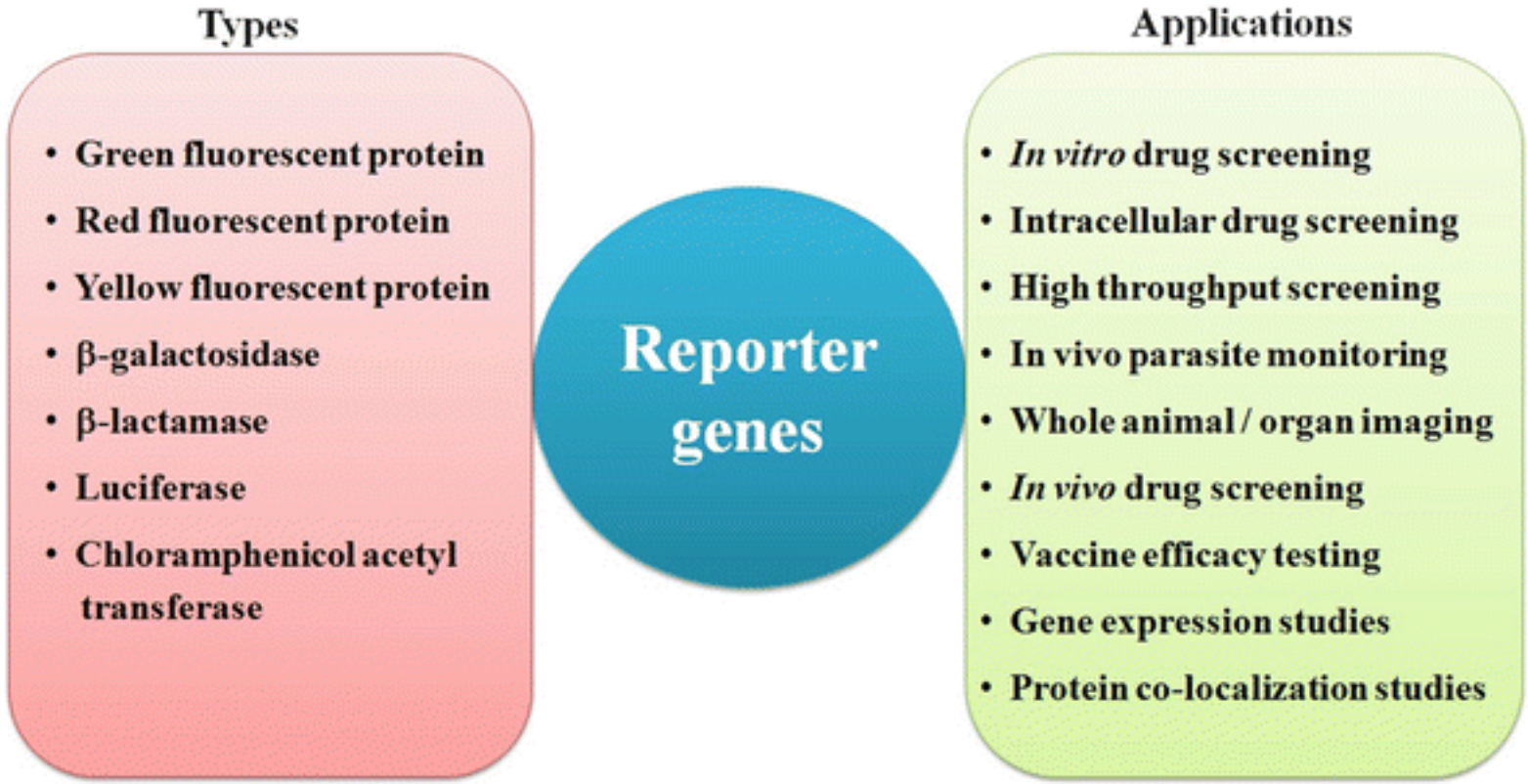
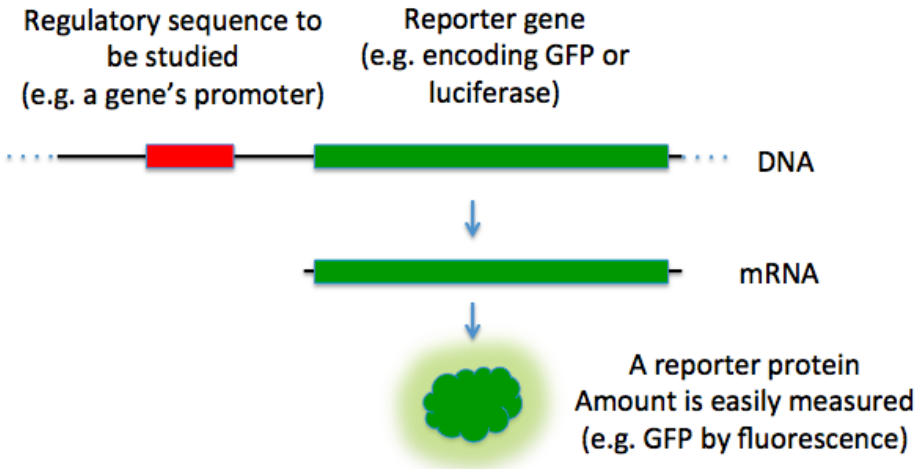
Vetores virais para marcação de células





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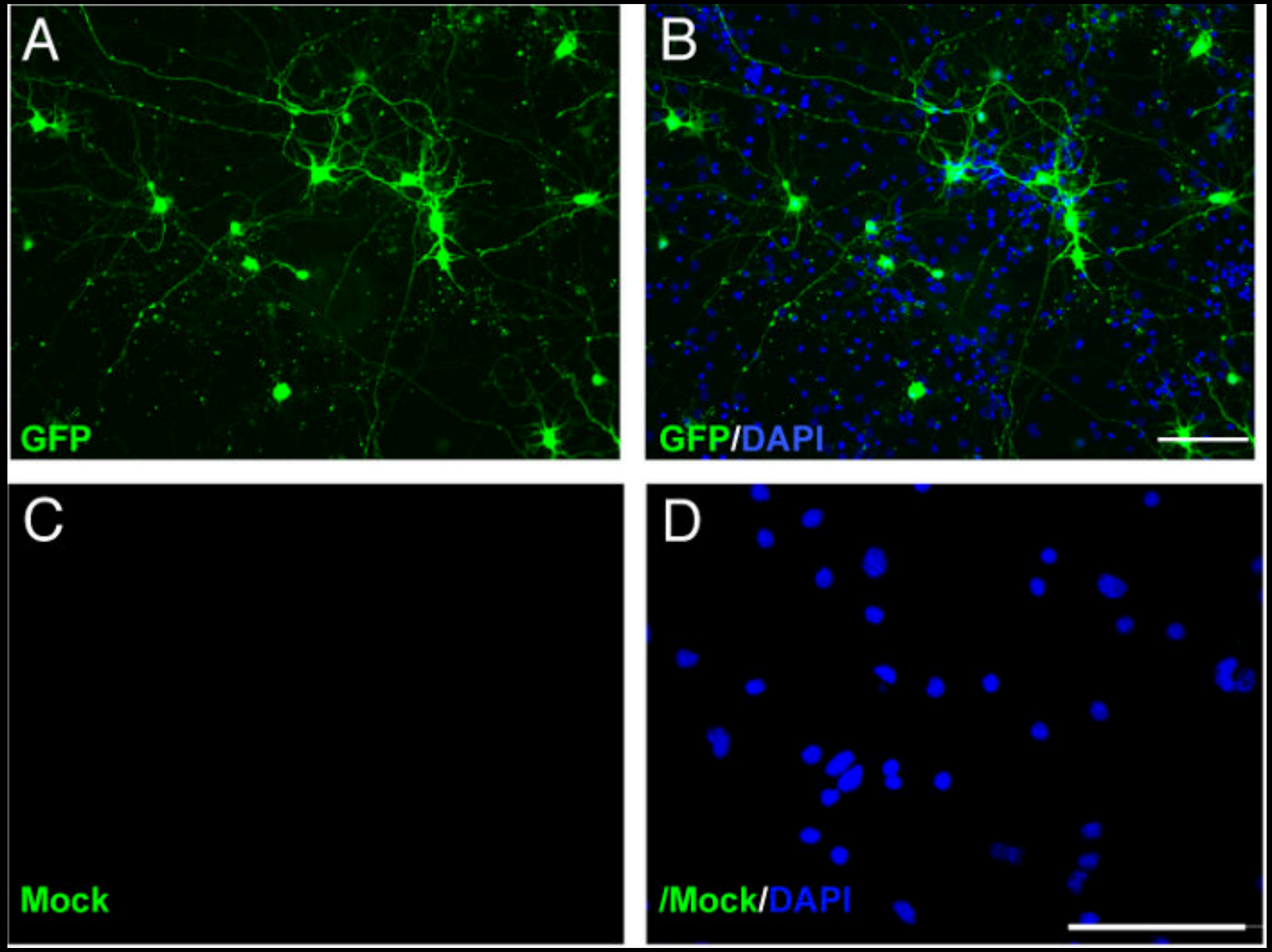

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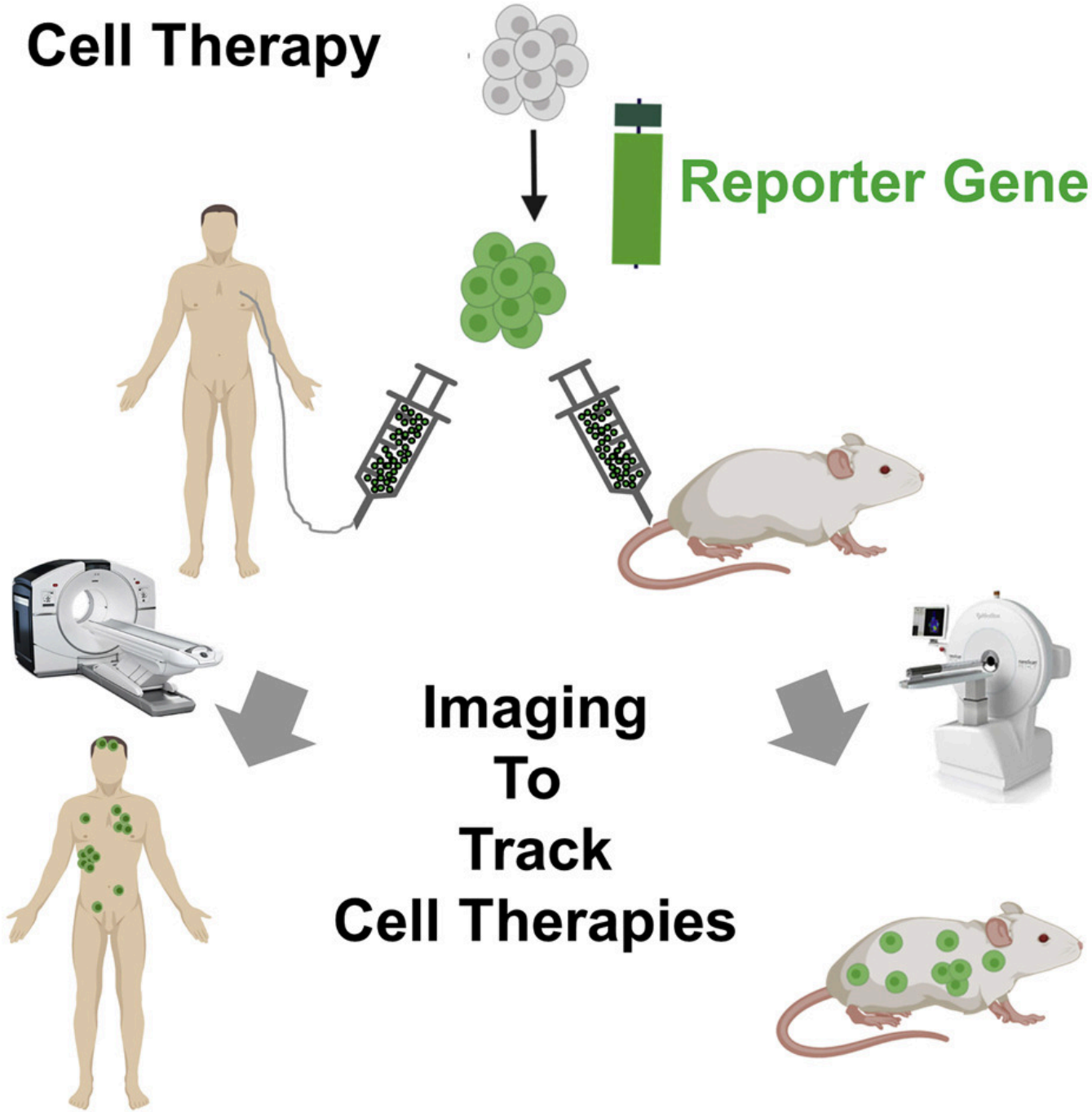

Disease	Vector	Transgene	Phase	Trial code
Ex vivo				
Alzheimer's disease	Retrovirus	NGF	I	US-0322
Metachromatic leukodystrophy	Lentivirus	ARSA	I, II	Biffi et al., 2013
Multiple sclerosis	Retrovirus	MBP	I, II	US-0851
Wiskott-Aldrich syndrome	Lentivirus	WASP	I, II	Aiuti et al., 2013
X-linked adrenoleukodystrophy	Lentivirus	ABCD1	I, II	Cartier et al., 2009
In vivo				
AADC deficiency	AAV	AADC	I, II	NCT01395641
Alzheimer's disease	AAV	NGF	I, II	NCT00087789, NCT00876863
Batten disease	AAV	CLN2	I	NCT00151216
Batten disease	AAV	CLN2	I, II	NCT01414985
Canavan disease	AAV	ASPA	I	Leone et.al., 2012
Giant axonal neuropathy	AAV	GAN	I	NCT02362438
Glioblastoma	Oncolytic poliovirus	-	I	NCT01491893
Glioblastoma multiforme (GBM), other gliomas	Oncolytic adenovirus	-	I	NCT00805376, NCT01956734, NCT02197169
Glioblastoma multiforme, other gliomas	Retrovirus	CD	I, II/III	NCT01470794, NCT02414165
Glioblastoma, other gliomas	Oncolytic HSV	-	I	NCT02031965
Glioblastoma, other gliomas	Oncolytic HSV	-	I	NCT00028158, NCT00157703
Leber's hereditary optic neuropathy	AAV	MT-ND4	I	NCT02161380
Metachromatic leukodystrophy	AAV	ARSA	I, II	NCT01801709
MPS IIIA (Sanfilippo Disease Type A)	AAV	SGSH, SUMF1	I, II	NCT01474343, NCT02053064
Parkinson's disease	AAV	GAD	I, II	NCT00195143, NCT00643890
Parkinson's disease	AAV	NTRN	I, II	NCT00252850, NCT00400634
Parkinson's disease	Lentivirus	TH, AADC, CH1	I, II	NCT00627588, NCT01856439
Parkinson's disease	AAV	GDNF	I	NCT01621581
Parkinson's disease	AAV	AADC	I, II	NCT02418598
Parkinson's disease	AAV	AADC	I	NCT00229736
Pompe disease	AAV	GAA	I, II	NCT00976352
Pompe disease	AAV	GAA	I	NCT02240407
Spinal muscular atrophy type 1	AAV	SMN	I	NCT02122952