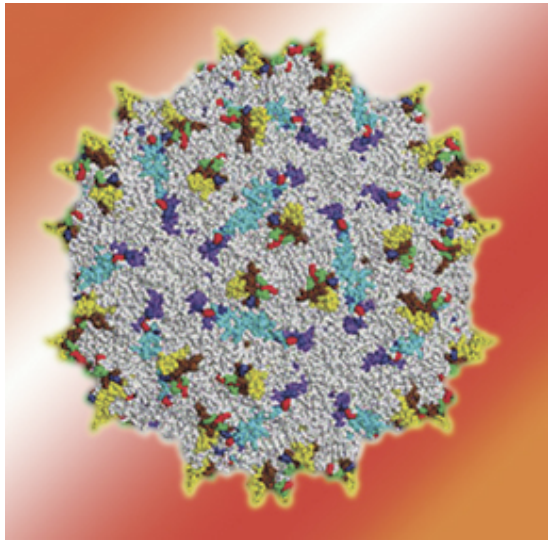
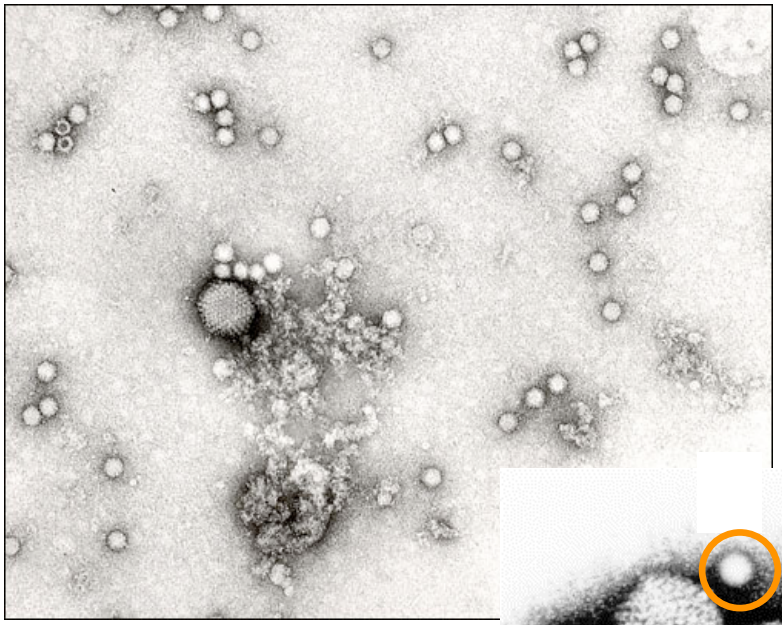
VIROLOGY

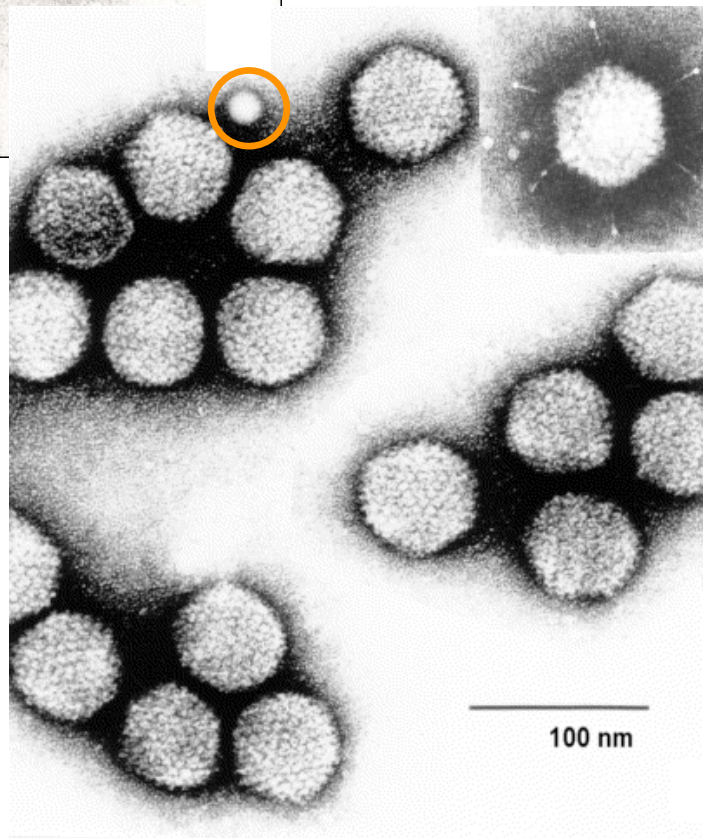
Engineering Viral Genomes: Adeno-Associated Vectors

Viral vectors

Virus	Insert size	Integration	Duration of expression	Advantages	Potential disadvantages
Adeno-associated virus	~4.5–9 (?) kb	Low efficiency	Long	Nonpathogenic, episomal, infects nondividing cells	Immunogenic, toxicity, small packaging limit
Adenovirus	2–38 kb	No	Short	Efficient gene delivery, infects nondividing cells	Transient, immunogenic
Alphavirus	~5 kb	No	Short	Broad host range, high level expression	Virulence
Epstein-Barr virus	~120 kb	No; episomal	Long	High capacity, episomal, long-term expression	
Gammaretrovirus	1–7.5 kb	Yes	Shorter than formerly	Stable integration	May rearrange genome, insertional mutagenesis require cell division
Herpes simplex virus	~30 kb	No	Long in central nervous system, short elsewhere	Infects nondividing cells; neurotropic, large capacity	Virulence, persistence in neurons, immunogenic
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; reversio to neurovirulence
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







Adeno-associated virus

Virus classification

Group:	Group II (ssDNA)
Family:	*Parvoviridae*
Subfamily:	*Parvovirinae*
Genus:	*Dependovirus*
Species:	*adeno-associated virus*

Parvoviruses: pathogenesis and diseases

Virus	Disease
B19 parvovirus	Erythema infectiosum (fifth disease) Aplastic crisis in patients with chronic hemolytic anemia Acute polyarthritis Abortion
Adeno-associated virus	Commonly infects humans, not associated with illness

Epidemiology

Transmission
- Respiratory and oral droplets

Distribution of virus
- Ubiquitous
- Fifth disease most common in late winter and spring

At risk or risk factors
- Children in elementary school (fifth disease)
- Parents of infected children
- Pregnant women (fetal infection and disease)
- Patients with chronic anemia (aplastic crisis)

Vaccines or antiviral drugs
- None

Disease mechanisms

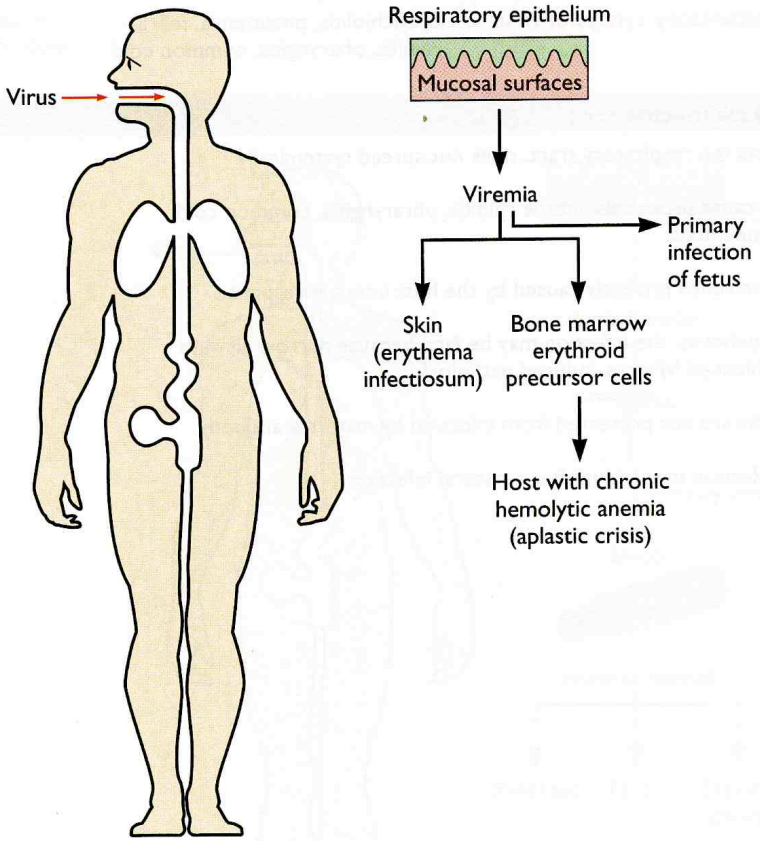
Transmitted by respiratory and oral secretions

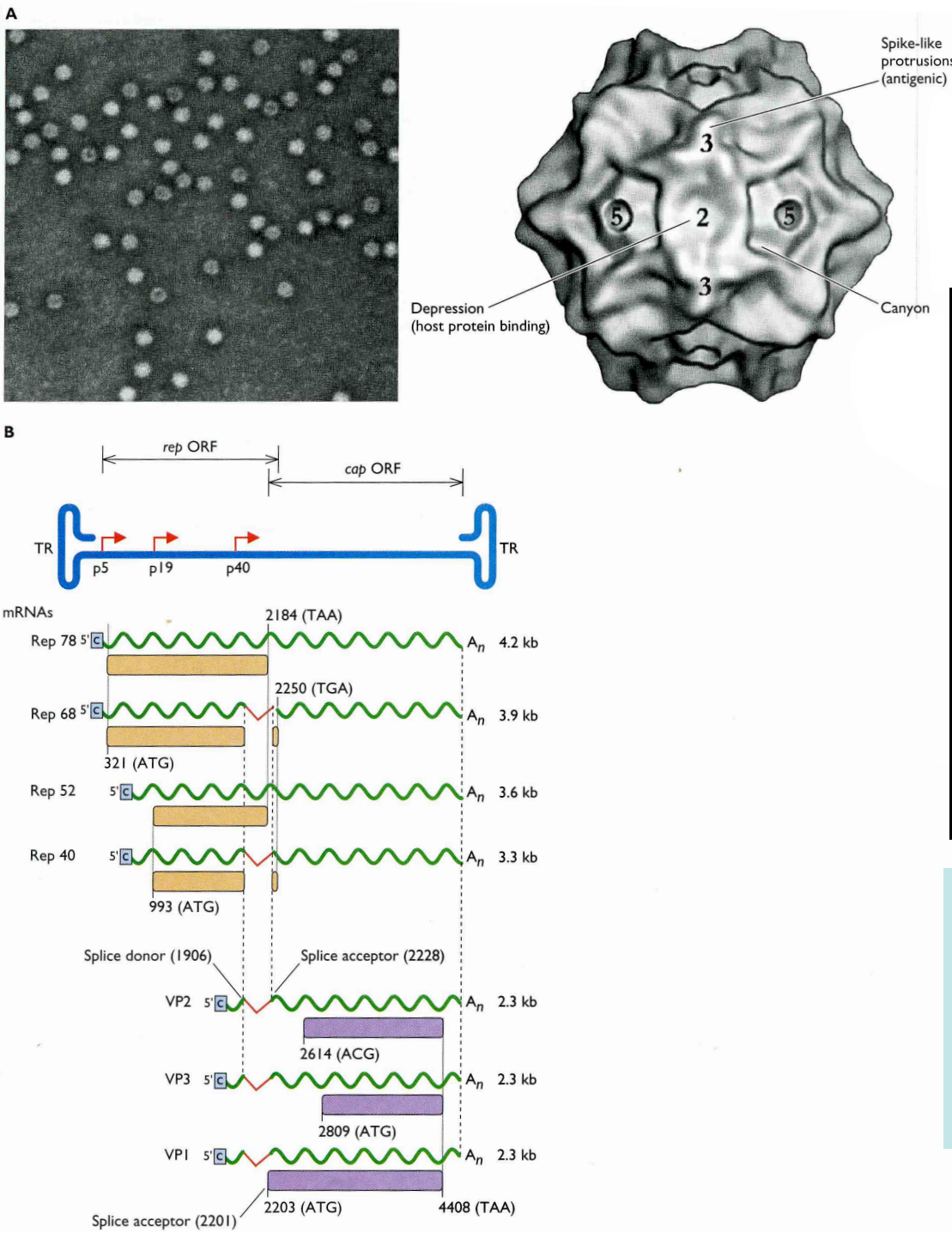
In utero infection

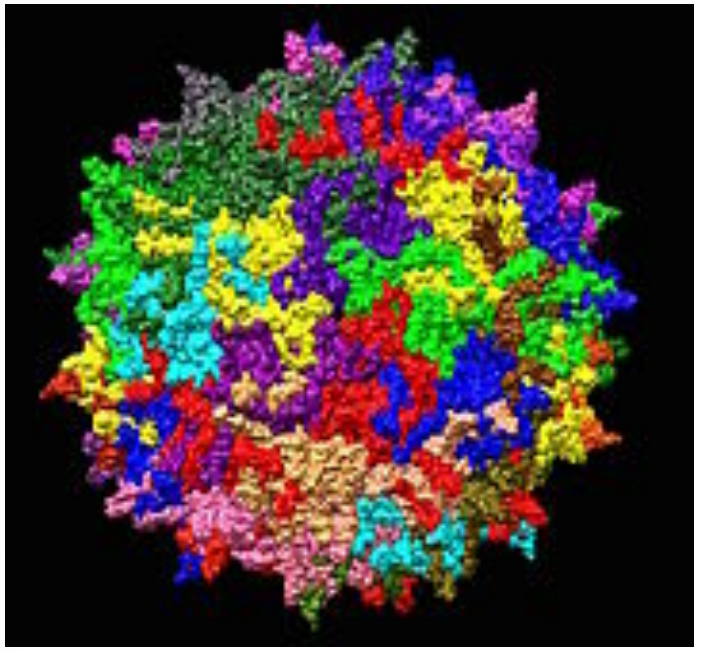
Virus infects mitotically active erythroid precursor cells in bone marrow

Biphasic disease
Flu-like phase, viral shedding during viremia
Later phase: erythematous maculopapular rash, arthralgia, and arthritis caused by circulating virus-antibody immune complexes

Aplastic crisis in patients with chronic hemolytic anemia is caused by depletion of erythroid precursors and destabilization of erythrocytes

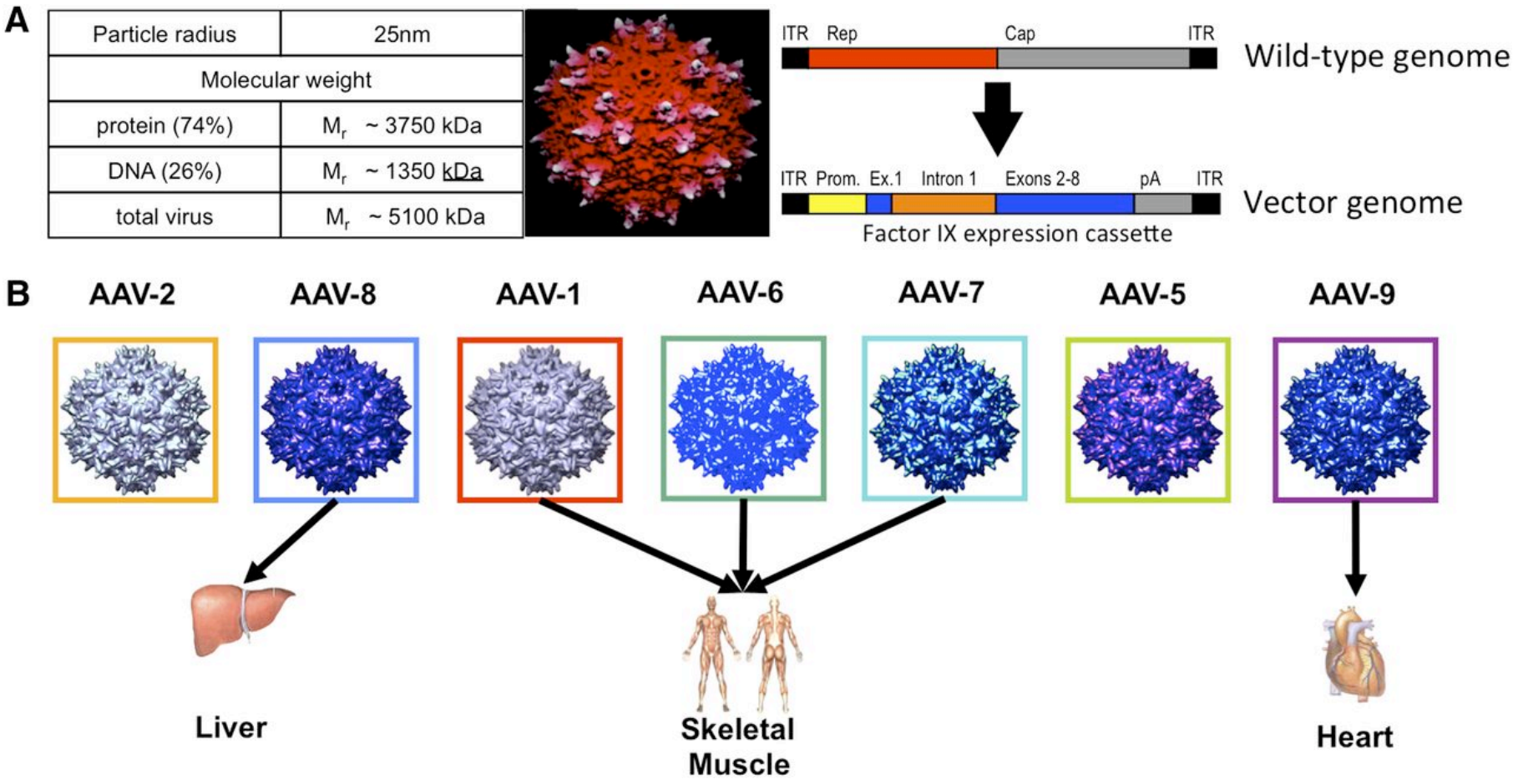




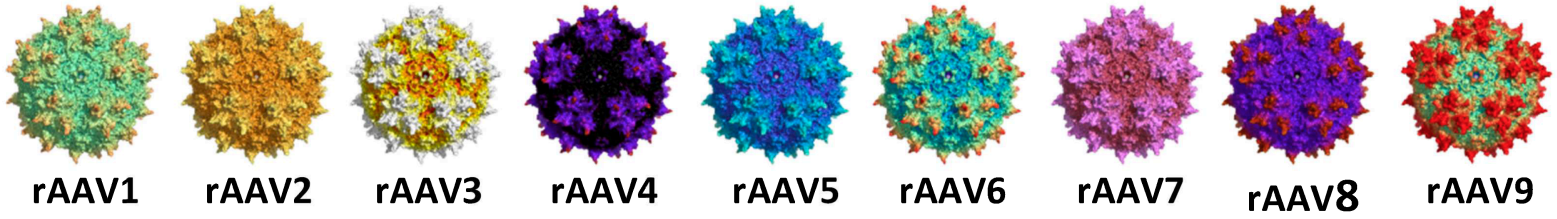


Structure and genome organization of adeno-associated virus type 2 (AAV2)

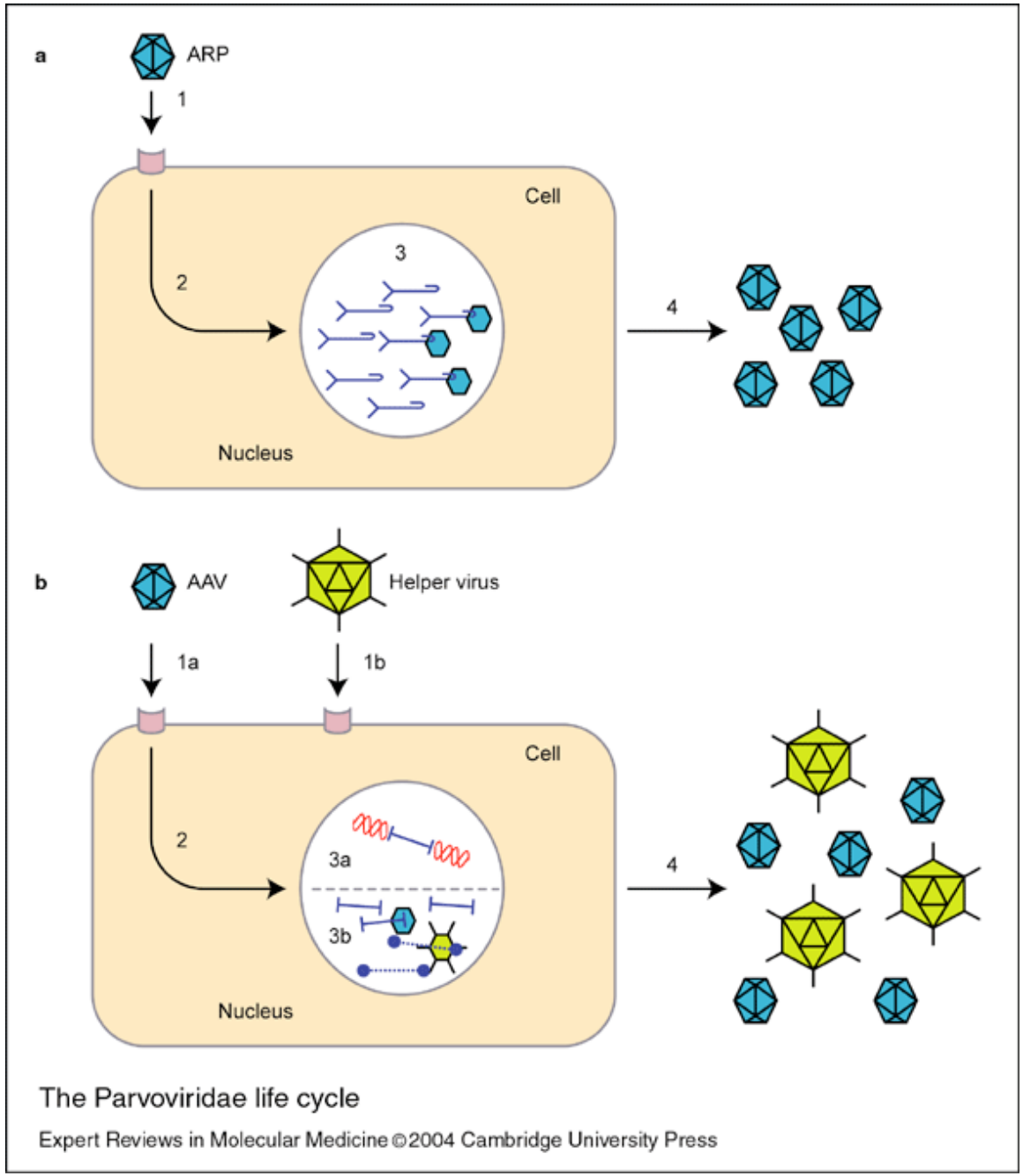
Structure and tropism of wild-type AAV and of recombinant AAV vectors.



Primary and secondary receptors used for AAV serotypes from 1 to 9 to infect and transduce cell types



	rAAV1	rAAV2	rAAV3	rAAV4	rAAV5	rAAV6	rAAV7	rAAV8	rAAV9
Primary receptor	N-linked sialic acid	HSPG	HSPG	O-linked sialic acid	N-linked sialic acid	N-linked sialic acid; HSPG	unknown	unknown	N-linked galactose
Secondary receptor	unknown	FGFR1, HGFR, integrins, CD9, LamR	FGFR1, HGFR, LamR	unknown	PDGFR	EGFR	unknown	LamR	LamR



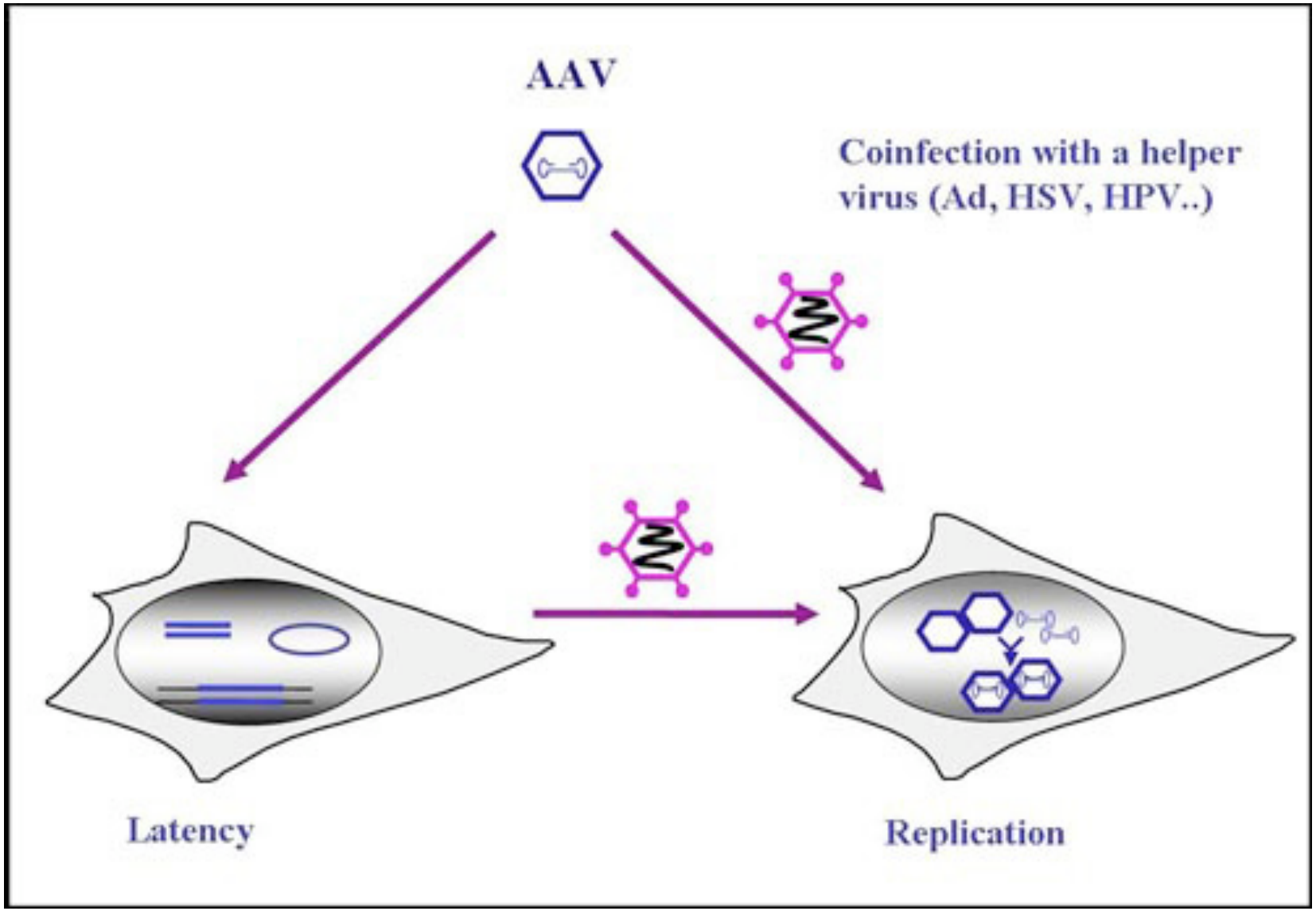
The Parvoviridae life cycle. Life cycle of (a) autonomously replicating parvovirus (ARP) and (b) adeno-associated virus (AAV).

Stages shown are:

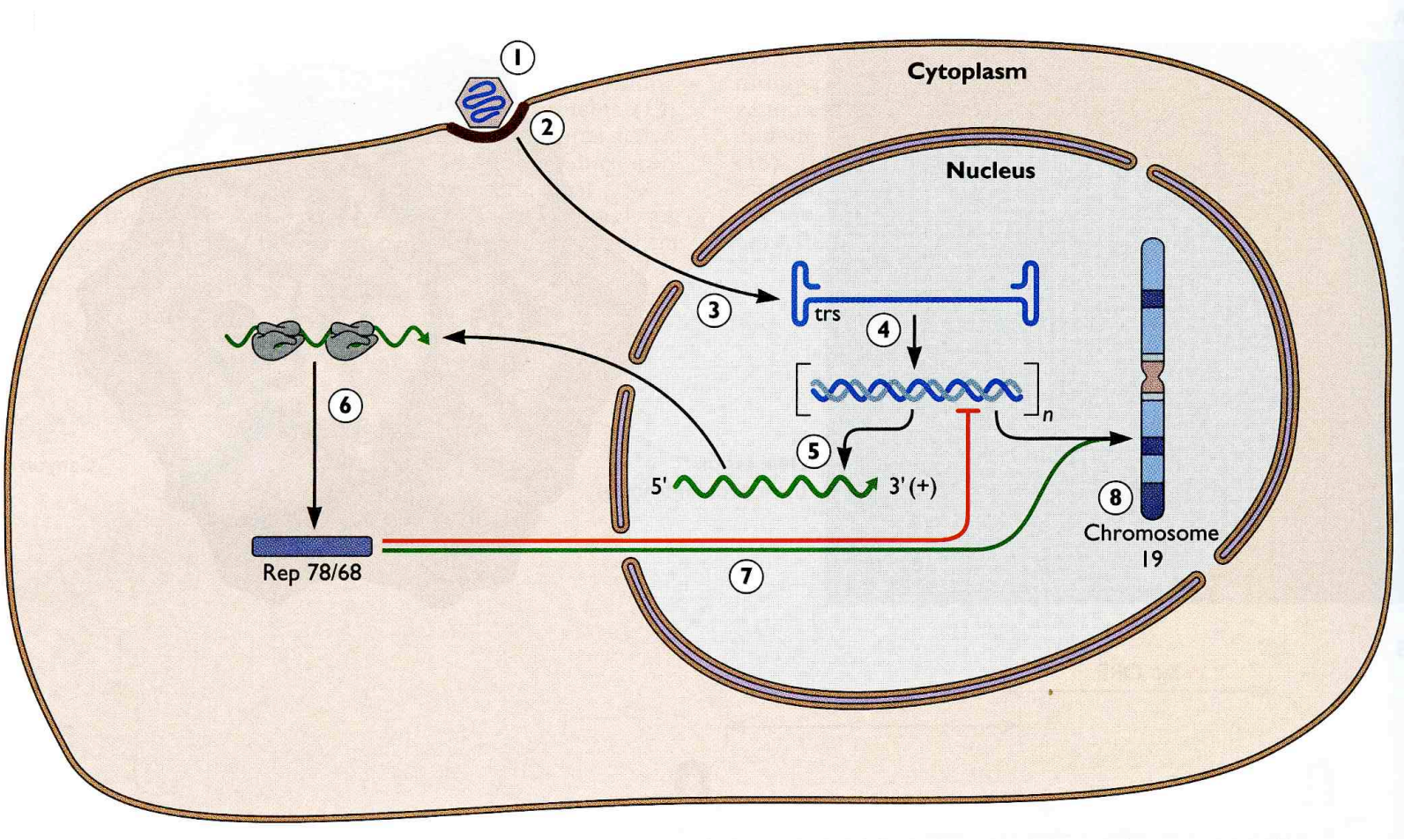
(1) virus binding and entry into the cell;
(2) intracellular trafficking;
(3) intracellular replication and virus production;
(4) release of intracellular viral particles.

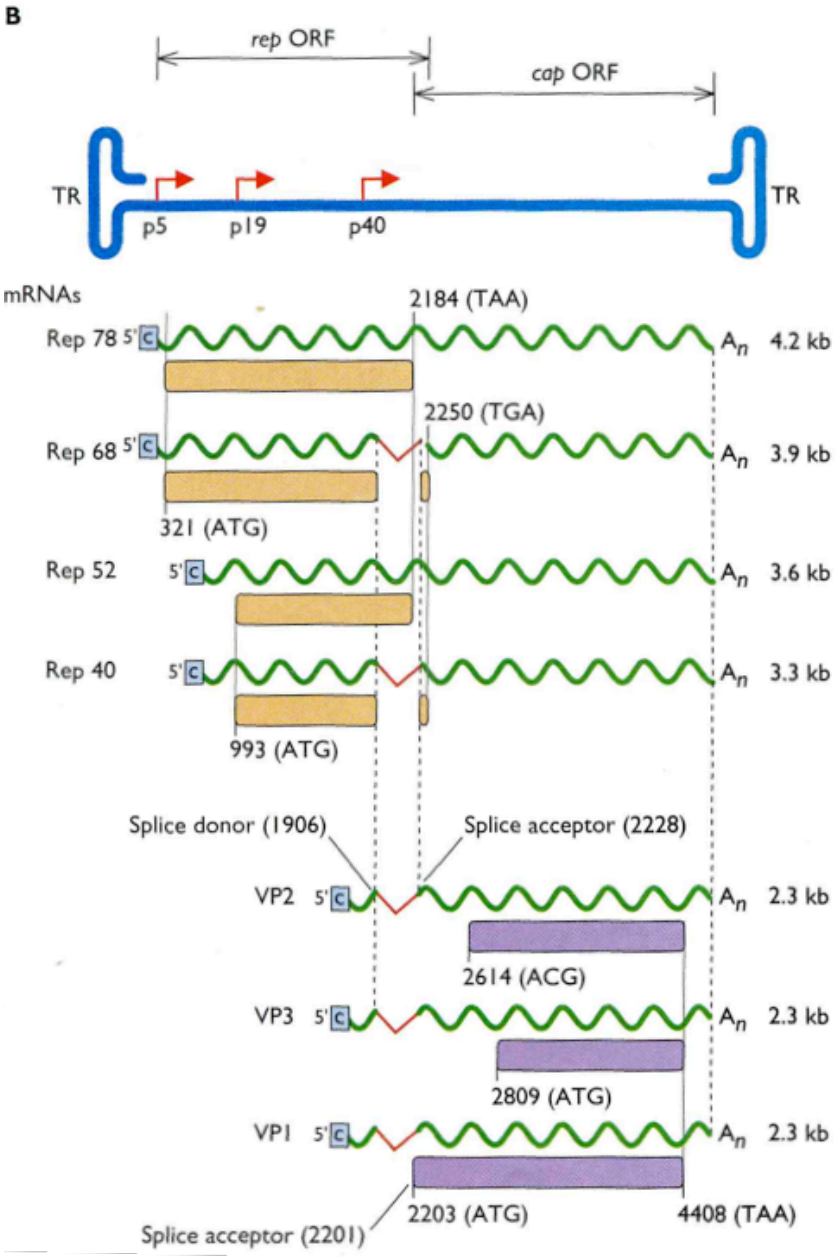
In the case of ARP, the virus replicates autonomously in the host cell nucleus.

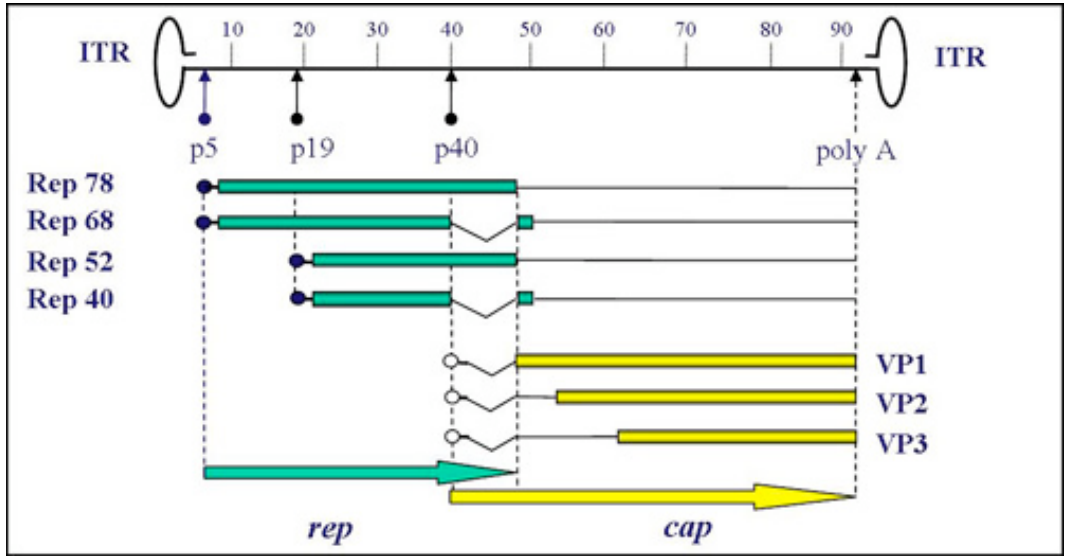
In the case of AAV, stages 1 and 3 include the absence (1a, 3a) or presence (1b, 3b) of helper virus; in the absence of helper virus, the AAV genome integrates into the host cell DNA in order to replicate.


AAV
Coinfection with a helper virus (Ad, HSV, HPV..)
Latency
Replication

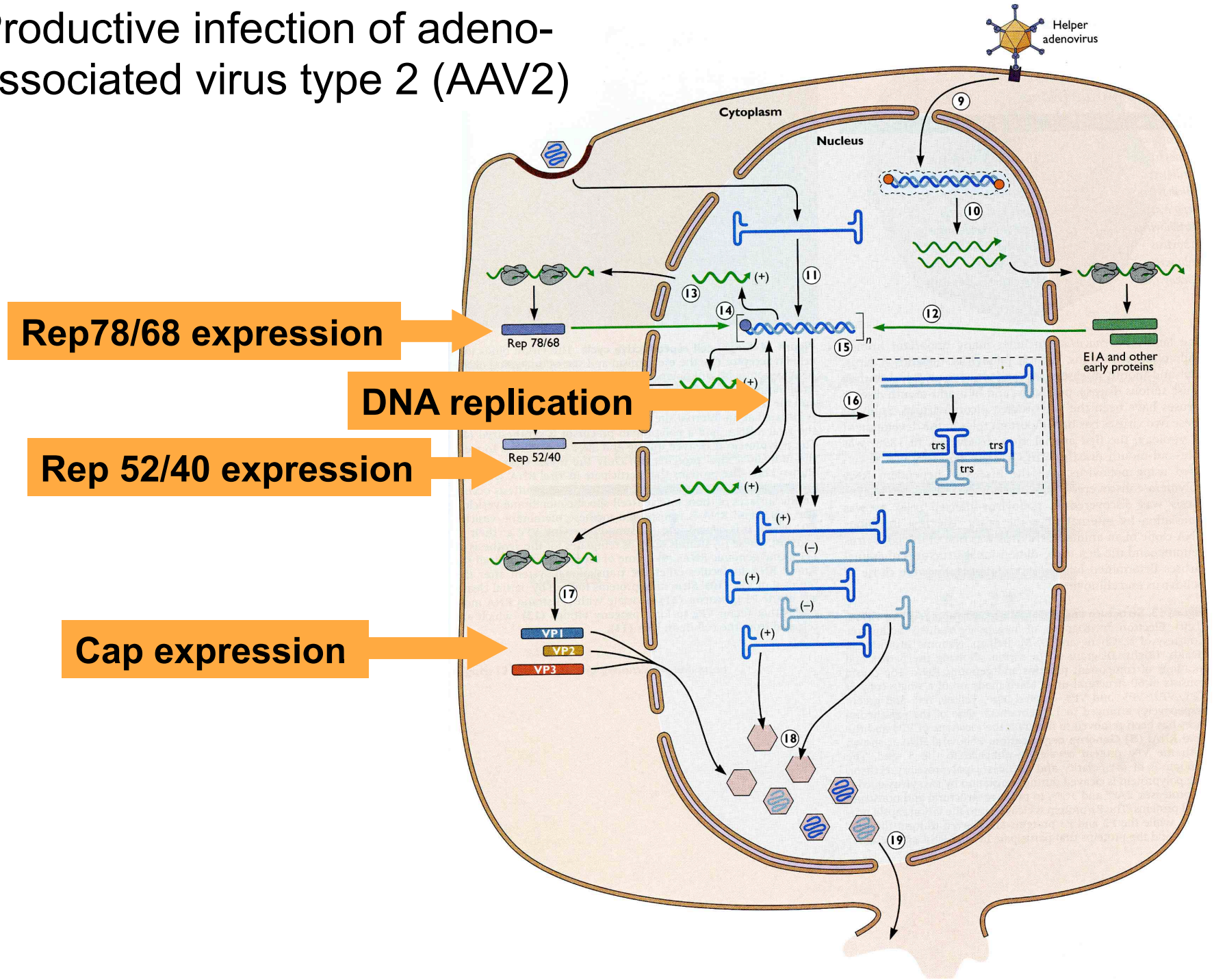
Latent infection of adeno-associated virus type 2 (AAV2)



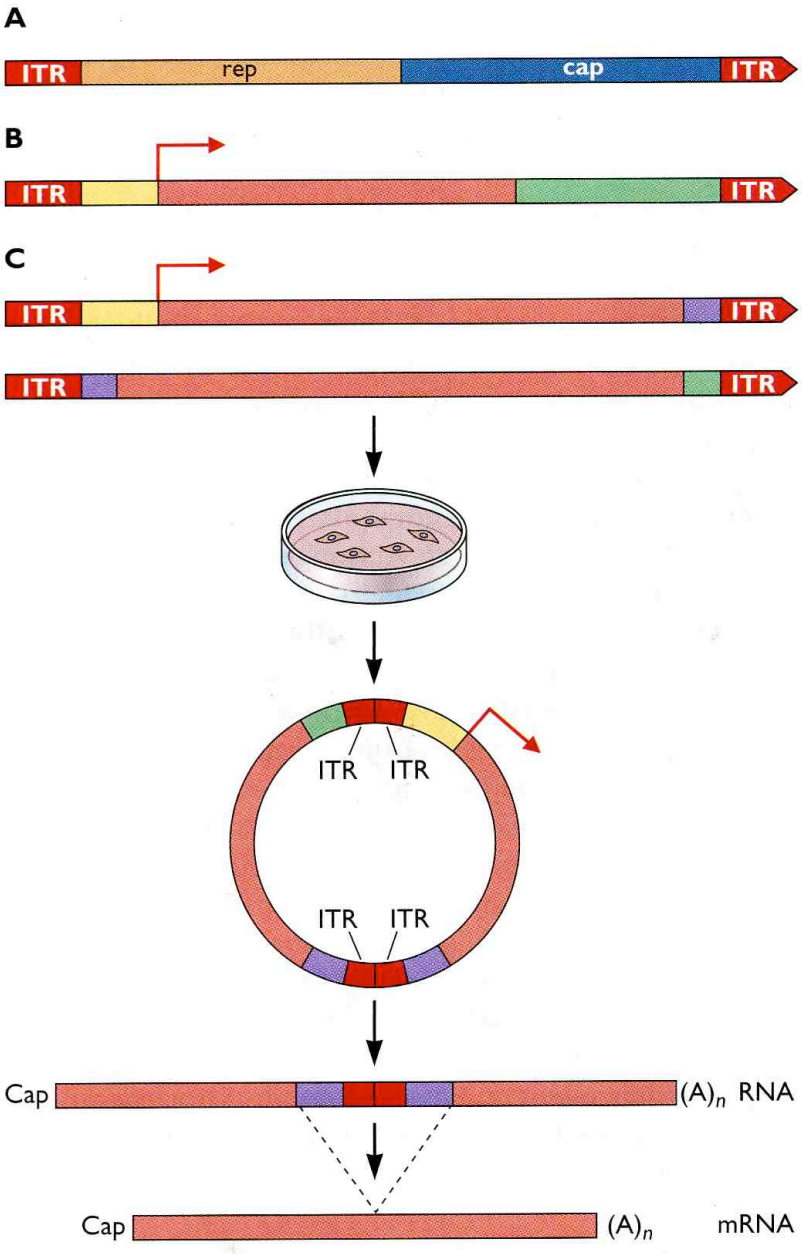

B
rep ORF
cap ORF
TR
p5
p19
p40
TR
mRNAs
2184 (TAA)
Rep 78 5'
An
4.2 kb
2250 (TGA)
Rep 68 5'
An
3.9 kb
321 (ATG)
Rep 52 5'
An
3.6 kb
Rep 40 5'
An
3.3 kb
993 (ATG)
Splice donor (1906)
Splice acceptor (2228)
VP2 5'
An
2.3 kb
2614 (ACG)
VP3 5'
An
2.3 kb
2809 (ATG)
VP1 5'
An
2.3 kb
2203 (ATG)
4408 (TAA)
Splice acceptor (2201)


ITR
10
20
30
40
50
60
70
80
90
ITR
p5
p19
p40
poly A
Rep 78
Rep 68
Rep 52
Rep 40
VP1
VP2
VP3
rep
cap

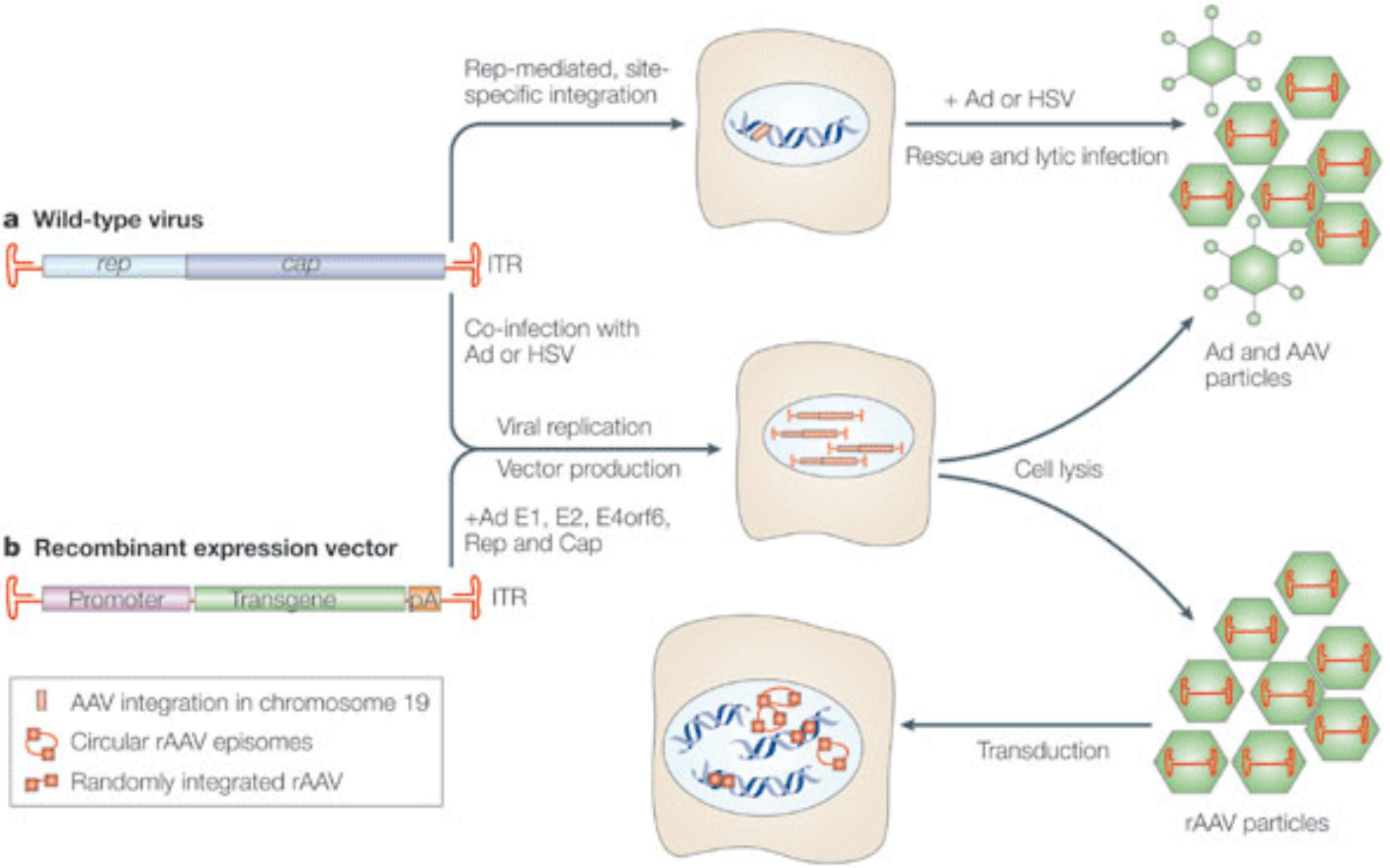
Productive infection of adeno-associated virus type 2 (AAV2)



Development of rAAV



Development of rAAV



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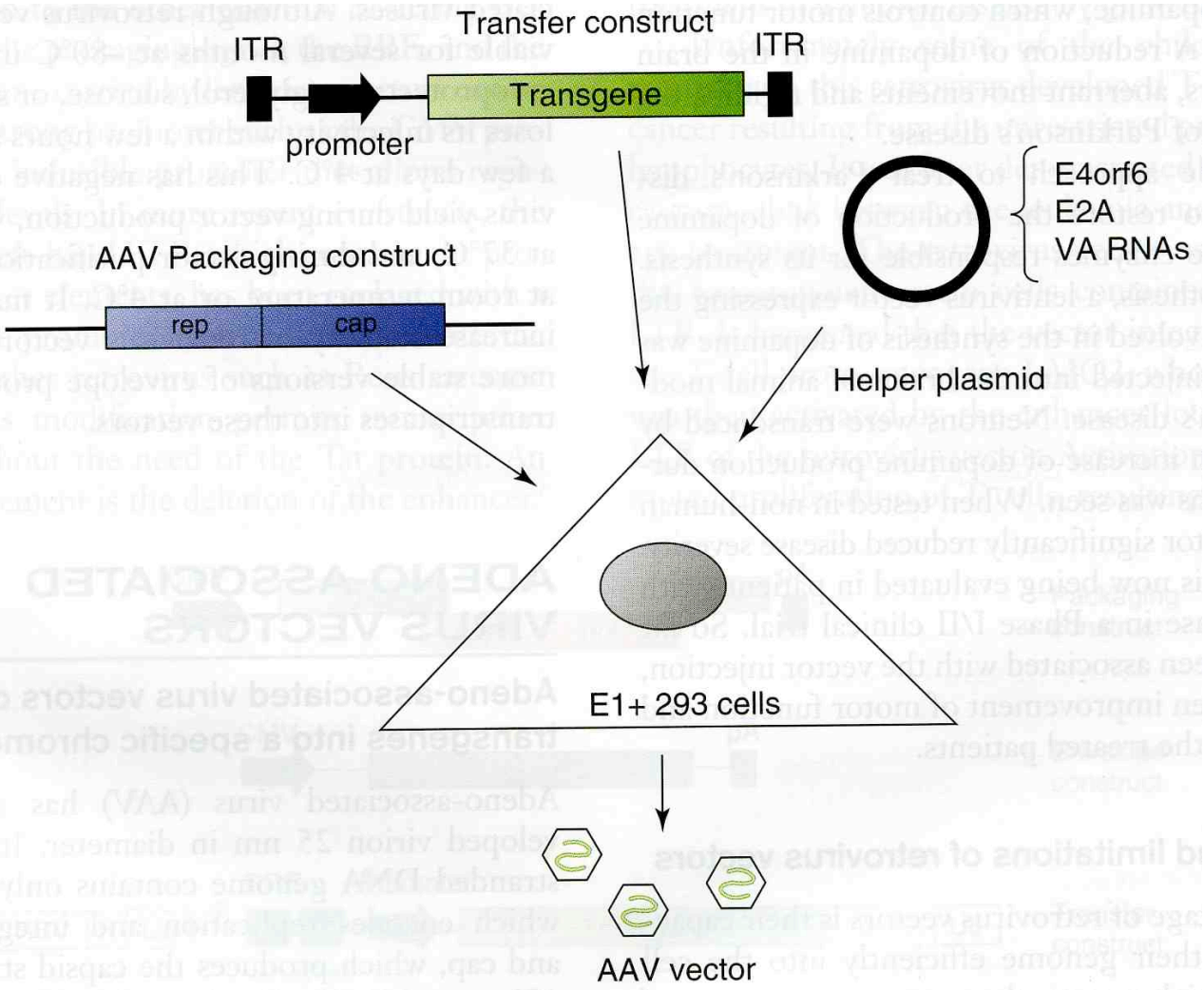
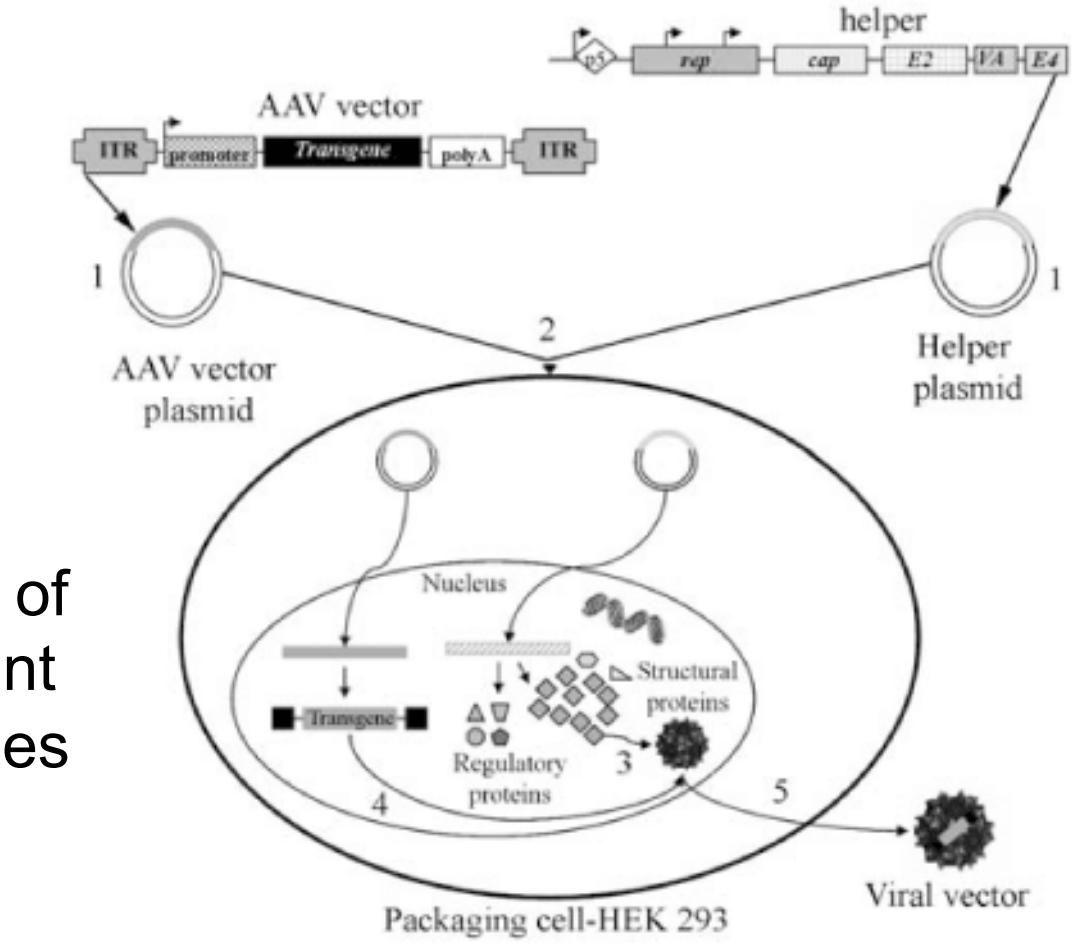
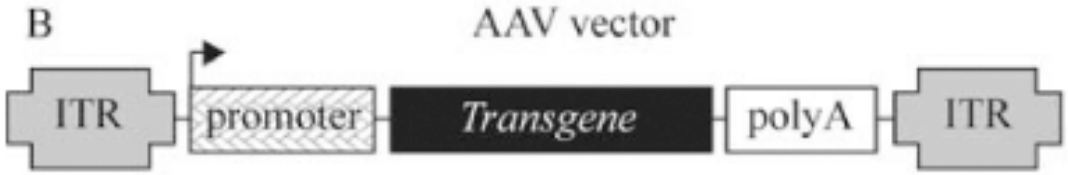
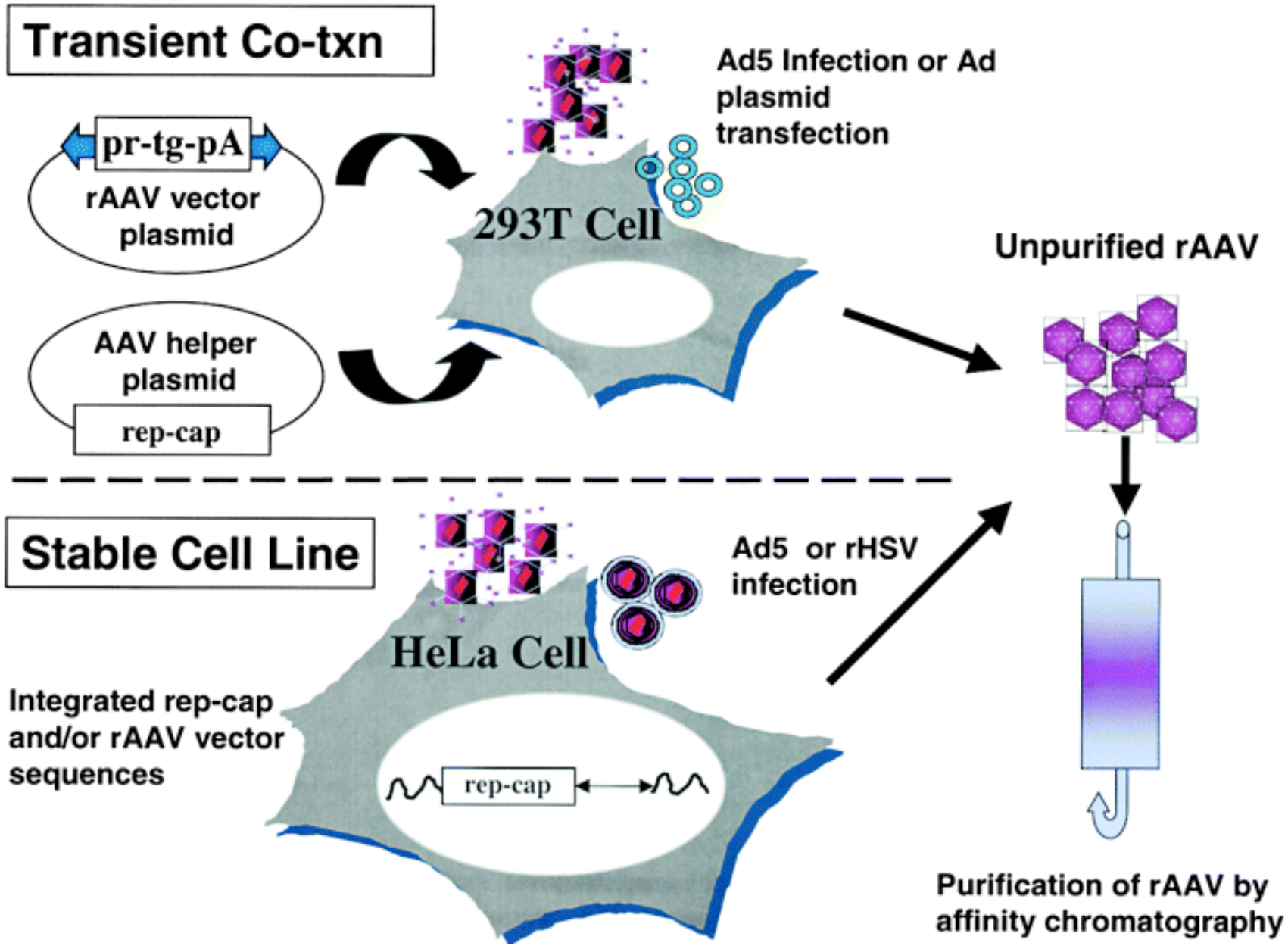
Development of rAAV



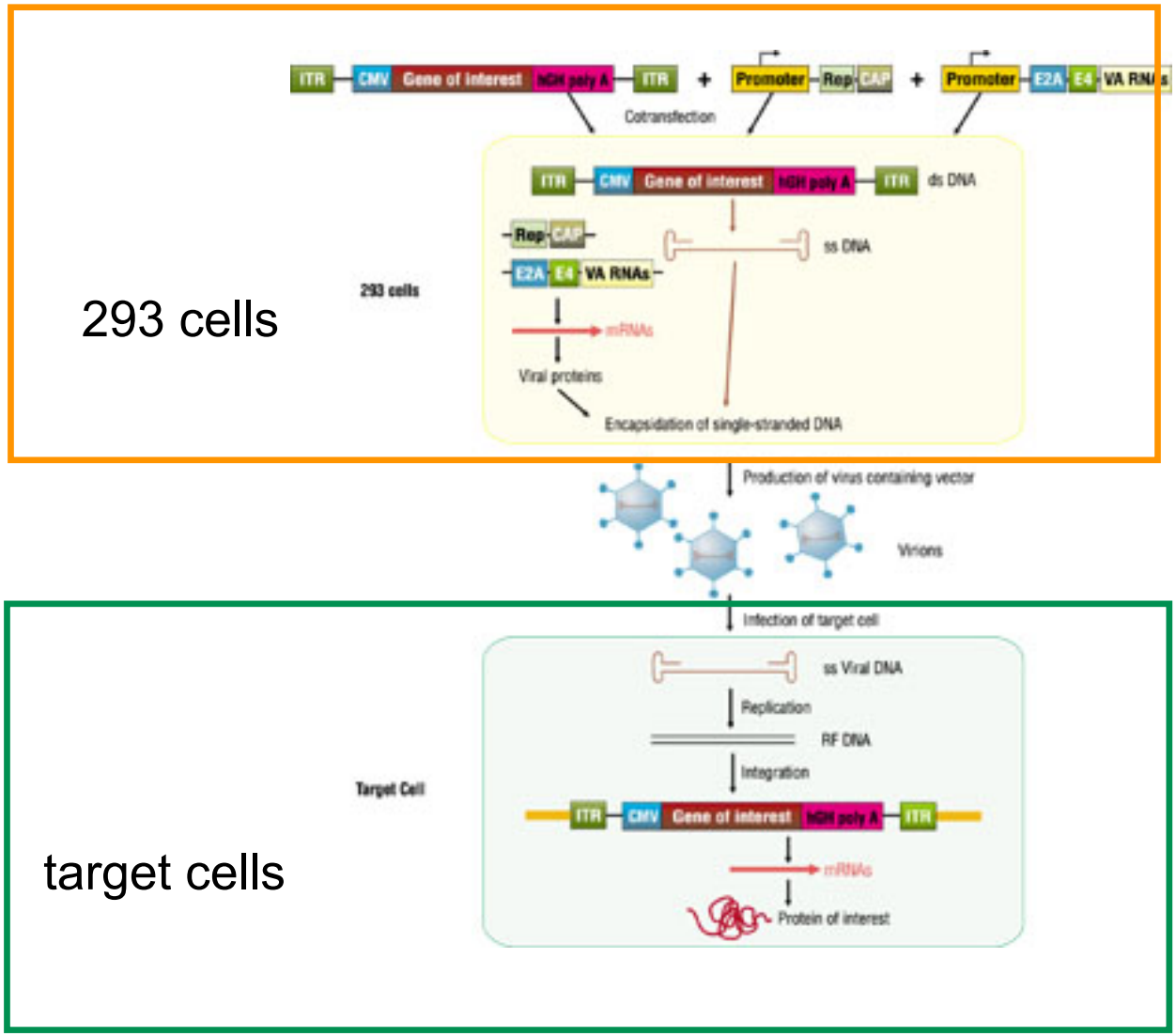
Production of recombinant AAV particles



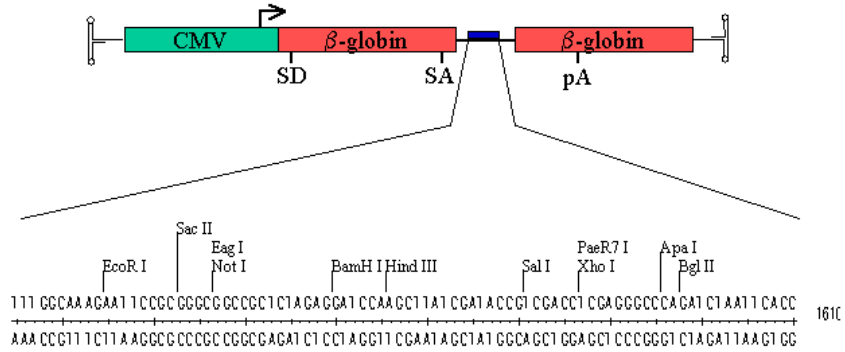



Transient Co-txn
pr-tg-pA
rAAV vector plasmid
AAV helper plasmid
rep-cap
293T Cell
Ad5 Infection or Ad plasmid transfection
Unpurified rAAV
Stable Cell Line
HeLa Cell
Ad5 or rHSV infection
Integrated rep-cap and/or rAAV vector sequences
rep-cap
Purification of rAAV by affinity chromatography

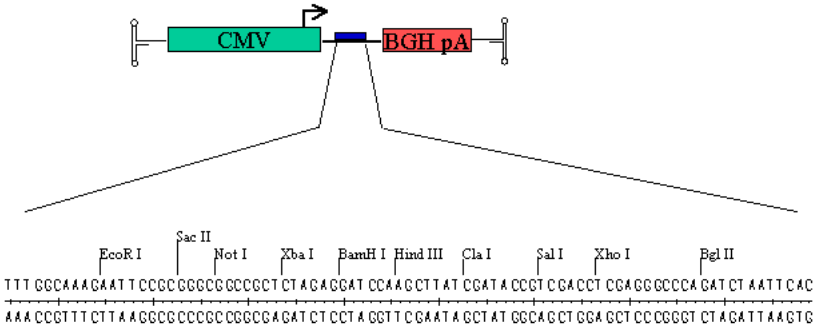
rAAV as vectors for transducing therapeutic genes



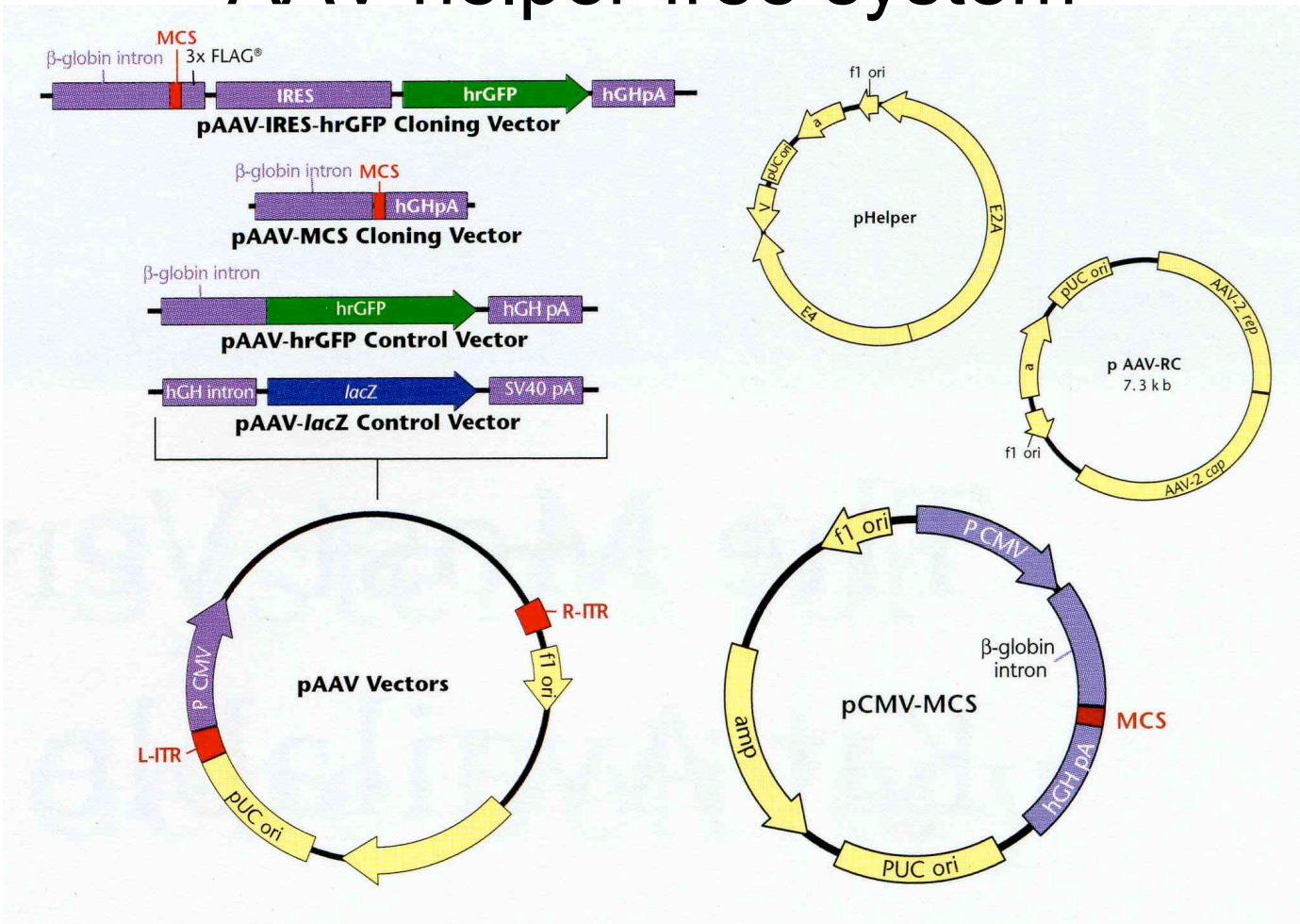
Structure of AAV-MCS Vector



Structure of AAV-MCS2

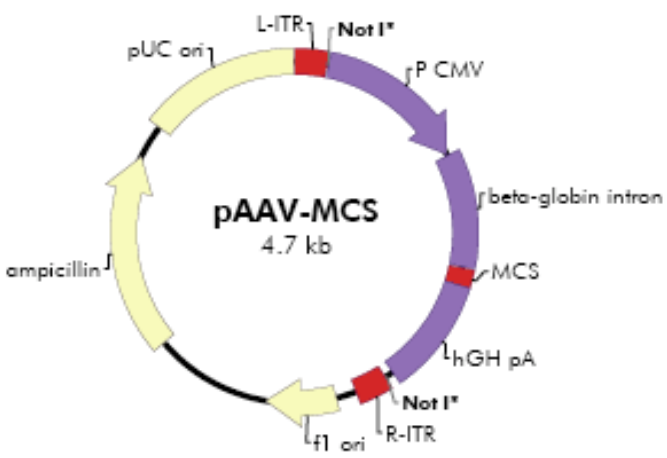


AAV helper-free system

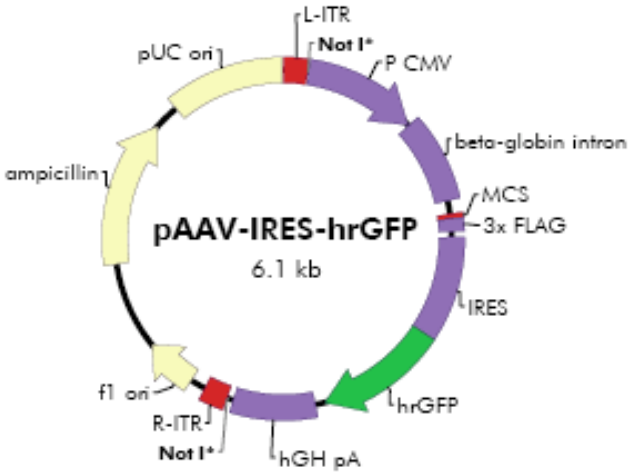


AAV vectors include pAAV or pCMV-MCS for cloning your gene of interest, pHelper for adenoviral production, and pAAV-RC for expression of capsid and DNA replication proteins.

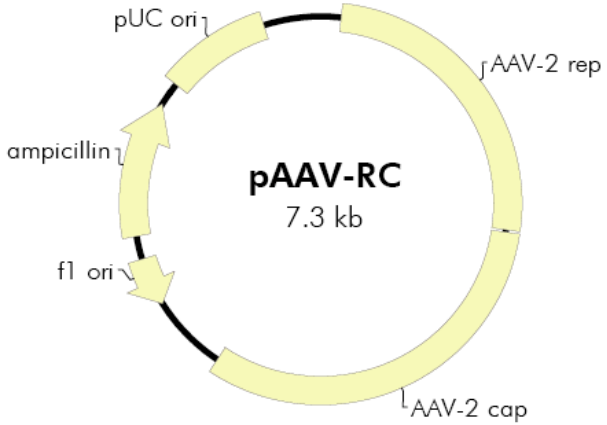
pAAV-MCS Vector



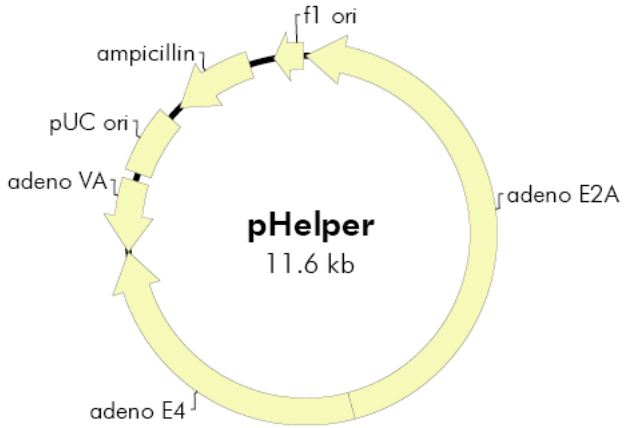
pAAV-IRES-hrGFP Vector



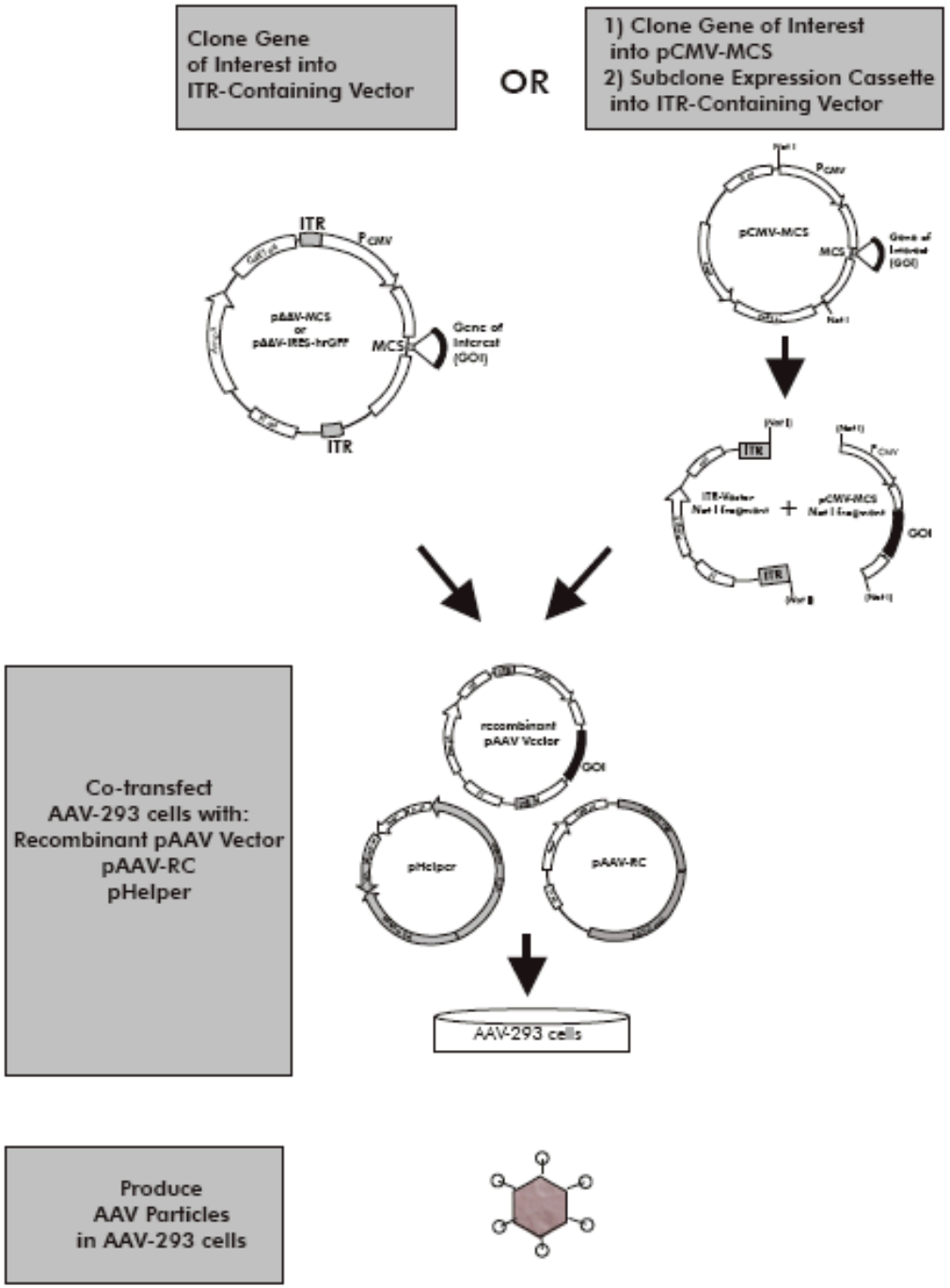
pAAV-RC Plasmid



pHelper Plasmid



Production of recombinant AAV particles



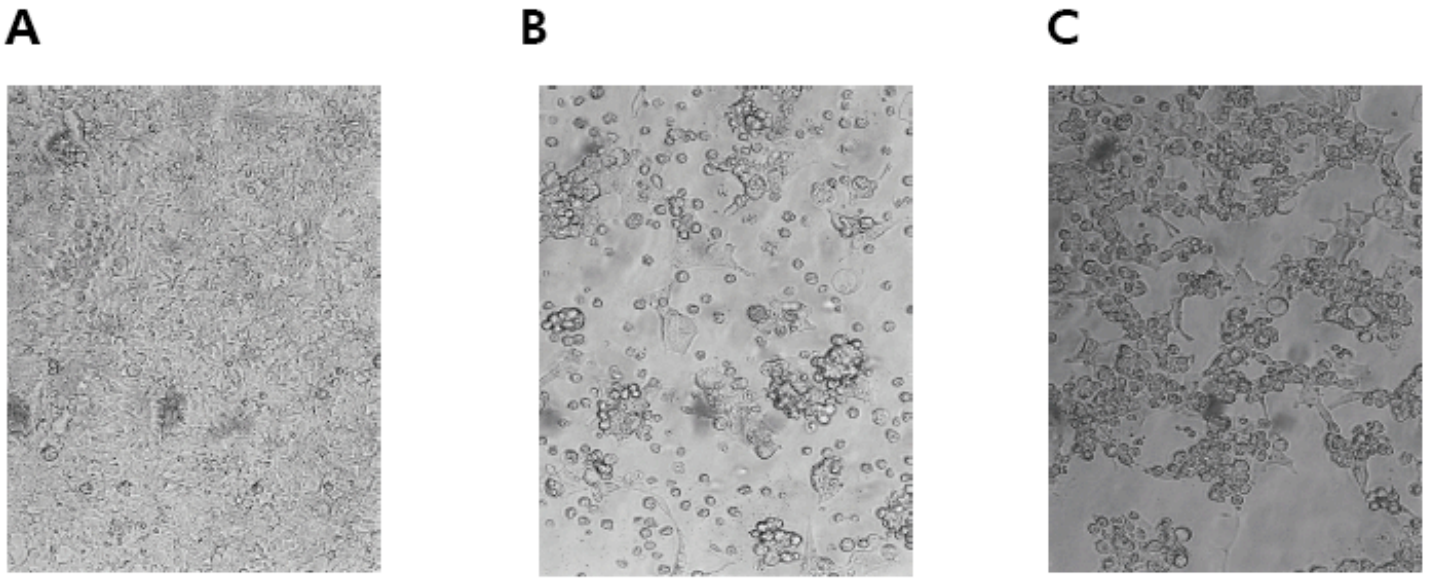
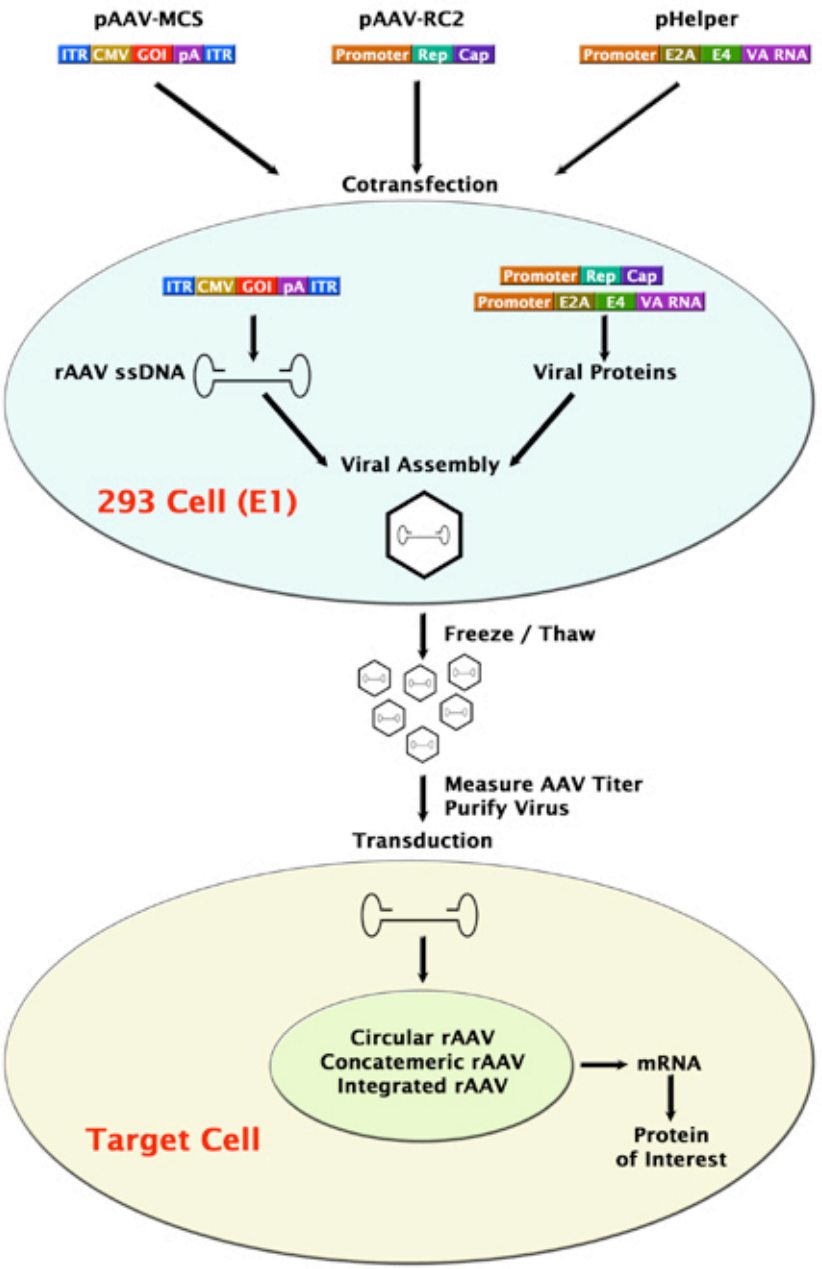


FIGURE 8 AAV particle production by the AAV-293 producer cells.

A AAV-293 cell morphology after performing the transfection protocol above with no DNA (virus production negative control). The photograph was taken at 100X magnification three days post-treatment.

B AAV-293 cell morphology after performing the transfection protocol above with pAAV-hrGFP, pAAV-RC, and pHelper. The photograph was taken at 100X magnification three days post-transfection without the removal of media and floating cells.

C AAV-293 cell morphology after performing the transfection protocol above with pAAV-LacZ, pAAV-RC, and pHelper. The photograph was taken at 200X magnification three days post-transfection following the removal of media and floating cells.



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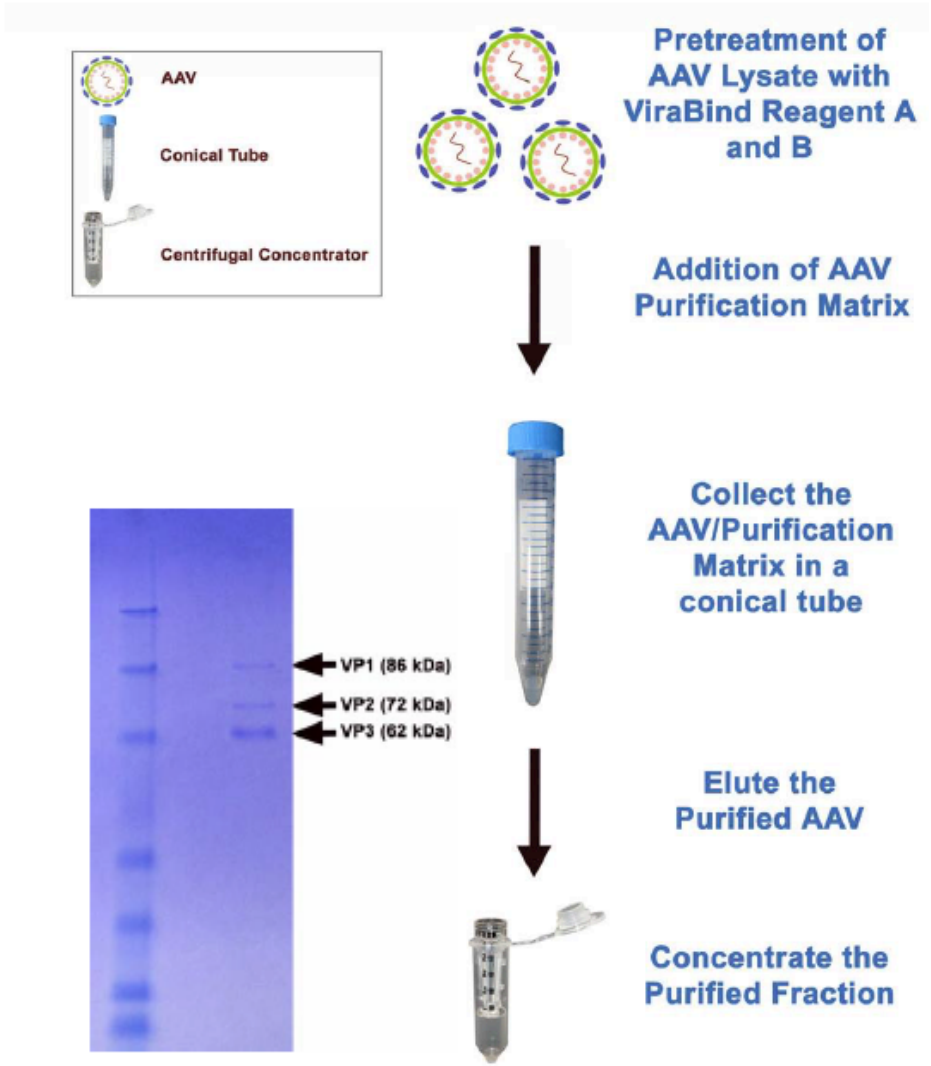
Creating Solutions for Life Science Research

Assay Principle



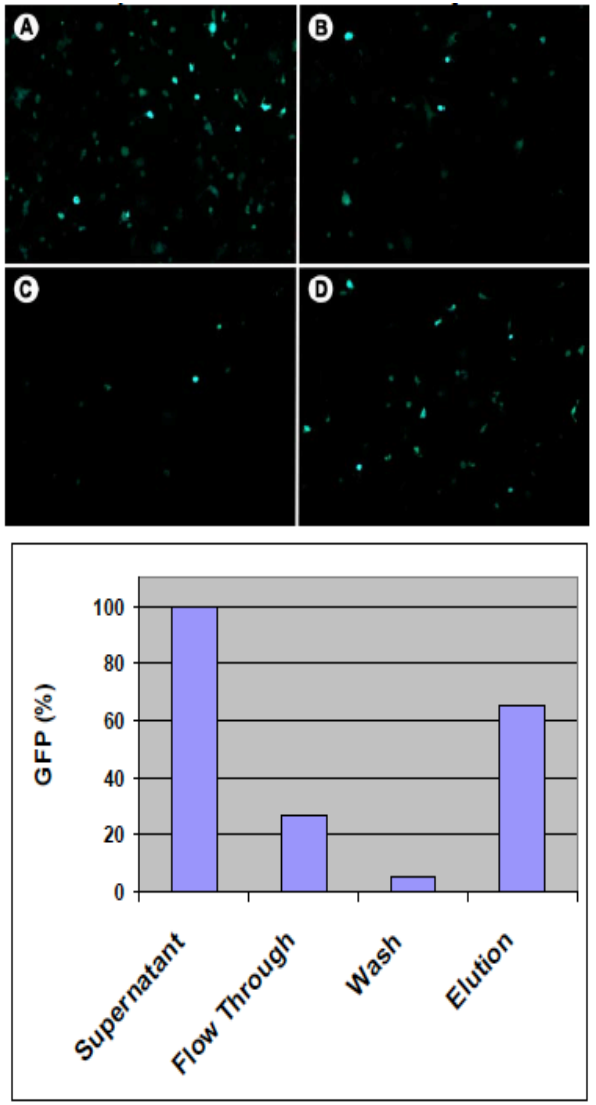


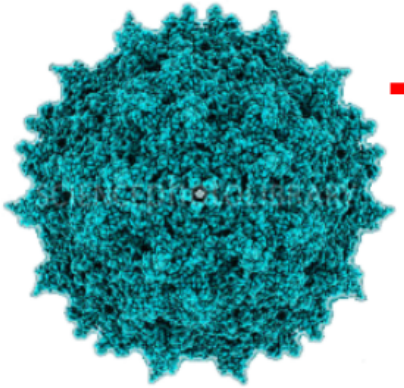
Figure 1: Purification of AAV2-GFP. AAV2-GFP was produced by a helper-free system in 293 cells. AAV supernatant was subjected to the purification steps. Samples from each fraction were used to infect 293 cells, GFP positive cells were scored by counting after three days. A: AAV Supernatant; B: Flow through; C: 1st wash; D: Elution.

Advantages of Adeno-associated Virus (AAV)

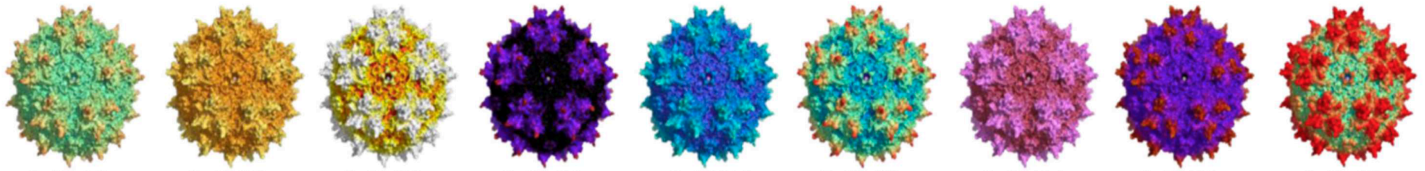
- Lack of pathogenicity
- Infect both dividing and non-dividing cells
- Long-term gene transfer and expression (epichromosomal)
- Stable integration into the host cell genome at a specific site (rare event)
- Unparalleled biosafety profile
- Low immunogenicity

Advantages of Adeno-associated Virus (AAV)

Recombinant AAV (rAAV) features

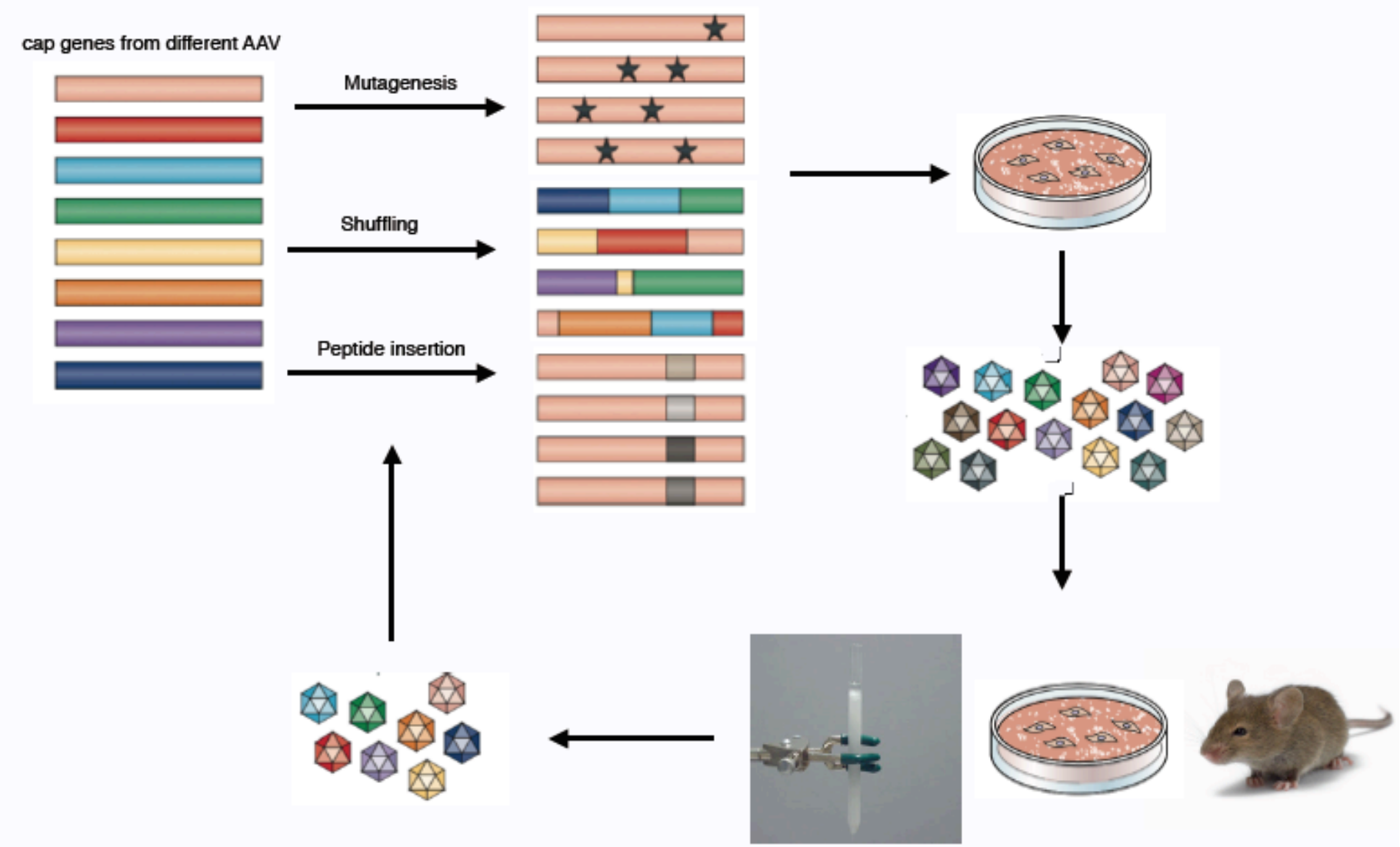


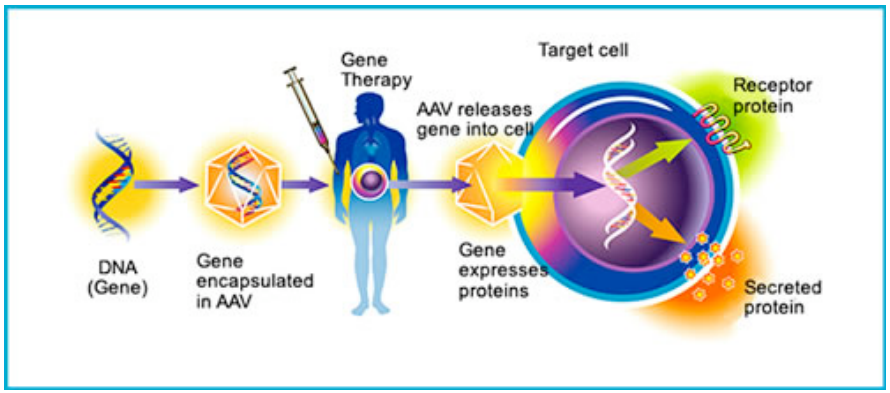
- Transfects both dividing & non-dividing cells
- No host-genome integration & Stable Expression
- Ease to produce at high viral titer (Helper Free)
- Do not elicit significant immune response *in vivo*
- Can be used for in vivo gene deliveries



	AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9
Large Animal	Heart, CNS and skeletal muscle	CNS, eye		Eye	CNS	Skeletal muscle and Heart, CNS		Eye, CNS	CNS, Heart and skeletal muscle

Modified AAV targeting by capsid modifications



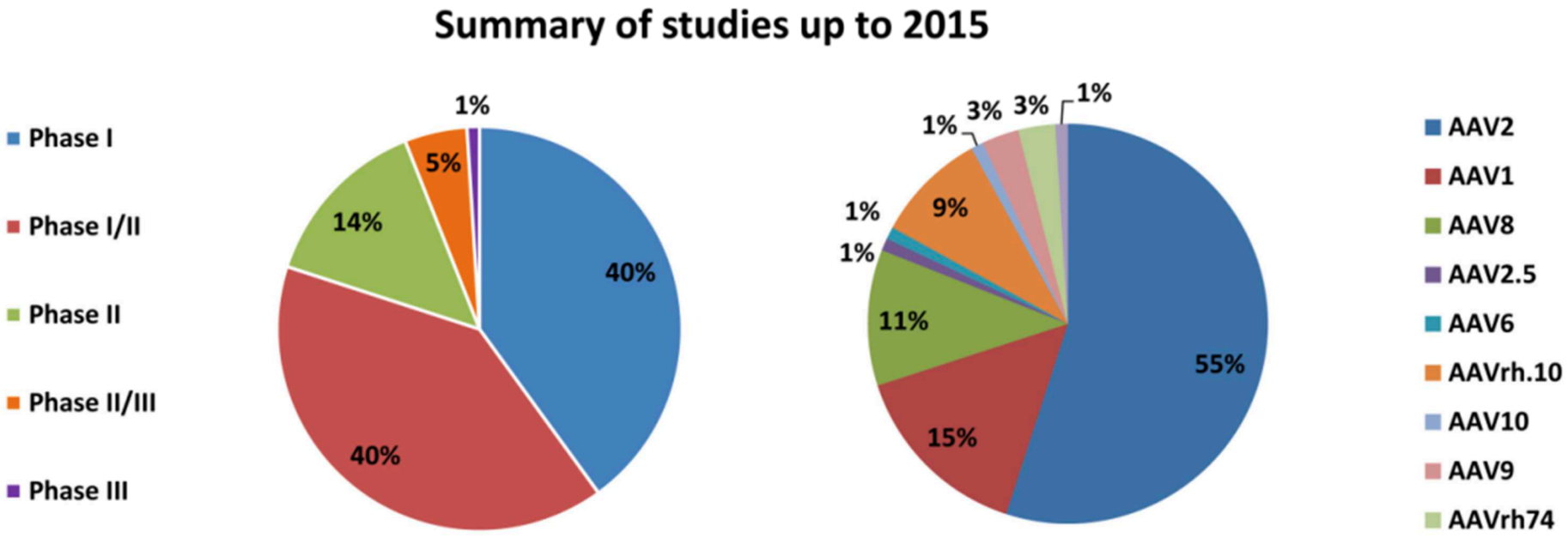


Selection of clinical trials using AAV-based vectors

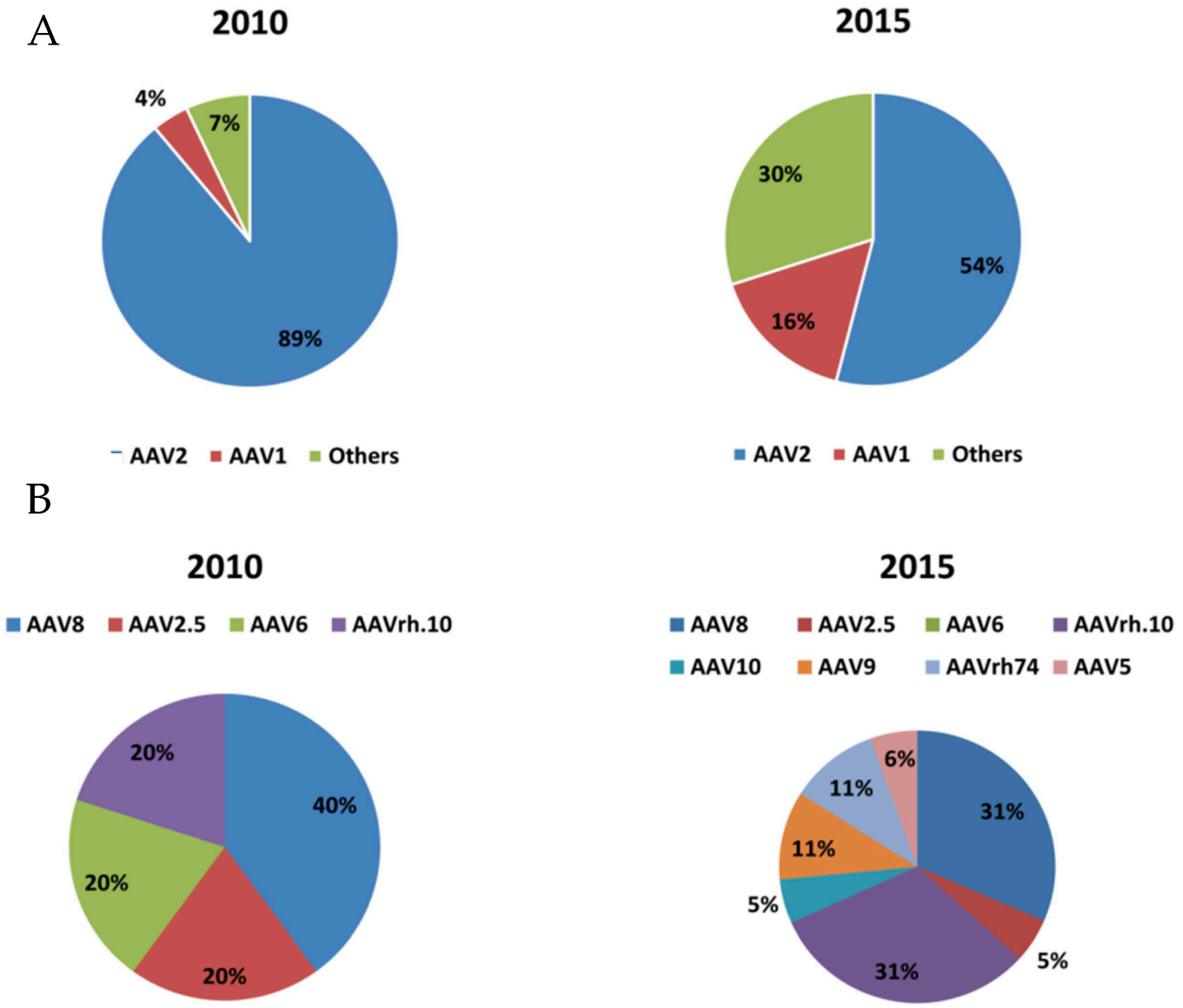
To date, AAV vectors have been used in over 117 clinical trials worldwide. (5.6%). Promising results have been obtained from Phase I and II trials for a number of diseases.

Indication	Gene	Route of administration	Phase	Subject number	Status
Cystic fibrosis	CFTR	Lung, via aerosol	I	12	Complete
	CFTR	Lung, via aerosol	II	38	Complete
	CFTR	Lung, via aerosol	II	100	Complete
Hemophilia B	FIX	Intramuscular	I	9	Complete
	FIX	Hepatic artery	I	6	Ended
Arthritis	TNFR:Fc	Intraarticular	I	1	Ongoing
Hereditary emphysema	AAT	Intramuscular	I	12	Ongoing
Leber's Congenital Amaurosis	RPE65	Subretinal	I-II	Multiple	Several ongoing and complete
Age-Related Macular Degeneration	sFlt-1	Subretinal	I-II	24	Ongoing
Muscular dystrophy	Sarcoglycan	Intramuscular	I	10	Ongoing
Parkinson's	GAD65, GAD67	Intracranial	I	12	Complete[19]
Canavan's	AAC	Intracranial	I	21	Ongoing
Batten's	CLN2	Intracranial	I	10	Ongoing
Alzheimer's	NGF	Intracranial	I	6	Ongoing
Congestive Heart Failure	SERCA2a	Intra-coronary	IIb	250	Ongoing

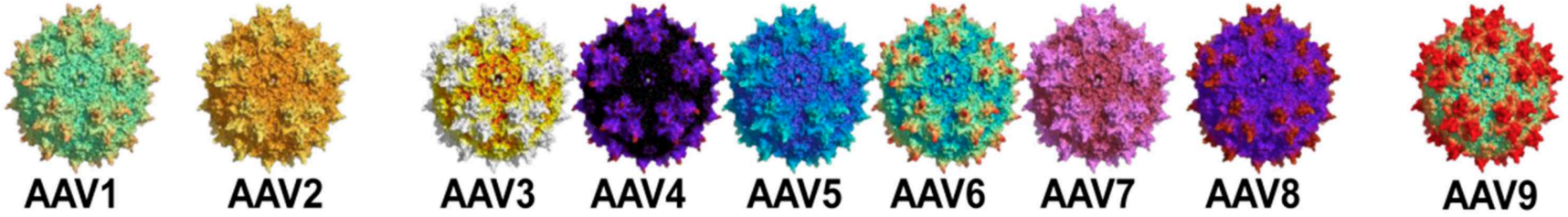
Clinical trials using rAAV technology



rAAV serotypes used in clinical trials

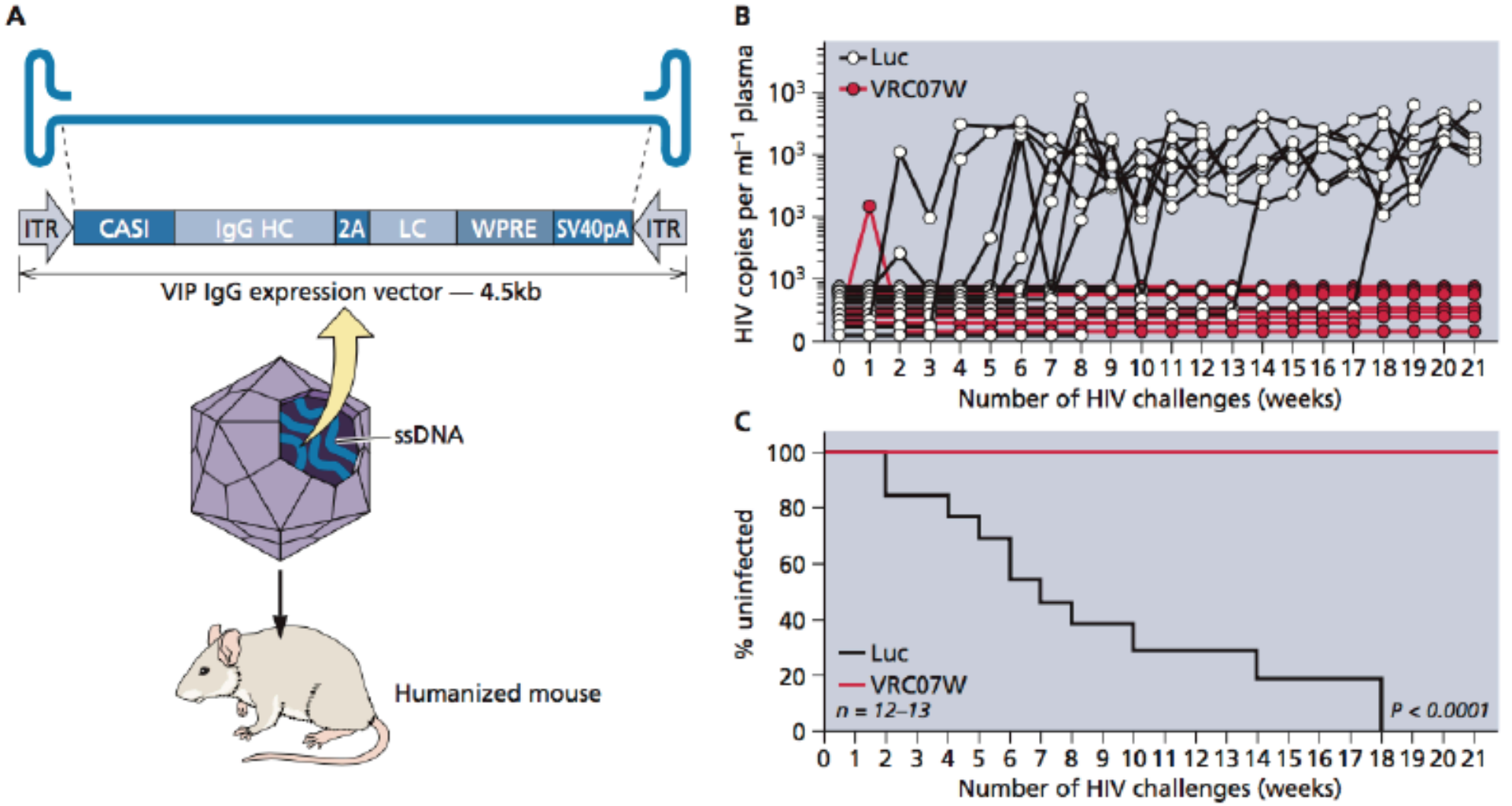


Diseases currently being tested in clinical trials with different rAAV serotypes



Condition to treat	AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9
	Congenital heart failure, cardiomyopathies, Limb Girdle Muscular Dystrophy Type 2C, muscular dystrophies, α-1 antitrypsin deficiency and Lipoprotein Lipase Deficiency.	Aromatic L-amino acid decarboxylase, Parkinson's, age-related macular degeneration, retinal degenerative disorders Leber's congenital amaurosis and choroideremia, Squamous Cell Head and Neck Cancer, Alzheimer's, Hemophilia B and HIV infection			Hemophilia B			Hemophilia B, hepatitis C, X-linked Retinoschisis, Galactosialidosis	Spinal Muscular Atrophy 1, Pompe Disease

AIDS Immunoprophylaxis with AAV

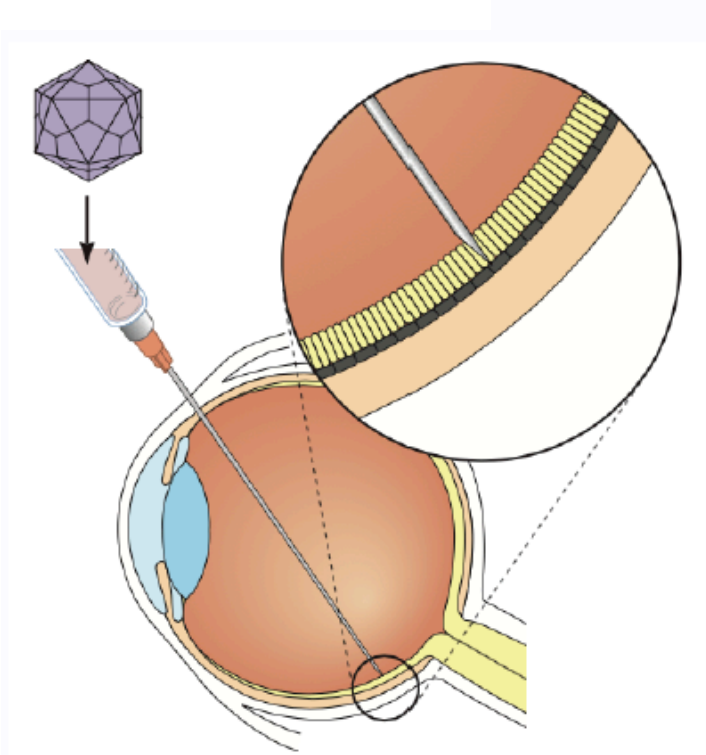


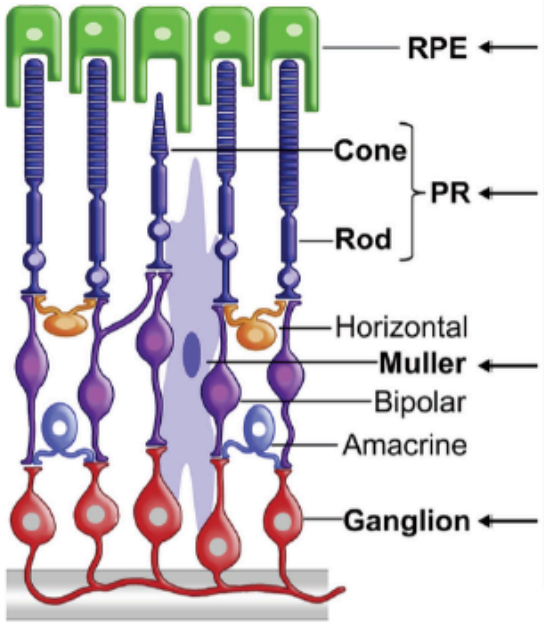
Disease	Transgene product	Serotype	Route of administration	Clinical trial	ClinicalTrials.gov identifier	Refs
AAV clinical trials for inherited diseases						
α1 antitrypsin deficiency	α1 antitrypsin	AAV2	Intramuscular	Phase I/II	NCT00377416	101,102
		AAV1			NCT00430768	
Batten's disease	CLN2	AAV2	Direct intracranial administration	Phase I	NCT00151216	90
		AAVrh10			NCT01161576	
Canavan's disease	Aspartoacylase	AAV2	Direct intracranial administration	Phase I	NA	89
Cystic fibrosis	CFTR	AAV2	Direct instillation to maxillary sinus, bronchoscopy to right lower lobe, aerosol to whole lung	Phase I/II	NCT00004533	154–158
Haemophilia B	Factor IX	AAV2	Intramuscular	Phase I/II	NCT00076557	36,39
			Hepatic		NCT00515710	
		AAV8	Intravenous		NCT00979238	
Leber's congenital amaurosis	RPE65	AAV2	Subretinal	Phase I/II	NCT00643747	4,7,17
					NCT00516477	
					NCT00481546	
LPL deficiency	LPL	AAV1	Intramuscular	Phase I/II	NCT01109498, NCT00891306	12,103,116
Pompe's disease	GAA	AAV1	Series of intradiaphragmatic injections	Phase I/II	NCT00976352	NA (unpublished)
Muscular dystrophy: Duchenne	Microdystrophin	AAV1–AAV2 hybrid	Intramuscular	Phase I	NCT00428935	97
Muscular dystrophy: limb girdle	α-sarcoglycan	AAV1	Two to six separate injections into the selected muscle	Phase I	NCT00494195	95,96
AAV clinical trials for acquired diseases						
Severe heart failure	SERCA2a	AAV1	Antegrade epicardial coronary artery infusion	Phase I/II	NCT00454818	159
		AAV6			NCT00534703	
Parkinson's disease	AADC	AAV2	Intracranial	Phase I/II	NCT00229736	64,65
	GAD				NCT00643890, NCT00195143, NCT01301573	66,69
	Neutrophin				NCT00252850, NCT00985517, NCT00400634	67,68
Age-related macular degeneration	sFLT01	AAV2	Intravitreal injection	Phase I	NCT01024998	NA (unpublished)
Rheumatoid arthritis	TNFR-Fc	AAV2	Intra-articular	Phase I	NCT00617032, NCT00126724	160–162

AADC, aromatic-L-amino-acid decarboxylase; AAV, adeno-associated virus; CFTR, cystic fibrosis transmembrane regulator; CLN2, also known as tripeptidyl peptidase 1 (TPP1); GAA, acid α-glucosidase; GAD, glutamic acid decarboxylase; LPL, lipoprotein lipase; NA, not available; RPE65, retinal pigment epithelium-specific protein 65 kDa; SERCA2a, sarcoplasmic reticulum calcium ATPase 2a; sFLT01, portion of the vascular endothelial growth factor natural receptor; TNFR-Fc, tumour necrosis factor receptor-immunoglobulin Fc fragment fusion protein.

Inherited retinopathies

- Common untreatable blinding conditions
- Monogenic, mutations in retinal photoreceptors and retinal pigment epithelium
- Many vectors tested in animal models, AAV most promising



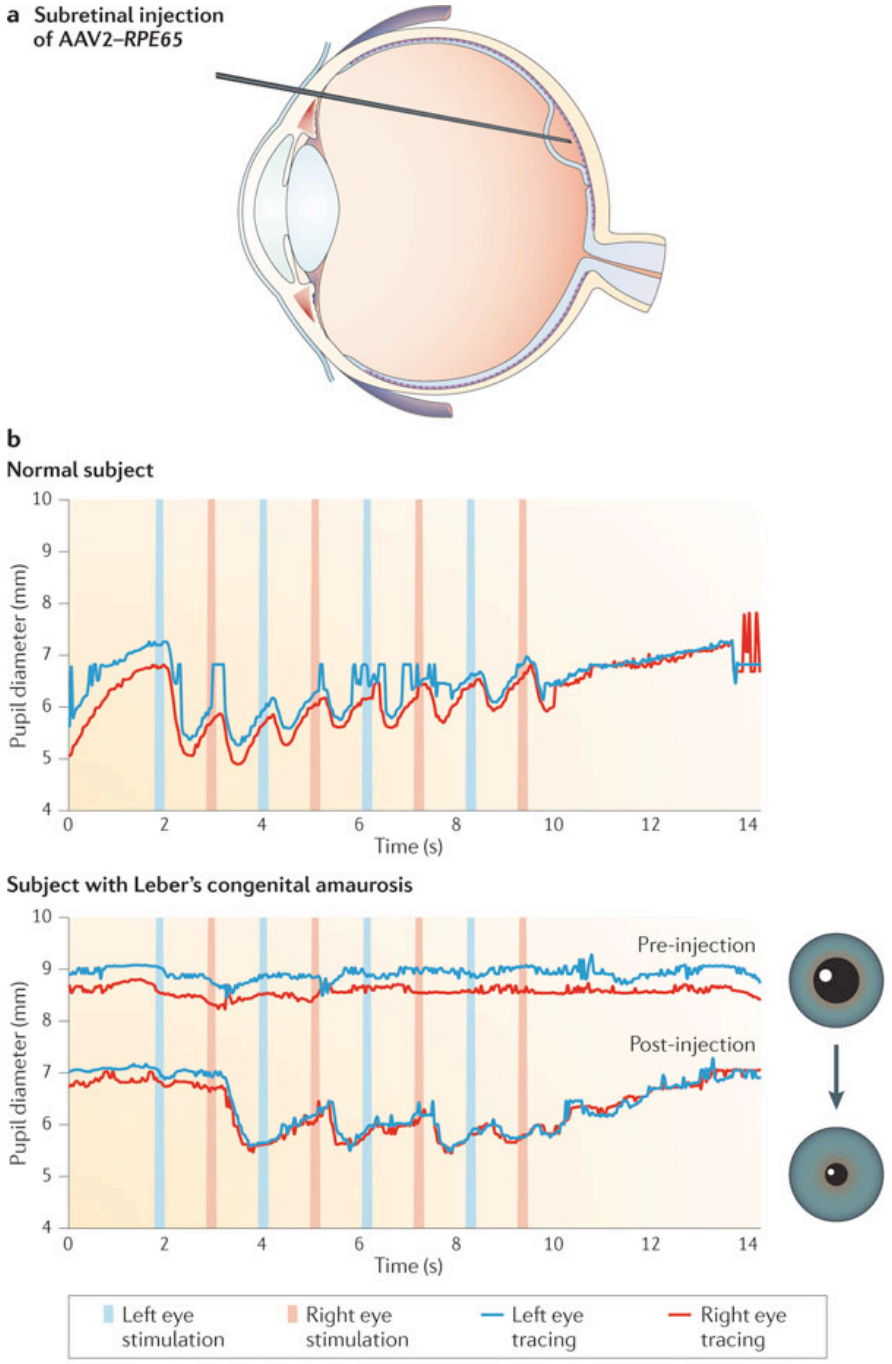


AAV	LV	Ad
2/1[1,2] 2/4[2,3] 2/6[4] *2/7m8[5] **2/Tyr mutant[6,7]	HIV-1-VSVG[1,17,18,19] HIV-1-Mokola[1,18] HIV-1-RRV[21] HIV-1-GP64[22] FIV-VSVG[23] SIV-VSVG[25,26,27,43] EIAV-VSVG[30,31,32] BIV-VSVG[28,29]	5[33,34,35,42] 2[22,38] 5/F35++[22] 5ΔRGD[37,41] 6[22] Ch30[22] Ch63[22] 5/F17[37] 5/F35[39]
2/5[1,8,9,10] 2/7[8,10] 2/8[8,10,11,12,13,14] 2/9[8] *2/7m8[5] **2/Tyr mutant[6,7]	HIV-1-VSVG[1,19,20] HIV-1-GP64[22] FIV-VSVG[24] EIAV-VSVG[31,32]	5[22,33,37,42] 5/F35[39] 5ΔRGD[37,41] *5/F37[40]
*2/2[1,10,15] *2/6[15] **2/8[8,10,14] **2/9[8,10] *2/ShH10[16] *2/7m8[5] **2/Tyr mutant[6,7]	HIV-1-VSVG[21] FIV-VSVG[23,24] EIAV-VSVG[31]	**5[22,33,36] 5/F37[41] 5/F17[37] 5/F35[39]
**2/2[1,10,15] *2/6[15] **2/8[10,14] *2/7m8[5] **2/Tyr mutant[6,7]	FIV-VSVG[23] EIAV-VSVG[31]	*5[40] 5ΔRGD[37]

Leber's congenital amaurosis

- Mutations in ***RPE65*** gene, encodes protein required for photoreceptor function
- Dog model: single subretinal injection of AAV vector with canine ***RPE65*** gene restores visual function
- Phase I/II trials, safe and leads to sustained (1.5 yr) visual improvement

Gene transfer of retinal pigment epithelium-specific protein 65 kDa (***RPE65***) using AAV leads to restoration of pupillary light response in affected individuals



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