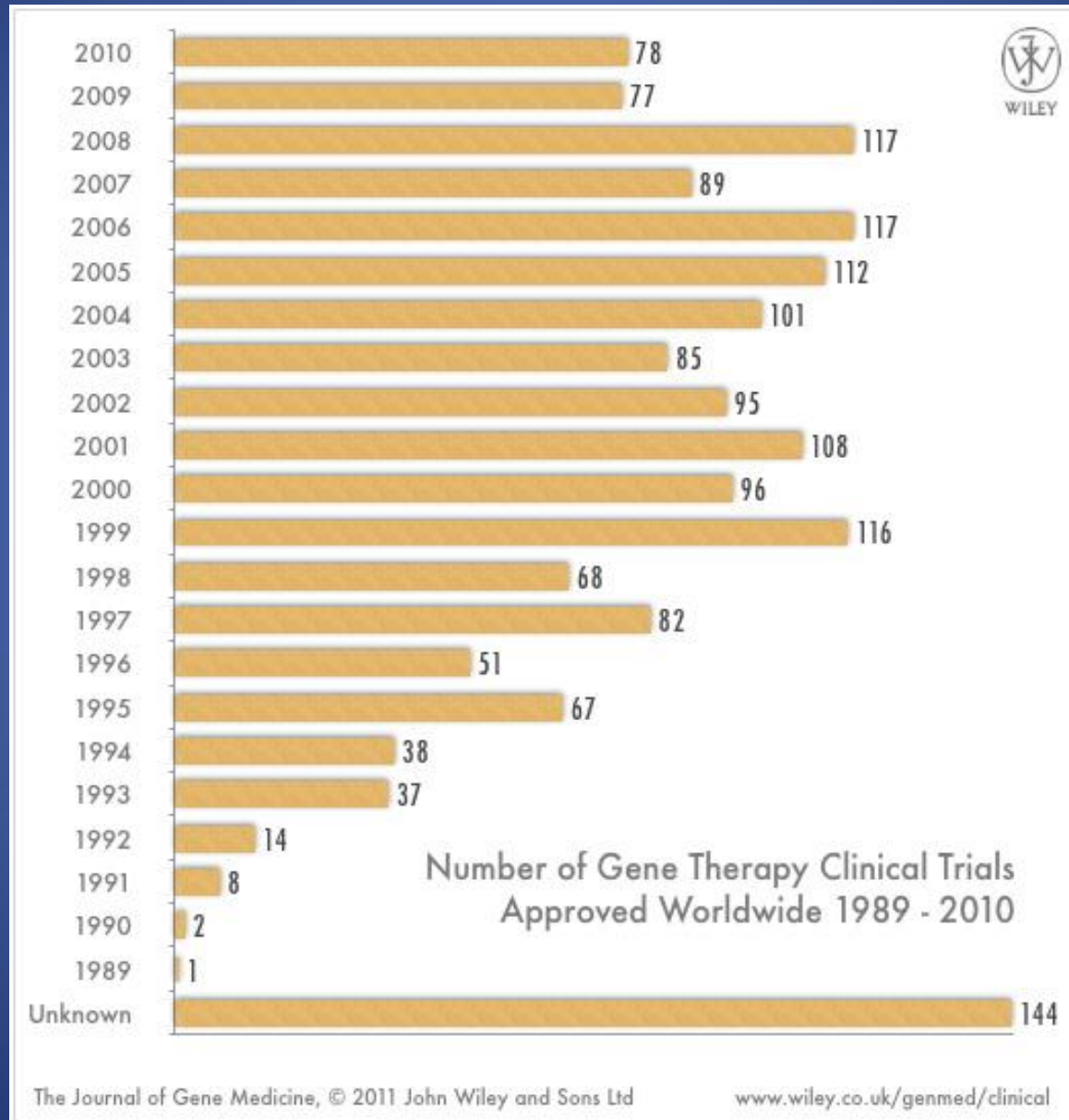


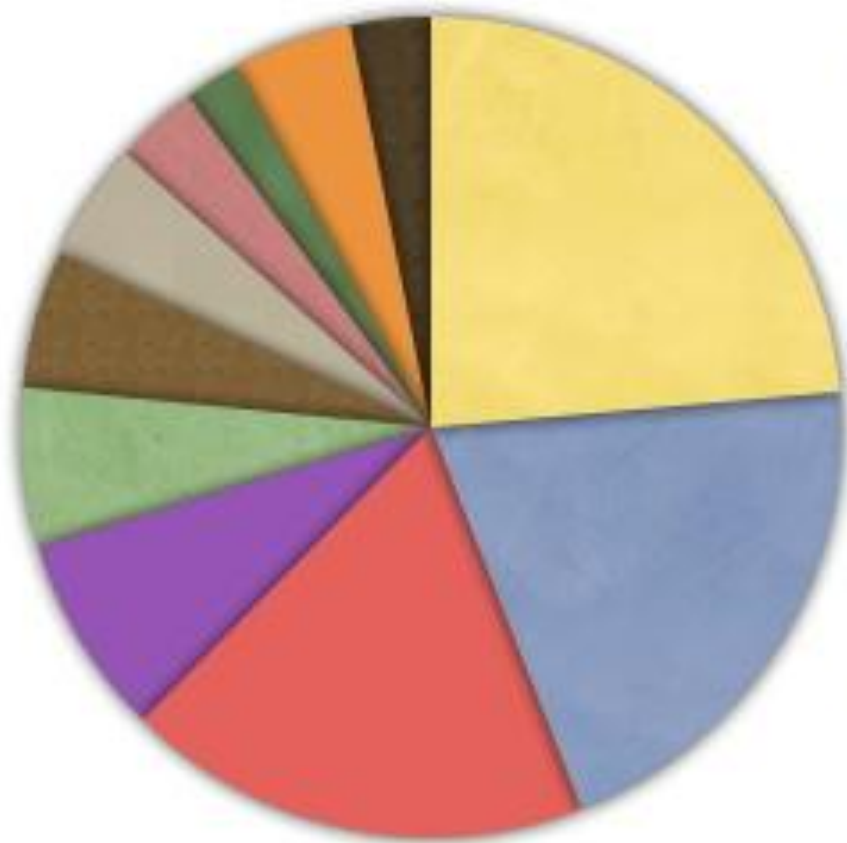
Choosing a Viral Vector System

Janet Douglas

20 years of Vector Development



Vectors Used in Gene Therapy Clinical Trials



- Adenovirus 24.1% (n=410)
- Retrovirus 20.8% (n=354)
- Naked/Plasmid DNA 18.7% (n=319)
- Vaccinia virus 8% (n=137)
- Lipofection 6.4% (n=109)
- Poxvirus 5.5% (n=94)
- Adeno-associated virus 4.8% (n=81)
- Herpes simplex virus 3.3% (n=57)
- Lentivirus 2.2% (n=38)
- Other categories 5.2% (n=89)
- Unknown 3.2% (n=55)

Considerations

- What are your target cells?
- Are they dividing or non-dividing?
- Do you want transient or long-term expression?
- Will an immune response to the vector affect your results?
- Is gene expression going to be evaluated in vitro or in vivo?
- Do you have access to and training for a BSL-2 lab?

	Adenovirus	AAV	Lentivirus	Retrovirus
Gene Expression	Transient	Transient or Stable	Transient or Stable	Stable
Infect Dividing Cells	Yes	Yes	Yes	Yes
Infect Non-Dividing Cells	Yes	Yes	Yes	No
Integration into Target Cell Genome	No	No*	Yes	Yes
Immune Response in Target Cells	High	Very Low	Low	Moderate
Relative Viral Titer	XXXX	XXX	XXX	XX
Relative Transduction Efficiency	XXXX	XXX	XXX	XX

**Native AAV will integrate, but recombinant AAV rarely does.*

Tropism

- Not all viral vectors are “designed” to infect all cell types...tropism of virus = tropism of vector
- Some are more versatile than others
 - Retroviruses/lentiviruses can be pseudotyped with a wide variety of envelope proteins to broaden tropism
 - Mouse specific...ecotropic MuLV envelope
 - Mouse and human...amphotropic MuLV envelope
 - Most vertebrates...VSV-G
 - AAV inherently broad tropism, but can increase with different serotypes
 - Adenovirus pretty broad, but needs CAR (receptor)
 - Low in human hematopoietic cells & mouse cells in general
 - Can swap fiber from other serotype

Promoter issues

- Tissue/cell type specific expression
 - ie. Liver, Endothelial cells, Cancer cells
- Inducible expression for toxic gene products
 - Tetracycline-operator
- High expression levels
 - Viral promoters (CMV; RSV; SV40)
 - Downside is they may be targeted by anti-viral mechanisms in vivo
- Consistent expression levels
 - Eukaryotic promoters (PGK; EF-1 α)
- Multiple genes
 - Multiple promoters
 - Internal ribosome entry site (ires)
 - Vector size restraints and variable expression
 - Self-cleaving 2A peptide fused to transgene product
 - Especially useful for AAV (small packaging size)

	Adenovirus	Adeno-asso- ciated virus	Alphavirus	Herpesvirus	Retrovirus / Lentivirus	Vaccinia virus
Genome	dsDNA	ssDNA	ssRNA (+)	dsDNA	ssRNA (+)	dsDNA
Capsid	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Complex
Coat	Naked	Naked	Enveloped	Enveloped	Enveloped	Enveloped
Virion polymerase	Negative	Negative	Negative	Negative	Positive	Positive
Virion diameter	70 - 90 nm	18 - 26 nm	60 - 70 nm	150 - 200nm	80 - 130 nm	170 - 200 X 300 - 450nm
Genome size	39 - 38 kb	5 kb	12 kb	120 - 200 kb	3 - 9 kb	130 - 280 kb

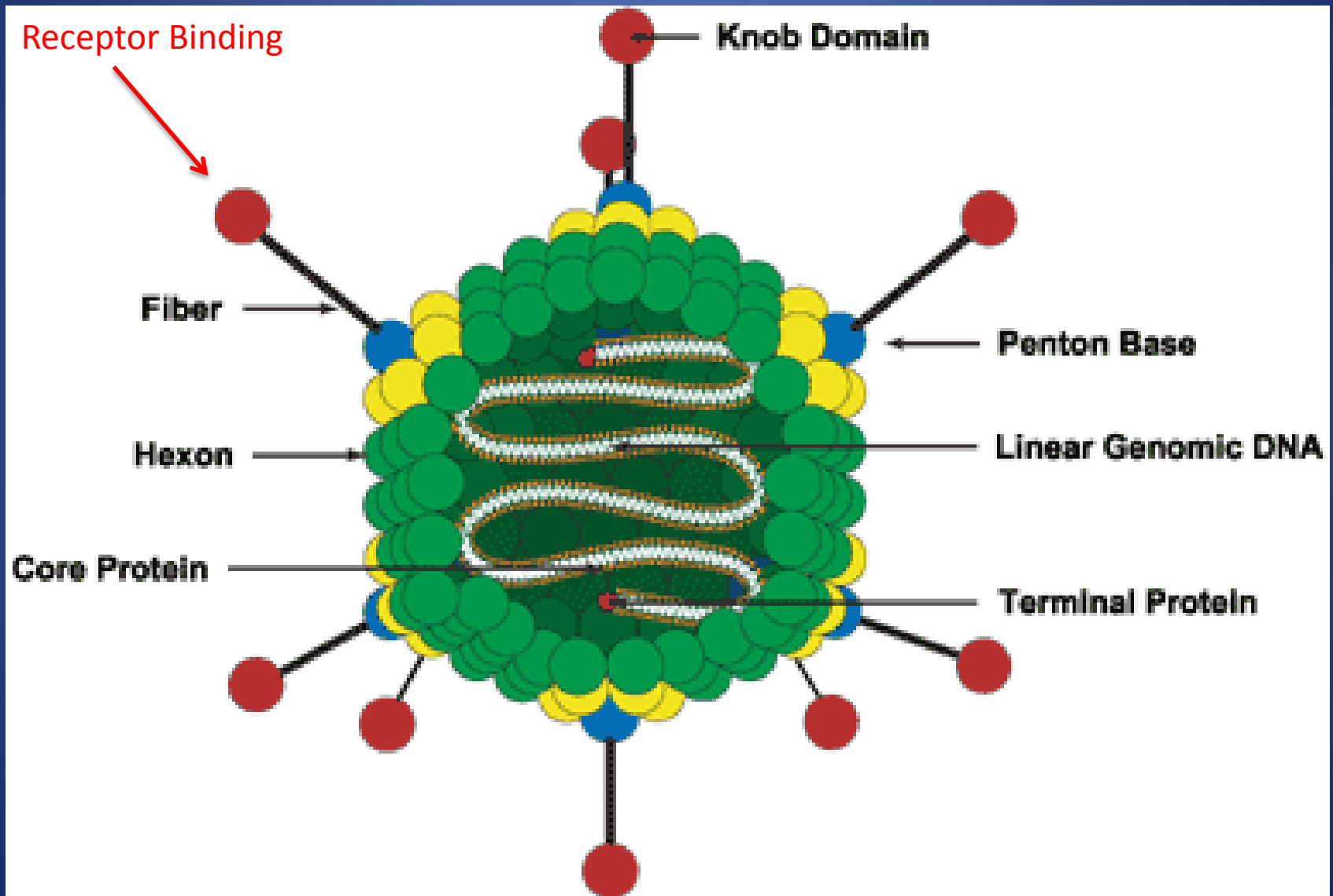


	Adenoviridae	Parvoviridae	Togaviridae	Herpesviridae	Retroviridae	Poxviridae
Family	<i>Adenoviridae</i>	<i>Parvoviridae</i>	<i>Togaviridae</i>	<i>Herpesviridae</i>	<i>Retroviridae</i>	<i>Poxviridae</i>
Infection / tropism	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non- diving cells	Dividing and non-diving cells	Dividing cells*	Dividing and non-diving cells
Host genome interaction	Non- integrating	Non- Integrating*	Non- integrating	Non- integrating	Integrating	Non- integrating
Transgene expression	Transient	Potential long lasting	Transient	Potential long lasting	Long lasting	Transient
Packaging capacity	7.5 kb	4.5 kb	7.5 kb	> 30 kb	8 kb	25 kb

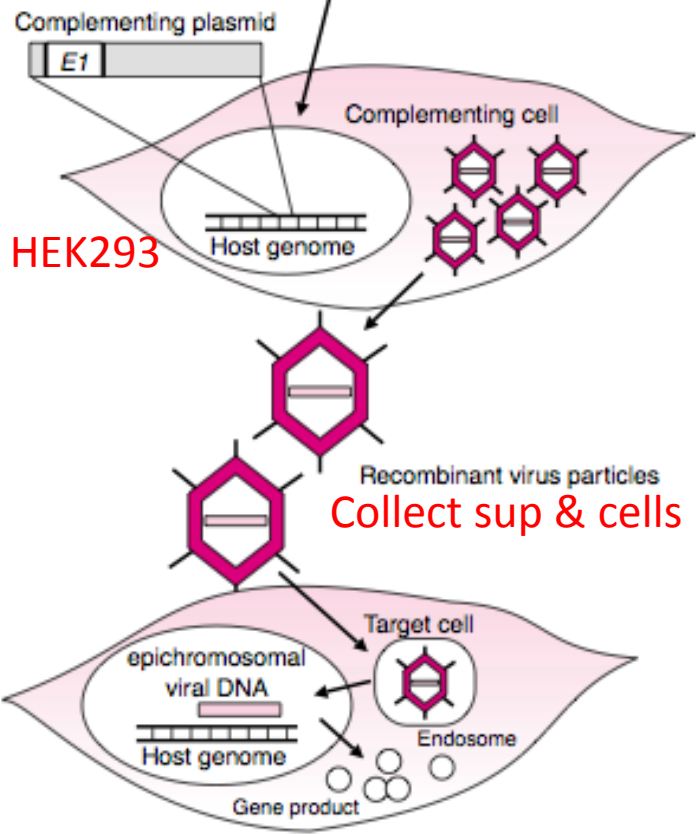
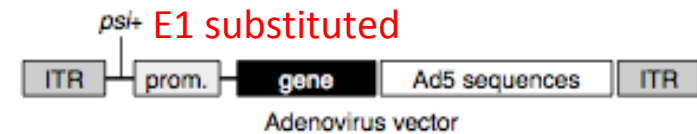
Recombinant Viral Vector Systems

- Vector has characteristics of parent virus
 - Capsid/Env dictates tropism
 - Viral genome maintenance dictates transient or long-term expression
 - Viral genome size dictates packaging size
 - Viral release determines vector preparation
 - Cell-associated and/or supernatant
- Safety Features
 - Packaging cell lines & helper constructs

Adenoviral particle

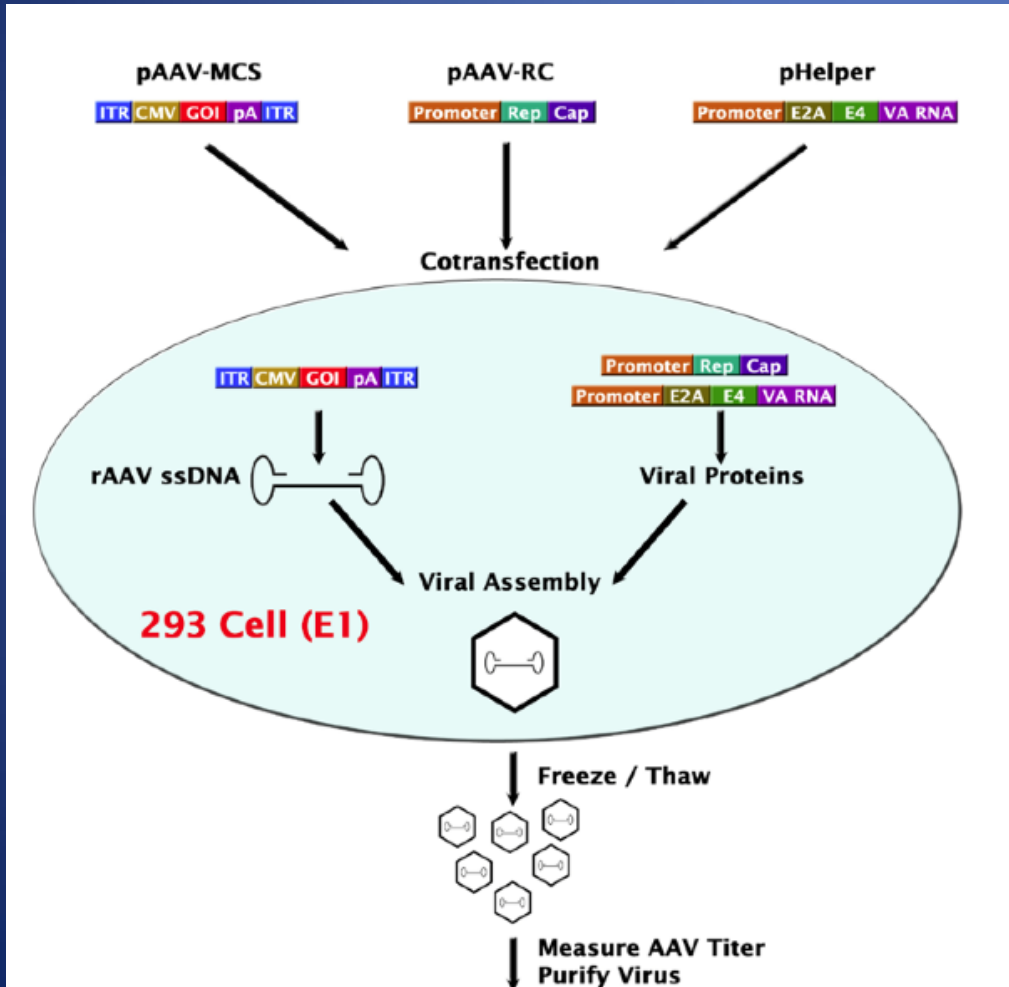


Recombinant Adenovirus



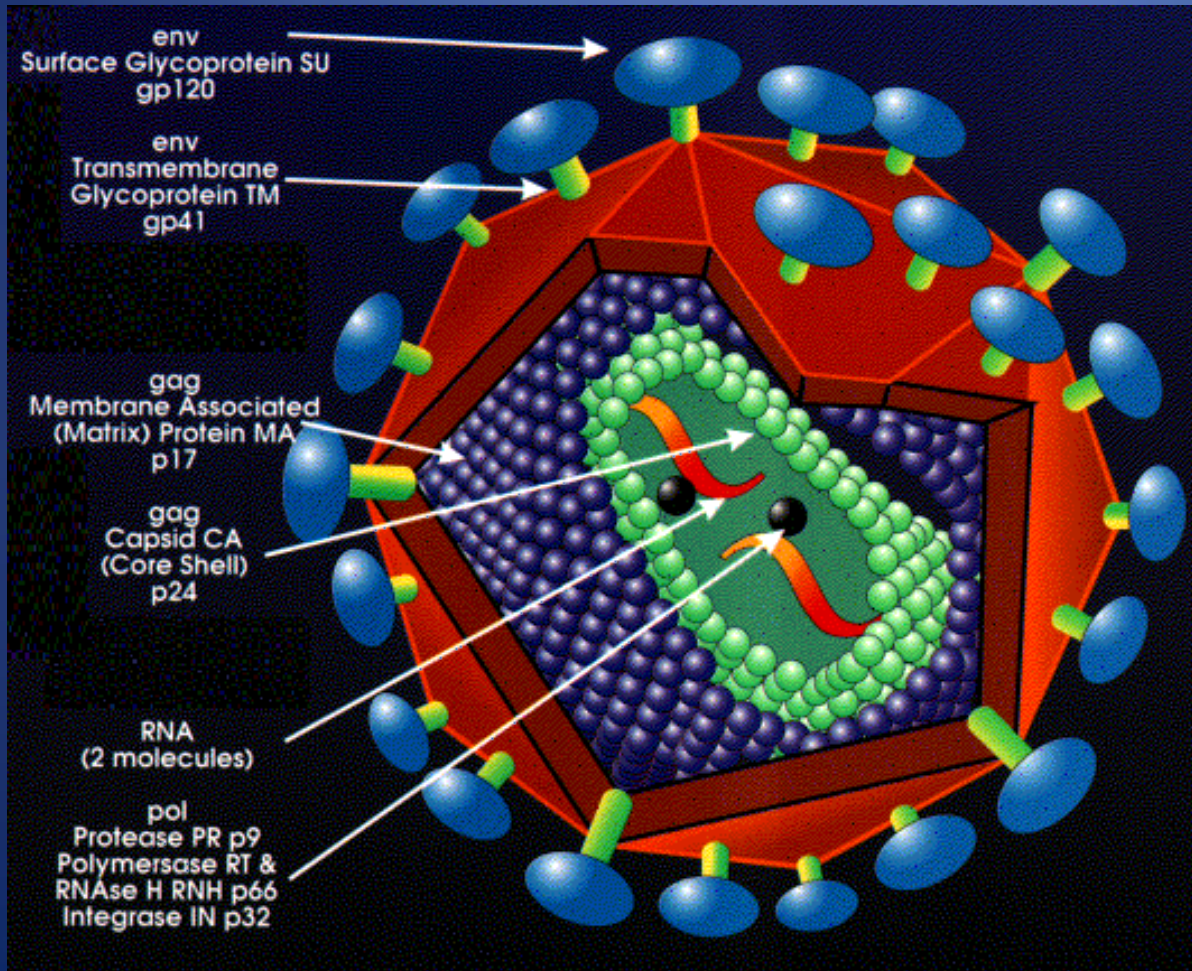
- Ad5-based vectors require Coxsackie-adenovirus receptor (CAR) on target cells
 - Low in human hematopoietic cells & mouse cells in general
- Transient gene expression
- High immune response
- High titers & transduction efficiency

Recombinant Adeno-associated virus (AAV)



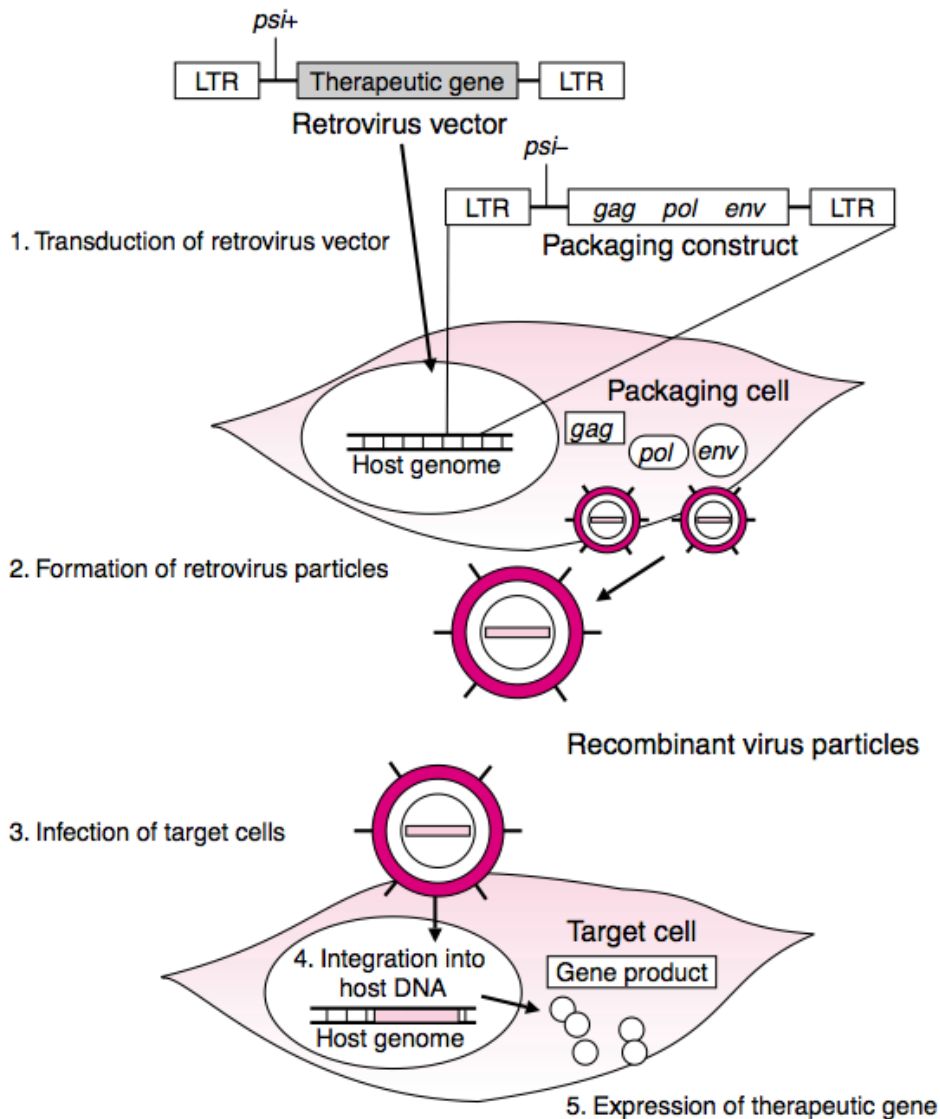
- Broad tropism
- Low immunogenicity
- Helper virus free systems
- BSL-1
- Disadvantage is small packaging size 4.5kb

Retroviral virion components



- Gag = structural proteins
 - Matrix
 - Capsid
 - Nucleocapsid
- pol=enzymes
 - Protease
 - Reverse Transcriptase
 - Integrase
- Env=surface glycoproteins
- Genome=RNA

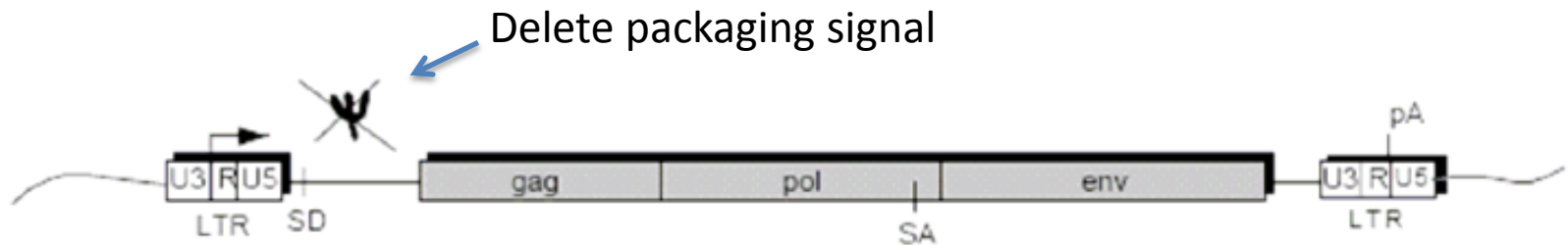
Recombinant Retroviruses



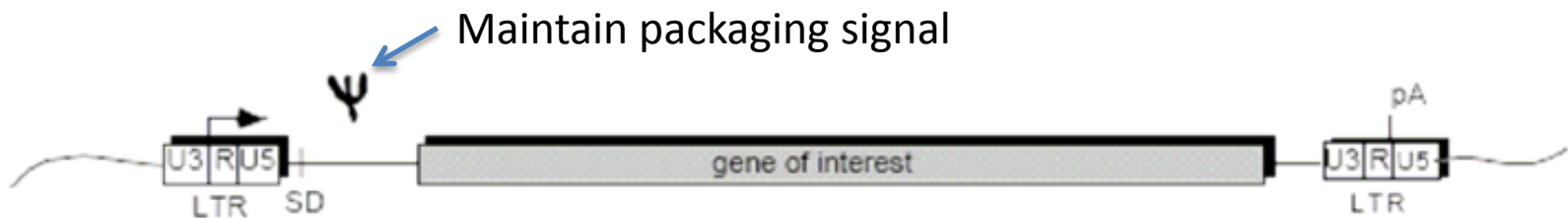
- Can be pseudotyped with various env proteins to broaden tropism
- Stable packaging cells
- Long-term gene expression through integration
 - Downside is insertional mutagenesis
- Disadvantage is only infects dividing cells

Retroviral vector design

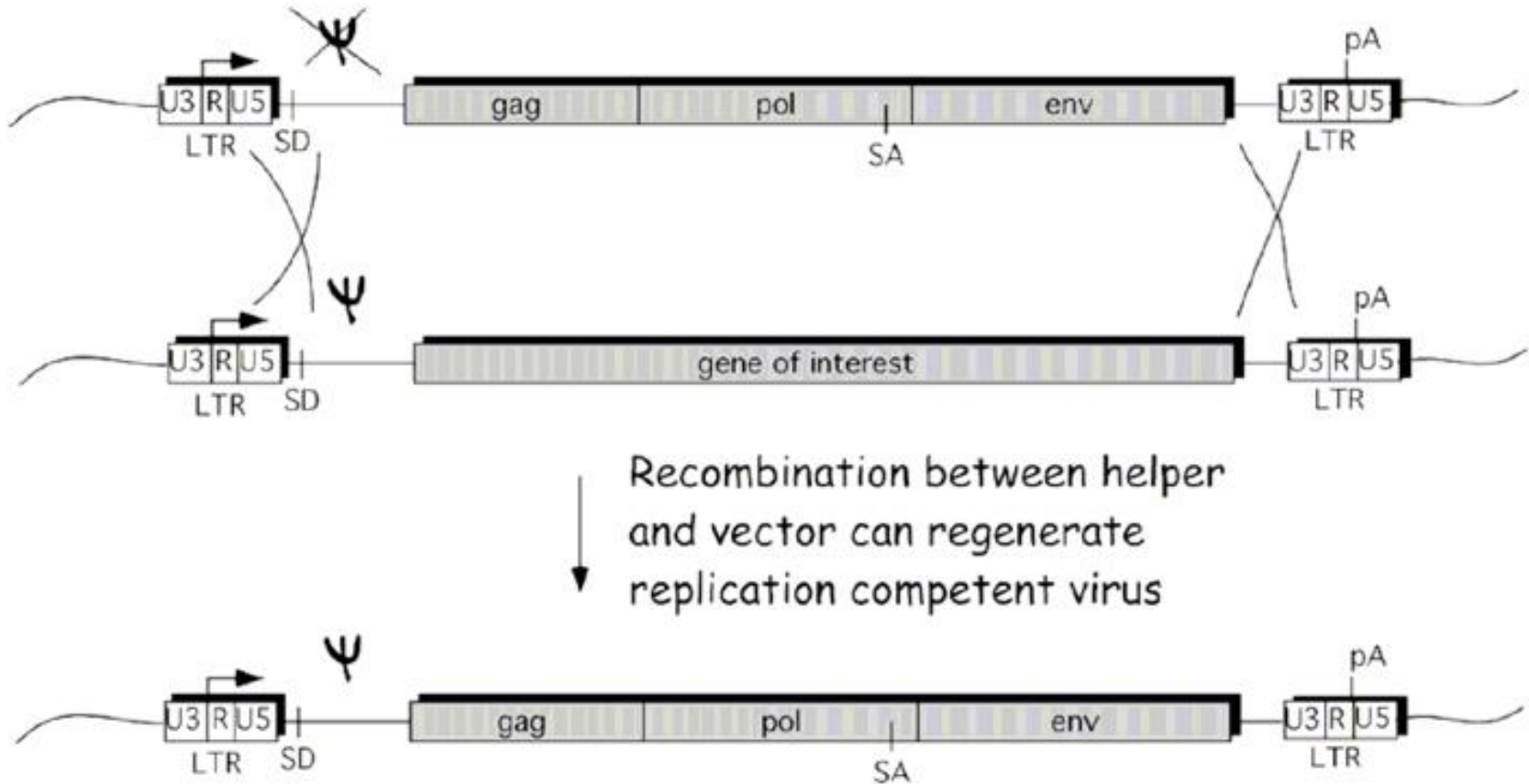
The Helper:



The Vector:



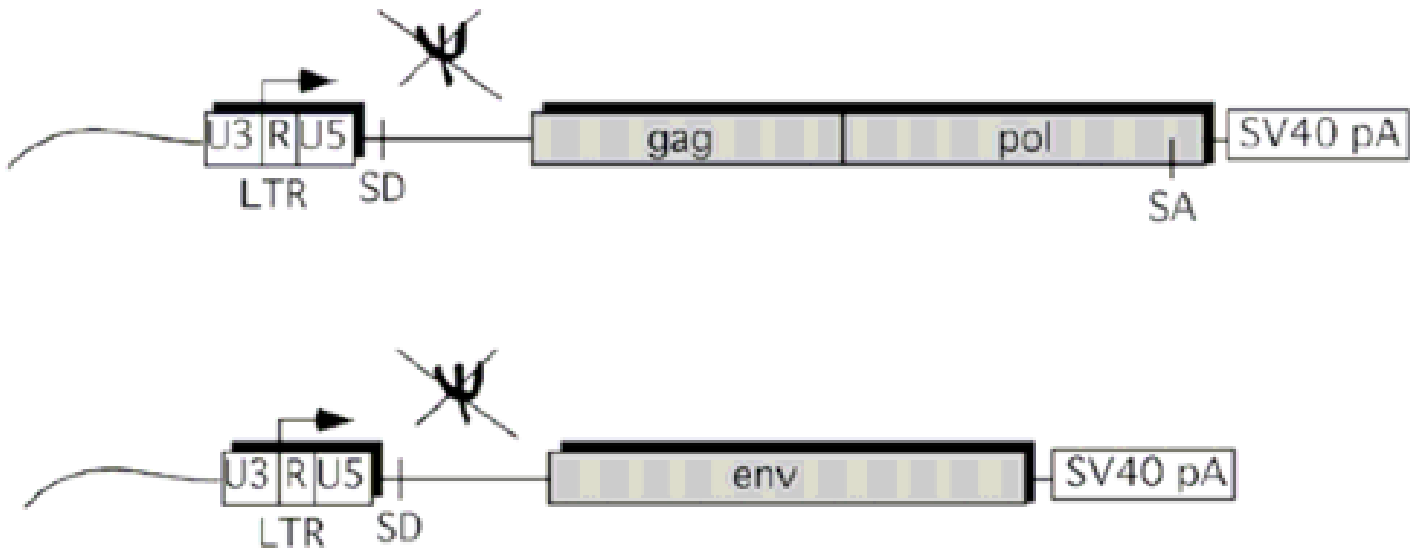
The Problem with recombination



- RCR-replication competent retrovirus

The Solution

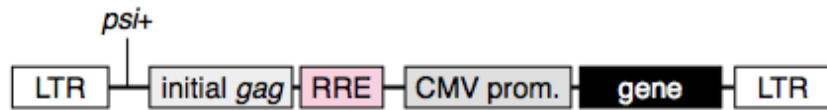
Split the Helper genome



Or...

Lentiviral vectors

Lentiviral vector



Lentiviral packaging constructs

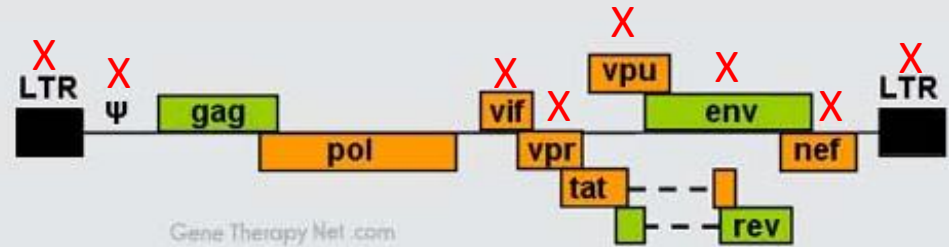
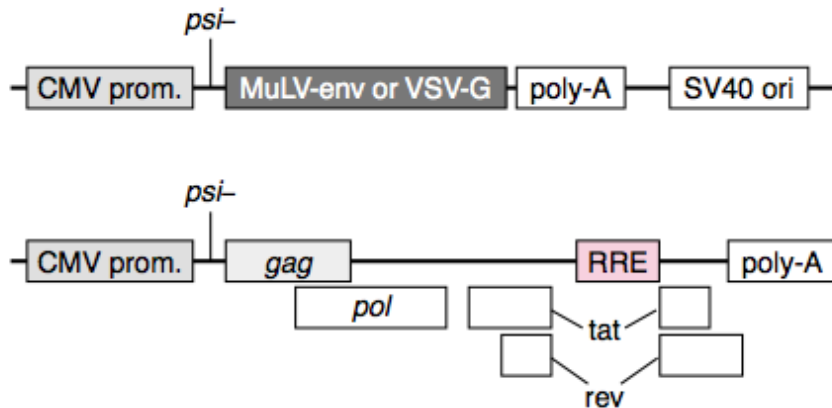


Figure 2. Genome organisation of lentiviruses.

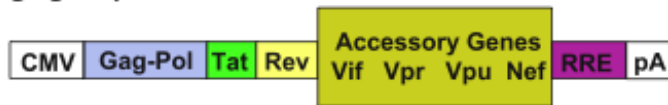
- Long-term gene expression like simple retrovirus
- Advantage is ability to infect non-dividing cells

- HIV-based systems
- Non-human based systems
 - Feline Immunodeficiency virus (FIV)
 - Equine infectious anemia virus (EIAV)
 - Simian immunodeficiency virus (SIV)

Evolution of Lentiviral Vector Development

Packaging Expression Cassettes

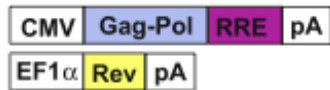
First Generation



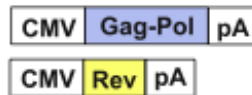
Second Generation



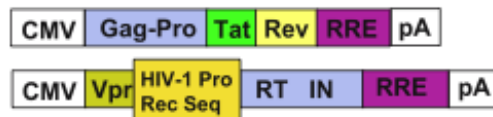
Third Generation



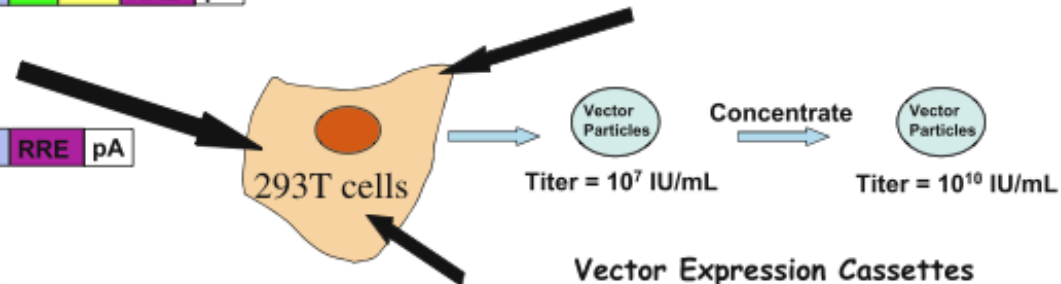
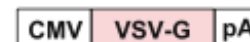
Codon Optimized Gag/Pol



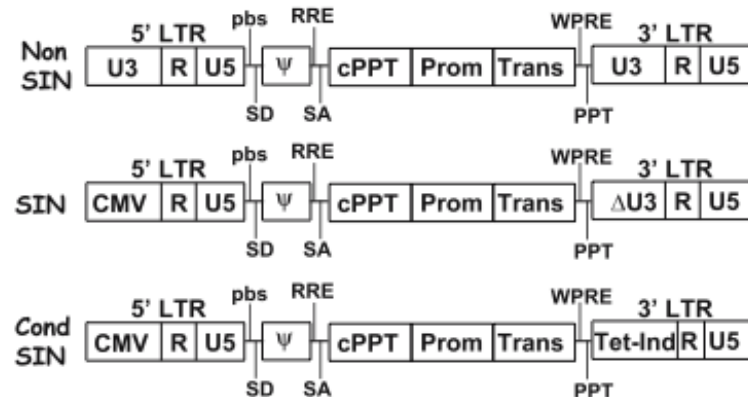
Split Gag/Pol



Envelope Expression Cassette



Vector Expression Cassettes



Overview

Table 1 | The main groups of viral vectors

Vector	Genetic material	Packaging capacity	Tropism	Inflammatory potential	Vector genome forms	Main limitations	Main advantages
Enveloped							
Retrovirus	RNA	8 kb	Dividing cells only	Low	Integrated	Only transduces dividing cells; integration might induce oncogenesis in some applications	Persistent gene transfer in dividing cells
Lentivirus	RNA	8 kb	Broad	Low	Integrated	Integration might induce oncogenesis in some applications	Persistent gene transfer in most tissues
Non-enveloped							
AAV	ssDNA	<5 kb	Broad, with the possible exception of haematopoietic cells	Low	Episomal (>90%) Integrated (<10%)	Small packaging capacity	Non-inflammatory; non-pathogenic
Adenovirus	dsDNA	8 kb* 30 kb [§]	Broad	High	Episomal	Capsid mediates a potent inflammatory response	Extremely efficient transduction of most tissues