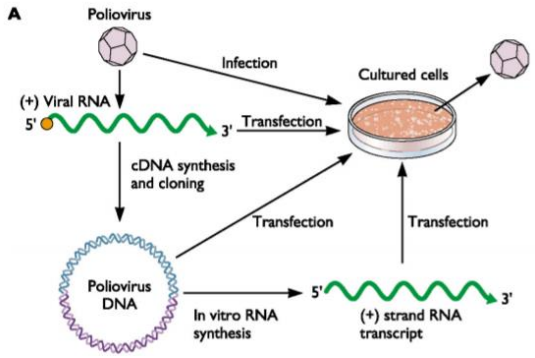
Viral vectors

Transfection & transduction

- **Transfection**: **naked nucleic acid** delivery system >> achieved through: 1. **Precipitate** **DNA** or **RNA** with CaPO4 (calcium phosphate) & deliver to cells 2. **Cationic** liposome – suspension of **DNA** or **RNA** & encapsulated into **cationic** **liposomes** that can fuse with cell membrane
- **Transduction**: **viral vector** delivery system >> e.g. adenovirus vector, it delivers nucleic acid directly into the nucleus hence, foreign nucleic acid not sensed by the host immune system



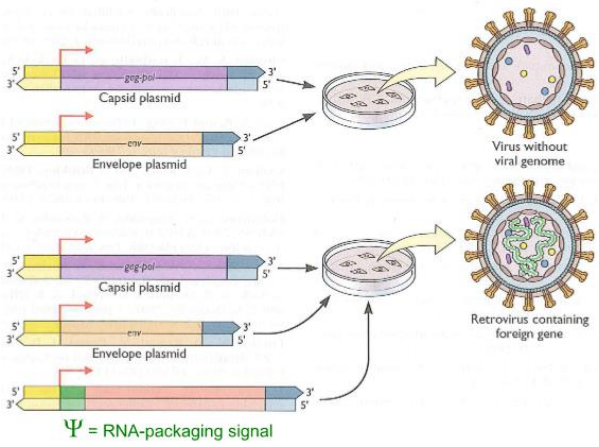
Culture poliovirus with possible transfections methods:

1. Extract **viral RNA** with **precipitation** or **cationic** **liposomes** method to deliver to cells
2. Clone **cDNA plasmid** from viral RNA with **RNA II promotor** placed on **suitable location** to transcribe same viral RNA (lacking 5' VPG)
3. **In vitro RNA synthesis** with **bacteriophage RNA polymerase** – **T7** or **SP6** RNA pol (lacking 5' VPG)

Viral vectors – harnessing the viral machine

<u>Viral vector design requirements</u>:

1. Removal of **viral sequence** responsible for **virulence factor** & **replication** >> viral particles must *infect* & *release* **transgene** (recombinant viral nucleic acid) into cells BUT must **NOT** be able to *produce* & *release* new infectious particles that could transfer **transgene**
2. Inclusion of 1. **Promotor elements** – **homologous** or **heterologous** (non-human) e.g. **CMV** **immediate-early** **promotor** uses ONLY cellular transcription factors >> immediately express **transgene** after infection 2. **PolyA signal** – terminate RNA transcription
3. Matching of **genome size** with **packaging limit of virus** – 1. Delete non-essential genes on the **transgene** 2. Select a virus that has a matching genome packing size for the **transgene**



Concept of retrovirus vectors:

1. Remove **viral sequence** responsible for **virulence factor** & **replication** – **a.** Package **Gag-pol** & **Env** gene with CMV promotors
2. **Separate** viral sequences required for replication & for production of viral particles – some vectors retain **replicative genes** in the vector construct
3. Flank transgene by essential **cis-acting sequence** & **packaging signal** – **b.** Package **transgene** with **RNA packaging signal**
4. Provide **viral proteins** required for **packaging** & **replication** in **packaging cell** – those proteins required to produce a viral vector

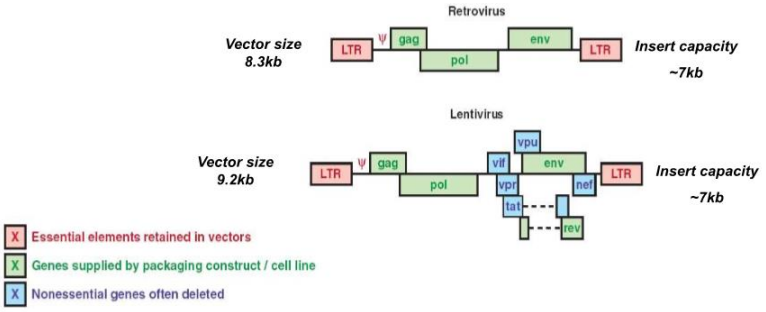
Comparison of different viral vectors:

Viral vector	Titres	Insert size	Manipulation of tropism	Immunogenicity	Infect NDV-cells
Adenovirus	10^{11}	2-38kb	Good	Very High	Yes
Retrovirus	10^{7}	1-7kb	Good	Low	No
Lentivirus	10^{7}	7-18kb	Good	Low	Yes
AAV	10^{7}	4.5kb	Not so good	Low	Yes
Herpesvirus	10^{7}	30kb	Not so good	Low	Yes
RNA replicons	10^{7}	3-8kb	Not so good	?	?

- *Adenovirus* gives the highest titres **10^11** with easy manipulation of **tropism** & high **immunogenicity** >> can infect non-dividing cells
- *Retrovirus* (simple) & *Lentivirus* (complex) both give **10^7** titre with easy manipulation of **tropism** & low **immunogenicity** >> ONLY Lentivirus can infect non-dividing cells
- *Adeno-associated virus* (AAV), *Herpesvirus* & both give **10^7** titre with poor manipulation of **tropism** & low **immunogenicity** >> can infect non-dividing cells

Lentivirus as a viral vector

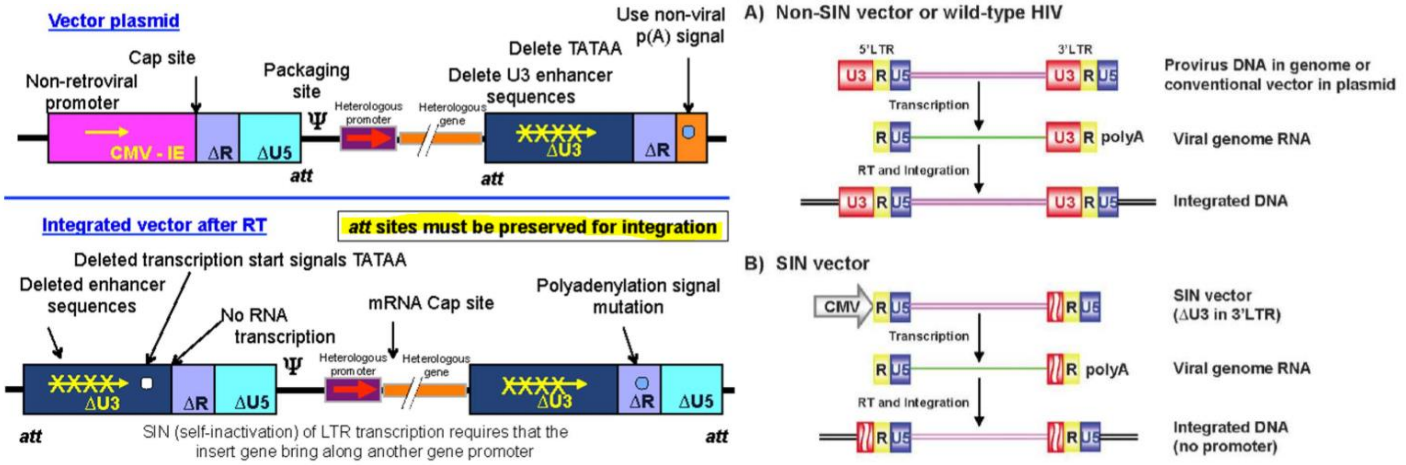
- It acquires its **envelope glycoprotein** passively >> glycoprotein from a different virus could be used to package lentivirus particles e.g. VSV-glycoprotein that binds almost every cell type
- Its genome can be permanently delivered to **non-dividing**, **terminally differentiated** **cells** >> it provides long-term therapeutic benefits



Process of lentivirus vector production:

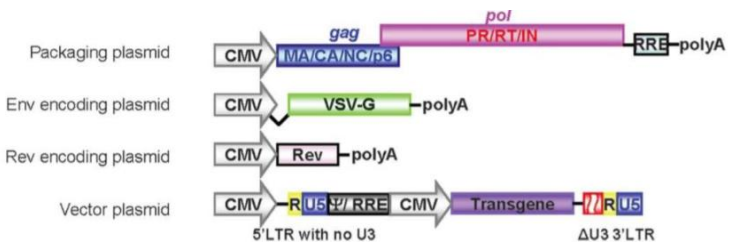
1. **Transgene** production: **Heterologous** genes are positioned in place of deleted gag, pol & env
2. **Vector particles** are produced by **transfection** (**naked nucleic acid** delivery system) of gag, pol & env into packaging cell line
3. **Vector particles** are harvested from cell culture

SIN vector:



- LTRs are modified to preserve **RT** & **integration**, but stop LTR transcription
  - **Viral plasmid**: sequence of engineered DNA – replace 5' U3 promotor with **CMV heterologous promotor**, 5' attachment sequence (**for integration**), packaging site, another heterozygous promotor, transgenes, 3' attachment sequence, mutated 3' U3 promotor & poly A signal
- After transcription of viral genome RNA from engineered DNA, only 5' R & U5 + **another** heterozygous promotor & transgenes + mutated 3'U3 & R left >> this RNA is then reverse transcribed back to cDNA
  - **Integrated vector after RT**: the 5' U3 promotor is mutated hence it cannot initiate a transcription >> therefore, LTR transcription is stopped >> start with **another** CMV promotor to transcribe **transgene**

Third generation of HIV vector:



- Requires 4 different plasmids:

1. A **packaging** plasmid – CMV, Gag, Pol & **RRE** sequence
2. A **Rev protein** plasmid – Rev sequence is essential for production of structural proteins
3. An **Env-encoding** plasmid – **VSV-G** sequence allows infection to almost every cell type
4. A packaging **plasmid**

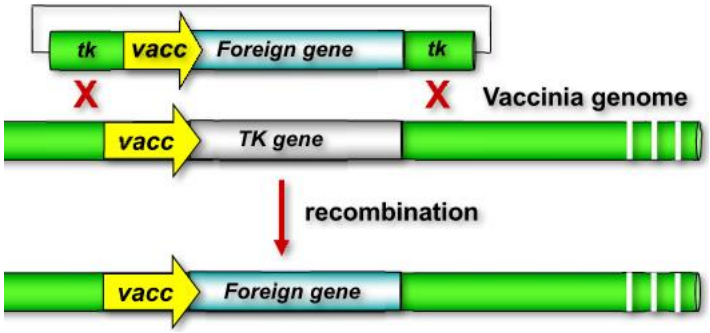
Issues with retroviral gene vector: 1. Packaging cells must NOT express endangers retroviruses 2. Better to remove Tat protein from HIV vectors 3. Insertional mutagenesis causes cancer 4. Heterologous gene **expression** wanes with time

Application of retroviral/lentiviral vector:

- Gene correction therapy: no vector-induced immune response or cytotoxicity, used for **X-CGD** (1. Mutation in gene of **NADPH oxidase complex** required for **microbe killing** 2. Therapy restores **defective phagocyte function** in **myeloid linages** 3. Insertional **activation** of **growth promoting genes** cause no malignancy) or **X-SCID** (T & NK cell development blocked from **mutation of cytokine receptor** >> restored function in but causing **leukemia** from insertion)
- Cancer therapy: transient high-level expression >> vector-induced or insert-induced cytotoxicity specific to tumour cells
- Vaccines: transient high-level expression >> strong activation of both innate & adaptive of immune responses

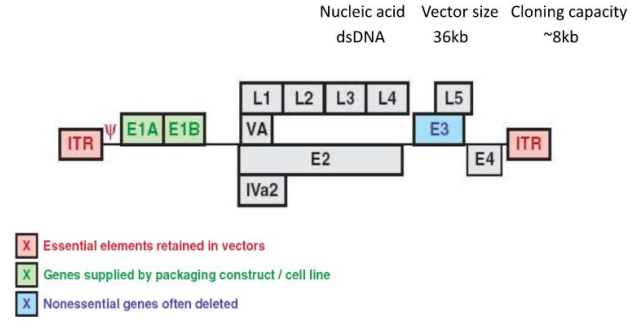
Adenovirus as a viral vector

- For DNA virus, its **genome** is beyond the size of a plasmid >> cannot only rely on encapsulation of virus based on its genome-expressing packaging signal >> **homologous recombination** is required between gene of interest & viral gene



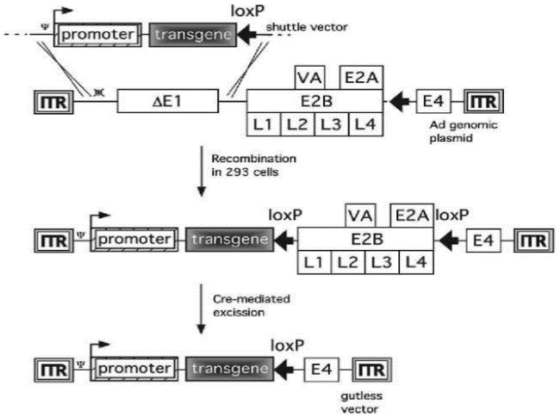
- It requires position **gene of interest** within viral DNA flanking sequence & under promotor control
- Co-transfection of **recombinant plasmid** & **viral DNA** OR transfection of **recombinant plasmid** into **virus-infected cells**

- Replication-**defective** forms of human adenovirus type 5 – Ad5 is most commonly used >> natural Ad5 infection typically occurs in **young children** with **no illness** or **mild respiratory diseases** >> approximately 40% **adults** have **neutralising Ab** indicating **previous infection** and **current protective immunity**
  - **Main features**: efficient transduction gives titres **10^11** with easy manipulation of **tropism** – penton spikes can be modified to tune tissue **tropism** & high **immunogenicity** >> can infect non-dividing cells
  - **Application**: 1. Gene correction therapy – limited application due to its Ad's transient expression 2. Cancer therapy – more suitable, as high titres of virus are **toxic** & Ads are **highly immunogenic** 3. Vaccine
  - **Limitation**: 1. Reduced efficiency of deliver due to pre-existing immunity 2. Short-term expression: strong T-cell responses to vector proteins produced in transduced cells >>> clearance of vector-transduced cells 3. Prevent re-immunization with vaccine: strong humoral response against viral capsid



Process of adenovirus vector production:

1. **Heterologous** genes are in place of deleted E1/3/4 genes
2. **Vector particles** are produced by **transfection** (**naked nucleic acid** delivery system) of E1 & E4 into packaging cell line (HEK 293 cells)
3. **Vector particles** are purified from cell by **cesium chloride gradient**

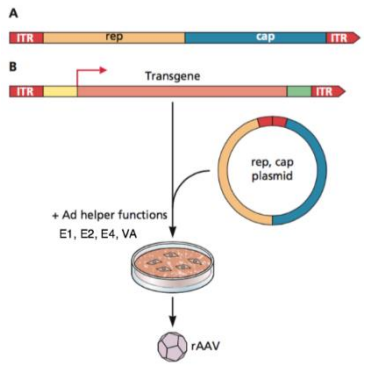


Gutless Adenoviral vectors:

- Pre-existing Abs against structural proteins cause problem
- Gutless adenoviral vectors lead to the deletion of all **structural protein** sequence on DNA:

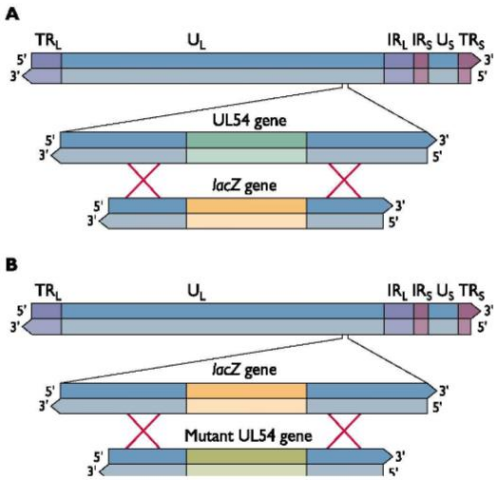
1. Homologous recombination between gene of interest & Ad DNA on the E1 sequence position
2. **Cre-protein mediated excision** cleaves structure genetic sequence between **LoxP sites**

AAV viral vector



- *Adeno-associated virus* (AAV) give **10^7** titre with poor manipulation of **tropism** & low **immunogenicity** >> can infect non-dividing cells
- Small single-stranded DNA (4.5kb) >> the genome can be concatenated to make longer fragments >> however, insertion is restricted to 4.7kb
- May integrate into host genomic DNA, sometimes as a concatemer, preferentially into human chromosome 19
- Co-transfect vector with plasmids expressing **replicase** & **capsid** proteins into the packaging cell line >> packaging proteins are provided by adenovirus
- **Advantage**: most viral genes removed, integration with specific site to give persistent expression, no **intestinal mutagenesis**. **Disadvantage**: genome size limitation, pow titres of virus, low level of gene expression, labour intensive to make & may link to a death

Herpesvirus viral vector

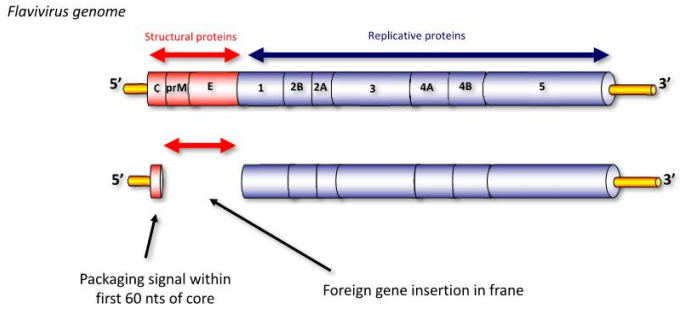


- *Herpesvirus* & both give **10^7** titre with poor manipulation of tropism & low **immunogenicity** >> can infect non-dividing cells
- Linear dsDNA (152kb) >> half of the total 81 genes are non-essential for virus replication >> 40 – 50kb foreign DNA can be accommodated
- **Homologous DNA recombination** >> herpesvirus vectors are produced by 1. Recombination with highly defective virus 2. Using large plasmids with an HSV Ori S & packaging sequence
- **Neurotropic virus** targets nervous system >> cause mild diseases in human hence no risk

RNA virus expression vectors

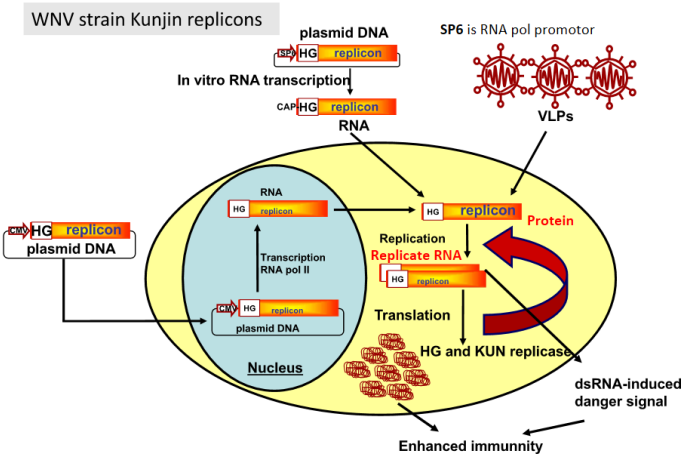
<u>RNA replicons</u>

- Self-replicating RNAs derived from RNA viral genomes & replication occurs in cytoplasm >> capable of high level expression of heterologous genes
- Can be delivered as **VLP**, naked **RNA** or naked **DNA** >> derived from (+)ssRNA viruses as vectors: 1. Alphavirus 2. Flavivirus
  - **Main features**: high level of cytoplasmic replication with no integration or recombination >> noncytopathic
  - **Application**: effective for vaccine & cancer therapy
  - **Limitation**: 1. VLPs have small cloning capacity 2. Pre-existing immunity – cross-reacting Abs to West Nile virus in Africa & America



Process of adenovirus vector production:

1. **Heterologous** genes are in place of deleted structural genes
2. **VLPs** are produced by RNA transfection into packaging cell line expressing structural genes
3. Three delivery models – DNA, RNA or VLPs



- **DNA**: plasmid enters nucleus to produce replicon RNA that is then exported into the cytoplasm >> translated to replicase >> amplifies replicon RNA level
- **RNA**: RNA enters cytoplasm >> translated to replicase >> amplifies replicon RNA level
- **VLP**: enters cytoplasm >> translated to replicase >> amplifies replicon RNA level

NOTE: large amount of dsRNA initiates dsRNA-induced danger signals >> good for vaccine

<u>VSV expression system & Ebola virus vaccine</u>:

- VSV RNA is integrated into a plasmid >> replace the VSV glycoproteins with Ebola glycoproteins >> hybrid viral particles
- Effective protection against Ebola