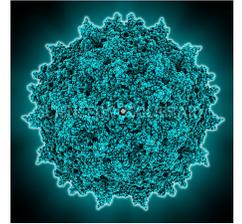


# Adeno-Associated Virus and AAV Vectors



Family: *Parvoviridae*

Genus: *Dependovirus*

Naked with icosahedral capsid

More than 100 serotypes, AAV2 well studied and frequently used as viral vector

Size: ~ 20 nm in diameter

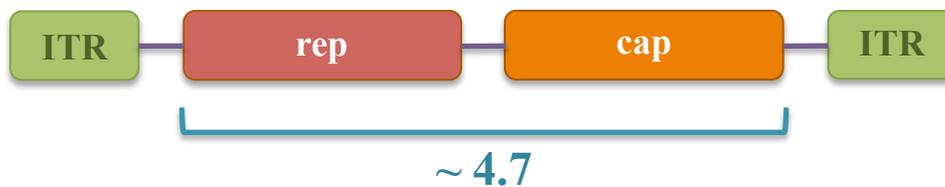
Genome: Linear, ~ 4.7 Kb ssDNA

Replication-deficient, requires helper virus (Ad, HSV, HPV)

Wide tropism for a variety of mammalian cells

Risk Group: 1

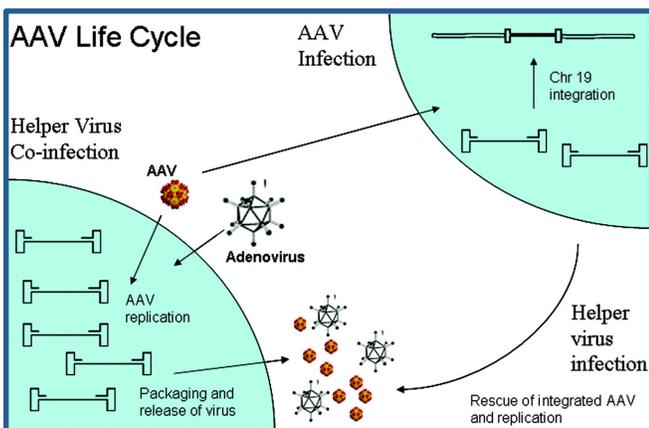
## Adeno-Associated Virus 2 (AAV2) Genome Structure



The genome of AAV2 consists of a single molecule of linear, single-stranded DNA with a size of 4,680 bp. The plus and minus strands are packaged into separate particles and organized into:

- Inverted Terminal Repeats (ITRs), which contain *cis*-elements required for replication and packaging and flank the two viral genes: *rep* and *cap*. The ITRs serve as origins of replication and play a key role in viral genome integration and subsequent rescue by helper virus.
- The *rep* (replication) gene encodes four non-structural proteins necessary for replication: Rep78, Rep68, Rep52, and Rep40.
- The *cap* (capsid) encodes three structural capsid proteins: VP1, VP2, and VP3.

## AAV Life Cycle



Wild-type AAV undergoes productive infection upon co-infection or super infection with a helper virus - such as adenovirus (Ad), Herpes Simplex Virus (HSV) or Human Papilloma Virus (HPV). This is characterized by DNA replication, viral gene expression and virion production.

When AAV infects human cells in the absence of a helper virus, its gene expression program is shut down and the virus remains latent by integrating its DNA into the AAVS1 region of chromosome 19. The AAV Rep proteins are

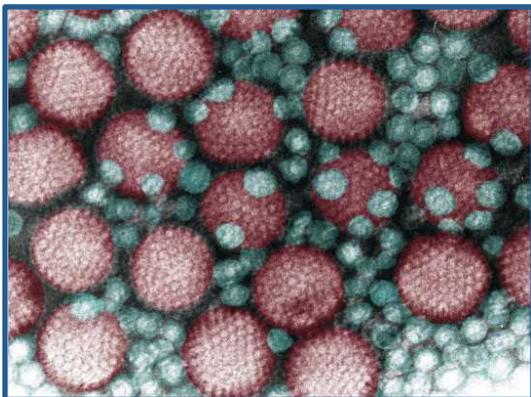
essential for targeted integration. Upon subsequent infection of the cell with a helper virus, the AAV genome is rescued from latency, which results in AAV DNA replication and the formation of new viral particles.

## Recombinant AAV Vectors

The production of recombinant AAV (rAAV) vectors is possible because the rep and cap genes can be deleted from the viral genome and provided in *trans*, leaving room for a small transgene. **However, integration of rAAV genomic sequences in the absence of the AAV Rep proteins is less efficient and not targeted to chromosome 19.** As with other integrating vectors, the potential with insertional mutagenesis may be a concern when working with AAV vectors.

Some of the inherent limitations of packaging genes into small rAAV genomes have been bypassed by manipulating the vector constructs. These include the use of dual vectors that expand the rAAV packaging capacity and self-complementary (scAAV) vectors that circumvent the requirement for dsDNA conversion.

## AAV Infection in Humans and Routes of Exposure in the Lab



The first human AAV was discovered in 1965 as a contaminant of adenovirus preparations. The picture on the left shows AAV particles (blue) and adenoviruses (red). Little is known about naturally occurring AAV infections, since AAV has not been associated with any pathology in humans and AAV particles have only been isolated in the context of acute adenovirus infection. In the presence of helper virus, AAV can replicate and generate up to  $1 \times 10^6$  copies per cell, thus killing them.

Approximately 80% of the population is seropositive for anti-AAV antibodies against serotypes 1, 2, 3 or 5.

Exposure to AAV in the lab may occur through skin or mucous membrane contact, accidental injection, inhalation and ingestion. **Though AAV is not known to cause disease in humans, precautions must be taken due to the possibility of insertional mutagenesis. In addition, helper viruses used to trigger AAV replication may cause disease.**

## Cell Tropism of AAV

Numerous serotypes of adeno-associated virus with different cell tropisms have been isolated. AAV can infect a wide range of dividing and non-dividing cells from many mammals. AAV genomes have been recovered from lung tissue, as can be expected given the respiratory route of infection. However, AAV has also been detected in other tissues derived from humans and non-human primates including bone marrow, brain, colon, heart, liver, lymph nodes, kidney and spleen. This suggests that the virus spreads through the body during infection. Besides integrated proviruses, episomal AAV genomes may persist for prolonged periods of time.

Pseudotyping of rAAV vectors is used to generate tropism-modified vectors, a.k.a. rAAV-targeting vectors. rAAV2 genomes can be packed into capsids derived from other AAV serotypes, thus narrowing or broadening the affinity of the new viral vector for specific cell types.

## Pros and Cons of AAV Vectors

ADVANTAGES	DISADVANTAGES
Wild-type AAV is not associated with any known disease	Small transgene capacity (~ 4.7 Kb)
Wide cell tropism	Potential for insertional mutagenesis
Efficient gene transfer	
Well tolerated → do not elicit a strong immune response	
Stable expression from integrated or episomal sequences	

## Environmental Stability of AAV Vectors

AAV particles are stable in a wide pH range (3 to 9) and can resist heating at 56°C for 1 hour. Due to the high stability of the capsid, AAV can remain infectious for at least a month at room temperature after desiccation or lyophilization.

Since AAV is a non-enveloped virus, it is very resistant to alcohol-based disinfectants. A freshly prepared 10% bleach solution should be used as a disinfectant instead.

## Biosafety Considerations for Work with AAV Vectors

Adeno-associated viruses are considered Risk Group 1 agents. Most work with AAV vectors can be conducted at BSL-1 or BSL-2 containment level, depending on a risk assessment.

## Resources

- <http://researchcompliance.uc.edu/training/aav-vectors/story.html>
- Smith, RH. Adeno-associated virus integration: virus versus vector. *Gene Therapy* 2008; 15, 817-822.
- Adeno-Associated Virus - Methods and Protocols. Editors: Richard O. Snyder & Philippe Moullier. Springer Science + Business Media, LLC. New York, 2011.