ADENO VIRUS

**Virus Characteristics:**

Adenoviruses are medium-sized (90–100 nm), non-enveloped (naked) icosahedral viruses composed of a nucleocapsid and a double-stranded linear DNA genome. They belong to the *Adenoviridae* family and infect various species of animals, including humans.

The viral capsid contains 252 protein components, the majority of which are three types of proteins: fiber, penton base and hexon. Fiber and penton base proteins are important in receptor binding and cell internalization, whereas hexon comprises the majority of the viral capsid.

The genetic material of the adenoviruses is not incorporated into the host cell's genetic material and is not replicated during cell division providing transient expression.

*Accumulation of virions in the nucleus of Vero cell*
Adenovirus Cycle and Genome

The adenovirus replication cycle occurs in the cell nucleus and is separated into two phases: an early and a late phase.

The early genes are responsible for expressing mainly non-structural, regulatory proteins. The late phase of the adenovirus life cycle is focused on producing sufficient quantities of structural protein to pack all the genetic material produced by DNA replication. Once the viral components have successfully been replicated, the virus is assembled into its protein shells and released from the cell as a result of virally induced cell lysis.

The adenoviral genome is large, consisting of a single double-stranded DNA molecule 36 to 38 kilobases in size. The genome is flanked by two inverted terminal repeat (ITR) regions of DNA which act as origins of replication.

Adenovirus Transmission and Clinical Manifestations

Adenoviruses are primarily spread via respiratory droplets, however they can also be spread by fecal routes. Most infections with adenovirus result in diseases of the upper respiratory tract. Also, the ocular and gastrointestinal tracts may be affected. A combination of conjunctivitis and tonsillitis is particularly common with adenovirus infections.
Different subgroups of adenovirus have affinity for different tissues.

The serotype 2 and 5 are commonly used for viral vector production. In these serotypes the attachment to the host cell primarily occurs via the Coxsackie-Adenovirus Receptor (CAR) on the surface of the host cell which is widely distributed in the host.

**Altering Cell Tropism - Pseudotyping**

Adenoviral vectors are capable of transducing a wide range of cell types. Although in many times this is an advantage, in other situations this lack of specificity is undesirable.

The original cell tropism of an adenoviral particle may be altered with the use of fiber/penton base proteins from a different serotype.

Other methods for retargeting adenoviruses, such as the use of bivalent antibody conjugates, can also retarget the vector and narrow its tropism range.
Adenoviral Vector Production

To create an adenoviral vector particle, at least two items need to be present: the Transfer Vector and a Packaging Cell.

**Transfer Vector (Viral Construct)**

This recombinant adenoviral DNA will most likely have a deletion of the E1 gene which will be replaced by the gene of interest (transgene) and possibly deletions of other genes depending on the vector generation.

The viral construct will always have the 2 viral ITRs and the packaging signal (Ψ). Packaging signal is the element required for encapsidation of the viral genome. It indicates in which genome structural proteins should be assembled.

_E1 is an essential adenovirus gene. Its products are necessary for viral growth. Without E1 gene the vector is rendered replication incompetent._

In addition to gene transfer, in specific cases such as treatment of cancer, the lytic properties of the virus is desired. This is when a replication-competent adenoviral vector targeting specific cells are considered. In this type of vector, transgenes are usually inserted in the E3 region.

**Packaging Cell (Helper Cell)**

In adenoviral vector production, a packaging cell is not only the location where the production of the vector takes place. Since most adenovirus transfer vectors lack the E1 gene, this essential gene needs to be provided in trans during vector packaging. Cell lines such as HEK 293, 911, PER.C6 and GH239 may be used as E1 gene source.

HEK293 is a cell line derived from human embryonic kidney cells grown in tissue culture. They are also known, more informally, as HEK cells. This particular line was initiated by the transformation and culturing of normal HEK cells with sheared fragments of human adenovirus type 5.

The transformation resulted in the incorporation of approximately 4.5 kilobases from the viral genome which contains the E1 gene into human chromosome 19 of the HEK cells.

_Colored scanning electron micrograph (SEM) of HEK cells._
Various adenoviral vector systems have been developed and are classified in generations:

**First-generation** - This vector has deletion of the E1 gene and may also contain deletion of the E3 gene (non-essential) to increase transgene capacity. Removal of the E1 region hampers the transcription of E2 and other viral genes and consequently blocks viral DNA replication.

**Second-generation** - This vector has deletions of the E1 regions plus other deletions such as E2a, E2b, or E4 genes.

**Third-generation (gutless or helper-dependent)** - In this type of vector, almost the entire genome is deleted (5’ and 3’ ITRs and packaging signal remain in this construct). During the vector production, a helper virus must be present (often a first generation adenoviral vector) so essential proteins can be produced.
When the viral construct has additional deletions, missing genes may be provided by Helper Plasmids and, in case of Gutless vectors, a Helper Virus must be included to provide all the absent coding genes.

Although vector is rendered replication-incompetent, it remains infective!

Final vectors lack the genetic information for self-propagation in cells, but retain the capacity of introducing genes of interest into the target cells through infection.

Replicating Competent Adenovirus (RCA):

One concern when working with these vectors is the possible occurrence of replication competent adenovirus (RCA) in a population of replication deficient adenoviral vectors.

The probability of acquisition of viral sequences (and loss of the transgene) from a complementing cell line or helper virus can be minimized if there are no overlapping sequences. For example, HEK293 cells carry 11% of the adenovirus genome containing the E1 cassette; this includes at least 800 bp of sequence present within most E1-deleted adenovirus vectors, providing the potential for recombination that restores the E1 region in the virus.

In contrast, PerC6 and similar cell lines have been engineered to express the minimal E1A and E1B genes from heterologous promoters, and thus have no sequence overlap with most newer E1-deleted vectors, greatly reducing the frequency of generating replication-competent virus.

Also, it has been demonstrated that after an increased number of passages in 293 cells the chance of emergence of RCA increases considerably. As a strategy, a pure master stock of recombinant adenovirus at a low passage number in 293 cells should be maintained.
Adenoviral Vector: Environmental Stability

Due to the lack of envelope, adenoviral vectors are relatively stable and resistant to dehydration. They are quite resistant to chemical agents (e.g. alcohol based disinfectants) and adverse pH conditions. These vectors are stable in the environment and can survive 3 to 8 weeks on work surfaces at ambient temperatures. In order to inactivate this agent a broader spectrum disinfectant should be considered (e.g. 10% household bleach).

![Adenoviruses exit cell by cell lysis.](image)

Surface Disinfection - Fresh solution containing 1 part of bleach and 9 parts of water.

70% alcohol is NOT effective against adenovirus.

Advantages

- Large transgene capacity (up to 37 kb – Gutless vectors)
- High transduction efficiency
- Broad Tropism - has the ability to infect most mammalian cell types (both dividing and non-dividing cells)
- Easily purified to high titers
- Does not integrate into the host chromosome not having intrinsic oncogenesis potential.

Disadvantages

- Transient expression:
  Since this agent does not integrate into the host chromosome, when cells divide genetic material is not transferred through generations

- Immunogenicity:
  Viral proteins elicit innate immune and inflammatory responses *in vivo*. More problematic in first-generation vectors and considerably decreased in gutless vectors. *This is a major concern when vectors are used in human trials.*
All human adenovirus serotypes belong to the Risk Group Classification 2 (RG2) and normally, guidelines recommend BSL2 practices and facilities for working with these agents.

Infected animals can excrete adenovirus, so cages and bedding are considered biohazardous for some period after viral vector administration.

Take precautions to avoid creating aerosols when emptying animal waste material.

The use of ventilation equipment such as a biosafety cabinet or changing station must be considered when emptying animal cages to minimize the creation of aerosols. Soiled cages should be disinfected prior to washing.

At the University of Cincinnati, animals must be housed at ABSL-2 housing for 72 hours post injection/exposure.
Exposure Risks

Transmission of adenoviruses can occur through inhalation of aerosolized droplets, mucous membrane contact, ingestion and accidental injection.

Respiratory Exposure - adenoviral vector manipulations should be conducted in a containment equipment such as a biological safety cabinet (BSC). When handling adenovirus outside a biosafety cabinet, a respirator (e.g. N95 mask) should be worn.

Mucosal Exposure - Laboratory personnel may also be exposed by direct contact of vector suspension (e.g. splash) with oral, ocular or nasal mucosa. Extreme care must be taken to avoid spilling and/or splashing infected materials, especially if working with high volumes (>10L).

Use aerosol containment devices when centrifuging. These include sealed canisters that fit in the centrifuge bucket, covers for the centrifuge bucket, heat sealed tubes, or sealed centrifuge rotors. Rotors should be removed and opened inside a BSC.

To determine proper fit, wearers must be fit tested to make sure they have selected the appropriate model and size. OSHA requires that every employee who wears a respirator receive an initial fit-test prior to using that respirator followed by annual tests.

The use of face shields provides protection against ocular, nasal and oral mucosa exposure. Also a combination of goggles and a respirator provides adequate protection (mucosal and respiratory).
**Adenovirus**

Ambikanandan Misra - Challenges in Delivery of Therapeutic Genomics and Proteomics Elsevier, Sep 10, 2010

http://en.wikipedia.org/wiki/Adenoviridae

https://microbewiki.kenyon.edu/index.php/Adenovirus_based_Gene_Therapy:_a_Promising_Novel_Cancer_Therapy

NIH Guidelines


Biosafety in Microbiological and Biomedical Laboratories (BMBL)

http://www.cdc.gov/biosafety/publications/bmbl5/

Genes in Cancer

http://atlasgeneticsoncology.org/Genes/Geneliste.html