

# GENE DELIVERY

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The ability to efficiently deliver genes to cells represents a powerful new therapeutic approach in treating ocular diseases. In addition, efficient gene delivery to cells in culture is an essential research tool. As a result, a module was established to assist with the construction of gene delivery vectors and prepare high titer stocks of these materials for use by researchers. The Core also will assist faculty in their efforts to use gene delivery methods.

Currently, four viral based vector systems are available including Adenovirus (AV), Adeno-Associated Virus (AAV), Herpes Simplex Virus (HSV), and Lentivirus (FIV and HIV based). The Gene Delivery module also has the capacity to produce high quality plasmid vectors. When plasmid DNA vectors are needed for clinical trials, the vector DNA's will be produced in the GMP facility.

## Consulting

Consultation is provided regarding the best type of vector system for each intended use. Consideration is given to the size of the expression construct, the level of expression desired, the length of time that expression is needed, potential use of tissue or cell-type specific promoters, the titers of vector needed, the potential for negative effects of the vectors (e.g. inflammation), and whether integration of the transgene is desirable.

## Vector construction

Module clients are assisted with construction of the desired vector or the Core will construct the vector for you. Some clients have expressed the desire to learn how to construct vectors themselves, or more importantly, to have a graduate student or post-doctoral fellow learn this valuable skill. In some instances, we will work side-by-side with these individuals to train them in the needed skills. The most efficient strategy usually is to construct the vector for the client.

## Primary culture preparation

The Core will provide assistance with the preparation of primary cultures of various ocular tissues. Many investigators have the need to test their vectors for expression and other properties in the specific cell types they are targeting *in vivo*. Therefore, there is a need to provide these cell types for such studies. This also is a quality control feature of the gene delivery module. To date, we have provided primary trabecular meshwork cells, primary human corneal keratocytes, and primary ciliary muscle cells for clients.

## High-titer vector stock preparation

- Adenovirus vector (AV) – AV is an intermediate sized non-enveloped DNA virus. AV vectors have received the greatest use to date, primarily because they were one of the earliest systems developed, they have been more readily available than other vectors, and they can be prepared at very high titers. AV vectors can efficiently deliver transgenes to the TM, Muller cells, and RPE so they are suitable for many projects. We use a commercial system, AdEasy, which allows production of transducing stocks that are free of helper virus.
- Adeno-associated virus vector (AAV) – AAV is a naturally replication deficient virus that normally requires Adenovirus for helper functions. With the discovery that AV E1, E2A, E4 and VA genes are the minimal required for the helper function, newer helper-AAV vector systems have become available, including one commercially available system by Stratagene which is used by the Core.
- Herpes simplex based vectors (HSV) – HSV is a large enveloped DNA virus that has a wide host range. HSV vectors have been predominantly used for neuronal gene delivery but Dr. Brandt has pioneered the use of HSV for ocular delivery. Two types of HSV vectors are available. The first is an attenuated whole virus system (hrR3 virus), which has the advantage of not requiring helper systems for packaging and can be prepared in titers as high as  $10^9$  to  $10^{10}$  transducing unit (TU) per ml. We have previously established that cells in the eyes of both rodents and non-human primates can be efficiently transduced with hrR3. Trabecular meshwork, ciliary body epithelial, and retinal ganglion cells are efficiently transduced, thus we have established the feasibility of the HSV vector for glaucoma gene therapy.

- We also have replication deficient HSV amplicon vectors, which are plasmids that carry the HSV origin of replication and packaging sequences but no other viral DNA. The advantages of this system include reduced toxicity, very large carrying capacity (up to 140 Kb), and the ability to rapidly construct the vectors. This system required helper functions for packaging the amplicons into viral particles.
- Lentiviral vector system (FIV and HIV) - The Vision Core has two available vector systems based on Lentiviral vectors. The first is a commercially available system based on Feline Immuno-deficiency Virus (FIV, Biosys, Inc.). The vector expresses the transgene as well as GFP to mark transduced cells. Separate promoters drive each gene. The second system is a self-inactivating Human Immuno-deficiency Virus (HIV) system. Separate control vectors expressing GFP or lac Z can be used as work cells for transduction. Both vectors are pseudotyped with the VSVG protein to expand the host range.