

## **Section 1**

### **Adenovirus and Adenoviral Vectors Information**

By checking this box you are stating that you have read and understand the facts that are listed in section I

#### **Description**

There are about 50 human serotypes as well as animal serotypes. They are medium sized (~90 nm), nonenveloped isohedral viruses containing double stranded DNA.

#### **Potential Health Hazards**

Adenovirus is a pathogen of respiratory and gastrointestinal mucosa as well as eye membranes. The virus does not need to be replication competent to cause corneal or conjunctival damage. It is very stable in the environment and is still infective after extraction with ether and/or chloroform.

The mode of transmission in research labs is by inhalation of aerosol droplets and mucous membrane contact, injection or ingestion. The incubation period is 1-10 days. The virus is communicable shortly prior to and for the duration of the disease.

#### **Basic Biology**

Transcription, replication and viral packaging take place in the nucleus of the infected cell. Transcription happens in two phases; early and late. Molecular biologists focus their manipulations on the early transcribed regions; E1, E2, E3, and E4. To ensure replication deficiency of the virus and to prevent cell lysis, first generation recombinant adenoviruses are E1 deleted. When packaged in a complementing cell line (ones that provide E1 e. g. HEK 293), viral replication will be enabled. Second and third generation vectors have additional deletions.

Types 5 and 2 are commonly used in research laboratories. Other human and animal types are in developmental stages.

#### **Some Advantages of using Recombinant Adenovirus**

- Can infect both replicating and non replicating mammalian cells
- Accommodate reasonably large transgenes (up to 7.5kb)
- Remains epichromosomal (does not integrate into the host chromosome so the vector won't inactivate genes or activate oncogenes)

#### **Some Disadvantages of using Recombinant Adenovirus**

- Replication deficient/incompetent vectors can be made competent by
  - Wild type adenovirus infecting a person or animal
  - Packaging cells lines or other cell lines in the lab with E1
- The Core Lab does not test for replication competency. (Section 15 – Administrative Controls – Biohazardous Materials Form) High titers would cause cell lysis and be detected by the Core.
- Laboratory acquired infections have been documented
- There is no specific vaccine or prophylaxis although cidofovir has shown promise in the treatment of ocular infections

#### **Transgenes and other foreign genetic elements**

May increase the risk associated with the vector

- Are any of the transgenes expressed toxins or oncogenes?
- Have foreign elements altered
  - Specificity
  - Host range
  - Stability
  - Titer of resulting vector
- What do you know about novel genes?

### **Control Measures**

- Biosafety Level 2 practices and containment facilities for all activities involving the virus, recombinant vectors and transfected material.
- Work with the virus or the vector is conducted in a biosafety cabinet.
- Centrifugation is done in a closed container using a sealed rotor or centrifuge safety buckets.
- Vacuum lines must be fitted with a HEPA filter if an aspiration flask is used to collect spent media.

### **Precautions with Animal Use (Rats and Mice only)**

Concurrent approvals are needed from the Biosafety Committee and the Animal Care and Use Committee.

Make advance arrangements with the Animal Facility Manager for housing and disposal of infected animals. Animals will be housed in filtered disposable microisolator cages. All non-disposable caging equipment may be wrapped and autoclaved before being brought into the wash room. Chemical disinfection may also be considered. Depending upon the route of introduction, infected animals have been shown to excrete adenovirus (especially the first 72 hours after infection).

### **Employee Exposures**

Rinse eyes for 15 minutes or wash skin with soap. Follow up with medical attention.

### **Disinfectants**

1-10% bleach with a minimum of 15 minutes contact time is preferred. Alcohols do not work.

During spill clean up, let aerosols settle for 30 minutes. Apply disinfectant to the outside of the spill and work in.

# Adenoviral Vector Core Facility Application

## Directions for Completing Application/Project Initiation

1.) Please type in the box to the right of the information requested. When entered, the text will wrap in the space provided. Please do not alter the formatting of the application. After completing the application, e-mail to [OUj@ioc.uh.edu](mailto:OUj@ioc.uh.edu). In addition PIs fax a copy of your IBC protocol approval to 6-7768.

2.) Once the completed application is received noting the desired shuttle vector, the AVCF will call the contact person to arrange for pick up of the shuttle vector at the Tupper facility.

3.) The Investigator will then clone the gene into the shuttle vector. The concentration of the shuttle vector containing the gene should be determined by A260. Please submit at least 20 ug of DNA with a purity of at least 1.8 (A260/A280) and a minimum concentration of 0.1 ug/ul.

4.) Please also submit a picture of an agarose gel of your sample with appropriate size and markers.

5.) It is the Investigator's responsibility to verify the construct by sequencing, PCR, restriction endonuclease analysis, etc. It is not the Core's responsibility if the vector is unsuccessful due to a faulty construct and the full charge will be incurred.

NOTE: Investigators who are using the AVCF for the amplification service only should complete sections II & III of this form and leave section IV blank.

## Section II General Information

Please provide all contact information, and be sure to check the box corresponding to the preferred method of contact

Date Application Submitted: \_\_\_\_\_

1.) Principal Investigator: \_\_\_\_\_ 4.) Phone: \_\_\_\_\_

2.) Campus Address: \_\_\_\_\_ 5.) E-Mail: \_\_\_\_\_

3.) Institution: \_\_\_\_\_ 6.) Fax: \_\_\_\_\_

7.) Contact Person: \_\_\_\_\_ 10.) Phone: \_\_\_\_\_

8.) Title: \_\_\_\_\_ 11.) E-mail: \_\_\_\_\_

9.) Campus Address: \_\_\_\_\_ 12.) Fax: \_\_\_\_\_

13.) Title of Grant: \_\_\_\_\_

14.) Grant Sponsor: \_\_\_\_\_

15.) NIH Grant Number (if applicable): \_\_\_\_\_

\*\* Needed for Core grant renewal purposes. (ex. R01 NHLBI 23456):

16.) Please provide a brief description of the project describing the gene (including size) to be expressed and a brief explanation of the work. Note whether transgenes and/or foreign genes express toxins **yes no** , oncogenes **yes no** , alter the specificity or host range of the vector?

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17a.) Approved animal protocol number: \_\_\_\_\_ 17b.) Date of protocol approval: \_\_\_\_\_

\*Note: Investigators using the Adenovirus for animal studies will receive a high titer virus (10<sup>12</sup> or 10x12 PFU/ml). A high titer adenovirus is very unstable at 4°C and the titer will decrease rapidly. Therefore, you need to aliquot it as soon as possible and store it at -80°C.

## Section III Billing Information

Please provide all contact information, and be sure to check the box corresponding to the preferred method of contact

- 1.) Billing Contact Person: \_\_\_\_\_ 4.) Phone: \_\_\_\_\_
- 2.) Title: \_\_\_\_\_ 5.) E-Mail: \_\_\_\_\_
- 3.) Campus Address: \_\_\_\_\_ 6.) Fax: \_\_\_\_\_
- 7.) Tufts Medical Cost Center: \_\_\_\_\_  
(Corp Code) (Acct. Unit) (Acct) (Activity) (Acct. Cat)
- 8.) Billing Reference Number: \_\_\_\_\_  
(Tufts PIs Only)
- 9.) By intialing here, I authorize the direct transfer of funds from the above noted cost center \_\_\_\_\_  
to the Adenovirus Core Facility for the above order placed by my laboratory.  
(Tufts Medical Center PIs only – Tufts PIs will receive an invoice.)

## Section IV Vector Information

\* Please choose one of the 4 shuttle vectors which will be provided to you by the Core. Note the restrictions on  
construct size and GFP tracer inclusion

\* Click [here](#) to view the protocol for the AdEasy System

- 1.) Name: \_\_\_\_\_  
Shuttle Vector: \_\_\_\_\_  
Gene Construct: \_\_\_\_\_ Construc Size: \_\_\_\_\_  
By what method have you tested this construct?: \_\_\_\_\_  
(Sequencing, PCR, restriction endonucleasae analysis, ect.)
- 2.) Name: \_\_\_\_\_  
Shuttle Vector: \_\_\_\_\_  
Gene Construct: \_\_\_\_\_ Construc Size: \_\_\_\_\_  
By what method have you tested this construct?: \_\_\_\_\_  
(Sequencing, PCR, restriction endonucleasae analysis, ect.)
- 3.) Name: \_\_\_\_\_  
Shuttle Vector: \_\_\_\_\_  
Gene Construct: \_\_\_\_\_ Construc Size: \_\_\_\_\_  
By what method have you tested this construct?: \_\_\_\_\_  
(Sequencing, PCR, restriction endonucleasae analysis, ect.)