



Policy: Use of Viral Vectors

Introduction

Viral vectors are a fundamental tool for biological research. The National Institutes of Health (NIH) have published guidelines for the use of recombinant and synthetic nucleic acid molecules in research. Montana State University (MSU) aims to provide researchers with information to safely work with viral vectors. Each vector system is listed with a suggested biological safety containment level. A higher-containment level may be required in specific cases. The biological safety containment level is ultimately determined by the Institutional Biosafety Committee (IBC) and/or Biological Safety Officer (BSO).

Scope

This policy applies to Principal Investigators (PI), staff, students and animal care staff who perform animal research involving viral vectors.

References

- [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules \(NIH Guidelines\)](#)
- [Novel and Exceptional Technology and Research Advisory Committee](#)
- [MSU Biosafety Program](#)

MSU Institutional Biosafety Committee Policy

All work involving Recombinant or Synthetic Nucleic Acid Molecules and/or viral vectors must be approved by the MSU IBC. Because the University receives funding from NIH grants for research involving recombinant/synthetic nucleic acids, all research conducted at the University must comply with the NIH Guidelines and University policies.

The Biosafety Level (BSL) of a viral vector defaults to the Risk Group (RG) of the wild type viral strain from which the vector and/or genes of interest are derived, whichever is higher RG. The Biosafety Level for replication incompetent viral vectors must be followed during preparation, during use in cell culture, and for the first 72 hours after inoculation into animals.

See specific viral vector information below.

General Biosafety for All Viral Vectors

Assume all viral vectors are infectious. Wear all required PPE (e.g., disposable gloves, lab coat, and safety glasses).

Adenovirus Vectors

General Description

Adenoviruses are linear, non-enveloped, icosahedral, double-stranded DNA viruses of approximately 36kb with a lytic infection cycle. There are 57 immunologically distinct types of adenoviruses that can cause human infection. Recombinant adenoviruses used for biomedical research are primarily based on Adenovirus 5.

First generation adenoviral vectors result from the deletion of the E1 region. The 1st generation adenoviral vectors can be packaged using HEK293 or PER.C6 cell lines which stably express the viral E1A and E1B proteins. Second generation adenoviral vectors additional deletions of E2 and/or E4 function. Deletion of the adenoviral E3 gene accommodates increased transgene packaging (>8 Kb). Adenoviral transgenes reside episomally in the host nucleus. This mitigates the risk of insertional mutagenesis but results in transient transgene expression.

Potential Health Hazards

Exposure to adenovirus can cause a wide range of symptoms including bronchitis (coughing, shortness of breath), sore throat, fever, diarrhea, and pink eye. Infections can be more severe in very young and immunocompromised individuals. NOTE: Adenoviral vectors do not have to be replication competent to cause corneal and conjunctival damage.

Biosafety Containment

- BSL-2/ABSL2 for all activities with materials and cultures known or reasonably expected to contain adenoviral vectors and for activities for experimentally infecting animals.
- Adenovirus must be administered to animals at ABSL-2. After infection, animals must be housed at ABSL-2 for 72 hours. After 72 hours animals may be moved to ABSL-1, however the IBC may raise containment if deemed necessary.

Decontamination

- Animal cages/bedding from the first cage change and any additional cage changes taking place in the first 72 hours post vector administration must be disposed of as biohazardous waste.
- Adenovirus is susceptible to:
 - 0.5% sodium hypochlorite (1:10 house hold bleach:water), 2% glutaraldehyde, 5% phenol; accelerated hydrogen peroxide (e.g., Rescue)
 - Autoclave for 60 minutes at 121°C
- Adenovirus is not susceptible to:
 - Alcohol disinfectants

Additional Information

- [MSU Adenovirus PSDS](#)

Adeno-Associated Virus (AAV) Vectors

General Description

Adeno-associated viruses (AAV) are small, non-enveloped viruses with single-stranded linear DNA. They are members of the Parvoviridae family and there are 12 serotypes. AAV get their name because they are found in cells that are simultaneously infected with adenovirus. AAV is dependent on the presence of a helper virus to replicate, generally an adenovirus or herpes virus. In the absence of a helper virus, AAV will stably integrate into the host genome. Of the most commonly used viral vectors in research, AAV are some of the smallest (<4.8 Kb) with small packaging capacities. AAV vectors are non-pathogenic and can infect dividing and nondividing (quiescent) cells making them preferred viral vectors for many applications.

Potential Health Hazards

There are no known health hazards for wild type AAV. Insertional mutagenesis is theoretically possible due to wild type AAV ability to integrated into the human genome.

Biosafety Containment

- BSL-2 for construction/packaging of AAV in human cell lines.
- BSL-2/ABSL2 for all activities with materials and cultures if in the presence of a helper virus, or if inserted genes code for an oncogene or toxin.
- AAV vectors may be administered to animals at ABSL-1 if no oncogene, toxin, or helper virus is present. The IBC may raise containment if deemed necessary.

Decontamination

- Animal cages/bedding from the first cage change and any additional cage changes taking place in the first 72 hours post vector administration must be disposed of as biohazardous waste.
- AAV is susceptible to:
 - 0.5% sodium hypochlorite (1:10 house hold bleach:water), 2% glutaraldehyde, 5% phenol
 - Autoclave for 60 minutes at 121°C
- AAV is not susceptible to:
 - Alcohol disinfectants

Lentivirus Vectors

General Description

Lentiviruses are enveloped retroviruses that are characterized by a long incubation period, immune evasion, and persistent infections in their natural hosts. They have single stranded and linear RNA that is reverse transcribed to produce DNA upon entry into the host cell. This DNA transcript then integrates into the host's genome. Lentiviruses have the ability to integrate into the genome of non-dividing cells, a feature that distinguishes lentiviruses from other retroviruses. Lentiviral vector systems can include viruses of non-human/non-primate origin (feline immunodeficiency virus, equine infectious anemia virus) as well as simian viruses (simian immunodeficiency virus) and human immunodeficiency viruses (HIV).

There are three generations of lentiviral vectors. Third-generation systems are currently the safest to use because the virus production is split across four plasmids.

Potential Health Hazards

Lentiviruses are transmitted via direct exposure to infected bodily fluids, sexual contact, and sharing unclean needles. Lentiviruses persist lifelong – being both a function of their ability to integrate into the host chromosome and ability to evade host immunity. Lentiviruses replicate, mutate, and undergo selection by host immune responses. The clinical manifestations of infection include non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue.

The use of lentiviruses also presents the risk of insertional mutagenesis, potentially leading to cancer. The nature of the transgene may pose additional risk.

Biosafety Containment

- BSL-2
- Lentivirus must be administered to animals under ABSL-2 conditions. Animals must be housed under ABSL-2 conditions for 72 hours after lentivirus administration, after which animals may be moved to ABSL-1 housing. The IBC may raise containment if deemed necessary

Decontamination

- Animal cages/bedding from the first cage change and any additional cage changes taking place in the first 72 hours post vector administration must be disposed of as biohazardous waste.
- Lentivirus is susceptible to:
 - 0.5% sodium hypochlorite (1:10 house hold bleach:water), 2% glutaraldehyde, formaldehyde, 70% ethanol
 - Autoclave for 60 minutes at 121°C

Additional Information

- [NIH Biosafety Considerations for Research with Lentiviral Vectors](#)
- [Addgene Lentiviral Guide](#)