**WHAT IS A VIRAL VECTOR?**

Viral vectors work like a “nanosyringe” to deliver nucleic acid to a target. They are often more efficient than other transfection methods, are useful for whole organism studies, have a relatively low toxicity, and are a likely route for human gene transfer.

All viral vectors require a host for replication. The production of a viral vector is typically separated from the ability of the viral vector to cause disease. While viral vectors are not typically considered infectious agents, they do maintain their ability to “infect” cells. Viral vectors just don’t replicate (although there are some replicating viral vectors in use) under experimental conditions. An HIV-based lentiviral vector no longer possesses the ability to establish disease in an inoculated individual, but it does maintain the ability to enter a cell and express genetic information. This is why viral vectors are useful, but also require caution. If a viral vector can transfect a human cell line on a plate, it can also transfect YOUR cells if accidentally exposed.

**SAFETY CONSIDERATIONS FOR ALL VIRAL VECTORS**

1. **What potential does your method of viral vector production have to generate a replication competent virus?**
   
   a. The generation system of a viral vector refers to the number of recombination events required to form a replication competent virus. For example, if you’re using a lentiviral vector that is split up between 4 plasmids (gag/pol, VSV-g, rev, transgene), 3 recombination events must take place to create a replication competent virus; therefore, this is a 3rd generation lentiviral production system.

2. **What specific hazard(s) does the transgene pose?**
   
   a. If your transgene is GFP, the hazard is relatively low. If your transgene is KRAS (an oncogene), the hazard is much greater.

3. **What is the tropism of the virus?**
   
   a. Ecotropic viral vectors have a narrow host range and can only infect mouse or rat cells. Amphotropic viral vectors have a wide host range and are capable of infecting numerous species or cell lines. Pantropic (VSV-G pseudotyped) virus can infect all mammalian cells.
ADENO-ASSOCIATED VIRUS

GENERAL DESCRIPTION

AAV are parvoviridae, icosahedral, single-stranded DNA viruses with a protein capsid. At only 22nm in diameter, it is one of the smallest viruses known. Adeno-Associated Virus (AAV) is most often found in cells that are simultaneously infected with adenovirus. Wild typed adenovirus or herpesvirus must be present for AAV to replicate. If these helper viruses are not present, AAV will stably integrate into the host cell genome. Co-infection with helper virus triggers a lytic infection cycle.

There are at least 11 natural serotypes of AAV. These serotypes differ in their tropism, or the types of cells they infect, making AAV a very useful system for preferentially transducing specific cell types.

Recombinant AAV vectors are typically packaged through plasmid co-transfection of HEK293 cells (which inherently express adenovirus E1):

1) Transgene flanked by inverted terminal repeats
2) AAV Rep/Cap proteins*
3) Helper plasmid encoding adenoviral E4, E2a and VA*

* The AAV Rep/Cap and adenoviral helper factors can be combined in a single plasmid vector.

Alternately, AAV vectors can be produced through adenovirus or herpesvirus co-infection. Large-scale packaging of AAV often utilize a recombinant herpes simplex virus platform.

POTENTIAL HEALTH HAZARDS

- There are no known health hazards associated with AAV.
- Contrary to wild-type AAV which readily integrate into the AAVS1 site on human chromosome 19, recombinant AAV which lack the Rep protein maintained extrachromosomally. As such, recombinant AAV have a very low likelihood of non-homologous integration.
- The low immunogenicity of AAV leads to long-term gene expression, the effects of which are not entirely understood.

LABORATORY HAZARDS

- Inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion.
- No specific treatment for infection with AAV.

BIOSAFETY CONTAINMENT

- Construction of AAV with helper virus should be performed at BSL2 within BSC.
- Once constructed, AAV may be manipulated at BSL1.
- PPE required for manipulation at BSL1 or BSL2: eye protection, lab coat, disposable gloves.

ANIMAL BIOSAFETY CONTAINMENT

- Animal procedures may be conducted using ABSL1 practices and containment.
- Animal housing must be maintained at ABSL-1.
- ABSL-2 containment is required if helper virus is present.

DISINFECTION

Adapted from the UK Biological Safety Department
• Susceptible to:
  o 0.5% Sodium hypochlorite
  o 2% Glutaraldehyde
  o 5% Phenol
  o Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure.
• Freshly prepared 10% household bleach recommended.
• Alcohol is **NOT** an effective disinfectant against AAV.

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**Adenovirus**

**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 adenovirus that can cause human infection. Recombinant adenoviruses used for biomedical research are primarily based on Adenovirus 5.

First generation adenoviral vectors results 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).

Contrary to integrative viral vectors, adenoviral transgenes reside episomally in the host nucleus. This mitigates the risk of insertional mutagenesis, but results in transient transgene expression.

**POSSIBLE HEALTH HAZARDS**

Adenovirus is a pathogen of respiratory, gastrointestinal mucosa, and mucous membranes. Symptoms of respiratory illness resulting from adenovirus infection can range from the common cold to pneumonia, croup, and bronchitis. Additional clinical symptoms include conjunctivitis (pink eye), cystitis, gastroenteritis (stomach flu), tonsillitis, rash-associated illness, and rare cases of severe disease (especially in immune compromised individuals). Adenoviral vectors **DO NOT** have to be replication competent to cause corneal and conjunctival damage.

**LABORATORY HAZARDS**

• Routes of exposure include inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion.

• Adenovirus is unusually stable in the environment. Adenovirus can still be infective after having been extracted with ether and/or chloroform.

• First generation systems may recover replication competent virus.

**BIOSAFETY CONTAINMENT**

• BSL-2
• NO open bench work.
• Biological Safety Cabinet (BSC) required.
• Eye protection, disposable gloves, laboratory coat required.
• When centrifuging adenovirus, rotors/buckets must be loaded/unloaded in the BSC and wiped down with appropriate disinfectant.
• Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.
• Adenoviral vector must be administered under ABSL-1+ for injections (the ‘+’ requiring the use of safe sharps, mucous membrane protection, and wiping of the injection site), but may be enhanced, depending upon the transgene.

• Animals may shed/excrete adenovirus for some time post-administration.

• Animals must be housed at ABSL-1 containment, but may be enhanced, depending upon the transgene.

DISINFECTION

• Susceptible to: 0.5% Sodium hypochlorite, 2% Glutaraldehyde, 5% Phenol, or Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure.

• Freshly prepared 10% household bleach recommended.

• Alcohol is **NOT** an effective disinfectant against adenovirus.

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**Baculovirus**

GENERAL DESCRIPTION

Baculoviruses are lytic DNA viruses that are primarily pathogenic for insects. The nucleocapsids of Baculoviruses are rod-shaped and enveloped, with circular genomes of double-stranded DNA, ranging in size from 80-180kbp.

Baculoviruses produce two distinct types of virions: occlusion-derived virions (ODV), embedded in large protein crystals called occlusion bodies, and budded virions (BV). ODV are responsible for horizontal transmission between insects, whereas BV help spread infection from cell to cell.

There have been more than 500 baculovirus isolates identified based on hosts of origin. Apart from their utility as gene expression vectors, they are also useful as biological pesticides. The two most common isolates used for gene expression are *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) and *Bombyx mori* (silkworm) nuclear polyhedrosis virus (BmNPV).

POTENTIAL HEALTH HAZARDS

Wild-type baculoviruses are typically not capable of replicating in vertebrate cells, and therefore do not pose much risk to laboratory personnel. Baculoviruses for gene expression which utilize polyhedrin or p10 promoters will only transfect insect cells. Baculoviruses that have been engineered with mammalian specific promoters do achieve expression of foreign genes in mammalian cell lines and primary cell cultures.

LABORATORY HAZARDS

• Budded virions (BV) are not infectious to insect hosts, minimizing potential spread to the environment.

• Baculovirus is very sensitive to human complement. Should exposure occur, rapid inactivation of the virus is expected.

• Pseudotyping with VSV-G may confer increased resistance to complement inactivation.

BIOSAFETY CONTAINMENT

• Baculoviruses with insect specific promoters (i.e. polyhedrin or p10) may be handled at BSL1.

• Baculoviruses with mammalian specific promoters must be handled at BSL-1.

• PPE required for manipulation at BSL1 or BSL2: eye protection, lab coat, disposable gloves.
ANIMAL BIOSAFETY CONTAINMENT

- Baculovirus with mammalian specific promoters may be administered using ABSL-1 practices and containment.
- Animals may be housed at ABSL1 containment.

DISINFECTION

- Susceptible to:
  - 70% Ethanol
  - 0.5% Sodium hypochlorite
  - Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach is recommended.

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**Epstein-Barr Virus**

GENERAL DESCRIPTION

Epstein-Barr virus (EBV) is a ubiquitous B-lymphotrophic herpesvirus. EBV causes the common childhood disease mononucleosis. It is an icosahedral, lipid enveloped, double-stranded DNA virus sized 120-150 nm in diameter. EBV has been found in the tumor cells of a heterogeneous group of malignancies (i.e. Burkitt's lymphoma, lymphomas associated with immunosuppression, other non-Hodgkin's lymphomas, Hodgkin's disease, nasopharyngeal carcinoma, gastric adenocarcinoma, lymphoepithelioma-like carcinomas, and immunodeficiency-related leiomyosarcoma). 80-90% of adults worldwide are infected with EBV.

POTENTIAL HEALTH HAZARDS

- Infectious mononucleosis – acute viral syndrome with fever, sore throat, splenomegaly and lymphadenopathy; lasting one to several weeks; rarely fatal.
- Burkitt's lymphoma – monoclonal tumors of B cells; typically involving children; jaw involvement also common; hyperdemic in highly malarial areas.
- Nasopharyngeal carcinoma – malignant tumor of epithelial cells of the nasopharynx; usually involving adults between 20 and 40 years of age.

LABORATORY HAZARDS

- Inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion.
- Cell lines are often immortalized by transformation with EBV. EBV is classified as a group 1 carcinogen by the International Agency for Research on Cancer

BIOSAFETY CONTAINMENT

- BSL-2.
- NO open-bench work.
- Eye protection, disposable gloves, laboratory coat required in addition to BSC.
- When centrifuging EBV, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC.
- Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.
**ANIMAL BIOSAFETY CONTAINMENT**

- EBV vector must be administered using ABSL-2 practices and containment.
- Animals must be housed at ABSL-2.

**DISINFECTION**

- EBV is susceptible to
  - 0.5% Sodium hypochlorite
  - 70% Ethanol
  - Glutaraldehyde
  - Formaldehyde
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

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**Herpes Virus**

**GENERAL DESCRIPTION**

Herpes Simplex Virus (Types I and II) are icosahedral, lipid enveloped, double-stranded linear DNA viruses approximately 110-200nm in diameter. HSV types I and II can be differentiated immunologically. HSV-I is herpes gingivostomatitis; whereas HSV-II is herpes genitalis, or genital herpes. HSV-derived vectors are unique in that the vectors have a wide host range and cell tropism in dividing and non-dividing cells, and are able to infect almost every cell type in most vertebrates. HSV has a dual life cycle – a lytic growth cycle in epithelial cells and latent infection of neuronal cells. This latency in neuronal cells leads to persistent, long-term expression.

**POTENTIAL HEALTH HAZARDS**

- Oral herpes – primary infection is typically mild and occurs in early childhood; reactivation of latent infection results in fever blisters or cold sores, usually on the face and lips, which crust and heal within a few days; possible CNS involvement (meningoencephalitis), 70% mortality rate if left untreated; causes approximately 2% of acute pharyngotonsillitis.
- Genital herpes – sexually transmitted, associated with aseptic meningitis; vaginal delivery may pose risk to newborn (encephalitis and death).
- Both HSV-I and HSV-II are capable of infecting the genital tract or oral mucosa.
- Latency and activation from latency are not well understood.

**LABORATORY HAZARDS**

- Inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion.
- Only treatment available is anti-viral drug therapy for symptoms.

**BIOSAFETY CONTAINMENT**

- BSL-2.
- NO open-bench work.
- Eye protection, disposable gloves, laboratory coat required in addition to BSC.
When centrifuging HSV, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC.

Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.

ANIMAL BIOSAFETY CONTAINMENT

- HSV must be administered using ABSL-2 practices and containment.
- Animals must be housed at ABSL-2.

DISINFECTION

- HSV is susceptible to:
  - 0.5% Sodium hypochlorite
  - 70% Ethanol
  - Glutaraldehyde
  - Formaldehyde
  - Iodine solutions containing ethanol

- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

Lentivirus

GENERAL DESCRIPTION

The genus of the family Retroviridae consists of non-oncogenic retroviruses that produce multi-organ diseases characterized by long incubation periods and persistent infection. There are five (5) serotypes recognized, based upon the mammalian hosts with which they are associated: Bovine, Equine, Feline, Ovine/Caprine, and Primate.

Most lentiviral vectors in use today are HIV-derived vectors. The cis- and trans- acting factors of the lentiviruses are often on separate plasmid vectors, with packaging being provided in trans. The vector constructs contain the viral cis elements, packaging sequences, the Rev response element (RRE), and a transgene. Lentiviral vectors can transfect dividing and non-dividing cells. Replacing the HIV envelope glycoprotein with VSV-G allows a broad host-range for the vectors, allows the viral particles to be concentrated via centrifugation, and alters the mode of transmission.

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 present the risk of insertional mutagenesis, potentially leading to cancer. The nature of the transgene may pose additional risk.

LABORATORY HAZARDS

- Direct contact with skin and mucous membranes, parenteral injection, or ingestion.
- Exposures associated with a hazardous transgene (i.e. an oncogene or toxin), should consider use of an antiretroviral agent (reverse transcriptase and integrase inhibitors, not protease inhibitors).

BIOSAFETY CONTAINMENT

- BSL-2+.
- NO open-bench work.
- Biological Safety Cabinet (BSC) required.
- Eye protection, disposable gloves, laboratory coat required in addition to BSC.
- When centrifuging Lentivirus, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC.
- Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.

### ANIMAL BIOSAFETY CONTAINMENT

- Lentivirus must be administered using ABSL-2+ (the ‘+’ requiring the use of safe sharps, mucous membrane protection, and wiping of the injection site) practices and containment.
- Animals must be housed at ABSL-2.
- If a 3rd generation system is used for packaging or negative RCV data is provided, animal procedures and housing may be reduced to ABSL-1+ and ABSL-1, respectively.

### DISINFECTION

- Lentivirus is susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

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**Poxviruses**

### GENERAL DESCRIPTION

The Poxviridae family is divided into two subfamilies: **Chordopoxviridae**, with a vertebrate host range, and **Entomopoxviridae**, with an insect host range. **Chordopoxviridae** is further broken down into eight genera: **Avipoxvirus**, **Capripoxvirus**, **Leporipoxvirus**, **Molluscipoxvirus**, **Orthopoxvirus**, **Parapoxvirus**, **Suipoxvirus**, and **Yatapoxvirus**.

Poxviruses are enveloped, with a double-stranded DNA genome with hairpin loops at each end and a lytic infection cycle. Poxviruses do not integrate into the hosts' genome because they remain in the cytoplasm and utilize virally encoded polymerases to carry out replication and transcription. Members of the **Orthopoxvirus** genus have both narrow and broad host range. **Variola**, the agent of smallpox, only infects humans. The absence of other host species has made the eradication of smallpox possible. On the other hand, Vaccinia virus has a very broad host range. Vaccinia is used as a live vaccine for protection against smallpox. Vaccinia's large genome (approximately 190kb) allows for the stable insertion of DNA as large as 25kb.

### POTENTIAL HEALTH HAZARDS

Unlike other viral vectors utilized, vaccinia is a replication competent vector. Vaccinia virus presents varying levels of health risk to laboratory personnel, depending on the strain utilized. Highly attenuated strains are typically unable to replicate or replicate poorly in human cells. Non-highly attenuated strains can replicate in human cells and pose a health risk. The classical symptom of poxvirus infection is a vesicular or pustular lesion on the skin at the inoculation site. Vaccinia can cause severe disease in people with active skin disorders (i.e. eczema, psoriasis), pregnant women, and immune compromised individuals.

### LABORATORY HAZARDS

- Ingestion, parenteral inoculation, droplet or aerosol exposure to mucous membranes or exposure to broken skin.
- Vaccinia and other poxviruses are stable at ambient temperatures when dried and can remain infectious for long periods of time.

### BIOSAFETY CONTAINMENT

- BSL2 containment and practices are required, unless otherwise stated in the IBC approval letter.

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Adapted from the UK Biological Safety Department
• PPE Required for BSL2 work: eye protection, disposable gloves, and laboratory coat in addition to BSC.

ANIMAL BIOSAFETY CONTAINMENT

• Poxviruses must be administered using ABSL-2 practices and containment, unless otherwise stated in the IBC approval.
• Animals must be housed at ABSL-2, unless otherwise stated in the IBC approval.

DISINFECTION

• Poxviruses are susceptible to:
  o 0.5% Sodium hypochlorite o Formaldehyde
  o 70% Ethanol o Iodine solutions containing Ethanol.
  o Glutaraldehyde
• Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

Rabies virus

GENERAL DESCRIPTION

Rabies virus is a member of Rhabdoviridae family. These are bullet shaped enveloped, single-stranded, negative-sense RNA viruses. The viruses have affinity for neurons and spread within and between neurons in a retrograde fashion. Because of this unique property, the recombinant vectors are often used to track neuronal circuits.

Rabies glycoprotein (G) is essential for its packaging and trans-synaptic spread. The viral genome encodes for 5 proteins. Initially, the viral coat is removed in the cytoplasm of infected cells. Individual viral proteins are transcribed with the help of viral RNA-dependent RNA polymerase. Replication produces a positive strand RNA which, in turn makes the negative strand RNA. The complete life cycle occurs in the cytoplasm.

Use of recombinant rabies virus with deleted G-glycoprotein (RVΔG) limits its trans-synaptic neuronal spread. RVΔG can be pseudotyped with VSV-G (Vesicular Stomatitis Virus Glycoprotein), EnvA and EnvB from avian sarcoma leucosis virus or HIV to incorporate non-native envelope proteins and change its tropism. The virus can be labelled with reporter genes (e.g. GFP, mCherry etc.) to allow tracking of the virus in infected cells.

POTENTIAL HEALTH HAZARDS

The most likely sources for exposure of laboratory and animal care personnel are accidental parenteral inoculation, cuts, or needle sticks with contaminated laboratory equipment, bites by infected animals, and exposure of mucous membranes or broken skin to infectious tissue or fluids.

Potential inhalation exposure when working with high concentrations of infectious aerosols.

Rabies viral vectors are non-integrative, presenting little risk of insertional mutagenesis.

LABORATORY HAZARDS

• Direct contact with skin and mucous membranes, parenteral injection.

BIOSAFETY CONTAINMENT

• BSL-2 or BSL-1, depending upon the viral vector tropism. The final decision will be present in the approval letter from the IBC.
• Eye protection, disposable gloves, laboratory coat required in addition to BSC.
- When centrifuging Rabies viral vectors, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC.
- Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.

**ANIMAL BIOSAFETY CONTAINMENT**

- Administration may be done using ABSL-2 or ABSL-1 containment, dependent on the viral vector tropism.
- Animals must be housed at ABSL-1 or 2.

**DISINFECTION**

- Rabies viruses are susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended

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**Murine Leukemia Virus**

**GENERAL DESCRIPTION**

Murine Leukemia Virus (MLV) is an enveloped, icosahedral, single-stranded virus with a linear RNA genome, approximately 100nm in diameter. MLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection.

The host range of MLV is dependent on the specificity of the viral envelope. The ecotropic env gene produces particles that infect only rodent cells. Amphotropic env gene allows infection of both murine and non-murine cells, including human. VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells.

**POTENTIAL HEALTH HAZARDS**

Recent data suggests a pathogenic mechanism by which chronic productive retroviral infection allows insertional mutagenesis leading to cell transformation and tumor formation. The nature of the transgene or additional introduced genetic element(s) may pose additional risk. The provirus integrates randomly into the genome which can lead to inactivation of genes for protein expression. The 5’ and 3’LTRs have promoter functions that can deregulate the expression of genes.

**LABORATORY HAZARDS**

- In mice, virus is transmitted via blood from infected mother to offspring; may also occur via germline infection.
- In vivo infection in humans appear to require direct parenteral injection with amphotropic or pseudotyped MLV.
- Exposures associated with a hazardous transgene (i.e. an oncogene or toxin) should consider the use of an antiretroviral agent (reverse transcriptase and integrase inhibitors, not protease inhibitors).

**BIOSAFETY CONTAINMENT**

- BSL1 containment for ecotropic MLV.
- PPE required for BSL1 work: eye protection, lab coat, disposable gloves.
- BSL2 containment for amphotropic or pseudotyped MLV.
- Biological Safety Cabinet (BSC) required.
- Eye protection, disposable gloves, laboratory coat required in addition to BSC.
- When centrifuging MLV, rotors/buckets must be loaded/unloaded within BSC and wiped down with appropriate disinfectant prior to being removed from BSC.
- Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.

Adapted from the UK Biological Safety Department
### ANIMAL BIOSAFETY CONTAINMENT

- Animals administered ecotropic MLV may be housed under ABSL1 conditions.
- Administration of amphotropic/pseudotyped MLV must done using ABSL2 containment and practices. Subsequent housing may be at ABSL1.

### DISINFECTION

- MLV is susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.
REPLICATION COMPETENCY TESTING

Most viral vectors used today are disabled such that replication competent viruses are not readily formed by any biological process that might occur in normal hosts. The Department of Biosafety encourages the use of such vectors in all relevant applications. In particularly sensitive applications, demonstrating that the viral stock used has no apparent contamination with replication competent vectors is essential. The issue is not whether replication competent virus (RCV) is present, but how much replication competent virus is present. Of course, assays for replication competence will never be perfect or absolute, so the Institutional Biosafety Committee (IBC) asks that one use a current procedure of demonstrated sensitivity and specificity.

In general, the IBC will require the use of such an assay whenever viruses or virus-infected cells are used in whole animals. Even more rigorous testing may be required in some instances, such as a vector bearing a pathogenic gene, in human gene transfer, or in any materials that could be released to the environment. Replication competency testing is not generally required if experiments are conducted entirely in tissue culture.

CONSIDERATIONS FOR AN APPROPRIATE RCV TEST...

1. The test is an experiment
   - Any experiment should have adequate controls. A positive control is necessary to demonstrate that you would have detected replication competent virus if it were present.

2. The test must be quantitative
   - What is the level of detection for replication competent virus? Typically, this requires running a dilution curve. This should also be reported with the results of your test.

3. What is an appropriate cut-off for declaring it is safe to use this virus in animals?

SETTING AN APPROPRIATE CUT-OFF...

1. FDA requirements for human use of recombinant adenovirus are less than 1 replication competent virus per 10^13 viruses
   - This is a very strict requirement because the quantity of virus used is high, containment is very difficult, and accidental release potentially disastrous

2. When working with small, easily sequestered animals (i.e. rats, mice), the cut-off can be considerably less
   - One replication competent virus per 10^6 viruses is common, but is this appropriate?
   - How many virus particles are being introduced into an animal?
   - Best estimate for number of replication competent virus in that bolus?
   - How many animals are likely to receive replication competent virus?
   - What is an acceptable risk?

CONSIDERATIONS WHEN DESIGNING THE TEST...

1. What does a recombinant virus need to regain replication competency?
   - Adenovirus needs E1
   - Lentivirus needs gag, pol, env

2. Where can recombinant virus pick up the sequences needed to regain replication competency?
   - Adenovirus has easy access to E1 in HEK293 cells
   - Retroviruses may pick up “assets” from endogenous retroviruses

3. How does a replication competent virus present itself?
### METHODOLOGICAL APPROACHES

- **Plaque Assays** (for lytic viruses, i.e. adenovirus)*
  - Must screen more viruses than the cut-off limit
    - If <1 in $10^6$ is the cut-off, must screen >$10^6$ viruses
- **ELISA** for production of viral protein essential for replication
  - p24 assay for HIV (lentivirus)
  - Sensitivity poor. Attempt amplification in a competent host
- **Quantitative PCR** for an essential viral gene
  - Very sensitive
  - Problem with background

### THINGS TO REMEMBER...

- Confirmation of absence of RCV must be documented by researcher *prior* to use of animals
  - Documentation of methodology and results must be made available to Department of Biosafety staff on request
- Procedure must be of demonstrated sensitivity and specificity
- Must use a positive control

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<td>Test for RCV by PCR for E1a</td>
<td>Dion DL, Fang J, Garver RI. 1996 Supematant rescue assay vs. polymerase chain reaction for detection of wild type adenovirus-contaminating recombinant adenovirus stocks, J Virol Methods 56:99-107</td>
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