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Lentiviral Vectors

Background

Lentiviruses are enveloped viruses in the family Retroviridae, composed of a nucleocapsid containing two copies of single-stranded, positive-sense RNA. Lentiviral vectors can deliver significant amounts of genetic information into the genome of the host cell. Lentiviruses have the ability to integrate DNA into the host genome of dividing and non-dividing cells, allowing for the viral DNA to be passed on to progeny cells after cell division. While viral genomic integration is essential to obtain a stable expression of the gene of interest, it may potentially contribute to insertional mutagenesis. The major safety concerns in using lentiviral vectors is the potential for generation of replication competent virus (RCV) and for the genome insertion to be oncogenic. Transmission is primarily spread through direct contact of the virus with mucous membranes and broken skin. Percutaneous exposure (i.e. needle stick) is also a common route of transmission.

The genome of lentiviruses contain essential genes (gag, pol, env, tat, rev) and accessory genes (vif, vpr, vpu, nef). Lentiviral vector systems have been refined over time through different "generations" to improve their safety and performance. One major feature for improved safety is separation of essential genes onto different plasmids so that multiple recombination events would be required to create RCVs. First generation lentiviral vectors are comprised of three plasmids, the first plasmid being the transfer vector with the gene of interest, the second comprising of all of the essential genes except the env, and the third containing the env. Second generation vectors are comprised of the same number of plasmids, with the difference that the accessory genes are completely removed. Third generation vectors eliminate the transactivator gene (*tat*) and split the vector into 4 total plasmids to further reduce the recombination potential, and retaining only three genes required for transgene expression. Third generations also have a self-inactivating (SIN) mutation that significantly reduces the level of expression from the viral promoter after integration, further reducing the risk of generating RCVs. Any viral stock that is first or second generation lentivirus must be tested for RCV.

Some lentiviral vectors can be pseudotyped with a murine envelope to allow infection of murine cells. This would decreased exposure risks as these viral vectors would have decreased chances of infected human cells. Other lentiviral vectors can be pseudotyped using the different envelope proteins, such as Vesicular Stomatitis Virus (VSV) G protein. This increases the tissue tropism as the VSV-G protein binds to a broad range of cell types and species. This also increases risk of infection following an accidental exposure as the virus will be able to infect any cell at the site of inoculation. Pseudotyping lentivirus also increases the stability of the viral particles to enable concentration of virus via ultracentrifugation.

Symptoms of Exposure

Acute infection with Lentivirus can cause "flu-like" symptoms including fever, nausea, vomiting, and myalgia. Following an accidental exposure, a lentiviral vector could potentially infect the lab worker. This could result in permanent transgene expression in the worker as well as result in activation (or inhibition) of host genes due to insertional mutagenesis. Activation of oncogenes or inactivation of tumor suppressor genes could lead to the development of cancer.

Environmental Stability

Enveloped viruses are rapidly inactivated when exposed to drying environments. However, it remains prudent practice in BSL-2 to disinfect with a broad-spectrum disinfectant, such as sodium hypochlorite (i.e. 1:10 bleach solution). This solution must be made daily.

Modes of Transmission

Lentiviruses and lentiviral vectors are transmissible through injection, ingestion, exposure to broken skin or contact with mucous membranes of the eyes, nose and mouth.

Host Range

Lentiviral vector systems are based on the genome of HIV, but many are pseudotyped to have a broad range of cell tropism for infection. Lentiviruses can infect dividing and non-dividing cells like neurons, macrophages, hematopoietic cells, muscle and liver cells.

Approvals

Experiments using any lentiviral vector, regardless of generation, require IBC approval before initiation of experiments. First and Second generation lentiviral systems require RCV testing.

Test Methods for Replication Competent Virus

**If vectors are being obtained from a commercial supplier, please check the manufacturer's information as to the quality control concerning replication competent viruses. This information should be supplied with the IBC application.

Lentivirus vectors can be tested for replication competent viruses by serial transfer and by ELISA assay for p24 antigen (Dull et al, 1998). The viral vector stock should be tested at a sensitivity limit of 1 infectious unit per mL. Vectors used for *in vitro* studies must be tested every six months to ensure no replication competent particles are being produced.

Laboratory Practices

Lentiviruses are classified as a **Biosafety Level 2** (BSL-2) organisms. Lentiviruses require BSL2 practices and procedures for all work with virus and Animal Biosafety Level 2 (ABSL-2) for all animal manipulation as well as animal housing. At the discretion of the IBC, experiments may need to be conducted at Biosafety Level -3 (BSL3). In the IBC application, the PI must justify that the gene to be expressed is not particularly harmful, and include citations to support these statements. The generation of the lentiviral system (i.e. second generation), as well as the transgene being inserted, must be factored into the risk assessment for use.

1. No work with lentiviruses is permitted on the open bench.

2. A certified Class II biosafety cabinet must be used for all manipulations including (but not limited to):
   - Pipetting
   - Harvesting infected cells for RNA
   - Purification of virus
   - Infection of cell culture
   - Infection of animals

3. Centrifugation must be done in closed containers with **sealed rotors or safety cups**. Safety cups are to be loaded and unloaded inside the biosafety cabinet.

4. All vacuum lines must be fitted with a HEPA filter (an example is the "Vacushield ™" inline hydrophobic filter, Product # 4402 from Gelman Science , Millex FH vacuum line protector Millipore (Fisher) cat # SLFH05010, or "HEPA-VENT™" inline hydrophobic filter, Catalog # 6723-5000 from Whatman).

5. All laboratory staff working with or supervising work with lentiviruses must be made aware of the hazards associated with the work, required safety practices and procedures, and proper handling of the agent, as well as be current on required laboratory safety and biosafety trainings.

6. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.

7. Special training must be given to all animal husbandry personnel on lentiviruses, the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment. This training would be provided by animal facility supervisors in consultation with REHS.

8. Signs and labels (universal biohazard symbol) must be placed to indicate each area where lentiviruses are used or stored (including biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.) The signs should include the name of the agent, emergency contact information and a biohazard sticker.

9. All work and manipulations of lentivirus must be conducted in a certified Class II biological safety cabinet. If there are extenuating circumstances or a biosafety cabinet is unavailable, please contact REHS (at the numbers listed at the end of this SOP) as additional precautions may be required.

Personal Protective Equipment

1. Disposable gloves.

2. Disposable gown or equivalent when introducing vector into animals or performing necropsies. Lab coats are adequate for tissue culture manipulations.

3. Eye Protection.

Instructions in the Event of Employee Exposure

- **EXPOSURE FROM SPLASH OR AEROSOLS – INHALATION**

  Report the incident to your supervisor and refer to the Rutgers Emergency Action Plan for further instructions. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **EXPOSURE FROM SPLASH OR AEROSOLS – EYE CONTACT, SKIN AND/OR MUCOUS MEMBRANE**

  Rinse a minimum of 15 minutes in eye wash or flush area with water, report the incident to your supervisor and refer to the Rutgers Emergency Action Plan posted in the lab for further

instructions. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **NEEDLESTICK AND/OR SHARPS EXPOSURE**

  Contaminated skin should be thoroughly scrubbed for several minutes with soap or a 10% povidone solution (Betadine) and copious amounts of water. Report the incident to your supervisor and REHS immediately after scrub. Seek medical attention at Campus Employee Health Services/Occupational Medicine Services. Refer to Rutgers Emergency Action Plan posted in the lab for after-hours exposure. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **EMERGENT EXPOSURES**

  For situations in which exposure to lentivirus occurred and medical treatment is an emergency, personnel should report to the Emergency Room, and ensure their supervisor completes incident report through https://MyREHS.rutgers.edu to document the event.

Decontamination

The most effective disinfectant against lentiviruses is a 1:10 sodium hypochlorite (bleach) solution that is made fresh daily.

- To make this solution, dilute 1 part bleach to 9 parts tap water.
- Ensure a 15 minute contact time.
- Use this disinfectant for treatment of reusable equipment, surfaces, and liquid waste (final volume 1% bleach).

Autoclaving for 1 hour at 121°C or 250°F (15 lbs psi of steam pressure).

- Use this disinfection method for reusable equipment, liquid waste or solid waste.

Animal Practices

1. When animals are infected with lentivirus or lentiviral vectors, an Animal Biosafety Level - 2 (ABSL-2) area must be used and approved by the animal facility staff and REHS for the procedure. Concurrent approvals are needed from the Institutional Biosafety Committee (IBC) and the Institutional Animal Care and Use Committee (IACUC).
2. All bedding, waste and animals shall be treated as biohazardous. Cage changing and husbandry must be performed according to the hazard sign provided by REHS. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal.
3. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.
4. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.

5. The following information must be posted on the door of the animal room. REHS will provide a sign template to the animal facility staff for this purpose.
    - A description of special housing required to ensure safety of animal facility personnel, such as ventilated cabinets or filtered cages.
    - A label on the animal cage indicating the hazardous materials to be administered to live animals. (i.e., lentivirus vector)
    - The name of individual(s) responsible for handling the materials (i.e., Drs. X, Y and Z and Technicians A and B as per protocol #00000) and emergency contact information
    - A description of how to handle animals, carcasses, and contaminated cages and bedding

References

- Braun, A. 2006. "Biosafety in Handling Gene Transfer Vectors." Current Protocols in Human Genetics. 12.1-12.18.
- CDC-BMBL, 5th ed., www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf
- Dull, T, Zufferey, R, Kelly M, mandel, RJ, Nguyen M, Trono D, Naldini L. 1998. A third generation lentivirus vector with a conditional packaging system. J Virol. 72: 8463-8471.
- Forestell, SP, Nando, JS, Bohnlein, E and Rigg, RJ. 1996. Improved detection of replication competent retrovirus. J Virol Methods. 60:171-178.
- Stanford University, "Working with Viral Vectors," https://ehs.stanford.edu/topic/biosafety-biosecurity/viral-vectors
- Wilson, CA, Ng TH, and Miller AE. 1997. Evaluation of recommendations for replication competent retrovirus testing associated with use of retroviral vectors. Human Gene Therapy. 8(7): 869-874.
- Young, L.S., Searle, P.F., Onion, D., and V. Mautner. 2006. "Viral gene therapy strategies: from basic science to clinical application." J. of Pathology. 208:299-318.

Standard Operating Procedures

Acknowledgement Page

I, _____, have read the SOP for working with _____ Viral Vector. The following people will be conducting experiments using these vectors. The staff members know where to find a copy of this SOP in the laboratory and they understand the hazards and safe work practices as detailed therein.

Name	**Job Title**	**Initials**

Principal Investigator (print): _____

Principal Investigator (Signature):_____