

Retroviral Vectors

Background

Retroviral systems are typically designed from murine retroviruses and can be grouped into one of three classes; ecotropic (infecting only murine cells), amphotropic (can infect human cells) or pseudotyped (vector particles express glycoproteins and can infect human cells). The most common pseudotyped virus utilizes a glycoprotein from vesicular stomatitis virus (VSV-g). All of these vectors have oncogenic potential and can integrate into transduced cells with high frequency.

One of the most commonly used retrovirus are the Lentiviruses. These viruses are able to infect and integrate into the chromosome of non-replicating cells. This family of viruses includes those of non-human (FIV, SIV) and human origin (HIV).

Currently, most retroviral vector systems place the components needed for recombination and replication (*gag*, *pol*, *env*) on different plasmids, thereby decreasing the possibility of a recombination event. Use of a four plasmid system in experiments is less risky than using a two plasmid system.

Symptoms of Exposure

Retroviral infection will manifest as an illness with nonspecific symptoms including anorexia, chronic diarrhea, weight loss, fever, opportunistic infections and malignant diseases without a cause for immune deficiency. Retroviral infections persist lifelong because the virus incorporates its nucleic acid into the host cell chromosome

Modes of Transmission

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

Host Range

Retroviral vector systems are usually based on murine viruses, but some have the potential to infect human cells as well. Retroviruses can infect dividing and non-dividing cells like neurons, macrophages, hematopoietic cells, muscle and liver cells.

Approvals

Experiments using retroviral vectors require local IBC approval before initiation of experiments.

Test Methods for Recombinant Virus-QC Tests

**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.

Retrovirus vectors (ecotropic and amphotropic) can be tested by amplifying any replication-competent virus (RCV) in permissive cell lines and then screening by an appropriate replication competent retrovirus (RCR) detection assay (i.e. PG-4 S⁺L⁻ assay, or the marker rescue assay). The vector stock should be tested at a sensitivity limit of 1 infectious unit per mL. (Wilson et al, 1997 and Forestell et.al 1996).

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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.

Murine Retrovirus (amphotropic or VSV-G Pseudotyped) vectors can be tested by marker rescue, antibiotic selection, PG3S⁺L⁻, PERT or infectivity RT-PCR assays. 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

Generally, retroviruses are classified as a **Biosafety Level 2** (BSL-2) organisms. Retroviruses require BSL2 practices and procedures for all 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.

1. No work with retroviruses 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 retroviruses 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 retroviruses, 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 EOHSS.
8. Signs and labels (universal biohazard symbol) must be placed to indicate each area where retroviruses 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.

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

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 Emergency Response Guide flip chart 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 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 Emergency Response Guide flip chart 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 AAV 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 retroviruses is a 10% sodium hypochlorite (bleach) solution that is made fresh daily.

- ♦ To make this solution, dilute 1 part Clorox to 5 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).

Disinfectant alternatives include 70% ethanol.

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 retrovirus, an Animal Biosafety Level - 2 (ABSL-2) area must be used and approved by the animal facility staff and EOHSS 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 infected with retrovirus shall be treated as biohazardous and handled in a biosafety cabinet. **Animals infected with retroviruses can shed the virus for up to 2 days post infection.** After all animals are removed from their primary enclosure immediately autoclave the empty cage. After autoclaving, dump the cage contents into medical waste and begin cleaning the cage for re-use. All waste must be decontaminated by autoclaving or chemical disinfectant 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 in the animal room. EOHSS 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., retrovirus 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 the hazardous materials are to be used in the protocol. See the following example: *“The protocol will be conducted at Biosafety Level 2 (BSL2) and Animal Biosafety Level 2 (ABSL2). Staff will use Class II biosafety cabinets for procedures that have the potential to generate aerosols, splash or spray. Personnel working on the protocol will be trained in the hazards of working with the recombinant retroviral vectors and the measures they need to take to protect themselves. They will wear lab coats or gowns, protective gloves and protective eyewear for all aspects of the protocol.”*

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,”

http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf

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.

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Acknowledgement Page

I, _____, have read the SOP for Retroviral Vectors. The following people will be conducting experiments using the Retroviral 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): _____

Contact Information:

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