

SOP: Working with Lentiviral Vectors

*All labs working with retroviral vector must have blood borne pathogens exposure control plan (BBP-ECP) as required by California/OSHA blood borne pathogen standard 8CCR Sec. 5193* [***http://www.dir.ca.gov/title8/5193.html***](http://www.dir.ca.gov/title8/5193.html)***.*** *All personnel handling such materials must take the Bloodborne Pathogens training course, provided via UCLC, annually.*

## Lab Contacts

Principal Investigator:

Phone:

Email:

Alternate:

Phone:

Email:

IBC Protocol #

IACUC Protocol # (if applicable)

Lab location:

Containment level: *(BSL1, BSL2, BSL2+)*

No one is allowed to work with lentivirus without having prior training by the Principal Investigator (PI) who supervises their work, or their designated technical expert. The worker should demonstrate good microbiological and tissue culture technique and an understanding of this Standard Operating Procedures (SOP) prior to being permitted to work with lentivirus. SOP for the planned procedures must be written and shall be present in the laboratory at all times. All staff involved with the handling and administration of lentiviral vectors must receive lab core safety training, blood borne pathogen, medical waste management and viral vector training provided by UCI EH&S and lab specific training provided by the PI or the lab supervisor.

*Please refer to the lab BBP-ECP for spill procedure, medical emergency contact information, reporting and documentation of injury and post exposure evaluation and follow-up.*

Background

Lentiviral vectors are based on Human Immunodeficiency Virus (HIV) which is the virus responsible for the development of Acquired Immunodeficiency Syndrome (AIDS). Lentiviruses are a subclass of retroviruses which are able to infect both proliferating & non-proliferating cells. Lentiviral vectors have been modified to provide a safer version of the HIV virus in which the viral replication genes have been removed. During infection, there is a possibility that the lentivirus may convert to a replication competent state. Although this scenario is highly unlikely, monitoring for such a possibility is encouraged, since such a conversion could compromise laboratory safety.

The major risks to be considered for research with HIV-1 based lentivirus vectors are the potential for generation of replication-competent lentivirus (RCL), and the potential for oncogenesis via random chromosomal integration. The nature of the transgene must also be considered in assessing risk. These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector (e.g., expression of a known oncogene with a constitutive strong promoter may require heightened safety precautions).

The potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are the number of recombination events necessary to reassemble a replication competent virus genome and the number of essential genes that have been deleted from the vector/packaging system. On this basis, later generation lentivirus vector systems are likely to provide a greater margin of personal and public safety than earlier vectors, because they use a heterologous coat protein (e.g., VSV-G) in place of the native HIV-1 envelope protein, thus reducing the risk of RCL generation. (It should be noted, however, that pseudotyping with coat proteins such as VSV-G may broaden the host cell and tissue tropism of lentivirus vectors, which will be considered in the overall safety assessment by the IBC). Later generation vector systems also separate vector and packaging functions onto three or four plasmids and they include additional safety features such as the deletion of Tat, which is essential for replication of wild-type HIV-1, and altered 3’ LTR that renders the vector “self-inactivating” (SIN). In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCL.

Type of lentiviral vector used in the lab: *description of lentiviral vectors and how they were generated*

Type of insert: *For example: Fluorescent protein, non-oncogene, oncogene, gene that knocks out tumor suppressor gene, toxic gene*

Modes of Transmission

The most probable route of exposure for this work would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure of the eyes, nose, and mouth. Another route would be inhalation via aerosols depending on the use of equipment such as centrifuges or vortex mixers.

Biosafety requirements and procedures

1. Physical Containment. All work with lentiviral vectors must be performed in a BSL2 laboratory or BSL2+ laboratory if the insert is an oncogene or involves deletion of tumor suppressor gene. This includes but is not limited to a room suitable for tissue culture and equipped with a certified Class II Biosafety Cabinet (BSC). Access to the laboratory must be limited when the agent is in use. Vacuum lines to be used for aspiration must be equipped with an in-line HEPA filter and a vacuum flask. If virus will be concentrated in an ultracentrifuge, rotors must be equipped with features (e.g., sealing o-rings) to minimize the risk of aerosol generation. Low-speed swinging-bucket centrifuge buckets must be equipped with aerosol-tight safety covers. Microcentrifuges must have aerosol-tight rotors capable of being removed while sealed so that the rotor can be opened/closed in the BSC. Doors must always be kept closed to maintain the BSL2/2+ containment. Please refer to Section IV of the CDC BMBL guidelines for BSL2 and BSL3 (for BSL2+) requirements.
2. Personal Protective Equipment (PPE). The following PPE must be worn when working with lentiviral vectors: gloves (double gloves for BSL2+) and lab coat (dedicated lab coat for BSL2+). A surgical mask and eye protection (goggles) or face shield is optional, but recommended any time there is a risk of aerosol/splash/spray of lentiviral particles to the face outside the BSC. In some cases a N95 respirator (annual fit testing required) might be required.
3. Spill Kit. The lab must have a spill kit, or the components of such readily accessible in the event of a spill. This comprises: an easy-to-read outline of the spill response SOP; gloves, masks, goggles, clean lab gown or lab coat, paper towels to absorb contaminated liquids, disinfectant, tongs or forceps to pick up broken glass and a red biohazard bag.
4. General Procedures for working with Lentivirus: Standard BSL2 practices should be employed, including a prohibition of eating, drinking, food storage, handling of contact lenses, applying lipstick or lip balm, mouth pipeting, and a requirement of appropriate PPE. Additional practices include the following recommendations:

* 1. Biosafety Cabinet: If the blower on the BSC is not left on continuously, it should be turned on and run for 3 minutes to allow complete exchanges of air before work can begin. At the beginning of the work session, plastic-backed absorbent toweling can be placed on the work surface (optional), but not obstructing air flow. Alternatively, the stainless steel work surface can be wiped down with 70% Ethanol. At the end of the work session, all items to be removed from the BSC must be decontaminated. The surface of the BSC must be wiped down with an effective disinfectant including 10% fresh bleach followed by 70% Ethanol.
  2. Sharps should be avoided whenever possible. Plastic aspirating pipets (<http://www.thomassci.com/Supplies/Pipets/_/Plasteur-Plastic-Pasteur-Pipets/> ) should be substituted for glass Pasteur pipets. If needles are required, they must never be re-capped, and must be disposed of in a sharps waste container immediately after use. While working with sharps inside the BSC, the sharps container must be kept inside the BSC.
  3. Solid Waste: Everything that comes in contact with the lentiviral material must be decontaminated before exiting the biosafety cabinet. Solid waste including pipet tips and tubes can be collected in a biohazard bag inside the Biosafety Cabinet. The biohazard bag must be inside a leak-proof, rigid container with a tight-fitting lid and labeled Biohazard. Another option is to fill a plastic container with some bleach and put the solids inside this container while carrying out the procedure. At the end of the work session, the biohazard bag will be closed, outside will be sprayed with 70% Ethanol and deposited into a biohazardous waste container. In case of the plastic container with bleach, pretreatment vessel, the bleach can be dumped down the sink with copious amount of water and the solid waste can be dumped in the biohazardous waste container.

* 1. Liquid Waste is aspirated into a vacuum flask containing 1/10 volume concentrated bleach. The vacuum flask must have a final concentration of at least 10% bleach, for a minimum time of 30 minutes prior to drain disposal. Liquid waste may also be collected in the hood in a simple 500 ml bottle (like a bottle used to store cell culture medium) that contains 50 ml concentrated bleach (10% final v/v). Allow a minimum of 30 minute incubation before pouring down the drain.
  2. Centrifugation. Centrifuge tubes should be prepared and sealed/loaded and unloaded in the rotor/buckets in the biosafety cabinet. This includes methods to ensure tubes are properly balanced (unless the balance tube contains no infectious material). At the end of the procedure, rotors and/or buckets must be decontaminated.
  3. Vortexing must be done in the BSC.
  4. If any materials infected/transduced with viral vectors are to be transported (from one location to another) a secondary container is required. The secondary container must be leak proof, rigid container, with a tight fitting lid and labeled biohazardous. A simple food storage container with a latchable lid and labeled with a biohazard sticker would suffice as a secondary transport container.
  5. Storage of lentiviral stocks must be in leak-proof secondary containers (i.e. freezer boxes) in a -80° freezer clearly marked with a warning label to indicate that lentivirus is present.
  6. Animal Work: Injections of lentiviral particles into rodents present a potential autoinoculation hazard. Injected rodents must be housed at ABSL2.

1. Please refer to the BBP-ECP for spill procedures, medical emergency contact information, reporting and documentation of injury and post exposure evaluation and follow-up

Procedure

Please include the lab specific procedure here

References

Biological Safety Principles and Practices, 3rd edition, 2000. ASM Press. Edited

by Diane O. Fleming, Ph.D, and Debra Hunt, Dr.P.H.

*Biosafety in Microbiology and Biomedical Laboratories*, 5th edition, Dec 2009.

Centers for Disease Control. <https://www.cdc.gov/labs/BMBL.html>

California/OSHA bloodborne pathogens standard 8CCR Sec. 5193

<https://www.dir.ca.gov/title8/5193.html>

Canadian Laboratory Centre for Disease Control Material Safety Data Sheets. [www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html](http://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html)

*Guidelines for Research Involving Recombinant DNA Molecules,* April 2019.

National Institutes of Health. <https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.html>

UCI Biosafety manual

<https://www.ehs.uci.edu/programs/biosafety/BiosafetyManual.pdf>

Acknowledgement

**As the Principal Investigator, it is your responsibility to ensure that all individuals listed in the IBC application are taught correct procedures for the safe handling of hazardous materials involved in this study. It is also your responsibility to assure that your personnel attend all the required training. Both PI and all persons associated with the protocol must sign the following acknowledgement: *I have read, asked questions, and understand the hazards of and safe working procedures for the activity/materials described herein.***

PI Signature DATE

Other Personnel (add more rows as needed):

Name/ Signature DATE

Name/Signature DATE

Name/Signature DATE

Name/Signature DATE