General Guidelines on Research Projects Involving Lentivirus

1. Introduction

Lentiviruses comprise a genus of the Retroviridae family and include bovine lentiviruses (e.g., Bovine immunodeficiency virus, Jembrana disease virus); equine lentiviruses (e.g., Equine infectious anemia virus); feline lentiviruses (e.g., Feline immunodeficiency virus); ovine/caprine lentivirus (e.g., Caprine arthritis-encephalitis virus, Ovine lentivirus, Visna virus); and primate lentiviruses (e.g., Human immunodeficiency virus (HIV) types 1 – 3, Simian AIDS retrovirus SRV-1, Human T-cell lymphotropic virus type 4, and Simian immunodeficiency virus).

Viral vectors have become a fundamental tool in the study of many aspects of cell and molecular biology. Lentiviral vector have proven to be very productive in terms of transduction due to their ability to infect both replicating and non-replicating cells and for short-interfering RNA (siRNA) delivery. Due to the increased use of lentiviral vector constructs in research applications, the following guidelines are established for laboratory users to understand and protect themselves from related exposure hazards.

2. Risk Assessment for Lentiviral Vectors

Two major risks that need to be considered for research involving lentivirus vectors include:

1. The potential for generation of replication-competent lentivirus (RCL), and
2. The potential for oncogenesis via random chromosomal integration.

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).

Risk Exposure Level of Lentiviral Vectors

	Higher Risk Exposures	**Lower Risk Exposures**
Vector Design	➢ Vector Packaging functions on two plasmids ➢ Expression of viral genes	➢ Vector and packaging functions separated onto multiple plasmids ➢ Deletion of viral genes
Transgene	Oncogene	Non-oncogene
Vector Generation	Large scale (>10 L)	Laboratory scale
Animal Hosts	➢ Permissive host ➢ Animals engrafted with human cells	Non-permissive host
Animal manipulations	Vector administration (e.g. use of sharps during injection)	Housing and husbandry (no use of sharps)
Modes of Transmission	➢ Skin puncture or injection ➢ Ingestion ➢ Contact with mucous membranes (eyes, nose, mouth)	➢ Bite from a recently infected animal ➢ Percutaneous contact with body fluids from a recently infected animal ➢ Inhalation via aerosols

3. Required Control Measures

3.1 Formal Approval

All investigators who intend to start their research activities involving lentiviruses at Hong Kong Baptist University must submit their research plan and protocols to the University's Laboratory Safety Sub-committee (LSSC) for expert review. A formal approval from the LSSC and their respective departmental/faculty/school laboratory safety committee (where applicable) must be obtained before commencement of research.

3.2 Vector Choice

The potential for generation of RCL from lentiviral vectors depends upon 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 or packaging system.

In order to reduce the exposure risk, the following approaches should be implemented when choosing the lentiviral vectors:

a. Segregate the lentivirus into vectors and packaging replication functions onto four or more plasmids.
b. Use HIV – 3rd Generation Packaging or HIV – 4th Generation Packaging System.
c. Use non-native env or a heterologous coating protein (e.g. VSV-G) in place of the native HIV-1 envelope protein. (However, the use of certain coating proteins, such as VSV-G may broaden the host cell and tissue tropism of lentivirus vectors.)
d. Delete genes essential for replicating wild-type HIV-1 such as *tat*.

3.3 Laboratory Facilities

With reference to National Institutes of Health (NIH) Guidelines for Research involving recombinant DNA molecules, activities involving the production of research-laboratory scale quantities of HIV or other blood borne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, should be performed in Biosafety Level 2 (BL2) laboratory facilities (see *Appendix 1*).

Biosafety Level 3 (BL3) laboratory facilities are required when activities involving the preparations of concentrated HIV (see *Appendix 2*).

3.3.1 Use of Biological Safety Cabinets

Class II Biological Safety Cabinet is required for handling lentivirus and lentiviral vector works. All vacuum lines must be fitted with a HEPA filter. No work with lentivirus is permitted on the open bench. All manipulations including (but not limited to) pipetting, harvesting infected cells for RNA/DNA/proteins, loading and opening containers and initial delivery of vector in animal hosts should be performed inside the Class II Biological Safety Cabinet.

3.4 General Laboratory Practices

a. In general, **Biosafety Level 3 (BL3) special practices** will be required for all experimental procedures in the lentivirus laboratory (see *Appendix 3*). Besides, since the animal rearing facilities in the University are not qualified for handling lentiviral-vector treated animals, all experiments on lentivirus vectors should be confined to be *in vitro*; thus, animals are not allowed in the lentivirus laboratory.

b. A designated laboratory for the sole use of such materials is preferable. **In a university setting with special conditions (e.g. with open access to the general public and/or with nearby classrooms/student function rooms), more stringent biosafety requirements may be required.**

c. Each department/division/faculty/school should designate a faculty member who is expert of the field to be in charge of the management of both facilities and research activities being involved in lentivirus experimentations.

d. Close supervision by the laboratory in-charge should be enforced and inventory on the storage, use and disposal of such materials should be properly recorded.

e. Access of the laboratory where lentivirus is used or stored should be limited to well-trained personnel only, such as senior postgraduate students and postdoctoral fellows; undergraduate students should be prohibited to undergo experiments involving lentiivirus.

f. Each research team should designate only 1 (2 at most) research personnel to work in the lentivirus laboratory in order to reduce access rate.

g. All procedures involving manipulation of the virus or viral vector or infectious materials should be conducted within a certified class II biosafety cabinet or other physical containment device.

h. Warning signage should be placed to indicate each area where lentivirus is used or stored including, but not limited to, biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.

i. Personal protective equipment to reduce the potential for mucosal exposure, splash to the face, and exposure of hands must be worn, including:
    - Gloves,
    - Wrap around outer clothing when introducing vector into *in vitro* systems. Laboratory coats are adequate for tissue culture manipulations. (Laboratory coat worn inside the lentivirus laboratory should not be used in other areas outside),
    - Safety Goggles, and
    - N-95 respirator, to be used with concentrated titers and highly aerosolizing procedures outside of the Biological Safety Cabinet.

j. Centrifugation should be done in closed containers and using sealed rotors. Rotors should be opened in a Biosafety cabinet.

k. Avoid use of needles and sharps where possible.

3.5 Transportation

All materials should be transported in a double-sealed leak-proof container. The waste container should be labeled with a biohazard symbol, the name of the agents, the quantity, name of the users and the room number of the laboratory.

3.6 Waste and Decontamination

All cultures, stocks, other biological wastes and small reusable apparatus should be decontaminated before disposal or storage. Two methods are approved for decontamination before disposal:

1. Autoclaving for 30 minutes at 121℃, and
2. Decontaminating with a solution of 1% Sodium hypochlorite, 2% Glutaraldehyde and 5% Phenol for 15 minutes contact time.

4. Contingency and Operational Continuity Plan

In the event of an accidental exposure or injury, the protocol is as follows:

Skin Exposure	Immediately go to an emergency shower and thoroughly wash the wound with soap and running water for 15 minutes with gentle messaging. Decontaminate any exposed skin surfaces with an antiseptic scrub solution.
Skin Wound	Immediately go to a sink and thoroughly wash the wound with soap and water for 15 minutes and pat dry.
Splash to Eye(s), Nose or Mouth,	Immediately go to an emergency eye wash and flush the area with running water for at least 15 minutes. Remember to remove your gloves before using your fingers to keep your eyes open.
Splash Affecting Garments	Remove garments that may have become soiled or contaminated and place them in a biomedical waste bag (minimum gauge of 150 micrometer for low density polyethylene or 75 micrometer for high density polyethylene or polyethylene) which should be decontaminated before disposal.

In the event of spills, the following procedures should be followed:

1) Wear personal protective clothing;
2) Allow aerosols to settle;
3) Gently cover spill with paper towel;
4) Apply approved disinfectants for decontamination of the spilled and cleaning materials (starting at perimeter and working towards the center):

    1% Sodium hypochlorite, 2% Glutaraldehyde and 5% Phenol

5) Allow sufficient contact time about 15 minutes before cleaning up;
6) Decontaminated spilled and cleaning materials should be sealed in biomedical waste bags and transported in a rigid container for disposal.

5. Reference

1. NIH Guidelines for Research Involving Recombinant DNA Molecules, Department of health and Human Services, National Institutes of Health, October 2011.
2. Biosafety Considerations for Research Involving Lentiviral Vectors, Recombinant DNA Advisory Committee.

Appendix 1: Laboratory Facilities for Biosafety Level 2

1. The laboratory is designed so that it can be easily cleaned.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
4. Each laboratory contains a sink for hand washing.
5. An autoclave for decontaminating laboratory wastes is available.

Reference:

G-II-B-4. Laboratory Facilities (BL2) of the National Institutes of Health (NIH) Guidelines for Research involving recombinant DNA molecules

Appendix 2: Laboratory Facilities for Biosafety Level 3

1. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.
2. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.
3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
4. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.
6. Windows in the laboratory are closed and sealed.
7. Access doors to the laboratory or containment module are self-closing.
8. An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.
9. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. The direction of the airflow (into the laboratory) should be verified by trained personnel. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.
10. The high efficiency particulate air/HEPA filtered exhaust air from Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner that avoids any interference with the air balance of the cabinets or building exhaust system.

Reference:

G-II-C-4. Laboratories Facilities (BL3) of the National Institutes of Health (NIH) Guidelines for Research involving recombinant DNA molecules

Appendix 3: Special Practices for Biosafety Level 3

1. The laboratory should have a designated laboratory-in-charge to oversee the management of both facilities and research activities.
2. A biosafety manual should be prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.
3. The laboratory-in-charge should establish policies and procedures to restrict the access of the laboratory to persons whose presence is required for research activities or support purposes. The laboratory-in-charge has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
4. Only persons being advised of the potential biohazard, met any specific entry requirements (e.g., immunization), and complied with all entry and exit procedures can enter the laboratory or animal rooms.
5. When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory-in-charge or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.
6. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.
7. Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
8. Molded surgical masks or respirators are worn in rooms containing experimental animals.
9. All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.
10. Animals and plants not related to the work being conducted are not permitted in the laboratory.
11. Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units, open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and –bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

    Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

12. Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.
13. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
14. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.
15. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinnoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
16. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.
17. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.
18. Laboratory doors are kept closed when experiments are in progress. A pest control programme for the laboratory is in effect.
19. Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules should be immediately reported to laboratory-in-charge.

Reference:

G-II-C-2. Special Practices (BL3) of the National Institutes of Health (NIH) Guidelines for Research involving recombinant DNA molecules