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Introduction

The use of Lentiviral vector systems has become increasingly common due to the attractive features that these systems possess. However, research using these vectors has raised biosafety issues. Many of these systems derive from HIV-1 and the use of these systems has the potential for the generation of replication competent lentivirus (RCL) and oncogenesis due to positional insertion. The newer generation of vectors have a number of features incorporated into them that enhance biosafety (for example, the removal of the tat gene needed for replication of wild type HIV-1 or the separation of vector and packaging elements onto four or more plasmids). However, due to the ease with which these vectors can transduce human cells, a through risk assessment must be carried out each time a new vector system is manipulated.

Associated Procedure

General Criteria for Risk Assessment of Lentivirus Vectors

- The nature of the vector system and the potential for the generation of RCL using the vector components.
- The nature of the insert(s) e.g. is the insert a known oncogene.
- Vector titer and total amount of vector.
- Biological containment level of any animals that may be used-Use of permissive/non-permissive animals.

Laboratory Containment Considerations

When using the newer generation of Lentiviral vectors, either BSL-2 or enhanced BSL-2 containment (the use of BSL-3 techniques, performed in a BSL-2 environment) is usually most appropriate. However, which level is most appropriate should be determined only after completing a through risk assessment and in consultation with the Research Biosafety Committee (RBC). Note that only BSL-1, BSL-2 and enhanced BSL-2 are permitted at the LKSKI.

It is strongly advised that all Lentiviral work be done during regular working hours. This is to ensure the worker's safety in the event of an accident.

All Lentiviral work will be performed in room 5-047. Access to this room, is by key card and access will only be given to those workers that have satisfactorily completed Lentiviral training. Proper signage, preventing unauthorized entry to the lab must be posted on the outer door when work is in progress. Pam Plant maintains a Lentivirus BSC calendar. Please consult with her regarding its location and use.

Housekeeping staff will be prohibited from entering the room. Therefore all users of the room must participate in the cleaning of the room. On a monthly basis, or as needs arise, users must clean and disinfect the floors.

Personal Protective Equipment

All persons working with Lentiviruses must wear a lab coat at all times. Lab coats with elasticized cuffs must be worn to ensure that unprotected skin is not exposed. These lab coats are not to be worn outside of the Lentivirus room and must be laundered on a regular basis. In the event of a spill onto the lab coat, it must be autoclaved first, before being laundered.

All workers must double glove when working with virus and the outer pair should be replaced regularly. The gloves must extend to cover the cuff of the lab coat. Do not spray gloves with 70% ethanol or other chemicals as this has been shown to increase the permeability of gloves, which compromises their protective ability.

Laboratory appropriate clothing only is permitted in the Lentivirus room. No shorts, open toed shoes or sandals are permitted. Hair must be tied back and absolutely no eating or drinking is allowed. Eye protection must be worn. Eye goggles and face masks are provided in the room.

At the end of the experiment, lab coats must be hung up on the hooks provided, hands must be washed and you must ensure that all doors are thoroughly closed and locked.

Prior to commencing experimental work

- Turn on BSC and clean the inside surfaces with 70% Ethanol or diluted Virox (1:10).
- Allow the BSC to run for at least 5 minutes.
- Disinfect the exterior of all material going into the BSC with 70% Ethanol.
- Place biohazard bags, waste containers, paper towels, tape, a spray bottle containing diluted Virox (1:10 dilution) in the BSC.
- Note: Virox is made from a concentrate then diluted to the appropriate dilution. It has a shelf life of only 30 days in its diluted state. Please put date of preparation on any container of diluted Virox. Discard any old Virox when preparing a fresh batch.

- All work in the hood must be conducted on absorbent pad (plastic side down). Ensure that enough pads are present in the BSC to cover the area of your work.

Note that the use of sharps (glass pipettes, needles etc) in the BSC is prohibited.

When using Lentiviral vectors

Avoid the generation of aerosols. The use of the BSC vacuum is prohibited as this is a likely source of aerosols.

Do not touch anything outside of the BSC with dirty gloves. Replace gloves with clean gloves, inside the hood, discarding the dirty gloves in the biohazard bag, before touching anything outside of the BSC e.g. an incubator.

All liquid waste must be thoroughly bleached inside the BSC before being discarded. Liquid waste is decanted into a large bottle containing at least 100mls of undiluted bleach. Once all liquid waste has been generated and discarded, add an additional 100ml, close off the bottle, ensure the exterior surface has been decontaminated, then place bottle on the right hand side of the sink. Ensure that the bottle has been labeled with your name, date and time at which the decontamination was started. Bottles should be left for 24 hours to ensure complete deactivation of the virus. Liquids that have been thoroughly bleached can be disposed of down the sink with large quantities of water. Please be considerate to others working in the room when discarding bleach as it is very volatile.

All solid waste should be placed into a plastic bag in the hood. Pipette tips should be placed into a plastic box (e.g. a P1000 tip box) then placed in the bag. Serological pipettes should be placed back in their sleeves then into the bag. Once all solid waste has been disposed of, the bag should be sealed, placed into a second bag, which is then sealed and its exterior disinfected with 70% Ethanol. This bag can then be autoclaved. Do not autoclave any item that contains bleach, as this will liberate Chlorine gas.

After removal of all garbage from the hood, it should be washed down with diluted Virox: allow the surface to remain wet for at least 30 seconds, and then wipe down the hood. UV irradiation can be used to ensure microbial decontamination, but note that UV irradiation has no effect on Lentiviruses.

Transport of Virus

Any cells that are with or are producing viral vectors should be carried to and from the BSC in a sealable container, lined with absorbent material. Inside of incubators, the lid can be opened to allow gaseous exchange.

If cells have to be removed from room 5-047, it is advisable to only move them 3 days post infection. This is the theoretical lifespan of the virus in solution. Before moving the cells however, remove all medium and thoroughly wash the cells (5 times), taking care to treat all washes as contaminated waste and decontaminate it according to the previous guidelines.

Use of Incubators

There are two incubators in the lentivirus room. One is a Heracell 150i and the other a NuAire Autoflow..

All users will be assigned shelf space within incubators and these space allocations will be posted on the incubator doors, so that any problems can be reported to the appropriate individual.

All work should be done in vented tissue culture flasks. However, if tissue culture plates are used, they must be stored in the incubators in sealable containers, which can be opened in the incubators to allow gaseous exchange.

The incubators will be decontaminated and cleaned every 6 months.

Spill Procedure

Small Scale spill

- Advise all those in the area that a spill is present.
- Ensure that you are wearing all necessary protective clothing, gloves and eye protection.
- Allow 30 mins for aerosols to settle.
- Gently cover the spill in paper towels starting from the outside and working your way in.
- Remove any spray attachments from the diluted Virox bottle and apply the Virox onto the paper towels, again working from the outside towards the centre.
- Allow 2mins, before carefully discarding the paper towels into a biohazard bag. Dry any remaining liquid with more paper towels. Discard them as before.
- Apply more Virox to the area. Allow it to stand for 5mins then dry with paper towels, discarding as before.
- Wash area with 70% Ethanol. Dry with paper towels discarding them into waste as before.
- Discard outer gloves into waste bag then put fresh pair on.
- Autoclave garbage bag immediately.

Large Scale Spill (Code Brown)

- In the event of a catastrophic failure of the ultracentrifuge or a spill of large volumes of Lentiviral Vector:-
- Advice all those in the area that a major spill is present, then leave the area immediately.

- Leave your lab coat in the room and wash your hands thoroughly.
- Contact the Biological Safety Office (x77534) and advise them of the incident. Give your name, where you are and the location and type of spill.
- Wait for the BSO to arrive. Do not let anyone enter the room.
- Any accidents that are life threaten should be handled by the fire service or paramedics. Call 9-911!

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