A. Design and Objectives

Lentivirus production will be performed according to the protocol developed by the Transgenic Core Facilities of the Ecole Polytechnique Fererale de Lausanne ([http://tcf.epfl.ch/page-75906-en.html)](http://tcf.epfl.ch/page-75906-en.html%29). See attached protocol. \*\*\*\*Note, Step 15 (Concentration by ultracentrifugation) will be performed in a Sorvall Legend XFR Centrifuge, using a sealed Thermo Scientific F14-6x250 LE basket in Amicon Ultra-15 Centrifugal Filter Units.\*\*\*\*

The purpose of the proposed research is to create human mast cell lines expressing one of the following three proteins: Green Fluorescent Protein, TRAIL or the SV40 Large T antigen. These cell lines will be used in the development of a mast cell-based cancer therapy as well as for general research concerning human mast cells.

B. Potential environmental impact

None.

C. Description of safety precautions

**Lentiviral Biosafety Manual**

**Standard Operating Procedure: Lentiviral-based vectors**

## Lab Contacts and Training

**Principal Investigator: Dr. Chris Kepley**

**Contact Information:**

 **Lab Location: Dept. of Nanoscience**

 **Office Phone: 336-285-2865**

 **e-mail: clkepley@uncg.edu**

 **24/7 contact telephone/pager:**

**Lentiviral-specific Training:** No one is allowed to work with lentivirus without having prior training by the Principal Investigator who supervises their work, or their designated technical expert. The worker should demonstrate good microbiological and tissue culture technique and an understanding of this SOP prior to being permitted to work with lentivirus.

## Background

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.

## Exposure risk

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. Care must be taken when pipeting in order to avoid splashing or generation of aerosols. Immunocompromised individuals should not work with lentivirus.

## Inactivation and Surface Decontamination

Lentiviral particles can be inactivated with a number of reagents, including (final concentrations) 10% bleach\*, 5% Amphyl (phenolic), 0.5% Wescodyne (iodophor). This SOP has been written for the use of bleach, but alternative disinfectants can be substituted, provided they are known to be effective for lentivirus.

\***A note on bleach**

Bleach is effective, inexpensive, but volatile and corrosive. Bleach-soaked paper towels should not be autoclaved because autoclaving releases chlorine, a chemical hazard, and could corrode the autoclave over time. 10% (0.5% final chlorine concentration) bleach solutions should be prepared fresh prior to each work session. If 10% bleach is used to decontaminate a spill within the Biosafety Cabinet (BSC), once the spill has been absorbed on paper towels and disinfected with 10% bleach, the BSC should be wiped down with 70% EtOH in order to remove residual bleach.

## Biosafety Requirements and Procedures

1. **Physical Containment.** In general, all work with lentiviral vectors must be performed in a BSL2 laboratory. This includes but is not limited to a room suitable for tissue culture with negative pressure and a closing door, and equipped with a certified Class II Biosafety Cabinet (BSC), and a dedicated tissue culture incubator. During work with viral particles, a warning sign must be posted on the door alerting personnel of the presence of lentiviral particles. Vacuum lines to be used for aspiration must be equipped with an in-line HEPA filter and a vacuum flask (two flasks connected in series are recommended, but not required), containing 10% bleach. 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 unloaded in the BSC.
2. **Personal Protective Equipment (PPE).** The following PPE must be worn when working with Lentiviral vectors: gloves; lab coat. A surgical mask and eye protection (goggles) or face shield is optional, but recommended any time there is a risk of a splash of lentiviral particles to the face outside the BSC. It is suggested, although not required, that double gloves be worn, with particular attention to ensuring bare skin at the wrists is covered. Another suggestion is the use of gloves with longer than standard wrists, and tucking the cuffs of the lab coat sleeves into the gloves. Remove potentially contaminated gloves and replace them with new gloves before touching anything outside the BSC, such as the refrigerator, centrifuge, or incubator.
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, clean scrubs and spare slip-on shoes (Crocs are not recommended because they do not fully enclose the feet) in case clothing not covered by lab coat becomes contaminated; paper towels to absorb contaminated liquids; disinfectant (e.g., 10% bleach); tongs or forceps to pick up broken glass; a biohazardous waste container large enough to handle wet, contaminated paper towels.
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. Whenever possible, work with lentiviral vectors during normal working hours, to enable adequate response to a severe adverse incident.
	2. Biosafety Cabinet: If the blower on the BSC is not left on continuously, it should be turned on and run for 5 min. to allow several 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% EtOH. When double-gloving (optional), remove the outer pair of gloves and deposit in a solid waste bag before removing hands from the BSC. 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 70% EtOH, and the sash lowered.

* 1. Sharps should be avoided whenever possible. Plastic aspirating pipets (e.g., Corning cat. # 4975; Falcon cat # 3575; Fisher Cat # 13-675-123) should be substituted for glass Pasteur pipets. If needles are required, they must never be re-capped, and must be disposed of in a rigid red sharps waste container. Reminder: syringes without needles can be discarded in either a biohazard bag or a biohazardous sharps container, but must never be discarded in regular trash. (**Note:** **West Campus labs must always discard syringes in sharps containers, with no exceptions**)
	2. Solid Waste: Everything that contacts virus-containing solutions or vessels must be decontaminated or contained before exiting the biosafety cabinet. Solid waste can be collected in a biohazard bag inside the Biosafety Cabinet. Pipet tips can be collected in a disposable plastic box (e.g., an empty P-1000 box), and the box closed and deposited into the biohazard bag (in the Biosafety Cabinet!) at the end of the work session. At the end of the work session, the biohazard bag will be closed, sprayed with 70% EtOH, and deposited into a biohazardous waste container.

* 1. Liquid Waste is normally aspirated into a vacuum flask containing 1/10 volume concentrated bleach, or 1/20 volume Amphyl, or 1/40 volume Wescodyne. A common practice is to anchor the end of the vacuum tubing to the outside of the sash or frame of the Biosafety Cabinet. For lentiviral work, engineer a way to anchor the free end of the vacuum tubing inside the Biosafety cabinet. At the end of the work session, aspirate 25-50 ml of concentrated bleach through the vacuum tubing, into the vacuum flask. 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 that is not aspirated must be treated with bleach, to a final concentration of at least 10%, in the hood, allowing a minimum time of 30 minutes to inactivate virus. A simple 500 ml bottle with 100 ml concentrated bleach may be suitable to collect non-aspirated liquid waste.
	2. Centrifugation. Centrifuge tubes should be prepared and sealed in the biosafety cabinet. This includes methods to ensure tubes are properly balanced (unless the balance tube contains no infectious material). Fixed angle rotors should be loaded in the BSC as well, and the entire rotor sprayed with 70% EtOH before removal of the rotor from the BSC. For ultracentrifugation with swinging bucket rotors (e.g., SW28), individual buckets can be prepared in the BSC, securely closed, wiped down with 70% EtOH, and then transported to the centrifuge in the respective rack for those buckets . When safety cups are used (for low-speed centrifugation to clarify viral supernatants), the aerosol-tight safety cups must be loaded, closed, wiped down with 70% EtOH prior to removal from the BSC; they must also be unloaded in the BSC. After centrifugation, the centrifuge lid must be opened cautiously, and the rotor quickly visually inspected for a failure which could have generated aerosols in the centrifuge chamber. The rotor and chamber must be misted with 70% EtOH, and the rotor (or swinging buckets/safety cups) transported into the BSC for further work. At the end of the procedure, rotors and/or buckets must be decontaminated.
	3. Vortexing must be done in the BSC.
	4. If tissue culture dishes are used for Lentiviral production, they must be transported to an incubator (clearly marked with a warning label to indicate that lentivirus is present) in a secondary, closed container in case liquid media sloshes out of the dishes during transport (see Accidents and Spills). A tupperware-type container will work, and the lid of the tupperware container can be removed or left ajar once the container is in the incubator, to enable gas exchange. To remove the tissue culture dishes from the incubator, close the Tupperware container with the lid before taking the dishes out of the incubator.
	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.

**j.**  Animal Work:Injections of lentiviral particles into rodents do not present a potential hazard other than autoinoculation during injection. Injected rodents can be housed at ABSL1. However, some experiments may call for transduction of human cells *in vitro*, followed by injection of the human cells into rodents. Because the human cells could allow replication of RCL which might conceivably be present, these animals should be housed in ABSL2.

1. **Accidents and spills**
	1. Spills in the BSC. First lower the sash for 5 minutes to allow the blower to move aerosols through the HEPA filter. During this time, check to see if the spill is fully contained within the BSC, if any PPE has become contaminated, or if any breach of containment has occurred (e.g., a splash where droplets have escaped the BSC and fallen on the floor). If there has been a breach of containment, response should be as for a spill outside the BSC. Small spills (<25 ml) can be decontaminated by layering paper towels soaked in 10% bleach on top of the spill, allowing 20 min. for the bleach to inactivate virus, then depositing the paper towels in the solid waste bag in the BSC. Residual bleach can be wiped off with paper towels sprayed with 70% EtOH, and the towels deposited in the solid waste bag. Small spills in the BSC that do not involve exposure do not require notification of the IBC Biosafety Officer, but do require notification of the PI, who will direct further training (e.g. retraining on pipeting techniques, or organization of materials and instruments in the BSC) to minimize the risk of recurrence. Note: a spill of media or buffer not containing virus is not a biohazard per se, but paper towels used to wipe it up should be deposited in the biohazard bag in the BSC.

Large spills (over 25 ml, with likely splattering of droplets outside the BSC) should be treated more cautiously. Leave the BSC running, and evacuate all personnel from the room (remove gloves (or outer gloves, if double-gloved) before touching the door knob). Close the door to the room as you leave, remove PPE and any contaminated clothing (check the sleeves of your lab coat), and place it in sealable plastic containers or a biohazard bag. Everyone in the room at the time of the spill should thoroughly wash their hands and face, using disinfectant soap. Post a warning sign on the door of the lentiviral room advising personnel not to enter. Notify the PI. If you are absolutely sure that there has been no exposure and no breach of containment, proceed as for a small spill in the BSC. [If there has been overt exposure (e.g., actual contact of bare skin with virus), wash skin with soap and water for 15 minutes, and contact an OHSU EHRS Biosafety advisor (503-494-0655, 503-494-2580). After hours, contact the OHSU Public Safety Office at OHSU for assistance (503-494-4444)]. Allow 30 min. for possible aerosols to settle. Don clean PPE, re-enter the room, cover the spill with paper towels, soak with 10% bleach (or 5% Amphyl, or 2.5% Wescodyne), starting at the perimeter and working inward toward the center. Allow 20 min. to inactivate the virus. Deposit soaked towels in biohazardous waste\*. The interior of the BSC should be decontaminated by wiping down the walls, sash, and equipment with disinfectant (70% EtOH). Autoclavable equipment (e.g., racks, some pipetors, and tube containers) should be autoclaved, if feasible. If the spill has inundated the BSC drain pan, more extensive decontamination must be carried out. The drain pan should be emptied into a collection vessel containing disinfectant. A hose barb and flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. The drain pan should be decontaminated with 5% Amphyl or 2.5% Wescodyne, flushed with water and the drain tube removed. Again, after decontamination with corrosive disinfectants, remember to wipe down the BSC with 70% EtOH to remove residual chemicals. If no overt exposure has occurred, and the spill was completely contained within the BSC, the biosafety advisor/IBC does not need to be informed. The PI should review the incident to revise procedures to minimize the risk of recurrence.

* 1. Minor spills **outside** **the BSC**. A minor spill is defined as a spill with low potential to aerosolize, presents no inhalational hazard and no endangerment to people or the environment. As a practical consideration, volumes less than 10 ml fall into this category. First, ascertain the extent of the spill. (Simply dropping a 150 mm dish contained inside a closed Tupperware container does not constitute a spill outside the BSC, since there is no breach of containment—as long as the Tupperware container stays closed). If other personnel are present, alert them immediately. Keep in mind: spills generate aerosols. Quickly check to ascertain the extent of the spill: if PPE is contaminated, (gloves, lab coat, pants cuffs, and especially shoes!), bare skin is exposed, or if liquid has splashed over a large area. If shoes are visibly contaminated, decontaminate them with 10% bleach, or other disinfectant, then evacuate the room (remove gloves (or outer gloves, if double-gloved) before touching the door knob), closing the door. Remove any potentially contaminated PPE, place it in a biohazard bag, wash hands and face thoroughly. Post a sign on the door warning personnel not to enter. Allow 30 min. for aerosols to settle. During this time, notify the PI and the Biosafety advisor. After 30 min., don fresh PPE, re-enter the room, cover the spill with paper towels, then soak them with 10% bleach (or 5% Amphyl, or 2.5% Wescodyne), starting at the periphery and moving inward toward the center. Be sure to check for and to decontaminate small splashes beyond the main affected area. Leave the soaked towels in place for 20 min. to inactivate virus. Leave the room during this time. After the 20 min. inactivation time Transfer soaked paper towels to biohazardous waste\*. Wipe up the residual spill with more paper towels. Give the area a final wipe-down with paper towels sprayed with 70% EtOH.

* 1. Major spills **outside the BSC**. A major spill is defined as a spill that spreads rapidly, presents an inhalational hazard, endangers people or the environment, and/or involves personal injury or rescue and should be handled as an emergency. In practical terms, this might be a spill of more than 10 ml splattering over a large area, thus presenting the possibility of aerosolization and widespread contamination. (Dropping a tightly closed Tupperware container with six 150 mm dishes of HEK293T cells producing Lentiviral particles does not constitute a spill as long as the tupperware container does not come open—just carry the container back to the BSC prior to decontamination.) If other personnel are present, alert them immediately. Keep in mind: spills generate aerosols. Ascertain the extent of the spill: possible overt exposure, splash on shoes or soles of shoes, contamination of PPE. If shoes are contaminated, disinfect them before evacuating the room (if shoes are extensively contaminated, you should remove them as you leave the room). After removing gloves (or outer gloves, if double-gloved), evacuate the room, closing the door as you leave. Remove PPE. Wash hands and face thoroughly. Post a sign on the door warning personnel not to enter. Allow 30 min. for aerosols to settle. During this time, notify the PI and the Biosafety advisor. If the spill is too difficult to manage alone, seek help from the Biosafety advisor. After 30 min. don fresh PPE, re-enter the room, cover the spill with paper towels, and soak the towels with 10% bleach (or 5% Amphyl, or 2.5% Wescodyne), working from the outside toward the center. Allow 20 min. for virus to be inactivated. If there is any broken glass associated with the spill, pick it up with tongs or forceps, and transfer it to a biohazardous broken glass container. Pick up soaked paper towels, and transfer to a biohazard bag. Wipe up residual spill with more paper towels. Give the area a final wipe-down with paper towels sprayed with 70% EtOH.

All Spills outside of the BSC that involve breach of containment, regardless of exposure, must be reported to a Biosafety Officer.

* 1. Accidents include release of virus due to equipment failure (e.g. tube failure in the centrifuge), needle-sticks, or other injuries concomitant with a breach of containment of virus.
		1. Centrifugation. If tube failure is suspected (sudden clunking or automatic shut-down due to imbalance), leave the centrifuge lid closed for 30 min. to allow aerosols to settle. During this time, notify the PI. Open the lid cautiously to check the integrity of the rotor/tubes. If the rotor looks intact, spray the rotor with 70% EtOH, and transport it into the BSC before unloading centrifuge tubes. If a tube has cracked or collapsed within a swinging bucket (e.g., SW28), decontaminate the tube and bucket inside the BSC (Use your own judgment regarding recovery of viral pellets). If there appears to be a leak or spill inside the centrifuge, decontaminate the centrifuge chamber by cautiously opening the centrifuge, adding paper towels to soak up any contaminated liquids, then liberally spraying disinfectant onto the walls and inside lid of the centrifuge, so that disinfectant pools at the bottom of the chamber. (e.g., about 0.5-1 liter). Close the centrifuge for 20 min. Clean up the soaked paper towels as for a major spill outside the BSC. In the event of a catastrophic failure in the centrifuge (e.g., swinging bucket coming off the rotor at 22,000 rpm, damaging the centrifuge, and releasing virus into the centrifuge chamber), keep the centrifuge lid closed for 30 min. During this time, notify the PI and contact the Biosafety advisor. If the contamination is too extensive to manage alone, ask the Biosafety advisor for assistance. Decontamination is similar to a major spill outside the BSC. Lay paper towels inside the centrifuge chamber, and soak with 10%bleach (or 5% Amphyl, or 2.5% Wescodyne). Spray the inside of the centrifuge jacket with 70% EtOH. Close the lid for 20 min. to inactivate virus. Clean up as for a major spill outside the BSC.
		2. Sharps should be avoided whenever possible for work on lentiviral production and delivery. However, if there is a needle-stick, briefly bleed the wound (squeeze it to produce a couple of drops of blood), then wash thoroughly with soap and water for 15 min. Report the incident to the PI and the Biosafety advisor.
		3. Other accidents might include slips, falls, or collisions with other personnel, leading to spills of virus. Additional help may be required in the event of personal injury, in which case assisting personnel must be made aware of the presence of uncontained virus so that they can respond appropriately. In the event of a major spill involving serious personal injury or requiring rescue, call Public Safety, a Biosafety advisor, and contact the PI.

**f.** Follow-up of exposures or injuries involving Lentiviral particles

Lentiviral Productionn RE, pMD2.G, pCMVR8.74, In the event of an exposure or injury the following steps should be followed:

1. Determine the severity of the injury/exposure. If it is life-threatening, call Public Safety immediately. They will help you decide whether to go directly to the ER or wait for emergency responders.

2. If the injury/exposure is non-life-threatening, call Employee Health. Over the phone, they can help you with first-aid advice, and also help you decide whether to go to the ER or whether to come to Employee Health for follow-up treatment. After hours, call Public Safety non-emergency help at.

3. If you are injured/exposed and need to leave the lab for treatment, delegate responsibility to a colleague for any clean-up or decontamination that may be necessary. Have the colleague document what happened.

4. Monitoring after potential exposure to a potentially infectious agent (e.g., a needlestick with uncharacterized primary human cells or blood) can be conducted by Employee Health.

5. The NIH/OBA independently requires notification (via the IBC) of overt exposures to BSL-2 agents, or spills outside the BSC. The following table, from the OHSU Biosafety Manual presents the OHSU IBC reporting time-frame.

|  |  |
| --- | --- |
| **Type of spill or exposure** | **Reporting time frame** |
| Spill outside the BSC of a BSL-2 agent | Incident Report Form must be submitted to the IBC within 10 days. |
| Documented exposure to a BSL-2 agent | Report immediately to the Biosafety Officer (by phone call, preferably). Submit Incident Report Form to the IBC within 5 days. |
| Potential or documented exposure to a BSL-3 agent |
| Spill outside the BSC of a BSL-3 agent  |

**Note:**

 Some incidents that do not require reporting to the NIH/OBA, such as a spill inside a BSC that is properly decontaminated, should nevertheless be reported to the PI/supervisor in order to review or revise SOPs so as to minimize recurrence of the event, or to prompt refresher training of personnel. An FAQ document on reporting can be found at the OBA website:

<http://oba.od.nih.gov/oba/ibc/FAQs/FAQS%20about%20Incident%20Reporting.pdf>

##

## Appendix I: Spill Response Cue Cards

**(Should be posted in the work area)**

**SPILLS IN THE BSC**

**A. Clean up of a small spill (<25 ml):**

1. Make sure the cabinet continues to operate. Wait 5 min. to allow aerosols to be pulled through the HEPA filter.

2. Decontaminate the surfaces within the cabinet, wearing protective clothing, gently cover the spill with absorbent paper towel and apply 10% bleach (or 5% Amphyl, or 2.5% Wescodyne) starting at the perimeter and working towards the center; allow sufficient contact time (20 min) before clean up.

3. Discard soaked paper towels in a biohazard bag in the BSC. Wipe up residual mess.

 Wipe down surfaces with 70% EtOH, discarding towels in biohazard bag.

4. Lower sash for 5 min. with blower on to circulate air through the HEPA filter.

**B. Clean up of a large spill (>25 ml):**

1. Evacuate the room, breathing as little as possible of any aerosols.

2. Close the door to the room.

3. Remove PPE and any contaminated clothing and place it in sealed plastic containers.

4. Post warning sign: DO NOT ENTER—lentivirus spill!

5. Everyone in the room at the time of the spill should thoroughly wash their hands and face, using disinfectant soap.

6. Wait 30 min. for aerosols to settle. Meanwhile, notify PI. If the spill has escaped the BSC, proceed as for a spill outside the BSC.

7. Proceed with clean-up as for minor spill; decontaminate all equipment supplies, or surfaces that were potentially contaminated.

8. If a large quantity is spilled, the entire cabinet, including fans, filters, airflow plenums, will need to be decontaminated.

**SPILLS OUTSIDE THE BSC**

**A. Clean-up of minor spill (<10 ml, localized to a small area):**

1. Alert personnel in the vicinity.

2.Ascertain extent of spill—check shoes! Decontaminate if necessary.

3. Evacuate the room. Close door. Discard potentially contaminated PPE and remove any contaminated clothing. Wash hands and face thoroughly.

4. Post warning sign: DO NOT ENTER—Lentivirus spill!

5. Wait 30 min. Meanwhile, notify PI.

6. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.

7. Re-enter the room, cover spill with paper towels.

8. Soak paper towels with 10% bleach, from perimeter toward the center.

9. Allow 20 min contact time.

10. Continue clean-up: soaked towels go in biohazard bags. Pick up sharps with tongs.

11. Wipe up residual mess with paper towels. Discard in biohazard bag.

12. Wipe down spill area with 70% EtOH.

13. With PI, write up a report to submit to the Biosafety Advisor.

**SPILLS OUTSIDE THE BSC**

**B. Clean-up of major spill (>10 ml, splattered over a large area):**

1. Alert personnel in the vicinity.

2. Ascertain extent of spill—check shoes! Decontaminate if necessary.

3. Evacuate the room. Close door. Discard potentially contaminated PPE and remove any contaminated clothing. Wash hands and face thoroughly. If eyes have been exposed, flush in eye station.

4. Post warning sign: DO NOT ENTER—Lentivirus spill!

5. Wait 30 min. Meanwhile, notify PI and Biosafety advisor.

6. If assistance is needed, discuss with Biosafety Advisor.

7. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.

8. Re-enter the room, cover spill with paper towels.

9. Soak paper towels with 10% bleach, from perimeter toward the center.

10. Allow 30 min contact time.

11. Continue clean-up: soaked towels go in biohazard bags. Pick up sharps with tongs.

12. Wipe up residual mess with paper towels. Discard in biohazard bag.

13. Wipe down spill area with 70% EtOH.

14. With PI, write up a report and submit to the Biosafety advisor.

**Special Situations:**

**In the incubator**:

Contamination of water pan—add bleach to 10% final; 30 min.

Decontaminate incubator as for a minor spill outside the BSC.

After decontamination procedure, follow regular incubator decontamination procedure.

As for spills outside BSC, submit a report for Biosafety Advisor

**Centrifuge**: Open lid of centrifuge slowly.

1. If no breach of containment, spray rotor with 70% EtOH, unload rotor in BSC. If inside of rotor is contaminated, decontaminate in the BSC. As a precautionary measure, decontaminate the centrifuge chamber.
2. If rotor or buckets are damaged, close centrifuge lid.

Alert personnel in the vicinity. Evacuate room. Post Warning Sign: DO NOT ENTER--Lentivirus Spill! Wait 30 min. Meanwhile, notify PI and Biosafety advisor. If assistance is needed, discuss with Biosafety advisor. Open lid slowly, add paper towels, flood chamber with disinfectant to saturate paper towels. Spray walls of chamber and rotor with 70% EtOH. Close fuge lid for 20 min contact time. Finish centrifuge clean-up as for major spill outside the BSC. Transport rotor to BSC. Open and decontaminate rotor/buckets in the BSC.

With PI, write up a report and submit to the Biosafety advisor.

##