Modification Form for Permit BIO-RRI-0055

Permit Holder: John MacDonald

Approved Personnel
(Please stroke out any personnel to be removed)

Gang Lei

Cristi Orth

Jillian Belrose

YuFeng Xie

Michael Jackson

Oies Hussein

Kai Yang

Lidia Brades

Natalie Lavine

Additional Personnel
(Please list additional personnel here)

Fabiana Andrade Caetano

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. Give the full name - do not abbreviate.**
Approved Microorganisms	E.coli; DH5 - Alpha, RosettaBlue	
Approved Primary and Established Cells	[Primary] - (Rodent): Mouse Brain/. [Established] - (Human): HEK 293. (Avian) : DT - 40	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmids] - pcDNA, pGEX, pLB, pSMPUW, pRSV(Rev), pCgpV, pCMV(Eco)	pFUGW (lenti expression vector)
Approved Use of Animals		
Approved Biological Toxin(s)	Tetrodotoxin, Tetanus toxin, Botulinum neurotoxin A	

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF..*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: J MacDonald

Current Classification: 2 **Containment Level for Added Biohazards:** _____

Date of Last Biohazardous Agents Registry Form: Sep 8, 2009

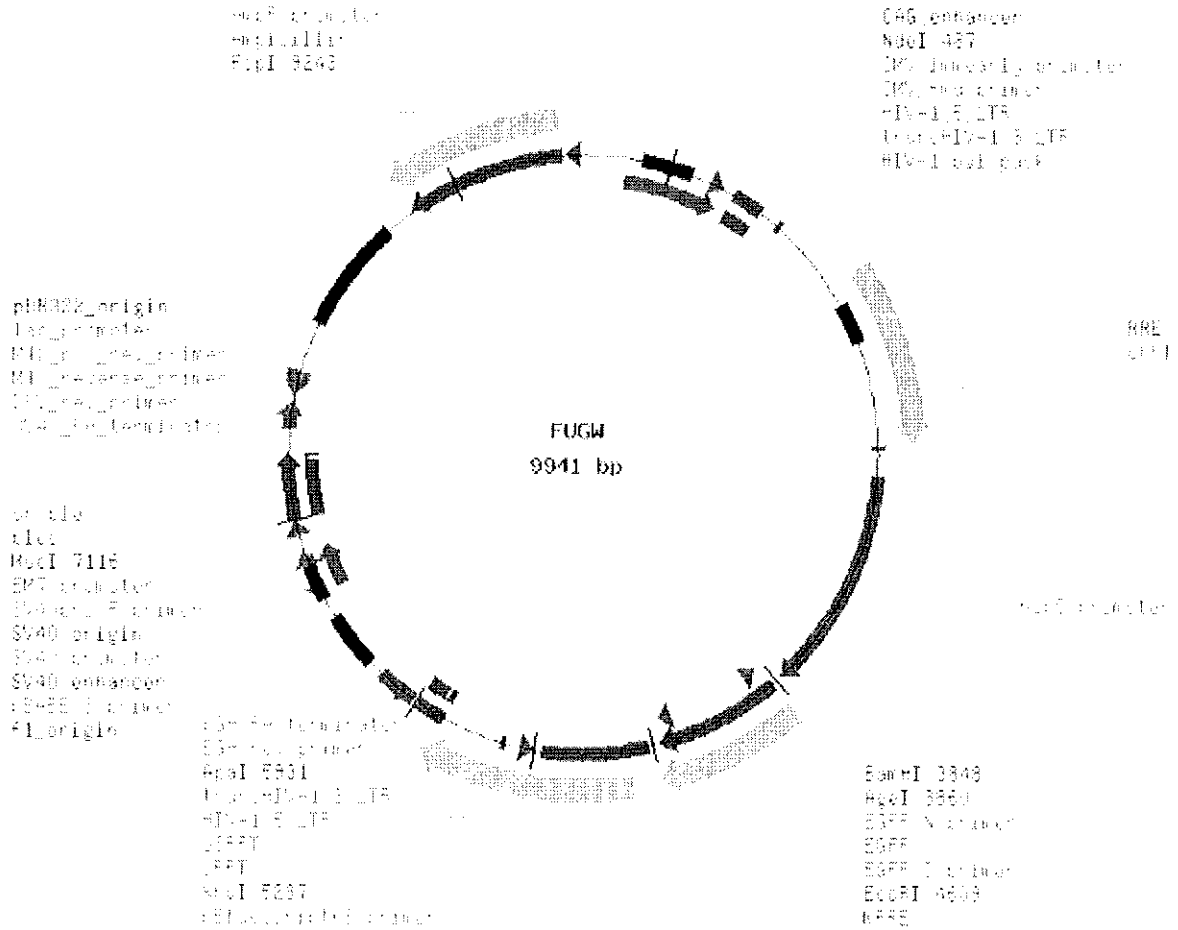
Date of Last Modification (if applicable): Aug 19, 2010

BioSafety Officer(s): Ronald Nozick NOV.25,2010

Chair, Biohazards Subcommittee: _____ **Date:** _____

We wish to add the plasmid pFUGW to our biohazard permit. Plasmid pFUGW is a transfer vector that will be used to express full length proteins after transduction within primary cultured neurons (mouse or rat). pFUGW will be packaged within replication deficient lentivirus following co-transfection of HEK 293 cells with packaging vectors (3rd generation) already listed on our biohazard permit (pRSV, pCgpV and pCMV(Eco)).

pFUGW map:



Modification Form for Permit BIO-RRI-0055

Permit Holder: John MacDonald

Approved Personnel
(Please stroke out any personnel to be removed)

Rohit Kesarwani

Gang Lei

Cristi Orth

Jillian Belrose

YuFeng Xie

Michael Jackson

Oies Hussein

Kai Yang

Lidia Brades

Natalie Lavine

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. Give the full name - do not abbreviate.**
Approved Microorganisms	E.coli; DH5 - Alpha, RosettaBlue	
Approved Primary and Established Cells	[Primary] - (Rodent): Mouse Brain/. [Established] - (Human): HEK 293. (Avian) : DT - 40	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmids] - pcDNA, pGEX, pLB, pSMPUW, pRSV(Rev), pCgpV, pCMV(Eco)	
Approved Use of Animals		
Approved Biological Toxin(s)	Tetrodotoxin	Tetanus toxin, Botulinum neurotoxin A

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.
** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF.

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: _____

Current Classification: 2 **Containment Level for Added Biohazards:** 2

Date of Last Biohazardous Agents Registry Form: Sep 8, 2009

Date of Last Modification (if applicable): Apr 9, 2010

BioSafety Officer(s): Ron Noseworthy J Stanley Aug 17/10

Chair, Biohazards Subcommittee: Susan Kovak **Date:** August 19, 2010

* Workers need to have their tetanus vaccinations up to date. Contact Workplace Health at x82047 for more information or to get an appointment.

Subject: Re: biosafety mod: MacDonald Lab
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Tue, 17 Aug 2010 09:48:50 -0400
To: Mike Jackson <mjackson@robarts.ca>
CC: Kristine Brown <kbrown3@uwo.ca>, S Siu <srscons@on.aibn.com>

Thanks Mike -
The people working with the toxin should be sure to have their tetanus vaccinations up-to-date (unless there are contraindications, etc). For more information, please contact Workplace Health.
Regards,
Jennifer

On 8/16/2010 10:11 AM, Mike Jackson wrote:

> Hi Jennifer,
>
> I got your message concerning our biosafety mod;
>
> In response to your questions: the personnel on the form lists everyone in the lab that may broadly have to deal with some elements of permit (ttx, lenti or TeTx, etc). Not all of those names will be specifically involved with experiments using tetanus toxin. For those, I would only list Natalie and Kai Yang, they will be the only users of tetanus toxin (at least initially).
>
> I can make sure their immunization is up to date,
>
> Let me know if you have any questions,
>
> take care, mike

Experimental plan:

The clostridial neurotoxins, tetanus toxin (TeTx) and botulinum neurotoxin A (BoNT/A), are endowed with metalloprotease activity targeting specific proteins involved in SNARE-dependent exocytosis. BoNT/A specifically cleaves the 25 kDa synaptosomal-associated protein (SNAP25) while TeTx targets the vesicle-associated membrane protein (VAMP)/synaptobrevin. Importantly, SNARE family proteins have been shown to contribute to the regulation of surface expressed excitatory amino acid neurotransmitter receptors (EEARs: including the AMPARs and NMDARs). TeTx and BoNT/A have therefore been successfully utilized as experimental tools to dissect the molecular mechanisms by which specific signaling pathways alter the surface expression of EEARs. We have recently demonstrated that Epac (exchange protein directly activated by cAMP) increases NMDAR current in acutely isolated cells. In addition, biochemical studies showed that Epac also enhances the surface expression of NMDAR. In order to investigate if NMDAR trafficking also contributes to the enhancement of NMDAR current in acutely isolated cells, we intend to pretreat acutely isolated cells with BoNT/A or Tetanus Toxin to cleave SNAP25 or VAMP2 respectively. These experiments will allow us to elucidate whether increased NMDAR surface expression, through SNARE-dependent exocytosis, contributes to the potentiation of NMDAR currents by Epac.

Handling, storage and use:

Protective clothing (lab coat, eye protection, gloves and masks) will be used at all times when handling TeTx or BoNT/A. Personnel will use proper glove removal techniques and hands will be washed after handling. The toxins, obtained as a lyophilized powder, will be reconstituted in a biological safety cabinet (BSC) by adding distilled water to the vial supplied by the manufacturer. Stock solutions will then be in stored at 2-8°C in a locked cabinet. A record of each use of the toxins will be maintained (noting the date, quantity used and signed by the user). After each use of the toxin, the BSC will be decontaminated using 70% ethyl alcohol. Spills will be handled according to procedures outlined in the University of Western Ontario biosafety guidelines and procedures manual. In brief, a paper towel will be placed over the liquid and a strong disinfectant will be poured around, but on the spill. The laboratory will be evacuated for a time expected to be sufficient for decontamination of the mixed material (~20 minutes). The paper will be placed in a bag for incineration.

The information relating to Section 8.0 of UWO's biological agents registry form on biological toxins is included below:

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? *YES* (If no, please proceed to Section 9.0)

8.2 If YES, please name the toxin(s): Tetanus *toxin (TeTx)_and Botulinum neurotoxin A (BoNT/A)*

Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD50 (specify species) of the toxin: *TeTx = <2.5 ng/kg (LD_{100}); BoNT/A = 1 ng/kg*

8.4 How much of the toxin is handled at one time*? As outlined in 8.5, after reconstitution, only single aliquots, containing no more than 100 ng of toxin, will be used at one time.

8.5 How much of the toxin is stored*? 10 µg (Note: this is the smallest quantity that can be purchased). After reconstitution, toxin stock solutions will be stored in screw cap vials with no more than 100 ng per vial.

8.6 Will any biological toxins be used in live animals? ***NO***

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

LIST BIOLOGICAL LABORATORIES, INC.
540 DIVISION STREET • CAMPBELL • CALIFORNIA 95008-6906 • USA
408-866-6363 • 800-726-3213 • FAX 408-866-6364 • EMAIL info@listlabs.com
WEBSITE www.listlabs.com

Product #190

MATERIAL SAFETY DATA SHEET
Tetanus Toxin

Hazardous Ingredients:

Tetanus toxin is a 150,000 dalton protein and is one of the most potent toxins known. The solvent is 20 mM HEPES, pH 7.4 and 1.25% lactose, which represents approximately 85% of the mass.

Physical Properties:

The product is provided as a white lyophilized powder. It forms a suspension in water, but is not completely soluble.

Fire and Explosion Hazard Data:

Tetanus toxin is combustible but not flammable. Use any commercial fire extinguisher.

Health Hazard:

The LD_{100} in unvaccinated humans is estimated at <2.5 ng/kg (Gill, D.M., *Microbiol. Rev.* **46**, 86, 1982). It is a powerful neurotoxin which may be fatal if inhaled or introduced into a wound. It causes muscle rigidity or spasms, paralysis, and death. If contact occurs, flush eyes, skin or wounds thoroughly with water. Seek medical attention, since supportive therapy will be required if symptoms occur. Immune globulin may also be a part of the medical treatment.

Reactivity Data:

This product is stable for years in the dried form, when stored at 4-7°C. No incompatibilities nor hazardous decomposition products are known. Hazardous polymerization will not occur.

(continued)

Spill or Leak Procedures:

If a spill occurs, cover with a damp cloth or paper towel. Wipe up and autoclave this material. Further, clean the area with 5% bleach. Solutions may be inactivated by boiling at 100°C for 30 minutes, or by autoclaving at 121°C and 15 psi for 15 minutes.

Special Protection Information:

Wear safety glasses, protective clothing, and rubber or latex gloves. When handling the product while in the lyophilized form, wear a face mask. Avoid inadvertent self inoculation when handling hypodermic needles. Do not pipette by mouth. Avoid inhalation of this product.

Special Procedures:

Persons handling this product and contaminated glassware should have a current tetanus vaccination, and their serum level should be greater than 1.0 international unit per milliliter.

This product is to be used by skilled personnel in a laboratory setting only. Good laboratory technique should be employed. This product is for research purposes only. It is not for use in humans and is not to be used as a diagnostic agent.

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WEBSITE www.listlabs.com

Product #130A, 130B

MATERIAL SAFETY DATA SHEET
Botulinum Neurotoxin A

Hazardous Ingredients:

Neurotoxin type A from *Clostridium botulinum* is a 150,000 dalton protein and is one of the most potent toxins known. This formulation also includes 1.25% lactose.

Physical Properties:

This product is provided as a white lyophilized powder. It is soluble in distilled water.

Fire and Explosion Hazard Data:

Neurotoxin type A is combustible but not flammable. Use dry chemical, halon, carbon dioxide, polymer foam or water fire extinguisher. Wear self-contained breathing apparatus and full protective clothing.

Health Hazard:

The LD_{50} in humans is estimated at 1 ng/kg (Gill, D.M., *Microbiol. Rev.* **46**, 86, 1982). It is a very potent neurotoxin which may be fatal if inhaled, ingested, injected, or introduced into a wound. The incubation period is usually 4 hours to 8 days. Symptoms include muscle weakness (especially in the face and neck early on, then the upper extremities, and finally the lower extremities), fatigue, dizziness, incoordination, an extremely dry mouth, blurred vision, sensitivity to bright lights, difficulty swallowing (drooling), difficulty speaking clearly (slurring), difficulty breathing, nausea, abdominal bloating, constipation, and difficulty urinating. There are no mentation nor sensory abnormalities and no fever.

If contact occurs, flush the eyes, skin, mouth (if conscious), or wounds thoroughly with water. For ingestion, dilute with water and induce vomiting. Seek medical attention, since supportive therapy will be required if symptoms occur. Immune serum (available from the U.S. Public Health Service, Centers for Disease Control, at 404-639-3311 days or 404-639-2888 after hours) may also be a part of the medical treatment.

(continued)

Reactivity Data:

This product is stable under normal handling procedures. No incompatibilities are known. Hazardous polymerization does not occur. Decomposition products include carbon monoxide and carbon dioxide.

Spill or Leak Procedures:

If a spill occurs, cover with a cloth or paper towel. Flood the area with a solution of 0.1 N NaOH. Wipe up and autoclave this material at 121°C and 15 psi for 15 minutes. Further, clean the area with a 5% bleach solution. Solutions may be inactivated by boiling at 100°C for 30 minutes or by autoclaving. Follow federal, state and local laws for disposal.

Special Protection Information:

Wear safety glasses, protective clothing, and rubber or latex gloves. Avoid inhalation when handling the product by using a glove box, biological safety cabinet, or OSHA approved respirator. Avoid inadvertent self inoculation when handling hypodermic needles. Do not pipette by mouth. Wash thoroughly after handling neurotoxin A.

Special Procedures:

Persons handling this product and contaminated glassware should be immunized with the pentavalent (ABCDE) botulinum toxoid which is available as an investigational new drug from the Centers for Disease Control.

This product is to be used by skilled personnel under the direction of a trained principal investigator in a laboratory setting only. Good laboratory technique should be employed.

This product is for research purposes only. It is not for use in humans and is not to be used as a diagnostic agent.

The above information is believed to be correct, but is not purported to be all inclusive. List Biological Laboratories, Inc. shall not be held liable for any damage resulting from the handling of this product.

Modification Form for Permit BIO-RRI-0055

Permit Holder: John MacDonald

Approved Personnel
(Please stroke out any personnel to be removed)

~~Michael Jackson~~
~~Oies Hussein~~
~~Hongbin Li~~
~~Xuanmao Chen~~
~~Bikram Sidhu~~
Kai Yang
Michelle Olah
Lidia Brades
Natalie Lavine

Additional Personnel
(Please list additional personnel here)

Yu-Feng Xie
Jillian Belrose
Cristi Orth
Gang Lei
Rohit Kesarwani

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	~~E.coli: DH5 - Alpha, RosettaBlue~~	lentivirus
Approved Cells	[Primary] - (Rodent): Mouse Brain/. [Established] - (Human): HEK 293. (Avian) : DT - 40	
Approved Use of Human Source Material		
Approved GMO	[Plasmids] - pcDNA, pGEX	- pLB, pSMPUW (lenti expression vectors) - pRSV(Rev), pCgpV, pCMV(Eco) (lenti packaging vectors)
Approved use of Animals		
Approved Toxin(s)	~~Tetrodotoxin~~	

PLEASE ATTACH A SEPARATE SAFETY DATA SHEET FOR EACH AGENT FOR NEW BIOHAZARDS
PLEASE ATTACH A BRIEF DESCRIPTION TO THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY ARE BEING USED

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: J J Thuett

Classification: 2

Date of Last Biohazardous Agents Registry Form: Sep 8, 2009

Date of Last Modification (if applicable):

BioSafety Officer(s): J Stanley Apr. 9, 2010 Ronald Moscarello Apr. 05/10 (Pending Level 2+ Inspection)

Chair, Biohazards Subcommittee: S.M. Calder

→ Pending Level 2+ inspection

→ Alternatives to bleach should be found - as bleach is corrosive to equipment such as biological safety cabinets

→ The portable autoclave must be used on site.

MacDonald Lab, RRI, Room 7260C-1

Proposed experimental use of lentivirus

Primary cultured neurons are notoriously difficult to transfect by traditional means (e.g. lipofectamine, $CaPO_4$, etc). Moreover, although these approaches may be successfully utilized, the efficiency achieved (usually ~2-3%) is insufficient for biochemical studies and the reliability of the procedure is too variable. In contrast, we have routinely achieved >80% efficiency using lentivirus-mediated gene transduction. We will generate lentivirus, utilizing the two expression vectors listed in the modification form, for shRNA-mediated gene silencing (pLB vector) or for overexpression of genetically modified proteins (pSMPUW) in primary murine cultured neurons. Proteins targeted for silencing include EPAC (exchange protein directly activated by cAMP), DISC1 (disrupted-in-schizophrenia 1), TRPM2 (transient receptor potential, melastatin 2) and NMDA receptor subunits (GluN1, GluN2A and GluN2B). For protein overexpression, HA- or FLAG-tagged TRPM2 will be expressed in our cultured neurons for immunostaining, immunoprecipitation and electrophysiological experiments.

Protocol for Handling Recombinant Replication-deficient Lentiviruses

Lentiviral vectors are different from the commonly used adenovirus based gene delivery systems because the gene of interest becomes stably integrated into the host cell's genome. The efficiency of lentiviral systems are due to the fact that they are actively imported into the nuclei of dividing, as well as non-dividing cells, as opposed to traditional retroviruses.

The lentiviral genome contains nine genes but only three of those are required to generate a replication-deficient virus. The three essential genes are Gag, Pol and Env and they can all be provided in trans. Gag encodes a capsid protein and Pol is required for the viral polymerase, RNase, protease and integrating functions. The Env, or, envelope gene encodes a transmembrane glycoprotein that also determines the tropism of the viral particle (ie. the specificity of the virus for a particular host cell). **In the ViraSafe Ecotropic Packaging system from Cell Biolabs, Inc. (which we will be using), the Env gene encodes a glycoprotein from Murine Leukemia Virus, thus providing a viral particle that can transduce only mouse and rat cells with high efficiency.** The remaining viral genome (*ie.* cis-elements only) are used to construct different Lentiviral cloning vectors and when the cloning vectors are transfected into packaging cell lines (usually 293 cells) also expressing the gag, pol and env protein in trans, replication-deficient Lentivirus particles can be generated that are carrying the gene of interest in the viral RNA genome.

Note! Only laboratory personnel that have been informed about safety precautions and working routines, and have permission from the person in charge are allowed to enter room 7260C-1 during Lentiviral work production. This also includes cleaners and service-personnel.

Principle:

All procedures for handling or manipulating Lentivirus should be carried out at Biosafety Level 2 (BL2) with the use of Containment Level 3 operational practices. All work will be done in a biological safety cabinet (BSC) by authorized personnel wearing coveralls, gloves, safety glasses and shoe covers (ie. full coverage protective clothing). Personal items (eg. purses) will not be brought into the containment room. All protective clothing will be removed upon completion of the work and left in the room or disposed of as waste (shoe covers, gloves). Protective items to be re-used will be autoclaved within room 7260C-1 using a portable autoclave. Coveralls will be kept on a coat rack within the containment room. No work with these viral vectors is permitted on the open bench.

Laboratory Facility:

The Principal Investigator has designated Room 7260C-1 for periodic lentiviral work, which contains a handwashing sink, biological safety cabinet (BSC), incubator, microscope, and CO2 source. This room is an inner lab with 2 doors between the BSC and the hallway and restricted entry to the lab. A sign stating that viral vectors are present, entry is restricted to authorized personnel, and doors are to remain closed will be posted on the laboratory door.

Working precautions for handling Lentivirus:

1. All experimental materials shall be handled with care.
2. The door to the containment room shall remain locked.
3. Within the BSC:
   a. For small quantities of low (cell lysate) and high (purified) titer Lentivirus, use sterile, aerosol barrier-containing pipette tips.
   b. For larger amounts (more than 1ml) of low titer lysates use sterile serological disposable pipettes.
   c. The maximum amount of infected growth media handled at one time should never exceed 500 ml.
4. Using a dunk tank, plastics will first be either filled (eg. pipet tips and serological pipettes) or rinsed (eg. plates and flasks) with Wescodyne Solution (20% Wescodyne/40% ethanol/40% water), drained, and then put into a high-density 4mil polyethylene plastic biohazard bag lined with a cardboard box prior to autoclaving.
5. Concentration of the viral particles will be done using Amicon Ultra-15ml 100k MWCO centrifugal filter devices. All centrifugation shall be done in closed buckets with aerosol-tight lids. Loading and unloading of samples into the sealed buckets will be done in the BSC. Buckets will be sprayed with 70% ethanol before removing from BSC.
6. Sharps shall be eliminated from experimental procedures to prevent injuries. No needles or Pasteur pipettes will be used in the production and use of lentivirus.
7. Gloves shall be worn at all times when working with viral vectors. Gloves will be sprayed with 70% ethanol and then removed by using the inside-out technique before disposing into biohazard waste to be autoclaved. Wash hands immediately after removing gloves and before

leaving work area. Never wear gloves outside of the laboratory, or touch things with gloved hands.

8. During any lentiviral work, signs and labels shall be placed to indicate each area where viral vectors are used and stored (BSC, incubators, freezer, laboratory entrance doors, etc.)

Decontamination and disposal procedures:

All materials that come in contact with viral particles must be properly decontaminated prior to disposal. Note that autoclaving of all materials will first be done within room 7260C-1 using a portable autoclave. Solid waste will then be autoclaved again through the central autoclave facility at Robarts.

1. ***Disposal/decontamination of solid waste such as, paper tissues, pipette tips, etc.***: All solid waste (including disposable plastic wares) should be discarded in biohazard bags for the appropriate treatment (autoclaving) according to institutional practices and guidelines prior to disposal.
2. ***Disposal/decontamination of liquid waste***: All liquid materials (Lentivirus-containing media, buffers, washes) should be decontaminated inside safety cabinet by addition of Sporgon Solution prior to autoclaving.
3. ***Work surfaces inside cabinets*** should be decontaminated with Sporgon Solution, followed by 70% ethanol.
4. ***Instruments, equipment*** and any other items that are not disposable and contact Lentivirus will be decontaminated with Sporgon Solution and/or autoclaved.
5. ***Routine laboratory cleaning*** will be done by lab personnel within the containment room.

Accidents:

Spills:

Effective disinfectants (10% bleach, Wescodyne or Sporgon Solution) will be made available in the laboratory at all times and for immediate use. In the event of a spill or container breakage resulting in the unintentional release of a biological agent:

(i) Place bleach soaked paper towel or absorbent on the liquid
(ii) pour a strong disinfectant solution (i.e. 10% bleach) around, but not on the spill, and mix the disinfectant with the spilled material cautiously;
(iii) evacuate the laboratory for a time expected to be sufficient for decontamination of the mixed material, normally 20 minutes;
(iv) pour a strong disinfectant solution (i.e. 10% bleach) around, but not on the spill, and mix the disinfectant with the spilled material cautiously;
(v) carefully place paper into a bag for incineration;
(vi) decontaminate all surfaces exposed to the spill with the disinfectant.

If aerosols may have been created in the spill or unintentional release, evacuate the laboratory for a time sufficient for most aerosols to settle, be dispersed, or removed by the ventilation system, usually 20-30 minutes. The use of respiratory protection should be considered for re-entry. Then proceed with items (i)-(v) above. During an emergency, the first priority is the protection of the health and safety of personnel, followed by the environment (i.e. sewer drains), followed by equipment or property.

Spills within a biological safety cabinet

- Leave the ventilation on
- All items within the cabinet should be disinfected (Walls and surfaces wiped down, equipment wiped down and/or autoclaved)
- Cover the spill area with paper towels or absorbent material
- Soak the spill area with an appropriate disinfectant (i.e. 10% bleach, Wescodyne or Sporgon Solution) Pour the disinfectant from the outside surface of the absorbent material towards the inside, surrounding the spill. Leave on for 20 to 30 minutes
- Pick up with absorbent material and place in biohazard bag to be then autoclaved
- Ventilation should run 10-15 minutes before continuing work in BSC

Spills within an incubator

- All shelves and walls within the incubator should be disinfected (walls and surfaces wiped down, and/or autoclaved)
- Cover the spill area with paper towels or absorbent material
- Soak the spill area with an appropriate disinfectant (i.e. 10% bleach, Wescodyne or Sporgon Solution) Pour the disinfectant from the outside surface of the absorbent material towards the inside, surrounding the spill. Leave on for 20 to 30 minutes (close the door of the incubator during the disinfection time)
- Pick up with absorbent material and place in biohazard bag to be then autoclaved
- Finish by wiping the incubator with 70% ethanol

Inhalation:

In case of inhalation, personnel should be directed to employee health for observation and maintained under medical surveillance. Cuts and abrasions should be treated as appropriate, according to their severity. Minor cuts should be treated with the Lab first Aid Kit (disinfectant wipe and band aid), otherwise personnel should be taken to emergency room for appropriate medical evaluation and care. Written records of all incidents should be maintained.

Eye exposure from splash or aerosol:

Rinse a minimum of 15 minutes in eye wash or flush with water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.

Skin exposure:

Contaminated skin should be scrubbed with germicidal soap and copious amounts of water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.